REMOVAL OF PYRIDINE BY ADSORPTION IN CONTINUOUS REACTOR AND BY BIODEGRADATION USING BACILLUS SUBTILIS

A DISSERTATION

Submitted in partial fulfilment of the requirements for the award of the degree of

MASTER OF TECHNOLOGY

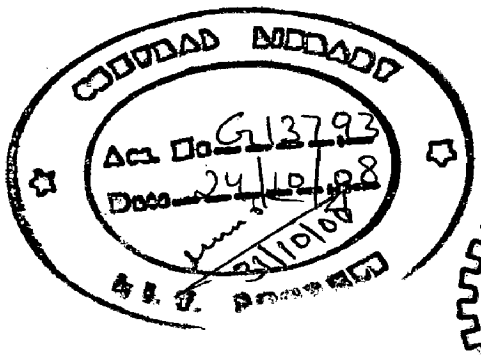
in

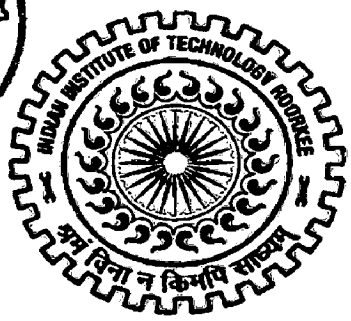
CHEMICAL ENGINEERING

(With Specialization in Industrial Pollution Abatement)

By

SOUMYA JYOTI CHOUDHURY





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CANDIDATE'S DECLARATION

I hereby declare that the work which is being presented in this dissertation titled **"REMOVAL OF PYRIDINE BY ADSORPTION IN CONTINUOUS REACTOR AND BY BIODEGRADATION USING BACILLUS SUBTILIS"**, in partial fulfillment of the requirements for the award of the degree of Master of technology in Chemical Engineering with specialization in "**Industrial Pollution Abatement**", and submitted to the Department of Chemical Engineering, Indian Institute of Technology, Roorkee, is an authentic record of the work carried out by me during the period July 2007 to June 2008, under the guidance of **Dr. C. BALOMAJUMDER** and **Dr. ARVIND KUMAR**. The matter embodied in this work has not been submitted for the award of any other degree.

Date: June 30, 2008
Place: IIT, Roorkee

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CERTIFICATE

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

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ABSTRACT

Pyridine (Py) and its derivatives are volatile, toxic and flammable with a pungent and unpleasant odor. Pyridine is the parent of a series of chemicals, and is used in many industries like paint, rubber, insecticides and herbicides. Pyridine and its derivatives are also used to make different products such as medicines, vitamins, food flavorings, dyes, adhesives, and in water proofing for fabrics. When heated to decomposition, pyridine emits highly toxic vapors of NO_x in an oxidative atmosphere. It has very high half-life when released in the atmosphere. It is also mildly toxic by inhalation; its vapour is skin and severe eye irritant and exposure to it can cause depression, gastrointestinal upset, liver and kidney damage, headache, nervousness, dizziness, insomnia, nausea, anorexia, frequent urination and dermatitis. The typical concentration of Pyridine in wastewaters is in the range of 20-300 mg/l. However, the vapor (odor) concentration may be as low as 7 µg/l, while the threshold odor concentration can be ~0.3 µg/l.

The various physical methods which are available for treatment of pyridine from various sources are thermal catalytic incineration, deep well injection, soil percolation, ultrafiltration, chemical coagulation and adsorption on various materials. Various chemical methods, required for the treatment of pyridine are based on UV/ ozone gas scrubbing, chemical/ photochemical oxidation and wet oxidation etc. Advanced oxidation processes (AOPs) are another major group of chemical treatment processes used for treatment of heterocyclic compounds like pyridine. The AOPs are associated with generation of highly reactive radical intermediates, mainly the hydroxyl radicals. Another attractive method to eliminate pyridine is biodegradation in which the organic compound is essentially converted to harmless compounds such as CO_2, H_2O, and NH^{2-} etc. In literature different aerobic and anaerobic microorganisms have been reported for degradation of pyridine.

In the present study pyridine contaminated air stream had been treated in continuous fixed bed column reactors. Soybean and puffed rice which have no mention of use in literature as adsorbents had been chosen as the adsorbents. The characterizations of these compounds were also performed. The data from adsorption study was used to validate Yoon and Nelson model for gas phase continuous reactor. In the next part of the present work where pyridine had been treated by biodegradation, *Bacillus Subtilis* was chosen as the microorganism to be used. Parameters like pH and temperature were

optimized through batch studies for degradation by *B. Subtilis*. The growth of microorganism was studied for various initial pyridine concentrations and subsequently the growth kinetics was also studied. Finally continuous biotrickling filter was run at a definite contaminant load, flow rate and empty bed residence time. Soybean was used as the packing material.

The adsorption study of pyridine laden air stream was carried out in continuous reactors by varying inlet concentration and total flow rate to the reactor. Soybean was found to give better results than puffed rice. The validation of Yoon and Nelson model showed that rate constant as well as proportionality constant reduced with increase in concentration for both adsorbents. When the model was validated for variation in flow rate the rate constant increased for soybean but decreased for puffed rice with increase in flow rate. The proportionality constant though decreased in both cases. The optimization study for biodegradation of pyridine by *B. Subtilis* showed that optimum temperature was 30 ^{O}C and optimum pH was 7. At this optimized pH and temperature conditions the growth of *B. Subtilis* was studied for initial pyridine concentration ranging from 50 to 500 ppm. The specific growth rate appeared to increase with increase in concentration thus indicating Monod growth kinetics. Hence double reciprocal plot was plotted to find the maximum specific growth (μ_{max}) rate as 0.0349 hr^{-1} and Half saturation constant (K_s) as 121.65 mg/l or ppm. The continuous process with soybean packing though did not work as soybean started to rot in 3 days and the bed also collapsed.

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INTRODUCTION

1.1 General Introduction:

Air and water are the most important matters required for sustainability and healthy growth of all living beings on earth. Our natural resources are needed to be conserved and preserved for this purpose. Waste discharges in both gaseous and liquid form from domestic and industrial activities, and from various urban utilities agglomerates into the atmosphere, thus affecting the air and water quality. The rapid industrialization with the use of the various types of raw materials for the production of final products is resulting in waste discharge with various inorganic and organic components. These components have different physico-chemical, thermal and toxic characteristics. The indiscriminate, somewhat reckless and ill-treated waste discharges affect the quality of natural resources and therefore the water and air as well. Chemical, petrochemical and agro-based chemical industries are the sources of various pollutants. Several of these industries are the sources of generating toxic materials and hazardous wastes. The waste emanating from such industries find their way into natural sources in various material states viz., solid, liquid, vapour or gas thereby adversely affecting the environment.

Toxics in wastewater often pass through effluent treatment plants (ETPs) which are not designed to remove them. They can also interfere with the operation of ETPs. It is therefore, necessary to properly reduce or minimize the pollution load at the source and then treat the wastewater either at the source or at the treatment facility (end of pipe, EOP treatment). Similarly many toxics are released in to the atmosphere via the air source. In a industry many pollutants are present in the air stream that comes out through the chimney or stacks following various stages of industrial processes. Industrial processes like painting unit, thermal treatment units or many other units and processes where fumes or vapour or smoke is generated are the prime source of gaseous pollutants from industry.

There was not much attention being paid to the serious implementation of regulatory rules regarding control of gaseous pollutants in industry till recently. Due to this tendency of ignorance even the treatment methods of gaseous pollutants did not receive much attention till now. But now due to serious efforts by different national and international regulatory agencies and also because of the serious problem of global warming, lot attention has now been diverted towards removal of gaseous pollutants coming out of industry. The minimization of toxicity at source can be achieved by following the rules of the pollution regulating agencies regarding the limiting pollutant discharges to the atmosphere via gaseous and water streams. The reduction of the load at the source can be achieved by minimizing the use and/or spillage and the proper inventory control of the toxics, proper and adequate treatment prior to toxics discharge, recycling and reuse of waste by-products, changes in the manufacturing processes-both the use of raw materials and the unit operations. There are some organic compounds which when present in wastewater streams and/or air, are a cause of major emission concern as they are considered to be toxic and hazardous to various target organisms including human beings.

1.2 Volatile Organic Compounds (VOCs):

EPA has defined "VOC" very broadly. In effect, "any volatile compound of carbon" is classified as a VOC for regulatory purposes, unless specifically exempted. EPA's VOC regulations, however, do not always apply to all compounds that meet the very broad definition. For example, for regulations involving paints and coatings, there is a specific test method, known as Test Method 24, that generally determines what is to be treated as VOC. Test method 24 is a collection of ASTM test methods that collectively defines the VOC content of a coating formulation [**www.americansolventcouncil.org**].

Denomination of volatile organic compounds (VOCs) refers to organic compounds whose vapour pressure is at least 0.01 kPa at 20 °C. VOCs are also characterized by their low water solubility. A large quantity of volatile organic compounds (VOCs) is released from various industrial plants and processes. Hence the

waste air contains a variety of VOCs, usually at low concentration. From an environmental point of view, it is necessary to limit and control vapour emissions because they affect the change of climate, the growth and decay of plants and the health of human beings and all animals. For example, according to a report of the National Academy of Sciences, the release of chlorofluoromethanes and chlorine-containing compounds into the atmosphere increases the absorption and emission of infrared radiation. The VOCs include aromatic and chlorinated species [**Iranpour et al.2005**]. These VOCs have already been found to play an important role in the atmospheric chemistry. The boiling point of VOCs ranges from less than -50°C to over 200°C. The maximum 3 hour concentration of hydrocarbon content is $1.6*10^{-4}$ kg/m (0.24 ppm) which must not be exceeded for more than once a year in an area. Reduction of VOCs emissions that exceeds the current national ambient air quality standard for ozone of 0.12 ppm is mandated under Title I of the US Clean Air Act Amendment of 1990. In addition, Title III of the amendments requires reduction of the emissions of 189 hazardous air pollutants, most of which are included under the definition of VOCs as well [**Ruddy & Carroll, 1993**]. The recently passed European Community stage emissions limit is 35 grams total organic compounds (TOC) per cubic meter gasoline loaded (35 g TOC/m3). The German TA-Luft Standard, the most stringent known gasoline emission regulation, has set an emissions limit to 150 mg TOC (excluding methane) per cubic meter of loaded product (0.15 g TOC/m3) [**USEPA,1991; Ruddy & Carroll,1993; Pezolt et al.1997**]. In India though there is no separate regulation as such to control VOCs emission.

VOCs are a major group of pollutants; this has now become a cause of concern worldwide. In North America and Europe the removal of VOCs from contaminated airstreams has become a major air pollution concern since 1990[**Lahane et.al.2007**]. VOCs are harmful to ecosystem, human health and atmosphere [**Srivastava et. al.2005, Pandey et. al.2005, Adam et. al.2001**]. Some VOCs (such as benzene, toluene, ethylbenzene and o-xylene (BTEX)) are important industrial solvents that are frequently encountered in industrial operations and contaminated sites. Many governmental agencies and local authorities in various developed and fast developing countries have introduced stringent and strict guidelines and standards for these VOCs. For instance, the South Coast Air Quality Management District for the California South Coast Air Basin has

several programs (Regulations XIII New Source Review; Regulations XIV Toxic Air Contaminants) that aim at reducing toxic VOC emissions from industrial sources. Throughout the world in order to abide by the regulations of the local regulation authorities and governments on the protection of the environment and air quality, industrial plants need to eliminate or reduce the content of toxic chemicals in gaseous effluents prior to their release into the atmosphere [**Mathur et. al.2007**].

As a result, the viability and efficiency of long existing air emission control technologies such as incineration, condensation, absorption and adsorption, and recent techniques, including biological processes, have become a major preoccupation of environmental organizations and those industries dealing with such emissions [**Ruddy and Carroll. 1993, Hounsell 1995**]. Common challenges to be faced in these technologies are the wide variety of the VOCs to be abated and the variety of the conditions, often strongly changing with time, under which VOCs must be eliminated[**Pina et. al.1997**] . Purification processes based on the ability of some micro-organisms (mainly bacteria) to degrade a variety of inorganic and organic compounds are currently an interesting environmental control option. Under aerobic conditions, these micro-organisms are able to oxidize the compounds concerned (substrate) into mineral end-products. Moreover, some of the organic compounds are transformed into new cell material [**Dicks and Ottengraf,1991**].

Sl No.	Volatile Organic Compound	Mol. Weight	Boiling point (^{o}C)	Vap. Pr. (mm-Hg) @ 23 ^{o}C
1	Propane	44.1	-44	
2	N-Butane	58.12	-0.48	
3	N-Octane	114.23	125.8	12.554
4	Ethene	28.05	-104	
5	1-Butene	56.11	-6.1	
6	Styrene	104.15	145.3	5.408
7	Benzene	78.11	80.2	86.554
8	Toluene	92.14	110.8	25.641
9	Acetylene	26.04	-84	

10	Methanol	32.04	64.7	
11	Isobutyle Alcohol	74.12	82.6	36.795
12	Acrylic Acid	72.06	139	
13	Propionic Acid	74.08	141.1	
14	Formaldehyde	30.03	-18.95	3652.842
15	Acetaldehyde	44.05	20.5	835.88
16	Aldehyde	86.14		
17	Acrolein	56.06	52.84	252.631
18	Methyl Ethyl Ketone	72.11	79.8	86.952
19	Methyl Isobutyl Ketone	100.16	116.7	17.716
20	Phenol	94.11	182	0.453
21	Nitrobenzene	123.11	210.9	
22	Vinyl Chloride	62.5	-13.4	
23	Methyl Bromide	94.95	3.6	
24	Chloroform	119.39	74.1	
25	TLEV Exhaust-LPG	14.86	-42.1	
26	TLEV Exhaust-CNG	15.22		

Table 1.1: List of VOCs as per EPA List of VOC

1.3 VOC Threshold Limit:

The VOC when come out in the atmosphere, they can be harmful to the surrounding, the humans and also to all other living beings in the environment. These VOC need not always be directly harmful to us. They can often cause indirect effects to the surrounding by reacting with other compounds in the atmosphere under suitable conditions and concentration. Thus they create secondary pollutants in the environment which can be directly harmful to us. One such example is the formation of smog.

Hence government and environmental agencies of various developed nations have come out with their standard allowable limits for the presence of these VOCs in the atmosphere. One such example of environmental agency is The **American Conference of Governmental Industrial Hygienists** (ACGIH). It has set established the threshold

dose for many the VOCs in air called the Threshold Limit Value (TLV). The TLV refers to the airborne concentrations that correspond to conditions where no adverse effects are normally expected during a worker's life time. TLV was formerly called the maximum allowable concentration. The United States **Occupational Safety and Health Administration** (OSHA) has defined its own threshold dose called a permissible exposure level (PEL). PEl values are not as numerous and are not updated as frequently. The TLV values are often somewhat conservative. The following table shows the TLV and PEL values of some of the VOCs.

Sl. No	Substance	TLV-TWA at 25°C (mg/m^3)	PEL at 25 °C (mg/m^3)
1	Acetaldehyde	180	180
2	Acrolein	0.25	0.25
3	Acrylic Acid	6	------
4	Benzene	30	30
5	Butane	1900	------
6	Chloroform	50	10
7	Formaldehyde	1.5	1.5
8	Isobutyle Alcohol	150	150
9	Methyl Alcohol	260	260
10	Methyl Bromide	20	20
11	Methyl Ethyl Ketone	590	590
12	Nitrobenzene	5	5
13	Octane	1450	1450
14	Phenol	19	19
15	Pyridine	15	15
16	Styrene	215	215
17	Sulfur Dioxide	5	5
18	Toluene	375	375
19	Vinyl Chloride	10	10

20	Xylene	435	435

Table 1.2: List of VOCs with their Threshold Limits Values

1.4 Hetaroaromatic Compounds:

Hetaroaromatic compounds with nitrogen in their structure like pyridine is receiving immense attention now days, due to their presence in environment and since they are toxic and carcinogenic in potential and have hazardous effect on our environment. Furthermore some of the pyridine derivatives are toxic to certain life forms [**Sims and Loughlin 1989; Padoley et al. 2008**]. Two-thirds of organic chemicals known to us contain heterocyclic structures [**Kuhn and Suflita 1989 ; Lettau 1980**]. Heterocyclic aromatic compounds exist naturally in biological systems as electron carriers, nucleotides, energy storage molecules, mycotoxins, and alkaloids. Synthetic heterocyclic aromatic compounds are used as industrial solvents, dyes, explosives, pharmaceuticals, and pesticides. Industries and industrial operations such as the mining industry, coal tar- and oil shale-processing operations, wood-preserving facilities, and chemical manufacturing plants produce a huge amount of waste water during their functioning which contain a lot of hetaroaromatic compounds which can be potentially harmful to life systems. Because of their heterocyclic structure, these chemicals are more soluble in water than their homocyclic analogs and can be more easily transported to groundwater. Though the potential harmful effects that these hetaroaromatic compound can incur upon human and other living beings through contaminated air and water is well known, insufficient research has been conducted upon them to find out their fate in the environment [**Berry et al. 1987; Bollag and Kaiser 1991; Grbic-Galic 1989**]. Therefore, it is important that we gain a clearer understanding of the processes by which they can be eliminated from waste streams of any sort. These hetaroaromatic compounds have also been found to be biodegradable and hence can be treated by biological methods known to us. Hence it becomes important that we garner a clear knowledge of the processes by which they are degraded and the types of microorganisms involved in these degradation processes [**Kaiser et al. 1996**].

Pyridines are rated as priority pollutants by United States Environmental Protection Agency (USEPA) [**Richards and Shieth 1986**]. These compounds can occur naturally in the environment and are also produced in large quantities as a result of

industrial activities as well [**Sims and Loughlin 1989, Richards and Shieth 1986, Fetzner 1998**]. Once the pyridine compounds enter the environment, they can persist for long periods [**Rogers et al. 1985**].

1.5 VOCs Control Techniques:

There are two forms of applicable air emission control. Source control involves the reduction of emissions through raw product substitution, reduction or recycling. However these reduction mechanisms may reduce the quality of the product or may increase the cost. Secondary control involves treatment of the waste gas after it has been produced. The choice of the technology is often dictated by economical and ecological constraints.

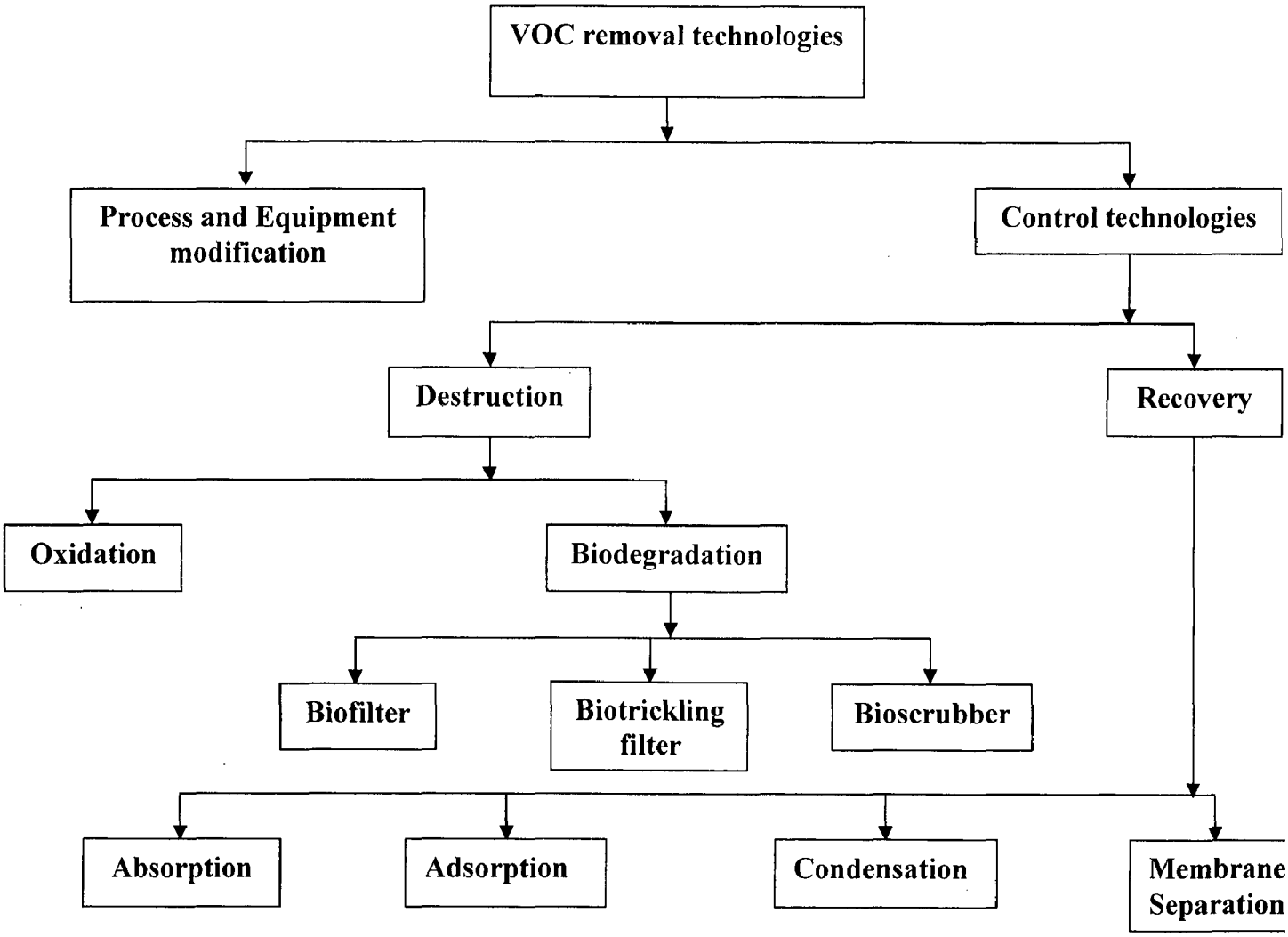


Fig 1.1: Classification of VOC Control Techniques

1.5.1 Absorption:

Absorption removes the waste contaminat with a scrubbing solution. The contaminant enter a large contactor where the gaseous pollutants are transferred to a liquid phase. Efficient gas-liquid mass transfer may be accomplished through the use of packed or bubble column or a venturi contactor. Success is dictated by the affinity of the pollutant for liquid phase. Water is the most frequently used scrubbing solution and the pH can be adjusted to increase the solubility acidic or basic gases. Once the pollutant transfer has occurred, additional treatment of the liquid phase may be necessary. This may be achieved by desorbing the pollutant at high temperatures and incinerating the vapors. If the scrubbing solution is water, the wastewater may be directed to a treatment plant.

1.5.2 Adsorption:

Adsorption generally occurs on a fixed or fluidized bed of material such as active carbon or zeolite and is most efficient for treatment of low concentration vapors and liquids. The effectiveness of an adsorption system for a particular waste stream is function of the air flow rate, the total VOC loading of the stream and the individual components of the VOC stream. Moisture is also one of the crucial parameters to dictate the efficiency and effectiveness of adsorption. Adsorption is generally used for controlling VOCs with low vapor pressure and high molecular weight. Physical adsorption has been found to be more significant in the case of separation processes. Physical adsorption is again classified into Thermal Swing Adsorption (TSA) and Pressure Swing Adsorption (PSA).

1.5.3 Condensation:

Waste contaminants that are concentrated and have a high boiling point may be partially recovered by simultaneous cooling and compressing of the gaseous phase. This technique is only economical for concentrated vapour where there is some recycle or recovery value. If the waste is a mixed pollutant stream, recycling will be virtually impossible and incineration of the condensed liquid may be required. This technique

must often be followed by additional removal technologies for compliance with regulatory emission standards.

1.5.4 Membrane Separation:

Membrane separation system can be used to transfer VOCs from air stream to a water phase. In a membrane separation system, compression and condensation of the emission stream is followed by membrane separation. By compressing the emission stream to approximately 310 to 1400 KPa, a higher vapor pressure can be maintained on the air-feed side than on the permeate side of the membrane. This pressure differential drives the membrane separation process. The compressed mixture can be processed through a condenser where portions of the organic vapors can be recovered. The remaining contaminant stream is then passed across the surface of a microporous hydrophobic membrane constructed of material such as polyethylene and polypropylene. The membrane provides high gas permeability without allowing bulk permeates transport across the membrane. The resulting products are a permeate stream containing the majority of the organic compounds and a stream containing residual organic compounds. The process then requires treatment of the liquid permeate for final VOC disposal or recycling. In this process gas permeation and reverse osmosis are the techniques used solvent recovery. Hydrocarbon mixture, Gasoline etc. can be separated effectively using aromatic poly membrane [**Khan et. al.2000**].

1.5.5 Incineration:

Thermal and catalytic oxidations are widely used and effective treatment process for waste streams. Thermal oxidation involves the combustion of pollutants at temperatures of 700 to 1400 °C. Catalytic oxidation allows process temperature between 300 to 700 °C with catalysts such as platinum, palladium, rubidium etc. Oxidation is the most widely used secondary technique, but costs are high for low concentration pollutant vapors because of the need for large amount of fuel. Regenerative or recuperative heat systems are often used as an attempt to reduce these fuel operating costs. Production of nitrogen oxides and some dioxins is also possible during oxidation. In general this technology is more suitable for concentrated stream with moderate flow rates.

1.5.6 Biodegradation:

Biotechniques are relatively cheap because of the low investment and operational costs and can show a good operational stability, are environment friendly (they lead to the formation of non dangerous products), and require ambient conditions for destruction of pollutants [**Spigno et. al.2003**]. Biological reactors utilize microbial metabolic reaction to treat contaminated stream. Biological treatment is effective and economical for low concentrations of contaminat in large quantities of air. The treatment is carried out at ambient temperature and it does not generate secondary waste stream. In case of gaseous contaminants, the contaminants are sorbed from a gas phase to an aqueous phase where microbial attack occurs. VOC containing air streams are transported to the air/biofilm interface where they are absorbed into the biofilm and employed as carbon or energy source by microorganism [**Mathur et.al.2006**]. These pollutants may be either organic or inorganic and are used as energy and sometimes as carbon source for maintenance and growth by the microorganism population. In general the microbes used for biological treatment are organisms that are naturally occurring. These microbial populations may be dominated by one particular microbial species or may interact with numerous species to attack a particular type of contaminant synergically. Microbes within these biological treatment systems are also engaged in many of the same ecological relationships that are typical to microorganisms. Such relationships are necessary to provide an important balance within the system.

The particular contaminants of interest must be biodegradable and non toxic for biological air treatment to be successful. The most successful removal in gas phase bioreactors occurs for low molecular weight and highly soluble organic compounds with simple bond structures. Compounds with complex bond structures generally require more energy to be degraded and this energy is not always available to the microbes. Hence little or no biodegradation of these compounds occur. Instead, microbes degrade those compounds that are readily available and easy to degrade. Organic compounds such as alcohols, aldehydes, ketones and some simple aromatics demonstrate excellent biodegradability. Some compounds that show moderate to slow degradation include phenols, chlorinated hydrocarbons, polyaromatic hydrocarbons and highly halogenated

hydrocarbons. Inorganic compounds such as hydrogen sulfide and ammonia are also biodegraded well.

Biotrickling Filters:

In biotrickling filter gas contaminants are absorbed in a free liquid phase prior to biodegradation by either suspended or immobilized microorganisms. For biotrickling filters suspended microbes fixed to an inorganic packing material and suspended microbes in the water phase degrade the absorbed contaminants as they pass through the reactor. Biotrickling filters operate with the air and water phases moving either counter-currently or co-currently, depending upon the specific operation. As the water is recirculated, nutrients, acids or bases may be added by the operator to regulate the environment for optimal pollutant removal. Biotrickling filters are governed by many of the same phenomena as biofilters. Most importantly a biotrickling filter reactor must host a thriving microbiological population while avoiding conditions that promote excessive biomass growth and clogging conditions. Various types of packing materials have been used in biotrickling filters. They are random dump plastic packing, lava rock, structured packing and open pore polyurethane foam. The high porosity of these packing causes less head loss compared to that of biofilters with organic packing though biotrickling filters are operated at higher gas velocity.

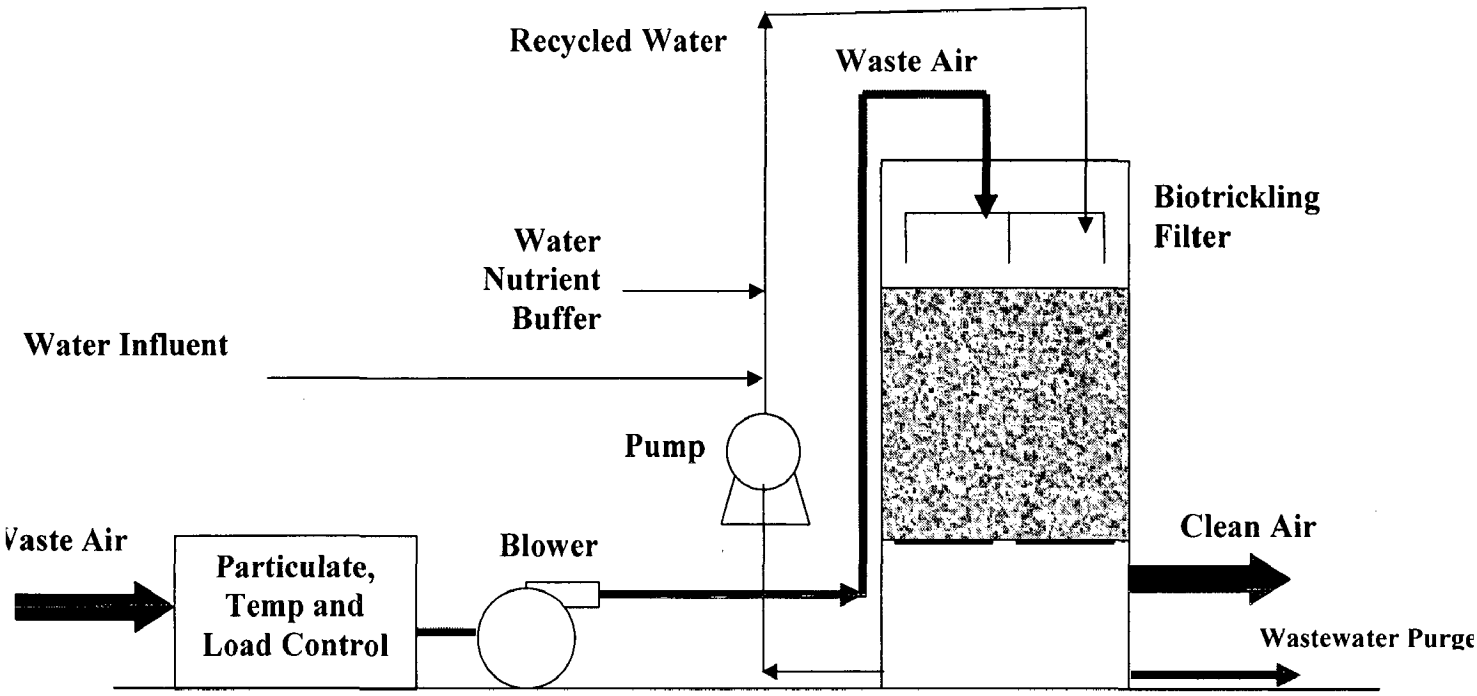


Fig 1.2: Schematic Diagram of Biotrickling Filter

1.6 Objective of Present Work:

It is well known that pyridine is a substance with extensive use in industry and laboratory experimental purpose. Pyridine and its derivative come out of these establishments in quite significant amount as gaseous and liquid pollutant. No work has been found in literature where pyridine adsorption was studied in a continuous gas phase system. Also no work was found on biological treatment of pyridine using *Bacillus Subtilis* in either liquid or gas phase. Based on extensive literature survey on adsorptive and biological treatments carried on pyridine Soybean and puffed rice has been selected as the adsorbents for carrying out adsorption study on continuous gas phase removal of pyridine and following aim and objectives have been fixed for the present work:

1. Various characteristics of the adsorbents are to be determined to characterize the adsorbents.
2. Adsorption of pyridine on continuous gas phase system is to be carried out using soybean and puffed rice in column reactor

3. Two process parameters namely inlet concentration of pyridine and flow rate of inlet gas stream is to be varied to find out their effect on adsorption of pyridine.
4. The data obtained by the experiments are to be validated using Yoon and Nelson model for adsorption of gaseous pollutants in continuous reactors.
5. The effect of temperature and initial pH on biodegradation of pyridine by *Bacillus Subtilis* is to be studied to optimize the biodegradation process.
6. Study is to be carried on biodegradation of pyridine by *bacillus Subtilis* for various concentrations of pyridine and the data thus obtained is to be validated to find out the growth kinetics which is best suited for the degradation process.
7. Continuous phase biodegradation of gaseous pyridine using *bacillus Subtilis* is to be carried out in trickling bed biofilter using soybean as the packing material.

PYRIDINE AND ITS CHARACTERISTICS

2.1 Pyridine:

Pyridine (Py) is a volatile, toxic and flammable compound with a pungent and unpleasant odour. Pyridine is the parent of a series of chemicals like picoline, quinoline, alkyl pyridine etc. It is a solvent used in paint and rubber industry. It is also used as an intermediate in making insecticides and herbicides for agricultural applications and in research laboratories for functions such as extracting plant hormones. It is also used directly in the denaturation of alcohol. Pyridine has a huge application in various industries where it is used extensively to make many different products such as medicines, vitamins, food flavorings, dyes, adhesives, and in water proofing for fabrics **[Kirk and Othmer, 1982; Yates, 1984; Kumar et al., 1995; Mall et al., 2003; Lataye et al., 2006]**. Industrial wastewater containing pyridine and its derivatives possess toxic threat to aquatic life and create nuisance because of their malodorous and pungent smell **[Lataye et al., 2006]**. Py is a colourless liquid with penetrating, empyresmatic odour. It mixes very easily with water and is also soluble in alcohol, ether, and benzene. It is a fire and explosion hazard in the form of vapour, when exposed to flame or spark. When heated to decomposition, it emits highly toxic vapours of NO_x in an oxidative atmosphere. It has very high half-life when released in the atmosphere. It is also mildly toxic by inhalation; its vapour is skin and severe eye irritant and exposure to it can cause depression, gastrointestinal upset, liver and kidney damage, headache, nervousness, dizziness, insomnia, nausea, anorexia, frequent urination and dermatitis **[Lewis, 2004]**.

Wastewaters containing pyridine or its derivatives is generated from pyridine manufacturing units, pharmaceutical units etc. The typical concentration of Py in wastewaters produced in a multidrug intermediates-product plant manufacturing Py and its derivatives and other products is in the range of 20-300 mg/l. During emergency spills, the concentration may be as high as 600-1000 mg/l. At a Py concentration of 0.82 mg/l in wastewaters, unpleasant odor is detectable **[Baker, 1963]**. However, the vapor (odor) concentration may be as low as 7 μg/l, while the threshold odor concentration can be ~0.3 μg/l. Pyridine does not have any BOD or COD. Although no concentration limit has been

prescribed in the industrial wastewaters for their safe discharge into sewers or on land, it is imperative that the wastewater should not have more than 1 mg/l of Py, so as to minimize the toxicity of wastewater and reduce the odor emanating from it. The plants related to pyridine are plagued with the problem of intense odour emanating either from the wastewater or from the handling and storage facilities. In a typical plant producing pyridine and its derivatives and other associated chemicals, wastewaters are concentrated in evaporators and then incinerated. This method of treatment is capital-intensive and requires fuel to be used to effect evaporation, concentration and incineration. Any problem arising in the incineration system forces the plant to store the concentrated wastewater, as no other alternative treatment system, generally, exists in the plant. Removal of pyridine from the wastewater stream is therefore of vital importance.

S. No.	Property	Pyridine
1	Synonyms	Azabenzene, Azine, Piridina, etc.
2	Chemical Formula	C_5H_5N
3	Molecular Weight	79.11
4	Physical Properties	Colourless liquid, sharp penetrating empyeumatic odor, burning taste
5	Density (g cm^{-3})	0.98
6	Boiling Point (°C)	115.3
7	Freezing Point (°C)	-42
8	Flash Point (°C)	68
9	Soluble in	water alcohol, ether
10	IDLH (Immediately Dangerous to Life or Health), mg dm^{-3}	1000
11	OSHA PEL (mg dm^{-3})	TWA 5
12	ACGIH TLV (mg dm^{-3})	TWA 5 (Proposed: TWA 1 mg dm^{-3} Confirmed Animal Carcinogen)
13	UN Hazard Class	3

14	Safety Profile	Poison by intraperitorial route, moderately toxic by ingestion, skin contact, intravenous and subcutaneous routes. Mildly toxic by inhalation, skin and severe eye irritant
15	Health Effects	Can cause CNS depression, gastrointestinal upset, and liver and kidney damage

Table 2.1: Properties of Pyridine

2.2 Sources of Pyridine:

a. Natural sources:

Pyridine ring is inherently present in many natural compounds. They are found most commonly in organic compounds which are important for performing important biological functions. Pyridine and its derivatives exist as electron carriers (NADP/ NADPH), flavin nucleotides (FAD/ FADH), as constituents of RNA and DNA and in energy storage molecules like ATP and GTP. Pyridine and its derivatives can also be found in various food products and in certain vegetables, like artichokes, cocoa and coffee [**Buttery et al. 1978, Vizthum et al. 1975, Stuerner et al. 1982**].

b. Anthropogenic sources:

Various industries such as pharmaceuticals, dyes, industrial solvent manufacturing, pesticides coal processing, chemical manufacturing etc use heterocyclic compounds with nitrogen source as a very important constituent in the processes involved. These industries produce a lot pyridine and its other derivatives as waste. Table 2.2 shows the various industries, industrial process sources for generating pyridine as a waste.

Pharmaceutical industries manufacture various products like Sulphamethoxazole, Isoanizide (a therapeutic agent used in the treatment of tuberculosis), cetylpyridine

(antiseptic), coramine (respiratory stimulant) (nicotinic acid, N,N-dimethyl amide), a- & b-eucaine (local anesthetic agents) and Demerol (analgesic) etc. Processes which are used to produce these important and valuable medicines generate wastewater containing appreciable concentration of pyridine and its derivatives [**Sims and Loughlin 1989, Fetzner 1998, Sims et al. 1986**]. Coal tar, coke and tar-based chemicals are the major products that are produced in coal processing industry. Production of these products involves use of following processes- coal carbonization, coal gassification and coal liquification. Coal carbonisation leads to the generation of wastewater containing pyridine [**Fetzner 1998, Stuerner et al. 1982, Pereira et al. 1983**]. Shale oil production generates almost equal quantity of wastewater containing pyridine and its derivatives [**Fetzner 1998, Steven and Robert 1984, Dobson et al. 1985**]. Dye manufacturing industry and chemical manufacturing industries are the other major sources of pyridine discharge. Dye is known to produce a very high amount of highly toxic and odorous waste discharge which is very injurious to human health.

Reference	Industries	Source of pyridine formation
Fetzner (1998) [23], Sims and Loughlin (1989)[77], Sims and Sommers (1986) [76]	Pharmaceutical Industry	Cetyl pyridine (anticeptic), Isoanizide (antituberculosis), Coramine (nicotinic acid, N,N-dimethyl amide)
Fetzner (1998) [23], Stuerner and Morris (1982)[81]	Coal processing	Coal carbonization, Coal gasification, Coal liquification
Steven and Robert (1984) [79], Sims and Loughlin (1989)[77], Lee et al. (1994)[45]	Shale oil processing	Retorting of oil shale to release product oil
Sims and Loughlin (1989)[77], Fetzner (1998)[23]	Pesticide	Picloram, diquat, paraquat, fluridone, nitrapyrin
Sims and Loughlin	Dye	Fabric, cyamoalphyes of clinical use

(1989)[77]		
Sims and Loughlin (1989)[77]	Chemical manufacturing industries	Nitrogenous compounds, Water repellent, Detergent, Corrosion inhibitor

Table 2.2: Various industrial and process sources of Pyridine

2.3 Physical properties of Pyridine:

Pyridine structure comprises of a stable, cyclic, 6-π-electron, π-deficient, aromatic structure containing a ring nitrogen atom. This ring nitrogen posses more electro negativity than the ring carbons, making the two-, four- and six-ring carbons more electropositive than otherwise would be expected from knowledge of benzene chemistry. The lone pair of electrons on the nitrogen atom does not need to take part in the process due to the 6π electron structure; hence the terms weakly basic and π-deficient are used to describe pyridine compounds. Pyridine has a boiling point 35.2 °C higher than benzene (115.3 vs. 80.1 °C), and unlike benzene, it is miscible with water in all proportions at ambient temperatures. The higher boiling point and water solubility of pyridine is attributed to the fact that it has a much higher dipole moment in comparison to benzene. Benzene and pyridine are aromatic compounds having resonance energies of similar magnitude, and both are miscible with most other organic solvents. Pyridine is a weak base (pea = 5.22), being both an electron-pair donor and proton acceptor, whereas benzene has little tendency to donate electron pairs or accept protons. Pyridine is less basic than aliphatic, tertiary amines. Table 2.1 shows the properties of Pyridine.

