BIODEGRADATION DYNAMICS OF PHENOLIC WASTEWATERS USING *GLIOMASTIX INDICUS* MTCC 3869

A THESIS

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DEEPIKA ARYA

DEPARTMENT OF CHEMICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE-247 667 (INDIA)

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INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **"Biodegradation dynamics of phenolic wastewaters using *Gliomastix indicus* MTCC 3869"** in partial fulfilment of the requirement for the award of the Degree of Doctor of Philosophy and submitted in the Department of Chemical Engineering of the Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out during a period from January 2007 to March 2012 under the supervision of Dr. Surendra Kumar, Professor, and Dr. (Mrs.) Shashi Kumar, Associate Professor, Department of Chemical Engineering, Indian Institute of Technology Roorkee, Roorkee, India.

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

Dpika
DEEPIKA ARYA

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

Shashi
(Shashi)
Supervisor

(Surendra Kumar)
Supervisor

Date: 26.3.2012

The Ph.D. Viva - Voce Examination of **Ms. Deepika Arya**, Research Scholar, has been held on 14.5.2012 at 12.00 noon

Sign. of Supervisors

Sign. Chairman SRC

Arunjay Kumar
Sign. of External Examiner

Head of the Department/Chairman ODC

ABSTRACT

Environmental pollution due to phenolic compounds is a major ecological problem as a result of their use in various industries. Phenol, resorcinol, and p–cresol are the phenolic compounds found in the effluents of many industries. They have harmful effects on every living being. Phenolic compounds present in a mixture cause problem during their biodegradation because different operating conditions like pH and temperature may be required for the biodegradation of different substrates. Hence, the analysis of substrate interaction taking place among these multiple substrates is a crucial step. Besides, the biodegradation technology fulfils the need of an economical and ecofriendly technology for the proper treatment of phenolic wastewaters. Use of biological treatment facility provides advantage of low capital and operating cost, no harmful by-product formation with simple installation of the treatment unit. Hence, in the present research work the biodegradation technique has been used for the removal of three phenolic compounds phenol, resorcinol, and p–cresol.

Literature sources revealed that most of the authors reported the biodegradation of phenolic compounds using bacteria and fungi. The studies involving fungi for biodegradation have been conducted for the detection of metabolic pathway and only a few authors have worked on biodegradation kinetics. Most of the research on biodegradation kinetics is centred on biomass growth kinetics only. There is a lack of quantitative analysis of substrate expenditure for maintenance of biomass, effect of maintenance energy expenditure on biomass growth yield and the biodegradation dynamics of substrates. The aim of this research work was to investigate these aspects of biodegradation of phenol, resorcinol, and p–cresol in single and dual substrate biodegradation systems.

The batch biodegradation experiments were conducted for phenol, resorcinol, and p–cresol as single and dual substrate systems using pure culture of filamentous fungus *Gliomastix indicus* MTCC 3869. Modified czapeck medium was used to carry out the experiments under the pH of 6 and temperature 28 °C. Biodegradation of phenol, resorcinol, and p–cresol was studied up to the initial concentration of 1000, 1300, and 700 mg/L, in the single substrate biodegradation system, starting from the

lowest concentration of 10 mg/L. The dual substrate biodegradation was carried out using the three substrates as phenol – p–cresol and phenol – resorcinol, in three combinations for each dual substrate system (100 mg/L phenol in presence of 300 mg/L p–cresol/resorcinol, 200 mg/L phenol in presence of 200 mg/L p–cresol/resorcinol, 300 mg/L phenol in presence of 100 mg/L p–cresol/resorcinol). Initially the fungus was acclimatized, supplying 2% glucose in the medium with the toxic substrates. 5 % V/V inoculums was taken in 250 mL flasks with working volume of 50 mL for biodegradation kinetic experiments. The lag phase was found to be completed within 19, 15, and 24 h for phenol, resorcinol and p–cresol respectively.

Five single substrate inhibition growth kinetic models were applied for the analysis of biomass growth kinetics of the fungus for the biodegradation of phenol, resorcinol, and p–cresol as single substrates. Predictions of Andrews and Noack model were found in best agreement with the experimental data of specific growth rate, for phenol and p–cresol. For resorcinol Yano model was found to be best fit to specific growth rate data. During the experimentation phenol, resorcinol, and p–cresol were observed inhibitory to biomass growth and self biodegradation beyond initial concentration of 70, 90, and 50 mg/L respectively. The maximum specific growth rate value 0.129 h^{-1} at 70 mg/L of phenol, 0.132 h^{-1} at 90 mg/L of resorcinol, and 0.102 h^{-1} at 70 mg/L of p–cresol were achieved. Similarly maximum observed biomass growth yield values of 0.437 g/g, 0.443 g/g and 0.31 g/g were obtained for phenol, resorcinol, and p–cresol at their respective inhibitory initial concentrations. Eight models (M1, M2, M3, M4, M5, M6, M7, and M8) analogous to growth inhibition kinetic models were used to examine the single substrate degradation kinetics of phenol. M1 to M4 are specific degradation rate models and M5 to M8 are initial specific degradation rate models. M1 and M8 were found to be best fit to the experimental degradation data of phenol. Generally, initial specific degradation rate models are applicable when the substrate degradation rate is too slow that was not the case here, therefore only the specific degradation rate models were considered for resorcinol and p–cresol. The specific degradation rate models M3 and M1 were best fit models for the experimental degradation rate data of resorcinol and p–cresol respectively. A maintenance energy model for the estimation of substrate consumption for biomass maintenance was proposed. The minimum value of maintenance energy coefficient 0.020 h^{-1} at 70 mg/L of phenol, 0.0135 h^{-1} at 90 mg/L of resorcinol, and 0.0229 h^{-1} was obtained at 50 mg/L

of p–cresol. The maximum values of maintenance energy coefficient were obtained at 1000 mg/L, 1300 mg/L, and 700 mg/L for phenol, resorcinol, and p–cresol respectively. Beyond the inhibitory initial concentration the maintenance energy coefficient values tend to increase with the initial concentration of the substrate due to the substrate inhibition. Therefore, it was found that the specific growth rate and observed biomass growth yield values are reduced beyond the inhibitory initial concentration of the substrate due to the increased substrate consumption for biomass maintenance during biodegradation process of toxic substrates as phenol, resorcinol, and p–cresol. Three models model – a, model – b and model – c were developed to investigate the biodegradation dynamics of single substrate degradation. The set of mathematical equations corresponding to each model were solved to get computed profiles of substrate biodegradation with time. The effect of variation in maintenance energy expenditure and observed biomass growth yield was incorporated in model – a. Model – b was based on the initial biodegradation rates and the variation of maintenance and observed biomass growth yield was not considered in case of model – c. Predictions of model – a were identified in best agreement with the experimental data in entire range of initial substrate concentration for phenol, resorcinol, and p–cresol. Predictions of model – b were not found in agreement with the experimental data of degradation for any of the substrate. Predictions of model – c showed a bit agreement with experimental data of phenol and p–cresol in lower substrate concentration range only. For resorcinol, the predictions of model – c were not in close agreement with the experimental degradation data. Hence, the effect of maintenance energy variation is important to consider, for the study of biodegradation dynamics of inhibitory substrates as phenol, resorcinol, and p–cresol.

The experiments on the two dual substrate biodegradation systems, phenol – p–cresol, and phenol – resorcinol were carried out to study the substrate interaction during the biodegradation of the two substrates. The substrate interaction between phenol and p–cresol was studied using three combinations of these substrates; 100 mg/L phenol with 300 mg/L p–cresol, 200 mg/L phenol with 200 mg/L p–cresol, and 300 mg/L phenol with 100 mg/L p–cresol. Similarly, three combinations of phenol and resorcinol were used to study the substrate interaction between them. A model to describe the specific growth rate and substrate interaction was derived and solved by Levenberg-Marquardt nonlinear regression technique. Four types of substrate inhibitions were

tested. Interaction parameter values ($I_{a,1}$= 0.044, $I_{a,2}$ = 1.17) revealed that the phenol inhibited the degradation of p–cresol more than the p–cresol caused inhibition to phenol degradation in the medium. For phenol – resorcinol degradation system the interaction parameter values ($I_{a,1}$= 1.09, $I_{a,2}$ = 0.052) indicated that the resorcinol posed stronger inhibition to the phenol degradation in comparison to the inhibition caused by phenol to resorcinol degradation. The competitive cross inhibition was observed to be involved during the biodegradation of two homologous substrates phenol and p–cresol, phenol and resorcinol. For the study of substrate degradation kinetics with the substrate consumption as maintenance energy expenditure in dual substrate systems, a conceptual model was developed which incorporates the variation of maintenance energy expenditure and specific growth rate with the variation in the concentrations of the two substrates in their mixture. To the best of our knowledge, there is no other study available in the literature for the estimation of maintenance energy expenditure in the dual substrate systems. The work presented here, is a reasonable starting point for the development and validation of mathematical model to describe the maintenance energy expenditure and thereby the specific degradation rate for mixtures of two homologous substrates. The specific degradation rate values were estimated for each substrate phenol, resorcinol, and p–cresol in the two dual substrate degradation systems. The model has provided excellent predictions of substrate degradation rate with the variation in maintenance energy expenditure. Biodegradation of phenol, resorcinol, and p–cresol was modelled in dual substrate degradation system. A set of equations was developed by incorporating the maintenance energy and growth yield variation, and solved to get the computed time profiles of substrate degradation. The model predictions were very close to the experimental data well.

It is our view that the proposed models for the biodegradation of phenolic compounds provide in depth knowledge of the biodegradation of organic pollutants, prediction of microbial growth, and substrate degradation dynamics for phenolic waste-water treatment in single as well as in the dual substrate systems, which may be useful for the design of a biodegradation facility.

