STUDIES ON MICROBIAL PRODUCTION OF CELLULASES BY FERMENTATION PROCESS

A THESIS

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by

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CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in this thesis entitled **"STUDIES ON MICROBIAL PRODUCTION OF CELLULASES BY FERMENTATION PROCESS"**, in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy and submitted in the Department of Paper Technology of Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out during a period from July, 2004 to July, 2011 under the supervision of Dr. M.C.Bansal, Professor and Dr.Vivek Kumar, Assistant Professor, Department of Paper Technology, Indian Institute of Technology Roorkee, Roorkee.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

(NITIN VERMA)

This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

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The Ph.D. Viva-Voce Examination of **Mr. NITIN VERMA**, Research Scholar, has been held on..Jan!!..20.12..

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ABSTRACT

Cellulases have a wide range of applications in various process industries. The major application of cellulases are in the biomass conversion industry for bioethanol production. Ethanol produced from renewable resources is being considered globally as the most prominent and possible substitute for fossil fuel. Today the production cost of bioethanol from lignocellulose is still too high. Cellulase production is the most expensive step during ethanol production from cellulosic biomass and it accounted for approximately 40% of the total cost. Therefore efforts are to be intensified to produce ethanol efficiently through improved and cost effective cellulase production technologies. Cellulase also plays a vital and integral role in waste paper recycling. Lignocellulosic biomass is the most abundant solid waste in the world thus it can provide some of the most promising feedstocks for the production of energy, enzymes and chemicals. Most microbial cellulases are inducible enzymes, and like other extracellular enzymes, they are secreted when the microorganisms are grown on cellulose or cellulosic components present in the raw materials. Inducers play a vital role in cellulase biosynthesis by microorganisms..

Three different types of morphology such as filamentous mycelia, pulpy and pelleted forms were observed with *T.reesei*, *N crassa* and *A. niger* fungal strains. 30^0C was found most effective temperature with majority of the fungal strains used in growth studies.

Maximum growth rate has been observed for *Aspergillus* strains with glucose (11.26g/L), xylose and maltose(10.68g/L) sugars, whereas *Trichoderma* strains showed highest growth in glucose(12.88g/L) and closely followed in lactose(10.83g/L) sugar.From the above experimental observation we suggests that sugar uptake capacity was much faster in *Neurospora crassa* for maltose, sucrose and glucose in comparison to lactose and xylose. *Trichoderma ressei* (9.88g/L)showed higher growth as well as *Aspergillus niger* (9.20g/L) exhibited highest growth and growth rate under whey based culture medium.on the other hand *Neurospora crassa* illustrated least growth and growth rate under such industrial waste containing culture medium.

On the other hand it has been observed that growth and growth rate was much better in boiled bagasse containing medium for most of the culture excluding *T. viride*(6.28g/L). The sugars present in these industrial waste were utilized by fungal strains makes the culture development process more cost effective and environment friendly.

Significant improvement in the cellulases activities have been achieved with *T.reesei, N.crassa* and *A. niger* respectively using experimental design or set of combinations of process parameters. It has

also been observed that inoculum dosages was the least effective parameters in most of the cases, while temperature was found much effective parameters in most of the cases.

Maximum cellulase activity was observed with cellulose sugar while least cellulase activity was observed with arabinose sugar by *T.reesei.*

Higher cellulase activity was observed with meat peptone, proteose peptone and yeast extract for *Aspergillus* strains while *N.crassa* showed higher activities with meat extract and proteose peptone.On the other hand higher cellulase activity was observed with Oil of olive and Tween 80 surfactants while least cellulase activity was observed with Triton X-100 and oil of castor.

Higher cellulase activity was observed with 30% dosages of whey while increasing the dosages cellulase activity was not significantly increases with *T.reesei*, while *N.crassa* showed least cellulase activity under whey based medium.

Higher cellulase activity was observed with wheat starch hydrolysates mixed with 30% whey based by *Trichoderma reesei* ,while potato starch hydrolysates was found much effective with *N.crassa.* On the other hand boiled bagasse and bagasse hydrolysates containing medium showed significant activity with *T.reesei* while wheat straw hydrolysates was found not much effective for most of the strains.Wheat bran bed was found much effective bed material followed by ground nut shell bed.

For the effective utilization of few conventional raw materials acid or alkali pretreatment technology have been introduced. Alkali treatment was found much effective in cellulase production with bagasse raw material Mixed raw material strategy has been also used for cellulase production. Improvement in cellulase activities was observed with (B+GSW+WB) combination as compared to bagasse was taken singly.

Mixed microbial cultures strategies (*Trichoderma* and *Aspergillus* strains) have been used for the production of complete set of cellulases. It was observed that *T.reesei* and *A.niger* (1:1) was found much effective combination to produces significantly higher cellulase and cellobiase activity.

Higher cellulase activities were observed with proteose peptone and yeast extract for *Aspergillus* strains while meat extract and proteose peptone were much effective for *N.crassa.*It has also been observed that higher dosages of nitrogen sources was not much effective in wheat bran and GSW bed as compared to bagasse and wheat straw solid bed. Keeping the view of waste management acid treated egg shell waste has been used as calcium salts in cellulase production. Significant cellulase activity was observed with *N.crassa* strain having egg shell waste containing media.Higher increment in cellulase activities was observed with wheat starch hydrolysates for *T.reesei* and *A.niger* while *N.crassa* showed significant activity with potato starch hydrolysates.5%

dosages of hydrolysates was found much effective for most of the strains. 5% H_2SO_4 treated wheat straw as well as 10% H_2SO_4 treated bagasse hydrolysate were found much effective for microbial growth and cellulase production.Cellulase activity was significantly higher for sugarcane bagasse than wheat straw hydrolysates used. Mutagenic agents such as UV and Etbr on microbial cultures growth, sustainability as well as on cellulase production have been investigated. Significant improvement in growth was observed with mutant, while slight improvement in cellulase activities were also observed. Unconventional and novel raw materials such as pea peel waste and others peel wastes have been used in solid state fermentative production for making cost effective and efficient approach. It has been observed from the experimental results that *Trichoderma* strains produces significant cellulase activity (2.86IU/mL) with non conventional raw materials such as pea peel waste.It was observed that incorporation of whey and starch hydrolysates significantly enhances the cellulase activities with *T.reesei* compared to other strains.*Luffa cylindrica* peel bed was found quite effective raw materials as compared to others.

Cellulase production by *Aspergillus niger* strain using surface adheshion fermentation (biofilm fermentation) was investigated. Significant improvement in the cellulases were observed with muslin cloth with 3 hole and polyester sheet based biofilm fermentation as compared to liquid state fermentation without solid support.

It has been also observed that cellulases from *T. viridae* was found better strains for the enzymatic deinking with their better treated handsheet properties in terms of brightness and tensile strength as compared to chemically deinked pulp.

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RESEARCH PUBLICATIONS

(A) Journal:

International (Published):

1.Nitin Verma,Mukesh C.Bansal, Vivek kumar, *Pea peel waste : A lignocellulosic waste and its utility in cellulase production by Trichoderma reesei under solid state cultivation* ***,Bioresources, ISSN1930-2126, (SCI Indexed) ,6(2),*** **2011,** 1505-1519.

2.Nitin Verma,M.C.Bansal,Vivek Kumar,*Studies on pelleted form of growth morphology achieved by Aspergillus strains with different sugars treatment under submerged cultivation,****Journal of Pure and Applied Microbiology, ISSN 0973-7510, (SCI Indexed)*** Vol-3(2),Oct-2009,559-565.

3.Nitin Verma,M.C.Bansal,Vivek Kumar,*Comparative studies on biofilm development by Aspergillus niger on polyester sheet and muslin cloth,,* ***Journal of Biochemical Technology,ISSN 0974-2328,*** Vol3(2),Aug 2010.

4.Nitin Verma, M.C.Bansal, Vivek Kumar,*Mathematical modelling aspects of Trichoderma reesei system:A review*, ***Chemical Engineering Transactions, ISSN 1974-9791,***Vol-14,2008,137-144 .

5.Nitin Verma,M.C.Bansal, Vivek Kumar,*Protoplast fusion Technology and Its Biotechnological applications*, ***Chemical Engineering Transactions, ISSN 1974-9791,***Vol-14,2008,113-120.

6. Nitin Verma, Mukesh C.Bansal, Vivek kumar, *Scanning electron microscopic analysis of industrially and environmentally significant Aspergillus niger growth under various process conditions,****International Journal of Microbiological Research, ISSN 2079-2093,****2(1)8-11,2011.***

7. Nitin Verma,M.C.Bansal,Vivek Kumar, Enzymatic and other recent approaches in waste paper recycling, ***Journal of Industrial Research and Technology(Malaysia)***, Vol1,2011.

8. Nitin Verma,M.C.Bansal,Vivek Kumar,*Utilisation of waste news paper hydrolysates in growth and production system of Neurospora crassa* ***,International Journal of Applied Environmental Sciences(IJAES), ISSN 0974-0260,***Vol 5,No 3,2010,331-335.

International (Accepted):

1. Nitin Verma, Vivek Kumar,M.C.Bansal, Utilisation of egg shell waste in cellulase production by Neurospora crassa under wheat bran based solid state cultivation, ***Polish Journal of Environmental studies,(SCI Indexed) ISSN 1230-1485,*** **13 pages.**

2.Nitin Verma,M.C.Bansal,Vivek Kumar,*Enzymatic deinking with cellulases:A review,****Journal of Solid Waste Management and Technology(USA) ISSN: 1088-1697,*** Published in 2011,10-11 (approx).

3.Nitin Verma,Mukesh C.Bansal, Vivek kumar. *Enzymatic deinking of old news paper by cellulases produced by various fungal strains,* ***Journal of Pure and Applied Microbiology, ISSN 0973-7510,(SCI Indexed),*** Vol-5(2),2011.

4. **Nitin Verma**,M.C.Bansal,Vivek Kumar, *Effects of sugars replacement on the growth of Aspergillus and Neurospora strain under submerged cultivation*, ***Journal of Pure and Applied Microbiology, ISSN 0973-7510,(SCI Indexed)***, Vol-5(1),2011.

International (Communicated)

1.Nitin Verma, Vivek Kumar,M.C.Bansal, *L.cylindrica* an agricultural waste and its utility in cellulase production,***Industrial Crops and Products***,2011,15 pages.

2.Nitin Verma,Mukesh C.Bansal, Vivek kumar,Fermentative production of cellulases by renewable bioresources,***Forestry Studies in China***,2011,16 pages.

3.Nitin Verma, M.C.Bansal, Vivek Kumar, Utilisation of Industrial waste in growth and production system of *Neurospora* and *Aspergillus* strains, ***Journal of Industrial Research and Technology(Malaysia)***,2011, 6 pages.

(B)Article in Book (Published)

1.Nitin Verma, Mukesh C.Bansal, Vivek Kumar, *Pretreatment Technology:An approaches for effective utilization of lignocellulosic biomass.****Industrialisation, Ecofriendly Industries and Climate Changes,,ISBN*** **978-81-7625-627-8**., Apr 2010,Published by Sarup and Sons, New Delhi.

2.Nitin Verma,M.C.Bansal,Vivek Kumar, Effect of different fermentation vessels on the growth and production system of *Aspergillus niger* under solid state cultivation,113-117, Published in ***Advances in Chemical Engineering ,MacMillan Advanced Research Series,ISBN CORP000185,MacMillan Publishers India Pvt Ltd.***

3.Nitin Verma,M.C.Bansal,Vivek Kumar, Effect of surfactants on the growth and production system of *Aspergillus niger* and *Neurospora* strains under submerged condition,118-124, ***Advances in Chemical Engineering ,MacMillan Advanced Research Series,ISBN CORP000185,MacMillan Publishers India Pvt Ltd.***

4.Nitin Verma,M.C.Bansal,Vivek Kumar,C.B.Mazumder, Effect of moisture percentage on *Aspergillus niger* and *Neurospora* strains growth and production system using wheat bran solid support,144-148, ***Advances in Chemical Engineering ,MacMillan Advanced Research Series,ISBN CORP000185,MacMillan Publishers India Pvt Ltd.***

5. Nitin Verma, Vivek Kumar, M.C.Bansal, Influence of nitrogen sources on the growth and production system of *Aspergillus* strains under submerged cultivation,125-129, ***Advances in Chemical Engineering ,MacMillan Advanced Research Series,ISBN CORP000185,MacMillan Publishers India Pvt Ltd.***

(C)Conferences/Symposia/Seminar
(In Proceedings)
International (Published):

1.Verma Nitin,Kumar Vivek,Bansal M.C.,*Importance of Cellulases in Bioethanol from Biomass:A review,*pp 208-219,***Proceedings of 5th International biofuels conference,***held on Feb 7-8,2008,organized by ***Winrock International ,New Delhi,India.***

2.Nitin Verma, Mukesh.C.Bansal, Vivek Kumar, *Growth and Morphological Studies of Trichoderma and Aspergillus and its coculture for effective utilization of lignocellulosic biomass"* , 77-81,***Proceddings of International Conference on Challanging Environmental trends and Sustainable Environment (CETAS-2009)*** held at ***Environmental Science and Enginerring Department,GJUS& T,Hisar.***

3.Nitin Verma, M.C.Bansal, Vivek Kumar, *Comparative Batch Growth Kinetic Studies of Trichoderma,Aspergillus and Neurospora strains under Submerged Cultivations,*892-897, ***Proceedings of International conference on Emerging Technologies in Environmental Science and Engineering*** ,held on Oct 26-28,2009,organized by ***Aligarh Muslim University (AMU)India in collaboration with Toledo University,U.S.A.***

4.Nitin Verma, Vivek Kumar, M.C.Bansal, Various *Inducers involved in environmental viable cellulase biosynthesis* ,1690-1695,***Proceedings of International conference on Emerging Technologies in Environmental Science and Engineering*** ,held on Oct 26-28,2009,organized ***by Aligarh Muslim University (AMU)India in collaboration with Toledo University,U.S.A.***

5.Nitin Verma,Vivek Kumar,Mukesh C.Bansal,*Studies on development of biofilm on muslin cloth by Aspergillus niger,*880-884,***Proceedings of International Conference on Recent Advances in Environment Protection(RAEP-2009),***held on Dec 17-19,2009 at Agra.

6.Nitin Verma, ,M.C.Bansal, Vivek Kumar, *Bagasse hydrolysate:Utility in Trichoderma growth and production system,*885-889,***Proceedings of International Conference on Recent Advances in Environment Protection(RAEP-2009),***held on Dec 17-19,2009 at Agra.

7.Nitin Verma,Vivek Kumar,Mukesh C.Bansal,*Utility of egg shell wastes in environmentally important Neurospora crassa growth system,*890-894,***Proceedings of International Conference on Recent Advances in Environment Protection(RAEP-2009),***held on Dec 17-19,2009 at Agra.

8.Nitin Verma, Vivek Kumar, M.C.Bansal ,*Fungal Cellulases: Production ,Applications and Recent Approaches,*717-720, ***Proceedings of International conference on Emerging Technologies for Sustainable Environment,*** held on Oct 29-30,2010,organized ***by Aligarh Muslim University (AMU)India in collaboration with La Sierra University, U.S.A. and Asia Pacific International University, Thailand.***

9. M.C.Bansal,Vivek Kumar, **Nitin Verma.,** *Studies on effect of pretreated starch on Aspergillus niger system,*721-723, ***Proceedings of International conference on Emerging Technologies for Sustainable Environment*** ,held on Oct 29-30,2010,organized ***by Aligarh Muslim University***

(AMU)India in collaboration with La Sierra University, U.S.A. and Asia Pacific International University, Thailand.

10.Nitin Verma,M.C.Bansal,Vivek Kumar, *Utilization of wheat straw hydrolyzates in Trichoderma reesei growth and production system,*714-716, ***Proceedings of International conference on Emerging Technologies for Sustainable Environment,*** held on Oct 29-30,2010,organized ***by Aligarh Muslim University (AMU)India in collaboration with La Sierra University, U.S.A. and Asia Pacific International University, Thailand.***

11. Nitin Verma,M.C.Bansal,Vivek Kumar, *Growth and morphological studies of Trichoderma strains under submerged cultivation,*711-713,***Proceedings of International conference on Emerging Technologies for Sustainable Environment*** , held on Oct 29-30,2010,organized ***by Aligarh Muslim University (AMU)India in collaboration with La Sierra University, U.S.A. and Asia Pacific International University, Thailand.***

National (Published):
(In Proceddings)

1.Nitin Verma,Mukesh.C.Bansal,Vivek Kumar.,*Waste Paper Recycling:An Enzymatic Approach for Effective Environmental Management,*249-255,***Proceedings of All India Seminar on "Advances in Environmental Science and Technology"*** ,held on Feb15-16 organized by ***Institution of Engineers (India),Aligarh Local Centre,Aligarh and Aligarh Muslim University(AMU),Aligarh,U.P.,India.***

2.NitinVerma,Vivek Kumar, Mukesh C.Bansal, *Application of Trichoderma,Aspergillus and Neurospora in Bioremediation Process: A review* ,363-371,***Proceedings of All India Seminar on "Advances in Environmental Science and Technology"*** ,held on Feb15-16 organized by ***Institution of Engineers (India),Aligarh Local Centre,Aligarh and Aligarh Muslim University(AMU),Aligarh,U.P.,India.***

3.Nitin Verma,Mukesh.C.Bansal,Vivek Kumar.,*Solid state fermentation :A Generalized and Engineering Aspect* ,355-362,***Proceedings of All India Seminar on "Advances in Environmental Science and Technology"*** ,held on Feb15-16 organized by ***Institution of Engineers (India),Aligarh Local Centre,Aligarh and Aligarh Muslim University(AMU),Aligarh,U.P.,India.***

4.Nitin Verma, Vivek Kumar, M.C.Bansal, *Growth Studies of Neurospra crassa for Effective Utilisation of Lignocellulosic Waste Biomass,*126-131,***Proceedings of National Conference on "Recent Advances in Waste Management,*** held at ***Chemical Engineering Department of Institute of Technology ,Banaras Hindu University(BHU),Varanasi*** on Feb,20-21,2009.

5.Nitin Verma , M.C.Bansal, Vivek Kumar, *Waste Paper Recycling with Cellulases : An Effective and Environmental Friendly Approaches for Solid Waste Management,*118-125,***Proceedings of National Conference on "Recent Advances in Waste Management"*** held at ***Chemical Engineering Department of Institute of Technology ,Banaras Hindu University(BHU),Varanasi*** on Feb 20-21,2009.

6. **Nitin Verma,**M.C.Bansal,Vivek Kumar,*Effects of mutagens on growth of environmentally important Neurospora and Aspergillus strains,148-150,* ***Proceedings of National conference on environmental degradation:Effects ,controls and remedies(EDECR),held on Feb-25-27 at Sirsa(Haryana),India.***

7.**Nitin Verma,** Vivek Kumar, M.C.Bansal,*Effects of wheat bran particle size and their combinations on the growth and production system of Aspergillus niger,124-125,* ***Proceedings of National conference on environmental degradation:Effects ,controls and remedies(EDECR),held on Feb-25-27 at Sirsa(Haryana),India.***

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CHAPTER I
INTRODUCTION

Cellulases are the multi component enzymes which act synergistically for the complete hydrolysis of cellulose. Cellulases are currently the third largest industrial enzymes worldwide. There is an increasing demand for the cellulases in the market for various application. Cellulase production is a major area of research globally. The demand for cellulases is consistently on the rise due to its diverse applications in various process industries such as textiles, detergent, juice and beaverge, bioconversion as well as pulp and paper industry. There are several companies involved in cellulase production for textile detergent, paper and feed industries. Globally, there are two major players known for cellulase production for biomass conversion "Genencor" and "Novozyme". Both the companies have played a significant role in bringing down the cost of cellulase several folds by their active research and are continuing to bring down the cost by adopting novel technologies. World wide consumption of cellulase from submerged fermentation is roughly 23000 tons annually. Cellulases are accounted for approx 20% of world enzyme market. The demand for highly active enzymes is growing rapidly and projected world market for industrial enzymes is rapidly growing at an annual rate of about 7.6% and is estimated to be $ 6 billion by 2012.

Lignocellulosic biomass is the most abundant solid waste in the world, thus it can provide some of the most promising feedstocks for the production of energy, enzymes and chemicals. It generally contains up to 75% of cellulose and hemicellulose, which are the potential source for the production of glucose and other chemicals. Most microbial cellulases are inducible enzymes, and like other extracellular enzymes, they are secreted when the microorganisms are grown on cellulose or cellulosic components present in raw materials. Cellulose is a linear homopolymer of anhydro D glucose units linked together by β-1,4 glucosidic bonds. Hydrogen bonding between cellulose molecules results in the formation of highly ordered crystalline regions that are not readily accessible to water. The crystalline cellulose can account for approximately 50–90% of the total cellulose, the remainder being composed of more disorganised amorphous cellulose. Hemicellulose is a heterogeneous polymer of the lignocellulosic matrix.The most common hemicellulose pentosans found in straws and grasses are xylan, arabino-4-*O*-methyl-glucurono-xylan, arabino-glucuronoxylan, arabino-xylan, glucurono-xylan and galacto-arabino- glucorono xylan. The primary hemicellulose hexosans found in herbaceous plants are mannans and glucans. The hemicellulose forms hydrogen bonds with the cellulose microfibrils, increasing the stability of the cellulose–hemicellulose– lignin matrix. Lignin, the third most common polymer found in plant

cell walls, functions as a binding and encrusting material. The lignin composition varies depending on the source. For example, the lignin in gymnosperms is composed of dehydrogenation polymers of coniferyl alcohols, while angiosperm lignin contains mixed dehydrogenation polymers of coniferyl and sinapyl alcohol. Commercial bioconversion of lignocellulosic waste requires the most economical methods of cellulase production.

Cellulases are multi component enzyme systems which act synergistically for the complete hydrolysis of cellulose and they belong to glycosyl hydrolase (EC 3.2.1.X) family. Cellulase enzyme system consist of at least three enzymes: Endoglucanase or CMCase (E.C. 3.2.1.4), Exoglucanase or cellobiohydrolase or Avicelase (E.C. 3.2.1.91) and β- glucosidase or cellobiase (E.C. 3.2.1.21).

A general feature of most cellulases is a modular structure often including both catalytic and carbohydrate binding modules (CBMs).The CBMs effect binding to the cellulose surface, presumably to facilitate cellulose hydrolysis by bringing the catalytic domains in close proximity to the insoluble substrate cellulose. Both bacteria and fungi can produce cellulases for hydrolysis of lignocellulosic materials. These microorganisms can be aerobic, anaerobic, mesophilic, thermophilic. Bacteria produces cellulase in smaller amount (<0.1g/l) and degrade cellulose by the action of cell bound enzyme whereas fungi secrete most of the enzyme into surrounding growth medium by utilizing cellulose as an energy source. Bacterial cellulase system differs from fungal cellulase, by forming aggregates of multienzyme system complex called cellulosomes on their cell surface rather than existing as individual extracellular enzymes. Fungal cellulases often act in a strongly synergistic manner. *Trichoderma*, *Aspergillus* and *Neurospora* play a vital and significant role in environmental waste managment through the production of a wide range of commercial enzymes namely, cellulases, hemicellulases, and β-1,3-glucanase. The best characterized and most widely studied organisms for cellulase systems are the fungus *Trichodermma reesei* and *Aspergillus niger*. Cellulase from *T.reesei* produces at least two exoglucanases (CBHI and CBHII), five endoglucanase (EG I-V) and two β –glucosidases. CBHI and CBHII are the principle components of *T.reesei* cellulase systems representing 60 and 20 % of the total cellulase produced by the fungus on a mass basis. EGI and EGII are the major species and represent less than 20% of total cellulase produced by the fungus on a mass basis. *T reesei* produces β-glucosidases at low levels compared to other fungi such as *Aspergillus*. *Aspergillus* sp are known to be good producers of β-glucosidase. *Neurospora crassa* was recently reported to produce high yields of CMCase and β-glucosidase when grow in solid substrate Cellulase produced by filamentous fungus have a high

industrial interest. Therefore it is necessary to study the growth rate and their morphological pattern of cellulase producing fungus. Due to the filamentous and non homogenous nature of the moulds growth, the analysis of the growth characteristic and growth curve is very difficult. Due to all these complications, it is better to estimate fungal growth in terms of cell dry weight (cell biomass). The fungal morphological form vary from mycelial pulpy to pelleted structure.

Inducers play a vital role in cellulase biosynthesis by microorganisms. Sugars, surfactants and various other components have been reported as inducers in cellulase biosynthesis and numbers of inducers are being searched for making cellulase production process more cost effective. Synthesis of cellulase can be induced by many oligomeric and dimeric sugars. Cellulose and cellulosic components in the lignocellulosic substrate are essential for the formation of m–RNA to form cellulases at the transcription level. Glucose on the other hand represses cellulase synthesis by a catabolite repression mechanism at the translational level. Bacterial cellulase are enzyme produced constitutively whearas fungal cellulase is produced only in the presence of cellulose as a substrate. Cellulose itself is practically unable to trigger the induction because of its low solubility, therefore a basal level of constitutive cellulase might degrade cellulose into soluble cellooligosaccharides thereby allowing these sacharides to enter the cell and can be converted into an inducer that triggers induction.

Cellulase can be produced either by solid state fermentation (SSF) or submerged fermentation (SmF). The most important advantage associated with the SSF as compared with SmF are improved product recovery, reduced energy requirement and the simplicity of the equipments used. Surface adhesion fermentation, a different approach has been recently used in cellulase production. Filamentous fungi are naturally adapted to growth on the surfaces and in those conditions they showed a particular physiological behavior, which is different to that in submerged cultures, thus they can be considered as biofilm forming organisms. Although fungal biofilms are less known than the bacterial biofilms, they can be used for cellulase production as it has been recently reported. Both SSF and biofilm fermentation depends upon surface adhesion, hence a new fermentation category named as surface adhesion fermentation (SAF) was established and first introduced by Gutieroz correa and Villenea.

Although cellulose forms a distinct crystalline structure, cellulose fibers in nature are not purely crystalline. Crystalline cellulose is highly resistant to microbial attack and enzymatic hydrolysis, whereas amorphous cellulose is degraded at much faster rate. As crystallanity increases, cellulose becomes increasingly resistant to further attack. The rate of microbial hydrolysis of cellulose is

dependent upon several structural features of the cellulose, which affect the rate of hydrolysis such as crystallanity of cellulose, molecular structure of cellulose, surface area of cellulosic fibers, degree of swelling of crystalline fibers, degree of polymerization, protection of cellulose by lignin, the heterogenous character of the biomass particles and cellulose sheathing by the hemicellulose associated lignin or other materials. Due to this structural complexity of the lignocellulosic matrix, cellulase production from raw materials requires effective pretreatment. The pretreatment results in enlargement of the inner surface area of substrate particles, accomplished by partial solubilization and/or degradation of hemicellulose and lignin. This causes the swelling and fractionation of the three components leading to an increase in the internal surface area, opening of cellulose structure, increase of porosity, separation of structural linkage between lignin and carbohydrates, disruption of lignin structure as well as removal of lignin and hemicellulose, reduction of cellulose crystallanity can significantly improve the microbial hydrolysis. Effective pretreatment must meet the many important requirements such as process should improve the formation of sugars or the ability to subsequently form sugars by enzyme hydrolysis, should avoid the degradation or loss of carbohydrate, should avoid the formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes and should be cost effective. Few literatures have reported the utilization of hydrolysate for growth and production system of microbes. Keeping in view the complete utilization of lignocellulosic biomass, the pretreated solid biomass have been used as solid bed for cellulase production and remaining hydrolysates after detoxification is also used for cellulase production under submerged condition.

Soluble carbon sources such as lactose, cellobiose or whey give significantly lower cellulase activity compared to pure insoluble cellulosic substances such as cellulose, avicel, solka floc at the same concentration. For large scale production of enzymes pure cellulose would be too much expensive, therefore lignocellulosic waste materials such as wheat bran, wheat straw, rice straw, baggase, vegetable peels etc, would be preferred substrate and carbon source because they are relatievely cheap and are easily available.

Dairy industry is another example of process industry where a number of effluents are generated, some of which contain nutritionally valuable constituents such as proteins, lactose and fats etc. One such effluent is whey. Due to the higher biochemical oxygen demand (BOD_5) of whey, it poses a major world-wide disposal and pollution problem, thus an effective and permanent solution is urgently needed. The utilization of whey as an inducer in the cellulase production as well as its utility in fungal growth could make the cost effective process. As suggested in the literature, the

water-soluble acid hydrolyzed starch also serves as an excellent inducer for the growth and cellulase production. It induces the enzymes to the same extent as pure cellulose. Since pure starch is expansive therefore the utilization of waste starch hydrolysates would be a great approach in cellulase production. Keeping the view of waste management process, utilization of acid hydrolysates of egg shell waste in cellulase production could be a novel approach.

There are various factors which directly or indirectly affect the microbial growth and enzyme production. Temperature is the critical and crucial parameter that has to be controlled and varied from organism to organism. Studies indicate that the most favorable temperature for cellulase producing microorganism is within the range of 25^0-35^0C but few organisms perform best at elevated temperatures from 37^0-45^0C. pH is yet another vital parameter for enzyme production. Most microorganisms are able to grow in a range of pH 5-7. The size of inoculum seems to have a profound effect on microbial growth and enzyme production. Inoculum size influences the carbon and nitrogen utilization in the medium by microbial sources. Moisture content is a critical factor on SSF processes because this variable has influence on the growth, biosynthesis as well as secretion of enzymes. Fungi may grow at very low levels of air humidity if water is available on the surface.

The demand for cellulases is consistently on the rise due to its diverse applications in various process industries such as textiles, detergent, juice and beaverage, bioconversion as well as pulp and paper industry. There are several companies involved in cellulase production for textile detergent, paper industries and other industries.

Table 1.1 List of cellulases manufacturers

Company	**Location**	**Trade name**
Worthington Biochemical Corporation	New Jersey,USA	-
Novo Nordisk A/S	Bagsvaerd, Denmark	Celluclast
Biocon Limited	India	Biocellulase
Amano Enzyme Inc	Nagova,Japan	Amano
EDC Enzyme Development	New York,USA	Enzeco cellulase
Rohm GmbH	Darmstadt, Germany	Rohament CT,CA,CW
Iogen Corporation	Ottawa,canada	-
Rohm Finland	Finland	-

Due to the accelerating accumulation of CO_2 and other green house gases may lead to adverse climatic changes that would seriously endanger the sensitive ecological balance of the earth. Energy shortages in the world coupled with environmental considerations have directed applied research towards the development of newer processes to produce renewable fuels with a special emphasis on fuel ethanol production from cellulosic materials. Today the production cost of bioethanol from lignocellulose is still too high. According to literature cellulase production is the most expensive step during ethanol production from cellulosic biomass and it accounted for approximately 40% of the total cost. Significant cost reduction is required in order to enhance the commercial viability of cellulase production technology. Therefore efforts are to be intensified to produce ethanol efficiently through improved and cost effective cellulase production technologies.

On the other hand fast depletion of forest resourses, its impact on the ecological balance, galloping rise in prices of fibrous raw materials and waste disposal problem have forced the paper industry to turn to the utilization of secondary fibers or waste paper. Waste paper is the single largest component of the solid waste stream and has a great effect on the environment. Recycling of paper not only saves energy and forest recourses for pulping and paper making but also reduce the cost of waste disposal. Cellulase plays a vital and integral role in waste paper recycling. Cellulases are considered to alter fiber surfaces by chemical bond modification in the vicinity of ink particles, there by ink particle on the surface gets released by washing and flotation. Cellulases also peel fibrils from fiber surfaces, thereby freeing ink particles for dispersal in suspension. This peeling mechanism has also been implicated as the pulp freeness increases after enzymatic treatment of secondary fibers. Most commercial cellulases used for deinking are a mixture of several components, therefore the deinking effect of commercial cellulase on recycled paper has not been optimized. Compared with conventionally deinked pulp, we observed that cellulolytic deinked pulp will lead to improved pulp and paper properties, lower residual ink contents, better machine runnability, reduced power consumption. A proper combination of enzymes depending upon the type of waste paper processed under proper operating conditions shall give better results.

Application of mixed cultures is an another strategy to improve the cellulase activity. The mixed culture of *T.reesei* and *A.phoenicus* could produce complete set of cellulase with a high level of β-glucosidase. Mixed cultures of *T.reesei* and *A.niger* inoculated at the same time gave hypercellulase activity. The highest filter paper and β-glucosidase activity in mixed culture representing approx 3 and 6 fold increase over the activities attained in single culture SSF. Strain improvement has been achieved by mutation, selection or genetic recombination .Strain

improvement is a traditional method used with great success for isolating mutants with high productivity. In many cases mutations are harmful but occasionally it may lead to better adopted organisms to its environment with improved biocatalytic performance.

Keeping in view the high demand of cellulase in various process and bioconversion industry, effective, efficient and economical production of cellulases is of paramount importance. Utilization of cheaper, abundantly available lignocellulosic biomass for cellulase production, becomes the promising alternative to make the process cost effective and environmentally viable. Therefore intensive search is being carried out for cheaper carbon source such as non conventional raw materials, vegetable peels as well as novel inducers such as whey, boiled baggase acid and alkali treated hydrolysates could be utilized in cellulase biosynthesis. The production cost and low yields of cellulases are the major impediment for industrial application. To overcome these problems, much work has been emphasized on using cheap, cost effective and abundantly available raw materials with improved fermentation conditions.

The present research project comprises of cost effective and efficient cellulase production technology and their application in waste paper recycling. Keeping the view of high demand of cellulases in various process industries, their cost effective production is utmost importance. In the present research work following experimental strategies have been used in microbial production of cellulases.

To evaluate the growth rate and their morphological pattern of cellulase producing fungus, fungal growth has been estimated in terms of cell dry weight (cell biomass) utilizing pure sugars as well as waste material hydrolysates (Dairy waste whey and boiled bagasse).

To perform the optimization of different process parameters such as temperature, pH, moisture percentage, particle size of raw materials, inoculum dosages and incubation period for cellulase production by various microbes, response surface methodology (box benkhem design) have been exercised.

To investigate the cellulase production under submerged conditions various insoluble and soluble sugars such as cellulose, lactose, xylose, sorbitol etc have been used as carbon source.

To explore a recently introduced surface adheshion fermentation (biofilm fermentation) has been investigated for cellulase production by *Aspergillus niger* strain.

To perform the utilization of various conventional raw materials such as wheat bran, bagasse, wheat straw and groundnut shell with or without pretreatment have been used as solid bed for solid state fermentation(SSF).For the effective utilization of few conventional raw materials acid or

alkali pretreatment technology have been introduced. For the overall exploitation of raw materials, Pretreated grounded, sieved solid biomass used as solid bed as well as remaining detoxified hydrolysates have been used as carbon source under submerged cultivation.

To investigate mixed raw material strategy for cellulase production.

To introduce the exploitation of non conventional and novel raw materials such as Pea peel (*Pisum sativum*), *Luffa cylindrica* peel, *Lagenaria sineraria* peel, *Litchi cinensis* peel have been also incorporated for solid state fermentative production of cellulases.

To investigate the mixed microbial cultures strategy (*Trichoderma* and *Aspergillus* strains) for the production of complete set of cellulases under wheat bran based solid state cultivation.

To explore the consumption of dairy industry waste whey, poultry industry waste egg shell waste and starch hydrolysates in cellulase production.

To study the mutagenic effect on microbial cultures growth and their sustainability under such condition as well as on cellulase production different mutagenic agents such as UV light as well as ethidium bromide have been used.

To investigate the application of crude cellulases produced from various fungal strains in the deinking of old news paper.

Keeping the view of high cost of cellulases, intensive search regarding some other novel, cheap, lignocellulosic waste material as well as inexpensive inducers which serve as both carbon source and inducer should be investigated in the future research.

CHAPTER II

LITERATURE REVIEW

Cellulase technology would substainly contribute to solving the impending energy and waste problem of mankind. Cellulolytic enzymes are essential for the maintenance of the global carbon cycle, since they initiate the degradation of cellulose, major individual biomolecules in nature. Lignocellulosic biomass is the most abundant solid waste in the world, thus it can provide some of the most promising feedstocks for the production of energy, food, and chemicals. Lignocellulose comprises more than 60% of plant biomass produced by photosynthesis on the earth. It generally contains up to 75% of cellulose and hemicelluloses. Conversion of these feedstocks into biofuels is an important choice for the exploitation of alternative energy sources and reduction of polluting and green house gases. Cellulase and hemicellulases make up of 15 of 70 identified glycosyl hydrolase families, Individual enzymes are not able to degrade cellulose completely, while mixture of enzymes enhance efficiency of saccharification. Lignocellulosic materials do not contain monosaccharides readily available for bioconversion. Instead they contain polysaccharides, such as cellulose and hemicelluloses, which have to be hydrolyzed, by means of acids or enzymes, to fermentable sugars. Enzymatic hydrolysis is a promising way for obtaining sugars from lignocellulosic materials, but this is a key problem for biomass conversion. The production cost, low yield of these enzyme as well as low enzymatic accessibility of the native cellulose are the major impediment for their industrial application. To overcome these problems much work has been emphasized on using cheap, cost effective and abundantly available raw materials with improved fermentation conditions. Cellulose in plants is closely associated with hemicelluloses and lignin. The lignin is partly covalently associated with hemicelluloses, thus preventing the access of hydrolytic agents to cellulose. In addition the crystalline structure of cellulose itself represents an extra obstacle to hydrolysis. A pretreatment is required for removing lignin and hemicelluloses, reducing cellulose crystallinity and increasing the porosity of the material, hence ultimately beneficial for easier cellulose availability for microbial attack. In this chapter a brief review of the status of applied research covering major aspects related to cellulase technology, attempted by various researchers either done in laboratory or pilot plant scale are discussed .

2.1. MICROORGANISMS PRODUCING CELLULASES

A large number of microbes have the ability to produce cellulases. In filamentous fungi and actinomycetes cellulases are mostly secreted as free molecules.Cellulases in certain anaerobic cellulolytic bacteria like *Clostridum thermocellum* are organized into high molecular weight complexes called cellulosomes [158]. Anaerobic bacterial cellulose are complexed called cellulosomes whereas aerobic bacteria and fungal cellulase are noncomplexed. However few are capable of producing all the necessary enzymes for degradation of complete cellulose. Most commonly studied cellulolytic organisms are *Trichoderma* [3, 9, 21, 112, 146, 149, 167, 200, 249, 295, 206], *Aspergillus* [201, 115, 126] *Penicillium* [81, 130] *Neurospora* [212], *Fusarium* [182], *Bacillus* [128], *Streptomyces*[113, 121], *Micromonospora* [49].*Trichoderma ressei* is one of the most extensively studied and best known cellulase producing organism having a complete set of cellulases [206, 249, 295]. *T.reesei* produces at least three endoglucanases, two exoglucanases and one β-glucosidase [146].Other species of *Trichoderma* have also been used for cellulase production like *Trichoderma viridae* [249], *Trichoderma lignorum* [295], *Trichoderma harzianum* [206]. Literature revealed that cellulase from *Aspergillus* strains are high in β-glucosidase activity [238, 281] .

2.1.1. Non complexed cellulase systems

The enzymes in the fungal system penetrate cellulosic substrate through hyphal extensions therefore presenting their cellulase systems in confined cavities within the cellulosic particles [73].The enzymes of the fungal systems also do not form stable high molecular weight complexes therefore called non complexed systems. Cellulase from *T.reesei* produces at least two exoglucanases or cellobiohydrolases (CBHI and CBHII), five endoglucanase (EGI-V) and two β – glucosidases. CBHI and CBHII are the principle components of *T.reesei* cellulase system denoting 60 and 20 % respectievely, of the total cellulase produced by the fungus on a mass basis[293].CBHI are particularly preferences reducing ends,whereas CBHII preffered non reducing ends of cellulose chain for their action [159].Crystallographic studies suggested that CBHI contains four surface loops that provide a tunnel of 50A whereas CBHII contains two surface loops with a tunnel of 20A.These cellobiohydrolase tunnels are essential for the processive cleavage of cellulose chains[65]. EGI and EGII are the major species represents less than 20% of total cellulase produced by the fungus on a mass basis.Endoglucanse structure comprises of the presence of groove rather than tunnel which presumably initiate entry of cellulose chain for subsequent

cleavage .At least two β-glucosidase produced by *Trichoderma reesei* assists the hydrolysis of cellobiose and other oligosacharrides to glucose,Both BGLI and BGLII have been isolated from the culture supernatants ,but the large fractions of these enzymes may remained cell wall bound[271]. Low levels of β-glucosidases produced by *T reesei* compared to other fungi such as *Aspergillus*. β-glucosidase of *T.reesei* are glucose intolerant whereas those of *Aspergillus* are more glucose tolerant. The levels of *Trichoderma* β –glucosidase are presumably sufficient for growth on cellulose, but not sufficient for extensive in vitro saccharification of cellulose, therefore *T.reesei* cellulase supplemented with *Aspergillus* are often used in cellulose saccharification on industrial scale [45 , 94, 207, 271, 94].

2.1.2. Complexed cellulase system

On the other hand anaerobic bacteria lack the ability to effectively penetrate cellulosic material and therefore they used an alternative mechanisms for degrading cellulose through development of complexed cellulase systems called cellulosomes. Cellulosomes are the protruberances produced on the cell wall of cellulolytic bacteria when growing on cellulosic materials.Electron microscopy reaveled that cellulosomes are compact "fist" like structures that open when attacking to microcrystalline cellulose [25, 110].

2.2. NATURE OF CELLULASES

2.2.1. Components of cellulases

Cellulases are multicomponent enzymes consist of three basic components and plays a vital role in biomass conversion process.It belongs to glycosyl hydrolase (EC 3.2.1.X) families. They are endo-β-glucanase (E.C. 3.2.1.4), exo-β-glucanase (E.C. 3.2.1.91) and β-glucosidase (E.C. 3.2.1.21).With particular components performing its particular function.

- Endo-β-4 glucanase randomly cut an internal amorphous sites in the cellulose chain into glucose and cello-oligosaccharides.
- Exo-β- 4 glucanase attacks the non reducing and of cellulose chain with cellobiose as the primary product.
- β- glucosidase hydrolyzes cellobiose into glucose.

The individual cellulase component however differ in their specificity and activity towards different cellulosic substrates and thus enzyme preparation enriched in or lacking a particular enzyme component would be useful for instance for the optimization of process developed for lignocellulose modification [67, 144].

2.2.2. Molecular aspects of cellulases

Cellulase are distinguished from other glucoside hydrolases by their ability to hydrolyse β-1,4 glucosidic bond between glucosyl residues, this breakage in cellulose proceeds through an acid hydrolysis mechanism, using a proton donor and a nucleophile or base[32, 291]. A general features of most cellulases is a modular structure often including both catalytic and carbohydrate binding modules (CBMs).The CBMs effects binding to the cellulose surface, presumably to facilitate cellulose hydrolysis by carrying the catalytic domains in close proximity to the insoluble cellulose.The presence of CBMs is particularly important for the initiation and processivity of exoglucanases. Cellulases systems are not only the agglomeration of three enzymes groups with or without CBMs, but rather act in a coordinated manner to efficiently hydrolyse cellulose[259, 263] Cellobiohydrlase Cel 7B had greater activity than endoglucanase against crystalline cellulose, whereas endoglucanase are more active towards amorphous cellulose.Cellulose binding module (CBM) improved the hydrolytic potential of each enzyme.It had a greater effect on the endoglucanase Cel 45A and Cel 7A than the cellobiohydrolase Cel 7B especially against crystalline cellulose[250].

2.2.3. Proposed induction and regulation of cellulase biosynthesis

Cellulase is a multienzyme complex and regulated by induction and repression in both bacteria and fungi.Synthesis of cellulase can be induced by many oligomeric and dimeric sugars. Cellulose and cellulosic component in the lignocellulosic substrate are essential for the formation of m–RNA to form cellulases at the transcription level. Glucose on the other hand represses cellulase synthesis by a catabolite repression mechanism at the translational level .*Aspergillus niger* produces strong activity of β-glucosidase which cause deglucosylation of substrates and produces gentiobiose which act as an strong inducer for cellulose [97]. Sophrose has been considered as the natural inducer of cellulase because it has been formed by trans glycosylation via β-glucosidase or EGI [129].Bacterial cellulase are enzyme produced constitutively by *Clostridium thermocellum* and *Cellulomonas flavogina* whearas fungal cellulase is produced only in the presence of cellulose as a substrate. Cellulose itself practically unable to trigger the induction because of its low solubility, therefore a basal level of constitutive cellulase might degrade cellulose into soluble cellooligosaccharides there by allowing these sacharides to enter the cell and can be converted into an inducer that trigger induction. Once the inducers enter in the cell it triggers full scale

transcription of cellulase gene mediated by activator protein and activating element [240].The mechanism controlling the cellulase biosynthesis varies greatly in various microorganisms. Inducible biosynthesis has been described in *Trichoderma reesei* and *Aspergilus terreus*, whilst *Monilla* sp synthesizes cellulase constitutively [13]. Studies indicated that cellulases are an inducible enzyme but its substrate cellulose is insoluble, fungus may contain low constitutive cellulase levels which attack on cellulose wherever it becomes available and this attack lead to the formation of inducer of the cellulase biosynthesis. Elevated level of cellobiohydrolases II may be beneficial to the initial attack of crystalline cellulose molecule. Cellobiohydrolases II synthesis occurs during the conidiation process. The predominance of cellobiohydrolases II on the conidial surface also leads to speculation on a major role of this in the initial attack of cellulose [129]. Todays evidence suggests that regulation of cellulase formation occurs at a pre transcriptional level and leads to cellulase gene transcription within 20 minute after addition of inducer. According to the synergistic action of CBHI&CBHII the major end product formed by the initial attack is cellobiose.This disaccharides is the first soluble compound formed from cellulose and its appearance in the cell should specifically indicate the presence of extracellular cellulase, therefore could be the logical inducer of further cellulase biosynthesis.As the end product of cellulose hydrolysis glucose inhibits further cellulase synthesis (in bacteria this phenomenon termed as carbon catabolite repression).In this a glucose catabolite interacts with a cellulase protein and this complex than interferes with gene transcription. Classical secretary pathway starting from endoplasmic reticulum(ER) and flowing via golgi bodies and vesicles to the plasma membrane[13, 51, 129, 240].In overall there are 3- stages involved in the cellulase biosynthesis in fungi: Expression at a basal level, mass secretion induced by inducers, Glucose or catabolite repression [240].The various transcriptional activators ie XlnR, ACEI and ACEII and catabolite repressors such as Cre proteins plays a important role in cellulase regulation [18]. Glucose does not represses cellulase production in *T.reesei* RUTC30 because it is acre1 mutated strain,however only low level of cellulase can be produced on glucose because of the lack of inducers[111]. Many cellulases, hemicellulases and pectinase genes in *Aspergillus* to be regulated by Cre proteins. XlnR of *A.niger* was the first transcriptional activator controlling expression of xylanolytic and cellulolytic genes in filamentous fungi[111, 240].

2.2.4. Cellulase adsorption

In the initial phase of hydrolysis, the adsorption of enzymes on the cellulose results in low free enzyme concentration, later when the cellulose is hydrolyzed the enzymes are released into the

medium. However lignocellulosic materials also contains lignin which is able to adsorbs the enzymes.This adsorption is considered partly irreversible as only around 50% of the added enzyme can be recovered after complete hydrolysis of cellulose fraction.In the cellulose hydrolysis enzyme firstly adsorbed on the cellulose molecules. The adsorption equilibrium is described empirically by a Langmuir Adsorption Isotherm[144, 264] .

$P_{ads} = K_P.P_{max}.P_L/1+K_P.P_L$

Where P_{ads} = adsorbed protein, mg/g cellulose. P_{max} =Saturation constant,mg/g cellulose

K_P = binding condtant, ml/mg, P_L = Protein in solution, mg/ml.

Cellulases enzyme components adsorb onto lignin as well as cellulose. Lignin act as a competitive inhibitor or adsorbant for cellulase, with the results enzymatic hydrolysis are diminished by the presence of lignin even in the absence of any steric effect [144].

2.3. VARIOUS INDUCERS INVOLVED IN CELLULASE BIOSYNTHESIS

Inducers play a vital role in cellulase biosynthesis by microorganisms.Sugars, surfactants and various other componant have been reported as inducers in cellulase biosynthesis and numbers of inducers is being searched for making cellulase production process more cost effective.

2.3.1. Sugars act as inducers

Sugars play a significant role in cellulase biosynthesis and it acts as an inducer. Cotoras and Agosine [51] investigated that brown rot fungus *Gloeophyllum trabeum* produced endoglucanases in the presence of monosaccharide such as glucose or mannose as the sole carbon source, but the expression of these enzymes was 4-5 times higher in the presence of cellulose or cellobiose, considered that endoglucanase production by brown rot fungi is inducible by cellulose and not subject to catabolite repression. Cellobiose is the most effective inducer of the system. Morikova *et al* [163] suggested lactose as an inducer for cellulase formation in *T.reesei* PC-3-7. Schaffner and Toledo [218] reported that cellulase activity was higher when media containing xylose supplemented with sorbose compared to xylose only as the carbon source by *Trichoderma reesei.* Janas *et al.*[106] reported that enzymatic activities of cellulases obtained by *T.reesei* M-7 in presence of lactobionic acid and lactose were about 20% higher than presence of only lactose.Thirumale *et al* [261]first time reported trehalose as an inducer in cellulase biosynthesis. Trehalose has been investigated as the best inducers as compared to the lactose and cellobiose. Sehnem *et al.*[220] also suggested lactose as inducer in cellulase biosynthesis by *Penicillium echinulatum* 9A0251.Cultures grown in lactose produced low activity of FPA (0.1FPU/ml) while

showed enzyme activity (1.5 FPU/ml) grown on lactose alongwith cellulose containing medium suggesting the possibility of increasing lactose/cellulose ratio results in a proportional increase of enzymatic activity.Morrison *et al*[164]reported that *Taloromyces emersonioi* CBS 814.70 was capable of growth on lactose containing media where lactose serve as inducers.Kamagata *et al* [114] reported cellulase production by *Penicillium purpogenum* strain P-26, that was strongly induced by cellobiose octaacetate (COA) in the presence of microcrystalline cellulose as a cosubstrate.A fraction of cello-oligosacharidses with a high degree of polymerization (GX) was found to be a potent cellulase inducers in *T.Koninngii* G-39[283]. Sophrose metabolism plays an important role in cellulase induction by *Trichoderma reesei* [142].The cellulose degradation product such as cellobiono1-5 lactone or oxidized cellulose have also been demonstrated to enhance the formation of cellulase in *T.reesei*[51].Induction was caused by any soluble substrate having β-1-4 glucan linkage ie carboxymethyl cellulose and cellobiose, but these are the weaker inducer than cellulose itself. Cellobiose the smallest cellooligosaccarides with β-1-4 linkage has been reported to induce cellulase biosynthesis. Surprigingly sophrose, which is a disaccharides of glucose with β-1-2 linkage can induce 2500 fold more cellulase than cellobiose in *T.reesei.* Gentiobiose a disaccharides of glucose with a β-1-6 linkage, found to induce 50 times more cellulase in *Penicillium* [240].Cellulase produced on lactose was more than the cellobiose.Cellobiose is an excellent growth substrate for these fungi and is rapidly consumed ,whereas lactose which differs from the cellobiose only in configuration around the number four carbon in the glucoside ring is a poor growth substrate for fungi and is poorly consumed.For most of the cellulase producers the inducing rate of the inducers for cellulase production are as follows Cellulose>cellobiose octa acetate>lactose>cellobiose. Binder and Ghosh [31] claims that the actual contact between hyphae and cellulose is essential for cellulase induction. Sophrose is produced by the acid hydrolysis of starch, it is a potant inducer of cellulase in *T.reesei.* Both cellobiose and lactose act as inducer but its high initial concentration to be much effective. During growth on cellobiose products of glucosyl transfer are found in the medium and they are reported to be better inducers than cellobiose .Cellulase production system of *T.viridae and N.crassa* is also differ.Only sophrose and cellulose significantly induces CMCase activity. CMC, cellobiose, lactulose did not induces CMCase, it induces β-glucosidase[31].Lactose is the important carbon source for the cellulolytic enzyme production by fungus *T.reesei*,Although lactose metabolism is slow than cellulose.*Trichoderma* hydrolyses lactose by an extracellular β-galactosidase .β-galactosidase, bgal transcript in *H. jacorina* showed that it was induced by arabinose,lactose,galactose,xylose and

xylitol[284]. Ju and Afloabi [112] evaluated cellulase inducing capability of waste paper hydrolysate and used as soluble substrate for cellulase production in continuous culture of *Trichoderma reesei*. Wyman and Chen[285] investigated soluble inducers derived from whole wheat flour for cellulase production by *Trichoderma reesei*. Chen and Wyman[294] also reported cellulase production by carbon source derived from waste newspaper (Discarded office paper) which act both raw material and inducing agent. Lactose is a low commercial cost disaccharides and also act as the source of carbon and inducer for the cellulase production by fermentation[220].

2.3.2. Surfactants act as inducer

Surfactants also plays a vital role in cellulase induction. Sukan *et al* [239] investigated the effect of natural oils and surfactants on cellulase production and activity. It was found that in general, emulsification leads to higher cellulase activites in both cultures, variations in enzyme levels depends upon the presence or absence of Tween-80 and different oils in growth media as well as the substrate used for cellulase activity.Deshpande *et al* [58] reported the effect of fatty acid on cellulase production by *Penicillium funiculosum* and its mutants. Addition of 0.1% oleic acid, linoeic acid and linolineic acid resulted in significant increase in extracellular exoglucanase and β-glucosidase in wild and N4 mutants whereas no appreciable increment was noticed in BU-36. However BU-36 showed positive response with 0.1% palmitic and stearic acid so these results indicates that fatty acids also have a significant role in release of cell bound enzymes. Pardo[187] reported the effect of different surfactants (nonionic surfactants such as Tween-80, Tween-20, TritonX-100, PEG-6000) surfactants on cellulase production by *Nectria Cataliansis* found that Tween-80 gave the highest yield of endoglucanase, exoglucanase and cellobiase at the 20th day of growth, on the other hand Tween-20 and Triton X-100 inhibited growth and cellulolytic enzyme production.

2.4. BATCH GROWTH AND MORPHOLOGICAL STUDIES OF CELLULASE PRODUCERS

Trichoderma, *Aspergillus* and *Neurospora* plays a vital and significant role in environmental solid waste management as they produces a wide range of commercial enzyme namely cellulases, hemicellulases and β-1,3-glucanase and have the ability to invade substrate has motivated their extensive use for the production of industrial enzymes[221]. *Aspergillus* species are widley used in the fermentation industry for acids and enzyme production [197]. *Aspergillus* sp are known to be a good producers of β-glucosidase.Intensive agitation altered the morphology of *Aspergillus* strain and reduced β-glucosidase activity[177]. *Neurospora crassa* (the common pink, red bread mould

and mesophilic fungus) is a filamentous ascomycete and true cellulolytic producer, secrete high levels of all the three enzymes components involved in the cellulose degradation[196].The genus name meaning "nerve spore" refers to the characteristic striations on the spores that resemble axon.Aerial growth and conidiation of *Neurospora crassa* occur when conidia are used to inoculate on solid media.When mycelia mat of *N.crassa* is exposed to the air controlled chamber, aerial growth is induced and synchronous condition is obtained. In exploring the mycelium to the air, growth ceases and the part of mycelium is transferred into the aerial hyphae and conidia.The 3 fractions (Arieal, upper and lower mycelia) were analysed [153]. *Neurospora crassa* was recently reported to produced high yields of CMCase and β-glucosidase when grow in solid substrate [147]. The best characterized and most widely studied organism for cellulase systems are the fungus *Trichodermma reesei.*Cellulase produced by filamentous fungus have a high industrial interest. During the growth and development of *Trichoderma reesei* cells, several morphological and physiological changes takes place. These changes (long hyphae appearance, sporulation etc) influenced cellulase production [273] .The fungal morphological form varies from mycelial pulpy to pellets structure. The filamentous growth characteristic creates a number of process engineering problems attributed to the morphological change.Rheology–morphology relationships are particular relevant in fermentations involving filamentous fungi and bacteria. Excessive hydrodynamic shear stresses are known to damage mycelial hyphae and form pellets. In such fermentations the mass transfer of oxygen and nutrients is considerably better and the subsequent separation of the pellets from the medium is simpler. Since agitation and aeration is much easier in such system, the power input therefore the operating cost is lower [67, 117, 122] .Growth of *A. niger* mycelium as large pellets was associated with lower specific protease activities and increased specific glucoamylase activities[160]. Owing to the filamentous and non homogenous nature of the moulds growth, the analysis of the growth characteristic and growth curve is very difficult. Although determining the rate of colony extension will provide certain measurement of fungal growth [305].Due to all these complications, it is better to estimate fungal growth (cell biomass) in terms of cell dry weight. These fungal strains showed distinctive growth phases such as lag, log, stationary and death phases.There are variety of pure sugars as well as industrial waste whey and hydrolysates have been used for the fungal growth studies.

2.5. METHODS OF ENZYME PRODUCTION

2.5.1. Solid state fermentation

Solid state fermentation refers the growth of microorganisms without the presence of free liquid, while the presence of moisture is necessary.The solid substrate act as a source of carbon, nitrogens, minerals as well as growth factors.The oxygen requirenments for the growth and metabolism of the cultures derived largely from gaseous state and to lesser extent from that dissolved in water associated with the solid[17, 265].The high product concentration,higher productivity, lower downstream processing, direct use of fermented product as crude enzymes and utilization of byproduct make solid state fermentation, a promising technology but one of the most critical problem associated with solid state fermentation is related to heat transfer and this can be solved by applying periodical dyanamic change of air (Including air pressure pulsation and internal circulation of air) instead of agitation and rotation[173, 184, 234, 256, 257, 267].Tengerdy indicated that there was about a 10 fold reduction in the production cost in SSF then SmF[257].The different fermentation mode and magnitude required for effective cellulase production are stated in Table 1-4.

2.5.2. Submerged fermentation

The process of submerged fermentation involves the growth of microorganisms in liquid media.Submerged fermentation also known as liquid fermentation in which the nutrients and cultures are present in the dissolved form in a large body of water. Low productivity, less concentrated products, tedious and costly process are the major drawbacks of the submerged fermentation [219].

2.5.3. Surface adhesion fermentation

Surface adhesion fermentation, a novel approach has been recently used in cellulase production [52]. Filamentous fungi are naturally adapted to growth on the surfaces and in those conditions they showed a particular physiological behavior, which is different to that in submerged culures. Although fungal biofilms are less known than the bacterial biofilms and they can be used for cellulase production.Both SSF and biofilm fermentation depends upon surface adhesion, hence a new fermentation category named as surface adhesion fermentation (SAF) was established and first introduced by Gutieroz correa and Villenea [275]. Biofilm formation process are made up of adheshion and subsequent gene expression to generate phenotype distinct from those of free living microorganisms [276]. In biofilm formation, spore adheshion depends both on its rough surface and adhesive substrates that form a pad between spore and support. An extracellular polymer

matrix surroundings also involved in the formation of biofilms [277]. *A niger* is better to known in the form of pelletized growth under submerged cultivation, But recent literature also suggested the growth in the biofilm form under the solid support. There are a major differences between the pellets and biofilm formation.Biofilm mycelia showed an orderly distribution forming surfaces and inner channels, while pellets showed highly intermined superficial hyphae and a densely packed deep mycelium.Actually once spores are adsorbed to the support they grow and attached to it and thus forming a biofilm.Literature also suggested that biofilm fermentation produces higher enzyme yields than SmF with lower biomass yields suggesting differential gene expressing mechanisms related to cell adheshion [276].

2.6. DESIGN AND OPTIMISATION OF PROCESS PARAMETERS

The statistical design of experiments (DOE) is a collection of predetermined settings of the process variables of interest, which provides an efficient procedure for planning experiments .Statistical analysis of bioprocess with repeated measurements can help to investigate the environmental factors and effects, affecting physiological and bioprocesses in analyzing and optimizing production process. Designed experiments are less time consuming and less expensive than haphazard ones. This method is more satisfactory and effective than other methods, such as classical, one at a time or mathematical methods. In most bioprocesses such as fermentation, there are no true theoretical or mathematical models that can describe the whole process with 100% certainty. Because of this limitation arising from the incredible complexity of cellular metabolism, efficient empirical approaches to explain these processes are necessary to solve research problems. The optimization of fermentation conditions, particularly physical and chemical parameters are of primary importance in the development of any fermentation process[78]. The relationship between the response and the independent variables is usually unknown in a process, therefore the first step in RSM is to approximate the function (response) through analyzing factors (independent variables). Usually this process employes a low order polynomial equation in a predetermined region of the independent variables [7][304]. Some of the popular choices in the statistical design includes the Plackett – burman design, Central composite design, the Box-behnken design and the Gracco-latin square design [306]. Box-behnken designs are response surface designs especially made to require three levels, coded as -1, 0, +1.They are formed by combining two level factorial designs with incomplete block designs. A box-behnken design was employed to analyze the interactive effects of these parameters and to arrive at an optimum. The base points for the design were selected from a single parameter study[78]. In box behnken design the treatment

combinations are at the midpoints of edges of the process space and at the centre, these designs are rotatable and required three levels of each factor [307]. Statistical optimization method for fermentation process could overcome the limitations of classical empirical methods and has been proved to be a powerful tool for the optimization of cellulase production and also to determine the optimum operating conditions necessary for the scale up of the process and to reduce the number and cost of experiments[138].

2.7. CHARACTERIZATION OF LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is a heterogenous complex of carbohydrates polymers and lignin (a complex polymer of phenylpropanoid units) .Cellulose like a starch is a polymer of glucose.The other carbohydrates component in the lignocellulosics are hemicelluloses[165].Hemicelluloses are consist of non cellulosic polysaccharides (D-xylose, L-arabinose, D-mannose ,D-glucose ,D-galactose and D-glucoronic acid) with repeated substances (hexauronic acid and its byproducts).Lignin is linked to hemicellulose and cellulose forming an impermeable rigid barrier preventing the enzymatic activity. Lignin backbone are made up of p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol [192].The major components present in the lignocellulosic are described as follows.

2.7.1. CELLULOSE

The cellulose molecules are bundled together and tightly packed in such a way that neither water nor enzyme can penetrate through the structure [253]. Cellulose molecules are produced in the biosphere (100 billion dry tons/year).Cellulose molecules are very stable, with a half life of 5-8 million years for β-glucosidage bond cleavage at 25^0C [194].

2.7.1.1. Crystallanity of cellulosic substrates

The crystallinity of cellulose, accessible surface area, protection of cellulose by lignin, the heterogenous character of the biomass particles and cellulose sheathing by the hemicellulose all contribute to the recalcitrance of lignocellulosic biomass to hydrolysis.The effective pretreatment causes disruption of these barriers so that hydrolytic enzymes can penetrate and cause hydrolysis [161].Cotton is almost pure α-cellulose and insoluble in 17.5% NaOH.Wood and other plant celluloses generally contains β-cellulose, a material soluble in 17.5% NaOH solution.The arrangement of cellulose molecules in the fibrillar bundles is so regular that cellulose has a crystalline X-ray diffraction pattern. The individual cellulose chains are packed in groups of about 30 to form elementary fibrils (microfibrils) and approximately 100 microfibrils are packed to form fibrils.These fibrils are further packed and converted into cellulose fibers[289].Cellulose is known to subsist in four structures (cellulose I-IV)[20, 33]. Cellulose I is the native and most abundant

form of cellulose in nature.Cellulose I has two distinct crystalline forms alpha (Iα) and beta (Iβ).Iα is dominant in bacterial and algal cellulose whereas Iβ prevailing in higher plants [20]. The degree of crystallanity of cellulose depends vastly on origin and type of pretreatment. It may vary from 0% (amorphous and acid swollen cellulose) to approximately 100% (algal cellulose from *Valonia macrophysa*) [26].

Table 2.1.Crystallanity index of various pure cellulose[194]

Substrates	CrI (Crystallanity index)
Cellodextrins(soluble)	NA
CMC(soluble)	NA
Cotton	0.8-0.95
Whatman number -1 filter paper	0.45
Bacterial cellulose	0.8-0.95
Microcrystalline cellulose(Avicel)	0.5-0.6
Phosphorus swallone cellulose (PASC)(Treated with 85% phosphoric acid)	0
Pulp (Solka floc)	0.4-0.7
Pretreated cellulosic substrates	0.4-0.7

Crystallanity of various pure cellulose molecules describes by Table 2.1. Crystalline cellulose is highly resistant to microbial attack and enzymatic hydrolysis whereas amorphous cellulose is degraded as much faster rate [73].As Table 2.1 indicates that cotton and bacterial cellulose are highly crystalline cellulose. Microcrystalline cellulose, filter paper alpha cellulose and pretreated cellulosic substrates have modest crystallanity and can be considered as a combination of crystalline and amorphous fraction.Whereas phosphorous acid swollen cellulose (PASC, Walseth cellulose) is considered as an amorphous cellulose due to having nil crystallanity. There are various ways of cellulose attack by microbial or enzymatic system. Either by the available or accessible surface area or by the pore structure of cellulosic biomass.Pore structure are more important in the natural cellulosic biomass than purified cellulose.

2.7.2. HEMICELLULOSE

Hemicellulose serves as a connection between lignin and cellulose fibers and it is readily hydrolyzed by dilute acid or base, as well as hemicellulase enzyme [253]. Glucuronoxylans (O-acetyl-4-O-methylglucuronoxylan) are the main hemicellulose of hardwoods. In hardwoods, GX represent 15– 30% of their dry mass and consist of a linear backbone of β-D-xylopyranosyl units (xylp) linked by β-(1,4) glycosidic bonds[195]. Galactoglucomannans (O-acetyl-galactoglucomannans) are the main hemicelluloses of softwoods, accounting up to 20–25% of their dry mass. GGM consist of a linear backbone of β-D-glucopyranosyl and β-D-mannopyranosyl units, linked by β-(1,4) glycosidic bonds[88, 195]. On the other hand arabinoglucuronoxylans

(arabino-4-O-methylglucuronoxylans) are the key components of non-woody lignocellulosic such as agricultural crops as well as they present as a minor component in softwoods.They consist of a linear β-(1,4)-D-xylopyranose backbone having 4-O-methyl-α-D-glucopyranosyl uronic acid and α- L-arabinofuranosyl units bonded by α-(1,2) and α-(1,3) glycosidic bonds [262, 292], whereas arabinoxylans (AXs) signify the major hemicellulose structures of the cereal grain cell walls. AXs are similar to hardwoods xylan with higher amount of L-arabinose [36, 226]. Cereals, seeds, gum exudates and mucilages contain structurally more complex heteroxylan.

2.7.3. LIGNIN

Lignin is covalently linked to cellulose and xylan such that lignin–cellulose–xylan interactions exert a great influence on digestibility of lignocellulosic materials[253]. Lignins of herbaceous crops are structurally different from those of softwood or hardwoods such as straw lignins possessing characteristic alkali solubility. Availability of alkali-labile ester bonds between hydroxycinnamic acids and hemicelluloses (arabinoxylans) in lignin/phenolics–carbohydrate complexes determines their alkali soluble nature.Hydroxycinnamic acids (*p*-coumaric and ferulic acid) are also attached to lignin via acid-labile ether bonds and serve as bridges between lignin and hemicelluloses. Straw and wood lignins also differs by their monomeric composition. Straw lignins contain all three H, G, and S subunits whereas wood lignins contain mainly G and S subunits [16, 199].The presence of lignin and hemicelluloses makes the access of microbes to cellulose difficult, thus reducing the efficiency of the hydrolysis. Removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase of porosity in pretreatment processes can significantly improve the hydrolysis.

2.8. PRETREATMENT TECHNOLOGY

Lignocellulosic biomass represents a rather unused or untrapped source of energy for fuels, feeds and chemicals production. Each pretreatment has its own effect(s) on the cellulose, hemicellulose and lignin, the three main components of lignocellulosic biomass. The crystalline structure and lignin existence in lignocellulosic materials prevent attack by microorganisms.Microbial systems readily degrades more easily accessiable amorphous portions but are unable to attack the less accessiable crystalline portion of cellulose. In order to developed an economically feasible and viable technology, these difficulties must be overcome by a suitable mechanical, physical and chemical pretreatment. The goal of any pretreatment process is to alter or remove structural and compositional impediments by breaking the lignin seal thereby seperating the carbohydrates from lignin matrix as well as disrupting the crystalline structure of cellulose. In other words pretreatment

is an important tool for practical cellulose conversion process by altering the structure of cellulosic biomass and making it more accessible for the microbial attack. An efficient pretreatment must free the highly crystalline structure of cellulose and extend the amorphous areas [132, 158, 165] .Each individual lignocellulosic substrate requires a specific pretreatment in order to achieve the maximum microbial as well as enzymatic hydrolysis and also reinforcing the concept that the nature of the native lignocellulosic matrix is a major limiting factor in the enzymatic hydrolysis[209].

Table 2.2: Various methods applied in pretreatment of lignocellulosic biomass.

Pretreatment methods	Agents	Procedure	Temperature /Pressure	Reaction Time (min)	Pretreated materials	Remarks
Physical Methods						
Mechanical Comminution	-	Chipping,grinding,milling	-	-	Wood,Forestry waste,Cane baggase,Alfalfa	Vibratory ball mill(size 0.2-2 mm)Or hammer mill (3-6 mm)
Pyrolysis	-	Intense heating,Cooling, Condensing	>300^{0}C	-	Wood,Corn stover	Formation of volatile products and char
Physico-chemical methods						
Steam explosion	Saturated steam	Saturated steam treatment then decompression	160-300^{0}C, 0.69-4.85MPa	Sec or min	Poplar,eucalyptus ,soft wood,Bagasse,straw	It can handle high solid loads,Inhibitors formation,Lignin is not solubilized,80-100% hemicelluloses hydrolysis
Liquid hot water	Hot water	Pressurized hot water	170-230^{0}C,p>5MPa	1-45 min	Bagasse,Alfalfa ,Corn stover	Cellulose depolymerization,80-98%xylose recovery,no formation of inhibitors,partial solubilization of lignin
Ammonia fiber explosion	Ammonia	1-2 kg ammonia /kg dry biomass	90^{0}C,1.12-1.36MPa	30 min	Switch ,Bermuda grass,news print,MSW,Bagasse,straw	Ammonia recovery is required,No inhibitors formation,10-20% lignin solubilization
CO_2 explosion	CO_2	4 kg CO_2/kg fiber	5.62 MPa	-	Bagasse,recycled paper	Cellulose conversion can be >75%,No inhibitors formation
Chemical methods						
Dilute acid hydrolysis	0.75-5%H_2SO_4,HCl or HNO_3	Continuous process for low solids loads ,Batch process for high solids load	120-200^{0}C, 1MPa	2-10 min	Poplar wood,Bagasse,grass,wheat straw	Lignin is not solubilized but it is redistributed,pH neutralization is required
Conc Acid hydrolysis	10-30%H_2SO_4	Same as dilute hydrolysis,1:1.6 solid :liquid ratio	170-190^{0}C	-	Bagasse,saw dust	Residence time greater,acid recovery is required
Alkaline hydrolysis	Dilute NaOH, $Ca(OH)_2$	Dilute NaOH,$Ca(OH)_2$ addition,H_2O_2 added at 35^{0}C	60^{0}C for NaOH,120^0 for $Ca(OH)_2$	-	Hardwood,Bagasse	Reactror cost lower compared to acid ,Cellulose swelling,24-55% lignin removal for hard wood ,lesser for soft wood
Biological methods						
Fungal treatment	Brown, White and Softrot fungi	Fungi produces cellulase ,hemicellulases,ligninase	-	-	Corn stover ,Wheat straw	Brown rot fungi degrades cellulose,white and soft rot degrades cellulose and lignin.

Source: [22, 165]

Various methods applied in pretreatment process such as physical, chemical, physicochemical and biological are described by Table 2.2. The lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further transformation. Because lignin is believed to be a major hindrance to enzymatic hydrolysis, its removal enhances cellulose digestion and also reduces non-productive binding of cellulose to lignin. Lignin and its derivatives are known to inhibit microbes and lignin removal improves microbial activity [199]. Minimization of lignin or its constituents phenol from the liquor may provide favorable condition for microbial growth and production system. Basheer and Farooqui treated lignin degradation product (phenol) with enzymes for their removal[24]. Pre-treatment of raw materials have often been found useful to improve its digestibility and easy access for microbial attack (by removing core and noncore lignin fractions) The pretreatment results in enlargement of the inner surface area of substrate particles, accomplished by partial solubilization and/or degradation of hemicellulose and lignin[184, 253]. Due to this structural complexity of the lignocellulosic matrix, cellulase production from raw materials requires at least effective pretreatment. Unlike sucrose or starch, lignocellulosic biomasses need to be pretreated to make cellulose accessible for efficient microbial and enzymatic depolymerization.

2.9. UTILIZATION OF LIGNOCELLULOSIC WASTES AS CARBON SOURCE

Soluble carbon sources such as lactose, cellobiose give significantly lower cellulase activity compared to pure insoluble cellulosic substances such as cotton, avicel, solka floc at the same concentration[163, 28]. *Trichoderma reesei* RUT C30 has proved to be one of the best cellulase producers and extensive research has been carried out to find a suitable but inexpensive carbon source. A number of different substrates have been utilized for the fermentative production of cellulase. Various purified cellulose such as Avicel, Solka floc, cotton have been used as carbon source for cellulase production[11, 218].The results showed that pure cellulose is the best carbon source among the insoluble materials.However, Solka Floc is quite expensive.

Table 2.3: Cellulase production by fermentation using pure cellulosic substrate[28, 163].

Strain	Substrate	Cultivation type	FPU/ml	Productivity FPU/lit.hr	Yield FPU/g
Trichoderma reesei QM 9414	3% cellulose	Batch	7.2	44	240
Trichoderma reesei MCG 80	5% Lactose	Continuous	6	168	120
Trichoderma reesei RUT C 30	2% Solka floc 2% Solka floc 5% Solka floc	Continuous Batch Fed Batch	2.1 4.2 -	976 29 -	105 210 160
Trichoderma reesei L-27	8% Avicel	Batch	18	94	225
Trichoderma reeseiCC 4/30	2% Lactose	Fed Batch	10.8	83	540

Utilisation of pure sugars as carbon source for cellulase production as shown by Table 2.3.For large scale production of enzyme pure cellulose would be too much costly and expensive to be applied in large scale process. Enormous amount of agricultural, industrial and municipal lignocellulosic wastes are constantly piled up and these materials are particularly attractive in the bioconversion process because of their low cost and plentiful supply. Therefore lingocellulosic waste material acts as a cheaper carbon source for cellulase production. The utilization of economically cheap lignocellulosic wastes for cellulase production could be a valuable approach in solid waste management[76].Literature account for various conventional and novel lignocellulosic waste as a substrate for cellulase production such as wheat straw[107, 149, 212],wheat bran[37] bagasse [87, 93, 146], rice straw [49], rice husk [130] corn syrup liquor (CSL) with native sugar cane baggase pith [126] steam pretreated willow [206] banana fruit stalk and its agro waste [21, 128], waste paper hydrolysate [112] coconut coir pith [167], corn stover[182]. Palm tree compound leaves [249] corn fiber fraction [139] corn cob residue [137] dairy manure [286, 287] whole wheat flour [285] corn fiber [279]OCC (Old corrugated card board) [251] water hyacinth biomass [12, 166] etc.Alam et al[9]utilized domestic waste water sludge alongwith wheat flour as a co-substrate for cellulase production by *Trichoderma* harzianum. Saw dust, bagasse, corn cob and computer cords were also used as substrate for cellulase production [115][178].Nawawi et al[172] utilized sugarcane bagasse as a raw material for cellulase production. Ground nut shell waste used as lignocellulosic substrate for cellulase production by Vyas *et al.*[280, 281], Juhasz *et al.*[111] stated β-glucosidase production by utilizing waste paper as carbon source. Couri *et al.* [53] investigated cellulase production using mango peels as a substrate in the presence of 0.2% cellobiose. Elishashuii *et al.*[72] utilized mandarine peels and its tree leaves for lignocellulolytic

enzyme production under submerged fermentation whereas Umikalsomms *et al*[270] used oil palm empty fruit bunch fiber as a substrate for cellulase production. Chandra *et al.* [42] have used marc of *Artemisia annua* for cellulase production by *Trichoderma citrinovirdae*. Pretreated willow has been used for cellulase and β-glucosidase production by *Trichoderma atroviridae* as investigated by Kovacs *et al*[127].Damato *et al*[54] utilized olive processing residue for cellulase production under submerged cultivation.Verma *et al*[274] utilized pea peel waste as a suitable raw material for cellulase production by *Trichoderma reesei* under solid state cultivation. Franceschin et al utilized waste news paper as carbon source for biofuel production.[79] Alverez and Sanchez [14]utilized orange peel as a substrate for enzyme production. Banana peel was also used for enzyme production by Kumar and Mandal[135]. A comparative studies on utilization of various lignocellulosic substrate for cellulase production through different microbes are briefly described in Table 2.4-2.7.

Table 2.4. Comparison of various waste biomass used as carbon source for cellulase production by *Trichoderma* strains.

Microorganism	Substrate	Temperature (°C)	pH	Incubation Time (in days)	Fermentation Mode	Magnitude	Enzymes Activity				Reference
							FPA	CMCase	Avicelase	β-Glucosidase	
Trichoderma reesei QM 9414	Alkali treated baggase	26	7.0	5	SSSF	Flask	10.3U/ml	24.9 U/ml	16.3U/ml	6.8 U/ml	146
	Alkali treated corncobs	26	7.0	5	SSSF	Flask	7.8 U/ml	19.8 U/ml	11.9 U/ml	4.7 U/ml	146
	Alkali treated computer cards	26	7.0	5	SSSF	Flask	8.0 U/ml	21.1 U/ml	12.3 U/ml	4.6 U/ml	146
	Alkali treated sawdust	26	7.0	5	SSSF	Flask	6.6. U/ml	18.9 U/ml	9.7 U/ml	3.3 U/ml	146
Trichoderma reesei TISTR 3080	Palm oil mill condensate	30	5.0	3	SmF	Shake Flask	0.156U/ml	0.79U/ml	-	-	200
Trichoderma reesei	Alkali + Steam treated wheat straw	35	5.5	8	SmF	Shake Flask	0.17IU/ml/h	0.48IU/ml/h	-	-	149
Trichoderma viride NCIM1051	Coconut coir pith	-	-	7	SSF	Flask	4.27IU/g	12.05IU/ g	-	-	167
		-	-	8	SSF	Flask	-	-	-	1.8IU/ g	167
Trichoderma reesei ZU02	Corncob residue from xylose industry	-	5.5	6	SSF	Shallow tray fermentor	158 IU/g koji	-	-	-	295
		-	-	5-6	SSF	Deep trough fermentor	128 IU/g koji	-	-	-	
Trichoderma reesei	Steam pretreted willow (SPW)	-	-	3	-	22 Ltr fermentor	108FPU/g cellulose	-		-	206
Trichoderma reesei Rut C30	Carbon source 20g/l (50% SPW + 50% CF)	30	6	8	SmF	Shake Flask	1.79 IU/ml	-	-	-	249
	Carbon source 10g/l (50% SPW + 50% CF)	30	5.8	8	SmF	Shake Flask	-	-	-	0.47IU/ml	249
Trichoderma reesei RUT C 30	Waste paper hydrolysate	25	5.0	-	Continuous	Bioreactor	12.20FPU/g.h*	-	-	-	112
Trichoderma reesei ATCC 26921	Water hyacinth biomass	31	4.8	8	SmF	Shake Flask	0.12 U/ml	0.44 U/ml	-	0.11 U/ml	166
Trichoderma reesei ZU-02	Corncob residue	~30	4.8	4	SmF	30 ltr Stirred tank fermentor	5.48 IU/ml	-	-	0.25 IU/ml	137
Trichoderma reesei RUT C30	Old corrugated card board (OCC)	-	5.0	7	SmF	Shake Flask	1.17 FPU/ml	-	-	-	251
		28	5.5	3	SmF	30 ltr bioreactor	2.27 FPU/ml	-	-	-	

Table 4 (Continued)

Microorganis	Substrate	Temperatu (°C)	pH	Incubation T (in days)	Fermentatic Mode	Magnitud	FPA	CMCase	Avicelase	β-Glucosidas	Referenc
Trichoderma reeseiQM 9414	CCF (Crude cornfiber)	28	4.2	8	SmF	Shake flask	0.22U/ml	6.25U/ml		0.43U/ml	139
	DSCF (De- starched corn fiber)	28	4.2	8	SmF	Shake flask	0.28U/ml	9.52U/ml	-	0.64U/ml	139
	CFC (Corn fiber with cellulose enriched	28	5.4	8	SmF	Shake flask	0.20U/ml	5.65U/ml	-	0.27U/ml	139
	CFAX (Corn fiber arabinoxylose	28	4.2	8	SmF	Shake flask	0.11U/ml	1.76U/ml	-	0.39U/ml	139
Trichoderma reesei RUT C30	Dairy manure	25.5	5.7	6-8	-	-	1.74U/ml	12.22U/ml	-	0.0978U/m l	286
Trichoderma viride	Palm tree compound leaves	30	8.0	4	SmF	Shake Flask	35.7U/ml	48.0 U/ml	-	-	3
Trichoderma sp strain 414	Corn Fibers	29	4.5	2	SmF	5 Ltr Fermantor	-	1.55U/ml	-	-	279
Trichoderma reesei	Wheat flour hydrolysates	-	-	~6	-	-	2.52IU/ml	-	-	0.28IU/ml	285
Trichoderma harzianum	Domestic waste water sludge (0.75%w/w +Wheat flour(2%)	32.5	5	3	SmF	Shake Flask	10.2FPU/ml	-	-	-	9
Trichoderma reesei NCIM 1052	Water hyacinth (*Eichhornia crassipes)*1%w/v with 1%NaOH treated	30	6.0-7.5	15	SSF	Flask	73.3IU/g cellulose	-	-	-	59
Trichodrma citrinovirdae	Sugar cane straw	30	6.0	4-5	SSF	Flask	16.22U/g	35.00U/g	-	26.00U/g	93
Trichodrma citrinovirdae	Citronella(*Cymbopogon winterianus*)1%w/v	28	5.6	-	SmF	Shake flask	~8.0 IU/g	~70.0 IU/g	-	81.86IU/g	42
	Marc of Artimisia(*Artemicia annua*1%w/v						~12.0 IU/g	~103.0 IU/g	-	102.4IU/g	
	Garden waste (*cynodon dactylon)*1%w/v						~11.0 IU/g	~82.0 IU/g	-	101.33IU/g	
Trichoderma reesei RUTC-30	Kinnow pulp+WB(4:1)	30	6.0	4	SSF	Flask	13.4 IU/gds	-	-	-	176
	Kinnow pulp+WB(3:2)			3			-	25.2 IU/gds	-	-	
	Kinnow pulp+WB(3:2)			4			-	-	-	18.0 IU/gds	
Trichoderma reesei NCIM-1186	Pea peel wastes	30	5.0	6	SSF	Flask	2.86 IU/mL	-	-	-	274

Table2.5. Comparison of various waste biomass used as carbon source for cellulase production by *Aspergillus* strains.

Microorganism	Substrate	Temperature (°C)	pH	Incubation Time (in days)	Fermantation Mode	Magnitude	Enzymes Activity				Reference
							FPA	CMCase	Avicelase	β- Glucosidase	
Aspergillus niger	Alkali treated baggase	26	7.0	5	SSSF	Flask	7.6 U/ml	21.5 U/ml	12.1U/ml	12.2 U/ml	146
	Alkali treated corncobs	26	7.0	5	SSSF	Flask	5.0 U/ml	17.6 U/ml	8.9U/ml	8.0 U/ml	146
	Alkali treated computer cards	26	7.0	5	SSSF	Flask	5.5 U/ml	18.1 U/ml	9.7U/ml	9.5 U/ml	146
	Alkali treated sawdust	26	7.0	5	SSSF	Flask	4.2 U/ml	14.8 U/ml	6.3U/ml	6.5 U/ml	146
Aspergillus niger 38	Wheat straw / Wheat bran	30	5	4	SSF	Flask	-	14.80 IU/ml	-	-	107
Aspergillus terreus	Alkali treated Sugarcane baggase	35	4.5	7	SmF	Shake Flask	0.08- 0.10 U/ml	0.85- 1.2 U/ml	-	-	172
Aspergillus flavus Linn isolate NSPR 101	Sawdust	35	-	0.5	Batch	7.5 ltr fermentor	0.0743IU/ml	-	-	-	178
	Bagasse	35	-	0.5	Batch	7.5 ltr fermentor	0.0573IU/ml	-	-	-	178
	Corncob	35	-	0.5	Batch	7.5 ltr fermentor	0.0502IU/ml	-	-	-	178
Aspergillus niger KK2	Rice straw	28	7.0	4	SSF	Flask	19.5 IU/g	-	-	-	115
		28	7.0	5-6	SSF	Flask	-	129.0 IU/g	-	100.0 IU/g	115
Aspergillus niger C-6	Corn syrup liquor + Sugar cane baggase pith	29	5.5	3	SmF	2 Ltr Microferm fermentor	-	-	-	7.2 IU/ml	126
Aspergilluse terreus AV 49	Groundnut shell	28	4.0	8	SSF	Flask	-	0.451 U/ml	0.037 U/ml	-	281
	Alkali treated groundnut shell	28	4.0	8	SSF	Flask	-	1.147U/ml	0.175U/ml	-	281
	Acid treated groundnut shell	28	4.0	8	SSF	Flask	-	1.023 U/ml	0.089U/ml	-	281
Aspergillus niger 3T588	Mango peel + 0.2% cellobiose	32	-	-	SSF	Tray fermentor	3.5U/ml	-	-	-	53
Aspergillus fumigatus	1%w/v wheat straw in 50 ml medium	30	-	7	SmF	Shake flask	-	321.0IU/ml	-	-	217
	Wheat straw				SSF	Flask	-	1044.0IU/ml	-	-	
Aspergillus phoenicis	Grape waste+P(10:5)	30	-	5	SmF	Shake Flask	0.106IU/ml	0.615 IU/ml	-	46.41 IU/ml	60
	Grapewaste+P(15:7.5)						0.095 IU/ml	0.666 IU/ml		43.56 IU/ml	
	Grape waste+P(10:5)						0.081 IU/ml	0.567 IU/ml	-	51.76 IU/ml	

Table 2.6. Comparison of various waste biomass used as carbon source for cellulase production by other microbial strains.

Micro organism	Substrate	Temperapture (°C)	pH	Incubation Time (in days)	Fermantation Mode	Magnitude	Enzymes Activity				Reference
							FPA	CMCase	Avicelase	β-Glucosidase	
Micromonospora sp	Alkali treated Baggase	37	7.2	3	SSF	Flask	--	23.7 U/ml	0.59 U/ml	-	49
	Alkali treated Rice straw	37	7.2	3	SSF	Flask	--	33.8 U/ml	0.94U/ml	-	49
	Alkali treated Jutestick	37	7.2	3	SSF	Flask	--	10.6 U/ml	0.28U/ml	-	49
	Alkali treated Sawdust	37	7.2	3	SSF	Flask	--	5.0 U/ml	0.20U/ml	-	49
*Myceliopthora thermophila*IFO31843	Palm cake	30	6.5	7	SmF	Shake flask	-	1.60 U/ml	-	0.141U/ml	201
	Palm cake	30	5.3	20	SSF	Flask	-	27.7 U/g	-	0.59 U/g	201
Penicillium citrinum	Untreated Ricehusk	28	5.0	12	SSF	Flask	15.3 U/g	-	-	-	130
	Alkali treated Ricehusk	28	5.0	12	SSF	Flask	36.9 U/g	-	-	-	130
Penicillium decumbans	Steam treated Wheat Straw/ Wheat bran(8:2)	-	4.5	2-3	SSF	Tray reactor	20.4IU/g	-	-	-	81
Neurospora crassa	Wheat straw	30	6.5	4	SmF	Shake Flask	1.33IU/ml	-	-	-	212
Bacillus subtilis CBTK 106	Banana fruit stalk waste	35	7.0	3	SSF	Flask	2.8IU/gds^{-1}	9.6 IU/gds^{-1}	-	4.5 IU/gds $^{-1}$	128
Chaetomium globosum Kunze	HNO_3 treated OPEFB	30	5.5	-	SmF	Shake Flask	0.95U/ml	15.80U/ml	-	7.60U/ml	270
Fusarium oxysporium	Corn stover	27	7.0	-	SSF	10ltrbioreactor	-	4.10U/g	-	0.140U/g	182
Pleurotus dryinus IBB903	Mandarine peels	27	6.0	12	SmF	Shake flask	4.0	47.7	-	-	72
	Mandarine leaves	27	6.0	12	SmF	Shake flask	4.2	47.0	-	-	72
Funalia trogii IBB146	Wheat straw(withPeptone)	27	6.0	-	SSF	Flask	18.0 U/g	322.0 U/g	-	-	116
	Beech tree L(withPeptone)						11.0 U/g	87.0 U/g	-	-	
Lentinus edodus IBB 363	Wheat straw(withPeptone)					Flask	23.0 U/g	93.0 U/g	-	-	
	Beech tree L(withPeptone)						19.0 U/g	158.0 U/g	-	-	
Pleurotus dryinus IBB903	Wheat straw(withPeptone)					Flask	43.0 U/g	369.0 U/g	-	-	
	Beech tree L(withPeptone)						32.0 U/g	284.0 U/g	-	-	
P.tuberregium IBB 624	Wheat straw(withPeptone)					Flask	24.0 U/g	49.0 U/g	-	-	
	Beech tree L(withPeptone)						17.0 U/g	51.0 U/g	-	-	

Table 2.7. Comparison of various waste biomass used as carbon source for cellulase production by mixed microbial cultures.

Micro organism	Substrate	Temperapture (°C)	pH	Incubation Time(in days)	Fermantation Mode	Magnitude	Enzymes Activity				Reference
							FPA	CMCase	Avicelase	β- Glucosidase	
Mixed culture *Trichoderma reesei* QM 9414, *Aspergillus niger*	Alkali treated baggase	26	7.0	5	SSSF	Flask	12.8U/ml	30.5 U/ml	21.7U/ml	13.3 U/ml	146
	Alkali treated corncobs	26	7.0	5	SSSF	Flask	9.8 U/ml	25.7 U/ml	17.1U/ml	11.6 U/ml	146
	Alkali treated computer cards	26	7.0	5	SSSF	Flask	10.5U/ml	27.1 U/ml	17.6U/ml	11.4 U/ml	146
	Alkali treated sawdust	26	7.0	5	SSSF	Flask	9.2 U/ml	24.8 U/ml	15.0U/ml	10.0 U/ml	146
Mixed culture *Aspergillus niger* BKMF 1305, *Trichoderma reesei* RUT C 30	Waste paper	30	6.0	7	SmF	Shake Flask	-	-	-	3.07 IU/ml	111
Mixed culture *Trichoderma reesei, Aspergillus phoenicis* QM 329	Dairy manure	27	5.5	10	SmF	Shake Flask	1.54 U/ml	-	-	-	287
		27	5.5	9	SmF	Shake Flask	-	-	-	0.64 U/ml	287
Mixed culture *Aspergillus terrus: Trichodermma viride*	Alkali treated ground nut shell waste	28	4.8	14	SSF	Flask	-	4.536IU/ml	0.457FPU/ml	-	280
Mixed culture *Trichodermma reesei* LM-U14 :*Aspergillus phoenicis* QM 329	Ammonia treated bagasse	30	-	4	SSF	Flask	18.7IU/g dry wt	-	-	38.8 IU/g dry wt	68
Mixed culture *Aspergillus niger*RK3 :*Trichoderma reesei* MTCC 164(3 :1)	10%alkali treated *Eicchornia crassipes*	30	5.0	18	Semisolid state	Flask	-	33.95U/g	22.89U/g	10.44U/g	133

Abbrevations: CCF = crude corn fibers; DSCF = De starched corn fiber; CFC = corn fiber with cellulose enriched CF AX = corn fiber arabinoxylose: SPW = steam Pretreated willow: POMC = Palm oil mill condensate: CCP = coconut coir pith : CCR = corn cob residue:WB=Wheat bran, WHB = water Hyacinth biomass: WPH = waste paper Hydrolysate: OCC = Old Corrugated Card board, OPEFB= Oil palm empty fruit bunch, CF = Consternate Filtrate,P=Peptone, SmF = Submerged fermentation, SSF = Solid State fermentation, SSSF = Semi Solid State fermentation.,Beech tree L:Beech tree leaves,CSL: Corn steep liquor,*Fagus sylvatica* TL: Fagus sylvatica tree leaves.

2.9.1 CONVENTIONAL LIGNOCELLULOSIC WASTES

2.9.1.1. Wheat bran

Wheat is one of the cereals used extensively in the many parts of the world for preparation of bread and many bakery products [169]. Wheat bran is the hard outer layer of grain and consists of combined aleurone and pericarp. Removing the wheat bran from grains signifantly reduce a portion of their nutritional value [34]. Bran is particularly rich in dietary fibers and contains significant quantities of starch, proteins and vitamin [309]. Industrial wheat bran usually accounts for 14–19% of the grain and comprises the outer coverings, the aleurone layer and the remnants of the starchy endosperm and has the potential to serve as low-cost feedstock to increase the production of commodity products [180].Wheat bran contains 38% (w/w) glucuronoarabinoxylan, 16% cellulose, 6.6% lignin, 25% protein, starch and some β-D-glucans and arabinoxylans from the aleurone layer. Wheat bran is also a good source of nitrogen due to the presence of protein content as well as good source of hemicellulose in overall it is a good inducer of cellulolytic enzyme system[37]. Wheat bran is composed predominantly of nonstarch carbohydrates (~58%), Starch (~19%) and crude protein (~18%).In non starch carbohydrates cellulose composition is about ~ 25%. High amount of protein present in the wheat bran may also reduce the cellulase biosynthesis but the soluble oligosaccharides, starch and cellulose present in the wheat bran significantly impact on the cellulase production [248].

2.9.1.2. Bagasse

Sugarcane (*Saccarhum officinarum*) bagasse is a residue produced in large quantities by sugar industries, In general one ton of sugarcane generates 280 Kg of bagasse, the fibrous byproduct remaining after sugar extraction from sugarcane[57].Relatively high crude fiber contant in sugar cane bagasse correlated positively with increase in the xylose contant [6].Bagasse consists of approximately 50% cellulose and 25% each of hemicellulose and lignin. Because of its low ash content, bagasse offers numerous advantages in comparison to other crop residue such as rice straw and wheat straw for usage in bioconversion processes using microbial cultures[185][228][235].

2.9.1.3. Wheat straw

Wheat (*Triticum aestivum* L.) is the world's most widely grown crop and cultivated in more than 115 nations,whereas wheat straw is annually generated worldwide (529 million tons/year)[199][124]. Wheat straw contains lower amounts of lignin with higher levels of cellulose and hemicelluloses. The overall chemical composition of wheat straws could slightly differ depending on wheat species, soil and climate

conditions. Cellulose, hemicellulose and lignin content of wheat straw are in the range of 33–40, 20–25, and 15–20 (%w/w), respectively [253][199]. In wheat straw cell walls, the majority of lignin is directly linked to arabinose sidechains of xylan by ether bonds without hydroxycinnamic acids.Marginal percentage of wheat straw lignin is also directly linked to glucuronic acid or 4-*O*-methylglucuronic acid by ester bonds[246]. Guaiacyl unit is the main component of the wheat straw lignin[247]. The removal of this particular lignin generally increases the digestibility of the material and enhances further microbial bioconversion.

2.9.1.4. Ground nut shell waste

Ground nut shell (*Arachis hypogea* L) is an important oilseed crop of india.The pod or dry pericarp contains about 25-40 percent shell. Chemical composition of groundnut shell is as follows: cellulose, 65.7; carbohydrates, 21.2; proteins, 7.3; minerals, 4.5; and lipids[280], 1.2%.Ground nut shell is used as a substrate for termite culture[1], for mushroom cultivation, production of extracellular enzymes and used as a manure.[260, 281]

2.9.2. NOVEL AND NON CONVENTIONAL RAW MATERIALS

2.9.2.1. *Pisum sativum*

Pea peel waste is an outer covering of pea (*Pisum sativum L*) vegetable, not the peel of seed. It is cheap and easily available lignocellulosic biomass that can be serve as potential raw material for cellulase production. Pea (*Pisum sativum L*) is an annual plant of cool season, belongs to the family leguminosae and grown in many part of the world[48]. Pea (*Pisum sativum*) is the second most important food legume worldwide after common bean (*Phaseolus vulgaris* L.).It is cultivated over 5.9 million hectares with a production of about 11.7 million tons. In India it is grown over 0.7 million hectares yielding about 0.6 million tons. [5, 225]. Pulse crops are an excellent source of protein, carbohydrates, as well as fiber and provide many essential vitamins and minerals [269]. Literature stated that pea cotyledon cell walls are mainly composed of arabinose-rich pectins (26%) and hemicelluloses (22%), whereas hulls contain primarily cellulose (69%)[213].Soluski and Wu[230] also reported that pea hulls contained 82.3%(DMB) total dietary fiber with 8.2% hemicelluloses and 62.3% cellulose.The influence of meterological conditions such as cultivation years ,soil characteristic of the cultivation area and their nutrient and antinutrient composition strongly affect the composition of pea . Gunaseelara *et al* [95] reported the composition *Pisum sativum* L (garden pea) pods without seeds in terms of lignin and cellulose (g/g volatile solid) 0.087 and 0.174 respectively. Peas have the potential of providing high

antioxidant compounds other than polysaccharides, proteins and micronutrients. Generally phenolics found in fruits and vegetables are bonded to dietary fibers, proteins or to sugars in plants to form complex structures [288]. Sultana and Anwar [244]reported the presence of flavanols such as (kaempeferol, querecetin and myricetin) in the pea seeds. Flavanols are naturally occurring phenolic substances.

2.9.2.2. *Luffa cylindrica*

L. cylindrica is a common cucurbitaceous vegetable crop grown abundantly in tropical and subtropical countries.The plant is climber with fruits, which are berry, elongated and cylindrical [27].Recently, *L. cylindrica* sponges have been applied as cell carriers in bioreactors, scaffolds for tissue engineering and for the development of biofiber rein forced composites, due to the presence of fibrous vascular system[35]. *L. cylindrica* sponge was found to be a good matrix for microbial and plant cells immobilization because of its high porosity, high specific pore volume, stable physical properties, biodegradability, non toxicity and low cost [140].Phytochemical investigation have shown the presence of alkaloids, saponins, D- mannitol, betulic acid and β-sitosterol in these peels. Amount of total polyphenols (mg gallic acid equivalent/100g dry weight) and flavonoids (mg/100gdry weight) in the peel extracts of *L. cyli* are 35.82 and 31.30 respectively [66].Du and Wang (2007)[69] reported the presence of p-coumaric acid, 1-O–feruloyl-β–D glucose, 1-O–p-coumarolyl-β-D glucose, 1-O caffeoyl-β-D glucose and 1-O –(4-hydroxybenzoyl)-glucose in *L. cyli* peels. Du et al(2006)[70] also reported the presence of antioxidant constituents in the fruits of the vegetable *L. cyli* Roem. Chemical composition of *L. cyli* comprises as holocellulose % 82.4, cellulose 63.0%, hemicelluloses 19.4%,lignin,11.2%,extractives 3.2% and ashes 0.4%.Certainly, the *L. cylindrica* composition will be very much dependent upon various factors, such as species, variety, soil type, whether conditions, plant age etc [254].

2.9.2.3. *Lagenaria sineraria*

L. sineraria is a large pubescent, climbing or tailing herb found throughout india.The cultivated form of *L. sineraria* is considered to be of African and Asian origins. Bitter fruits mainly contains cucurbitacins B are known to be cytotoxic[310].The plant contains tri terpenoid, Cucurbitacins, flavones, C-glycosides, β- glycosides,Vit C and fair source of ascorbic acid in fruits.The edible portion also contains thiamin ,riboflavin and niacin. *L. sic* is a rich source of vitamins, irons and minerals as well as an excellent diet for people having digestive problems. It contains higher concentrations of dietry fiber, Vit A, Vit K, Vit B_6, Folate, Potassium, Manganese, Protein, Vit E, Thiamin, Riboflavin, Pantothenic acid,

Calcium, Iron, Magnesium, Phosphorus and selenium [131]. The composition of various fibers and other constituents are as follows in bottle guard (g/100g dry basis): Neutral detergent fiber:Acid detergent fiber: Total detergent fiber: hemicelluloses:cellulose: lignin: pectin: 23.65: 17.10: 24.14: 6.55: 16.90: 0.20: 0.49.[205] .

2.9.2.4. (*Litchi chinensis Sonn.*)

(*L. chinensis* Sonn.,) is a subtropical to tropical fruit of high commercial value in international trade, originated from China is cultivated all over the world in warm climates [301].It belongs to the family Sapinndaceae and possesss a clear pulp containing 80% water, 0.4-0.9 g/kg of vitamin, 0.2-1.1 % acidity and 11.8-20.6% total soluble solids. The red colour is due to the presence of anthocyanins .Cyanidin -3 rutinoside and cyaniding 3-glucoside have been reported as the major pigments in the pericarp. The main postharvest problems of *L. chin* fruit are loss of red colour by high dehydration rates.It has been reported that enzymatic degradation of the anthocyanins plays a key role in browning when these pigments are hydrolysed by anthocyanase to anthocyanidins.The anthocyanidins and phenolic compounds can be oxidized by polyphenol oxidases (PPO) and /or peroxidases (POD) to o-quinones[154].Polyphenol oxidases (PPO) catalyses the hydroxylation of monophenol into o-diphenol which is oxidized into the corresponding o-quinones and subsequently polymerized into brown, red or black pigments[245]. *L. chinensis* peels showed the best potential also in terms of heating value (19.6 MJ/kg). Theoratically, there is a considerable potential of *L. chinensis* as renewable energy resources in global scenario due to their availability and fuel properties [168]. Knowing the cell wall polysaccharides of *L. chinensis* would provide avenues for its potential use in commercial food, cosmetics and medicinal or industrial products[100]. *L. chinensis* skins contain significant amounts of Polyphenolic compounds. Fresh pericarps also contains major pigments ,tannins and anthocyanins[215].

2.10. UTILISATION OF OTHER WASTE MATERIAL IN CELLULASE PRODUCTION

2.10.1. Starches

Starches from many sources have long been used in tablet formulations as a diluent, binder, and disintegrant. Potatoes are the world's fourth largest food crop followed by rice, wheat and maize. Every year nearly 24 Million metric tones potato is produced [311].Starch molecules arrange themselves in the plant in semi-crystalline granules. Each plant species has a unique starch granular size. Rice starch is relatively small (about 2μm) while potato starches have larger granules (up to 100μm). Although in

absolute mass only about one quarter of the starch granules in plants consist of amylose. Amylose is a much smaller molecule than amylopectin.Wheat starch grains are bimodal in size, B-starch (15 - 20%) is 2 – 15mm diameter and the larger A-starch granules (80 - 85%) are 20 – 35mm. B-starch is always highly contaminated with pentosans, fine fibres, lipids and protein.[312, 314, 315]. As the literature suggested that the water soluble acid hydrolyzed starch is an excellent inducer for growth and cellulase production, which induces the enzymes to the same extant as pure cellulose[285, 283],Since pure starch is expansive therefore the utilization of waste starch would be a great approach.

2.10.2. Dairy industry waste (whey)

Dairy industry is one of the example of process industry where a number of effluents are generated, some of which contain nutritionally valuable constituents such as proteins, lactose, fats etc. One such effluent is whey. Whey is liquid generated during manufacture of Cheese. Whey have a biological oxygen demand in the range of 35000 to 40000 mg/L[15, 155]. The production of cellulases can be induced by the dimeric sugars (lactose) which is the major constituents of whey.High biochemical oxygen demand (BOD_5) of whey poses a major world-wide disposal and pollution problem for which an effective and permanent solution is urgently needed [155]. The whey contains proteins and half the solids present in the original whole milk, including most of the lactose (5%), minerals and water soluble vitamins [316].Valorization of industrial waste or effluents in to formation of valuable product are a better approach in liquid waste management [76].

2.10.3. Poultry industry waste (egg shell waste)

Recently the egg shell wastes in the poultry industry has been highlighted because of its reclamation potential [29, 119]. Egg shell waste is available in huge quantity from food processing, egg breaking and hatching industry. About 250,000 tons of egg shell waste is produced annually world wide by food processing industry only. Fresh egg shell waste is approximately 95% calcium carbonate crystals stabilized by a protein matrix [82, 171, 317]. The average egg shell contain about 95% of the calcium carbonate, 0.3% phosphorus, 0.3% magnesium and traces of sodium, potassium, Zn, Mn, Fe, Cu. Egg shell waste composed of calcium carbonate, protein membrane, amino acid and collagen[318]. Most of the egg shell waste is commonly disposed in landfills without any pretreatment because it is traditionally useless and ultimately creates serious environmental problems[317]. Therefore proper treatment is required to recover valuable calcium ions from egg shell waste.

2.11. UTILITY OF HYDROLYSATE IN PRODUCT FORMATION

2.11.1. Acidic hydrolysates

Few literatures have been reported on the utilization of hydrolysate for growth and production system of microbes[55]. Gonazalez *et al* [89] have been carried out hydrolysis of wheat straw with sulphuric acid at 34 and 90^{0}C and found that treatment at 90^{0}C yields complete solubilisation of hemicellulose to xylose and arabinose without significant amounts of furfural. The acidic medium attacks the polysaccharides, especially hemicelluloses that are easier to be hydrolyzed than cellulose. Therefore, the cellulose and lignin fractions remain almost unaltered in the solid phase and can be further processed. Depending on the operational conditions acidic hydrolysates contained sugars such as xylose, glucose and arabinose as well as decomposition products of hemicellulose (such as acetic acid, furfural, 5-hydroxymethylfurfural (HMF) and dehydration product of pentoses and hexoses)[39, 253].Based on the dosages of acid used in the preprocess, acid catalysed hydrolysis can be divided into two types: Concentrated and dilute-acid hydrolysis.

2.11.1.1. Concentrated acid hydrolysis

In this type of hydrolysis biomass is treated with high concentration of acids at ambient temperatures, which results in the high yield of sugars [88]. This process has few drawbacks including high acid and energy consumption, equipment corrosion and longer reaction time as well as obligation for acid recovery after treatment that largely limit its application.

2.11.1.2. Dilute acid hydrolysis

Dilute acid hydrolysis,In first stage typically employ a dilute acid (0.5–1% H_2SO_4) under moderate temperature conditions (140–160^{0}C) to release the pentoses whereas in the second stage the temperatures are raised to 200–240^{0}C to facilitate the hydrolysis of cellulose and recovery of six carbon sugars[55]. High temperature is favorable to attain acceptable rates of cellulose conversion to glucose, hemicellulose sugar decomposition [253]. Both cellulose and hemicellulose components can be hydrolysed using dilute acid catalysed two step-hydrolysis processes. The difference between these two steps is mainly the operational temperature [88]. Xylose yields of 75–90% are possible if this process is done effectively.Delgenes *et al*, treated wheat straw with 72% (w/v) for H_2SO_4, 30 min at 30^{0}C and obtained 11.1 g monomeric sugars in total from 18.8 g dry raw material[55].The main drawbacks of this method are the formation of many inhibiting byproducts such as furan derivatives and other unidentified toxic products. The acidic treatment removed nearly all the hemicelluloses [245, 253]. Literature also indicates

that the hydrolysates obtained from the enzymatic hydrolysis of lignocellulose have been shown to have cellulase inducing capability. However the fundamental knowledge on its induction and its growth supporting characteristics is lacking. The use of the hydrolysates as soluble inducing substance for cellulase production deserves more study.

2.11.2. Alkaline hydrolysates

Alkaline treatments are similar to those used in kraft paper pulping processes and compared to acid pretreatments they are more focused on the solubilisation and removal of lignin from the biomass rather than hydrolysis of hemicellulose which tends to remain insoluble, however few acetyl and uronic acid groups are removed from hemicelluloses [53].Alkaline process is based on utilization of dilute bases in pretreatment of lignocellulosic feedstocks. Alkaline pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies and well suited for agricultural residues [253]. All of the ester-linked substituents of the hemicelluloses and other cell wall components can be cleaved by alkali treatment. The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds crosslinking xylan hemicellulose and other components. Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in crystallinity, separation of structural linkage between lignin and carbohydrates and disruption of the lignin structure[16, 199].

2.11.3. Detoxification of hydrolysates

During pretreatment of lignocellulosics, in addition to the sugars, aliphatic acids (acetic, formic and levulinic acid) furan derivatives furfural and HMF (2-furaldehyde and 5-hydroxymethyl-2-furaldehyde) and phenolic compounds are formed.These compounds are known to affect microbial growth and product fermentation performance.Furfural could be generated as a degradation product from pentoses[39, 246]. Another inhibitory substances founded in lignocellulolisc is acetic acid. Acetic acid can be generated when the hydrolysis reaction takes place at the acetyl group of hemicelluloses. Several detoxification methods like neutralization, overliming with calcium hydroxide, activated charcoal, ion exchange resins [40, 191] and enzymatic detoxification using laccase [43] are known for removing various inhibitory compounds from lignocellulosic hydrolysates. Overliming the hydrolysate has been effective as a detoxification process due to partial removal of toxic inhibitors, such as furfural and 5-hydroxymethylfurfural, although the whole mechanism is not well understood. Another potential drawback of overliming is sugar loss due to hydroxide-catalysed degradation reactions and conversion of

sugars into unfermentable compounds [210]. Chandel et al.[43] have also demonstrated that acetic acid concentration is not altered using overliming but this method led to the removal of furans (45.8%) and phenolics (35.87%).After detoxification, the concentrations of all those toxic compounds were lower than the reported levels causing, lesser inhibition of microorganism metabolism [210]. An inhibitory effect of hydrolysates on microbial activity was observed but it could be effectively relieved by using the some other strategy such as large inoculum, diluted hydrolysate solution, tolerant strains or a combination of the three [302]. Biglow and Wyman [30] stated that few microbial strains were also able to consume or convert essentially all furans, aldehydes and acetic acid which released in the hydrolysates after hydrolysis process.

2.12. FACTORS AFFECTING GROWTH AND CELLULASE PRODUCTION

2.12.1. Temperature

Temperature is the critical and crucial parameter that has to be controlled and varied from organism to organism. Studies indicate that the most favorable temperature for cellulase producing microorganism within the range of 25^0-35^0C but few organisms such as *Trichoderma lignorum* [21] *and Micromonospora sp* [49] perform best at elevated temperatures from 37^0-45^0C. Temperature above or below 30 °C was somewhat unfavorable for cellulase production by *T. reesei*, which might be due to their physiological nature and protein synthesis capability. At higher temperature, significant decrement in the enzyme activity has been observed, which might be due to the fact that at higher temperature enzyme biosynthesis decreases due to thermal deactivation, while at lower temperature the transport of nutrients in the cells is hindered, and due to this the microbial enzyme production capability is decreased. The most effective temperature for cellulase production by different microorganisms is explained in Table 2.4-2.7.

2.12.2. pH

pH is yet another vital parameter for enzyme production. Most microorganisms are able to grow in a range of pH 5-7. Only some microorganisms require pH lesser than 5 for better cellulase production. *Micromonospora sp* is most effective at pH 7.2 [49].The most effective pH for cellulase production is illustrated in Table 2.4-2.7.