2.4 Harmful Effects of Pyridine:

According to Agency for Toxic Substances and Dieses Registry ToxFAQ (ATSDR) pyridine has CAS no 110-86-1. Everyone is exposed to very low levels o pyridine in air, water, and food. Workers who make or use the chemical may be exposed to higher levels of it. Studies in people and animals suggest that pyridine may damage th

liver. This chemical has been found in at least 11 of 1,416 National Priorities List sites identified by the Environmental Protection Agency. In the air, it may take several months or years until it breaks down into other compounds. In water or soil, it may be broken down in a few days to a few months by microscopic organisms. Pyridine sticks to soil particles.

Everyone is exposed to very low levels of pyridine in air, water, and food. Workers may be exposed in industries that make pyridine or use it to make other products by breathing it in air or by touching it. People may breathe pyridine when it is released into the air from burning cigarettes and from hot coffee. People who live near hazardous waste sites or landfills where pyridine exists may be exposed to it by breathing contaminated air or by drinking contaminated water. People who live near hazardous waste sites or landfills where pyridine exists may be exposed to it by breathing contaminated air or by drinking contaminated water. Very little information is available on the health effects of pyridine. Animal studies and some limited case reports in people have noted liver damage from exposure to pyridine. Harmful effects to the liver were seen in rats and mice that were given pyridine for three months. Headaches, giddiness, a desire to sleep, quickening of the pulse, and rapid breathing occurred in adults who breathed an unknown amount of pyridine for an unknown length of time. Mild skin irritation and eye irritation were seen in rabbits when pyridine was placed on their skin or in their eyes. We do not know whether pyridine affects the ability of men and women to have children or whether it causes birth defects. The Department of Health and Human Services, the International Agency for Research on Cancer, and the Environmental Protection Agency (EPA) have not classified pyridine as to its human carcinogenicity.

2.5 Removal techniques of pyridine:

Industrial waste discharge can be treated in two ways: (a) primary control at the source and (b) secondary control. Secondary control involves treatment of the wastewater after its generation. Different physical, chemical and biological methods are available for treatment of wastewaters containing pyridine.

2.5.1 Physical and chemical techniques:

The various physical methods are available for treatment of pyridine in wastewater discharges from various sources. Available methods are thermal catalytic incineration, deep well injection, soil percolation, ultrafiltration, chemical coagulation and adsorption on various materials. Various chemical methods are also available for the treatment of pyridine containing waste stream, which are based on UV/ ozone gas scrubbing, chemical/ photochemical oxidation and wet oxidation etc. Advanced oxidation processes (AOPs) are another major group of chemical treatment processes used for treatment of heterocyclic compounds like pyridine. The AOPs are associated with generation of highly reactive radical intermediates, mainly the hydroxyl radicals [**Prousek 1996**]. The active hydroxyl oxidants are considered to be capable of decomposing almost all types of organic contaminants; AOPs are receiving a lot of attention from researchers as promising technologies for degrading toxic and hazardous wastewater. The advanced oxidation processes can be divided into two major categories viz. photo oxidation and wet-oxidation [**Padoley et al. 2008**].

2.5.2 Biological methods:

An attractive method to eliminate pyridine is biodegradation in which the organic compound is essentially converted to harmless compounds such as CO_2, H_20, and NH^{2-} etc. In literature different aerobic and anaerobic microorganisms have been reported for degradation of pyridine. This biological technology is also a desirable method for the cleaning up of subsurface and ground water, when they are contaminated by pyridine or its derivatives as pyridine degrading microorganisms have been found to grow in this place too[**Thomas and Ward 1989**]. Immobilized phase degradation experiments have yielded much more improved results than suspended phase ones [**Bettmann and Rehm 1985, Westmeier and Rehm 1987, Hallas et al. 1992, Ferschl et al. 1991, Rhee et al. 1996**]. In case of derivatives of pyridine, the nature and position of ring substituents affect the biodegradation [**Sims et al. 1986, Sims and Sommers 1985, Naik et al. 1972**]. According to literature reports degradation of unsubstituted pyridine occurs within 8 days in soil suspensions, where as hydroxypyridines require 32–64 days and pyridine carboxylic acids are reported to be most labile group susceptible for aerobic degradation usually getting degraded in 4–16 days in soil suspensions [**Sims and Somers 1985**].

ADSORPTION AND BIODEGRAFDATION FUNDAMENTALS

3.1 Adsorption:

Adsorption is a surface phenomenon. It is used as a separation process where a species present in a fluid phase is transferred to the solid phase and gets attached to the solid surface, if the concentration of the species in the fluid-solid boundary region is higher than that in the bulk of the fluid [**Tien, 1994**]. In an adsorption process, molecules or atoms or ions in the fluid phase get concentrated or accumulated on the surface of a solid, where they bond with the solid surface or are held there by weak inter-molecular forces. The accumulated or concentrated species on the surface of solid is called the adsorbate, and the porous solid material is known as an adsorbent.

3.1.1 Adsorption Diffusion Study:

Studies carried out by **Boyd et al. [1947]** and **Reichenberg [1953]** with regard to relation between adsorption, the particle, and film diffusion etc have created the foundations of sorption kinetics. The rate of adsorption in kind of adsorption process is influenced heavily by the mass transfer between solute or adsorbate and adsorbent particle. Hence it becomes absolutely essential to study the rate at which the solute is removed from aqueous solution, i.e. gets adsorbed by the adsorbent, in order to apply adsorption by solid particles to industrial uses. It is also necessary to identify the step that governs the overall removal rate in the adsorption process in order to interpret the experimental data. There are essentially four steps in the adsorption of a solute from the bulk of fluid to the adsorbent.

1. From the bulk of the fluid solute is transported to the external film of fluid surrounding the adsorbent particle(assumed to be very fast in agitated systems)
2. Then the adsorbate diffuses from external bulk fluid layer adjacent to the adsorbent to the external surface of the adsorbent particle (film diffusion;

the resistance could be neglected for properly mixed/agitated vessels)

3. Adsorbate is diffused gradually from the poremouth of the adsorbent particle through the pores to the immediate vicinity of the internal adsorbent surface (pore, surface and molecular diffusion)
4. Finally the adsorbate is adsorbed onto the interior surface of the adsorbent.

In the overall sorption of the solute from the bulk fluid phase to the internal surface of an adsorbent al the above mentioned steps play a very key role. The transport resistance of the adsorbate from the bulk phase to the exterior film surrounding the adsorbent may be small and can be neglected. In addition, the adsorption of adsorbate at surface sites (step 4) is usually very rapid, and thus offers negligible resistance in comparison to other steps, i.e. steps 2 and 3. Thus, these processes usually are not considered to be the rate-limiting steps in the sorption process.

In most cases, steps (2) and (3) may control the sorption phenomena. For the remaining two steps in the overall adsorbate transport, three distinct cases may occur:

Case I: external transport resistance > internal transport resistance

Case II: external transport resistance < internal transport resistance

Case III: external transport resistance ≈ internal transport resistance

In cases I and II, the overall rate is governed by the film diffusion and diffusion in the pores, respectively. In case III, the transport of solute to the boundary may not be possible at a significant rate, thereby, leading to the formation of a liquid film with a concentration gradient surrounding the adsorbent particles.

3.1.2 Factors Controlling Adsorption:

There are number of factors that control the adsorption process from bulk of fluid phase to the adsorbent phase. These controlling factors are-

3.1.2.1 Nature of Adsorbent:

The adsorption capacity of an adsorbent depends upon its physicochemical characteristics, specific surface area and its affinity for the adsorbate. Adsorption capacity is directly proportional to the exposed surface of the adsorbent. For the non-

porous adsorbents, the adsorption capacity is inversely proportional to the particle diameter whereas for porous material it is practically independent of particle size. However, for porous substances particle size affects the rate of adsorption. For substances like GAC, the breaking of large particles to form smaller ones open up previously sealed channels making more surface accessible to the adsorbent. Pore sizes are classified in accordance with the classification adopted by the International Union of Pure and Applied Chemistry (IUPAC) [IUPAC, 1982], that is, micro-pores (diameter (d) < 20 Å), meso-pores (20 Å < d < 500 Å) and macro-pores (d > 500 Å). Micro-pores can be further divided into ultra-micropores (d < 7 Å) and super micro-pores (7 Å < d < 20 Å). For liquids, the adsorption of organic molecular would generally be facilitated by mesopores, which effect faster intra-particle migration of the adsorbate.

3.1.2.2 Adsorbent dosage:

As the adsorbent dose icreases the removal of adsorbate also increases rapidly. An increase in the magnitude of sorption with an increase in the adsorbent dose can be attributed to the increase in the mesoporous surface area available for sorption, and hence, the availability of more adsorption sites. However, the unit adsorption decreases with an increase in dosage. The decrease in sorption capacity per unit weight of adsorbent is because of the fact that an increase in the sorbent dose at a constant concentration and volume leads to the saturation of sorption sites through the sorption process [**Yu et al., 2003; Lataye et al., 2006**]. Also, particle-particle interaction such as aggregation at higher *m* leads to a decrease in the total surface area of the sorbent and an increase in the diffusional path length [**Shukla et al., 2002, Ozacar 2005**]. The incremental uptake of adsorbate is very small after a particular dose, as the surface concentration and the bulk solution concentration of adsorbate come to equilibrium to each other.

3.1.2.3 pH of Solution:

The surface charge as well as the degree of ionization is affected by the pH of the solution. Since the hydrogen and hydroxyl ions adsorb readily on the adsorbent surface, the adsorption of other molecules and ions is strongly affected by pH. Generally a surface adsorbs anions favourably at low pH and cations at high pH.

3.1.2.4 Contact Time:

In physical adsorption, most of the adsorbate species are adsorbed within a short interval of contact time. However, strong chemical binding of adsorbate with adsorbent requires a longer contact time for the attainment of equilibrium. Available adsorption results reveal that the uptake of adsorbate species is fast at the initial stages of the contact period, and thereafter, it becomes slower near the equilibrium. In between these two stages of the uptake, the rate of adsorption is found to be nearly constant. This may be due to the fact that a large number of active surface sites are available for adsorption at initial stages and the rate of adsorption is a function of available vacant site. During the course of adsorption, the concentration of the available vacant sites decreases and the repulsion between solute molecules on the surface and solution increases thereby reducing the adsorption rate.

3.1.2.5 Initial Concentration of Adsorbate:

A given mass of adsorbent can adsorb only a fixed amount of adsorbate. So the initial concentration of the adsorbate in the solution is very important. The amount adsorbed decreases with an increase in the adsorbate concentration as the resistance to the uptake of solute from the solution decreases with an increase in the solute concentration. Therefore, the rate of adsorption increases because of the increasing driving force.

3.1.2.6 Temperature:

Temperature dependence of adsorption is of complex nature. Adsorption processes are generally exothermic in nature and the extent and rate of adsorption in most cases decreases with increasing temperature. This trend may be explained on the basis of rapid increase in the rate of desorption or alternatively explained on the basis of Le-Chatelier's principle.

Some of the adsorption studies show increased adsorption with increasing temperature. This increase in adsorption is mainly due to an increase in number of adsorption sites caused by breaking of some of the internal bonds near the edge of the

active surface sites of the adsorbent. Also, if the adsorption process is controlled by the diffusion process (intraparticle transport-pore diffusion), then the sorption capacity increases with an increase in temperature due to endothermicity of the diffusion process. An increase in temperature results in an increased mobility of the ions and a decrease in the retarding forces acting on the diffusing ions. These result in the enhancement in the sorption capacity of the adsorbents.

3.1.3 Model of Yoon and Nelson:

A model which can be used to analyze breakthrough curves in continuous adsorption process has been developed by Yoon and Nelson [Yoon and Nelson, 1984] and has been found to be used effectively in literature [note, 1999]. Breakthrough curve can be described as follows using this model with an applicable approximation to fit the process of adsorption in gas phase. If a fixed bed with adsorbent packing is considered where gas flows continuously, the adsorption kinetics of the bed would be such that some amount of adsorbate will get adsorbed on active sites of adsorbent material when it comes in contact, whereas the remaining would pass as unadsorbed. P as probability of breakthrough and Q as probability of adsorption are two parameters introduced by Yoon and Nelson. The relationship between them is considered as follows-

$$P = 1-Q = C_b/C_i \qquad [3.1]$$

Where,

C_b is the effluent breakthrough concentration of adsorbate

C_i is the influent initial concentration of adsorbate

As it is known that rate of adsorption is proportional to rate of molecular collisions with unoccupied adsorption sites [A. W. Adamson, 1990] it is reasonably assumed that the rate of change in the breakthrough concentration, dC_b/dt, is proportional to C_b and to the number of adsorptive sites. Hence from the above understanding one can obtain that

$$-dQ/dt = k'Q\,(1-Q) \qquad [3.2]$$

Where;

k' is the Rate constant ($time^{-1}$)

Equation 2 is the differential form, which can be simplified by integration to obtain more explicit form. The equation will thus appear of the form

$$\ln (Q/ (1-Q)) = k' (\tau - t) \qquad [3.3]$$

where;

τ is the time needed to obtain 50% breakthrough (ie Q= 0.5)

t is the breakthrough time

By replacing equation 1 in equation 3 and by further simplifying the following linear expression has been derived for breakthrough time (t) which would in terms of initial influent concentration (C_i) and breakthrough concentration (C_b). the expression will be of the form:

$$t = \tau + (1/k') \ln(C_b/ (C_i-C_b)) \qquad [3.4]$$

As per equation 4 value of k' can be obtain by ploting a graph between t and $\ln(C_b/ (C_i-C_b))$ and by calculating the slope of the straight line thus obtained. Value of τ which represent 50% breakthrough time can also be calculated from this equation once k' value is known by determining the time needed to reach Q= 0.5. After calculating values of k; and τ, Yoon and Nelson introduced another parameter which can be calculated from this model. This can be found from the following relationship:

$$k = k' \tau \qquad [3.5]$$

where;

k is the proportionality constant for given adsorbate and adsorbent.

Yoon and nelson reported that k is independent of adsorbate concentration and flow rate. On the other hand though both k' and τ are dependent on the concentration of adsorbate and the flow rate.

3.2 Biodegradation:

Biological reactors utilize microbial metabolic reaction to treat contaminated waste stream. Biological treatment is effective and economical for low concentrations of contaminat in large quantities of air or water waste stream. The treatment is carried out at ambient temperature and it does not generate secondary waste stream. The contaminants are sorbed from a gas phase to an aqueous phase in case of gas phase biotreatment, where microbial attack occurs. VOC containing air streams are transported to the air/biofilm interface where they are absorbed into the biofilm and employed as carbon or energy source by microorganism [**Mathur et.al.2006**]. Through oxidative and occasionally reductive reactions, the by products are generated. The by-products of microbial oxidation are primary water, carbon dioxide (in case of gaseous pollutants), mineral salts, some volatile organic compound and microbial biomass [**Nicolai et al.2006**]. The most successful removal in gas phase bioreactors occurs for low molecular weight and highly soluble organic compounds with simple bond structures. Compounds with complex bond structures generally require more energy to be degraded and this energy is not always available to the microbes. Hence little or no biodegradation of these compounds occur. Instead, microbes degrade those compounds that are readily available and easy to degrade.

In aerobic environments, the pyridine compounds normally get activated by hydroxylations, and metabolised to formic acid. Hydroxylation is initiated at a position adjacent to the N-hetero atom and this is true for pyridine and derivatives. The incorporation of oxygen is generally derived from water, and a number of molybdenum-containing hydroxylases catalysing these hydroxylation reactions have been characterized thoroughly [**Romao and Huber 1998, Hille 1996**]. The pyridine ring is electron-deficient at ortho(2) and para(4) position. As a consequence the ring is more electronegative at these positions and thus resists electrophilic attack and thus favours nucleophilic attack. This characteristic property is exploited by microorganisms for the degradation of pyridines [**Sims and Loughlin 1989**]. However, the nature and position of the ring substituents alter the pattern of degradation. Two general strategies of ring metabolism reported in most of the studies are (i) the pathway involves initial hydroxylation of the ring and (ii) ring reduction or fission not involving hydroxylation.

Though the mechanism of pyridine degradation involving initial hydroxylation is not clearly demonstrated, several workers have reported initial hydroxylation of the pyridine ring as the mechanism of biodegradation, for example 2-hydroxypyridine [**Cain et al. 1974, Gupta and Shukla 1975**], 3-hydroxypyridine [**Cain et al. 1974, Khanna and Shukla 1977**]. The intermediate products that are being formed during aerobic degradation of pyridine as well as its derivatives are reported to be dihydropyridine, trihydropyridine and maleamic acid and the –OH group for the hydroxylation is derived from water and not from O_2 and this was demonstrated using $_{18}O$ labelled water and O_2 [**Hunt et al. 1958**]. The second pathway involving aerobic reduction is not initiated by hydroxylation [**Sims and Loughlin 1989**].

There are number of microorganisms present in the nature which can treat or degrade pyridine and its derivatives in anaerobic conditions also with very high efficiency. The anerobic microbial degradation of pyridine is however, an important area of research and deserves significance [**Padoley et al. 2008**]. By definition when the demand of oxygen in a certain environment is more than its availability, the environment is termed as anaerobic which means that there is shortfall between supply and demand of O_2. This is a common phenomenon in many natural environments like flooded soils, sediments, landfills, lagoons and some groundwaters [**Berry et al. 1987**]. In anaerobic environments, it is the availability of electron donors and acceptors that play a very crucial role in microbial activity and diversity. Heterocyclic aromatic nucleus can be metabolized by microorganisms under anaerobic conditions.

3.2.1 Factors controlling Biodegradation:

Many physico-chemical and operational factors influence performance, treatment costs and stability of microorganism in biodegradation process. In general, three most important parameters for an efficient biodegradation are medium moisture content (for continuous process), pH, and temperature. Other factors are also important but they influence medium lifetime or removal performance to a lesser extent than do these three factors. The fundamental means of treatment in biodegradation is the action of pollutant degrading microorganisms.

3.2.1.1 Water content of medium:

The amount of water in a biofilters is perhaps the most important parameter under the control of the operator. Neglect of the water content or difficulties in controlling it are the most common cause of poor biofilter operation. Microorganism cannot be active without it. Its presence affects the transfer of contaminant from the air and the physical properties of the medium. When water is first circulated in the media, before the surface is covered water activity is primarily controlled by the strength of attraction of the water molecules to adsorption sites on the bare surface. The amount of water required to complete a monolayer of water on media surface varies with the surface area of the medium. Microorganisms cannot survive where the soil suction exceeds their tolerance limits. Even within the range where they can survive, a greater suction means they must expend greater energy to draw water into cell, and growth will be reduced. The most biologically relevant parameter describing water content is the chemical potential because it is direct measure of hoe much energy the organisms must expend to collect water. Unlike fixed reactors used for water treatment gas phase reactors may include a film of standing water outside the biofilm. This could be the case if the water content of the biofilter is increasing, so that the thickness of the water film is growing faster than the thickness of the biofilm. Microorganisms can swim in this water and biofilters can maintain communities of free-swimming organisms which are not seen in other reactors.

3.2.1.2 Temperature:

Microbial activity and biodegradation success are strongly influenced by temperature. A microorganism is a tiny bag of chemicals and enzymes and life is a myriad of chemical reactions that run faster as the temperature rises. Successful microorganisms must coordinate these reactions. If some run ahead of others, excess and shortage of compounds will develop, threatening the organism. Each species is adapted to control its reactions within a certain temperature range, synthesizing and activating enzymes as needed to maintain control. Most reaction rates approximately double when the temperature raises 10°C and microbial activity will increase proportionately through the range in which the coordination of the reactions can be maintained. High temperature may make some reactions occur so rapidly that metabolic coordination is disrupted.

Enzymes are made of proteins. Each enzyme has a temperature limit beyond which it is denatured and is no longer effective. If temperatures decline the metabolism of the cell will slow, reducing the rate at which treatment proceeds. The combination of these factors produces a temperature activity curve for a given cell which typically rises with temperature to a limiting value then falls rapidly. The effects of temperature are strongly dependent on time in a continuous reactor like biofilter. A biofilter which operates continuously at high temperature (say 60°C) may develop a healthy community of active thermophiles which will rapidly degrade the slower but still support a healthy community of microorganisms which can make the system successful. While a warmer biofilter generally support more active organisms, the physico-chemical effects of higher temperatures are usually unfavorable.

3.2.1.3 pH:

The effects of pH and temperature on success of biodegradation process are analogous in several ways. Each species of microorganism is most successful over a certain range of pH and will be inhibited or killed if conditions move outside this range. The ranges for different species may be narrow or broad. Some species do well at high pH and some at low pH but species tolerant of moderate pH are probably more common. Rapid changes in pH are damaging to most species. Microbial ecosystems, however adapt to slow changes in pH: species tolerant of the new conditions replace those which are not. Most bioreactors are designed for operation near pH 7. This is generally accepted as a benign condition and designers are most familiar with it. The *a priori* assumption that a given compound is best treated at pH 7 is commonly chosen as a conservative guess, but it is often not supported by actual data.

3.2.1.4 Nutrient:

The microorganisms in biodegradation process consume contaminants for the energy and carbon they provide; however they also need mineral nutrients: nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, sodium, iron and many others. Some species may require special compounds, such as vitamins, that they cannot synthesize for themselves. Successful biodegradation process requires that the needed nutrients be

provided in the form and quantities that will support vigorous microbial activity. The nutrient needs of microorganisms are similar to those of plants, so compounds and elements are released during degradation in the approximate proportions appropriate for cell growth. However, it is possible that the rate of degradation and therefore the rate at which suitable nutrients are generated can be too slow. Inorganic media such as rock, activated carbon, plastic shapes or polyurethane foam when used in continuous biodegradation processes as packing, do not contain an appropriate supply of nutrients. These must be added when reactor is put into operation. Ideally a bioreactor has stationary water phase and a steady state microbial eco-system, so that it might be expected that the nutrient content would be maintained and continually recycled. Degradation of the biomass releases the nutrient in soluble form, where they can be taken up again by growing cells.

3.2.1.5 Contaminant concentration:

It is the concentration of substance to be degraded present in the system. In batch studies the concentration of pollutant is varied at the very beginning of the process and the effect of this initial contaminant concentration is monitored throughout. The mass of contaminant entering the bioreactor per unit time and per unit volume is the contaminant load. It has major effects on biodegradation and it is important to interpret the results of experiments and operational experience in light of the load being treated. Biofilter employed for odor control purposes, where concentrations are typically a few parts per billion, may treat less than 1 $gm^{-3}h^{-1}$ of contaminant. At the other extreme, bioreactors treating industrial effluents or soil vapor extraction off-gases may see concentrations in the thousands of parts per million and load above 100 $gm^{-3}h^{-1}$ of contaminant. Removal efficiency may be excellent when loads are low but poor when loads are high.

3.2.1.6: Oxygen limitation:

Oxygen limitation is a major issue in biodegradation process in batch as well as continuous process systems. In batch process the system is kept air tight to keep it free of contamination. This sometimes lead to shortage of oxygen in the flasks. In high performance biofilters, oxygen limitation may occur in the biofilm. Note that the term

"oxygen limitation" does not necessarily imply that oxygen is completely depleted in the biofilm, but refers t the general situation where the rate of biodegradation is affected by the concentration of oxygen. At first, the existence of oxygen limitation in an air biofilter might sound contradictory because air contains 21% oxygen. However the reason for oxygen limitation is that the oxygen gas-liquid partition coefficient is 33.5, meaning that most of the oxygen is in gas phase rather than dissolved.

3.2.2 Monod Growth Kinetics:

If concentration of one essential medium constituent is varied while the concentrations of all other medium components are kept constant, the growth rate typically changes in a hyperbolic way. A functional relationship between the specific growth rate μ and an essential compound's concentration was proposed by Monod in 1942. of the same form as the Langmuir adsorption isotherm and the standard rate equation for enzyme catalysed reaction with a single substrate (Michelis-Menten, 1913), the Monod equation states that

$$\mu = \frac{\mu_{max} S}{K_S + S}$$

Here μ_{max} is the maximum growth rate achievable $s >> K_s$ and the concentrations of all other essential nutrients are changed. K_s is that value of the limiting nutrient concentration at which the specific growth rate is half its maximum value, roughly speaking, it is the division between the lower concentration range, where μ is strongly (linerly) dependent on s, and the higher range, where μ becomes independent of s.

3.2.3 Biofilter Terminology:

To describe mechanisms of biofiltration clearly general terminology pertinent to the field should be well defined. There are certain terms in the field of biofiltration that deserve special mention because of their common and sometimes confusing use within biofilter publication and presentation.

Empty Bed Residence Time:

The term "empty bed residence time" and "empty bed detention time" relates the flow rate to the size of the biofilter. It is defined as the empty bed filter volume divided by the air flow:

$$\mathbf{EBRT} = \frac{\mathbf{V_f}}{\mathbf{Q}}$$

Where **EBRT** = Empty bed residence time (Seconds minutes)

$\mathbf{V_f}$ = Filter Bed Volume (m^3, ft^3)

Q = Air flow rate ($m^3\ h^{-1}$)

Mass Loading Rate:

The mass loading rate (either surface or volumetric) is the mass of the contaminant entering the biofilter per unit area or volume of filter material per unit time, often expressed as grams per m^2 or m^3 of filter material per hour. Because flow remains constant through a filter bed, the mass loading along the length of the bed will decline as contaminant is removed. However, generally an overall mass loading rate for a system is defined as:

$$\textbf{Mass loading (Surface)} = \frac{\mathbf{Q} * \mathbf{C_{Gi}}}{\mathbf{A}}$$

Where $\mathbf{C_{Gi}}$ = Inlet concentration ($g\ m^{-3}$)

$$\textbf{Mass loading (Volumetric)} = \frac{\mathbf{Q} * \mathbf{C_{Gi}}}{\mathbf{V_f}}$$

Removal Efficiency and Elimination Capacity:

Removal efficiency and elimination capacity are used to describe the performance of a biofilter. Removal efficiency (RE) is the fraction of the contaminant removed by the biofilter, expressed as percentage:

$$\textbf{Removal efficiency} = \left(\frac{C_{Gi} * C_{Go}}{C_{Gi}}\right) * 100$$

Where C_{Gi} = Inlet concentration (ppmv, gm^{-3})

C_{Go} = Outlet concentration (ppmv, gm^{-3})

Elimination capacity is the mass of contaminant degraded per unit volume of filter material per unit time. Typical units for elimination capacity are grams of pollutant per m^3 of filter material per hour. An overall elimination capacity is generally defined:

$$\textbf{Elimination capacity} = \frac{(C_{Gi} - C_{Go}) * Q}{V_f}$$

Elimination capacity = Volumetric mass loading * RE

LITERATURE REVIEW

4.1 Biological removal of Pyridine:

Pyridine and its derivatives can be removed or degraded by using biological methods of treatment. The critical review on the biological treatment of pyridine and its derivatives has been discussed in this section.

In the study carried out by **Mathur et al. (2008 a)**, two bacterial strains capable of utilizing pyridine as a sole carbon source were isolated from biofilters. Based on the biochemical test, the organisms were identified as *Shewanella putrefaciens* and *Bacillus sphaericus*. In liquid cultures, *S. putrefaciens* and *B. sphaericus* degraded pyridine quite effectively up to 500 mg L^{-1}. *S. putrefaciens* degrades 500 mg L^{-1} of pyridine completely within 140 h, whereas the *B. sphaericus* degrades 500 mg L^{-1} of pyridine only nearly 75% and takes long duration of 150 h. *S. putrefaciens* used pyridine as sole carbon and energy source better than *B. sphaericus*.

In the study by **Mathur and Majumder (2008 b)**, a new strain namely *Shewanella putrefaciens* was used for biofiltration of pyridine laden air stream in a corn-cob packed biotrickling filter. In the tested biotrickling filter, *S. putrefaciens* exhibited that the maximum removal of pyridine is determined to be 100% at less than the average inlet concentration of 0.653 g m^{-3} and more than 93% at higher average inlet concentration of 1.748 g m^{-3} with an empty bed residence time (EBRT) of 106 s. But when the biotrickling filter was operated at nearly same higher average inlet concentration of 1.752 g m^{-3} with an EBRT of 53 s, the removal was not attained greater than 85%. The maximum elimination capacity (EC) of the biotrickling filter was 102.34 g m^{-3} h^{-1} at inlet pyridine load of 119.62 g m^{-3} h^{-1} with an EBRT of 53 s in phase VII. The maximum deviation of EC from 100% conversion line was varied from 0.257 to 10.166% from phase I to VIII. Kinetic analysis showed that the maximum removal rate (μmax) and saturation constant (Ks) values of pyridine were calculated as 0.24 g m^{-3} h^{-1} and 6.44 g m^{-3}, respectively with correlation coefficient (R^2) of 0.9939 and stadradard deviation of error of 23.94% of pyridine.

In the study by **Mudliar et al. (2008),** they reported removal of pyridine in a rotating rope bioreactor with removal efficiency of 85%. The culture used for this purpose was *Psedomonas pseudoalcaligenes*-KPN isolated from garden soil.

In the study by **Padoley et al. (2006),** the biodegradation of pyridine in a completely mixed activated sludge process was observed. A potential bacterial culture (P1), isolated from garden soil and identified as *Pseudomonas pseudoalcaligenes*–KPN, was used as a starter seed to develop the biomass in a completely mixed activated sludge (CMAS) reactor and the system was evaluated for treatment of wastewater containing pyridine. The results of this study indicate that pyridine could be degraded efficiently at a loading of 0.251 kg pyridine kg $MLSS^{-1}$ d^{-1} (0.156 kg TOC kg $MLSS^{-1}$ d^{-1}) and at an optimal hydraulic retention time (HRT) of 24 h. Pyridine was used as the sole source of carbon and nitrogen by the biomass. Ammonia-nitrogen (NH_3-N) was formed due to the metabolism of the pyridine ring. In this investigation, the performance of CMAS with reference to pyridine biodegradation and the bio-kinetic constants for the biodegradation of pyridine, in a continuous system, were computed. The results indicate that a CMAS system inoculated with *P. pseudoalcaligenes*–KPN, under optimum conditions of HRT and pyridine loading, gives a yield coefficient of (Y) 0.29, decay coefficient (K_d) 0.0011 d^{-1}, maximum growth rate constant (lmax) 0.108 d^{-1} and saturation rate constant (K_s) 5.37 mg l^{-1} for pyridine. It is reported that the isolated *Pseudomonas pseudoalcaligenes*–KPN could degrade pyridine efficiently in the concentration range of 50-300 mg l^{-1}.

In the study by **Mohan et al. (2003)**, the microbial degradation of pyridine by *Pseudomonas* (PI2), isolated from the mixed population of the activated sludge unit was studied. Aerobic degradation of pyridine was studied with the isolated strain and the growth parameters were evaluated. The process parameters like biomass growth and dissolved oxygen consumption were monitored during pyridine degradation. In order to conform with the plasmid capability to degrade pyridine, the requisite plasmid was isolated and transferred to DH 5a *Escherichia coli.*

In the study by **Lee et al. (2001),** they studied the degradation of Cells of 3-methylpyridine and 3-ethylpyridine on *Gordonia nitida* LE31. Cyclic intermediates were not detected, but formic acid was identified as a metabolite. Degradation of levulinic acid was induced in cells grown on 3-methylpyridine and 3-ethylpyridine. Levulinic aldehyde dehydrogenase and formamidase activities were higher in cells grown on 3-methylpyridine and 3-ethylpyridine than in cells grown on acetate.

In the study by **O'Loughlin (1999),** a bacterium was isolated which was capable of degrading 2-methylpyridine by enrichment techniques from subsurface sediments collected from an aquifer located at an industrial site that had been contaminated with pyridine and pyridine derivatives. The isolate, identified as an *Arthrobacter* sp., was capable of utilizing 2-methylpyridine, 2-ethylpyridine, and 2-hydroxypyridine as primary C, N, and energy sources. The isolate was also able to utilize 2-, 3-, and 4-hydroxybenzoate, gentisic acid, protocatechuic acid and catechol, suggesting that it possesses a number of enzymatic pathways for the degradation of aromatic compounds. Degradation of 2-methylpyridine, 2-ethylpyridine, and 2-hydroxypyridine was accompanied by growth of the isolate and release of ammonium into the medium. Degradation of 2-methylpyridine was accompanied by overproduction of riboflavin. A soluble blue pigment was produced by the isolate during the degradation of 2-hydroxypyridine, and may be related to the diazadiphenoquinones reportedly produced by other *Arthrobacter* spp. when grown on 2-hydroxypyridine. When provided with 2-methylpyridine, 2-ethylpyridine, and 2-hydroxypyridine simultaneously, 2-hydroxypyridine was rapidly and preferentially degraded; however there was no apparent biodegradation of either 2-methylpyridine or 2-ethylpyridine until after a seven day lag. The data suggest that there are differences between the pathway for 2-hydroxypyridine degradation and the pathway(s) for 2-methylpyridine and 2-ethylpyridine.

In rge study by **Uma and Sandhya (1998),** they used *Bacillus coagulans* strain isolated from contaminated soil immobilised on activated carbon for degradation of pyridine, toluene and methylene chloride containing synthetic wastewaters in a packed bed reactor. Pyridine was supplied as the only source of nitrogen in the wastewaters. Continuous runs in a packed bed laboratory reactor showed that immobilized *B. coagulans* can degrade pyridine

along with other organics rapidly and the effluent ammonia is also controlled in presence of "organic carbon". About 644 mg dm^{-3} of influent TOC was efficiently degraded (82.85%) at 64.05 mg l^{-1} h^{-1}.

In he study by **Uma and Sandhya (1997)** on the degradation and heterocyclic nitrification of pyridine by *Bacillus coagulans*, the gram-positive, pyridine-degrading microorganism were isolated from contaminated soil by enrichment culture technique. Pyridine was used as source of carbon, nitrogen and energy. *Bacillus coagulans* reduce nitrogen from aromatic ring to ammonia and subsequently heterotrophically to nitrite and nitrate. The maximum degradation of pyridine was 94.1 % within 72 h at 30 °C with a 7.57 h doubling time.

In th study by **Rhee et al. (1997)**, the anaerobic and aerobic degradation of pyridine by a newly isolated denitrifying bacterium was monitored. New denitrifying bacteria that could degrade pyridine under both aerobic and anaerobic conditions were isolated from industrial wastewater. The successful enrichment and isolation of these strains required selenite as a trace element. These isolates appeared to be closely related to *Azoarcus* species according to the results of 16S RNA sequence analysis. An isolated strain, pF6, metabolized pyridine through the same pathway under both aerobic and anaerobic conditions. Since pyridine induced NAD-linked glutarate-dialdehyde dehydrogenase and isocitratase activities, it is likely that the mechanism of pyridine degradation in strain pF6 involves N–C-2 ring cleavage. Strain pF6 could degrade pyridine in the presence of nitrate, nitrite, and nitrous oxide as electron acceptors. In a batch culture with 6 mM nitrate, degradation of pyridine and denitrification were not sensitively affected by the redox potential, which gradually decreased from 150 to 2200 mV. In a batch culture with the nitrate concentration higher than 6 mM, nitrite transiently accumulated during denitrification significantly inhibited cell growth and pyridine degradation. Growth yield on pyridine decreased slightly under denitrifying conditions from that under aerobic conditions. Furthermore, when the pyridine concentration used was above 12 mM, the specific growth rate under denitrifying conditions was higher than that under aerobic conditions. Considering these characteristics, a newly

isolated denitrifying bacterium, strain pF6, has advantages over strictly aerobic bacteria in field applications.

In the study by **Rhee et al. (1996)**, they investigated the effect of the presence of supplementary glucose or acetate on the growth and pyridine-degrading activity of freely suspended and calcium-alginateimmobilized *Pimelobacter* sp. Although the supplementary carbon sources could be degraded simultaneously with pyridine, *Pimelobacter* sp. exhibited a preference for pyridine over supplementary carbon sources. Thus, the pyridine-degrading activity of the freely suspended cells was not decreased significantly by the addition of either glucose (1.5-6 mM) or acetate (6-24 mM) to the pyridine (6-24 mM). In the semi-continuous immobilized cell culture, immobilized cells also exhibited a preference for pyridine over supplementary carbon sources and did not switch their substrate preference throughout the culture. Owing to a high cell concentration, the volumetric pyridine degradation rate at 24 mM pyridine in the immobilized cell culture was approximately six times higher than that in the freely suspended cell culture. Furthermore, the immobilized cells could be reused 16 times without losing their pyridine-degrading activity during the culture period tested. Taken together, the use of immobilized *Pimelobacter* sp. For the degradation of pyridine is quite feasible because of the preference for pyridine over supplementary carbon sources, the high volumetric pyridine degradation rate, and the reusability of immobilized cells. They Concluded that, the use of immobilized *Pimelobacter* sp. for the degradation of pyridine in retort water is feasible because of the preference for pyridine over supplementary carbon sources, the high volumetric pyridine degradation rate, and the reusability of the immobilized cells.

The biodegradation of pyridine was studied by **Lee et al. (1994)** by using freely suspended and Ca-alginate-immobilized cells of *Pimelobacter* sp. When the pyridine concentration was up to 2 g dm^{-3}, freely suspended cells completely degraded pyridine regardless of the initial cell concentrations used. However, when the pyridine concentration increased to 4 g l^{-1}, the initial cell concentration in freely suspended cell culture should be higher than 1.5 g dry cell weight dm^{-3} for complete degradation of pyridine. In addition, a freely suspended cell culture with a high initial cell concentration resulted in a high

volumetric pyridine-degradation rate, suggesting the potential use of immobilized cells for pyridine-degradation. When the immobilized cells were used for pyridine-degradation, neither specific pyridine-degradation rate nor tolerance against pyridine was improved. However, a high volumetric pyridine-degradation rate in the range 0.082-0.129 g l^{-1} hr^{-1} could be achieved by the immobilized cells because of the high cell concentration. Furthermore, when the immobilized cells were reused in degrading pyridine at a concentration of 2-4 g l^{-1} they did not lose their pyridine-degrading activity for 2 weeks. The authors found that Pyridine at the concentration up to 6 g dm^{-3} could be degraded completely within 70 h. However, when the pyridine concentration increased to 10 g l^{-1}, immobilized cells, like freely suspended cells, could not degrade pyridine completely.

In the study by **Sims et al. (1986)** the degradation of pyridine by *Micrococcus luteus*, isolated from soil by enrichment culture was studied. The organism oxidized pyridine for energy and released N contained in the pyridine ring as ammonium. The organism could not grow on mono- or disubstituted pyridinecarboxylic acids or hydroxy-, chloro-, amino-, or methylpyridines. Cell extracts of *M. luteus* could not degrade pyridine, 2-, 3-, or 4-hydroxypyridines or 2, 3-dihydroxypyridine, regardless of added cofactors or cell particulate fraction. The organism had a NAD-linked succinate-semialdehyde dehydrogenase which was induced by pyridine. Cell extracts of *M. luteus* had constitutive amidase activity, and washed cells degraded formate and formamide without a lag. Cells of *M. luteus* were permeable to pyridinecarboxylic acids, monohydroxypyridines, 2, 3-dihydroxypyridine, and monoamino- and methylpyridines. The results provide new evidence that the metabolism of pyridine by microorganisms does not require initial hydroxylation of the ring and that permeability barriers do not account for the extremely limited range of substrate isomers used by pyridine degraders.