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DEEPIKA ARYA

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NOMENCLATURE

Abbreviations

PSSH	pseudo-steady state hypothesis
SKIP	Sum Kinetics with Interaction Parameters

Notations

f	[–]	Substrate interaction coefficient
$I_{a,1}$	[–]	Inhibition to the degradation of substrate 1 in the presence of substrate 2
$I_{a,2}$	[–]	Inhibition to the degradation of substrate 2 in the presence of substrate 1
$I_{b,1}$	[L/mg]	Inhibition to the degradation of substrate 1 in the presence of both the substrates 1 and 2
$I_{b,2}$	[L/mg]	Inhibition to the degradation of substrate 2 in the presence of both the substrates 1 and 2
K_{41}	$[mg/L]^2$	Substrate interaction coefficient for substrate 1 as defined in Eq. (5.37)
K_{42}	$[mg/L]^2$	Substrate interaction coefficient for substrate 2 as defined in Eq. (5.38)
K_{51}	[mg/L]	Substrate interaction coefficient for substrate 1 as defined in Eq. (5.37)
K_{52}	[mg/L]	Substrate interaction coefficient for substrate 2 as defined in Eq. (5.38)

K_i	[mg/L]	Inhibition constant
K_{i1}	[mg/L]	Inhibition constant for substrate 1
K_{i2}	[mg/L]	Inhibition constant for substrate 2
K_S	[mg/L]	Saturation constant or half velocity constant
K_{S1}	[mg/L]	Saturation constant of biomass growth for substrate 1
K_{S2}	[mg/L]	Saturation constant of biomass growth for substrate 2
k	[–]	Constant used in Eq. (4.7)
k_{11}	[mg/L]	Ratio of k_{4b} to k_4
k_{12}	[mg/L]	Ratio of k_{10b} to k_{10}
k_{21}	[mg/L]	Ratio of k_{5b} to k_5
k_{22}	[mg/L]	Ratio of k_{11b} to k_{11}
k_{31}	[mg/L]	Ratio of k_{6b} to k_6
k_{32}	[mg/L]	Ratio of k_{11b} to k_{11b}
k_2, k_8	[h^{-1}]	Reaction rate constants
k_{i1}	[mg/L]	Ratio of k_{11b} to k_{11b}
k_{i2}	[mg/L]	Ratio of k_{9b} to k_9
k_j	[mg.h/L]$^{-1}$	Forward reaction rate constant, j = 1, 3, 4, 5, 6, 7, 9, 10, 11, 12
k_{jb}	[h^{-1}]	Backward reaction rate constant, j = 1, 3, 4, 5, 6, 7, 9, 10, 11, 12

m_1	[h^{-1}]	Constant component of maintenance energy coefficient
m_2	[h^{-1}]	Growth dependent component of maintenance energy coefficient
m_S	[h^{-1}]	Maintenance energy coefficient
m_{Si}	[h^{-1}]	Maintenance energy coefficient for substrate i
q_S	[h^{-1}]	Specific degradation rate
q_{S1}	[h^{-1}]	Specific degradation rate of substrate 1
q_{S2}	[h^{-1}]	Specific degradation rate of substrate 2
q_{Si}	[h^{-1}]	Specific degradation rate of substrate i
$q_{S_{max}}$	[h^{-1}]	Maximum specific degradation rate
q_{S_o}	[h^{-1}]	Initial specific degradation rate
$q_{S_{o\max}}$	[h^{-1}]	Maximum initial specific degradation rate
S	[mg/L]	Substrate concentration
S_1	[mg/L]	Concentration of substrate 1
S_2	[mg/L]	Concentration of substrate 2
S_i	[mg/L]	Concentration of substrate i
S_o	[mgL^{-1}]	Initial substrate concentration
t	[h]	Time
X	[mg/L]	Biomass concentration

X_o	[mg/L]	Initial biomass concentration
X_T	[mg/L]	Total biomass concentration
$(Y_{X/S})_o$	[g/g]	Observed growth yield coefficient
$(Y_{X/S})_T$	[g/g]	True growth yield coefficient
$(Y_{X/S})_{T1}$	[g/g]	maximum growth yield coefficient with respect to substrate 1
$(Y_{X/S})_{T2}$	[g/g]	Maximum growth yield coefficient with respect to substrate 2
$(Y_{X/S})_{Ti}$	[g/g]	maximum growth yield coefficient with respect to substrate i

Greek letters

μ_g	[h^{-1}]	Specific growth rate
$\mu_{g_{max}}$	[h^{-1}]	Maximum specific growth rate
μ_{gi}	[h^{-1}]	Specific growth rate for substrate i
μ_{g1}	[h^{-1}]	Specific growth rate for substrate 1
μ_{g2}	[h^{-1}]	Specific growth rate for substrate 2

CHAPTER I

INTRODUCTION

Water is most precious natural resource for life on the earth. For establishment of a stable community, availability of safe and reliable water resource is essential. The rapid industrial development, urbanization, lack of effective environmental protection and continuous population growth are affecting water bodies such as rivers, lakes, and coastal waters severely. Factors responsible for the depletion of water resources are uneven distribution of water resources, low rate of natural replenishment and frequent droughts [Mohan and Pittman (2006)]. The pollutants present in water make it unsuitable for drinking, irrigation and many other beneficial purposes.

General components of wastewater are 95% water, pathogens, organic chemicals and inorganic substances like metals. The presence of pathogens and hazardous substances, e. g. Cu, Co, Mn, Ni, and Zn in limited quantities, is beneficial and essential for life because they give water a pleasant taste and have a positive effect on health [Gupta (2004)]. Pollutants generated from various sources contaminate the natural water resources and the polluted water enters into the earth surface for reuse. Waste compounds introduced into natural water bodies undergo biochemical reactions involving oxidation, because the natural water bodies are rich with microorganisms and nutrients. These biochemical reactions lead to the deficiency of dissolved oxygen into the water bodies, whereas the oxidisable chemicals as reducing agents, initiate chemical reactions demanding increased amount of oxygen in the water [Srinivas (2008), Tchobanoglous *et al.* (2003)].

On the basis of the origin, wastewater can be classified as follows [Srinivas (2008)]:

(i) The black water comes from lavatories, cesspit leakage, sewage treatment plant discharge and septic tank discharge, while the washing water (personal, clothes, floors, dishes etc.) is known as grey water.

(ii) Wastewater from rainfall contains oil and fuel residues, rubber residues, metal from vehicle exhaust, animal and plant waste.

(iii) Wastewater from domestic sources contains liquids like drinks, cleaning liquids, pesticides, lubricating oil, cooking oil, paint etc.

(iv) The wastewater coming from industries has significant amount of toxic chemicals. Industrial wastewater contains alkali, oil, sand, biocides and other organic-inorganic chemicals which cause extreme pH changes in wastewater.

Therefore, considering the limited availability of fresh water for living beings the wastewater must be treated and recycled. The wastewater treatment strategy includes four steps for water purification [Srinivas (2008)].

(i) Preliminary treatment of wastewater is physical operation for the removal of floating and heavy settleable solids of wastewater. Grease and oil are removed in skimming tanks for improvement of flow characteristics of water; it also prevents the clogging of sewers and pumps.

(ii) Inorganic substances and organic settleable solids are removed by primary treatment process.

(iii) For the removal of organic matter from wastewater, biological and chemical processes are used during secondary treatment. This step involves coagulation and stabilization of colloidal solids and organic matter.

(iv) The wastewater is subjected to the treatment by the combination of unit operations and processes in tertiary treatment process of wastewater for the removal of residual nitrogen, phosphorous, and chlorides.

In the biological treatment of wastewater, organic matter present in wastewater can be degraded by microorganisms as it serves as a source of carbon and energy. Use of microorganisms for this purpose is economical and ecofriendly too. The types of biological treatment of wastewater are, namely aerobic, anaerobic, and photosynthetic. Oxygen is required for aerobic treatment of wastewater; it involves oxidation of toxic substrates for its metabolism by the microorganisms. It results into the formation of carbon dioxide, water, and energy molecules in the microorganisms. Anaerobic

treatment of wastewater occurs in the absence of oxygen. Anaerobes oxidize organics in the complete absence of dissolved oxygen, using oxygen bound in other compounds such as nitrate, sulphate, and degrade complex organic pollutants into methane and carbon dioxide (biogas). Anaerobic wastewater treatment is a slow process compared to aerobiosis. Anaerobes are more sensitive to toxic compounds and are much sensitive to pH changes than aerobes. In photosynthetic treatment process, the microorganisms utilize solar energy to meet required energy demands. In addition to this, since the cellular materials of microorganisms are organic in nature, the microorganisms undergo self oxidation in the absence of carbon and energy source. The self oxidation is known as endogenous respiration utilizing own body cells. During self oxidation, microorganisms produce non-biodegradable materials that are relatively stable.

There is a variety of aliphatic and aromatic toxic compounds present in wastewater. In the present work, our focus is on the biological treatment of aromatic, phenolic compounds like phenol, resorcinol, and p–cresol.