2.12.3. Incubation period

Incubation period is also one of the key factors for enzyme production. Literature suggests that most of the microorganisms require 5-8 days of incubation period for higher cellulase production but few strains

of *Trichoderma, Aspergillus* and other microorganisms like *Bacillus subtilis, Penicillum decumbans, Micromonospora* require lesser time period 0.5-4 days. Besides that, there are few other strains of *Trichoderma, Aspergillus* and *Penicillium* grown on different substrate which require more time period for higher yield such as 10-14 days. The different incubation time period required for effective cellulase production are summarized in Table 2.4-2.7.

2.12.4. Substrate concentration

Substrate concentration is one of the main factors that affect the yield and initial rate of enzymatic hydrolysis of cellulose. At low substrate levels, an increament in substrate concentration normally consequences in an increase of the yield and reaction rate of the hydrolysis, conversely high substrate concentration can cause substrate inhibition, which significantly lowers the rate of hydrolysis and the degree of substrate inhibition depends up on the ratio of total substrate to total enzyme[101][193].

2.12.5. Fermentation mode and magnitude

Commercially cellulase production has been tried by either solid state fermentation [107, 149, 167] or by submerged fermentation including batch [9, 127, 139] fed batch [85] and continuous mode[112, 218].Several authors have reported cellulase production at flask level [200, 166, 251] whereas production in large stirred tank fermentor and in tray fermentor is also reported [53, 126, 137,178, 182, 295].Surface adhesion fermentation a novel approach has also been used in cellulase production [275]. The high product concentration, lower downstream processing, direct use of fermented product as crude enzymes and utilization of byproduct makes solid state fermentation, a promising technology. But one of the most critical problem associated with solid state fermentation is related to heat transfer [162] and this can be solved by applying periodical dyanamic change of air (Including air pressure pulsation and internal circulation of air) instead of agitation and rotation [173, 184, 234, 256, 257]. The different fermentation mode and magnitude required for effective cellulase production are stated in Table 2.4-2.7.

2.12.6. Role of inoculum dosages in microbial growth and cellulase production

The size of inoculum seems to have a profound effect on microbial growth and enzyme production. Inoculum sizes influences the carbon and nitrogen utilization from the medium by microbial sources. There are two type of effects have been associated with the inoculum size and microbial growth. Smaller inoculum sizes produced a transient mycelial stage with the mycelium length inversely proportional to inoculum size.This phenomenon termed as "inoculum size effect". Many fungal spores also exhibit a crowding effect in which the spores contain a prepackaged self-inhibitor that prevents germination under

crowded, high-cell-density conditions. These self-inhibitors usually are phenolic molecules, such as *cis*-ferulic acid methyl ester or *cis*-3,4-dimethoxycinnamic acid methyl ester[99].Alam *et al*[10] studied the effects of inoculum sizes 5-25% (w/v) on cellulase production .Cellulases activity increases with higher inoculum size,except in day 3 where 5 % of inoculums size gave the highest cellulase yield of 0.0413 U/ml. The effect of inoculum size on the enzyme activity was also studied by Dhillon *et al* [63] they stated that maximum enzyme activity was observed using 5% inoculum. An increase in inoculum size from 5% showed a progressive decrease in enzyme activity reaching the lowest at 20% inoculums. A higher inoculum concentration becomes favorable, probably because of the reduction in the lag phase caused by highly concentrated inocula. Decrease in its production on increasing the inoculum size could be due to competition between microorganism colonies for nutrients and probably the non-availability of nutrients for the large population limits the fungal growth[150]. Inoculum size could reduce the time at which the maximal conidial productions were achieved with no effect on variables related to conidia quality[174]. Therefore a suitable and appropriate inoculums size or dosages required for healthier fungal propagation and their enzyme production for better and improved solid waste management.

2.12.7. Effect on moisture content on growth and production

Fungi may grow at very low levels of air humidity if water is available on the surface. Thus, repeated or persistent moisture condensation or water leakage is sufficient for fungal germination [190]. Moisture content is a critical factor on SSF processes because this variable has influence on the microbial growth, biosynthesis as well as secretion of enzymes. According to researchers higher moisture levels can cause a reduction in the enzyme yields due to the steric hindrance of the growth of producer strain by reduction in the porosity (interparticle space of the matrix), thus interfering oxygen transfer. Alam *et al*[10] reported the optimal moisture content in the solid substrate appears to be at 50%, under this condition a cellulase activity of 0.0433 units was obtained. Gervais *et al*[83] who worked with *Penicillium roqueforti* and *Trichoderma viride* stated that a value of a_w close to 1.0 was the best for fungal growth. Solid-state fermentation allows the manipulation of a_w values in order to adjust this parameter to particular requirements by fungal strain.

2.12.8. Effect of nitrogen sources on growth and production

Nitrogen sources plays a very essential and fundamental role in the fungal growth and production system.Each nitrogen source having diverse and distinctive type of valuable components required for

fungal growth system. Meat extract is one of the important nitrogen sources The amount of valuable components present in the chief meat extracts are describes in Table 2.8.

Table 2.8. Various componants present in chief meat extracts[319]

Chief meat extracts	Water	Proteins and gelatine	Extractives	Nitrogen from substances	Salts
Armour's Extract.	24.3	160	20.5	200	19.0
Hipi(mutton preparation)	35.0	38.0	16.0	1.5	8.4
Liebig's Extract	20.0	-	557	0.9	24.0
Oxine Extract	62.9	13.0	4.5	-	19.6

On the other hand yeast extract is the water-soluble portion of autolyzed yeast [170].A yeast extract composition satisfies all the requirements that the contents of mannitol, glutamic acid, alanine, 5'-IMP, and 5'-GMP[208]. It is rich in nucleic acids, consisting of mainly ribonucleic acid[320].The typical composition of yeast extract is (expressed on dry matters basis) : total nitrogen content : 8 to 12 %, corresponding to a protein content of 50 to 75 % amino nitrogen content : 3.0 to 5.2 %, total carbohydrate content : 4 to 13 % lipid content : none or very little. Whereas beef extract powder is a dehydrated extract of bovine tissue used in preparing microbiological culture media. Beef extract powder provides nitrogen, amino acids, nucleotides, vitamins, and carbon [90, 321].Proteose-peptone is a heat-stable and acid-soluble protein fraction of milk that has important functional properties. Component 3, which is the most hydrophobic fraction, appears to be largely responsible for the physicochemical properties of proteose-peptones and for their important biological role in milk. A complex mixture of glycoproteins, phosphoproteins, and peptides, the PP fraction is thought to contain at least 38 components. The component 3 fraction, however, includes different glycoproteins and hydrophobic constituents. The PP3 is of great interest because of its functional properties, particularly its emulsifying power and biochemical role, such as spontaneous lipolysis regulation in milk[105].While soybean peptones Type III and IV (Sigma) can serve as excellent nutrients for this fermentation[214].

2.12.9. Role of surfactant in growth and cellulase production.

The presence of low concentration of fatty acids or other surfactants have been reported to inhibit or stimulate microbial growth and / or product formation. Many reports have shown the stimulatory effects of surfactants on enzyme production by microorganisms in submerged or solid state fermentation.Most of the surfactant used were chemically synthesized surfactants such as Tween-80,Tween-60,Tween-20,TritonX-100,Polyethylene glycol ,Sodium taurocholate.These surfactants had various effects on different enzymes and the most commonly used surfactants are Tween -80[141]. Apparently the main effect of surfactant are an increase in cell membrane permeability, the release of cell bound enzyme and

the diminution in mycelia growth caused by a decrease in oxygen supply, by increasing the fungal growth.The surface active agents which effects the cell permeability promote the release of cellulases from the medium[58].Tween-80 could provok an increase in cell membrane permeability leading to a more efficient nutrient uptake,without significant alteration in oxygen supply. Conversely Tween-20 and Triton X-100 clearly diminished the growth of *N.catalinensis*, probably decreasing oxygen availability as reported by Pardo[187]. Yezdi *et al*[300]also found a similar effect of Triton X-100 on *Neurospora crassa* The surfactant could change the nature of substrate by increasing the available cellulose surface or by removing inhibitory lignin[98].Karr and Holtzapple was found that surfactants could promote the availability of reaction sites, which would increase the hydrolysis and uptake rate[118]. Triton surfactants are not found suitable for large scale because of the environmental effects due to the presence of aromatic ring in the surfactant whereas Tween surfactants are non toxic and suitable for biotechnical use.Non ionic surfactants have been shown to be the most effective surfactants in improving cellulose conversion[120]. A plausible explanation for surfactant effect on lignocellulose hydrolysis is that the hydrophobic part of the surfactant binds through hydrophobic interactions to lignin on the lignocelluose fibers and the hydrophilic head group of the surfactant prevents unproductive binding of celluloses to lignin[74].Natural oils exist in abundance as agricultural products, but they differ widely in their effectiveness in foam suppression. Microbial hydrolysis of natural oil leads to a mixture of fatty acids and glycerol [91].Literature also reported that stimulating influence of natural oil may be changed into an inhibitory effect for enzyme synthesis as the concentration of natural oils increases. This inhibitory effect can be due to concomitant changes in the cell permeability; following the adsorption of fatty acids on the cell surface .Fatty acids also exhibiting surfactant properties alter the interfacial tension between cell membrane and bulk aqueous solution. It has also been reported that the mass transfer in detergent or surfactant based systems is very fast[222].

2.13. APPLICATION OF MIXED CULTURE STRATEGY

Application of mixed cultures is another strategy to enhance cellulase activity. The mixed culture of *T.reesei* and *A.phoenicus* could produce complete set of cellulase with a high level of β-glucosidase.Mixed cultures of *T.reesei* and *A.niger* inoculated at the same time gave hypercellulase activity. The highest filter paper and β-glucosidase activity in mixed culture representing approx 3 and 6 fold increases over the activities, attained in single culture SSF.*T.reesi* produces a high level of total cellulaes with very low level of β-glucosidase on the other hand total cellulase produced by *Aspergillus*

strain was very low whereas β-glucosidase activity was high.The mixed culture resulted in a relatively high FPA and β-glucosidase, however its FPA was 15% lower than the pure culture of *T.reesei* and β-glucosidase activity was 18% lower than the pure culture[133, 287, 280]. Table 2.7 describes the application of mixed culture for cellulase production.

2.14. APPLICATION OF MUTAGENS IN STRAIN IMPROVEMENT

Strain improvement has been achieved by mutation, selection or genetic recombination.Strain improvement is a traditional method used with great success for isolating mutatnts with high productivity[4].In many cases mutations are harmful but ocassionly it may lead to better adopted organisms to its environment with improved biocatalytic performance.The potential of a microorganisms to mutate is an important property conferred by DNA,since it creates new variation in the gene pool. UV and Ethidium bromide are important inducers of strain mutations.The pyrimidins (thymine and cytocine) are especially sensitive to modification by UV rays absorption.This may results in the production of thymine dimmers that divert the DNA halix and block future replication [189].Modifiction of cell membrane can also promote release of the metabolites /secondary proteins /enzymes from the cell [61]. Dhawan et al [62] studied the effect of ethidium bromide, a DNA intercalating agent, on laccase production from *Cyathus bulleri* and found that EtBr treated white-rot fungus *Cyathus bulleri* produced a sixfold increase in laccase production.The mutants resulted in higher cellulase activity than the wild type. Successive mutation by EMS treatment with UV irradiation and selection results in isolation of two promising mutants, both mutants produced two times higher FPA and CMCase activities than the parent strain[4]. Gherbawy studied the effect of gamma radiation on the cell wall degrading enzymes[87]..Mutation studies also carried out on *Aspergillus terreus* ATCC 52430 with successive UV and nitrosoguanidine treatments resulted in isolation of strain UNGI-40 having 3.5,4.6 and 3.3 fold increase in filter paper, β-glucosidase and carboxymethyl cellulase activity respectively compared to parental strain[44, 19]

2.15. ECONOMICS OF CELLULASE

The demand of cellulases has been increasing in the global market, including in the food processing and energy fields[176].In fact, enzyme industry sales are expected to increase to 2.2 billion dollars by 2010 [136]. Cellulases are well known for industrial applications and are indispensible for ethanol production from lignocellulosics. They contribute 43.5% to the total cost of cellulosic ethanol, when procured from external sources but the cost is drastically reduced once the in house produced crude enzymes are used

for saccharification purpose. The economics and environmental sustainability of enzymatic lignocelluloses to ethanol conversion processes are adversely affected by the use of purchased cellulase preparations. Using the crude extract avoids several processing steps associated with commercial cellulase preparations (Purification, concentration, addition of buffers, stabilizers and preservatives, freeze drying and packaging).These factors have led to the suggestion that using the crude unprocessed cellulase extract from solid state fermentation may be relatively a simple method of providing cellulase on site for the cellulose to ethanol conversion process or other application[136]. Production cost of cellulases in the crude fermentation by SmF was about $20/Kg, it was only $0.2/Kg when in situ SSF was used[172].Asahi Breweries, Ltd a major Japanese brewery, announced on September 25, 2009, that it has successfully developed the technology to produce cellulase at a low cost. The technique developed by Asahi allows the use of large amounts of wastepaper such as copypaper, newspaper, and cardboard from offices and households as a culture for producing cellulase. The price of enzymes is often controlled by the method of production and more importantly the purification method[324].Various carbohydrases primarily amylases and cellulases, used in various industries such as the starch, textile, detergent and baking industries, represent the second largest group. Overall, the estimated value of the worldwide use of industrial enzymes has grown from $1 billion in 1995 to $1.5 billion in 2000. The enzyme industry as we know it today is the result of a rapid development seen primarily over the past four decades [125].The demand for cellulases is consistently on the rise due to its diverse applications. There are several companies involved in cellulase production for textile detergent, paper industries and other industries. Globally, there are two major players known for cellulase production for biomass conversion"Genencor" and "Novozyme". Both the companies have played a significant role in bringing down the cost of cellulase several folds by their active research and adopting novel technologies. Recently, Genencor has launched Accelerase®1500, a cellulase complex intended specifically for lignocellulosic biomass processing industries. Accelerase®1500 is produced with a genetically modified strain of *T. reesei*. This enzyme preparation is claimed to contain higher levels of β-glucosidase activity than all other commercial cellulases available today, so as to ensure almost complete conversion of cellulose to glucose .Genencor has also launched Accelerase® XC is an accessory xylanase/cellulase enzyme complex that contains a broad profile of hemicellulase and cellulase activities and enhances both xylan (C5) and glucan (C6) conversion when blended with other Accelerase® enzyme products. Novozymes also have a diverse range of cellulase preparations available based on application as

Cellusoft®AP and Cellusoft®CR for bioblasting in textile mills, Carezyme® and Celluclean for laundry in detergent, Denimax® 6011 for stonewash industry at low temperature as well as many others specific for particular application. National Renewable Energy Laboratory's (NREL) estimates the minimal cost of enzyme by a novel *on site* SF production as the unit cost of enzyme is US$ 0.38/100,000 FPU. In comparison, a commercial enzyme, Novo-Nordisk (Novozyme) Celluclast 1.5 l would have a unit cost of US$ 16/100,000 FPU, From this preliminary estimate it is evident that the SSF enzyme is about half as costly as the most optimistically estimated SF enzyme[258].

2.15.1. Companies engaged in cellulases and bioethanol production

There are several companies in all over world involved in cellulase and bioethanol production. The first pilot plants for second-generation bioethanol production have been commissioned by Novozymes and ready to deliver its cellulase enzymes[325].Danisco's American division Genencor has launched the first commercial enzyme for the production of cellulose-based bioethanol under the name Accellerase [326]. Novozymes, Denmark, and Broin, US, are expanding their collaboration in the development of a second-generation process for the production of ethanol from biomass[327].Novozymes, Denmark, launched three new enzymes which increase the efficiency of ethanol production from wheat, rye, and barley by up to 20%, thereby creating the possibility of boosting the production of bioethanol in Europe.These preparations, known as Viscozyme Wheat, Viscozyme Rye, and Viscozyme Barley [328].

2.15.2. Manufacturers of cellulases

World wide consumption of cellulase from submerged fermentation is roughly 23000 tons annually[264]. Cellulases are accounted for approx 20% of world enzyme market [28].The demand for more stable, highly active and specific enzymes is growing rapidly and projected world market for industrial enzymes is rapidly growing at an annual rate of about 7.6% and is estimated to be $ 6 billion by 2012[329]. Current international players in the production of commercial cellulases are Genencor and Novozymes. Genencor had recently announced the launch of first ever commercial enzyme product for cellulosic ethanol. Similarly collaborative subcontract between novozymes and NRELs has been able to reduce the cost of cellulases for biomass to bioethanol, which is almost 30 fold reduction from estimated cost in 2001[330].The enzymes from Novozymes has been produced second generation bioethanol by 2010. The company also has announced setting up of on $ 80-100 million production facility in Nebraska for cellulase production [331].

2.16. Application of cellulases in various process industries

2.16.1. In Textile industry

In textile industry cellulases have been used as biostoning agents. Cellulase gained a foothold in the industry by producing the softness and appearance of jeans washed with pumice stones. The original cellulases used in denim washing were the crude enzymes of *Trichoderma* and *Humicola* referred to as acid and neutral cellulases respectively based on the optimum pH range of use of the enzymes, which was pH 4-5 for acid cellulase and 6-7 for neutral cellulase. *Trichoderma* cellulase, comprising the more complete set of EG and CBH components capable of the full hydrolysis of cellulose. *Trichoderma* cellulase also achieves certain desired "finishes" (appearances)[28, 264].

2.16.2. In Detergent industry

Cellulase in laundry detergent removes the hairs, known as pills, that occur on the cotton cloth after repeated wearing and machine washing. Cellulase also enhances the softness and removal of soil from the garment. *Trichoderma* cellulases have not been successful in this application[264].

2.16.3. In Feed industry

The primary use of cellulase in the feed industry has been in barley and wheat based feeds for broiler chickens and pigs. The barley and wheat contain soluble β-glucans that increases the viscosity of the feed in the gut of the animals. This in turns, causes an uptake of water which decreases the amount of carbohydrate and vitamins that the animal obtains from the feed, as well as causing sticky stool and related problems of disease and effluent disposal. Application of cellulases in the feed, helps to overcome these problems. This application is carried out with the crude cellulases of *Trichoderma* and *Aspergillus*[**152**, 264]

2.16.4. In Textile biopolishing

The enzyme removes pills from the fabric, restoring its appearance and conditioning it for resistance to further pilling. The preferred method of removing the pills is to treat the pilled fabric with cellulase enzymes. Cellulase treatment is typically for 15-30 min at 40-50 0C, pH 5.0.This application has been primarily carried out using acidic cellulase from *Trichoderma.*[264]

2.16.5. Juice and Beverage industry

Cellulase enzymes break down cellulose and β-glucan associated with the cell walls, thereby decreasing the viscosity of the mash and increasing the ease of juice recovery. The enzyme treatment can also increases the clarity of the juice by solubilizing small particles. Most cellulase used in juice industry is

Trichoderma cellulase. Apple juice is the juice most commonly produced using cellulase enzymes[28, 264].

2.16.6. Baking

Enzyme action desired in baking is very mild. Cellulases is used to break down gums in the dough structure, so as to allow a more even dough rise and flavor distribution. *Aspergillus* is the most widely used cellulase in baking[264].

2.16.7. In bioethanol production

The accelerating accumulation of CO_2 and other green house gases may lead to adverse climatic changes that would seriously endanger the sensitive ecological balance of the earth. Energy shortages in the world coupled with environmental considerations have directed applied research towards the development of newer processes to produce renewable fuels with a special emphasis on fuel ethanol production from cellulosic materials [123]. Ethanol produced from renewable resources is being considered globally as the most prominent and possible substitute for fossil fuel[234]. To reduce the net contribution of GHGs to the atmosphere, bioethanol has been recognized as a potential alternative to petroleum-derived transportation fuels [22].On the other hand, renewable energy is more environment friendly in term of emission compared to fossil fuels. Hence, renewable energy is seen as the long-term solution for reliable and infinite source of energy supply in the future. Cellulases play a very vital and significant role in bioethanol production from cellulosic biomass. It accounts approximately 45% of ethanol production costs. Generally, biofuels such as bioethanol is derived from agricultural products, for instance corn, sugarcane, palm oil, rapeseed or soybean. Collectively, these biofuels from food sources are known as first-generation biofuels.Second-generation bioethanol only requires inexpensive cellulosic biomass as feedstock, non-edible sources, wasted crops such as lignocellulose biomass, which is plentiful and easily obtainable throughout the world.

2.16.7.1. Economics of bioethanol production

Today the production cost of bioethanol from lignocellulose is still too high, which is the major reason why bioethanol has not became commercialized till date. By using cheaper waste products from forestry, agriculture and industry, the costs may be lowered. Approximately 60% of the production cost of bioethanol comes from raw materials.The key barrier of current technology to produce cost-effective ethanol from cellulose is the high cost of cellulase enzymes needed to hydrolyze the cellulose to glucose[307]. Researchers pointed out that a 10-fold increase in enzyme specific activity could lead to

production costs savings of more than 15.85 US cents per liter of ethanol[22, 123].The evaluation showed that currently available industrial cellulases accounts for 43–45% of ethanol production costs, and therefore, a 10-fold reduction in the cellulase costs and a 30% reduction in capital costs are required in order to reach ethanol production costs competitive with starch ethanol .The cost of the cellulase enzymes is a critical features to improve the economics of ethanol production[104].

2.16.8. In waste Paper recycling

The fast depletion of forest resourses, its impact on the ecological balance, galloping rise in prices of fibrous raw materials and waste disposal problem have forced the paper industry to turn to the development of fast growing species, utilization of nonwood fibrous materials especially to the use of secondary fibers or waste paper. Consumption of recovered paper is estimated to be 4-5 million tones in india only. India is currently using both imported & domestic waste paper [50]. The critical major barriers in the profitable conversion of this relatively abundant and inexpensive raw material into quality products are the ink and contaminants removal [108].This requires an effective, efficient and more economical deinking technology for waste paper recycling. Chemical deinking is not an effective means for deinking of non impact paper such as xerographic and laser printed paper [148]. Thus removal of ink remains a major technical obstacle for a greater use of recycled paper. It has also been reported that in conventional method, lesser improvement in brightness, freeness and strength occurred, besides this the effluent released by this process is having a higher COD load[56]. Therefore enzymatic deinking may provide a means to meet these needs and seems to be a innovative and novel solution to overcome these problems through which the impact on environment is bare minimum. Several enzymes such as cellulases, hemicellulasses, Pectinase, lipase, easterse, α-amylase and lignolytic enzymes have been used for deinking of various recycled fibers [23]. Hemicellulases, cellulases and lignolytic enzymes can be attacked on fiber surface.

2.16.8.1. Cellulolytic deinking mechanism

There are several mechanisms which have been proposed for enzymatic deinking. Nine different possible mechanism for enzyme deinking have been reported by Welt and Dinus[290] .Enzyme partially hydrolyze and depolymerize cellulose molecule at fiber surfaces, thereby weakning bonds between fiber and freeing them from one another. Cellulases peel fibrils from fiber surfaces, thereby freeing ink particles for dispersal in suspension. This peeling mechanism has also been implicated as the pulp freeness increases after enzymatic treatment of secondary fibers. Xia *et al*[296] pointed out that cellulase

reaction are mainly confined to fiber surfaces rather than extending into fiber walls however fiber length distribution and individual fiber length are unaffected. When cellulases and hemicellulases are used, the release of ink particles into suspension is generally attributed to the cellulose hydrolysis on the fiber/ink inter bonding regions which facilitates ink detachment. Additionally, these enzymes can remove small fibrils from the surface of the ink particles[145, 181]. Understanding the mechanism of enzymes towards the fibers is essential to minimize the related negative impact on the strength of the paper and its quality [282]. According to Jefferis et al cellulases having high filter paper degrading activities are effective in deinking[108]. Whereas other researchers have suggested that the filter paper degrading activities of cellulase complex from *Aspergillus* L22 and *Trichodermma pseudokoningi* S 28 had detrimental effect on the paper strength[204]. Gubitz *et al*[92] treated laser printed waste paper individually and with the combinations of purified endoglucanases from *Gleophillum sepiarium* and *Gleophillum trabenum*, found that pure endoglucanase are responsible for most of the success in deinking.

2.16.8.2. Effect of cellulases on pulp and paper properties

Literature suggests that application of cellulase effects pulp and paper properties in terms of brightness and tensile strength. Treatment of 0.05% cellulase IUZ342 along with 0.001% amylase BAN 240 gave the greatest efficiency (96%). The blend gained 3% points over the treatment with cellulase alone (93%) and seven points over the treatment with amylase alone (89%)[71]. Individual mono components of cellulase having a single EG (endoglucanase) effectively remove inks from MOP fiber. CBD (Cellulase Binding domain) have negative impact on deinking of MOP. Literature showed that cellobiohydrolase (CBH) hydrolyze cellulose from the ends of cellulose chain because cellulose chain ends are limited so it is not much effective for removal of ink from the cellulose fiber and is also does not affect significantly the strength property of paper whereas endoglucanase have ability to hydrolyze the internal portion of cellulose chain so it is much more effective. Endoglucanase has positive effect on deinking, where as exoglucanase has a negative impacts on deinking efficiency [23, 290].Vyas and Lachke[282] investigated that alkaline active extracellular enzymes from alkalotolerant *Fusarium sp* gave better results for deinking of MOW in terms of brightness. Jefferies *et al* [108] compared the enzymatic deinking with conventional deinking of xerographic and laser printed paper, found that enzyme sample A (1500CMC U/g and 35 FPU/ml) is more effective in terms of ERIC value. Soni *et al*[229] reported that three isolates *Aspergillus sp* AMA, *Aspergillus terreus* AN1 and *Myceliopthora fergusii* T41 showed significant deinking of composite wastepaper. These cultures were found to produce multiple

endoglucanase, which may be responsible for better deinking efficiency. Table 2.9 describes the effect of cellulases alone or alongwith other enzymes and surfactants on pulp and paper property of various grades of papers.

2.16.8.3. Other recent approaches in enzymatic deinking

Colour offset news print, laser ink and toner of non impact printing papers which are difficult to deink broken down by ultrasound to a suitable size for flotation [297].A new process engineering introduced by Sarri for deinking using ultrasound and magnetic separations. Large toner particles broken down by ultrasound into a suitable sizes for flotation in the complete absence of chemicals. Magnetic separation improves the efficiency of ink removal due to the magnetic content of toner. When both of these process as are combine with enzymatic deinking they produce better results [216]. Combined deinking technology with ultrasonic, UV irradiation and enzymes was investigated Zhenyieng *et al*[303]. Result showed that the brightness of the combined deinking of ultrasonic and enzymatic is slightly higher than the brightness of cellulase + amylase treated, while the dirt area is comparatively lower. Performance and the efficiency of old news paper deinking by combining cellulase, hemicellulase with laccase violuric acid system was investigated by Xu *et al*[298], Results gave 20% and 13% higher tensile and tear strength respectively. The brightness of LVS–deinked pulp was 4.2 % ISO higher than that of the control. Experiments with cellulase mixture and purified cellulase monocomponents have shown that endoglucanase plays a major role in the improvement in recycling process (deinking and drainage of recycled pulp). Individual cellulase monocomponents can be produced by genetic engineering via overexpression of desired gene and /or deletion of undesired gene.Diends *et al*[64] produced individual monocomponents by repression of undesired gene using recombinants of *Trichoderma reesei* strains to produce intact Cel 7B (endoglucanase I,EGI) and catalytic core of Cel 7B selectively under glucose repression of other cellulase components. The culture filtrate containing intact or core Cel 7B applied for secondary fiber treatments showed improved drainage rate with 16-17 % decrement in Schopper – Rieglervalues.

Table 2.9. Effect of cellulases on pulp and paper properties of various grades of waste papers

Waste Papers	Enzyme Dose	Particulars	Brightness ISO %	Residual Ink Area%	ERIC ppm	Opacity	Burst Index	Tenlsile Strength	Tear index	Breaking length(m)	Freeness (CSF),ml	Refrences.
Black and white letter Press printed news print wastes	-	Control	52.5	0.71	-	-	1.26	26.15	8.71	2670	368	203
	0.2 CM case U/g OD Pulp	Preparation I	56.0	0.47	-	-	1.37	27.90	9.15	2780	450	203
	0.2 CM caseU/g OD Pulp	Preparation II	55.8	0.40	-	-	1.43	28.33	9.58	2890	440	203
	100 xylanaseU/g OD Pulp	Preparation III	56.5	0.52	-	-	1.60	30.55	10.4	3110	420	203
	19.34xylanaseU/g OD Pulp	Preparation IV	57.5	0.51	-	-	1.37	27.20	9.15	2780	460	203
	Enzyme activities(U/ml) – Preparation I (CMCase – 6.0, Xylanase – 10.0, Filter Paper activity -42), Preparation II(CMCase -6.0,Xylanase-6.0,FPA activity-1.0),Preparation III(CMCase – 0.20, Xylanase – 200, Filter Paper activity -0.005), Preparation IV(CMCase – 26, Xylanase – 580, FPA activity -55),											
Laser printed Papers	-	Control	90.25	-	123.45	-	1.28	43.02	4.67	-	~442	232
	0.5 IU/g X+0.001 U/g. C	Cartazyme PS 10	93.45	-	67.23	-	1.41	38.74	3.73	-	480	232
	0.05 IU/g. C	Cartazyme MCX-A	92.28	-	76.32	-	1.53	40.83	5.16	-	520	232
	0.05 IU/g. C	Cartazyme EG	92.05	-	80.30	-	1.30	39.24	4.41	-	510	232
	Enzyme used :Cartazyme PS 10(Xylanase0.5IU/g+ Cellulase0.001 IU/g) ,Cartazyme MCX-A(Cellulase 0.05U/g),Cartazyme EG (Cellulase 0.05 IU/g)											
Office waste papers	-	Control	8pts	258*	-	-	2.20	0.410	4.28	-	510	109
	-	Enzyme I	9pts	173*	-	-	2.42	0.430	4.39	-	570	109
	-	Enzyme II	10pts	26*	-	-	2.34	0.0412	4.25	-	565	109
	Enzyme used: Microbial cellulase , Enzyme run I at pH 8.4,Enzyme run II at pH 7.9.Here * shows residual ink area for 0.04-5mm ink size.											
Old News Paper (ONP)	-	Control	39.8	-	717.4	65.0	-	-	-	-	550	231
	-	A	40.9	-	643.8	68.7	-	-	-	-	610	231
	-	B	44.8	-	614.1	69.8	-	-	-	-	650	231
	-	C	43.1	-	678.3	66.4	-	-	-	-	630	231
	-	D	46.2	-	645.9	67.2	-	-	-	-	720	231
	Enzyme Used = Sample A (Cartazyme PS - 0.1IU/g Xylanase, 0.02 IU/g Cellulase), Sample B Double the Dose of A, Sample C (Cartazyme MCX- 0.1 IU/g Cellulase), Sample D (Cartazyme MCX)											
Mix Office Waste	0.04% w/w	Control	64.5	52*	-	-	2.2	34.0	11.0	-	465	229
	0.04% w/w	Enzyme I	66.5	62*	-	-	2.4	38.0	11.6	-	480	229
	0.04% w/w	Enzyme II	61.0	75*	-	-	2.2	36.0	12.3	-	440	229
	Enzyme used = Enzyme I= Cellulase (Imported), Enzyme II = Cellulase and Xylanase (Indigenous) ,Here * shows residual ink area for 20-80μm sub visible ink.											

Burst index in KP_am^2/g,Tear index in $mN/m^2/g$,Tensile index in Nm/g,ERIC shows effective residual ink content.

Compared with conventionally deinked pulp, we conclude from this study that cellulolytic deinked pulp will lead to improved pulp and paper properties, lower residual ink contents, better machine runnability, reduce power consumption. A proper combination of enzymes depending upon type of waste paper processed under proper operating conditions shall give better results. Waste water produced by this process shall have lower COD which ultimately reduces the load on effluents treatment systems.

CHAPTER III
MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Chemicals

Chemicals used in the experimental studies were AR grade and of Himedia, Merck and Sigma Aldrich make.

3.1.2. Microorganisms

Five standard fungal strains, *Trichodema reesei* NCIM 1186, *Trichodema viride* NCIM 1195, *Aspergillus niger* NCIM 777, *Aspergillus fumigatus* NCIM 902 and *Neurospora crassa* NCIM 1021 were procured from National Chemical Laboratory (NCL), Pune, India.

3.1.3. Substrates preparation

All the substrates were procured from local market utilized as substrates and carrier for solid state fermentation. All the raw materials were grounded and sieved with different mesh screen. Grounded raw materials were put into plastic bags and kept for further studies.

3.1.4. Media

The following media were used in the present study depending on the nature of experiments:

3.1.4.1. Media for culture development

Potato dextrose agar (PDA) medium was used for culture development and maintenance of *Trichoderma* and *Aspergillus* strains, whereas *Neurospora* strain was developed and maintained on M_2 agar medium as recommended by National Chemical Laboratory, Pune.

PDA medium containing (g/L) Peeled Potato, 200; Dextrose, 20; Yeast extract, 0.1; Agar 15 at pH 5.6. M_2 agar medium containing (g/L) Glucose, 10; Glycerine, 10; Yeast extract, 5; KH_2PO_4, 0.3; $MgSO_4.7H_2O$, 0.1; Agar, 20 at pH 6.8.

3.1.4.2. Production media for solid state fermentation

In (g/L) Urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.3; Peptone, 1.0; Tween80, 0.2; $FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$,0.0016; $ZnSO_4.7H_2O$; 0.0014; $CaCl_2.2H_2O$; $CoCl_2.6H_2O$, 0.02 and solid bed of raw material 9.0., pH 5.0 except in the case where the effect of variable pH were studied.

3.1.4.3. Production media for submerged fermentation

In (g/L) Urea, 0.3; $(NH_4)_2SO_4$,1.4; KH_2PO_4,2.0; $MgSO_4.7H_2O$, 0.3; Peptone, 1.0; Tween80, 0.2; $FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$; 0.0014; $CaCl_2.2H_2O$; $CoCl_2.6H_2O$, 0.02 and pure sugar 10.0., pH 5.0 except in the case where the effect of variable pH were studied.

3.2. METHODS

3.2.1. Microbial growth and production system

3.2.1.1. Maintenance of stock culture

The stock culture of *Trichodema, Aspergillus* and *Neurospora* strains were maintained on potato dextrose agar slants and M_2 agar slant respectively. The same media were also used for subculturing of the respective fungal strains. The procured fungal stock was kept at 4^0 C in 20% (v/v) glycerol.

3.2.1.2. Inoculum development

For inoculum development experiments have been performed in a 250 ml Erlenmeyer flasks containing 100 ml of Potato Dextrose Broth (PDB) medium (In g/l Peeled Potato, 200; Dextrose, 20; and Yeast extract, 0.1) and M_2 broth medium in which 5 loopfull cultures of fungal spores or mycelial conidia were added and shaken at 180 rpm at 30^0 C in an incubator shaker for 3-4 days (Domingues *et al.*2004) . A definite volume of prepared cultures in PDB or M_2 broth suspension (having 0.56 g/L cell dry weight) was used as inoculum for further production studies.

3.2.1.3. Cell biomass determination for batch growth studies

For the study of growth rate and morphology of cellulase producing cultures, batch experiments were carried out in 250 ml Erlenmeyer flasks (total 16 flasks) containing 50 ml of PDB for *Trichoderma, Aspergillus* and isolated strains while M_2 broth media for *Neurospora crassa.* A definite volume of earlier prepared cultures in PDB or M_2 broth suspension (containing 0.56 g/L cell dry weight) were added to each flask containing their respective culture media. Samples (As whole flask containing 50 ml, due to nonuniform nature of growth in fungal system) were taken at every 6 h interval till 84 h.

3.2.1.4. Solid State Fermentation

Separate set of batch experiments were carried out in 250 ml Erlenmeyer flasks containing sieved raw material as carbon source, which was impregnated with following production media in (g/L) Urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.3; Peptone, 1.0; Tween80, 0.2; $FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$; 0.0014; $CaCl_2.2H_2O$; $CoCl_2.6H_2O$, 0.02. Raw material having soaked with basal salt media were autoclaved, cooled and then inoculated with specific volume having (0.56 g/L cell dry weight) of PD and M_2 broth culture solution of respective strains. The autoclaved and inoculated flasks were placed in incubator at 30^0C for 6 days.

3.2.1.5. Submerged Fermentation

Separate set of batch experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml of production media having components in (g/L) Urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $MgSO_4.7H_2O$,0.3; Peptone, 1.0; Tween80, 0.2; $FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$; 0.0014; $CaCl_2.2H_2O$; $CoCl_2.6H_2O$, 0.02; Pure sugars 10., Flasks containing production media were autoclaved ,cooled and then inoculated with specific volume having (0.56 g/L cell dry weight) of PD and M_2 broth culture solution of respective strains. The autoclaved and inoculated flasks were placed in incubator shaker at 30^0C with 180 rpm for 5 days.

3.2.2. Pretreatment of materials

3.2.2.1. Pretreatment of starch

Acid pretreatment of starch have been carried out by using 2%, 5% and 10% HCl (v/v) solution.10 g of each (potato, wheat and rice) powdered starch were taken and 40 mL of diluted HCl solution with specific strength were added to maintain the slurry of about 25%. Afterwards these starch slurries were subjected to steam treatment under pressure of 15lb at 121^0C for 1 h and 3 h duration. The treated starch slurries were used in the production medium, either as pure hydrolysates or as hydrolysates alongwith lactose.

3.2.2.2. Pretreatment of lignocellulosic waste materials

Raw materials were collected form the local market. Chopped and grounded raw materials of a definite particle size (850μm) were used for the acid and alkali pretreatment. Acid and alkali treatment of raw materials have been performed with 5% H_2SO_4 (v/v) and 5% NaOH (w/v) solution with maintaining definite solid liquid ratio. Further these solutions were kept at room temperature for 1h and then subjected to steam treatment under pressure of 15 lb at 121^0C for 1h duration. After steam treatment solutions, were cooled out and filtered with muslin cloth. Pretreated raw materials were washed several times with distilled water to maintained their pH neutral.

3.2.2.3. Preparation of lignocellulosic hydrolysates

Raw materials were collected form the local market. Chopped and grounded raw materials of a definite particle size (850μm) were used for the acid pretreatment. Acid treatment of raw materials have been performed with 5, 10 and 20% H_2SO_4 (v/v) solution with maintaining solid liquid ratio of about 1:20. Further these solutions were kept at room temperature for 1h and then subjected to steam treatment under pressure of 15 lb at 121^0C for 1h duration. After steam treatment solutions, were cooled out and filtered with muslin cloth. Due to acid treatment at higher temperature several

toxic components were released in the hydrolysates. To minimize or overcome these toxic substances, overliming process has been conducted. Calcium salt solution was used to perform overliming process. The hydrolysates were added into calcium salt solution and boiled for 1h and kept at room temperature for next 1h. The resulting detoxified hydrolysates suspension were diluted to 100 mL with distilled water and make up the resulting solution at pH 7.0 with diluted NaOH solution. The resulting solution which has been obtained under these treatments was used in the production medium, either as pure hydrolysates or in combination with pure sugar.

3.2.2.4. Pretreatment of waste news paper

The hydrolysates of waste news paper were prepared by acid digestion.Old news paper (Times of India) were collected form the local market. Before acid pretreatment, deinking process of the chopped and grounded waste news paper have been performed by using effective chemicals and surfactants (Tween-80). Deinked waste news papers were further used for the acid pretreatment. Acid treatment of WNP (Waste news paper) have been performed with 20-70% H_2SO_4 (v/v) solution with maintaining the definite solid liquid ratio.Waste news paper were soaked in acid, at room temperature for one hour. These solution were cooled out, filtered and maked up the resulting solution pH 7.0 by dilute NaOH solution.The resulting hydrolyzates was used in the production medium as carbon source.

3.2.2.5. Pretreatment of egg shell wastes

Egg shells were collected from the local bakeries. To remove impurity and the interference material, the egg shells were rinsed several times into the deionised water (Stadelman N.J,2000). Chopped and grounded egg shells waste (0.4, 0.8 and 1.2g) with definite particle size were used for the acid pretreatment. Acid treatment have been performed with 5, 10 and 15% HCl (v/v) solution with maintaining the desired solid liquid ratio, soaked at room temperature for two hour. The resulting solution was diluted upto a desired level and used for the growth and production studies and make up the resulting solution at pH 7.0 by dilute NaOH solution.

3.2.3. Developmant of UV and ETBR mutants

To check the growth capability through mutagenesis, two mutagens UV (Ultra violet light) irradiation and ETBR (Ethidium bromide) were used to obtain the mutants. First set of experiments have been performed by taking freshly prepared *A.niger* PDA and *Neurospora crassa* M_2 plates and were irradiated with UV irradiation at a distance of 11 cm with treatment periods 30, 40 and 50 min.While other set of experiment has been performed by taking 40 min UV irradiated cultures,

subsequently treated with ethidium bromide of concentration 150 µg/mL. All the mutated strains with their respective cell dry weight were inoculated in a 250 mL Erlenmeyer flasks containing 100 ml of Potato Dextrose broth (PDB) medium for *Aspergillus* and *Trichoderma* strains and M_2 medium for *N.crassa* were shaken at 180 rpm at 30^0 C in an incubator shaker for 96 h to study the growth of mutated cultures.

3.2.4. Biofilm development

Biofilm formation of *Aspergillus niger* was studied by adding washed, sterilized muslin cloths and polyester sheet. Muslin cloth with 3 holes and 9 holes of 0.5 cm diameter each was added to the first two Erlenmeyer flasks. Muslin cloth without holes was added to the third flask. Polyester sheet was added to the fourth flask. Fermentation was carried out in all the four conical flasks containing lactose based production media having components in (g/L) Urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2.2H_2O$, 0.4; $MgSO_4.7H_2O$, 0.3; Peptone, 1.0; Tween-80, 0.2; $FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$, 0.0014; $CoCl_2.6H_2O$, 0.02 with lactose 10, as carbon source[275]. Liquid media were adjusted to an initial pH 5.0 and sterilized for 15 min at 121°C. An inocula of 4 mL culture broth having 0.56 g/L cell dry weight were added in production media and shaken on rotary shaker at 180 rpm at 30^0 C for 4 days.

3.2.5. Proximate analysis of raw materials

3.2.5.1.Estimation of pentosans in non wood raw materials

Pentosans in non wood raw materials was estimated by following procedure described as: 3-5 g of sample on O.D. basis were taken in a round bottomed flask of pentosan appatarus in which 300 ml hydrochloric acid (13.5%) was added .Further flask was connected to the pentosan apparatus and boiled the solution. Distilate was collected in a 250 mL conical flask . Acid level in a round bottomed flask was maintained by adding it drop by drop continuously through seperating funnel. Around 320 mL of distillate was collected. Make up the final volume of distillate upto 500 mL with distilled water in a volumetric flask. Absorbance of distillate was measured at 280 nm using UV-Vis spectrophotometer. Pentosan percentage was calculated by the following stated equation.

Pentosans % = (Absorbance at 280 nm x Dilution x 1.563 x 0.5 x 100) / (151 x O.D.weight of sample).

3.2.5.2.Estimation of holocellulose in non wood raw materials

This method was used to determine the total carbohydrate contents of non-wood materials. Holocellulose in non-wood raw materials was estimated by the TM1-A-9 test method mentioned in the laboratory manual of Central Pulp and Paper Research Institute (CPPRI) Saharanpur, U.P.,

India. 5 g of ground sample on O.D. basis (passed through mesh size 40) was taken in a 250 mL conical flask, wetted thoroughly with 10 mL of distilled water, followed by the addition of 1.5 g of sodium chlorite along with 0.5 mL of acetic acid. The mixture was incubated in a conical flask for one hour at 70 ^{0}C in a water bath. After cooling the conical flask, the supernatant was transferred to a tared crucible (W_1). Treatment of water, sodium chlorite, and acetic acid was repeated. The filtered contents of the conical flask were transferred into a tared crucible, followed by washing the residue with acetone. The solids were dried it in oven at 105°C for 2 h, and the filtering crucible was weighed with its contents (W_2). Holocellulose percentage was evaluated by the following equation (Laboratory manual 2001).

Holocellulose % = ($W_2 - W_1$) x 100 / O.D. weight of sample.

3.2.5.3.Estimation of lignin in non wood raw materials

Acid-insoluble lignin was estimated as per the TM1-A-7 test method mentioned in the laboratory manual of CPPRI [335]. It involves addition of 2 mL 72% H_2SO_4 into 1 g of oven dry basis sample in a 100 mL beaker, followed by addition of 13 mL of 72% H_2SO_4. The mixture was kept in the beaker in a water bath at 20°C with continous stirring for 2 h. The contents of the beaker were filtered through a tared G2 crucible that weighed (W_1). The residue was dried with the crucible in an oven at 105 °C overnight and weighed (W_2). The acid-insoluble lignin was assessed by the following expression:

Acid insoluble lignin %= (W_2-W_1) x 100/ O.D weight of sample

The filtrate obtained from the G2 crucible was kept for soluble lignin estimation, which was measured spectrophotometrically at 280 nm. Acid soluble lignin can be estimated through the following expression [335]

Acid soluble lignin % ={ (Absorbance at 280 nm x Dilution factor / 20) x 100} /1000 x O.D weight of sample.

3.25.4. Determination of Ash Content

5g of ground OD sample (passed through mesh size 40) were taken in a sintered crucible and then placed inside a muffle furnance at 600 °C for two hours, followed by cooling in a desicator. Ash content was calculated by the following expression

Ash % = W_2(weight of ash with crucible) – W_1(weight of only crucible) x 100/O.D weight of sample.

3.2.5.5.Measurment of moisture content

The moisture content of the medium was estimated by drying 5 g of the wet sample to a constant weight at 105^0 C and the dry weight was recorded.

3.2.6.Analytical Methods

3.2.6.1.Extraction of enzyme

Distilled water was added to fermented samples (in a 1:5 proportion in erlenmeyer flasks and the extraction was done after shaking in shaker at 30^0 C and 200 rpm for 1 h.The samples were then filtered through musclin cloth and the extract obtained was centrifuged at 8000 rpm for 15 min. by centrifuge and further resulting supernatant was stored and used as enzyme source. All extractions were conducted in duplicate.

3.2.6.2.Total cellulase activity (Filter Paper Activity)

Filter paper activity (FPA) was determined by the method recommended by Ghose (1987)[12]. as follows: 0.5 mL of culture supernatant was added to 1 mL of 0.05M citrate buffer, pH 4.8 and filter paper (50 mg, Whatman filter paper no#1), mix well and incubated at 50^0 C for 60 min. The enzymatic reaction was terminated by addition of 3 mL dinitrosalicylic acid reagent (DNSA). All reducing sugar determinations were performed by the 3,5-dinitrosalicylic (DNS) method at 540 nm. One unit of enzyme activity was defined as the amount of enzyme that released 1μmole of reducing sugar equivalent to glucose / min under the assay conditions.

3.2.6.3.CMCase activity

Carboxymethyl cellulase activity was determined by the method recommended by Ghose (1987), as follows 0.5 mL of culture supernatant, diluted with citrate buffer was added to 1 mL 2% carboxymethyl cellulose solution prepaerd in 0.05M citrate buffer, pH 4.8, mix well and incubated at 50^0 C for 30 min. The enzymatic reaction was terminated by addition of 3 mL dinitrosalicylic acid reagent (DNSA). All reducing sugar determinations were performed by the 3,5-dinitrosalicylic (DNS) method at 540 nm.

3.2.6.4.Cellobiase activity

One ml of test enzyme solution was added to 1 mL of 15 mM cellobiose solution prepaerd in 0.05M citrate buffer, pH 4.8, mix well, capped tightly and incubated at 50^0 C for 30 min. The enzymatic reaction was terminated by the addition of 3 ml dinitrosalicylic acid reagent (DNSA). All reducing sugar determinations were performed by the 3,5-dinitrosalicylic (DNS) method at 540 nm.

3.2.6.5.Soluble protein determination

The soluble protein was determined by the method of Lowry et al, using bovine serum albumin as standard.

3.2.6.6.Assay of lactose

Reducing sugar lactose was estimated by dinitro salicylic acid (DNS) method and expressed as reducing sugar glucose. Lactose content of the cheese whey was estimated by colorimetric method. Panesar et al.[186] described the steps of this method, whereby 5 ml of whey sample was prepared by prior treatment with zinc acetate–phosphotungstic acid reagent for removal of proteins and sodium hydroxide. This was further added with 5 ml of glycine–NaOH buffer and 0.5 ml each of methylamine–HCl and sodium sulfite solution followed by thorough mixing and kept at 65°C in a water bath for 25 min. The sample mixture was cooled immediately in an ice-water bath for 2 min and the absorbance was measured at 540 nm.

3.2.6.7.Dry weight determination

A 5.0 ml of sample was taken from the fermentation and was centrifuged at 8000 rpm. The solids collected were washed thoroughly with cold distilled water and then further washed with 5.0 ml of 0.9% NaOH soltion. Then filtered on a dried and preweighted whatman filter paper no 1. The filter with mycelium was then dried for 24 h at 105 ^{0}C and weighed. The growth as determined by cell dry weight were expressed as the mean of three independent readings.

3.2.6.8.FTIR spectral analysis

FTIR spectroscopy is a promising tool for the rapid, noninvasive and multiparameter analysis of the samples. The fourier transform infrared spectroscopy is used for identifying the structure of constituents of lignocellulosic structure. FTIR spectroscopy was performed by using a spectrophotometer. Samples were oven dried at 105^{0}C for 4 h, mixed with KBr in the ratio of 1:200 mg (raw material: KBr) and pressed under vaccum to form the pellets. Absorbance was measured over a range from 4000-500 cm^{-1}.

3.2.6.9.XRD analysis

XRD determined the crystalline content of raw materials. XRD diffraction of samples was recorded on a Phillips X-ray diffractometer, having copper target with a scanning rate of 2^{0}/min operating at 40 KV and 30 mA. The sample was mounted horizontally while the Geiger counter moved in a vertical arc. The samples were scanned in the range from 0^{0}-70^{0} angle.

Scanning electron microscopy was used to investigate the surface as well as morphological properties of different raw materials. In this study, the samples were coated with a gold film. The samples were then examined using scanning electron microscope model LEO-435 VP.

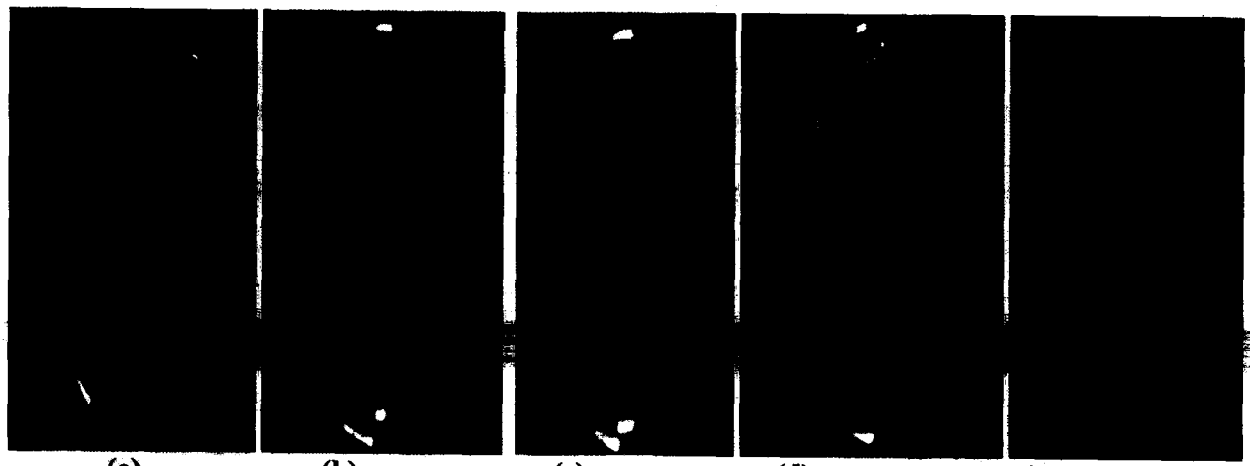
3.2.7. Deinking experiments

Old news papers were individually torn in approximate one inch square for the preparation of pulp. In all the experiments pulping in the hydrapulper was carried out using 525 gm air dry mass at 6 % consistency with approximately 6% moisture content at 65^0C. For chemical deinking chemicals were added on the basis of oven dry weight as follows NaOH: 2%, Sodium silicate; 2.5%, H_2O_2; 1%, DTPA; 0.5%, EDTA; 0.2% with Tween–80 surfactant. Retention time in hydrapulper was about 15 min. Pulping experiments were carried out in laboratory helicon pulper supplied by Universal Engineering Corporation, Saharanpur. It has provision for controlling rotor speed and temperature at varying conditions. It was adapted to operate at rotor speed ranging from 0-650 rpm. After the completion of pulping process, slurry from the hydrapulper was sent to the second stage of flotation deinking in case of chemical deinking, whereas in enzymatic deinking pulp was treated with different dosages of crude enzymes produced by various fungal strains with Tween-80 surfactant for 3 h contact time. After that this enzyme treated pulp with 1% cy was sent to flotation cell for further processing. In the flotation stage, the deinking was performed in a laboratory flotation cell supplied by Universal engineering corporation Saharanpur. In flotation process about 100 g OD pulp of the repulped stock from the hydrapulper was diluted to 1% cy. About 10 litre diluted stock was then sent in the batch flotation cell .The agitation speed was fixed at 2000 rpm Retention time in flotation cell was about 10 min. The optical and strength properties were measured from handsheets with a basis weight of 60 g/m^2 prepared after flotation on the British standard handsheet machine according to Tappi standard method T-205. Brightness was measured on the both sides of sheet and reported as an average of two readings. ISO brightness and Tensile strengths have been measured by using the instruments supplied by fibertech (ISO 9001, 2000) Lorentzen and welfre (075/70).

The data shown in the experimental results is the average of three replicates of experiments.The graphical plots are drawn around the mean value.

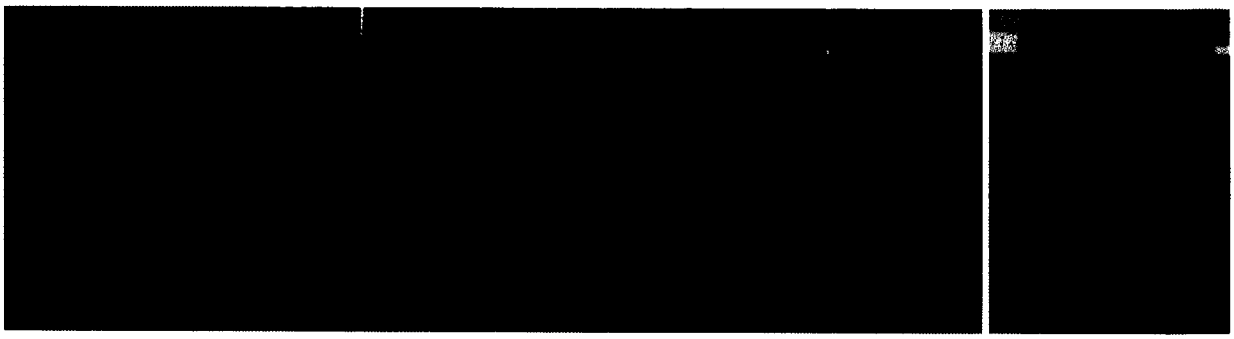
CHAPTER IV
RESULTS AND DISCUSSION
SECTION I
GROWTH AND MORPHOLOGICAL STUDIES OF CELLULASE PRODUCING STRAINS
4.1.1. GROWTH AND MORPHOLOGICAL STUDIES OF FUNGAL STRAINS

The present section describes the morphological nature of the *Trichoderma*, *Aspergillus* and *Neurospora* strains and their growth pattern under the pure sugars as well as industrial wastes (whey and boiled bagasse) containing culture broth medium.



Figs. 4.1.1 Growth of *Trichoderma reesei* ,*Trichoderma viridae*, *Aspergillus niger*,*Aspergillus fumigatus* in Potato Dextrose agar (PDA) solid culture media and *Neurospora crassa* in M_2 agar solid media at 30^0C respectively.

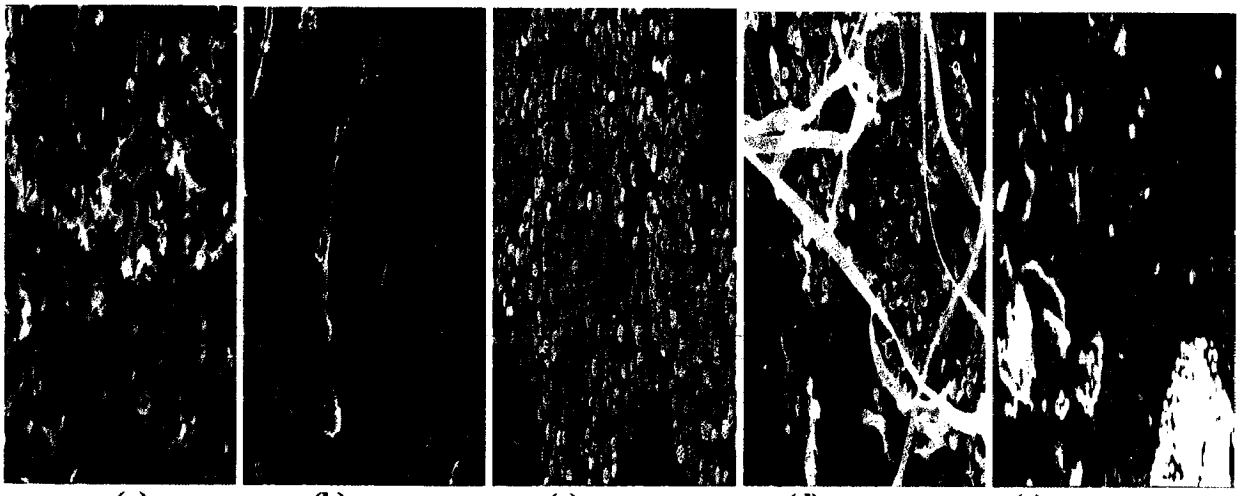
From the above observation we found that *Trichodermma reesei* showed a yellowish whitish growth on potato dextrose agar plate, whereas whitish circular and compact growth was observed with *Trichoderma viride* strain on potato dextrose agar plate as shown in Figs. 4.1.1a and 4.1.1b, But the growth patterns changed in potato dextrose broth culture medium. Yellowish small filamentous mycelium (with branched active, inactive hyphae and hyphal tips) type of morphology has been observed from *T.reesei* as shown by Fig. 4.1.2a. SEM figure of *T.reesei* also confirms their filamentous mycelial nature as observed from Fig. 4.1.3a. whereas *Trichoderma viridae* showed the growth in the form of big round shaped mycelial structure having compact central core and a loosely fluffy packed filamentous outer zone, looked like a big pellet as observed from Fig. 4.1.2b.



(a) (b) (c) (d) (e)

Figs.4.1.2 Growth of *Trichoderma reesei* NCIM1086, *Trichoderma viride*, *Aspergillus niger*NCIM777, *Aspergillus fumigatus* in Potato Dextrose Broth (PDB) media and *Neurospora crassa* NCIM1021 in M_2 media at 30^0C and 180 rpm respectively.

On the other hand when we compared the growth of *Aspergillus* strains, we observed that *Aspergillus niger* exhibits blackish sporangial nature of growth when cultivated on potato dextrose agar plate, while under potato dextrose broth liquid cultivation media the nature of growth has changed, it appeared as medium sized spherical compact solid pellets with no lateral growth as have been observed from Fig. 4.1.1c and 4.1.2c. It has also been proved by SEM Fig. 4.1.3d, that large number of spores are scattered around the major section and having vague holes.



(a) (b) (c) (d) (e)

Figs:4.1.3 Scanning electron micrograph of the growth of *Trichoderma reesei,Trichoderma viridae*, *Aspergillus niger*NCIM777,*Aspergillus fumigatus* and *Neurospora crassa* NCIM 1021 respectively.

Aspergillus fumigatus showed, whitish growth on PDA plate. When this strain was cultivated on potato dextrose broth under submerged condition, it appears as bigger virtually round shaped diffused, fluffy spherical branched type of growth having central core with interwoven network of tentacles around the whole periphery, as has been seen by Fig. 4.1.1d and 4.1.2d. Their channeled structure can be seen by SEM Fig. 4.1.3d.

On the other hand *Neurospora crassa* showed dissimilar kind of growth and morphology. They confirmed the pinkish aerial nature of growth which covered the whole M_2 agar plate. Sometimes the aerial mycelia comes outside the plates as viewed from photograph 4.1.1e. A thick mycelial pulpy nature of growth has been observed in M2 broth culture media. Fig. (4.1.2e) represents their pulpy nature of growth. SEM Fig. (4.1.3e) of *Neurospora crassa* showed clear view of their morphological structure. There are three types of morphologies that have been achieved by used fungal strains such as filamentous mycelium, pelleted forms and pulpy types. This morphological information might be helpful for getting the better and industrial suitable morphology with their improved productivity. The filamentous growth characteristic creates a number of process engineering problems attributed to the morphological changes accounted during the fermentation process in large scale which affects on the productivity. As the literature suggested that, morphological state of *T. reesei* influences the growth and cellulase production. Manori et al[151] suggested that polygalacturonose (PG) synthesis increases with the degree of compactness of mycelium. Due to thick and pulpy type of morphology, organisms also suffer mass, heat and O_2 transfer limitation which might be affect the overall productivity of the product produced by fermentation process. It can also be stated that growth of *Aspergillus niger* having medium sized pellets was much more intense with respect to *Aspergillus fumigatus* culture having bigger pellets, which closely resembled the findings of Feng *et al*[77] who suggested that larger or very smaller pellets size gave poorer yield in comparison to medium sized pellets. It has been concluded that fungal growth in pellet form is a favorable alternative which benefits most of the liquid fungal fermentations since it not only makes repeated-batch fungal fermentation possible but also significantly improves the culture rheology, which results in better mass and oxygen transfer into the biomass and lower energy consumption for aeration and agitation. Hence the morphology of the culture, which affects growth, productivity provides some solid evidence regarding their application in batch liquid fermentation therefore it needs to be controlled. In industrial applications pellet morphology is usually preferred in fermentations and in downstream processing due to their non viscous rheology of the broth. Both *Aspergillus strains* showed the pelletized morphology, in which *Aspergillus niger* pellets are more compact in nature and also medium sized with respect to *A.fumigatus*. By knowing the morphological pattern of the filamentous organisms, a controlled morphological growth could be obtained, for productivity. Keeping the view of broth rheology finally pelleted nature of growth may be beneficial followed by filamentous mycelia and pulpy forms, for their extensive use in liquid state fermentation. This morphological information

might be helpful in knowing the mass scale production of these organisms under liquid state and also their on product formation capability.

4.1.2. BATCH GROWTH STUDIES OF FUNGAL STRAINS UNDER VARIOUS SUGARS TREATMENT IN CULTURE MEDIUM

Owing to the filamentous and non homogenous nature of the moulds growth, the analysis of the growth characteristic and the growth curve is very difficult. Although determining the rate of colony extension will provide us, measure of fungal growth but it will not necessarily be equivalent to the increase in the biomass of the fungus because hyphae will be growing down through the agar medium as well as across its surface. Due to all these complications, it is better to estimate fungal growth (cell biomass) in terms of cell dry weight.

4.1.2.1. Effect of temperature on the growth of fungal strains under glucose based culture medium

To study the effect of temperature on the batch growth of fungal strains separate set of experiments have been performed at different temperatures such as 25, 30, 35^{0}C and 180 rpm under glucose containing culture medium.

Table 4.1.1. Biomass in terms of cell dry weight (g/L) of various fungal strains under glucose containing culture medium at different temperature (^{0}C).

T (h)	Biomass in terms of cell dry weight (g/L) under glucose based media at different temperature and 180 rpm.														
	Trichoderma reesei			*Trichoderma viridae*			*Aspergillus niger*			*Aspergillus fumigatus*			*Neurospora crassa*		
	25	30	35	25	30	35	25	30	35	25	30	35	25	30	35
0	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
6	1.98	4.20	2.34	0.80	0.95	1.02	0.86	0.87	0.98	0.67	0.85	0.90	0.98	0.66	0.58
12	2.78	5.40	3.56	0.98	1.76	1.78	1.43	1.68	1.73	1.31	1.74	1.88	1.23	4.34	1.31
18	3.03	5.54	4.55	1.76	3.88	3.90	1.98	2.02	2.12	1.54	1.92	2.02	3.43	5.82	2.32
24	4.11	8.04	5.98	3.98	6.58	4.23	2.21	2.52	2.78	1.98	2.47	3.41	4.67	7.04	3.01
30	6.34	8.54	6.87	4.54	7.32	6.12	2.98	3.86	3.43	2.11	3.64	4.63	4.98	7.12	4.46
36	7.86	9.42	8.01	5.67	8.29	6.66	3.87	5.12	4.31	2.23	4.93	5.89	5.32	7.42	5.11
42	8.11	10.36	8.43	6.88	9.12	6.98	4.89	6.64	4.89	4.78	6.55	6.98	5.98	7.66	5.55
48	8.85	10.60	8.98	7.12	9.33	7.32	6.78	7.78	5.12	5.87	8.55	7.03	6.09	8.16	5.76
54	9.05	11.42	9.21	7.35	10.06	7.77	7.21	8.50	7.54	6.63	9.35	7.56	6.54	8.86	6.01
60	9.54	12.88	9.65	7.41	10.93	8.12	7.98	8.94	8.21	6.93	9.83	7.88	7.03	8.42	6.34
66	9.87	12.64	9.89	7.89	11.03	8.31	8.32	9.60	8.88	7.16	10.36	8.23	7.83	8.16	6.73
72	9.98	11.30	10.01	8.05	11.52	8.76	8.53	9.94	9.10	7.79	10.63	8.89	7.79	7.02	6.34
78	10.21	11.68	10.78	8.31	11.63	8.89	9.45	11.26	10.45	8.67	11.91	9.41	7.71	6.92	6.39
84	9.67	11.02	10.65	8.81	11.81	9.83	10.06	10.91	10.21	9.87	11.00	10.52	7.60	6.76	6.11
90	9.73	9.72	10.71	8.64	10.66	9.65	9.98	9.42	10.01	9.79	9.76	9.67	7.64	6.55	5.98

T: Time (h)

It has been observed from Table 4.1.1. and Fig 4.1.4. that 30^{0}C was most suitable temperature for the growth of *Trichoderma* strains. Temperature above or below 30^{0}C was somewhat unfavorable for their growth. When compared the growth under lower and higher temperature to 30^{0}C, it was observed that lower temperature was much more discouraging for the growth of *Trichoderma*

strains as compared to their growth on higher temperature, which may be due to their physiological nature. Growth rate and growth was highest at 30^0C in comparison to lower and higher temperature.

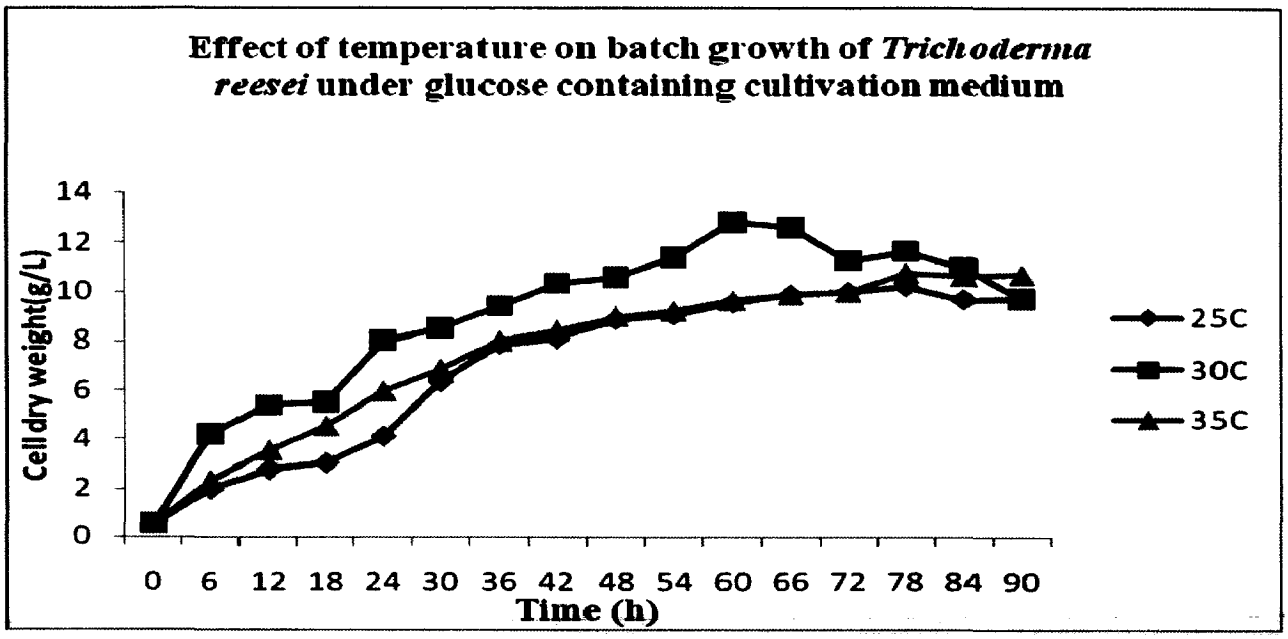


Fig.4.1.4. Effect of temperature on the batch growth of *Trichoderma reesei* under glucose containing potato broth medium at 180 rpm.

Maximum cell dry weight (g/L) attained by *Trichoderma reesei* and *Trichoderma viride* were 10.21, 12.88, 10.78, 8.81, 11.81, 9.83 at 78, 60, 78, 84, 84, 84h at temperatures 25, 30, 35^{0}C respectively, which indicates that maximum growth achieved by *Trichoderma* strains was at 30^0C. It has also been examined that *Trichoderma reesei* required shorter incubation period (60h) for reaching their highest growth at 30^0C in comparison to 25^0C and 35^0C as observed from Table 4.1.1. and Fig 4.1.4.While *Trichoderma viride* required same incubation period (84h) for reaching their maximum growth at all the three temperatures used in experimental study, but their highest growth in terms of cell dry weight achieved at 30^0C as has been shown in Fig.4.1.5.

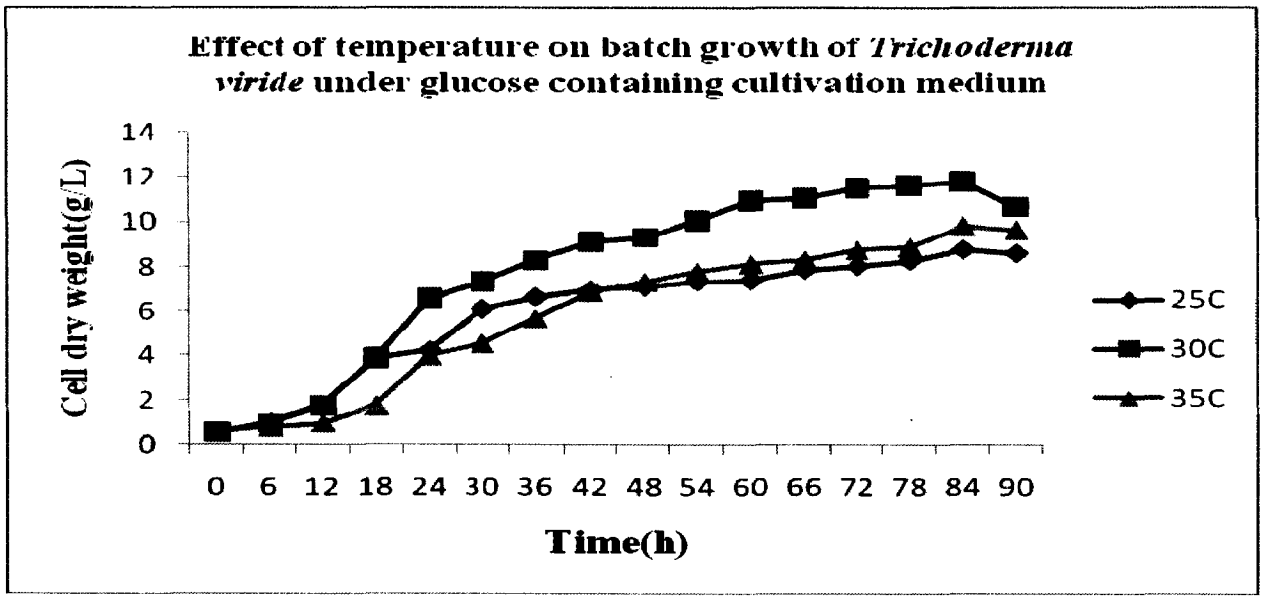


Fig.4.1.5. Effect of temperature on the batch growth of *Trichoderma viride* under glucose containing potato broth medium at 180 rpm.

On the other hand *Aspergillus* strains attained maximum growth at 30^0C whereas growth rate was observed faster at 35^0C, which indicates that temperature higher than 30^0C was somewhat beneficial for their growth. Temperature below 30^0C was found unpromising for both growth as well as growth pattern as observed from Table 4.1.1 and Fig 4.1.6 and 4.1.7.

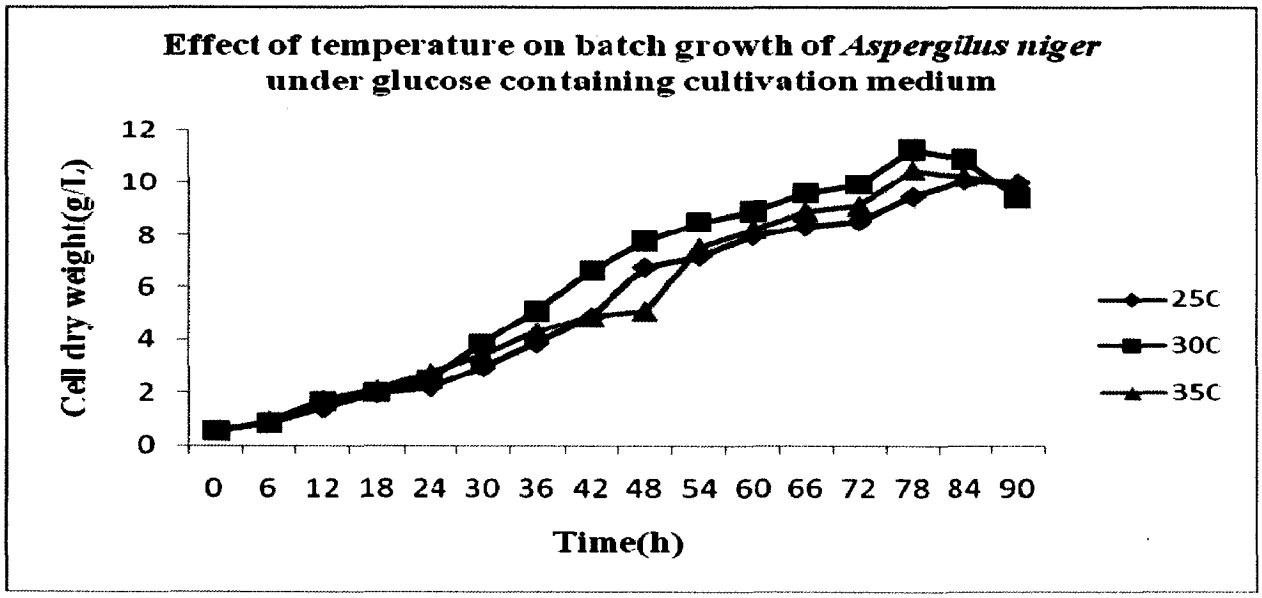


Fig.4.1.6. Effect of temperature on the batch growth of *Aspergillus niger* under glucose containing potato broth medium at 180 rpm.

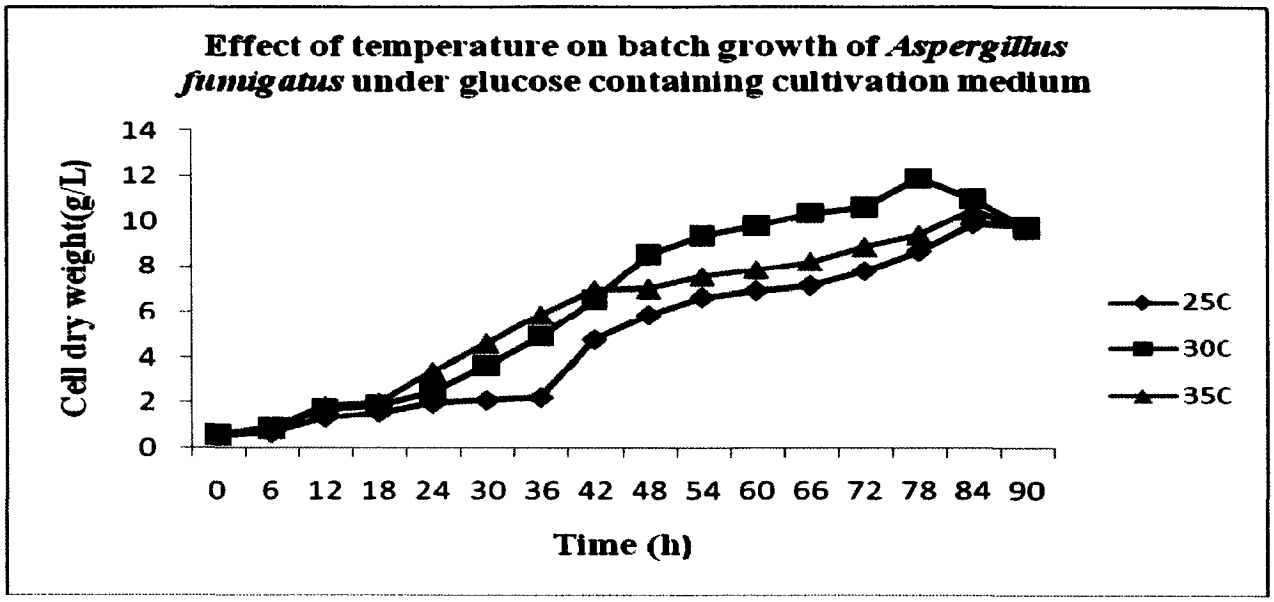


Fig.4.1.7. Effect of temperature on the batch growth of *Aspergillus fumigatus* under glucose containing potato broth medium at 180 rpm.

Maximum cell dry weight (g/L) attained by *Aspergillus niger* and *Aspergillus fumigatus* were 10.06, 11.26, 10.45, 9.87, 11.91, 10.52 at 84, 78, 78, 84, 78, 84h at temperatures 25, 30, 35^{0}C respectively, which confirms that both *Aspergillus* strains required shorter incubation period (78h) for reaching their highest growth at 30^{0}C in comparison to 25^{0}C and 35^{0}C as observed from Table 4.1.1.

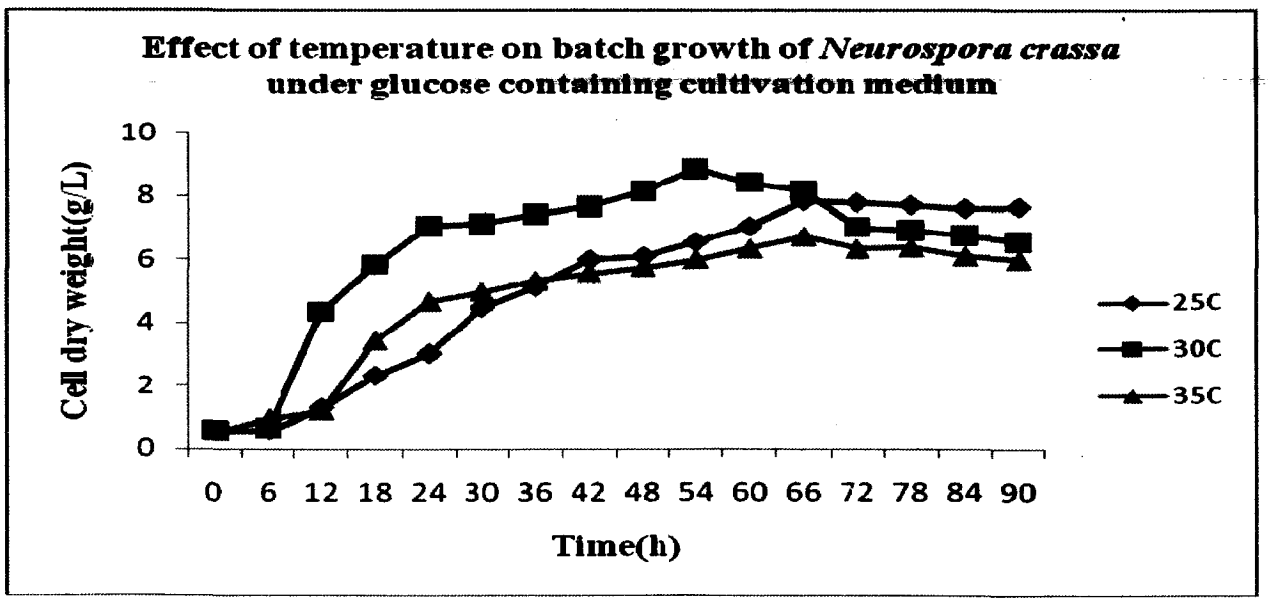


Fig.4.1.8. Effect of temperature on the batch growth of *Neurospora crassa* under glucose containing potato broth medium at 180 rpm.

Both growth and growth rate of *Neurospora crassa* was maximum at 30^0C. Temperature above 30^0C was somewhat unfavorable for the growth of *Neurospora crassa*, but lower temperature was found encouraging for the growth of *Neurospora crassa* strain in comparison to higher temperature. Maximum cell dry weight (g/L) attained by *Neurospora crassa* was 7.83, 8.86, 6.73 at 66, 54, 66 h at temperatures of 25, 30, 35^0C respectively as observed from Table 4.1.1 and Fig 4.1.8. *Neurospora crassa* achieved their maximum growth in shorter incubation time at 30^0C.

4.1.2.2. Studies on growth phases achieved by various fungal strains under glucose containing culture medium

To study the growth pattern and the effect of pure sugars on the growth of cellulase producing organisms separate sets of batch experiments have been performed. It has been observed from Fig 4.1.9. that different fungal strains showed diverse type of growth phases. Lag phase has been observed upto 6 h in case of *Aspergillus niger* and *Neurospora crassa* while for *Trichoderma viride* as well as *Aspergillus fumigatus* required somewhat longer lag phase (9 and 12 h) whereas *Trichoderma reesei* entailed very short lag phase (approx 2-3 h). In the lag phase microbes acclimate to food and nutrients in their new habitat. The longer and stable lag phase in *Trichoderma viride*, *Aspergillus fumigatus* and *Neurospora crassa* shows that this microbe takes much more time for reaching their active state in comparison to *Trichoderma reesei.* In *Trichoderma,* after 2 h a steep rise in the growth was observed till 24 h and the exponential or log phase (as in this phase substrate conversion and cell mass reached to their maximum values) also started. A sharp increment in the growth has been observed after 12 h and progresses upto 30 h in case of *Trichoderma viride.* whereas in *Aspergillus* strains no sharp increment have been observed only steady increment was observed up to 70 h, which indicates that growth rate was much slower in case of *Aspergillus* strains. It has also been viewed from Fig 4.1.9. that growth rate of *Aspergillus fumigatus* was much faster after 48 h of incubation than *Aspergillus niger.* Whereas in the case of *Neurospora crassa,* growth sharply increased till 18 h (exponential phase) afterwards they showed steady increment with the progress of fermentation upto 48 h. The period of exponential phase was observed differently for each fungal strain.

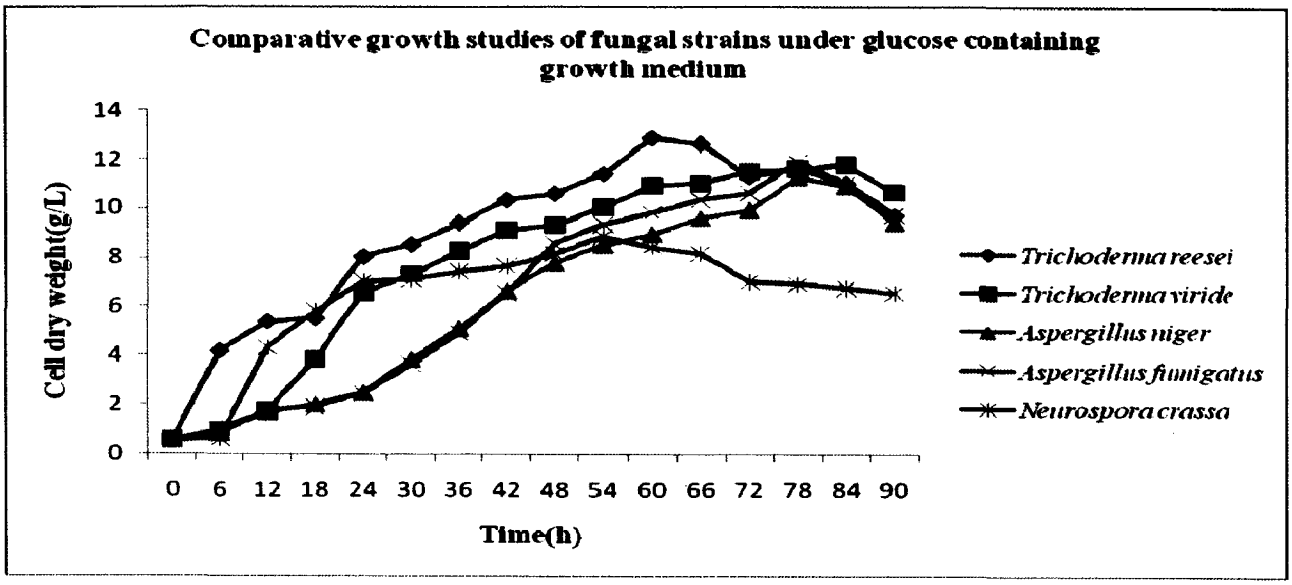


Fig.4.1.9. Comparative batch growth curve of various fungal strains in glucose containing respective culture medium at 180 rpm and 30^{0}C.

The maximum cell dry weight (g/L) attained by *Trichoderma reesei*, *Trichoderma reesei* *Aspergillus niger, Aspergillus fumigatus* and *Neurospora* were 12.88, 11.81, 11.26, 11.91, 8.86 at 60, 84, 78, 78 and 54h respectively, which indicates that the growth and growth rate both were much faster in case of *Trichoderma reesei.* Growth rate was lowest in case of *Trichoderma viride. Aspergillus* strains also showed good growth but lower growth rate whereas *Neurospora crassa* showed deprived growth but was having fastest growth rate as observed from Fig 6. After the above mentioned time period, no significant increment in cell biomass by each microbe has been observed probably due to the depletion of nutrients and carbon source in the media and therefore booming growth had stopped, which indicates starting of their stationary phase. Whereas in the case of *Trichoderma reesei* a slight increment in growth (11.30 g/L) has been observed during fermentation period between 72 h to 78h.This might be due to the formation of secondary mycelium. Secondary mycelia having much growing hyphal tips and has much more tendency for protein synthesis and enzyme production. After this time period cell growth was moves towards stationary and death phase .As in death phase toxic waste products build up, food is depleted and the microbes begin to die.

4.1.2.3. Effect of pure sugars on the growth of *Trichoderma* strains under submerged cultivation

To study the effect of pure sugars on the growth of various fungal strains separate sets of batch experiments have been performed. As observed from Table 4.1.2 and Fig 4.1.10. *Trichoderma reesei* showed fastest lag phase in glucose based culture media followed by xylose, maltose and

lactose based culture medium. *Trichoderma reesei* showed slowest growth in the sucrose based medium. Extended lag phase associated with sucrose based medium corroborates that sucrose is not easily utilizable sugar by *Trichoderma reesei.*Exponential phase was sustained upto 60, 60, 72, 72 and 78h in glcose, xylose, maltose, sucrose and lactose based medium respectively by *Trichoderma reesei.* The maximum cell dry weight (g/L) attained by *Trichoderma reesei* was 12.88, 11.54, 10.65, 8.48, 10.83 at 60, 60, 72, 72 and 78h in glucose, xylose, maltose, sucrose and lactose based medium respectively which indicates that growth was much faster in glucose, xylose and lactose based media whereas growth rate was highest in glucose and xylose based medium. Lower growth and growth rate was observed by *Trichoderma reesei* in sucrose based medium as observed from Table 4.1.2.Whereas *Trichoderma viride* showed different growth and their pattern as observed by Table 4.1.2 and Fig 4.1.11. Shorter lag phase has been observed in glucose and maltose based culture medium. Exponential phase has been viewed upto 84, 78, 84, 66 and 84 h by *Trichoderma viridae* in glucose, xylose, maltose, sucrose and lactose based medium respectively.

Table 4.1.2: Biomass in terms of cell dry weight (g/L) of *Trichoderma* strains under various pure sugars containing potato dextrose broth culture medium at 30^0C and 180 rpm.

Time (h)	Biomass in terms of cell dry weight(g/L)									
	Glucose based		Xylose based		Maltose based		Sucrose based		Lactose based	
	T.reesei	*TV*	*T.reesei*	*TV*	*T.reesei*	*TV*	*T.reesei*	*TV*	*T.reesei*	*TV*
0	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
6	4.20	0.95	1.68	0.63	1.35	0.75	1.02	0.59	1.20	0.60
12	5.40	1.76	2.68	0.98	2.12	1.02	1.32	0.63	2.02	0.73
18	5.54	3.88	3.56	1.93	2.56	1.36	2.32	0.98	2.60	0.98
24	8.04	6.58	5.76	2.67	4.87	3.64	3.45	1.21	3.98	1.56
30	8.54	7.32	7.23	4.76	6.76	5.45	4.55	2.02	5.89	2.98
36	9.42	8.29	8.98	5.98	7.89	7.11	5.96	2.95	7.56	4.56
42	10.36	9.12	9.08	6.32	8.35	7.34	6.89	3.33	8.53	5.11
48	10.60	9.33	10.21	6.52	8.80	7.89	7.04	4.21	8.98	5.23
54	11.42	10.06	10.76	7.32	9.93	8.02	7.56	4.78	9.32	6.89
60	**12.88**	10.93	**11.54**	7.65	10.12	8.86	7.85	5.94	9.43	7.21
66	12.64	11.03	11.39	7.89	10.43	8.98	8.21	**6.68**	9.87	7.34
72	11.30	11.52	11.23	7.98	**10.65**	9.06	**8.48**	6.46	9.90	7.67
78	11.68	11.63	11.25	**8.56**	10.21	9.11	8.12	6.21	**10.83**	7.88
84	11.02	**11.81**	10.89	8.32	10.28	**9.36**	8.15	6.12	10.54	**8.53**
90	9.72	10.66	9.56	8.02	10.08	9.23	7.96	5.02	10.61	9.21

TV: *Trichoderma viride*

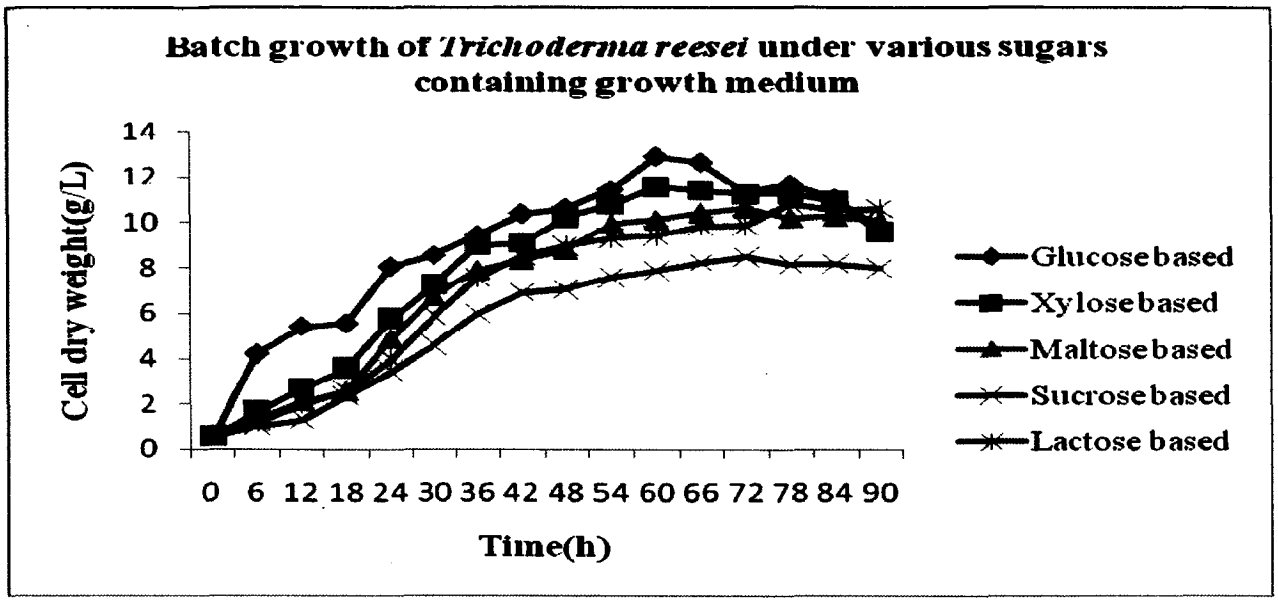


Fig.4.1.10. Batch growth curve of *Trichoderma reesei* under glucose, xylose, maltose, sucrose and lactose containing potato broth medium at 180 rpm and 30^{0}C.

The maximum cell dry weight (g/L) attained by *Trichoderma viridae* was 11.81, 8.56, 9.36, 6.68, 8.56 at 84, 78, 84, 66 and 84 h in glucose, xylose, maltose, sucrose and lactose based medium respectively which indicates that growth and growth rate were much faster in glucose and maltose in comparison to xylose, lactose and sucrose based media. Lowest growth and growth rate was observed by *Trichoderma viride* in sucrose based medium as observed from

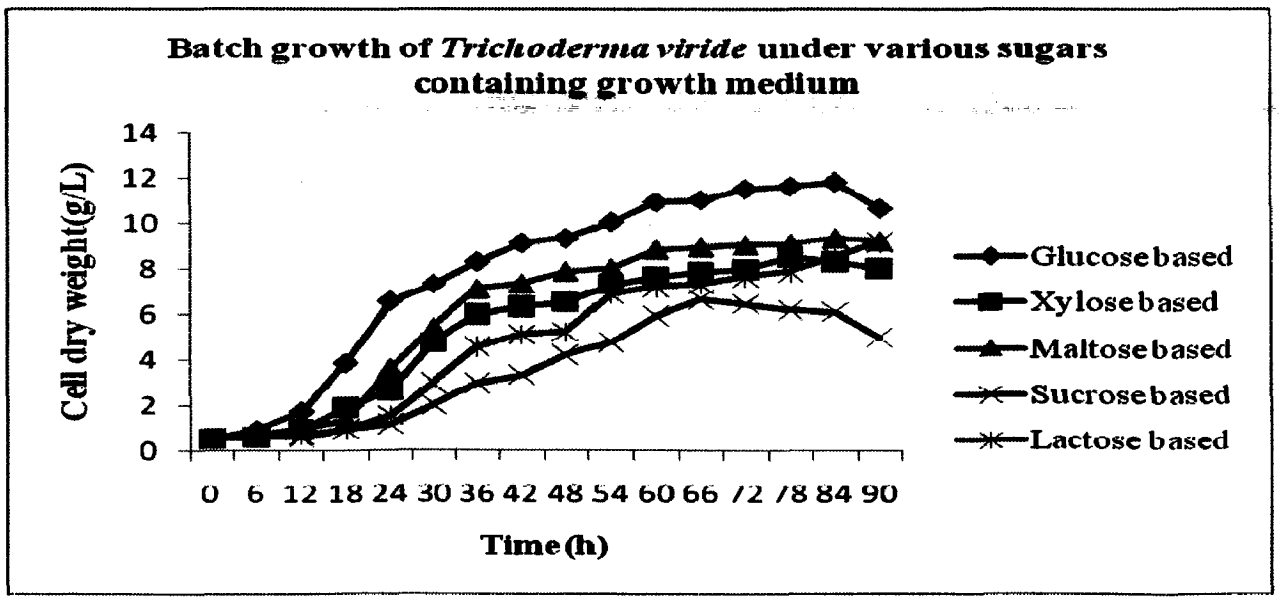


Fig.4.1.11. Batch growth curve of *Trichoderma viride* under glucose, xylose, maltose, sucrose and lactose containing potato broth medium at 180 rpm and 30^{0}C.

Table 4.1.2 It can be inferred from the above experimental observation that sucrose is not the suitable carbon source for both of the *Trichoderma* strains.

4.1.2.4. Effect of pure sugars on the growth of *Aspergillus* strains under submerged cultivation

To study the effect of pure sugars on the growth of *Aspergillus* strains, different sugars used separately as carbon source potato broth medium. It is evident from Table 4.1.3 and Fig. 4.1.12. that lag phase was observed upto 6 h in most of the sugar containing cultivation media by *Aspergillus niger*. As in the lag phase microbes acclimate to food and nutrients in their new habitat.

Table 4.1.3: Biomass in terms of cell dry weight (g/L) of *Aspergillus* strains under various pure sugars containing potato dextrose broth culture medium at 30^0C and 180 rpm.

Time (h)	Biomass in terms of cell dry weight(g/L)									
	Glucose based		Xylose based		Maltose based		Sucrose based		Lactose based	
	A.niger	*AF*	*A.niger*	*AF*	*A.niger*	*AF*	*A.niger*	*AF*	*A.niger*	*AF*
0	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
6	0.87	0.85	0.85	0.60	0.75	0.76	0.72	0.67	0.60	0.61
12	1.68	1.74	2.00	0.71	1.36	0.98	1.30	0.99	1.13	0.76
18	2.02	1.92	3.46	0.79	1.96	1.45	1.93	1.54	1.90	0.89
24	2.52	2.47	4.32	1.33	2.26	1.87	2.60	1.97	2.12	1.24
30	3.86	3.64	5.10	2.87	3.78	2.89	4.21	2.32	3.42	2.53
36	5.12	4.93	7.36	3.90	5.00	3.67	5.98	3.34	4.89	2.78
42	6.64	6.55	**11.93**	4.98	6.50	4.32	**10.36**	4.43	6.02	2.97
48	7.78	8.55	11.80	5.89	8.60	6.98	10.20	5.31	**9.34**	4.21
54	8.50	9.35	11.68	6.90	**10.58**	7.34	10.00	7.32	9.00	5.11
60	8.94	9.83	11.20	7.20	10.46	8.04	9.96	8.08	8.98	6.78
66	9.60	10.36	11.02	8.11	10.31	9.88	9.88	**9.66**	8.76	7.98
72	9.94	10.63	10.90	9.78	10.02	**10.68**	9.72	9.57	8.70	**8.54**
78	**11.26**	**11.91**	10.60	**10.68**	9.98	10.33	9.42	9.43	8.10	8.34
84	10.91	11.00	10.23	10.53	9.62	9.97	9.30	9.21	8.03	8.20
90	9.42	9.76	9.25	9.97	9.32	9.76	9.21	8.98	7.98	7.98

AF: *Aspergillus fumigatus*.

As the time proceeds beyond 6 h, exponential phase started, which was almost similar in the glucose, maltose, lactose and sucrose based potato broth medium although *Aspergillus niger* growth was faster with higher biomass in case of xylose based culture media. As in this phase substrate conversion and cell mass reached to their maximum values. After 36 h, steep increment in the growth was observed till 42 h, in case of sucrose and xylose based culture media.The maximum cell dry weight (g/L) attained by *Aspergillus niger* was 11.26, 11.93, 10.58, 10.36, 9.34 at 78, 42, 54, 42 and 48 h in glucose, xylose, maltose, sucrose and lactose based culture media respectively, which indicates that growth rate was much faster in xylose, maltose and sucrose based media with respect to glucose and lactose based culture media. But finally the highest growth has been achieved by *Aspergillus niger* in glucose based medium.

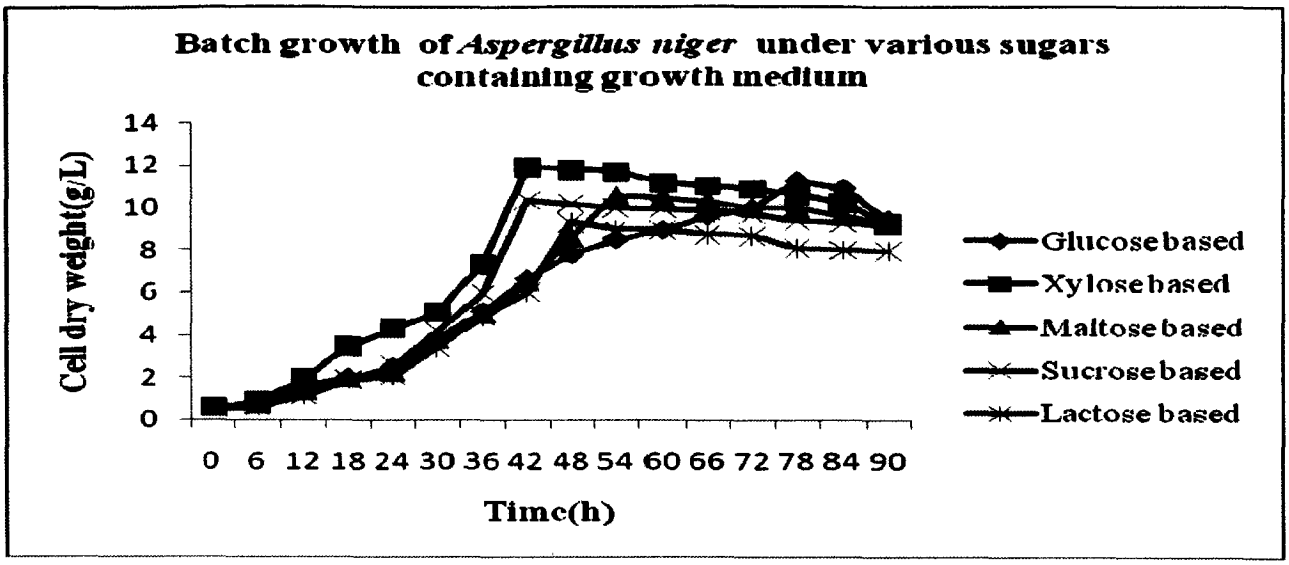


Fig.4.1.12. Batch growth curve of *Aspergillus niger* under glucose, xylose, maltose, sucrose and lactose containing potato broth medium at 180 rpm and 30^0C.

Margaris et al[152] have investigated the *Aspergillus* growth under different carbon source and observed that maltose supported growth substantially more than sucrose.The maximum growth has been observed in case of glucose and xylose based media. Prathumpai et al [202] have reported that the activities of the key enzymes for xylose metabolism increased only when the effects of glucose repression had been relieved. *A.niger* preferentially utilized D-xylose in the mixture of xylose and xylulose, which proves the easier uptake of xylose sugars by this microbes. After 42 h, in xylose and sucrose based culture media, there was no significant increment in cell biomass, probably due to the depletion of nutrients and carbon source in the media therefore booming growth stops whereas in case of lactose, maltose and glucose based culture media this phase was sustained up to 48, 54 and 72 h respectively, as has been observed in Table 4.1.3.After that cell growth moves towards stationary and death phase. As in death phase toxic waste products build up, food is depleted and the microbes begin to die. It has been inferred that under xylose and sucrose based media growth rate was faster and closely resemble to growth in glucose containing media which indicates that this strain of *Aspergillus* having much faster xylose and sucrose exhausting capacity, as almost similar to glucose.

Whereas in case of *Aspergillus fumigatus* longer lag phase has been observed which proves that microbes have taken extra time for reaching their dynamic stage as observed from Table 4.1.3. and Fig 4.1.13. When compared the growth in different sugars than we viewed that in xylose and lactose containing culture medium *Aspergillus fumigatus* having much prolonged lag phase which imply that these sugars are not easily taken up by microbes. As the time elapses beyond 12 h in

case of glucose based and after 24 h in the rest of the sugars containing potato dextrose broth medium.

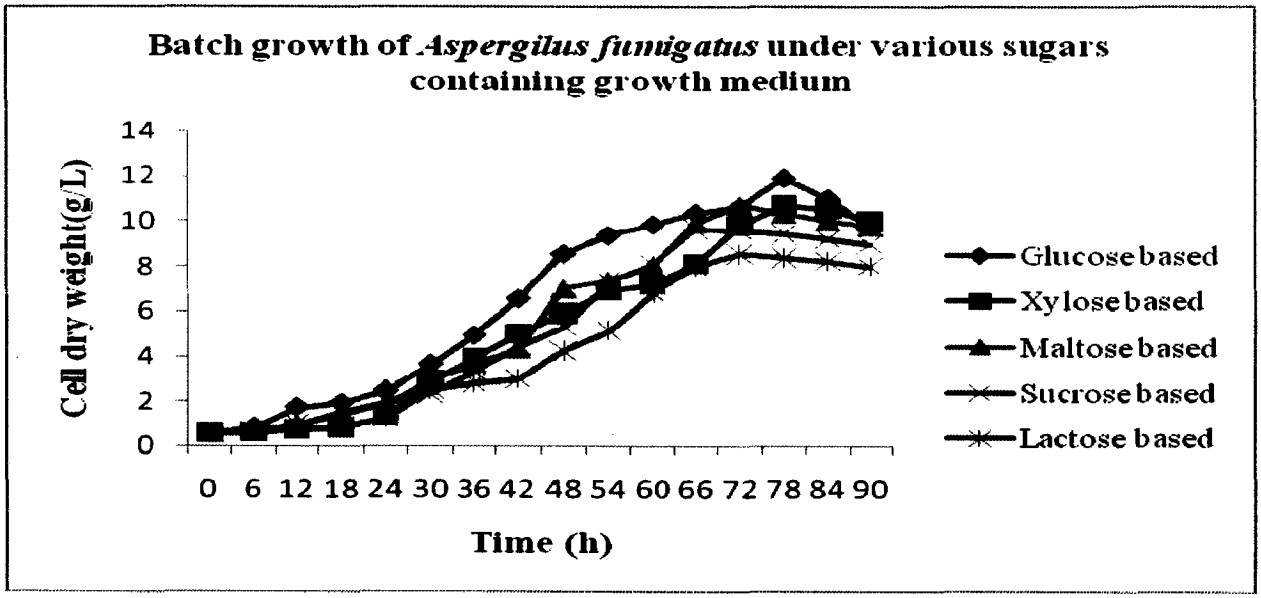


Fig.4.1.13. Batch growth curve of *Aspergillus fumigatus* under glucose, xylose, maltose, sucrose and lactose containing potato broth medium at 180 rpm and 30^{0}C.

The maximum cell dry weight (g/L) attained by *Aspergillus fumigatus* was 11.91, 10.68, 10.68, 9.66, 8.54 at 78, 78, 72, 66 and 72 h in glucose, xylose, maltose, sucrose and lactose based culture media respectively, which indicates that growth rate was much faster in xylose, maltose and sucrose based media with respect to lactose based culture media, which indicates that *Aspergillus fumigatus* strain attained much faster growth and growth rate in glucose as well as maltose based culture medium. Under lactose based culture media microbes revealed lowest growth and growth rate as shown by Table 4.1.3.

4.1.2.5. Effect of pure sugars on the growth of *Neurospora crassa* strains under submerged cultivation

On the other hand in *Neurospora* distinctive situation has been observed, as shown by Table 4.1.4. and Fig 4.1.14. The stable lag phase has been observed in *Neurospora crassa* which indicates that this microbes takes much more time for reaching their active state. Afterward a sharp inclination has been seen in sucrose, glucose and maltose based M_2 media whereas much lesser growth observed in xylose based culture media. Exponential phase was observed upto 36 h in maltose and sucrose based culture media whereas in glucose based media this was observed till 54 h. On the other hand in lactose and xylose based culture media exponential phase was much smaller with lesser growth. The maximum cell dry weight(g/L) attained by *Neurospora crassa* was 8.86, 3.28,

8.11, 8.24 and 5.25 at 54, 36, 54, 36 and 54 h in glucose, xylose, maltose, sucrose and lactose based culture media respectively as shown by Fig 4.1.14.

Table4.1.4 Biomass in terms of cell dry weight (g/L) of *Neurospora crassa* under various pure sugars containing M2 broth culture medium 30^0C and 180 rpm.

Time (h)	Biomass in terms of cell dry weight (g/L)				
	Neurospora crassa				
	Glucose based	Xylose based	Maltose based	Sucrose based	Lactose based
0	0.56	0.56	0.56	0.56	0.56
6	0.66	0.58	0.62	0.60	0.58
12	4.34	1.02	4.20	4.00	2.32
18	5.82	2.10	5.22	5.66	3.86
24	7.04	3.12	6.63	6.96	4.00
30	7.12	3.26	6.98	7.82	4.13
36	7.42	**3.28**	7.02	**8.24**	4.45
42	7.66	3.16	7.10	8.20	4.80
48	8.16	2.98	7.90	8.02	5.00
54	**8.86**	2.93	7.96	7.98	**5.25**
60	8.42	2.83	**8.12**	7.63	5.20
66	8.16	2.76	7.98	7.34	5.02
72	7.02	2.59	7.33	6.98	4.98
78	6.92	2.53	6.83	6.75	4.93
84	6.76	2.41	6.59	6.32	4.83
90	6.55	2.40	6.43	6.20	4.80

After 36 h in xylose and sucrose based culture media no significant increment in the growth has been observed probably due to the depletion of nutrients and carbon source in the media whereas such condition has been observed, after 54 h in glucose, maltose and lactose based culture media. After that cell growth has been shifted towards stationary and death phase.

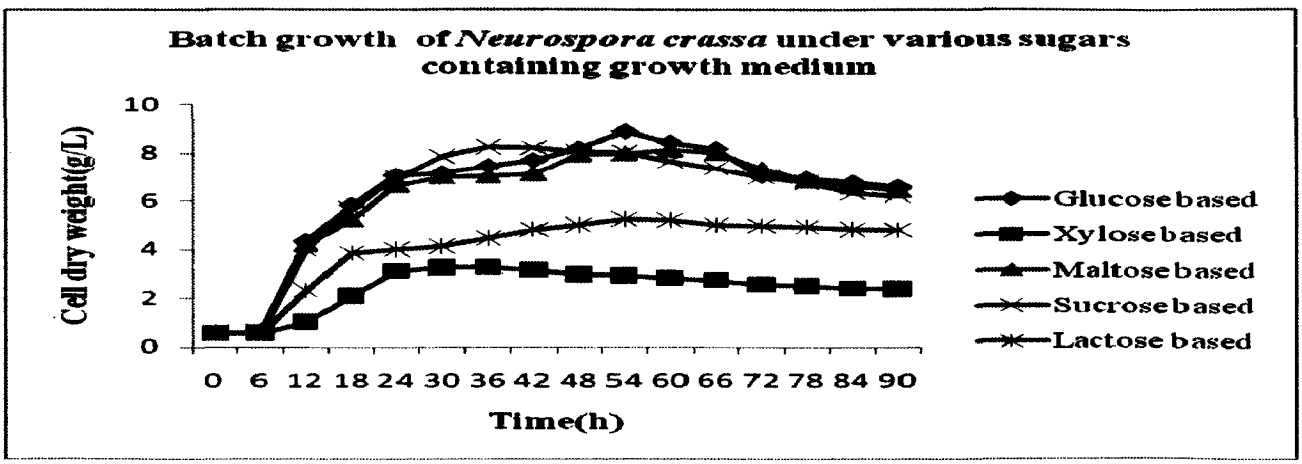


Fig.4.1.14. Batch growth curve of *Neurospora crassa* under glucose, xylose, maltose, sucrose and lactose containing potato broth medium at 180 rpm and 30^0C.