In the study by **Rogers et al. (1985),** they have studied the microbiological degradation of a mixture of alkylpyridines in groundwater maintained under aerobic and anaerobic conditions. A marked difference was observed between the aerobic and anaerobic degradation rates. In the presence of a soil inoculum and under aerobic conditions, the residual alkylpyridine concentrations generally approached zero concentration within 10 to

31 days of incubation, whereas under anaerobic conditions the concentrations of residual alkylpyridines only decreased between 40 and 80 % over a 33 day incubation period. Biodegradation rates under aerobic conditions were greatly affected by the specific ring substitution of structural isomers within a given weight class. A similar effect was not observed for anaerobic degradation rates.

In the study by **Watson and Cain (1975),** they used *Bacillus* sp. and *Nocardia* sp. (strain ZI), isolated from soil by enrichment with 0.1 % (v/v) pyridine and grew rapidly on this compound as sole C, N and energy source. Treatment of cells with toluene led to rapid loss of the ability to oxidize pyridine. In the presence of 10mM-semicarbazide at pH 6.0, *Nocardia* Zl accumulated a semialdehyde identified as its 2,4-dinitrophenylhydrazone by chromatography, mixed melting point, mass spectrometry and isotope trapping from [2,6-'4 C] pyridine as glutarate semialdehyde. Extracts of this bacterium prepared from cells grown with pyridine or exposed to the gratuitous inducer 2-picoline, contained high activities of a specific glutarate semialdehyde dehydrogenase. Cells grown with pyridine or glutarate also contained a glutaric dialdehyde dehydrogenase, an acyl-CoA synthetase and elevated amounts of isocitrate lyase but no glutaryl-CoA dehydrogenase. Bacillus 4 accumulated in the presence of 10 mM-semicarbazide several acidic carbonyl compounds from pyridine among which was succinate semialdehyde. Extracts of this bacillus after growth of the cells with pyridine contained an inducible succinate semialdehyde dehydrogenase in amounts at least 50-fold over those found in succinate-grown cells. Two mutants of this bacillus, selected for their inability to grow on pyridine were deficient in succinate semialdehyde dehydrogenase. In the presence of 0.2 mM-KCN, washed suspensions of Bacillus 4 accumulated formate and possibly formamide from pyridine. The use of [14C] pyridine showed that formate was derived from C-2 of the pyridine ring. The organism had a specific formamide amidohydrolase cleaving formamide quantitatively to formate and NH_3. Formate was further oxidized by the particle fraction.

In the study by **Watson et al. (1974),** Pyridine degradation was tested by three types of assay: (i) pyridine disappearance was followed by ΔE_{257} (e for pyridine=32401Pmol^{-1} cm^{-1}) or manometrically by 02 uptake; (ii) NAD^+ or $NADP^+$ was included and increase in ΔE_{340}

or 02 uptake determined; or (iii) NADH or NADPH were included and $-\Delta E_{340}$ determined in the presence and absence of pyridine. Only high-speed supernatant could be tested by this spectrophotometric method because of the high NAD (P) H oxidase activity of crude extracts. Pyridine oxidation could be measured manometrically when the flasks contained a reduced nicotinamide nucleotide-regenerating system.

In the study by **Cain et al. (1974)**, they studied the microbial metabolism of 2 and 3-hydroxypyridines by the maleamate pathway in *Archromobacter* sp. They used washed suspensions of two *Achromobacter* species (G2 and 2L), capable of growth upon 2- and 3-hydroxypyridine respectively as sources of C and N, rapidly oxidized their growth substrate pyridine-2, 5-diol (2, 5-dihydroxypyridine) and the putative ring-cleavage product maleamate without a lag. Extracts of both bacteria oxidized pyridine-2, 5-diol with the stoicheiometry of an oxygenase forming 1 mol of NH_3 mol^{-1} of substrate. Heat treated extracts, however, formed maleamate and formate with little free NH_3. The conversion of maleamate into maleate plus NH_3 by extracts of strain 2L, fractionated with $(NH_4)_2SO_4$, and the metabolism of maleamate and maleate to fumarate by extracts of both strains demonstrated the existence of the enzymes catalysing each reaction of the maleamate pathway in these bacteria. The pyridine-2, 5-diol dioxygenase (mol.wt. approx. 340000) in extracts of these *Achromobacter* species required Fe^{2+} (1.7 pM) to restore full activity after dialysis or treatment with chelating agents; the enzyme from strain 2L also had a specific requirement for L-cysteine (6.7 mM), which could not be replaced by GSH or dithiothreitol. The oxygenase was strongly inhibited in a competitive manner by the isomeric pyridine-2, 3- and -3, 4-diols.

In the study by **Houghton and Cain [1972]**, the degradation of pyridine and the three isomeric hydroxypyridines was studied by micro-organisms that were capable of utilizing pyridine compounds as carbon and energy source isolated from soil and sewage. Three species of *Achromobacter* produced pyridine-2, 5-diol from 2- or 3-hydroxypyridine whereas an uncommon *Agrobacterium* sp. (N.C.I.B. 10413) produced pyridine-3, 4-diol from 4-hydroxypyridine. On the basis of chemical isolation, induction of the necessary enzymes in washed suspensions and the substrate specificity exhibited by the isolated bacteria, the initial transformations proposed are: 2-hydroxypyridine → pyridine-2,5-diol; 3-

hydroxypyridine → pyridine-2,5-diol and 4-hydroxypyridine → pyridine-3,4-diol. A selected pyridine-utilizer, *Nocardia* Zl, did not produce any detectable hydroxy derivative from pyridine, but carried out a slow oxidation of 3-hydroxypyridine to pyridine-2, 3-diol and pyridine-3, 4-diol. Addition of the isomeric hydroxypyridines to a model hydroxylating system resulted in the formation of those diols predicted by theory.

4.2 Physico-Chemical Removal of Pyridine:

Pyridine and its derivatives can also be removed by using physico=chemical methods of treatment. The critical review on the physico-chemical treatment of pyridine and its derivatives has been discussed in this section.

Lataye et al. (2007) reported the use of BFA and RHA the agri-wastes, abundantly available at a throw-away price from the particulate collection equipment attached to the flue gas line of the boilers/furnaces operating on bagasse and rice husk as fuels as adsorbents for the removal of pyridine and its derivative 2-picoline. These agri-wastes have excellent mesoporous structure and very high affinity towards a large number of solutes (adsorbates) in aqueous solutions and waste waters. The authors also reported the use of granular activated carbon as an adsorbent for the removal of pyridine and picoline. The overall adsorptive uptake was in the order of BFA > GAC > RHA for that of pyridine and its derivatives.

Lataye et al. (2006) studied the adsorption of pyridine on low cost adsorbent which is available easily and abundantly. The authors studied the adsorption of pyridine from aqueous solutions, using BFA using batch adsorption system. The influence of various parameters, such as initial pH (pH_0), adsorbent dose (m), contact time (t), initial concentration (C_0), and temperature (T), on the removal of Py from the aqueous solutions have been studied. The study was performed in the wide range of initial concentration i.e. 50 – 600 mg dm^{-3}. The maximum removal of Py is determined to be 99% at lower concentrations (<50 mg l^{-1}) and 95% at higher concentrations (600 mg l^{-1}), using a BFA dosage of 25 kg m^{-3} at normal temperature. They conducted the studies on Py adsorption equilibrium and kinetics. The adsorption equilibrium analyses were performed, using the Langmuir, Freundlich, Redlich-Peterson, and Temkin isotherm equations. The authors found

that the Langmuir equation is determined to be the best to represent the equilibrium sorption data. They have also reported the thermodynamic studies, revealed that the adsorption of Py on BFA is endothermic in nature and that the isosteric heat of adsorption decreases as the equilibrium uptake of Py on the BFA surface increases (269.92 and 187.63 kJ kg^{-1} for the equilibrium Py concentration on the adsorbents in the range of 4-20 mg g^{-1}). The authors also studied the desorption of Py from Py-loaded BFA with several solvents, showed that only 68.70% and 51% of Py could be recovered, using ethyl alcohol and 0.1 N H_2SO_4, respectively.

Mohan et al. (2005) studied the ability of activated carbons developed from coconut shell to adsorb 2Pi, 3-picoline, and 4Pi from aqueous solution. The developed carbons were designated as SAC (activated carbon derived from coconut shells with out any treatment) and ATSAC (activated carbon derived from acid treated coconut shells). They characterized and utilized the carbons for the sorption of α-picoline, β-picoline, and γ-picoline at different temperatures, particle size, pH and solid to liquid ratio. The Langmuir and Freundlich isotherm models were applied and the Langmuir model was found to best report the equilibrium isotherm data. The adsorption of α-picoline, β-picoline, and γ-picoline followed the pseudo-second order rate kinetics. Authors have concluded that in majority of cases, the adsorption is controlled by particle diffusion at temperatures 10 and 25 °C while at 40 °C it is controlled by film diffusion mechanism. Similarly at concentrations 25 and 50 mg l^{-1} the adsorption was governed by particle diffusion in most of the cases while at > 50 mg l^{-1} it was film diffusion controlled. The overall capacity of ATSAC was higher than SAC. The sorption capacity of γ-picoline was found more followed by α-picoline and β-picoline. The authors reported that the sorption followed the order α-picoline < β-picoline < γ-picoline Further the sorption followed the order a-picoline The authors reported that, 40-50% of the ultimate adsorption occurs within the first hour of contact for the adsorption of α-picoline, β-picoline and γ-picoline and the saturation is reached in 48 h.

Mohan et al. (2004) developed carbons; FAC (activated carbon derived from coconut fibres), SAC (activated carbon derived from coconut shells), ATFAC (activated carbon derived from acid treated coconut fibres) and ATSAC (activated carbon derived from acid

treated coconut shells) and used in the adsorption study. They studied the adsorption in the lower range of pyridine concentration (1-100 mg l^{-1}). The authors found that the Langmuir adsorption isotherm model fits the data better as compared to the Freundlich adsorption isotherm model. The adsorption of pyridine follows the first order rate kinetics. They found the effective diffusion coefficient D_i, increasing with temperature in the range of 45.27 x 10^{-10} – 237.12 x 10^{-10} m^2s^{-1} for all the adsorbents. The activation energy for FAC, SAC, ATFAC and ATSAC was found to be 3.66, 5.34, 18.73 and 26.18 $kJmol^{-1}$ respectively. The authors have concluded that the adsorption occurs through a particle diffusion mechanism at temperatures 10 and 25 °C while at 40 °C it occurs through film diffusion mechanism. Similarly at concentrations 25 and 50 mg l^{-1} the adsorption is particle diffusion controlled while at $\leq$ 50 mg l^{-1} it is film diffusion controlled.

Diez and Amalvy (2003) studied the interaction of pyridine, 2-vinylpyridine, and 4-vinylpyridine with silica surfaces and found that pyridine interacts more strongly with silica clusters than its derivatives, whereas 2-vinylpyridine, in its turn, exhibits a larger interaction energy than 4-vinylpyridine. They also found that the interaction pattern is dominated in all cases by the formation of a hydrogen bond between the nitrogen atom and a hydroxyl group of the silica cluster, a second, very weak hydrogen bond is formed in some of the systems, though. It is proposed that the trend of not forming nanocomposites when using 2-vinylpyridine is mainly due to the involvement of its vinyl group in a weak hydrogen bond to an oxygen atom of the silica surface avoiding polymerizing and neither due to a weak acid–base interaction nor steric effects.

Tadjeddine et al. (1999) have investigated the geometry of the adsorption of pyridine on gold single-crystal electrode by in situ difference frequency generation (DFG) in aromatic ring spectral range, by using the CLIO free electron laser. Pyridine adsorbs via the nitrogen lone pair, the molecular plane being tilted. The potential dependence of the vibrational modes showed evidences of a reorientation of the molecule around the potential of zero charge.

Niu et al. (2002) developed a technique with high-area carbon cloth (C-cloth) electrodes as quasi-3-dimensional interfaces, coupled with in situ UV–Vis spectrophotometric techniques and scanning kinetics for quantitatively monitoring the adsorption/ desorption processes. The conversion of Py to PyH+, which decreases the removal efficiency, takes place in non-buffered electrolyte solution due to pH changes but is eliminated in a HOAc+NaOAc buffer (pH 6.8) solution. Rapid and complete removal of Py is then achieved by anodic polarization.

Bludau et al. (1998) monitored the sorption and desorption kinetics of pyridine into and out of mordenite, ZSM-5 and silicate. Sorption and desorption of pyridine was measured through the intensity changes of the IR bands typical of sorbed pyridine. At sufficiently high temperatures 525-575 K the uniform diffusion coefficients for the whole sorption process was $D = 1x10^{-12}$ and $D = 6x10^{-11}$ cm^2s^{-1} for H-MOR at 525 K and H-ZSM-5 at 575 K respectively. The slow transport of pyridine into the micropores is the rate-determining step, which may indeed be described as diffusion, but followed by a rapid reaction with the acidic Brönsted and/or Lewis sites. The values were found for the kinetics of populating both the Brönsted centres (indicated through the change in the absorbance of the typical PyB band at 1540 cm^{-1}) and the Lewis sites (monitored by the increase of the absorbance of the typical PyL band at 1450 cm^{-1}). Isosteric heat of adsorption of pyridine in Li-ZSM-5 and Na-ZSM-5 were determined to 195-155 $kJmol^{-1}$ and 120 $kJmol^{-1}$ and were thus significantly higher than for benzene in H-ZSM-5 (65 ± 5 $kJmol^{-1}$).

Stern et al. (1997) reported batch ozonation and biological treatment method for the removal of 3-Methylpyridine (MP) and 5-ethyl-2methylpyridine (EMP). They found that 90 % methylpyridines were removed. 5 moles of ozone were needed per mol of MP removed. EMP was initially oxidized much faster but the reduction of DOC only 5% when EMP was removed by 90%. 4 moles of ozone were required to remove one mole of EMP.

Kumar et al. (1995) reported the adsorption of toxic and odorous pyridine on activated carbon in batch and column system. The batch study was performed in the initial concentration range of 50-250 mg dm^{-3} of pyridine. At an initial concentration of 200 mg l^{-1}, 96.4 per cent (q_e = 1.203 mg g^{-1}) removal of pyridine was obtained. Equilibrium adsorption data represented by both the Freundlich and Langmuir model.

Akita and Takeuchi (1993) reported thattThe sorption of pyridines can be best represented by Langmuir isotherm on strong acid ion-exchange resin and Freundlich isotherm adsorption can be used for sorption of pyridines on both weak acid ion-exchange and porous resins. The pyridines were sorbed on the resins in the sequence of pyridine < 3-picoline < 3, 5-lutidine where the selectivity increased as follows: strong acid ion-exchange resin (200C) <weak acid ion-exchange resin (IRC-50) < porous resin (XAD-4). The monolayer adsorption capacity reported in the study is 4.47 mg g^{-1} for pyridine, 4.48 mg g^{-1} for 3-picoline and 4.2 mg g^{-1} for that of 3-, 5- lutidine on the resins.

Martin et al. (1992) studied the adsorption of pyridine, formic acid, and acetic acid on MgO, MoO_3 and MoO_3/MgO (Mo: Mg = 1:15) samples calcined at 773 K (where no extensive reaction between the two oxides seems to take place) or 1100 K (where the $MgMoO_4$, species is formed) by FIT-IR spectroscopy. Found that pyridine get sorbed on the lewis sites on the surface of magnesia developed by molybdena. Pyridine get physisorbed shown by the bands at 1442 and 1598 cm^{-1}.

Zaki et al. (1989) studied the adsorption of pyridine onto ceria surface and found that the IR spectrum resulting from Py adsorption on Al/Ce displays, in addition to the characteristic bands of Py bonded to Lewis site, a unique band at 1534 cm^{-1}. Thus the band occurs in the frequency range (1439-1550 cm^{-1}). When the band occurs at $\geq$ 1631 cm^{-1} it indicates the formation of Py bonded to a Brönsted site. Doping of ceria with Na^+, Al^{3+} and Cr^{3+} ions (at 1 atom dopant per Ce atom) largely modified the Py adsorption capacity and

surface reactions, thus revealing disparate impacts on acid/base properties of the surface. They concluded that (1) ceria surfaces, produced by the thermal decomposition at 400 °C of diammonium hexanitratocerate, exposes Lewis acid sites of at least two different acidity strengths as well as two types of OH^- groups. (2) high-temperature calcination of ceria (at 800 °C) weakens the surface acidity and improves the chemical reactivity of basic sites. (3) Na^+ ions have a very strong poisoning effect on the overall acid/base properties of ceria. Cr^{3+} ions have a promoting effect, whereas Al^{3+} ions help to generate Brönsted acid sites.

Zhu et al. (1988) used Rundle spent shale for the adsorption of pyridine from dilute aqueous solution. They reported that at pH 8, Rundle-Lurgi spent shale adsorbs a significant amount of pyridine. The adsorption of pyridine from aqueous solutions onto the solids generated by the processing of Rundle oil shale showed the adsorption isotherm of Langmuir type (L-4) with two plateaux.

Luh and Baker (1971) also studied the desorption of pyridine from loaded clay in aqueous solution as a function of solution pH, time, the number of stages of desorption with transfer to fixed volumes of solution and with transfer to solutions of varying volume. For kaolinite no pyridine desorption occurred at pH 9 or 11 over 7 days after the initial pyridine release in 17 min. For the montmorillonite, maximum desorption occurs in 17 min at pH 11. In this case, desorption is greater (89 %) than that for kaolinite (66 %) at the same pH. For both clays, minimum desorption occurs at pH 5 which is to be expected since maximum sorption occurs in this range. For the montmorillonite, significant desorption was noted at pH 9 as a function of time. At pH 1, both clays exhibited significant pyridine desorption. Previously sorbed pyridine desorbs from the clays as a function of time and solution pH with maximum desorption at pH 1 and 11 and minimum near pH = pK_a = 5.25. Pyridine desorption was found to be much slower than sorption at comparable pH and clay-pyridine ratio. The extent of desorption is also directly related to the number of stages and/or the volume of solution.

Reference	Method	Important considerations	Type of wastewater
Ross (1965)	Thermal catalytic Incineration	oxides of nitrogen along with CO_2 are generated, fuel Consumption is large, risk of further air pollution remains due to incomplete combustion	Multi-component wastewaters containing nitrogenous compounds
Lowry (1973), Stuerner and Morris (1982)	Deep well injection	Ground water pollution	Coal carbonization effluents
Lowry (1973)[52], Shanker et al. (1991), Fetzner (1998)	Soil percolation	desired microbial population should be able to degrade in subsurface environment	Coal carbonization effluents
Klemetson (1979)	Ultrafiltration and hyperfintration	Membrane filtration processes are highly expensive	Carbonization effluents Containing pyridine and its derivatives
Fox et al. (1980), Bell et al. (1984)	Activated carbon	Source and preparation process of adsorbent influences removal	Shale oil retort wastewater
Zhu et al. (1988), Zhu et al. (1995)	Spent crude oil shale	Type and concentration of exchangeable cations influences adsorption Adsorption in high concentration range is not reversable	Pyridine in retort water
Baker and Luh	Clay (Mont-	Mechanism is pH sensitive	Pyridine in aqueous

(1971)	Morillonite and Kaolinite)		solution
Technical report, NEERI (2004)	Chemical Coagulation	Low removal efficiency, Further treatment may be required	Pyridine wastewater from chemical manufacturing industry

Table4.1: Physical methods for removal of pyridine

Reference	Method	Mechanism	Reactants	Important considerations
Demetes (1999)	Chemical scrubbing	suitable sorbent with acid/alkali for scrubbing	Sodium hypochlorite, hydrogen peroxide, titanium dioxide at a suitable pH	Optimal contact time between contaminant and sorbent
Cocquet et al. (2000), Waki et al. (2000)	Photochemical Process	Bond between carbonyl group 'C' atoms and 'N' atom of adjacent groups are cleaved	Hydrogen peroxide, titanium dioxide, iron compounds	Paticle surface which is positively charged due to generation of excess proton adsorbs the organic compound
Technical Report, NEERI (2004)	Wet oxidation with Fenton reagent	Iron acts as a catalyst and hydrogen peroxide acts as an oxidant in the form of OH radical	Feric ion, hydrogen peroxide	The reaction leads to creation of further waste. Recovery of catalyst also produces unwanted chemicals needing further treatment
Engwall et al. (1999), Mueller et al. (1989)	Photo fenton reaction	ferric ion and hydrogen peroxide react to give fenton reagent in presence of UV light	Feric ion, hydrogen peroxide, UV light	Ammonium ions with nitric and nitrus acid are generated as end product. Toxicity is almost nil in

				outlet stream
Dupont et al. (1990)	UV–visible radiation	Solar irradiation (photolytic reaction) decontaminates the soil	Light of lower intensity than sunlight	Photolysis products require secondary treatment
Low et al. (1991), Legrini et al. (1993), Abramovi´c et al. (2004)	Photo catalytic oxidation	TiO_2 coated on borosilicate glass acts as the oxidant in presence of UV light	Titanium dioxide, UV light (20 W fluorescent tube), borosilicate glass spiral wound	Illumination time, initial solute concentration and pH influences formation ammonium and nitrate ions as end product
Andreozzi et al. (1992), Stern et al. (1997)	Ozonation	the oxidation mainly proceeds through ozone attack on the homocyclic ring with the cleavage of the 5, 6 and 7, 8 bonds.	Ozone and UV light	Direct attack of ozone leads to formation of secondary products arising from the oxidative cleavage of the N-heterocyclic ring.
Allen et al. (1994)	Wet air oxidation	The principle is same as that of photo-fenton reaction and proceeds via formation of hydroxy radicals	----	The initial concentration of Discharge is critical for induction and total reaction time

Table 4.2 Chemical methods for removal of pyridine

Reference	Compound	Microorganism	Result achieved
Lee et al. (1994)	Pyridine	*Pimelobactor sp.*	Freely suspended cells degraded upto 2g/l pyridine. Immobilized cells degraded in the range of 0.082-.129 g/l/hr
Rhee et al. (1997)	Pyridine	*Azoarcus evansii pF6*	Above pyridine concentration of 0.9492 g/l growth of bacteria was inhibited
Uma et al. (2002)	Pyridine	*Bacillus consortia*	Biodegradation of pyridine contained in the live pharmaceutical wastewater have been studied and discussed
Shukla and Kaul (1986)	Pyridine-N-oxide and pyridine	*Nocardia sp.*	Ring cleavage initiated via a hypothetical 1-4 dihydropyridine intermediate. Formamide and succinic semialdehyde were observed to be the intermediate products of degradation
Mathew et al. (1988)	3-Cyanopyridine	*Rhodococcus rhodochrous*	Degradation of 3 cyanopyridine yielded valuable product like nicotinic acid
Houghton and Cain (1972)	2-Hydroxy and 3-hydroxy Pyridine	*Achromobacter sps.*	The degradation proceeded via initial hydroxylation. A maximum concentration of 200 mg/L could be treated in shake flask culture experiments
Korosteleva et al. (1981)	Pyridine and 3-Methylpyridine	*Pseudomonas sps*	Biodegradation of 3 methylpyridine yielded

			corresponding carboxylic acid
Lee et al. (2001)	3-Methylpyridine and 3-ethylpyridine	*Gordonia nitida LE 31*	Compounds were degraded via a new pathway involving C-2 and C-3 pathways. Cells grown on 3-methylpyridine degraded 3-ethylpyridine without lag time and vice versa. Little more than 1mM alkyl pyridine was completely degraded in about 120 mins
Uma and Sandhya (1997)	Pyridine	*Baccilus coagulans*	Bacteria used Py as a sole source of carbon, nitrogen and energy. Maximum degradation of 94.1% within 72 h at 30 °C
Kim et al. (2006)	Pyridine	*Pseudomonas putida MK1*	Presence of calcium alginate immobilized cells offer higher resistence to phenol. 1g/l Py solution was completely degraded even at a phenol concentration of 2g/l in 38 h
Cain et al. (1974)	2 and 3-hydroxypyridine	*Achromobacter species (G2 and 2L)*	Rapidly oxidized their growth substrate to 2, 5-dihydroxypyridine and the putative ring-cleavage product maleamate without a lag.
Watson and Cain (1975)	Pyridine	*Bacillus sp. and Nocardia sp*	With 0.1 % (v/v) pyridine solution, cells grew rapidly on the compound as sole C, N and energy source
Rogers et al. (1985)	Alkylpyridine		With soil inoculum and aerobic conditions, the residual

			alkylpyridine approached zero concentration within 10 to 31 days, under anaerobic conditions it only decreased between 40 and 80 %
Sims et al. (1986)	Pyridine	*Micrococcus luteus*	The results proved that the metabolism of pyridine by microorganisms does not require initial hydroxylation of the ring
Rhee et al. (1996)	Pyridine	*Pimelobacter sp.*	Bacteria exhibited a preference for pyridine over supplementary carbon sources. Degradation rate in imobilezed phase was upto six times faster
Uma and Sandhya (1998)	Pyridine with toluene and methyl chloride	*Bacillus coagulans*	About 644 mg dm^{-3} of influent TOC was efficiently degraded (82.85%) at 64.05 mg dm^{-3} h^{-1}
O'Loughlin et al. (1999)	2 methyl, ethyl & hydroxyl-pyridine	*Arthrobacter sp*	2hydroxy pyridine was degraded immediately but others were degraded after 7 day lag period
Mohan et al. (2003)	Pyridine	*Pseudomonas (PI2)*	Maximum of 90% removal was achieved in 8 days
Padoley et al. (2006)	Pyridine	*Pseudomonas pseudoalcaligenes–KPN*	pyridine was degraded efficiently at loading of 0.251 kg Py kg $MLSS^{-1}$ d^{-1} (0.156 kg TOC kg $MLSS^{-1}$ d^{-1}) at an optimal hydraulic retention time (HRT) of 24 h. Pyridine was used as the sole source of

			carbon and nitrogen by the biomass
Stobdan et al. (2007)	Pyridine and 4-methylpyridine	*Gordonia terrea IIPN1*	Upto 70 mM of pyridine was completely degraded at rate of0.69 mg/g/h by growing cells
Lodha et al. (2008)	Pyridine	Isolated consortium	At 25% bio-augmentation 100% of 20 mg/l pyridine solution was degraded
Feng et al. (1994)	2, 3, 4-ethylpyridine	Mixed culture	At 28 °C and 7 pH, the culture can degrade maximum concentration of 1000 mg/l 2-ethylpyridine, 200 mg/l 3-ethylpyridine, 500 mg/l 4-ethylpyridine
Brinkman and Babel (1996)	Pyridine	*Rhodococcus opacus UFZ B 408*	the maximum rate of pyridine degradation achieved was 0.27 g/l/h in comparison to 0.210 g/l/h with growth on pyridine as single substrate
Adav et al. (2007)	Pyridine	----	Maximum of 1 g/l pyridine was completely degraded in 38 h in presence of 1.5 g/l phenol
Li et al. (2001)	Pyridine	----	pyridine with maximum initial concentration 0.1 g/l degraded slightly in first 12 h, but degraded at a higher rate from there on to complete degradation in 24 h.
Mathur et al. (2007)	Pyridine	*Bacillus spaericus, Swenahella puteiaciens*	*putrefaciens* degrades 0.5 g/l of pyridine completely in 140 h, whereas the *B.*

			sphaericus degrades 0.5 g/l of pyridine only nearly 75% in 150 h
Mudliar et al. (2008)	Pyridine	*Psedomonas pseudoalcaligenes-KPN*	Degradation of pyridine with removal efficiency of more than 85% at higher pyridine concentration (up to 1 g/l) and loading [up to 0.4 g/m2/h (66.86 g/m3/h)], with a shorter hydraulic retention time of 9–18 h

Table 4.3: Biodegradation of pyridine by various microorganism

4.3 Pyridine Biodegradation Pathways:

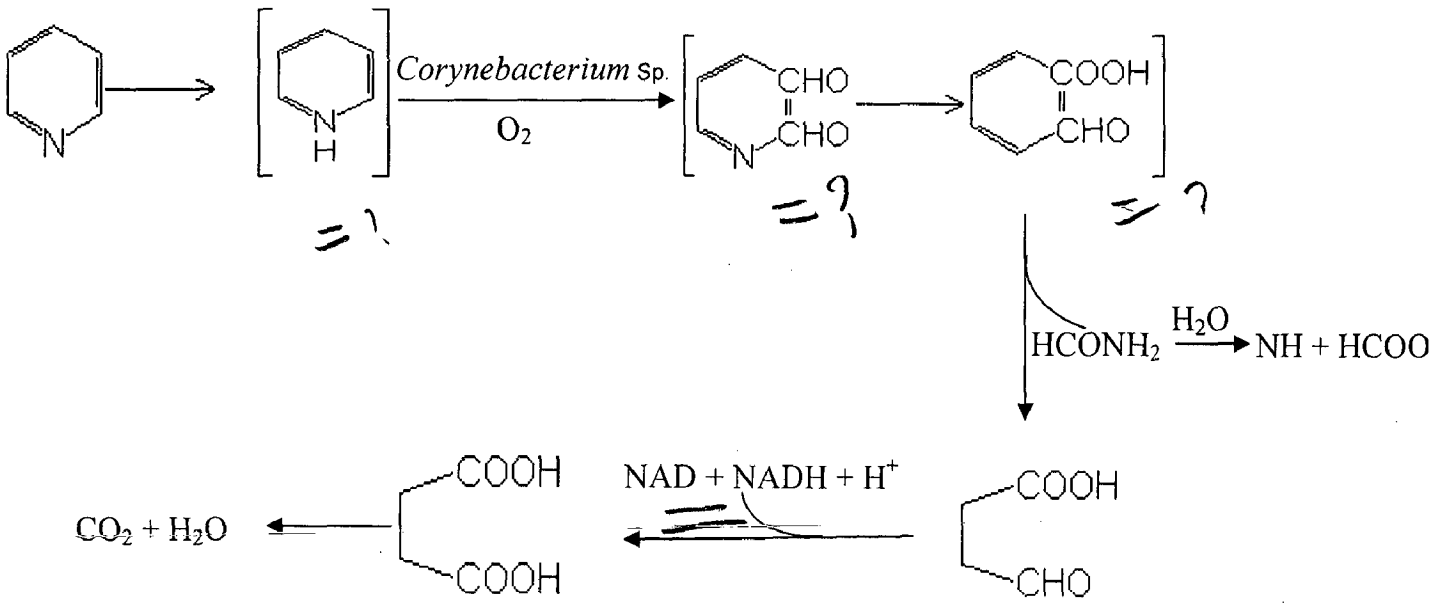


Fig 4.1: Pathway for pyridine metabolism involving *Coryebacterium* (Padoley et al., 2008)

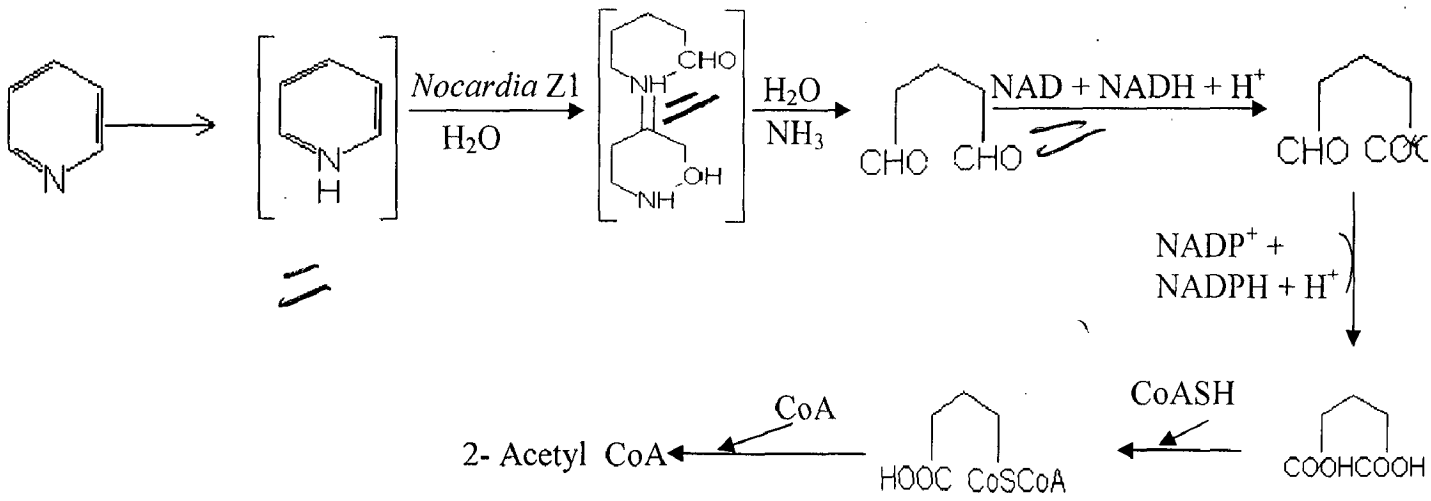


Fig 4.2: for pyridine metabolism involving *Nocardia* Z1 ((Padoley et al., 2008))

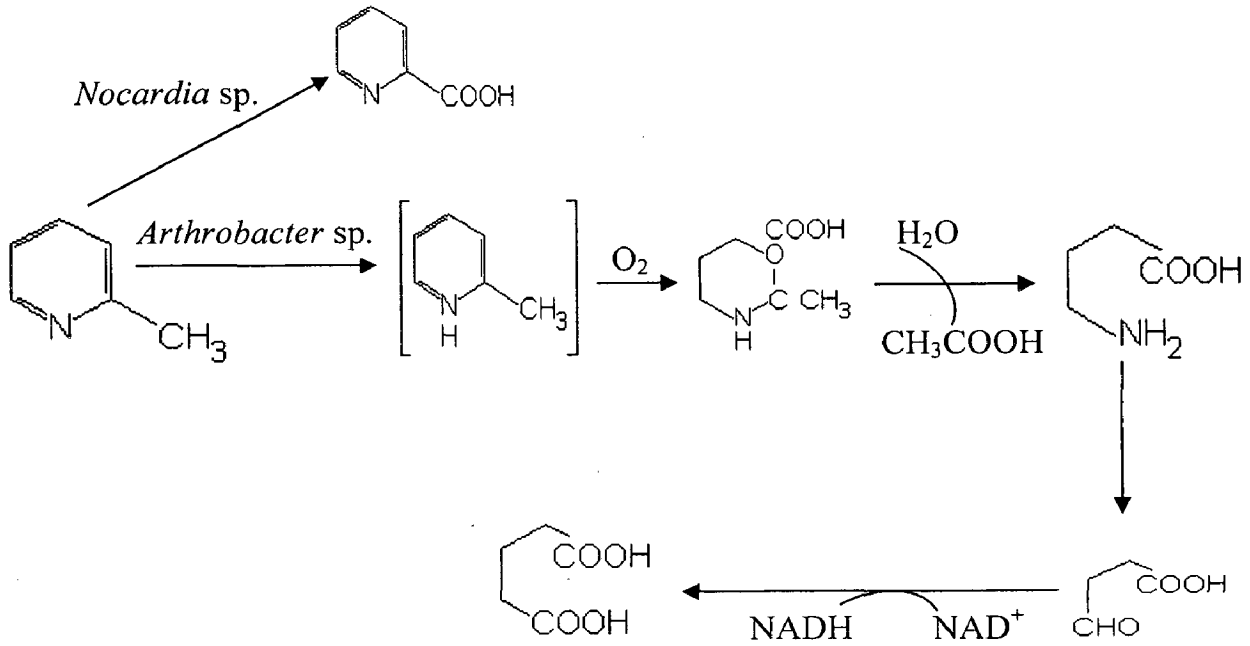


Fig 4.3: Pathway for 3- methylpyridine metabolism involving *Nocardia* sp. And *Arthrobacter* sp. ((Padoley et al., 2008))

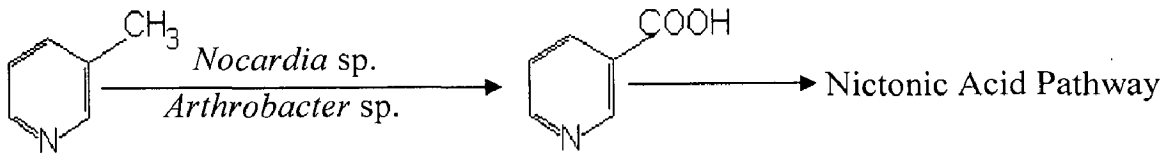


Fig 4.4: Pathway for 2- methylpyridine metabolism involving *Nocardia* sp. and *Arthrobacter* sp. ((Padoley et al., 2008))

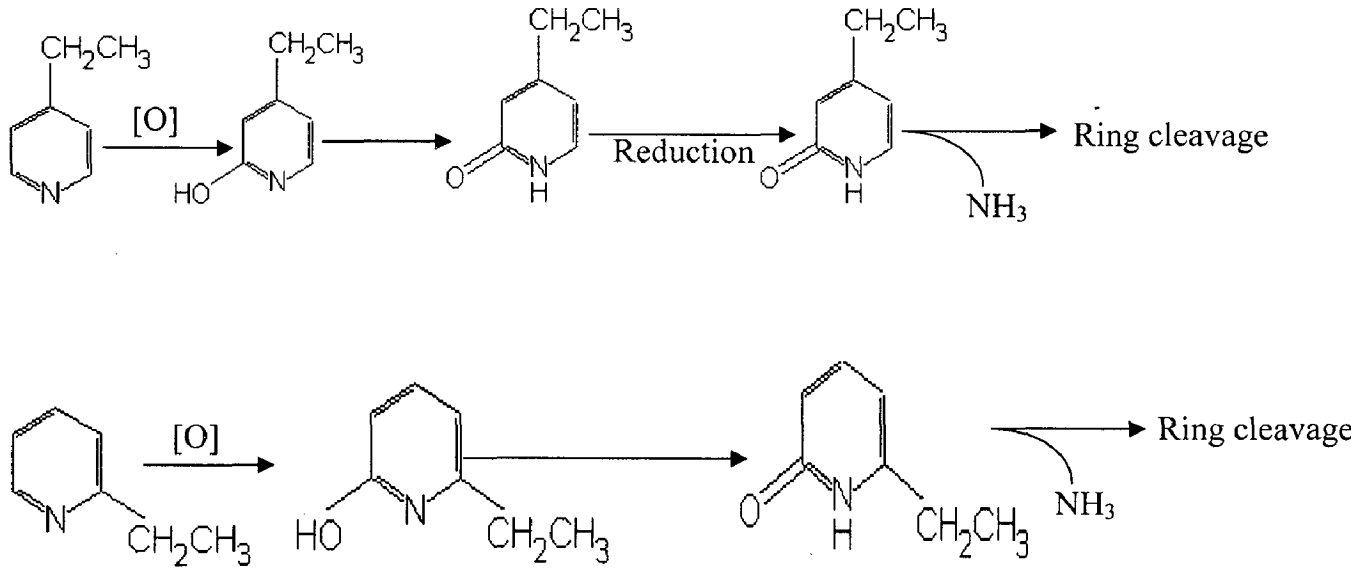


Fig 4.5: Proposed pathway of 2 & 4- ethylpyridine metabolism by a mixed culture under aerobic condition (Feng et al., 1994)