1.1 PHENOLIC COMPOUNDS IN WASTEWATER

Phenolic compounds enter into the environment from both the natural as well as xenobiotic sources [Wang (2007)]. They contain one or more hydroxyl groups that are attached to a benzene ring. These compounds constitute a large group of pollutants in the wastewater. Environmental pollution due to phenolic compounds is a major problem, which is being faced by industrialized countries today. Phenol and its derivatives such as resorcinol, and cresols are widely found in the effluents of many industries. The xenobiotic sources of phenols are waste derived from oil refineries, ceramic plants, steel plants, coal conversion process plants, and textile, phenolic resin, paper and pulp, pharmaceutical, fertilizers, pesticides, plastic, petrochemical, explosive production, rubber industries. Besides, phenol is used in the manufacturing of nylon, polycarbonates, hydraulic fluids, heavy duty surfactants, as a disinfectant in household cleaning and consumer products like mouthwashes, gargles, and throat sprays. Adhesives, creams, lotions for skin, and biological glues contain 0.5 - 2 % resorcinol. It is found in the mainstream of cigarette smoke in the range of 0.0008 – 0.008 mg per cigarette [Rustemeier *et al.* (2002)]. p–Cresol is used in manufacturing of disinfectants, fumigants, photographic developers, and explosives [Tallur *et al.* (2006)]. The discharge range of phenolic compounds depends upon the type of industry. Although

there is a lack of quantitative data on the range of discharge concentrations of resorcinol, and p–cresol in industrial wastewater, the discharge ranges of concentration of phenol, resorcinol and p–cresol from a few industries have been summarized in the Table 1.1 [González-Muñoz *et al.* (2003), Gonzalez *et al.* (2001), Kira *et al.* (2000), Phutdhawong *et al.* (2000), Kumaran and Paruchuri (1997), Babich and Davis (1981)]. Once the phenolic compounds present in untreated industrial effluents, are released into the environment, they persist in the water for a week or more and adversely affect life forms. They react with metal ions and other compounds present in the waste resulting into the formation of more toxic complex compounds [Sufit (1978)].

Table 1.1: Discharge ranges of phenol, resorcinol, p-cresol in industrial wastewaters

Industries	Discharge range (mg/L)		
	phenol	resorcinol	p–cresol
Gas plant effluent	≤ 131.25	≤ 11.25	≤ 114.5
Low temperature carbonization effluent	≤ 3395	≤ 770	≤ 1425
Coke oven effluent	≤ 283.5	≤ 14.8	≤ 95
Manufacturing of phenolic resin	≤ 10000	≤ 10000	≤ 10000
Manufacturing of plastic	600 – 2000		
Petrochemical industry	50 – 600	–	–
Production of stocking	≤ 6000		
Oil refineries	10 – 100		
Manufacturing of fiber glass	40 – 400		
Domestic wastewater	0.1 – 1		
Coal fuel conversion plant	9 – 680	≤ 1000	≤ 1423
Pharmaceutical, plastics, wood products, paint, paper and pulp industries	0.1 – 1600		
Steel industry	500 – 1000		

Phenol can be detected in water by its odor and taste in the concentration range of 0.01 – 0.1 mg/L. Short term exposure to high levels of phenolic compounds cause headaches, irritation of eyes, skin and respiratory tract, while long term exposure has adverse effect on detoxification system of body affecting kidney, liver, lungs, and lead to the damage of heart, red blood cell, central nervous system, spleen, and

gastrointestinal system with cardiovascular disease [Pohanish (2011), Shawabkeh *et al.* (2007), Nuhoglu and Yalcin (2005), González *et al.* (2001)].

1.1.1 Regulations for Phenolic Compounds

Environmental Protection Agency (EPA) has included a list of eleven substituted phenols in the federal register [Khleifat (2006)]. These phenols have hazardous effects on human health. Ministry of Environment and Forest (MOEF), Government of India has set standards for discharge of phenolic compounds into environment: the concentration should not exceed 1 mg/L in case of surface water, and should not be more than 5 mg/L in case of public sewers, marine coastal areas, and on land for irrigation [Aksu and Bulbul (1998)]. Phenol and resorcinol are not potential carcinogens. But United States Environmental Protection Agency has classified p–cresol as pollutant of group C (possible human carcinogens) and listed it as priority pollutant [ATSDR (1990), Fawell and Hunt (1988), Buckman *et al.* (1984)]. Maximum permissible concentrations of phenol in nonchlorinated and chlorinated water are 0.1 and 0.001- 0.002 mg/L respectively [Eksperiandova *et al.* (1999)]. On chlorination, phenol at a concentration of 0.005 mg/L imparts smell, therefore, World Health Organization (WHO) has set a limit level of 0.001 mg/L to regulate the concentration of these phenolic compounds in drinking water [WHO (1963)]. It is recommended that human exposure should not be more than 20 mg of phenol in an average working day [Hannaford and Kuek (1999)]. The European Union listed phenol among the "substances undesirable in excessive amounts" and has decided that its amount in waters (lakes, streams) should be limited to 0.3 mg/L to protect human health from the possible harmful effects on exposure to phenol by drinking water and eating contaminated water plants [Zuane (1997)].

1.2 REMOVAL TECHNOLOGIES

Various treatment technologies have been studied for the treatment of wastewater contaminated with phenols in order to reduce the toxicity of wastewater and thereby to reduce the environmental load of harmful phenolic compounds. Among these technologies, the most commonly used physico-chemical techniques include adsorption, incineration, solvent extraction, ion exchange, chlorination, chemical precipitation, ozonation etc. The use of these techniques has limitations of high energy

consumption, high start-up and running costs of wastewater treatment units. Besides, the treatment process results into the production of secondary effluents such as cyanates, chlorinated phenols, hydrocarbons etc.

Technique of adsorption uses adsorbent like activated carbon for the removal of toxic compounds. The used activated carbon is subjected to the process of incineration which gives rise to many new compounds such as dioxins and furans having severe consequences on human health. The non-adsorbable contaminants cannot be removed by this technique. Solvent extraction involves the use of solvents, namely benzene, isopropyl ethyl, and butyl acetate for recovery of phenolic compounds which remain present into the water with residual phenols. Hence the water is still not safe for use even after the treatment and also the solvents being used are costly [Behera and Jena (2009)]. Ion exchange process uses lime soda to maintain the pH above 10 during the wastewater treatment. Calcium ions in excess, released from lime soda remain present into the water even after the treatment. Chlorination generates chlorinated phenols and leads to severe odour problem [Nemerow (1971)]. Technique of chemical precipitation suffers from the drawbacks like pH fluctuations, generation of chemical sludge that must be treated before disposal, insufficient efficiency for removal of toxic compound, and high operation cost [Lettner *et al.* (2007)]. Wastewater treatment by ozonation does not remove dissolved minerals, salts from water and generates harmful by-products such as bromate. Ozone is highly corrosive especially for steel and iron [Rakness (2005)].

There is a need of an economical and ecofriendly technology for the proper treatment of phenolic wastewaters. In this regard, the biodegradation technique can replace physico-chemical methods because of certain advantages: simple installation of a biological treatment unit, lower capital and operating cost, and no harmful by-product formation [Shawabkeh *et al.* (2007), Khleifat (2006)]. The disadvantages associated with the technique of biodegradation are slow rate of biochemical reactions, nutrient depletion, pH changes, and the presence of toxic or inhibitory chemicals which reduce the viability of microorganisms beyond a certain concentration of toxic substrate. The rate of biochemical reactions can be improved using the technique of microbial immobilization [Behera and Jena (2009)]. Although there are many microorganisms reported in the literature (and described in the Chapter II) which survive in the presence

of high concentrations of toxic chemicals, but a combination of both physico-chemical and biological treatment techniques can be suggested as an economical option to achieve complete and ecofriendly degradation of toxic compounds present in the wastewater. The intermediate concentration of 5 – 500 mg/L for phenols in wastewater, is generally found suitable for biodegradation. The wastewater with higher concentrations (beyond 500 mg/L) of phenols must be treated with the techniques as solvent extraction, and for the lower concentrations (1 – 5 mg/L) ozonation and/or adsorption techniques can be used [Patterson (1985)].

As stated earlier, there are two types of biodegradation techniques in use; aerobic and anaerobic. In aerobic biodegradation, oxygen is used as an electron acceptor and microorganisms utilize this oxygen to oxidize organic substances to obtain the energy. In anaerobic processes, the microorganisms utilize nitrates, sulphates, ferrous salts and other hydrogen (electron) acceptor to obtain energy for the synthesis of cellular material from organic substances. The rate of anaerobic biodegradation is slow in comparison to aerobic biodegradation process. Usually aerobic microorganisms are preferred for wastewater treatment process, because they are more efficient at biodegradation of toxic compounds and they grow faster in comparison to anaerobes, transforming organic compounds into carbon dioxide, water etc. which are further utilized by several other organisms too [Kim *et al.* (2002)]. For the success of biodegradation strategies, understanding of the environmental factors affecting the rate of biodegradation is necessary. Some intrinsic factors of microbial cells alter the rate of uptake of toxic compounds and microbial metabolism. Therefore, to achieve better biodegradation efficiency, few factors affecting the rate of biodegradation are summarized in Table 1.2 [Srinivas (2008), Wang (2007)]. A variety of microorganisms are available to consume toxic substrates as carbon and energy source, in the wide range of environmental conditions for the treatment of large volumes of phenolic wastewater. Various microorganisms studied for biodegradation of phenolic compounds so far are Bacteria, Yeast, and Fungi. Table 1.3 provides a few microorganisms being used for biodegradation of phenol and its derivatives [Jain (2001)].