It has been observed from Fig 4.1.14 that growth rate of *Neurospora crassa* was much faster in maltose and sucrose based culture media which closely resemble to growth in glucose based

culture media hence we can say that sugar uptake capacity was much faster in *Neurospora crassa* for maltose, sucrose and glucose in comparison to lactose and xylose.

4.1.2.6. Utilization of industrial wastes as sugar source on the growth of fungal strains under glucose based culture medium.

Dairy as well as sugarcane industry waste, whey and bagasse respectively are renewable, produced annually in great quantities and usually disposed to the environment causing pollution load. Therefore it is indispensable to utilize whey and boiled bagasse syrup rather than pure glucose for the growth of these organisms. In the present experiments we have used whole bagasse (not depithed) to utilized substantial quantity of sugars content from bagasse. Separate set of batch experiments were performed to study the utilization of industrial wastes as carbon source for making the cost effective process.

Table 4.1.5. Biomass in terms of cell dry weight (g/L) of various fungal strains in industrial waste material containing respective culture medium.

Time (h)	Biomass in terms of cell dry weight(g/L)									
	Trichoderma reesei		*Trichoderma viridae*		*Aspergillus niger*		*Aspergillus fumigatus*		*Neurospora crassa*	
	WB	*BBB*	*WB*	*BBB*	*WB*	*BBB*	*WB*	*BBB*	*WB*	*BBB*
0	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
6	0.89	0.62	0.57	0.57	0.58	0.60	0.60	0.58	0.58	0.59
12	1.23	1.14	0.62	0.69	1.08	1.00	0.71	0.89	2.12	2.93
18	1.87	1.45	0.76	0.91	1.83	1.81	1.02	1.04	3.72	3.66
24	2.31	2.11	0.98	0.98	2.10	2.10	1.11	1.86	3.88	4.96
30	2.56	2.41	1.21	1.89	3.30	4.02	1.89	2.12	3.93	5.72
36	3.43	3.23	2.32	2.41	4.80	5.21	2.12	2.98	4.02	**6.28**
42	3.98	4.89	3.42	3.56	6.00	**9.76**	2.45	3.31	4.13	6.20
48	5.67	5.78	3.77	4.54	**9.20**	9.59	2.98	4.11	4.76	6.00
54	6.87	6.14	5.11	4.98	8.88	9.41	3.43	4.97	4.83	5.91
60	7.90	7.13	5.34	5.23	8.63	8.98	5.48	6.32	**4.91**	5.83
66	8.32	**7.97**	5.97	5.61	8.51	8.60	5.87	6.89	4.86	5.71
72	8.96	7.82	6.06	**6.33**	7.72	8.42	6.02	7.03	4.63	5.62
78	**9.88**	7.78	6.78	6.02	7.48	8.31	**7.93**	**8.98**	4.32	5.55
84	9.67	7.89	**7.21**	5.98	7.70	8.22	7.88	8.93	4.22	5.43
90	9.70	7.55	7.10	5.91	7.43	8.10	7.67	8.84	4.04	5.40

WB: whey based, BBB: boiled bagasse based

When we compared the growth of various fungal strains under whey containing culture medium.It has been observed that the growth of *Trichoderma reesei* was found maximum followed by *Aspergillus niger* in whey containing culture medium. Least growth has been observed by *Neurospora crassa* under this medium, which strongly intimates that the major sugar (lactose) present in the whey are not easily utilizable by *Neurospora crassa* in contrast to their utility by *Trichoderma reesei*. The maximum cell dry weight (g/L) attained in whey containing culture

medium was 9.88, 7.21, 9.20, 7.93 and 4.91 at 78, 84, 48, 78 and 60 h by *Trichoderma reesei*, *Trichoderma viride*, *Aspergillus niger*, *Aspergillus fumigatus*, *Neurospora crassa* respectively. It can be observed from Table 4.1.5. and Fig 4.1.15. that *Trichoderma ressei* showed higher growth as well as *Aspergillus niger* exhibited highest growth and growth rate under whey based culture medium. On the other hand *Neurospora crassa* illustrated least growth and growth rate under such industrial waste containing culture medium.

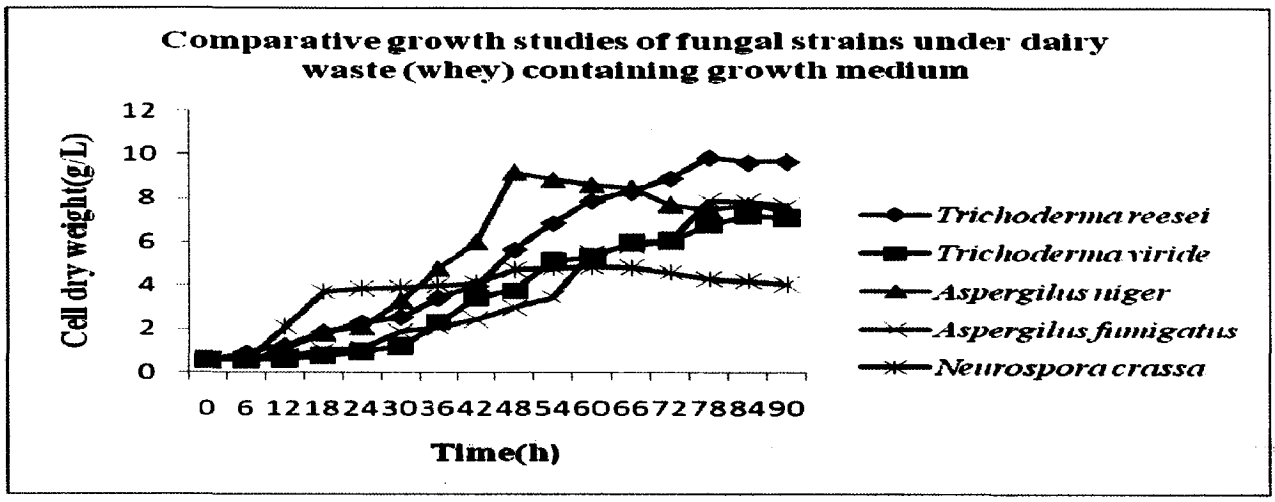


Fig.4.1.15. Comparative batch growth curve of various fungal strains in dairy industry (whey) containing respective culture medium at 180 rpm and 30^{0}C.

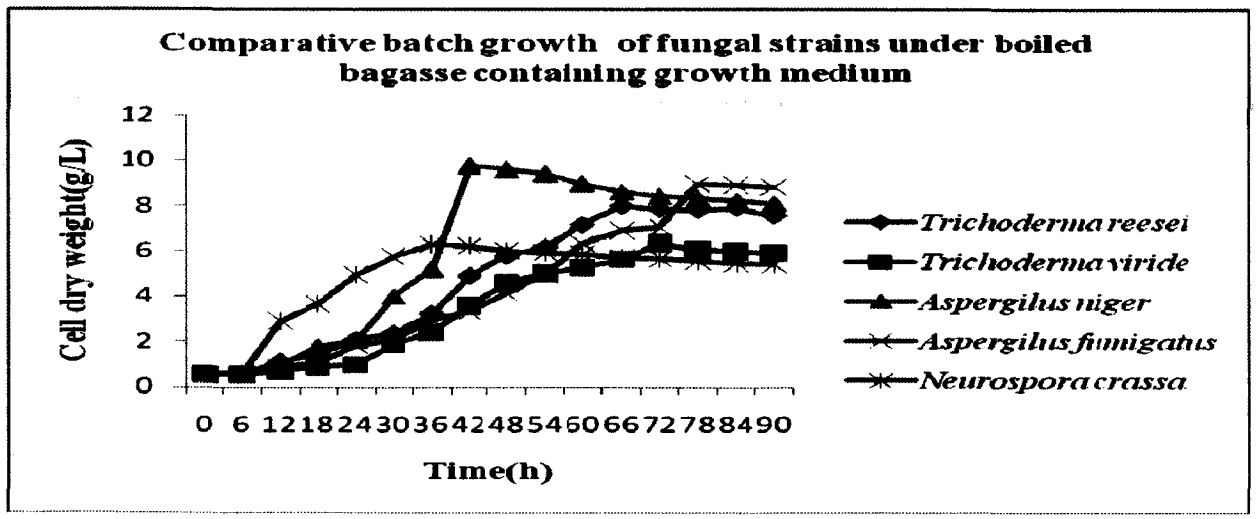


Fig.4.1.16. Comparative batch growth curve of various fungal strains in boiled bagasse solution containing respective culture medium at 180 rpm and 30^{0}C.

It has been observed that various fungal strains exhibited distinctive type of growth pattern under boiled bagasse based culture medium. It has been monitored by Table 4.1.5 that *Aspergillus niger* showed significantly higher growth and growth rate under boiled bagasse based culture media

compared to whey based culture media. On the other hand *Trichoderma* strains showed significantly lower growth and growth rate in boiled bagasse based culture media as obsereved from Fig 4.1.16. Good growth has also been observed by *Neurospora crassa* as well as *Aspergillus fumigatus* under such medium, which might be due to the presence of easily utilizable sugars. The maximum cell dry weight (g/L) attained in boiled bagasse containing culture medium was 7.97, 6.33, 9.76, 8.98 and 6.28 at 66, 72, 42, 78 and 36 h by *Trichoderma reesei*, *Trichoderma viride*, *Aspergillus niger, Aspergillus fumigatus, Neurospora crassa* respectively. It has been inferred from the above observation that growth and growth rate was much better in boiled bagasse containing medium for most of the culture excluding *Trichoderma* strains. The sugars present in these industrial waste were utilized by fungal strains for their growth makes the culture development process more cost effective and environment friendly.

SECTION II
OPTIMIZATION OF PROCESS PARAMETERS FOR CELLULASE PRODUCTION

4.2. OPTIMIZATION OF PROCESS PARAMETERS

To determine the much effective and optimum conditions as well as to detect the frequent interactions occurring between two or more factors statistical optimization methodology has been used. The primary goal of designing an experiment statistically is to obtain valid results at a minimum of effort, time and resources. Design based optimization for fermentation process could overcome the limitations of classical empirical methods and has been proved to be a powerful tool for the optimization of cellulase production as well as also to determine the optimum operating conditions necessary for the scale up of the process and to reduce the number and cost of experiments. Designed experiments use a small set of carefully planned experiments. This method is more satisfactory and effective than other methods, such as classical one, at a time or mathematical methods, because it can study many variables simultaneously with a low number of observations, saving time and costs. Single variable optimization methods are not only tedious, but also can lead to misinterpretation of results, especially because the interaction between different factors is overlooked. The present section describes the box behnkem design of experiments for cellulase production by *Trichoderma*, *Neurospora* and *Aspergillus* fungal strains to get their effective and valuable conditions.

4.2.1. Optimization of physical and chemical parameters for cellulase production by *Trichoderma reesei.*

Temperature, pH, initial moisture content of substrate, inoculums size of fungal strains, particle sizes of raw material, incubation period were identified as the most influential among physical and chemical parameters. A box behnken design was used to analyze the interactive effect of these parameters and to arrive at an optimum. The base points for the design were selected from a single parameter study (data not shown). A summary of the variables and their variation levels is given in Table 4.2.1.

Table 4.2.1. Variables used in box–behnken design for the optimization of process parameters such as temperature, pH and inoculum dosages.

Factor	Basic level	Variation interval	Value of the factor	Coded value
Temperature (^{0}C)	30	5	25	-
			30	0
			35	+
pH	5	2	3	-
			5	0
			7	+
Inoculum dosages(g/L)	0.56	0.18	0.38	-
			0.56	0
			0.74	+

Table 4.2.2. Different set of combinations using temperature, pH and inoculum dosage as parameters for the response of cellulase activity.

Medium code	Temperature(^{0}C)	pH	Inoculum dosages(g/L)	Total cellulase activity FPA (IU/mL)
A	25	3	0.56	2.36
B	25	5	0.38	3.10
C	25	5	0.74	1.69
D	25	7	0.56	2.32
E	30	3	0.38	3.24
F	30	3	0.74	1.58
G	30	5	0.56	4.01
H	30	7	0.38	3.48
I	30	7	0.74	1.63
J	35	3	0.56	2.51
K	35	5	0.38	3.35
L	35	5	0.74	1.14
M	35	7	0.56	1.97

Different sets of combination have been used for cellulase production by *T.reesei*. It was observed from Table 4.2.2 and Figs 4.2.1 and 4.2.2. that temperature at 30^{0}C, pH at 5 and inoculums dosages of 0.56g/L was found quite effective set of combination for cellulase activity (4.01 IU/mL) as compared to other.

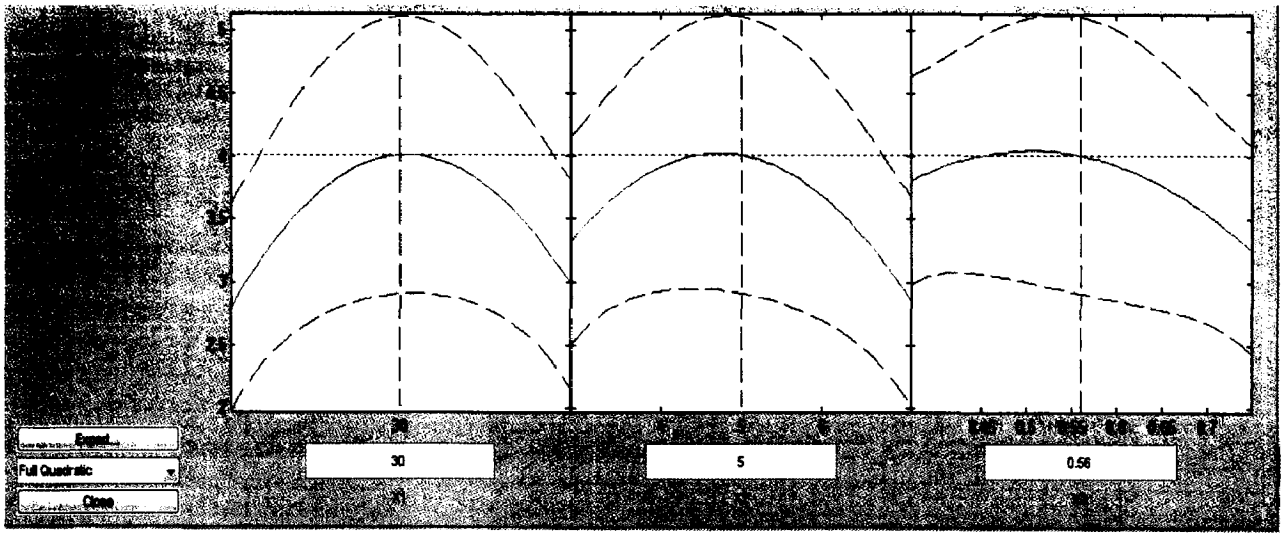


Fig 4.2.1. Cellulase activity as a response (Y1) of three parameters temperature(X1), pH(X2) and Inoculum dosage(X3).

It was also observed from Fig that rate of decrement of enzyme activity was somewhat lower towards temperature more than 30^0C, pH lesser than 5 and inoculum dose lower than 0.56g/L.

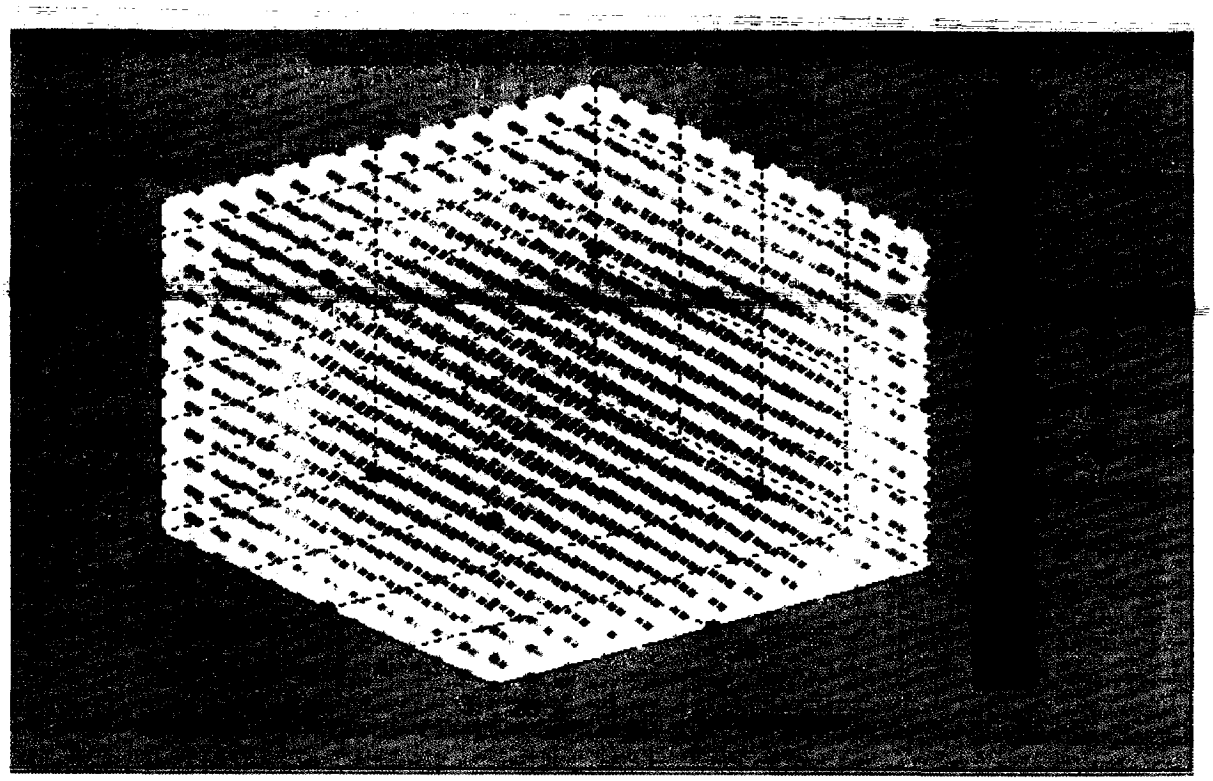


Fig. 4.2.2. Quadratic response surface model with cellulase activity as a response of three parameters temperature, pH and Inoculum dosage.
Cellulase dosage represents the cellulase activity(IU/mL).

As observed from Fig 4.2.2 that higher cellulase activity region lie in the centre of the graph which proves the significantly better optimization of process parameters.

Table 4.2.3. Comparative experimental and predicted values of cellulase activity (IU/mL) under different set of combination.

Medium code	T	pH	Inoculum dosages	FPA (IU/mL)E	T	pH	Inoculum dosages	FPA (IU/mL)P
A	25	3	0.56	2.36	-1	-1	0	1.258606
B	25	5	0.38	3.10	-1	0	-1	4.023431
C	25	5	0.74	1.69	-1	0	1	1.477622
D	25	7	0.56	2.32	-1	1	0	2.437406
E	30	3	0.38	3.24	0	-1	-1	4.367511
F	30	3	0.74	1.58	0	-1	1	1.277202
G	30	5	0.56	4.01	0	0	0	4.174276
H	30	7	0.38	3.48	0	1	-1	4.564311
I	30	7	0.74	1.63	0	1	1	1.438002
J	35	3	0.56	2.51	1	-1	0	2.221106
K	35	5	0.38	3.35	1	0	-1	4.548431
L	35	5	0.74	1.14	1	0	1	0.877622
M	35	7	0.56	1.97	1	1	0	2.399906

E: Experimental value (exp); P: Predicted value (pre)

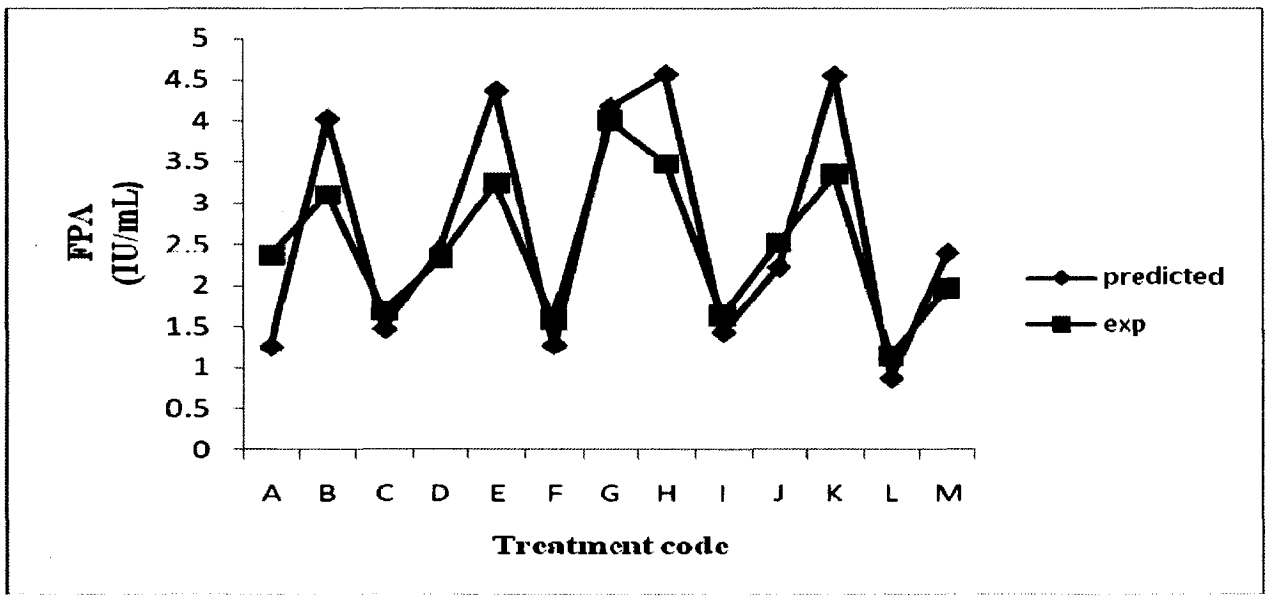


Fig.4.2.3. Comparative graph of experimental and predicted values of cellulase activity (FPA).

The observed and predicted values are quite close to each other which represents the good correlation as shown in Table 4.2.3 and Fig 4.2.3.

Table 4.2.4. Variables used in box–behnken design for the optimization of process parameters such as particle size, moisture percentage and incubation period.

Factor	Basic level	Variation interval	Value of the factor	Coded value
Particle size (μm)	850	400	450	-
			850	0
			1250	+
Moisture percentage (%)	61	5	55	-
			61	0
			66	+
Incubation period(days)	6	3	3	-
			6	0
			9	+

Table4.2.5. Different set of combinations using particle size, moisture percentage and incubation period as parameters for the response of cellulase activity.

Medium code	Particle size(μm)	Moisture percentage (%)	Incubation period(days)	Total cellulase activity FPA(IU/mL)
A	450	61	3	2.69
B	850	55	3	2.17
C	850	66	3	2.05
D	1250	61	3	3.19
E	450	55	6	2.34
F	450	66	6	2.18
G	850	61	6	4.72
H	1250	55	6	2.85
I	1250	66	6	2.65
J	450	61	9	2.96
K	850	55	9	2.49
L	850	66	9	2.37
M	1250	61	9	3.53

Different sets of combination have been used for cellulase production by *T.reesei*. It was observed from Table 4.2.4-4.2.5. and Figs 4.2.4-4.2.5. that particle size of 850 μm, moisture percentage 61, and incubation period of 6 days was found quite effective set of combination for cellulase activity (4.72 IU/mL) as compared to other.

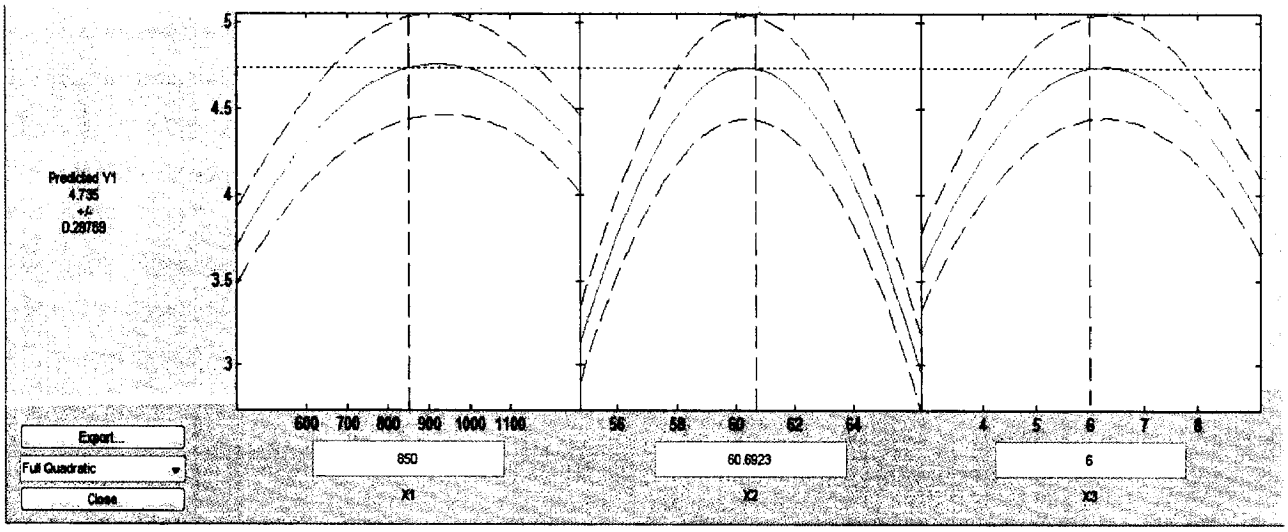


Fig 4.2.4. Cellulase activity as a response (Y1) of three parameters particle size(X1), moisture percentage (X2) and incubation period (X3).

It was also observed from Fig 4.2.4. that rate of decrement in enzyme activity was somewhat lower towards particle size bigger than 850 μm, both higher and lower moisture percentage than 61, bigger incubation period than 6 days.

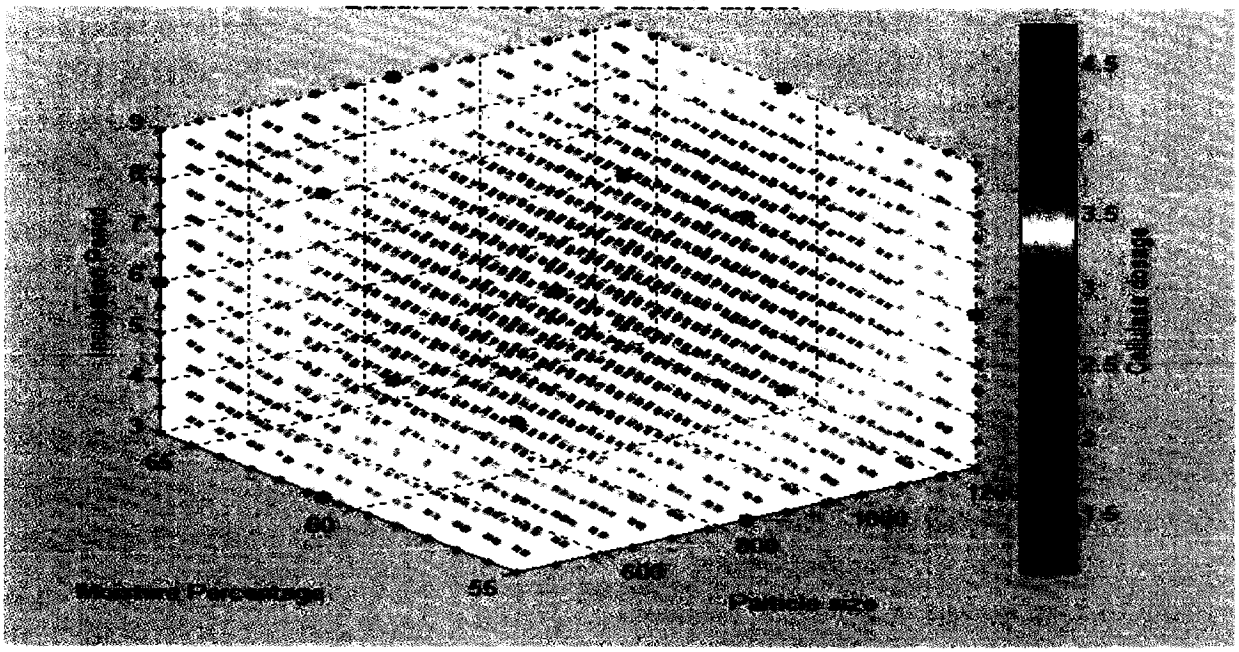


Fig. 4.2.5. Quadratic response surface model with cellulase activity as a response of three parameters particle size, moisture percentage and incubation period.

Cellulase dosage represents the cellulase activity(IU/mL).

As observed from Fig 4.2.5 that higher cellulase activity region lie in the nearby centre of the graph which proves significantly good optimization of process parameters.

Table 4.2.6. Comparative experimental and predicted values of cellulase activity (IU/mL) under different set of combination.

Medium code	Particle size	MP	IP	Total cellulase activityFPA (IU/mL)	Particle size	MP	IP	Predicted value FPA (IU/mL)
A	450	61	3	2.69	-1	0	-1	3.618463
B	850	55	3	2.17	0	-1	-1	2.881086
C	850	66	3	2.05	0	1	-1	3.088698
D	1250	61	3	3.19	1	0	-1	4.013295
E	450	55	6	2.34	-1	-1	0	3.006647
F	450	66	6	2.18	-1	1	0	3.232605
G	850	61	6	4.72	0	0	0	5.516143
H	1250	55	6	2.85	1	-1	0	3.371527
I	1250	66	6	2.65	1	1	0	3.561757
J	450	61	9	2.96	-1	0	1	3.81397
K	850	55	9	2.49	0	-1	1	3.026628
L	850	66	9	2.37	0	1	1	3.235203
M	1250	61	9	3.53	1	0	1	4.109922

MP: Moisture percentage, IP: Incubation period (in days)

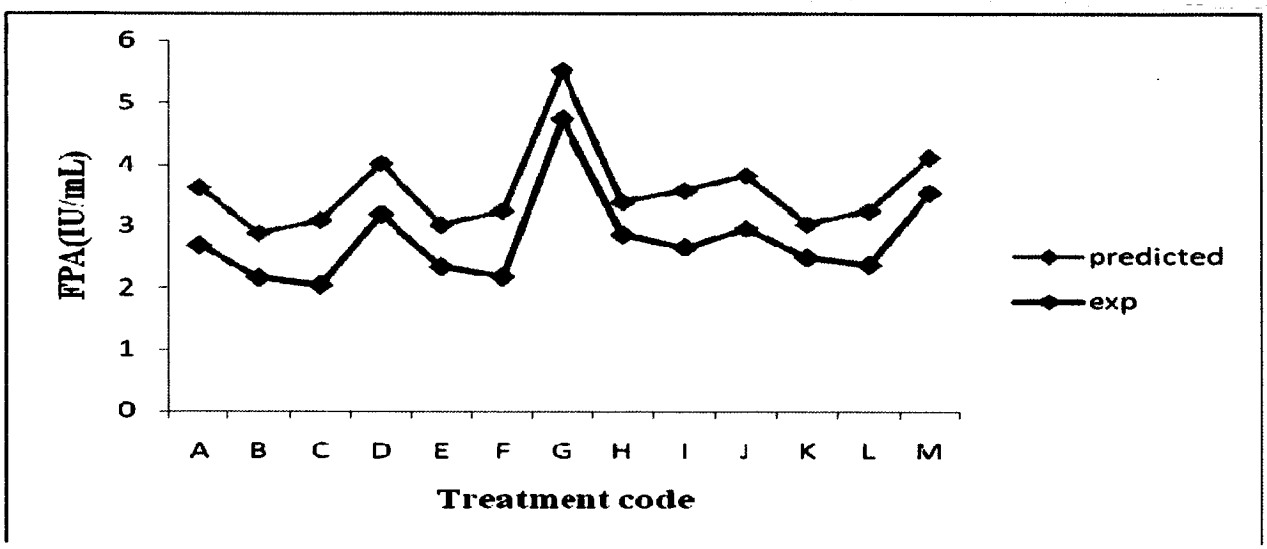


Fig.4.2.6. Comparative graph of experimental and predicted values of cellulase activity (FPA).

The observed and predicted values of cellulase activity are very close to each other which represents the good correlation as observed from Table 4.2.6 and Fig 4.2.6

4.2.2. Optimization of physical and chemical parameters for cellulase production by *Neurospora crassa.*

Separate set of design experiments have been performed to study the optimization of process parameters.

Table 4.2.7. Variables used in box–behnken design for the optimization of process parameters such as temperature, pH and inoculum dosages.

Factor	Basic level	Variation interval	Value of the factor	Coded value
Temperature (^{0}C)	30	5	25	-
			30	0
			35	+
pH	6	2	4	-
			6	0
			8	+
Inoculum dosages(g/L)	0.56	0.14	0.42	-
			0.56	0
			0.70	+

Table 4.2.8. Different set of combinations using temperature, pH and inoculum dosage as parameters for the response of cellulase activity.

Medium code	Temp(^{0}C)	pH	Inoculum dosages(g/L)	Total cellulase activityFPA(IU/mL)
A	25	4	0.56	1.35
B	25	6	0.42	1.40
C	25	6	0.70	1.28
D	25	8	0.56	1.18
E	30	4	0.42	1.43
F	30	4	0.70	1.26
G	30	6	0.56	1.93
H	30	8	0.42	1.26
I	30	8	0.70	1.11
J	35	4	0.56	1.08
K	35	6	0.42	1.15
L	35	6	0.70	1.00
M	35	8	0.56	0.946

Different sets of combination have been used for cellulase production by *N. Crassa.* It was observed from Table 4.2.7-4.2.8 and Fig 4.2.7-4.2.8. that temperature at 30^{0}C, pH at 6 and inoculums dosages of 0.56g/L was found quite effective set of combination for cellulase activity (1.93 IU/mL) as compared to other.

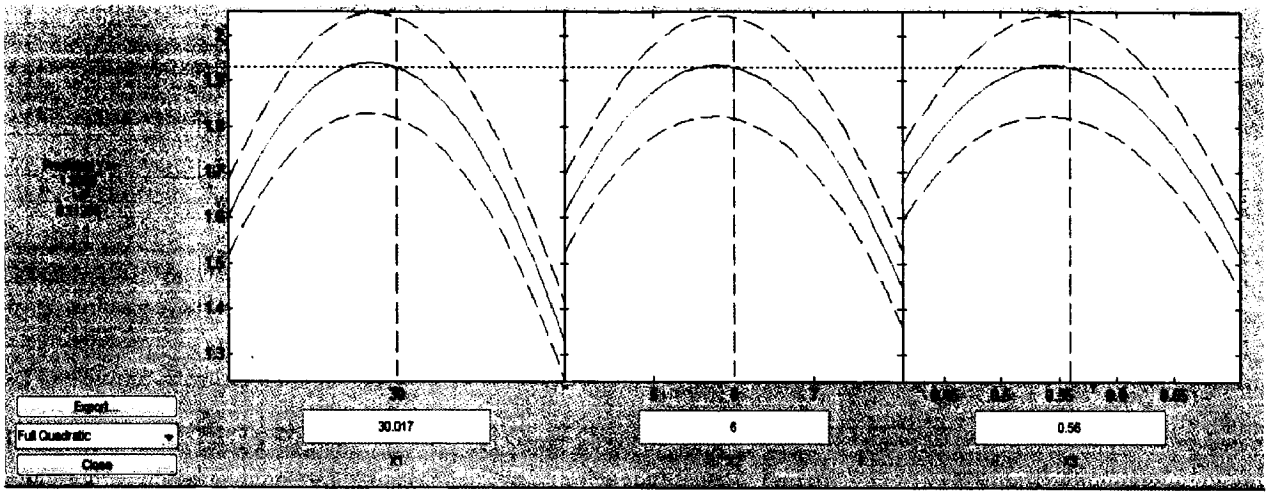


Fig 4.2.7. Cellulase activity as a response (Y1) of three parameters temperature(X1), pH(X2) and Inoculum dosage(X3).

It was also observed from Fig 4.2.7.that rate of decrement in enzyme activity was somewhat lower towards temperature lower than 30^0C, pH lower than 6 and inoculum dose lower than 0.56g/L.

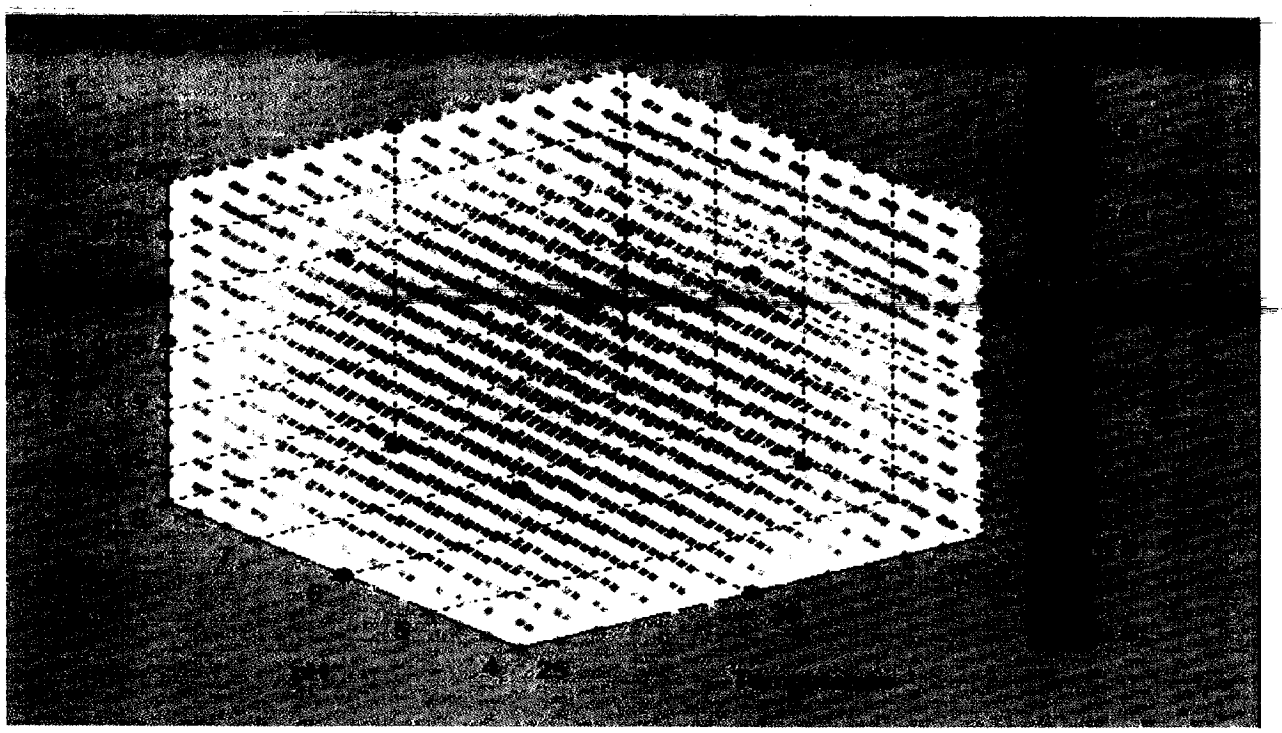


Fig. 4.2.8. Quadratic response surface model with cellulase activity as a response of three parameters temperature, pH and Inoculum dosage.
Cellulase dosage represents the cellulase activity(IU/mL).

As observed from Fig 4.2.8 that higher cellulase activity region lie in the centre of the graph which proves the significantly better optimization of process parameters.

Table 4.2.9. Comparative experimental and predicted values of cellulase activity (IU/mL) under different set of combination.

Medium code	Temp	pH	Inoculum dosages(g/L)	FPA (IU/mL) E	Temp	pH	Inoculum dosages(g/L)	FPA (IU/mL) P
A	25	4	0.56	1.35	-1	-1	0	1.691181
B	25	6	0.42	1.4	-1	0	-1	1.630877
C	25	6	0.7	1.28	-1	0	1	1.650396
D	25	8	0.56	1.18	-1	1	0	1.45307
E	30	4	0.42	1.43	0	-1	-1	1.733673
F	30	4	0.7	1.26	0	-1	1	1.784189
G	30	6	0.56	1.93	0	0	0	2.313885
H	30	8	0.42	1.26	0	1	-1	1.51956
I	30	8	0.7	1.11	0	1	1	1.558081
J	35	4	0.56	1.08	1	-1	0	1.57468
K	35	6	0.42	1.15	1	0	-1	1.507376
L	35	6	0.7	1	1	0	1	1.576895
M	35	8	0.56	0.946	1	1	0	1.37257

E= Experimental values, P = Predicted values.

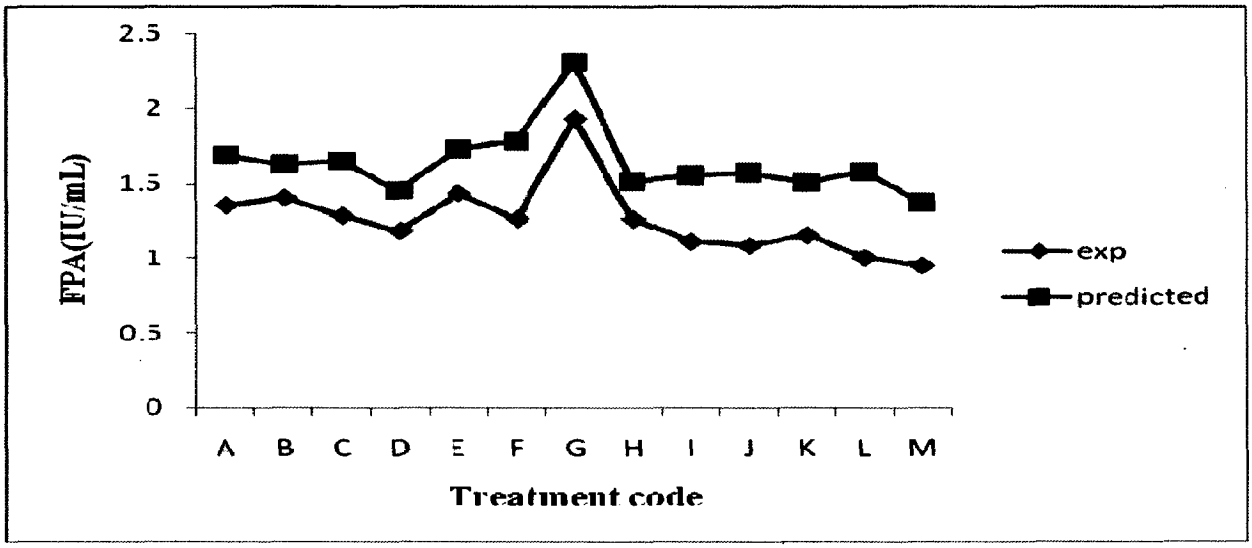


Fig.4.2.9. Comparative graph of experimental and predicted values of cellulase activity (FPA).

The observed and predicted values of cellulase activity are very close to each other which represents the good correlation as observed from Table and Fig 4.2.9

Table 4.2.10. Variables used in box–behnken design for the optimization of process parameters such as particle size, moisture percentage and incubation period.

Factor	Basic level	Variation interval	Value of the factor	Coded value
Particle size (μm)	850	400	450	-
			850	0
			1250	+
Moisture percentage (%)	66	5	61	-
			66	0
			71	+
Incubation period(days)	10	5	5	-
			10	0
			15	+

Table 4.2.11. Different set of combinations using particle size, moisture percentage and incubation period as parameters for the response of cellulase activity.

Medium code	Particle size	Moisture percentage	Incubation period	Total cellulase activity FPA(IU/mL)
A	450	66	5	1.65
B	850	61	5	1.85
C	850	71	5	1.58
D	1250	66	5	1.72
E	450	61	10	1.81
F	450	71	10	1.14
G	850	66	10	2.36
H	1250	61	10	1.51
I	1250	71	10	1.42
J	450	66	15	1.05
K	850	61	15	0.98
L	850	71	15	0.87
M	1250	66	15	1.07

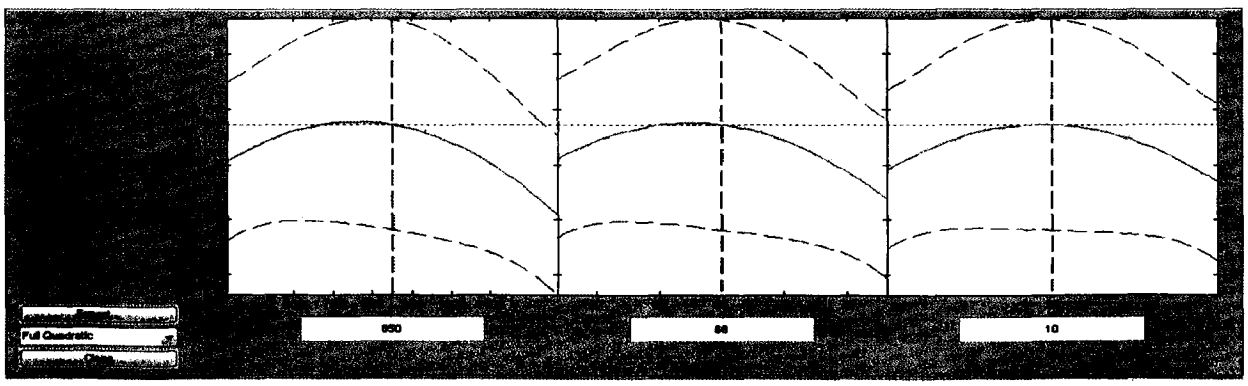


Fig 4.2.10. Cellulase activity as a response (Y1) of three parameters particle size(X1), moisture percentage (X2) and incubation period (X3).

Different sets of combination have been used for cellulase production by *N. crassa*. It was observed from Table and Fig 4.2.10-4.2.11. that particle size of 850 μm, moisture percentage 66, and incubation period of 10 days was found quite effective set of combination for cellulase activity (4.72 IU/mL) produced by *N. crassa* as compared to other. It was also observed from Fig that rate of decrement in enzyme activity was somewhat lower towards smaller particle size than 850 μm, lower moisture percentage than 66 and smaller incubation period than 10 days.

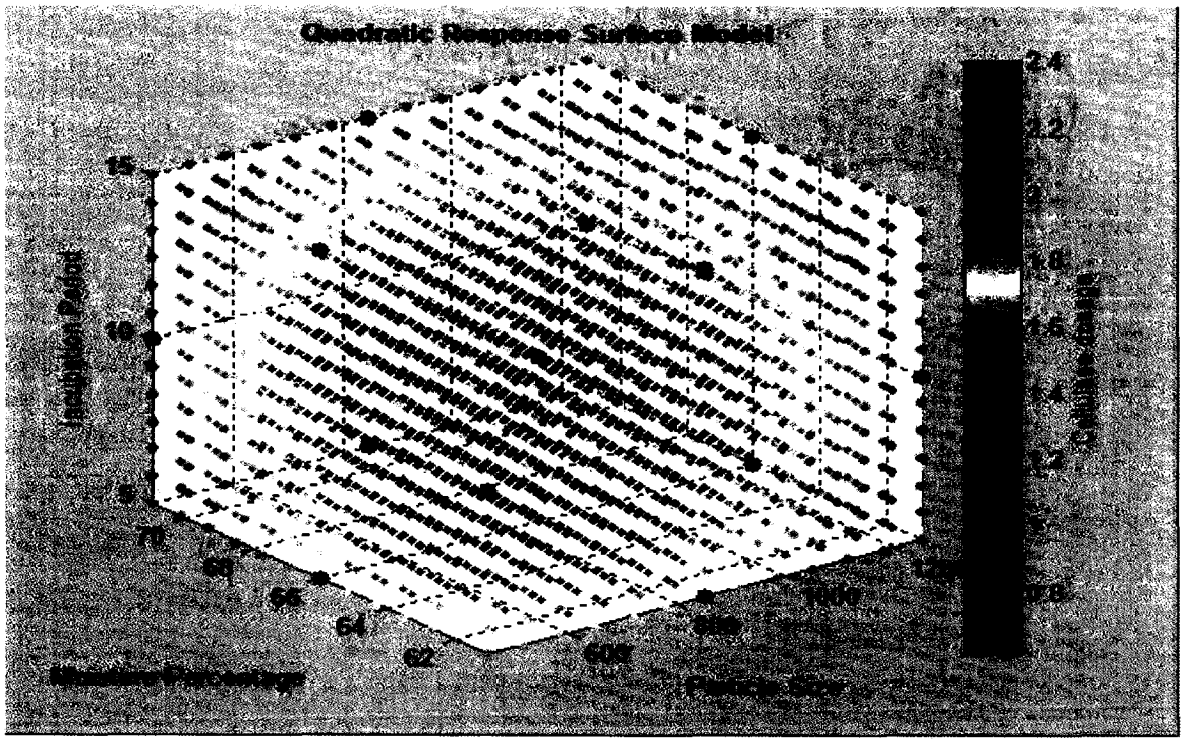


Fig. 4.2.11. Quadratic response surface model with cellulase activity as a response of three parameters particle size, moisture percentage and incubation period.
Cellulase dosage represents the cellulase activity(IU/mL).

As observed from Fig 4.2.11 that higher cellulase activity region lie in the nearly centre of the graph which proves the significantly good optimization of process parameters.

Table 4.2.12. Comparative experimental and predicted values of cellulase activity (IU/mL) under different set of combination.

Medium code	Particl e size	MP	IP	Total cellulase activity FPA(IU/mL)	Particle size	MP	IP	Total cellulase activity FPA (IU/mL)
A	450	66	5	1.65	-1	0	-1	1.784503
B	850	61	5	1.85	0	-1	-1	2.018746
C	850	71	5	1.58	0	1	-1	1.696059
D	1250	66	5	1.72	1	0	-1	2.316303
E	450	61	10	1.81	-1	-1	0	1.632143
F	450	71	10	1.14	-1	1	0	1.069268
G	850	66	10	2.36	0	0	0	1.753605
H	1250	61	10	1.51	1	-1	0	0.768943
I	1250	71	10	1.42	1	1	0	0.686068
J	450	66	15	1.05	-1	0	1	1.006908
K	850	61	15	0.98	0	-1	1	0.086339
L	850	71	15	0.87	0	1	1	-0.23672
M	1250	66	15	1.07	1	0	1	-0.81629

MP: Moisture percentage, IP: Incubation period (in days)

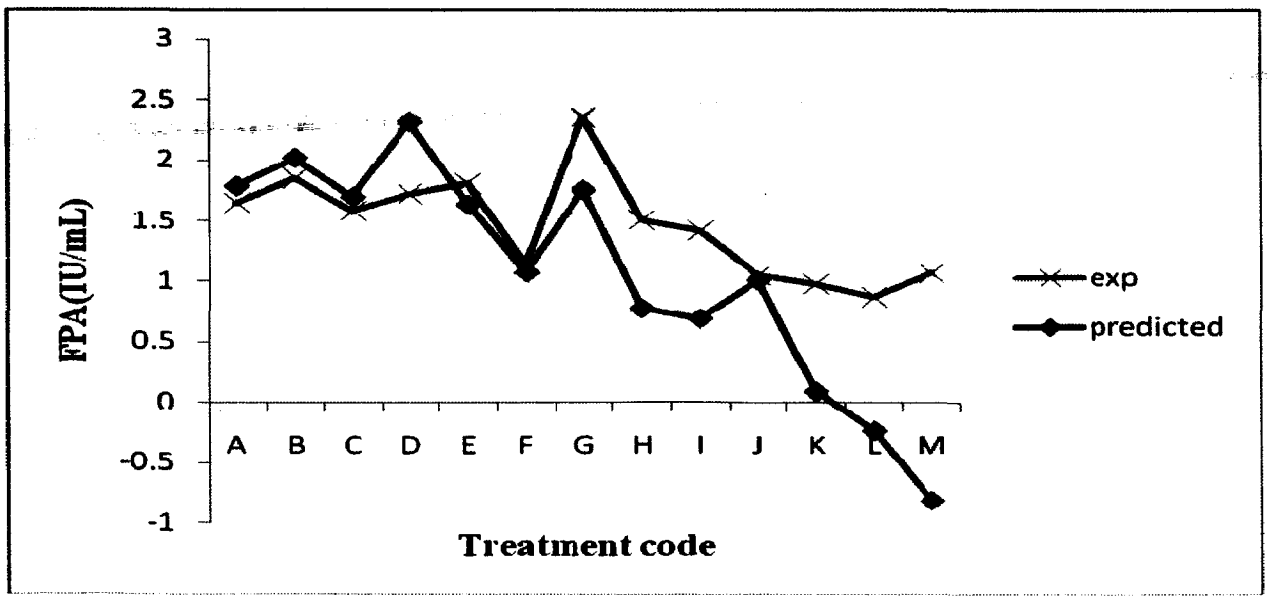


Fig.4.2.12. Comparative graph of experimental and predicted values of cellulase activity (FPA).

The experimental and predicted values of cellulase activity are close as well as distant to each other which represents the nearly good correlation to each other as observed from Table and Fig 4.2.12.

4.2.3. Optimization of physical and chemical parameters for cellulase production by *Aspergillus niger.*

Table 4.2.13. Variables used in box–behnken design for the optimization of process parameters such as temperature, pH and inoculum dosages.

Factor	Basic level	Variation interval	Value of the factor	Coded value
Temperature (^{0}C)	30	5	25	-
			30	0
			35	+
pH	5	2	3	-
			5	0
			7	+
Inoculum dosages	0.56	0.24	0.32	-
			0.56	0
			0.80	+

Table 4.2.14. Different set of combinations using temperature, pH and inoculum dosage as parameters for the response of cellulase activity.

Medium code	Temp(^{0}C)	pH	Inoculum dosages	FPA(IU/mL)
A	25	3	0.56	0.518
B	25	5	0.32	0.646
C	25	5	0.80	0.578
D	25	7	0.56	0.442
E	30	3	0.32	0.622
F	30	3	0.80	0.554
G	30	5	0.56	0.980
H	30	7	0.32	0.583
I	30	7	0.80	0.476
J	35	3	0.56	0.578
K	35	5	0.32	0.544
L	35	5	0.80	0.646
M	35	7	0.56	0.510

Different sets of combination have been used for cellulase production by *A. niger*. It was observed from Table and Fig 4.2.13-4.2.14 that temperature at 30^{0}C, pH at 5 and inoculums dosages of 0.56g/L was found quite effective set of combination for cellulase activity (0.980 IU/mL) produced by *A. niger* as compared to other.

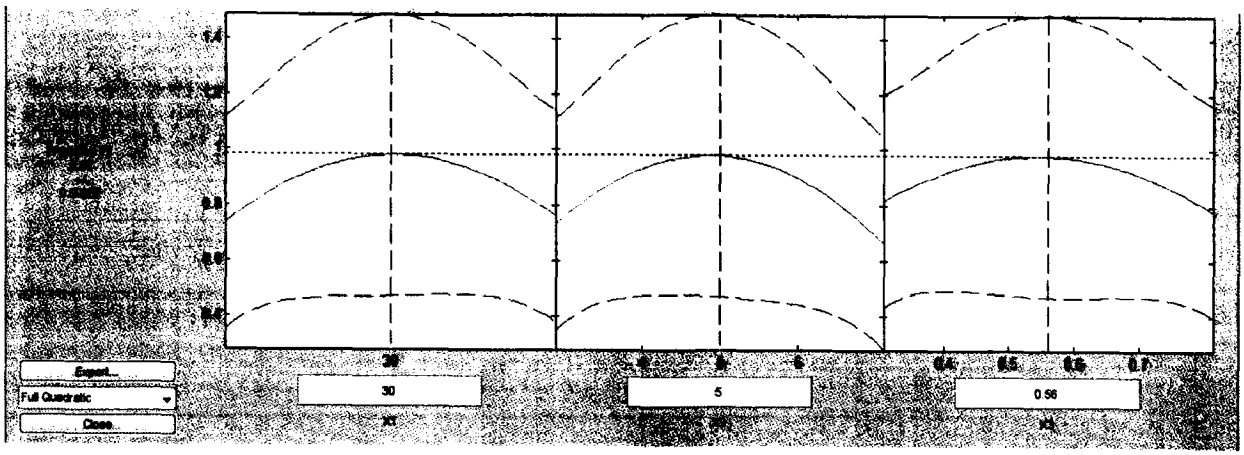


Fig 4.2.13. Cellulase activity as a response (Y1) of three parameters temperature(X1), pH(X2) and Inoculum dosage(X3).

It was also observed from Fig 4.2.13 that rate of decrement in enzyme activity was somewhat lower towards both higher and lower temperature than 30^0C, pH lower than 5 and inoculum dose both lower and higher than 0.56g/L.

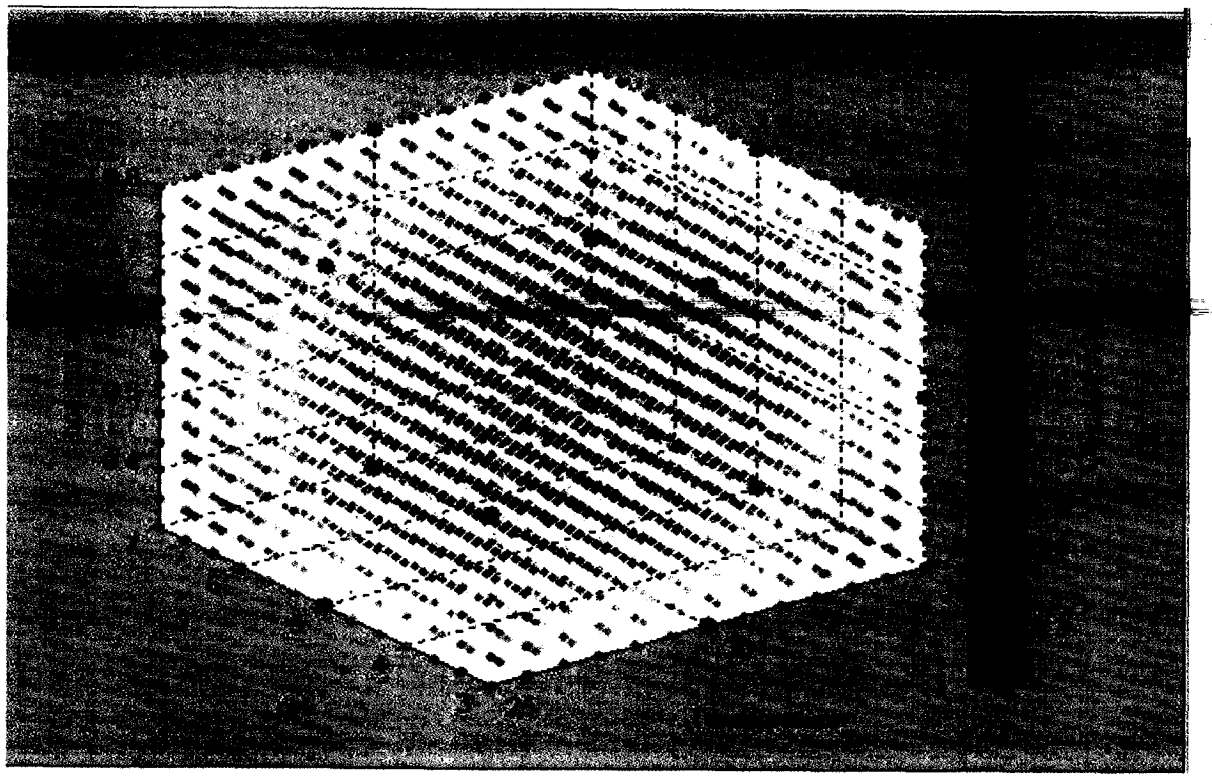


Fig. 4.2.14. Quadratic response surface model with cellulase activity as a response of three parameters temperature, pH and Inoculum dosage.
Cellulase dosage represents the cellulase activity(IU/mL).

As observed from Fig 4.2.14. that higher cellulase activity region lie in the centre of the graph which proves the significantly better optimization of process parameters.

Table 4.2.15. Comparative experimental and predicted values of cellulase activity (IU/mL) under different set of combination.

Medium code	T	pH	Inoculum dosages	Total cellulase activity FPA(IU/mL) Exp(E)	T	pH	Inoculum dosages	Total cellulase activityFPA(IU/mL) Pre(P)
A	25	3	0.56	0.518	-1	-1	0	0.345249
B	25	5	0.32	0.646	-1	0	-1	0.79221
C	25	5	0.8	0.578	-1	0	1	0.136993
D	25	7	0.56	0.442	-1	1	0	0.400984
E	30	3	0.32	0.622	0	-1	-1	0.613322
F	30	3	0.8	0.554	0	-1	1	-0.12464
G	30	5	0.56	0.98	0	0	0	0.695444
H	30	7	0.32	0.583	0	1	-1	0.639056
I	30	7	0.8	0.476	0	1	1	-0.0309
J	35	3	0.56	0.578	1	-1	0	0.049683
K	35	5	0.32	0.544	1	0	-1	0.549388
L	35	5	0.8	0.646	1	0	1	-0.20332
M	35	7	0.56	0.51	1	1	0	0.113418

T: Temperature (^{0}C),E:Experimental, P:Predicted

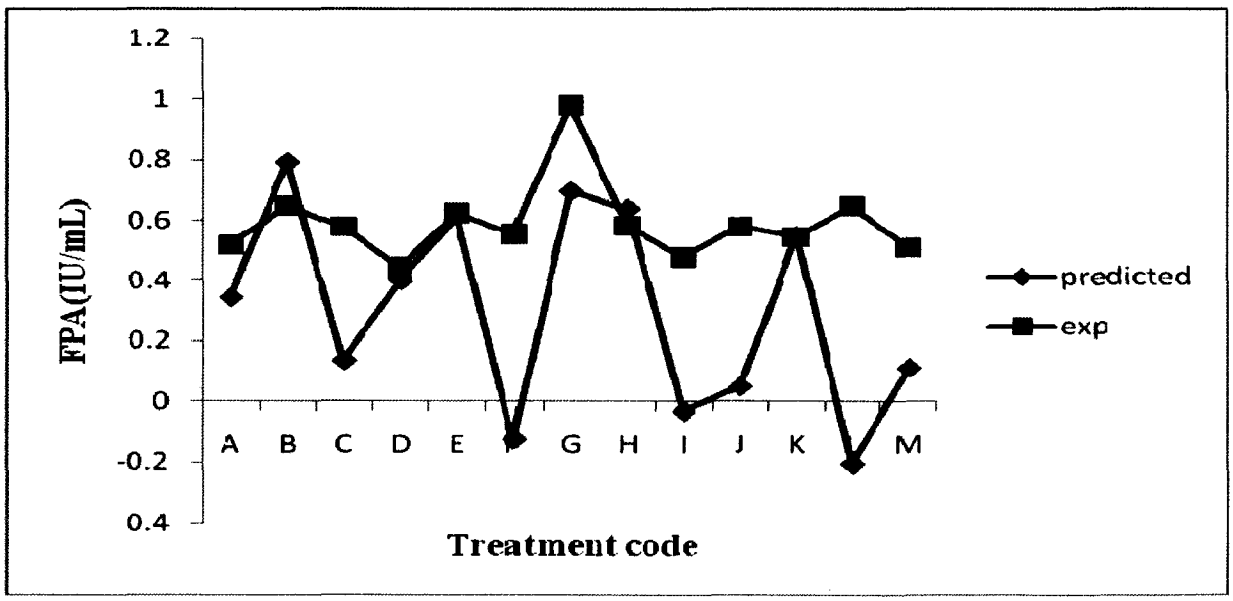


Fig.4.2.15. Comparative graph of experimental and predicted values of cellulase activity (FPA)

The experimental and predicted values of cellulase activity are nearly close to each other which represents the nearly good correlation as observed from Table and Fig 4.2.15.

Table 4.2.16. Variables used in box–behnken design for the optimization of process parameters such as particle size, moisture percentage and incubation period.

Factor	Basic level	Variation interval	Value of the factor	Coded value
Particle size	850	400	450	-
			850	0
			1250	+
Moisture percentage(%)	76	5	71	-
			76	0
			81	+
Incubation period	6	3	3	-
			6	0
			9	+

Table 4.2.17. Different set of combinations using particle size, moisture percentage and incubation period as parameters for the response of cellulase activity.

Medium code	Particle size	Moisture percentage	Incubation period	Total cellulase activity(FPU/ml)
A	450	76	3	0.672
B	850	71	3	0.567
C	850	81	3	0.672
D	1250	76	3	0.567
E	450	71	6	0.735
F	450	81	6	0.840
G	850	76	6	1.29
H	1250	71	6	0.630
I	1250	81	6	0.735
J	450	76	9	0.798
K	850	71	9	0.696
L	850	81	9	0.801
M	1250	76	9	0.683

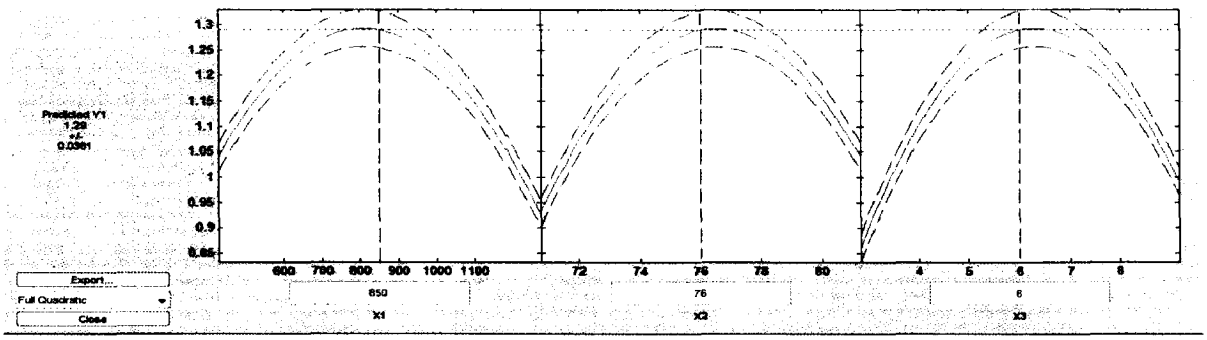


Fig 4.2.16. Cellulase activity as a response (Y1) of three parameters particle size(X1), moisture percentage (X2) and incubation period (X3).

Different sets of combination have been used for cellulase production by *A. niger*. It was observed from Table and Fig 4.2.16-4.2.17 that particle size of 850 μm, moisture percentage 76, and incubation period of 6 days was found quite effective set of combination for cellulase activity (1.29 IU/mL) produced by*A. niger* as compared to other. It was also observed from Fig 4.2.16. that rate of decrement in enzyme activity was somewhat lower towards smaller particle size than 850 μm, higher moisture percentage than 76 and higher incubation period than 6 days.

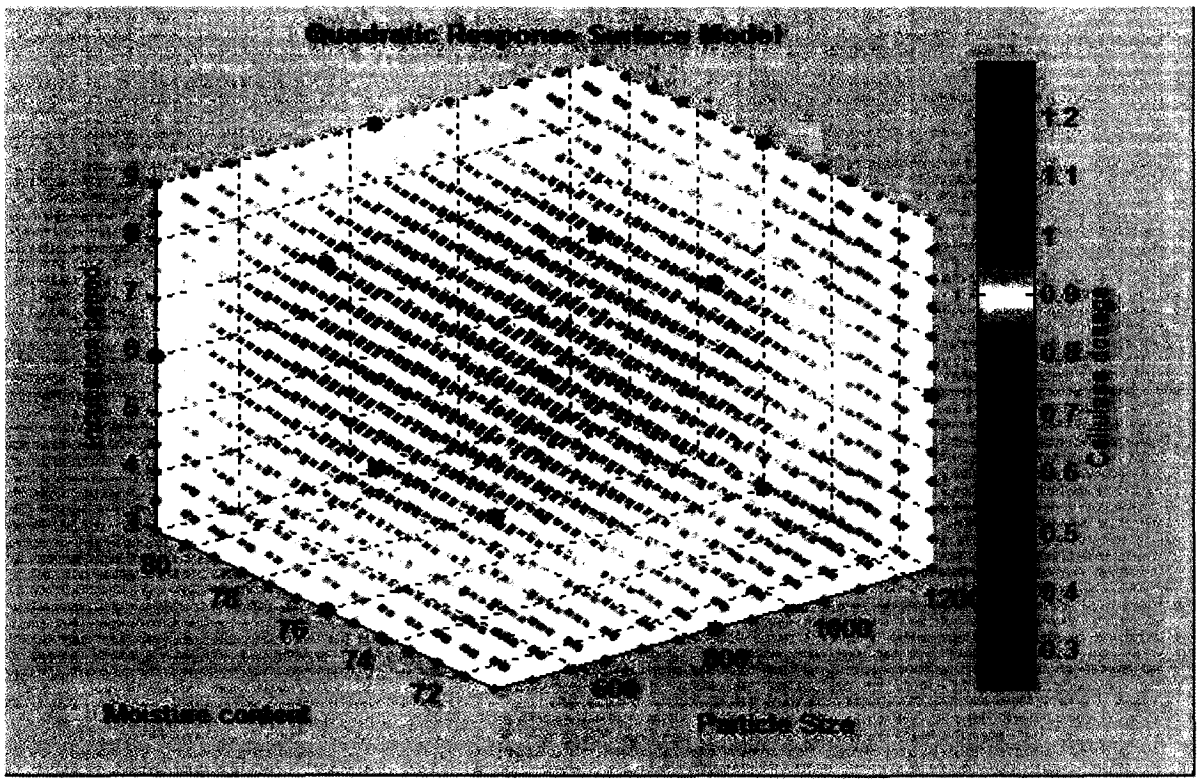


Fig. 4.2.17. Quadratic response surface model with cellulase activity as a response of three parameters temperature, pH and Inoculum dosage.
Cellulase dosage represents the cellulase activity(IU/mL).

As observed from Fig 4.2.17. that higher cellulase activity region lie in the centre of the graph which proves the significantly better optimization of process parameters.

The observed and predicted values of cellulase activity are extremely close to each other which represents the fine correlation between them as observed from Table and Fig 4.2.18.

Table 4.2.18. Comparative experimental and predicted values of cellulase activity (IU/mL) under different set of combination.

Particle size	Moisture percentage	Incubation period	Total cellulase activity FPA (IU/ml)(E)	Particle size	Moisture percentage	Incubation period	Total cellulase activity FPA(IU/ml) (P)
450	76	3	0.672	-1	0	-1	0.680729
850	71	3	0.567	0	-1	-1	0.60796
850	81	3	0.672	0	1	-1	0.712898
1250	76	3	0.567	1	0	-1	0.647129
450	71	6	0.735	-1	-1	0	0.75069
450	81	6	0.84	-1	1	0	0.855565
850	76	6	1.29	0	0	0	1.333828
1250	71	6	0.63	1	-1	0	0.71709
1250	81	6	0.735	1	1	0	0.821965
450	76	9	0.798	-1	0	1	0.815566
850	71	9	0.696	0	-1	1	0.74286
850	81	9	0.801	0	1	1	0.847673
1250	76	9	0.683	1	0	1	0.781966

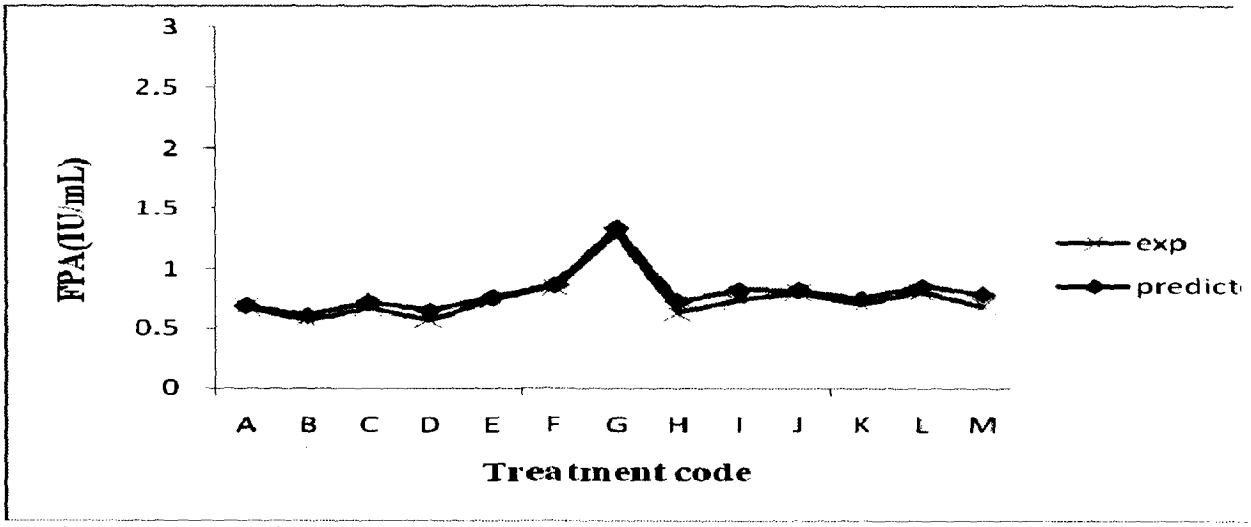


Fig.4.2.18. Comparative graph of experimental and predicted values of cellulase activity (FPA)

Qudratic polynomial equation used for determining the values are stated by following equation
$b0 + b\,1x1 + b2\,x2 + b\,3x\,3 + b12\,x1\,x2 + b\,13\,x1\,x3 + b23\,x2\,x3 + b11\,x1^2 + b22\,x2^2 + b3$
$x3^2$
In each sets total 10 coefficients are generated as observed from Table 4.2.19.

Table 4.2.19. Coefficient values under different sets of condition.

Trichoderma reesei		***Neurospora crassa***		***Aspergillus niger***	
Set 1, Trail 1	Trail 2	Set 2, Trail 1	Trail 2	Set 3, Trail 1	Trail 2
Temp, pH, inoculums dosages	Particle size, moisture percentage, incubation period	Temp, pH, inoculums dosages	Particle size, moisture percentage, incubation period	Temp, pH, inoculums dosages	Particle size, moisture percentage, incubation period
Coefficients	Coefficients	Coefficients	Coefficients	Coefficients	Coefficients
-42.2368	-188.437	-20.0693	-72.7166	-8.51818	-65.765
2.46125	0.008198	0.99295	0.001051	0.489417	0.002848
2.141875	6.143066	1.02425	2.25315	0.63	1.68554
15.73225	1.282604	16.73036	0.347988	2.171528	0.463271
-6.59E-16	-4.06E-06	0.0009	6.00E-05	0.0002	-5.14E-19
-0.025	1.46E-05	-0.01071	-3.75E-06	0.035417	-2.08E-06
-0.3125	-2.06E-05	0.017857	-0.0003	-0.02031	-1.61E-16
-0.0405	-4.35E-06	-0.01697	-3.27E-06	-0.00847	-1.75E-06
-0.20813	-0.05085	-0.09169	-0.0177	-0.06409	-0.01102
-13.2716	-0.10347	-15.2168	-0.0168	-2.86241	-0.03672

It can be concluded that in most of the sets optimization was found quite effective, as well as in most of the cases experimental and predicted values were very close to each other, representing better correlation between them. An optimized sets of condition have been achieved and this optimized conditions were used in further production studies.

SECTION III
CELLULASE PRODUCTION THROUGH LIQUID STATE FERMENTATION

4.3. CELLULASE PRODUCTION UNDER SUBMERGED FERMENTATION

Sugar play a vital and critical role in cellulase biosynthesis as it acts as an inducer. Soluble carbon sources such as lactose, cellobiose give significantly lower cellulase activity compared to pure insoluble cellulosic substances such as cotton, avicel, solka floc at the same concentration [28, 163]. A number of different substrates have been utilized for the fermentative production of cellulase. Various purified cellulose such as Avicel, Solka floc, cotton have been used as carbon source for cellulase production [11, 218]. For large scale production of enzyme, pure cellulose would be too costly and expensive to be applied in large scale process. The present section evaluates the utility of pure sugars in the cellulase production by various fungal strains.

4.3.1.Effect of cellulose as carbon source on cellulase production under submerged cultivation

Separate sets of batch experiments have been performed to study the effect of pure sugar such as cellulose (10g/L) on cellulase production by various fungal strains under submerged cultivation. It is observed from Table 4.3.1.and Figs 4.3.1(a), 4.3.1(b) that cellulose is very effective carbon source for cellulase production. This might be due to their insoluble nature which makes it a better inducer for cellulase production. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei, T.viride, N.crassa, A.niger* and *A.fumigatus* were 3.29, 2.96; 1.63, 4.18; 1.59, 3.52; 0.801, 5.86; 1.05, 4.97 at120, 96; 120, 144; 144, 120; 96, 72; 96,72 h at 30^0C temperature respectively under cellulose based fermentation medium. It has been observed from Fig 4.3.1(a) that *T.reesei* produced higher FPA under cellulose based fermentation medium as compared to the other fungal strains. Steep rise in FPA activity was observed after 48h by *T.reesei* while *T.viride* produced FPA with steady increment. On the other hand higher CMCase was achieved by *Aspergillus* strains followed by *T.viride* .Sharp increment in the CMCase activities were observed upto 60 h by *Aspergillus* strains while steady increment has been observed in CMCase activity till 144 h as viewed from Fig 4.3.1(b).

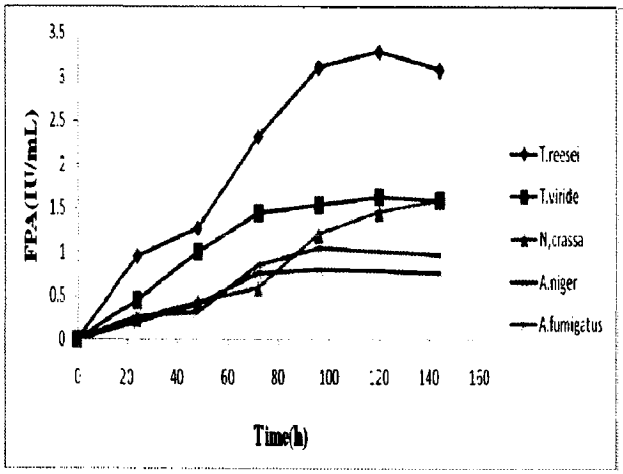


Fig 4.3.1(a)

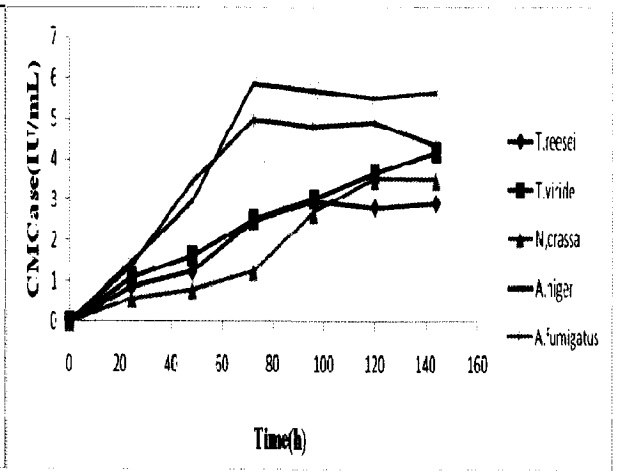


Fig 4.3.1(b)

Fig 4.3.1.Comparative FPA and CMCase achieved by various fungal strains under cellulose based production medium at 30^0C and pH 5.0

4.3.2. Effect of lactose on cellulase production under submerged cultivation

Separate sets of batch experiments have been performed to study the effect of pure sugar such as lactose (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.1.and Figs 4.3.2(a), 4.3.2(b) that fungal strains produced significantly good cellulase activities under lactose based fermentation medium. When compared, the cellulase activity under lactose and cellulose based medium then it was observed that fungal strains produced significantly lesser cellulase activity under lactose based medium as observed from Table 4.3.1.This might be due to the soluble nature of lactose disaccharide as literature reported that soluble carbon sources give significantly lower cellulase activity compared to pure insoluble cellulosic substances at the same concentrations (28, 163).

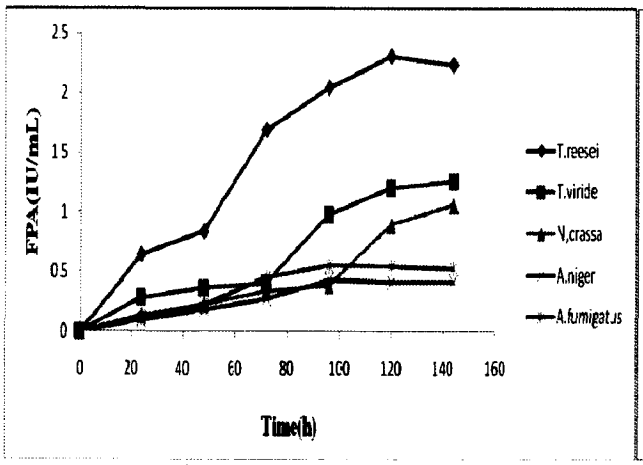


Fig 4.3.2(a)

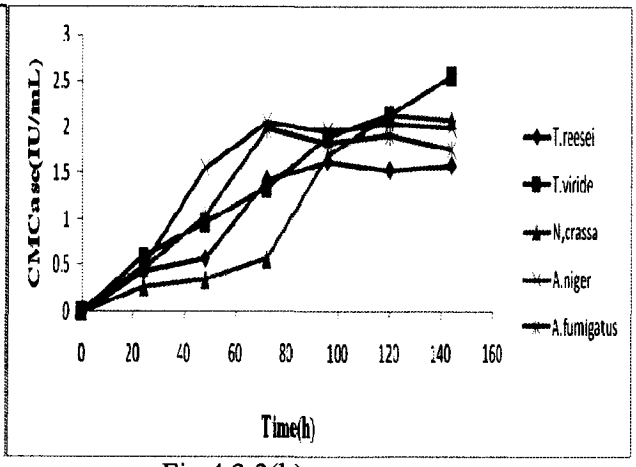


Fig 4.3.2(b)

Fig 4.3.2.Comparative FPA and CMCase achieved by various fungal strains under lactose based production medium at 30^0C and pH 5.0

The maximum cellulase activities (IU/mL) in terms of FPA and CMCase achieved by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 2.31, 1.63; 1.26, 2.55; 1.06, 2.13; 0.433, 1.96; 0.556, 1.98 at 120, 96; 144, 144; 144, 120; 96, 72; 96, 72 h respectively under lactose based fermentation medium. It has been observed from Fig 4.3.2(a) that *T.reesei* produced higher FPA activity under lactose based fermentation medium as compared to the other fungal strains, which states that lactose act as a better inducer for cellulase production by *T. reesei*. Sharp increment in FPA activities were observed after 48, 72 and 96 h by *T.reesei*, *T.viride* and *N.crassa* strains. On the other hand higher CMCases were achieved by *T.viride* followed by *N.crassa* and *A.niger* fungal strains. A sharp increment has been observed in CMCase activity till 72h by *A.niger* while a steep increment has been observed in CMCase after 72 h as viewed from Fig 4.3.2(b).

4.3.3. Effect of CMC as carbon source on cellulase production under submerged cultivation

Individual sets of batch experiment have been performed to study the effect of carboxy methyl cellulose (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.1.and Figs 4.3.3(a), 4.3.3(b) that various fungal strains produce significantly better cellulase activities under carboxymethylcellulose based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 2.46, 1.83; 1.31, 3.08; 0.986, 1.89; 0.523, 2.41; 0.603, 2.23 at 120, 96; 120, 144; 144, 120; 120, 120; 96, 120 h respectively under carboxymethylcellulose based fermentation medium. It has been observed from Fig 4.3.3(a) that *T.reesei* and *T.viride* produces higher FPA under CMC based fermentation medium as compared to the other fungal strains. A steep increment in FPA was observed after 72h by *T.reesei* which suggests that upto 72h, *T.reesei* utilized energy for their growth and after this period microbes utilized all the energy for enzyme production and reached their maximum at 120 h of fermentation period. On the other hand higher CMCases were achieved by *T.viride* followed by *Aspergillus* strains. A steep increment in the CMCase have been observed till 72 h by *T.viride* and *Aspergillus* strains as viewed from Fig 4.3.3(b).

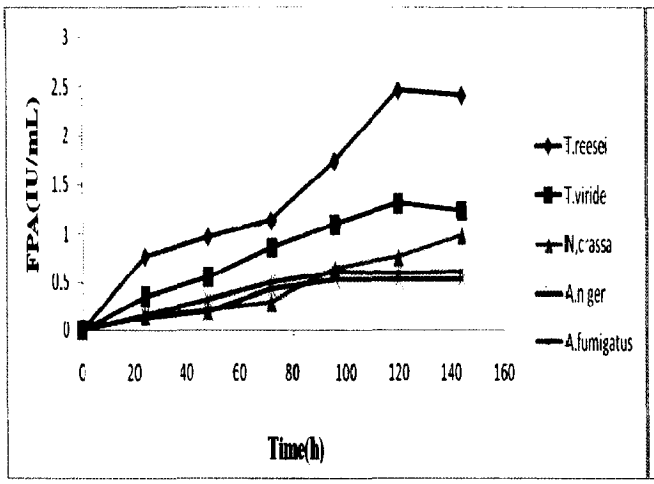


Fig 4.3.3(a)

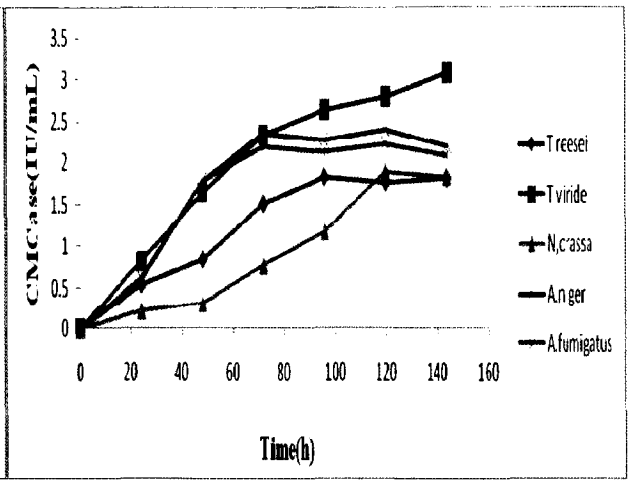


Fig 4.3.3(b)

Fig 4.3.3.Comparative FPA and CMCase achieved by various fungal strains under CMC based production medium at 30^0C and pH 5.0

4.3.4. Effect of cellobiose on cellulase production under submerged cultivation

Individual sets of batch experiment have been performed to study the effect of cellobiose (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.1.and Figs 4.3.4(a), Fig 4.3.4(b) that various fungal strains produce significant amount of cellulase activities under cellobiose based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 2.02, 1.75; 1.08, 2.81; 0.955, 1.92; 0.795, 3.72; 0.885, 3.19 at 120, 96; 120, 144; 144, 120; 96, 72; 120; 96, 120 h respectively under cellobiose based fermentation medium. It has been observed from Fig 4.3.4(a) that *T.reesei* produces higher FPA activity under cellobiose based fermentation medium as compared to the other fungal strains but when compared the cellulases produced by *T.reesei* under cellulose, lactose and CMC, somewhat lower activity was observed under cellobiose based fermentation medium as compared to other sugars based medium. FPA produced by *Aspergillus* strains were something higher under cellobiose based medium as compared to other sugars while the performance of *N.crassa* strain was found something lower in cellobiose based fermentation medium as compared to other sugar based medium as observed from Fig 4.3.4(b).

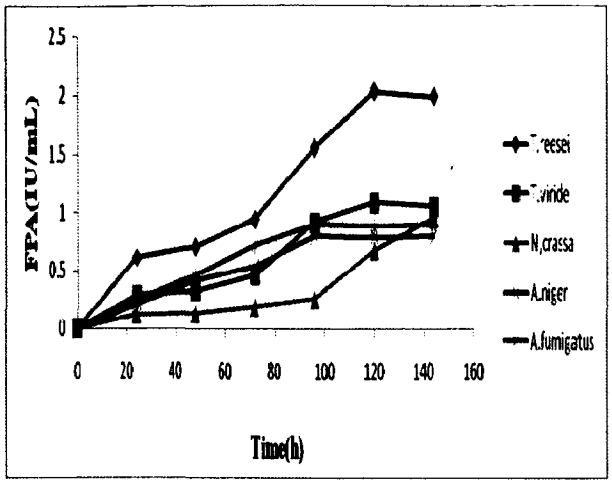


Fig 4.3.4(a)

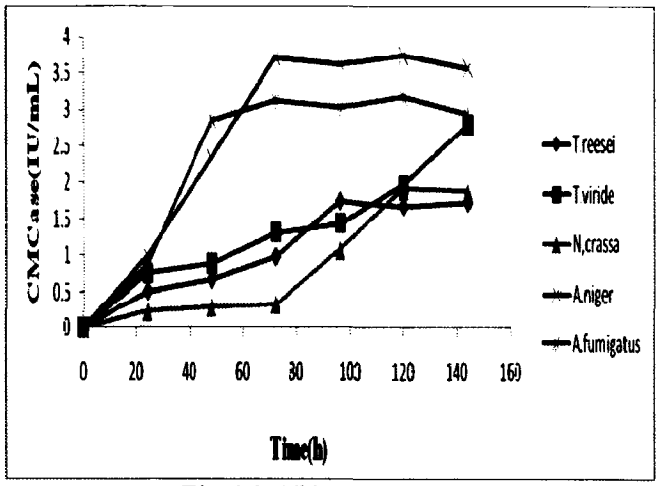


Fig 4.3.4(b)

Fig 4.3.4. Comparative FPA and CMCase achieved by various fungal strains under cellobiose based production medium at 30^0C and pH 5.0

4.3.5. Effect of sucrose sugar on cellulase production under submerged cultivation

Seperate sets of batch experiment have been performed to study the effect of sucrose (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.1 and Figs 4.3.5(a), 4.3.5(b) that various fungal strains produces significant cellulase activities under sucrose based fermentation medium.

Table 4.3.1. Effects of pure sugars as carbon source on the cellulase production (IU/mL) by various fungal strains under submerged fermentation at 30^0C and pH 5.F
FPA represents filter paper activity and CMCase represents carboxymethyl cellulase activity

Sugars	*Trichoderma reesei*			*Trichoderma viridae*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
	Time (h)	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
Cellulose	24	0.963	0.873	0.451	1.08	0.244	0.574	0.201	1.46	0.274	1.37
	48	1.28	1.23	1.01	1.63	0.441	0.786	0.408	2.97	0.321	3.43
	72	2.32	2.49	1.45	2.51	0.596	1.23	0.759	5.86	0.859	4.97
	96	3.11	2.96	1.54	3.03	1.21	2.68	0.801	5.69	1.05	4.81
	120	3.29	2.81	1.63	3.67	1.46	3.52	0.788	5.50	1.01	4.90
	144	3.08	2.90	1.59	4.18	1.59	3.49	0.757	5.63	0.978	4.34
Lactose	24	0.646	0.443	0.291	0.596	0.141	0.276	0.097	0.491	0.122	0.465
	48	0.843	0.578	0.368	0.967	0.229	0.349	0.184	1.56	0.231	1.04
	72	1.69	1.43	0.411	1.33	0.341	0.567	0.276	2.05	0.453	1.98
	96	2.05	1.63	0.983	1.89	0.389	1.69	0.433	1.96	0.556	1.83
	120	2.31	1.54	1.20	2.13	0.894	2.13	0.410	2.03	0.547	1.91
	144	2.24	1.59	1.26	2.55	1.06	2.07	0.419	1.99	0.531	1.76
CMC	24	0.762	0.549	0.353	0.825	0.147	0.226	0.138	0.616	0.165	0.594
	48	0.967	0.843	0.552	1.65	0.211	0.313	0.198	1.79	0.321	1.79
	72	1.13	1.51	0.854	2.34	0.297	0.778	0.432	2.37	0.498	2.20
	96	1.74	1.83	1.09	2.65	0.654	1.18	0.519	2.29	0.602	2.13
	120	2.46	1.77	1.31	2.81	0.765	1.89	0.523	2.41	0.589	2.23
	144	2.41	1.81	1.23	3.08	0.986	1.83	0.521	2.21	0.597	2.09
Cellobiose	24	0.610	0.519	0.289	0.761	0.128	0.230	0.214	0.996	0.248	0.867
	48	0.702	0.675	0.321	0.897	0.131	0.287	0.412	2.34	0.458	2.87
	72	0.934	0.986	0.456	1.32	0.189	0.333	0.532	3.72	0.712	3.13
	96	1.54	1.75	0.912	1.45	0.256	1.08	0.795	3.63	0.885	3.04
	120	2.02	1.68	1.08	1.98	0.675	1.92	0.789	3.75	0.878	3.19
	144	1.98	1.73	1.01	2.81	0.955	1.88	0.793	3.56	0.890	2.95
Sucrose	24	0.463	0.384	0.212	0.541	0.135	0.236	0.086	0.394	0.101	0.374
	48	0.678	0.578	0.513	0.987	0.213	0.554	0.095	0.986	0.205	0.788
	72	0.975	1.23	0.733	1.34	0.567	0.898	0.116	1.97	0.289	1.81
	96	1.67	1.67	0.912	1.87	0.981	1.56	0.401	1.87	0.483	1.73
	120	1.93	1.71	1.01	2.06	1.01	2.36	0.394	1.92	0.476	1.80
	144	1.87	1.61	0.989	2.46	1.18	2.31	0.388	1.56	0.455	1.69
Maltose	24	0.180	0.187	0.101	0.267	0.131	0.240	0.091	0.446	0.110	0.437
	48	0.251	0.244	0.230	0.456	0.344	0.789	0.189	1.71	0.254	1.87
	72	0.434	0.987	0.289	0.897	0.767	1.32	0.386	2.81	0.512	2.59
	96	0.867	1.21	0.342	1.23	0.980	1.78	0.571	2.73	0.685	2.51
	120	1.13	1.11	0.698	1.56	1.09	2.78	0.543	2.64	0.671	2.60
	144	1.09	1.18	0.689	1.91	1.43	2.69	0.555	2.69	0.678	2.43

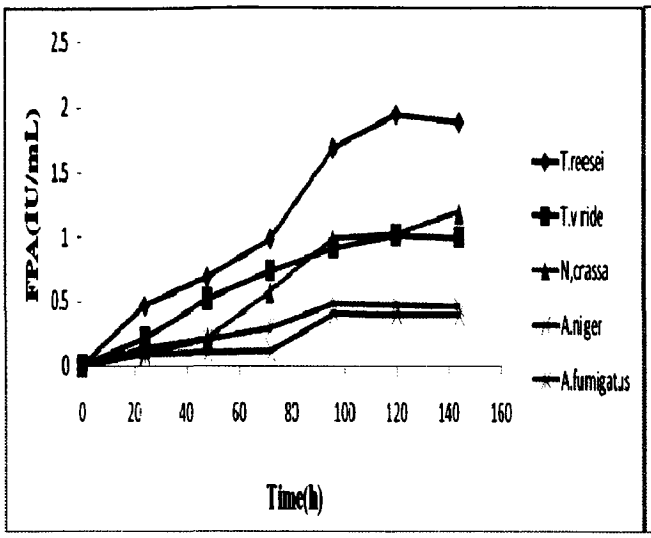


Fig 4.3.5(a)

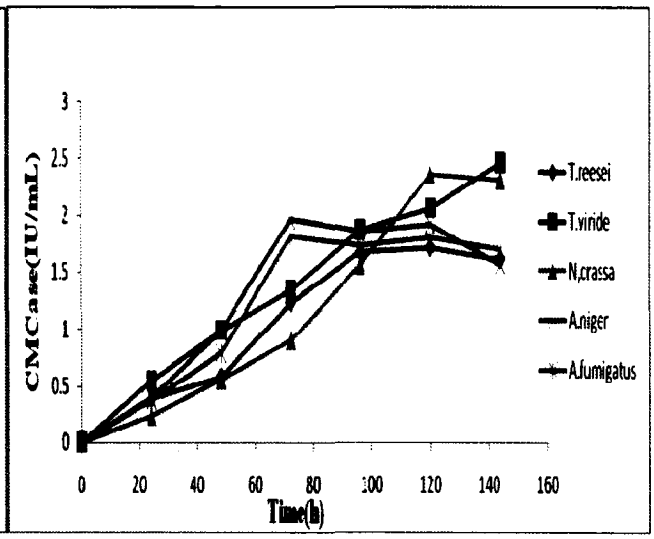


Fig 4.3.5(b)

Fig 4.3.5.Comparative FPA and CMCase achieved by various fungal strains under sucrose based production medium at 30^0C and pH 5.0

Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei, T.viride, N.crassa, A.niger* and *A.fumigatus* were 1.93, 1.71; 1.01, 2.46; 1.18, 2.36; 0.401, 1.97; 0.483, 1.81 at 120, 96; 120, 144; 144, 120; 96, 72; 96, 72 h respectively under sucrose based fermentation medium. It has been observed from Fig 4.3.5(a) that *T.reesei, T.viride* and *N.crassa* yield higher FPA activity under sucrose based fermentation medium as compared to *Aspergillus* strains. On the other hand higher CMCases were achieved by *T.viride* and *N.crassa* strains under sucrose based fermentation medium as viewed from Fig 4.3.5(b). Overall we can infer that *Trichoderma* as well as *Neurospora* strains performed better under sucrose based fermentation medium in terms of cellulase production as compared to *Aspergillus* strains.

4.3.6. Effect of Maltose sugar on cellulase production under submerged cultivation

Separate sets of batch experiment have been performed to study the effect of maltose (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.1 and Figs 4.3.6(a), 4.3.6(b) that various fungal strains produces significantly better cellulase activities except *Trichoderma* under maltose based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei, T.viride, N.crassa, A.niger* and *A.fumigatus* were 1.13, 1.21; 0.698, 1.91; 1.43, 2.78; 0.571, 2.81; 0.685, 2.51 at 120, 96; 120, 144; 144, 120; 96, 72; 96, 72 h respectively under maltose based fermentation medium. It has been observed from Fig 4.3.6(a) that *N.crassa* produces higher FPA activity under maltose based fermentation medium as compared to the other fungal strains. Good improvement in FPAs activity of *Aspergillus* strains were observed maltose based medium

as compared to sucrose based fermentation medium as shown in Table 4.3.1. On the other hand higher CMCases were achieved by *Aspergillus* as well as *Neurospora* strains.A steep increment in the CMCase have been observed till 48h by *Aspergillus* strains as viewed from Fig 4.3.6(b).

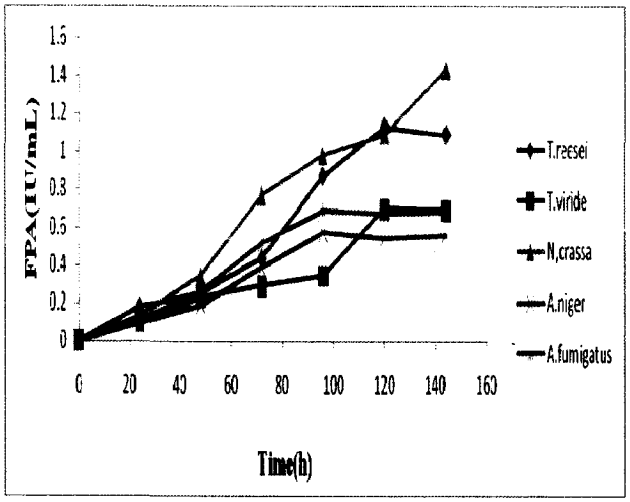


Fig 4.3.6(a)

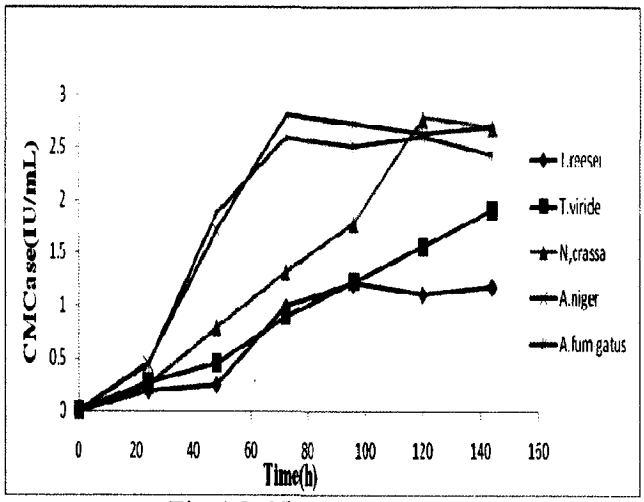


Fig 4.3.6(b)

Fig 4.3.6.Comparative FPA and CMCase achieved by various fungal strains under maltose based production medium at 30^0C and pH 5.0

4.3.7.Effect of xylose as carbon source on cellulase production under submerged cultivation

Individual sets of batch experiments have been performed to study the effect of xylose (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.2 and Figs 4.3.7(a), 4.3.7(b) that various fungal strains produces significantly lesser cellulase activities except *Trichoderma* strains. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 1.53, 1.15; 0.780, 1.54; 0.639, 1.30; 0.239, 1.21; 0.341, 1.29 at 120, 96; 96, 120; 144, 144; 96, 72; 96, 72 h respectively under xylose based fermentation medium. It has been observed from Fig 4.3.7(a) that higher FPA activity was produced by *T.reesei* under xylose based fermentation medium as compared to the other fungal strains. A steep increment in FPA was observed after 96h by *T.reesei* which suggests that upto 96h *T.reesei* utilized energy for their growth and after this period microbes utilized all the energy for enzyme production and reached their maximum at 120 h of fermentation period. On the other hand, higher CMCases were achieved by *T.viride*. A steady increment in the CMCase has been observed till 120 h by *T.viride* strain as viewed from Fig 4.3.7(b).

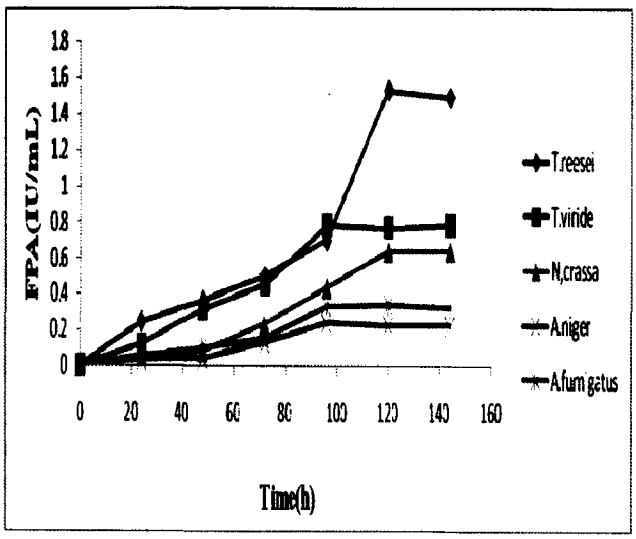


Fig 4.3.7(a)

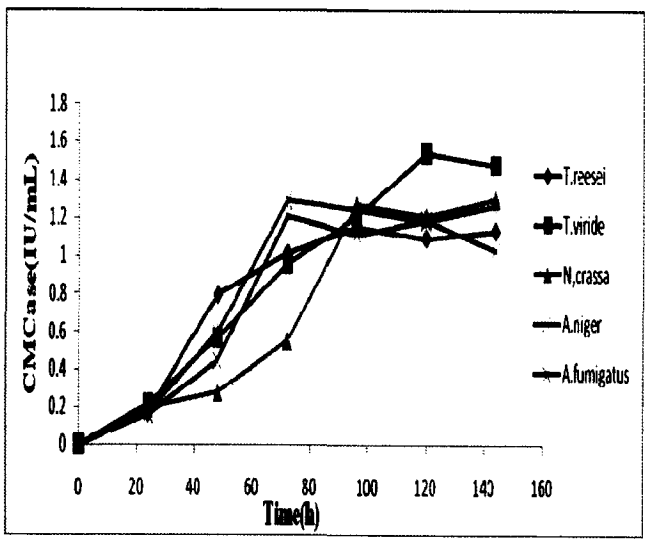


Fig 4.3.7(b)

Fig 4.3.7. Comparative FPA and CMCase achieved by various fungal strains under xylose based production medium at 30^0C and pH 5.0

4.3.8. Effect of arabinose as carbon source on cellulase production under submerged cultivation

Separate sets of batch experiments have been performed to study the effect of arabinose pure sugar (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.2 and Figs 4.3.8(a), 4.3.8(b) that various fungal strains produce significantly reduced cellulase activities under arabinose based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei, T.viride, N.crassa, A.niger* and *A.fumigatus* were 0.693, 0.490; 0.402, 0.886; 0.351, 0.755; 0.203, 1.02; 0.231, 0.896 at 120, 96; 120, 144; 144, 120; 120, 96; 120, 72 h respectively under arabinose based fermentation medium. It has been observed from Fig 4.3.8(a) that higher FPA activity was produced by *T.reesei* under arabinose based fermentation medium as compared to the other fungal strains. A steep increment in FPA was observed after 72 by *T.reesei*. On the other hand higher CMCases were achieved by *Aspergillus* strains followed by *T.viride* and *N.crassa*. A sharp increment in the CMCase have been observed till 72 h by *Aspergillus* strains while CMCase produced by *T.viride* at a steady rate till 120 h after that period a sharp increment has been observed as viewed from Fig 4.3.8(b).

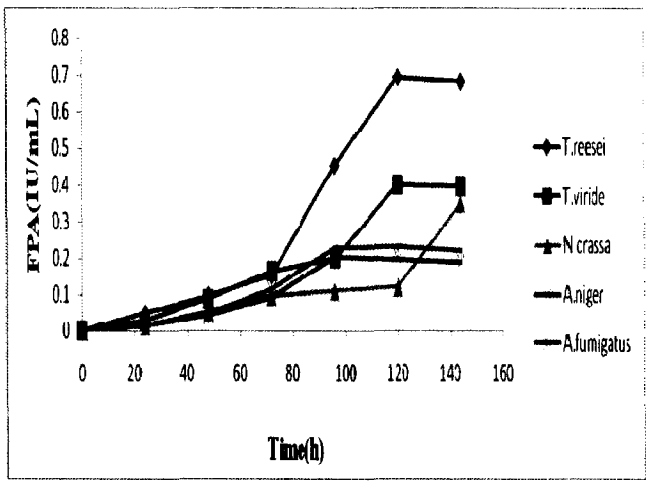


Fig 4.3.8(a)

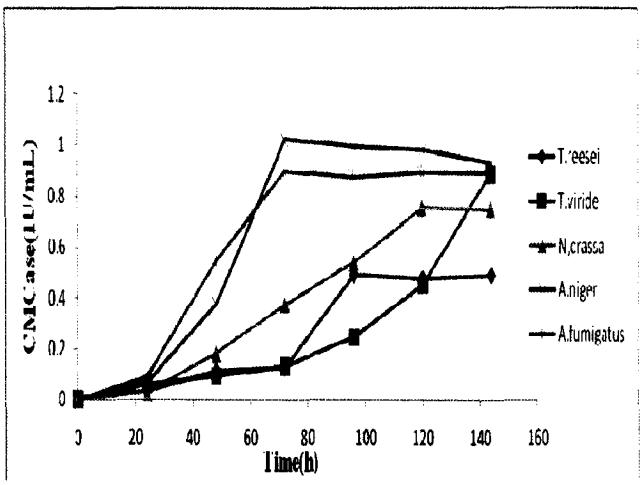


Fig 4.3.8(b)

Fig 4.3.8.Comparative FPA and CMCase achieved by various fungal strains under arabinose based production medium at 30^0C and pH 5.0

4.3.9.Effect of Trehalose as carbon source on cellulase production under submerged cultivation

Seperate sets of batch experiments have been performed to study the effect of trehalose sugar (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.2. and Figs 4.3.9(a), 4.3.9(b) that various fungal strains produce significantly lower cellulase activities under trehalose sugar based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 0.831, 0.589; 0.482, 1.07; 0.158, 0.302; 0.434, 2.18; 0.439, 1.19 at 120, 96; 120, 144; 144, 120; 96, 120; 96, 72 h respectively. Higher FPA activity was observed by *T.reesei* followed *T.viride* and *Aspergillus* strains under trehalose based fermentation medium. A steep increment in FPA activity was observed after 72h by *T.reesei* and *A. fumigatus* strain as observed from Fig 4.3.9(a). On the other hand higher CMCases were achieved by *Aspergillus niger* followed by *Aspergillus fumigatus* strain while *N.crassa* showed lower CMCase activity under trehalose based fermentation medium. A steep increment in the CMCase have been observed after 24 h by *Aspergillus* strains as viewed from Fig 4.3.9(b).

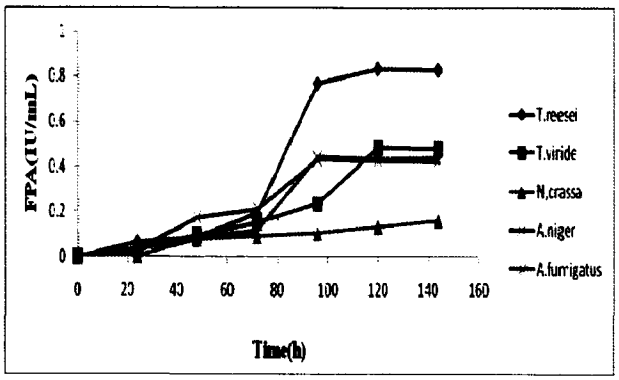


Fig 4.3.9(a)

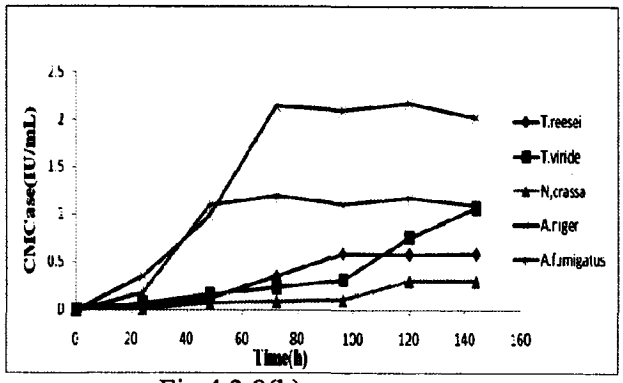


Fig 4.3.9(b)

Fig 4.3.9.Comparative FPA and CMCase achieved by various fungal strains under trehalose based production medium at 30^{0}C and pH 5.0

4.3.10. Effect of sorbitol on cellulase production under submerged cultivation

Individual sets of batch experiments have been performed to study the effect of sugar alcohol such as sorbitol (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.2 and Fig 4.3.10(a), 4.3.10(b) that various fungal strains produce significantly reduced cellulase activities under sorbitol based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 0.709, 0.506; 0.312, 0.786; 0.423, 0.795; 0.278, 1.18; 0.239, 0.911 at 120, 96; 120, 144; 144, 120; 96, 120; 96, 120 h respectively. Higher FPA was observed by *T.reesei* followed *N.crassa* and *T.viride* strains under sorbitol based fermentation medium. A steep increment in FPA activities were observed after 48h by *T.reesei*, while smooth increments were observed after 96 and 120h by *T. viride* and *N.crassa* strain respectively. On the other hand higher CMCases were achieved by *Aspergillus strains* followed by *T.viride and N.crassa* strains under sorbitol based fermentation medium as viewed from Fig 4.3.10(b).