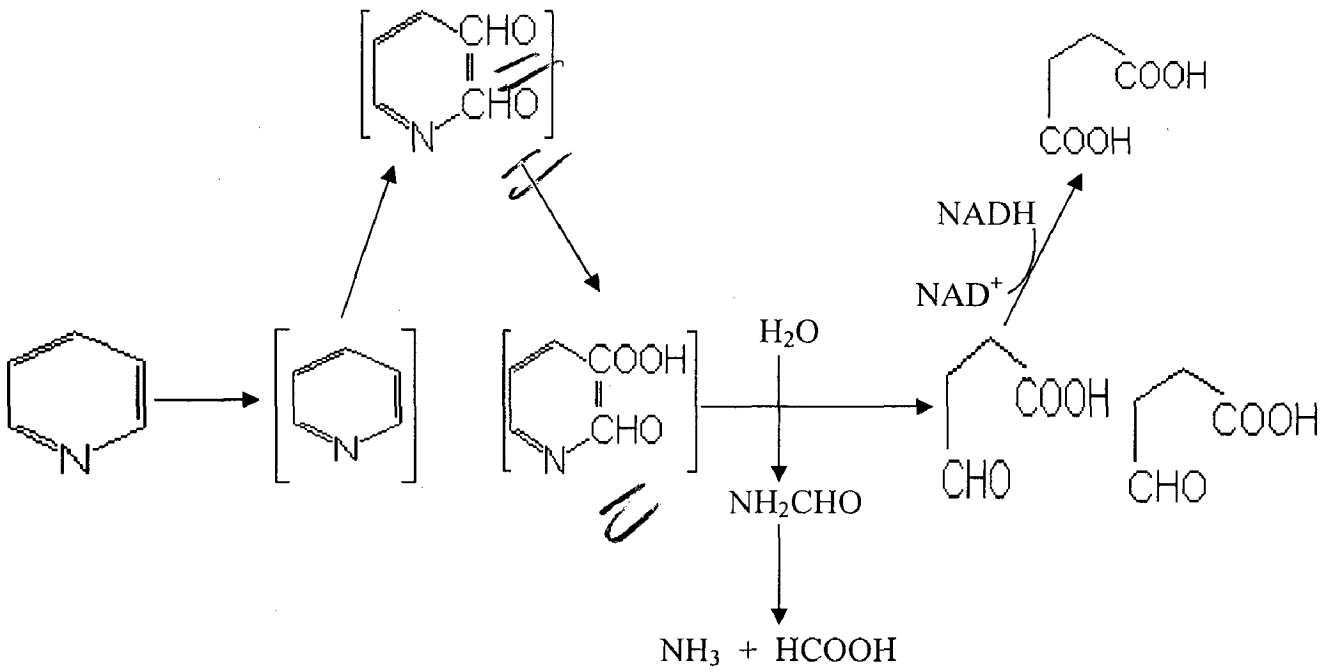


Fig 4.6: Proposed pathway for pyridine metabolism by *Micrococcus luteus* (Sims et al., 1986)

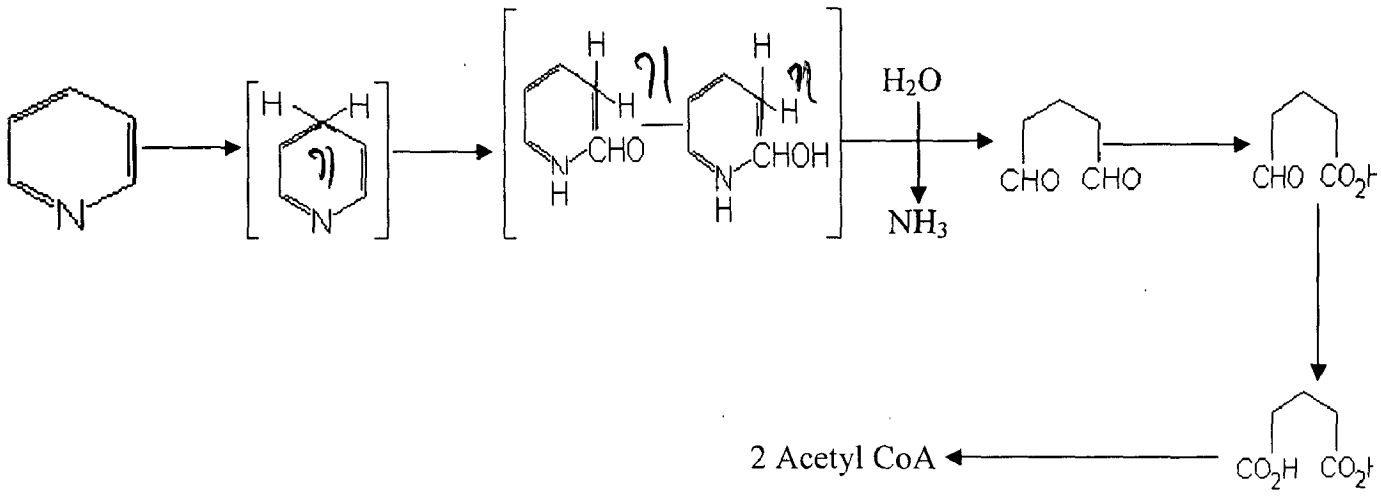


Fig 4.7: Proposed pathway for pyridine metabolism by *Azoarcus* sp. (Watson & Cain, 1975)

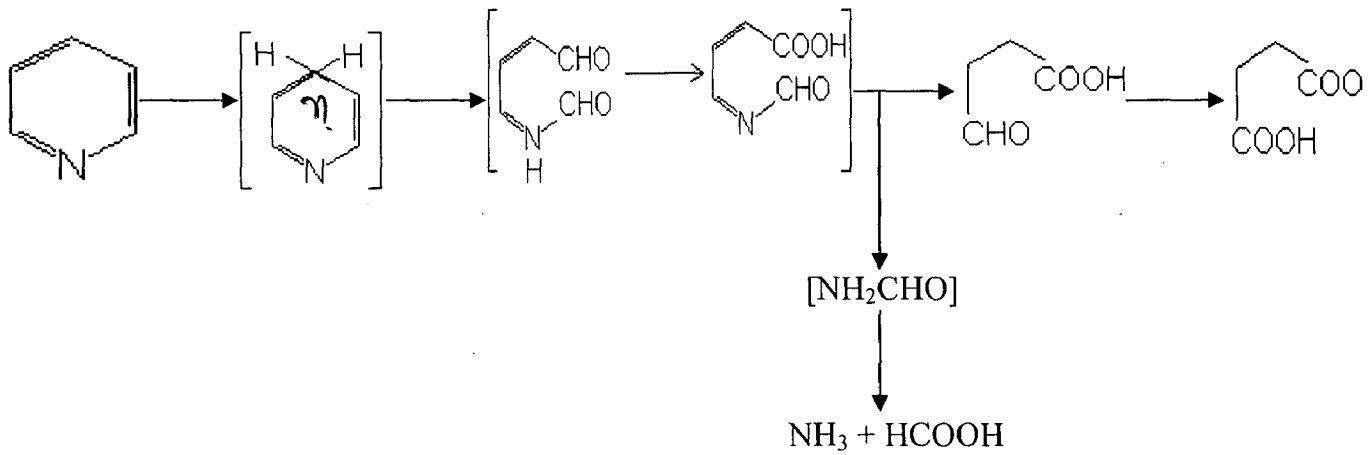


Fig 4.8: Pathway for pyridine metabolism by *Bacillus strain* (Watson & Cain, 1975)

Author	Packing media	Temperature	pH	VOC(s) treated	Result
J.M. Barnes et al. 1995	Wod compost with wood chip	**37^{O}C**	**6-7**	Oxides of nitrogen	90% removal
M. Mohseni & D. Grant Allen. 2000	Oak & maple wood chips with perlite	39-40 ^{O}C	**7.5-7.8**	Methanol & α-pinene	90-95% removal of both
C. Lu et al. 2001	Coal particles	23-27 ^{O}C	7	Ethylacetate	95% & 90% removal for loading up to 490 and 810 $gm^{-3}h^{-1}$
Chang et al. 2001	Coal particles	25 ^{O}C	7	Ethylacetate & Xylene	70 to 97% removal with changing loading and EBRT
M. Delhomenie et al. 2002	Vegetable & sewage compost with binder	21-25.7 ^{O}C	7	Toluene	Up to 95% removal
H.H.J. Cox, M.A. Deshusses. 2002	Polypropylene	25 ^{O}C	4.5 & 7	H_2S & Toluene	100% removal of H_2S in all condition and toluene at pH 7
Y. Liu et al. 2002	Lava, compost , soil at ratio of 3:5:2	20-25 ^{O}C	5.5-7.08	Ethylacetate & Toluene	Up tp 90% removal of both
G. Spigno et al. 2003	Sterilized granular expanded clay	30 ^{O}C	7	Hexane	Up to 95% removal in combination
W.M. Moe, B. Qi. 2004	Polyurethane foam	23 ^{O}C	5	n-butyl acetate, methyl ethyl ketone, methyl propyl ketone, toluene	Over 90% removal of all
L. Otten et al. 2004	mature compost & perlite	40 ^{O}C	6.7	Butyric acid	Up to 100% removal
N. Khamar et al. 2004	Peat & activated carbon	20-25 ^{O}C	7	methanol, acetone, MEK, MIBK, ethyl acetate, butyl acetate, toluene,	80% removal

				ethylbenzene, p-xylene, 1.2-dichloroethane, dichloromethane	
R. Dehghanzadeh et al. 2005	Yard waste compost mixed with shredded hard plastics (25:75)	30 ^{O}C	6.9	Styrene	88% removal
D. Kim et al. 2005	Palletized diatomaceous earth	20 ^{O}C	7	Styrene, toluene, MEK, methyl isobutyl ketone	Up to 99% removal
L. Li, J.X. Liu. 2006	Foam cubes	15 to 33 ^{O}C	4.5-6.5	Styrene	up to 90%
A.K. Mathur et al. 2006	Coal with activated sludge from sewage	25 ^{O}C	7-7.5	Mono chlorobenzene	up to 90% removal
E. Pagans et al. 2006	organic fraction of municipal sold waste, raw sludge, anaerobically digested wastewater sludge, animal by products & bulking agent	35 ^{O}C	8.7-9	General compost VOCs	82 to 97% removal
B. Qi, W.M. Moe. 2006	Polyurethane foam	25 ^{O}C	3	acetone, MEK, toluene, ethylbenzene, p-xylene	Overall removal of 90%
S. Potivichayanon et al. 2006	Plastic Packing	25-28 ^{O}C	6.4-7.2	H_2S	Up to 98% removal
Y. Jin et al. 2007	Pall ring	20-25 ^{O}C	2	H_2S & methanol	100% removal
M. Koutinas et al. 2007	Pall ring	30 ^{O}C	6.95-7.05	Fluorobenzene, 1,2-dichloroethane	Up to 92% removal

A.K. Mathur et al. 2007	Compost, sugar cane bagasse, granulated activated carbon (55:30:15)	28-32 ^{O}C	7	benzene, toluene, ethyl benzene and o-xylene (BTEX)	99% removal of all
F. Lahane et al. 2007	Polypropylene	25 ^{O}C	7	Methanol, MEK, MIBK, ethylacetate, butyl acetate, ethylbenzene, xylene, 1,2-dichloroethane	Up to 80% for mixture
D.R. Nielsen et al. 2007	----------	30 ^{O}C	6.6	Benzene	100% removal

Table 4.4: List of Biological Treatment Processes Applied for various VOCs

Chapter 5

EXPERIMENTAL PROGRAMME

This chapter details the experimental procedure adopted, analytical methods followed during the analysis of experimental samples and various materials used for the experiment. Column study was carried out to understand the affectivity of soybean and puffed rice as a gas phase adsorbent. Then for understanding the biodegradation of pyridine by *bacillus Subtilis* the effect of temperature, pH and initial concentration was studied. Then attempt was made to treat gas phase pyridine in a biotrickling filter using soybean as the packing material.

5.1 Materials:

5.1.1 Adsorbate:

The chemical used in the study were of analytical reagent (AR) grade. The adsorbate pyridine (Synonym: Azobenzene) with molecular weight of 79.1 and chemical formula of C_5H_5N was obtained from Rankem of Ranbaxy ltd.

5.1.2 Adsorbent:

Soybean and puffed rice were being selected as the adsorbent for gas phase adsorption process. Both the materials were obtained from local market at throwaway prices. They were made dust free by sieving. The powder was also removed by this process.

5.1.3 Stock solution:

Stock solution of pyridine was prepared by dissolving 1.02 ml of pyridine in water to make 1000 ml solution. Thus stock solution of 1000 ppm concentration was obtained.

5.1.4 Microorganism:

A pure culture of *Bacillus Subtilis* was obtained in slant form in a test tube from department of Biotechnology, IIT Roorkee.

5.1.5 Growth medium:

Apart from the carbon source, the bacteria require many macro and micronutrients. for these purpose Nutrient agar and nutrient broth medium was used

5.1.6 Revival of culture:

The culture thus obtained could not have been used directly for experimental work and degradation studies and hence needed to be revived. The culture was revived as per the standard procedure using sterilized nutrient broth solution.

5.1.7 Basal salt media:

The basal salt medium used for the present study is given in the following table . The final volume of the BSM after addition of salts was 1 l. The pH of the medium was adjusted to 7.0 ±0.1.

K_2HPO_4	0.91 gm/l
$Na_2HPO_4, 2H_2O$	2.39 gm/l
$FeSO_4, 7H_2O$	0.2 gm/l
$MgSO_4, 7H_2O$	2 gm/l
$MnSO_4, 7H_2O$	0.0009 gm/l
$Na_2MoO_4, 2H_2O$	0.001 gm/l
$CaCl_2$	0.003 gm/l
$ZnSO_4, 7H_2O$	0.00004 gm/l

Table 5: Composition of Basal salt medium (BSM)

5.2 Methods:

5.2.1 Determination of pyridine concentration in gas phase:

Concentrations of pyridine gas was analyzed using a Netel India Limited (model- MICHRO 9100) gas chromatograph equipped with a capillary column of type HP5 (30m * 0.249mm * 0.25μm film thickness) and a flame ionization detector. The injector, oven and detector temperature were maintained at 210 °C, 60 °C, and 230 °C, respectively. The hydrogen gas was used as the fuel and nitrogen was used as the carrier gas at a flow rate of 20 mL/min. The calibration curve was prepared by injecting known amounts of the pyridine into a sealed bottle equipped with a Teflon septum according to the standard procedure [Lodge 1989]. The injected amount of pyridine was allowed to evaporate in the air space within the bottle at the experimental temperature (30 °C). For the calibration, air samples were drawn from the bottle by a 1 mL gas tight syringe (Hamilton-Bonaduz-Schweiz) and analyzed by gas chromatograph. Gas samples were collected at regular intervals of time from the inlet, outlet sampling ports provided at reactor by using an airtight syringe and analyzed for residual pyridine.

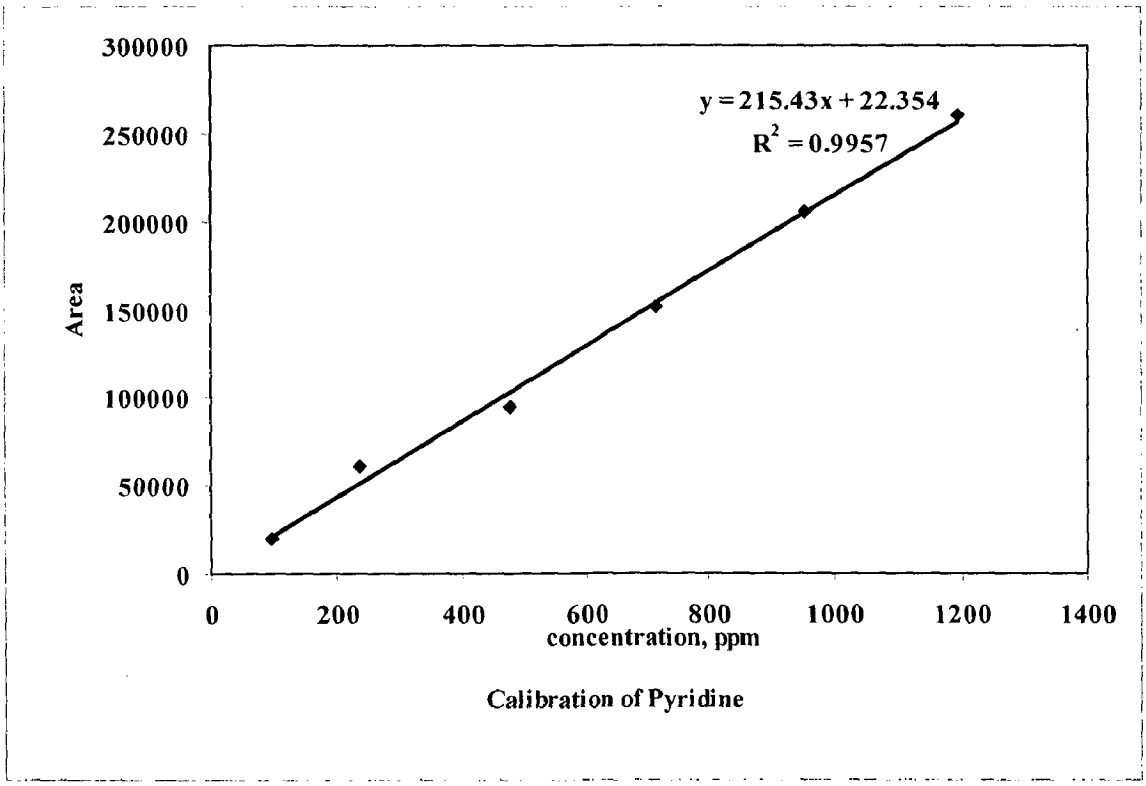


Fig 5.1: Calibration curve for determination of pyridine concentration in gas chromatography

5.2.2 Determination of pyridine in liquid phase:

The concentration of the pyridine in the solutions was measured by using UV/VIS Spectrophotometer [Model Perkin Elmer]. The calibration curve was plotted by reading the absorbance at 256 nm for pyridine. The calibration curve for pyridine was prepared by using pyridine of known concentration from the stock solution. The samples were diluted to bring the concentration of compound to the straight line portion of the absorbance plot. pH and dilution do not affect the absorbance. Filtration of samples did not influence the measurements.

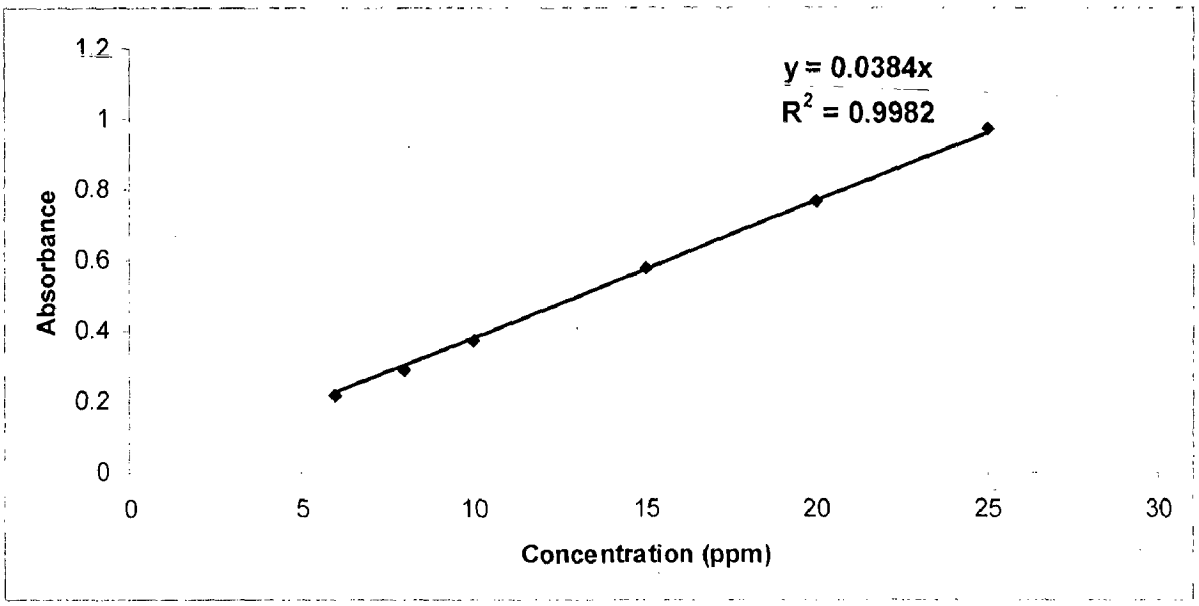


Fig 5.2: Calibration curve for determination of pyridine concentration in UV/VIS Spectrophotometer

5.2.3 Sterility:

All the glassware along with the solid and liquid medium were sterilized in a autoclave at 1.05 kg/cm^2 for 20 min. Transfer of bacteria and media were all done in an inoculation chamber.

5.2.4 Method of transfer:

Liquid medium containing composition of nutrient growth medium was prepared in distilled water in two 250 ml conical flasks. For solid medium appropriate quantity of agar was added to one of the flask containing liquid medium. Both these flasks were autoclaved for 20 min at 1.05 kg/cm^2. While hot, the conical flask containing agar medium was poured into sterilized Petri dishes. On cooling, the liquid medium with agar was solidified. Inoculation was done in the inoculation chamber

5.2.5 Maintenance of isolated culture:

Maintenance of isolated strain was done by periodical transfer onto nutrient agar slant on Petri plates and was allowed to grow by leaving the plates at 30 ^{O}C for 24 hours. The plates were then stored at 4 ^{O}C for further study. Nutrient agar solution was prepared by mixing 2% agar with nutrient broth and then the mixture was autoclaved and cooled after pouring in Petri plates.

5.2.6 Acclimatization of the culture:

The culture was cultivated in 250 ml flask containing 100 ml of the BSM with pyridine as the sole carbon. The cultures were acclimatized to pyridine by exposing the culture in a series of shake flasks. The startup of acclimatization was obtained by inoculating 100 ml of BSM (pyridine concentration, 10 mg l^{-1}) with *B. Subtilis* from nutrient agar slants, under sterile conditions. After 48 hours of incubation at 30 ^{O}C, 5 ml of this culture was added to fresh BSM (10 mg l^{-1} pyridine) as inoculum. 48 h later, a third fresh BSM was also inoculated with 5 ml of the last culture to ensure that the bacteria were already adapted to pyridine.

5.2.7 Cell growth:

The cell growth was monitored by measuring the optical density in a UV spectrophotometer at a wavelength of 340 nm. For the separation of biomass the samples were centrifuged at approximately 8000 rpm for 10 min. The supernatant was decanted and used for the determination of the Pyridine concentration.

5.3 Adsorbent Characterization:

5.3.1 Proximate Analysis:

A small amount of the adsorbent was finely ground and a representative sample was taken for analysis. Sample was divided into two portions. The first portion of the sample was placed in a silica crucible and its moisture was determined. To determine the moisture content, sample was weighed and kept in oven at 105 ^{0}C, for 1 h. After 1 h the dry weight of sample was taken and % moisture was determined from the difference of initial weight and final weight (dry weight). After that, the sample was heated to 750 ^{0}C in a muffle furnace and maintained at this temperature for 2 h or more till a constant weight of the residue was obtained. The weight of the residue represented the ash content of the adsorbent.

5.3.2 Bulk density:

Bulk density of the adsorbents was determined by using MAC bulk density meter.

5.3.3 Scanning Electron Microscopic analysis:

To understand the morphology of the adsorbents before and after the adsorption of pyridine, the scanning electron microscope (SEM) micrographs were taken using LEO, Model 435 VP, England. The adsorption particles were first gold coated to make the sample conductive, using Sputter Coater, Edwards S150, then the SEMs were taken at various magnification.

To understand the morphology of the packing materials and microorganisms before and after the experimentation, samples were analysed using scanning electron microscope (SEM, LEO 435 VP, UK). For SEMs, the packing materials and microorganisms were fixed with 2% (v/v) glutaraldehyde aqueous solution for 2 h at less than 20 ^{o}C, washed with phosphate buffer and then dehydrated with ethyl alcohol. Dehydration is accomplished by passing the fixed specimen through a graded series of increasing concentration of dehydration agent (ethanol) in water. The samples were treated with the alcohol gradients of 30%, 50%, 70%, 90% and 100% for dehydration.

Samples were kept for 15 min each upto 70% alcohol gradient, there after treated for 30 min each for subsequent alcohol gradients. After passing through 100% alcohol, samples were dried and given a metal coating with gold using Sputter Coater, Edwards S150, which provides conductivity to the samples, and then the SEMs were observed under various magnifications.

5.3.4 Pore size distribution:

Textural characteristics of adsorbents were determined by nitrogen adsorption at 77.15 K to determine the specific surface area and the pore diameter using an ASAP 2010 Micromeritics instrument and by Brunauer-Emmett-Teller (BET) method (Brunauer et al., 1938), using the software of Micromeritics. Nitrogen was used as cold bath (77.15 K).

5.3.5 Fourier Transform Infra red (FTIR) spectral analysis:

FTIR spectrometer (Thermo nicolet, Model Magna 760) was employed to determine the presence of functional groups in the adsorbents before and after the adsorption of pyridine at room temperature. Pellet (pressed-disk) technique has been used for this purpose. The sample was mixed with KBr (IR spectroscopy, grade) thoroughly and pellet was made by using a special mold provided to make pellet under the pressure of 15 ton. The spectral range was from 4000 to 400 cm^{-1}.

5.4 Experimental Details:

5.4.1 Continuous column reactor:

The rector consists of Perspex pipe with internal diameter of 5 cm and effective packing bed height of 90 cm. Total length of the pipe is 126 cm and total volume is 1.767 L. The reactor consists of three sections each of which consists of 30 cm length section where the adsorbent material had been packed. An estimated 15 cm length had been left open at the bottom and top of the reactor for easy entry and exit of the gaseous flow. An acrylic sieve mesh had been provided at the bottom of each section for holding up the packing material in each section. A 3 cm plenum is being provided between every 2

sections for sampling of gaseous sample for analysis. The compressed air was being passed through cleaning process to free it from any moisture, oil and particulate matter if any were present. The filtered and purified air stream was split in to two halves, major and minor halves. The flow rates of both streams were controlled by rotameters (JTM, Japsin Industrial Instrumentation, India). Rotameter used for higher flow rate can be varied between 1-10 l/min. Rotameter used for minor flow rate can be varied between 0-1 L/min. For producing pyridine loaded air stream of required concentration, the minor was made to pass through glass bottle containing pyridine and through the humidifier. These two streams were again mixed further in a third bottle called mixing bottle. The stream out of this mixing bottle was then mixed with the major stream of purified air. This was final stream of desired pyridine concentration that was fed to the bottom of the reactor. Pyridine concentration was further controlled by the bras control valve provided at the inlet of the bottle containing pyridine.

5.4.2 Biological batch studies:

Batch studies of biodegradation process were carried out in 250 ml flasks taking 100 ml samples. This enabled us to understand the effect of temperature, pH and initial concentration of pyridine on biodegradation process. These batch studies also allowed us to understand the growth kinetics.

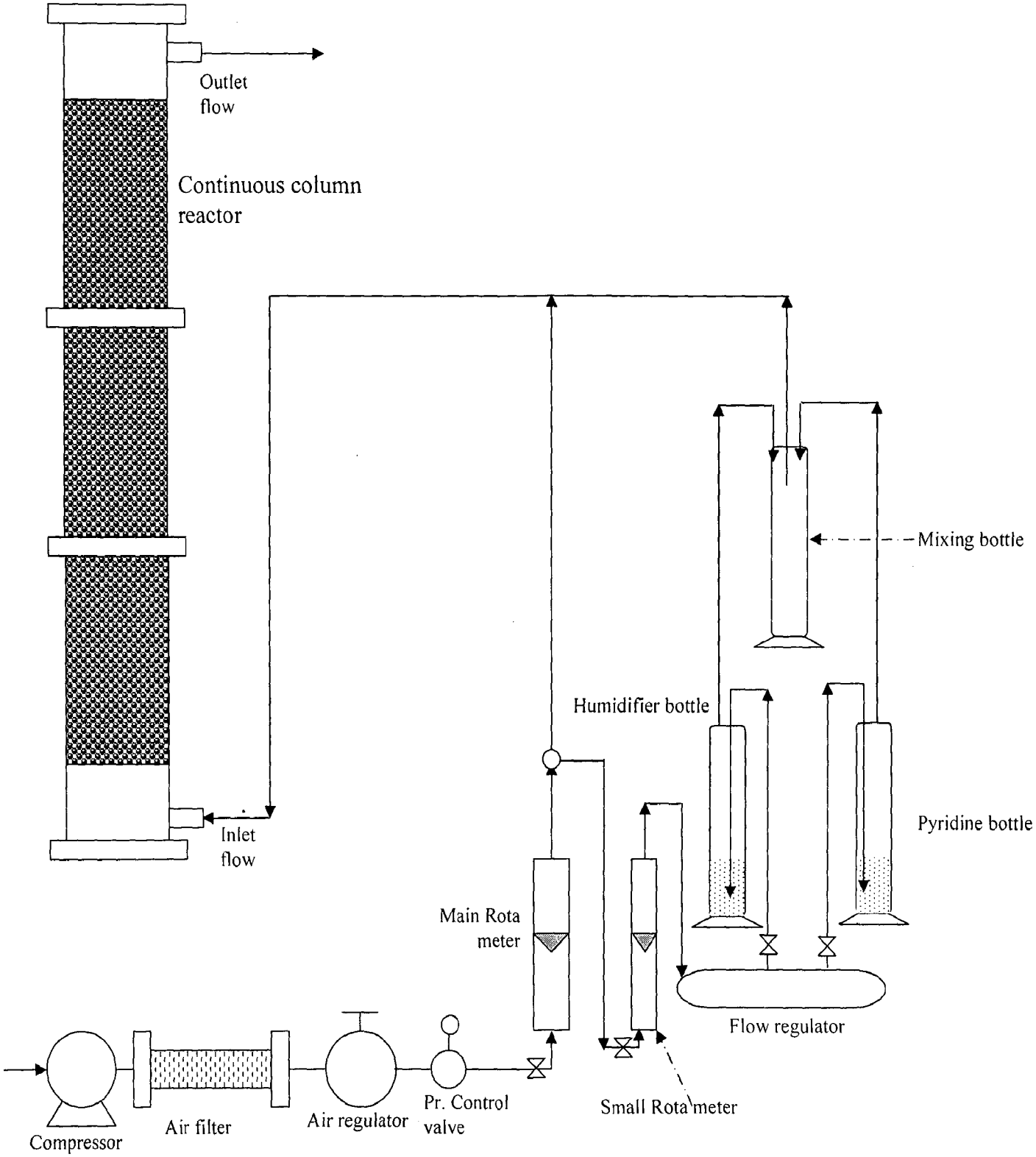


Fig 5.3: Schematic diagram of continuous reactor set up for adsorption study

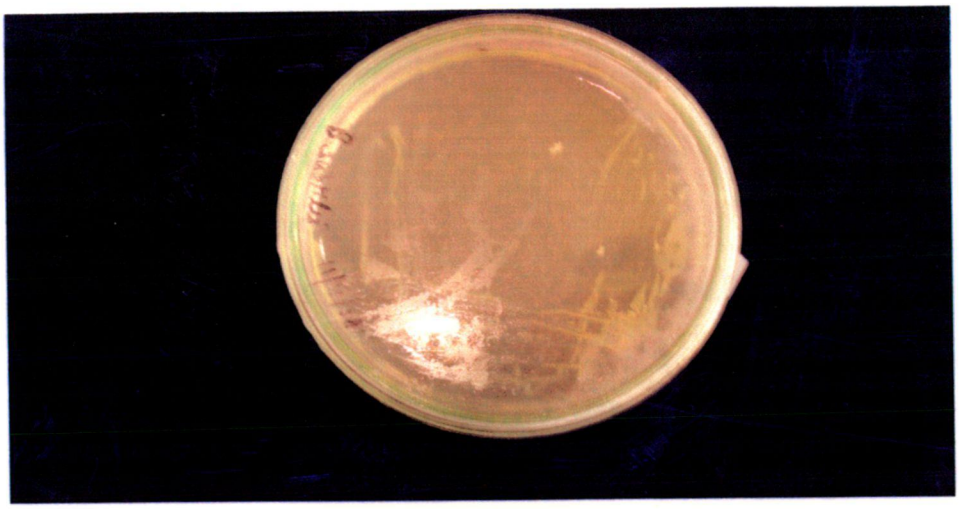


Fig 5.4: Plate with *Bacillus Subtilis* grown on nutrient agar

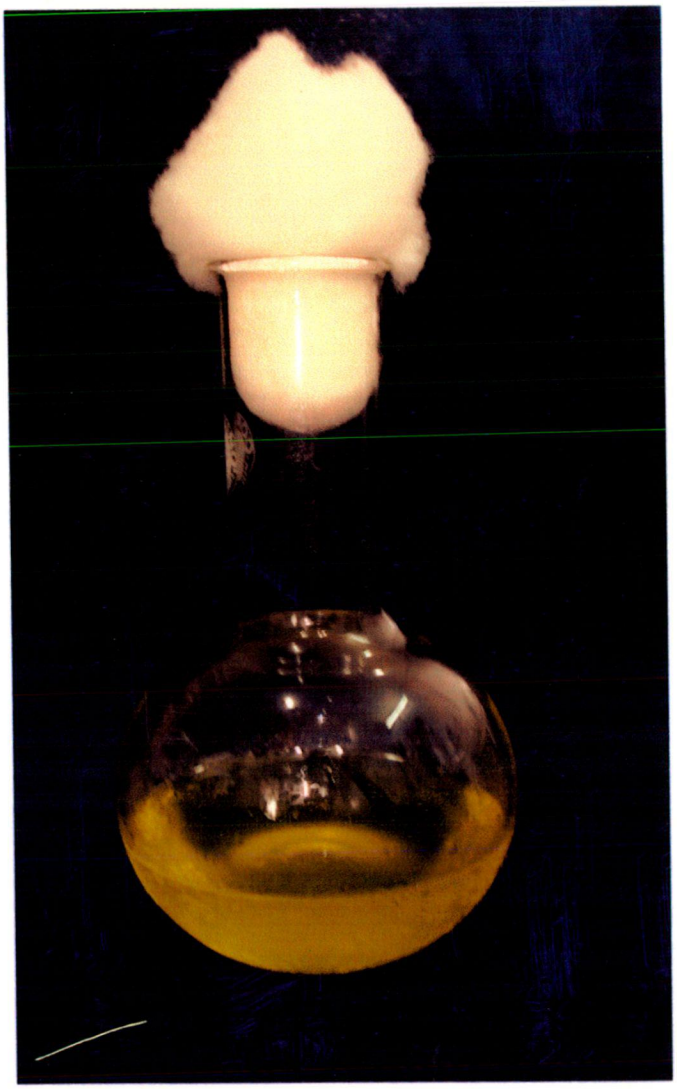


Fig 5.5: *Bacillus Subtilis* grown on nutrient broth solution

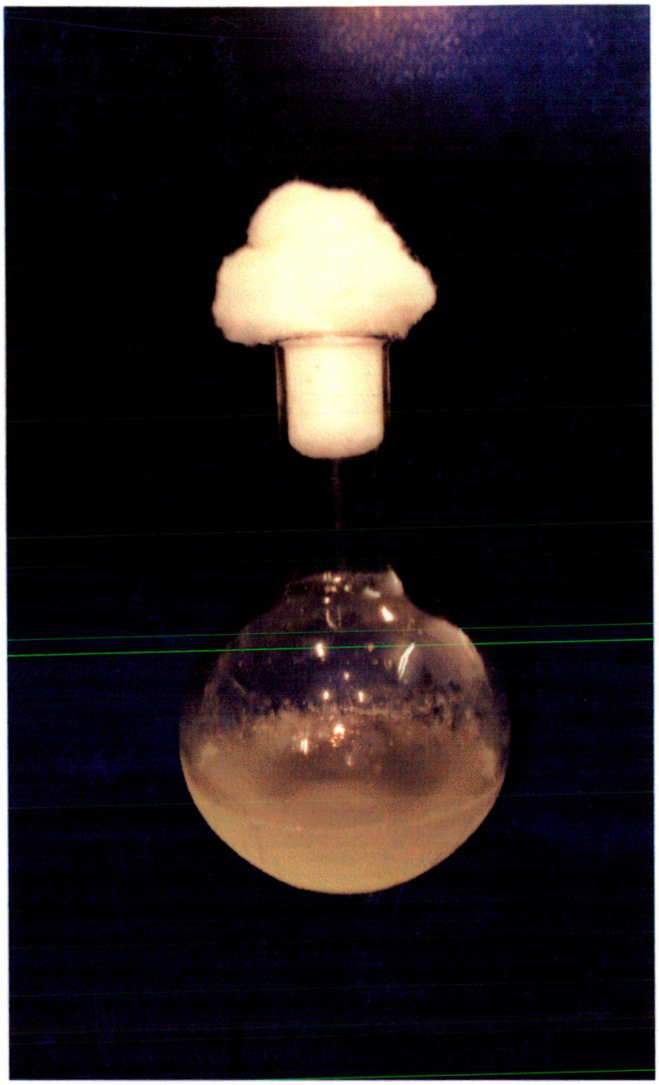


Fig 5.6: *Bacillus Subtilis* grown on basal salt medium solution

RESULT AND DISCUSSION

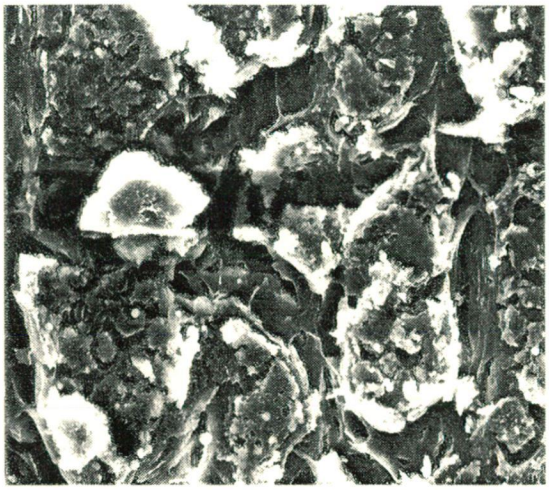
6.1 General:

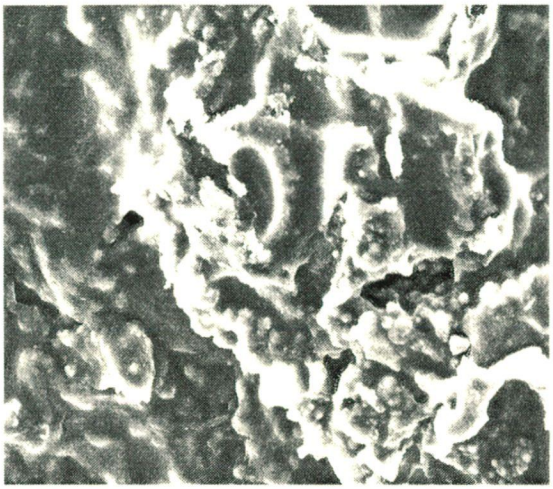
The aim of the dissertation is to remove pyridine in continuous gas phase system as well as to find out the affectivity and efficiency of *bacillus Subtilis* in biologically degrading pyridine as sole carbon source. In the first part the aim has been to carry out continuous adsorption of pyridine in gas phase in a fixed bed reactor using soybean and puffed rice as the adsorbents for gas phase adsorption. Attempt has been made to characterize these two adsorbents as well. The continuous adsorption process has been studied for both the adsorbents for varying inlet concentration and inlet flow rate. The resultant breakthrough curves have been studied for the usefulness and affectivity of these adsorbents at various inlet conditions.

In the second part of this dissertation pure isolated strain of *bacillus Subtilis* has been used to treat pyridine and an attempt has been made to find out the efficiency of this bacteria. The batch experiments conducted in present study include optimization of temperature, pH and pyridine concentration. The growth kinetics that best fits the biodegradation process has also been found and then corresponding constants have been calculated. In the last part the continuous reactor has been used as the biotrickling filter with soybean as the packing material. The solution of basal salt media has been supplied from the top of the reactor and gaseous pyridine has been supplied from the bottom of the reactor at desired inlet concentration and inlet flow.

The chapter has been divided into following parts:

1. Characterization of the adsorbents
2. Continuous adsorption study of gaseous pyridine in column reactor with soybean and puffed rice as adsorbents. The following parameters are being studied-
    a) Inlet concentration
    b) Inlet flow rate
3. Optimization of parameters for biodegradation of pyridine by *Bacillus Subtilis* in batch studies and thus finding the growth kinetics suiting the degradation process.