Table 1.2: Factors affecting the rate of biodegradation

Factors	Mechanism and implications
Electron acceptors	Oxygen is used as an electron acceptor in aerobic biodegradation. Nitrates, sulphates, ferrous salts and the hydrogen (electron) acceptor are used to obtain energy in anaerobic processes.
Microbial populations degrading phenols	Microbial populations need to get adapted for utilization of toxic chemicals. Therefore, the acclimatization is a significant step before the microorganism is exposed to toxic environment.
Molecular structure of phenols	The benzene ring with hydroxyl/methyl group, or with both methyl and hydroxyl groups is susceptible to enzymatic cleavage and further to be metabolized by microorganisms.
Bioavailability of compound	The solubility of toxic substrate into water affects the bioavailability of the compound.
Added nutrients	The addition of the background nutrients (N, S, P, K, Fe etc) must be sufficient in the medium to maintain the biodegradation rate.
Temperature	5° to 40° C is the most suitable temperature for soil bacteria and fungi to flourish. In general as the temperature increases, the microbial activity increases exponentially, but optimal temperature is necessary to maintain the enzyme activity and microbial metabolism.
pH	Most microorganisms have their peak activity around a neutral pH of 6 to 9. Moulds and yeasts prefer acidic range while sulphur bacteria (Thiobacillus) prefer a pH around 1. Methane fermentors cease to act when the pH falls below 6.
Salinity	Saline environments adversely affect the biodegradation rate.

Table 1.3: Microorganisms in use for biodegradation of phenols

Bacteria	*Pseudomonas putida, Acetobacter, Pseudomonas flurescence, Azotobacter, Rhodococcus erythro, Acinetobacter calcoaceticus, Bacillus sp., Methanobacillus omelianskii*
Yeast	*Candida tropicalis, Deberomyces subglobosus, Trichosporon cutaneum, Ochromonas danica, Basidiomycota, Candida maltose, Aureobasidium pullulans, Candida maltose, Rhodotorula glutis, Trichosporon cutaneum*
Fungus	*Aspergillus sp., Pencillium sp., Neurospora crassa, Phanerochate crysosporium, Scedosporium apiospermum, Mortierella sarnyensis, Fusarium flocciferum, Penicillium sp.*

1.3 FUNGUS IN BIODEGRADATION

Fungi are widely spread in nature and are capable to degrade complex natural substances such as lignin, cellulose, and chitin [Stanchev *et al.* (2008), Juang and Tsai (2006)]. They are multicellular, nonphotosynthetic, heterotrophic eukaryotes. Most fungi are either strict or facultative aerobes, which reproduce sexually or asexually, by fission, budding, or spore formation. Molds or true fungi produce microscopic units "hyphae" which collectively form a filamentous mass called mycelium. Yeasts are fungi that cannot form a mycelium, and are therefore unicellular.

1.3.1 Advantages of using Fungi [Tchobanoglous *et al.* (2003)]

The use of fungal cultures provides advantages over bacterial cultures as given below.

(i) Fungi have ability to grow under low moisture, low nitrogen condition and can tolerate an environment with relatively low pH and temperature changes, while bacterial cultures are much sensitive to pH and temperature changes and conditions of nutrient limitation. The ability of fungi to survive under low pH and nitrogen limiting conditions coupled with their potential to degrade cellulose, makes them very important in the composting of sludge.

(ii) Fungi are enzyme rich, therefore, they possess higher potential for biodegradation in comparison to bacteria.

(iii) Fungi are eukaryotes, more developed organisms than bacteria (prokaryotes), and are ubiquitous in nature.

(iv) Fungi are more efficient in assimilating and storing the nutrients. They show higher cellular activity and high salt tolerance in comparison to bacteria.

(v) Survival chances of fungal cultures are more than bacteria because their cell wall is made up of chitin and melanin polymers, which make them resistant to degradation by other microorganisms. Bacterial membranes are made up of energy rich phospholipid molecules, and function as rich food source for a wide range of microorganisms.

A large number of reports on the biodegradation of phenolic compounds by bacteria are available in the literature [Bajaj *et al.* (2009), Wang *et al.* (2008), Bai *et al.* (2007), Juang and Tsai (2006), Khleifat (2006), Kumar *et al.* (2005), Jiang *et al.* (2005), Arutchelvan *et al.* (2005), Alexievaa *et al.* (2004), Tchobanoglous *et al.* (2003)]. But, the application of yeast and fungal strains has not been studied in detail. However, the filamentous fungi are being used in many biological processes like production of organic acids, enzymes, hormones, antibiotics, steroids, and treatment of environmental problems [Luke and Burton (2001), Gerin *et al.* (1995)]. In recent years the research interest has been focused on the degradation of industrial effluents by the fungi in order to solve the environmental problems. In the present research programme *Gliomastix indicus* strain MTCC 3869 has been used for biodegradation of phenol, resorcinol, and p–cresol in synthetic wastewaters.

1.3.2 Morphology of Filamentous Fungus *Gliomastix indicus*

Gliomastix indicus MTCC 3869 is a filamentous fungus, isolated by Nagalakshmi *et al.* (2009), from a wasteland soil sample at Tiruchengode, Tamilnadu. The fungus was assigned to the genus *Gliomastix* because of the presence of catenulate amerospores on undifferentiated, sporogenous cell. It is a fast growing fungus on all common mycological media like malt extract, potato-dextrose agar in the temperature range of 25 – 30 °C. Fig. 1 shows the mycelium of *G. indicus* with conidiophores. The

mycelium is hyaline in the beginning but soon becomes ropy, radiating from the centre and turns to sooty black with abundant sporulation. The mycelium is branched, septate and pale brown in colour. Conidia are one celled, oval to spherical or oblong, catenulate, and brown in colour. One or two vacuoles are present per conidium. Spherical conidia are 11 μm in diameter, oval conidia measure 13.8 – 18.5 × 7 – 9 μm and oblong conidia are 14 – 17.5 × 6.5 – 11 μm in size.

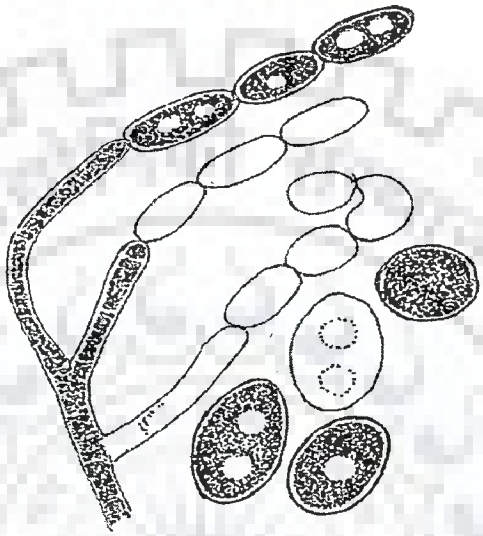

Figure 1.1: Mycelium of filamentous fungus *Gliomastix indicus* with conidia

Reverse side of colony is black and the margin is off white. *G. indicus* has potential to biodegrade the paper mill effluent and can produce α amylase also [Nagalakshmi *et al.* (2003)].

1.4 BIODEGRADATION OF PHENOLIC COMPOUNDS IN WASTEWATER

The technique of biodegradation uses microorganisms for the removal of toxic compounds such as phenols. In batch biodegradation studies the toxic substrates with nutrients present in the culture medium are consumed by microorganisms as carbon and energy source for biomass synthesis, extra-cellular product formation and for maintenance activity of the cells. Hence, the concentrations of nutrients, biomass and toxic substrate vary with time as the microbial growth proceeds. The maintenance activity includes motility, repair and resynthesis, osmotic regulation, transport and heat loss [Shuler and Kargi (2003)]. When the number of cells produced is plotted as a function of time, the batch growth pattern of microorganism under cultivation is

obtained. This growth pattern has five growth phases, namely lag phase, exponential growth phase, deceleration phase, stationary phase, and death phase.

The lag phase takes place just after the inoculation of the medium with microbial culture. This phase is for the adaptation of microbial cells to the new environmental conditions. The number of cells does not increase but a little increase may occur in the cell mass. After the adaptation period, cell mass and number both grow exponentially, therefore, the concentration of biomass increases continuously during the exponential growth phase. The exponential growth phase is the period of balanced growth in which all components of a cell grow at the same rate with increase in the biomass concentration but the average composition of a cell remains constant. Experimental data of biomass growth and substrate degradation are taken during exponential growth phase, because biomass growth and substrate degradation take place during this phase only. The deceleration phase occurs for a very short period of time, and follows exponential growth phase. In this phase, the rate of microbial growth decelerates due to either the depletion of nutrients or the accumulation of toxic by-products in the medium. The biomass concentration, cell number, and cell size change during the deceleration phase. At the end of the deceleration phase the stationary phase starts where no further growth occurs and the number of cells remains stationary but the number of viable cells decreases due to cell lysis. At this phase cell growth rate becomes equal to the cell death rate. To generate new energy producing monomers and building blocks cells catabolise their cellular reserves. The death phase occurs after stationary phase, where only the cell death takes place.

During single substrate biodegradation of a toxic compound, the biomass growth inhibition occurs, due to the enhanced substrate toxicity, beyond a certain initial concentration in the culture medium. This substrate inhibition effect results into the slow biomass growth rate, lower biomass yield, and increase in the energy expenditure for maintenance of the cells. Usually the industrial effluents contain a mixture of pollutants. The occurrence of phenolic compounds in a mixture of pollutants in wastewater creates problem for their biodegradation because different operating conditions such as pH and temperature may be required for the biodegradation of different compounds. Besides, the biodegradation of one substrate may be inhibited by the presence of other substrates present in the wastewater. The interaction among these

multiple substrates is complex due to their toxicity and competition for microbial enzymes and cofactors [Hu *et al.* (2005)]. Therefore, it is necessary to study the way of interaction of substrates with each other and its effect on their degradation. However, the mixture of pollutants may contain inorganics, heavy metals, radionuclides, the readily degraded compounds such as a mixture of sugars (from fermentation), other than the toxic organic chemicals. Some of these pollutants may serve as the growth substrate, supplying carbon and energy to the microorganisms which are responsible for the biodegradation and they may induce the production of enzymes required for biodegradation of the recalcitrant, nongrowth organic chemicals. But most of the workers have reported negative interactions only [Reardon *et al.* (2000)].