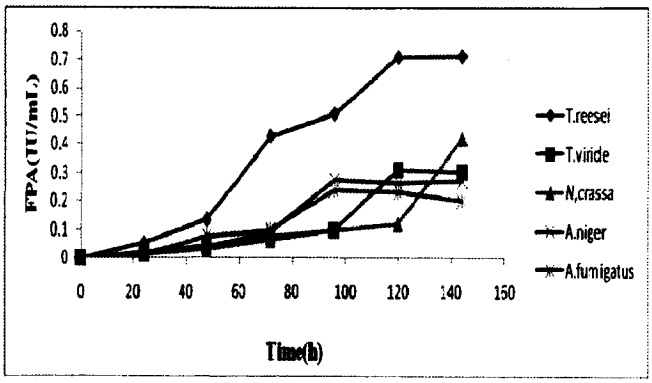


Fig 4.3.10(a)

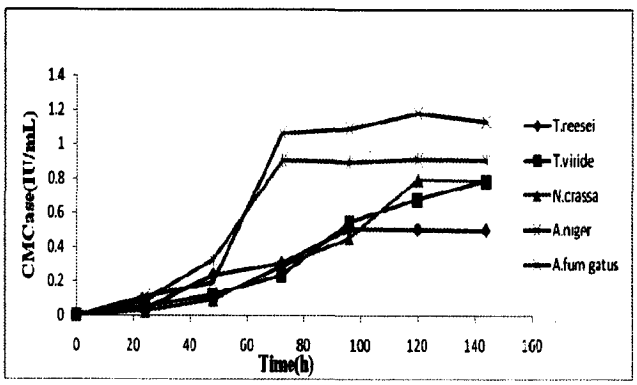


Fig 4.3.10(b)

Fig 4.3.10.Comparative FPA and CMCase achieved by various fungal strains under sorbitol based production medium at 30^{0}C and pH 5.0

Table 4.3.2. Effects of pure sugars as carbon source on the cellulase production (IU/mL) by various fungal strains under submerged fermentation at 30^0C and pH 5.

Sugars	*Trichoderma reesei*			*Trichoderma viridae*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
	Time (h)	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
Xylose	24	0.245	0.166	0.117	0.215	0.060	0.196	0.031	0.160	0.047	0.168
	48	0.361	0.789	0.311	0.564	0.078	0.276	0.043	0.443	0.098	0.587
	72	0.498	1.01	0.452	0.955	0.231	0.549	0.128	1.21	0.156	1.29
	96	0.697	1.15	0.780	1.21	0.437	1.27	0.239	1.11	0.336	1.24
	120	1.53	1.09	0.767	1.54	0.636	1.21	0.226	1.19	0.341	1.18
	144	1.49	1.13	0.777	1.48	0.639	1.30	0.221	1.03	0.321	1.26
Arabinose	24	0.051	0.033	0.028	0.058	0.015	0.027	0.013	0.064	0.015	0.090
	48	0.098	0.111	0.088	0.091	0.054	0.179	0.043	0.376	0.048	0.543
	72	0.154	0.125	0.165	0.131	0.097	0.376	0.091	1.02	0.112	0.896
	96	0.453	0.490	0.201	0.243	0.112	0.540	0.203	0.993	0.228	0.875
	120	0.693	0.476	0.402	0.453	0.121	0.755	0.197	0.977	0.231	0.891
	144	0.681	0.487	0.397	0.886	0.351	0.748	0.189	0.928	0.221	0.888
Trehalose	24	0.064	0.042	0.036	0.071	0.006	0.013	0.028	0.346	0.030	0.174
	48	0.087	0.116	0.097	0.157	0.076	0.071	0.173	0.982	0.087	1.09
	72	0.189	0.343	0.151	0.233	0.089	0.089	0.212	2.15	0.112	1.19
	96	0.767	0.589	0.235	0.313	0.103	0.106	0.434	2.10	0.439	1.10
	120	0.831	0.581	0.482	0.765	0.131	0.302	0.430	2.18	0.431	1.17
	144	0.826	0.585	0.477	1.07	0.158	0.298	0.423	2.03	0.436	1.09
Sorbitol	24	0.053	0.035	0.021	0.052	0.015	0.025	0.018	0.108	0.015	0.087
	48	0.134	0.233	0.043	0.117	0.034	0.093	0.039	0.187	0.077	0.321
	72	0.431	0.301	0.077	0.233	0.065	0.289	0.091	1.06	0.099	0.906
	96	0.510	0.506	0.098	0.543	0.098	0.456	0.278	1.09	0.239	0.897
	120	0.709	0.501	0.312	0.678	0.121	0.795	0.267	1.18	0.231	0.911
	144	0.713	0.496	0.303	0.786	0.423	0.789	0.273	1.13	0.241	0.903
Mannitol	24	0.040	0.028	0.017	0.042	0.009	0.020	0.005	0.024	0.004	0.088
	48	0.176	0.045	0.033	0.078	0.045	0.080	0.009	0.203	0.032	0.119
	72	0.213	0.189	0.056	0.287	0.068	0.451	0.034	0.401	0.067	0.311
	96	0.432	0.435	0.101	0.443	0.091	0.498	0.102	0.398	0.083	0.301
	120	0.602	0.423	0.267	0.501	0.106	0.530	0.094	0.411	0.054	0.306
	144	0.597	0.431	0.259	0.678	0.274	0.521	0.087	0.391	0.011	0.297

4.3.11. Effect of mannitol as carbon source on cellulase production under submerged cultivation

Separate sets of batch experiments have been performed to study the effect of sugar alcohol such as mannitol (10g/L) as carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.2 and Fig 4.3.11(a), 4.3.11(b) that various fungal strains produce least cellulase activities under mannitol based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 0.602, 0.435; 0.267, 0.678; 0.274, 0.530; 0.102,

0.411; 0.083, 0.311 at 120, 96; 120, 144; 144, 120; 96, 120; 96, 72 h respectively. It has been observed from Fig 4.3.11(a) that *T.reesei* produces higher FPA activity under mannitol based fermentation medium as compared to the other used fungal strains. On the other hand significantly good CMCases were observed by all the used fungal strains except *Aspergillus fumigatus* strain as viewed from Fig 4.3.11(b).When compared, the cellulase activities produced by various fungal strains under sorbitol and mannitol based medium, it can infer that sorbitol based medium was found much suitable for cellulase production compared to mannitol.

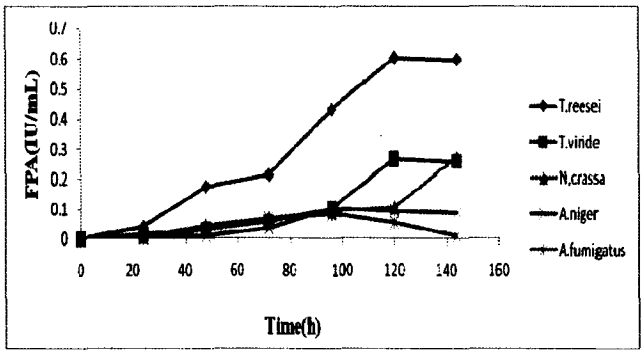


Fig 4.3.11(a)

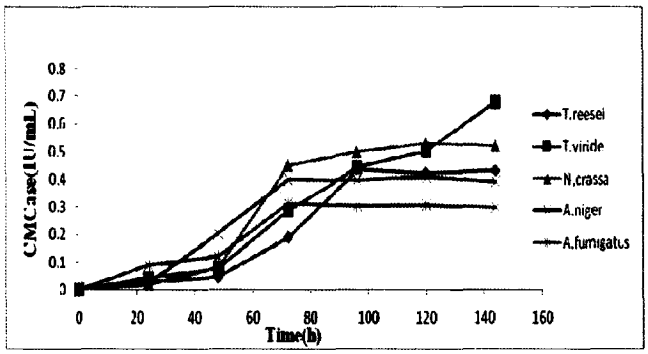


Fig 4.3.11(b)

Fig 4.3.11.Comparative FPA and CMCase achieved by various fungal strains under mannitol based production medium at 30^0C and pH 5.0

4.3.12. Effect of mixed pure sugars as carbon source on cellulase production under submerged cultivation

Individual sets of batch experiments have been performed to study the effect of mixed pure sugars (Total 10g/L) such as cellulose + lactose (5+5), cellulose + xylose (5+5), cellulose + sorbitol(5+5), cellulose+ lactose + xylose (4+3+3), cellulose+ lactose + sorbitol (4+3+3), cellulose +lactose+xylose +sorbitol (2.5+2.5+2.5+2.5) in g/L as mixed carbon source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.3 and Figs 4.3.12(a) (b), 4.3.13(a) (b), 4.3.14(a) (b), 4.3.15(a) (b), 4.3.16(a) (b), 4.3.17(a) (b) that various fungal strains produces significantly better cellulase activities under carbon sources used as mixed condition than taken singly as lactose, sorbitol and xylose sugars. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 2.93, 2.59; 1.43, 3.17; 1.39, 2.75; 0.670, 4.03; 0.833, 3.46 : 2.29, 2.19; 1.26, 2.96; 1.08, 2.45; 0.593, 3.75; 0.711, 3.11 : 1.91, 1.78; 1.01, 2.41; 0.987, 2.19; 0.523, 3.64; 0.634, 3.01 : 2.47, 2.11; 1.51, 3.02; 1.29, 2.41; 0.541, 3.87; 0.765, 3.32: 2.20, 1.96; 1.31, 2.67; 1.13, 2.34; 0.592, 3.70; 0.751, 3.08 : 2.04, 1.61; 1.09, 2.28; 0.968, 1.96; 0.469, 2.78; 0.557, 2.49 under mixed sugars condition such as (L + C), (X + C), (S + C), (L +X + C), (L + S + C), (L + X + S + C) respectively.

Table 4.3.3. Effects of mixed pure sugars as carbon source on the cellulase production (IU/mL) by various fungal strains under submerged fermentation at 30^0C and pH 5.

Mixed Sugar	*Trichoderma reesei*			*Trichoderma viridae*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
	Time (h)	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
C+L	**24**	0.840	0.688	0.383	0.843	0.198	0.398	0.159	0.987	0.200	0.864
	48	1.26	0.984	0.414	1.43	0.204	0.666	0.212	1.89	0.311	1.93
	72	1.48	1.31	0.511	1.56	0.651	0.675	0.311	4.03	0.643	3.43
	96	1.67	2.46	0.943	1.97	0.739	0.945	0.670	3.97	0.833	3.36
	120	2.93	2.59	1.46	2.06	0.911	2.71	0.656	4.09	0.821	3.46
	144	2.87	2.41	1.41	3.17	1.39	2.75	0.674	4.00	0.829	3.21
C + X	**24**	0.651	0.460	0.264	0.612	0.130	0.286	0.124	0.829	0.154	0.723
	48	0.721	0.543	0.312	0.934	0.159	0.564	0.137	1.89	0.176	1.45
	72	0.932	0.988	0.422	1.21	0.232	0.897	0.213	3.75	0.432	3.21
	96	1.42	2.19	0.878	1.76	0.868	1.54	0.593	3.67	0.711	3.13
	120	2.29	2.11	1.26	1.95	0.911	2.45	0.584	3.51	0.700	3.08
	144	2.25	2.16	1.21	2.96	1.08	2.41	0.589	3.65	0.703	3.16
C + S	**24**	0.364	0.333	0.177	0.432	0.095	0.209	0.079	0.689	0.102	0.496
	48	0.393	0.363	0.198	0.543	0.121	0.311	0.084	1.04	0.199	0.941
	72	0.411	0.941	0.211	0.654	0.223	0.398	0.211	3.64	0.204	3.01
	96	0.567	1.78	0.433	0.598	0.198	0.987	0.523	3.56	0.634	2.87
	120	1.91	1.59	1.01	1.23	0.766	2.19	0.501	3.51	0.626	2.81
	144	1.86	1.63	0.989	2.41	0.987	2.09	0.513	3.59	0.630	2.91
C+L+ X	**24**	0.624	0.523	0.354	0.718	0.165	0.291	0.119	0.783	0.175	0.796
	48	0.831	0.789	0.378	0.876	0.178	0.311	0.198	1.17	0.208	1.41
	72	0.989	1.23	0.467	0.911	0.231	0.365	0.209	3.83	0.318	3.32
	96	1.76	2.11	0.987	1.54	0.554	1.54	0.538	3.71	0.765	3.21
	120	2.47	2.01	1.51	1.97	0.876	2.41	0.541	3.87	0.758	3.29
	144	2.41	1.96	1.47	3.02	1.29	2.32	0.533	3.65	0.751	3.35
C+L+ S	**24**	0.519	0.471	0.276	0.609	0.136	0.354	0.136	0.929	0.152	0.605
	48	0.734	0.543	0.311	0.777	0.198	0.411	0.145	1.23	0.187	1.04
	72	0.967	0.945	0.454	0.809	0.456	0.487	0.186	3.70	0.343	3.08
	96	1.17	1.96	0.678	0.987	0.756	0.912	0.592	3.65	0.751	2.97
	120	2.20	1.89	1.31	1.45	1.01	2.34	0.573	3.61	0.734	2.83
	144	2.11	1.81	1.23	2.67	1.13	2.25	0.581	3.67	0.741	2.92
C+L+X +S	**24**	0.471	0.384	0.237	0.509	0.118	0.243	0.109	0.669	0.110	0.489
	48	0.498	0.411	0.332	0.678	0.176	0.299	0.212	1.73	0.132	0.898
	72	0.543	0.675	0.438	0.897	0.197	0.432	0.333	2.78	0.390	2.49
	96	1.23	1.61	0.932	1.54	0.346	0.985	0.469	2.63	0.557	2.38
	120	2.04	1.53	1.09	1.67	0.676	1.96	0.451	2.54	0.534	2.25
	144	1.97	1.57	1.03	2.28	0.968	1.89	0.462	2.69	0.551	2.41

C+L: cellulose + lactose; C+X: cellulose + xylose; C+S: cellulose + sorbitol; C+L+X: cellulose + lactose+ xylose; C+L+S: cellulose + lactose+ sorbitol; C+L+X+S: cellulose + lactose+ xylose+sorbitol.

Whereas maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 2.31, 1.63; 1.26, 2.55; 1.06, 2.13;

0.433, 1.96; 0.556, 1.98 : 1.53, 1.15; 0.780, 1.54; 0.639, 1.30; 0.239, 1.21; 0.341, 1.29 : 0.709, 0.506; 0.312, 0.786; 0.423, 0.795; 0.278, 1.18; 0.239, 0.911 under single sugars containing fermentation medium such as lactose, xylose and sorbitol respectively. It has been observed from Table 4.3.3 and Figs 4.3.12(a) (b), 4.3.13(a) (b), 4.3.14(a) (b), 4.3.15(a) (b) that cellulases activity achieved by used fungal strains under (lactose + cellulose), (lactose + xylose + cellulose) ; (xylose + cellulose) ; (sorbitol + cellulose) were somewhat higher than lactose, xylose and sorbitol based medium taken singly and separately, which suggests that under mixed sugars condition microbes are able to take both types of sugars required for growth as well as cellulase induction capability rather than sugars taken singly in fermentation medium having only growth inducing with reduced induction capability.

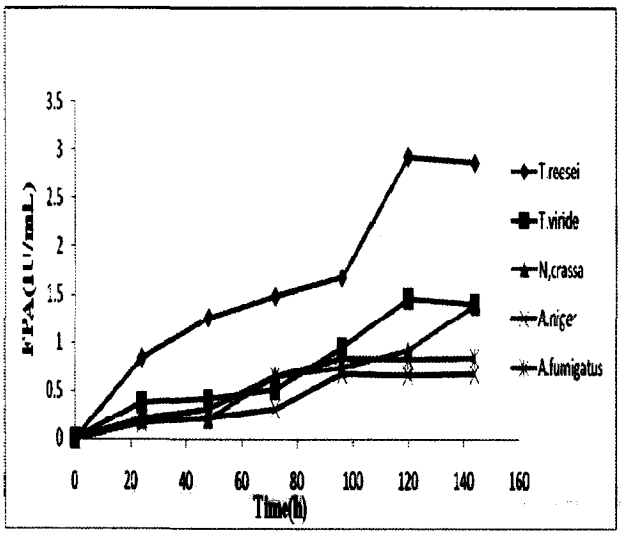


Fig 4.3.12(a)

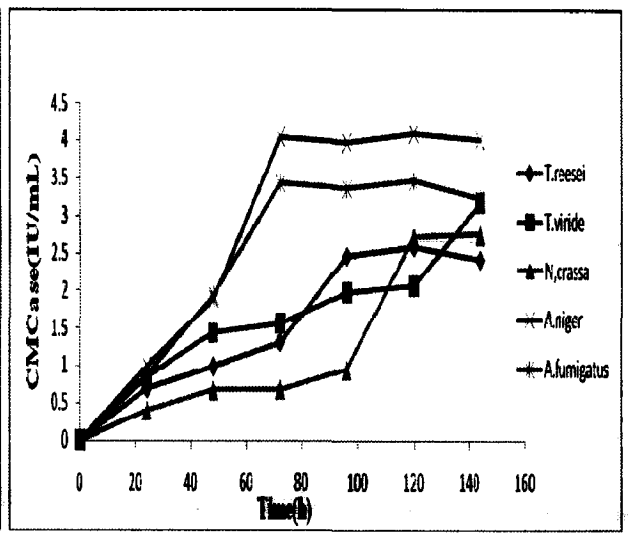


Fig 4.3.12(b)

Fig 4.3.12.Comparative FPA and CMCase achieved by various fungal strains under cellulose + lactose based production medium at 30^{0}C and pH 5.0

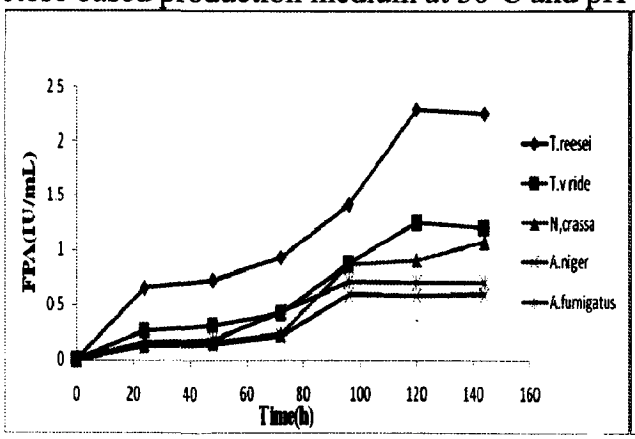


Fig 4.3.13(a)

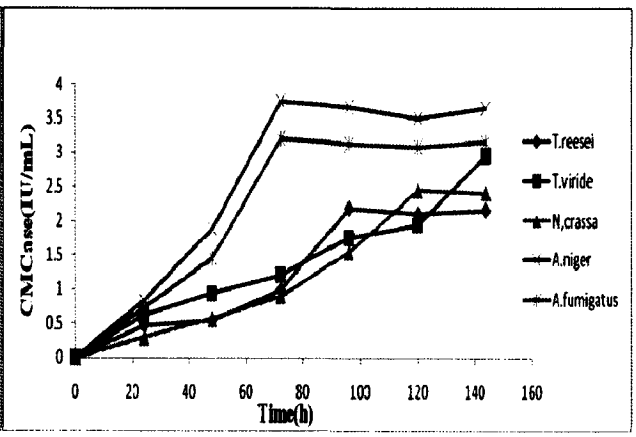


Fig 4.3.13(b)

Fig 4.3.13.Comparative FPA and CMCase achieved by various fungal strains under cellulose + xylose based production medium at 30^{0}C and pH 5.0

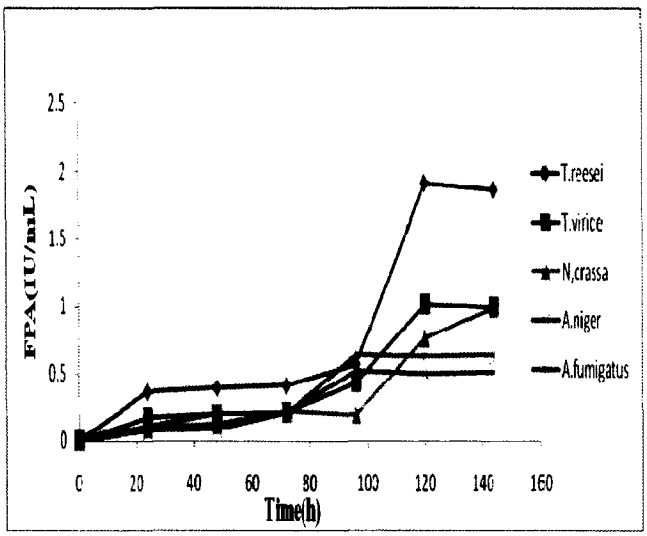


Fig 4.3.14(a)

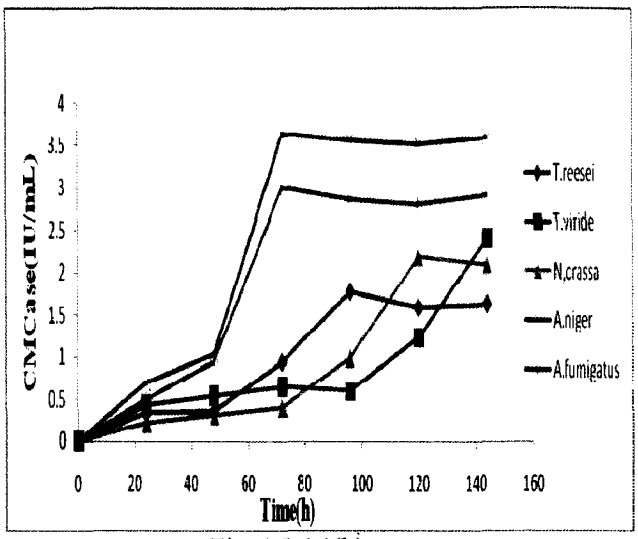


Fig 4.3.14(b)

Fig 4.3.14.Comparative FPA and CMCase achieved by various fungal strains under cellulose + sorbitol based production medium at 30^0C and pH 5.0

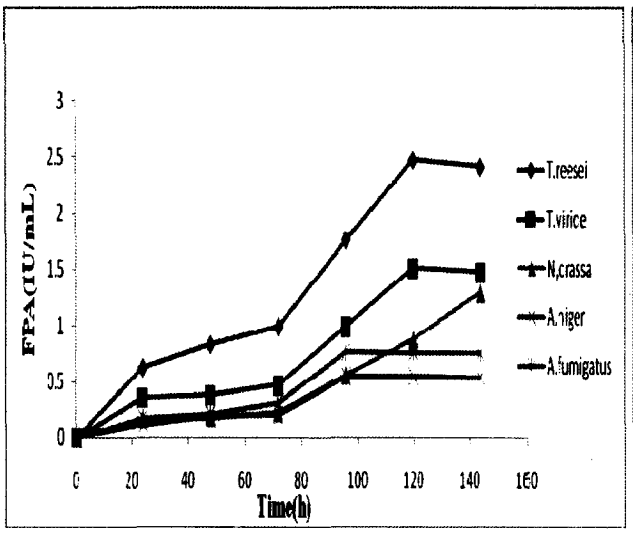


Fig 4.3.15(a)

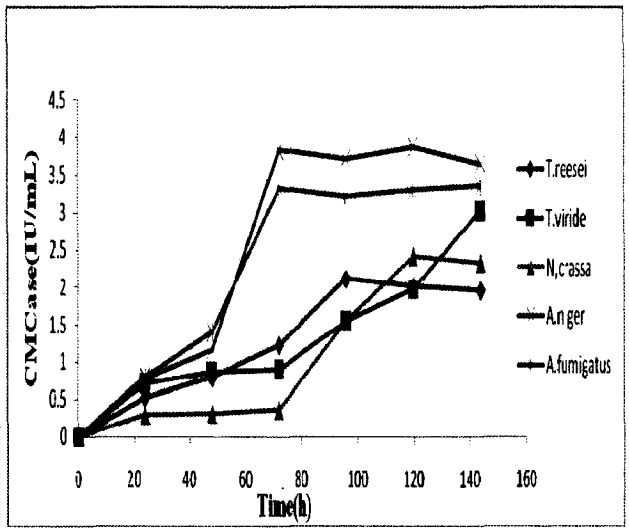


Fig 4.3.15(b)

Fig 4.3.15.Comparative FPA and CMCase achieved by various fungal strains under cellulose + lactose + xylose based production medium at 30^0C and pH 5.0

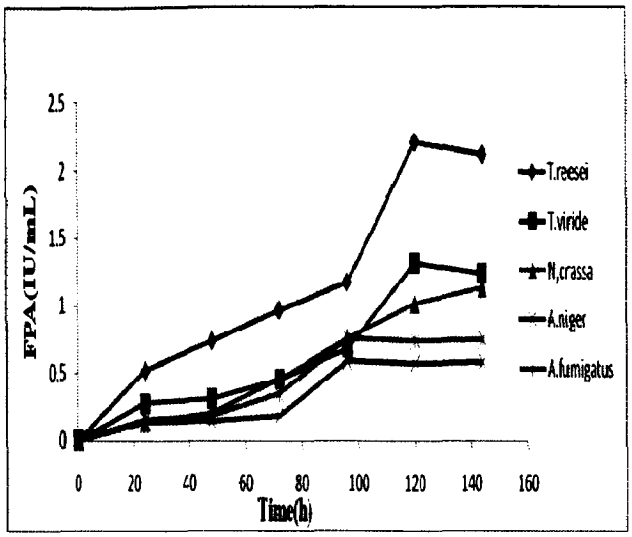


Fig 4.3.16(a)

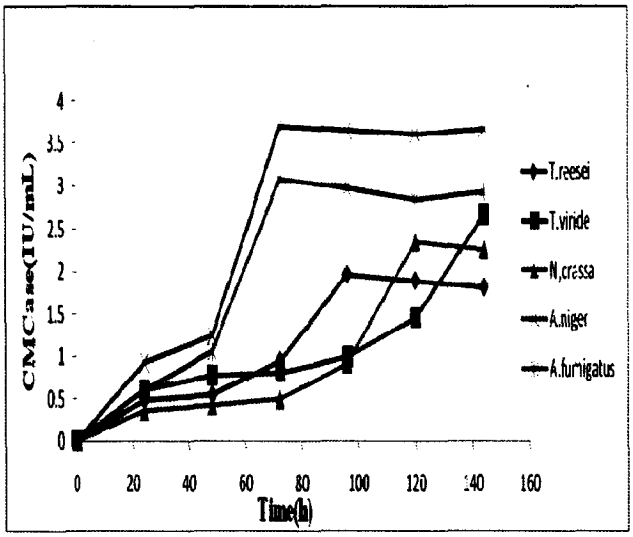


Fig 4.3.16(b)

Fig 4.3.16.Comparative FPA and CMCase achieved by various fungal strains under cellulose + lactose + sorbitol based production medium at 30^0C and pH 5.0

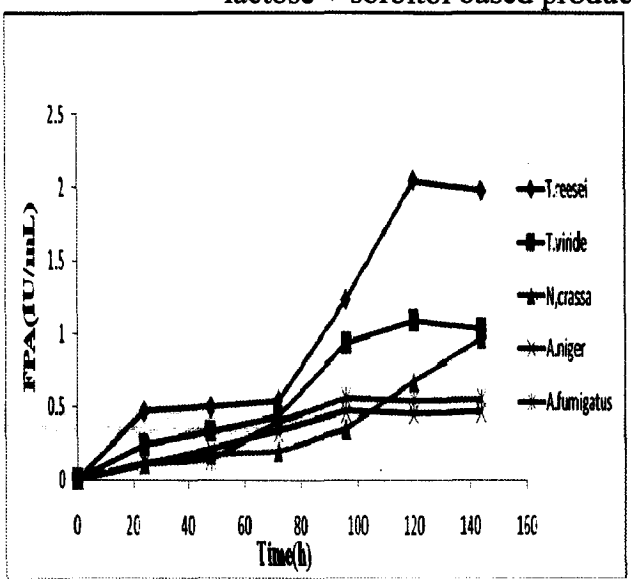


Fig 4.3.17(a)

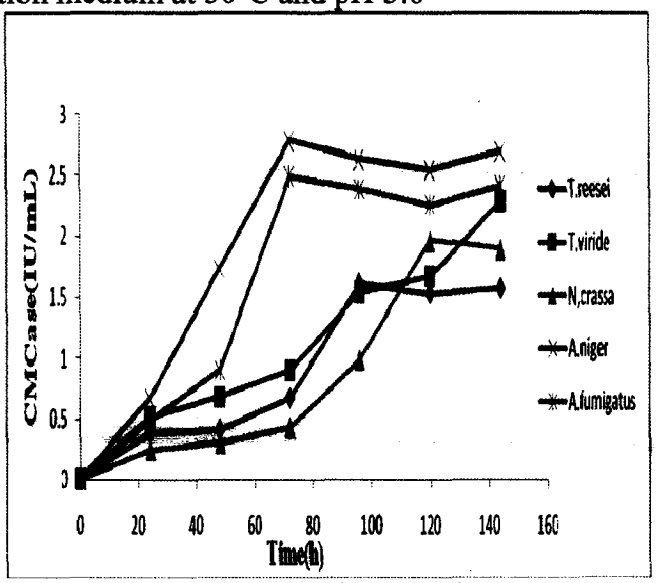


Fig 4.3.17(b)

Fig 4.3.17.Comparative FPA and CMCase achieved by various fungal strains under cellulose + lactose + sorbitol + xylose based production medium at 30^0C and pH 5.0

4.3.13. Effects of peptone type of nitrogen sources on cellulase production under submerged cultivation

Individual sets of batch experiments have been performed to study the effect of peptone type of nitrogen sources such as proteose peptone, soya peptone and meat peptone (1g/L) as nitrogen source on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.4 and Figs 4.3.18, 4.3.19, 4.3.20, 4.3.21, 4.3.22 that various fungal strains produce significantly better cellulase activities under peptone type of nitrogen sources based fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase

attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 3.29, 2.96; 1.63, 4.18; 1.59, 3.52; 0.801, 5.86; 1.12, 5.03 at 120, 96; 120, 144; 144, 120; 96, 72; 96, 72 h under proteose peptone and meat peptone based fermentation medium respectively as observed from Table 4.3.4. Proteose peptone was found much suitable nitrogen source for almost each used fungal strains compared to other peptone based nitrogen sources. When compared the cellulase activities produced by various fungal strains, it was found that higher cellulase activity achieved by *T.reesei*, *T.viridae* and *N.crassa* under proteose peptone based fermentation medium followed by meat peptone, whereas lower activity has been achieved by *Trichoderma* strains under soya peptone based medium as observed from Figs 4.3.18, 4.3.19, 4.3.20. In contrast higher cellulase activitiy is accomplished by *A. niger* strain under proteose and meat peptone nitrogen source based fermentation medium, whereas *A. fumigatus* showed higher activities under meat peptone based fermentation medium as observed from Figs 4.3.21, 4.3.22.

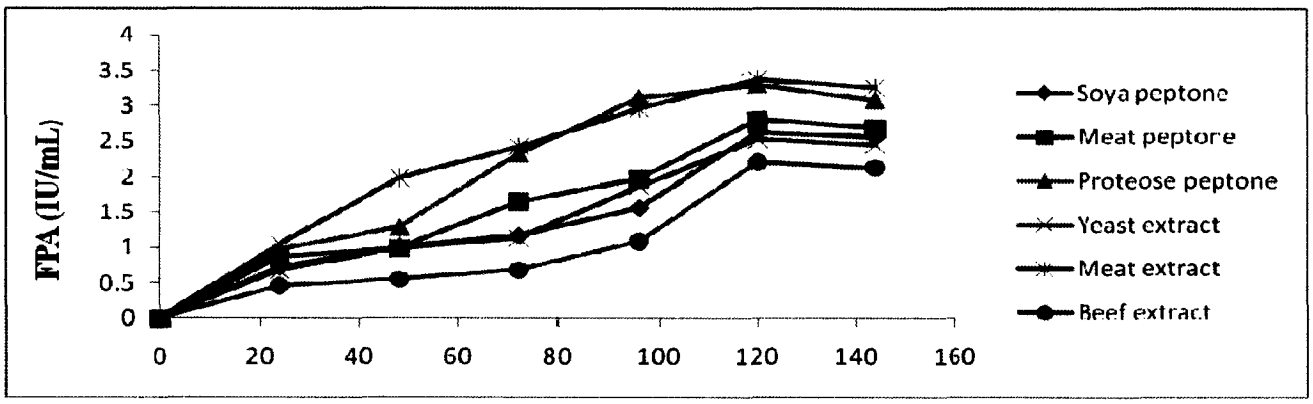


Fig 4.3.18.Comparative FPA achieved by *T.reesei* under various nitrogen sources based production medium at 30^0C and pH 5.0

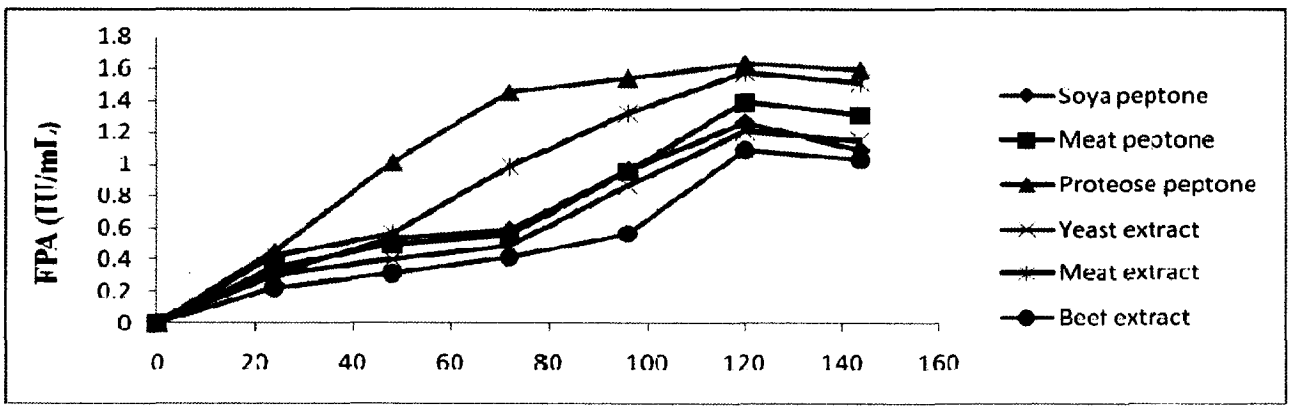


Fig 4.3.19.Comparative FPA achieved by *T.viride* under various nitrogen sources based production medium at 30^0C and pH 5.0.

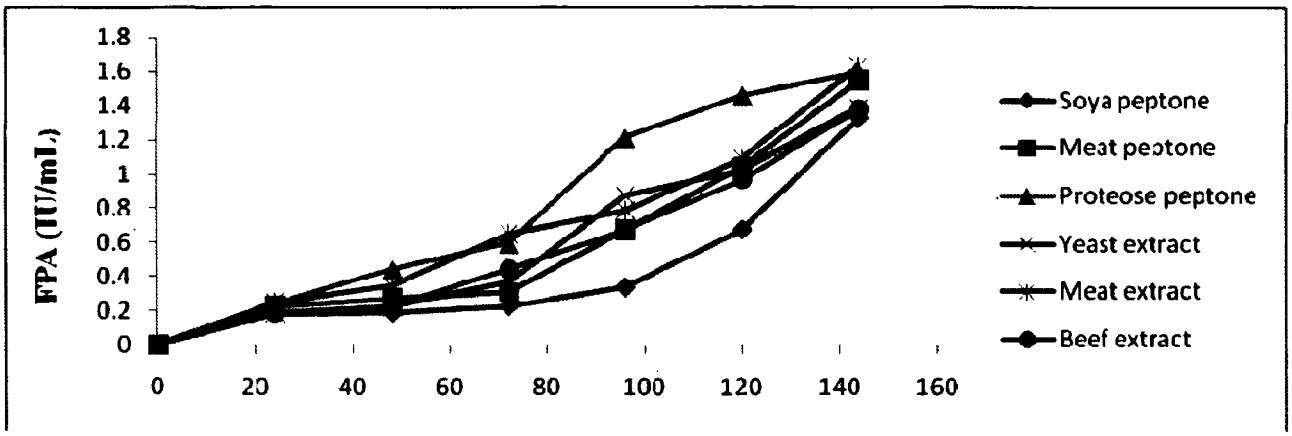


Fig 4.3.20.Comparative FPA achieved by *N.crassa* under various nitrogen sources based production medium at 30^{0}C and pH 5.0.

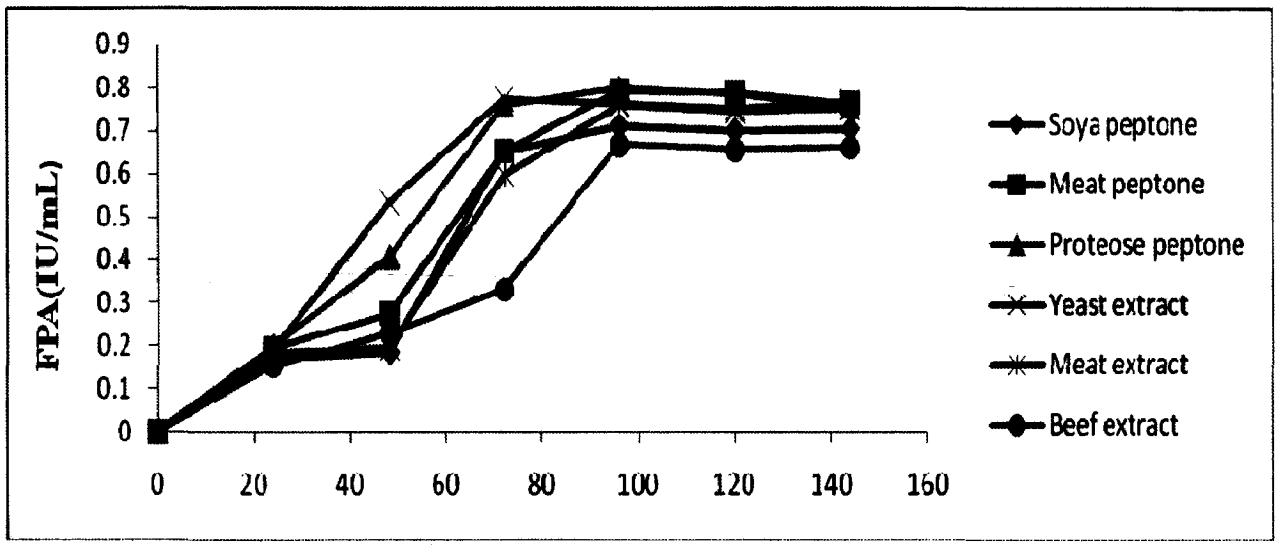


Fig 4.3.21.Comparative FPA achieved by *A.niger* under various nitrogen sources based production medium at 30^{0}C and pH 5.0.

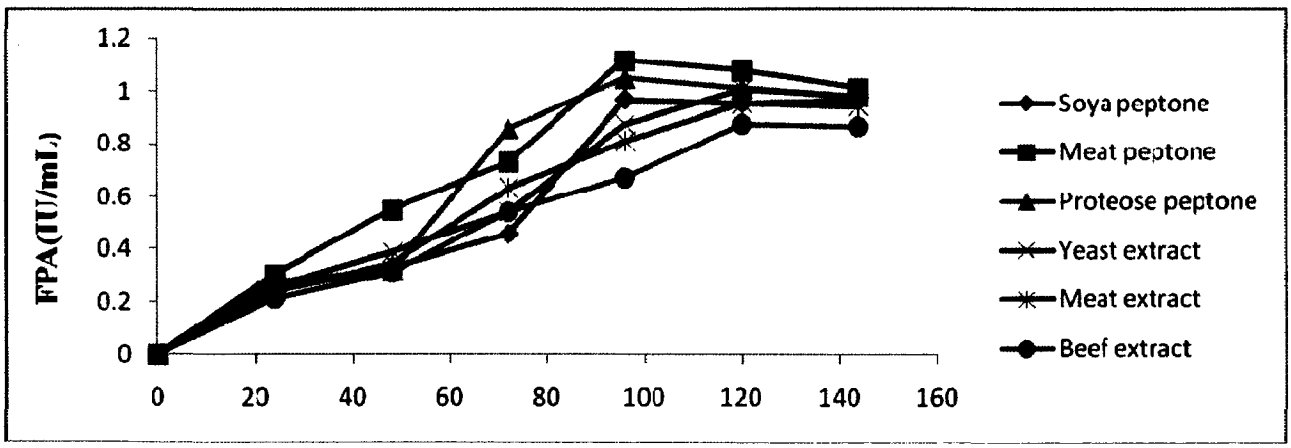


Fig 4.3.22.Comparative FPA achieved by *A. fumigatus* under various nitrogen sources based production medium at 30^{0}C and pH 5.0

Table 4.3.4. Effects of nitrogen sources on cellulase production (IU/mL) by various fungal strains under submerged fermentation at 30^0C and pH 5.

N_2 sources	*Trichoderma reesei*		*Trichoderma viridae*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
	Time (h)	**FPA**	**CMCase**	**FPA**	**CMCase**	**FPA**	**CMCase**	**FPA**	**CMCase**	**FPA**	**CMCase**
Soya peptone	**24**	0.723	0.653	0.311	0.793	0.186	0.439	0.169	1.38	0.244	1.01
	48	1.01	0.876	0.534	0.832	0.193	0.678	0.187	2.34	0.333	1.96
	72	1.16	1.23	0.589	0.998	0.233	0.956	0.654	4.93	0.458	4.02
	96	1.56	2.42	0.964	1.87	0.342	1.09	0.712	4.87	0.967	3.95
	120	2.63	2.35	1.26	2.21	0.678	2.91	0.701	4.81	0.953	3.68
	144	2.56	2.39	1.09	3.43	1.33	2.83	0.705	4.90	0.961	3.81
Meat peptone	**24**	0.871	0.781	0.347	0.908	0.229	0.559	0.195	1.32	0.304	1.39
	48	0.987	0.985	0.498	1.11	0.278	0.711	0.276	3.04	0.551	2.98
	72	1.65	1.32	0.554	1.89	0.309	0.987	0.653	5.71	0.732	5.03
	96	1.98	2.65	0.956	2.34	0.675	1.77	0.793	5.54	1.12	4.91
	120	2.81	2.57	1.39	2.66	1.04	3.39	0.789	5.65	1.08	4.67
	144	2.70	2.61	1.31	3.67	1.55	3.44	0.765	5.61	1.01	4.85
Proteose peptone	**24**	0.963	0.873	0.451	1.08	0.244	0.574	0.201	1.66	0.274	1.37
	48	1.28	1.23	1.01	1.63	0.441	0.786	0.408	2.97	0.321	2.13
	72	2.32	2.49	1.45	2.51	0.596	1.23	0.759	5.86	0.859	4.97
	96	3.11	2.96	1.54	3.03	1.21	2.68	0.801	5.69	1.05	4.81
	120	3.29	2.81	1.63	3.67	1.46	3.52	0.788	5.80	1.01	4.90
	144	3.08	2.90	1.59	4.18	1.59	3.49	0.757	5.63	0.978	4.34
Yeast extract	**24**	0.687	0.565	0.302	0.729	0.194	0.491	0.192	1.51	0.262	1.31
	48	0.987	0.664	0.401	0.883	0.232	0.654	0.534	3.88	0.389	1.87
	72	1.13	1.09	0.488	1.67	0.367	1.23	0.775	5.32	0.543	2.67
	96	1.87	2.12	0.865	1.98	0.876	1.93	0.763	5.21	0.876	4.85
	120	2.54	2.03	1.21	2.32	1.02	3.09	0.751	5.09	1.01	4.74
	144	2.45	2.09	1.15	3.17	1.39	3.01	0.769	5.28	0.978	4.61
Meat extract	**24**	1.04	0.887	0.426	1.07	0.253	0.634	0.185	1.55	0.244	1.14
	48	1.98	1.07	0.566	1.91	0.345	0.897	0.196	3.08	0.345	1.87
	72	2.43	1.88	0.984	2.45	0.654	1.64	0.597	5.55	0.632	2.43
	96	2.96	3.01	1.32	3.42	0.789	1.92	0.759	5.43	0.811	4.12
	120	3.38	2.96	1.58	3.78	1.09	3.63	0.743	5.32	0.954	4.03
	144	3.26	2.91	1.51	4.07	1.63	3.54	0.751	5.51	0.941	4.09
Beef extract	**24**	0.464	0.374	0.223	0.575	0.193	0.454	0.157	1.21	0.212	0.792
	48	0.554	0.498	0.312	0.786	0.231	0.678	0.231	2.76	0.311	0.981
	72	0.686	0.878	0.412	0.987	0.444	0.897	0.332	4.35	0.543	1.94
	96	1.09	1.87	0.567	1.27	0.675	1.87	0.669	4.21	0.671	3.87
	120	2.21	1.76	1.09	1.56	0.967	3.03	0.658	4.32	0.875	3.78
	144	2.13	1.69	1.03	2.98	1.38	2.96	0.663	4.29	0.863	3.63

4.3.14. Effects of meat extract, beef extract and yeast extract as nitrogen source on cellulase production under submerged cultivation

Individual sets of batch experiments have been performed to study the effect of other nitrogen sources such as meat extract, beef extract and yeast extract (1g/L) on cellulase production by various used fungal strains under submerged cultivation. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 3.38, 3.01; 1.58, 4.07; 1.63, 3.63; 0.775, 5.32; 1.01, 4.85 at 120, 96; 120, 144; 144, 120; 72, 72; 120, 96 h under meat extract and yeast extract based fermentation medium respectively as observed from Table 4.3.4 and Figs 4.3.18, 4.3.19, 4.3.20, 4.3.21, 4.3.22. Meat and yeast extracts were found much suitable nitrogen sources for cellulase production by using fungal strains compared to beef extract. Higher cellulase activities were achieved by *T.reesei* and *T.viridae* under meat extract based fermentation medium, whereas lower activity has been achieved by *Trichoderma* strains under beef extract containing medium as observed from Figs 4.3.18, 4.3.19. In contrast higher cellulase activities are accomplished by *A. niger* and *A. fumigatus* strain under yeast extract based fermentation medium as observed from Figs 4.3.21, 4.3.22. On the other hand beef extract was found quite suitable nitrogen source for cellulase production by *N.crassa* compared to other used fungal strains. Higher cellulase activity was achieved by *N.crassa* under meat extract based fermentation medium followed by yeast and beef extact as observed from Fig 4.3.20.

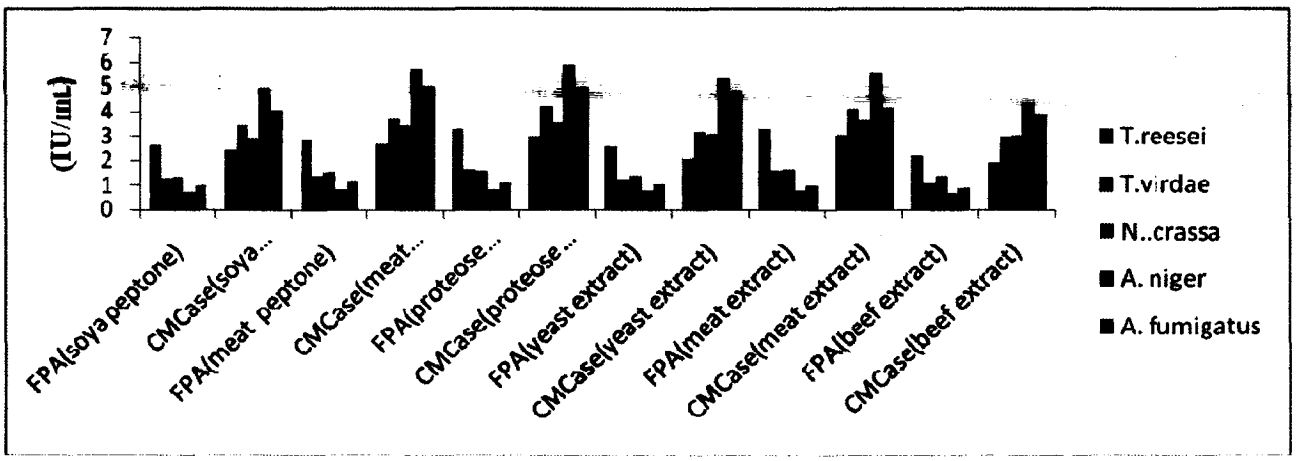


Fig 4.3.23. Comparative FPA and CMCase activities achieved by various fungal strains under different nitrogen source based fermentation medium at 30^0C and pH 5.0

When compared the overall performance of each fungal strains on different nitrogen sources, it was observed that meat extract and proteose peptone were found much suitable nitrogen sources for cellulase production by *Trichoderma* strains, whereas meat extract, proteose peptone and meat peptone were found quite favorable nitrogen sources for cellulase production by *N.crassa* fungal

strain. In contrast yeast extract, proteose peptone and meat peptone were found much constructive nitrogen sources for cellulase production by *Aspergillus* strains as viewed from Fig 4.3.23. Beef extract was rather a poor nitrogen source for any of the fungal growth and metabolic system, this may be due to the components present beef extract are either not much nutritious (required and useful constituent are in lesser amount whereas unuseful components are in higher amount) or not easily utilizable by fungal system.

4.3.15. Effect of Tween series and Triton X-100 surfactants on cellulase production under submerged cultivation

Separate sets of batch experiments have been performed to study the effect of surfactants such as Tween series and Triton X-100 (1g/L) on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.5 and Figs 4.3.24, 4.3.25, 4.3.26, 4.3.27, 4.3.28.that various fungal strains produce significantly better cellulase activities under Tween series based fermentation medium compared to Triton X-100. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 3.29, 2.96; 1.63, 4.18; 1.59, 3.58; 0.801, 5.86; 1.12, 5.03 at 120, 96; 120, 144; 144, 120; 96, 72; 96, 72 h under Tween-80 based fermentation medium respectively as observed from Table 4.3.5. Tween-80 was found quite suitable surfactant for almost each used fungal strains compared to Tween 60, Tween-20 and Triton X-100. When compared the cellulase activities produced by various used fungal strains under different surfactants based fermentation medium, it was found that higher cellulase activity achieved by *T.reesei* under Tween-80 and Tween-20 based fermentation medium, whereas *T.viridae* and *N.crassa* showed higher cellulase activities under Tween-60 and Tween-80 based fermentation as observed from Figs 4.3.24, 4.3.25, 4.3.26. On the other hand higher cellulase activities are accomplished by *A. niger* strain was also under Tween-60 and Tween-80 based fermentation medium, whereas *A. fumigatus* showed higher activities under Tween-60, Tween-80 and Triton X-100 based fermentation medium as observed from Figs 4.3.27, 4.3.28. It can be concluded that in between Tween series surfactants Tween-60 and Tween-80 were found much suitable for cellulase production by used fungal strains, whereas Tween-20 was found somewhat unfavorable for cellulase production by almost each fungal strain except *T.reesei*. On the other hand Triton X-100 was found fairly unsuitable surfactant for cellulase production by most of the fungal strains may be due to the presence of aromatic ring in the surfactant which inhibits the fungal growth.

Table 4.3.5. Effects of surfactants on cellulase production by various fungal strains under submerged fermentation at 30^{0}C and pH 5.

Surfactan	*Trichoderma reesei*		*Trichoderma viridae*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
	Time (h)	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
Tween-20	24	0.881	0.793	0.365	0.943	0.223	0.482	0.151	1.19	0.132	0.943
	48	1.09	0.896	0.564	1.11	0.298	0.522	0.191	2.23	0.165	1.23
	72	1.87	1.43	0.598	1.65	0.543	0.943	0.221	4.78	0.321	3.39
	96	2.13	2.66	0.897	1.98	0.844	1.43	0.711	4.85	0.847	3.31
	120	3.10	2.57	1.41	2.04	1.18	1.87	0.701	4.67	0.832	3.46
	144	3.01	2.61	1.33	3.80	1.09	3.02	0.681	4.71	0.811	3.23
Tween-60	24	0.867	0.641	0.421	1.02	0.287	0.571	0.191	1.42	0.223	1.19
	48	0.912	0.883	0.621	1.43	0.336	0.765	0.321	2.09	0.543	1.98
	72	0.876	1.21	0.987	1.89	0.412	1.89	0.545	5.43	0.611	4.21
	96	1.32	2.39	1.05	2.54	0.976	3.58	0.789	5.32	0.976	4.11
	120	2.88	2.48	1.58	3.09	1.51	3.43	0.775	5.51	0.967	4.29
	144	2.74	2.31	1.51	4.01	1.45	3.51	0.779	5.39	0.971	4.09
Tween-80	24	0.963	0.873	0.451	1.08	0.244	0.574	0.201	1.66	0.274	1.37
	48	1.28	1.23	1.01	1.63	0.441	0.786	0.408	2.97	0.321	2.13
	72	2.32	2.49	1.45	2.51	0.596	1.23	0.759	5.86	0.859	4.97
	96	3.11	2.96	1.54	3.03	1.21	2.68	0.801	5.69	1.05	4.81
	120	3.29	2.81	1.63	3.67	1.46	3.52	0.788	5.80	1.01	4.90
	144	3.08	2.90	1.59	4.18	1.59	3.49	0.757	5.63	0.978	4.34
Triton X-100	24	0.710	0.619	0.276	0.793	0.211	0.421	0.178	1.02	0.208	1.04
	48	0.803	0.787	0.231	0.954	0.221	0.621	0.321	1.87	0.411	2.11
	72	0.908	1.05	0.432	1.21	0.345	0.878	0.412	4.29	0.621	4.01
	96	1.32	2.31	0.654	1.78	0.605	1.35	0.679	4.08	0.932	3.96
	120	2.60	2.22	1.02	2.09	1.03	1.98	0.630	4.16	0.921	3.89
	144	2.51	2.34	0.997	2.97	0.998	2.76	0.639	4.23	0.927	3.93
Oil of olive	24	0.985	0.963	0.332	0.834	0.251	0.563	0.221	1.59	0.263	1.32
	48	1.47	1.45	0.431	0.936	0.342	0.987	0.333	3.02	0.411	2.03
	72	2.09	1.98	0.675	1.05	0.564	1.11	0.454	5.78	0.867	4.56
	96	2.59	3.14	0.987	2.13	0.897	1.87	0.810	5.47	1.01	4.61
	120	3.37	3.01	1.32	2.85	1.47	2.33	0.789	5.65	0.981	4.43
	144	3.29	2.93	1.27	3.57	1.31	3.49	0.795	5.53	0.967	4.49
Oil of castor	24	0.701	0.564	0.301	0.811	0.201	0.521	0.193	1.27	0.187	0.989
	48	0.723	0.611	0.410	0.988	0.311	0.666	0.222	2.89	0.323	1.23
	72	0.798	0.967	0.559	1.07	0.897	0.897	0.331	5.02	0.444	3.78
	96	1.32	2.12	0.876	1.54	1.01	1.54	0.734	4.95	0.902	3.61
	120	2.56	2.02	1.21	2.05	1.29	1.93	0.710	4.83	0.909	3.71
	144	2.29	2.06	1.10	3.28	1.08	3.21	0.729	4.98	0.897	3.53

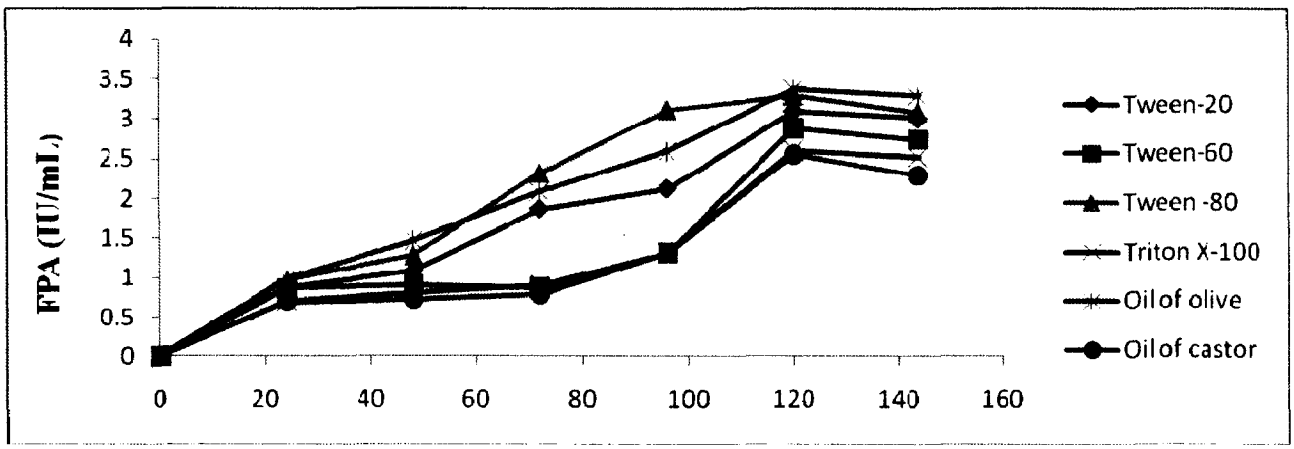


Fig 4.3.24. Comparative FPA achieved by *T.reesei* under various surfactants based production medium at 30^0C and pH 5.0

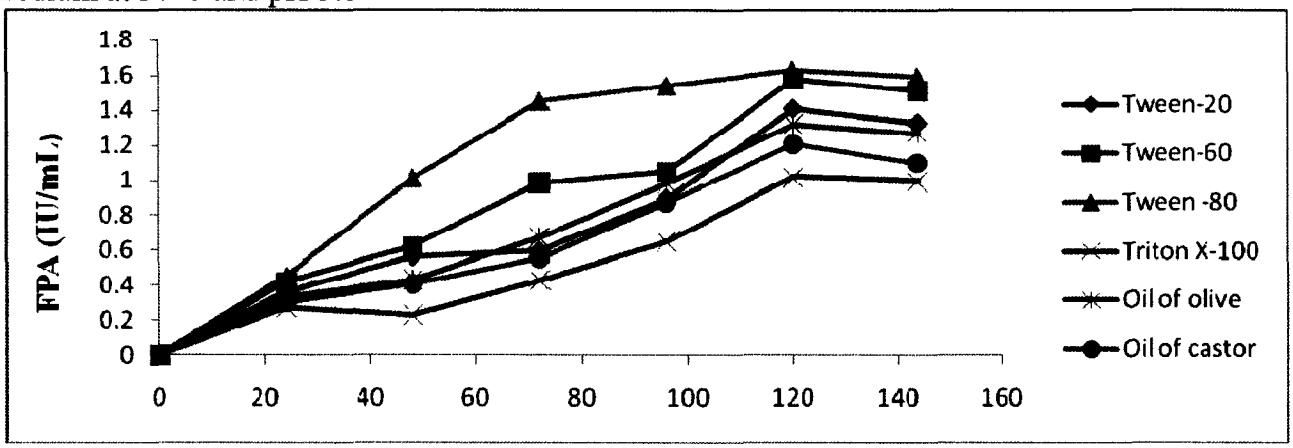


Fig 4.3.25. Comparative FPA achieved by *T.viride* under various surfactants based production medium at 30^0C and pH 5.0

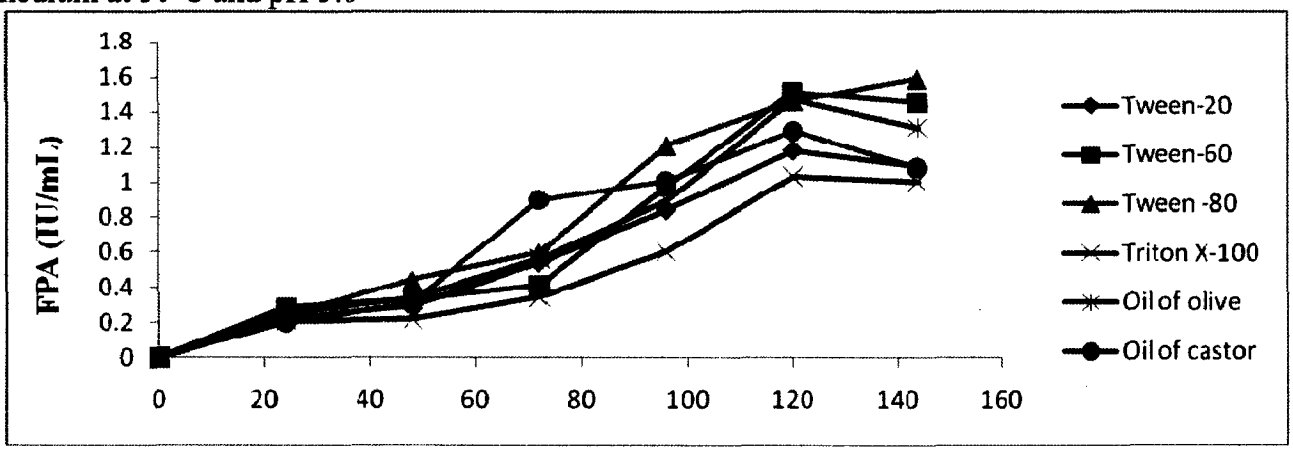


Fig 4.3.26. Comparative FPA achieved by *N.crassa* under various surfactants based production medium at 30^0C and pH 5.0.

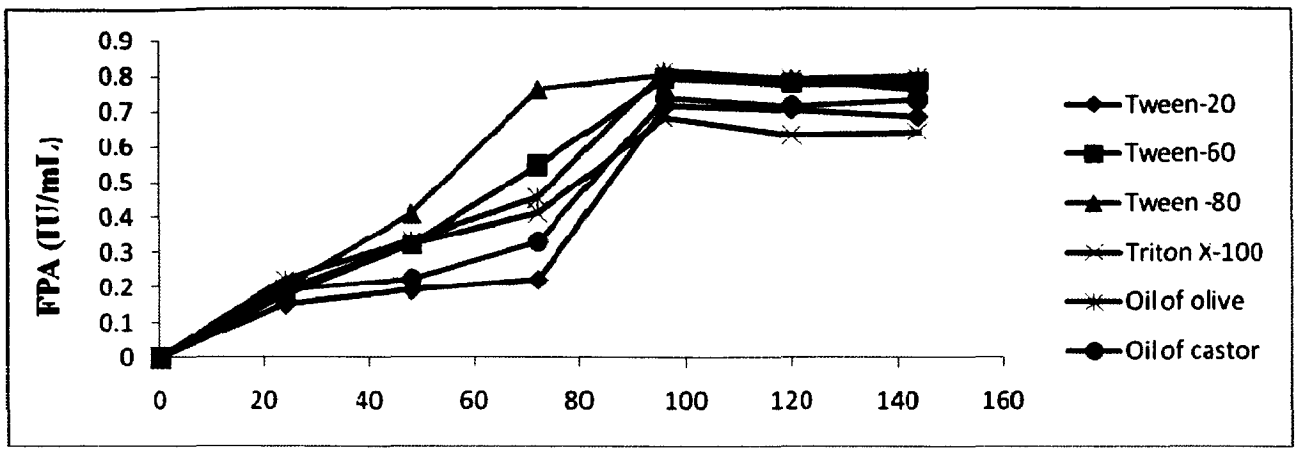


Fig 4.3.27.Comparative FPA achieved by *A.niger* under various surfactants based production medium at 30^0C and pH 5.0

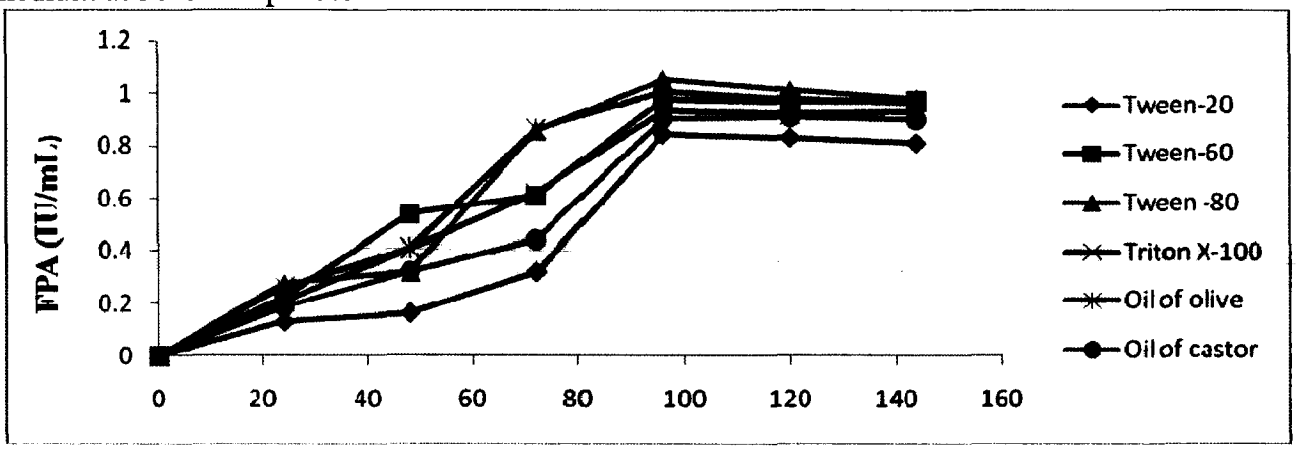


Fig 4.3.28.Comparative FPA achieved by *A. fumigatus* under various surfactants based production medium at 30^0C and pH 5.0.

4.3.16. Effect of olive and castor oil as surfactants on cellulase production

Individual sets of batch experiments have been performed to study the effect of different oil based surfactants such as oil of olive and castor oil (1g/L) on cellulase production by various fungal strains under submerged cultivation. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 3.37, 3.14; 1.32, 3.57; 1.47, 3.49; 0.810, 5.78; 1.01, 4.61 at 120, 96; 120, 144; 120, 144; 96, 72; 96, 96 h under oil of olive based fermentation medium respectively as observed from Table 4.3.5 and Figs 4.3.24, 4.3.25, 4.3.26, 4.3.27, 4.3.28. Oil of olive was found much suitable surfactant for cellulase production by almost each fungal strains compared to oil of castor. Since oil of olive is metabolizable and the resulting product from their hydrolysis is a mixture of fatty acids, which significantly affects the membrane permeability of fungal system as well as olive oil also serves as other carbon source for fungal growth. Higher cellulase activities achieved by each fungal strains

were under oil of olive based fermentation medium, whereas somewhat lower activities have been achieved by fungal strains under oil of castor based medium. This may be due to presence of certain microbial growth inhibitory components in oil of castor. Although lower activity has been observed with oil of castor but *Aspergillus* strains showed significant activity under this oil based fermentation as observed from Figs 4.3.27, 4.3.28.When compared the overall performance of each fungal strains under different surfactant based medium, It was observed that Tween-80, Tween-20 and oil of olive were found much suitable surfactant for cellulase production by *Trichoderma* strains, whereas Tween-80, Tween-60 were found much favorable surfactants for cellulase production by *T. viride* fungal strain. On the other hand *N.crassa* and *A. niger* showed better cellulase activity under Tween-60, Tween-80 and oil of olive based fermentation. In contrast *A. fumigatus* showed higher activities under Tween-60, Tween-80 and Triton X-100 based fermentation medium as viewed from Fig 4.3.29.

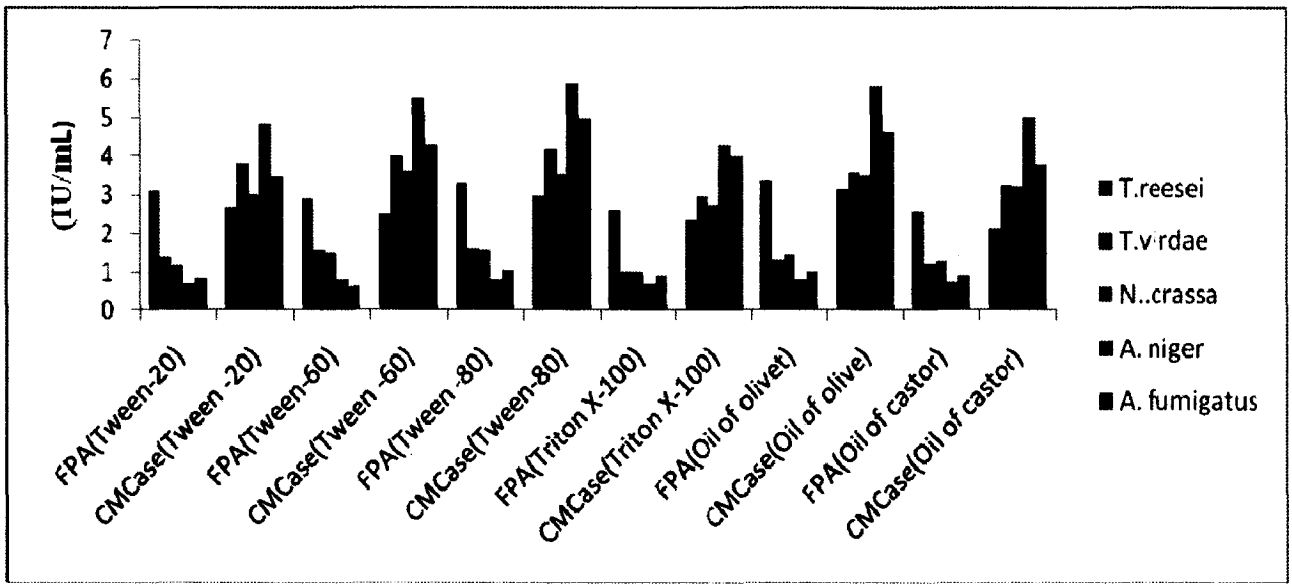


Fig. 4.3.29. Comparative FPA and CMCase activities achieved by various fungal strains under different surfactants based fermentation medium at 30^0C and pH 5.0.

4.3.17. Effect of rpm on cellulase production under cellulose and lactose based submerged fermentation

Individual sets of batch experiments have been performed to study the effect of rpm (120, 180, 250) on cellulase production by various fungal strains under cellulose based fermentation. It was observed from Table 4.3.6. and Figs 4.3.30, 4.3.31, 4.3.32, 4.3.33, 4.3.34 that various fungal strains produce significantly higher cellulase activities under 180 rpm. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger*

and *A.fumigatus* were 3.29, 2.96; 1.63, 4.18; 1.59, 3.58; 0.801, 5.86; 1.12, 5.03 at 120, 96; 120, 144; 144, 120; 96, 72; 96, 72 h respectively at 180 rpm under cellulose based fermentation medium. It has been observed from Figs 4.3.30 and 4.3.31 that *T.reesei* and *T.viride* produce higher FPA activity at 180 rpm, which suggests that agitation at 180 rpm provides much favorable condition such as better mass, heat and nutrient transfer with reduced cell damage state, required for good fungal growth and enzyme production. When compared, the enzyme activities attained by used fungal strains at 120 and 250 rpm, we have observed that at both lower and higher rpm enzyme activities were reduced as compared to 180 rpm. It has been also observed that *T.reesei* produced higher enzyme activities at 250 rpm as compared to 120 rpm this might be due to the fact that at higher mechanical agitation, more number of branched hyphae with larger number of tips per hyphae have been generated, which result to produce higher activity but at this rpm enzyme activities were also reduced may be due to the cell damage therefore overall enzyme activity at this agitation was somewhat lower than 180 rpm. Lesser enzyme activities have also been observed at 120 rpm by *T.viridae*, *N. crassa* and *Aspergillus* strains may be due to the reduced nutrient transfer. At higher agitation (250 rpm) enzyme activities produced by *T.viridae*, *A. fumigatus* and *A.niger* were also improved may be due to mechanical agitation size of pellets were reduced from bigger as well as medium sized pellets which provides favorable condition for cellulase production but at this agitation some of the pellets also damage due to the high shear stress, therefore enzyme activities were somewhat reduced at this agitation compared to 180 rpm as observed from Figs 4.3.31, 4.3.33, 4.3.34. In contrast *N.crassa* showed lesser activities at higher agitation (250 rpm) compared to lower agitation (120 rpm) which might be due to the pulpy nature of their growth morphological appearance. At higher rpm pulpy nature of growth appearance converted into clumpy nature which provides a little bit unfavorable condition for easier nutrient and mass transfer, therefore at higher rpm activities have been reduced compared to 120 rpm as viewed from Fig 4.3.32.

Table 4.3.6. Effects of rpm on the cellulase production by various fungal strains under cellulose based fermentation media at 30^0C and pH 5.

Rotation per minute (rpm)		Time (h)	*Trichoderma reesei*		*Trichoderma viridae*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
			FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
120	Cellulose based	24	0.509	0.493	0.257	0.523	0.169	0.420	0.083	0.615	0.093	0.491
		48	0.704	0.987	0.310	0.981	0.198	0.891	0.097	1.86	0.123	0.580
		72	1.13	1.54	0.431	1.42	0.234	0.995	0.138	2.36	0.168	1.96
		96	1.67	1.76	0.945	1.97	0.679	1.85	0.376	2.31	0.403	1.91
		120	1.82	1.67	1.01	2.21	0.831	2.43	0.383	2.46	0.397	1.73
		144	1.71	1.71	0.978	2.26	1.06	2.49	0.351	2.20	0.410	1.88
180	Cellulose based	24	0.963	0.873	0.451	1.08	0.244	0.574	0.201	1.66	0.274	1.37
		48	1.28	1.23	1.07	1.63	0.441	0.786	0.408	2.97	0.321	2.13
		72	2.32	2.49	1.45	2.51	0.596	1.23	0.759	5.86	0.859	4.97
		96	3.11	2.96	1.54	3.03	1.21	2.68	0.801	5.69	1.05	4.81
		120	3.29	2.81	1.63	3.67	1.46	3.52	0.788	5.80	1.01	4.90
		144	3.08	2.90	1.59	4.18	1.59	3.49	0.757	5.63	0.978	4.34
250	Cellulose based	24	0.734	0.701	0.343	0.777	0.101	0.292	0.148	0.986	0.151	0.728
		48	0.976	0.911	0.561	0.811	0.187	0.323	0.159	1.41	0.187	0.934
		72	1.63	1.14	0.663	0.945	0.234	0.876	0.187	1.97	0.221	1.78
		96	1.98	2.43	0.976	1.73	0.671	1.23	0.332	3.51	0.243	2.71
		120	2.66	2.51	1.32	2.89	0.712	1.95	0.613	3.43	0.596	2.63
		144	2.59	2.39	1.25	3.05	0.869	1.87	0.618	3.49	0.603	2.69

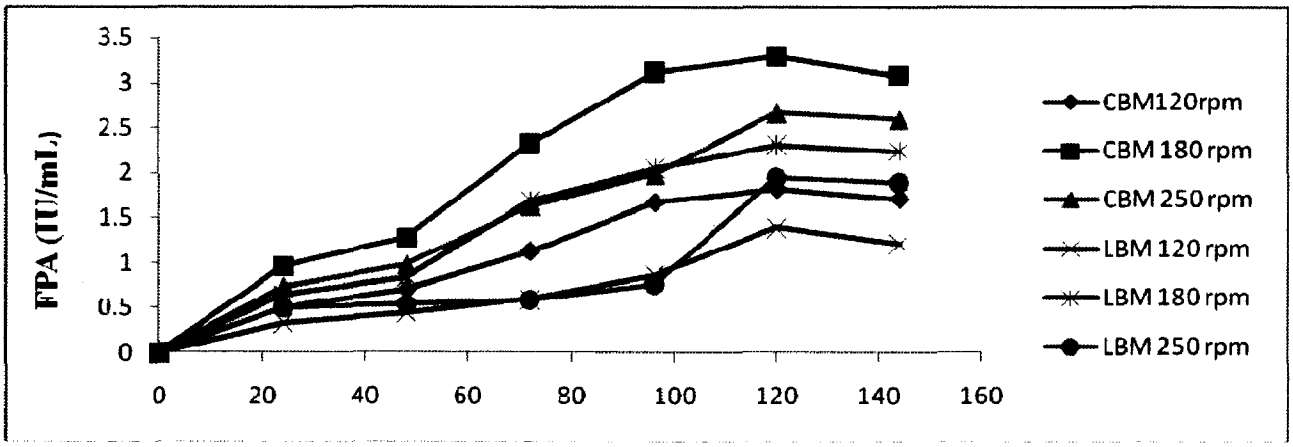


Fig 4.3.30. Comparative FPA achieved by *T.reesei* under cellulose and lactose based production medium at different rpm.(CBM:Cellulose based medium,LBM:Lactose based medium).

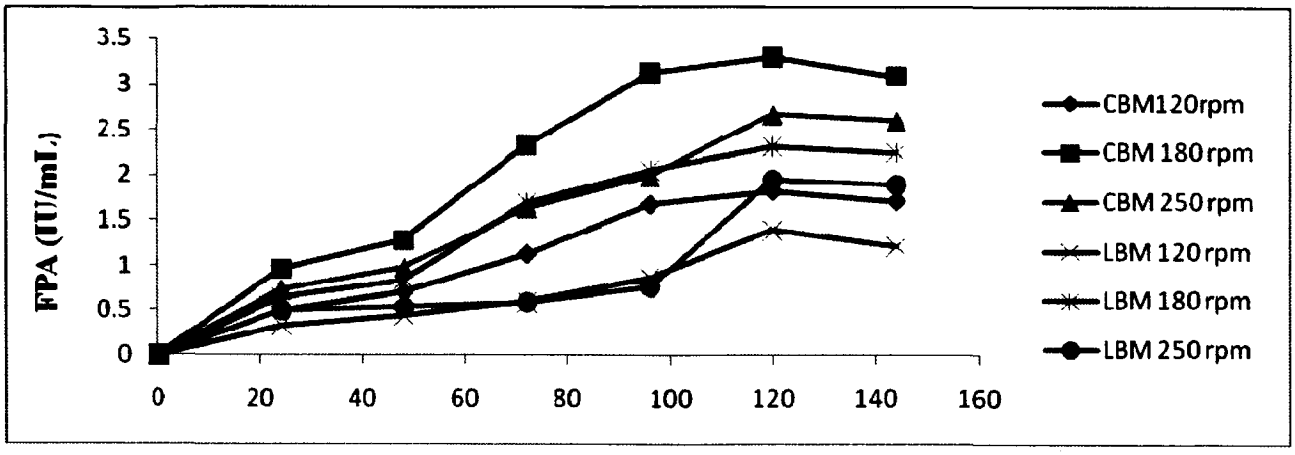


Fig 4.3.31.Comparative FPA achieved by *T.viride* under cellulose and lactose based production medium at different rpm. (CBM:Cellulose based medium,LBM:Lactose based medium).

Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 2.31, 1.63; 1.26, 2.55; 1.06, 2.13; 0.433, 1.96; 0.556, 1.98 at 120, 96; 144, 144; 144, 120; 96, 72; 96, 72 h respectively at 180 rpm under lactose based fermentation medium as observed from Table 4.3.7.

Table 4.3.7.Effects of rpm on the cellulase production by various fungal strains under lactose based fermentation media at 30^{0}C and pH 5.

Rotation per minute (rpm)		Time (h)	*Trichoderma reesei*		*Trichoderma viridae*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
			FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
120	Lactose based	24	0.336	0.249	0.179	0.354	0.119	0.178	0.058	0.249	0.063	0.273
		48	0.451	0.429	0.230	0.433	0.249	0.191	0.097	0.367	0.123	0.883
		72	0.597	1.01	0.431	0.945	0.367	0.298	0.146	1.19	0.153	1.13
		96	0.865	1.13	0.710	1.18	0.678	0.867	0.287	1.03	0.302	1.19
		120	1.39	1.09	0.857	1.36	0.754	1.32	0.293	0.976	0.312	1.03
		144	1.21	1.15	0.843	1.51	0.825	1.27	0.279	1.09	0.307	1.10
180	Lactose based	24	0.646	0.443	0.291	0.596	0.141	0.276	0.097	0.491	0.122	0.465
		48	0.843	0.578	0.368	0.967	0.229	0.349	0.184	1.56	0.231	1.04
		72	1.69	1.43	0.411	1.33	0.341	0.567	0.276	2.05	0.453	1.98
		96	2.05	1.63	0.983	1.89	0.389	1.69	0.433	1.96	0.556	1.83
		120	2.31	1.54	1.20	2.13	0.894	2.13	0.410	2.03	0.547	1.91
		144	2.24	1.59	1.16	2.55	1.06	2.07	0.419	1.99	0.531	1.76
250	Lactose based	24	0.507	0.381	0.229	0.483	0.074	0.132	0.076	0.418	0.081	0.318
		48	0.545	0.411	0.321	0.498	0.098	0.221	0.111	0.532	0.123	0.665
		72	0.591	0.523	0.388	0.539	0.113	0.323	0.178	1.78	0.201	1.43
		96	0.765	1.52	0.786	0.612	0.321	0.843	0.346	1.56	0.367	1.37
		120	1.95	1.43	0.996	1.56	0.456	1.11	0.332	1.65	0.381	1.51
		144	1.89	1.48	0.893	1.93	0.598	1.04	0.351	1.61	0.351	1.29

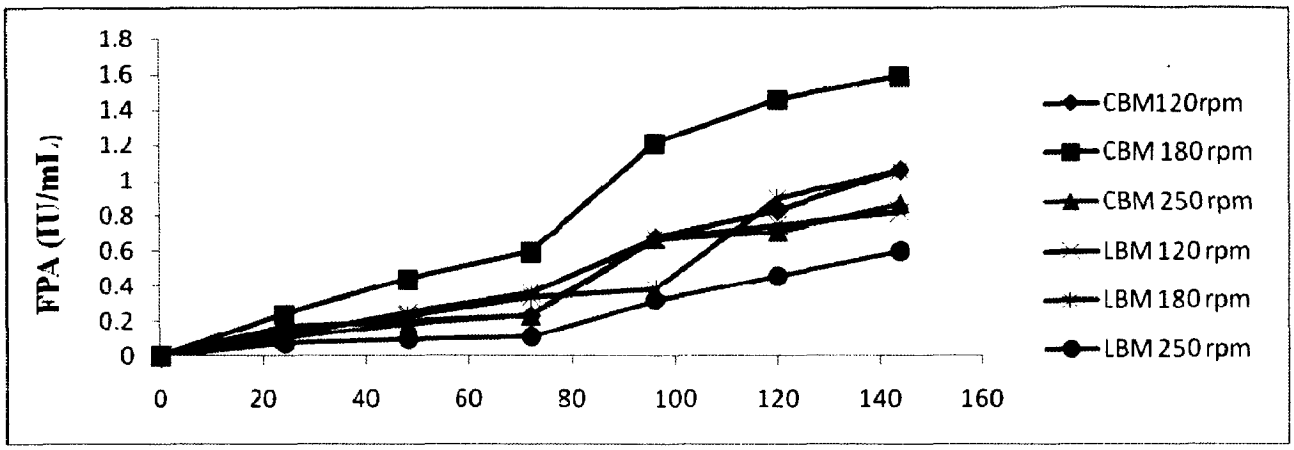


Fig 4.3.32.Comparative FPA achieved by *N.crassa* under cellulose and lactose based production medium at different rpm. (CBM:Cellulose based medium,LBM:Lactose based medium).

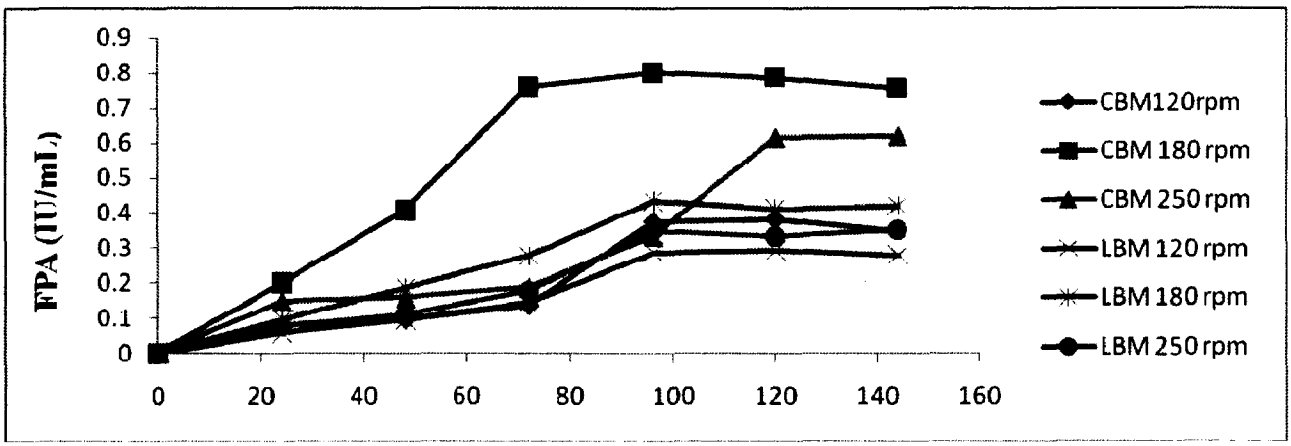


Fig 4.3.33.Comparative FPA achieved by *A. niger* under cellulose and lactose based production medium at different rpm. (CBM:Cellulose based medium,LBM:Lactose based medium).

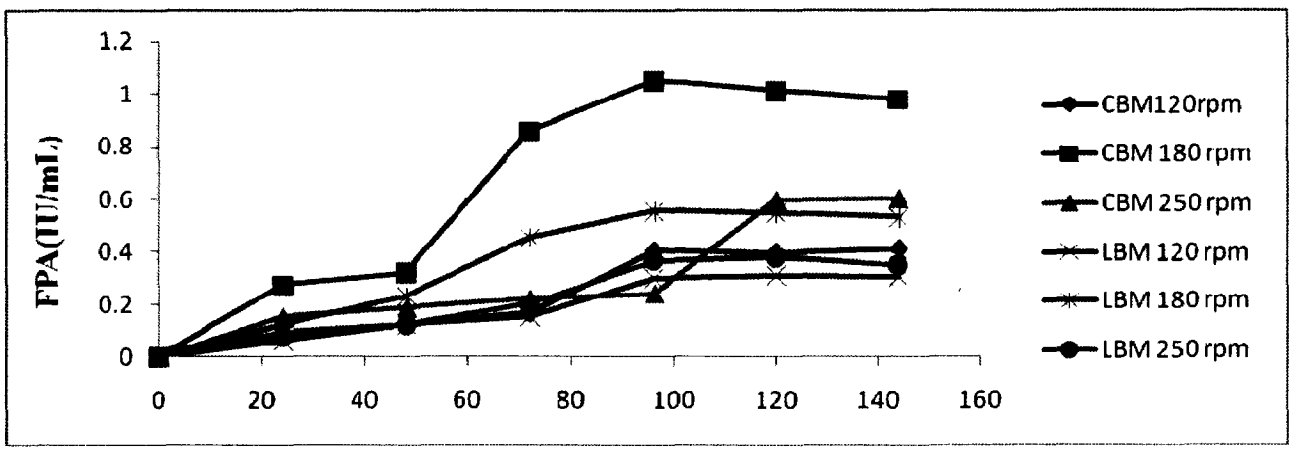


Fig 4.3.34.Comparative FPA achieved by *A. fumigatus* under cellulose and lactose based production medium at different rpm. (CBM:Cellulose based medium,LBM:Lactose based medium).

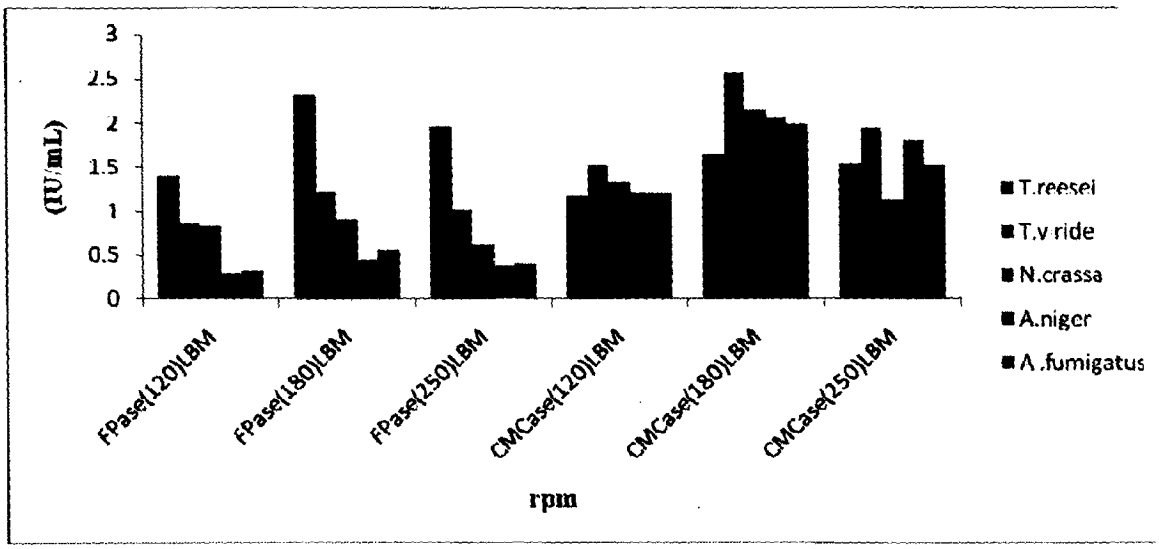


Fig 4.3.35. Comparative FPA and CMCase activities achieved by various fungal strains und cellulose and lactose based fermentation medium at different rpm. (CBM:Cellulose bas medium,LBM:Lactose based medium).

When compared the cellulase activities produced by various fungal strains under lactose an cellulose based fermentation medium, it has been observed that at 120 rpm decrement rate o enzyme activities was found somewhat higher in cellulose based fermentation as compared tc lactose based fermentation which might be due to the fact that at lower agitation proper nutrient and mass transfer has not taken place because of insoluble nature of cellulose, hence the cellulases activities were reduced. On the other hand at higher agitation (250 rpm) decrement rate of cellulases activities, due to high shear stress was somewhat lower under cellulose based fermentation as compared to lactose based fermentation because of the presence of insoluble molecules in the fermentation system, which reduces the effect of shear stress due to high agitation as observed from Fig 4.3.35.

4.3.18.Effect of dairy industry waste whey as carbon source on cellulase production under submerged fermentation

Separate sets of batch experiments have been performed to study the effect of dairy industry waste, whey 15, 30 and 50% (v/v) as carbon source separately and also alongwith pure sugars such as cellulose and lactose on cellulase production by various fungal strains under submerged cultivation. It was observed from Table 4.3.8 and Fig 4.3.36. that fungal strains produced

significantly good cellulase activities under 30 and 50% (v/v) whey based fermentation medium. Cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 2.77, 1.96; 1.45, 3.07; 1.07, 2.19; 0.521, 2.43; 0.671, 2.28 respectively at 180 rpm under 30% (v/v) whey based fermentation. When compared the cellulase activities produced by different fungal strains under various concentrations of whey 15, 30 and 50% (v/v), It is observed that 30%(v/v) of whey based fermentation medium was found much suitable for higher cellulase activities with higher incremental rate as compared to 15% and 50% (v/v) of whey based fermentation medium as observed from Table 4.3.8 and Fig 4.3.36. At higher concentration of whey (50%v/v), there was no significant improvement in the cellulases which might be due to the higher sugar present in the whey which becomes inhibitory or microbial system becomes saturated for taking further sugars for cellulase production.

Table 4.3.8. Effects of whey as carbon source on the cellulase production (IU/mL) by various fungal strains under submerged fermentation at 30^0C and pH 5.

Pure Lactose/Dairy waste (Whey)		*Trichoderma reesei*		*Trichoderma viride*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
		FPA	**CMCase**	**FPA**	**CMCase**	**FPA**	**CMCase**	**FPA**	**CMCase**	**FPA**	**CMCase**
Whey	15%(v/v)	1.40	1.02	0.731	1.58	0.628	1.17	0.259	1.26	0.345	1.20
	30%(v/v)	2.77	1.96	1.45	3.07	1.07	2.19	0.521	2.43	0.671	2.28
	50%(v/v)	2.88	2.06	1.57	3.12	1.10	2.15	0.531	2.51	0.679	2.34
C+ W	5(g/L)+30 % (v/v)	3.01	2.59	1.59	3.81	1.39	3.02	0.642	4.21	0.857	3.75
L +W	5(g/L)+30 % (v/v)	2.83	1.97	1.39	2.96	1.02	2.10	0.495	2.39	0.634	2.25

C+W:Cellulose + whey; L+W:Lactose + Whey

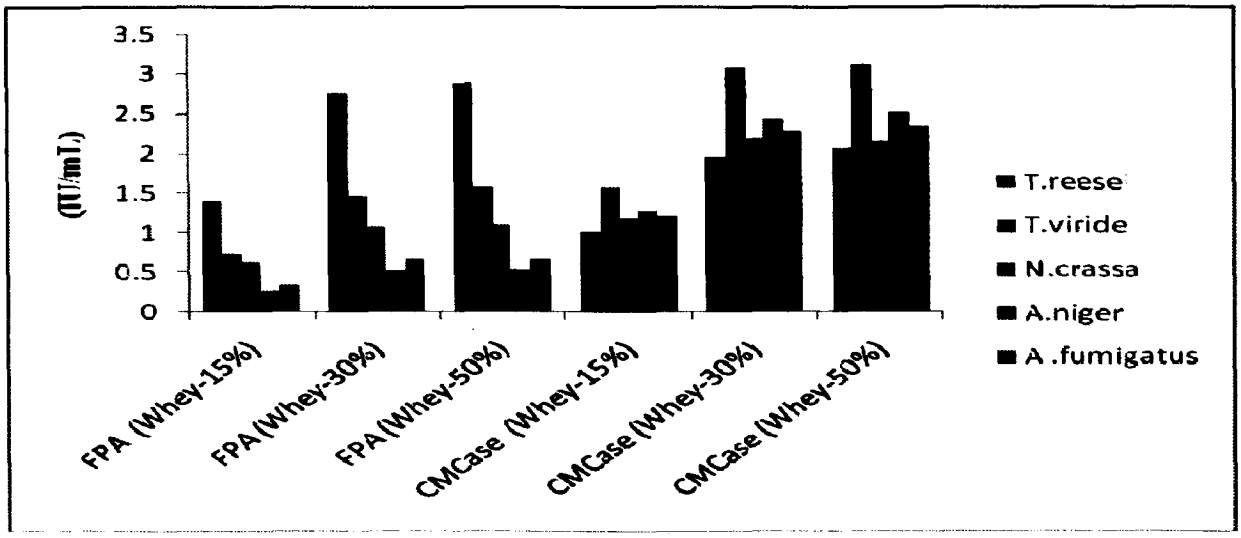


Fig 4.3.36. Comparative FPA and CMCase activities achieved by various fungal strains under different concentration of whey based fermentation medium at 30^0C and pH 5.0

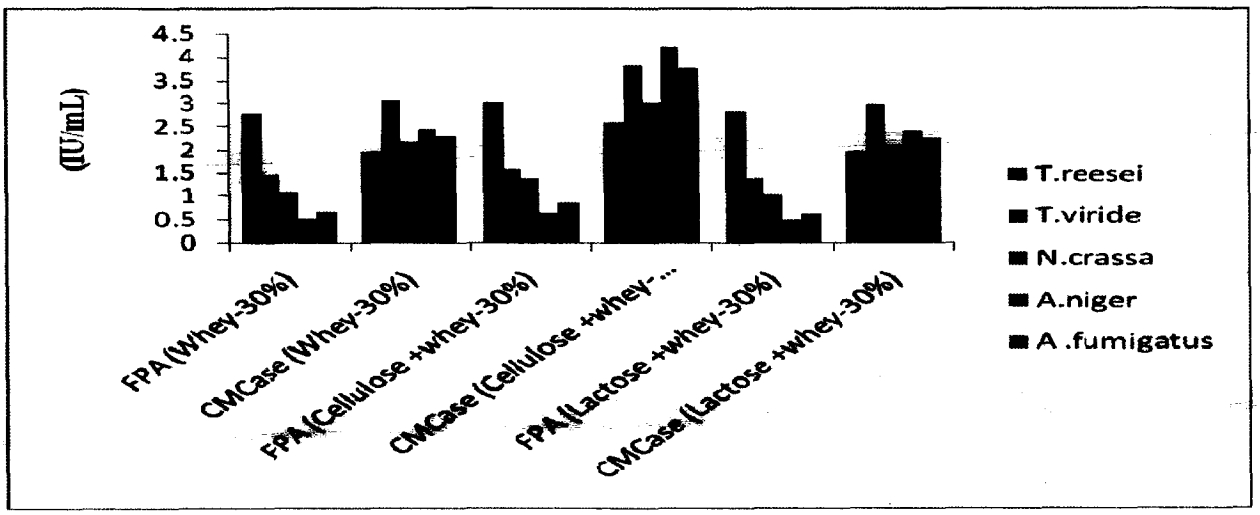


Fig 4.3.37. Comparative FPA and CMCase activities achieved by various fungal strains under 30% whey alongwith cellulose and lactose based fermentation medium at 30^0C and pH 5.0

When compared, the cellulase activities produced under 30% whey along with lactose and cellulose based medium then it was observed that fungal strains produced significantly higher cellulase activities under 30% whey with cellulose based fermentation medium as observed from Table 4.3.8.This might be due to the intensification of cellulase induction rate in the presence of whey alongwith cellulose as compared to whey alongwith lactose containing fermentation medium. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase achieved by *T.reesei*, *T.viride*, *N.crassa*, *A.niger* and *A.fumigatus* were 3.01, 2.59; 1.59, 3.81; 1.39, 3.02; 0.642, 4.21;

0.857, 3.75 respectively under whey (30% v/v) alongwith cellulose based fermentation medium as observed from Table 4.3.8 and Fig 4.3.37.

4.3.19. Effect of starch hydrolysates on the growth of fungal strains under submerged cultivation

To investigate the role of starch hydrolysates in cellulase production, separate set of experiments were performed. Pretreatment of starches were performed by taking 2 and 5% HCl with varying pretreatment time of 1 and 3h. From the above experimental observation we found that after hydrolysis potato and wheat starch becomes more soluble than rice starch. 2% HCl treatment was more favorable than 5% HCl for starch hydrolysis. Under 5% HCl treatment overhydrolysis of starch molecules occurred, Upon increasing the pretreatment time, condition becomes more extreme and unfavorable for microbial system, as the essential components or sugars present in the starch solution were destroyed or burnt out. Pretreatment of wheat starch with 2% HCl having pretreatment time of 1 h was found most suitable for the *Trichoderma* growth in comparison to higher acid dosages with higher pretreatment time. Therefore 5% HCl treated starch with 3 h pretreatment time was unsuitable for the microbial growth as has been observed from Table 4.3.9.

Table 4.3.9. Visual observation of growth of various used fungal strains under acid pretreated wheat, potato and rice hydrolysates containing production media by at 48 h of incubation period under submerged cultivation.

Starch type	Acid treatment dosages	Pretreatment time(h)	*Trichoderma reesei* growth in pure starch hydrolysate based medium	*Aspergillus niger* growth in pure starch hydrolysate based medium	*Neurospora crassa* growth in pure starch hydrolysate-based medium
Potato	2%HCl	1	Very good growth	Very good growth	Good growth
		3	Good growth	Very light growth	Light growth
	5%HCl	1	Very light growth	Very light growth	Very light growth
		3	Least growth	Not suitable	Not suitable
Wheat	2%HCl	1	Good growth	Good growth	Good growth
		3	Very light growth	Very light growth	Good growth
	5%HCl	1	Very light growth	Very light growth	Very light growth
		3	Not suitable	Not suitable	Not suitable
Rice	2%HCl	1	Good growth	Moderate growth	Very light growth
		3	Very light growth	Very light growth	Least growth
	5%HCl	1	Least growth	No growth	Least growth
		3	Not suitable	Not suitable	Not suitable

4.3.20. Effect of starch hydrolysates on cellulase production under submerged fermentation

Individual sets of batch experiments have been performed to study the effect of untreated as well as 2% and 5% HCl treated wheat starch hydrolysate as carbon source on cellulase production by various fungal strains under submerged cultivation at 180 rpm. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 1.40, 1.24; 0.184, 0.375; 0.118, 0.849 respectively under 2% wheat starch hydrolysate based fermentation medium. It was observed from Table 4.3.10 and Fig 4.3.38 and 4.3.39. that fungal strains produce significantly higher cellulase activities under 2% HCl treated wheat starch hydrolysate based fermentation medium as compared to untreated and 5% HCl treated one. Presumably due to the release of some dimeric sugars (sophrose) in the hydrolyzates, which induces more cellulase production rather than growth enhancement. The inducing ability of the acid-hydrolyzed starch may be attributed to the reversion product of glucose, by increasing the acid strength, resulting in more reversion products of glucose in the hydrolyzates, which are responsible for the induction capability of the starch. But when the starch is overhydrolyzed, other byproducts may be generated, which might have served as inhibitors for cellulase production [47].When compared, the effectiveness of wheat starch hydrolysates for various used fungal strains than it has been observed that *T.reesei* and *A. niger* performed much better in terms of cellulase activity under wheat starch hydrolysate based fermentation medium as compared to *N.crassa* fungal strain.

Table 4.3.10. Effects of wheat starch hydrolysates as carbon source on the cellulase production by various fungal strains under submerged fermentation at 30^{0}C and pH 5.

	Wheat Starch hydrolysates (WSH)	*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
UTR	Only starch	0.601	0.498	0.122	0.186	0.052	0.241
	Starch +30% Whey	3.43	2.79	1.18	2.25	0.603	2.84
	Starch +50% L	3.19	2.32	1.13	2.34	0.535	2.52
2%HCl	Pure Starch HYL	1.40	1.24	0.184	0.375	0.118	0.849
	Starch HYL(5%)+30% Whey	4.21	3.29	1.27	2.46	0.640	3.12
	Starch HYL+50% L	3.79	3.13	1.31	2.51	0.564	2.88
5%HCl	Pure Starch HYL	0.789	0.776	0.086	0.127	0.073	0.298
	Starch HYL +50% Whey	3.55	2.94	1.11	2.28	0.619	2.88
	Starch HYL+50% L	3.27	2.56	1.16	2.12	0.527	2.49

WSH:Wheat starch hydrolysate; UTR:Untreated; HYL: Hydrolysate; L:Lactose

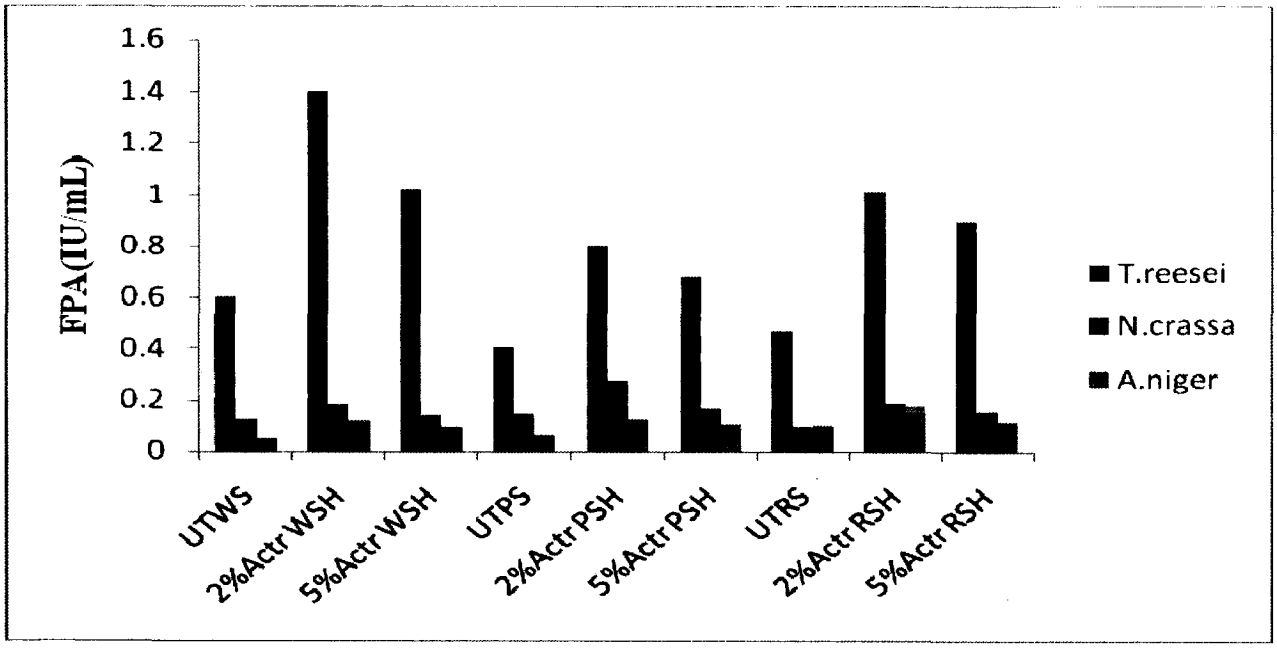


Fig 4.3.38. Comparative FPA achieved by various fungal strains under acid treated starch hydrolysate based fermentation medium at 30^0C and pH 5.0.

On the other hand maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 0.802, 0.670; 0.275, 0.543; 0.121, 0.567 respectively under 2% potato starch hydrolysate based fermentation medium. It has been observed from Table 4.3.11 that *N.crassa* showed higher cellulase activities under potato starch based fermentation medium compared to wheat starch based medium, whereas *T.reesei* and *A. niger* showed somewhat lower cellulase activity under potato starch hydrolysate based medium as observed from which might be due to the fact sugars released after hydrolysis are growth inducing nature rather than cellulase inducing for *T.reesei* and *A. niger* system Table 4.3.11 and Fig 4.3.38 and 4.3.39.

Table 4.3.11. Effects of potato starch hydrolysate as carbon source on the cellulase production by various fungal strains under submerged fermentation at 30^{0}C and pH 5.

Potato Starch hydrolysates (PSH)		*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
UTR	Only starch	0.403	0.319	0.142	0.189	0.061	0.251
	Starch +50% Whey	3.23	2.40	1.29	2.51	0.578	2.64
	Starch +50% Lactose	2.89	2.26	1.34	2.46	0.523	2.39
2%HCl	Pure starch HYL	0.802	0.670	0.275	0.543	0.121	0.567
	Starch HYL +50% Whey	3.51	2.53	1.39	2.84	0.606	2.91
	Starch HYL +50% Lactose	3.03	2.21	1.36	2.75	0.540	2.47
5%HCl	Pure starch HYL	0.518	0.421	0.157	0.276	0.097	0.332
	Starch HYL +50% Whey	3.36	2.43	1.26	2.61	0.540	2.60
	Starch HYL +50% Lactose	3.00	2.12	1.23	2.65	0.503	2.23

PSH:Potato starch hydrolysate; UTR:Untreated; HYL: Hydrolysate

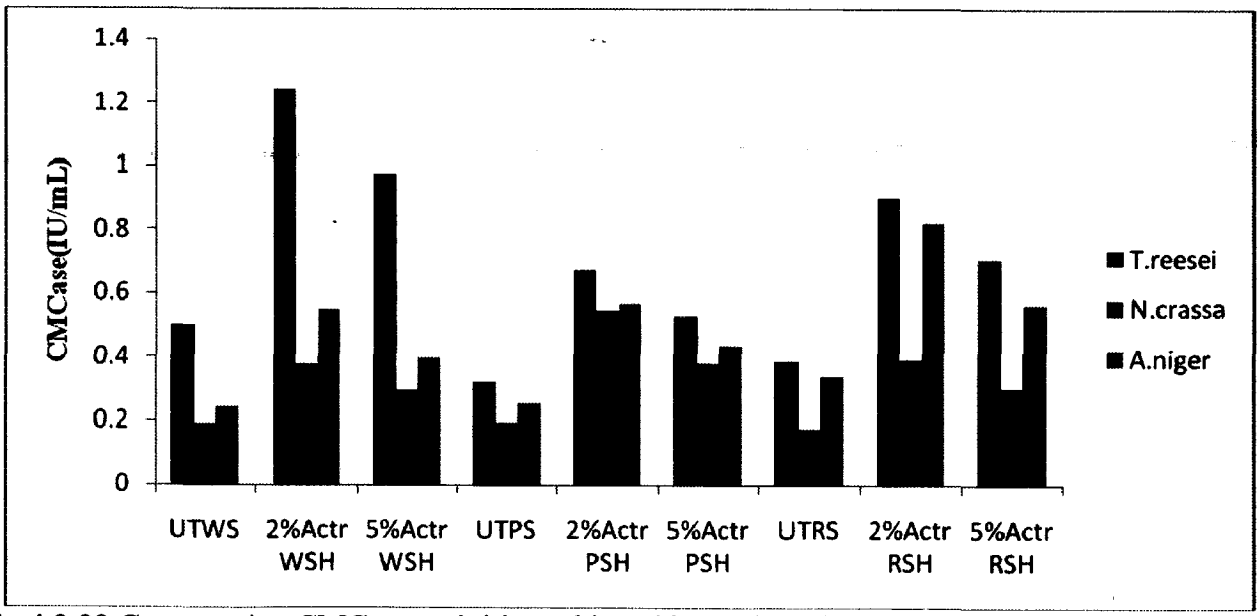


Fig 4.3.39. Comparative CMCase activities achieved by various fungal strains under acid treated starch hydrolysate based fermentation medium at 30^{0}C and pH 5.(UTWS:Untreated wheat starch; UTPS:Untreated Potato starch; UTRS:Untreated rice starch; Actr:Acid treated).

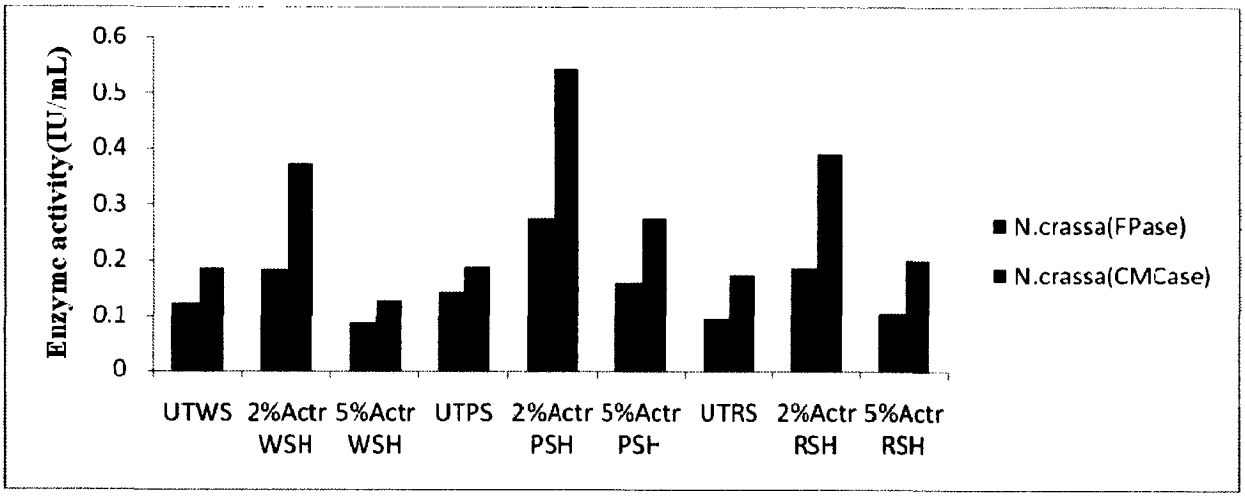


Fig 4.3.40. Comparative FPA and CMCase activities achieved by *N. crassa* under acid treated starch hydrolysate based fermentation medium at 30^0C and pH 5.0.(UTWS:Untreated wheat starch; UTPS:Untreated Potato starch; UTRS:Untreated rice starch; Actr:Acid treated).