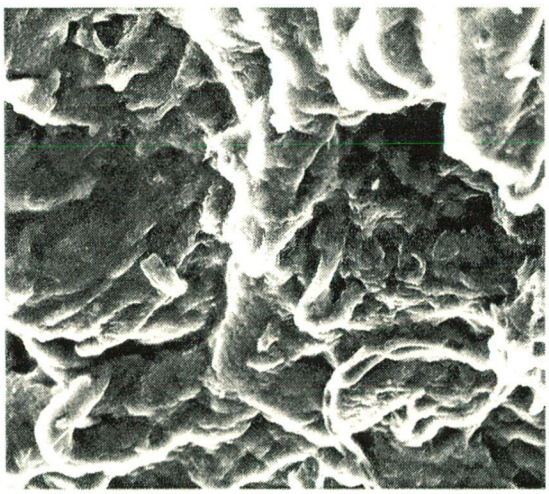


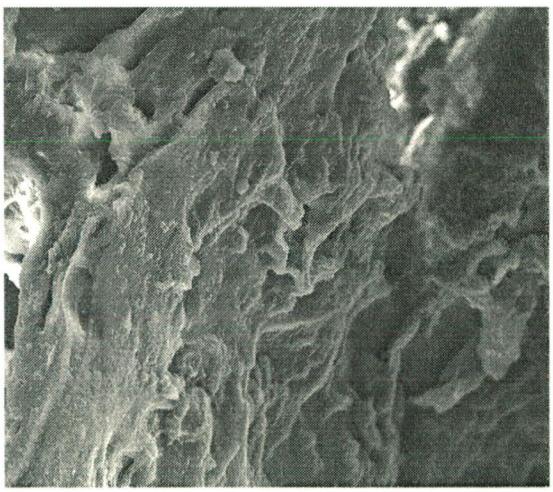


Puffed rice- Blank

Puffed rice- Pyridine

Fig 6.1: SEM of blank and pyridine loaded puffed rice at 1000X

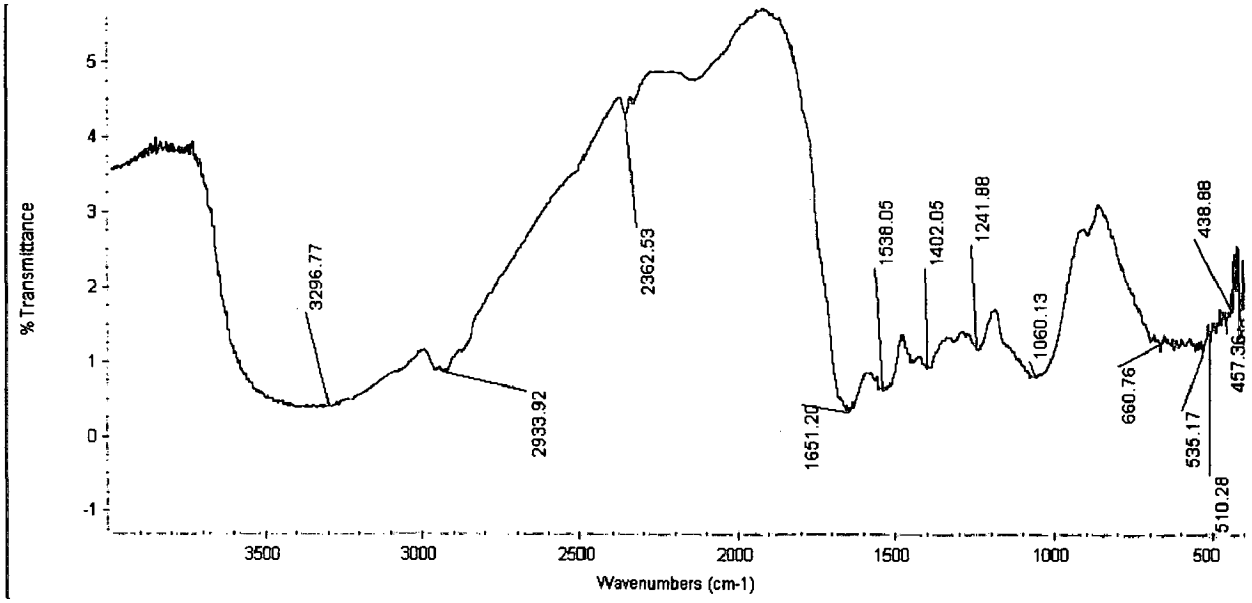




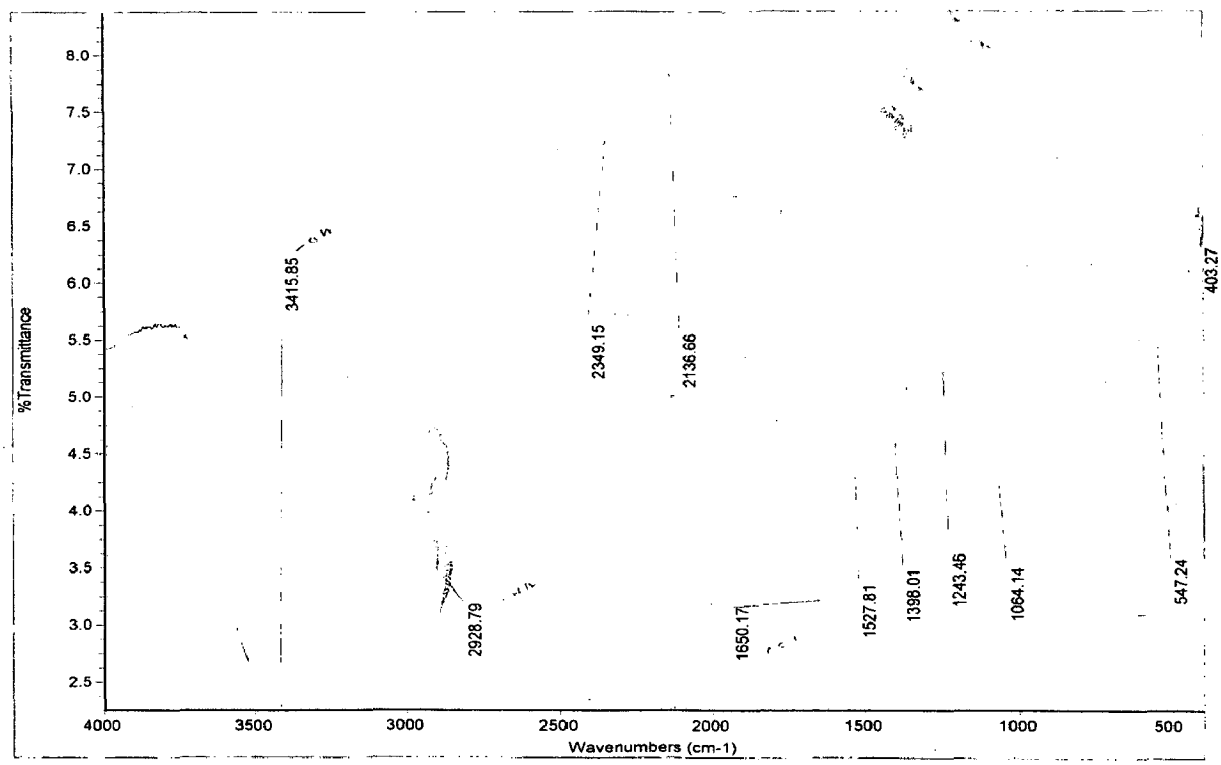
Soybean- Blank

Soybean- Pyridine

Fig 6.2: SEM of blank and pyridine loaded soybean at 1000X

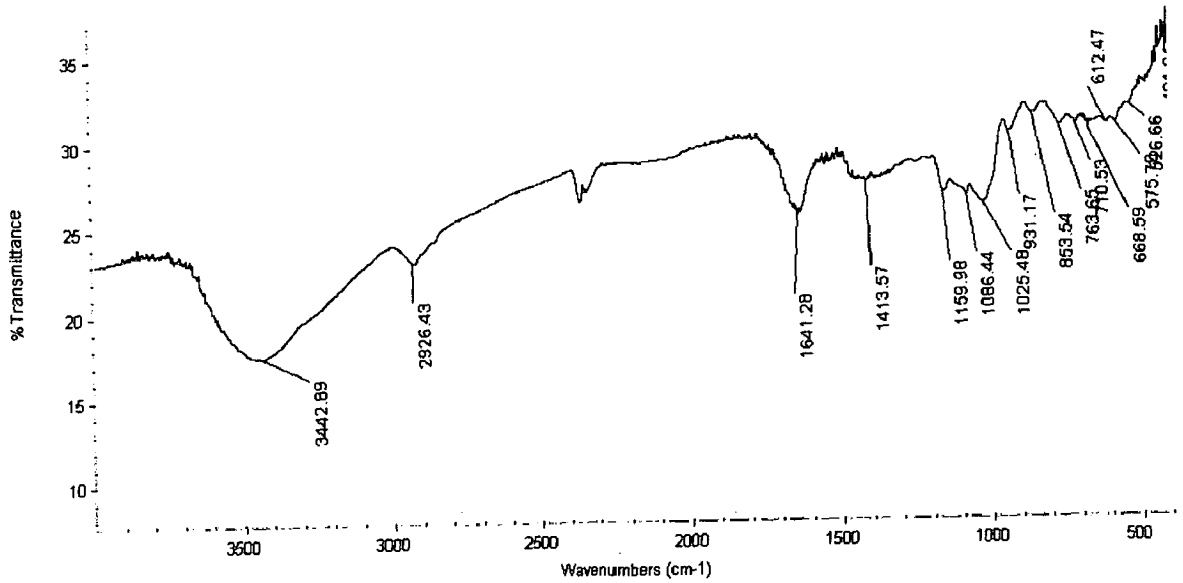


Soybean- After adsorption

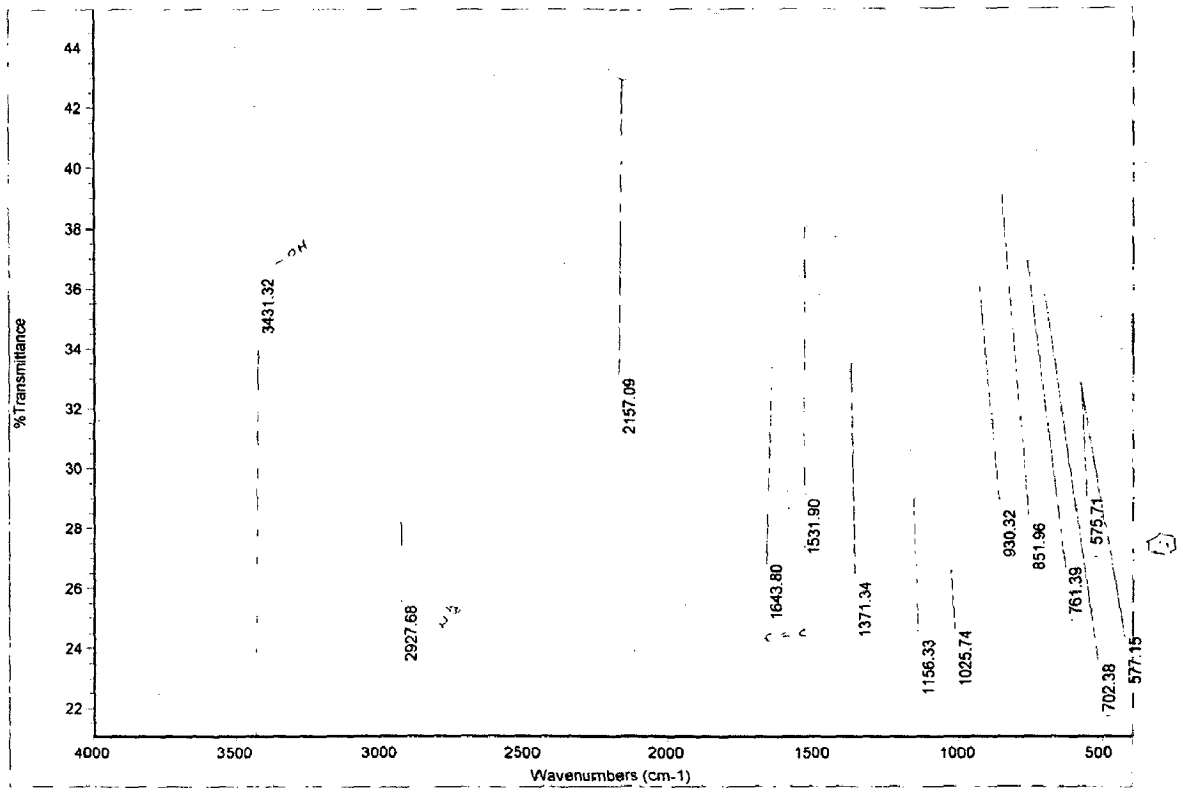


Soybean- Blank

Fig 6.3: FTIR spectra of soybean after and before adsorption

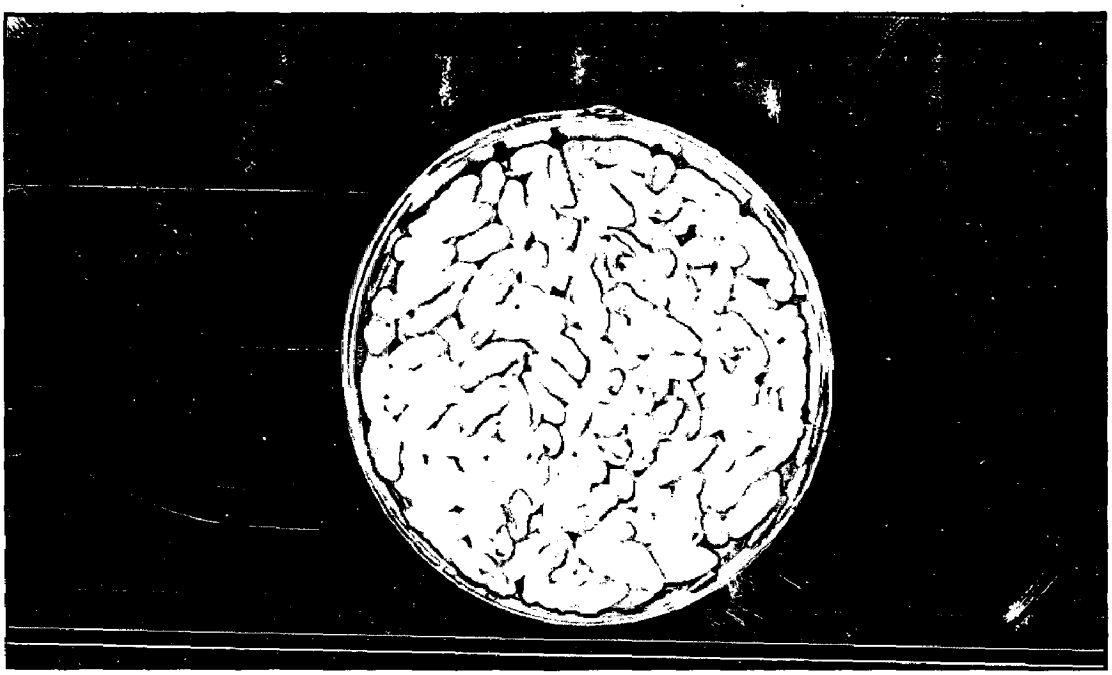


Puffed rice- After adsorption

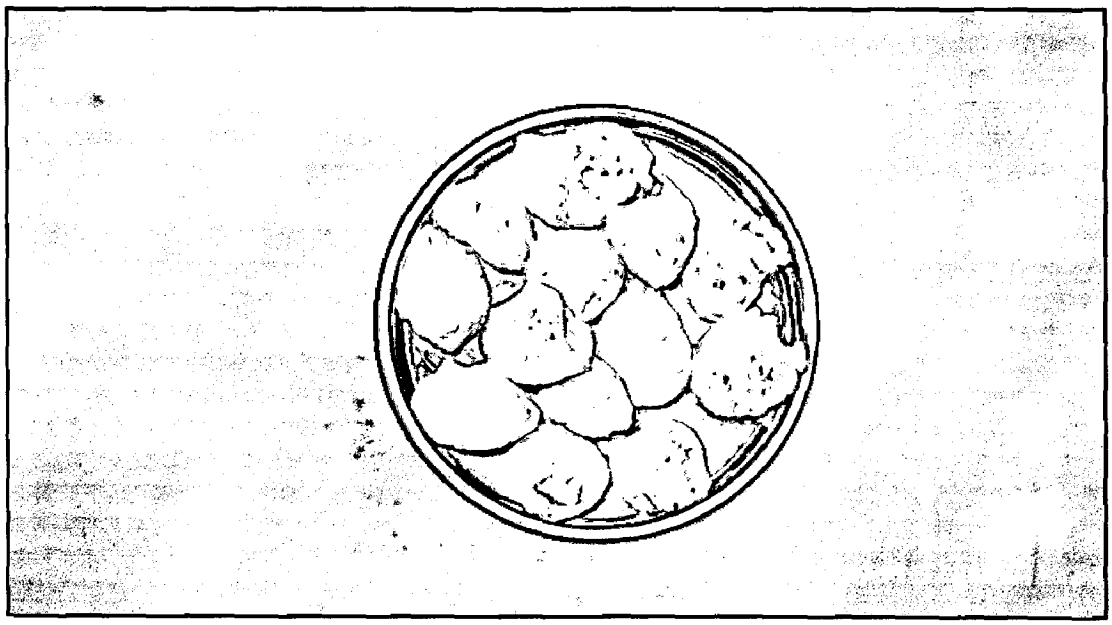


Puffed rice- Blank

6.4: FTIR spectra of puffed rice after and before adsorption



Puffed rice



Soybean

Fig 6.5: Photographs of Puffed rice and Soybean respectively

The pH value of both the adsorbents was found to be slightly towards the acidic side. The pH of soybean though was slightly more acidic than puffed rice. The proximate analysis of the adsorbents revealed that soybean has slightly more moisture content than puffed rice again. Further analysis of soybean revealed that it has ash content of 75.58%. Bulk density of soybean measured using MAC bulk density meter showed that soybean has density value which is more than double that of puffed rice. Chemical composition analysis carried out by EDAX presented that both the compounds have similarly high carbon content in them. But then puffed rice showed higher amount of nitrogen whereas soybean showed much higher amount of oxygen compared to the other. Very small amounts of other metallic elements were detected in the analysis in both the adsorbents. BET surface area analysis showed slightly higher surface area and monolayer volume for soybean that puffed rice. The details of these above mentioned properties are being present in table 6.1. The SEM image of adsorbents taken before and after adsorption process showed the change that had occurred in their surface due to continuous adsorption performed in the reactor (Fig 6.1 and 6.2).

Another important analysis has been the FTIR spectra analysis of the adsorbents. Analysis performed before the adsorption process, i.e. for the blank samples revealed the structural detail of these organic compounds. The FTIR spectra analysis of blank soybean (Fig 6.3) showed the structural features of it. It appeared to have an O-H and N-H group together followed by aromatic C-H group. There appeared to be a C=O group around the wavelength of 1650.17. There was also an aromatic C=C in the wavelength of 1527.81. Towards the end of spectra there appeared a vinyl ether type formation along with 1^{O} alcohol together with bending aromatic C-H structure. Similarly in case of puffed rice (Fig 6.4) it also appeared to have an O-H and N-H group together followed by aromatic C-H group. There appeared to be a C=O group around the wavelength of 1643.8. There was also an aromatic C=C in the wavelength of 1531.71. Towards the end of spectra there appeared a vinyl ether type formation along with 1^{O} alcohol together with bending aromatic C-H structure. A striking similarity was found in presence of functional groups for both the adsorbents. The spectra analysis of soybean after adsorption showed remarkable change in the O-H and N-H group bonding site. Slight change could be seen

in C=O group as well. Similarly there was slight change in the C=C group bonding region. Again towards the end of the spectra major changes were detected in the spectra where vinyl ether type formation along with 1^{O} alcohol together with bending aromatic C-H structure is present. In case of puffed rice the changes were slightly different though. Here also change was visible in the region where O-H and N-H groups were present. Minor changes were also seen in the regions where C-O and C=C groups were present. In case of puffed rice the changes in various functional groups were not as clear as in soybean till the last section of spectra. In the last section of spectra where vinyl ether type formation along with 1^{O} alcohol together with bending aromatic C-H structure is present, major changes can be seen in the FTIR spectra.

6.3 Adsorption Study in Continuous Reactor:

The reactor which is divided in to three sections was filled with cleaned and dust free Soya bean. Total amount of Soya bean in the reactor was 250 grams. Each section was filled with Soya bean of amount 86.36, 71.46, 92.5 grams respectively starting from bottom. The pyridine inlet concentration was fixed at a desired concentration using the various control valves installed and the amount of inflow concentration was measured using gas chromatography, mentioned earlier. Humidified Pyridine flow was achieved passing compressed, clean and dust free air through pyridine bottle and humidifier and mixing them together. Similarly total amount of puffed rice in the reactor was 133.65 grams. Each section was filled with puffed rice of amount 43.43, 37.86, 52.36 grams respectively starting from bottom. For variation of inlet concentration total inlet flow rate was fixed at 2 LPM. Similarly for the second part where the total inlet flow rate to the reactor was varied to 4 different values the inlet concentration of pyridine was kept fixed at 100 ppm

6.3.1 Variation in Concentration:

Sl. No.	Inlet Concentration (ppm)	Time needed to achieve 100% breakthrough (hr)	
		Soybean	Puffed rice
1	100	14	12
2	200	11	10
3	400	9	8
4	500	7	5

Table 6.2: Comparison of breakthrough times for variable inlet concentrations

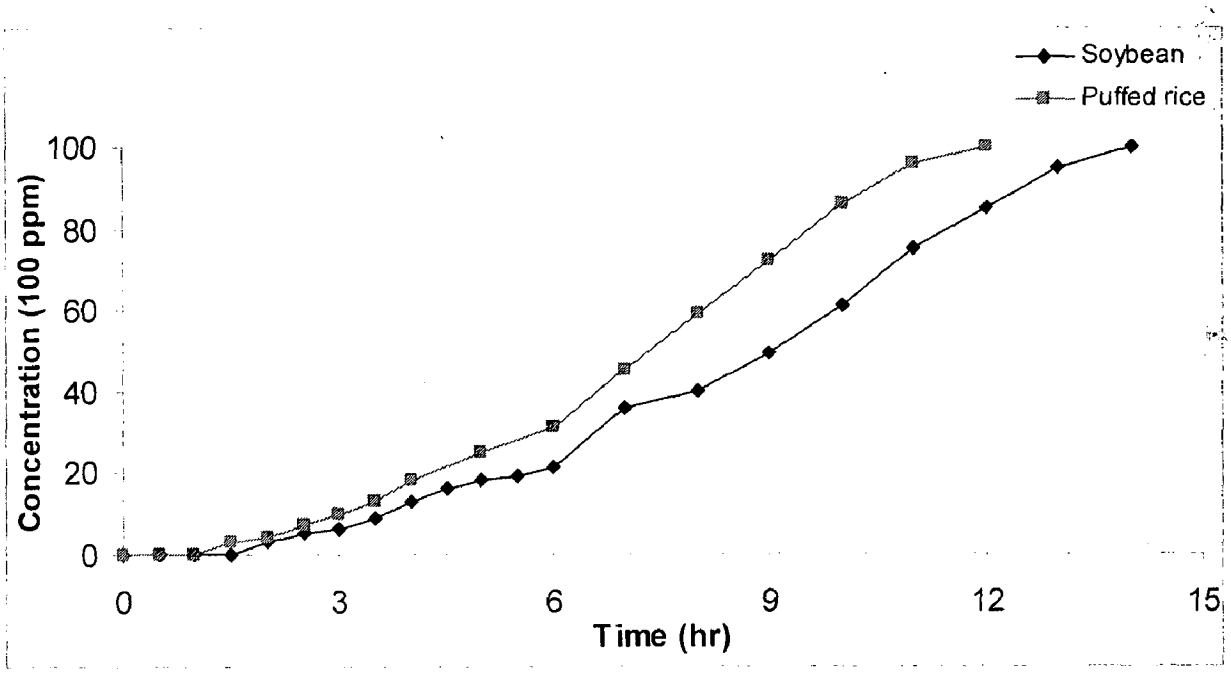


Fig 6.6: Comparison of breakthrough times for adsorption of 100 ppm inlet flow

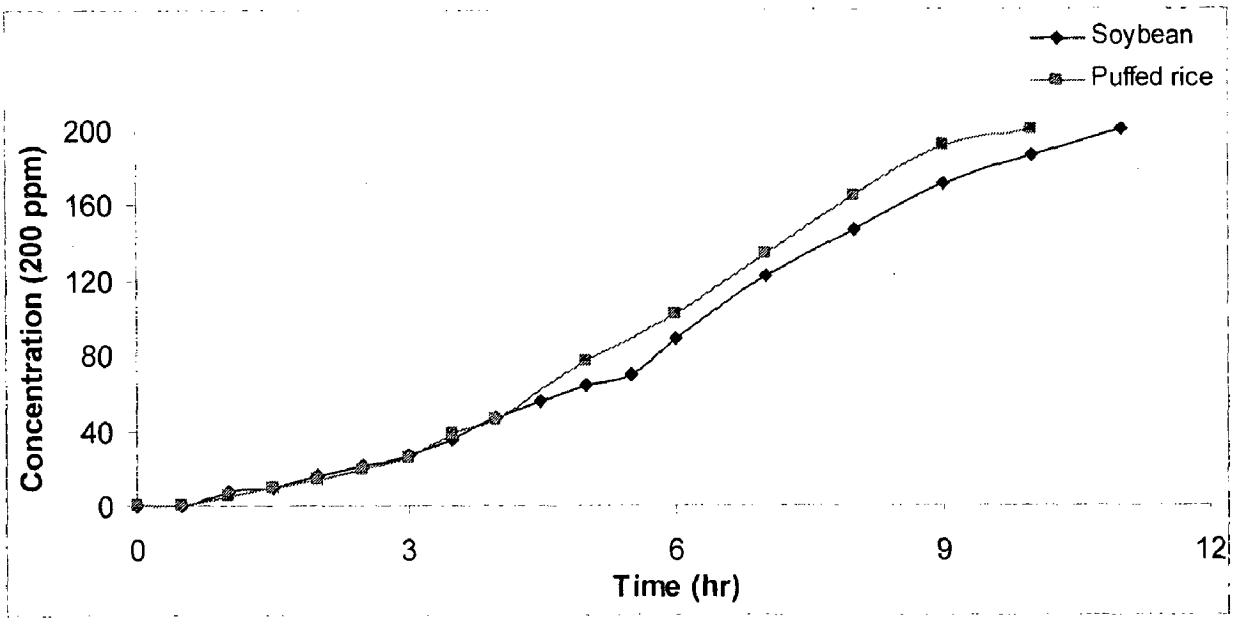


Fig 6.7: Comparison of breakthrough times for adsorption of 200 ppm inlet flow

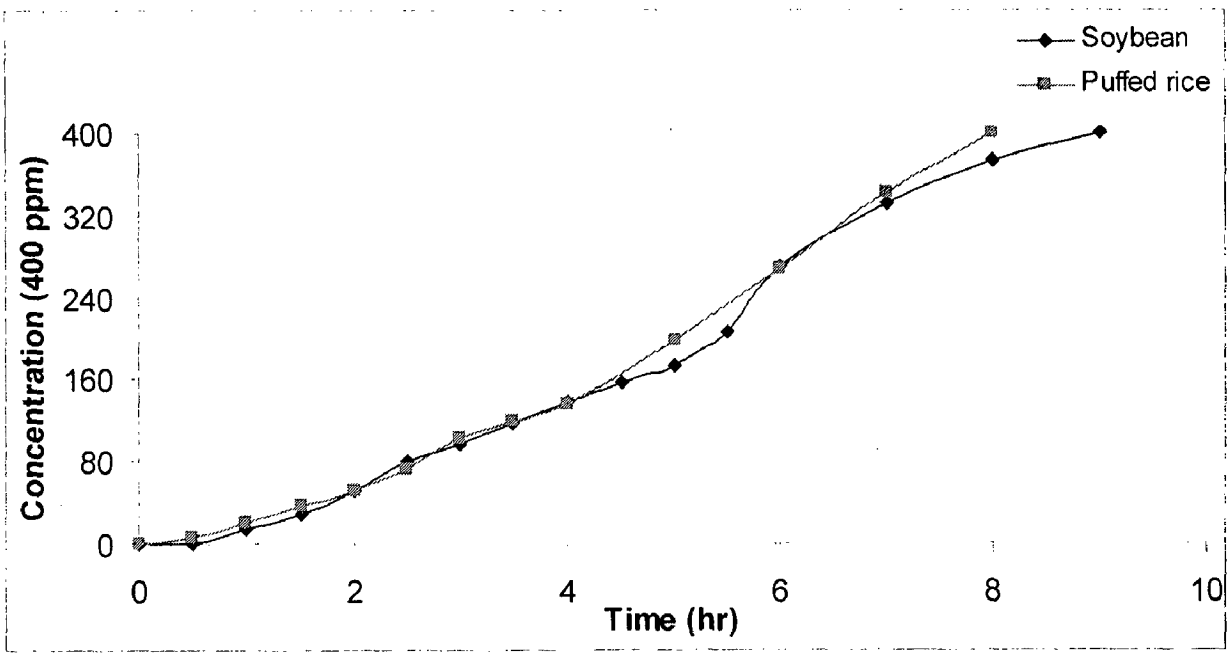


Fig 6.8: Comparison of breakthrough times for adsorption of 400 ppm inlet flow

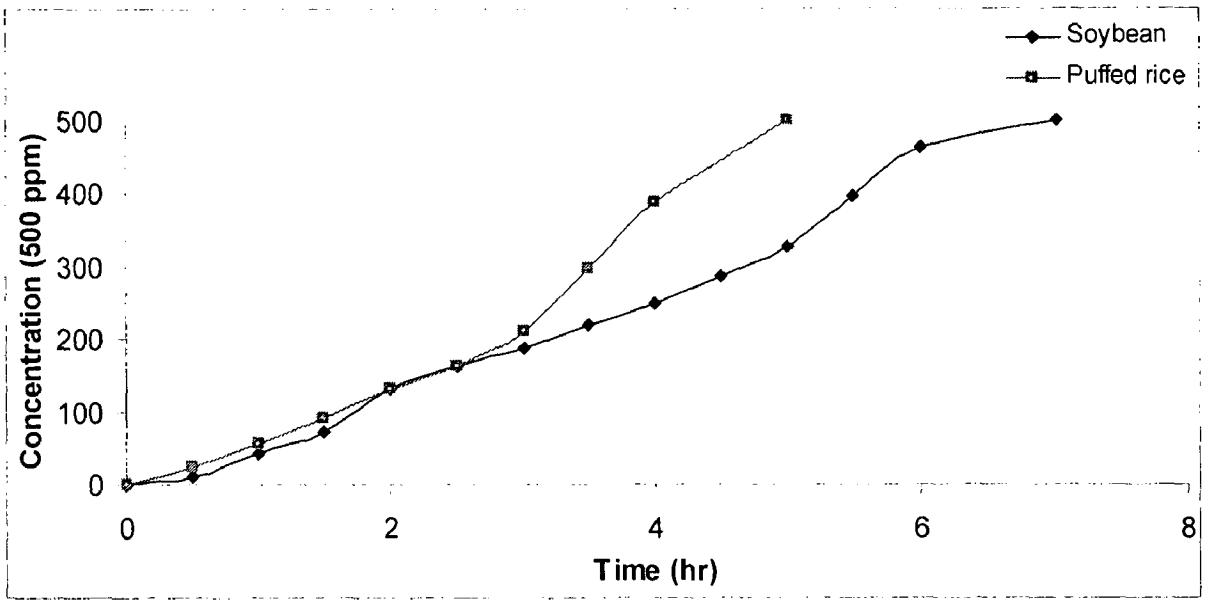


Fig 6.9: Comparison of breakthrough times for adsorption of 500 ppm inlet flow

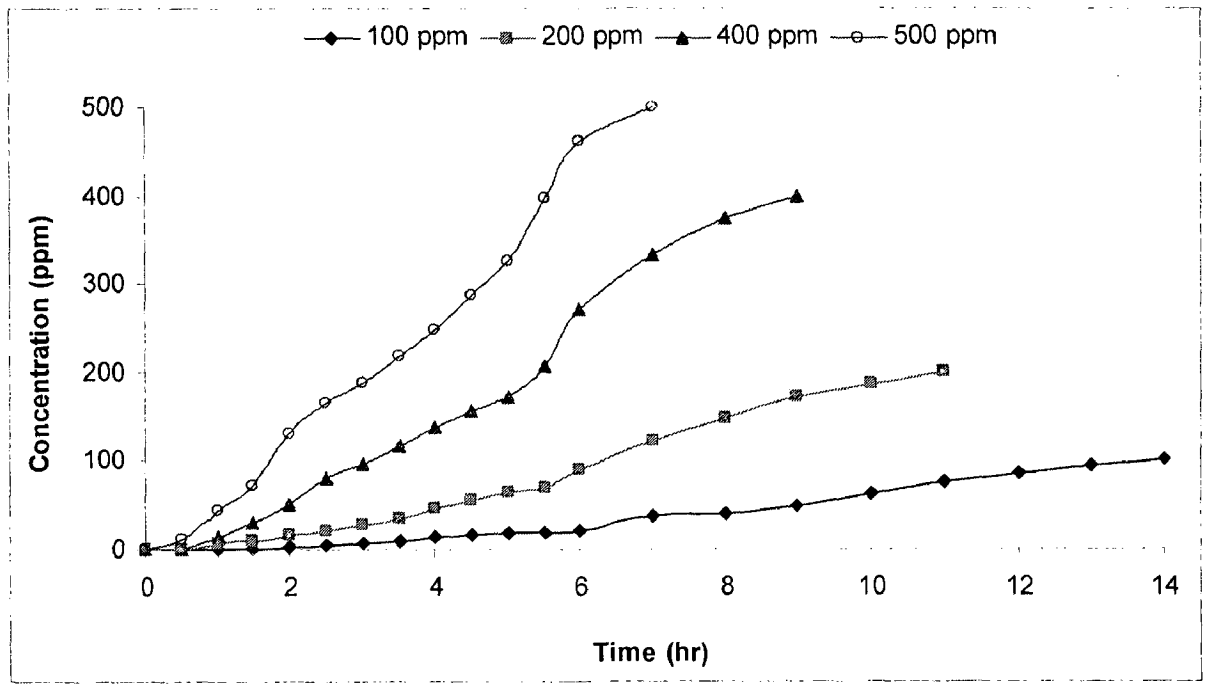


Fig 6.10: Breakthrough curves for removal of pyridine of various concentrations using soybean as adsorbent

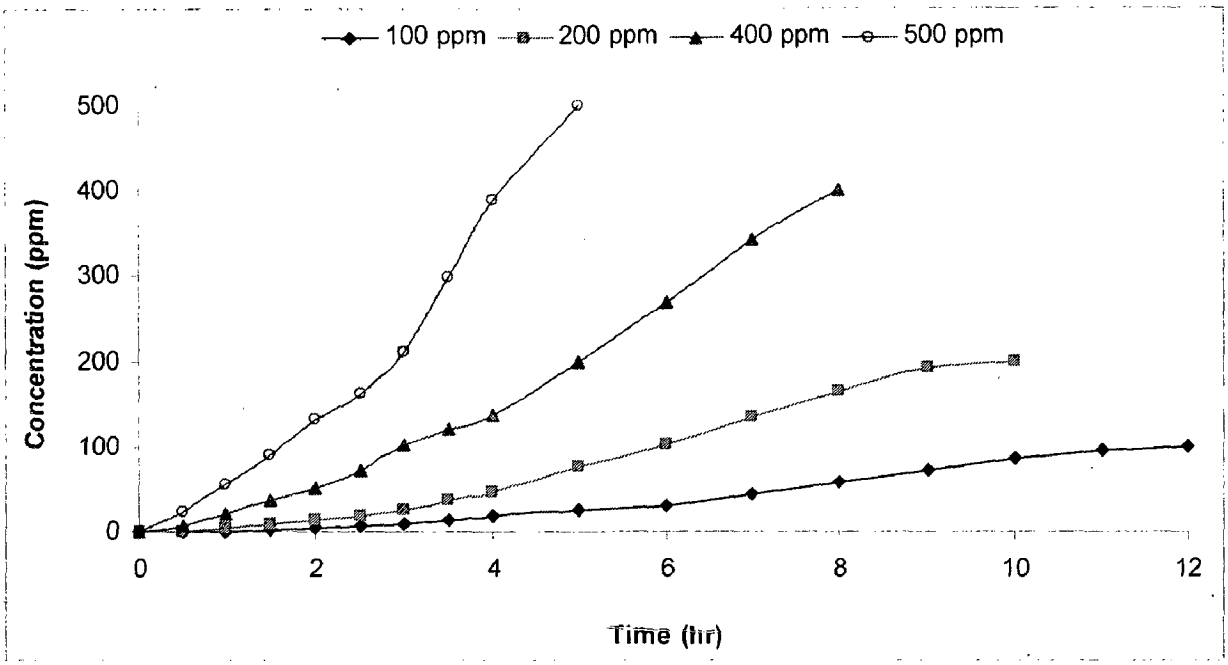


Fig 6.11: Breakthrough curves for removal of pyridine of various concentrations using puffed rice as adsorbent

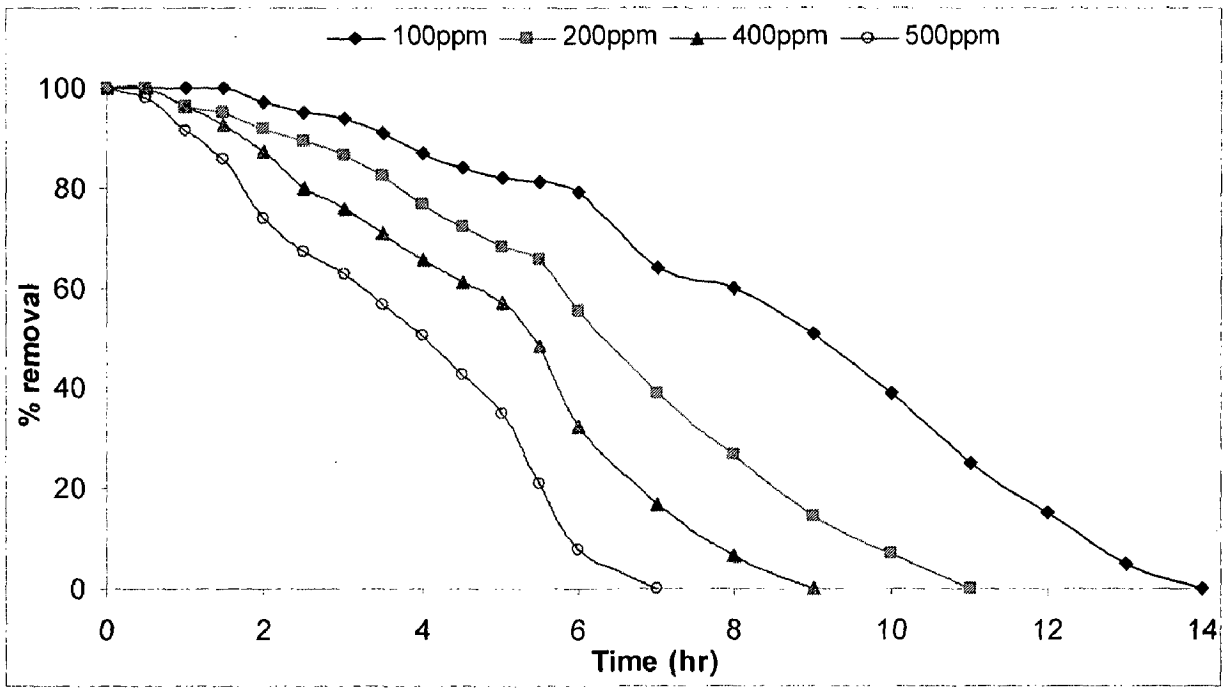


Fig 6.12: Percentage removal of pyridine over the period of time at different concentrations using soybean as adsorbent

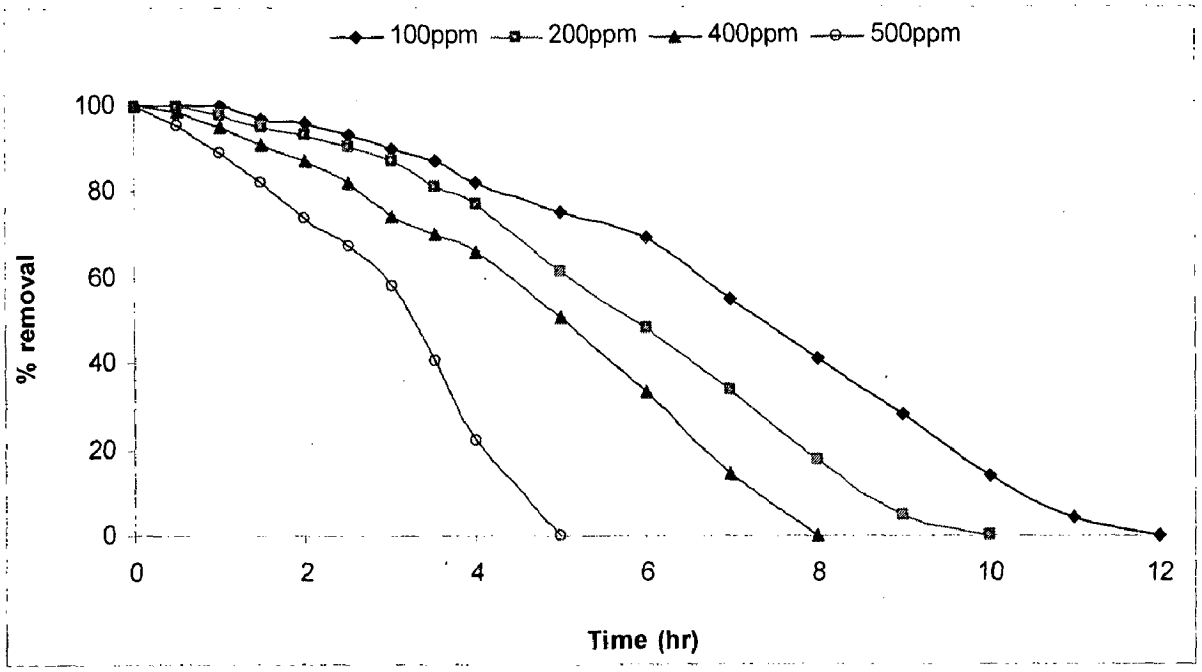


Fig 6.13: Percentage removal of pyridine over the period of time at different concentrations using puffed rice as adsorbent

For 100 ppm inlet concentration it can be observed (Fig 6.6) from the figure that initially there was full adsorption for both the adsorbents. As time progressed full adsorption pyridine was no more observed first for puffed rice and then for soybean. Even then outlet concentration of pyridine in the reactor remained similar for both adsorbents till 3 hours. After 3 hours a difference in adsorption ability was clearly evident. As s result the breakthrough curve started to split away from each other clearly indicating that amount of pyridine being adsorbed by puffed rice was reducing at a much quicker rate compared to soybean with time. Finally 100% breakthrough was achieved in 12 hours for puffed rice compared to 14 hours needed by soybean.