In dual substrate system the biodegradation rate remains low, usually due to the substrate toxicity, competitive inhibition, and the formation of toxic intermediates by non specific enzymes [Juang and Tsai (2006), Mendonca *et al.* (2004), Alexievaa *et al.* (2004), Katayama *et al.* (1994)]. Various types of substrate interaction patterns including competitive inhibition and non-competitive inhibition have been observed in different dual substrate biodegradation systems [Wang and Loh (1999), Kar *et al.* (1997), Hutchinson and Robinson (1988), Meyer *et al.* (1984)]. The pollutants like phenol, resorcinol, and p–cresol are toxic to growing cells. They damage the cells inhibiting their metabolism and stop their growth when present in higher concentrations. Such inhibitory substrates can be degraded as dual substrate by the microorganisms, because enzymes and co-factors being utilized by microorganisms to degrade one toxic substrate do not act with specificity, therefore, structurally similar chemical compounds can be degraded in mixtures [Doran (2005), González-Muñoz *et al.* (2003), Eksperiandova *et al.* (1999)]. The structurally related compounds have an effect on the overall metabolic rates of compounds in a multisubstrate environment [Latkar and Chakrabarti (1994)]. Therefore, the microorganisms being used for biodegradation must be able to degrade and survive in the presence of all the pollutants in addition to the phenols.

In majority of biodegradation studies research workers have frequently reported biomass growth kinetics and substrate degradation kinetics without taking into account the changes in maintenance energy requirement of the culture. A significantly higher amount of maintenance energy is required during the biodegradation of toxic

substrates, in comparison to other cultures where energy providing substrate is non-toxic substance like glucose, fructose, molasses etc. Experimentally it has been observed that the biomass growth yield and requirement of maintenance energy of microorganisms vary with the different concentrations of toxic substrate in the medium [Jiang *et al.* (2007b), Hao *et al.* (2002), Banerjee *et al.* (2001)]. The energy requirement for the cell maintenance depends upon the microorganism and the toxic substrate under biodegradation. The operating conditions such as temperature, concentration of nutrients, and pH value in the medium also affect the maintenance energy requirement of microorganisms. The cells under biodegradation studies require a minimum, constant and continuous amount of maintenance energy to tolerate the toxicity of the substrate and for their survival at each growth phase. This amount of maintenance energy is consumed by the cells for maintenance activities while the rest of the maintenance energy is produced for the growth of microorganisms. Further, when substrate inhibition to biomass growth takes place in the medium, the degree of toxicity of substrate including the production of various intermediates and extracellular products, affect the biomass growth yield adversely and lead to the higher requirement of energy for maintenance of the cells [Minkevich *et al.* (2000), Allsop *et al.* (1993)]. Therefore, the biomass growth yield and the substrate degradation are not directly proportional to each other. Substrate degradation takes place even though the biomass growth yield is low, because the consumed substrate is utilized for more energy generation to be utilized for higher maintenance of microbial cells at enhanced concentrations of the toxic substrates like phenol, resorcinol, and p–cresol, causing the inhibition to biomass growth and to their own degradation. Hence, the concept of energy expenditure for maintenance of cells is needed to provide proper description of biodegradation dynamics. The knowledge of biodegradation dynamics is significant to design the biodegradation unit and to predict the changes in the concentration of a component in the wastewater during its removal by biodegradation technology.

1.5 OBJECTIVES

In view of the reviewed literature, the main objectives of present research work are as follows:

(A) EXPERIMENTATION

(i) To procure the filamentous fungus *Gliomastix indicus* MTCC 3869 and to acclimatise it for high concentrations of phenol, resorcinol and p–cresol separately.

(ii) To measure the biomass growth and substrate concentrations at different time intervals using phenol, resorcinol, and p–cresol as single substrate.

(iii) To measure the biomass growth and concentrations of the two substrates (phenol and p–cresol) at different time intervals using combinations of various concentrations of phenol and p–cresol in dual substrate degradation study.

(iv) To measure the biomass growth and concentrations of two substrates (phenol and resorcinol) at different time intervals using combinations of various concentrations of phenol and resorcinol in dual substrate degradation study.

(B) COMPUTATION

a. Single Substrate Biodegradation

(i) To select the single substrate growth kinetic model on the basis of statistical analysis by fitting the experimental data and to estimate the model parameters for phenol, resorcinol, and p–cresol as single substrate using appropriate tool in MATLAB.

(ii) To model the biomass growth yield and maintenance energy expenditure for phenol, resorcinol, and p–cresol as single substrate.

(iii) To propose biodegradation kinetic models for phenol, resorcinol and p–cresol as single substrate.

(iv) To select the best fitted biodegradation kinetic model on the basis of statistical analysis and to estimate the model parameters for phenol, resorcinol, and p–cresol as single substrate using appropriate tool in MATLAB.

(v) To describe the biodegradation rate by incorporating the variation in growth yield and maintenance energy expenditure.

(vi) To develop mathematical models by considering different conditions of yield and maintenance energy to illustrate the single substrate biodegradation dynamics.

(vii) To solve the developed mathematical models by using appropriate equation solver tool in MATLAB and to compute the single substrate biodegradation profiles with time, for phenol, resorcinol, and p–cresol as single substrate.

(viii) To validate the model predictions with experimental results in order to select the best mathematical model with suitable growth and maintenance conditions to illustrate degradation dynamics for phenol, resorcinol, and p–cresol.

b. Dual Substrate Biodegradation

(i) To select multi-substrate growth kinetic model and to estimate the model parameters by fitting the experimental data for dual substrate systems, namely phenol – p–cresol, and phenol – resorcinol, by using optimization tool in MATLAB.

(ii) To select the multi-substrate degradation kinetic model by incorporating the yield and maintenance energy variations for two dual substrate systems: phenol – p–cresol, and phenol – resorcinol.

(iii) To develop the mathematical model to illustrate the dynamics of substrate degradation in dual substrate system.

(iv) To solve the model equations using appropriate equation solver tool in MATLAB for dual substrate systems, viz. phenol – p–cresol, and phenol – resorcinol

(v) To validate the model predictions with experimental results for dual substrate systems, namely phenol – p–cresol, and phenol – resorcinol, to illustrate the dual substrate degradation dynamics.

1.6 ORGANIZATION OF THE THESIS

The thesis has been organised into five Chapters. Chapter I introduces the background information on water pollution due to phenolic compounds, phenol,

resorcinol, and p–cresol, their discharge ranges from various industries, regulations, the removal technologies, significance of biodegradation technology over other techniques in use, the importance of fungi for biodegradation, microbial growth phases, biomass yield, maintenance energy expenditure and biodegradation dynamics. Chapter II presents detailed review of literature on single substrate and dual substrate biodegradation studies of phenolic compounds with the studies on maintenance energy expenditure during the uptake of organic compounds. The Chapter III focuses on the acclimatization and substrate degradation experiments of the three substrates in single and dual substrate degradation system using fungal strain *Gliomastix indicus* MTCC 3869. The computational techniques have been presented for the analysis of experimental data. Chapter IV has been devoted to the kinetics for biomass growth, variation in biomass growth yield, substrate consumption for maintenance activity and substrate degradation in single substrate biodegradation systems from theoretical point of view. After the single substrate biodegradation kinetics the results and discussion on the single substrate biodegradation of phenol, resorcinol, and p–cresol have been presented. Various models, used for biodegradation study have been solved by MATLAB and two other parameters, namely biomass growth yield coefficient and maintenance energy coefficient have also been calculated. Chapter V describes the results of dual substrate biodegradation study of two substrate systems, viz. phenol – p–cresol and phenol – resorcinol, which includes growth kinetics, substrate degradation kinetics, modelling of maintenance energy expenditure and biodegradation dynamics for the two dual substrate systems. Chapter VI highlights the main conclusions of the thesis and provides the recommendations for future work.

CHAPTER II

LITERATURE REVIEW

2.0 INTRODUCTION

The biological treatment of wastewater to remove phenolic compounds has been in use for past many years. The phenolic compounds present in the industrial effluents may differ in their biodegradability. Besides, due to the availability of high precision detection instruments and techniques, the pollution load of these compounds has been detected even up to nano levels. Therefore, from time to time the biological treatment of phenolic wastes has undergone several modifications with the aim of improving the extent of removal of phenolic compounds from wastewater within the permissible discharge limit. Hence, enormous literature is available on various aspects of removal technology of phenolic waste by biological treatment. This makes the task of writing a detailed literature review extremely difficult. Therefore, in this Chapter, only those aspects of biological treatment have been described and reviewed, which elucidate the biodegradation of phenolic compounds in batch cultures and are required for its modelling and simulation studies related to the objectives of the present research program, outlines in the Chapter I. The present research program has been focused on the biological treatment of aromatic compounds, viz. phenol, resorcinol, and p–cresol from wastewater in single and multisubstrate systems.

There are many studies available in the literature on single substrate biodegradation kinetics. Most of them are focused only on growth kinetics. In the present research program, growth kinetics of filamentous fungus *G. indicus* has been studied for the biodegradation of phenol, resorcinol and p–cresol in single and dual substrate biodegradation systems, along with the biodegradation kinetics and biodegradation dynamics, estimation of maintenance energy requirement. In the literature, biodegradation studies on phenolic wastes have been carried out widely on bacteria, and a few studies are reported on algae and fungi [Table 1.3 in Chapter I]. The

biodegradation kinetic studies reported on the growth kinetics of different phenolic compounds are briefly summarized in Table 2.1. The later sections have been devoted to present an overview of the experimental and modelling studies related to biodegradation of phenolic compounds in single substrate and multisubstrate systems along with the biomass yields and maintenance energy requirements.