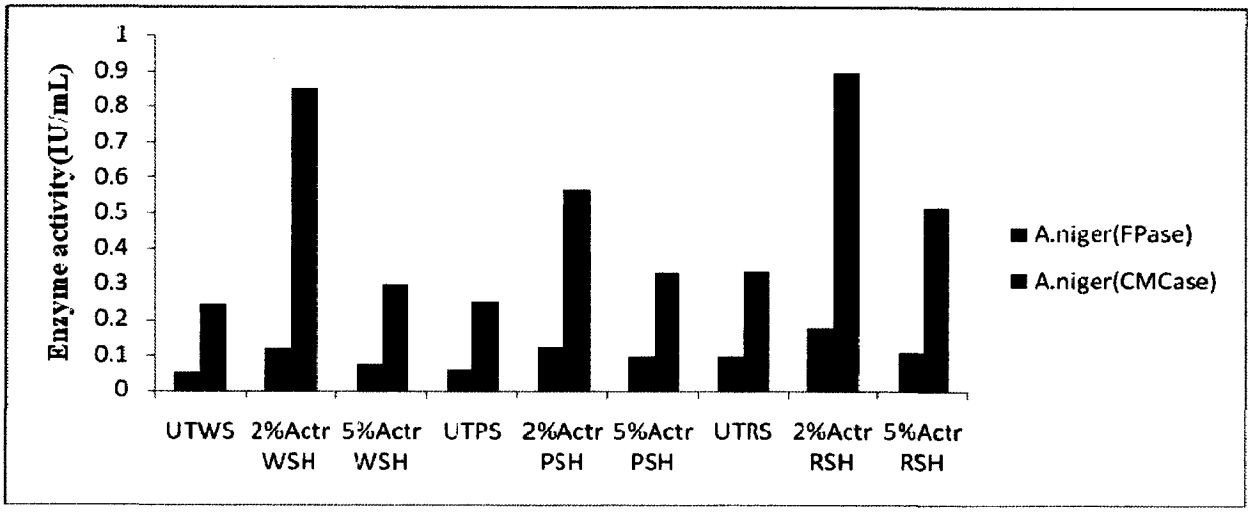


Fig 4.3.41. Comparative FPA and CMCase activities achieved by *A.niger* under acid treated starch hydrolysate based fermentation medium at 30^0C and pH 5.0. (UTWS: Untreated wheat starch; UTPS:Untreated Potato starch; UTRS:Untreated rice starch; Actr:Acid treated).

Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 1.01, 0.797; 0.186, 0.390; 0.174, 0.895 respectively under 2% rice starch hydrolysate based fermentation medium as shown by Table 4.3.12. It has been observed from Table 3 that *A. niger* showed higher cellulase activities under rice starch hydrolysate based fermentation medium compared to wheat and potato starch hydrolysate, whereas *N.crassa* showed somewhat lower cellulase activity under rice starch hydrolysate based medium as observed from Table and Figs 4.3.40 and 4.3.41.

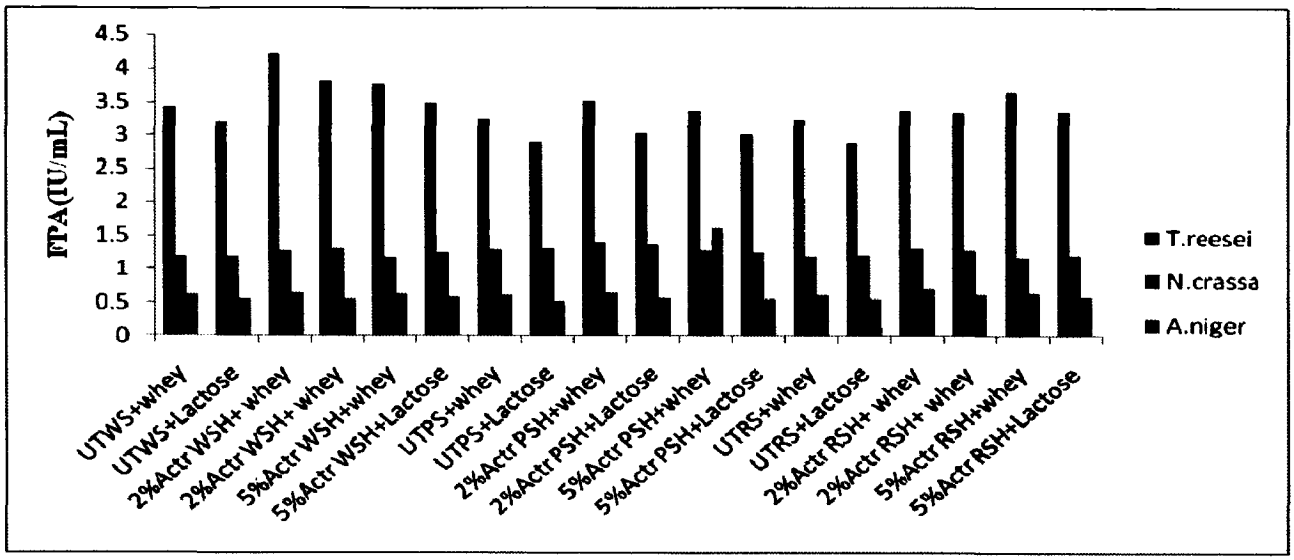


Fig 4.3.42. Comparative FPA achieved by various fungal strains under acid treated starch hydrolysate separately as well as alongwith cellulose and lactose based fermentation medium at 30^0C and pH 5.0.

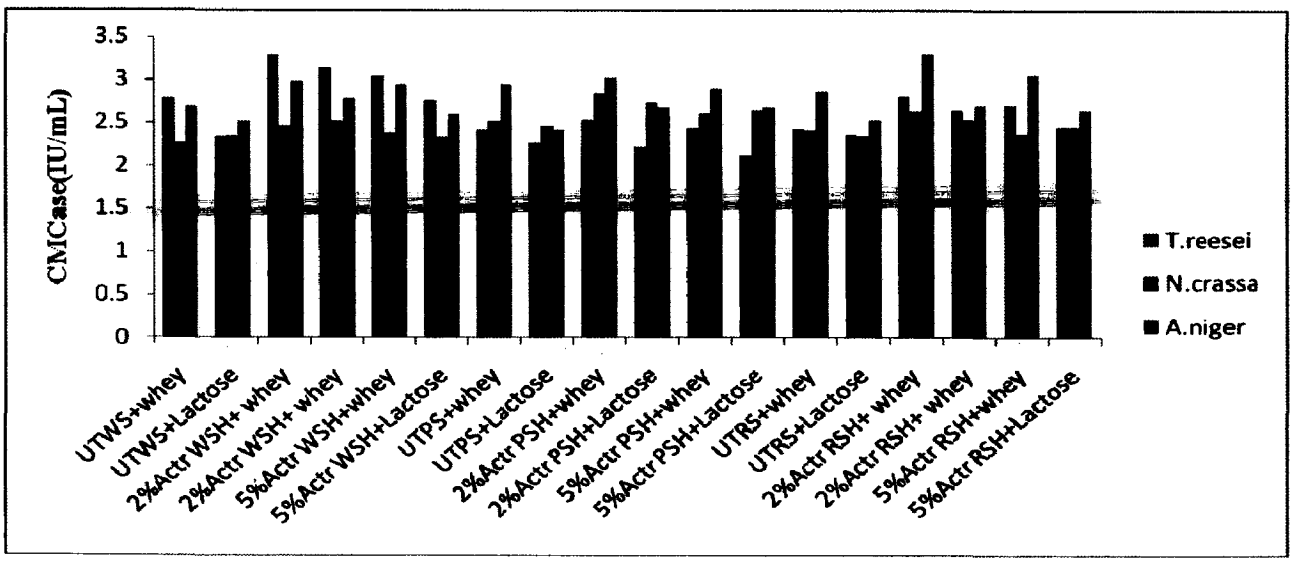


Fig 4.3.43. Comparative CMCase activitiy achieved by various fungal strains under acid treated starch hydrolysate separately as well as alongwith cellulose and lactose based fermentation medium at 30^0C and pH 5.

Table 4.3.12. Effects of rice starch hydrolysate as carbon source on the cellulase production by various fungal strains under submerged fermentation at 30^0C and pH 5.

Rice starch hydrolysates (RSH)		*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
UTR	Only starch	0.468	0.385	0.094	0.174	0.097	0.336
	Starch +50% Whey	3.21	2.42	1.18	2.40	0.609	2.93
	Starch +50% Lactose	2.87	2.35	1.20	2.34	0.532	2.53
2%HCl	Pure starch HYL	1.01	0.797	0.186	0.390	0.174	0.895
	Starch +50% Whey	3.58	2.81	1.31	2.64	0.701	3.44
	Starch +50% Lactose	3.32	2.65	1.26	2.53	0.629	2.92
5%HCl	Pure starch HYL	0.593	0.504	0.103	0.201	0.107	0.518
	Starch +50% Whey	3.45	2.70	1.17	2.37	0.612	3.00
	Starch +50% Lactose	3.35	2.45	1.10	2.29	0.567	2.57

RSH:Rice starch hydrolysate; UTR:Untreated; HYL: Hydrolysate

Inclusion of starch hydrolysate with whey as well as lactose based medium significantly increases the cellulases activity. 2% HCl treated starch hydrolysate with 30% (v/v) whey inclusion was found much effective in terms of enzyme activites as compared to starch hydrolysate with lactose inclusion as observed from Tables 4.3.10, 4.3.11, 4.3.12 and Figs 4.3.42, 4.3.43.

4.3.21. Effect of bagasse and wheat straw hydrolysates on the growth of fungal strains under submerged cultivation

To study the effects of bagasse and wheat straw hydrolysates on the growth and production system of fungal strains, acid pretreatment of bagasse and wheat straw have been performed with 5, 10 and 20% H_2SO_4 (v/v) solution with maintaining the solid liquid ratio of 1:25 and 1:20 respectively. The resulting detoxified hydrolyzates were used in the production medium, either as pure hydrolyzates or in combination with lactose. Growth of *Trichoderma reesei* has been observed in the form of tiny and minute filament under pure hydrolyzate system whereas larger and thick mycelial form of growth were observed under hydrolysate alongwith lactose based medium as evident from Table 4.3.13. Distinctly longer lag phase ~ 20h were observed in the cell growth under hydrolysate based medium presumably because of the presence of hydrolysis by products. On the other hand growth of *A.niger* was observed in the form of smaller round shaped beads, whereas *N.crassa*

showed diminished growth under hydrolysate based production medium. 20% H_2SO_4 treated wheat straw and 10% H_2SO_4 treated bagasse was found quite unsuitable for the growth as compared to lower acid dosages as observed from Table 4.3.13.

Table 4.3.13. Visual observation of growth of various used fungal strains under acid pretreted bagasse and wheat straw hydrolysates containing production media at 48 h of incubation period under submerged cultivation.

Raw materials	Acid treatment dosages	Growth and production medium	*Trichoderma reesei* growth	*Aspergillus niger* growth	*Neurospora crassa* growth
Wheat Straw	5%H_2SO_4	Pure wheat straw hydrolysate based	Good growth	Very good growth	Good growth
	10%H_2SO_4	Pure wheat straw hydrolysate based	Moderate growth	Good growth	Moderate growth
	20%H_2SO_4	Pure wheat straw hydrolysate based	Not suitable	Not suitable	Not suitable
Bagasse	10%H_2SO_4	Pure bagasse hydrolysate based	Good growth	Moderate growth	Good growth
	20%H_2SO_4	Pure bagasse hydrolysate based	Not suitable	Not suitable	Least growth

4.3.22.Effect of boiled bagasse syrup on cellulase production under submerged fermentation

Individual sets of batch experiments have been performed to study the effect of boiled bagasse syrup as carbon source on cellulase production by various fungal strains under submerged cultivation at 180 rpm. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 0.841, 0.709; 0.142, 0.187; 0.080, 0.298 respectively under boiled bagasse syrup based fermentation medium. It was observed from Table 4.3.14 and Fig 4.3.44 that *Trichoderma reesei* produces significantly higher cellulase activities as compared to *N.crassa* and *A.niger* under boiled bagasse syrup based fermentation medium .This might be due to the fact that upon boiling of bagasse some superficial sugars such as sucrose released in the medium which induces the cellulase production as well as growth enhancement. Incorporation of boiled bagasse syrup with lactose act as a better carbon source for cellulase production by fungal strains. Higher cellulase activities were observed with *T.reesei* and *N. crassa* while *A.niger* showed significantly lower activity under such condition as observed from Table 4.3.14 and Figs 4.3.44, 4.3.45.

Table 4.3.14. Effects of boiled bagasse syrup, bagasse and wheat straw hydrolysate as carbon source on the cellulase production by various fungal strains under submerged fermentation at 30^0C and pH 5.

Lignocellulosic Hydrolysates			*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
			FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
Boiled Bagasse syrup		BBS	0.841	0.709	0.142	0.187	0.080	0.298
		BBS+ 50%L	2.15	1.68	0.642	1.15	0.287	1.34
Bagasse hydrolysate	10%	BH	0.813	0.601	0.125	0.146	0.059	0.306
		BH+50%L	2.01	1.54	0.564	1.13	0.271	1.41
	20%	BH	0.201	0.193	0.036	0.072	0.012	0.101
		BH+50%L	1.23	1.01	0.296	0.931	0.204	1.04
Wheat straw hydrolysate	5%	WSH	0.568	0.522	0.045	0.091	0.047	0.257
		WSH+50%L	1.65	1.49	0.491	1.03	0.279	1.36
	10%	WSH	0.291	0.377	0.013	0.045	0.035	0.164
		WSH+50%L	1.21	1.08	0.432	0.981	0.256	1.13

BBS: Boiled bagasse syrup; BH: Bagasse hydrolysate; WSH: Wheat straw hydrolysate; L:Lactose

4.3.23. Effect of bagasse hydrolysates on cellulase production under submerged fermentation

Individual sets of batch experiments have been performed to study the effect of bagasse hydrolysates used separately or alongwith lactose pure sugar as carbon source on cellulase production by various fungal strains under submerged cultivation at 180 rpm. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 0.813, 0.601; 0.125, 0.146; 0.059, 0.306 respectively under only 10 % acid treated bagasse hydrolysates based fermentation medium. It was observed from Table 4.3.14 that *Trichoderma reesei* produces significantly higher cellulase activities as compared to *N.crassa* and *A.niger* under bagasse hydrolysates based fermentation medium. This might be due to the fact that acid hydrolysis of bagasse may release C-6 and C-5 sugars such as glucose, xylose, arabinose, mannose, galactose, as well as few dimers (cellobiose) and oligosaccharides. In all the released sugars few are involved in the enhancement of fungal growth as well as others have the ability to induce fungal system for cellulase production. In bagasse hydrolysate there may be possibility of releasing

more hemicellulose degradation products such as xylose and arabinose as compared to others, which ultimately affects the fungal growth and cellulase production system of different fungal strains. Xylose is also known to induce cellulase synthesis, although not as effectively as cellulose. The nonglucose sugars present in the hydrolysates also facilitated the induction for cellulase synthesis. Available literature shows that acidic attacks especially hemicelluloses portion of raw materials than cellulose and lignin [39, 253]. Hydrolysates with less than 20% of oligomers supported active cellulase biosynthesis. Incorporation of bagasse hydrolysates with lactose act as a better carbon source for cellulase production by fungal strains. Higher cellulase activities were observed with *T.reesei* and *N. crassa* while *A.niger* showed significantly lower activity under such conditions as observed from Table 4.3.14 and Figs 4.3.44, 4.3.45.

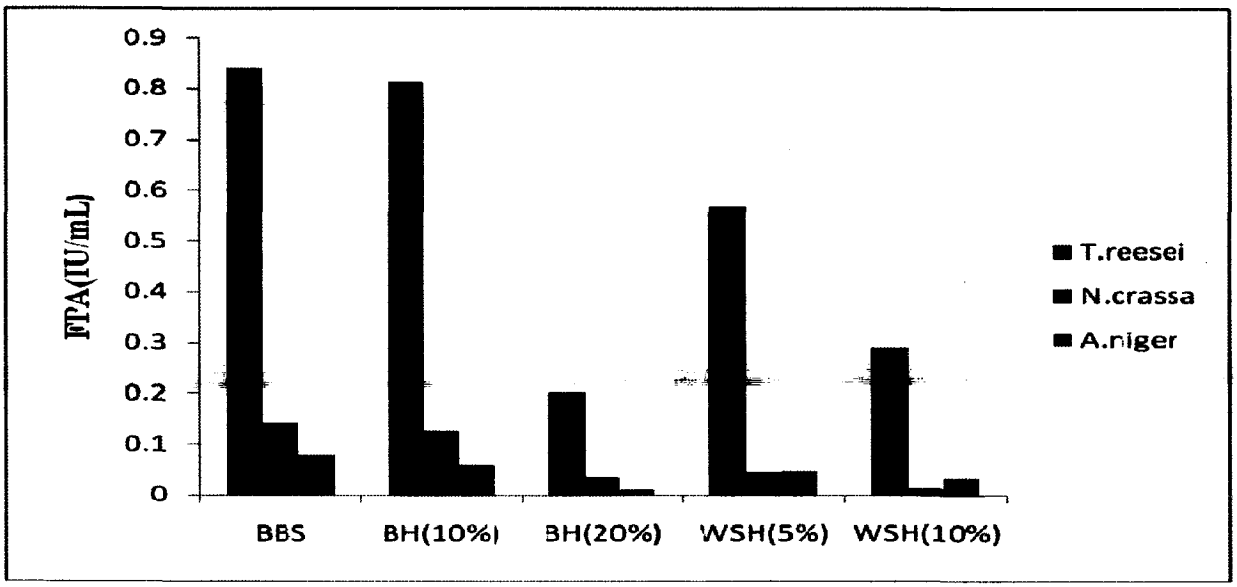


Fig 4.3.44. Comparative FPA activity achieved by various fungal strains under boiled bagasse syrup, bagasse and wheat straw hydrolysate based fermentation medium at 30^0C and pH 5.

4.3.24. Effect of wheat straw hydrolysates as carbon source on cellulase production under submerged fermentation

Individual sets of batch experiments have been performed to study the effect of wheat straw hydrolysate used separately or alongwith lactose pure sugar as carbon source on cellulase production by various fungal strains under submerged cultivation at 180 rpm. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 0.568, 0.522; 0.045, 0.091; 0.047, 0.257 respectively under 5% acid treated wheat straw hydrolysates based fermentation medium. It was observed from Table 4.3.14 and Figs 4.3.44, 4.3.45 that *Trichoderma reesei* produces significantly higher cellulase activities as compared to *N.crassa* and *A.niger* under bagasse hydrolysates based fermentation medium. Decremental rate of enzyme activities were somewhat higher in case of *N.crassa* followed by *A. niger* under wheat straw hydrolysate based fermentation medium.

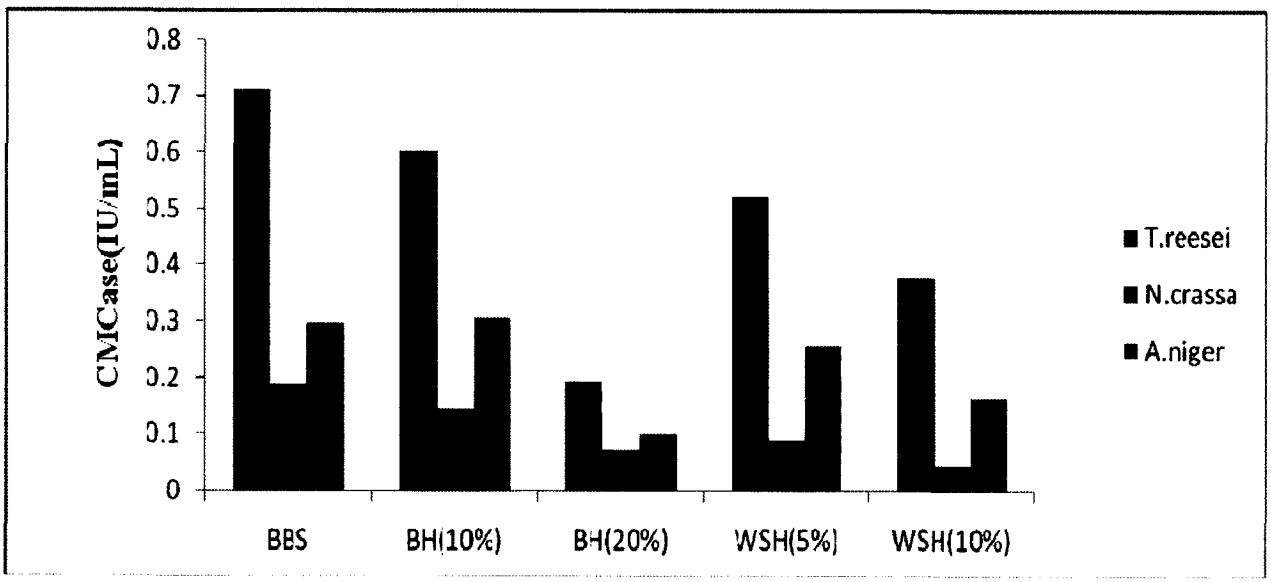


Fig 4.3.45. Comparative CMCase activity achieved by various fungal strains under boiled bagasse syrup, bagasse and wheat straw hydrolysate based fermentation medium at 30^0C and pH 5.

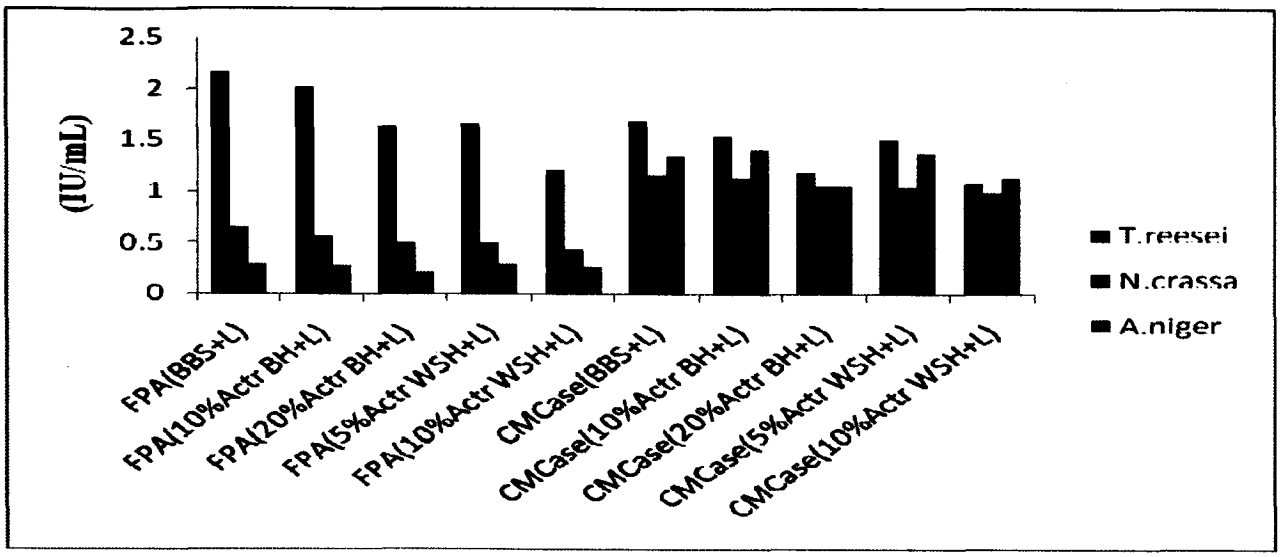


Fig 4.3.46. Comparative FPA and CMCase activities achieved by various fungal strains under boiled bagasse syrup, bagasse and wheat straw hydrolysate seperataly as well as alongwith lactose sugar based fermentation medium at 30^0C and pH 5.

Enzyme activities were increased under bagasse and wheat straw hydrolysates with lactose sugar as viewed from Fig 4.3.46. When compared the enzyme activities attained by fungal strains under wheat straw and bagasse hydrolysate based fermentation medium, it has been observed that bagasse hydrolysate based medium was found much suitable for growth and production system of fungal strain compared to wheat straw hydrolysate. 20% H_2SO_4 treated wheat straw and 10% H_2SO_4 treated bagasse were found quite unsuitable for the growth and cellulase production by fungal strains, which might be due to the fact that under higher acid dosages discharge of certain inhibitory, toxic, noxious and lethal components because of the overhydrolysis of monomers, dimmers and oligomeric sugars. In conclusion, despite the potentially inhibitory effects, the hydrolysates supported cell growth and induced active cellulase biosynthesis. Utilization of bagasse and wheat straw hydrolyzates for growth and production is a better approach for the complete utilization of waste raw material, as the pretreated solid waste biomass used as solid bed under solid state fermentation and on the other hand hydrolyzates released after treatment can also be used for cellulase production under submerged fermentation system, which revealed the complete utilization of sugars present in the waste lignocellulosic biomass.

4.3.25. Effect of waste news paper hydrolysates as carbon source on cellulase production under submerged fermentation

To investigate the effect of waste news paper hydrolysates as carbon source on cellulase production under submerged fermentation separate set of experiments have been performed with utilizing 20, 30, 40, 50 and 70% acid (H_2SO_4) treated waste news paper hydrolysate.

Table 4.3.15. Effects of waste news paper hydrolysate as carbon source on the cellulase production by various fungal strains under submerged fermentation at 30^{0}C and pH 5.

Waste news paper hydrolysates	*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
WNP(20% acid treated)	0.822	0.896	0.112	0.275	.0.089	0.537
WNP (30% acid treated)	0.923	0.977	0.196	0.328	0.101	0.585
WNP (40% acid treated)	0.986	1.04	0.216	0.390	0.121	0.605
WNP (50% acid treated)	0.602	0.627	0.106	0.219	0.095	0.267
WNP (70% acid treated)	0.176	0.133	0.029	0.102	0.015	0.131

WNP: Waste news paper hydrolysates

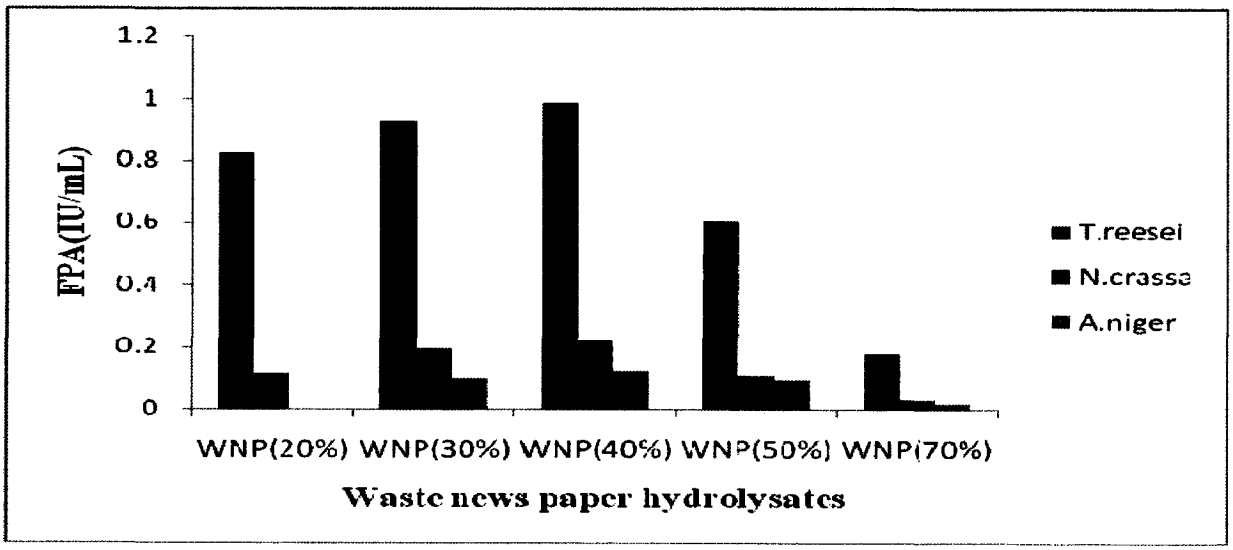


Fig 4.3.47. Comparative FPA activity achieved by various fungal strains under waste news paper hydrolysate based fermentation medium at 30^{0}C and pH 5.

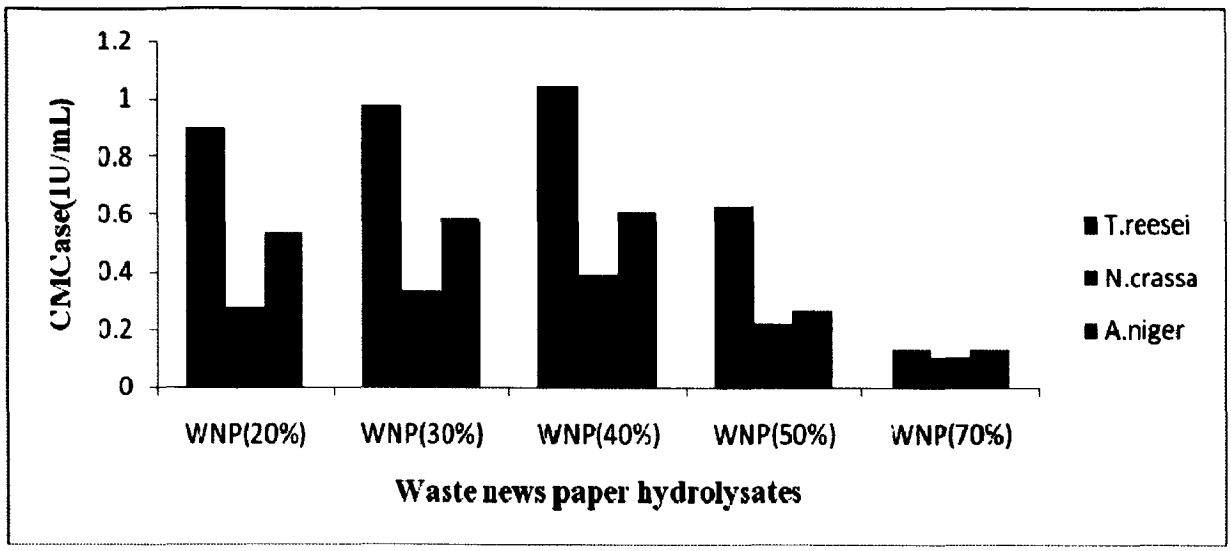


Fig 4.3.48. Comparative CMCase activity achieved by various fungal strains under waste news paper hydrolysate based fermentation medium at 30^0C and pH 5.

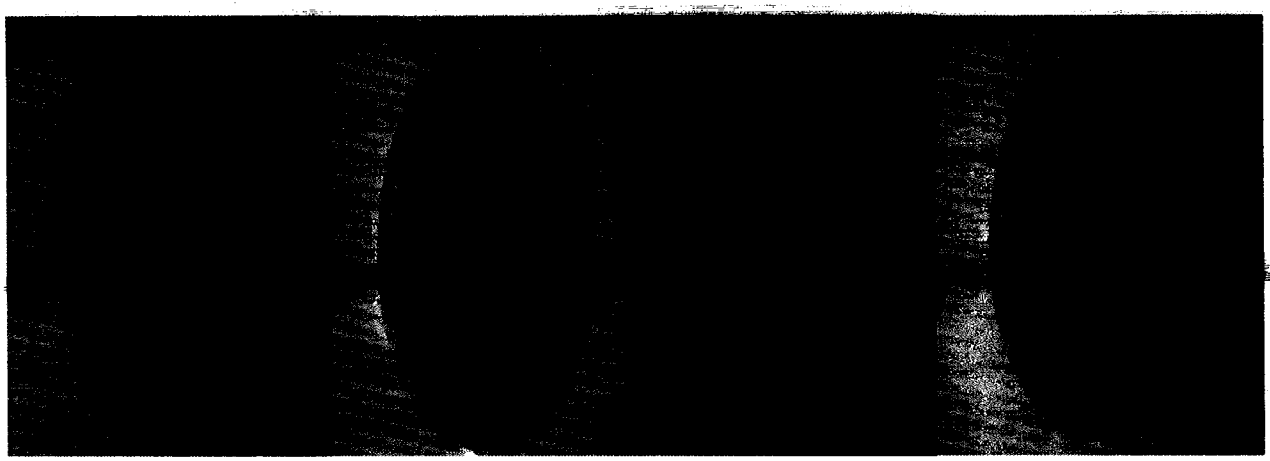
It has been observed from Table 4.3.15 and Figs 4.3.47, 4.3.48. that 40% acid treated waste news paper was found much more suitable for cellulase production by fungal strains as compared to higher acid dosages. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 0.986, 1.04; 0.216, 0.390; 0.121, 0.605 respectively under 40% acid treated waste news paper hydrolysates based fermentation medium. *Trichoderma reesei* showed higher cellulase activities followed by *N.crassa* under such fermentation medium this might be due to the fact that acid hydrolysis of waste news papers released sophrose, cellobiose, xylose and glucose etc, which may induces the cellulase production by fungal strains.

SECTION IV
UTILIZATION OF CONVENTIONAL RAW MATERIALS IN CELLULASE PRODUCTION

Lignocellulosic biomass represents a rather unused or untrapped source of energy for fuels, feeds and chemicals production. Various lignocellulosic waste materials like agricultural and forest wastes are rich in cellulosic components, hence can be used as raw materials for cellulase production. These lignocellulosic materials are particularly attractive because of their low cost, plentiful supply, continuous replenishment and environmental consideration. Considerable effort has gone into investigating the potential of different agricultural byproducts as feedstock for bio-production of enzymes and chemicals. The present section evaluates the utility of conventional raw materials such as wheat bran, sugar cane bagasse, wheat straw and ground nut shell waste in cellulase production by various fungal strains under solid state cultivation.

4.4.1. CHARACTERIZATION OF RAW MATERIALS

4.4.1.1. Visual observation of conventional raw materials



(4.4.1.1.a) (4.4.1.1.b) (4.4.1.1.c) (4.4.1.1.d)

Fig.4.4.1. Ground and sieved Wheat bran (4.4.1.1.a), Wheat straw (4.4.1.1.b), Sugar cane bagasse (4.4.1.1.c), Ground nut shell waste (4.4.1.1d).

Visual observations of ground and sieved conventional raw materials such as wheat bran, wheat straw, sugar cane bagasse and ground nut shell waste are shown by Fig 4.4.1.1. It was observed from the images that wheat bran particles were light brown and rounded in shape, whereas wheat straw particles were lighter and elongated in shape. Bagasse particles looked like white yellowish rough powder while ground nut shell waste particles were rough rectangular in shape.

4.4.1.2. Proximate analysis of conventional raw materials

To determine the suitability and effectiveness of raw materials for cellulase production, separate set of experiments and analysis have been performed. To resolve the major constituent of wheat bran proximate analysis have been executed.

Table 4.4.1. Content of Major Constituents of Ground, Sieved and Oven-dried wheat bran

Constituents	% composition
Holocellulose	**57.35±2.96**
Lignin	**7.12±1.18**
Ash	**1.20±0.56**
Moisture	**2.13±0.67**

Data are reported as mean ± standard deviation based on the repeated trails.

To resolve the major constituent of bagasse proximate analysis have been executed.

Table 4.4.2. Content of Major Constituents of Ground, Sieved, and Oven-dried bagasse

Constituents	% composition
Holocellulose	**63.40±4.91**
Lignin	**18.92±3.10**
Ash	**2.30±1.76**
Moisture	**3.03±0.58**

Data are reported as mean ± standard deviation based on the repeated trails.

To resolve the major constituent of wheat straw proximate analysis have been executed.

Table 4.4.3. Content of Major Constituents of Ground, Sieved, and Oven-dried wheat straw

Constituents	% composition
Holocellulose	**60.15±4.96**
Lignin	**20.82±1.18**
Ash	**5.20±1.66**
Moisture	**2.45±0.27**

Data are reported as mean ± standard deviation based on the repeated trails.

To resolve the major constituent of ground nut shell waste proximate analysis have been executed.

Table 4.4.4. Content of Major Constituents of Ground, Sieved, and Oven-dried ground nut shell

Constituents	% composition
Holocellulose	**66.11±4.91**
Lignin	**16.98±1.28**
Ash	**4.10±1.72**
Moisture	**3.41±0.23**

It has been observed from the Tables 4.4.1-4.4.4 that holocellulose and lignin are the major constituents present in almost every raw materials except wheat bran. When compared the raw

materials, we found that ground nut shell having (66.11±4.91) higher percentage of holocellulose composition, while lignin percentage (20.82±1.18) was observed much higher in oven dried wheat straw bed material as compared to others.

4.4.1.3. XRD pattern of non conventional raw materials

To determine the accessibility and nature of cellulose present in the nonconventional raw materials, XRD analysis have been executed.

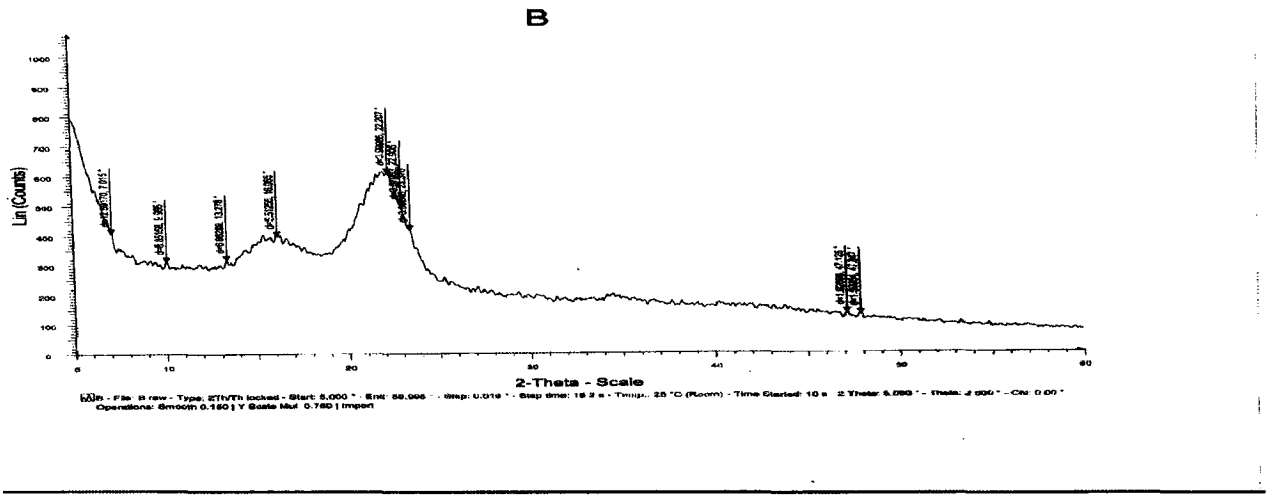


Fig 4.4.2. XRD pattern of sugar cane bagasse agricultural waste.

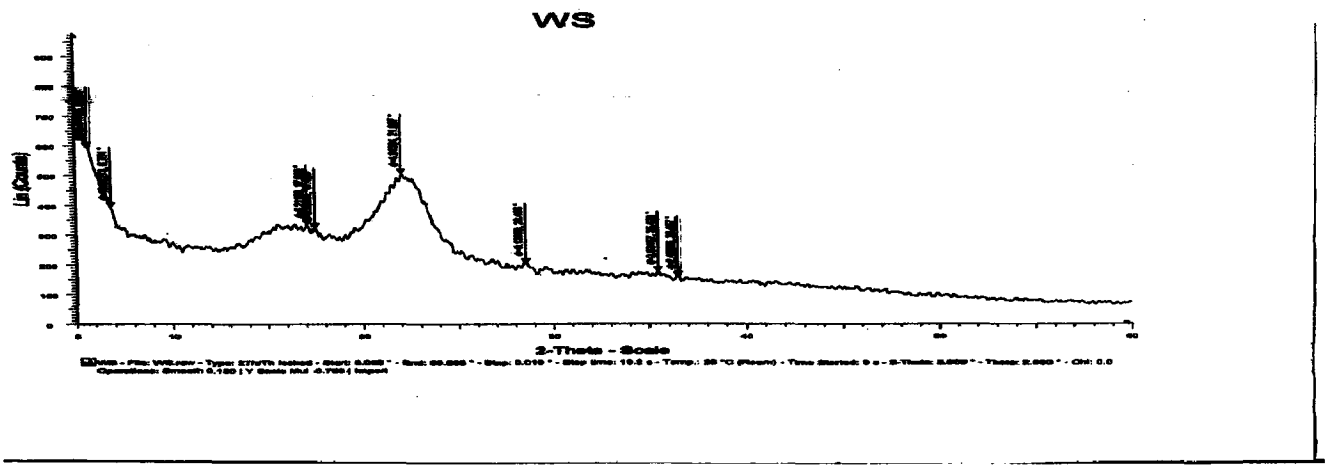


Fig 4.4.3. XRD pattern of wheat straw agricultural waste.

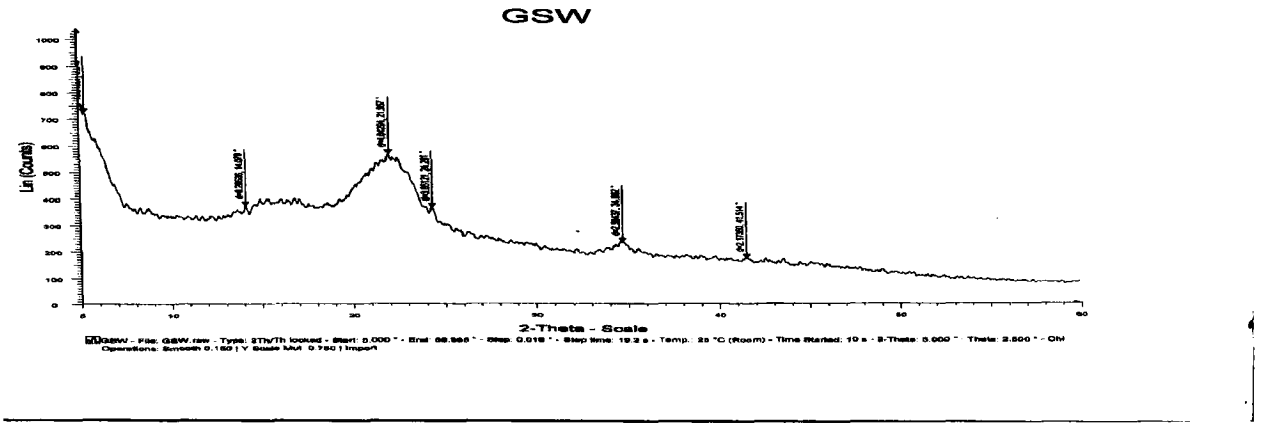


Fig 4.4.4. XRD pattern of ground nut shell waste.

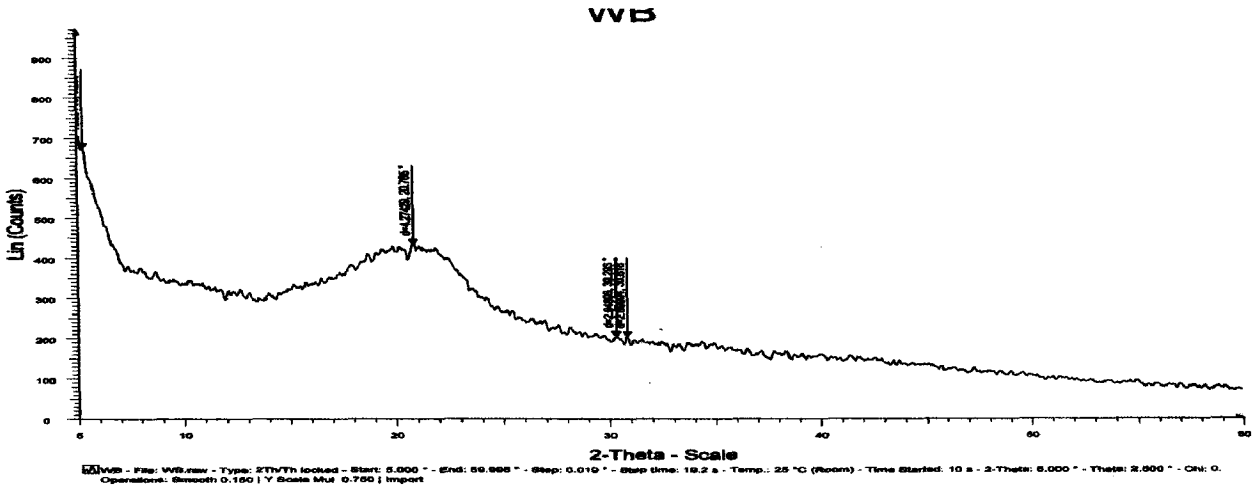


Fig 4.4.5. XRD pattern of wheat bran agricultural waste.

When we compared the XRD pattern of different conventional raw materials used in cellulase production, we have discerned that bagasse contains more number of peaks and a peak at 21 theta having respectable peak heights shows their random and higher crystalline nature, followed by wheat straw and ground nut shell waste having peak with slight reduced peak height, illustrates their something reduced crystallanity compared to bagasse. On the other hand XRD pattern of wheat bran represents much reduced peak height with larger area shows least crystalline nature of cellulose as observed from Figs 4.4.2-4.4.5. Therefore we can suggest that cellulose present in wheat bran are easily available for microbial attack compared to other raw materials such as bagasse, wheat straw and ground nut shell used in production study.

4.4.2. Utilization of conventional raw materials in cellulase production under solid state fermentation

To investigate the utility of conventional raw materials such as wheat straw, bagasse, wheat bran, groundnut shell waste as carbon source, separate set of experiments have been performed under solid state fermentation at 30^0C. It has been observed from Table 4.4.5 and Fig 4.4.6 that wheat bran was found much effective raw material for cellulase production as compared to other conventional raw materials, which might be due to the presence of soluble oligosaccharides, hemicellulose, starch and easily available cellulose which significantly induces the cellulase production. Oligosaccharides present in wheat bran may also be converted into strong inducer such as sophrose and gentiobiose by transglucosylation. As the literature reported that wheat bran is a good source of nitrogen due to the presence of protein content as well as it is also a good source of hemicellulose, as a whole it is a good inducer for cellulolytic enzyme system [37]. Although the cellulose percentage in wheat bran are low [248], but it is easily utilizable by microbes, this can be proved by XRD pattern of wheat bran. Lesser number of peaks with smaller peak height in the XRD pattern of wheat bran shown by Fig 4.4.5.revealed that cellulose present are easily available for microbial hydrolysis. Lower percentage of lignin may also provide fruitful condition for microbial growth as well as the easier uptake of cellulose and other inducers required for cellulase production. Ground nut shell waste was also found quite useful raw material for cellulase production compared to wheat straw and bagasse. As observed from Figs 4.4.2-4.4.4 that groundnut shell waste raw material having lesser crystallanity compared to wheat straw and bagasse which makes their cellulose much easier accessible for microbial hydrolysis. On the other hand proximate analysis data from Table 4.4.1-4.4.4 also showed that groundnut shell waste contained higher percentage of holocellulose which provides much favorable condition for fungal attack and cellulase production. Literature also reported the presence of significant amount of protein was well as few other inductive components, which makes it somewhat quite effective raw material for cellulase production under solid state fermentation [34].

On the other hand we have observed that wheat straw and bagasse were found somewhat less effective than wheat bran. Although the proximate analysis datas showed the presence of good percentage of cellulose but these cellulose are not easily accessible for microbial attack either due to lignin sheathing or their highly crystalline nature as proved by XRD pattern of these raw materials.

Table 4.4.5. Comparative enzyme activities (IU/mL) produced by various fungal strains under different conventional raw materials as carbon source.

Microbes	*T.reesei*		*T.viride*		*N.crassa*		*A. niger*		*A.fumigatus*	
Raw materials	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
Wheat straw	2.19	2.23	1.04	3.98	1.31	4.08	0.689	5.41	1.33	6.09
Bagasse	3.02	3.21	0.996	4.28	1.08	2.83	0.833	4.05	1.20	6.38
Wheat bran	4.72	5.02	2.23	7.74	2.36	6.36	1.29	10.10	1.68	9.49
Groundnut shell waste	2.97	3.05	1.56	4.59	1.18	3.52	1.00	8.05	1.09	7.43

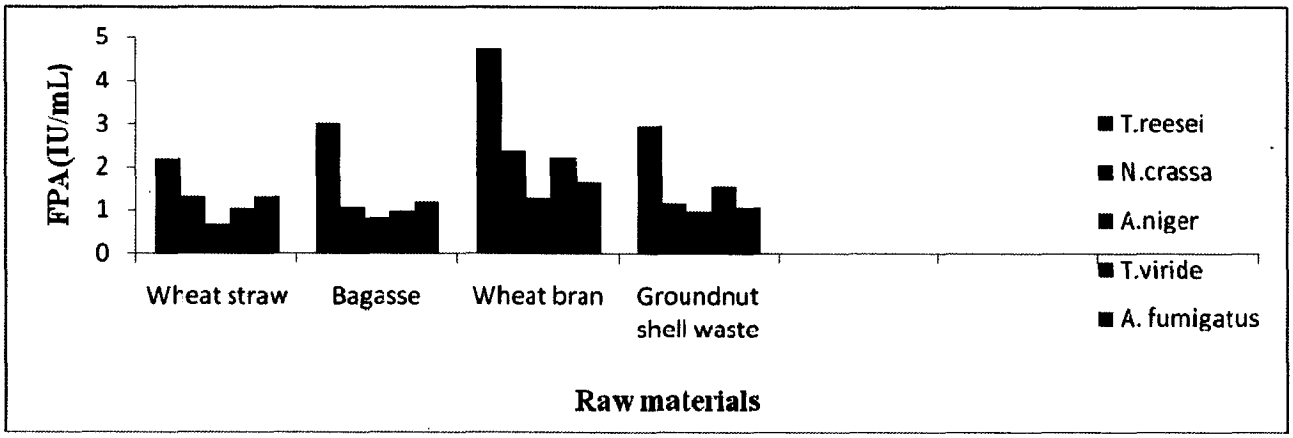


Fig 4.4.6. Comparative FPA achieved by various fungal strains under conventional raw materials as carbon source at 30^{0}C and pH 5.

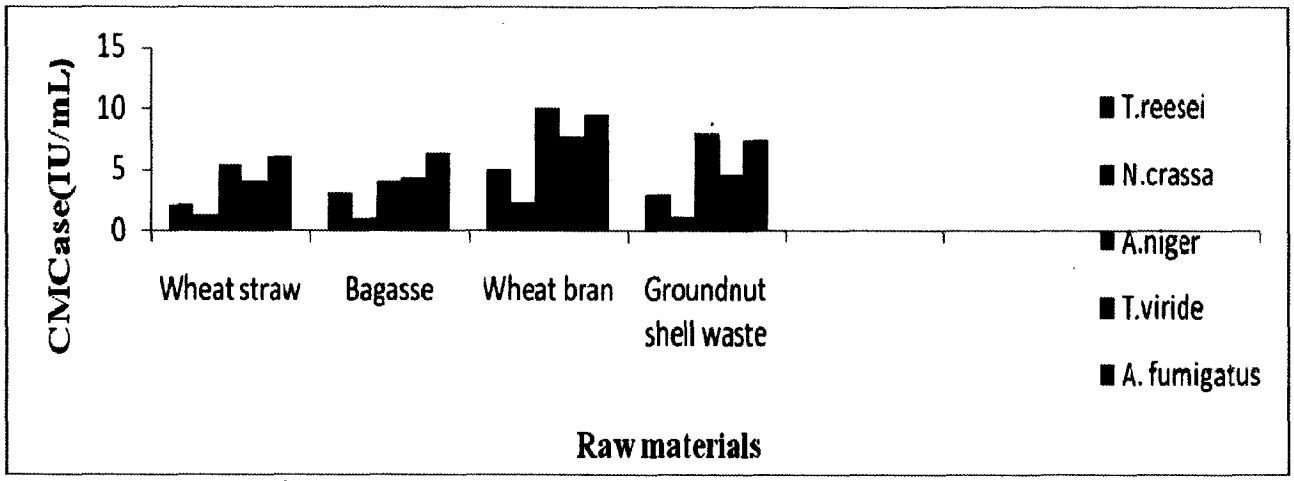


Fig 4.4.7. Comparative CMCase activity achieved by various fungal strains under conventional raw materials as carbon source at 30^{0}C and pH 5.

When we compared the enzyme activities achieved by different fungal strains under different raw material based fermentation than it was found that wheat bran, bagasse and ground nut shell waste

were quite effective raw materials for cellulase production as compared to wheat straw, whereas *T. viride* showed significantly higher cellulase activities under wheat bran and GSW based solid based solid state fermentation. *Neurospora crassa* showed somewhat higher cellulase activities under wheat bran followed by wheat straw based solid state fermentation. On the other hand higher cellulase activities were observed by *Aspergillus* strains under wheat bran and ground nut shell based solid state fermentation as observed from Table 4.4.5 and Figs 4.4.6 and 4.4.7. Scanning electron microscopic views also confirmed the effectiveness of raw materials for microbial growth and cellulase production as shown by Figs 4.4.8, 4.4.9, 4.4.10 and 4.4.11.

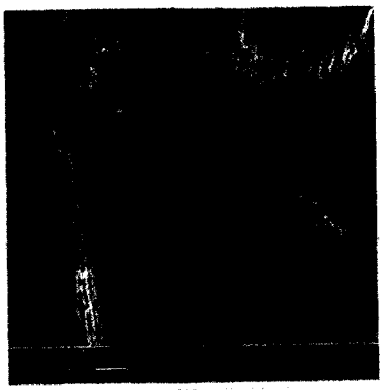


Fig 4.4.8. SEM micrograph of *A.niger* treated wheat bran solid bed under 1000X magnification

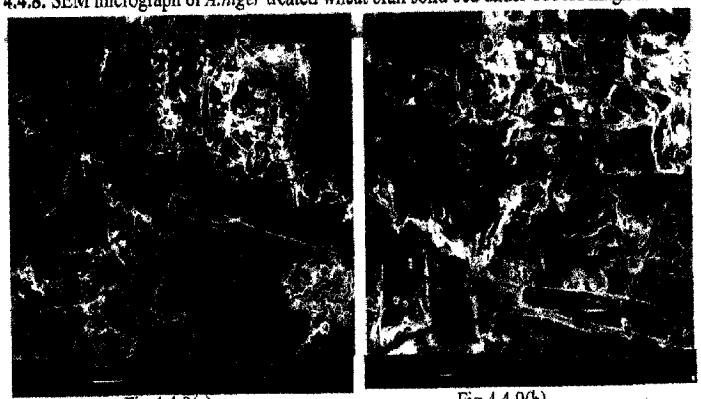


Fig 4.4.9(a) Fig 4.4.9(b)

Fig4.4.9. SEM micrograph of *A.niger* treated ground nut shell waste solid bed under 1000X 2000X magnification (4.4.9.a), (4.4.9.b) respectively.

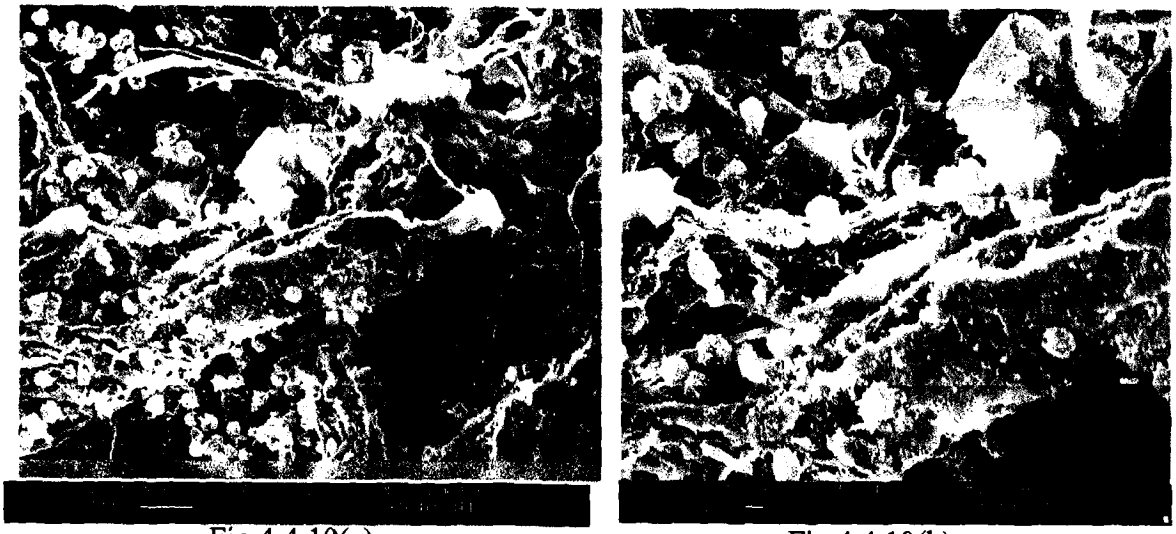


Fig 4.4.10(a) Fig 4.4.10(b)

Fig 4.4.10. SEM micrograph of *A.niger* treated bagasse solid bed under 1000X and 2000X magnification (4.4.10.a), (4.4.10.b) respectively.

Fig 4.4.11. SEM micrograph of *T. reesei* treated wheat straw waste solid bed under 1000X magnification.

4.4.3. Soluble protein determination of fungal strains under wheat bran based fermentation

Soluble protein was determined by prescribed Lowry et al method. It has been observed from Table 4.4.6 that *Trichoderma reesei* enzyme showed highest soluble protein (4.20 mg/mL) whereas least protein was observed with *Aspergillus niger* strain (0.986 mg/mL)

Table 4.4.6. Soluble protein and specific enzyme activity produced by fungal strains.

Fungal strains	Soluble protein(mg/mL)
Trichoderma reesei	4.20
Trichoderma viride	1.82
Neurospora crassa	1.95
Aspergillus niger	0.986
Aspergillus fumigatus	1.34

4.4.4. Application of pretreatment technology on cellulase production under bagasse, wheat straw and GSW based solid state fermentation

To investigate the effect of acid (H_2SO_4) (5%v/v) and alkali (NaOH) (5%w/v) pretreatments, separate sets of pretreatment experiments were carried out with acid (5%v/v) and alkali (5%w/v) on wheat straw, bagasse and ground nut shell waste. Acid and alkali pretreated raw materials were used separately for cellulase production under solid state fermentation at 30^0C.

Table 4.4.7. Comparative enzyme activities produced by *T.reesei, N.crassa* and *A. niger* under various pretreated conventional raw materials as carbon source.

Microbes / **Raw materials**	***T.reesei***		***T.viride***		***N.crassa***		***A. niger***		***A.fumigatus***	
	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
Wheat straw	2.19	2.23	1.04	3.11	1.31	4.08	0.889	5.41	1.33	6.09
Acid treated wheat straw	2.28	2.12	1.00	3.20	1.18	4.34	0.898	5.22	1.27	6.16
Alkali treated wheat straw	**2.95**	**2.88**	**1.12**	**3.63**	**1.73**	**4.93**	**1.13**	**5.98**	**1.54**	**6.67**
Bagasse	3.02	3.21	0.996	4.28	1.08	2.83	1.13	3.05	1.20	6.38
Acid treated	2.96	3.25	1.01	4.39	1.15	3.02	1.10	3.18	1.23	6.51
Alkali treated	**3.91**	**3.79**	**1.15**	**4.98**	**1.54**	**3.45**	**1.28**	**4.87**	**1.41**	**6.89**
Groundnut shell waste	2.97	3.05	1.56	4.59	1.18	3.52	1.00	8.05	1.09	7.43
Acid treated	2.95	3.08	1.50	4.51	1.23	3.56	1.04	8.13	1.06	7.40
Alkali treated	**3.63**	**3.76**	**1.76**	**4.98**	**1.67**	**3.82**	**1.29**	**8.64**	**1.10**	**7.65**

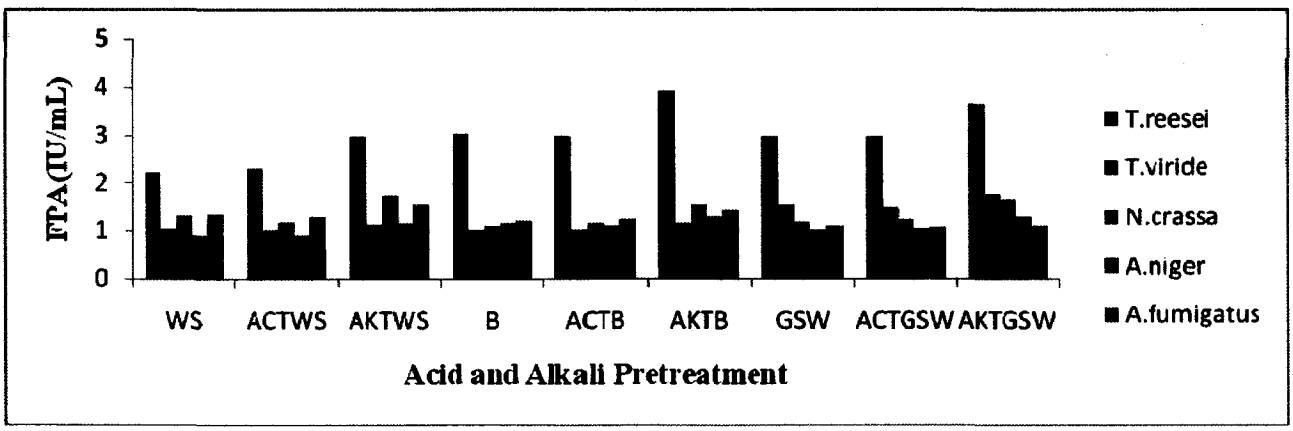


Fig 4.4.12. Comparative FPA achieved by various fungal strains under acid and alkali pretreated conventional raw materials as carbon source at 30^0C and pH 5.

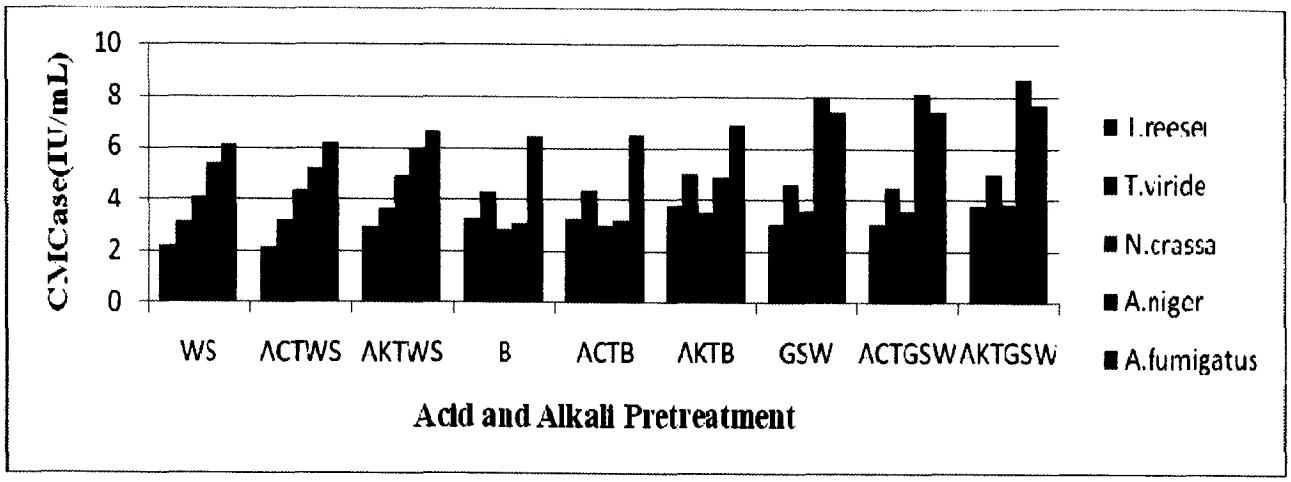


Fig 4.4.13. Comparative CMCase activity achieved by various fungal strains under acid and alkali pretreated conventional raw materials as carbon source at 30^0C and pH 5.

It has been observed from Table 4.4.7 and Figs 4.4.12 and 4.4.13 that fungal strains produced significantly higher cellulase activities under alkali pretreated raw materials based solid state fermentation as compared to acid pretreted.This might be due to the fact that alkali pretreatment are more focused on the solubilisation and removal of lignin from the biomass, which provides somewhat favorable conditions for fungal growth and cellulase production[55]. Due to the lignin removal more accessible surface areas of cellulose have been generated for fungal attack, therefore somewhat increase in the enzyme activities were observed. When compared the XRD patterns of untreated and alkali treated raw materials, we have observed that intensity of peak heights were increased in alkali pretreated raw materials as compared to untreated. This might be due to the fact that alkali treatment solubilize and removes major portion of lignin and because of this more crystalline nature of cellulosic biomass were exposed which may increased the peak heights as observed from Figs 4.4.14- 4.4.16. On the other reduction in lignin portion due to alkali pretreatment may also provides fruitful conditions for fungal growth and cellulase production.

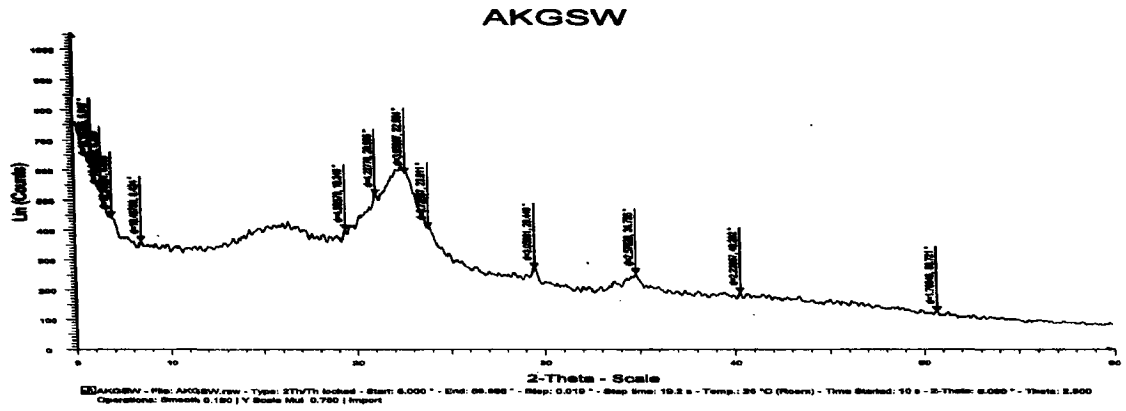


Fig 4.4.14. XRD pattern of alkali treated ground nut shell waste .

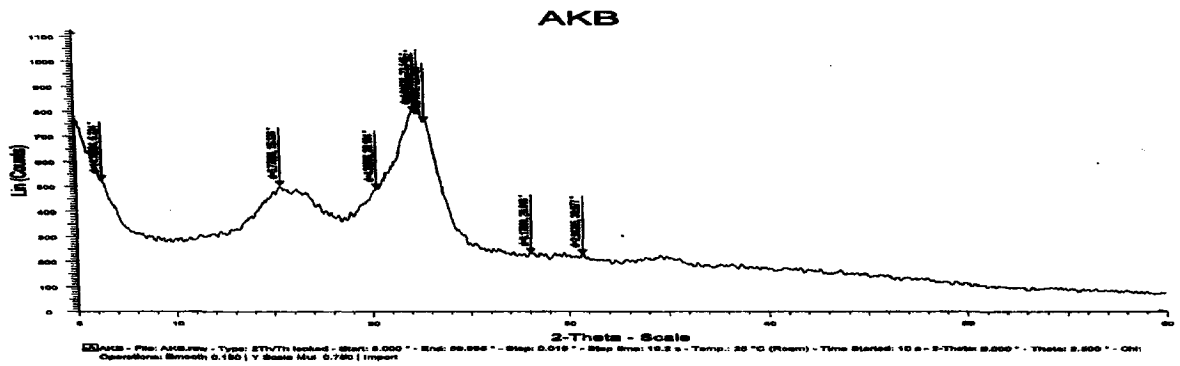


Fig 4.4.15. XRD pattern of alkali treated bagasse

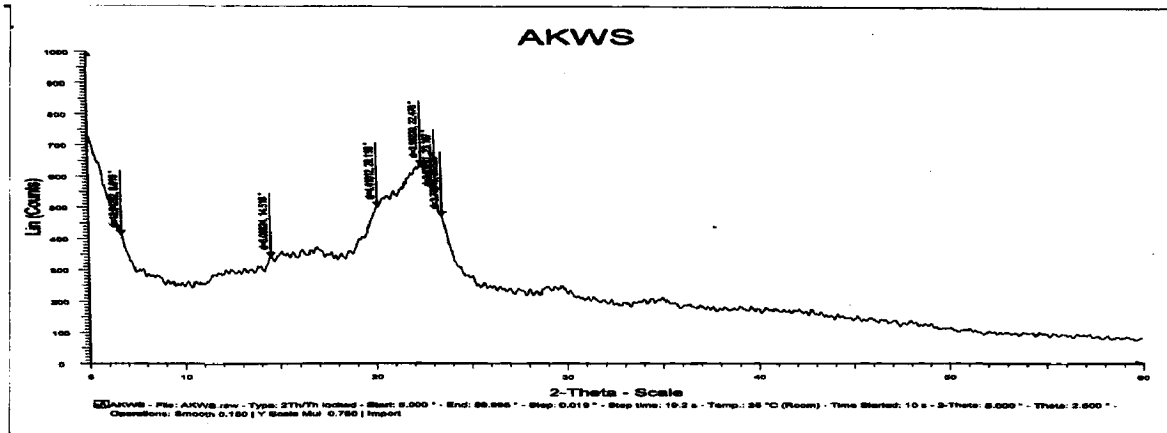


Fig 4.4.16. XRD pattern of alkali treated wheat straw

4.4.5. Application of mixed microbial cultures in cellulase production under wheat bran based solid state cultivation

To investigate the application of mixed microbial cultures in cellulase production separate set of experiments have been performed under wheat bran based solid state fermentation by using mixed fungal strains in the inoculums dosage ratio (1/2:1/2) and (1:1). It has been observed from Table 4.4.8 and Figs 4.4.17 and 4.4.18 that mixed microbial cultures of *Trichoderma reesei* and *Aspergillus niger* (1:1) was found much effective for both cellulase activities in terms of FPA (4.11IU/mL) and cellobiase (2.23IU/mL) as compared to the fungal strains *Trichoderma reesei* (FPA:4.72IU/mL; cellobiase: 0.986IU/mL) and *Aspergillus niger* (FPA:1.29IU/mL; cellobiase: 2.78IU/mL) used separately. As literature shows that for the complete saccharification of cellulosic biomass both FPA and cellobiase are required in sufficient ratio [146, 287], hence for the complete saccharification of cellulosic biomass cellulase produced by mixed microbial cultures would be a better option.

Table 4.4.8. Comparative FPA and cellobiase activities achieved by *Trichoderma* and *Aspergillus* strains based SSF.

Microbial strains	Inoculum dosages ratio	FPA(IU/mL)	Cellobiase(IU/mL)
Trichoderma reesei	1	4.72	0.986
Trichoderma viride	1	2.23	1.65
Aspergillus fumigatus	1	1.68	2.03
Aspergillus niger	1	1.29	2.78
Trichoderma reesei + ***Aspergillus fumigatus***	½: ½	3.51	1.37
Trichoderma viride + ***Aspergillus fumigatus***	½: ½	1.64	1.46
Trichoderma reesei + ***Aspergillus niger***	½: ½	**3.66**	**1.89**
Trichoderma viride + ***Aspergillus niger***	½: ½	1.41	1.94
Trichoderma reesei + ***Aspergillus fumigatus***	1:1	4.29	1.63
Trichoderma viride + ***Aspergillus fumigatus***	1:1	1.83	1.78
Trichoderma reesei + ***Aspergillus niger***	1:1	**4.11**	**2.23**
Trichoderma viride + ***Aspergillus niger***	1:1	1.68	2.36

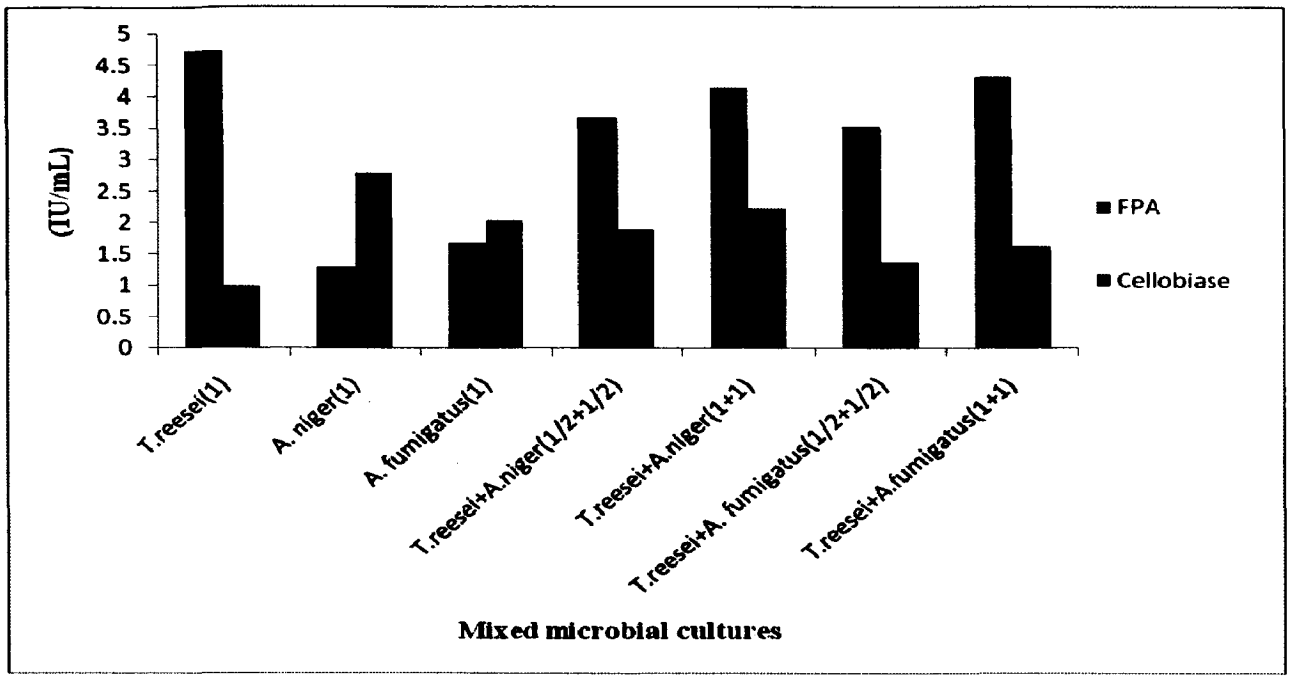


Fig 4.4.17.Comparative FPA and cellobiase activities achieved by *T.reesei* and *Aspergillus* strains based mixed microbial cultures

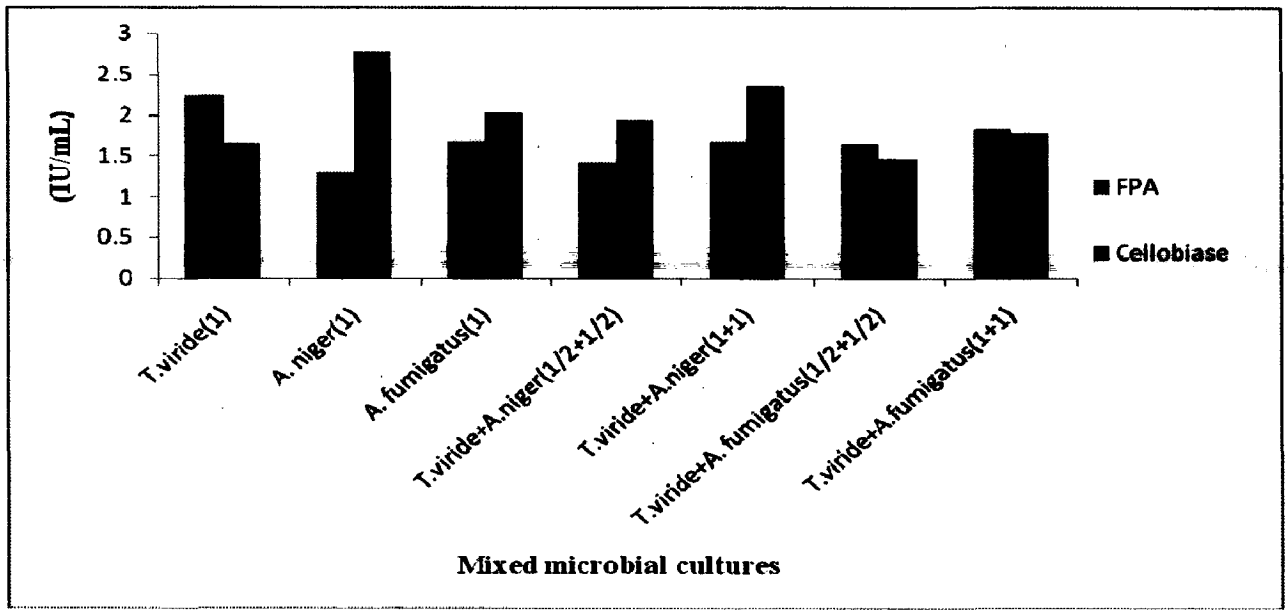


Fig 4.4.18. Comparative FPA and cellobiase activities achieved by *T.viride* and *Aspergillus* strains based mixed microbial cultures.

It has been also observed from Table 4.4.8 and Figs 4.4.17 and 4.4.18 that mixed microbial cultures with inoculum dosages ratio (1:1) were found much effective in terms of both FPA and cellobiase activities as compared with inoculum dosages ratio (1/2:1/2).*T.reesei* alongwith *A. fumigatus* was also found quite successful in terms of both FPA and cellobiase activities. In overall we can infer that mixed microbial cultures of *Trichoderma* and *Aspergillus* strains produced

complete set of cellulase activities in terms of both FPA and cellobiase reqiured for better utilisation of lignocellulosic biomass.

4.4.6. Utilization of mixed raw materials in cellulase production under solid state fermentation

To investigate the effectiveness of mixed raw materials in cellulase production under solid state cultivation, separate set of experiments have been performed using various raw materials in the ratio (g) 5+5, 3+3+4 and 2+3+2+3. It has been observed from Table 4.4.9 and Figs 4.4.19, 4.4.20, 4.4.21 and 4.4.22 that microbial strains produces significantly higher cellulase activities under mixed raw materials based fermentation compared to particular raw material taken singly. Cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei, T.viride, N.crassa, A.niger* and *A.fumigatus* were 3.87, 4.10: 3.42, 3.51: 3.02, 3.21; 1.60, 5.98: 1.45, 5.45: 0.996, 4.28; 1.75, 4.66: 1.59, 4.44: 1.08, 2.83; 0.774, 7.10: 0.956, 6.52: 1.13, 3.05; 1.47, 7.90: 1.43, 7.32: 1.20, 6.38 under (bagasse + wheat bran) (bagasse + wheat straw + wheat bran) and (only bagasse) based solid state fermentation correspondingly, on the other hand cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei, T.viride, N.crassa, A.niger* and *A.fumigatus* were 3.36, 3.55: 3.56, 3.71: 2.19, 2.23; 1.68, 5.85: 1.65, 5.35: 1.04, 3.11; 1.84, 5.25: 1.65, 4.34: 1.31, 4.08; 0.990, 7.83: 1.06, 7.15: 0.889, 5.41; 1.51, 7.78: 1.52, 7.68: 1.33, 6.09 under (wheat straw + wheat bran) (bagasse + wheat straw + wheat bran + ground nut shell waste) and (only wheat straw) based solid state fermentation. Fungal strains produces higher cellulase activity under bagasse mixed with wheat bran or bagasse blend with wheat straw and wheat bran as compared to only bagasse based solid state fermentation. On the other hand fungal strains also produces significantly higher activity under wheat straw mixed with wheat bran or wheat straw blend with bagasse, wheat bran and ground nut shell waste. It can be surmised from the above observation that effectiveness of particular raw material for product formation by microbial strains would be improved after inclusion of much effective raw materials to this.

Table 4.4.9. Comparative enzyme activity achieved by various fungal strains under mixed conventional raw materials based solid state fermentation at 30^{0}C and pH 5.

Mixed raw materials	Ratio in (g)	*T.reesei*		*T.viride*		*N.crassa*		*A. niger*		*A.fumigatus*	
		FPA	CMC ase	FPA	CMC ase	FPA	CMC ase	FPA	CMC ase	FPA	CMC ase
B+WB	5+5	3.87	4.10	1.60	5.98	1.75	4.66	0.774	7.10	1.47	7.90
WS+WB	5+5	3.36	3.55	1.68	5.85	1.84	5.25	0.990	7.83	1.51	7.78
GSW+WB	5+5	4.05	4.31	1.91	6.16	1.80	4.97	1.15	9.10	1.40	8.46
B+WS	5+5	2.60	2.76	1.02	4.15	1.20	3.45	0.760	4.76	1.19	6.25
B+GSW	5+5	3.06	3.15	1.28	4.40	1.12	3.18	0.921	6.10	1.16	6.90
WS+GSW	5+5	2.65	2.64	1.32	4.31	1.27	3.80	0.850	6.76	1.22	6.71
B+WS+WB	3+3+4	3.42	3.51	1.45	5.45	1.59	4.44	0.956	6.52	1.43	7.32
B+GSW+WB	3+3+4	3.95	4.01	1.59	5.56	1.53	4.21	1.04	7.50	1.35	7.80
WS+GSW+WB	3+3+4	3.46	3.59	1.63	5.61	1.61	4.71	1.00	7.92	1.39	7.61
B+WS+GSW	3+3+4	2.78	2.88	1.21	4.30	1.16	3.50	0.846	5.93	1.18	6.64
WS+WB+B +GSW	2+3+2+3	3.56	3.71	1.65	5.35	1.65	4.34	1.06	7.15	1.52	7.68

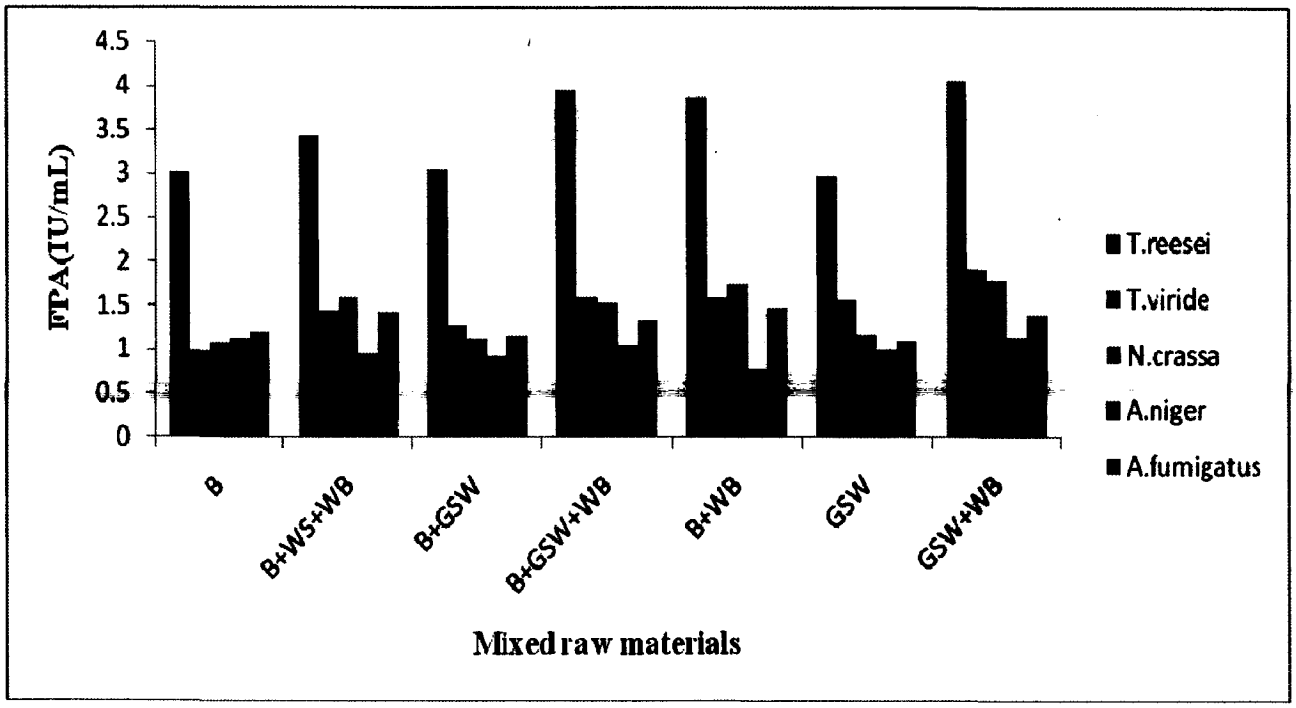


Fig 4.4.19. Comparative FPA achieved by various fungal strains bagasse based mixed conventional raw materials as carbon source at 30^{0}C and pH 5.

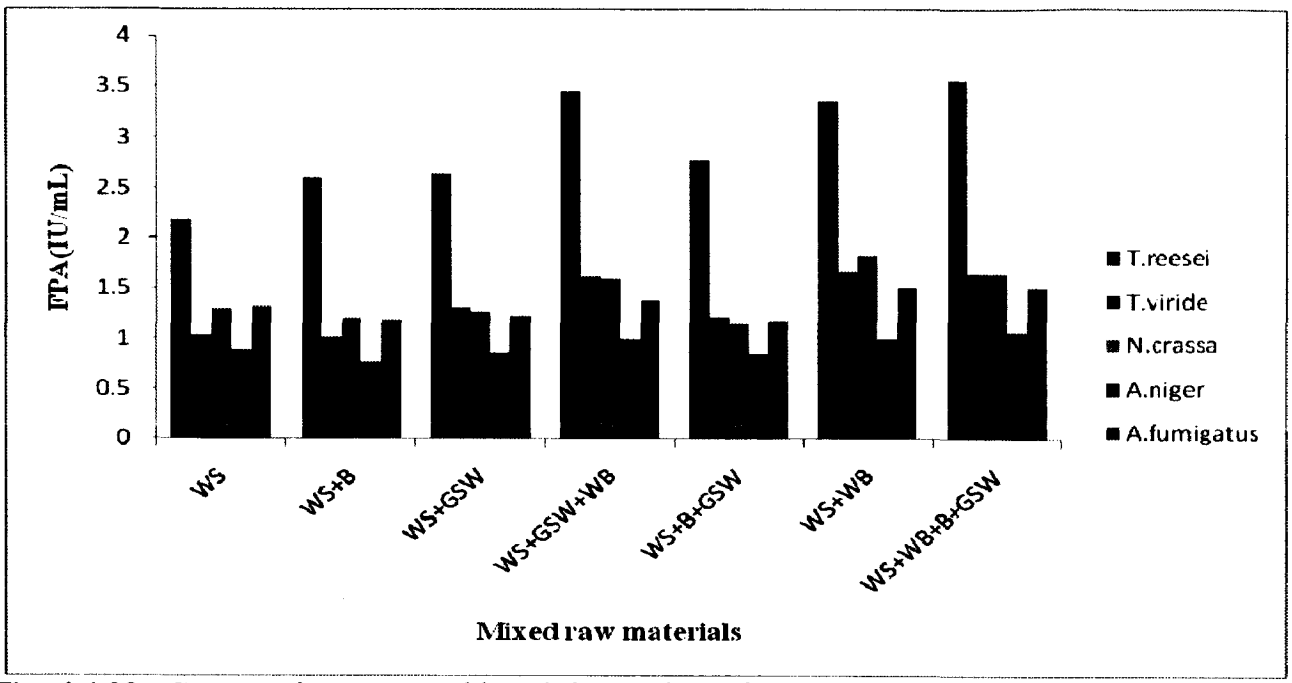


Fig 4.4.20. Comparative FPA achieved by various fungal strains under wheat straw based conventional raw materials as carbon source at 30^0C and pH 5.

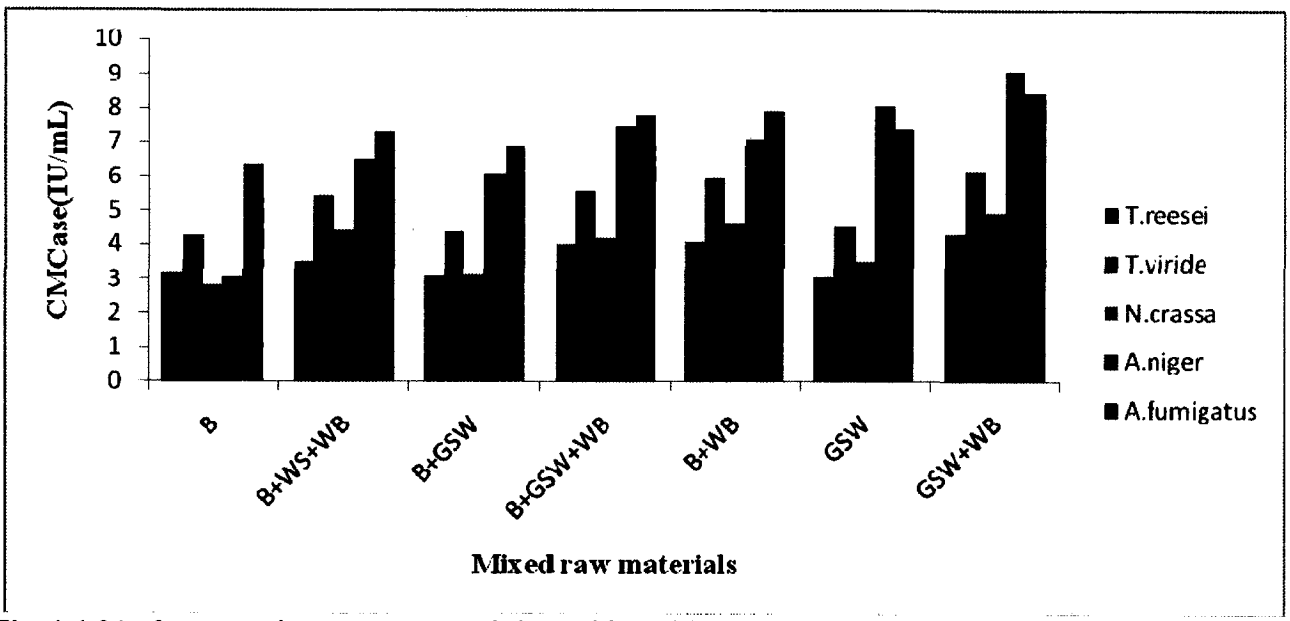


Fig 4.4.21. Comparative CMCase activity achieved by various fungal strains under bagasse based conventional raw materials as carbon source at 30^0C and pH 5.

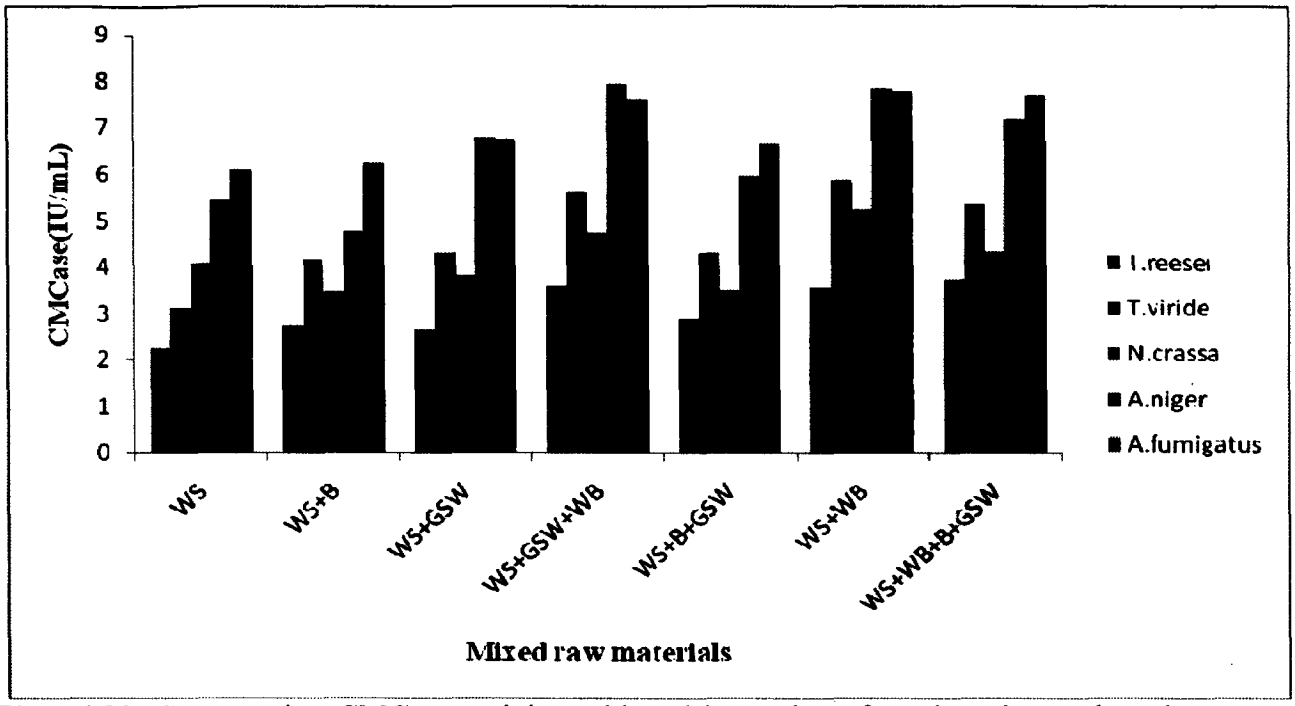


Fig 4.4.22. Comparative CMCase activity achieved by various fungal strains under wheat straw based conventional raw materials as carbon source at 30^0C and pH 5.

4.4.7. Effect of surfactants and their dosages on cellulase production under wheat bran based solid state fermentation

To explore the effect of surfactants and their dosages on cellulase production under wheat bran based solid state fermentation by various fungal strains, separate set of experiments have been performed using diverse type of surfactants with dosages (1g/L, 1.25g/L and 1.5g/L). It has been observed from Table 4.4.10 and Figs 4.4.23 and 4.4.24.that in between Tween series surfactants *T.reesei* produces higher cellulase activity under Tween-80 based solid state fermentation followed by Tween-20 surfactant whereas *T. viride* produces higher cellulase activity under Tween-80 based solid state fermentation followed by Tween-60. *N. crassa* showed higher cellulase activity under Tween-20 based solid state fermentation followed by Tween-80. On the other hand *Aspergillus* strains produces higher cellulase activity under Tween -80 followed by Tween-60.

Table 4.4.10. Comparative enzyme activity achieved by various fungal strains under various dosages of surfactant containing wheat bran based solid state fermentation at 30^0C and pH 5.

Surfactants used		*Trichoderma reesei*		*Trichoderma viride*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
		FPA	CMC ase	FPA	CMC ase	FPA	CMCase	FPA	CMC ase	FPA	CMC ase
T-80	1.0mL	4.72	5.02	2.23	7.74	2.36	6.36	1.29	10.10	1.68	9.49
	1.25mL	4.67	4.97	2.31	7.98	2.41	6.66	1.33	10.21	1.75	9.87
	1.50mL	4.58	4.79	2.27	7.80	2.29	6.53	1.39	10.25	1.63	9.55
T-60	1.0mL	4.05	4.33	2.08	7.11	2.03	5.82	1.12	8.32	1.41	8.02
	1.25mL	3.96	4.19	2.01	6.87	1.92	5.43	1.08	8.09	1.35	7.76
	1.50mL	3.79	4.03	1.98	6.56	1.84	5.02	1.01	7.89	1.28	7.61
T-20	1.0mL	4.21	4.51	1.95	6.33	2.45	6.81	1.03	7.94	1.30	7.11
	1.25mL	4.24	4.65	1.86	6.01	2.53	7.03	0.995	7.78	1.21	6.88
	1.50mL	4.10	4.33	1.79	5.68	2.31	6.55	0.932	7.54	1.10	6.59
Triton X-100	1.0mL	3.87	4.03	1.53	5.69	1.63	5.08	0.456	3.83	1.26	6.93
	1.25mL	3.67	3.87	1.47	5.12	1.49	4.77	0.401	3.38	1.21	6.71
	1.50mL	3.59	3.59	1.39	4.86	1.37	4.34	0.366	3.06	1.05	6.08
Oil of olive	1.0mL	4.91	5.32	1.89	7.08	2.29	6.18	1.41	10.86	1.59	8.87
	1.25mL	4.98	5.49	1.78	7.00	2.25	5.91	1.48	11.13	1.63	9.33
	1.50mL	5.06	5.81	1.71	6.77	2.18	5.73	1.43	10.97	1.51	9.02
Oil of castor	1.0mL	3.43	3.53	1.78	6.37	1.86	5.67	0.791	5.98	1.34	7.54
	1.25mL	3.15	3.02	1.61	6.11	1.77	5.43	0.721	4.88	1.21	7.11
	1.50mL	2.98	2.76	1.42	5.83	1.60	5.01	0.689	4.53	1.15	6.73

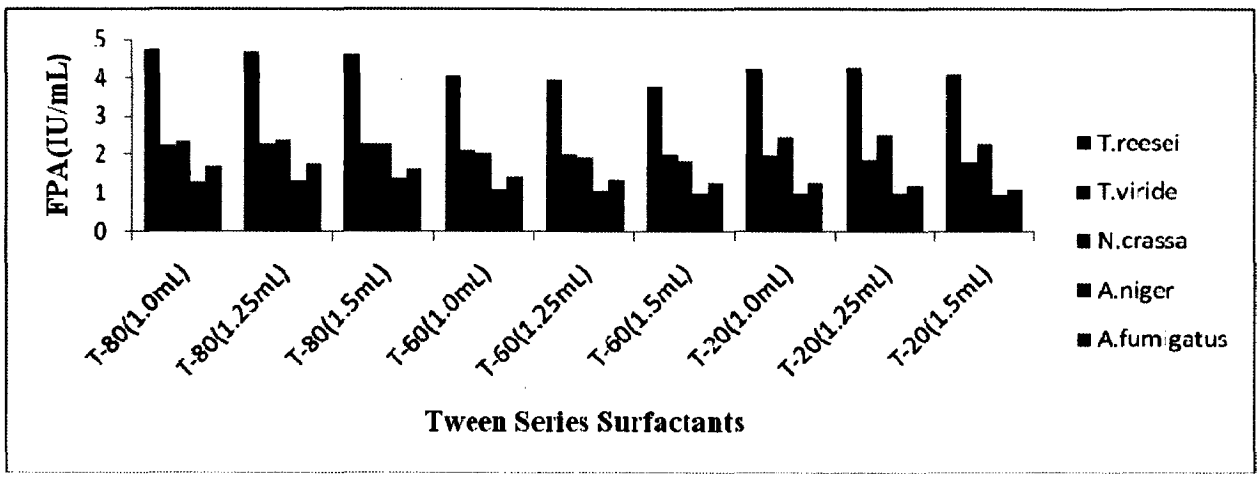


Fig 4.4.23. Comparative FPA achieved by various fungal strains under various dosages of Tween series based solid state fermentation at 30^0C and pH 5.

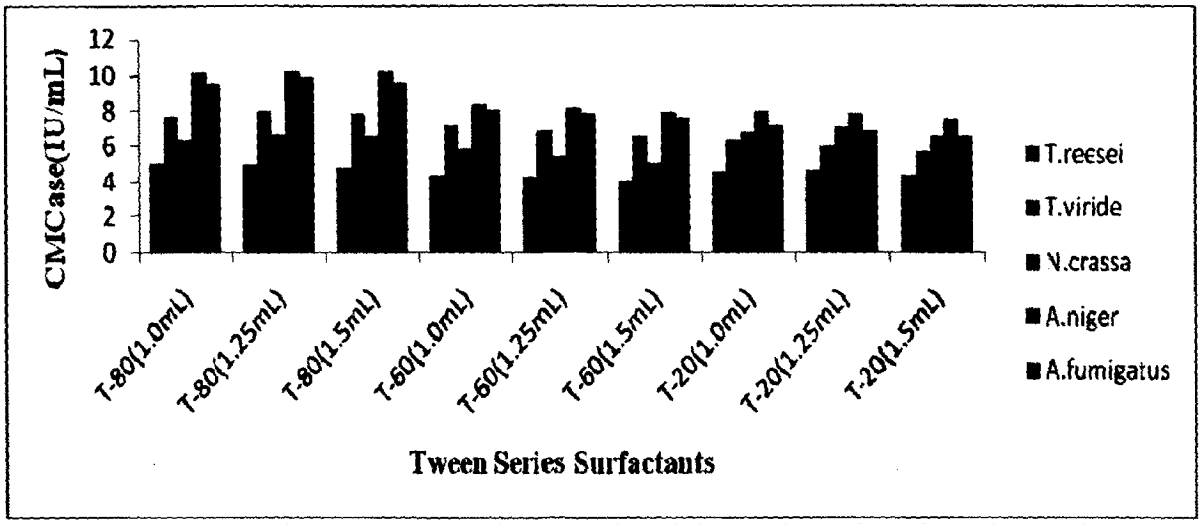


Fig 4.4.24. Comparative CMCase activity achieved by various fungal strains under various dosages of Tween series based solid state fermentation at 30^0C and pH 5.

When compared the cellulase activity produced by various fungal strains under oil of olive, oil of castor and Triton X- 100 based solid state fermentation, it was observed from Table 4.4.10 and Figs 4.4.25, 4.4.26 that *T.reesei* produces higher cellulase activity under oil of olive based solid state fermentation followed by Triton X- 100 whereas *T. viride, N.crassa* and *Aspergillus* strain produces significantly higher cellulase activity under oil of olive based solid state fermentation followed by oil of castor based solid state fermentation. Upon increasing the dosages of various surfactant under wheat bran based solid state fermentation, it has been observed that increment Tween-80 dosages was quite favorable for cellulase production by almost each strains except *reesei*. Reduction in enzyme activities were observed upon further increment in Tween-80 dosage except *A.niger* strain, underwhich enzyme activity was increased with the increment in Tween-dosages, whereas no significant improvement in the enzyme activity were observed with increasing the dosages of Tween-60 and Tween-20 surfactants under wheat bran based solid state fermentation for almost each fungal strains except *N.crassa*. Increment in the enzyme activities were observed with the increment in the Tween-20 dosages under wheat bran based solid state fermentation by *N.crassa*. It has also been also observed from Table 4.4.10 that further improvement significantly reduces the cellulase activity. Significant reduction in the cellulase activity has been observed with Triton X-100 based solid state fermentation by each strain. Decrement rate in the enzyme activity was somewhat lower under Triton X-100 based fermentation of *A. fumigatus*, which indicates the better sustainability of *A. fumigatus* under Triton X-100 based fermentation. On the other hand increment in oil of olive dosages enhance

enzyme activities produced by each fungal strains under wheat bran based solid state fermentation but it has also been observed that further increment in the dosages decreases the cellulase activity except *T.reesei*. *T.reesei* showed increment in enzyme activity with further improvement in oil of olive dosages, whereas significant reduction in the enzyme activity was observed with increasing the dosages of oil of castor.

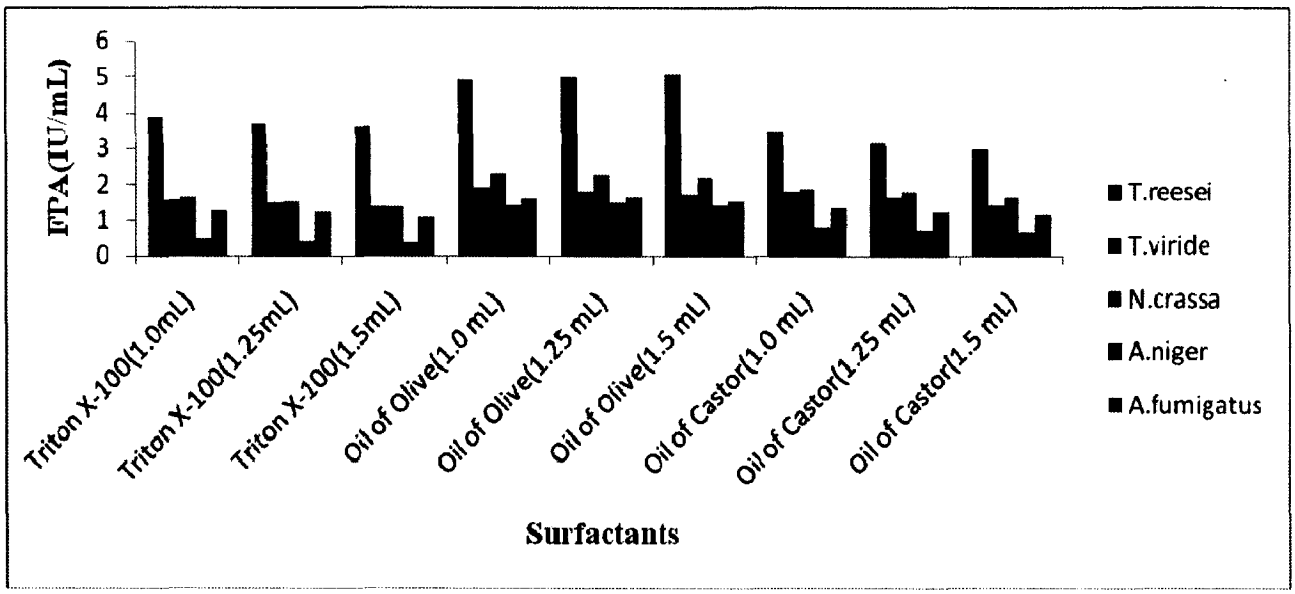


Fig 4.4.25. Comparative FPA achieved by various fungal strains under various dosages of surfactants based solid state fermentation at 30^0C and pH 5.

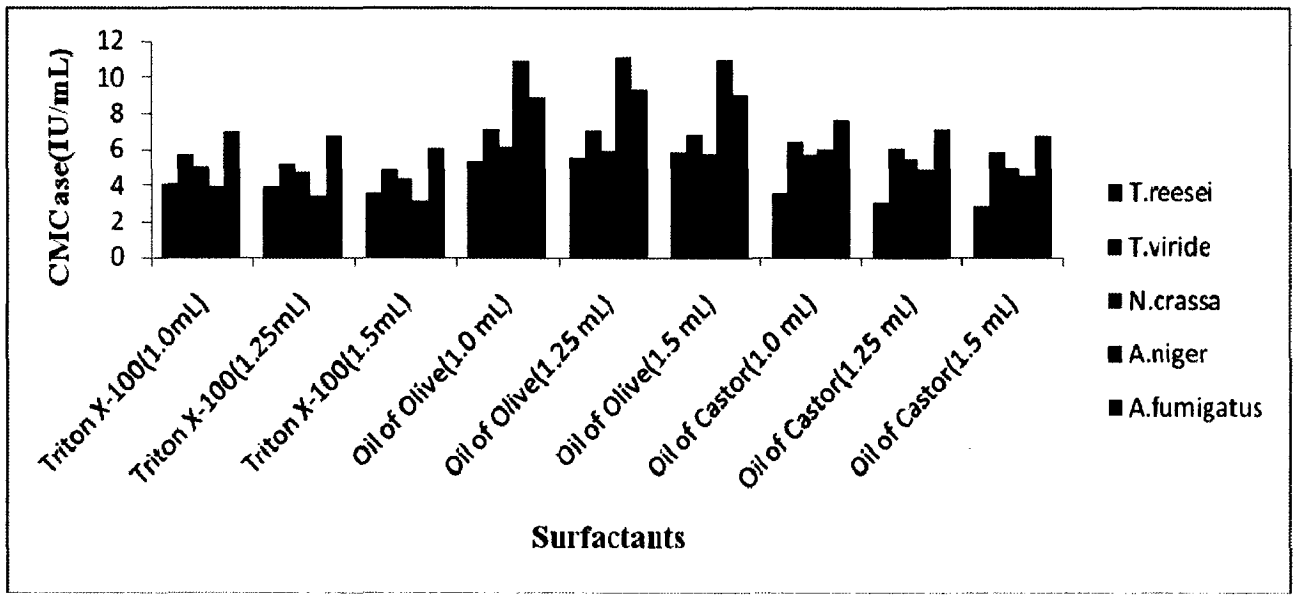


Fig 4.4.26. Comparative CMCase activity achieved by various fungal strains under various dosages of surfactants based solid state fermentation at 30^0C and pH 5.

4.4.8. Effects of nitrogen source and their dosages on cellulase production under wheat bran based solid state fermentation.

To investigate the effect of nitrogen sources and their dosages on cellulase production under wheat bran based solid state fermentation separate sets of experiments have been performed by using various types of nitrogen sources with different dosages (1g/L, 1.25g/L and 1.5g/L). It has been observed from Table 4.4.11 and Figs 4.4.27, 4.4.28 that meat extract and proteose peptone were found much effective nitrogen sources for cellulase production by *Trichoderma* strains under wheat bran based solid state fermentation. *N. crassa* showed better cellulase activity under meat peptone, meat extract and proteose peptone based solid state fermentation, on the other hand *Aspergillus* strains produced enhanced cellulase activity under meat peptone, proteose peptone and yeast extract based solid state fermentation. It has been also observed from Table 4.4.11 and Figs 4.4.27, 4.4.28 that upon increasing the dosages of nitrogen sources, significant reduction in the activities have been observed except yeast extract nitrogen source based fermentation which may be due the fact that wheat bran itself having good amount of protein hence increasing the dosages of nitrogen sources, fungal strains utilized excess nitrogen for fungal growth rather than for cellulase production, therefore enzyme activity has been reduced upon increasing the nitrogen sources under wheat bran based solid state fermentation. To minimize the cost of nitrogen source, cheap, natural, crude and raw baker's yeast has been used rather than pure yeast extract. It has been observed from Table 4.4.11 and Figs 4.4.29, 4.4.30. fungal strains especially *Aspergillus* strains produces significantly better cellulase activity under baker's yeast based solid state fermentation. Upon increasing the dosages of baker's yeast from 1g/L to 3g/L, significant improvement in the enzyme activity has been observed.

Table 4.4.11. Comparative enzyme activity (IU/mL) achieved by various fungal strains under various dosages of nitrogen sources containing wheat bran based solid state fermentation at 30^0C and pH 5.