For 200 ppm inlet concentration (Fig 6.7) 100 % removal was observed up to 30 minutes for both the adsorbents. After that for both compounds outlet concentration started to increase gradually. Even during this period it can be observed from the graph that the adsorption of pyridine was almost same for both adsorbents up to 3.5 hours. After this point there appeared a difference in adsorption ability of the compounds. Hence from

this point onward the breakthrough curves started to split away from each other similarly to 100 ppm curve. Eventually 10% breakthrough was achieved in 10 hors for puffed rice and in 11 hours for soybean

For 400 ppm inlet concentration (Fig 6.8) though no pyridine was detected at outlet for soybean there was detectable pyridine concentration for puffed rice. Like at 200 ppm concentration at this concentration also a striking similarity can be noticed in amount of pyridine adsorbed by both adsorbents for a substantial period of time. If we observe the graph it can be seen that up to 4 hours similar outlet concentration of pyridine was detected in the reactor. After that the outlet concentration started increase at slightly faster rate for puffed rice, but the graphs still remained in close proximity to each other. Eventually 100% breakthrough for puffed rice was achieved in 8 hours compare to 9 hours for soybean, but even towards the end it can be observed that at 8 hour point concentration of pyridine for soybean was close to 400 ppm.

For 500 ppm inlet concentration (Fig 6.9) at no time full adsorption was possible and there was detectable amount of pyridine in the outlet from very beginning for both adsorbents. At this concentration the outlet concentration of pyridine was always slightly higher for puffed rice that that for soybean. Beyond 2.5 hour point though the outlet concentration of pyridine started to increase drastically for puffed rice and reached 100% breakthrough in just 5 hours. In contrary the outlet concentration for soybean though increased quickly but it was still somewhat slower in comparison to puffed rice. Hence 100% breakthrough for soybean was achieved in 7 hours.

Figures 6.10 and 6.11 show the cumulative breakthrough curves at all inlet concentrations for soybean and puffed rice respectively. From these graphs we can get an idea of variation in time needed to reach 100% breakthrough at different inlet concentrations for soybean and puffed rice respectively. Similarly figures 6.12 and 6.13 show % removal of pyridine observed at different times for soybean and puffed rice respectively at all the concentrations. These graphs can be considered to be simplified form of previous graphs as figures 6.12 and 6.13, where % removal has been shown against time for various inlet concentrations, give a clear idea of how quickly removal has decreased with increasing concentrations.

6.3.2 Variation in total flow rate:

Sl. No.	Total inlet flow rate (LPM)	Empty bed residence time (EBRT)	Time needed to achieve 100% breakthrough (hr)	
			Soybean	Puffed rice
1	2	53	14	12
2	2.5	42	14	11
3	3	35	13	9
4	3.5	30	12	8

Table 6.3: Comparison of breakthrough times for variable total inlet flow rates

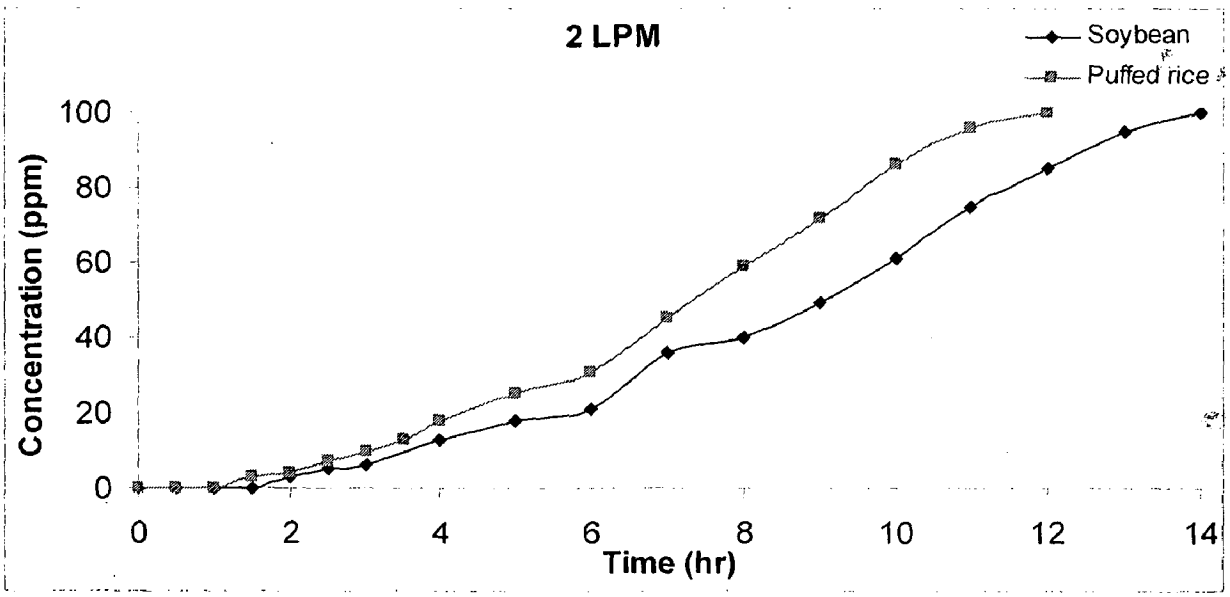


Fig 6.14: Comparison of breakthrough times for adsorption at total inlet flow rate of 2 LPM

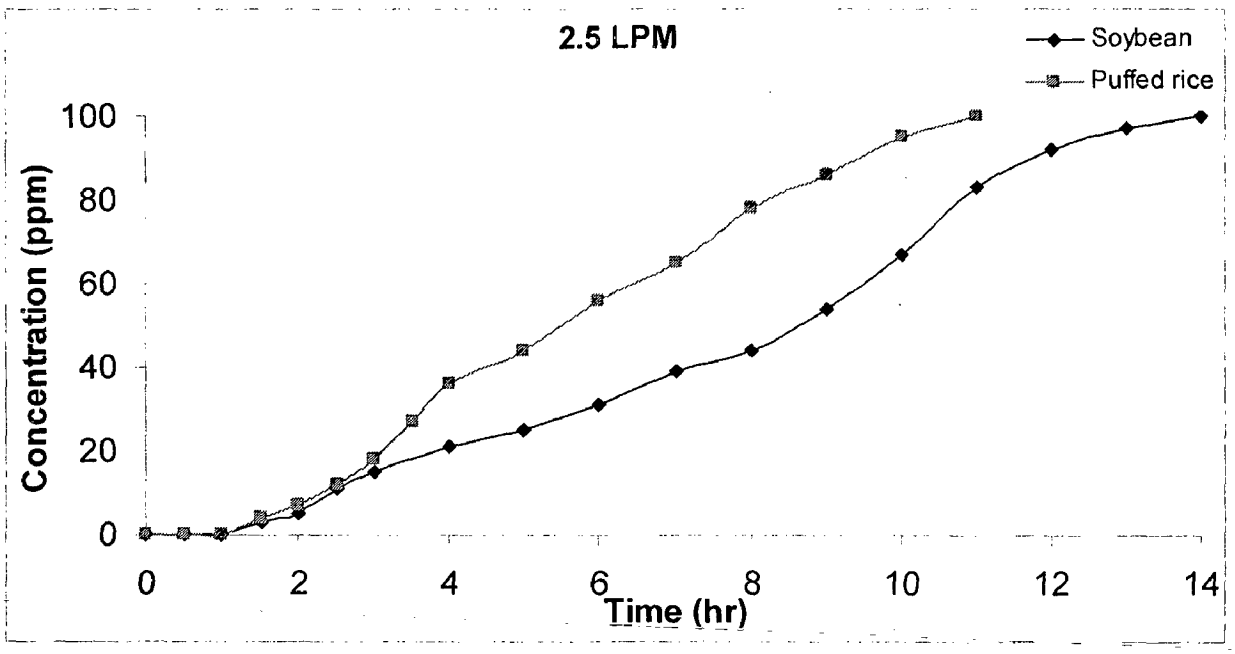


Fig 6.15: Comparison of breakthrough times for adsorption at total inlet flow rate of 2.5 LPM

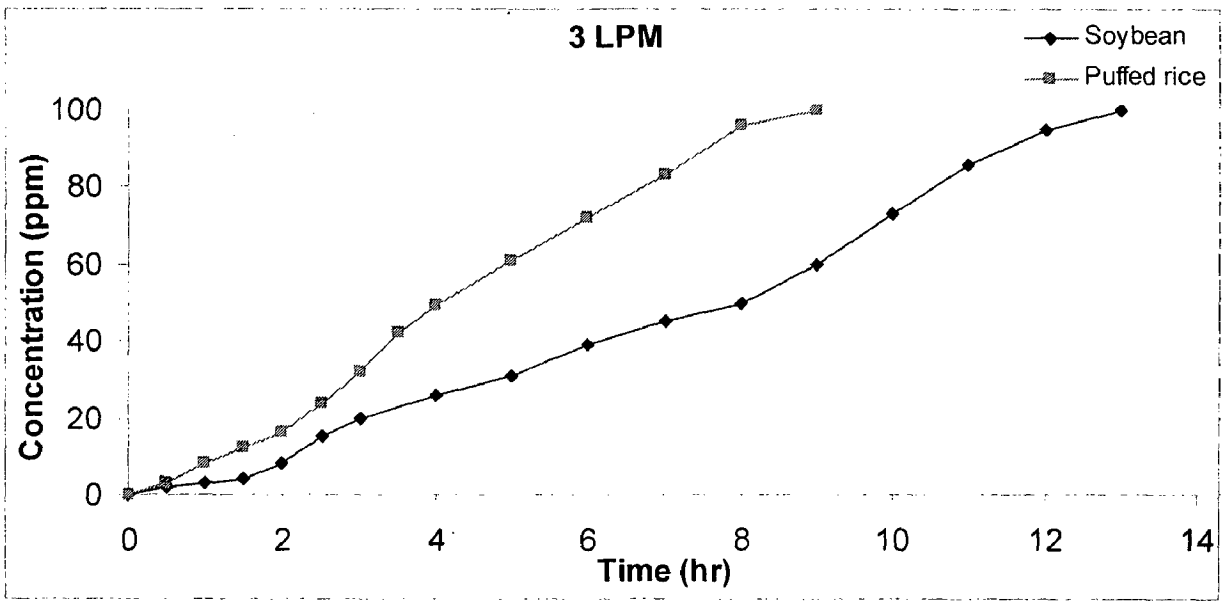


Fig 6.16: Comparison of breakthrough times for adsorption at total inlet flow rate of 3 LPM

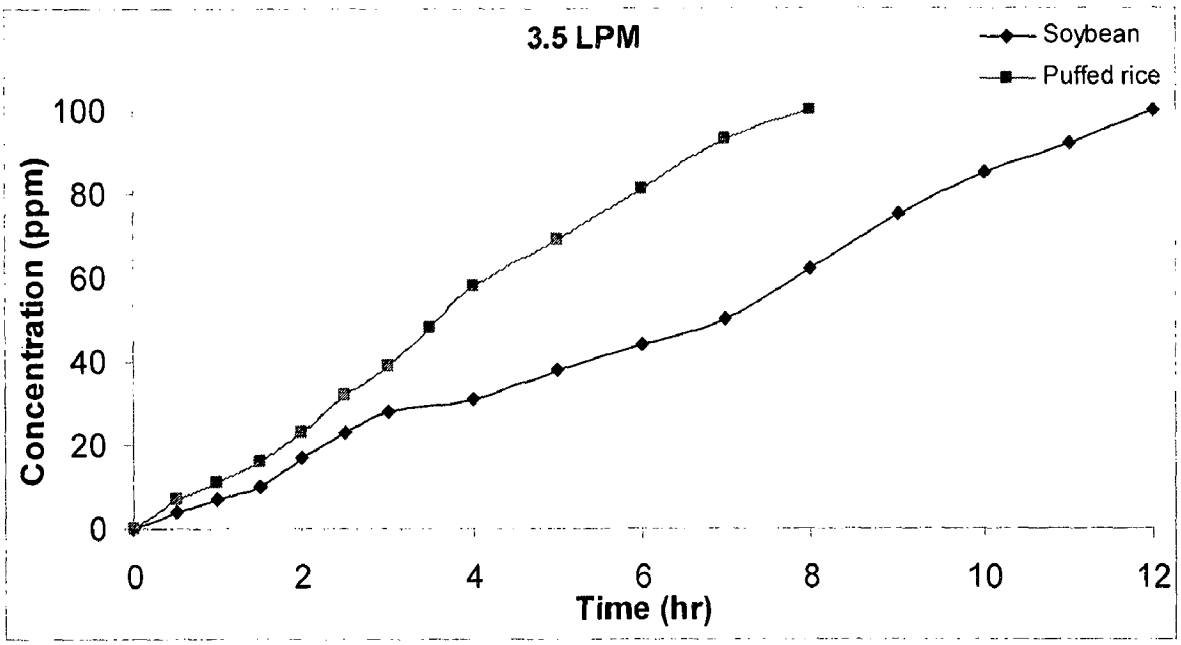


Fig 6.17: Comparison of breakthrough times for adsorption at total inlet flow rate of 3.5 LPM

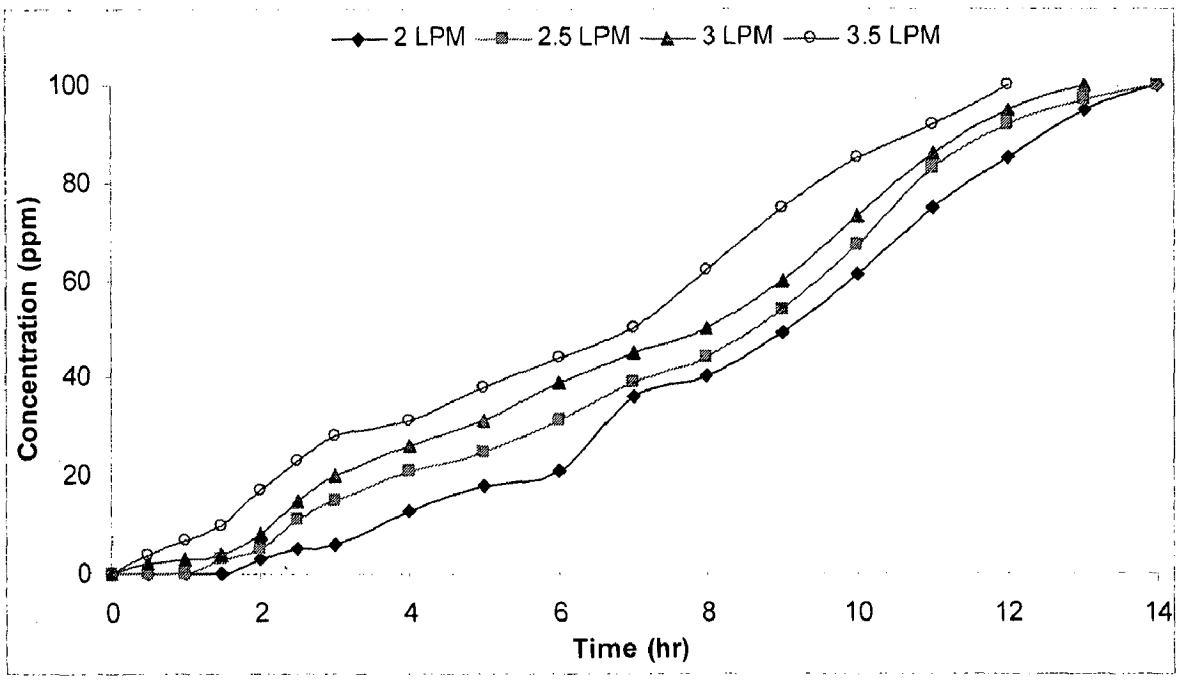


Fig 6.18: Breakthrough curves for removal of pyridine at various total inlet flow rates using soybean as adsorbent

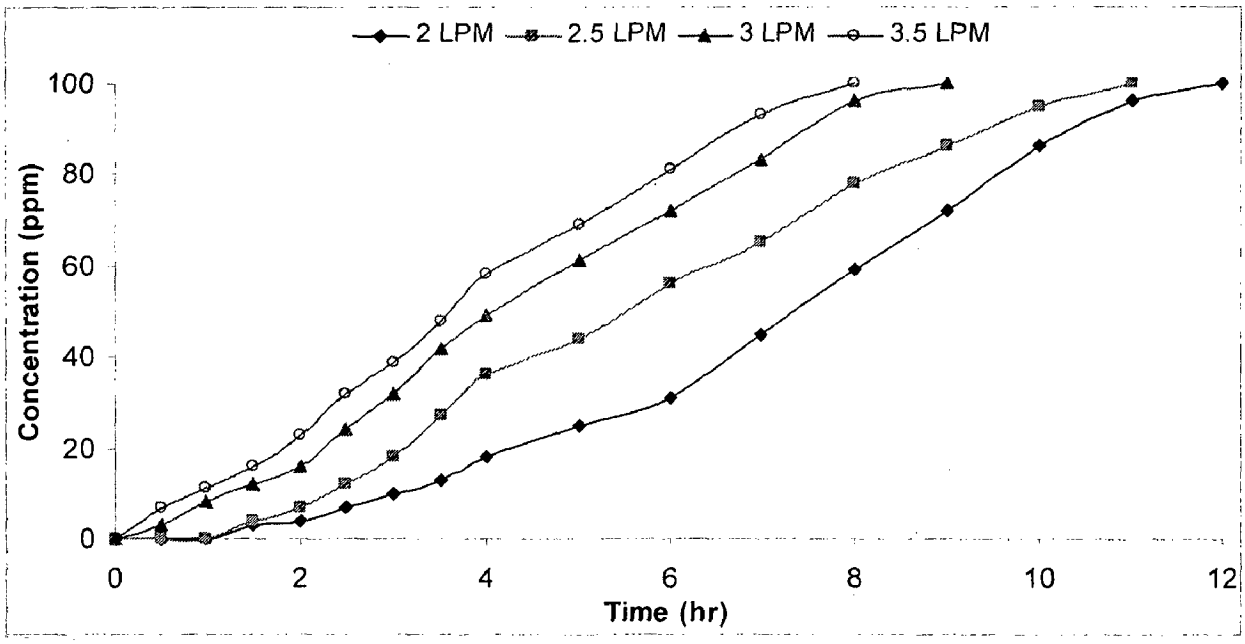


Fig 6.19: Breakthrough curves for removal of pyridine at various total inlet flow rates using puffed rice as adsorbent

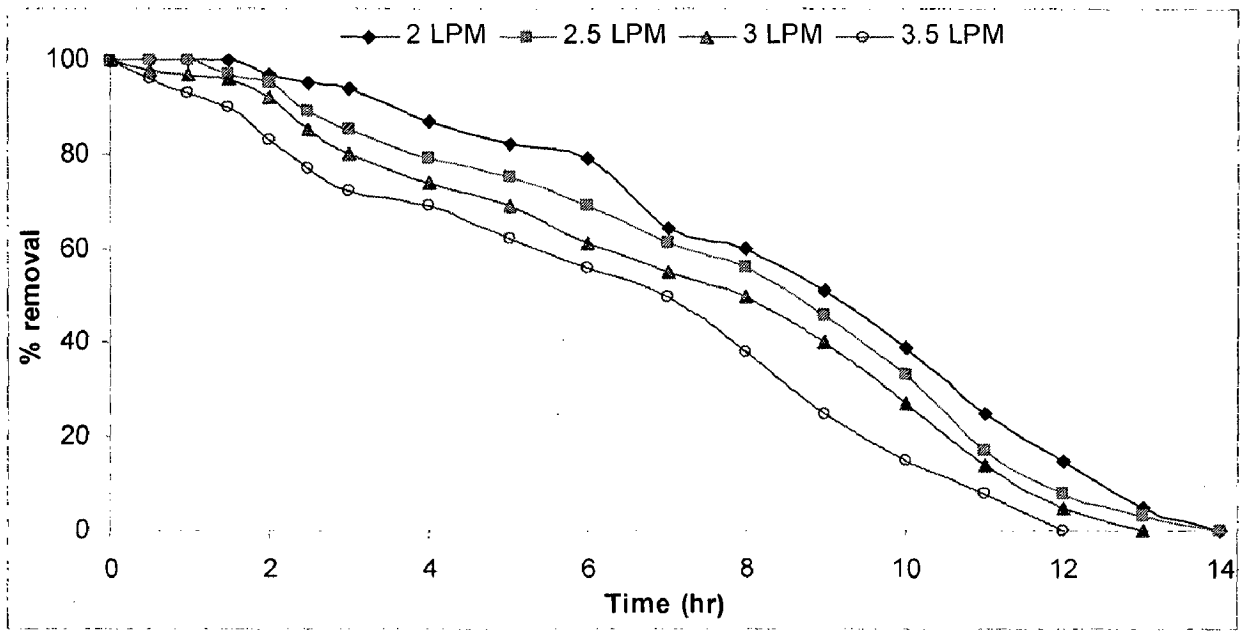


Fig 6.20: Percentage removal of pyridine over the period of time at different total inlet flow rates using soybean as adsorbent

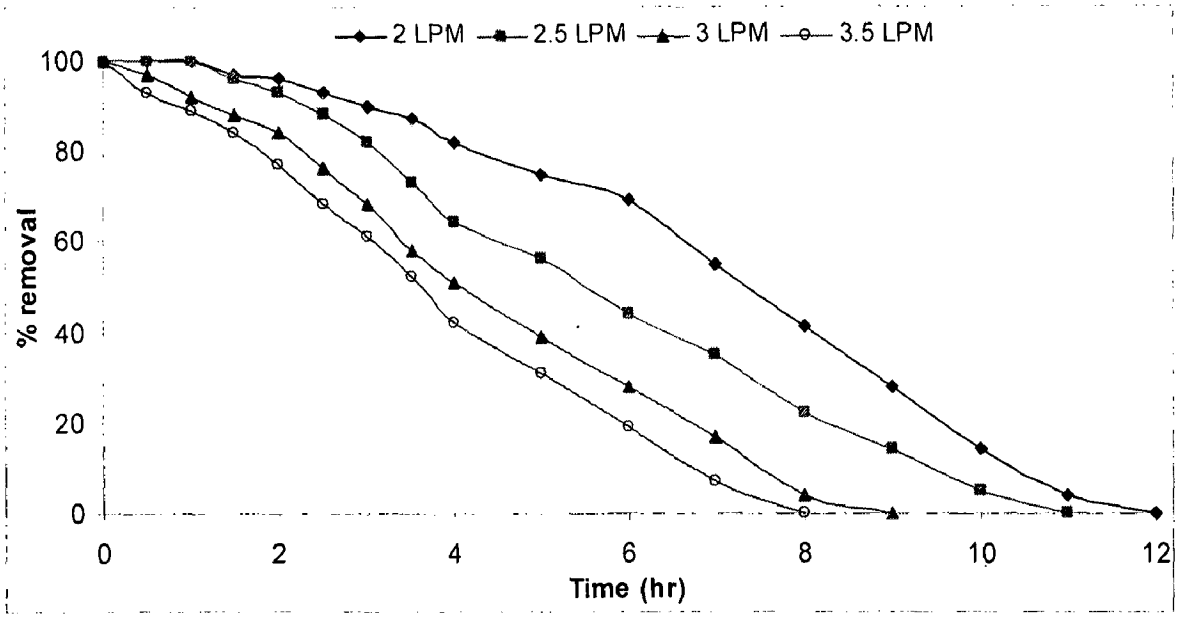


Fig 6.21: Percentage removal of pyridine over the period of time at different total inlet flow rates using puffed rice as adsorbent

At 2 LPM flow rate (Fig 6.14) 100% removal was obtained up to 1 & 1.5 hour for puffed rice and soybean respectively. From there on there was pyridine in the outlet of the reactor. Similar to concentration variation in case of 2 LPM flow rate too the two curves started to move away from each other as time progressed. It can also be seen that as time progress drop in rate of adsorption was higher in puffed rice compared to soybean. From the graph it can also be observed that beyond the 7 hour point the difference between the two graphs increased even further. Finally 100% breakthrough was achieved in 14 and 12 hours respectively at 2 LPM for soybean and puffed rice.

At 2.5 LPM flow rate (Fig 6.15) for both adsorbents full adsorption was achieved till 1 hour. After that the concentration of pyridine in the outlet started to increase gradually and the increase was found to be almost similar for both adsorbents till 2.5 hours. After that as time passed the increase in outlet concentration started to increase comparatively at much faster rate for puffed rice. In case of soybean it was observed that the pattern of increase in outlet concentration or in other words the approach toward the

100% breakthrough limit was almost similar to that for 2 LPM. Eventually 100% breakthrough for soybean and puffed rice was achieved in 14 and 11 hours respectively.

For 3 LPM flow rate (Fig 6.16) there was detectable outlet concentration of pyridine from very beginning which can be attributed to the fact that the flow rate now was higher than previous. Here as the time passed the outlet concentration of pyridine increased at a comparatively higher rate with respect to previous studies at lower flow rates. In case of soybean the increase was initially slow till 1.5 hours and it pick up pace from there on. In case of puffed rice the increase was gradual as well faster than soybean from the very beginning. With increase in time the rate of increase in pyridine concentration in the reactor outlet picked up for both adsorbents but again the pace of pick up being faster for puffed rice. At this flow rate 100% breakthrough was achieved in 13 and 9 hours respectively for soybean and puffed rice respectively. Hence at 3 LPM flow rate a striking difference can be observed in the time taken to reach 100% breakthrough for the two adsorbents.

At 3.5 LPM flow rate (Fig 6.17) the pattern of the graphs can be found to be somewhat similar to that in 3 LPM flow rate. At this flow rate a significant amount of pyridine was detected in the outlet at the earliest reading itself for both the adsorbents. At this flow rate the increase in outlet pyridine concentration was almost linear till 4 hours after which it started to increase at slightly slower rate to eventually attain 100% breakthrough in 8 hours. When the soybean graph is being observed it can be seen that in the early stages the rate of increase in concentration was the fastest. As time passed this rate slowed after 3 hours but again picked up after 7 hours to eventually reach 100% breakthrough in 12 hours.

Figures 6.18 and 6.19 show cumulatively the increase in outlet concentration at all flow rates for soybean and puffed rice respectively. When looked closely at the soybean graph it can be observed that the pattern of increase in outlet concentration was almost similar at all flow rates. Also the there was no change in total time with increase from 2 to 2.5 LPM. There was slight reduction in time needed to achieve 100% breakthrough at 3 and 3.5 LPM flow rates. In case of puffed rice though with each increase in flow rate the time needed for 100% breakthrough had reduced gradually and a significant decrease can be observed in breakthrough time between lowest and highest flow rates of 2 and 3.5

LPM respectively. The change in % removal of pyridine has been shown in figures 6.20 and 6.21 for soybean and puffed rice respectively.

6.3.3 Model of Yoon and Nelson:

6.3.3.1 Variation in Concentration:

Concentration (ppm)	R^2	1/k'	k' (hr^{-1})	τ (hr)	k
100	0.9772	0.5002	1.999	9.083	18.15692
200	0.9942	0.6176	1.619	4.167	6.746373
400	0.9709	0.7474	1.338	1.978	2.646564
500	0.9338	0.8964	1.116	1.107	1.235412

Table 6.4: Values of theoretical parameters k', τ, k for adsorption of pyridine on Soybean chunks for various inlet concentrations

Concentration (ppm)	R^2	1/k'	K' (hr^{-1})	τ (hr)	k
100	0.9487	0.6167	1.622	7.357	11.93305
200	0.9861	0.7483	1.336	5.92	7.90912
400	0.9588	0.7907	1.265	5.029	6.361685
500	0.9805	1.0947	0.9135	3.227	2.947865

Table 6.5: Values of theoretical parameters k', τ, k for adsorption of pyridine on puffed rice for various inlet concentrations

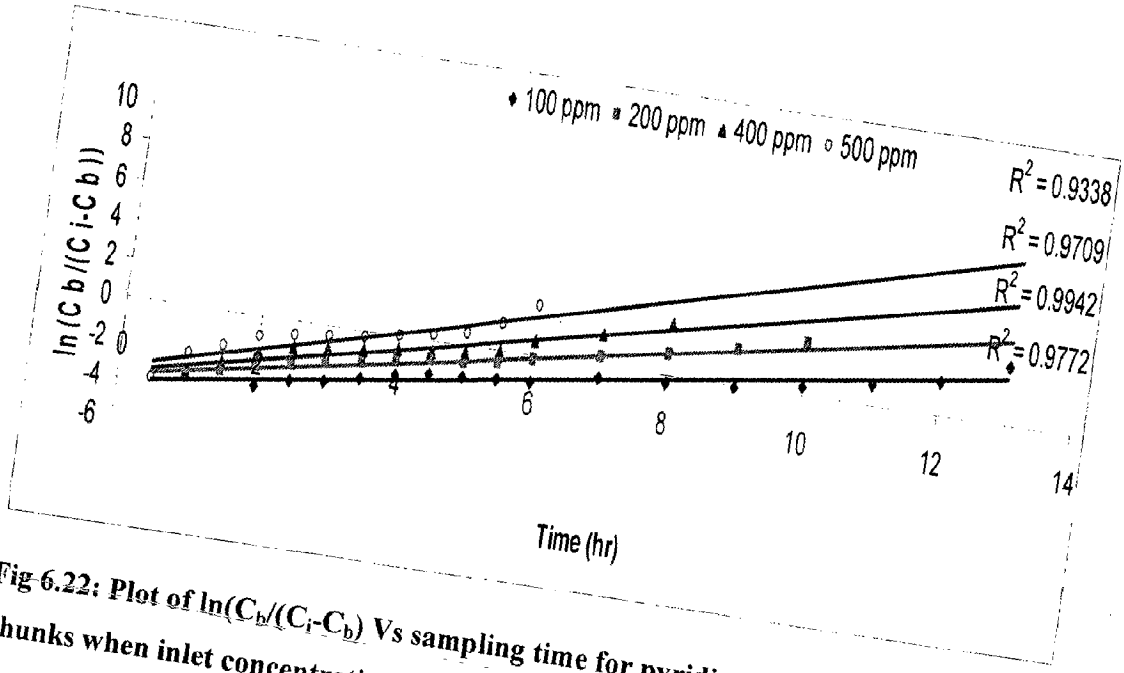


Fig 6.22: Plot of $\ln(C_b/(C_i-C_b)$ Vs sampling time for pyridine adsorption on soybean chunks when inlet concentration was varied (symbols: Experimental data, solid line: calculated from equation [3.4])

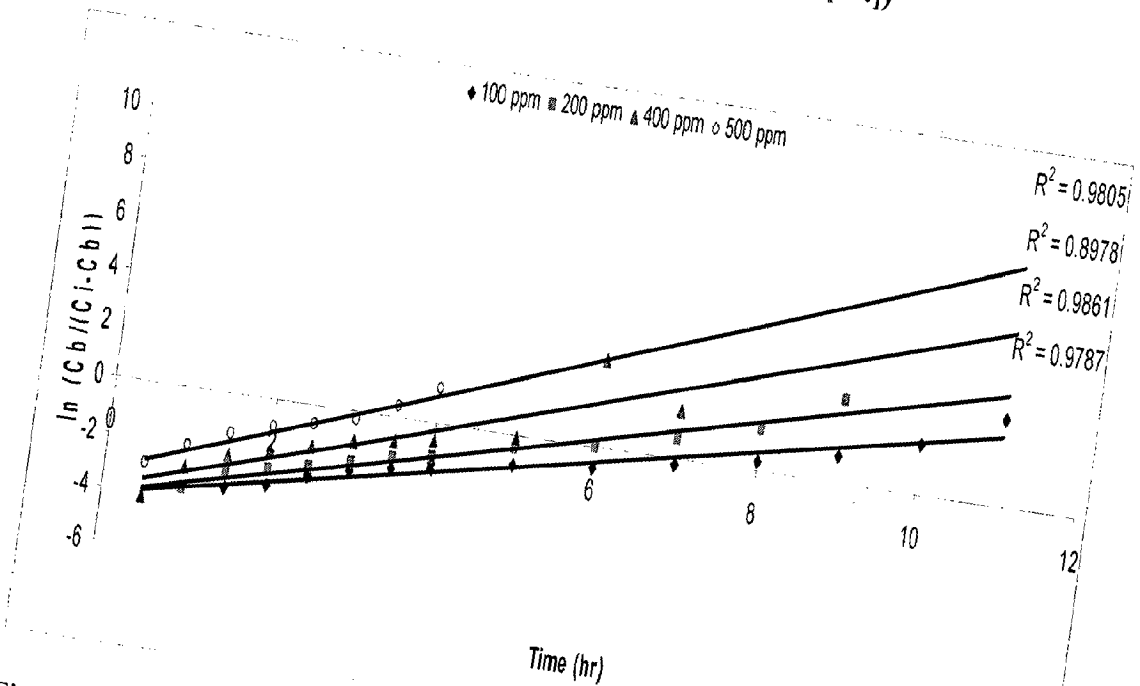


Fig 6.23: Plot of $\ln(C_b/(C_i-C_b)$ Vs sampling time for pyridine adsorption on puffed rice when inlet concentration was varied (symbols: Experimental data, solid line: calculated from equation [3.4])

It can be observe from the data, obtained from the analysis of the model for variable concentrations that for all the concentration ranges the experimental data obtained over a certain period of time was fitting in the model with appreciable accuracy. When the straight lines were being plotted to best fit the data using lest square method in fig no5, the lines thus obtained showed fairly high R^2 values which meant that the lines were being well fitted for the data obtained during experiments. The other important feature that can be observed was that the lines were fitting much better at lower concentrations and the R^2 value for 500 ppm reduced slightly compared to other concentrations. This gives an indication that at higher concentration of 500 ppm the rate at which breakthrough was attained may not be as linear as others. But still the value was very much within appreciable limits. The rate constants that were obtained using the model decreased gradually with increasing concentrations probably owing to the fact that with higher concentration the time duration of the adsorption process gradually came down. The rate constant values though decreased marginally with increasing concentrations. The 50% breakthrough time or τ also decreased with increasing concentration as the saturation limit for a given amount of adsorbent approached quicker with higher concentrations. τ decreased quite significantly from 100 to 200 ppm and then 400 ppm. But then difference between 400 and 500 ppm values were not that large. The final parameter which is suggested by this model is proportionality constant. The proportionality constant was very high for 100 ppm due to large τ value. The proportionality constant value reduced by almost 3 times as concentration changed from 100 to 200 and 200 to 400 ppm. The value drops marginally between 400 and 500 ppm concentrations.

Similarly it can be observe from the data, obtained from the analysis of the model for variable concentrations that for all the concentration ranges the experimental data obtained over a certain period of time was fitting in the model with appreciable accuracy. When the straight lines were being plotted to best fit the data using lest square method, the lines thus obtained showed fairly high R^2 values which meant that the lines were being well fitted for the data obtained during experiments. The other important feature that can be observed was that the lines were fitting much better at 200 and 500 ppm concentrations and the R^2 value for 100 and 400 ppm reduced slightly compared to other

concentrations. This gives an indication that at 100 and 400 ppm concentrations, the rate at which breakthrough was attained may not be as linear as others. But still the values were very much within appreciable limits. The rate constants that were obtained using the model decreased gradually with increasing concentrations probably owing to the fact that with higher concentration the time duration of the adsorption process gradually came down. The rate constant values though decreased marginally with increasing concentrations. The 50% breakthrough time or τ also decreased with increasing concentration as the saturation limit for a given amount of adsorbent approached quicker with higher concentrations. τ decreased quite significantly from 100 to 200 ppm and then from 400 to 500 ppm. But then difference between 200 and 400 ppm values were not that large. The final parameter which is suggested by this model is proportionality constant. The proportionality constant was very high for 100 ppm due to large τ value. The proportionality constant value reduced significantly as concentration changed from 100 to 200 and from 400 to 500 ppm. The value drops marginally between 200 and 400 ppm concentrations though.