2.1 SINGLE SUBSTRATE BIODEGRADATION STUDIES

In this section the studies reported on single substrate biodegradation have been summarized.

ClauBen and Schmidt (1998) isolated a fungus from soil and identified it as *Scedesporium apiospermum*. The purpose of this study was to discover the different metabolic pathways in the fungus, for the biodegradation of phenol and p–cresol. They used 5 mM/L phenol or p–cresol of the same concentration as carbon and energy source at temperature 37 °C and pH 7.3. The fungal strain degraded hydroquinone, 1, 2, 4-benzenetriol, catechol, 4-hydroxybenzylalcohol, 4-hydroxybenzaldehyde, 4-hydroxybenzoate, protocatechuate and 3-oxo-adipate besides phenol and p–cresol. Results obtained from these studies demonstrated that both the phenol and p–cresol induced appropriate enzymes for degradation of these compounds.

In this study p–cresol was found to be catabolised through a single pathway that resulted into the formation of 3-oxoadipate while phenol was degraded by two different enzymatic sequences. The methyl group of p–cresol was oxidised to 4-hydroxybenzylalcohol, 4-hydroxybenzaldehyde and 4-hydroxybenzoate that further resulted into the production of protocatechuate. Protocatechuate was channelled in to the *ortho*-pathway. The fungus degraded phenol into the sequence catechol and 3-oxo-adipate, while the other sequence involved was hydroquinone, 1, 2, 4-trihydroxybenzene, malelacitate and 3- oxo-adipate.

Wang and Loh (1999) worked over a wide range of initial phenol concentration using *Pseudomonas putida* ATCC 49451 in batch cultures. They used Haldane model to study growth kinetics but the model was found inadequate to predict the phenol biodegradation profiles specifically at high initial phenol concentrations.

Table 2.1 Summary of growth kinetics obtained in various studies on the biodegradation of phenolic wastes

S. No.	Microbial strain	Substrate	Concentration range (mg/L)	Kinetic Model	Kinetic model parameters			Temperature (°C)/pH	Reference
					μ_m (h^{-1})	K_S (mg/L)	K_i (mg/L)		
1	*Lysinibacillus cresolivorans*	m–cresol	0 – 1200	Haldane	0.89	426.3	51.26	35 / 7	Yao *et al.* (2011)
2	Mixed culture	Phenol	23.5 – 659	Haldane	0.309	0.793	6.88	25 / 7.2	Bajaj *et al.* (2009)
3	*Rhodococcus sp.* NJUST16	Picric acid	20 – 800	Haldane	0.236	9.91	362.7	30 / 7	Shen *et al.* (2009)
4	Mixed culture	Phenol	100 – 600 100 – 400	Haldane	0.48 0.185	171.2 65.1	294.7 243.56	27 /7	Saravanan *et al.* (2008a)
5	Mixed culture	Phenol	100 – 800	Haldane	0.308	44.92	525	27 /7	Saravanan *et al.* (2008b)
6	*Alcaligenes faecalis*	Phenol	10 – 1400	Haldane	0.15	2.22	145.4	30 /7.2	Bai *et al.* (2007)
7	*Pseudomonas putida* CCRC 14365	Phenol	0 – 416	Haldane	0.245	0.129	12.6	30 /7	Juang and Tsai (2006)
8	*Candida tropicalis*	Phenol	0 – 2000	Haldane	0.48	11.7	208	30 /6	Yan *et al.* (2006)

Contd…

Table 2.1 Summary of growth kinetics obtained in various studies on the biodegradation of phenolic wastes (Contd.)

S. No.	Microbial strain	Substrate	Concentration range (mg/L)	Kinetic Model	Kinetic model parameters			Temperature (°C)/pH	Reference
					μ_m (h^{-1})	K_S (mg/L)	K_i (mg/L)		
9	*Aspergillus awamori* NRRL 3112	Phenol	0 – 1000	Haldane	0.017	1	1.07	30/5.5	Stoilova *et al.* (2006)
10	Mixed culture	o–Cresol	30 – 600	Haldane	0.368	92.4	125.2	25/7	Maeda et al. (2005)
11	Mixed culture	Phenol	25 – 1450	Haldane	0.143	87.45	107.06	25/–	Nuhoglu and Yalcin (2005)
12	*Pseudomonas putida* MTCC 1194	Phenol Catechol	0 – 1000 0 – 500	Haldane	0.305 0.326	36.33 29.89	129.79 99.85	30 /7	Kumar *et al.* (2005)
13	*Pseudomonas putida* F1 ATCC 700007	Phenol	10 – 200	Haldane	0.051	18	430	30 /7	Abuhamed *et al.* (2004)
14	*Acinetobacter* sp.	Phenol	60 – 350	Haldane	0.83	1.5	250	30 /-	Hao *et al.* (2002)
15	*Pseudomonas putida* ATCC 49451	Phenol	25 – 800	Haldane	0.90	6.93	284.3	30 /6.5	Wang and Loh (1999)
16	*Arthrobacter* sp. MTCC 1553	Phenol p–cresol o–cresol	50 - 1200	Haldane	0.82 0.92 0.84	90 48 84	1000 800 1050	28 /7	Kar *et al.* (1997)

Authors reported that it was because of the inhibition of metabolic intermediates during phenol degradation and the variable cell mass yield. Therefore to overcome this shortcoming they proposed a new phenol degradation model incorporating the inhibition effects of metabolic intermediates and extended the model over a wide range of initial substrate concentration. 2 – hydroxyl muconic acid semialdehyde (2-HMAS) was found to be as major intermediate in phenol degradation and was found to be proportional to the amount of phenol degraded. The concentration of the 2-HMAS reached a maximum at around the time when phenol could not be detected in the medium. After that, its concentration decreased quickly to the extent of being undetectable. The value of yield coefficient varied between 0.94 to 0.43 g/g. The new model was compared with Haldane equation and it was concluded that the inhibition of metabolic intermediates played a crucial role in phenol degradation modelling, especially over a wide initial substrate concentration range.

Luke and Burton (2001) investigated the capacity of a fungus *Neurospora crassa* to produce oxidative enzymes. Their application in the biodegradation of phenolic compounds was demonstrated in static and shaken non-immobilized batch cultures, and by capillary membrane-immobilized biofilms. Two phenolic substrates, phenol and p–cresol, both common components of industrial effluent streams, were chosen as model pollutants for bioremediation studies using the *N. crassa* enzyme system. *N. crassa* cultures, in both immobilized and non-immobilized systems, were shown to produce intracellular and extracellular oxidase enzymes at high levels of activity. Immobilized cultures were capable of sustaining this enzyme production continuously whereas in flask cultures, 18 mg p–cresol and 23 mg phenol respectively were removed from 5 mM solutions/g wet biomass, over a 6 day period. Over the same time period, immobilized cultures were found to remove 10 mg p–cresol or 8 mg phenol per gram of biomass. The immobilized biomass in a continuous reactor was found to have the capacity to sustain this removal efficiency continuously for a 4-month period whereas the batch liquid culture systems remained active for approximately 8–15 days, and later cultures were no longer viable.

Santos and Linardi (2004) isolated thirty filamentous fungal strains from effluents of a stainless steel industry and tested them for phenol tolerance. The purpose of this

investigation was to isolate, characterize and screen mycelial fungi with potential for phenol degradation. To test the phenol tolerance, shake flask experiments were conducted and the medium containing phenol was used for culture growth. The fungal strains showing growth on phenol were further tested for the activity of enzymes involved in phenol biodegradation, namely Phenol hydroxylase, Catechol 1, 2-dioxygenase, and Catechol 2, 3-dioxygenase. Activities of enzymes Catechol 1, 2-dioxygenase, and Catechol 2, 3-dioxygenase were detected into the cell free extracts of fungal strain, measuring the absorbance at 260 and 375 nm spectrophotometrically, for the presence of *cis cis* – muconic acid and 2-hydroxymuconic semi aldehyde . The assay for phenol hydroxylase was conducted spectrophotometrically by monitoring absorbance decrease at 340 nm, as described by Jones *et al.* (1985). The presence of the formation of *cis cis* – muconic acid in the cell extracts suggested that phenol metabolism throughout occurred via *ortho* fission of catechol in this work.

Perron and Welander (2004) worked on single substrate biodegradation of o-, m- and p–cresol using fungus *Mortierella sarnyensis* with bacterial culture in the reactor. Their aim was to investigate the degradation of common pollutants (phenol, o–, m- and p–cresol) at temperatures prevailing in many groundwater aquifers. A suspended carrier biofilm process was used for the experiments because they were less sensitive to toxic compounds in comparison to suspended sludge processes like activated sludge.

Organic acids were produced during the degradation of phenolic compounds and lower pH was required for proper growth of fungus. Hence the fungus *M. sarnyensis* Mil'ko was taken as dominating part of biomass in reactor. Conclusion of this study was: the mixed culture was found capable to degrade a wide range of pollutants. During the biodegradation the fungus transformed the pollutants but did not mineralize in many cases. Therefore, fungus in combination with bacteria was very useful for complete mineralization of pollutants. The fungus performed the first step of degradation process, which was difficult for bacteria while the bacteria finalized the degradation of the intermediates formed.

Kumar *et al.* (2005) studied the biodegradation kinetics of phenol and catechol by a bacterial strain *Pseudomonas putida* MTCC 1194, in single substrate biodegradation system. In this shake flask study acclimatized bacterial strain was used for biodegradation of both the substrates. The biodegradation of initial phenol concentration of 1000 mg/L was completed in 162 h and the biodegradation of initial catechol concentration of 500 mg/L took 94 h. Authors used Monod and Haldane models for growth kinetic studies but during the experimentation it was observed that both the substrates caused inhibition to biomass growth and to self biodegradation after a particular initial concentration. Therefore, Haldane model was found more appropriate to describe the growth kinetics of both the substrates in the case of substrate inhibition. Authors estimated the value of decay coefficient as 0.0056 h^{-1} for phenol biodegradation and 0.0067 h^{-1} for catechol biodegradation. The values of observed growth yield coefficient on phenol and catechol were found to be 0.65 and 0.50 g/g respectively.