Nitrogen sources used		*Trichoderma reesei*		*Trichoderma viride*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
		FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
Meat peptone	1.0g/L	4.63	4.91	2.11	7.25	2.39	6.31	1.21	9.45	1.73	9.63
	1.25g/L	4.57	4.61	2.05	6.61	2.41	6.27	1.17	9.15	1.76	9.59
	1.50g/L	4.32	4.36	2.08	6.73	2.23	5.91	1.10	8.75	1.61	9.05
Proteose peptone	1.0g/L	4.72	5.02	2.23	7.74	2.36	6.36	1.29	10.10	1.68	9.49
	1.25g/L	4.63	4.59	2.26	7.38	2.30	6.02	1.20	9.13	1.62	8.97
	1.50g/L	4.50	4.47	2.19	7.29	2.21	5.82	1.02	8.03	1.54	8.59
Meat extract	1.0g/L	4.81	5.14	2.18	7.43	2.47	6.59	1.08	8.29	1.53	8.67
	1.25g/L	4.73	4.79	2.08	6.81	2.38	6.09	1.03	7.98	1.41	8.35
	1.50g/L	4.63	4.67	1.97	6.34	2.21	5.76	0.998	7.81	1.37	7.97
Soya peptone	1.0g/L	4.51	4.79	2.01	6.96	2.07	6.28	1.10	8.56	1.47	8.41
	1.25g/L	4.39	4.31	1.92	6.29	1.98	5.29	1.05	8.09	1.51	8.55
	1.50g/L	4.36	4.34	1.83	6.10	1.75	4.89	0.993	7.73	1.27	7.42
Beef extract	1.0g/L	4.13	4.27	1.61	5.33	1.98	5.19	0.952	7.51	1.21	7.31
	1.25g/L	4.01	3.97	1.41	4.59	2.02	5.28	0.912	7.09	1.08	6.88
	1.50g/L	3.78	3.69	1.32	4.08	1.71	4.67	0.894	6.78	0.994	6.33
Yeast extract	1.0g/L	4.47	4.71	1.97	6.67	2.18	5.81	1.16	10.21	1.65	9.19
	1.25g/L	4.44	4.59	2.00	6.88	2.23	5.84	1.29	10.53	1.78	9.62
	1.50g/L	4.23	4.19	1.89	6.34	2.02	5.13	1.07	8.34	1.62	9.08
Baker's yeast	1.0g/L	3.48	3.73	1.33	4.62	1.64	4.58	0.967	7.41	1.02	6.77
	2.5g/L	4.06	3.97	1.56	5.11	1.98	5.11	0.995	7.89	1.53	8.56
	3.0g/L	4.21	4.32	1.88	6.39	2.03	5.21	1.19	10.17	1.76	9.71

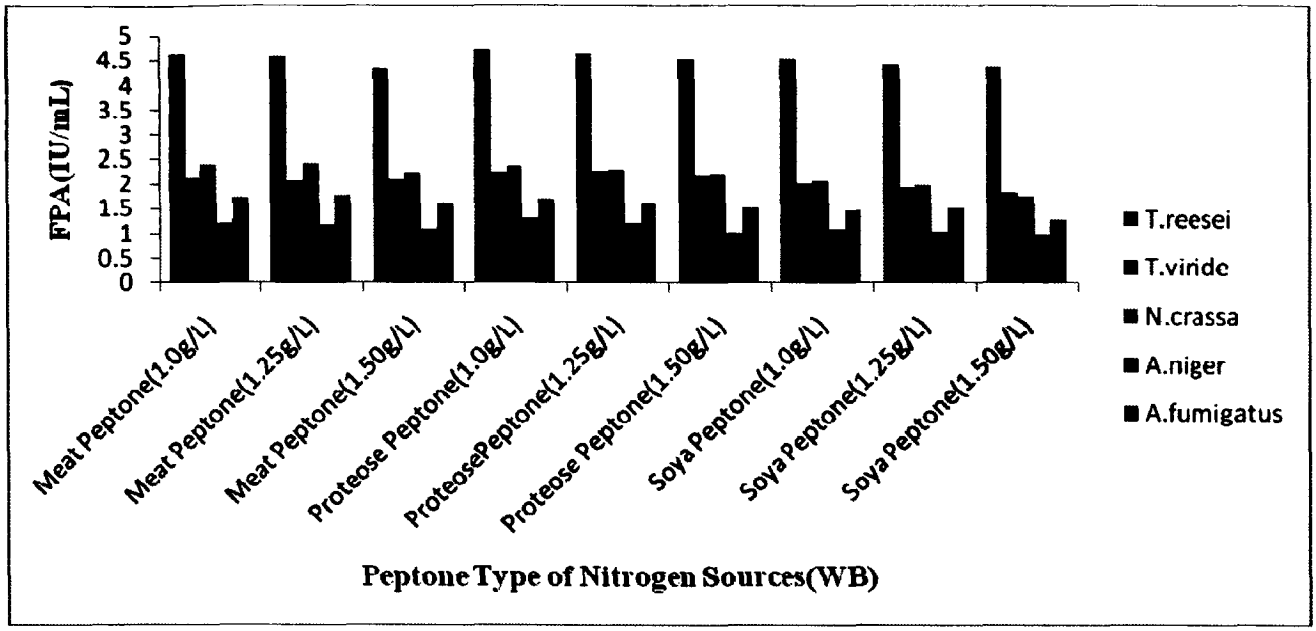


Fig 4.4.27. Comparative FPA achieved by various fungal strains using various dosages of peptone type of nitrogen sources under wheat bran based solid state fermentation at 30^0C and pH 5.

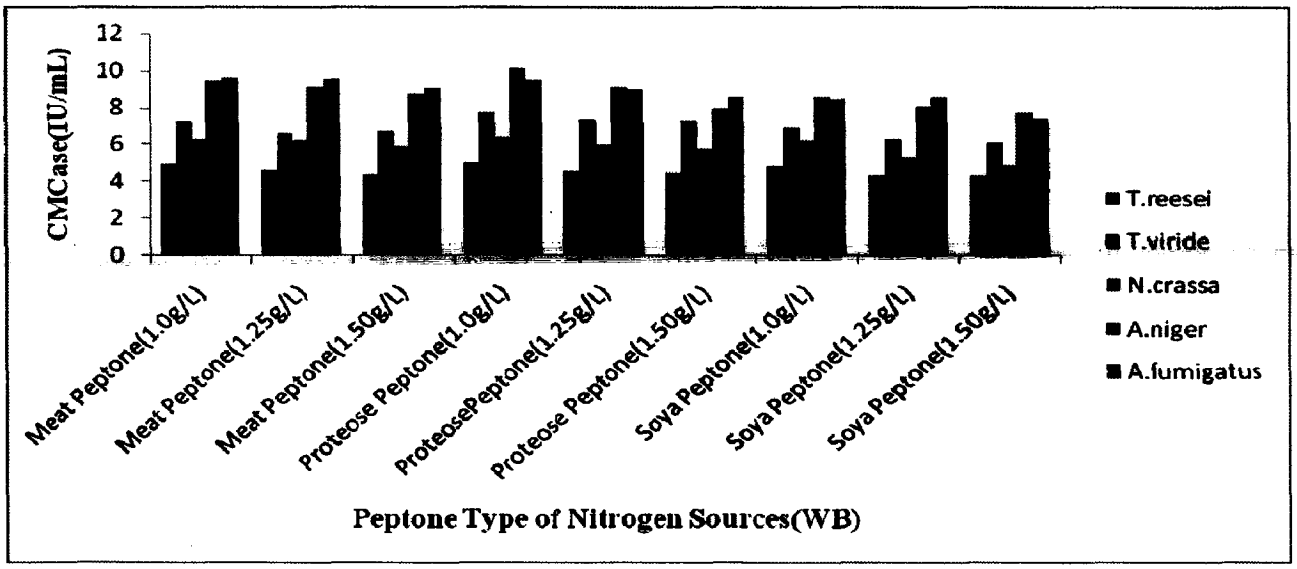


Fig 4.4.28. Comparative CMCase activity achieved by various fungal strains using various dosages of peptone type of nitrogen sources under wheat bran based solid state fermentation at 30^0C and pH 5.

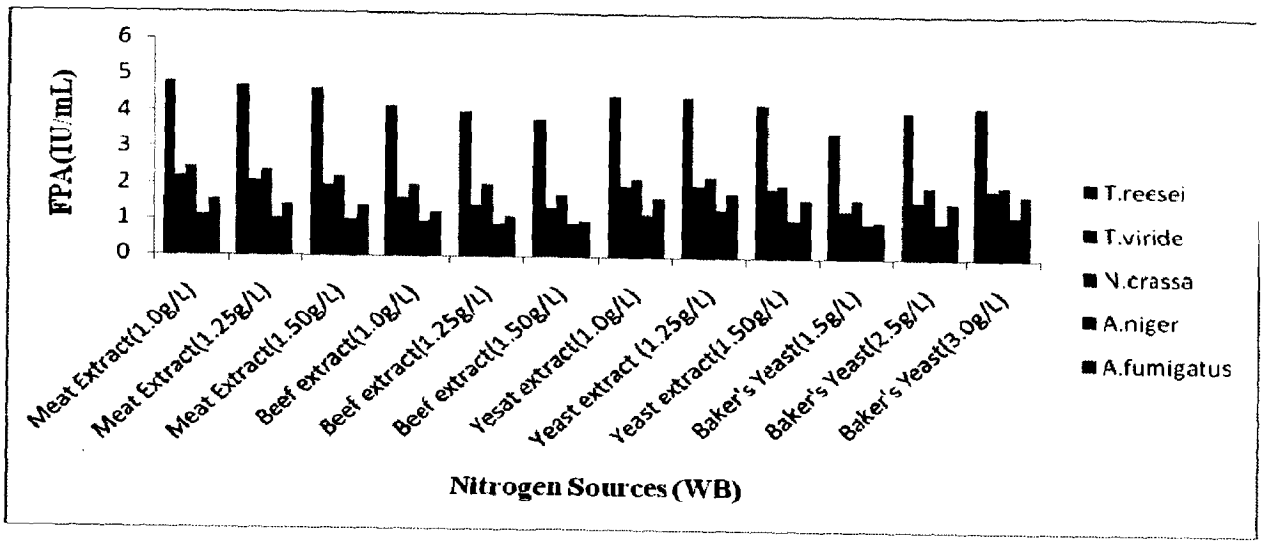


Fig 4.4.29. Comparative FPA achieved by various fungal strains using various dosages of othe type of nitrogen sources under wheat bran based solid state fermentation at 30^0C and pH 5.

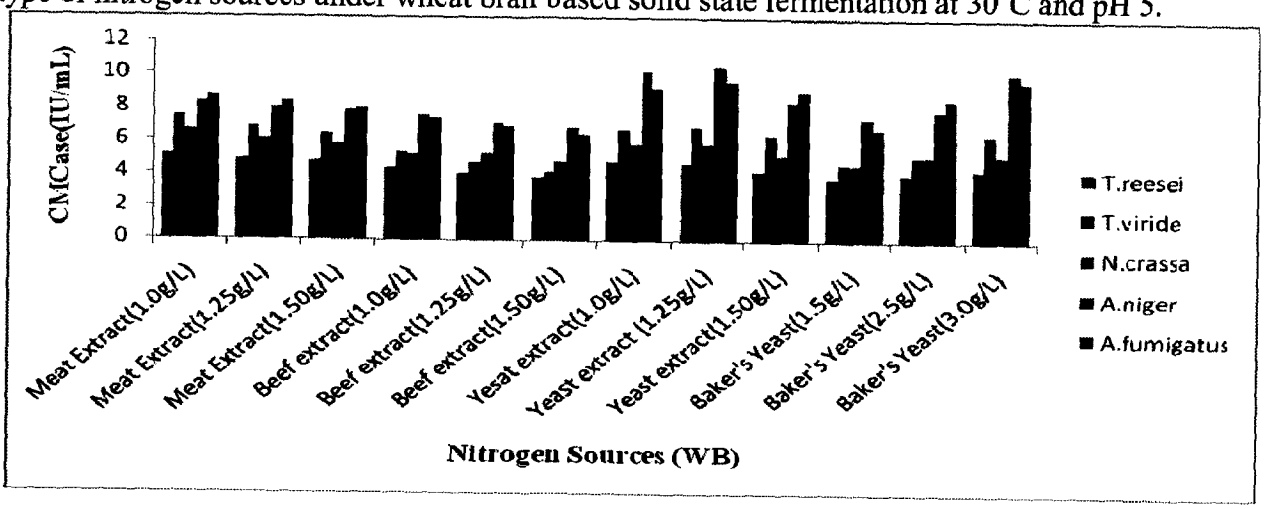


Fig 4.4.30. Comparative CMCase activity achieved by various fungal strains using various dosages of other type of nitrogen sources under wheat bran based solid state fermentation at 30^0C and pH 5.

4.4.9.Effects of nitrogen sources and their dosages on cellulase production under bagasse based solid state fermentation.

To investigate the effect of nitrogen sources and their dosages on cellulase production under bagasse based solid state fermentation separate sets of experiments have been performed by using various types of nitrogen sources with different dosages (1g/L, 1.25g/L and 1.5g/L). It has been observed from Table 4.4.12 and Figs 4.4.31, 4.4.32 that meat peptone, meat extract, yeast extract and proteose peptone were found quite effective nitrogen sources for cellulase production by *Trichoderma reesei* and *Aspergillus* strains under bagasse based solid state fermentation, whereas *T.viride* and *N. crassa* showed significantly better cellulase activities under meat peptone, meat extract and proteose peptone based fermentation. It has been also observed from Table 4.4.12 and

Figs 4.4.31-4.4.34 that upon increasing the dosages of nitrogen sources, significant increment in the activities have been observed except soya peptone and beef extract based fermentation. It has been observed from Table 4.4.12 and Figs 4.4.33- 4.4.34 fungal strains especially *Aspergillus* strains produces significantly better cellulase activity under baker's yeast based solid state fermentation. Upon increasing the dosages of baker's yeast from 1g/L to 3g/L, significant improvement in the enzyme activity has been observed for each fungal strains. It can be inferred that increment in the dosages of nitrogen sources are quite favorable for enhancement in the enzyme activity under bagasse based solid state fermentation as compared to wheat bran.

Table 4.4.12. Comparative enzyme activity (IU/mL) achieved by various fungal strains under various dosages of nitrogen sources containing bagasse based solid state fermentation at 30^0C and pH 5.

Nitrogen sources used		*Trichoderma reesei*		*Trichoderma viride*		*Neurospora crassa*		*Aspergillus niger*		*Aspergillus fumigatus*	
		FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase	FPA	CMCase
Meat peptone	1.0g/L	3.13	3.59	0.954	4.18	1.12	3.05	0.891	4.46	1.14	6.11
	1.25g/L	3.23	3.65	0.951	4.23	1.21	3.30	0.902	4.57	1.21	6.51
	1.50g/L	3.17	3.60	0.943	4.12	1.15	3.15	0.877	4.41	1.09	5.81
Proteose peptone	1.0g/L	3.02	3.21	0.996	4.28	1.08	2.83	0.833	4.05	1.20	6.38
	1.25g/L	3.10	3.61	1.07	4.71	1.19	3.29	0.840	4.23	1.23	6.61
	1.50g/L	2.96	3.43	0.983	4.33	1.14	3.08	0.828	4.21	1.14	6.23
Meat extract	1.0g/L	3.32	3.83	0.975	4.29	1.21	3.33	0.813	4.07	1.06	5.71
	1.25g/L	3.45	3.94	0.983	4.41	1.32	3.65	0.810	3.96	1.01	5.54
	1.50g/L	3.48	4.05	0.969	4.19	1.28	3.51	0.802	4.02	0.984	5.21
Soya peptone	1.0g/L	3.03	3.51	0.902	3.87	0.981	2.98	0.796	3.91	0.988	5.35
	1.25g/L	2.91	3.37	0.891	3.91	0.984	2.61	0.783	3.78	0.976	5.29
	1.50g/L	2.78	3.21	0.873	3.83	0.972	2.65	0.789	3.83	0.954	5.08
Beef extract	1.0g/L	2.29	2.51	0.772	3.29	0.935	2.55	0.602	3.11	0.922	4.89
	1.25g/L	2.01	2.26	0.751	3.11	0.923	2.51	0.583	2.93	0.876	4.72
	1.50g/L	1.97	2.11	0.719	3.01	0.911	2.46	0.554	2.55	0.855	4.31
Yeast extract	1.0g/L	2.96	3.43	0.839	3.71	1.02	2.87	0.826	4.25	1.35	7.28
	1.25g/L	3.01	3.52	0.835	3.67	1.13	3.01	0.839	4.37	1.46	7.79
	1.50g/L	2.78	3.20	0.821	3.51	1.08	2.92	0.833	4.31	1.41	7.47
Baker's yeast	1.0g/L	2.22	2.62	0.780	3.31	0.911	2.31	0.713	3.63	1.01	5.67
	2.5g/L	3.03	3.47	0.821	3.62	0.934	2.51	0.809	4.16	1.27	6.73
	3.0g/L	3.10	3.67	0.845	3.80	0.987	2.71	0.829	4.33	1.46	7.61

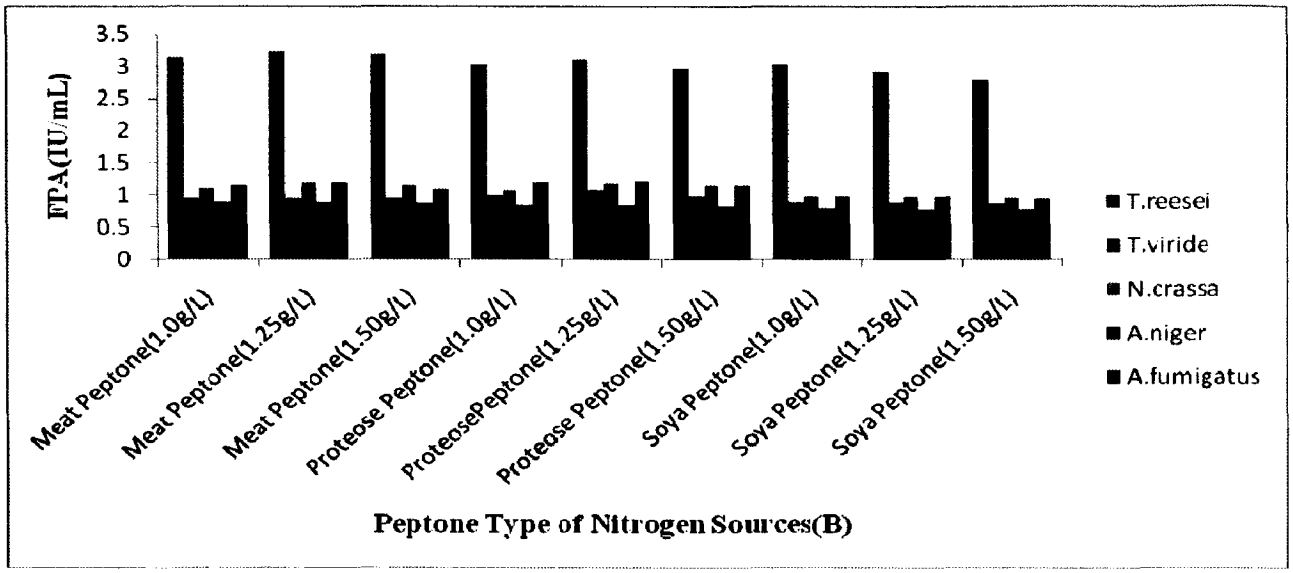


Fig 4.4.31. Comparative FPA achieved by various fungal strains using various dosages of peptone type of nitrogen sources under bagasse based solid state fermentation at 30^0C and pH 5.

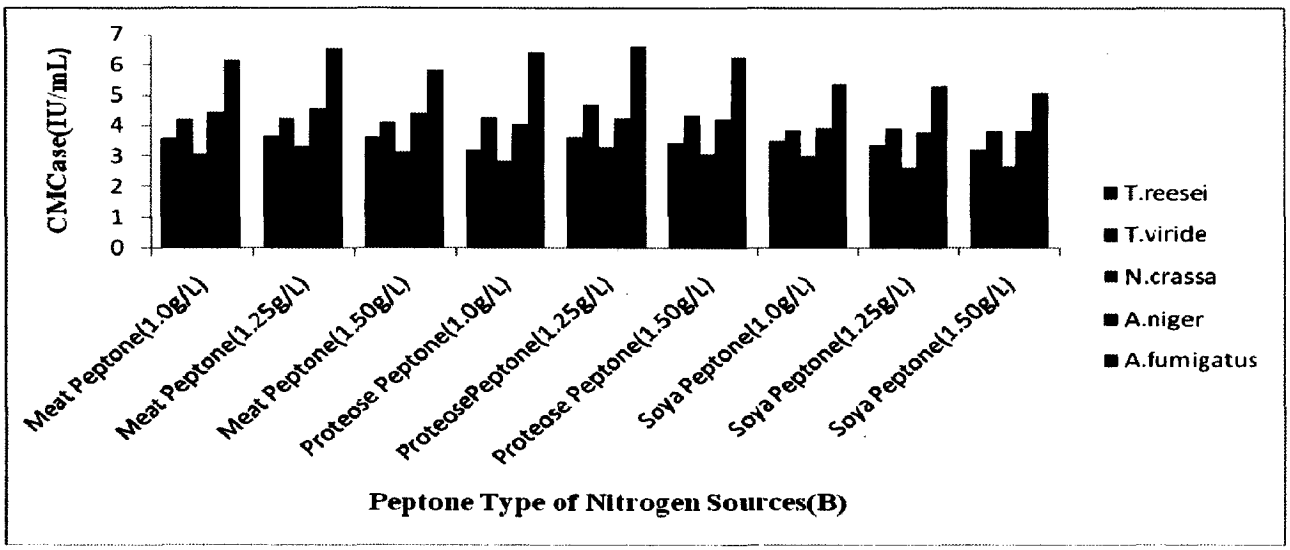


Fig 4.4.32. Comparative CMCase activity achieved by various fungal strains using various dosages of peptone type of nitrogen sources under bagasse based solid state fermentation at 30^0C and pH 5.

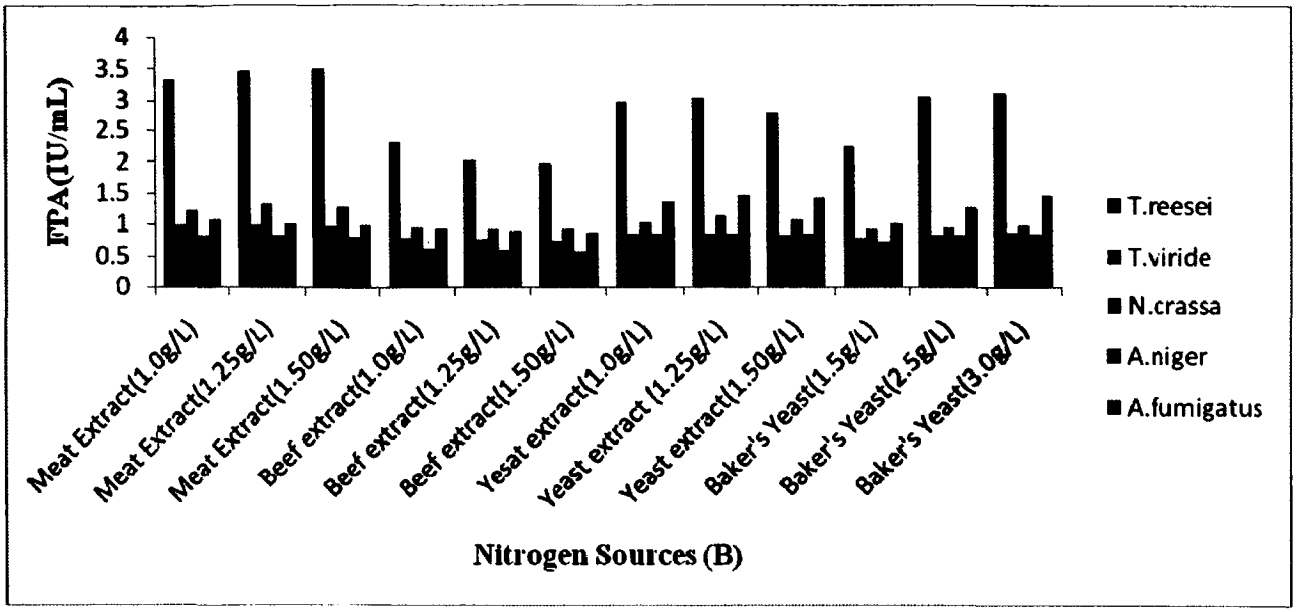


Fig 4.4.33. Comparative FPA achieved by various fungal strains using various dosages of other type of nitrogen sources under bagasse based solid state fermentation at 30^{0}C and pH 5.

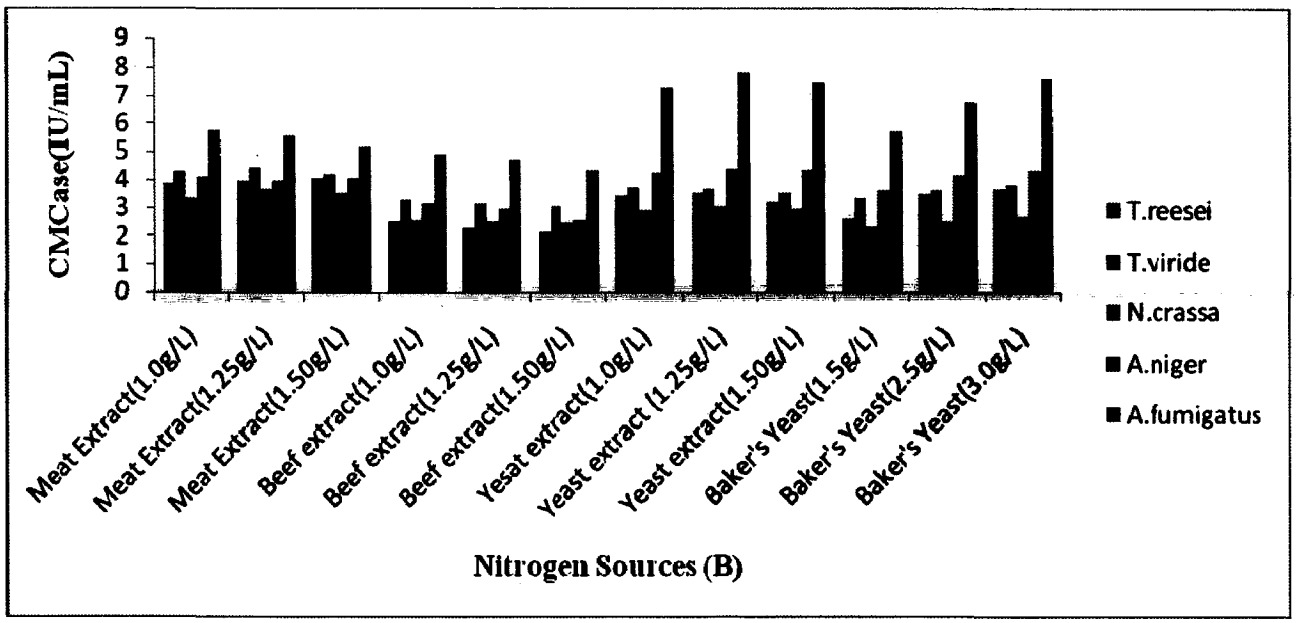


Fig 4.4.34. Comparative CMCase activity achieved by various fungal strains using various dosages of other type of nitrogen sources under bagasse based solid state fermentation at 30^{0}C and pH 5.

4.4.10.Effects of particle size and their combinations on cellulase production under wheat bran based solid state fermentation.

To investigate the effects of particle size and their combinations on cellulase production under wheat bran based solid state fermentation, separate sets of experiments have been performed by using different particle sizes of the wheat bran (9F, 9M, 9R, 6M+3R, 6M+3F, 6R+3M, 6F+3M,

3F+3R+3M) under solid state fermentation by using various fungal strains. It has been observed from the Table 4.4.13 and Fig 4.4.35 that 9F coded particle size of wheat bran was found not suitable for cellulase production by *T.reesei*, whereas 9R coded particle size of wheat bran was found quite unfavorable for cellulase production by *N.crassa* and *A.niger*. As literature reported that smaller and larger particle sizes of wheat bran is not quite suitable for the product formation by microbial system due to the fact that at smaller particle size, inter particle porosity is less while surface area is high, whereas in larger particle sizes, inter particle porosity is high with low surface area. On the other hand 9M coded particle size of wheat bran was found much suitable for the cellulase production this might be due to the fact that at medium sized particle both inter particle porosity and surface area is high, which provides a better situation for product formation by microbial strains due to better mass and heat transfer. The too smaller substrate particle sizes may also results in substrate accumulation, which may interfere microbial respiration/aeration and therefore result in poor growth [272]. As observed from the Table 4.4.13. 6M+3F combination of wheat bran particle size exhibited higher cellulase activity (1.36 IU/mL) by *Aspergillus niger*, whereas *T.reesei* (4.72IU/mL) and *N. crassa* (2.36 IU/mL) showed higher cellulase activity under 9M coded particle size of wheat bran. The order of the cellulase activity in terms of FPA produced by *T.reesei*, *N.crassa*, *Aspergillus niger* at different particle size and their combinations are as follows: 9M> 6M+3R> 6M+3F > 6R+3M> 9R> 3R+3M+3F> 6F+3M> 9F ; 9M > 6M+3F> 6F+3M> 9F> 3R+3M+3F> 6M+3R> 6R+3M> 9R; 6M+3F> 9M> 6R+3M> 3R+3M+3F> 9R> 6F+3M> 9F> 6M+3R. It has been concluded that medium sized (850μm) particle of wheat bran having better suitability cellulase production by almost each of the fungal strains.

Table 4.4.13. Effects of particle size combination on cellulase activity (IU/mL) under wheat bran solid bed by various fungal strains

Treatment	Particle sizes(μm)	*T.reesei*		*N. crassa*		*A. niger*	
		FPA	CMCase	FPA	CMCase	FPA	CMCase
9M(1)	850	4.72	5.02	2.36	6.36	1.29	10.10
9F(1)	<850	2.82	3.05	1.97	5.39	0.996	7.98
9R(1)	>850	3.69	3.98	1.17	3.01	0.645	4.97
6M+3F(2:1)	(850)+ (<850)	3.91	4.23	2.09	5.71	1.36	10.56
6M+3R(2:1)	(850)+ (>850)	4.36	4.78	1.69	4.45	1.06	8.39
6R+3M(2:1)	(>850)+(850)	3.89	4.06	1.28	3.29	0.856	6.43
6F+3M(2:1)	(<850)+(850)	3.21	3.35	2.01	5.56	1.13	8.89
3R+3F+3M(1:1:1)	(850)+(>850)+(<850)	3.53	3.81	1.79	4.87	0.970	7.32

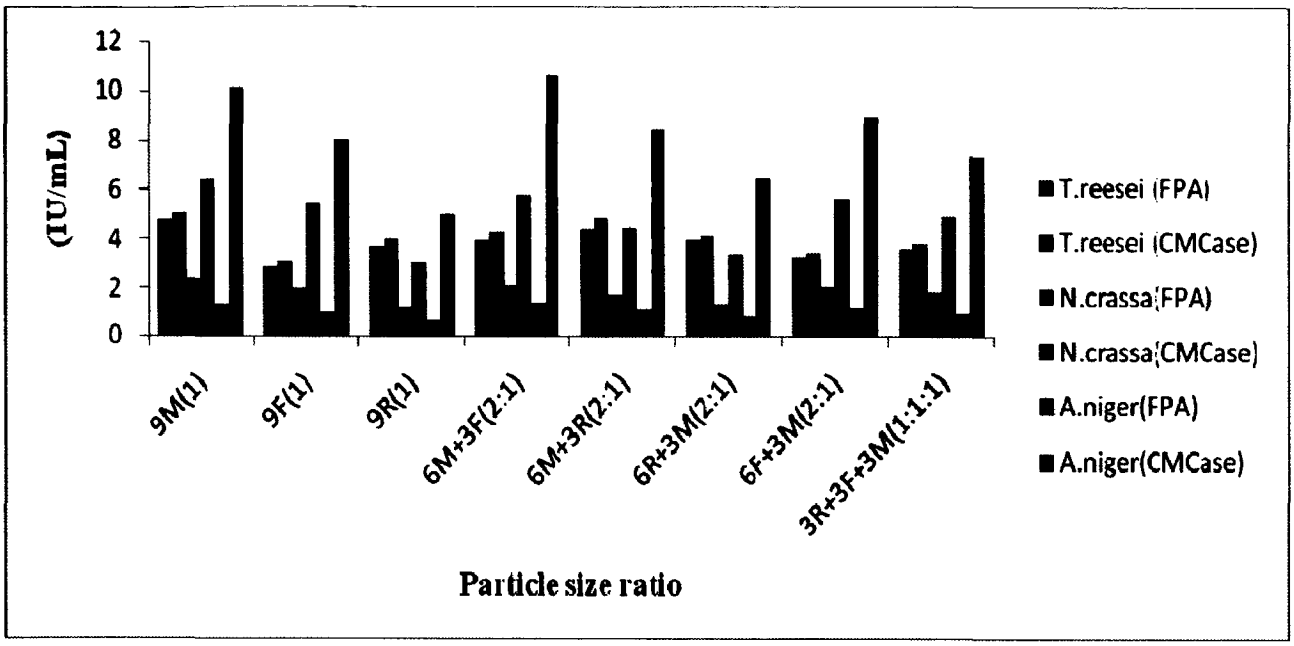


Fig 4.4.35. Comparative enzyme activities achieved by various fungal strains under different particle size of wheat bran and their combinations as carbon source at 30^0C and pH 5.

4.4.11. Effect of inclusion of pure sugars and sugars alcohol on cellulase production under wheat bran based solid state cultivation.

To explore the effect of pure sugars and sugars alcohol inclusion on cellulase production under wheat bran based solid state cultivation, separate set of batch experiments have been performed by incorporating pure sugars under wheat bran based solid state fermentation. It has been observed from Table 4.4.14 and Figs 4.4.36, 4.4.37, 4.4.38 that *T.reesei* showed better FPA and CMCase (IU/mL) activities under wheat bran based solid state fermentation supplemented with lactose (5.54, 5.51), carboxymethyl cellulose (5.47, 5.65), cellobiose (5.39, 5.60) and sucrose (5.23, 5.59) pure sugars respectively, whereas *N.crassa* showed better FPA and CMCase (IU/mL) activities under wheat bran based solid state fermentation supplemented with maltose (2.83, 7.37), sucrose (2.72, 7.18), carboxymethyl cellulose (2.67, 7.01) pure sugars respectively. On the other hand *Aspergillus niger* showed higher FPA and CMCase (IU/mL) activities under wheat bran based solid state fermentation supplemented with cellobiose (1.51, 11.38), maltose (1.45, 11.02) and trehalose (1.43, 11.08) pure sugars respectively.

Table 4.4.14. Comparative enzyme activities (IU/mL) achieved by various fungal strain under wheat bran incorporated with pure sugars based fermentation at 30^{0}C and pH 5.

Microorganisms	*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
Sugars/ Sugar alcohols	FPA	CMCase	FPA	CMCase	FPA	CMCase
WB	4.72	5.02	2.36	6.36	1.29	10.10
WB+Arabinose	4.83	5.11	2.45	6.56	1.34	10.40
WB+cellobiose	5.39	5.60	2.61	7.03	1.51	11.38
WB+CMC	5.47	5.65	2.67	7.01	1.41	10.91
WB+Trehalose	4.94	5.15	2.37	6.38	1.43	**11.08**
WB+Maltose	5.01	5.40	2.83	7.37	1.45	11.02
WB+Lactose	5.54	5.51	2.63	7.05	1.39	10.63
WB+Sucrose	5.23	5.59	2.72	7.18	1.36	10.78
WB+xylose	5.16	5.38	2.56	6.76	1.33	10.48
WB+Sorbitol	4.89	5.18	2.51	6.57	1.35	10.46
WB+Mannitol	4.75	5.09	2.41	6.48	1.31	10.16

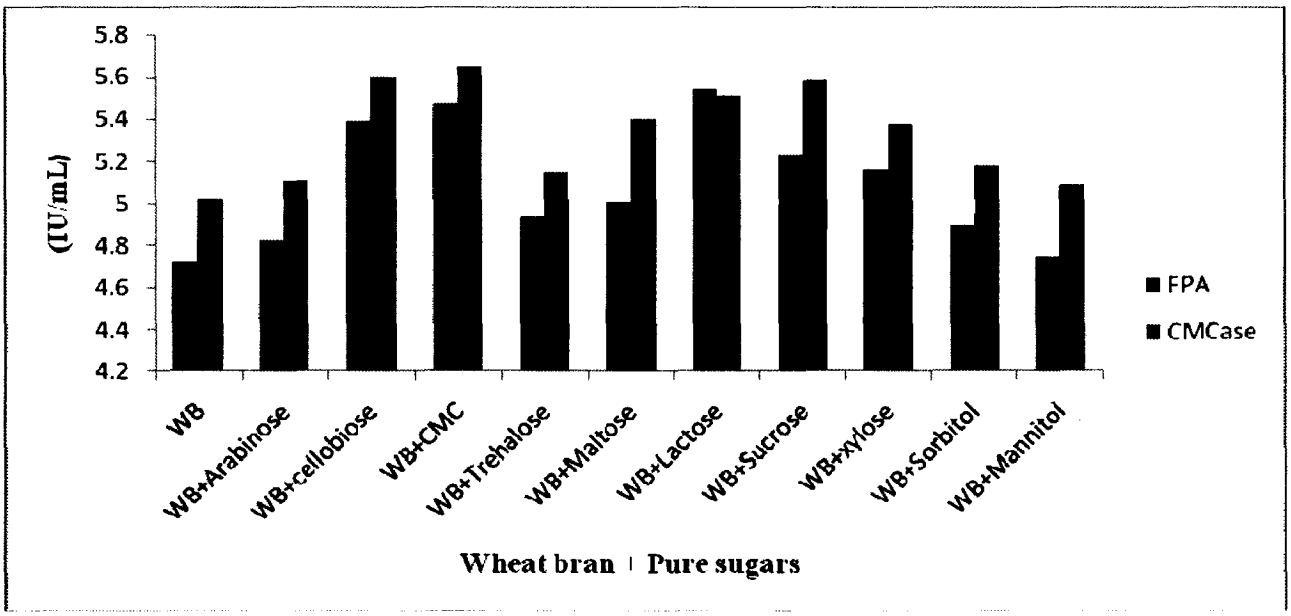


Fig 4.4.36. Comparative enzyme activities achieved by *Trichoderma reesei* strain under wheat bran incorporated with pure sugars based fermentation at 30^{0}C and pH 5.

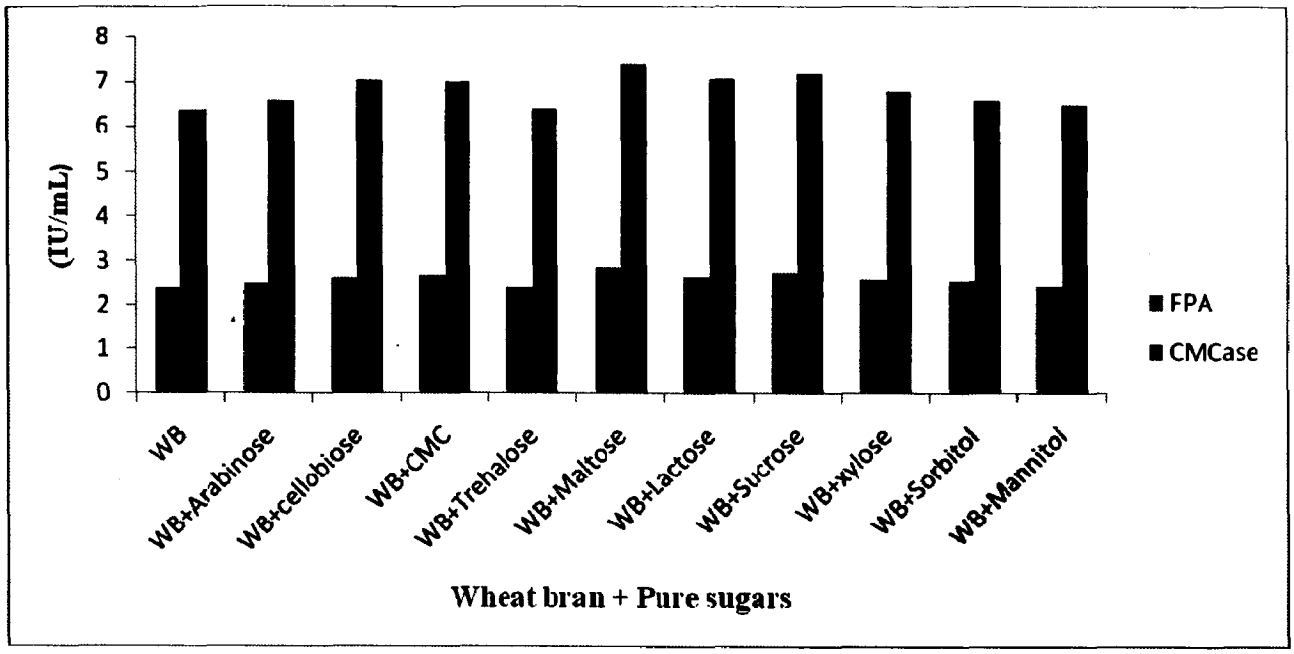


Fig 4.4.37. Comparative enzyme activities achieved by *Neurospora crassa* strain under wheat bran incorporated with pure sugars based fermentation at 30^0C and pH 5.

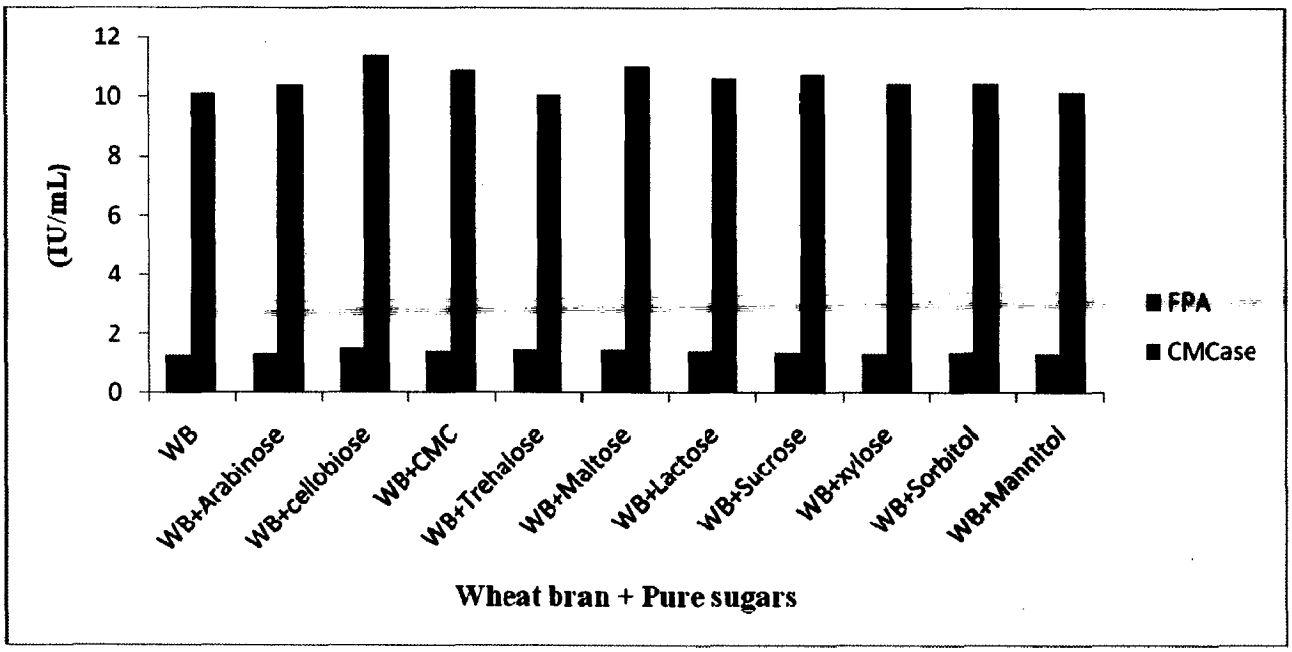


Fig 4.4.38. Comparative enzyme activities achieved by *Aspergillus niger* strain under wheat bran incorporated with pure sugars based fermentation at 30^0C and pH 5.

4.4.12. Application of egg shell waste in cellulase production under wheat bran based solid state cultivation.

Separate set of batch experiments have been performed for the cellulase production studies using egg shell waste in place of pure calcium salt by fungal strains under wheat bran based solid state fermentation. Acid treatments of egg shell waste have been performed with 5, 10 and 15% HCl

(v/v) solution with maintaining the desired solid liquid ratio, soaked at room temperature for two hours. The resulting solution was diluted up to a desired level and made up the resulting solution pH 7.0 with dilute NaOH solution. This solution was further used for the production studies. As observed from Table 4.4.15 that higher and lower dosages of acid concentration was not much suitable for the growth of used fungal strains. Pretreatment of egg shell waste with 10% HCl was found most suitable for the growth in comparison to lower and higher acid dosages. At lower acid concentration egg shell waste was not properly dissolved, therefore organisms were unable to take Ca^{++} ion from undissolved ($CaCO_3$) state, whereas higher acid concentration was not favorable for the growth may be due to higher acidity. Treated egg shell wastes are interested for their ability to support cell growth. As evidence suggested that most good quality of egg shell contains approximately 2.2 g of calcium in the form of calcium carbonate. Insoluble $CaCO_3$ are not useful for microbial growth, therefore it is necessary to convert it into soluble form. Insoluble egg shell waste calcium ($CaCO_3$) becomes soluble ($CaCl_2$) under the treatment with HCl, which provides soluble calcium ions required for microbial growth and production system.

Table 4.4.15. Suitability of acid treated egg shells waste for fungal growth.

Waste material	**Acid treatment dosages**	**Soaking time(h)**	***Neurospora crassa* growth**	***Trichoderma reesei* growth**	***Aspergillus niger* growth**
Egg shells waste	Untreated	2	Very light growth	Light growth	Light growth
	5% HCl	2	Light growth	Moderate growth	Moderate growth
	10% HCl	2	Good growth	Good growth	Good growth
	15% HCl	2	Very-very light growth	Least growth	Light growth

Table 4.4.16. Comparative enzyme activity (IU/mL) achieved by various fungal strains using egg shell waste under wheat bran based solid state fermentation at 30^0C and pH 5.

Egg Shell Waste		**Optimized acid treatment dosages**	**Vol of acid (mL)**	*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
				FPA	CMCase	FPA	CMCase	FPA	CMCase
Pure Calcium salt	0.4g/L	-	-	4.72	5.02	2.30	6.27	1.29	10.10
Egg shell waste	0.4g/L	10%HCl	25	3.96	4.45	1.89	5.17	1.05	8.11
	0.8g/L	10%HCl	50	4.49	4.98	2.11	5.83	1.19	9.43
	1.2g/L	10%HCl	75	4.17	4.55	1.96	5.38	1.10	8.57

When compared the cellulase activity under wheat bran based solid state fermentation having different concentrations of egg shell waste (0.4, 0.8 and 1.2g/L) in production media. We have

observed from Table 4.4.16 and Fig 4.4.39, 4.4.40, 4.4.41 that 0.8g/l egg shell waste containing wheat bran solid bed showed better FPA activity 4.49 IU/mL, 2.11 IU/mL, 1.19 IU/mL in comparison to other by *T.reesei*, *N.crassa* and *Aspergillus niger* strains respectively, which may possibly be due to the fact that acid treatment of 0.4g/L egg shell waste, may not provide the desired level of free calcium ions required for proper fungal growth and production, whereas at higher dosages much amount of calcium ions as well as acidity may inhibit the microbial growth.

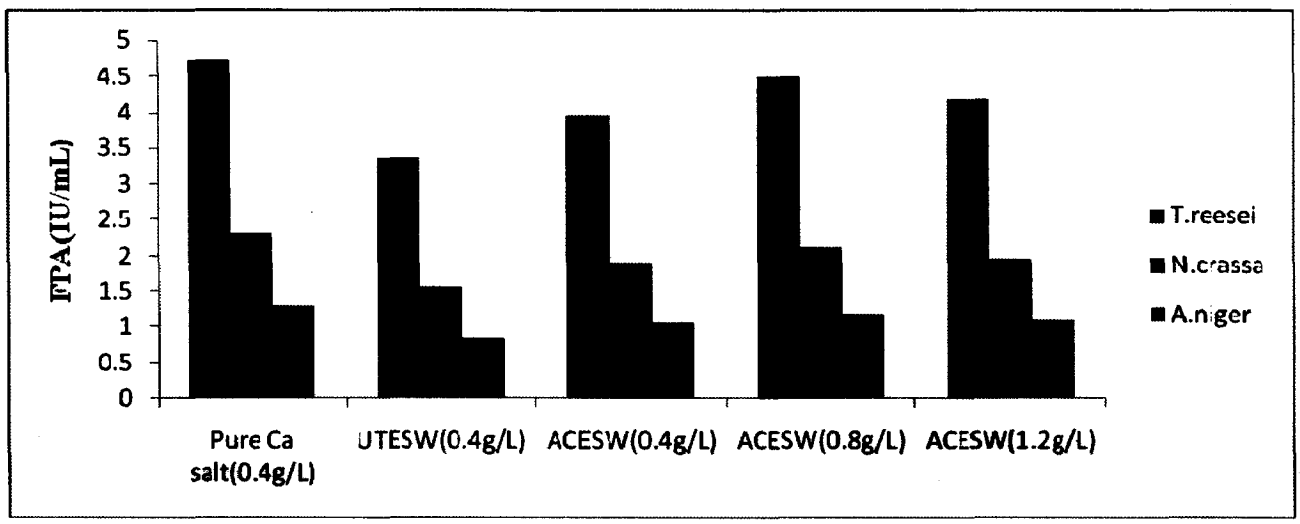


Fig 4.4.39. Comparative FPA achieved by various fungal strains using egg shell waste under wheat bran based solid state fermentation at 30^0C and pH 5.

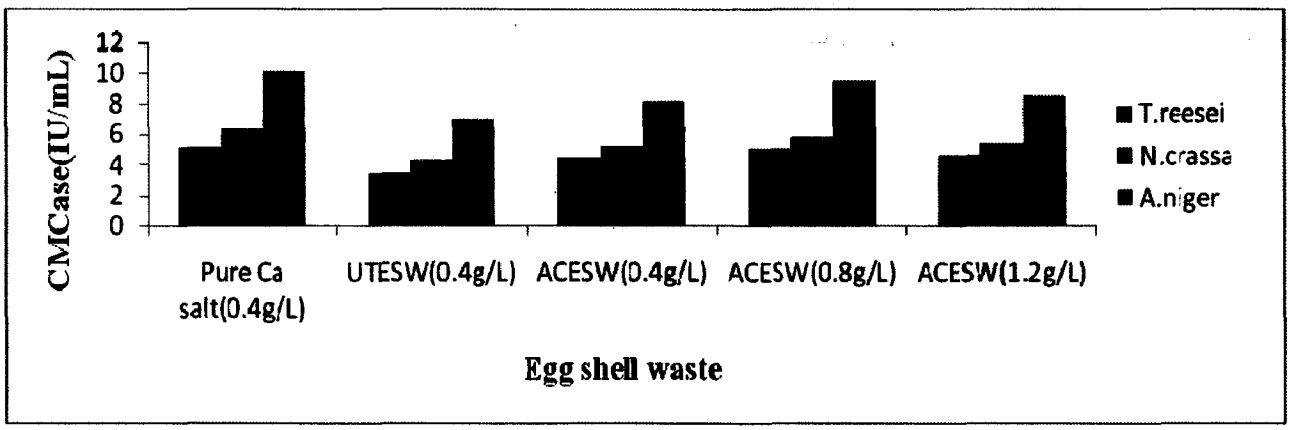


Fig 4.4.40. Comparative CMCase activity achieved by various fungal strains using egg shell waste under wheat bran based solid state fermentation at 30^0C and pH 5.

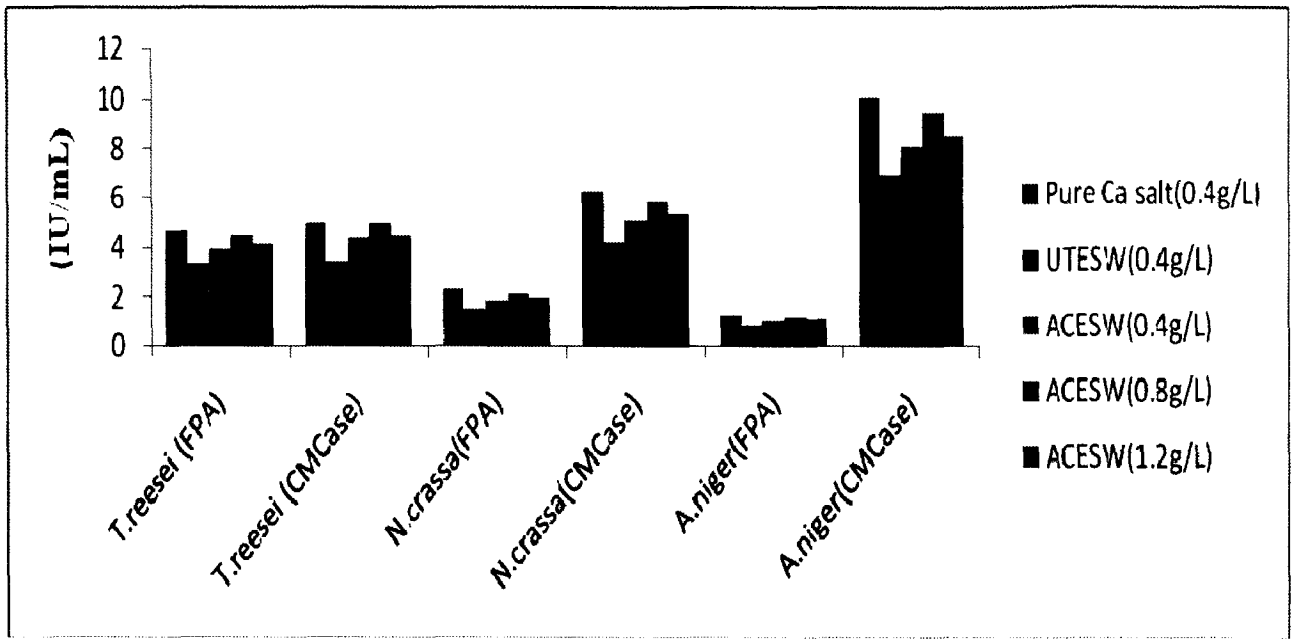


Fig 4.4.41. Comparative bar diagram of enzyme activities achieved by various fungal strains using egg shell waste under wheat bran based solid state fermentation at 30^0C and pH 5.

4.4.13. Effect of design of fermentation vessels on cellulase production under wheat bran based solid state fermentation

To investigate the effect of fermentation vessels design on cellulase production under wheat bran based solid state fermentation separate set of experiments have been performed under Erlenmeyer flask, petridish, beaker and culture tube type of fermentation vessels.

Table 4.4.17. Comparative enzyme activity (IU/mL) achieved by various fungal strains under different fermentation vessel based SSF at 30^0C and pH 5.

Fermentation vessel	**VOA/SBA***	***Trichoderma reesei***		***Neurospora crassa***		***Aspergillus niger***	
		FPA	CMCase	FPA	CMCase	FPA	CMCase
Erlenmeyer flask	Low/High	4.72	5.02	2.36	6.36	1.29	10.10
Petridish	Very low/High	3.38	3.61	1.69	4.53	1.06	8.13
Beaker	High /High	4.83	5.25	2.22	6.03	1.41	10.68
Culture tube	Low/Low	2.07	2.11	1.03	2.67	0.611	4.69

* VOA/SBA indicates vessel opening area / solid bed area.

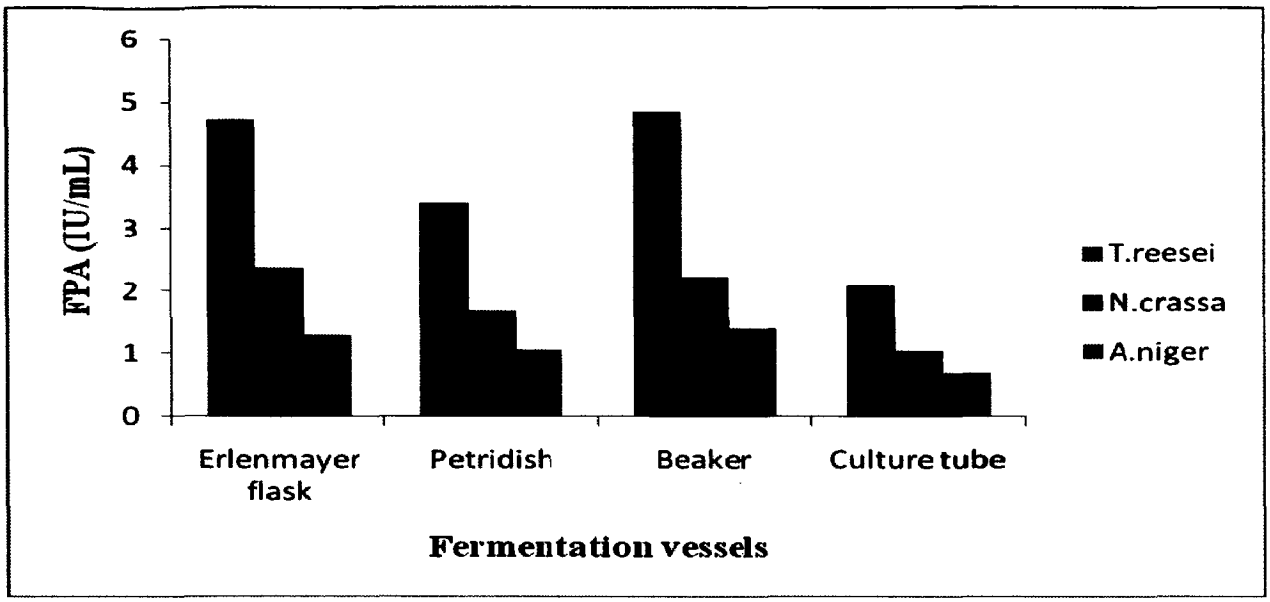


Fig 4.4.42. Comparative FPA achieved by various fungal strains under different fermentation vessel based SSF at 30^0C and pH 5.

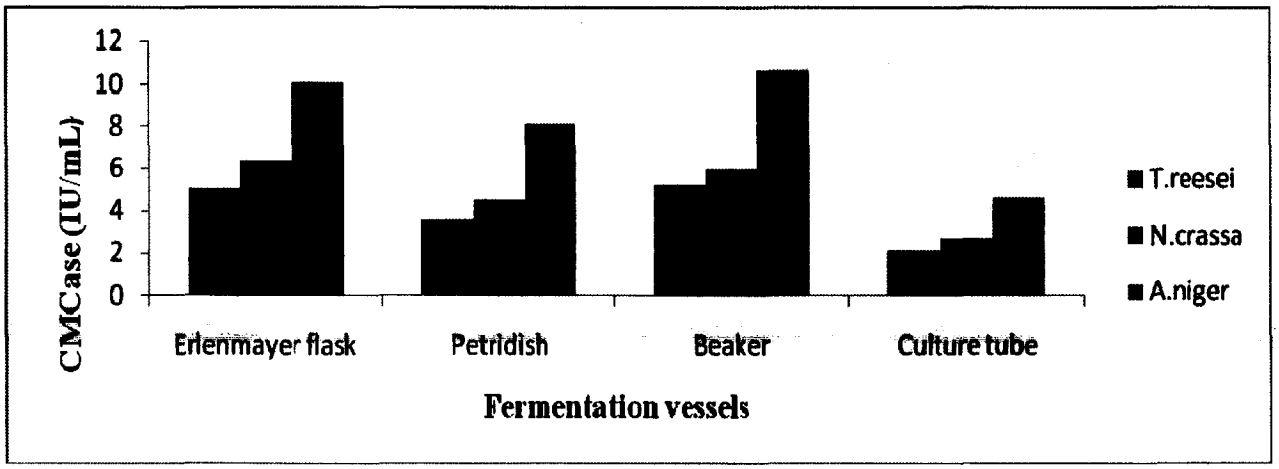


Fig 4.4.43. Comparative CMCase activity achieved by various fungal strains under different fermentation vessels based SSF at 30^0C and pH 5.

It has been observed from Table 4.4.17 and Figs 4.4.42, 4.4.43 that type of fermentation vessels plays a very crucial and significant role in the microbial growth and production system. In the present comparative study we had used four type of fermentation vessels such culture tube, beaker, petridish and Erlenmeyer flask for solid state production of cellulases by various fungal strains utilizing wheat bran as solid support. These fermentation vessels have dissimilar kind of solid bed area for microbial growth as well as having variable vessel opening area for aeration and transfer of gases required for microbial growth and production system. As observed from Table 4.4.17 and Figs 4.4.42, 4.4.43 that *Trichoderma reesei* and *Aspergillus niger* showed significantly higher

cellulase activity under beaker type of fermentation vessel based solid state fermentation vessel which may be due to that beaker type of vessel bears high vessel opening and solid bed area, which provides much favourable conditions (due to more surface for growth and better space for aeration, metabolic gas as well as heat transfer) for cellulase production by fungal strains. On the other hand *N. crassa* showed higher cellulase activity in erlenmayer flask type of fermentation vessels as compared to others. Growth of *A. niger* under different fermentation vessels was also confirmed by Fig 4.4.44.

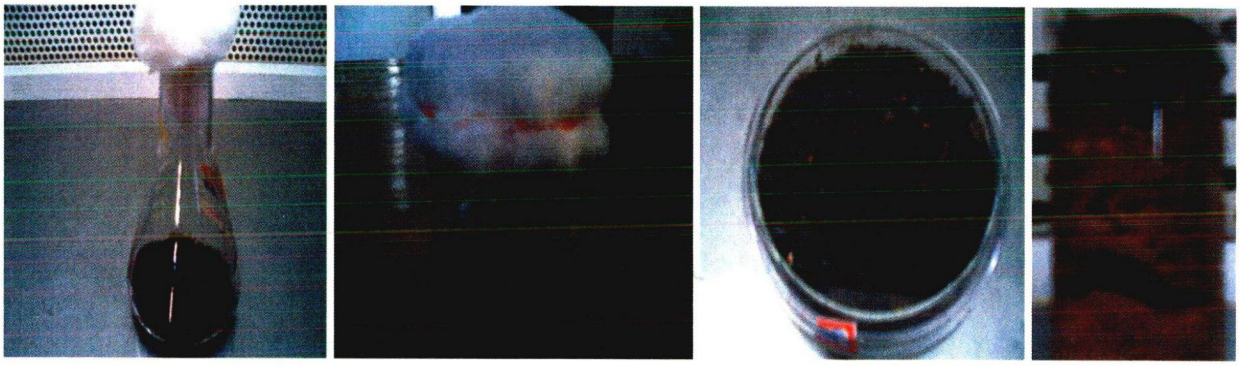


Fig 4.4.44. Comparative growth of *Aspergillus niger* in Erlenmayer flask,beaker,petriplate and culture tube type fermenation vessel using wheat bran solid support at 30^0C

Least cellulase activity has been observed in culture tube type of fermentation vessel by fungal strains, which might be due low vessel opening and solid bed area as compaerd to other fermentation vessels. Vessel opening as well as solid bed area both strongly influenced the fungal growth and production system. It can be inferred that sufficient surface area along with proper space for aeration metabolic gas transfer would be a better state for aerobic microbial growth.

4.4.14. Effect of Starch hydrolysates on cellulase production under wheat bran based solid state fermentation

To investigate the role of starch hydrolysates in cellulase production under wheat bran based solid state fermentation, separate set of experiments were performed. Pretreatment of starches were performed by taking 2 and 5% HCl with pretreatment time of 1h. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei* and *N.crassa* were 5.74, 5.98; 2.61, 6.51 respectively under 5%(v/v) dosages of 2% acid hydrolysed wheat starch hydrolysate based solid state fermentation, whereas *A. niger* showed maximum FPA (1.57IU/mL) and CMCase (12.45IU/mL) under 5%(v/v) dosages of 2% acid hydrolysed rice starch hydrolysate based solid state fermentation, which may be due to release of some dimeric sugars such as sophrose in the acid hydrolysates starches. It was also observed from Table 4.4.18 and Fig 4.4.45 and 4.4.46. that fungal strains produces significantly higher cellulase activities under 2% HCl treated wheat starch

hydrolysate based fermentation medium as compared to 5% HCl treated one, which may be due to the fact that upon increasing the acid strength starch is overhydrolyzed, other byproducts may be generated, which might have served as inhibitors for cellulase production[47].When compared the effectiveness of wheat starch hydrolysates for various used fungal strains than it has been observed that all the strains performed much better in terms of cellulase activity under wheat starch hydrolysate containing wheat bran based solid state fermentation. On the other hand *A. niger* performed better under rice hydrolysate containing wheat bran based fermentation. Potato starch hydrolysate was found less effective for cellulase activity enhancement by almost each fungal strains under wheat bran based solid state fermentation. It can be concluded that wheat starch hydrolysate was found quite effective for cellulase induction capability. We can also suggest that raw material composition also affects on the performance of starch hydrolysates or in other word they utilized better under less inhibitory environment.

Table 4.4.18. Comparative enzyme activities achieved by different fungal strains under various starch hydrolysates incorporated wheat bran based SSF at 30^{0}C and pH 5.

Starch hydrolysates (SH)		*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**
Untreated WS	2%(w/v)	4.78	4.95	2.40	6.17	1.32	10.29
2%HCl WSH	2%(v/v)	5.10	5.40	2.54	6.29	1.41	11.17
	5%(v/v)	5.74	5.98	2.61	6.51	1.53	11.87
5%HCl WSH	2%(v/v)	4.31	4.51	2.20	5.16	1.20	9.70
	5%(v/v)	4.27	4.45	2.16	5.08	1.18	9.51
Untreated PS	2%(w/v)	4.74	5.02	2.37	5.91	1.27	9.92
2%HCl PSH	2%(v/v)	4.86	5.25	2.44	6.18	1.38	10.70
	5%(v/v)	5.09	5.37	2.55	6.43	1.41	11.21
5%HCl PSH	2%(v/v)	4.28	4.56	2.25	5.23	1.16	9.32
	5%(v/v)	4.20	4.41	2.18	5.37	1.13	9.21
Untreated RS	2%(w/v)	4.80	4.98	2.41	6.09	1.33	10.53
2%HCl RSH	2%(v/v)	5.02	5.33	2.59	6.71	1.43	11.59
	5%(v/v)	5.25	5.57	2.63	6.89	1.57	12.45
5%HCl RSH	2%(v/v)	4.34	4.39	2.28	5.76	1.22	9.83
	5%(v/v)	4.37	4.31	2.31	5.81	1.20	9.67

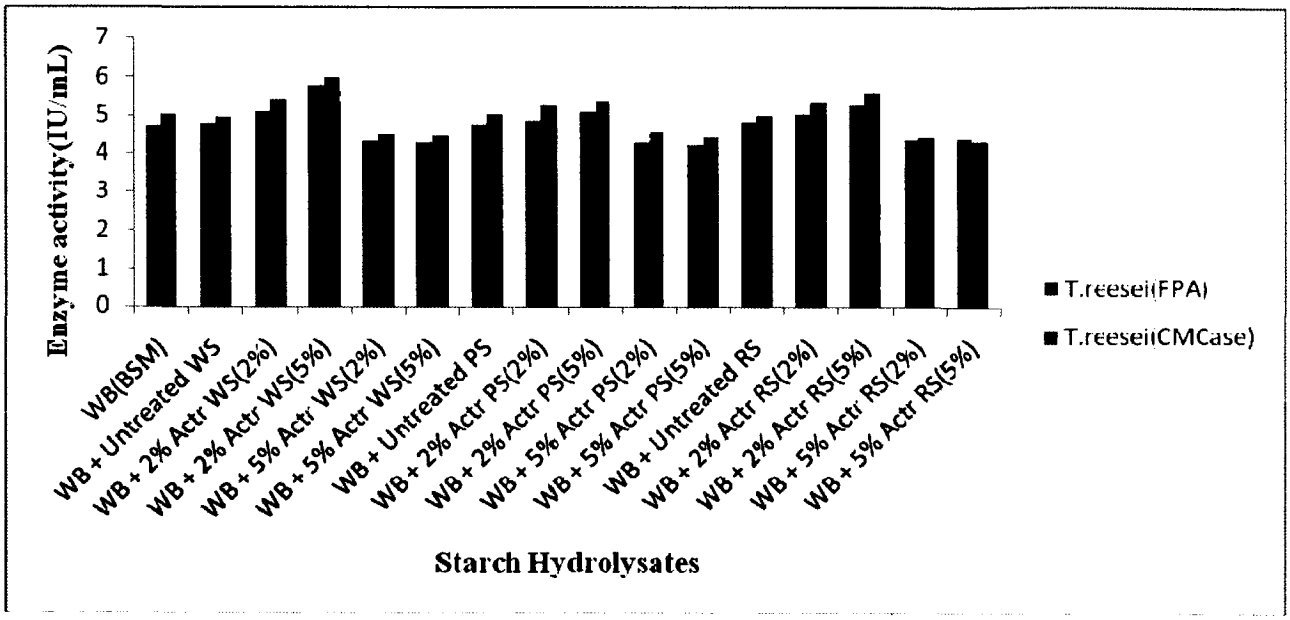


Fig 4.4.45. Comparative enzyme activities achieved by *Trichoderma reesei* under various starch hydrolysates incorporated wheat bran based SSF at 30^0C and pH 5.

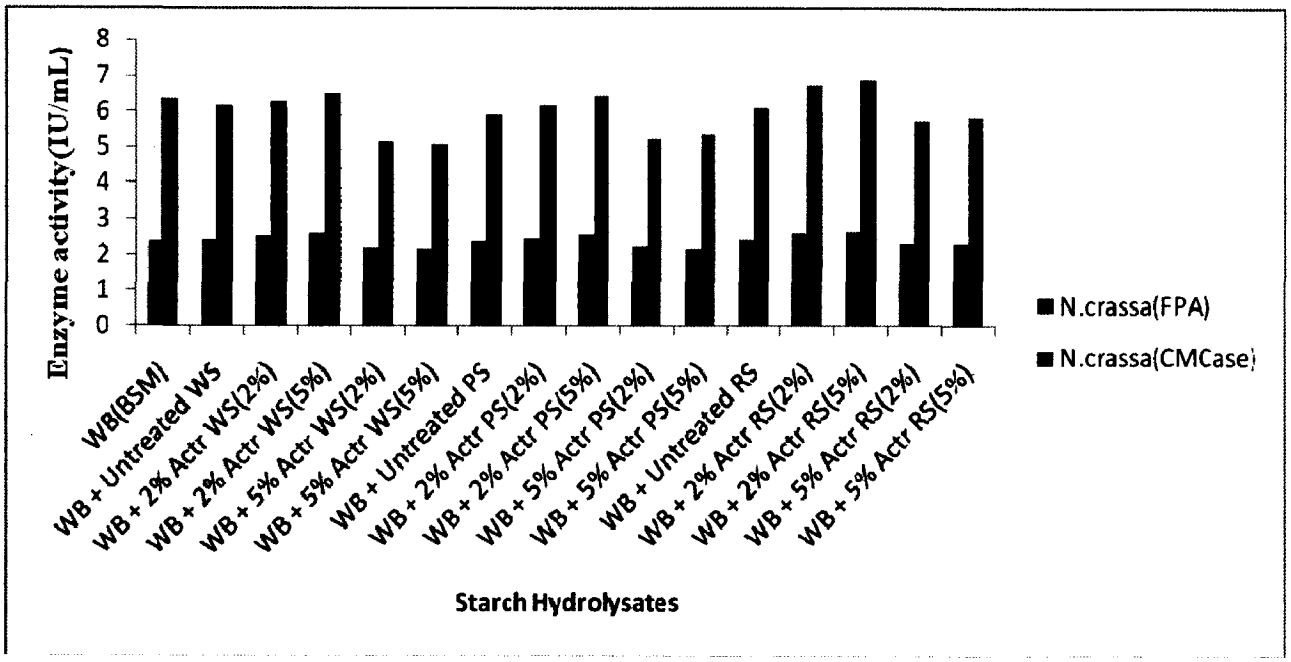


Fig 4.4.46. Comparative enzyme activities achieved by *N.crassa* under various starch hydrolysates incorporated wheat bran based SSF at 30^0C and pH 5.

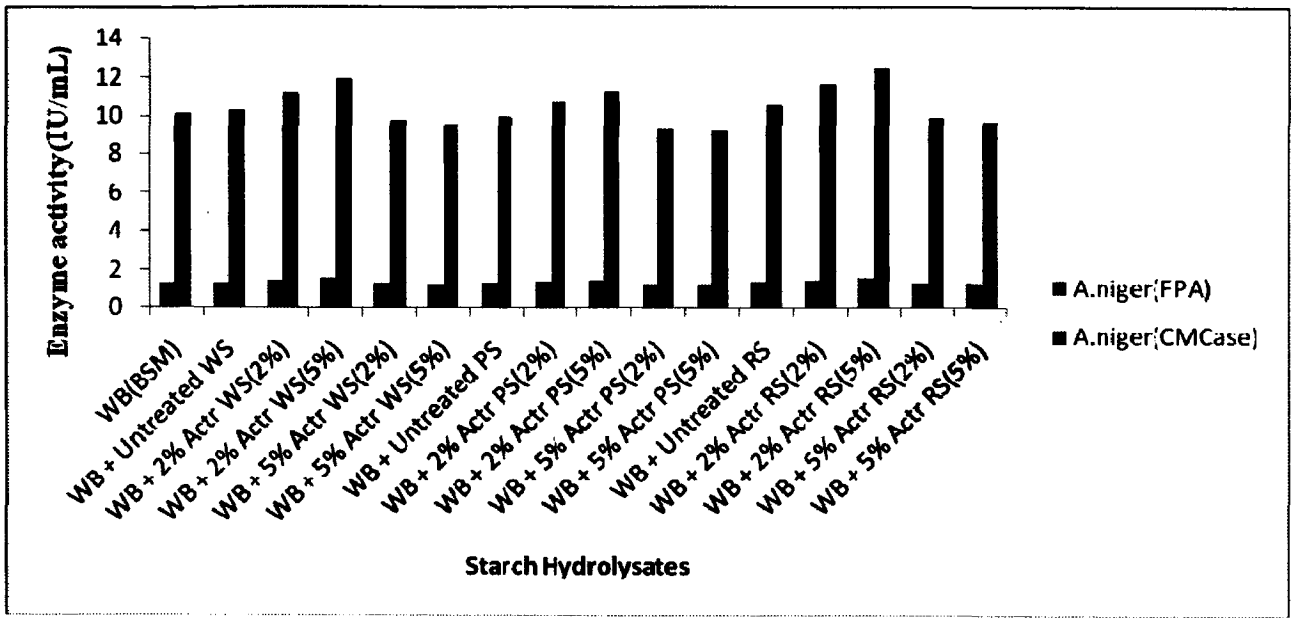


Fig 4.4.47. Comparative enzyme activities achieved by *A. niger* under various starch hydrolysates incorporated wheat bran based SSF at 30^0C and pH 5.

4.4.15. Effect of starch hydrolysates alongwith whey on cellulase production under wheat bran based solid state fermentation

To investigate the effect of starch hydrolysates alongwith whey on cellulase production under wheat bran based solid state fermentation, separate sets of batch experiments have been performed by taking 30% whey and starch hydrolysates. It has been observed from Table 4.4.19 and Fig 4.4.48, 30% (v/v) whey and 5% (v/v) wheat starch hydrolysate combination under wheat bran based solid state fermentation was found much effective for cellulase production by almost each strains. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 6.93, 7.29; 2.97, 7.43; 1.73, 12.87 respectively under 30% whey and 5% (v/v) acid hydrolysed wheat starch hydrolysate containing wheat bran bed. Increment rate in cellulase activity by *T.reesei* under such condition was much higher as compared to others, which may be due to the dual inductive effect of the sugars such as sophrose and lactose present in the medium.

Table 4.4.19. Comparative enzyme activities achieved by various fungal strains under various starch hydrolysates alongwith whey incorporated wheat bran based SSF at 30^{0}C and pH 5.

BSM +Whey + Starch hydrolysates		*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
Basal salt media + WB		4.72	5.02	2.36	6.36	1.29	10.10
BSM+WB+Whey		6.08	6.02	2.72	6.87	1.47	11.02
W+WSH	30%+UTWS 2% (w/v)	6.13	6.37	2.74	6.03	1.45	10.97
	30%+2%(v/v)	6.47	6.83	2.89	7.19	1.67	12.16
	30%+5%(v/v)	6.93	7.29	2.97	7.43	1.73	12.87
W+PSH	30%+UTPS 2% (w/v)	6.10	6.31	2.75	6.76	1.43	10.82
	30%+2%(v/v)	6.31	6.62	2.83	7.02	1.56	11.98
	30%+5%(v/v)	6.43	6.78	2.88	7.50	1.61	12.09
W+RSH	30%+UTRS (w/v)	6.15	6.29	2.78	6.84	1.52	11.84
	30%+2%(v/v)	6.39	6.68	3.01	7.67	1.62	12.28
	30%+5%(v/v)	6.60	6.97	3.10	7.89	1.73	12.76

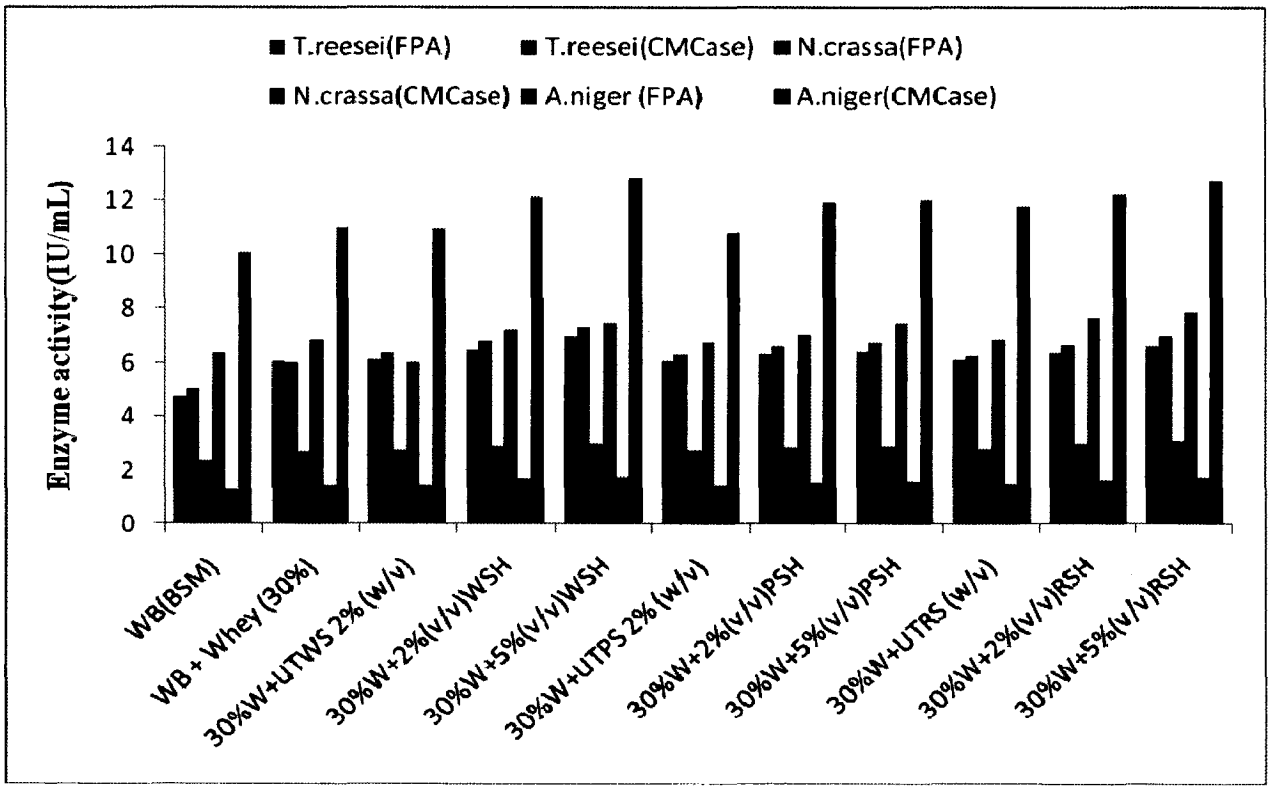


Fig 4.4.48. Comparative enzyme activities achieved by various fungal strains under various starch hydrolysates alongwith whey incorporated wheat bran based SSF at 30^{0}C and pH 5.

4.4.16. Effect of pure sugar lactose and dairy waste whey on cellulase production under wheat bran based solid state fermentation

To investigate the effect of pure sugar lactose and dairy waste whey on cellulase production under wheat bran based solid state fermentation separate set of experiments have been performed using pure lactose as well as 15%, 30% and 50%(v/v) whey. It has been observed from Table 4.4.20 and Figs 4.4.49, 4.4.50 that fungal strains showed better activity under 30% whey containing wheat bran as compared to other dosages of whey and pure lactose. The maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 6.08, 6.02; 2.72, 6.87; 1.47, 11.02 under 30% whey containing wheat bran based solid state fermentation. Lactose and other proteins present in the whey may provide favorable condition for better cellulase activity especially for *Trichoderma* strains.

Table 4.4.20. Comparative enzyme activity achieved by various fungal strains under different concentrations of whey as well as lactose pure sugars containing wheat bran based SSF at 30^0C and pH 5.

Pure Lactose/Dairy waste (Whey)		*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**
Pure Lactose	0.50g/L	5.10	5.32	2.45	6.48	1.34	10.41
	1.0 g/L	5.54	5.51	2.63	6.75	1.39	10.63
Whey	15%(v/v)	5.35	5.47	2.53	6.61	1.31	10.53
	30%(v/v)	6.08	6.02	2.72	6.87	1.47	11.02
	50%(v/v)	5.93	5.86	2.69	6.94	1.41	10.62

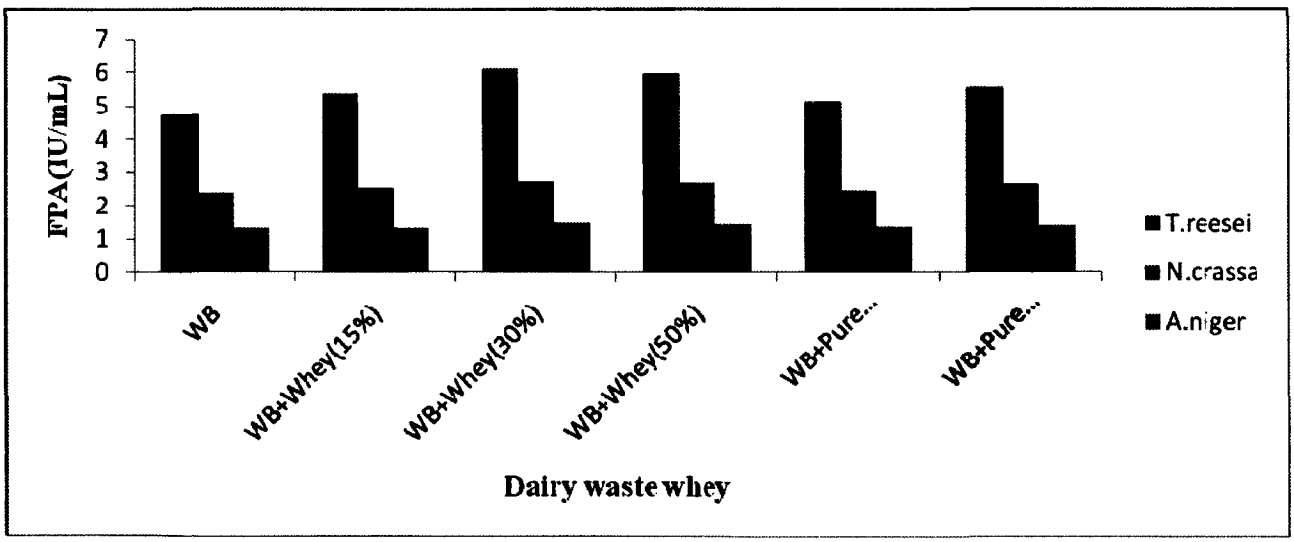


Fig 4.4.49. Comparative FPA achieved by various fungal strains under different concentrations of whey as well as lactose pure sugars containing wheat bran based SSF at 30^0C and pH 5.

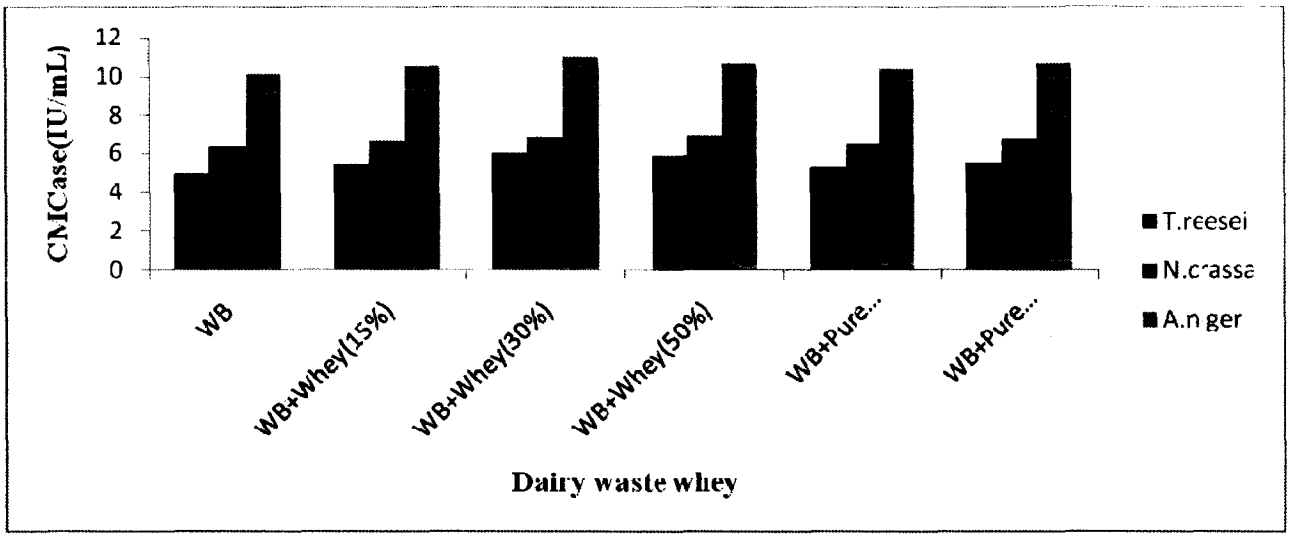


Fig 4.4.50. Comparative CMCase activity achieved by various fungal strains under different concentrations of whey as well as lactose pure sugars containing wheat bran based SSF at 30^0C and pH 5.

4.4.17. Effect of boiled bagasse syrup, bagasse hydrolysate and wheat straw hydrolysate on cellulase production under wheat bran based solid state fermentation

To explore the effect of boiled bagasse syrup, bagasse hydrolysate and wheat straw hydrolysate on cellulase production under wheat bran based solid state fermentation separate set of experiment have been performed. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 5.07, 5.30: 5.03, 5.25: 5.00, 5.28; 2.41, 6.42: 2.43, 6.46: 2.38, 6.39; 1.31, 10.23: 1.34, 10.28: 1.30, 10.26 under boiled bagasse syrup, 10% acid treated bagasse hydrolysate and 5% acid treated wheat straw hydrolysate containing wheat bran based fermentation respectively. It was observed from Table 4.4.21 and Figs 4.4.51, 4.4.52. that fungal strains produces significantly better cellulase activities under boiled bagasse syrup, 10% acid treated bagasse hydrolysate as compared to others. The higher cellulase activity produced by fungal strains under wheat bran bed incorporating with boiled bagasee syrup and 10% acid treated bagasse hydrolysate as compared to wheat bran bed solely, which may be due to the inclusion of some others inductive nature of sugars as well as lesser inhibitory environment of wheat bran, all these situation generates favorable condition for growth and enzyme production. It has been also observed that there was no significant improvement in the CMCase activity under hydrolysate based fermentation, which might be due to the fact that sugars presents in hydrolysate may induces FPA strongly than CMCase.

Table 4.4.21. Comparative enzyme activity achieved by various fungal strains under boiled bagasse syrup(BBS), bagasse hydrolysates(BH) and wheat straw hydrolysates(WSH) containing wheat bran based SSF at 30^0C and pH 5.

Lignocellulosic Hydrolysates		*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**
BBS		5.07	5.30	2.41	6.42	1.31	10.23
BH	10% H_2SO_4 treated	5.03	5.25	2.43	6.46	1.34	10.28
	20% H_2SO_4treated	4.78	5.08	2.32	6.40	1.27	10.01
	5% H_2SO_4treated	5.00	5.28	2.38	6.39	1.30	10.26
	10% H_2SO_4 treated	4.86	5.17	2.35	6.31	1.25	10.13

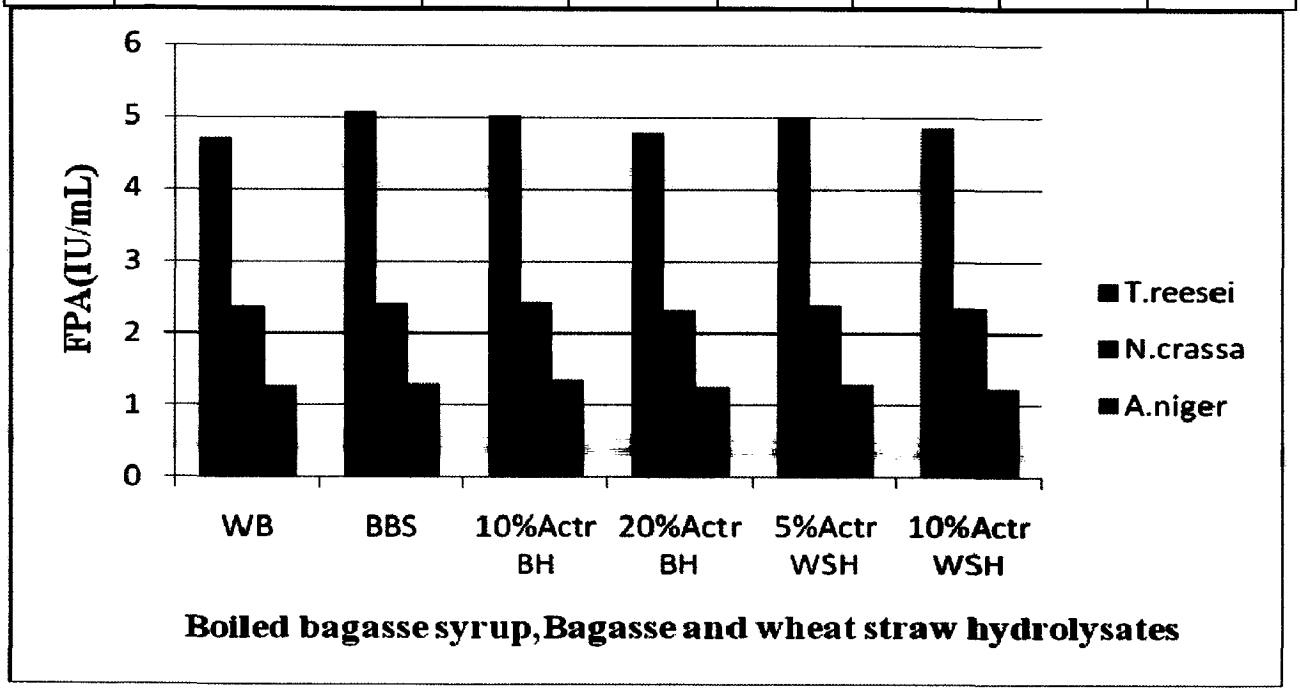


Fig 4.4.51. Comparative FPA achieved by various fungal strains under boiled bagasse syrup, bagasse hydrolysates and wheat straw hydrolysates containing wheat bran based SSF at 30^0C and pH 5.

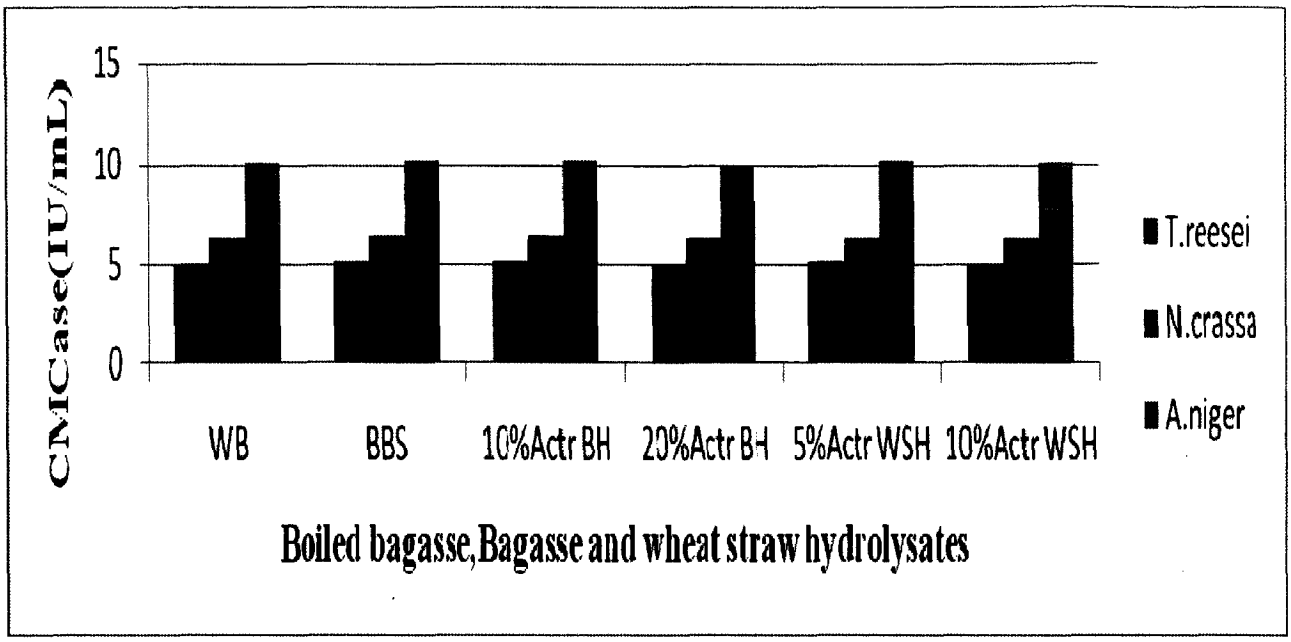


Fig 4.4.52. Comparative CMCase activity achieved by various fungal strains under boiled bagasse syrup, bagasse hydrolysates and wheat straw hydrolysates containing wheat bran based SSF at 30^0C and pH 5.

4.4.18. Effect of waste news paper hydrolysates on cellulase production under wheat bran based solid state fermentation

To explore the effect of acid treated waste news paper hydrolysate on cellulase production under wheat bran based solid state fermentation separate set of experiment have been performed using 20, 30, 40, 50 and 70% H_2SO_4 treated waste news paper hydrolysate. It has been observed from Table 4.4.22 and Figs 4.4.53, 4.4.54 fungal strains produces better cellulase activity under wheat bran solid bed incorporated with 40% acid treated waste news paper hydrolysate. Maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *T.reesei*, *N.crassa* and *A.niger* were 5.15, 5.45; 2.47, 6.51; 1.41, 10.48 under 40% acid treated waste news paper hydrolysate containing wheat bran bed. Improvement in the cellulase activities may be due to release of sophrose, cellobiose, xylose and glucose sugars, which induces the cellulase production by fungal strains. On the other hand lesser inhibitory condition provided by wheat bran bed generates favorable condition for fungal growth and activity enhancement under such condition.

Table 4.4.22. Comparative enzyme activity achieved by various fungal strains under waste news paper hydrolysates containing wheat bran based SSF at 30^{0}C and pH 5

Waste news paper hydrolysates	*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
WNP (20% acid treated)	4.87	5.26	2.38	6.39	1.27	10.15
WNP (30% acid treated)	4.96	5.31	2.41	6.43	1.31	10.19
WNP (40% acid treated)	5.15	5.45	2.47	6.51	1.41	10.48
WNP (50% acid treated)	4.82	5.18	2.38	6.41	1.30	10.11
WNP (70% acid treated)	4.69	4.95	2.33	6.30	1.26	10.02

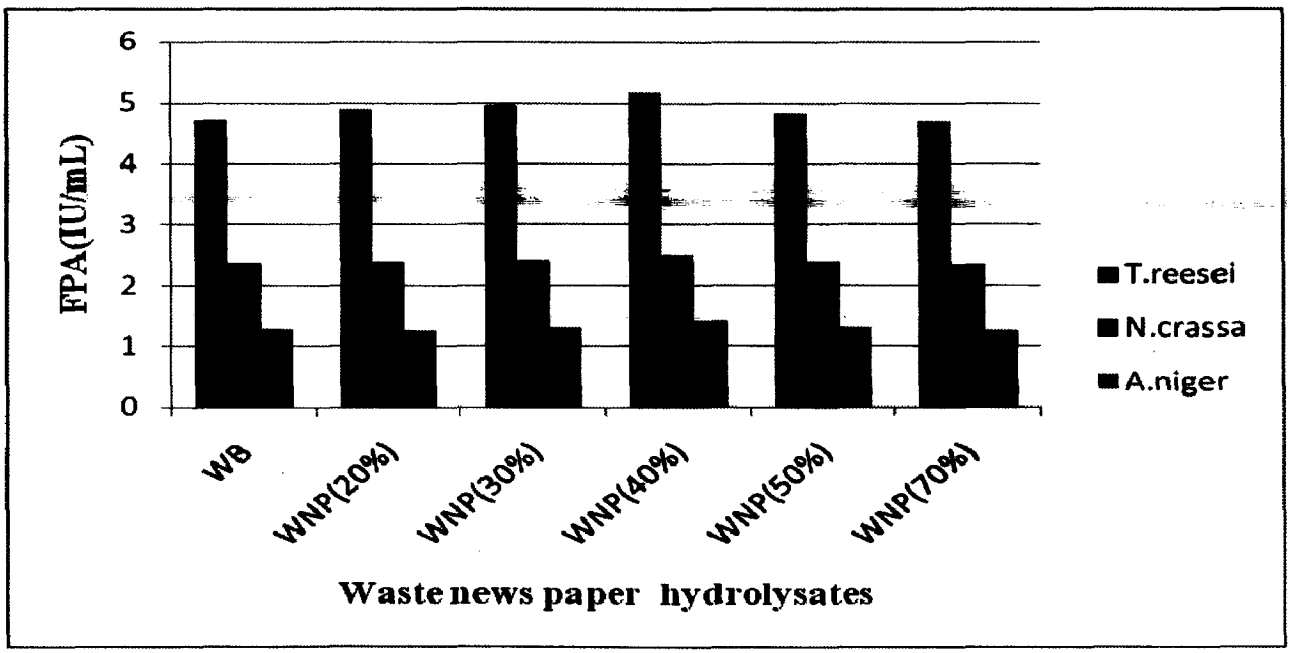


Fig 4.4.53. Comparative FPA achieved by various fungal strains under waste news paper hydrolysates containing wheat bran based SSF at 30^{0}C and pH 5.

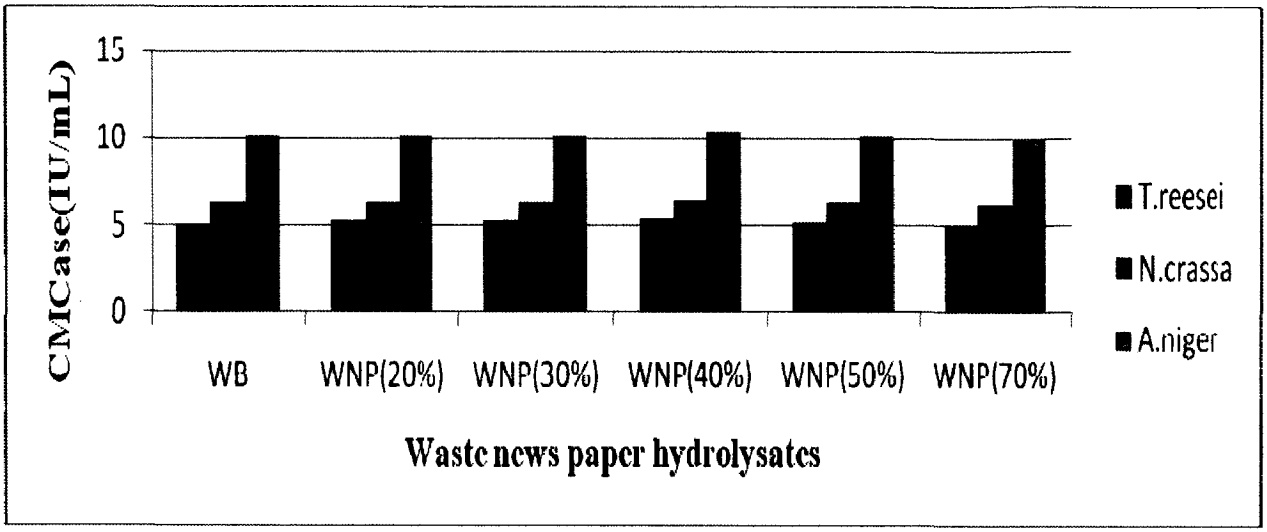


Fig 4.4.54. Comparative CMCase activity achieved by various fungal strains under boiled waste news paper hydrolysates containing wheat bran based SSF at 30^0C and pH 5.

4.4.19. Effects of physical and chemical mutagens on growth and production system of fungal strains under wheat bran based solid state cultivation on cellulase production

Separate sets of experiment have been performed to study the mutagenic effects of mutagens on wild fungal strains. Mutant strains of the *Trichoderma, Aspergillus* and *Neurospora* were cultured in PD and M_2 broth based culture media respectively. As the Table 4.4.23 suggests that the growth of wild strains of *Trichoderma reesei*, *Aspergillus* and *Neurospora* were in the form of filamentous mycelial, medium sized pellets and thick pulpy forms respectively whereas the mutated cultures *Trichoderma*, *Aspergillus* and *Neurospora* showed thick mycelia, bead and pulpy form of growth. UV irradiated (30min) *Aspergillus* mutant showed very–very dense growth, having both tiny and medium sized beads whereas UV irradiated (50min) *Aspergillus* mutant showed lesser growth with tiny particle sized beads. On the other hand UV irradiated alongwith Etbr treated *Aspergillus* mutants exhibited only medium sized pellets having blackish central core with transparent solution as represented by Fig 4.4.55. On the other hand 30 min UV irradiated *N. crassa* mutant showed pulpy growth, while UV + EtBr mutated *Trichoderma* showed thick mycelia growth. These strains exhibited good increment in growth than the wild strains, which reveals the potential use mutant strains in the industry. Mutagens plays a major role in internal variations in the microbes and these changes may or may not be beneficial for the microorganisms growth and production capacity.

Table 4.4.23. Growth of various fungal strains after mutagen treatment

Mutagens	**Time period /conc**	***A. niger* morphological forms**	***N. crassa* morphological forms**	***T.reesei* morphological forms**
Wild type	Wild type	Pelleted forms(medium sized)	Dense pulpy growth	Filamentous mycelial growth
UV irradiation	30 min	Dense small sized pellet or bead form of growth	Pulpy	Mycelial growth
	40 min	Bead form	Pulpy	Dense mycelial growth
	50 min	Tiny sized bead	Pulpy	Thin mycelial growth
UV +EtBr	40min+150 µg/ml)	Medium sized pelletes with blakish core	Thin Pulpy growth	Thick mycelial growth

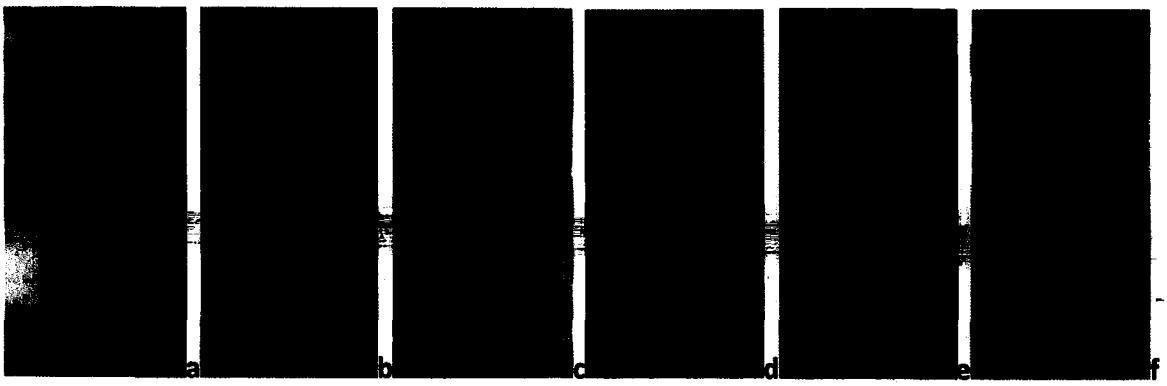


Fig 4.4.55. Growth of *Neurospora crassa & Aspergillus niger* under 30 min,50 min UV irradiation (Fig a,b, d & e respectively) whereas Fig c & f represents growth of above under 40 min UV irradiation alongwith 150 µg/ml ethidium bromide treatment in their respective culture media.

It has been observed from the Table 4.4.24 and Figs 4.4.56, 4.4.57 that 30 min of UV irradiation of the cultures were found better for growth and cellulase production by *T.reesei* and *A.niger* strains, under this condition the sustainable or survival capacity of organisms are high in comparison to the prolonged UV irradiation, whereas 30 min of UV irradiation of *N.crassa* culture was found much effective for growth and FPA activity but 50 min UV irradiated wild *N.crassa* strain produces significantly higher CMCase activity. On the other hand both UV irradiated (40 min) alongwith ethidium bromide (150µg/mL) treated mutants of *T.reesei* and *N.crassa* showed significant improvement in enzyme activities whereas UV irradiated (40 min) alongwith ethidium bromide(150µg/mL) treated mutants of *A. niger* showed somewhat higher activities compared to wild strain but also showed significantly lesser enzyme activities as compared to 30 min UV irradiated mutant strain. Fig 1-6 represents the growth nature and the sustain capacity of mutant strains.

Table 4.4.24. Comparative enzyme activity achieved by various mutated fungal strains wheat bran based SSF at 30^{0}C and pH 5.

Mutagens	Time period /conc	*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
		FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
Wild type	Wild type	4.72	5.02	2.36	6.36	1.29	10.10
UV irradiation	30 min	4.81	5.13	2.18	6.49	1.48	11.09
	40 min	4.65	4.88	2.30	6.22	1.21	9.34
	50 min	4.20	4.39	2.47	6.19	0.986	7.53
UV +EtBr	40min+150 µg/ml)	4.98	5.27	2.55	6.86	1.35	10.36

The enhancement in the cellulase activity might be due to the fact that some amino mutations in the cellulase monocomponent proteins caused by base mutations, could lead to enhanced cellulase activity because cellulase enzyme was a complex enzyme not single one, there were many genes to code cellulase proteins. 30 min of UV irradiation alone and 40 min UV irradiation alongwith ethidium bromide treatment are better mutagenic condition for the growth of microbes. Internal variations and the survival capacity of the mutant strains of *Neurospora* and *Aspergillus* and *Trichoderma* strains were also effective at these conditions therefore this could be beneficial for the industrial and environmental application of these microbes.

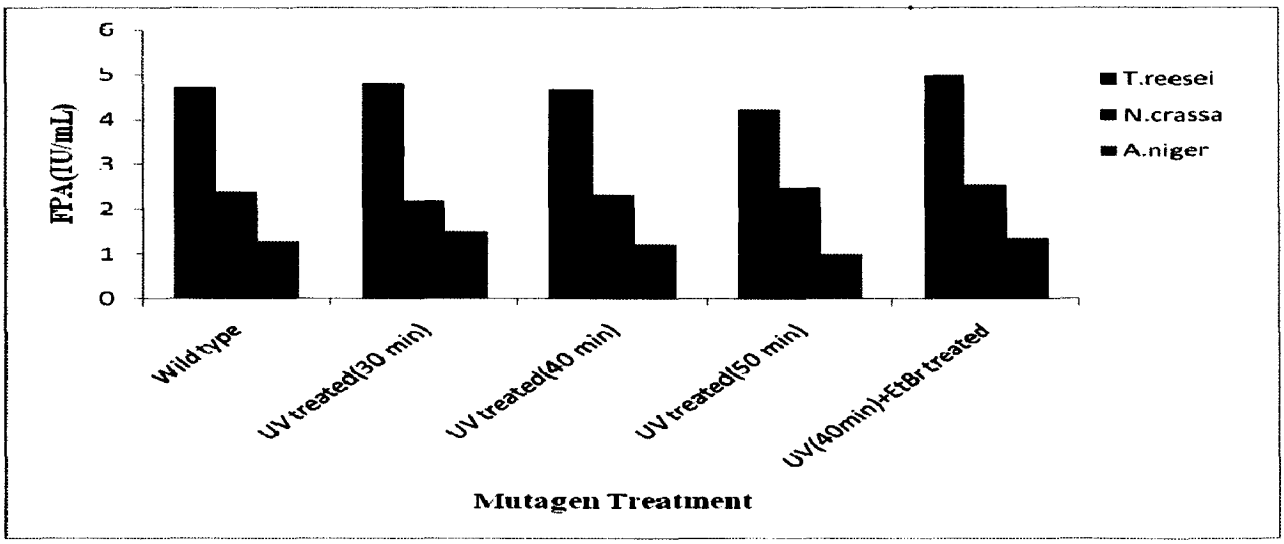


Fig 4.4.56. Comparative FPA achieved by various mutated fungal strains wheat bran based SSF at 30^{0}C and pH 5.

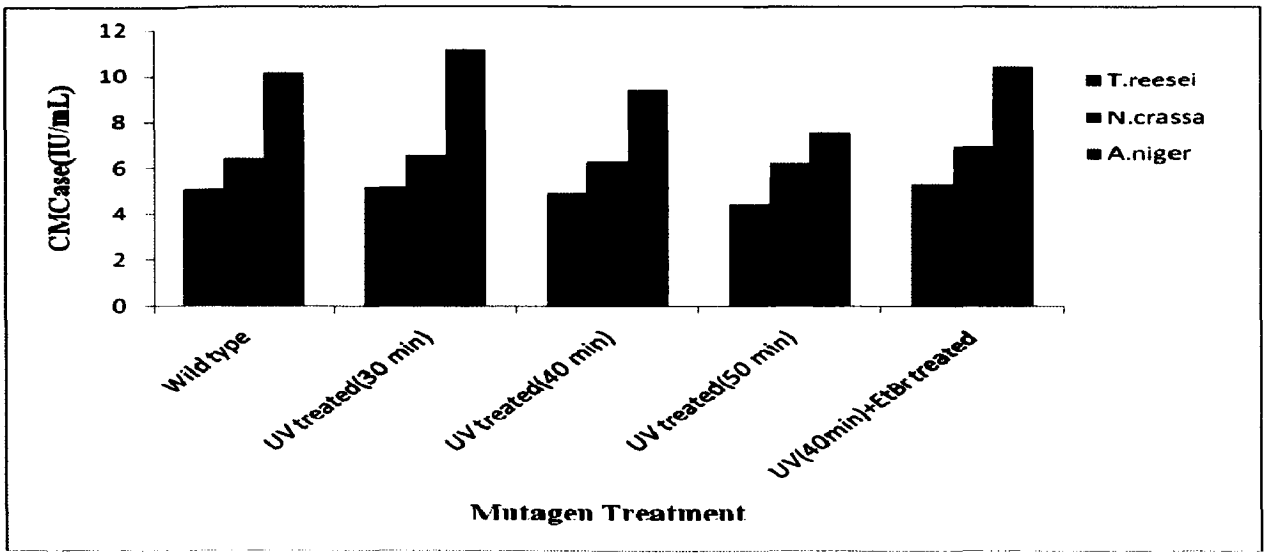


Fig 4.4.57. Comparative CMCase activity achieved by various mutated fungal strains wheat bran based SSF at 30^0C and pH 5.