6.3.3.2 Variation in flow rate:

Flow rate (LPM)	R^2	1/k'	k' (hr^{-1})	τ (hr)	k
2	0.9781	0.5075	1.97	9.08	17.8876
2.5	0.9525	0.4971	2.012	8.6	17.3032
3	0.9482	0.4951	2.02	8	16.16
3.5	0.959	0.4509	2.218	7	15.526

Table 6.6: Values of theoretical parameters k', τ, k for adsorption of pyridine on Soya bean chunks for various flow rates

Flow rate (LPM)	R^2	1/k'	k' (hr^{-1})	τ (hr)	k
2	0.9787	0.6167	1.6215	7.357	11.92938
2.5	0.9724	0.6336	1.5783	5.5	8.68065
3	0.9716	0.7594	1.3168	4.083	5.376494
3.5	0.9917	0.748	1.3369	3.6	4.81284

Table 6.7: Values of theoretical parameters k', τ, k for adsorption of pyridine on puffed rice for various flow rates

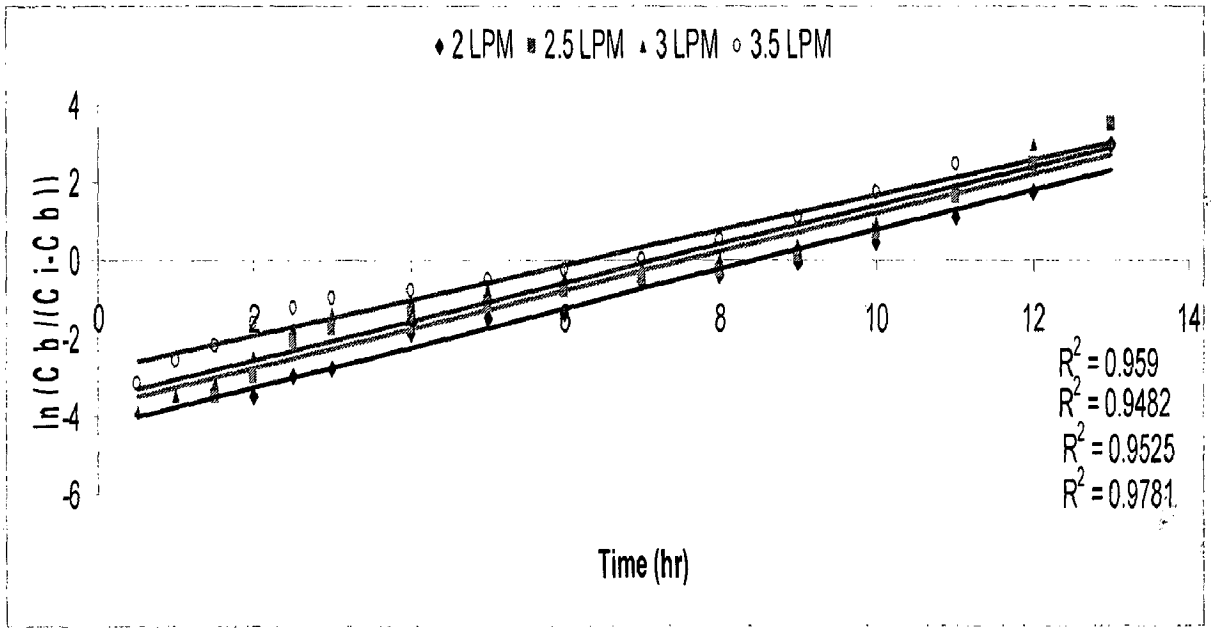


Fig 6.24: Plot of $\ln(C_b/(C_i-C_b)$ Vs sampling time for pyridine adsorption on soybean chunks when inlet concentration was varied (symbols: Experimental data, solid line: calculated from equation [3.4])

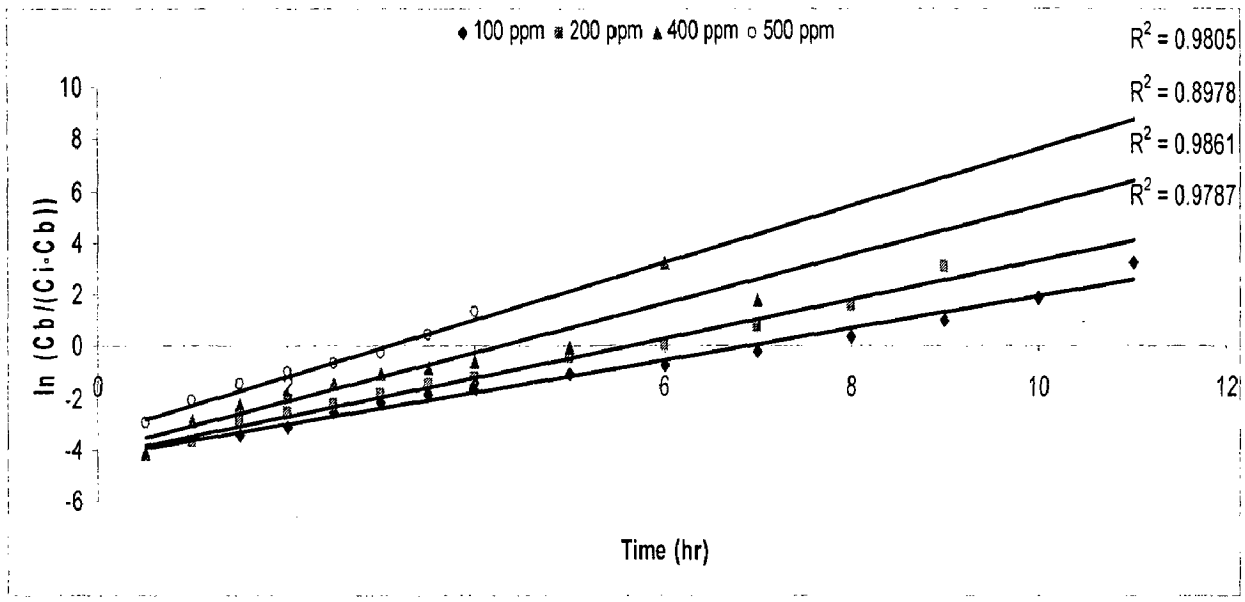


Fig 6.25: Plot of $\ln(C_b/(C_i-C_b))$ Vs sampling time for pyridine adsorption on soybean chunks when inlet concentration was varied (symbols: Experimental data, solid line: calculated from equation [3.4])

For study of variation in flow rate the inlet concentration was being kept at 100 ppm throughout. When the data obtained for various flow rates were being plotted on best fitted straight line in fig no 6 using least square method we could observe that the values were fitting nicely for all flow rates. Also the R^2, k' and τ values were in close proximity for all the flow rates. The rate constant values increased very little as the flow rates increased. The 50% breakthrough time decreased very little with each increasing flow rate. Finally the proportionality constant values were found to be almost same for 2 and 2.5 liter per minute (LPM) flow rate. It decreased marginally for 3 LPM and again was found to be in close proximity for 3 and 3.5 LPM flow rates. Thus it was observed that as the flow rate was varied at certain fixed concentration the constant values calculated from the model did not change to much and remained in close proximity with one another.

For study of variation in flow rate the inlet concentration was being kept at 100 ppm throughout. When the data obtained for various flow rates were being plotted on best fitted straight line using least square method we could observe that the values were

fitting nicely for all flow rates. Also the R^2, k' and τ values were in close proximity for all the flow rates. The rate constant values increased to some extent as the flow rates increased. The 50% breakthrough time though decreased appreciably with each increasing flow rate. Finally the proportionality constant values were found to be high for 2 liter per minute (LPM) flow rate. It decreased noticeably for 2.5 and 3 LPM flow rates and then again decreased marginally for 3.5 LPM but was found to be in close proximity for 3 and 3.5 LPM flow rates. Thus it was observed that as the flow rate was varied at certain fixed concentration the constant values calculated from the model did change appreciably but not too drastically.

6.4 Biodegradation Studies using *Bacillus Subtilis*:

6.4.1 Batch Study:

Batch test	Parameter varied	Temperature (^{O}C)	pH	Contaminant load (ppm)	Reaction time (hr)
1	Temperature	20, 25, 30, 35, 40	7	50, 100	24
2	pH	30	5, 6, 7, 8, 9	100	48
3	Contaminant concentration	30	7	50, 100, 200, 400, 500	120

Table 6.8: Batch studies of pyridine used to test optimum degradation conditions

Bacillus Subtilis was cultivated in 250 ml flask containing 100 ml of the basal salt medium with pyridine as the carbon source. The culture was acclimatized to pyridine by exposing the culture in series of shaking flasks. The startup of acclimatization was obtained by inoculating 100 ml basal salt medium along with 10 ppm pyridine with the bacteria from nutrient agar slant under sterile condition. After 48 hours of incubation at 30 ^{O}C, 5 ml of this culture was added to fresh basal salt medium (with 10 ppm pyridine) as inoculum. 48 hours later, a third fresh basal salt medium was also inoculated with 5 ml of the last culture to ensure that the bacteria get adapted to pyridine.

In order to understand the growth pattern of the bacteria, the effect of various operating parameters like temperature, pH, and growth study was performed in basal salt medium with various concentrations of pyridine. For each batch experiment, one of the following parameters was varied while others were kept constant: temperature, pH and pyridine concentration. Therefore an experimental program was designed to avoid volatilization of pyridine to conduct the batch experiments. During the biodegradation studies experiments were conducted with only 100 ml working volume in a 250 ml volume to avoid the deficit of oxygen.

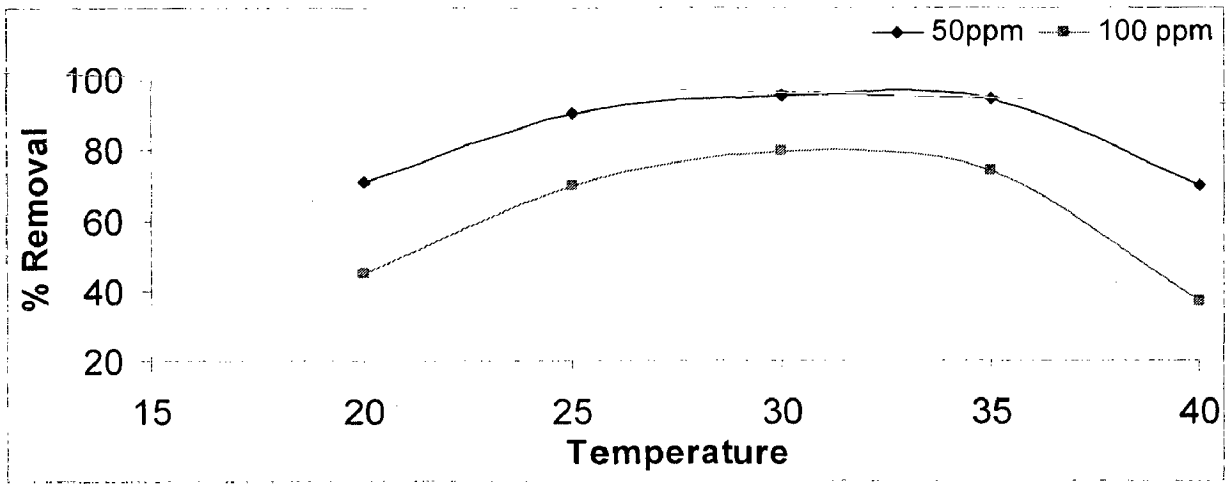


Fig 6.26: Effect of temperature on pyridine removal in batch studies by *B. Subtilis* at low (50 ppm) and high (100 ppm) concentration at pH 7.0 for 24 hours

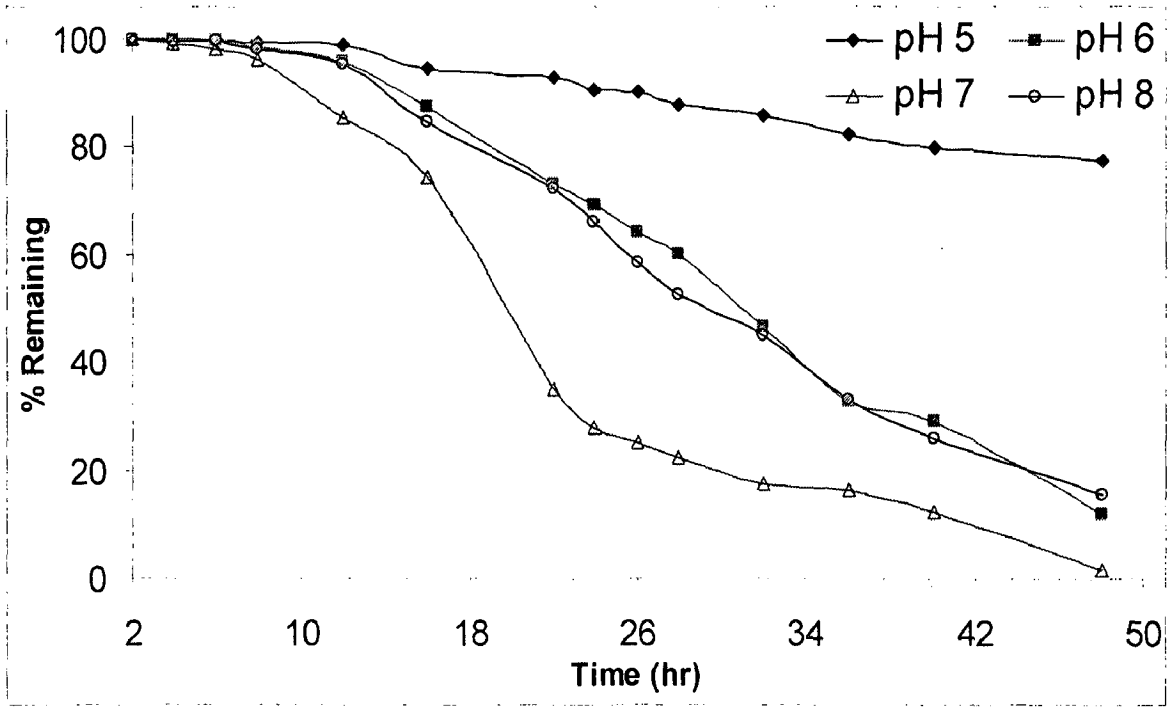


Fig 6.27: Effect of pH on removal of pyridine in batch studies by *B. Subtilis* at 100 ppm concentration at 30 ^{O}C for 48 hours

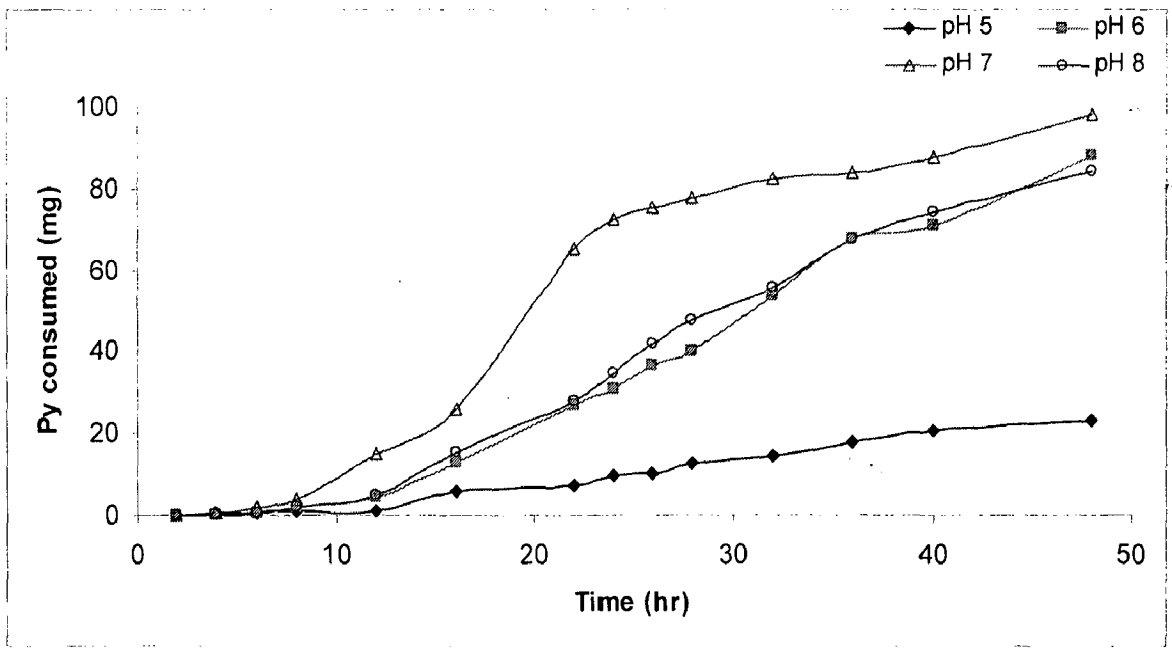


Fig 6.28: Consumption of pyridine by B. Subtilis at 100 ppm concentration for varying pH values

6.4.1.1 Effect of temperature on the biodegradation of pyridine by *Bacillus Subtilis*:

Figure 6.26 shows the effect of temperature on biodegradation of pyridine by *B. Subtilis*. It shows the % removal of pyridine by *B. Subtilis* for low (50 ppm) and high (100 ppm) concentration as a function of temperature at fixed pH of 7. The results were obtained by measuring % removal of pyridine at certain temperatures for a period of 24 hours. The temperatures were increased at step of 5 ^{O}C over a temperature range of 20 to 40 ^{O}C. it was observed that the % removal of pyridine by the bacteria gradually increased with increase in temperature. Figure shows that for both concentrations, there was a sharp increase in % removal as temperature changed from 20 to 25 ^{O}C. Between 25 and 30 ^{O}C temperature, the increase in % removal was marginal for both concentrations. As temperature was further increased to 35 ^{O}C a marginal drop in %removal was observed for 50 as well as 100 ppm concentrations. Beyond this temperature the drop in % removal was quite severe for both concentrations. At the temperatures of 25, 30 and 35 ^{O}C the % removal attained in 24 hour were 90.21, 95.181, 94.277 for 50 ppm and 70.029, 79.819, 73.946 for 100 ppm respectively. It is clear from the figure that biodegradation of pyridine increased with temperature between 20 and 30 ^{O}C but started to reduce thereafter for high as well as low concentrations, thus suggesting that the optimum temperature for biodegradation of pyridine by *B. Subtilis* is 30 ^{O}C.

6.4.1.2 Effect of pH on biodegradation of pyridine by *B. Subtilis*:

pH has a significant effect on biodegradation of pyridine by microbs. The removal of pyridine of 100 ppm concentration by *B. Subtilis* at various initial pH values are being shown in fig 6.27. The temperature was kept constant at 30 ^{O}C throughout the study. The readings at various pH values were taken for 48 hours. From the figure it can be seen that in 48 hours *B. Subtilis* removed pyridine up to 22.804, 88.091, 98.269, and 84.369% for pH values of 5, 6, 7, and 8 respectively. % removal increased with pH and reached the maximum value at pH 7 and hen started to reduce again. Hence percentage removal reached maximum value at pH 7, specifically between the optimum pH of 6.0-8.0. Degradation is comparatively less outside this range. This was attributed mainly to the inhibitory effect of superacidity and superalkalinity on the activity of intracellular activity

of bacteria. Figure 6.28 shows the consumption of pyridine by bacteria during 48 hours at various pH values.

6.4.1.3 Growth of *Bacillus Subtilis* in pyridine:

Growth of *Bacillus Subtilis* in presence various concentrations of pyridine ranging from 50 ppm to 500 ppm is being reported in fig 6.29. The growth of the microorganism was found to increase with increasing concentration of pyridine. But at the same time the lag phase increased with increasing concentrations of pyridine, which demonstrated the positive correlation between cell biomass and pyridine. In the batch studies, the biomass concentrations of *B. Subtilis* was initially low, but later on growth increased exponentially. The result showed in the lag period, the biodegradation time and the maximum microorganism concentration increased on increasing the substrate concentration. Biodegradation rates were calculated as the degradation of substrate concentration per total degradation time and cell concentration obtained. It is clear from the figure (Fig 6.29) that pyridine was utilized by *B. Subtilis* effectively up to 500 ppm concentration. At 200, 400 and 500 ppm concentrations the exponential phase appear to start comparatively late and the lag period was found to be increasing with increase in concentration.

6.4.1.4 Pyridine biodegradation by *Bacillus Subtilis* in batch culture:

The result of batch studies for pyridine biodegradation using *Bacillus Subtilis* in basal salt medium is shown in fig 6.30. *Bacillus Subtilis* degrades about 100, 100, 100, 88.722, and 77.245 % of pyridine with an initial concentration of 50, 100, 200, 400 and 500 ppm. Times needed for complete consumption of pyridine at 50, 100 and 200 ppm were 47, 54 and 72 hours respectively. At higher concentration it was observed that towards the end of the substrate consumption curve, there is a region of relatively reduced rate of substrate removal. Results show that the degradation time of pyridine was low at low substrate concentrations since degradation rate is high at these concentrations. It also shows that at higher concentrations of pyridine degradation rate is low and degradation time is high. Following reasons can be attributed to this occurrence-

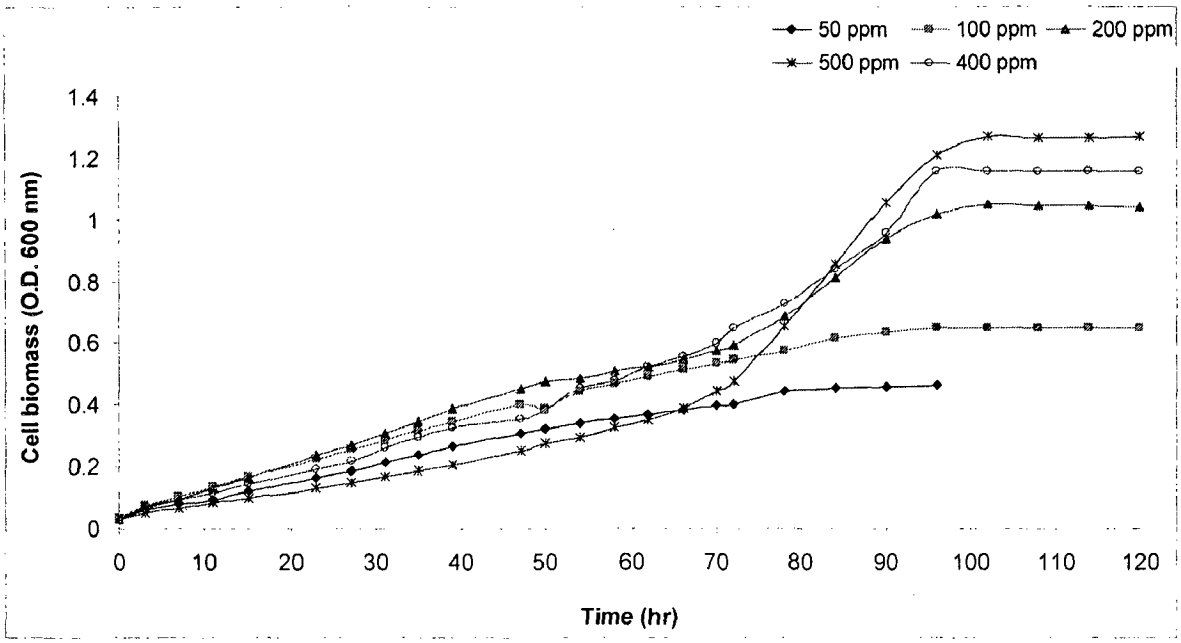


Fig 6.29: Growth of *B. Subtilis* in pyridine (Temperature= 30±1 ^{O}C, pH= 7.0)

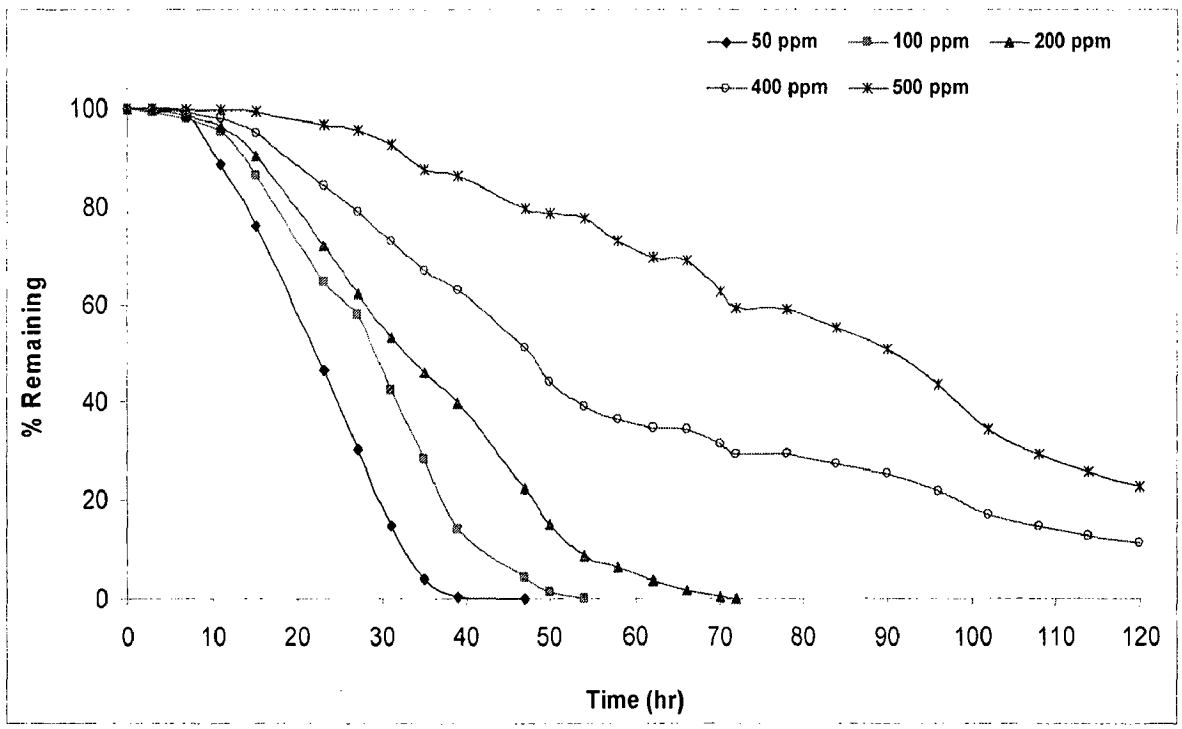


Fig 6.30: Biodegradation of pyridine by *B. Subtilis* in batch culture (Temperature= 30±1 ^{O}C, pH= 7.0)

a) These experiments were performed in 250 ml flasks with 100 ml working volume. Hence it is clear that this culture was not able to degrade efficiently at high concentration of pyridine under hypoxic conditions in bottle [**Morgan et al. 1993**].

b) In exponential phase, a drop in oxygen concentration may be a possible reason for low growth rate [**Yang and Humphrey, 1975**].

c) The slight fall in pH of the solution over time may be another reason, since pyridine is a heterocyclic aromatic compound and is a week organic base [**Blanch and clark, 1996**].

6.4.1.5 Growth kinetics:

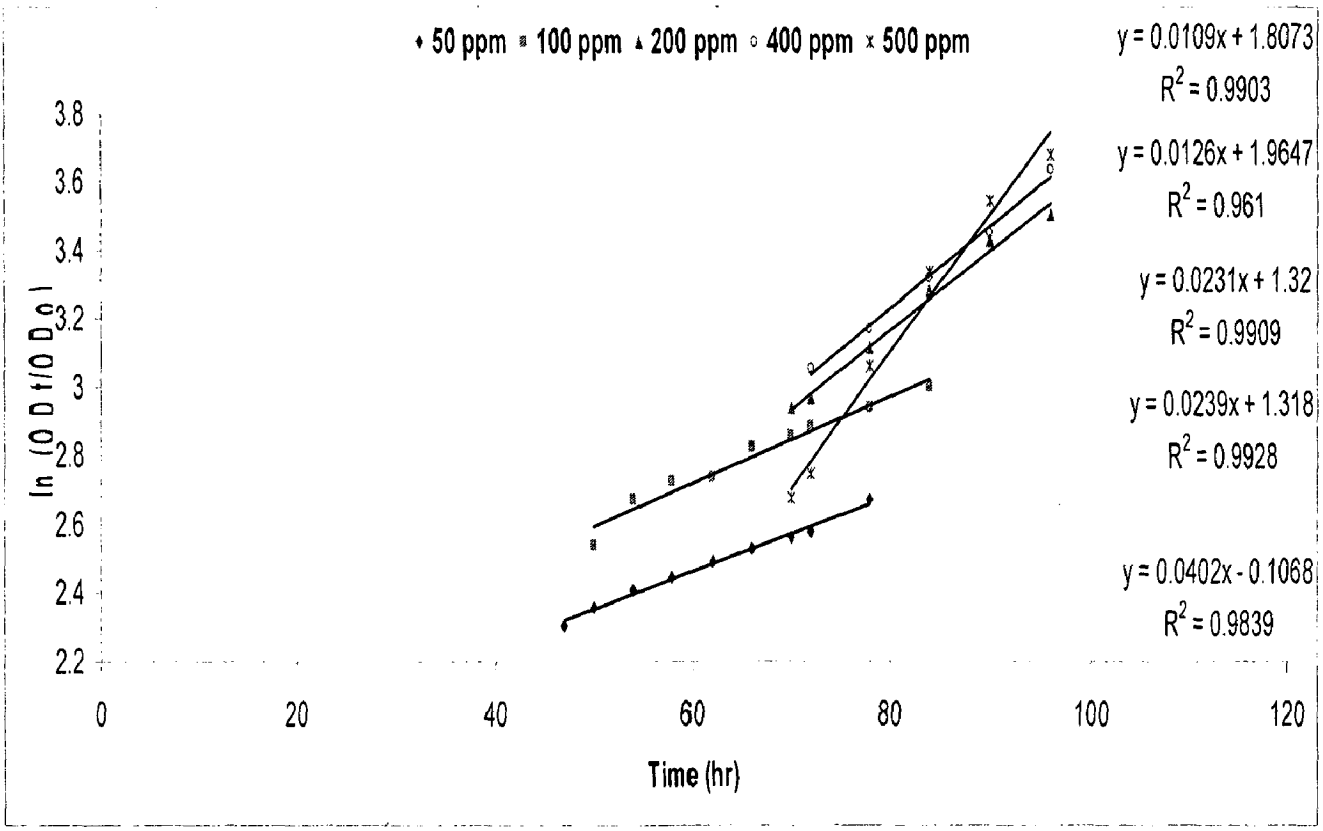


Fig 6.31: Plot of $\ln(OD_t/OD_0)$ Vs time (hr)

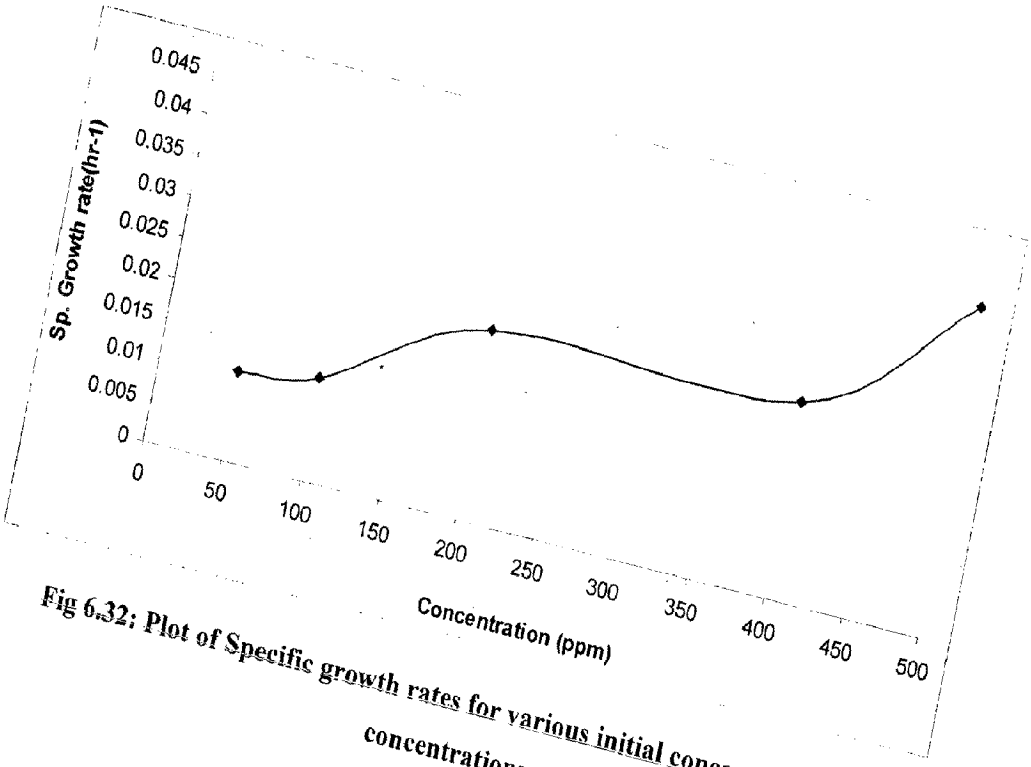


Fig 6.32: Plot of Specific growth rates for various initial concentrations Vs concentrations

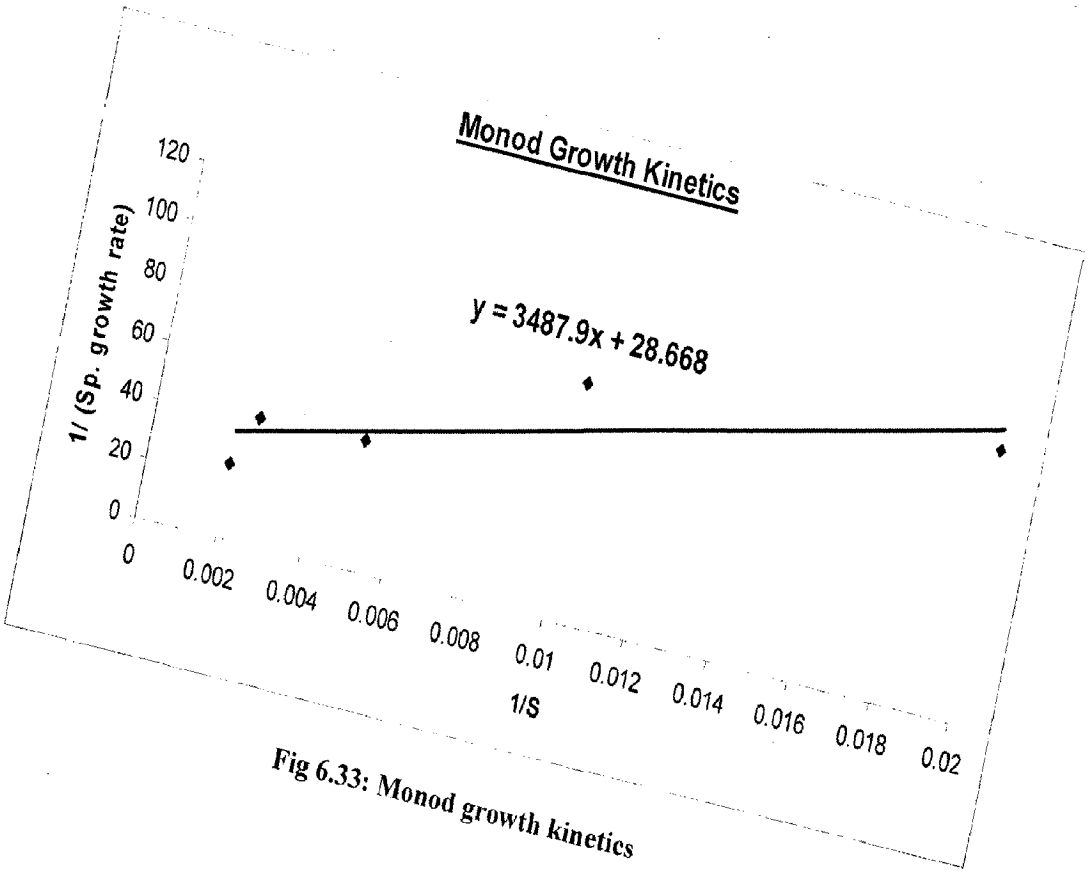


Fig 6.33: Monod growth kinetics

The specific growth rate (μ) of *B. Subtilis* and corresponding initial concentration of pyridine has been plotted in fig 6.32. Here specific growth rate increased slowly initially then a large increase can be seen between 100 and 200 ppm. The rate remains almost same between 200 and 400 ppm concentration. Again at 500 ppm concentration a large increase in specific growth rate can be seen. Hence it can be observed that the specific growth rate was increasing throughout the study with increase in initial concentrations. Therefore this growth rate is exhibiting the Monod growth kinetics. Hence the constants of Monod kinetics, namely maximum specific growth rate (μ_{max}) and half saturation constants (K_s) can be found out for this batch study by plotting reciprocals of specific growth rate and initial substrate concentration. From fig 6.33 the maximum specific growth (μ_{max}) rate is **0.0349 hr^{-1}**. Half saturation constant (K_s) obtained is **121.65 mg/l or ppm**.

6.4.2 Continuous study:

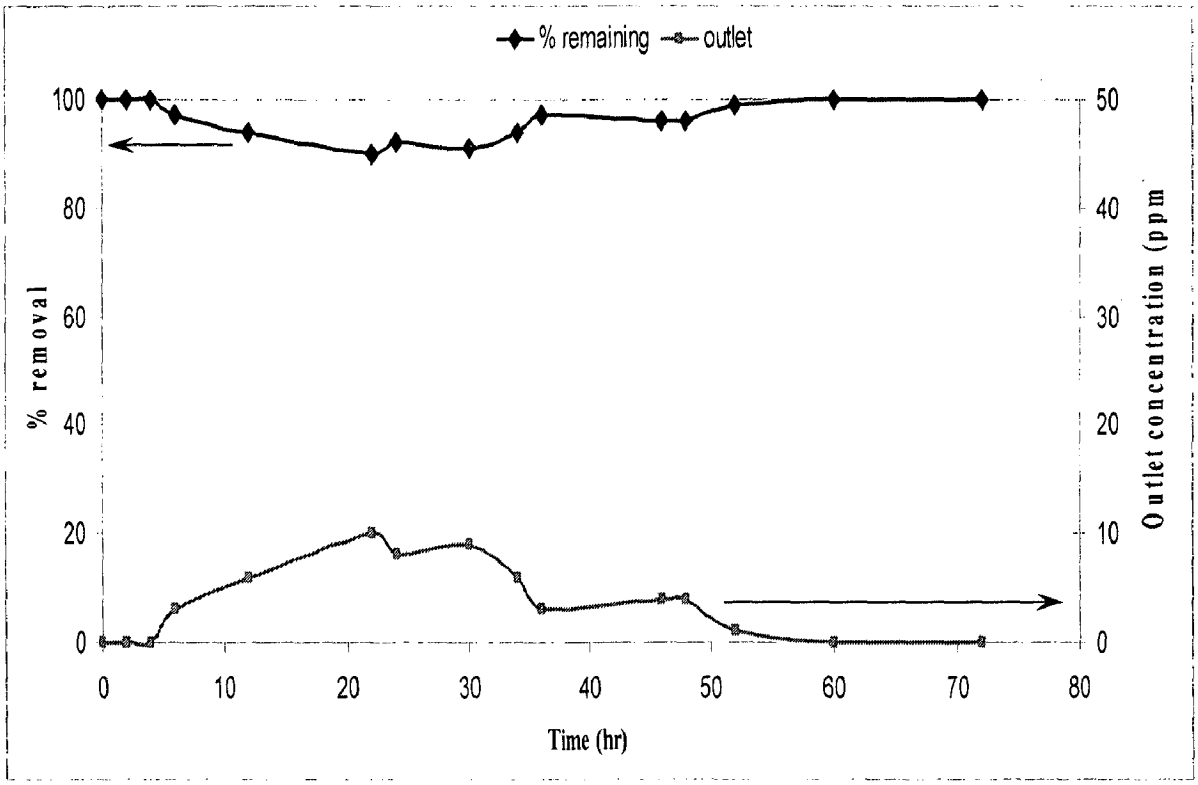


Fig 6.34: Performance of continuous biotrickling filter in removal of pyridine with time

The biotrickling filter was sterilized by passing steam. Steam from autoclave was directly passed from the bottom of biotrickling filter twice for 20 min at 15 psi to avoid any type of contaminations. After sterilization, the pyridine adopted culture (*S. Subtilis*) was introduced from the top of the biotrickling filter and recirculated for 24 hours. After the immobilization, the biotrickling filter was fed with nutrient solution continuously from the top at a rate of 4-5 ml/min by a Peristaltic pump and pyridine vapour laden air from the bottom of the reactor. To evaluate the biotrickling filter performance the reactor which was packed with autoclaved soybean was run at a flow rate of 1LPM with an empty bed residence time of 106 seconds. The concentration of pyridine in the inlet gas flow was 0.177 g/m^3 or 50 ppm and average volumetric loading rate was 6.01 g/m^3-h. The nutrient (without any carbon source) was supplied from the top of the reactor by using a perilstatic pump. As the process bean no pyridine concentration was observed in the outlet of the reactor. This continued till the 4^{th} hour reading. After this initial period the concentration of pyridine in the outlet started to increase with time and reached a concentration of 10 ppm. Thus the % removal dropped to 90% at this time. This could be attributed to the fact that a certain amount of pyridine gets adsorbed by soybean in the initial period. Pyridine is also known to be soluble in the nutrient solution that was being used in the reactor. Hence some amount of the gaseous pyridine got dissolved in the nutrient solution as well. But later on there was some release of pyridine which lead to decrease in removal efficiency. That was why the outlet concentration of pyridine increased between 6^{th} and 22^{nd} hour reading. The nutrient medium was recirculated over the biotrickling filter to avoid more dissolve of pyridine in the liquid medium. The recirculated nutrient medium was refreshed in a semi continuous manner by replacing 10% of the solution every day. The removal efficiency started to stabilize after this period of fluctuation and steadily moved towards 100% efficiency. It finally attained 100% removal efficiency in 60 hours. But as the time progressed and culture media grew on the surface of the packing media, the media started to rot after 2 days of operation. Though the reactor still continued to operate in efficient manner but the bed started to contract and collapse. Also there was foul smell due to rotting of the reactor. The bed completely collapsed after 3 days due to rotting and bed pressure could also be a reason for this failure.