Nuhoglu and Yalcin (2005) studied phenol biodegradation in a batch reactor using mixed culture. In this work they assessed the prediction adequacy of the model employing Haldane equation with respect to initial phenol concentrations and evaluated the requirement of a new model. Haldane model was used to predict the relation between specific growth rate and initial substrate concentration. They observed that Haldane model was adequate to reflect the phenol concentration profile for low initial phenol concentration up to 100 mg/L, but for higher initial phenol concentrations it was not accurate. For initial phenol concentrations of 250 to 680 mg/L Haldane model predicted shorter complete degradation times than those measured experimentally. They reported that during the phenol degradation at higher initial concentrations various metabolic intermediates like 2-hydroxy muconic acid semialdehyde, a major intermediate, were produced and accumulated. The model should take the effect of intermediates into account for accurate prediction of complete biodegradation time. Therefore, the Haldane model was not good for prediction of complete biodegradation time at high initial substrate concentrations. A model incorporating the effect of metabolic intermediates of phenol degradation was developed by Wang and Loh (1999) and it was successfully applied to pure cultures degrading wide range of phenol concentrations. Authors also applied the same model for their study and observed that the new model was able to make adequate

predictions of complete biodegradation times for a wide range of initial phenol concentration.

Yan *et al.* (2005) isolated strain *Candida tropicalis* from acclimated activated sludge and used its pure culture for biodegradation of initial phenol concentrations in the range of 1600 – 2000 mg/L. The strain showed the capability to degrade initial phenol concentration 2000 mg/L in 66 h. High inoculum volume decreased the toxic effect of phenol for biomass and increased phenol biodegradation velocity or rate. However for a certain starting inoculum, with the step increase in initial phenol concentration, the substrate inhibition was also enhanced. More phenol was not only consumed by the cells for growth and biomass production but to overcome the strong substrate inhibition effect too. In this batch culture study the cell growth and phenol degradation kinetics of *C. tropicalis* was investigated up to the initial phenol concentration of 2000 mg/L using Haldane model. They represented specific substrate utilization rate in terms of specific biomass growth rate. The values of maintenance energy coefficient and true growth yield coefficient were observed as 0.277 h^{-1} and 1.215 g/g.

Maeda *et al.* (2005) proposed the three – phase gas – liquid – solid slurry bioreactor for batch biodegradation of o–cresol. The biodegradation kinetics of o–cresol was examined in batch experiments at varying initial o–cresol concentrations (30 to 600 mg/L), waste activated sludge concentrations (1000 to 11500 mg/L), and aeration rates (0.05 to 1.0 L/ min). The kinetic parameters of o–cresol aerobic biodegradation were estimated using Haldane substrate inhibition model with the correlation factor of approximately 0.95. The oxygen consumption rate was adequately described by the Haldane type model. The biodegradation of o–cresol by waste activated sludge and the change of dissolved oxygen concentration in the slurry bioreactor were simulated successfully. The initial decrease in dissolved oxygen due to oxygen consumption by the biological degradation of o–cresol and the quick increase in dissolved oxygen after the completion of o–cresol biodegradation were satisfactorily described by the model predictions. Information about the kinetics of o–cresol biodegradation and the oxygen consumption is useful for optimal design and operation of aerobic biological treatment reactions.

Tallur *et al.* (2006) used a *Bacillus* sp. Strain PHN 1 to study the biodegradation of p–cresol. The strain was isolated from phenol contaminated soil. The bacterial strain showed degradation activity for phenol, o–cresol, m–cresol, 4-hydroxy benzoic acid, and gentisic acid too. They worked on the pathway detection for the utilization of p–cresol by the bacterial strain. The batch culture experiments including enzyme assay were conducted for the purpose. The study concluded that p–cresol was first degraded to 4-hydroxy benzoic acid and further it was metabolized by gentisate pathway.

Stoilova *et al.* (2006) investigated the fungus *Aspergillus awamori* NRRL 3112 for its ability to degrade phenol, catechol, 2, 4-dichlorophenol and 2, 6-dimethoxyphenol at high concentrations. The organism had mineralized phenol concentration of 300 mg/L in 60 h, 600 mg/L in 72 h and 1000 mg/L in 7 – 8 days. *A. awamori* fully degraded catechol concentration of 1000 mg/L in 82 h, 2000 mg/L in 108 h and 3000 mg/L in 124 h. Five days were sufficient for complete biodegradation of 2000 mg/L of 2, 4-dichlorophenol. The degradation of 2, 6-dimethoxyphenol was slow and only concentration of 1000 mg/L was fully degraded in 7 days. Activity of enzymes Phenol hydroxylase and Catechol 1, 2-dioxygenase was determined to detect the pathways involved in the biodegradation of these phenolics compounds. As a result, ortho – cleavage pathway was detected. To study the microorganism growth kinetics Haldane type inhibitory growth model was used. The values of decay and yield coefficients for all phenols at various concentrations were also determined.

Jiang *et al.* (2007) isolated and characterized *Alcaligenes faecalis* to investigate its phenol biodegradation potential at high initial concentrations larger than 1200 mg/L. They isolated the microorganism from acclimated activated sludge and screened on the bases of its phenol tolerance capacity and maximum phenol degradation velocity among other microorganisms. They analyzed the BIOLOG and 16S renal sequence to identify the strain as *Alcaligenes faecalis*. To confirm the activities of enzymes (Phenol hydroxylase, Catechol 1, 2-dioxygenase and Catechol 2, 3-dioxygenase) involved in phenol biodegradation, they performed enzyme assay and detected the enzyme activity in cell free extracts spectrophotometrically. The phenol hydroxylase activity was detected by measuring the absorbance of NADPH at 340 nm. They found the activity of enzyme

Catechol 1, 2-dioxygenase in cell free extract that confirmed phenol degradation by ortho – fission pathway in the microorganism. Activity of enzymes phenol hydroxygenase and catechol 1,2-dioxygenase were observed maximum at the late phase of the exponential which led to a higher phenol – degrading potential of the fungus at the late phase of the exponential stages. Haldane model was selected for the assessment of the dynamic behavior of *A. faecalis* grown on phenol and studied phenol degradation kinetics assuming a constant growth yield. During experimentation they observed that in case of the degradation of toxic substrates like phenol, energy requirements were high to overcome the substrate inhibition effect especially at high initial concentrations. Thus, both specific growth rate and biomass yield were low at the initial phase of biodegradation, and with the consumption of phenol these increased as a result of the declining inhibition effect of phenol. They observed gradual increase in phenol biodegradation capacity of strain with the augmentation of inoculum concentration. This indicated that there was no essential association between cell growth and phenol degradation at high initial phenol concentrations. The production and accumulation of various intermediates might be the reason of no essential relation between cell growth and phenol degradation. This also caused decrease in biomass yield, although phenol was consumed mainly for assimilation into biomass and for the cell growth and maintenance.

Saravanan *et al.* (2008a) investigated the phenol degradation capacity of a mixed culture isolated from sewage treatment plant. No inhibitory effect of phenol up to the initial concentration of 400 mg/L was observed. Specific growth rate attained the maximum value at this initial phenol concentration. Haldane and Han–Levenspiel models were used to represent growth kinetics of the mixed culture and the Han–Levenspiel model was found more accurate to predict the growth kinetics. They reported that Han–Levenspiel model was based only on the effect of a product that might be formed during degradation while Haldane model is based on the effect of substrate on culture growth. Further it was concluded that the phenol utilization by the mixed culture was also associated with some products formed during its degradation but this aspect was needed to be verified experimentally.

Stanchev *et al.* (2008) worked on biodegradation of high initial catechol concentrations using *Aspergillus awamori*. The dynamics of biodegradation was investigated at initial catechol concentrations of 1000 to 3000 mg/L. The values of the kinetic model parameters for specific growth rate at different initial conditions were determined. At initial catechol concentration of 1000 mg/L, the biodegradation process proceeded in the conditions of substrate limitation while at higher catechol concentrations (2000 and 3000 mg/L) a presence of substrate inhibition was established. Haldane and Harris model was used to study the growth kinetics and as the initial catechol concentration was increased the specific growth rate value was decreased. This observation indicated that high initial catechol concentrations exhibit toxic effect on microbial cells. The optimal value of the ratio catechol/biomass was determined to maximize the specific catechol degradation rate. Authors reported that this parameter could serve as a starting base for the determination of initial conditions for a batch process, for specifying the moment of feeding for a fed-batch process, and for monitoring and control of a continuous process from the point of view of time-optimal control. This study concluded that the process control on the basis of this parameter would minimize the time interval for reaching a steady-state at a maximum value of the specific catechol degradation rate.

Saravanan *et al.* (2009) investigated the batch growth kinetics of a mixed microbial culture isolated from a sewage treatment plant, utilizing m–cresol as single carbon and energy source. They conducted shake flask experiments for m–cresol biodegradation in the range of concentration 100 to 900 mg/L. The culture took 136 h for complete degradation of the initial m–cresol concentration of 900 mg/L. To study the growth kinetics of the culture, authors used growth-associated, non-growth-associated models and a three-half-order kinetic model. Among the models tested, only non-deterministic three-half-order kinetic model was found suitable for the experimental data. They stated that the three-half-order kinetic model integrated both the kinetics of substrate degradation and growth of the culture, while other models do not consider these aspects simultaneously. The maximum observed degradation rate was 0.585 h^{-1} at initial m–cresol concentration of 200 mg/L. The inhibition effect due to the enhanced toxicity of m–cresol started to take place in the medium at the initial concentrations higher than 200 mg/L. Further, Edward, Haldane, Luong, Han-Levenspiel, and Yano-Koga models were used to

explain substrate inhibition on the growth of microbial culture, using specific growth rate and specific degradation rate data. Luong and Han-Levenspiel models were found best fitted to the experimental data.