4.4.20. Thermo stability of cellulases produced by different fungal strains under solid state cultivation

To investigate the thermostability of cellulases produced by different fungal strains separate sets of activity measurement experiments were performed at 40, 50, 60, 70, 80 and 90^0C reaction mixture temperature under specified reaction conditions. It has been observed from Table 4.4.25 and Figs 4.4.58, 4.4.59, 4.4.62 cellulases produced by *T. viride* (51.4%) was more thermostable followed by *A. fumigatus* strain (48.8%) as compared to others. Least thermostability was observed with *A. niger* cellulases (32.0%).

Table 4.4.25. Comparative thermostability of cellulases produced by various fungal strains

Temperature(^{0}C)	*T.reesei*	*N.crassa*	*A.niger*	*T.viride*	*A.fumigatus*
	Relative activity (percentage)				
40	83.3	76.8	74.3	68.4	69.2
50	100.0	100.0	100.0	100.0	100.0
60	85.0	76.4	69.1	73.5	72.8
70	79.2	63.8	57.0	68.7	67.2
80	63.5	54.0	42.6	56.9	53.9
90	39.8	35.7	32.0	51.4	48.8

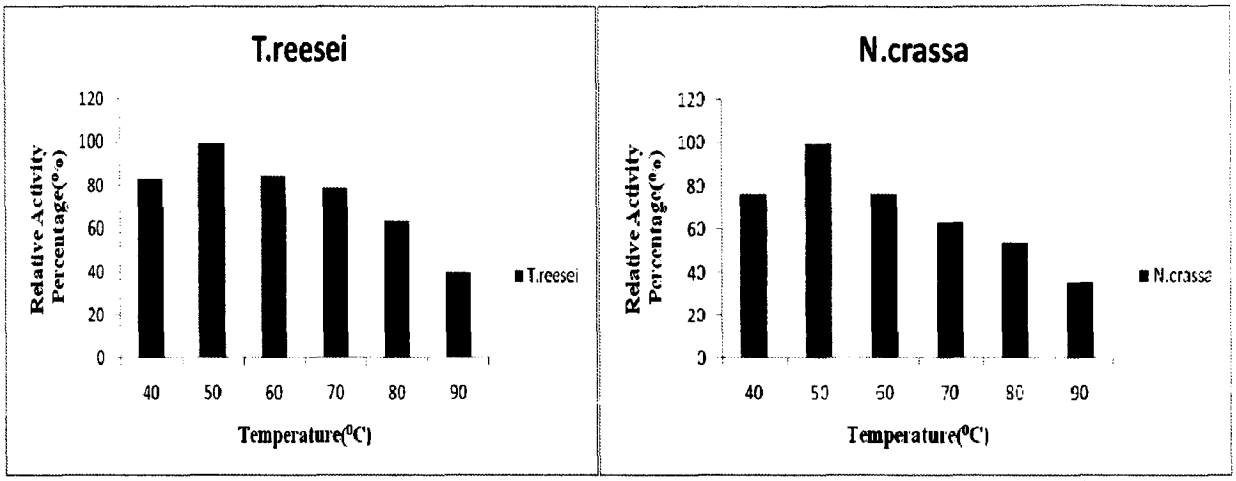


Fig 4.4.58. Comparative thermostability of cellulases produced by *T.reesei* and *N.crassa* fungal strains

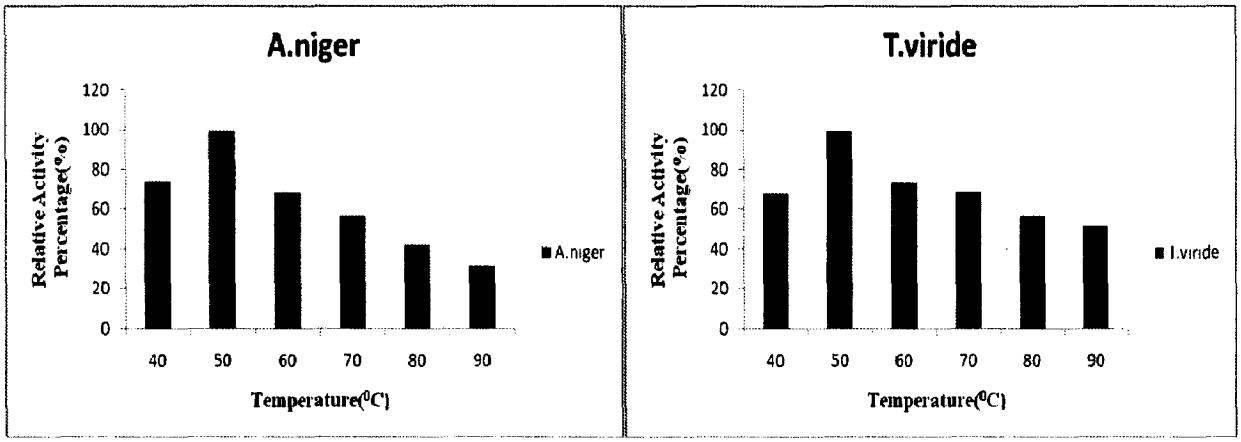


Fig 4.4.59. Comparative thermostability of cellulases produced by *A. niger* and *T.viride* fungal strains

4.4.21. pH stability of cellulases produced by different fungal strains under solid state cultivation

To investigate the pH stability of cellulases produced by different fungal strains separate sets of activity measurement experiments were performed with different buffer solutions having pH 3.0, 4.0, 4.8, 6.0, 9.2 and 10.0 under specified conditions.

Table 4.4.26. Comparative pH stability of cellulases produced by various fungal strains

pH	*T.reesei*	*N.crassa*	*A.niger*	*T.viride*	*A.fumigatus*
	Relative activity (percentage)				
3.0	86.9	83.0	85.6	78.2	74.6
4.0	93.6	91.2	92.1	81.9	78.7
4.8	100.0	100.0	100.0	100.0	100.0
6.0	85.0	89.7	78.6	86.9	83.4
9.2	49.7	59.0	40.9	62.6	61.1
10.0	36.1	40.6	32.0	49.7	51.8

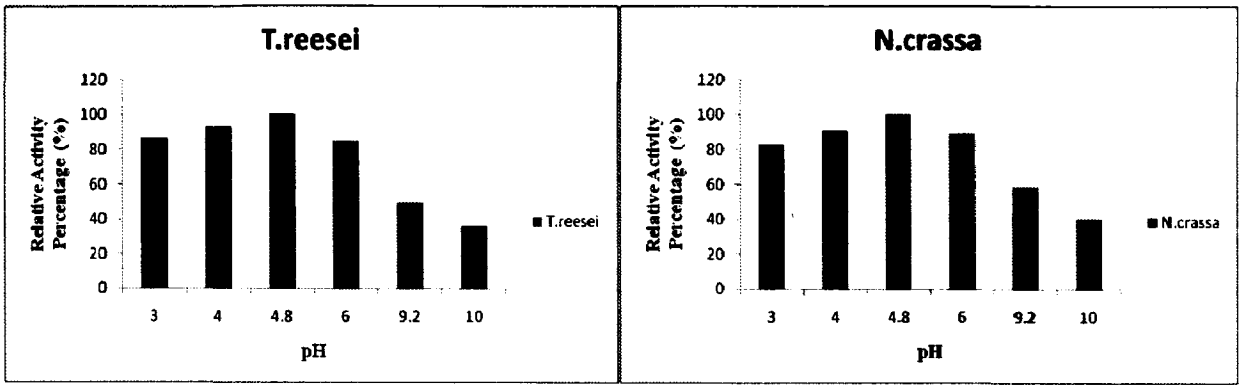


Fig 4.4.60. Comparative pH stability of cellulases produced by *T.reesei* and *N.crassa* fungal strains

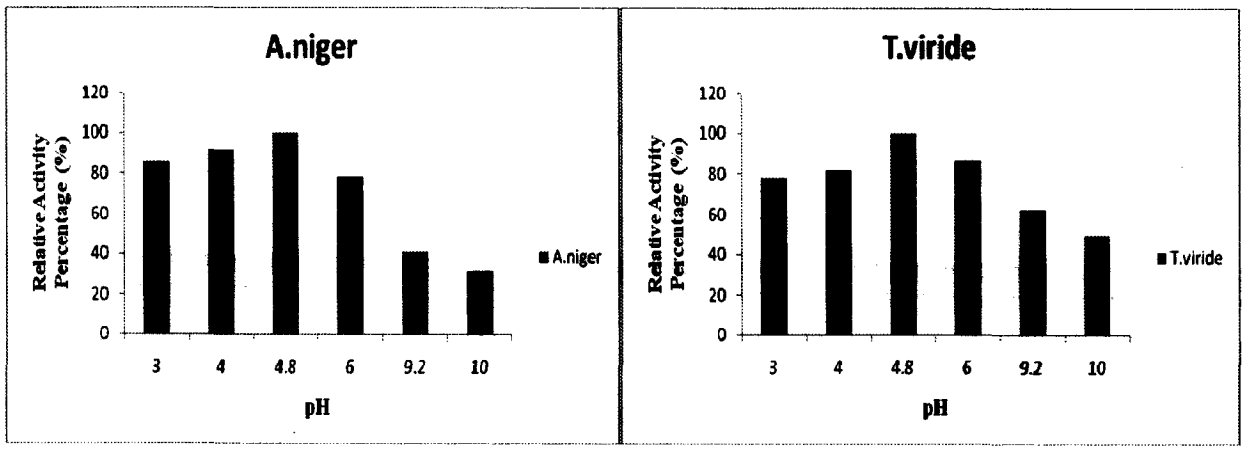


Fig 4.4.61. Comparative pH stability of cellulases produced by *A. niger* and *T.viride* fungal strains

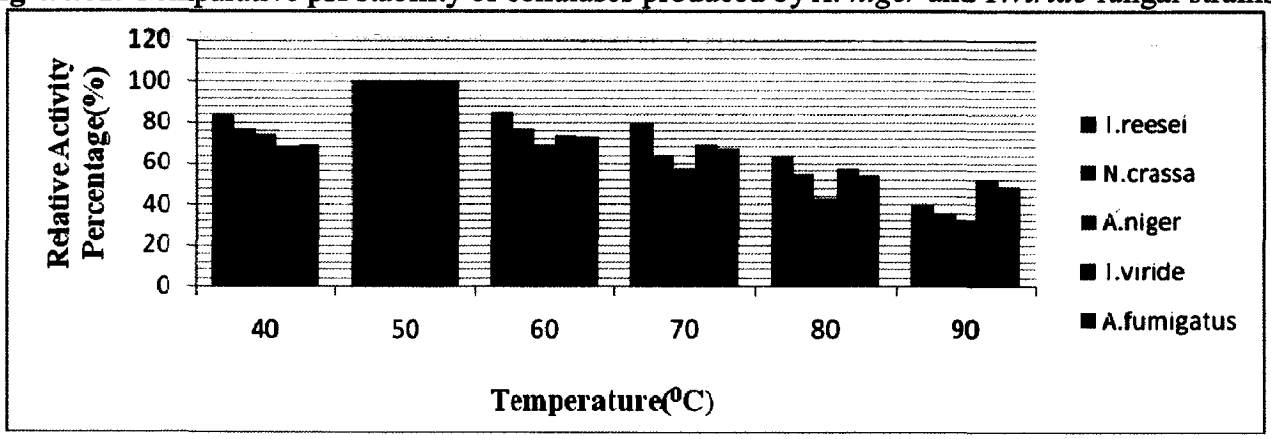


Fig 4.4.62. Comparative bar diagram of thermostability achieved by various fungal strains

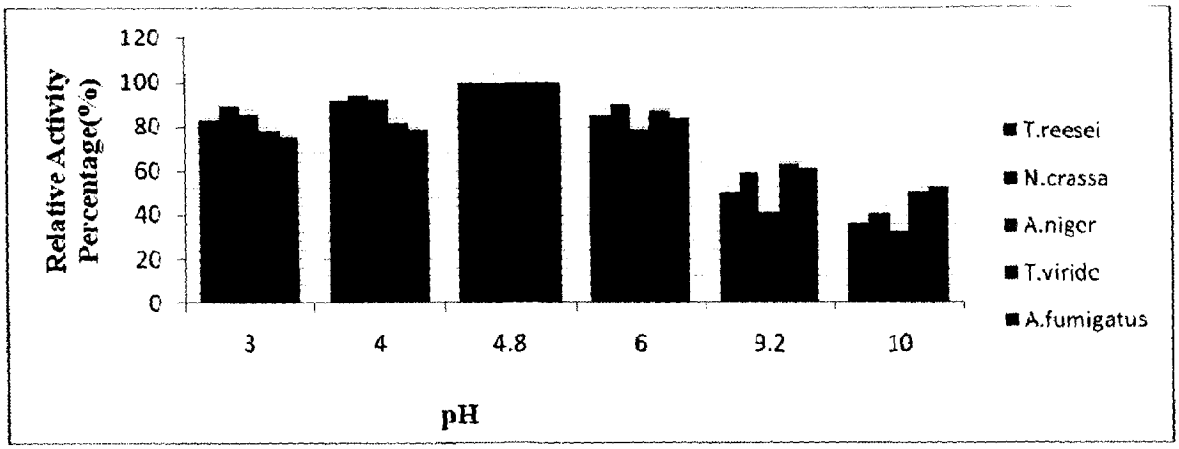


Fig 4.4.63. Comparative bar diagram of pH stability achieved by various fungal strains.

It has been observed from Table 4.4.26 and Fig 4.4.60, 4.4.61, 4.4.63 that cellulases produced b *A. fumigatus* (51.8%) was more alkali pH stable followed by *T. viridae* (49.7%) and *N.crass* (40.6%) as compared to others. Least alkali pH stability was observed with *A. niger* cellulas (32.0%). On the other hand higher acidic pH stability was observed with *T.reesei* (86.9) follow by *A. niger* (85.6).

SECTION V
UTILIZATION OF NON CONVENTIONAL RAW MATERIALS IN CELLULASE PRODUCTION

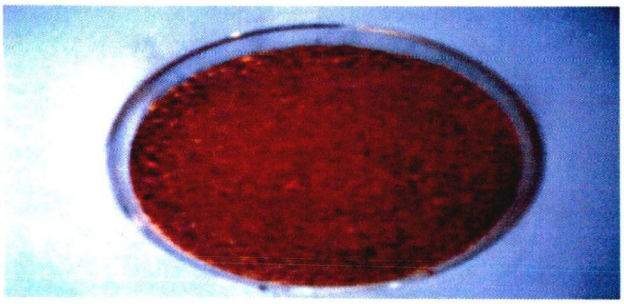
Lignocellulosic biomass is the most abundant solid waste in the world, thus it can provide some of the most promising feedstocks for the production of cellulases. A wide variety of waste bioresources are available on our planet for conversion into bioproducts. In the biological systems, microorganisms are used to utilize waste as an energy source for the synthesis of valuable products such as biomass proteins and enzymes. The large quantities of byproducts generated during the processing of plant food involve economic and environmental problems due to their high volumes and elimination costs. After isolation of the main constituent, there are abundant remains which represent an inexpensive material that has been undervalued until now.

The present section evaluates the utility of non conventional waste raw materials such as *Pisum sativum* peel, *Luffa cylindrica* peel, *Lagenaria cineraria* peel and *Litchi cinensis* peel waste in cellulase production by various fungal strains under solid state cultivation.

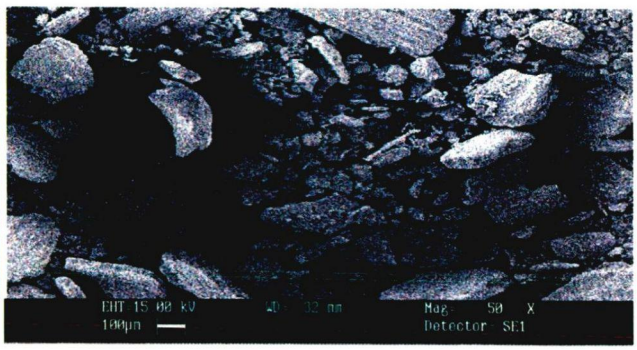
4.5.1. CHARACTERIZATION OF RAW MATERIALS

4.5.1.1. Scanning electron microscopic studies of non conventional raw materials

To investigate the configuration, shape, pattern as well as surface properties of different grounded and sieved raw materials, scanning electron microscopy was performed. Fig (4.5.1.a), Fig (4.5.2.a), Fig (4.5.3.a), Fig (4.5.4.a) illustrate the visual observation of ground and sieved *Luffa cylindrica* peel waste, *Lagenaria cineraria* peel waste, *Litchi cinensis* peel waste, *Pisum sativum* peel waste respectively.From the SEM images of *Luffa cylindrica* peel waste, *Lagenaria cineraria* peel waste, *Litchi cinensis* peel waste we have observed that they showed rough surfaces with almost rounded and elliptical configures.

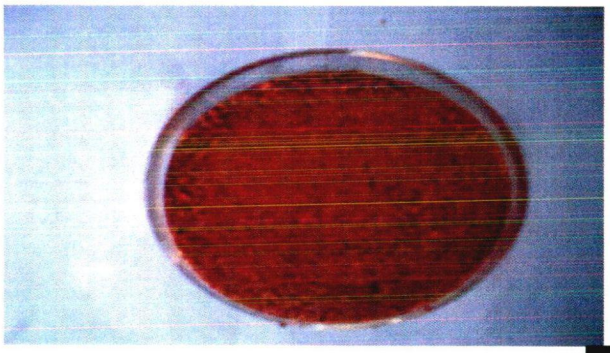


(4.5.1.a)

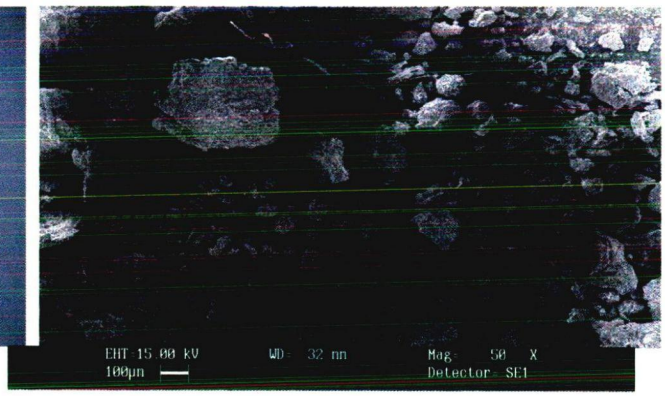


(4.5.1.b)

Fig.4.5.1. Ground and sieved raw *Luffa cylindrica* peel waste (4.5.1.a), SEM photograph of Ground and sieved raw *Luffa cylindrica* peel waste (4.5.1.b)

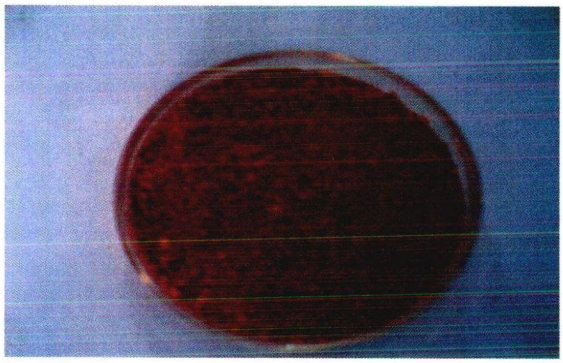


(4.5.2.a)

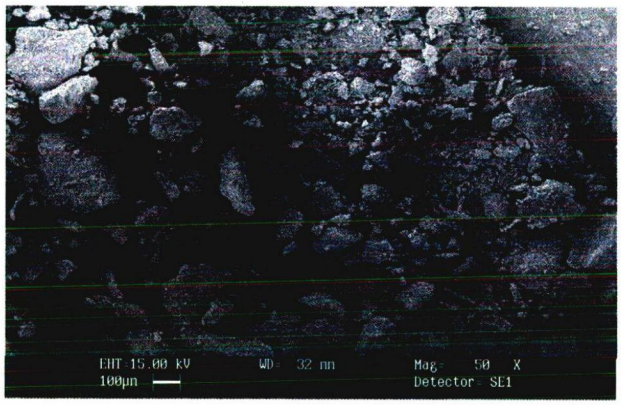


(4.5.2.b)

Fig.4.5.2. Ground and sieved raw *Lagenaria cineraria* peel waste (4.5.2.a), SEM photograph of Ground and sieved raw *Lagenaria cineraria* peel waste (4.5.2.b)



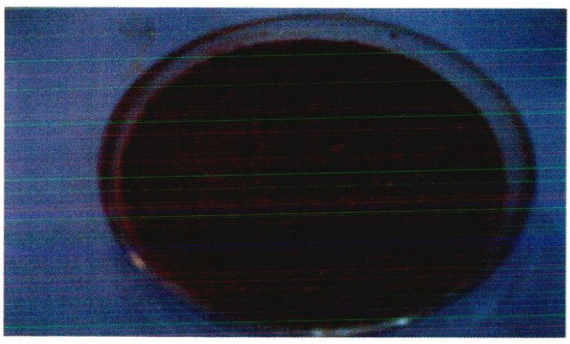
(4.5.3.a)



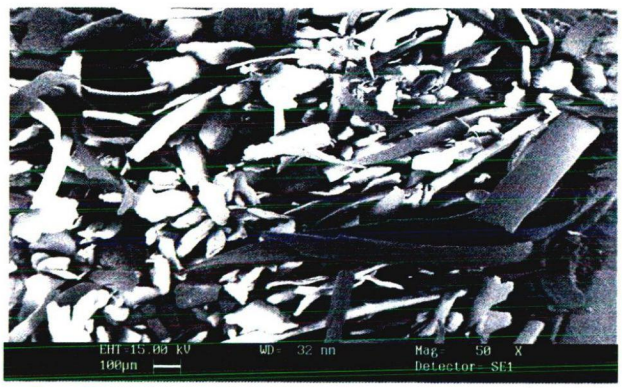
(4.5.3.b)

Fig.4.5.3. Ground and sieved raw *Litchi cinensis* peel waste (4.5.3.a), SEM photograph of Ground and sieved raw *Litchi cinensis* peel waste (4.5.3.b)

On the other hand SEM icon of *Pisum sativum* Peel waste demonstrate their rough and smooth surfaces with shapeless pointed arrangement as viewed from Fig (4.5.1.b), Fig (4.5.2.b), Fig (4.5.3.b), Fig (4.5.4.b) respectively.



(4.5.4.a)



(4.5.4.b)

Fig.4.5.4. Ground and sieved raw *Pisum sativum* peel waste (4.5.4.a), SEM photograph of Ground and sieved raw *Pisum sativum* peel waste (4.5.4.b).

4.5.1.2. Proximate analysis of non conventional raw materials

To determine the suitability and effectiveness of raw materials for cellulase production, separate set of experiments and analysis have been performed. To resolve the major constituent of raw materials, proximate analysis of different raw materials have been executed separately.

Table 4.5.1. Major Constituents of Ground, Sieved, and Oven-dried *Pisum sativum* peel waste

Constituents	% composition
Holocellulose	**61.35±4.96**
Lignin	**22.12±3.18**
Ash	**4.80±1.76**
Moisture	**3.53±0.67**

Data are reported as mean ± standard deviation based on the repeated trails.

Table 4.5.1 describes the major constituents as holocellulose and lignin present in the dried and grounded peapeel waste. The rest of the valuable constituents present in this raw material, may be pectins and antioxidants.

To resolve the major constituent of *Luffa cylindrica* peel, proximate analysis have been executed.

Table 4.5.2. Major Constituents of Sieved, and Oven-dried *Luffa cylindrica* peel

Constituents	% composition
Holocellulose	**70.53±3.46**
Lignin	**16.96±2.21**
Ash	**2.60±1.12**
Moisture	**3.16±0.97**

Data are reported as mean ± standard deviation based on the repeated trails.

To resolve the major constituent of *L. siceraria* peel, proximate analysis have been executed.

Table 4.5.3. Major Constituents of Ground, Sieved, and Oven-dried *Lageneria siceraria* peel

Constituents	% composition
Holocellulose	**66.35±4.66**
Lignin	**21.80±3.38**
Ash	**3.07±1.86**
Moisture	**3.53±0.89**

Data are reported as mean ± standard deviation based on the repeated trails.

To resolve the major constituent of *Litchi sinensis* peel, proximate analysis have been executed.

Table 4.5.4. Major Constituents of Ground, Sieved, and Oven-dried *Litchi cinensis* peel

Constituents	% composition
Holocellulose	**59.95±4.79**
Lignin	**25.92±3.43**
Ash	**4.50±1.66**
Moisture	**2.53±0.67**

Data are reported as mean ± standard deviation based on the repeated trails.

It has been observed from the Tables 4.5.1-4.5.4 that holocellulose and lignin are the major constituents present in almost each raw material. When we compared the holocellulose composition, we found that *Luffa cylindrica* peels having (70.53±3.46) higher percentage of this, while lignin percentage (25.92±3.43) was found much higher in *Litchi cinensis* bed material as compared to others.

4.5.1.3. XRD pattern of non conventional raw materials

To determine the availability and nature of cellulose present in the nonconventional raw materials, XRD analysis have been executed.

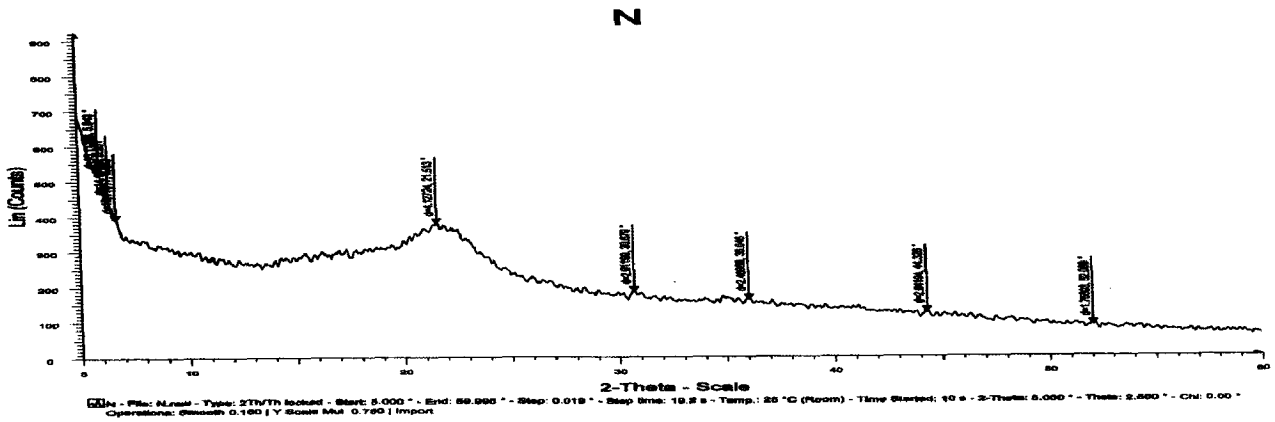


Fig 4.5.5. XRD pattern of *Luffa cylindrica* peel waste

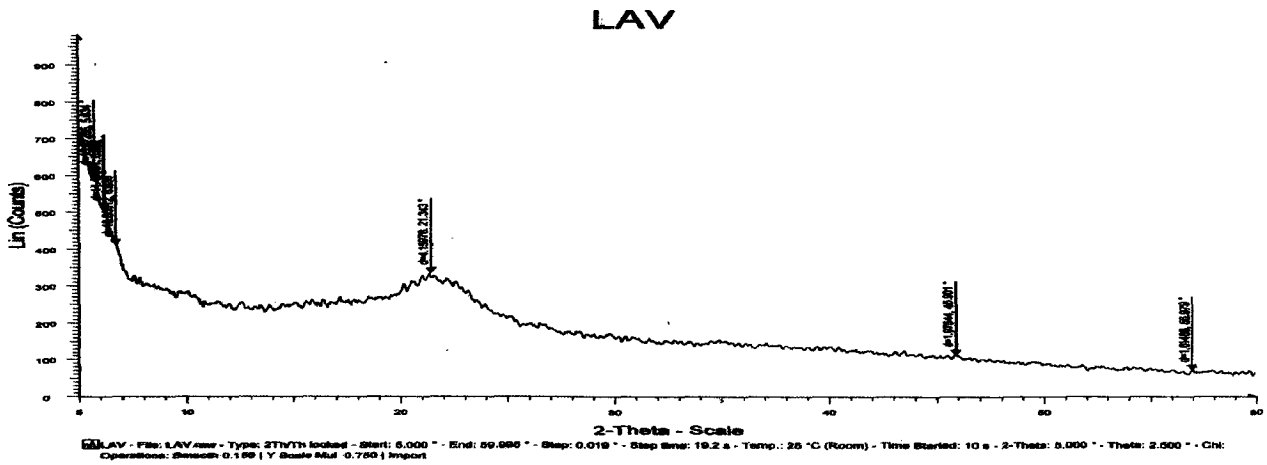


Fig 4.5.6. XRD pattern of *Lagenaria cineraria* peel waste

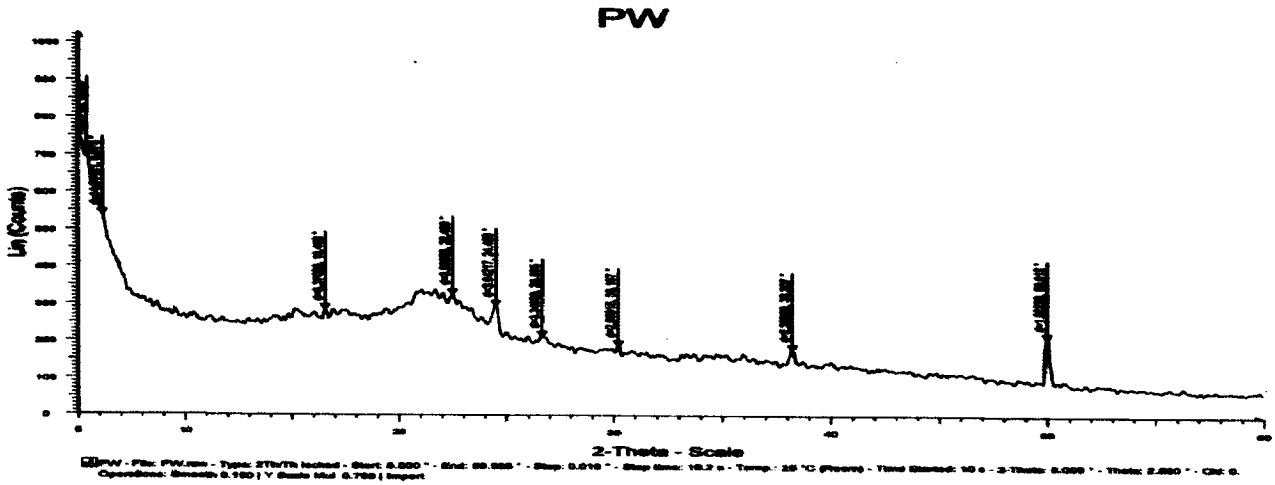


Fig 4.5.7. XRD pattern of *Pisum sativum* peel waste

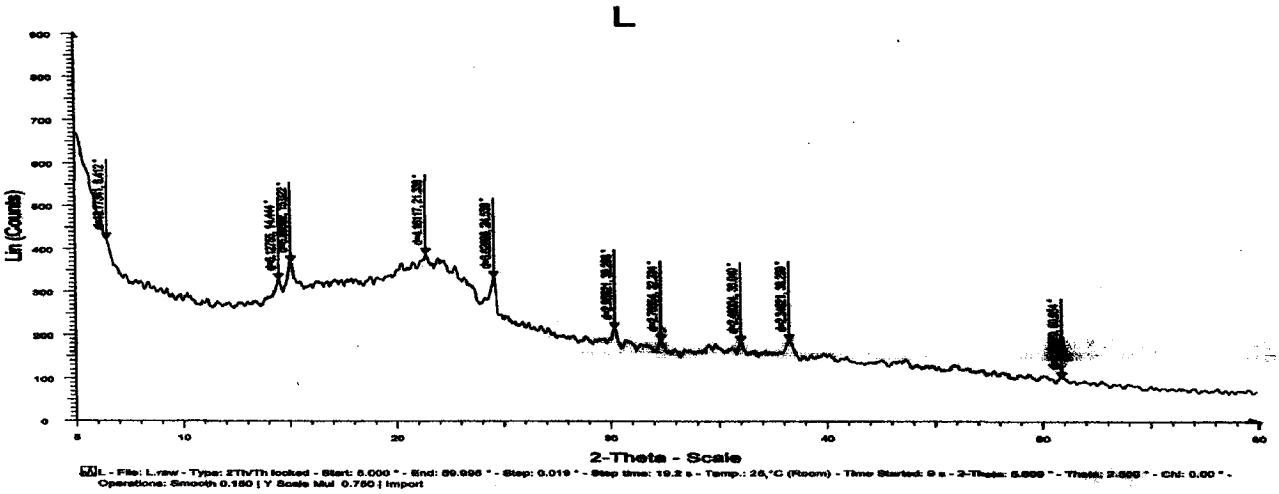


Fig 4.5.8.XRD pattern of *Litchi cinensis* peel waste

When we compared the XRD pattern of different non conventional raw materials used in cellulase production, we have discerned that *Litchi cinensis* peels have much number of peaks with reasonable peak heights followed by *Pisum sativum* peel having peaks with modest peak heights respectively shows their random crystalline nature as observed from Fig 4.5.7 and 4.5.8.

On the other hand *Lagenaria cineraria* and *Luffa cylindrica* peels show lesser number of peaks with much reduced peak heights proves their lesser crystalline nature as viewed from Fig 4.5.5 and 4.5.6.Therefore we can suggest that cellulose present in *Luffa cylindrica* and *Lagenaria cineraria*

peels are easily available for microbial attack compared to other non conventional raw material used in production study.

4.5.1.4. FTIR spectra of non conventional raw materials

To identify the constituents of lignocellulosic waste materials FTIR spectroscopy have been performed.This is a well established analytical method for process monotiring and identifying the chemical species. It gives total simultaneous chemical analysis of lignocellulosic waste material.

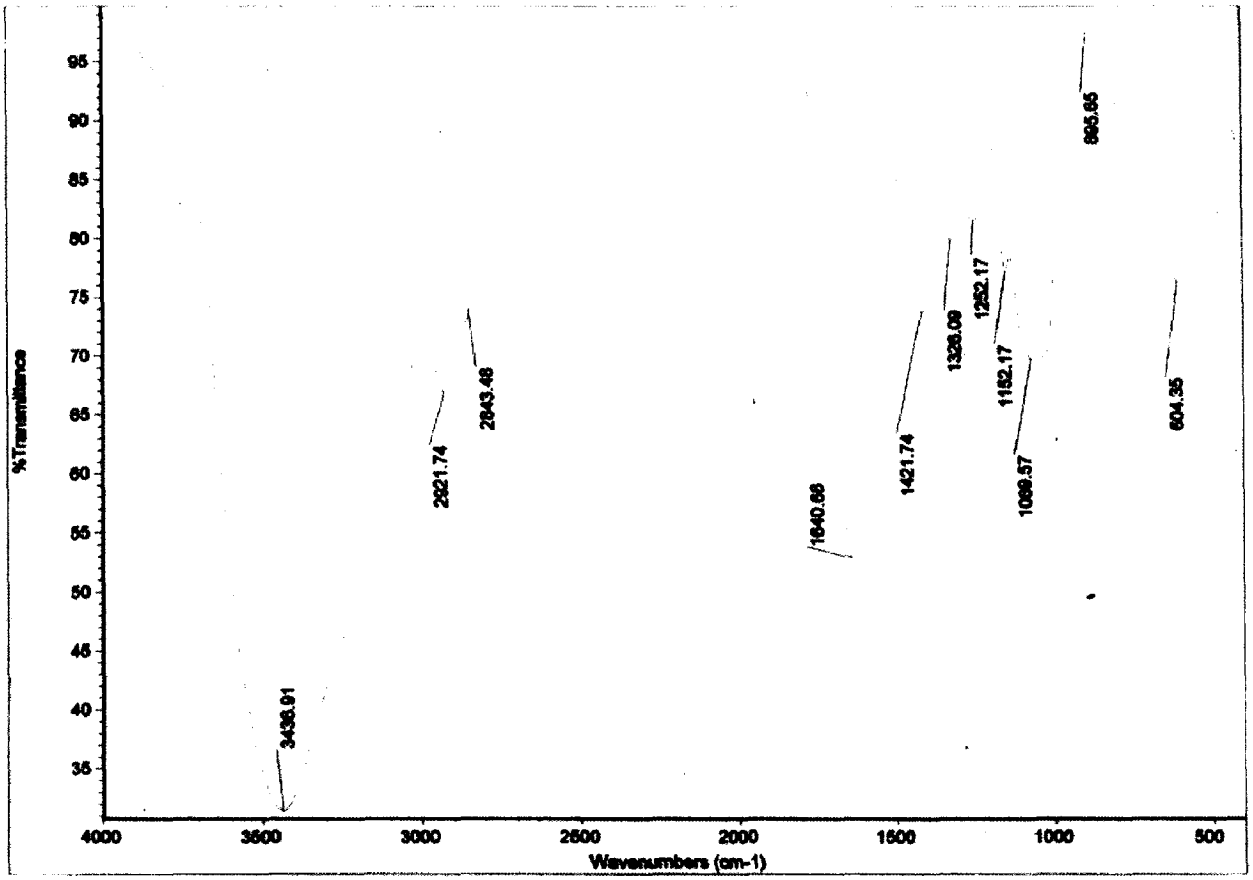


Fig 4.5.9. FTIR spectral diagram of untreated dried, grounded *Pisum sativum* peel waste

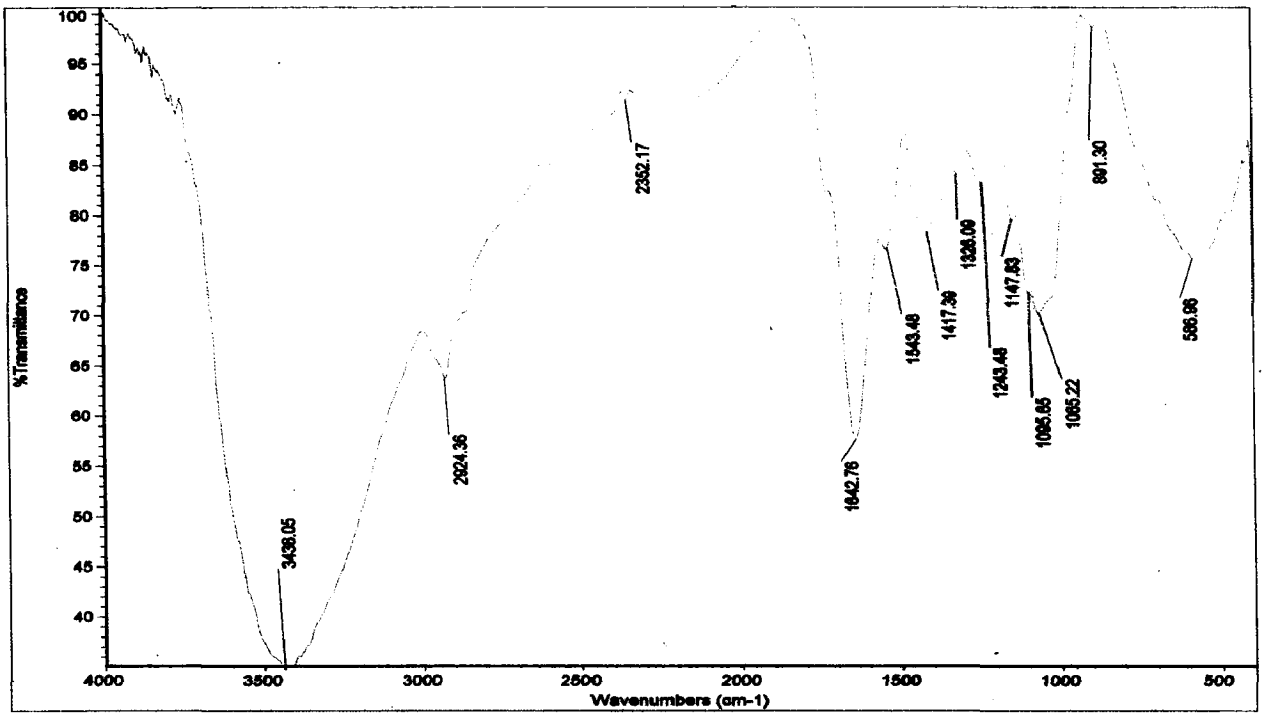


Fig 4.5.10.FTIR spectral diagram of dried, grounded *Luffa cylindrica* peel waste.

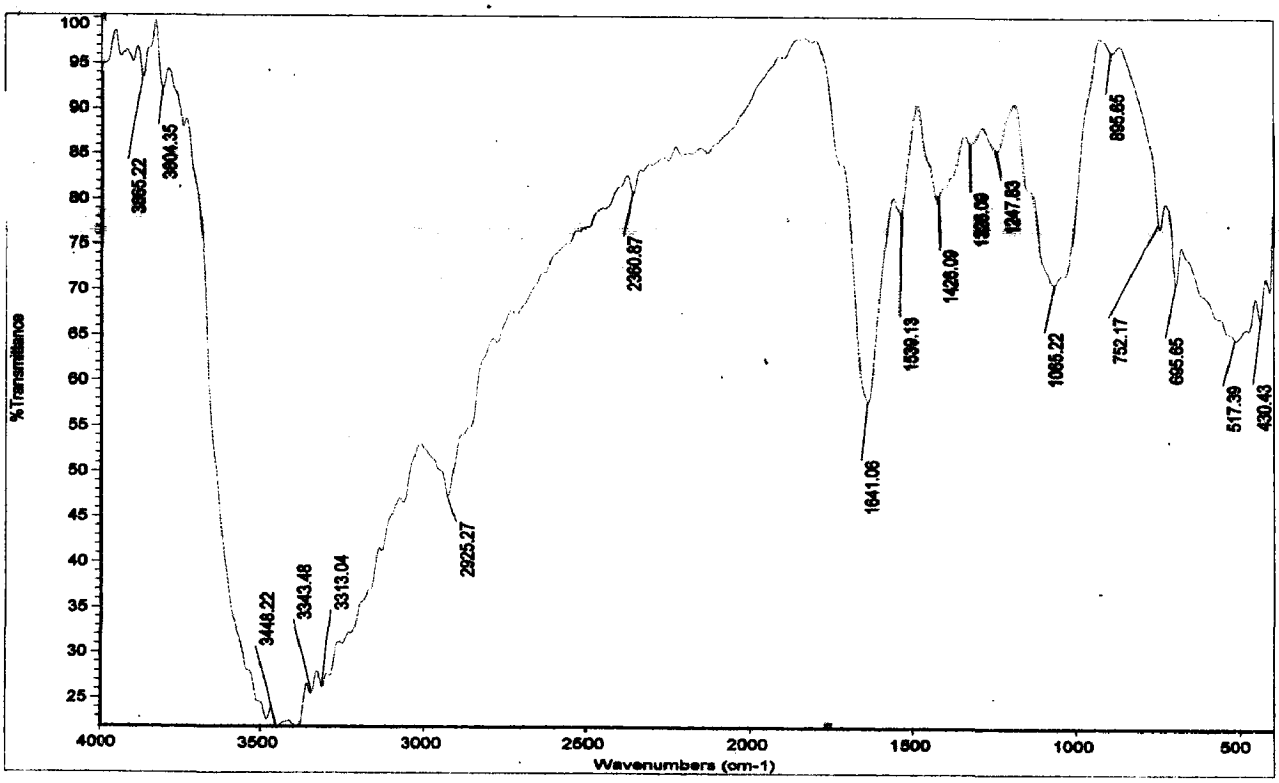


Fig 4.5.11.FTIR spectral diagram of dried, grounded *Lagenaria cineraria* peel waste.

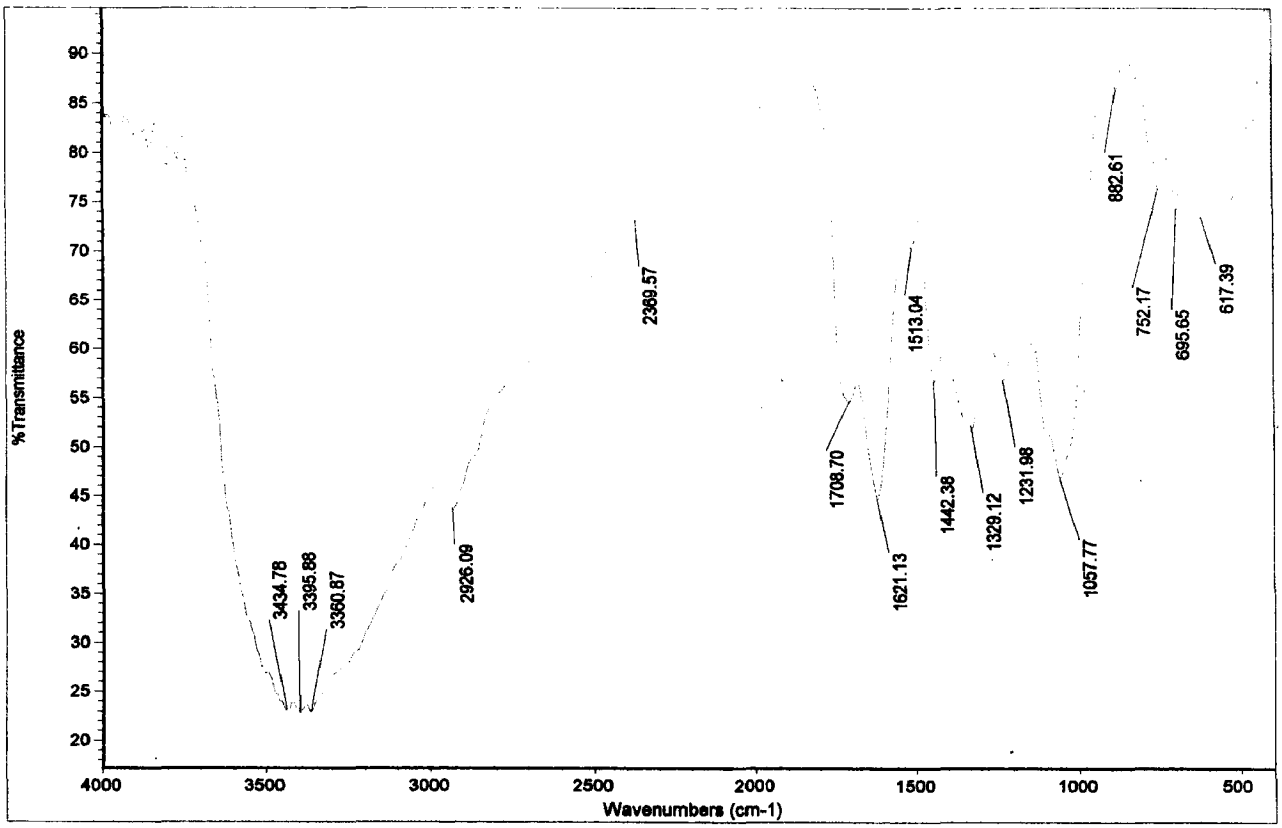


Fig 4.5.12.FTIR spectral diagram of dried, grounded *Litchi cinencis* peel waste.

FTIR spectroscopy was used for identifying the constituents of lignocellulosic structure. The lignocellulosic composition of different raw materials could be identified from the peak presence between 3434 cm^{-1} and 882 cm^{-1}. FTIR spectra of raw materials showed several peaks (3436 cm^{-1}, 2921cm^{-1}, 1640 cm^{-1}, 1421 cm^{-1}, 1326 cm^{-1}, 1252 cm^{-1}, 1152 cm^{-1},1069 cm^{-1}, and 895 cm^{-1}; 3436 cm^{-1}, 2924 cm^{-1}, 2352 cm^{-1},1642 cm^{-1},1417 cm^{-1},1326 cm^{-1},1243 cm^{-1},1147 cm^{-1},1065 cm^{-1},891 cm^{-1};3448 cm^{-1},3343 cm^{-1},3313 cm^{-1},2925 cm^{-1},2360 cm^{-1}, 1641 cm^{-1},1426 cm^{-1}, 1326 cm^{-1}, 1247 cm^{-1}, 1065 cm^{-1},895 cm^{-1};3434 cm^{-1},3395 cm^{-1},3360 cm^{-1}, 2926 cm^{-1},2369 cm^{-1},1708 cm^{-1},1621 cm^{-1},1442 cm^{-1},1329 cm^{-1},1231 cm^{-1},1057 cm^{-1}, 882 cm^{-1} etc in this region for , *Luffa cylindrica*, *Lagenaria cineraria* and *Litchi cinensis* peels waste material respectively as shown in Fig 4.5.9-4.5.12.

It was observed that *Trichoderma* and *Aspergillus* and *Neurospora* grew well with most of the non conventional solid bed material except *Litchi cinensis* solid bed. *Neurospora* growth was very poor with least cellulase activities under *Litchi cinensis* solid bed followed by *Pisum sativum* solid bed whereas *A.niger* shows boomed growth with significantly higher cellulases under *Lagenaria cineraria* based solid bed as shown by Figs 4.5.13, 4.5.14, 4.5.17, 4.5.21 and 4.5.25.These observations prove that *T. reesei* and *A.niger* fungal strains have the ability to grow and produce cellulases under raw materials having phenolics constituents on the other hand *N.crassa* shows lesser tolerance under such harsh conditions. During thermal treatment in autoclaving a blanching process occurred, which disrupted the cell membranes, cell walls, and hydrolysed the bonds, making more available the cellulosic as well as other constituents such as antioxidant and phenolics. As literature suggested, *T. reesei* have the tolerance to grow in the phenolic environment up to a certain level [223].

When we compared the capability of various raw materials for fungal attack, growth and cellulase production, we have observed that *Luffa cylindrica* peel solid bed has much higher potential for fungal growth and cellulase production as compared to others as observed from Figs 4.5.17 a, 4.5.17 b and 4.5.17 c. Scanning electron microscopic analysis also proves enhanced growth of *T.reesei* and *A.niger* under *Luffa cylindrica* based solid state fermentation as shown by Figs 4.5.18.a and 4.5.18.b respectively. Higher FPA and CMCase activities were observed by *T.reesei* (4.04IU/mL, 3.58IU/mL); *N.crassa* (2.07 IU/mL, 5.49 IU/mL and *A.niger* (1.04IU/mL, 8.66IU/mL) under utilizing *Luffa cylindrica* solid bed solid support whereas lower growth and cellulase production in terms of FPA and CMCase (1.98IU/mL, 1.44 IU/mL; 0.911 IU/mL, 2.08 IU/mL; 0.532 IU/mL, 4.06 IU/mL) were discerned by *T.reesei*, *N.crassa, A.niger* respectively under *Litchi cinensis* solid bed as viewed by Table 4.5.7. and Fig 4.5.13.Significant amount of cellulase activities were observed by *T.reesei* (3.38IU/mL, 2.78IU/mL; 2.86 IU/mL, 2.31 IU/mL) *N.crassa* (1.52 IU/mL, 4.42 IU/mL; 1.47 IU/mL, 3.89 IU/mL) and *A.niger* (0.898 IU/mL, 8.25 IU/mL; 0.768 IU/mL, 5.87IU/mL) under *Lagenaria cineraria* and *Pisum sativum* solid bed respectively.

Table 4.5.7. Comparative enzyme activities produced by *T.reesei, N.crassa* and *A. niger* under various non conventional raw materials as carbon source.

Microrganisms / Enzyme activities / BSM + Raw materials	*Trichoderma reesei*		*Neurospora crassa*		*Aspergillus niger*	
	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
Basal salt media + *Pisum sativum* **peel waste**	2.86	2.68	1.47	3.89	0.768	5.87
Basal salt media + *Luffa cylindrica* **peel waste**	4.04	4.51	2.07	5.49	1.04	8.66
Basal salt media + *Lagenaria cineraria* **peel waste**	3.38	3.45	1.52	4.42	0.898	**8.25**
Basal salt media + *Litchi cinensis* **peel waste**	1.73	1.38	0.911	2.08	0.532	4.06

BSM: Basal salt media

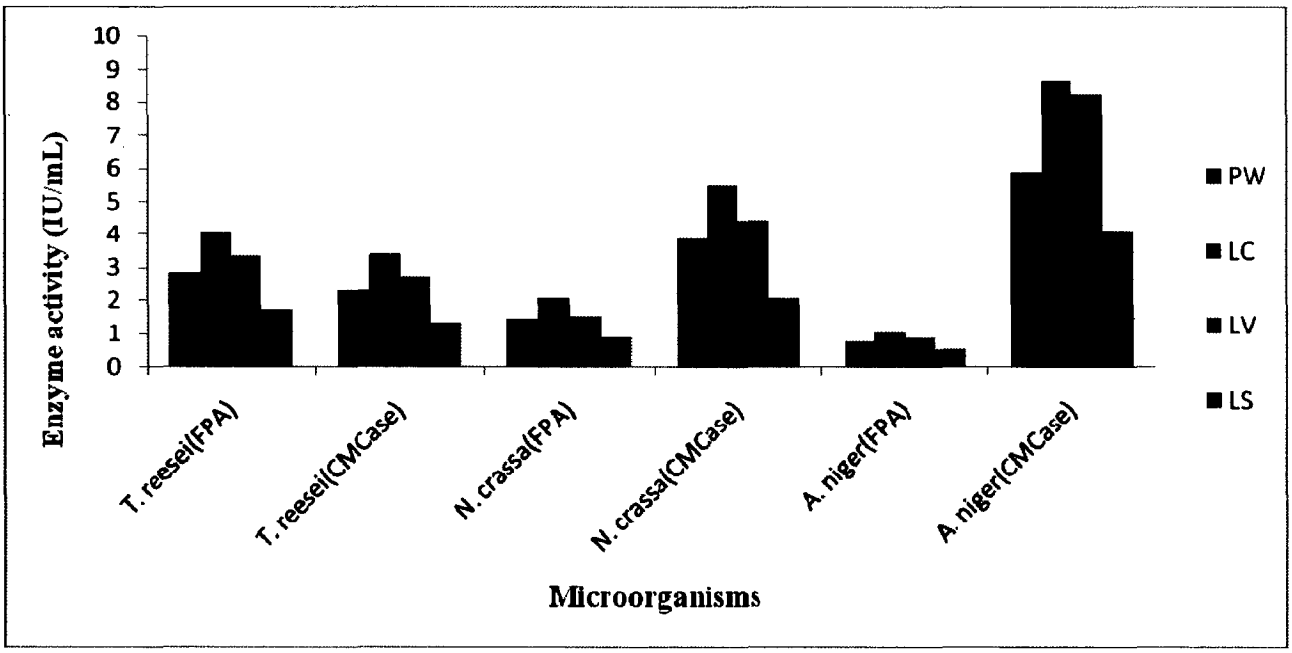


Fig.4.5.13. Comparative bar diagram of enzyme activities produced by *T.reesei, N.crassa* and *A. niger* under PW (*Pisum sativum)*, LC (*Luffa cylindrica)*, LV(*Lagenaria cineraria)* and LS (*Litchi cinensis)* raw materials as carbon source.

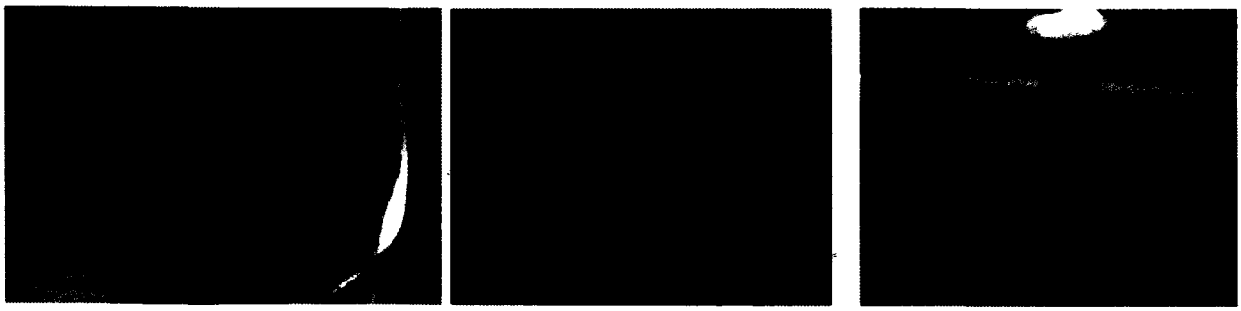
As observed from Figs 4.5.5-4.5.8 that *Luffa cylindrica* and *Lagenaria cineraria* peels raw materials having lesser crystallanity compared to *Litchi cinensis* and *Pisum sativum* peel raw materials which makes their cellulose much easier accessible for microbial hydrolysis. On the other hand proximate analysis datas from Table 4.5.1- 4.5.4, also show that *Luffa cylindrica* peel containing much higher percentage of holocellulose as well as lower percentage of lignin

constituents as compared to *Lagenaria cineraria*, *Pisum sativum* and *Litchi cinensis* peels provides much favorable condition for fungal attack and cellulase production. This situation also proved by FTIR spectral diagram of different raw material shown by Figs 4.5.9-4.5.12, which suggests that *Luffa cylindrica*, *Lagenaria cineraria* and *Pisum sativum* peels having sizeable amount of cellulose required for efficient cellulase production.

Table 4.5.8. Cellulase activities produced by *T. reesei, N.crassa* and *A. niger* on normal basal salt as well as modified basal salt media incorporated on *P. sativum* Peel Waste based Solid Bed at 30 °C and pH 5.0.

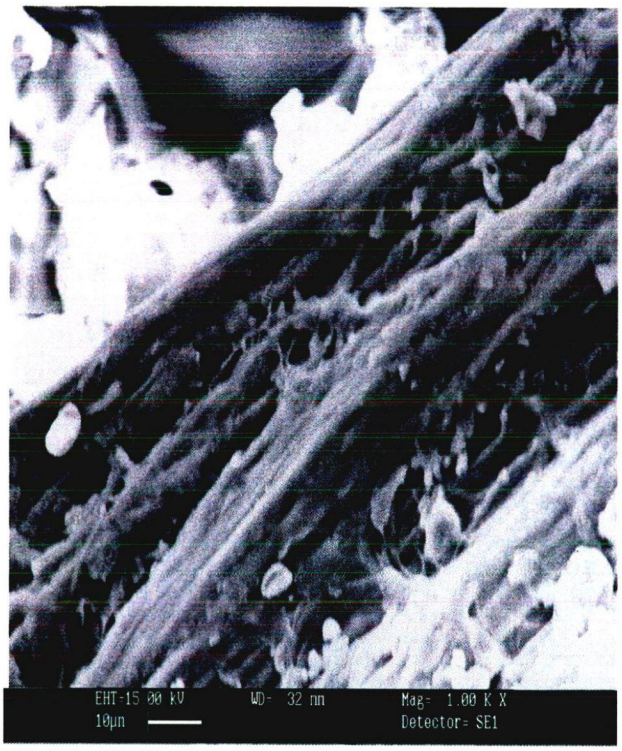
BSM +Whey + Starch hydrolysates		***Trichoderma reesei***		***Neurospora crassa***		***Aspergillus niger***	
		FPA (IU/mL)	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**
Basal salt media + *Pisum sativum* peel waste		2.86	2.68	1.47	3.89	0.768	5.87
BSM+ *Pisum sativum* peel+Whey		3.37	2.85	1.58	4.02	0.856	6.45
W+WSH	30%+UTWS 2% (v/v)	3.44	2.91	1.60	3.98	0.861	6.51
	30%+2%(v/v)	3.63	3.26	1.68	4.10	0.892	7.01
	30%+5%(v/v)	3.95	3.65	1.73	4.16	0.976	7.16
W+PSH	30%+UTPS 2% (v/v)	3.40	2.82	1.64	4.08	0.853	6.43
	30%+2%(v/v)	3.63	3.13	1.83	4.21	0.869	6.58
	30%+5%(v/v)	3.72	3.21	2.05	4.63	0.876	6.65
W+RSH	30%+UTRS (v/v)	3.42	2.94	1.61	4.04	0.864	6.49
	30%+2%(v/v)	3.69	3.09	1.75	4.16	0.898	6.88
	30%+5%(v/v)	3.83	3.37	1.98	4.51	0.997	7.01

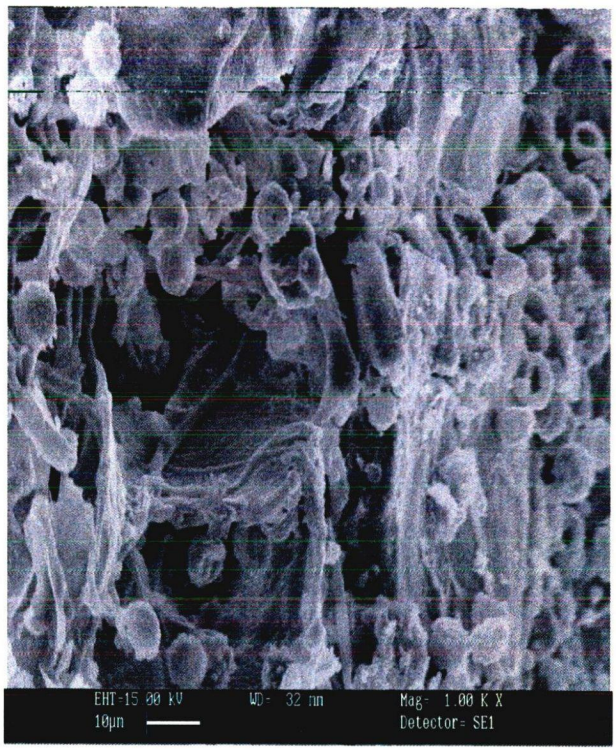
W: Whey, UTWS: Untreated wheat starch; UTPS: Untreated potato starch; UTRS: Untreated rice starch; WSH:Wheat starch hydrolysate; PSH: Potato starch hydrolysate; RSH: Rice starch hydrolysates.



(4.5.14.a) (4.5.14.b) (4.5.14.c)

Fig.4.5.14. Growth of *T. reesei*, *A.niger* and *N.crassa* on *Pisum sativum* peel waste based solid bed respectively at 30°C and pH 5.0 (4.5.14.a, 4.5.14.b, 4.5.14.c).





(4.5.15.a) (4.5.15.b)

Fig 4.5.15.: SEM micrograph of *T. reesei* and *A.niger* treated *Pisum sativum* peel waste solid bed under 1000X magnification (4.5.15.a), (4.5.15.b) respectively.

On the other hand fungal strains produced slightly lower cellulase activities under *Pisum sativum* peel waste which might be due to the presence of sizeable amount of lignin as well as moderate and random crystalline nature of this raw material. These circumstances jointly create few hindrances for microbial attack and cellulase production as observed from Table 4.5.1 and Figs 4.5.7, 4.5.9, 4.5.14.a, 4.5.14.b, 4.5.14.c.Even if under this critical situation microbes produces significant cellulase activities on *Pisum sativum* peel waste used as carbon source. Scanning electron micrographs of *T.reesei* and *A. niger* also proves their good growth under *Pisum sativum* based solid state fermentation as viewed by Figs 4.5.15.a, 4.5.15.b respectively.

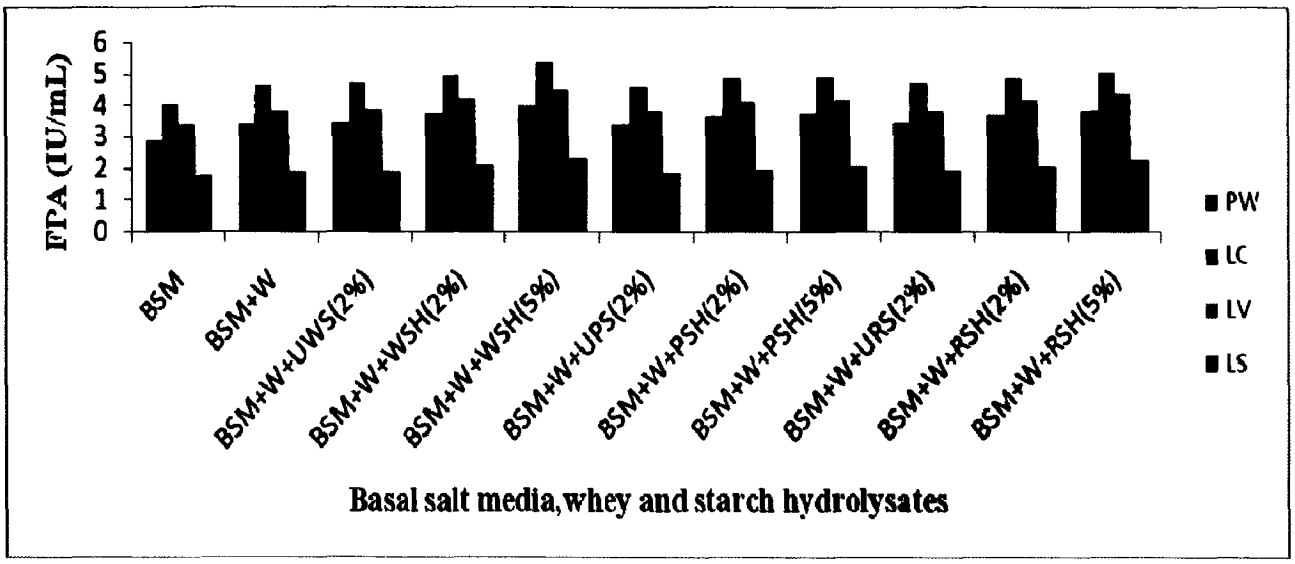
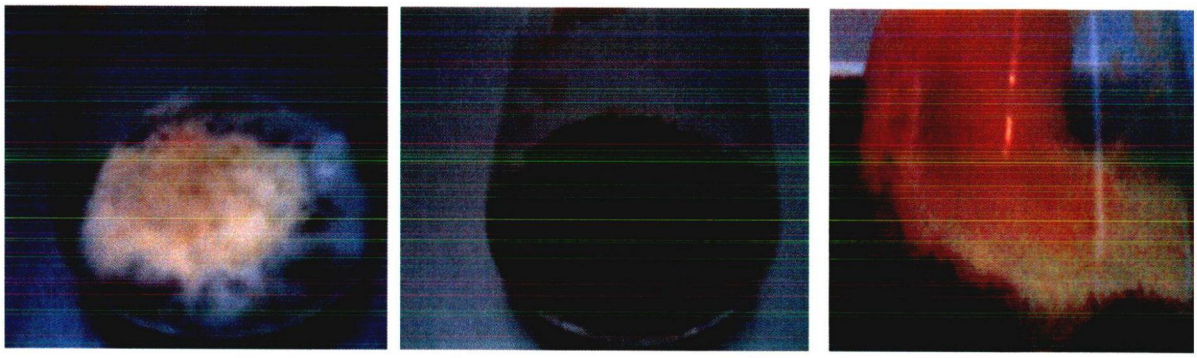


Fig.4.5.16. Comparative bar diagram of FPA produced by *T. reesei* on normal basal salt as well as modified basal salt media incorporated on PW(*Pisum sativum)*, LC(*Luffa cylindrica)*, LV(*Lagenaria cineraria)* and LS(*Litchi cinensis)* peel waste based solid bed at 30 °C and pH 5.0

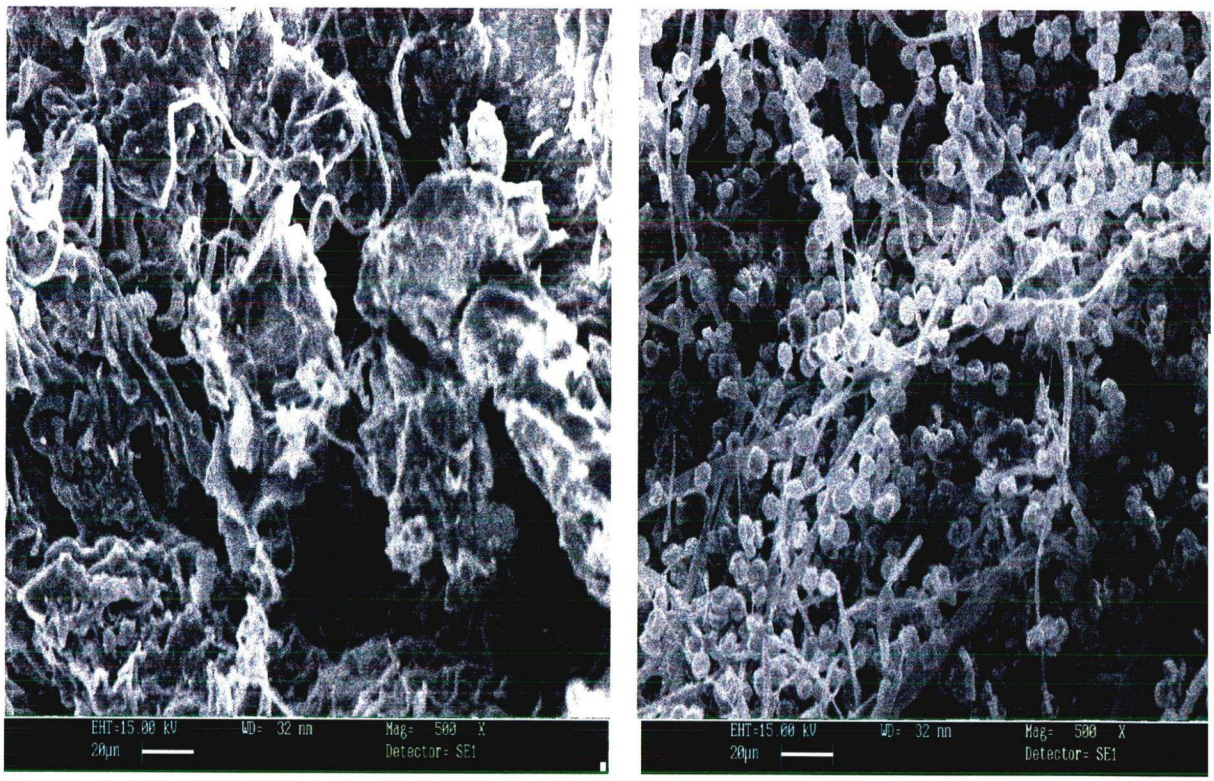
Table 4.5.9. Cellulase activities produced by *T. reesei, N.crassa* and *A. niger* on normal basal salt as well as modified basal salt media incorporated on *Luffa cylindrica* Peel Waste based Solid Bed at 30 °C and pH 5.0

BSM +Whey + Starch hydrolysates		***Trichoderma reesei***		***Neurospora crassa***		***Aspergillus niger***	
		FPA (IU/mL)	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**
Basal salt media + *Luffa cylindrica*		4.04	4.51	2.07	5.49	1.04	8.66
BSM+ *Luffa cylindrica* **peel+Whey**		4.64	4.70	2.25	5.66	1.13	9.02
W+WSH	30%+UTWS 2% (v/v)	4.69	4.75	2.28	5.53	1.17	9.12
	30%+2%(v/v)	4.95	5.16	2.39	5.86	1.21	9.65
	30%+5% (v/v)	5.39	5.62	2.44	5.93	1.28	10.02
W+PSH	30%+UTPS 2% (v/v)	4.62	4.73	2.36	5.70	1.11	9.00
	30%+2%(v/v)	4.85	5.01	2.61	6.13	1.16	9.09
	30%+5%(v/v)	4.91	5.17	2.78	6.29	1.18	9.17
W+RSH	30%+UTRS(v/v)	4.70	4.68	2.30	5.61	1.14	9.05
	30%+2%(v/v)	4.86	5.09	2.54	5.96	1.21	9.68
	30%+5%(v/v)	5.03	5.35	2.69	6.05	1.32	9.75



(4.5.17.a) (4.5.17.b) (4.5.17.c)

Fig.4.5.17. Growth of *T. reesei*, *A.niger* and *N.crassa* on *Luffa cylindrica* peel waste based solic bed respectively at 30^0C and pH 5.0 (4.5.17.a, 4.5.17.b, 4.5.17.c).



(4.5.18.a) (4.5.18.b)

Fig 4.5.18.: SEM micrograph of *T. reesei* and *A.niger* treated *Luffa cylindrica* peel waste solid under 500X magnification (4.5.18.a), (4.5.18.b) respectively.

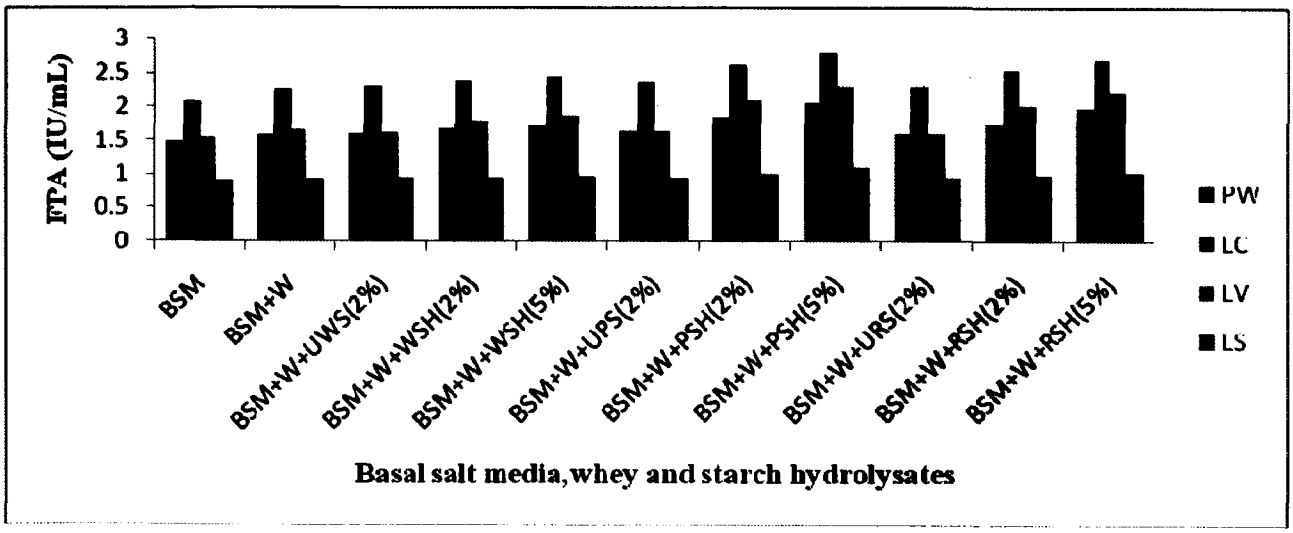


Fig.4.5.19. Comparative bar diagram of FPA produced by *N.crassa* on normal basal salt as well as modified basal salt media incorporated on PW(*Pisum sativum)*, LC (*Luffa cylindrica)*, LV(*Lagenaria cineraria*)and LS(*Litchi cinensis)* peel waste based solid bed at 30 °C and pH 5.0

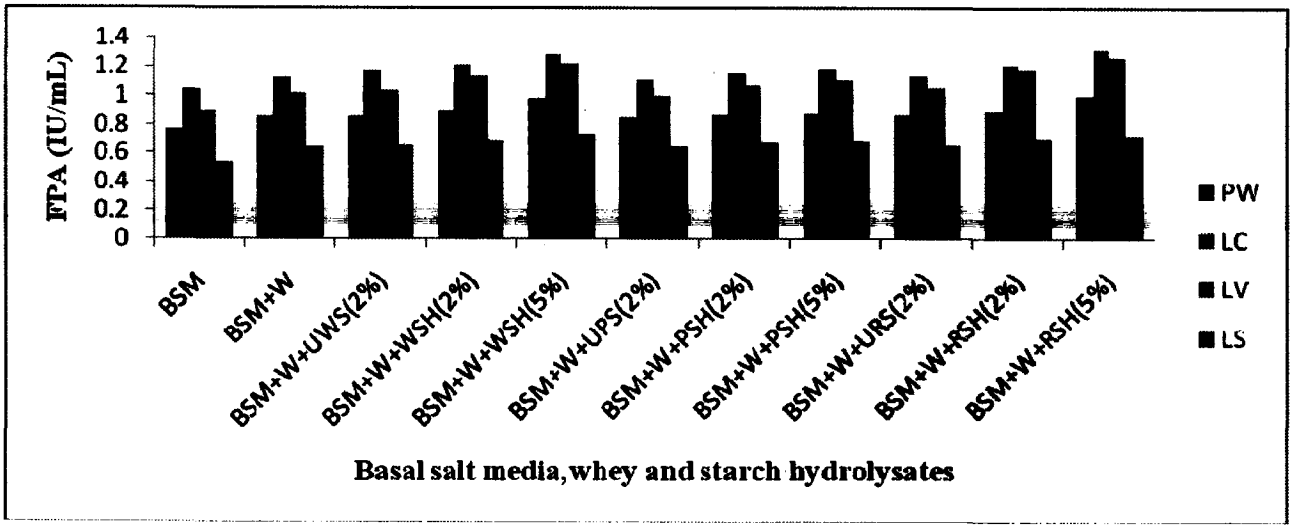
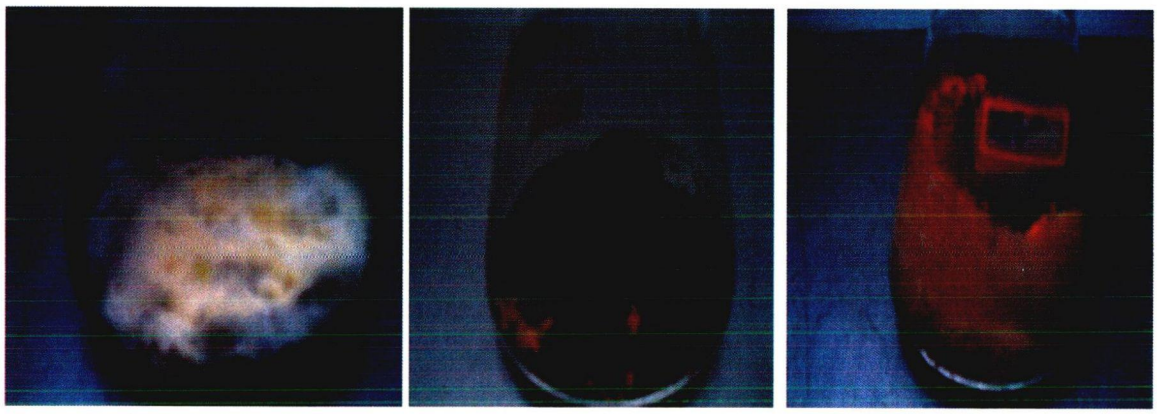


Fig.4.5.20. Comparative bar diagram of FPA produced by *A.niger* on normal basal salt as well as modified basal salt media incorporated on PW(*Pisum sativum)*, LC (*Luffa cylindrical)*, LV(*Lagenaria cineraria)* and LS(*Litchi cinensis)* peel waste based solid bed at 30 °C and pH 5.0

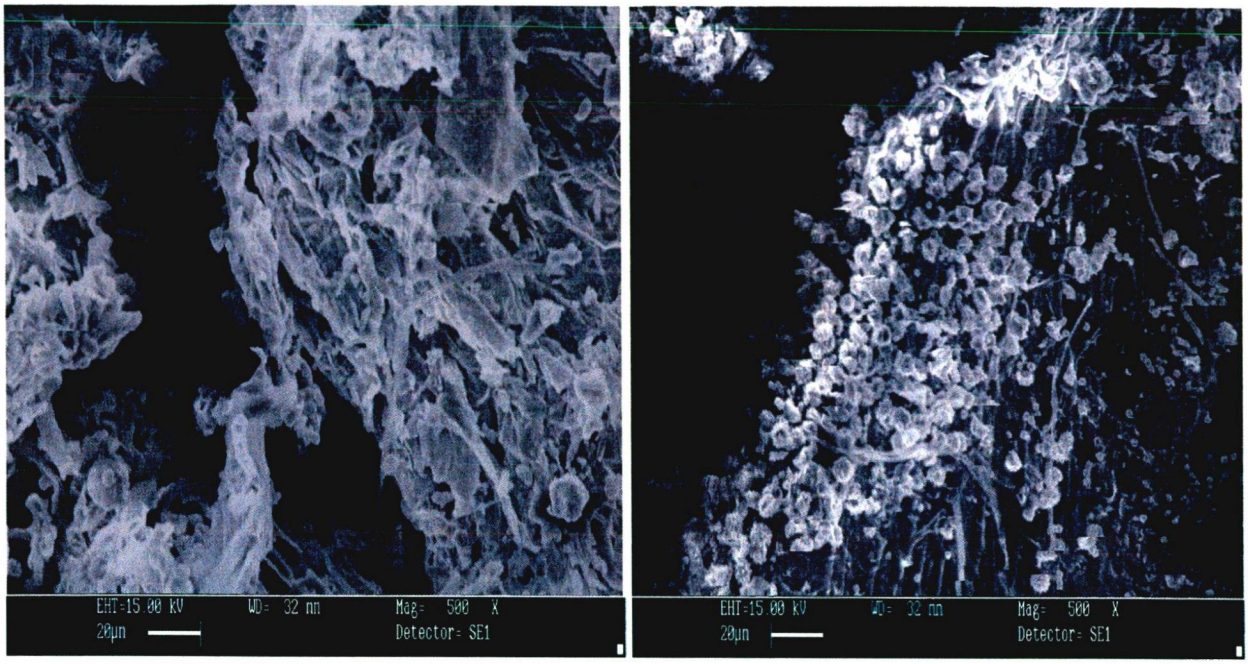
Table 4.5.10. Cellulase activities produced by *T. reesei, N.crassa* and *A. niger* on normal basal salt as well as modified basal salt media incorporated on *Lagenaria cineraria* Peel Waste based Solid Bed at 30 °C and pH 5.0.

BSM +Whey + Starch hydrolysates		***Trichoderma reesei***		***Neurospora crassa***		***Aspergillus niger***	
		FPA (IU/mL)	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**	**FPA (IU/mL)**	**CMCase (IU/mL)**
Basal salt media + *Lagenaria cineraria*		3.38	3.45	1.52	4.42	0.898	8.25
BSM+ *Lagenaria cineraria* **peel+Whey**		3.80	3.58	1.66	4.58	1.01	8.66
W+WSH	30%+UTWS 2% (v/v)	3.85	3.67	1.63	4.56	1.03	8.70
	30%+2%(v/v)	4.19	4.01	1.79	4.70	1.14	9.20
	30%+5%(v/v)	4.45	4.20	1.86	4.81	1.22	9.85
W+PSH	30%+UTPS 2% (v/v)	3.82	3.63	1.65	4.67	0.998	8.68
	30%+2%(v/v)	4.10	3.82	2.09	4.89	1.07	8.81
	30%+5%(v/v)	4.16	3.93	2.28	5.43	1.11	8.83
W+RSH	30%+UTRS (v/v)	3.84	3.65	1.60	4.59	1.05	8.67
	30%+2%(v/v)	4.16	3.88	2.01	4.83	1.18	8.89
	30%+5%(v/v)	4.37	4.05	2.21	5.33	1.26	9.35



(4.5.21.a) (4.5.21.b) (4.5.21.c)

Fig.4.5.21. Growth of *T. reesei*, *A.niger* and *N.crassa* on *Lagenaria cineraria* peel waste bas solid bed respectively at 30^{0}C and pH 5.0 (4.5.21.a, 4.5.21.b, 4.5.21.c).



(4.5.22.a) (4.5.22.b)

Fig 4.5.22. SEM micrograph of *T. reesei* and *A.niger* treated *Lagenaria cineraria* peel waste solid bed under 500X magnification (4.5.22.a), (4.5.22.b) respectively.

lthough *Lagenaria cineraria* peels having significant amount of lignin percentage which creates some unfavorable situations for microbial growth but sizeable percentage of easily available cellulose with low crystallanity counterattacks unfavorable situations as observed from Table 4.5.3 and Figs 4.5.6, 4.5.11.Therefore all these conditions may communally provide encouraging environment for microbial growth and cellulase production as shown by Figs 4.5.21.a, 4.5.21.b, 4.5.21.c.Scanning electron micrographic analysis also proves fruitful growth.of *T.reesei* and *A. niger* under *Lagenaria cineraria* based solid state fermentation as shown by Figs 4.5.22.a and 4.5.22.b.

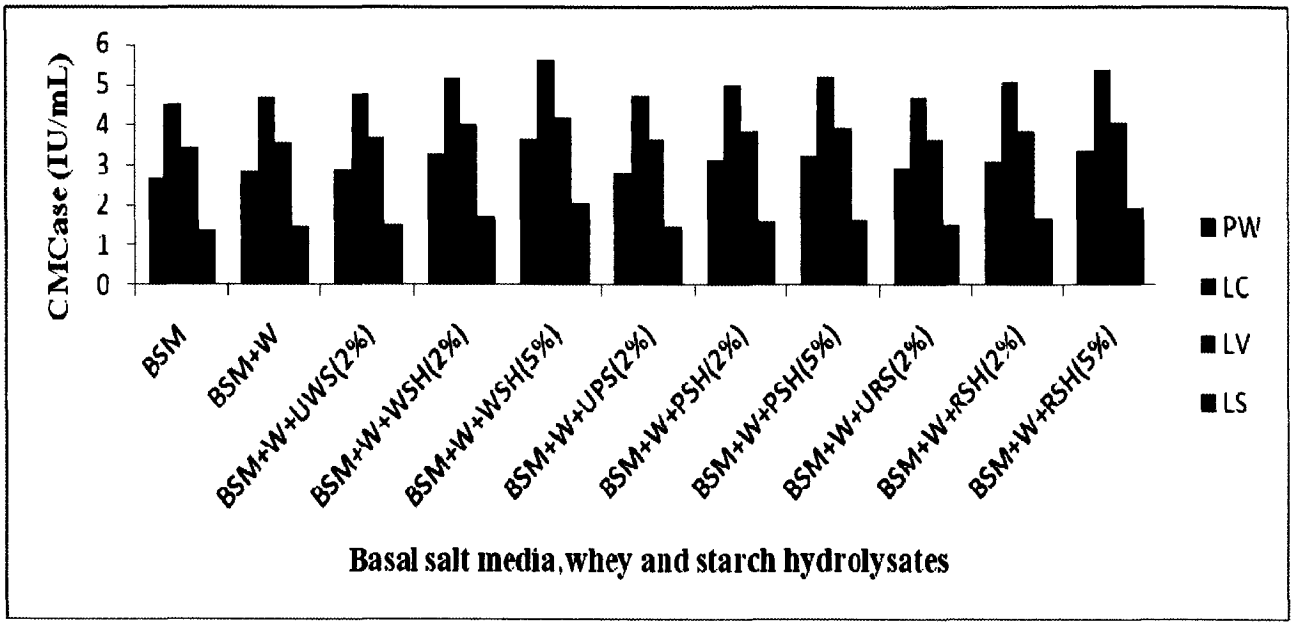


Fig.4.5.23. Comparative bar diagram of CMCase activities produced by *T. reesei* on normal basal salt as well as modified basal salt media incorporated on PW(*Pisum sativum)*, LC(*Luffa cylindrica)*, LV(*Lagenaria cineraria)* and LS(*Litchi cinensis)* peel waste based solid bed at 30 °C and pH 5.0

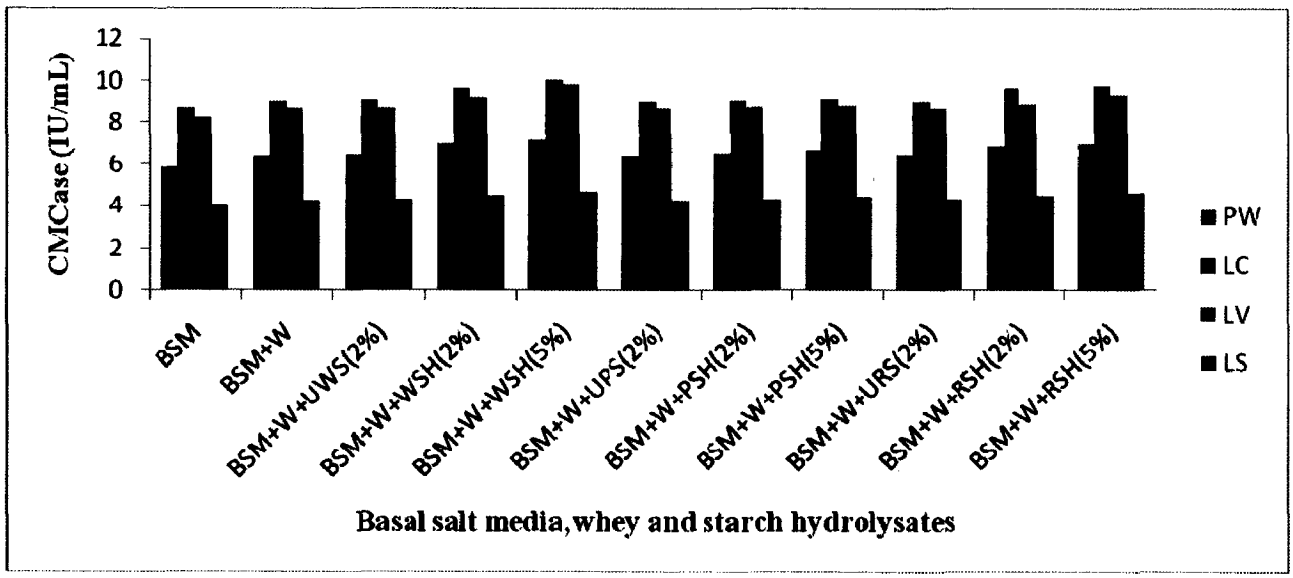
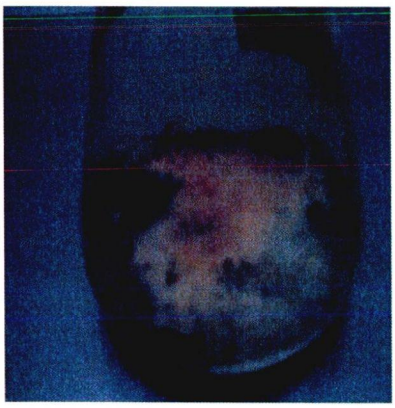


Fig.4.5.24. Comparative bar diagram of CMCase activities produced by *A. niger* on normal basal salt as well as modified basal salt media incorporated on PW(*Pisum sativum)*, LC(*Luffa cylindrica)*, LV(*Lagenaria cineraria)* and LS(*Litchi cinensis)* peel waste based solid bed at 30 °C and pH 5.0

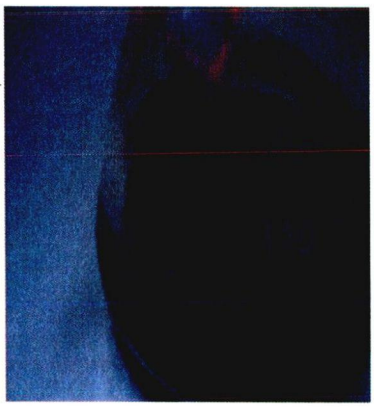
Table 4.5.11. Cellulase activities produced by *T. reesei, N.crassa* and *A. niger* on normal basal salt as well as modified basal salt media incorporated on LS(*Litchi cinensis)* Peel Waste based Solid Bed at 30 °C and pH 5.0

BSM +Whey + Starch hydrolysates		***Trichoderma reesei***		***Neurospora crassa***		***Aspergillus niger***	
		FPA	**CMCase**	**FPA**	**CMCase**	**FPA**	**CMCase**
Basal salt media + *Litchi cinensis* **peel**		1.73	1.38	0.911	2.08	0.532	4.06
BSM+ *Litchi cinensis* **peel+Whey**		1.89	1.47	0.937	2.15	0.643	4.29
W+WSH	30%+UTWS 2% (v/v)	1.93	1.52	0.940	2.11	0.649	4.32
	30%+2%(v/v)	2.08	1.75	0.946	2.19	0.681	4.51
	30%+5%(v/v)	2.35	2.07	0.962	2.26	0.721	4.70
W+PSH	30%+UTPS 2% (v/v)	1.86	1.49	0.945	2.19	0.638	4.27
	30%+2%(v/v)	1.94	1.57	1.01	2.51	0.667	4.34
	30%+5%(v/v)	2.05	1.61	1.10	2.83	0.678	4.47
W+RSH	30%+UTRS(2%v/v)	1.91	1.52	0.939	2.16	0.646	4.30
	30%+2%(v/v)	2.02	1.65	0.993	2.60	0.693	4.49
	30%+5%(v/v)	2.25	1.93	1.03	2.71	0.710	4.61



(4.5.25.a)

(4.5.25.b)



(4.5.25.c)

Fig.4.5.25. Growth of *T. reesei*, *A.niger* and *N.crassa* on *Litchi cinensis* peel waste based solid bed respectively at 30^{0}C and pH 5.0 (4.5.25.a, 4.5.25.b, 4.5.25.c).

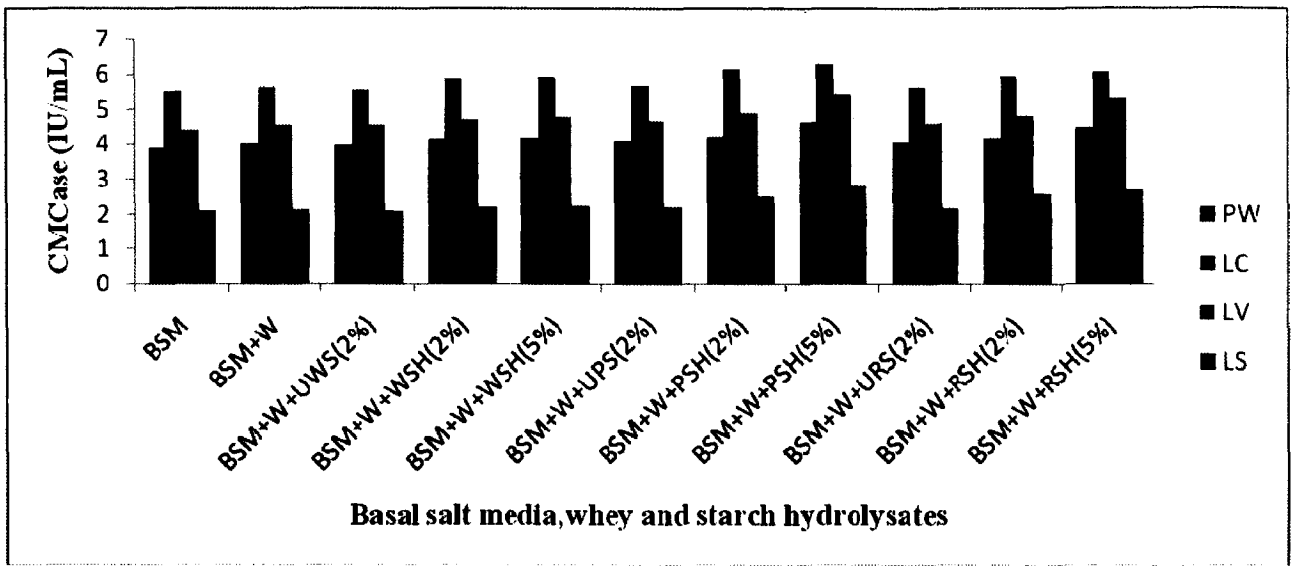


Fig.4.5.26. Comparative bar diagram of CMCase activities produced by *N.crassa* on normal basal salt as well as modified basal salt media incorporated on PW(*Pisum sativum)*, LC(*Luffa cylindrica)*, LV(*Lagenaria cineraria)* and LS(*Litchi cinensis)* peel waste based solid bed at 30 °C and pH 5.0.

Lower cellulase activities were observed by fungal strains under *Litchi cinensis* solid bed which might be due to the presence of higher percentage of lignin in its lignocellulosic composition as well as higher crystalline nature of raw material which collectively imparts something harmful and discouraging conditions for microbial attack and growth, hence these unfavorable surroundings hampers microbial system for cellulase production as observed from Table 4.5.4 and Figs 4.5.4, 4.5.12, 4.5.25.a, 4.5.25.b, 4.5.25.c.

To study the effects of whey and starch hydrolysates separate set of experiments have been performed by utilizing modified basal salt media. Regarding this 30% (v/v) whey were incorporated in the earlier used production media which was further used for impregnation of solid bed. On the other hand 2 and 5% (v/v) wheat, potato as well as rice starch hydrolysate used separately in 30% (v/v) whey based basal salt media which was further used for impregnation of different raw materials solid bed.

It has been observed from Tables 4.5.8-4.5.11 and Figs 4.5.16, 4.5.19 and 4.5.20 that FPA activities were enhanced by incorporation of 30% whey in BSM. Highest increment in FPA was observed by *T. reesei* (4.64IU/mL) strain followed by *A. niger* (1.13IU/mL) and least increment was observed with *N.crassa* (1.58IU/mL) on *Luffa cylindrica* peel based solid state fermentation, which suggests that *T. reesei* quite effectively utilized whey as inducer and carbon source while whey uptake, utilization and induction capacity was very low by *N.crassa* system. On the other

hand FPA activities were not significantly improved by most of the untreated starch except untreated wheat starch based production system in which nominal enhancement in activity was observed. They implies that untreated starch having very little or nearly zero cellulase induction capability.

It has been observed from Tables 4.5.8-4.5.11 and Figs 4.5.16, 4.5.19 and 4.5.20 that acid hydrolyzed starches (2% HCl treated with 1 h pretreatment time) were found much effective in cellulase enhancement. FPA (4.95IU/mL) of *T.reesei* was further enhanced by incorporation of (2%v/v) wheat starch hydrolysate in whey based basal salt medium. By increasing the wheat starch hydrolysate concentration (5%v/v) additional improvement in the FPA (5.39IU/mL) was observed, which suggests that wheat starch hydrolysates contains few sugars which induces the *Trichoderma reesei* cellulase production system. In all the hydrolyzates containing medium, growth and cellulase induction both take place, presumably due to the release of some dimeric sugars in the hydrolyzates which induces cellulase production[47]. In the previous study it was found that starch itself was poor inducer for the cellulase production, but it was rendered highly effective by acid hydrolysis. This was because of the acid hydrolysis of starch resulted in the formation of reversion products, such as sophrose, a disaccharide, formed which has been considered to be to the real inducers for the cellulase biosynthesis[47]. Reasonable improvement in FPA (5.03IU/mL) was also observed by *T.reesei* under (5%v/v) rice starch hydrolyzates medium containing *Luffa cylindrica* peel based solid state fermentation,while potato starch hydrolysates was found not much effective for FPA induction by *T.reesei*. No significant enhancement in FPA (4.85IU/mL) was observed under potato starch hydrolysate based production.

When studied the effects of acid hydrolysed wheat, rice and potato starch on the FPA produced by *Aspergillus niger* system under *Luffa cylindrica* based SSF, it was found that rice and wheat starch hydrolzates containing medium induces FPA (1.32IU/mL, 1.28 IU/mL) much higher compared to potato starch hydrolyzates containing medium (1.18 IU/mL), which states that sugars present in potato hydrolysates are much effective for fungal growth rather than induces enzyme activity by *A. niger*. Higher dosages (5%v/v) of potato starch hydrolyzates also improves *Aspergillus* growth rather than activity enhancement as observed from Table 4.5.9 and Fig.4.5.20.

On the other hand *N.crassa* system showed different behaviors for starch hydrolyzate based cellulase induction induction. It has been observed from Table 4.5.9 and Fig 4.5.19 that *N.crassa* showed satisfactorily improvement in the FPA activities (2.61IU/mL) (2.54IU/mL) under 2%v/v

potato and rice starch hydrolyzates containing *Luffa cylindrica* peel bed respectively, comparison to FPA activity (2.38IU/mL) under wheat starch hydrolysates based fermentation. Significant improvement in FPA activity (2.78IU/mL) was also observed by increasing potato starch hydrolysates dosages (5% v/v).These findings suggest that sugars present in the potato starch hydrolysates induces cellulase activity rather than its growth.

It has been observed from Table 4.5.9 and Figs 4.5.23, 4.5.24 and 4.5.26 that CMCase activities were not much enhanced by incorporation of 30% whey in BSM. Higher increment in CMCase activity (9.02IU/mL) was observed by *A. niger* system followed by CMCase activity (3.66IU/mL) produced by *T. reesei* under whey based solid state fermentation. On the other hand *N. crassa* showed poor increment in CMCase activity (5.66IU/mL).These finding insinuate that whey are not much effective inducers for CMCase in comparison to FPA activity. It has been observed from Table 4.5.9 and Figs 4.5.23, 4.5.24 and 4.5.26 that CMCase activities were satisfactorily enhanced by incorporation acid hydrolyzed starch in BSM. Higher increment in CMCase activity (10.02IU/mL) was observed by *A. niger* system followed by CMCase activity (4.51IU/mL) produced by *T. reesei* under wheat starch hydrolysate based solid state fermentation. On the other hand *N. crassa* showed significant increment in CMCase activity (6.29IU/mL) under potato starch hydrolysate based fermentation.

It has been observed from Table 4.5.8-4.5.11.that whey and acid hydrolysed starches induce cellulase activities diversely under *Pisum sativum*, *Luffa cylindrica*, *Lagenaria cineraria* and *Litchi cinensis* based solid state fermentation. Higher cellulase activities produced by *T. reesei* in terms of FPA (3.37, 3.95; 4.64, 5.39; 3.80, 4.45; 1.89, 2.35 IU/mL) and CMCase (2.51, 3.27; 3.66, 4.51; 2.86, 3.35; 1.46, 2.05 IU/mL) were observed under whey and wheat starch hydrolysate containing *Pisum sativum*, *Luffa cylindrica*, *Lagenaria cineraria* and *Litchi cinensis* solid bed based fermentation respectively, whereas higher cellulase activities produced by *A. niger* in terms of FPA (0.856, 0.997; 1.13, 1.23; 1.01, 1.26; 0.643, 0.721IU/mL) and CMCase (6.45, 7.16; 9.02, 10.02; 8.66, 9.85; 4.29, 4.70 IU/mL) were observed under whey and rice as well as wheat starch hydrolysate containing *Pisum sativum*, *Luffa cylindrica*, *Lagenaria cineraria* and *Litchi cinensis* solid bed based fermentation correspondingly. On the other hand higher cellulase activities produced by *N.crassa* in terms of FPA (1.58, 2.05; 2.25, 2.78; 1.66, 2.28; 0.937, 1.10 IU/mL) and CMCase (4.02, 4.63; 5.66, 6.29; 4.58, 5.43; 2.15, 2.83 IU/mL) were observed under whey and potato starch hydrolysates containing *Pisum sativum*, *Luffa cylindrica*, *Lagenaria cineraria* and

Litchi cinensis solid bed based fermentation respectively. From the above Tables 4.5.8-4.5.11 it was also viewed that *Luffa cylindrica* solid bed based fermentation produced higher cellulase activities with their higher increment rate as compared to *Lagenaria cineraria* and *Pisum sativum* based fermentation whereas lowest cellulase activities with lowest increment rate was observed with *Litchi cinensis* based solid state fermentation, which might be due to the presence of higher lignin percentage in their lignocellulosic composition. Therefore during autoclaving phenolics and other harmful components released according to their presence in the different raw materials which restricts the microbial growth as well as limits the inducing action of whey and starch hydrolyzates.

From the all the above observation we concludes that *Luffa cylindrica* peel bed was much effective for cellulase production followed by *Lagenaria cineraria* and *Pisum sativum* peel beds while *Litchi cinensis* peel bed was observed least effective for cellulase production by various fungal strains.

SECTION VI
SURFACE ADHESION FERMENTATION
(BIOFILM FERMENTATION)

4.6. SURFACE ADHESION FERMENTATION

The present section describes the surface adhesion fermentation, a innovative and distinctive approach that has been used in cellulase production by *Aspergillus* strain. It correlates the fungal growth achieved under submerged and surface adhesion fermentation as well as compares the cellulase production under such conditions. It also gives a detailed view of fungal biofilm fermentation attained by *Aspergillus* strain.

Filamentous fungi are naturally adapted to grow on the surfaces and in such conditions they have shown a particular physiological behavior, which is different to that in submerged cultures, thus they can be considered as biofilm forming organisms. Although fungal biofilms are less known than the bacterial biofilms, they can be used for cellulase production as it has been recently reported. Both SSF and biofilm fermentation depends upon surface adhesion, hence a new fermentation category named as surface adhesion fermentation (SAF) was established and first introduced by Gutieroz - Correa and Villena[275].

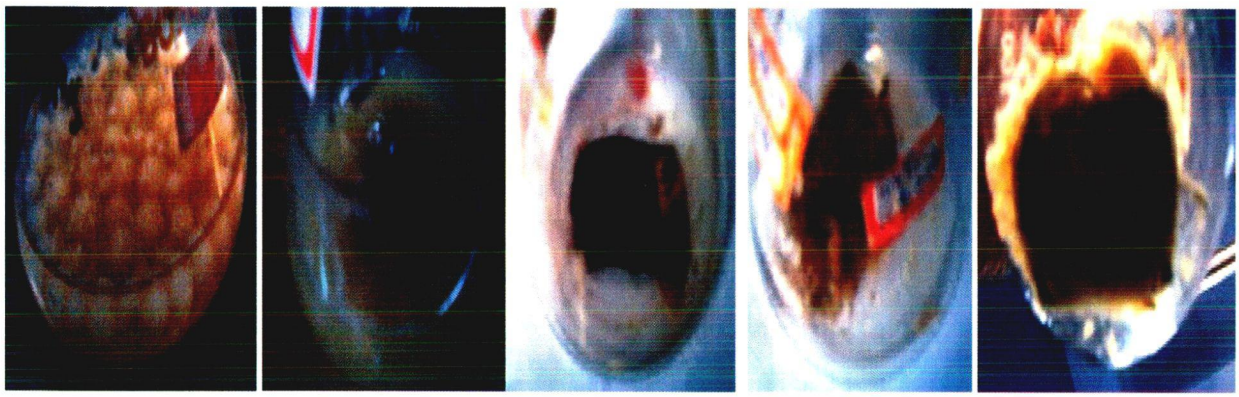
To evaluate the fungal biofilm fermentation separate set of batch experiments have been performed. Biofilm formation of *Aspergillus niger* NCIM 777 was studied by adding sterilized muslin cloth and polyester sheet as solid support. Muslin cloth with 3 holes and 9 holes of 0.5 cm diameter each was added to the first two Erlenmeyer flasks. Muslin cloth without holes was added to the third flask. Polyester sheet was added to the fourth flask and fifth flask was without any solid support.

4.6.1.Visual observation of *Aspergillus* growth under submerged and surface adheshion conditions

Heavy growth of *Aspergillus niger* was observed with muslin cloth with 3 holes when compared with muslin cloth with 9 holes or without hole. This result might be due to the fact that muslin cloth with hole has the capability for better aeration and mass transfer of the nutrients which required for microbial growth. But smaller biofilm has been observed under muslin cloth with 9 holes, may be due to loose support. Heavy growth has been observed with polyester sheet solid support. Polyester sheet acts as a fine support for *Aspergillus niger* when compared with muslin cloth as observed from Table and Fig 4.6.1. It provides a thick scaffold for fungal biofilm growth under the agitating condition. Polyester sheet with rough textures also provides a favorable condition for the biofilm development, due to better adhesion property.

Table 4.6.1. Growth of *Aspergillus niger* as biofilm and pellets nature.

Support materials	Growth and Production media	Fermentation time(h)	Nature of growth	Growth of *Aspergillus niger*
Muslin cloth without holes	Lactose based	96	Biofilm	Very light growth
Muslin cloth with 3 hole	Lactose based	96	Biofilm	Good growth
Muslin cloth with 9 hole	Lactose based	96	Biofilm	Light growth
Polyester sheet	Lactose based	96	Biofilm	Very heavy growth
Without solid support	Lactose based	96	Pelleted	Heavy growth



(1a) **(1b)** **(1c)** **(1d)** **(1e)**

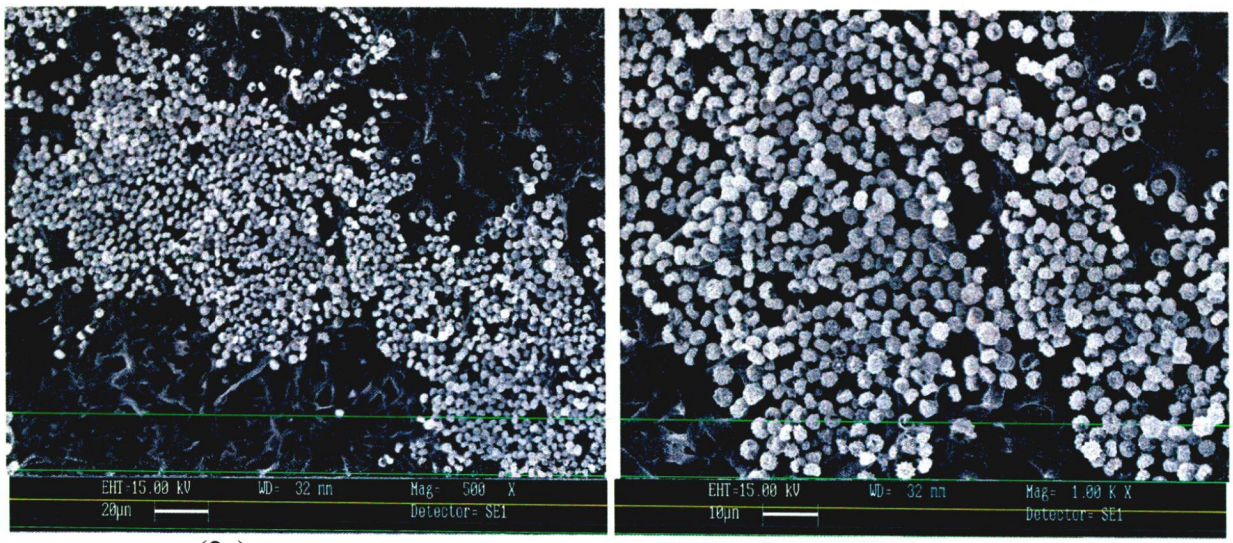
Fig 4.6.1. Pelleted form of growth, Biofilm growth on muslin cloth, Polyester sheet without biofilm, Polyester sheet with biofilm (front view), Polyester sheet with biofilm (backview) respectively.

Fig (4.6.1a) represents the growth of *Asprgillus niger* in the form of big pellets whereas Fig (4.6.1b) reveals biofilm development on muslin cloth. Fig (4.6.1c, 4.6.1d and 4.6.1e) describes the *Aspergillus niger* growth in the form of biofilm under polyester support.