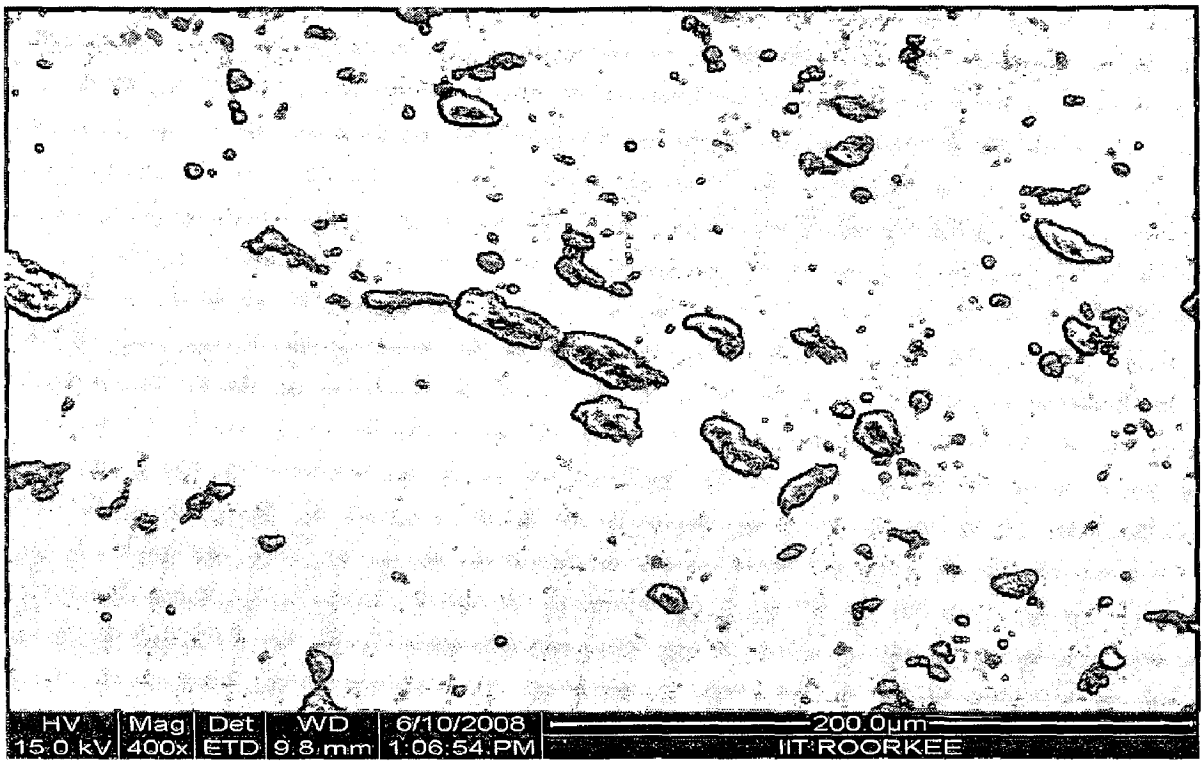


Fig 6.35: SEM image of *Bacillus Subtilis*

Fig 6.36: SEM image of *B. Subtilis* grown on soybean surface after 3 days

CONCLUSION and RECOMENDATIONS

7.1 Conclusion:

In the present study continuous adsorption process was carried out with two new adsorbents, namely soybean and puffed rice for gas phase removal of pyridine where the inlet concentration pyridine varied from 100 to 500 ppm. The flow rate of inlet gas was also varied between 2 and 3.5 LPM keeping the pyridine concentration constant. The results thus obtained have also been validated using Yoon and Nelson model for gas flow in continuous reactors. Again the pyridine was also treated using *Bacillus Subtilis*. Batch studies were conducted to optimize the parameters of temperature, pH and then growth of the bacteria was studied at optimized conditions by changing the initial concentration of pyridine between 50 and 500 ppm. The growth thus obtained has been analyzed for finding the suitable growth kinetics that best suits the growth process. Continuous biotrickling filter was also operated using soybean as the packing material and Bacillus Subtilis as the degrading microorganism. From the present study following conclusions can be derived-

1. Both soybean and puffed rice showed slightly acidic pH value and density lower than that of water. Soybean though was found to have more than twice the density value of puffed rice. Chemical composition by EDAX analysis showed that both have almost similar amount of carbon content. But soybean was found to have higher oxygen content and puffed rice had higher nitrogen content. Surface area analysis showed that soybean has higher surface area and monolayer volume.
2. FTIR spectra analysis of soybean showed that adsorption process effected major changes in the region where O-H and N-H groups and then again where vinyl ether type formation along with 1^{O} alcohol together with bending aromatic C-H structure were present. In case of puffed rice minor changes were seen in the region where O-H and N-H groups, then C-O and C=C groups were present. Major changes were visible though in the last section where vinyl ether type formation along with 1^{O} alcohol together with bending aromatic C-H structure were present

3. When concentration was varied by keeping flow rate fixed at 2 LPM, soybean attained 100% breakthrough in 14, 11, 9 and 7 hours for 100, 200, 400 and 500 ppm concentrations respectively.
4. when puffed rice was used as adsorbent in the same operating condition 100% breakthrough was observed in 12, 10, 8 and 5 hours for 100, 200, 400, 500 ppm respectively.
5. When flow rate was varied by keeping concentration fixed at 100 ppm, for soybean 100% breakthrough was observed in 14, 14, 13, 12 hours for 2, 2.5, 3 and 3.5 LPM flow rates respectively.
6. When adsorbent was changed to puffed rice 100% breakthrough was achieved in 12, 11, 9 and 8 hours for 2, 2.5, 3 and 3.5 LPM flow rates.
7. When Yoon and Nelson model was validated for variable concentration with soybean as adsorbent, the rate constant changed from 1.999 hr^{-1} for 100 ppm to 1.116 hr^{-1} for 500 ppm. The proportionality constant varied from 18.15692 for 100 ppm to 1.235412 for 500 ppm.
8. For variable concentration study with puffed rice as adsorbent, rate constant reduced from 1.622 hr^{-1} for 100 ppm to 0.9135 hr^{-1} for 500 ppm. In the same way proportionality constant changed from 11.93305 for 100 ppm to 2.947865 for 500 ppm.
9. When the model was validated for variable flow rates with respect to soybean as adsorbent, rate constant varied from 1.97 hr^{-1} for 2 LPM to 2.218 hr^{-1} for 3.5 LPM. The proportionality constant changed from 17.8876 to 15.526 as flow rate changed from 2 LPM to 3.5 LPM.
10. When the model was validated for variable flow rates with respect to puffed rice as adsorbent, rate constant varied from 1.6215 hr^{-1} for 2 LPM to 1.3369 hr^{-1} for 3.5 LPM. The proportionality constant changed from 11.92938 to 4.81284 as flow rate changed from 2 LPM to 3.5 LPM.
11. Parameters affecting biodegradation of pyridine by *Bacillus Subtilis* were optimized first. Temperature and pH were selected as the parameters to be optimized and after optimizing these parameters, the degradation efficiency of

Bacillus Subtilis was studied at various initial pyridine concentrations at these optimized conditions.

12. The effect of temperature on biodegradation of pyridine by *B. Subtilis* was studied by observing the degradation for 24 hours at temperatures ranging between 20 and 40 ^{O}C for 50 ppm and 100 ppm initial concentrations. The temperatures were increased by 5 ^{O}C to find out their effect on biodegradation process. B. Subtilis exhibited best result at 30 ^{O}C at which the % removal was found to be 95.181 for 50 ppm and 79.819 for 100 ppm.
13. The effect of pH was studied by observing the biodegradation for 48 hours at pH values of 5, 6, 7 and 8. Percentage removal was found to be 22.804, 88.091, 98.269 and 84.369 at pH of 5, 6, 7 and 8. Hence pH 7 was chosen as the most optimum pH.
14. The growth of *B. Subtilis* on pyridine as the sole carbon source and consequently removal of pyridine by the microorganism at various initial pyridine concentrations were now studied at these optimized conditions.
15. The growth of the microorganism was found to increase with increasing concentration of pyridine. But at the same time the lag phase increased with increasing concentrations of pyridine. The result showed in the lag period that the biodegradation time and the maximum microorganism concentration increased on increasing the substrate concentration. It is clear that pyridine was utilized by *B. Subtilis* effectively up to 500 ppm concentration.
16. The batch studies to find the effect of pyridine concentration were carried out for maximum of 120 hours. *Bacillus Subtilis* degrades about 100, 100, 100, 88.722, and 77.245 % of pyridine with an initial concentration of 50, 100, 200, 400 and 500 ppm. Times needed for complete consumption of pyridine at 50, 100 and 200 ppm were 47, 54 and 72 hours respectively. At higher concentration it was observed that towards the end of the substrate consumption curve, there is a region of relatively reduced rate of substrate removal.
17. The specific growth rate was found to be increasing with the increase in concentration when calculated for the batch study. Hence the process was found to follow Monod growth kinetics. Hence a double reciprocal plot was plotted

between inverse of specific growth rate and initial substrate concentration. The maximum specific growth (μ_{max}) rate was **0.0349 hr^{-1}**. Half saturation constant (K_s) obtained was **121.65 mg/l or ppm**.

18. Continuous run was carried out in biotrickling filter using soybean as the packing material and *B. Subtilis* as the microorganism. To evaluate the biotrickling filter performance the reactor was run at a flow rate of 1LPM with an empty bed residence time of 106 seconds. The concentration of pyridine in the inlet gas flow was 0.177 g/m^3 or 50 ppm and average volumetric loading rate was 6.01 g/m^3-h.
19. Though % removal of pyridine was quite satisfactorily high and it succeeded in obtaining 100% removal within 3 days, the process could not be continued longer as the bed collapsed and also rot thus producing foul smell.

7.2 Recommendations for future work:

On the basis of present study following recommendations can be made for future works-

1. The physico-chemical characteristics of soybean and puffed rice should studied in more detail and attempt can be made to standardize them.
2. The effect of other parameters like column diameter or height of adsorbent bed on continuous adsorption process should be studied.
3. Other models which are available for continuous gas phase reactors can be validated for this process.
4. The effect of biodegradation time should be studied for *Bacillus Subtilis*. The effect of initial contaminant concentration can also be studied for higher concentrations values.
5. Since not much work was found in literature regarding biodegradation of VOCs using *B. Subtilis*, it can be used for biodegradation of other VOCs as well.
6. Immobilized phase studies were not carried out in the present work. The efficiency should be compared in future works between suspended and immobilized phase biodegradation.
7. The failure of continuous process can be analyzed in more detail to ensure that there is more accuracy in selecting packing material.

PUBLICATIONS

Paper accepted in National/ International Conferences:

1. **S. J. Choudhury**, C. B. Majumder; Use of Biodegradation for Treatment of Volatile Organic Compounds; published in the proceedings of 23rd National Convention of Chemical Engineers, September 2007.

2. **S. J. Choudhury**, C. B. Majumder; Review Paper On Removal Of Volatile Organic Compounds (Vocs) By Pervaporation In Polydimethylsiloxane (Pdms) Membrane, selected in the International conference ICAPC-2008 at NEERI Nagpur.

Paper communicated to International Journals:

1. **S. J. Choudhury**, C. B. Majumder; Removal of Pyridine- A review; communicated to Journal Chemical Engineering Communication, Paper under review (Manuscript number assigned)

2. **S. J. Choudhury**, C. B. Majumder; Removal of Pyridine gaseous phase by Adsorption in Continuous reactor using puffed rice, communicated to Journal Chemical Engineering Communication, Paper under review ((Manuscript number assigned))

3. **S. J. Choudhury**, C. B. Majumder; Removal of Pyridine gaseous phase by Adsorption in Continuous reactor using puffed rice; communicated to Chemical Engineering Journal

REFERENCES

1. Abramovi´c B. F., Anderluh V. B., Topalov A. S., Gaál F. F. (2004). Titanium dioxide mediated photocatalytic degradation of 3-amino-2 chloropyridine, *Appl. Catalysis B: Environmental,* 48, 213–221
2. Adam J.H., Consatable J.V.H., Guenther A.B., Zimmerman P., An estimate of natural volatile organic compound emissions from vegetation since the last glacial maximum, Chemosph. Global Change Sci. 3(2001) 73–91.
3. Adamson A.W. (1990). Physical Chemistry of Surface, 5th Edition, Willey, New York
4. Adav S. S., Lee D. J., Ren N. Q. (2007). Biodegradation of pyridine using aerobic granules in the presence of phenol, *Wat. Res.*, 41, 2903 – 2910
5. Akita S., Takeuchi H., Sorption equilibria of pyridine derivatives in aqueous solution on porous resins and ion exchange resins. J. Chem. Eng. Jpn., 26, 237-241 (1993).
6. Allen D. W., Clench M. R., Morris J. (1994). Investigation of the products of oxidation of methylpyridines under aqueous conditions by gas chromatography–mass spectrometry, *Analyst*, 119, 903–907
7. Andreozzi R., Inzola A., Caprio A., D'Amore M. G. (1992). Quinoline ozonation in aqueous solution, *Wat. Res.*, 26, 639–643
8. Baker R. A. (1963). Threshold odors of organic chemicals, *J. Am. Water Works Ass.*, 55, 913-916
9. Barnes J.M., Apel W.A., Barrett B.K., Removal of nitrogen oxides from gas streams using biofiltration, J of Hazard. Mater., 41(1995) 315-326
10. Bell P. R. F., Greenfield P. F., Corney M., Moore A. J. (1984). Evaluation of rundle shale as an adsorbent for the treatment of retort water. In: *Proc. 17th Oil Shale Symposium,* Colorado School of Mines, Golden Colo

11. Berry, D. F., Francis A.J., Bollag J-M. (1987). Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions, *Microbiol. Rev.* 51, 43–59.

12. Bettmann H., Rehm H. J. (1985). Continuous degradation of phenol(s) by *Pseudomonas putida* p8 entrapped in polyacrylamide-hydrazide, *Appl. Microbiol. Biotechnol.*, 22, 389-39

13. Blanch H. W., Clark D. S., Biochem Eng, Marcel Dekker Inc., New York, (1996).

14. Bludau H., Karge h. G., Niessen W., Sorption, sorption kinetics and diffusion of pyridine in zeolites, Micropor. Mesopor. Mat., 22, 297-308 (1998).

15. Bollag, J.-M., Kaiser J. P. (1991). The transformation of heterocyclic aromatic compounds and their derivatives under anaerobic conditions, *Crit. Rev. Environ. Control,* 21, 297–329

16. Boyd G.E., Adamson A.W., Meyers L.S., The exchange adsorption of ions from aqueous solution by organic zeolites II Kinetics, J. Am. Chem. Soc., 69, 2836-2848 (1947).

17. Brinkmann U., Babel W. (1996). Simultaneous utilization of pyridine and fructose by *Rhodococcus opacus* UFZ B 408 without an external nitrogen source, *Appl. Microbiol. Biotechnol.*, 45, 217-223

18. Buttery R.G., Guadagni D.G., Ling L.C. (1978). Volatile components of cooked artichoke, *J. Agric. Food Chem.*, 26, 791.

19. Cain R.B., Houghton C., Wright K.A. (1974). Microbial metabolism of the pyridine ring. Metabolisms of 2- and 3-hydroxypyridine by maleamate pathway in *Achromobacter* sp., *Biochem. J.*, 140, 293

20. Chang K., Lu C., lin M-R., Treatment of volatile organic compounds from polyurethane and epoxy manufacture by a trickle-bed air biofilter, J of Biosci. & bioengg., 92(2001) 126-130

21. Cocquet G., Ferroud C., Guy A. (2000). A mild and efficient procedure for ring-opening reactions of piperidine and pyrrolidine derivatives by single electron transfer photooxidation, *Tetrahedron*, 56, 2975

22. Cox H.H.J., Marc A.D., Co-treatment of H2S and toluene in a biotrickling filter, Chem. Engg. J, 87(2002) 101-110

23. Dehghanzadeh R., Torkian A., Bina B., Poormoghaddas H., Kalantary A., Biodegradation of styrene laden waste gas stream using a compost-based biofilter, Chemosphere, 60(2005) 434-439

24. Delhomenie M.C., Bibeau L., Bredin N., Roy S., Broussau S., Brzezinski R., Kugelmass J.L., Heitz M., Biofiltration of air contaminated with toluene on a compost-based bed, Adv in Environ. Res., 6(2002) 239-254

25. Demetes, G. (1999). *The sweet smell of successful odor control.* Air Pollut. Consult. 9

26. Deshusses M.A., Biological waste air treatment in biofilters, Cur. Opin. in Biotech. 8(1997) 335-339

27. Dicks, R.M., Ottengraf, S.P.P., Process engineering aspects of biological waste gas purification. Environmental Technology. In: Proc. International Symposium on Environmental Biotechnology, Ostende, Belgium, Verachtert, H., Verstraete, W., Belgium: Royal Flemish Society of Engineers, pp. (1991) 353–368.

28. Diez P. R., Amalvy J. I., A density functional study of the adsorption of pyridine, 2-vinylpyridine, and 4-vinylpyridine onto a silica surface, J. Mol. Struc-Theochem, 634, 187–193 (2003).

29. Dobson K.R., Stephenson M., Greenfield P.F., Bell P.R.F. (1985). Identification and treatability of organics in oil shale retort water, *Wat. Res.* 19, 849

30. Dupont R. R., McLean J. E., Hoff R. H., Moore W. M. (1990). Evaluation of the use of solar irradiation for the decontamination of wood treating wastes, *J. Air Waste Manage. Assoc.*, 40, 1257–1265

31. Engwall M. A., Pignatello J. J., Grasso D. (1999). Degradation and detoxification of the wood preservatives creosote and pentachlorophenol in water by the photo-fenton reaction, *Wat. Res.*, 33, 1151–1158

32. Feng, Y., Kaiser, J.P., Minard, R.D., Bollag, J.M. (1994). Microbial transformation of ethylpyridines, *Biodegradation,* 5, 121–128.

33. Ferschl A., Loidl M., Ditzelmtiller G., Hinteregger C., Streichsbier F (1991). Continuous degradation of 3-chloroaniline by calcium alginate-entrapped cells of

Pseudomonas acidovorans CA28: influence of additional substrates, *Appl. Microbiol. Biotechnol.* 35, 544 550

34. Fetzner, S. (1998). Bacterial degradation of pyridine, indole, quinoline and their derivatives under different redox conditions, *Appl. Microbiol. Biotechnol.*, 49, 237–250

35. Fetzner, S. (1998). Bacterial degradation of pyridine, indole, quinoline and their derivatives under different redox conditions, *Appl. Microbiol. Biotechnol.*, 49, 237–250

36. Fox J.P., Jackson D.E., Sakaji R.H. (1980). Potential uses of spent shale in the treatment of oil shale retort water. In: *13th Oil Shale Symp. Proc.*, Colorado School of Mines, Golden, CO, 311–320

37. Grbic-Galic D. (1989). Microbial degradation of homocyclic and heterocyclic aromatic hydrocarbons under anaerobic conditions, *Dev. Ind. Microbiol.*, 30, 237–253.

38. Gupta R.C., Shukla O.P. (1975). Microbial metabolism of 2-hydroxypyridine, *Ind. J. Biochem. Biophys.*, 12, 296–298.

39. Hallas L. E., Adams W. J., Heitkamp M. A. (1992). Glyphosate degradation by immobilized bacteria: field studies with industrial wastewater effluent, *Appl. Environ. Microbiol.* 58, 1215-1219

40. Hille R. (1996). The mononuclear molybdenum enzymes, *Chem. Rev.*, 96, 2757–2816.

41. Houghton C., Cain R. B., Microbial metabolism of the pyridine ring, formation of pyridinediols (dihydroxypyridines) as intermediates in the degradation of pyridine compounds by micro-organisms, Biochem. J., 130, 879-893 (1972).

42. Hounsell, G., Case studies: selection of high efficiency VOCs removal technologies for process air streams. In: Proc. 88th Annual Meeting of the Air and Waste Management Association. Pfizer Inc., San Antonio, TX, pp. (1995) 1–24

43. Iranpour R., Cox H.H.J., Deshusses M.A., Schroeder E.D., Literature Review of Air Pollution Control Biofilters and Biotrickling Filters for Odor and Volatile Organic Compound Removal, Environ. Progress. 24(2005) 254-267

44. IUPAC Manual of Symbols and Terminology of Colloid Surface, Butterworths, London, 1 (1982).

45. Jin Y., Veiga M.C., Kennes C., Co-treatment of hydrogen sulfide and methanol in a single-stage biotrickling filter under acidic conditions, Chemosphere, 68(2007)

46. Kaiser J. P., Feng Y., Bollag J. M. (1996). Microbial Metabolism of Pyridine, Quinoline, Acridine, and their Derivatives under Aerobic and Anaerobic Conditions, *Microbiol. Rev.*, 483–498

47. Khammar N., Malhautier L., Degrange V., Lensi R., Fanlo J.L., Evaluation of dispersion methods for enumeration of microorganisms from peat and activated carbon biofilters treating volatile organic compounds, Chemosphere, 54(2004) 243-254

48. Khan I.F., Ghoshal A.K., Removal of Volatile Organic Compounds from polluted air, J Loss Preven. 13(2000) 527-545

49. Kim D., Cai Z., Sorial G.A., Impact of interchanging VOCs on the performance of trickle bed air biofilter, Chem. Engg. J, 113(2005) 153-160

50. Kim M. K., Singleton I., Yin C. R., Quan Z. X., Lee M., Lee S. T. (2006). Influence of phenol on the biodegradation of pyridine by freely suspended and immobilized *Pseudomonas putida* MK1, *Lett. Appl. Microbiol*, 42, 495–500.

51. Kirk R. E., Othmer D. F. (1982). *Encyclopedia of chemical technology*, 3rd ed., John Wiley Science, New York, 15, 454-480.

52. Klemetson, S.L. (1979). Ultrafiltration and hyperfiltration of phenoliccompounds in coal gasification wastewater, *Pollut. Abstract*, 10, 111-79-00632.

53. Korosteleva L. A., Kost A. N., Vorob'eva L. I., Modyanova L. V., Terent'ev P. B., Kulikov N. S. (1981). Microbiological degradation of pyridine and 3-methylpyridine, *Appl. Biochem. Microbiol.*, 17, 276

54. Koutinasa M., Baptistaa I.I.R., Meniconib A., Peevaa L.G., Mantalarisa A., Castroc P.M.L., Livingston A.G., The use of an oil–absorber–bioscrubber system during biodegradation of sequentially alternating loadings of 1,2-dichloroethane and fluorobenzene in a waste gas, chem. Engg. Sci., 62(2007) 5989-6001

55. Kuhn, E. P., Suflita J.M.. (1989). Microbial degradation of nitrogen, oxygen and sulfur heterocyclic compounds under anaerobic conditions.Studies with aquifer samples, *Environ. Toxicol. Chem.* 8, 1149–1158

56. Kumar R., Mishra I.M., Mall I.D. (1995). Treatment of pyridine bearing wastewater using activated carbon, *Res. and Industry*, 40, 33-37.

57. Lahane F., Malhautier L., Roux J.C., Fanlo J.L., Absorption of a mixture of volatile organic compounds (VOCs) in aqueous solutions of soluble cutting oil, Biores. Technol., (2007)

58. Lahane F., Malhautier L., Roux J.C., Fanlo J.L., Absorption of a mixture of volatile organic compounds (VOCs) in aqueous solutions of soluble cutting oil, Biores. Technol., (2007)

59. Lataye D.H., Mishra I.M., Mall I.D. (2006). Removal of pyridine from aqueous solution by adsorption on bagasse fly ash, *Ind. Eng. Chem. Res.*, 45(11), 3934-3943.

60. Lataye D.H., Mishra I.M., Mall I.D. (2008). Pyridine sorption from aqueous solution by rice husk ash (RHA) and granular activated carbon (GAC), *J. Hazrd. Mater.*, 154, 858-870

61. Lee J. J., Rhee S.-K, Lee S.T., Degradation of 3-methylpyridine and 3-ethylpyridine by *Gordonia nitida* LE31, Appl. Environ. Microb., 67(9), 4342–4345 (2001).

62. Lee S.T., Rhee S.K., Lee G.M. (1994). Biodegradation of pyridine by freely suspended and immobilized *Pimelobacter* species, *Appl. Microbiol. Biotechnol.*, 41, 652–657.

63. Legrini O., Oliveros E., Braun A.M. (1993). Photochemical processes for water treatment, *Chem. Rev.*, 93, 671–698.

64. Lettau, H. (1980). Chemie der Heterocyclen. Deutscher Verlag fu¨r Grundstoffindustrie, Leipzig, Germany

65. Lewis R. J. Sr. (2004). *Sax's Dangerous Properties of Industrial Materials*, Eleventh edition, John Wiley & Sons: Hoboken, New Jersey, 3106.

66. Li L., Liu J.X., Removal of xylene from off-gas using a bioreactor containing bacteria and fungi, Internl. Biodeter. & Biodegran., 58(2006) 60-64

67. Li Y., Gu G., Zhao J., Yu H. (2001). Anoxic degradation of nitrogenous heterocyclic compounds by acclimated activated sludge, *Process Biochem.*, 37 81-86

68. Liu Y., Quan X., Sun Y., Chen J., Xue D., Chung J.S., Simultaneous removal of ethyl acetate and toluene in air streams using compost-based biofilters, J of Hazard. Mater., B95(2002) 199-213

69. Lodha B., Bhadane R., Patel B., Killedar D. (2008). Biodegradation of pyridine by an isolated bacterial consortium/strain and bio-augmentation of strain into activated sludge to enhance pyridine biodegradation, *Biodegradation,* DOI 10.1007/s10532-008-9176-4

70. Low G. K.-C., McEvoy S. R., Mathews R. W. (1991). Formation of nitrate and ammonium ions in titanium dioxide mediated photocatalytic degradation of organic compounds containing nitrogen atoms, *Environ. Sci. Technol.*, 31, 3573–3580

71. Lowry H.H. (1973). *Chemistry of Coal Utilization, Supplementary.* Volume I. John Wiley and Sons Inc., London

72. Lu Ch., Lin M-R., Lin J., Chang K., Removal of ethylacetate vapor from waste gases by a trickle-bed air biofilter, J of Biotech., 87(2001) 123-130

73. Luh M. D., Baker R.A., Sorption and desorption of pyridine-clay in aqueous solution, Water Res. 5, 849-859 (1971).

74. Mall I.D., Tewari S., Singh N., Mishra I.M. (2003). Utilisation of bagasse fly ash and carbon waste from fertiliser plant for treatment of Py and 3-picoline bearing wastewater. *Proceeding of the Eighteenth International Conference on Solid Waste Technology and Management* held at Philadelphia, PA, USA, March 23-26.

75. Martin C., Martin I., Rives V., An FT-IR study of the adsorption of pyridine, formic acid and acetic acid on magnesia and molybdena-magnesia, Journal of Molecular Catalysis, 73, 51-63 (1992).

76. Mathew C. D., Nagasawa T., Kobayashi M., Yamada H. (1988). catalyzed production of nicotinic acid from 3-cyanopyridine in *Rhodococcus rhodochrous* J1, *Appl. Environ. Microbiol.*, 54, 1030

77. Mathur A. K., Majumder C. B. (2008a). Biofiltration of pyridine by Shewanella putrefaciens in a corn-cob packed biotrickling filter, Clean Soil Air Water
78. Mathur A.K., Majumdar C.B., Chatarjee S., Combined removal of BTEX in air stream by using mixture of sugar cane bagasse, compost and GAC as Biofilters media, J of Hazard. Mater.(2007).
79. Mathur A.K., Majumder C.B., Chatterjee S., Roy P. (2008 b), Biodegradation of pyridine by the new bacterial isolates *S. Putrefaciencs* and *B. Spaericus*, J. Hazard. Mater., doi:10.1016/j.jhazmat.2007.12.112
80. Mathur A.K., Sunaramurthy J., Majumder C.B., Kinetics of the removal of mono-chlorobenzene vapour from waste gases using a trickle bed Biofilters, J Hazard. Mater., B137(2006) 1560-1568
81. Moe W.M., Qi B., Performance of a fungal biofilter treating gas-phase solvent mixtures during intermittent loading, Water Res., 38(2004) 2259-2268
82. Mohan D., Singh K.P., Sinha S., Gosh D., Removal of pyridine derivatives from aqueous solution by activated carbons developed from agricultural waste materials, Carbon, 43(2005) 1680-1693
83. Mohan D., Singh K.P., Sinha S., Gosh D., Removal of pyridine from aqueous solution using low cost activated carbons derived from agricultural waste materials, Carbon, 42, 2409-2421 (2004).
84. Mohan S. V., Sistla S., Guru R. K., Prasad K. K., Kumar C. S., Ramakrishna S.V., Sarma P.N. Microbial degradation of pyridine using *Pseudomonas* sp. and isolation of plasmid responsible for degradation, Waste Manage. 23, 167–171 (2003).
85. Mohseni M., Allen D.G., Biofiltration of mixtures of hydrophilic and hydrophobic volatile organic compounds, Chem. Engg. Sci., 55(2000) 1545-1558
86. Morgan P., Lewis S. T., Watkinson R. J., Biodegradation of benzene, toluene, ethylbenzene and xylenes in gas-condensate-contaminated ground-water, Environ. Pollut. 82 (1993) 181–190.
87. Mudliar S. N., Padoley K. V., Pandey R. A., Bhatt P., Sureshkumar M., Lokhande S. K., Vaidya A. N., Pyridine bioegradation in novel rotating rope bioreactor, Biores. Techol., 95 (2007) 1044-1051

88. Mueller J. G., Chapman P. J., Pritchard, P. H. (1989). Creosote-contaminated sites – Their potential for bioremediation, *Environ. Sci. Technol.*, 23, 1197–1201

89. Naik M.N., Jackson R.B., Stokes J., Swaby R.J., (1972). Microbial degradation of picloram and other substituted pyridines, *Soil Biol. Biochem.*, 4, 313.

90. Nicolai R.E., Janni K.A., Biofilter media mixture ratio of wood chips and compost treating swine odors, Wat. Sci. Technol. 44 (2001) 261–267.

91. Nielsen D.R., Daugulis A.J., McLellan P.J., Dynamic simulation of benzene vapor treatment by a two-phase partitioning bioscrubbers Part I: Model development, parameter estimation, and parametric sensitivity, Biochem Engg J., 2007

92. Note (1999). Simplified description of adsorption breakthrough curves of 1,1-Dichloro- 1- fluoroethane (HCFC- 141b) on activated carbon with temperature effects, J. Col. Inter. Sci., 214, 455-458

93. O'Loughlin E. J., Sims G. K., Trainal S. J., Biodegradation of 2-methyl, 2-ethyl, and 2-hydroxypyridine by an *Arthrobacter* sp. isolated from subsurface sediment, Biodegradation, 10, 93–104, (1999).

94. Otten L., Afzal M.T., Mainville D.M., Biofiltration of odours- laboratory studies using butyric acid, Adv. in Environ. Res., 8(2004) 397-409

95. Ozacar M., Sengil I.A., Adsorption of metal complex dyes from aqueous solutions by pine sawdust. Biresource Technol., 791-795, (2005).

96. Padoley K V, Mudliar S N, Pandey R A, (2008). Heterocyclic nitrogenous pollutants in the environment and their treatment options – An overview, *Biores. Technol.* 99, 4029–4043

97. Padoley K.V., Rajvaidya A.S., Subbarao T.V., Pandey R.A., Biodegradation of pyridine in a completely mixed activated sludge process, Bioresource Technol., 97, 1225–1236 (2006).

98. Pandey P.K., Varshney C.K., Emission of volatile organic compounds (VOC) from tropical plant species in India, Chemosphere. 59(2005) 1643–1653.

99. Pereira, W.E., Rosad, C.E., Garbarino, J.R., Hult, M.F. (1983). Groundwater contamination by organic bases derived from coal-tar wastes, *Environ. Toxicol. Chem.*, 2, 283.

100. Pezolt D. J., Collick, S. J., Johnson, H. A., & Robbins, L. A., Pressure swing adsorption for VOC recovery at gasoline terminals. Chem. Engg. Progress, *16* (1997) 16

101. Potivichayanon S., Pokethitiyook P., Kruatrachue M., Hydrogen sulfide removal by a novel fixed-film bioscrubber system, Process Biochem., 41(2006) 708-715

102. Prousek, J. (1996). Advanced oxidation processes for water treatment. Chemical processes, *Chemicke Listy*, 90, 229–237.

103. Qi B., Moe W.M., Performance of low pH biofilters treating a paint solvent mixture- Continuous and intermittent loading, J of Hazard. Mater., B135(2006) 303-310

104. Reichenberg D., Properties of ion-exchange resin in relation to their structure. III. Kinetics of exchange, J. Am. Chem. Soc., 75, 589 (1953).

105. Rhee S. K, Lee G. M, Lee S. T. (1996). Influence of a supplementary carbon source on biodegradation of pyridine by freely suspended and immobilized *Pimelobacter* sp., *Appl. Microbiol. Biotechnol.* 44, 816-822

106. Rhee S.K., Lee G. M., Yoon J.H., Park Y.H., Bae H.S., Lee S.T., Anaerobic and Aerobic Degradation of Pyridine by a Newly Isolated Denitrifying Bacterium, Applied and Environmental Microbiology, 63(7), 2578–2585 (1997).

107. Richards, D.J., Shieth, W.K. (1986). Biological fate of organic priority pollutants in the aquatic environment, *Wat. Res.*, 20, 1077–1090

108. Rogers J.E., Rily R.G., Li S.W., O' Melley M.L., Thomas B.L. (1985). Microbial transformation of alkyl pyridine in ground water, *Wat. Air Soil Pollut.*, 24, 443–454.

109. Romao M.J., Huber R. (1998). Structure and function of the xanthineoxidase family of molybdenum enzymes, *Struct. Bond.*, 90, 69–95.

110. Ross, R.D. (1965). *Industrial Waste Disposal. Rein Hold Book.* Corpn. New York

111. Ruddy, E.D., Carroll, L.A., Select the best VOCs control strategy. Chem. Eng. Progress. 89(1993) 28–35.

112. Shanker R., Kaiser J.P., Bollag J.M. (1991). Microbial transformation of heterocyclic molecules in deep subsurface sediments, *Microb. Ecol.*, 22, 305–316

113. Shukla A., Zhang Y.H., Dubey P., Margrave J.L., Shukla S.S., The role of sawdust in the removal of unwanted materials from water, J of Hazard. Mater., 137-152 (2002).

114. Shukla O. P., Kaul S. M. (1986). Microbial transformation of pyridine-Noxide and pyridine by *Nocardia* sp., *Can. J. Microbiol.*, 32, 330

115. Sims G.K., Sommers L.E., Konopka A. (1986). Biodegradation of pyridine derivatives in soil suspensions. *Environ. Toxicol. Chem.*, 5, 503.

116. Sims, G.K., Loughlin, E.J.O. (1989). Degradation of pyridines in the environment, *Crit. Rev. Env. Ctrl.* 19, 309–340

117. Spigno G., Pagella C., Fumi M.D., Molteni R., De Faveri D.M., VOCs removal from waste gases- gas-phase bioreactor for the abatement of hexane by Aspergillus niger, Chem. Engg. Sci., 58(2003) 739-746

118. Srivastava A., Joseph A.E., Patila S., More A., Dixit R.C., Prakash M., Air toxics in ambient air of Delhi, Atmosp. Environ. 39(2005) 59–71.

119. Stern M., Heinzle E., Kut O. M., Hungerbuhler K., Removal of substituted pyridines by combined ozonation/fluidiaed bed biofilm treatment, Water Sci. Technol., 34(4), 329-335 (1997).

120. Steven B.H., Robert E.S. (1984). Emission of organic air pollutants from shale oil wastewaters, *Environ. Sci. Technol.*, 18, 483–490.

121. Stobdan T., Sinha A., Singh R. P. (2007). Degradation of pyridine and 4-methylpyridine by *gordonia terrea* IIPN1, *Biodegrdation,* DOI 10.1007/ s10532-007-9152-4

122. Stuerner D.H., Ng D.J., Morris C.J. (1982). Organic contaminants in ground water near an underground coal gassification site in northeastern Wyoming, *Environ. Sci. Technol.* 16, 582–587

123. Tadjeddine A., Le Rille A., Pluchery O., HeH bert P., Zheng W.Q., Marin T., Adsorption of pyridine on gold, studied by difference frequency generation (DFG) using the CLIO-FEL Nuclear Instruments and Methods in Physics Research A, 429, 481-484 (1999).

124. Thomas J.M., Ward C.H. (1989). In situ biorestoration of organic contaminants in the subsurface, *Environ. Sci. Technol.*, 23, 760-766

125. Tien C., Adsorption Calculations and Modeling, Butterworth-Heinemann, 1994.

126. Uma B., Sandhya S., Kinetics of pyridine degradation along with toluene and methylene chloride with Bacillus sp. in packed bed reactor, Bioprocess Eng. 18, 303-305 (1998).

127. Uma B., Sandhya S., Pyridine degradation and heterocyclic nitrification by Bacillus coagulance, Can. J. Microbiol., 43, 595-598 (1997).

128. Uma T. S., Sandhya S., Sathyanarayana S., Kaul S. N. (2002). Biodegradation of pyridine from pharmaceutical wastewater using *Bacillus* consortia, *J. IAEM*, 29, 76–80

129. USEPA. EPA handbook: control technologies for hazardous air pollutants USEPA: Office of Research and Development, Washington, DC. (1991)

130. Vitzthum O.G., Werkhoff P., Hubert P. (1975). Volatile components of roasted Cocoa: basic fraction, *J. Food Sci.* 40, 911.

131. Waki K., Zhao J., Horikoshi S., Watanabe N., Hidaka H. (2000). Photooxidative mechanism of nitrogen-containing compounds at TiO_2/H_2O interface: an experimental and theoretical examination of hydrazine derivatives, *Chemosphere*, 41, 337

132. Watson G. K., Cain R. B., Microbial metabolism of the pyridine ring, metabolic pathways of pyridine biodegradation by soil bacteria Biochem. J. 146, 157-172 (1975).

133. Watson G. K., Houghtont C. Cain R. B., Microbial metabolism of the pyridine ring the hydroxylation of 4-hydroxypyridine to pyridine-3, 4-diol (3, 4-dihydroxypyridine) by 4-hydroxypyridine-3-hydroxylase, Biochem. J. 140, 265-276 (1974).

134. Westmeier F., Rehm H. J. (1987). Degradation of 4-chlorophenol in municipal wastewater by adsorptive immobilized *Alcaligenes* sp. A 7 2, *Appl. Microbiol. Biotechnol.*, 26, 78-83

135. Yang R. D., Humphrey A. E., Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures, Biotechnol. Bioeng. 17 (1975) 1211–1235.

136. Yates, F.S. (1984). *Pyridine and their benzo derivatives: (vi) applications in: A.R.Katritzky, C.W. Rees Eds., Comprehensive Heterocyclic Chemistry: The Structure, Reaction, Synthesis and uses of Heterocyclic compounds,* Vol. 2, Part 2 A, Chapter 2.09, Pergamon Press, Oxford, 511-524,

137. Yu L.J., Shukla S.S., Dorris K.L., Shukla A., Margrave J.L., Adsorption of chromium from aqueous solutions by maple sawdust J. Hazard. Mater., 53-63, (2003).

138. Zaki M.I., Hussein G.A.M., Mansour S.A.A., El-ammawy H.A., Adsorption and surface reactions of pyridine on pure and doped ceria catalysts as studied by infrared spectroscopy, J. Molecular Catalysis, 51, 209 – 220 (1989).

139. Zhu S., Bell P.R.F., Greenfield P.F., Adsorption of pyridine onto spent rundle oil shale in dilute aqueous solution. Water Res, 22(10), 1331–1337 (1988).

140. Zhu S., Bell, P. R. F., Greenfield P. F. (1995). Quinoline adsorption onto combusted rundle spent shale in dilute aqueous solution at the natural pH 8, *Wat. Res.*, 29, 1396–1400.