4.6.2. Scanning electron micrographic studies of biofilm and pelleted form of growth

From the above observation, two types of growth morphology have been observed, pelleted (without any support containing medium) and biofilm (medium with solid support) form. Microbial biofilms and fungal pellets have been studied with increasing curiosity during the past few years. For the detailed morphological and structural studies, scanning electron micrography has been performed. Although the huge majority of published papers refer only to bacterial or yeast-like biofilms, filamentous fungi could be included as biofilm forming microorganisms, since they are naturally adapted. Scanning electron microscopy has proved to be a precious and invaluable tool for analyzing the structure and growth of fungal pellets and biofilms. Fungal pellets

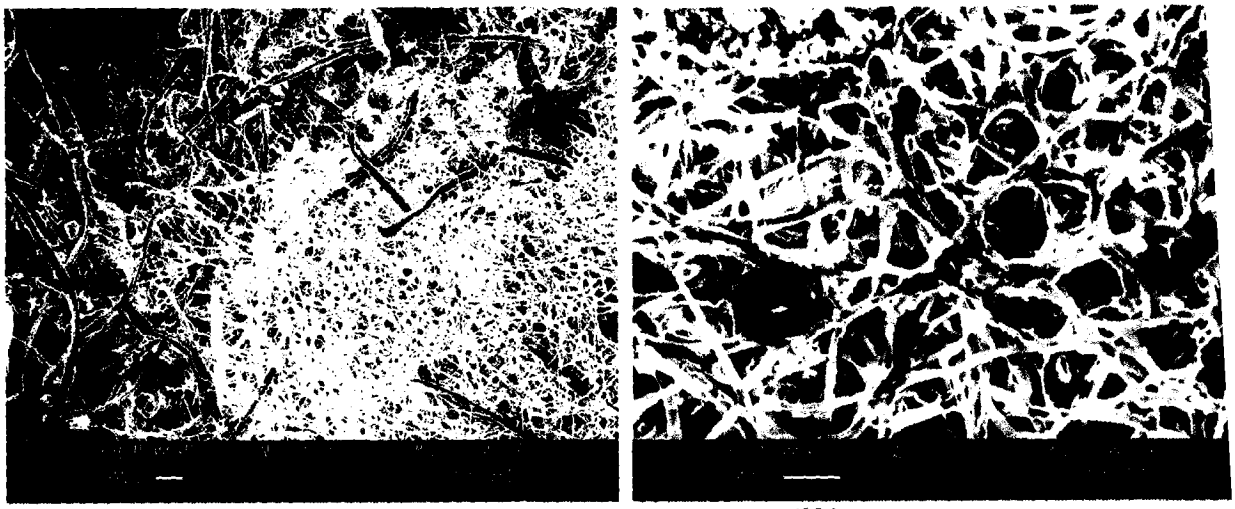
dispersed or hairy regions containing radially growing hyphae, which can vary in size between several hundred micrometers to several millimeters, whereas typical microbial biofilm structure includes a complex three dimensional structural design characterized by interstitial voids and water channels with cells usually encapsulated within an extracellular matrix[237].



(2a) (2b)

Fig. 4.6.2. Scanning electron micrograph of *Aspergillus niger* pellets at 500X and 1000 X magnification respectively.

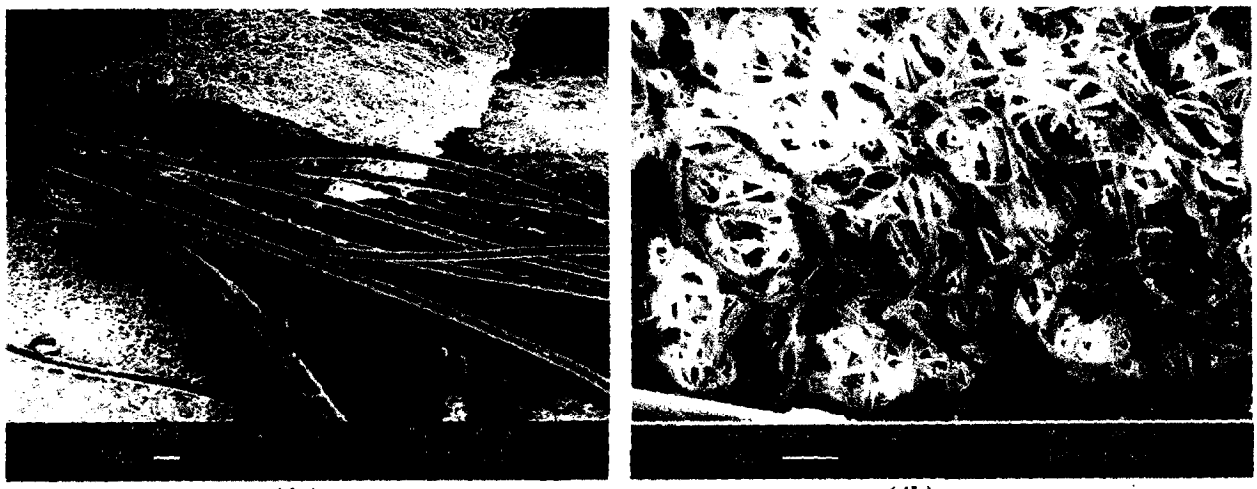
In a nut shell biofilm mycelia showed an orderly distribution forming surfaces and inner channels, while pellets showed highly intermined superficial hyphae and a densely packed deep mycelium [276].Pellets had vague holes which represented the lesser portion of the total area, however because they are not factual channels, compacting and stressed hyphae were manifest around them as evident from Figs 4.6.2(a and b). It has also been shown that large number of spores are scattered around major section. However, in culture growth systems, *Aspergillus* pellets consist of an outer shell of growing hyphae and an inner mass of non growing mycelium. The thickness of the outer growing layer of pellets is considered to be limited by nutrient diffusion rate. Researchers ave suggested that the formation of pellets originated from the adherence of germinated spores to id particles in medium .The attached solid particles were also digested during the fungal mentation and resulted in the formation of the smooth and hollow pellets [266].



(3a) (3b)

Fig 4.6.3: Scanning electron micrograph of *A. niger* biofilm on muslin cloth solid support at 50 and 500X magnification respectively.

Biofilms revealed surface heterogeneity and interactions voids as well as also exhibited w structured channels with swollen hyphae around as explained in Figs. 4.6.3(a, b) and 4.6.4(a, When compared the biofilms developed on solid support and pellets of *A.niger* we clearly obser a different spatial growth harmonization when fungus adhere to the surface. This synchronizati is the result of steric interactions between hypha and tips, when in contact with the surface. At ti distances, interactions between tip and hyphae involve a local spatial rearrangement, resulting reduction of the tip elongation rate. As a result, a control in maximum biomass surface density w observed, which provided a better condition for product formation.



(4a) (4b)

Fig 4.6.4: Scanning electron micrograph of *A. niger* biofilm on polyester sheet solid support at 50X and 500X magnification respectively.

Upon comparing the scanning electron micrograph of *A.niger* biofilm on muslin cloth and polyester solid sheet support, huge amount of growth has been observed with polyester solid sheet support having smaller and lesser number of interstitial voids incorporated with dense and compact network of inner channels whereas biofilm developed on muslin cloth holding bigger and more number of interstitial voids integrated with less dense network of inner channels as confirmed by Figs.4.6.4.(a and b)which might be due to hard and compact structure of polyester sheet required for better surface adhesion under agitating condition.The presence of compact network of inner channels with lesser number of interstitial voids, provide a favourable condition for the product formation. A channeled structure has a noticeable benefit in that it allows fluids and nutrients to pass through easily which ultimately enhances mass transfer which also proves the robust and sound relationship between growth morphology and product formation [278]. Therefore, fungal biofilms are considered more efficient than suspended mycelium in many production systems [80, 252].

4.6.3.Cellulase production under surface adhesion fermentation

Cellulase activities produced by *Aspergillus niger* was significantly higher under solid support containing liquid production media compared to cellulase activities (0.433 IU/ml and 2.05IU/ml) media without support as viewed from Table 4.6.2.

Table 4.6.2. Cellulase production by *Aspergillus niger* as biofilm and pellets nature.

Support materials	Fermentation time(h)	Nature of growth	Enzyme activities	
			FPA(IU/mL)	CMCase(IU/mL)
Without solid support	24	Pelleted	0.118	0.522
	48		0.216	1.56
	72		0.369	**2.05**
	96		**0.433**	1.97
	120		0.421	1.73
Muslin cloth without holes	24	Biofilm	0.143	0.713
	48		0.247	1.74
	72		0.376	2.39
	96		0.520	2.02
	120		0.467	1.80
Muslin cloth with 3 hole	24	Biofilm	0.159	0.805
	48		0.268	2.43
	72		0.493	**3.28**
	96		**0.714**	3.01
	120		0.663	2.79
Muslin cloth with 9 hole	24	Biofilm	0.150	0.769
	48		0.296	2.65
	72		0.432	2.97
	96		0.659	2.83
	120		0.641	2.32
Polyester sheet	24	Biofilm	0.163	0.965
	48		0.386	2.98
	72		**0.803**	**3.93**
	96		0.792	3.85
	120		0.730	3.31

Higher cellulase activities (0.714IU/ml and 3.28 IU/ml) with significant growth of *Aspergillus niger* was observed with muslin cloth having 3 holes when compared with muslin cloth with 9 holes or without hole. This result might be due to the fact that muslin cloth with holes has the capability for better aeration and proficient mass transfer of the nutrients through the hole which ultimately is beneficial for microbial growth and production system. But smaller biofilm and slight reduced cellulase activities (0.659IU/ml and 2.97IU/ml) have been observed in muslin cloth with 9 holes, may be due to loose support. Highest cellulase activities (0.803IU/ml and 3.93IU/ml) with profound growth were observed with polyester sheet solid support. This results also confirmed the finding of Villena and Gutieroz correa[275], as they reported that *Aspergillus* produces higher FPA under media having polyester sheet solid support compared to without support. Polyester sheet acts as a fine support for *Aspergillus niger* when compared with muslin cloth. It provides a thick scaffold for fungal biofilm growth under agitating condition. Polyester sheet with rough textures also provides a favorable condition for the biofilm development, due to better adhesion property. A

sharp increment in FPA of *Aspergillus niger* was observed after 48 h of fermentation time and this reached maximum at the 96 h in all the experimental set up except in polyester sheet containing production media which required only 72h of fermentation time for highest FPA (0.803IU/ml) as observed by Fig 4.6.5.

On the other hand *Aspergillus* produces significant CMCase activity in lesser fermentation time and reached their maximum at 72 h in each set up. It also suggests that *Aspergillus* strain starts CMCase synthesis in the early period of fermentation as has been observed by Fig 4.6.6. Maximum CMCase activity (3.93IU/ml) was observed by *Aspergillus niger* in polyester sheet under surface adhesion fermentation.

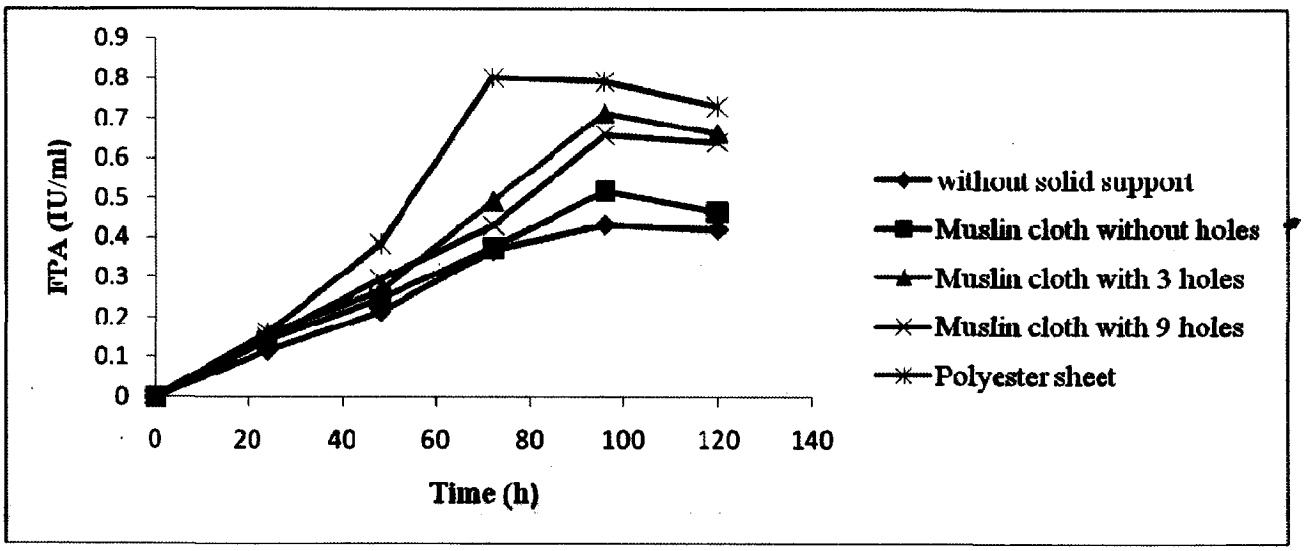


Fig 4.6.5. Comparative FPA achieved by *Aspergillus niger* under without and with support containing liquid fermentation medium.

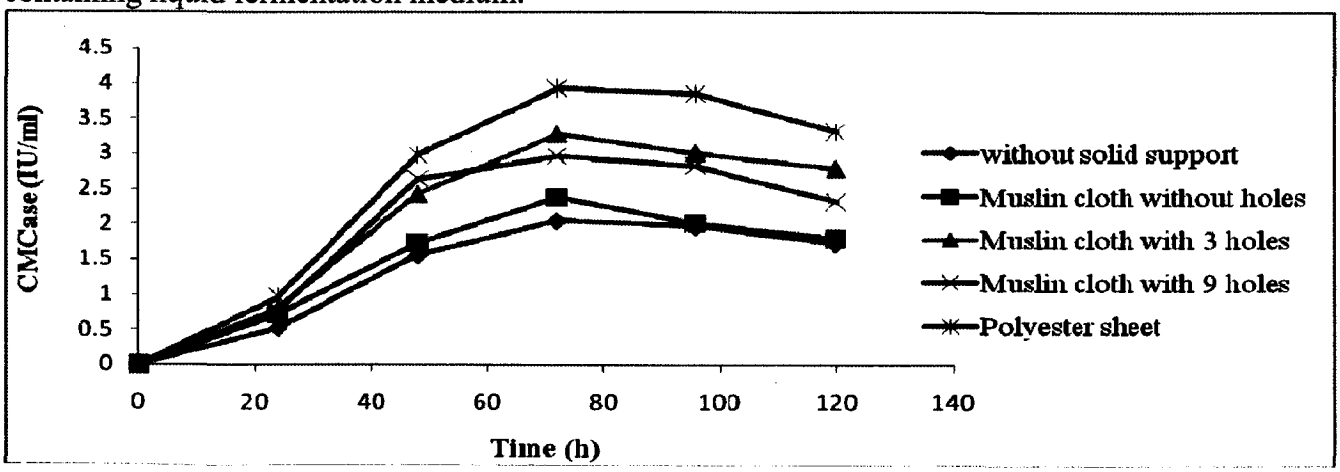


Fig 4.6.6. Comparative CMCase activities achieved by *Aspergillus niger* under without and with support containing liquid fermentation medium.

In a nutshell biofilms fermentation or surface adhesion fermentation developed on polyester sheet are massive in nature with lesser interstitial voids and more channeled structure. This structural arrangement provides improved nutrient and mass transfer compared to pelleted, filamentous mycelia or fungal biofilms having more interstitial voids and less dense channeled structure and ultimately these conditions offer improved fungal growth as well as higher product formation in terms of cellulase activity. Therefore biofilms fermentation or surface adhesion fermentation for *Aspergillus* system becomes much more advantageous than submerged fermentation for product formation.

SECTION VII
ENZYMATIC DEINKING OF OLD NEWS PAPER BY CELLULASES PRODUCED BY VARIOUS FUNGAL STRAINS

4.7. ENZYMATIC DEINKING OF WASTE NEWS PAPER

The present section describes the enzymatic deinking of old news paper (Times of India) by the crude cellulases produced by *Trichoderma reesei, Neurospora crassa, Aspergillus niger, Trichoderma viride* in terms of brightness and tensile strength. It also illustrates a comparative studies between enzymatic deinking with different fungal strains as well as with conventional deinking. The continuously growing paper manufacturing industry imposes a severe demand on green plants that forms the basic raw materials. Shortage of forest based raw materials and problem in processing agro residues are the major constrains in growth of production of paper industry. Waste paper is the single largest component of the solid waste stream and has a great effect on the environment. Recycling of paper saves energy and forest resourses for pulping and paper making. Consumption of recovered paper is estimated to be 4-5 million tones in india only[50]. Conventional deinking technology requires a large amount of chemical agents such as sodium hydroxide, sodium carbonate, sodium silicate, hydrogen peroxide which results in a high detrimental impact to the environment. In order to overcome these problems, enzymatic deinking becomes a better option, due to its high efficiency and low environmental impacts. Enzyme based deinking offers a potential means for reducing chemicals used in conventional deinking process. During the past few decades, a number of enzymes, including cellulase, xylanase, pectinase and lipase, have been evaluated for their potential to replace hazardous chemicals in deinking recycled paper. Conservation of wood products, toxic emissions from traditional paper making methods and new process technologies have made paper recycling a viable industry[198].

To evaluate the chemical as well as enzymatic deinking of old news paper separate set of pulping and flotation experiments have been performed.Production of cellulases have been done under wheat bran based solid state fermentation by various fungal strain at 30^0C. Cellulases produced by different fungal strains used separately as in crude form to carried out enzymatic deinking. Pulp getting after pulping process was introduced with crude enzymes. After the completion of pulping process slurry from the hydrapulper was sent to the second stage of flotation deinking in the case of chemical deinking, whereas in enzymatic deinking pulp was treated with different dosages of crude enzymes produced by various fungal strains alongwith Tween-80 surfactant for 3h contact time. After that this enzyme treated pulp with 1% consistency were sent to flotation cell for further

processing. The optical and strength properties such as brightness ISO% and tensile strength (Nm/g) were measured from prepared handsheets. Enzyme activities in terms of FPA and CMCase produced by various fungal strains used in deinking process are stated in Table 1.Maximum FPA activity was observed by *Trichoderma reesei* followed by *Trichoderma viride* and *Neurospora crassa* whereas highest CMCase was observed with *Aspergillus niger* followed by *Trichoderma viride* and *Neurospora crassa.*

Table 4.7.1. FPA and CMCase activities of different fungal strain.

Fungal strains	Enzyme activities	
	FPA(IU/mL)	CMCase(IU/mL)
Trichoderma reesei	4.72	5.02
Neurospora crassa	2.36	6.36
Aspergillus niger	1.29	10.10
Trichoderma viride	2.23	7.74

4.7.1. Comparative studies on brightness and tensile strength of enzymatically and chemically deinked old news paper.

It has been observed from Table 4.7.1 that enzymes produced from different fungal strains impact differently on the ONP brightness and tensile strength.When compared the optical sheet property such as brightness (ISO%) of prepared handsheet attained after chemical and enzymatic deinking, we have observed that there was no significant improvement in the brightness of hand sheet treated with *Trichoderma reesei* but on the other hand significant decrement in the tensile strength of the pulp was observed. This might be due to the presence of more FPA in comparison to the endoglucanase, which affect more frequently on the strength property compared to optical property. It is also reported that the main effect of cellulase is the hydrolysis and superficial degradation of cellulose that implies ink removal from fibers. Understanding the mechanism of enzymes towards the fibers is essential to minimize the related negative impact on the strength of the paper and its quality[282]. Higher dosages of enzyme gave slight improvement in brightness of the handsheet due to their endoglucanase action but at this dosages strength property was much affected might be due to more FPA action as observed from Fig 4.7.1 and 4.7.2. According to Jefferis et al cellulases having high filter paper degrading activities are effective in deinking[108]. Whereas other researchers have suggested that the filter paper degrading activities of cellulase complex from *Trichodermma pseudokoningi* S 28 had detrimental effect on the paper strength[204]. When examined the optical and strength properties of *Neurospora crasa* treated deinked handsheet, we found that significant improvement in the brightness has been occurred with

Table 4.7.2. Comparative brightness and tensile strength of chemical and enzyme deinked old news paper.

Deinking type	Cellulases from fungal strains	Enzyme dosages(mL)	Contact time (h)	Brightness (ISO %)	Tensile strength (Nm/g)
Chemical	-	-	-	55.0	37.19
Enzymatic	*Trichoderma reesei*	5	3	58.0	28.14
		10	3	58.2	27.76
		15	3	59.8	26.04
Enzymatic	*Neurospora crassa*	5	3	60.8	30.37
		10	3	60.5	29.41
		15	3	60.1	28.58
Enzymatic	*Aspergillus niger*	5	3	59.5	29.66
		10	3	60.5	27.61
		15	3	61.0	28.00
Enzymatic	*Trichoderma viride*	5	3	60.2	31.95
		10	3	61.4	31.31
		15	3	61.8	30.45

less affecting strength property this may be due to that *Neurospora* cellulases having more endoglucanase compared to FPA which ultimately improve their brightness with less affecting strength property.It has been observed from Fig 4.7.1 and 4.7.2 that lower dosages of *Neurospora* cellulases gave much satisfactory results compared to other dosages. As literature showed that cellobiohydrolase (CBH) hydrolyze cellulose from the ends of cellulose chain because cellulose chain ends are limited so it is not much effective for removal of ink from the cellulose fiber whereas endoglucanase have ability to hydrolyze the internal portion of cellulose chain so it is much more

effective. Endoglucanase has positive effect on deinking, where as exoglucanase has a negative impacts on deinking efficiency [23,84]. Whereas *Aspergillus niger* based cellulase treated pulp showed better increment in the brightness with good strength property probably due to the more endoglucanase activity. As viewed from Fig 4.7.1 and 4.7.2 that 10 mL of enzyme dosages was found better as compared to lower and higher dosages. On the other hand *Trichoderma viridae* produced cellulase treated pulp showed better results in paper properties. Handsheet obtained after *T.viridae* cellulase treatment showed improved brightness with less affected tensile strength certainly due to the presence of more endoglucanase as well as cellobiohydrolase activity. As literature reported that when cellulases and hemicellulases are used, the release of ink particles into suspension is generally attributed to the cellulose hydrolysis on the fiber /ink inter bonding regions which facilitates ink detachment [145].Lower dosages (5mL) of *Trichoderma viride* cellulases was found much suitable for handsheet tensile strength whereas higher dosages (15mL) *Trichoderma*

viride cellulases for handsheet brightness as observed from Fig 4.7.1 and 4.7.2. Marques et al have investigated the effect of glucanases secreted by *A.niger* CCMI 498 and *Trichoderma viride* CCMI 84 on enzymatic deinking of MOW(mixed office waste),an increment of 24% in ink removal was observed by *T.viride*, compared with control due to the fact that *T.viride* contained 4-5 fold more endoglucanase activity than *A terreus*[156].

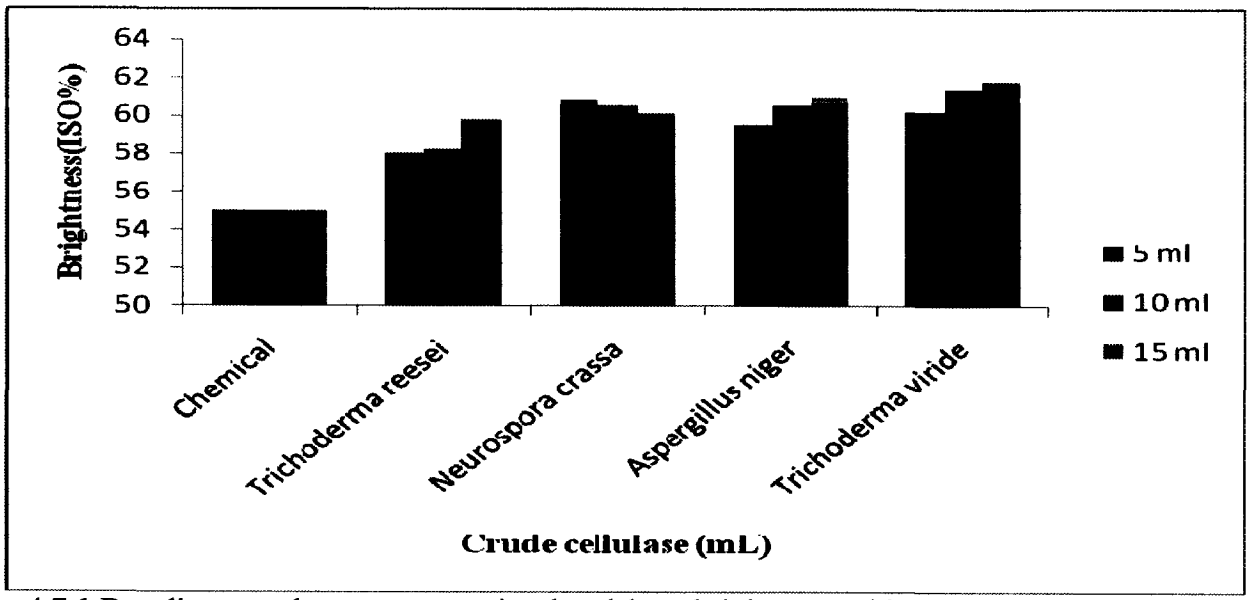


Fig 4.7.1.Bar diagram shows comparative handsheet brightness achieved by chemical as well as various dosages of enzyme treatment.

Decrement in the strength property has been observed in each enzyme deinked pulp, compared to chemical deinking, this can be overcome by reducing the contact time between enzyme and pulp. Literature suggests that application of cellulase in deinking increases brightness and decreases the ERIC (Effective residual ink content) value of ONP/OMG after flotation when added in low charge prior to repulping, at pH 5. The breaking length, burst index, tear index, tensile index also decreased by various degrees depending on the enzyme charges and contact time[203]. Chemical deinked pulp handsheet showed good strength property but lesser improvement in brightness as viewed by Fig 4.7.3. The role of cellulases in deinking is still not clear. Brightness and tensile strength of enzyme as well as chemical deinked pulp can be compared by Fig 4.7.1 and 4.7.2.

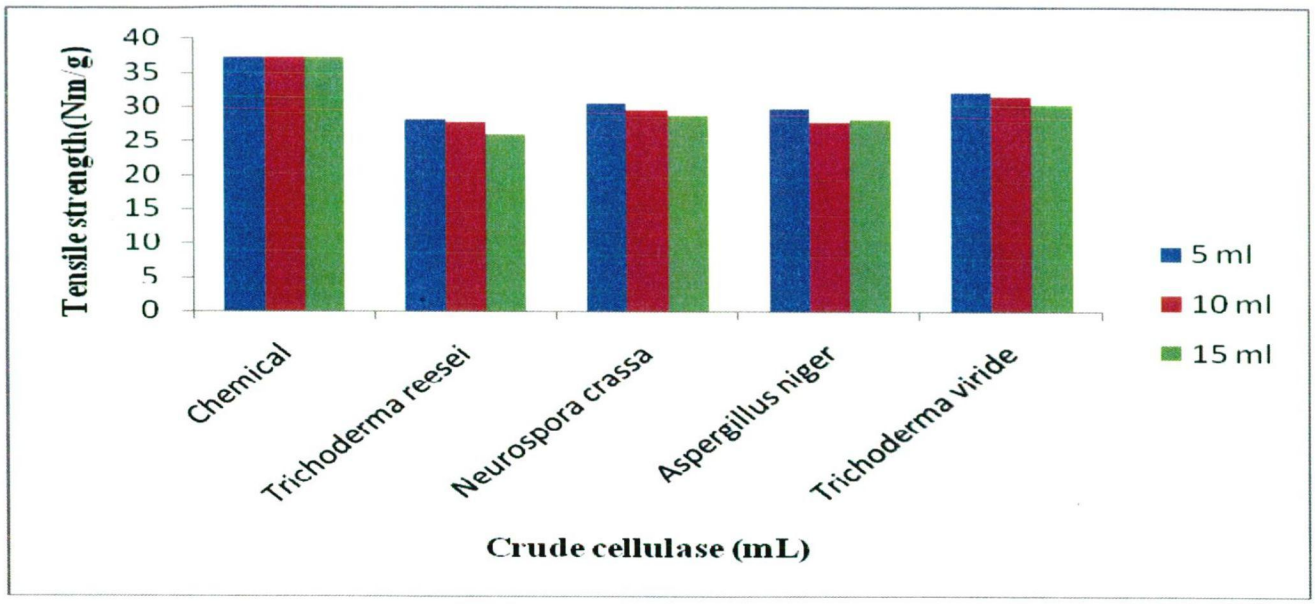
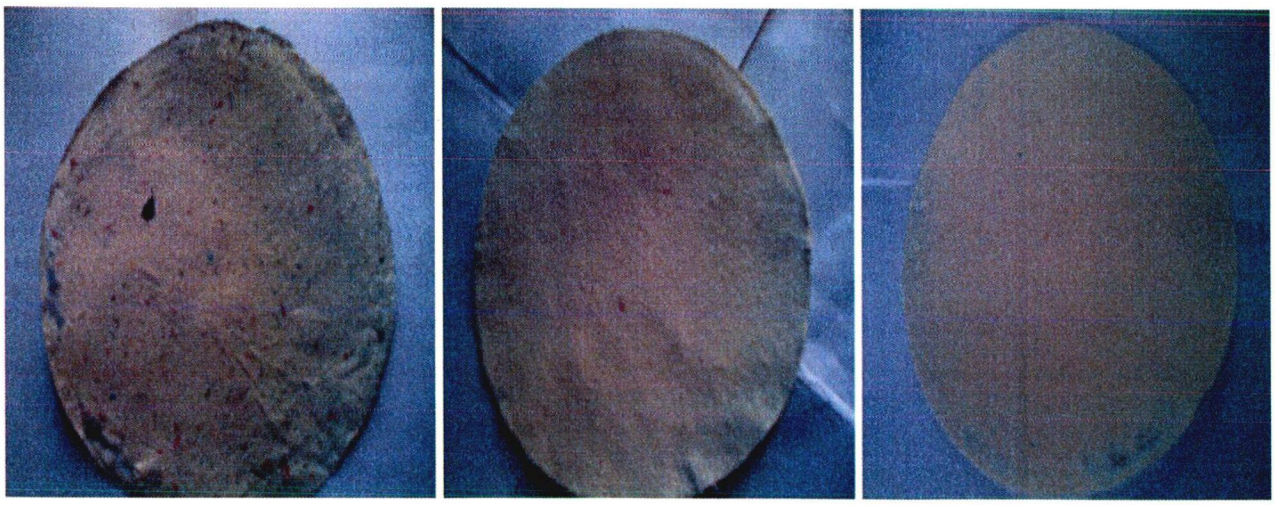


Fig 4.7.2. Bar diagram shows comparative tensile strength of handsheet achieved by chemical as well as various dosages of enzyme treatment.

4.7.2. Comparative studies of handsheet prepared from enzymatically and chemically deinked pulp

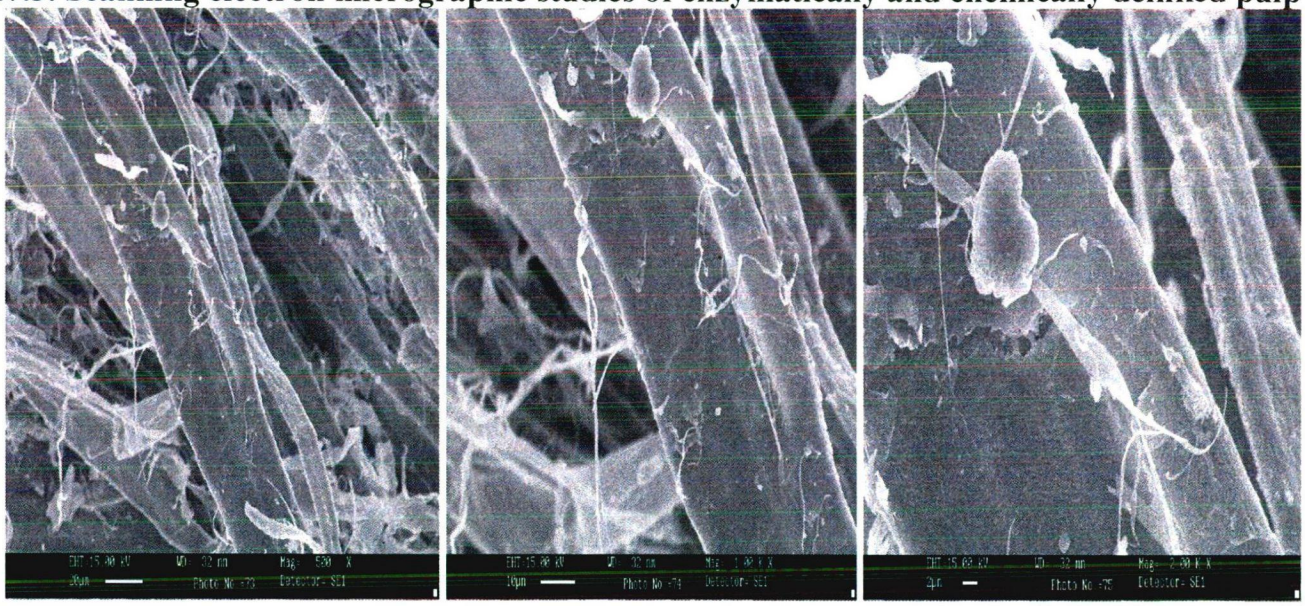
As observed from Fig 4.7.3a that handsheet prepared after chemical deinking having lesser brightness with higher strength property whereas enzymatically deinked handsheet (4.7.3b) shows good brightness with better strength. On the other hand enzymatically deinked handsheet (4.7.3c) shows better brightness with reduced strength.



(3a) **(3b)** **(3c)**

Fig 4.7.3. Handsheet photograph of chemically deinked old news paper (3a) and enzymatically deinked old news paper (3b) and (3c).

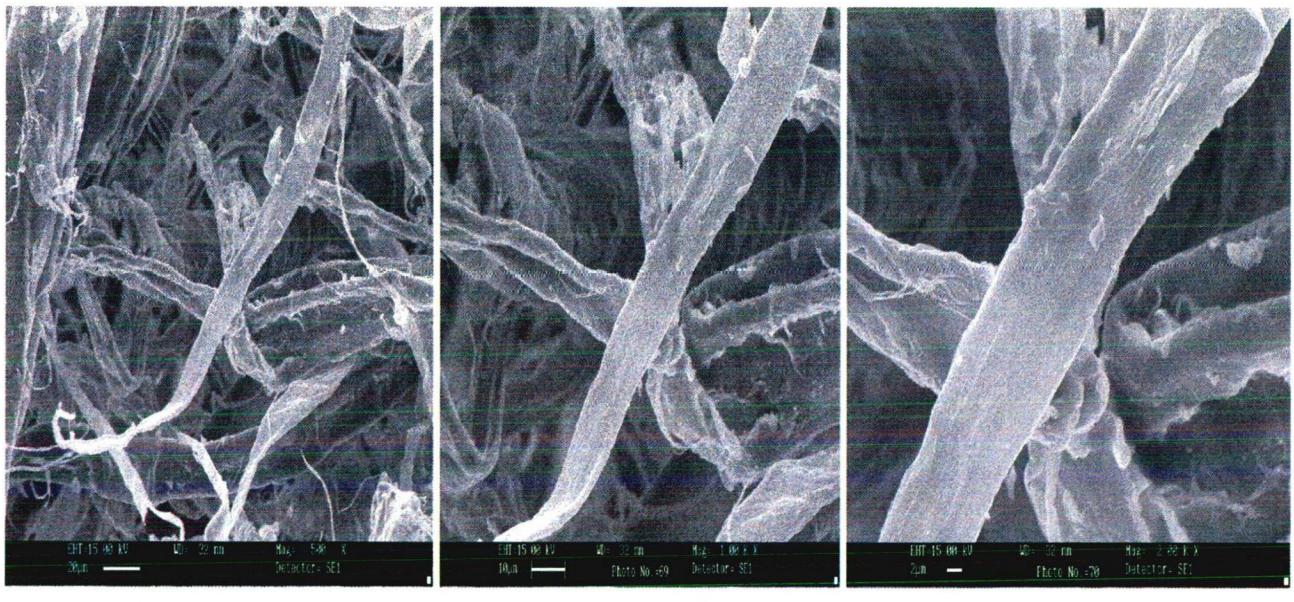
4.7.3. Scanning electron micrographic studies of enzymatically and chemically deinked pulp



(4a) (4b) (4c)

Fig. 4.7.4: Scanning electron micrograph of chemical deinked old news paper pulp at 500X(4a),1000X(4b) and 2000X(4c) respectively.

Scanning electron micrograph gives the magnified view of pulp fibers. Chemical deinked pulp shows fiber with higher strength but lesser brightness as viewd from Fig 4.7.4a, 4.7.4b and 4.7.4c. whereas scanning electron micrograph of enzymaticaly deinked pulp fibers shows higher brightness of pulp fiber .



(5a) (5b) (5c)

Fig 4.7.5: Scanning electron micrograph of enzymatic deinked old news paper pulp at 500X(5a),1000X(5b) and 2000X(5c) respectively.

The role of endoglucanase in the overall process of cellulose degradation is to breakdown the cellulose fibers into the several amorphous sites and generate innumerous reducing ends of the chain. This action enhances the loosening of fibers, which ultimately helps in releasing the ink particles from mixed office waste papers during flotation deinking process in the presence of surfactants[282]. Cellulases are widely reported to facilitate deinking of mixed office waste (MOW)[179]. Individual mono components of cellulase having a single EG (endoglucanase) effectively remove inks from waste fiber. CBP (Cellulase Binding domain) have negative impact on deinking of MOP[109].

Compared with conventionally deinked pulp, we conclude from this study that cellulolytic deinked pulp will lead to improved optical properties such as brightness, lower residual ink contents. A proper combination of enzymes depends upon type of waste paper processed under proper operating conditions shall give better results. Selective enzyme with its specific components or suitable enzyme mixtures at their optimal conditions would be more effective in particular type of paper in deinking process. In order to maintain better strength properties with improved brightness, proper balance must be needed between the enzyme dosages and pulp. Contact time between the enzyme and pulp also plays a very active role in the enzymatic process, therefore it should be optimized. It has been found that cellulases produced from *Trichoderma viridae*, *Neurospora crassa* and *A. niger* are better for the enzymatic deinking in terms of brightness, they strongly suggests that endoglucanase component of cellulases plays a vital role in enzymatic deinking. It has been also concluded that cellulases produced from *T. viridae* was found better for the enzymatic deinking in terms of both brightness and tensile strength as compared to chemically deinked pulp.

CHAPTER V

CONCLUSION

Keeping the view of production cost and high demand of cellulases in various process and bioconversion industry, effective, efficient and economical production of cellulases is of utmost importance. Utilization of cheaper, abundantly available lignocellulosic biomass for cellulase production, becomes the promising alternative to make the process cost effective and environmentally viable. Therefore intensive search is being carried out for cheaper carbon source such as non conventional raw materials as well as the utilization of other waste materials such as whey, acid hydrolysates of egg shell waste, acid hydrolysates of starch and acid hydrolysates of lignocellulosic biomass and boiled bagasse syrup for cellulase biosynthesis could make the cost effective cellulase production process.

Growth and morphology of the cellulase producers have a crucial effect on cellulase production. Therefore it is necessary to study the growth rate and pattern of these organisms. 30^0C was found quit suitable temperature for the growth of most of the fungal strains. Growth and growth rate both were much faster in case of *Trichoderma reesei* while *Trichoderma viride* showed lowest growth rate. *Aspergillus* strains also showed good growth but lower growth rate whereas *Neurospora crassa* showed deprived growth but fastest growth rate .*Trichoderma reesei* showed fastest growth phase in glucose based culture media followed by xylose, maltose and lactose based culture medium while lowest growth and growth rate were observed by *Trichoderma viride* in sucrose based medium. *A. niger* showed much faster growth rate in xylose, maltose and sucrose based media with respect to glucose and lactose based culture media. But finally the highest growth has been achieved by *Aspergillus niger* in glucose based medium while *Aspergillus fumigatus* strain attained much faster growth and growth rate in glucose as well as maltose based culture medium. Whereas growth rate of *Neurospora crassa* was much faster in maltose and sucrose based culture media which closely resemble to growth in glucose based culture media. Most of the microbial strains utilized sugars present in the industrial wastes whey and boiled bagasse. Growth and growth rate was much better in boiled bagasse containing medium for most of the culture excluding *Trichoderma* strains. *Trichoderma ressei* and *Aspergillus niger* showed higher growth and growth rate under whey based culture medium. On the other hand *Neurospora crassa* illustrated least growth and growth rate under such industrial waste containing culture medium. The utilization of dairy industry waste (whey) as well as sugarcane industry waste (bagasse) for the growth and

culture development of *Aspergillus* and *Neurospora* could be a novel approach which may reduce the culture development cost and pollution load.

Significant improvement in the cellulases activities were achieved by *T.reesei, N.crassa* and *A. niger* respectively using box behnkem experimental design. In most of the cases higher cellulase activity lie in the centre region of the quadratic response surface model of the graph which proves the significantly better optimization of process parameters. A good correlation was found in between experimental and predicted values of cellulase activities.

When compared the cellulase activity produced by fungal strains under pure sugars based liquid state fermentation, it was concluded that *Trichoderma* strains produces higher activity under cellulose, lactose, CMC and sucrose, xylose, trehalose and sorbitol based medium, while *Aspergillus* strains showed good activity under cellulose, maltose and cellobiose, xylose, trehalose based medium. On the other hand *Neurospora* showed higher activity under cellulose, sucrose and maltose and arabinose based medium while least activity was observed with lactose based media. Arabinose sugar was found least effective for cellulase production with *Trichoderma* and *Aspergillus* strains, while in between sugar alcohol sorbitol was found quite effective for cellulase production as compared to mannitol.

Proteose peptone and meat extract were established effective nitrogen source for most of the fungal strains. *Aspergillus* strains also showed better activity under yeast extract based production.Cheaper nitrogen source was found quite effective with *Aspergillus* strains, which also reduce the cellulase production cost upto some extant. In between various surfactant Tween -80 and oil of olive were found much effective for cellulase production by fungal strains.Good cellulase activity was observed with 30% whey based medium by *Trichoderma* and *Aspergillus* strains. 2% acid treated starch hydrolysate was found quite effective inducer for cellulase production by most of the fungal strain, in between starch hydrolysates wheat starch hydrolysate was found quite effective for cellulase producton followed by rice starch hydrolysate. Whey as well starch hydrolysate inclusion in the medium was found effective for cellulase production by *Trichoderma* strains. When compared the cellulase activity produced by fungal strains under lignocellulosic hydrolysate, boiled bagasse syrup as well as bagasse hydrolysate were found much effective for cellulase production as compared to wheat straw hydrolysate.

40% acid treated waste news paper hydrolysate was found quite effective for cellulase production as compared to other treatment dosages.

Cellulase activity produced under solid state fermentation was significantly higher than liquid state fermentatipon. Wheat bran was found much effective conventional raw material for cellulase production. It was also observed that fungal strains produced significantly higher cellulase activities under alkali pretreated raw materials based solid state fermentation as compared to acid pretreted.

Mixed microbial cultures with inoculum dosages ratio (1:1) were found much effective in terms of both FPA and cellobiase activities as compared with inoculum dosages ratio (1/2:1/2). we can infer that mixed microbial cultures of *Trichoderma* and *Aspergillus* strains produced complete set of cellulase activities in terms of both FPA and cellobiase reqiured for better utilisation of lignocellulosic biomass.

Microbial strains produces significantly higher cellulase activities under mixed raw materials based fermentation compared to particular raw material taken singly. It has been concluded that medium sized (850μm) particle of wheat bran having better suitability for cellulase production by almost each of the fungal strains.

Keeping the view of solid waste management, egg shell waste has been also used in cellulase production. 10% HCl treated egg shell waste with concentration 0.8 g/L was found quite effective for growth and cellulase production by microbial strains.

When compared the cellulase activity produced under different fermentation vessels, we have observed that erlenmayer flask and beaker type of fermentation vessels were found quite effective for cellulase production.

Significant improvement in cellulase activity was observed with the inclusion of whey, starch hydrolysate, lignocelluloses hydrolysate under wheat bran solid bed. Higher thermostable and pH stable cellulase produced by *T.viride* and *A. fumigatus* strains as compared to other.

Keeping the view of cost effective cellulase production process various non conventional and novel substrates have been used. *Luffa cylindrica* peel was found much effective raw material followed by *Lagenaria cineraria* and *Pisum sativum* peel. *Litchi cinensis* was observed least effective bed material.

Recently introduced surface adhesion fermentation or biofilm fermentation was also used for cellulase production study by *A. niger*. Significant improvement in the cellulase activity was observed under this category as compared to submerged fermentation. Higher cellulase activity was observed under polyester sheet based biofilm fermentation as compared to muslin cloth biofim fermentation as well as submerged fermentation.

Compared with chemically deinked pulp, cellulolytic deinked pulp showed improved brightness. In order to maintain better strength properties with improved brightness, proper balance must be needed between the enzyme dosages and pulp. Contact time between the enzyme and pulp also plays a very active role in the enzymatic process, therefore it should be optimized. It has been concluded that cellulases from *Trichoderma viridae* was found much suitable strain for the enzymatic deinking in terms of optical and strength properties.

It can be also concluded that *Trichoderma reesei* and *Neurospora crassa* as well as mixed culture of *Trichoderma reesei* and *Aspergillus niger* would be much effective strains and strain combination for their application in bioethanol production.

FUTURE STRATEGIES

The high production cost and low yields of these enzyme are the major impediment for industrial application. To overcome these problems, much work has been emphasized on using cheap, cost effective and abundantly available raw materials in the improved fermentation conditions, developing hyperproducing microbial strains. Intensive search regarding some other novel, cheap, lignocellulosic waste material as well as inexpensive inducers, which serve as both carbon source and inducer should be investigated in the future research.

Cellulase production can also be enhanced by applying some other novel approaches such as mutation, protoplast fusion and genetic engineering. The mutants resulted in higher cellulase activity than the wild type. The yields of enzyme can also be improved by using some other idea such as media and metabolic engineering. *Hypocrea jecorina* (*Trichoderma reesei*) is a filamentous fungi, known to produce high amounts of the enzyme cellulase. Although *Trichoderma* has long been identified as a high cellulase producer, not much has been done on the improvement of this organism to further improve cellulase production, at the molecular biology level. Complete set of cellulases having each component essential for biomass conversion in a single microbial source can be achieved by genetic engineering and protoplast fusion technology and should be investigated in the future research. Regarding the application of cellulases in deinking, microbial sources having more endoglucanases component should be investigated in the future research.

BIBLIOGRAPHY

[1] Abasiekong SF, Effect of termite culture on crude protein and crude fiber harvest residues", Bioresource Technol,62,55-57,(1997).

[2] Abdel-Hafez,S.T.I.,El-Said,A.H.M, and Gharbawy,Y.A.M.H, "Mycoflora of leaf surface, stem, bagasse and juice of adult sugarcane plant and cellulolytic ability in Egypt", Bull.Fac.Sci.,Assiut Univ,24,(2-D),153-179, (1995).

[3] Abulnaja KO, and Zaid AAZA. "Utilization of palm tree compound leaves in formation of cellulases".Bioresource Technol,44,255-257,(1993).

[4] Adsul M.G,Bastawdek B,Verma,A.J and Gokhle D.V, "Strain improvement of *Penicillium janthenellum*" ,Bioresource Technol,98,1467-1473.,(2007).

[5] Ahmad G., Mudasir., Kudesia, R., Shika and Srivastava, M. K. "Evaluation of genetic diversity in pea (*Pisum sativum*L) using RAPD analysis", Genetic Engg Biotechnol J. 16, 1-5,(2010).

[6] Airiksson, B,Rose, S.H,Vanzyl, W.H,Sjode, A,Nilverben,t N.O and Jonsson, L.J, "Cellulase production from spent lignocellulosic hydrolysate by recombinant *Aspergillus niger*",Appl Env Microbiol,75,2366-2374,(2009).

[7] Aktas N,Boyaci IH, Mutlu M and Tanyolac A, "optimization of lactose utilization in deproteinated whey by *Kluveromyces marxianus* using response surface methodology (RSM)",Bioresource Technol,97,2252-2259,(2006).

[8] Allan,A.L and Roche, C.D., "Effects of strain and fermentation conditions on production of cellulases by *T.reesei*".Biotechnol Bioengg,33,650-656,(1989).

[9] Alam MZ, Muyibi SA and Wahid R. "Stastical optimization of process conditions for cellulase production by liquid state bioconversion of domestic waste water sludge".Bioresource Technol,99,4709-4716,(2008).

[10] Alam Md Z,Muhammad N and Mahmat M.E., "Production of cellulose from oil palm biomass as substrate by solid state bioconversion" .,American J Appl Sci .,2(2) 569-572,(2005).

[11] Allan,A.L and Roche,C.D., "Effects of strain and fermentation conditions on production of cellulases by *T.reesei*" .Biotechnol Bioengg,33,650-656,(1989).

[12] Ali, S., Sayed, A., Sarkar, R.I.,and Alam, R, "Factors effecting cellulase production by A *Aspergillus terrus* using water hyacinth". World J Microbiol Biotechnol, 7, 62-66,(1991).

[13] Ali, S and Sayed, A, "Regulation of cellulase biosynthesis in *Aspergillus terreus*". World J Microbiol Technol, 8,73-75,(1992).

[14] Álvarez D. and Sánchez O., "Evaluation of orange peel and green soybean as a substrate for the production of α-galactosidase by a soil isolated *Aspergillus oryzae* in solid state fermentation", Chemical Engineering Transaction,(14),337-344,(2008).

[15] Anekar, S and Rao,C.R., "Ultra Filtration–Tool to Recover Valuable Constituent from Dairy Waste Water",J Appl Sci Environ Sanitation, 4 (2), 125-132.,(2009).

[16] Agblevor F.A,Cundiff J.S.,Mingle C and Li W. Storage and characterization of cotton gin waste for ethanol production,Resourse Conservation and Recycling, 46, 198-216(2006).

[17] Antier,P.,Minijares A.,Rainbault,M,Roussoss and Vinigera,G., "Pectinase hyperproducing mutants of *Aspergillus niger* C28B25 for solid state fermentation of coffee pulp",Enzyme Microbial Technol,15,254-260,(1993).

[18] Aro, N,Pakula, T and Pentilla, M, "Transcriptional regulation of plant cell wall degradation by filamentous fungi",FEMS Microbiol Rev, 25, (2004).

[19] Araujo A,Word OP and Disouza J. "Use of mutant strategies applied to *Aspergillus terreus* ATCC 52430 to obtain mutans with improved cellulase productivity".Biotechnology Technique,5:283-288,(1991).

[20] Atalla, R.H. and Vanderhart, D.L., "Native cellulose: A composite of two distinct crystalline forms",Science,223:283-285,(1984).

[21] Baig MMV. "Cellulolytic enzymes of *Trichoderma lignorum* produced in banana agro waste : Optimization of culture medium conditions", JSIR,64,57-60, (2005).

[22] Balat M, Balat H, and OZ C, "Progress in bioethanol processing",Progg Energy Combustion Sci ,34, 551-573, (2008).

[23] Bajpai P and Bajpai P. "Deinking with enzymes a review", TAPPI J, 81, 111,(1998).

[24] Basheer F and Farooqui I,H., "Enzymatic Treatment of Phenols", International Journal of Ecology, Environment and Conservation, vol. 13, no. 1(2007).

[25] Bayer, E.A, Chanzy, H., Lamed,R and Shoham,Y., "Cellulose, cellulases, cellulosomes", Cur Opin Struct Biol, 8, 548-557, (1998).

[26] Beguin P and Aubert JP, FEMS Microbiol Rev,13,25,(1994).

[27] Behra S,Mohanty R.C., and Ray ,R.C., "Ethanol production from mahua (*Madhuca lanifolia L.*) flowers with immobilized cells of *Saccharomyces cerevisiae* in *Luffa cylindrica* L. sponge discs", Appl Energy ,*88*,issue 1,212-215,(2011).

[28] Bhatt,M.K., "Cellulases and related enzyme in biotechnology".Biotechnol Adv,18,355-383,(2000).

[29] Biggs AR ,Ethholi MM,Etneshary S and Nickersson R, "Effects of Calcium salts on growth ,polygalacturonase activity and in infection of each fruit by *Monilia fructiola*" ,Plant Disease ,399-403, (1997).

[30] Biglow M and Wyman C.E,, "Cellulase production on bagasse pretreated with hot water" ,Appl Biochem Biotechnol, 98-100,(2002).

[31] Binder, A., and T. K. Ghose, "Adsorption of cellulose bv *Trichoderma viride*. Biotechnol Bioengg". 20,1187- 1199,(1978).

[32] BirsanC., Johnson,P., Joshi, M, Macleod,A., McIntosh,L., Monem,V., Nitz,M., Rose,D.R., Tull,D.Wakarchuck,W.W., Wang,Q., Warren,R.A,J., White,A and Withers,S.G., "Mechanism of cellulases and xylanases", Biochem Soc Trans, 26, 156-160, (1998).

[33] Blackwell J, In: Brown R.M Jr(ed). "Cellulose and other natural polymer systems biosynthesis, structure and degradation". Plenum, New York, 403,(1982).

[34] Borsa C.P and Palmarola-Adrados B, Galbe M, Zacchi G, Melzoch K and Rychtera,M, "Processing of wheat bran to sugars" ,J Food Engg,61,issue 4,561-565,(2004).

[35] Boynard C.A., and D'Almeida J.R.M., "Morphological characterization and mechanical behaviour of sponge guord (*Luffa cylindrica*) polyester composite materials" ,Polymer–Plastic Technol Engg ,*39*(3),489-499,(2000).

[36] Brillouet, J.M., Joseleau, J.P., Utille, J.P and Lelievre, D., "Isolation, purification, and characterization of a complex heteroxylan from industrial wheat bran". J Agri Food Chem. 30 (3), 488–495, (1982).

[37] Brijwani,K.;Oberoi.H.S. and Vadlani,P.V., "Production of cellulolytic enzyme system in mixed culture solid state fermentation of soyabean hulls supplemented with wheat bran",Process Biochem,45,issue 1,120-128, (2010).

[38] Buranov, Anvar U, and Mazza, *G.* "Lignin in straw of herbaceous crops",Indl Crops Products 28, 237–259,(2008).

[39] Cardona, C.A., Quintero, J.A., and Paz, I.C. "Production of bioethanol from sugarcane bagasse: Status and perspectives",Bioresource Technol 101,4754–4766,(2010).

[40] Carvalheiro, F., Duarte, L.C., Lopes, S., Parajó, J.C., Pereira, H., and Gírio, F.M., "Evaluation of the detoxification of brewery's spent grain hydrolysate for xylitol production by *Debaryomyces hansenii* CCMI 941". Process Biochem. 40, 1215– 1223, (2005).

[41] Chandra M,Kalra A,Sharma P.K and Sangwan R.S, "Cellulase production by six *Trichoderma* spp fermented" ,J Ind Microbiol Biotechnol, 36,605-609.,(2009).

[42] Chandra, M.,Kalra, A.,Sharma,P.K.,Kumar, H., and Sangwan, R.S, "Optimization of cellulases production by *Trichoderma citrinoviride* on marc of *Artemisia annua* and its application for bioconversion process",Biomass Bioenergy,1-7,(2010).

[43] Chandel, A.K., Kapoor, R.K., Singh, A.,and Kuhad, R.C.. "Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida shehatae* NCIM3501". Bioresource Technol. 98, 1947–1950.,(2007).

[44] Chad P,Aruna A,MaqsoodA.M and RaoL.V, "Novel mutation method for improved cellulase production",J Appl Microbiol ,96,318-323, (2005).

[45] Chen,H.Z, and Hayn,M,Esterbauer,H., "Purification and cvharacterization of two extracellular β-glucosidase from *Trichoderma reesei*",Biochem Biophys Acta,1121,54-60,(1992).

[46] Chen S and Wayman M, "Use of sorbose to enhance cellobiose activity in a *Trichoderma reesei* cellulase system produced on wheat hydrolysates" ,Biotechnol Technique,7,345-350,(1993).

[47] Chen, S., and Wayman, M. "Novel inducers derived from starch for cellulase production by *Trichoderma reesei*," Process Biochem. 27, 327-334(1992).

[48] Choi, W. S. and Han, K. J. H, "Physical and mechanical properties of pea-protein-based edible films", J. Food Sci,66,319-322,(2001).

[49] Chowdhary, N.A., Monruzzaman, M., Nahar, N.,and Chowdhary, N, "Production of celllulase and saccharification of lignocellulosic by *A. Micromonospora* Sp." World J Microbiol Biotechnol 7, 603-606,(1991)

[50] Chowdhary P., "What's available in deinking markets", IPPTAJ, 3, 111.,(2005).

[51] Cortoras, M and Agosin,E, "Regulatory aspects of endoglucanase production by the brown rot fungus *Gleophyllum trabenum*",Experimental Microbiol, 16,253-260,(1992).

[52] Correa, M. G and Villena, G.K. "Surface adhashion fermentation: A new fermentation category".Revista Peruna De Biologia,10,(2003).

[53] Couri, S.,Terzi, S.D.C,Pinto, G.A.S.,Freitas, S.P., and Costa, A.C.A.D, "Hydrolytic enzyme production in solid state fermentation by *Aspergillus niger* 3T588",Process Biochem,36,255-261,(2000).

[54] Damato G., Vivona G., Stoller M., Bubbico R and Bravi M., "Cellulase Production from Olive Processing Residues",Chemical Engineering Transaction,(20),73-78,(2010).

[55] Daniel J. Hayes, "An examination of biorefining processes, catalysts and challenges", Catalysis Today, 145, 138–151,(2009).

[56] Dash B and Patel M., "Recent Advances in deinking Technology", IPPTAJ, 9, 61-70,(1997).

[57] Daud S,Salleh MdS.S ,Salleh M.N,Kasim F.H and Saad S.A, "Analysis of chemical composition in sugarcane bagasse and rice straw for their suitability using in paper production",ICo SM,(2007).

[58] Deshpande,M.V.,Srinivasan, M.C. and Deshmukh, S.S., "Effect of fatty acids on cellulase production by *Penicillium funiculosum* and its mutants",Biotech Letts,Vol 9,no-5,301-304, (1987).

[59] Deshpande P,Nair S and Khedkar S, "Water hyacinth as carbon source for the production of cellulase by *Trichoderm reesei"*,Appl Biochem Biotechnol,158,552-560,(2009).

[60] Dedavide silva L.A.,Lopes F.C.,Silveira S.T. and Brandelli A, "Production of cellulolytic enzymes by *Aspergillus phoenicis* in grape waste using response surface methodology",Appl Biochem Biotechnol,152,295-305,(2009).

[61] Demain A.L and Birnbaum J,Curr Topics Microbiol Immunology,46,1-29,(1968).

[62] Dhawan S,Lal R and Kuhad RC, "Ethidium bromide stimulated hyper laccase production from bird's nest fungus *Cyathus bulleri"*,Letts Appl Microbiol,36(1)64-67,(2003).

[63] Dhillon S.S.,Gill R.K.,Gill S.S and Singh M., "Studies on the utilization of citrus peel for pectinase production using fungus *Aspergillus niger",*,Intl J Env Studies ,61(2),199-210,(2004).

[64] Dienes D,Borjessan J,Stalbrand H, and Reczy K. "Production of *Trichoderma reesei* Cel 7B and its catalytic core on glucose medium and its application for treatment of secondary fibers".Process Biochem ,41:2092-2096,(2006).

[65] Divne C,Stahlberg J,Reinikainen T,Ruohonen L,Pettersson G,Knowles JKC,Terri TT and Jones TA, "The three dimensional crystal structure of the catalatic core of cellobiohydrolase I from *Trichoderma reesei*".Science,265,524-528,(1994).

[66] Dixit Y and Kar A, "Antioxidative activity of some vegetables peels determined in vitro by inducing liver lipid peroxidation",Food Research Intl,42,1351-1354.,(2009).

[67] Domingues,F.C.,Queinoz,J.A.,Cabral,J.M.S.,Fonseca,L.P., "The influence of culture conditions on mycelial structure and cellulase production by *Trichoderma reesei* RUT C 30".Enzyme Microbial Technol,26,(2004).

[68] Duenas R, Tengerdy RP, Guiterrez and Correa M, "Cellulase production by mixed fungal solid substrate fermentation of sugarcane baggase".World J Microbiol Biotechnol,11,333-337,(1995).

[69] Du Q, and Wang K, "Preparative seperation of phenolic constituents in the fruits of *Luffa cylindrica* (L.) Roem, using slow rotary countercurrent chromatogarphy",J Liquid Chromatography Related Technol,*30*(13)1915-1922.,(2007).

[70] Du Q,Xu Y,Li,L, Zhao Y,Jerz G and Winterhalter P, "Antioxidant constituents in the fruits of *Luffa cylindrica*" ,J Agri Food Chem,54,(12) 4186-4190,(2006).

[71] Elegir G, Panizza E and Canetti M, "Neutral enzyme assisted deinking of xerographic office waste with a cellulase -amylase mixture", Tappi J (83) No 11, 71 ,(2000).

[72] Eliasashvili, V.,Penninckx, M.,Kachlishvili, E.,Astiani, M. and Kvesitadze G., "Use of *Pleurotus dryinus* for lignocellulolytic enzymes production in submerged fermentation of mandarine peels and tree leaves",Enzyme Microbial Technol ,38:998-1004,(2006).

[73] Eriksson K-EL.,Blanchette R.A, and Ander,P., "Microbial and enzymatic degradation of wood and wood componants",Springer,Berlin Heidelberg New York.

[74] Eriksson, T.,Borjesson, J. and Tjerneld, K., "Mechanism of surfactant effect in enzymatic hydrolysis of lignocelluloses",Enzyme Microbial Technol,31,353-364, (2002).

[75] Farid Talebnia, Dimitar Karakashev and Irini Angelidaki, "Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation", Bioresource Technol, 101 (2010) 4744–4753,(2010).

[76] Farooqui,I.H, "Solid waste management :A great perspective",National conference in environmental management,held, organized by civil engineering department, Aligarh Muslim University, India(2008).

[77] Feng, K. C., Rou, T. M., Liu, B. L., Tzeng, Y. M and Chang, Y. N., "Effect of fungal pellet size on the high yield production of destruxin B by *Metarhizium anisopliae"*, Enzyme Microbial Technol,34,22-25,(2004).

[78] Francis F,Sabu A and Nampoothiri KM, "Ramachandran S,Ghosh S,Szakacs G,PandeyA.Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillus oryzae"*,Biochem Engg J,15,107-115,(2003).

[79] Franceschin, G.,Favaron C and Bertucco A., "Waste paper as carbohydrate source for biofuel production :An experimental investigation",Chem Engg Transaction,(20),279-284,(2010).

[80] Fiedurek, J.,Production of gluconic acid by immobilized in pumice stones mycelium of *Aspergillus niger* using unconventional oxygenation of culture. Biotechnol Letts,23:1789–1792,(2001).

[81] Fujian X, Hozhang C and Zuahu L. "Effect of periodically Dynamic changes of air on cellulase Production in solid state fermentation". Enzyme Microbial Techonol, 30, 45-48,(2002).

[82] GayD.B and Richard D. M., Concepts of egg shell quality, www.afn.org/poultryfkmann4.htm.

[83] Gervais P, Molin P, Grajek W and Bensoussan M. "Influence of the water activity of a solid substrate on the growth rate and sporogenesis of filamentous fungi". Biotechnol Bioengg., 31, 457-463.,(1998).

[84] Geng X, Li K, Kataeva I.A., Li XL and Ljangdahl L.G., "Effects of two cellobio hydrolases CbhA and Cel K from *Clostridium thermocellum* on deinking of recycled mixed office Paper", Progg Paper Recycling,12 ,6-10 ,(2003).

[85] Ghose, T.K., and Sahai, V, "Production of celluleses by *Trichoderma reesei* Q M 9414 in fed batch and continuous flow culture with cell recycle", Biotechnol Bioengg 21, 283-296,(1979).

[86]Ghose, T. K. "Measurement of cellulase activities," Pure Appl. Chem. 59(2), 257-268(1987).

[87] Gherbawy,Y.A.M.H, (1998),"Effect of gamma irradiation on the cell wall degrading enzymes by *Aspergillus niger"*, Int J Food Microbiol, 40,127-131(1998).

[88] Gírio, F.M , Fonseca,C, Carvalheiro,F, Duarte,L.C. ,Marques,S, and Bogel-Łukasik, R., "Hemicelluloses for fuel ethanol: A review",Bioresource Technol 101,4775–4800,(2010).

[89] Gonzalez, G., Lopez-Santin, J.,Caminal,G and Sola,C., "Dilute acid hydrolysis of wheat straw hemicellulose at moderate temperature". Biotechnol Bioengg .,28,288-293.,(2004).

[90] Greenwood,D.A., Kraybill,T.H.R.,B. S and Schweiger,B.S., "Amino acid composition of fresh and cooked beef cuts" (Received for publication, May 23, 1951) 23-28.J Biol Chem.

[91] Greenway, D.L.A and Dyke, K.G.H., "Mechanism of the inhibitory action of linoleic acid on the growth of *Straphylococcus aureus"* ,J Gen Microbiol,115,233-245, (1979).

[92] Gubitz GM,Mansfield SD and Saddler JN., "Effectiveness of two endoglucanase from *Gleophyllum* sp in deinking of mixed office waste paper",Int conf Biotchnol Pulp Ind(47^{th}),65,209-215,(1998).

[93] Guerra G, Casado M.R.L.G,Arguelles J,Sanchez MAI,Manzano AM and Guzman T, "Cellulase production with sugarcane straw by *Trichoderma citrinoviride* on solid bed" ,Sugar Tech ,8(1),30-35,(2006).

[94] Gunata Z and Vallier M J, "Production of a highly glucose tolerant extracellular β-glucosidase by three *Aspergillus* strains",Biotech Letts,21,219-223,(1999).

[95] Gunaseelana, V. N. "Regression models of ultimate methane yields of fruits and vegetable solid wastes, sorghum and napier grass on chemical composition", Bioresource Technol, 98(6), 1270-1277(2007).

[96] Guo, G. L., Hsu, D. C., Chen, W. H., Chen, W. H., and Hwong, W. S., Charecterisation of enzymatic saccharification for acid pretreated lignocellulosic materials with different lignin composition," Enzyme Microbial Technol. 45(2), 80-87(2009).

[97] Hanif, A,Yasmeen, A and Rajoka, MI, "Induction,,repression and derepression of exoglucanase synthesis in *Aspergillus niger"* Bioresource. Technol, 63,1298-1306,(2004).

[98] Helle, S.S.,Duff, S.J.B. and Cooper, ,D.G., "Effect of surfactant on cellulose hydrolysis" ,Biotechnol Bioengg,42,611-617, (1993).

[99] Hornby,J.M, Jacobitz-Kizzier,S.M., McNeel,D,J., Jensen,E,C,Treves,D,S., Kenneth W. and Nickerson,K.W., "Inoculum Size Effect in Dimorphic Fungi: Extracellular Control of Yeast-Mycelium Dimorphism in *Ceratocystis ulmi"* , Appl Env Microbiol,70,(3), 1356-1359,(2004).

[100] Hsieh, Y. S.-Y. and Wong, C.-Y., "The composition of polysaccharides in primary walls of *litchi chinensis* sonn".J Food Biochem, 34, 5,971–982, (2010).

[101] Huang and Penner, 1991; Huang XL, Penner MH. "Apparent substrate inhibition of the *Trichoderma reesei* cellulase system". J Agric Food Chem 1991;39:2096–100,(1991).

[102] Ibrahim, M. N. M., Ahmed-Haras, M. R., Sipaut, C. S., Aboul-Encin, H. Y., and Mohamed, A. A. "Preparation and characterization of a newly water soluble lignin graft copolymer from oil palm lignocellulosic waste," Carbohydrate Polymers 80(4), 1102-1110(2010).

[103] Ibrahim, M. M., Dufresne, A., El-Zawawy, W. K., and Agblevor, F. A,. "Banana fibers and microfibrils as lignocellulosic reinforcements in polymer composites," Carbohydrates Polymers 81, 811-819(2010).

[104] Ingram LO and Doran JB, "Conversion of cellulosic materials to ethanol",FEMS Microbiol Rev,16,235-241.,(1995).

[105] Innocente,N.,Corradini,C.,Blecker,C and Paquot,M, "Dynamic surface properties of the proteose-peptone fraction of bovine milk",J Dairy Sci, 81:1833–1839 ,(1998).

[106] Janes, P,Targonski, Z and Mleko, S, "New inducers for cellulase production by *Trichoderma reesei* -7",Electronic J Polish Agri Uni,Food and Technology Series, 5,(2002).

[107] Jecu, L. "Solid state fermentation of agricultural wastes for endoglucanase Production", Ind. Crops Prod.11,1-5,(2000).

[108] Jeffries T.W, Klungness J.H., Sykes M.S. and Rutledge-Cropsey K.R.., "Comparison of enzyme enhanced with conventional deinking of xerographic and laser printed paper", TAPPI J, 77, 173-179,(1994).

[109] Jeffries T.W., Sykes M.S, Rutledge Cropsey. K, Klungness J.H. and Abubakr S., "Enhanced removal of toners from office waste paper by microbial cellulases", Proc of 6th Inter Conf on Biotechnol in the pulp and paper Industry. 141-144,(1995).

[110].Juhaz T, Egyrazi,A. and Reckzy,K.B., "β –glucosidase production by *Trichoderma reesei*."Appl Biochem Biotechnol, 121-124,243-254,(2005).

[111] Juhasz T, Kozma K, Szengyel Z and Reczey K, "Production of β – Glucosidase in Mixed culture of *Aspergillus niger* BKMF 1305 and *Trichoderma reesei* RUT C30", Food Technol Biotechnol,41,49-53,(2003).

[112] Ju L.K. and Afolabi O.A. "Waste paper hydrolysate as soluble inducing substrate for cellulase production in continous culture of *Trichoderma reesei"*, Biotechnol Progg,5,91-97,(1999).

[113] Jurg H and Chag KS, "Thermostable cellulase from *Streptomyces* Sp: Scale up production in a 50 L fermenter", Biotech Letts, 27,239-242,(2005).

[114] Kamagata, Y,Yachi, M,Kurasawa, T,Suto, M,Sasaki, H,Takao, S and Tomito F, "Cellulase induction by cellobiose octaacetate in *Penicillium purpogenum"*,J Fermentation Bioengg,72 , 217-220,(1991).

[115] Kang SW, Pork YS,Lee JS,Hong SE,Kim SW, "Production of cellulases and hemicellulases by lingocellulosic biomass", Bioresource Technol,91,153-156,(2004).

[116] Kachlishvili E,Penninckx,M.J.,Tsiklauri N and Elisashvili V, "Effect of nitrogen source on lignocellulolytic enzyme production by white–rot basidiomycetes under solid state cultivation",World J Microbiol Biotechnol,(2005).

[117] Karns, G,Dalchow, E, Klappacha, G and Meyer, D, "Formation and release of β-glucosidase by *Aspergillus Highzinet* 43746 in correlation to process operations", Acta Biotechnol,6, 355-359,(1995).

[118] Karr, W.E. and Holtzapple, M., "Benefits from Tween during enzymatic hydrolysis of corn stover", Biotechnol Bioengg,59,419-427, (1998).

[119] Kataria, M.C., Hazary, R.C. and Nath, M., "Response to long-term selection for part period egg production". XXI World Poultry Congress., Montreal, Canada, 20-24, (2000)

[120] Kaya, F.,Heitmann, J.A. and Joyce, T .W., "Influence of surfactants on the enzymatic hydrolysis of xylan and cellulose",TAPPI J,78,150-157, (1995).

[121] Keke BC and Pateron A. "Simaltaneous production and induction of cellulolytic and xylanolytic enzymes in *Streptomyces* sp" .World J Microbiol Biotechnol,483-487,(1992).

[122] Kelly,S, Grimmer, LH, Herysther, J, Schultheis, E ,Krull, R and Hempel, DC,2004, "Agitation effects on submerged growth and product formation of *Aspergillus niger*".Bioprocess Biosystem Engg,26,315-32,(2004).

[123] Kheshgi H.S and Rickeard D.J, "Greenhouse Gas Emissions from Bioethanol and Bio-Diesel Fuel Supply Systems",Green house Gas Control Technologies ,1419-1424.,(2003).

[124] Kim, S. and Dale, B.E., "Global potential bioethanol production from wasted crops and crop residues", Biomass Bioenergy, 26, 361–375,(2004).

[125] Kirk O,Borchert T,V. and Fuglsang C.C, "Industrial enzyme applications,Current Opinion in Biotechnology",13,345-351,(2002).

[126] Kirchner OG, Granados MS and Pascual PR. "Effect of media composition and growth conditions on production of β– Glucosidase by *Aspergillus niger* C-6". Appl Biochem Biotechnol,121-124,347-360,(2005).

[127] Kovas, K.,Megyeri, L., Szakacs, G.,Kubicek, C.P.,Galbe, M., and Zacchi G, "*Trichoderma atroviride* mutants with enhanced production of cellulase and_β-glucosidase on pretreated willow",Enzyme Microbial Technol,43,48-55,(2008).

[128] Krishna C. "Production of bacterial cellulases by solid state bio processing of banana wastes". Bioresource Technol,69, 231-239,(1999).

[129] Kubiecek, C P,Messener, R,Grauber, F,Mach, R L and Kubiek pranz EM, , "The *Trichodermma* cellulase regulatory puzzle:From the interior of life of a secretory fungus",Enzyme Microbial Technol, 15,90-99, (1993).

[130] Kuhad RC and Singh A. "Enhanced Production of cellulase by *Penicillium citrinum* in solid state fermentation of cellulosic residue". World J Microbiol Biotechnol,9,100-101,(1999).

[131] Kubde M.S., Khadabadi S.S., Farooqui ,I.A and Deore ,S.L, "*Lagenaria siceraria*: Phytochemistry,phamacognosy and pharmacological studies,Report and Opinion",2(3),91-98,(2010).

[132] Kumakura M,Kojima T and Koetsu I, "Pretreatment of lignocellulosic wastes by combination of irradiation and mechanical crushing" .,Biomass, 2,299-300,(1982).

[133] Kumar R and Singh R.P. "Semisolid state fermentation of *Eicchornia crassipes* bikomass as lignocellulosic biopolymer fro cellulose and β-glucosidase production by cocultivation of *Aspergillus niger* RK3 and *Trichoderma reesei* MTCC 164", Appl Biochem Biotechnol,71-82,(2001).

[134] Kumar, S., Negi, Y. S., and Upadhyaya, J. S., "Studies on characterization of corn cob based nanoparticles," Adv. Mat. Lett. 1(3), 246-253,(2010).

[135] Kumar R and Mandal SK, "Utility of banana peel waste in the hemicellulases production",Mapna Journal of Sciences,5(1),47-53,(2006).

[136] Lever M,Goen H, Cord-Ruwisch R , "Ethanol from lignocelluloses using crude unprocessed cellulase from solid state fermentation" , Article in press.

[137]Liming, X.,and Xueliang, S. "High yield cellulase production by *Trichoderma reesei* ZU–02 on corn cob residue". Bioresource Technol, 91,259-262,(2004).

[138]Li Y,Lin J,Meng D,Lu J,Gu G, and Mao Z, "Effect of pH,cultivation time and substrate concentration on the endoxylanase production by *Aspergillus awamori* ZH-26 under submerged fermentation using central composite rotary designs",Food Technol Biotechnol,44(4),473-477,(2006).

[139]Li, X.L., Dien, B.S., Cotta, M.A., WU, Y.V.,and Saha, B.C, "Profile of enzyme production by *Trichoderma reesi* Grown on corn fiber fractions", Appl Biochem Biotechnol. 121-124, 321-334, (2005).

[140]Liub Y-K,Seki M,Tanaka,H and Furusaki S, "Characteristics of Looffa (*Luffa cylindrica*) sponge as a carrier for plant cell immobilization",J Fermentation Bioengg ,85, 4,416-421,(1998).

[141] Liu, J.,Yuan, X.,Zeng,G.,Shi, J. and Chen, S., "Effect of biosurfactant on cellulose and xylanase production by *Trichoderma viride* in solid substrate fermentation" ,Process Biochem,41,2347-2351, (2006).

[142] Loewenberg, JR and Chapman, CM, "Sophrose metabolism and cellulase induction in *Trichoderma",* Archives Microbiol, 113 ,61-64,1997.

[143]Lowry, OH, Rosebrough , N.J.,Farr, AL.,Randall,R.J.,Protein measurement with folin phenol reagent, J. Biol. Chem.,193(1),265-275.,(1951).

[144] Lynd,L.R.,Weimer,P.J,Vanzyl,W.H. and Pretorious,I.S., "Microbial cellulose utilization:Fundamentals and Biotechnology". Microbiol Mol Biology Rev,66 ,506-577,(2000).

[145] Ma J.H. and Jiang C, "Enzyme applications in pulp and paper industry"., Progg Paper Recycling, 36-46, (2002).

[146] Madamwar, D. and Patel, S. "Formation of cellulases by co-culturing of *Trichoderma reesei* and *Aspergillus niger* on cellulosic waste", World J Microbiol Biotechnol,8,183-186,(1992).

[147] Macris, BJ, Kekos, D and Evangelidou, X, "Simple and inexpensive method for cellulase and β-glucosidase production by *Neurospora crassa",* Appl Microbiol Biotechnol,31,150-15,(1989).

[148] Magnin L, Lantto R and Delpech P, "Use of Enzymes for deinking of wood containing and wood free recovered Papers", Progg Paper Recycling, 13-20,(2002).

[149] Mahesweri DK, Jahan H, Paul J and Varma A. "Wheat Straw a potential substrate for cellulase production using *Trichodema reesei",* World J Microbiol Biotechnol,9,120-121,(1993).

[150] Mandhania, S., Jain,V and Malhotra,S.P., "Culture optimization for enhanced production of microbial pectin methylesterase under submerged conditions". Asian J. Biochem., 5: 12-22.,(2010).
[151] Manori,S.,Tatsuta,M. and Yamakawa,K.,Substrate permeability in pellets formed by *Aspergillus niger*.J Fermentation Bioengg,1995,79,387-390,(1995).

[152] Margaris N.S.,Mitrakos K and Markon ,Carbon sources for the *Aspergillus niger* growth under different shaking programmes, Folia Microbiol,19,394-396 (1974).

[153] Marzluf GA, "Regulation of nitrogen metabolism and gene expression in fungi", Microbiol review,45,437-451,(1981).

[154] Martinez-castellanos,G., Pelayo-Zaldivar C, Perez-Flores,L.J., Lopez-Luna.,A., Gimeno M,Barzana E. and Shirai K., "Postharvest litchi(*Litchi cinensis* Sonn.) quality preservation by *Lactobacillus*", Postharvest Biololgy and Technology,59,172-178,(2011).

[155] Marwaha, S.S and Kennedy, J.F., "Whey pollution problem and potential utilization",Int J Food Sci Technol,23,323-336,(2007).

[156] Marques, S., Pala, H.,Alves, L., Amarat-coilaco, M.T., Girio, F.M and Gino, F.M., Characterisation and application of glycanases secreted by *Aspergillus terreus* CCMI 498 and *Trichoderma viride* CCMI 84 for enzymatic de-inking of Mixed office waste paper, J Biotechnol,100,issue 3, 209-219(2003).

[157] Masenda E, "Groundnut shells", chapter 5,substrate, Mushroom growers handbook I,120-122(2004),www.mushworld.com/service/handbook/2004/chapter5-8pdf.

[158] Mathew,G.M.,Sukumaran,R.K.,Singhania,R.R. and Pandey,A., "Progress in research on fungal cellulases for lignocellulosic degradation", JSIR,67,898-907,(2008).

[159] MedveJ,Stahalberg J and Tjerneld F, "Adsorption and synergism of cellobiohydrolase I and cellobiohydrolase II from *Trichoderma reesei* on microcrystalline cellulose",Appl Biochem Biotechnol,66,39-56,(1997).

[160] Michael W. Harding, Lyriam L.R. Marques, Ronald J. Howard and Merle E. Olson, "Can filamentous fungi form biofilms?",Trends in Microbiology, 17(11),475-480.

[161] Mielenz, J. R. "Ethanol production from biomass: Technology and commercialization status", Cur. Opinion Microbiol.(4),324-329,(2001).

[162] Mishra P,K and Srivastava,K.K., "Variations of local heat transfer coefficients of gas fluidized bed", Procc of conference on Thermal systems at Institute of Technology, B.H.U, Varanasi(1995).

[163] Morikova Y,Ohashi T,Mantani O and Okada H , "Cellulase induction by lactose in *Trichoderma reesei* PC3-7", Appl Microbiol Technol ,44, 106-111, (1995).

[164] Morrision, J, MCaccarthy, U and Michal, A P, "Cellulase production by *Taloromyces emersonii* CBS 814.70 and a mutant UV7 during growth on cellulose ,lactose,and glucose containing media", Enzyme Microbial Technol, 9, 422-425,(1987).

[165] Mosier N,Wyman C,Dale B,Elander R,Lee YY,Holtzapple M and Ladisch M., "Features of promising technologies for pretreatment of lignocellulosic biomass"., Bioresource Technol ,96, 673-686,(2005).

[166] Mukhopadhyay, S.,and Nandi, B, "Optimization of cellulase production by *Trichoderma reesei* ATCC 26921 using a simplified medium on water hyacinth biomass", JSIR, 58,107-111,(1999).

[167]Muniswaran PKA and Charyulu CLN. "Solid Substrate fermentation of coconut coir pith for cellulase production". Enzyme Microbial Technol,16,436-440,(1994).

[168] Nagle M,Habasimbi K,Leis H,Mahayothee B,Janjai S,Haewsungcharen M and Muller J, "Availibility and potential of local biomass resourses as fuel for drying of tropical fruits in northern Thailand" ,Tropentag ,University of Kassel-Witzenhausen and University of Gottingen,Oct 9-11,(2007).

[169] Nandini, C.D and Salimath P.V, "Carbohydrate composition of wheat ,wheat bran,sorghum and bajra with good chapati/roti(Indian flat bread) making quality", Food Chem, 73,197-203,(2001).

[170] Nakajo, Yukihiro;Sano, and Hiroyuki,"Yeast extract composition, yeast for obtaining the same, and process for producing yeast extract composition",IPC: ***A23L 1/229*** (2006.01), ***A23L 1/30*** (2006.01), ***A23L 1/39*** (2006.01), ***C12N 1/16*** (2006.01).

[171]Nakano T,Ikawa NF and Ozienek C, "Chemical composition of chicken egg shells and shell membrane" , Poultry Sci,8213,510-514.,(2008).

[172]Nawwi KE and Kader AAE. "Production of single cell protein and cellulase from sugarcane bagasse : Effect of culture factors". Biomass Bioenergy, 11,361-364,(1996).

[173]Nigam,P., "Processing of agriculture wastes in solid state fermentation for cellulolytic enzyme production". JSIR,55, 457-463,(1996).

[174] Nunez-Gaona O, Saucedo-Castañeda G,Alatorre-Rosas R. and Loera O., "Effect of moisture content and inoculum on the growth and conidia production by *Beauveria bassiana* on wheat bran", Braz Arch Biol Technol.,53,4.,(2010).

[175] Nwodo-Chinedu S,Okochi,V.I.,Smith,HA,Okafor,U. and Omidiji,O.,(2007), "Effects of carbon sources on cellulase production *by Penicillium chrysogenum"*,Afr J Biochem Res,(1),6-10,(2007)

[176] Oberoi H.S, Chavan Y,Bansal S and Dhillon G.S, "Production of cellulases through solid state fermentation using kinnow pulp as major substrate",Food Bioprocess Technol,(2008).

[177] Ong, LGA ,Aziz, SA ,Narini, S,Karim, MIA and Hassan, MA, "Enzyme production and profile by *Aspergillus niger* during solid substrate fermentation using palm kernal cake as substrate" ,Appl Biochem Biotechnol ,118,73-79,(2004).

[178] Ojumu, T.V., Solomon, B, Betika, V.El., Layokun, S.K.,and Amigun B., "Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 fermented in sawdust, bagasse and corn cob", Afr J Biotechnol,2(6), 150-152,(2003).

[179] Palle, M and Zhang, X, Enzymes find their niche, Pulp & Paper Canada, (106) 6, 17-20 (2005).

[180] Palmarola-Adrados, B.,Choteborska, P.,Galbe, M and Zacchi, G., "Ethanol production from non starch carbohydrates of wheat bran",Bioresource Technol,96,843-850,(2005).

[181] Pala, H,Mota, M and Gama, F.M. "Enzymatic versus chemical deinking of non impact ink printed paper".J Biotechnol ,108,79-89,(2004).

[182] Panagiotou G,Kekos D,Macris BJ,Christakopoulas P. "Production of cellulolytic and xylanolytic enzymes by ***Fusarium oxysporum*** grown on corn stover in solid state fermentation".Ind Crops Prod;18:37-45,(2000.)

[183] Pandey,A, Soccol, L.R and Mitchell, D., "New development in solid state fermentation".Process Biochem.35,135-1153,(2000).

[184] Pandey,A , Soccol,C R, Nigam ,P and Soccol,V.T., "Biotechnological potential of agro-industrial residues. I: sugarcane bagasse", Bioresource Technol, 74, 69-80,(2000).

[185] Pandey Singh P, Nigam P and Pandey A, "Sugarcane Bagasse, Biotechnology for agroindustrial utilization", 239-252, ed-1,(2009).

[186] Panesar,P.S.,Kennedy, J.F.,Gandhi,D.N.,and Bunko,K, bioutilisation of whey for lactic acid production ,Food Chem J,105, 1-14,(2007).

[187] Pardo, AG, "Effect of surfactants on cellulase production by *Nectria cataliensis*",Curr Microbiol, 33,275-278,(1996).

[188] Pandey, A, Selvakumar, P, Soccol, C.R., Nigam, P,Solid state fermentation for the production of industrial enzymes,Current Science,(1999).

[189] Parikh,S.,Vinciv, A and Strobel, R.J, "Improvement of microbial strains and fermentation process",Appl Microbiol Biotechnol,54,287-303.,(2000).

[190] Pasanen, A.L,Kalliokoski P.,Pasanen P,Jantunen M.J and Nevalainen A., "Laboratry studies on the relationship between fungal growth and atmospheric temperature and humidity", Env Intl,17,issue 4 ,225-228.,(1991).

[191] Pattra, S., Sangyoka, S., Boonmee, M. and Reungsang, A., "Bio-hydrogen production from the fermentation of sugarcane bagasse hydrolysate by *Clostridium butyricum.*" Int. J. Hydrogen Energy 33, 5256–5265,(2008).

[192] Pejo ET, Oliva JM , Ballesteros M, "Realistic approach for full scale bioethanol production from lignocellulose :a review",JSIR, 67,874-884,(2008).

[193] Penner MH and Liaw ET. "Kinetic consequences of high ratios of substrate to enzyme saccharification systems based on *Trichoderma* cellulase". In: Himmel ME, Baker OJ, Overend RP, editors. Enzymatic conversion of biomass for fuels production. Washington, DC: American Chemical Society, 363–371.,(1994).

[194] Patel N, Choy V, Malouf P, Thibault J,Growth of *Trichoderma reesei* RUT C-30 in stirred tank and reciprocating plate bioreactors,Process Biochemistry,44,1164-1171, (2009).

[195] Piccolo, C, Fabrizio, B., A techno economic comparison between two technologies for bioethanol production from lignocelluloses, Biomass and Bioenergy, 33, 478-491(2009).

[196] Perkins, D and Davis,R, "Evidence for safety of *Neurospora* species for academic and commercial uses", Appl Environ Microbiol,66,5107-5109,(2000).

[197] Perrone, G, Susca, A, Cozzi, G, Ehrlich, K, Varga, J, Frisvad, JC, Meijer, M, Noonim ,P, Mahakarnchanakul, W and Samson, R A, "Biodiversity of *Aspergillus* species in some important agricultural products", Stud Mycol ,59,53-66,(2007).

[198] Pletka, J.,Chee, G.K.R.,Mcguire, J.P.,Prelich, J. and Groleau, L.,Interfacial effects of a poly alkylene oxide /fatty acid surfactant blend in flotation deinking of mixed office papers, Progg Paper Recycling,40-48(2000).

[199] Prasad,S., Singh,A, and Joshi, H.C, "Ethanol as an alternative fuel from agricultural, industrial and urban residues".Resources, Conservation and Recycling 50, 1–39, (2007).

[200] Prasertsan P, Kittikul AH and Chitmanee B. "Isolation and selection of cellulolytic fungi from palm oil mill effluent".World J Microbiol Biotechnol,8,614-617,(1992).

[201] Prasertsan P and Oi S, "Production of cellulolytic enzymes from fungi and use in the saccharification of palm cake and palm fiber", World J Microbiol Biotechnol,8,536-538,(1992).

[202] Prathumpai W,Mclntyre M and Nielsem J.The effect of CreA in glucose and xylose catabolism in *Aspergillus nidulans* , Appl.Microbiol Biotechnol,63,748-753, (2004).

[203] Prasad D.Y; Heitmann J.A. and Joyce T.W, Enzyme Deinking of black and white letterpre printed newsprint waste. Progg Paper Recycling, 21-30 (1992).

[204] Quin M,Guo P,Shao Z,Fu Y and Wang Q.,Enzymatic deinking of old newsprint,Zhongg Zaoahi(Chienese),19,7-13(2000).

[205] Rani B and Kawatra A, "Fiber constituents of some foods",Plant Foods Human Nutriti ,45,343-347.,(1994).

[206] Reczey K, Szengyel Z, Eklund R and Zacchi G, "Cellulase production by *reesei*", Bioresource Technol,57, 25-30,(1996).

[207] Reczey,K., Brumbauer A, Bollok,M, Szengyel,Z and Zacchi,G., "Use hemicelluloses hydrolysate for β-glucosidase fermentation",Appl Bioche Biotechnol,70-72,225-235,(1998).

[208] Revillion, J.P.D.P., Brandelli, A. and Ayub,M.A.Z., "Production of yeast extract from wh using *Kluyveromyces marxianus*", Braz. Arch. Biol. Technol., 46 no.1,(2003).

[209] Rivers DB. "Factors affecting the enzymatic hydrolysis of bagasse and rice stra .Biological Wastes ,26,85-95,(1998).

[210] Roberto, I.C., Lacis, L.S., Barbosa, M.F.S and Demancilha, I.M., "Utilization of sugarca bagasse hemicellulose hydrolysate *byPichia stipitis* for the production of ethanol". Proce Biochem,26, 15-21,(1991).

[211] Rockey-Salimi K. and Hamidi –Esfahani Z, "Evaluation of the effect of particle si ,aeration rate and harvest time on the production of cellulase by *Trichoderma reesei* QM 94 using response surface methodology", Food Bioproducts Processing,88,61-66,(2010).

[212]Romero D, Aguado J, Gonzaez L and Ladero M., "Cellulase production l *Neurospora crassa* on wheat straw". Enzyme Microbial Technol, 25, 244-250,(1999).

[213] Roy, F., Boye, J. I., and Simpson, B. K, "Bioactive protein and peptides in pulse crops: Pe chickpea and lentil",Food Res Intl. 43, 432-442,(2010).

[214] Rozman,D., Pertot,E., Beli,I and Komel,R, "Soybean peptones as nutrients in t fermentative production of clavine ergot alkaloids with *Claviceps fusiformis*.", Biotech Letts, vol no 8, 563-566.,(1985).

[215] Sarni Manchado-P ,Le R.E.,Le-Guerneve C,Lozano Y and Cheynier V, "Phenol composition of litchi fruit pericarp", J Agri Food Chem,48(12),5995-6002.,(2000).

[216] Sarri J, "New Process engineering for deinking Part -3:Ultrasound magnetic separation and enzymes".Paper Technol,4,:14-15,(2005).

[217] Saquib AA.N,Hassan M,Khan N.F and Baig S, "Thermostability of crude endoglucanase form *Aspergillus fumigatus* grown under solid state fermentation and submerged fermentation", Process Biochem,45,641-646,(2010).

[218] Schaffner, O W and Toledo,RT, "Cellulase production by *Trichoderma reesei* when culture on xylose based media supplemented with sorbose".Biotechnol Bioengg, 37, 12-16,(1991).

[219] Schmidt,O.,Angermann,H and Hoppe,K, "Experimental and theoretical investigations of submerged fermentation and synthesis of pectinolytic enzymes by *Aspergillus* sp"., Appl Microbial Biotechnol,43,424-430,(1995).

[220] Sehnem, N T,Bitteencourt, L R D,Camassola, M and Dillon, A J P, "Cellulase production by *Penicillium echinulatum* on lactose",Appl Microbial Biotechnol,72,163-167,(2006).

[221] Seidle, HF and Huber,RE, "Transglucosidic reactions of the *Aspergillus niger* family 3 β-glucosidae qualitative and quantitative analyses and evidence that the transglucosidic rates is independent of pH". Archives Biochem Biophys 436,254-264,(2005).

[222] Selber, K.,Tjernald, F.,Collen, A.,Hyytia, T.,Nakari –Setala, T.,Bailey, M.,Fagerstrom, R.,Kan, J.,Laan, J.V.D.,Pentilla, M. and Kula, M.R, "Large scale separation and production of engineered proteins designed for facilitated recovery in detergent based aqueous two phase extraction systems",Process Biochem,39,889-896, (2004).

[223] Selvakumar, G., Saha, S., and Kundu, S,. "Inhibitory activity of pine needle extracts on some agriculturally resourceful microbes," Indian J. Microbiol. 47(3), 267-270,(2007).

[224] Shiang, K,Linden, JC,Mohaghoghi, A,Grohmann, K and Himmel, ME, "Regulation of cellulase synthesis in *Acidothermus cellulolyticus*" ,Biotechnol Progg,7,315-322,(1991).

[225] Sharma, A., Hasseb, A., and Abujar, S. "Screening of field pea (*Pisum sativum*) selections for their reactions to root-knot nematode (*Meloidogyne incognita*)". J Zhejiang Univ Sci, 7(3), 209-214, (2006).

[226] Shibuya, N and Iwasaki, T., "Structural features of rice bran hemicelluloses".Phytochem, 24 (2), 285–289, (1985).

[227] Sciubba L, Diana Di Gioia, Leonardo Setti, Maurizio Ruzzi, Fabio Fava, "Bioconversion of ferulic acid obtained from wheat bran into vanillin", Chem Engg Transaction,(14)193-198,(2008).

[228] Singh I., Solomon,S., Srivastava,A. K., Singh, P.K. and Singh J, "Post harvest quality deterioration of cane juice: Physio biochemical indication",Sugar Tech, Vol 8(2 & 3),(2006).

[229] Soni, R.,Nazir, A.,Chadha, B.S. and Saini, H.S., "Novel sources of fungal cellulases for efficient deinking of composite paper waste", BioResources,3, 234-246,(2008).

[230] Sosulski, F. W., and Wu, K. K. "High-fiber breads containing field pea hulls, wheat, corn and wild oat brans", Cereal Chem.65, 186-191,(1988).

[231] Spiridon I and Anrade AMD, "Enzymatic deinking of old newspaper (ONP), Progg Paper Recycling" (14) , (2005).

[232] Spiridon I and Belgacem M.N., "Enzymatic deinking of laser printed paper", Progg Paper Recycling, 13, 12-15,(2004).

[233]Spiance, M. A. S., Lambert, C. S., Fermoselli, K. K. G., and De Paoli, M. A., "Characterization of lignocellulosic curaua fibers," Carbohyd. Poly. 77(1), 47-53,(2009).

[234]Srivastava KN, Srivastava,K.K, Gupta SN and Singh D, "Heat transfer from flat plate to water, ethanol under pool boiling",Proceedings on Thermal System, at Institute of Technology, B.H.U, Varanasi ,371,(1986).

[235] Srivastava A K and Solomon, "Mining of sugarcane residues for application in medicinal, biocidal and pharmaceutical", Sugar asia conference proceddings, New delhi,34-36,(2008).

[236] Stephen, A.M., "Other plant polysaccharides. In: Aspinall, G.O. (Ed.), The Polysaccharides". Academic Press, New York, 97–194, (1983).

[237] Stoodley P.,K. Sauer,D.G.Davies and J.W. Costerton,. Biofilms as a complex differentiated communities. Annual. Review of. Microbiology,56: 187–209,(2002).

[238] Sukumaran RK, Singhania RR and Pandey A. "Microbial cellulases production, applications & a challenges". JSIR, 64,832-844,(2005).

[239]Sukan, S.S,Guray, G and Sukan, FV, "Effects of natural oils and surfactants on cellulase production and activity",J Chem Technol Biotechnol ,46,179-187,(1989).

[240] Suto, M and Tomita, F, "Induction and catabolite repression mechanisms of cellulase in fungi", J Biosci Bioengg, 92,305-311,(2001).

[244] Sultana, B and Anwar, F, "Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants", Food Chem, 108, 879-884,(2008).

[245] Sun J,Shi J,Zhao M,Xue S.J.,Ren J.,Jiang Y, "A comparative analysis of property of lychee polyphenoloxidase using endogenous and exogenous substrates", Food Chem,108,818-823,(2008).

[246] Sun, R., Lawther, J.M., Banks, W.B., "A tentative chemical structure of wheat straw lignin", Ind Crops Prod, 6, 1–8, (1997).

[247] Sun, X., Sun, R., Fowler, P and Baird, M.S., "Extraction and characterization of original lignin and hemicelluloses from wheat straw", J Agri Food Chem. 53, 860–870, (2005).

[248] Sun, X.,Liu, Z.,Qu, Y.,Li, X, "The effects of wheat bran composition on the production of biomass hydrolyzing enzymes by *Penicilium decumbens*",Appl Biochem Biotechnol,146,119-128,(2008).

[249] Szengyle Z, Zacchi G, Reczey K, "Cellulase production based on hemicellulose hydrolysate from steam pretreated willow". Appl Biochem Biotechnol,63-65,351-363,(1997).

[250] Szizurto N,Silika –ahom,Tenkanen M,Alapuranen M,Vehmaunpera J,Reczey K and Vikari L, "Hydrolysis of amorphous and crystalline cellulose by heterologously produced cellulases of *Melano corpous albomyces*", J Biotechnol,136,140-147,(2008).

[251] Szijarto, N., Faigl, Z., Reczey, K., Mezes, M.,and Bersenyi, A, "Cellulase fermentation on a novel substrate (waste card board) and subsequent utilization of home produced cellulase and commercial amylase in a rabbit feeding trail". Ind Crops Prod, 20,49-57,(2004).

[252] Talabardon, M and S.T.Yang, Production of GFP and glucoamylase by recombinant and effects of fermentation conditions on fungal morphology and protein secretion. Biotechnol Progress, 21,1389–1400.,(2005).

[253] Talebnia F, Karakashev D and Angelidaki I, "Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation", Bioresource Technol, 101, 4744–4753,(2010).

[254] Tanobe V.O.A.,Sydenstricker T.H.D, Munaro M, and Amico S.C.,A comprehensive characterization of chemically treated brazilian sponge-gouards (*Luffa cylindrica*) ,Polymer Testing, *24*,474-482,(2005)

[255] Tandon R, Thakur V.V., Chisty M.H., Mathur R.M and Kulkarni A.G, "Enzymatic deinking an alternate technology for quality upgradation of mix office waste". IPPTAJ, (17) no3, 51-55 ,(2005).

[256] Tao S, Zuohu L and Deming L. "A novel design of solid state fermenter and its evaluation for cellulase production by *Trichoderma viride* SL -1", Biotech Letts, 10,889-895, (1996).

[257] Tengedy, R.P, "Cellulase production by solid state fermentation", JSIR. 55,313-316,(1996).

[258] Tengerdy,R.P. and Szakacs G, "Bioconversion of lignocelluloses in solid state fermentation", Biochem Engg J 13,169–179.(2003).

[259]Teri,T.T., KoiVula,A, Linder,M., Wohlfahrt,G., Divne, C and Jones, T.A., "*Trichoderma reesei* cellobiohydrolases: Why so efficient on crystalline cellulose",Biochem Soc Tran,26,173-178,(1998).

[260] Tewari HK, Marwaha SS and Singh L, "Studies on cellulase production from groundnut shell",J Res (PAU),25,424,432(1988).

[261] Thirumale, S, Rani, D S and Nand, K, "Control of cellulase formation by trehalose in *Clostridium papyrosolvens* CFR-703",Process Biochem, 37, 241-245,(2001).

[262] Timell, T.E.,. "Wood hemicelluloses". Adv. Carbohydr. Chem. Biochem. 20, 409–483, (1965).

[263] Tomme,P.,Warren,R.A.J and Gilkes,N.R., "Cellulose hydrolysis by bacteria and fungi",Adv Micro Physiol,37,1-81,(1995).

[264] Tolan,J.S. and Foody,B., "Cellulase from submerged fermentation", Adv Biochem Engg /Biotechnol,65, 42-54,(1999).

[265] Trejo-Hernandez,M.R.,Oriol,E.,Lopez-Canales,A.,Roussos S,Viniegra G., and Raimbault.M, "Production of pectinase by *Aspergillus* sp by solid state fermentation on support",Micol Neotrop.Appl,4,49-62,(1991).

[266] Troung, Q. T.,N. Miyata and K. Iwahori, Growth of *Aspergillus oryzae* during treatment of cassava starch processing waste water with high contents of suspended solids. J Bioscience Bioengg,97:329-335,(2004).

[267] Tsao G. T, Xia, L, Coo N and Gary CS, "Solid State fermentation with *Aspergillus niger* for cellobiose production".Appl Biochem Biotechnol,84/86: 743-749,(2000).

[268] Tserki, V., Matzinos, P., Kokkou, S., and Panayiotou, C,. "Novel biodegradable composites based on treated lignocellulosic waste flour as filler. Part I, Surface chemical modification and caracterization of waste flour," Composites Part A: Appl. Sci. Manufact. 36(7), 965-974,(2005).

[269] Tosh, S. M., and Yada, S. "Dietary fibers in pulse seeds and fractions, Characterization,functional attributes and applications", Food Research Intl. 43,450-460,(2010).

[270] Umikalsom, M.S, Arriff, A.B.,Zulkifli, H.S.,Tong, C.C.,Hassan, M.A., and Karim M.I.A, "The treatment of oil palm empty fruit bunch fiber for subsequent use as substrate for cellulase production by *Chaetomium globossum* Kunze",Bioresource Technol.62,1-9,(1997).

[271] Usami S,Kirimura K,Imura M,Morikova S., "Cellular localization of constitutive β-glucosidase in *Trichoderma viride*",J Ferment Bioengg,73,399-40,(1993).

[272] Vanajakshi J,Subhaker C and Jetty A,Media engineering for the production of cellulases by a novel strain *Aspergillus* sp IICT-F 141 using wheat bran as raw materials, J Pure Appl Microbiol,,Vol 3(2),477-483(2009).

[273] Velkovska, S, Marten, MR and Ollis, D F, "Kinetic model for batch cellulase production by *Trichodermma reesei* RUT C-30",J Biotechnol,54,83-94,(1997).

[274] Verma,N.,Bansal ,M.C. and Kumar V., "Pea peel waste: A lignocellulosic waste and its utility in cellulase production by *Trichoderma reesei* under solid state cultivation",Bioresources,6(2),1505-1519,(2011).

[275] Villena,G.K and Guiterrez–Correa M. "Production of cellulases by *Aspergillus niger* biofilm developed on polyester cloth",Letts Appl Microbiol,4,262-268,(2006).

[276] Villena,G.K and Guiterrez–Correa M, "Production of lignolytic enzymes by *Aspergillus niger* biofilms at variable water activites",Electronic J Biotechnol,10,(2007).

[277] Villena,G.K and Guiterrez–Correa M, "Morphological patterns of *Aspergillus niger* biofilms and pellets related to lignocellulolytic enzyme productivities",Letts Appl Microbiol,45,231-237,(2008).

[278] Villena, G.K.,T. Fujikawa, S. Tsuyumu, and M. Gutiérrez-Correa, Structural analysis of biofilms and pellets of *Aspergillus niger* by confocal laser scanning microscopy and cryo scanning electron microscopy, Bioresource Technol, 101, 1920–1926,(2010).

[279] Vlaev, S. D.,Djejeva, G.,Raykovska, V., and Schugerl, K, "Cellulase production by *Trichodermma* sp grown on corn fiber substrate".Process Biochem ,32,no-7,561-565,(1997).

[280] Vyas, A., and Vyas, D, "Production of fungal cellulase by solid state bioprocessing of ground nut shell waste", JSIR, 64, 767-770,(2005).

[281] Vyas A, Vyas D and Vyas KM, "Production and optimization of cellulase on pretreated ground nut shell by *Aspergillus terrus* AV 49". JSIR,64, 281-286, (2005).

[282] Vyas S and Lachke A. "Biodeinking of mixed office waste paper by alkaline active cellulases from alkalotolerant *Fusarium sp*" .Enzyme Microbial Technol,32, 236-245,(2003).

[283] Wang, C H,Hseu, T H and Huang, C M, "Induction of cellulases by cellooligosaccharides in *Trichoderma koninghii* G-39",J Biotechnol, 9, 47-60,(1988).

[284] Wang, C H and Reesei ET, "Lactose utilization by fungal system an approach",Enzyme Microbial Technol,(1994).

[285] Wayman, M., and Chen, S, "Cellulase production by *Trichodermma reesei* using whole wheat flour as a carbon source".Enzyme Microbial Technol,14,825-831,(1992).

[286] Wen, Z.,Liao, W.,and Chen, S, "Production of cellulase by *Trichoderma reesei* from dairy manure", Bioresource Technol,96,491-499,(2005).

[287] Wen, Z., Liao, W.,and Chen, S. "Production of cellulase/ β- glucosidase by the mixed fungi culture of *Trichoderma reesei* and *Aspergillus phoenicis* on dairy Manure". Appl Biochem Biotechnol.121-124:93-104,(2005).

[288] Wen, T. N., Prasad, K. N.,Yang, B., and Ismail A. "Bioactive substance contents and antioxidant capacity of raw and blanched vegetables", Innovative Food Sci. Emerging Technol, 11, 464-469,(2010).

[289] Weimer,P.J.,Critical Rev Biotechnol,12,189,(1992).

[290] Welt T. and Dinus R.J., Enzymatic deinking -A Review, Progg in Paper Recycling, 36-46, (1995).

[291]Withers,S.G.,"Mechanisms of glucosyl transfereses and hydrolases",Carbohydrates Polym,44,325-337,(2001).

[292] Woodward, J, "Xylanases: Functions, Properties and Applications". Ellis Horwood Ltd., Chichester, (1984).

[293] Wood T.M, "Fungal cellulases",Biochem Soc.Tran.,20,46-53.

[294] Wyman, M and Chen, S, "Cellulase production induced by carbon source derived from waste news paper",Process Biochem, 26, 93-100,(1991).

[295] Xia L and Cen P. "Cellulase production by solid state of fermentation on ligno cellulosic waste from the xylose industy", Process Biochem,34,909-912,(1999).

[296] Xia Z, Beaudry A and Bourbonnais S, "Effect of cellulases on the surfactant assisted acidic deinking of ONP and OMG", Prog. Paper Recycling, 46-58 , (1996).

[297] Xie Y,Wu H and Lai Y. "Deinking of colored offset newsprint with enzyme treatment in cooperation with ultrasonic wave".Cellulose ChemTechnol ,36,285-293,(2002).

[298] Xu, Q., Fu, Y., Gao, Y.,Qin, M., "Performance and efficiency of old news paper deinking by combining cellulase /hemicellulase with laccase-violuric acid system", Waste Management.,29 (5) 1486-1490.,(2009).

[299]Yang, S., Li, J., Zheng, Z., and Meng, Z., "Lignocellulosic structural changes of *Spartina alterniflora* after anaerobic mono and co digestion," Intl. Biodeterioration Biodegradation 63, 569-575, (2009).

[300] Yezdi, T.,Woodward, J.R. and Radford, A., "The cellulase complex of *Neurospora crassa* : activity,stability and release" ,J Gen Microbiol,136,1313-1319, (1990).

[301] Yueming Jianga, Xuewu Duana, Daryl Joycec, Zhaoqi Zhangd and Jianrong Lib, "Advances in understanding of enzymatic browning in harvested litchi fruit" ,Food Chem, 88, 3,443-446,(2004).

[302] Yu J and Kieko S, "Microbial utilization and biopolyester synthesis of bagasse hydrolysates" ,Bioresource Technol,99,8047-8048.,(2008).

[303] Zhenying S, Shijin D, Yan G, Junfeng L, Hongyan W and Zhang SX, "Combined de-inking technology applied on laser printed paper", Chem Engg Processing: Process Intensification, 48(2) 587-591,(2009).

[304] Zhi wenbo,Song J,Ouyang F and Bi J, "Application of response surface methodology to the modeling of α- amylase purification by aqueous two phase systems",J Biotechnol,118,157-165,(2005).

[305] Fungi on line, An introduction to fungal growth kinetics, http://www.fungionline.org.uk/5kinetics/kinetics_summ.html

[306] Engineering statstic hand book, response surface design,http//www.itl.nist.gov/div898/handbook/pri/section 3/pri335.htm.

[307] Engineering statstic hand book, response surface design,http//www.itl.nist.gov/div898/handbook/pri/section 4/pri335.htm.

[308] Pretreatment of lignocelluloses biomass. In: HimmelME,Baker JO,OverendRP, editors. Conversionof hemicellulose hydrolyzates to ethanol. Washington, DC: American Chemical Society Symposium,292–324,(1994).

[309] http://en.wikipedia.org/wiki/Bra
[310]].http://en.wikipedia.org/wiki/Lagenaria cinerria

[311].http://en.wikipedia.org/wiki/Wheat

[312] http://en.wikipedia.org/wiki/Potato.

[313] http://www.britannica.com/EBchecked/topic/502352/rice-starch

[314] International starch institute, Denmark, Technical memorandum on wheat starch,http://www.starch.dk/isi/starch/tm33wheat.asp.

[315] http://en.wikipedia.org/wiki/Rice
[316] http://en.wikipedia.org/wiki/Dairy

[317]www.free patents online.com/6899294.htm.Hatchery egg shell waste processing method and device ,US patent 6899294.

[318] http// ww.fgsc.net /asil206/2006 *Neurospora* 2006,poster abstracts.

[319] Food and feeding in health disease ", by Chalmers Watson. Also available from Amazon: Food and feeding in health disease with title foods prepared from meat.

[320] Surfactants and nitrogen source\Eurasyp - Yeast Extract.mht(European association for speciality yeasts products),, Position paper on the EU GM Regulations and yeast extracts, Background Genetically modified food and feed are regulated in the EU by: • Regulation (EC) No 1829/2003.of the Europe.

[321] BD ,Bionutrients ,Technical manual,Advanced Bioprocessing,Third edition,30-31.

[324] http://www.asahibeer.co.jp/english, The latest information on environmental topics from japan to world-Japan for sustainability.Asahi Breweries Produces World's First Cellulase Enzyme from Wastepaper.

[325] Novozymes expects to have enzymes ready for second-generation bioethanol production,Focus on Catalysts,(2008),3-4,2008.

[326] Genencor launches enzyme for cellulose-based bioethanol production ,Focus on Biocatalysts,2007(2007)3.

[327] Novozymes and Broin collaborate in bioethanol production,Focus on Catalyst,2006(2006)4.

[328] Novozymes launches three new enzymes for bioethanol production ,Focus on Catalysts,2006(2006) 4.

[329] World enzyme to 2011,Market study# 2229 by freedonia group:http# www.freedonia.com,2007.

[330]Novozymes and NREL reduce enzyme cost Science Direct –Focus on Catalysts ,4-4.,2005.

[331] Novozyme Press release,june 23,http;# www.novozymes .com /en/main structure /Press and publication /Press release /2008/New+Facility+in+Nebraska.htm.,2008.

[332] Increased cellulase production in *Trichoderma reesei* by metabolic engineering,Articles in the September 11,,issue of biofuels supplement news,Crop Biotech update,International Service for the Acquisition of Agro-Biotech Applications.,2009.

[333] http://links.JSTOR.org/sici, JSTOR, Proceedings of the Royal Society of London, Series B, Biological Sciences, Vol 166,1-3.

[334] World enzyme to 2011,Market study# 2229 by freedonia group:http# www.freedonia.com,2007.

[335] Laboratory Manual (2001). Laboratory Manual of Central Pulp and Paper Research Institute, Analysis of fibrous raw materials, proximate chemical analysis, Saharanpur, 247001(U.P.), India, "Estimation of acid insoluble lignin in. wood/nonwood," TM1A-7; "Estimation of acid soluble lignin in wood/nonwood," TM1A-8; "Estimation of holocellulose in wood/nonwood"; TM1-A9.