Shen *et al.* (2009) presented shake flask study on biological degradation of picric acid using *Rhodococcus sp.* NJUST16. To study the effect of initial substrate concentration on biomass growth, the specific growth rate data were taken and the maximum specific growth rate value was found on the initial substrate concentration of 60 mg/L. This suggested that picric acid was inhibitory substrate because the specific growth rate values tend to decrease at the concentrations higher than 60 mg/L. The values of Haldane kinetic model parameters led to the conclusion that *Rhodococcus sp.* NJUST16 degraded picric acid efficiently. The observed biomass growth yield value reported in this work varied between 0.1424 to 0.3215 g/g and the maximum observed biomass growth yield value was observed at the initial concentration of 60 mg/L. The value of endogenous decay coefficient was also estimated and it was obtained as 0.01713 h^{-1}. In this work authors proposed a model to predict the picric acid degradation profiles for initial substrate concentration ranging from 100 to 800 mg/L.

Bajaj *et al.* (2009) worked on biological degradation of phenol using a mixed bacterial consortium in batch aerobic reactor. They reported that since the inoculum used for the present study was well acclimatized to phenol, it did not seem to be accumulated as metabolic product during the degradation process as evident from biomass growth. Therefore, Haldane model was selected to calculate the kinetic parameters. The kinetic model constants indicated good tolerance of the mixed culture on phenol. The predicted values of phenol biodegradation that were calculated with the Haldane equation for the biokinetic parameters, agreed well with the experimental data. There was only a slight difference for low concentrations. These results could be significant for understanding the capacities of the mixed cultures for phenol degradation and operation of treatment systems dealing with phenolic wastewater.

Salehi *et al.* (2010) investigated the capability of *Ralstonia eutropha* H16 to degrade p-nitrophenol with or without a supplementary substrate glucose or yeast extract at

pH of 7 and temperature of 30 °C. A modified form of the Monod equation that considered substrate inhibition, was used to model the growth behavior of bacterium, when only p-nitrophenol was used as growth substrate. p-nitrophenol at initial concentration of 6 mg/L was degraded within 20 h. The biodegradation time of p-nitrophenol was reduced to half in the presence of yeast extract as supplementary carbon and energy source. In the presence of glucose in the medium, *R. eutropha* growth was not supported and PNP was degraded in about 14 h indicating degradation time reduced by one third of the complete biodegradation time. A kinetic model for PNP biodegradation was suggested.

Ucun *et al.* (2010) assessed the performance of a jet loop bioreactor (JLB) for the aerobic treatment of phenol. Authors determined the growth kinetic coefficients based on Haldane equation using phenol as the sole carbon source, and a mixed microbial culture. The mixed culture was found able to degrade the phenol concentration between 50 and 1000 mg/L. At low initial concentrations of phenol model predictions was found sufficient but there was a disagreement between measured and estimated values at the initial phenol concentration of 750 mg/L and 1000 mg/L.

2.2 DUAL SUBSTRATE BIODEGRADATION STUDIES

This section presents a brief summary of the research work on dual substrate biodegradation studies.

Hutchinson and Robinson (1988) worked on biodegradation kinetics of phenol and p–cresol in dual substrate system, using *Pseudomonas putida.* Authors developed a model to predict phenol and p–cresol levels during their biodegradation. They assumed that biomass growth yields for phenol and p–cresol were identical; therefore they considered the specific growth rate as constant. Simultaneous degradation of phenol and p–cresol was observed. Neither of the substrate was consumed preferentially. The rate of consumption of a particular organic compound was found related to its fraction of the total organic substrate present.

Kar *et al.* (1997) presented a study on biodegradation of phenol, p–creol and o–cresol in dual substrate system, using *Arthrobacter* species, MTCC 1553. Authors

observed simultaneous biodegradation of both phenol and cresol. To study the type of interaction between two compounds they used double reciprocal plot method. The competitive type inhibition pattern was found between p–cresol and phenol while there was null effect of phenol on o–cresol biodegradation. Phenol and p–cresol were biodegraded by the meta pathway. Therefore, competitive inhibition appeared to be consistent. Authors concluded that structurally related compounds had an effect on overall metabolic rates of compounds in a multisubstrate environment of industrial wastewater.

Reardon *et al.* (2000) studied the kinetics of *Pseudomonas putida* F1 growing on benzene, toluene, phenol and their mixtures, and compared the mathematical models to describe the results. Toluene and benzene were found to be better growth substrates in comparison to phenol. They resulted into higher growth and observed growth yield coefficient. Growth on toluene was slightly faster than growth on benzene. The growth kinetics of *Pseudomonas putida* F1 followed Monod kinetics for benzene and toluene while for phenol Andrews and Noack model was used as phenol was found inhibitory substrate. SKIP model was used to describe the growth and biodegradation kinetics in case of the growth on mixed substrate biodegradation system. All the substrates were metabolized by similar enzymes but the inhibitory interactions between these substrates were not competitive in nature. Toluene exerted largest degree of inhibition to the biodegradation of other substrates because it was being consumed preferentially. The SKIP model provided good prediction of biodegradation kinetics for the mixture of three substrates.

Yu and Loh (2002) used *Pseudomonas putida* ATCC 1748 to study the substrate interactions during cell growth on carbazole-containing mixtures with p–cresol and sodium salicylate. p–Cresol and sodium salicylate were utilized by microorganisms as growth substrate. Carbazole, p–cresol and sodium salicylate, showed strong substrate interactions. Sodium salicylate not only supported the cell growth but was also responsible for inducing some specific enzymes for carbazole degradation. The presence of p–cresol was found to inhibit the degradation of sodium salicylate and the time required for degrading sodium salicylate was prolonged with a concomitant decrease in the average transformation rate of sodium salicylate. In the case of carbazole degradation, the presence of p–cresol not only

retarded the rate of carbazole degradation but also reduced its extent of the degradation. This study suggests that p–cresol should first be removed before carbazole can be tackled, especially when p–cresol concentration in the wastewater is high.

Hao *et al.* (2002) investigated the degradation kinetics of self-inhibitory growth substrate (phenol) and non-growth substrate (4-chlorophenol) in single and dual substrate biodegradation system using *Acinetobacter* strain. During the exponential growth phase the mean yield value of 0.72 g/g was observed in case of phenol. They used Haldane model to predict the biodegradation kinetics of both the substrates phenol and 4-chlorophenol but this model was found to be inadequate at high substrate concentrations. During 4-chlorophenol biodegradation they found the likely involvement of oxygenize - mediated reactions and the concentration of 4-chlorophenol was immediately reduced to a lower level. The amount of the reduction was proportional to the initial amount of cell biomass used as inoculum. They concluded that due to the depletion of cell internal energy reserves the 'phenol induced *Acinetobacter* cells' utilized 4-chlorophenol. The 4-chlorophenol transformation rate was observed decreasing at higher initial 4-chlorophenol concentrations which indicated that 4-chlorophenol inhibited its own degradation at higher concentrations. Synchronous phenol and 4-chlorophenol utilization by the *Acinetobacter* isolate was observed when the initial phenol to 4-chlorophenol concentration ratio was sufficiently higher than 4:1 mg/L.

Abuhamed *et al.* (2004) used batch cultures of *Pseudomonas putida* F1 ATCC 700007 to degrade benzene, toluene and phenol. The biodegradation of the three substrates was studied in the initial concentration range of 3 – 187.7 mg/L for benzene, 2.76 – 160.3 mg/L for toluene, and 10 – 200 mg/L for phenol. The values of observed yield coefficient were 0.75 g/g for benzene, 0.58 g/g for toluene and 0.44 g/g for phenol. To investigate the interaction parameters of Benzene, Toluene and Phenol sum kinetic model proposed by Yoon *et al.* (1977) was used. They studied three combinations in dual substrate systems; benzene with toluene, benzene with phenol, and toluene with phenol. In this study Toluene and benzene were found to be better growth substrates for the microorganism than phenol; they result into faster microbial growth. It was found that toluene and benzene enhanced

the biodegradation of phenol but phenol inhibited the biodegradation of both benzene and toluene.

Yan *et al.* (2006) carried out the biodegradation of phenol and m–cresol in single as well as dual substrate system using the fungus *Candida tropicalis*. The results showed that *C. tropicalis* could degrade 2000 mg/L phenol alone and 280 mg/L m–cresol alone within 66 and 52 h respectively. The capacity of the strain to degrade phenol was obviously higher than that to degrade m–cresol. Because of the inhibition of phenol on the cell growth, the Haldane's equation was selected for assessing the dynamic behavior of *C. tropicalis* grown on phenol. Since m–cresol imposed a strong inhibition on cells, a Haldane type kinetic model was used to describe the behavior of m–cresol biodegradation. Authors modeled specific substrate utilization rate in terms of specific biomass growth rate and the value of maintenance energy coefficient for phenol and m–cresol were observed as 0.004 h^{-1} and 0.019 h^{-1} respectively while the true growth yield coefficient values were 0.637 g/g and 0.282 g/g for phenol and m–cresol respectively. In dual substrate system studies the presence of m–cresol intensely inhibited phenol biodegradation. Only 1000 mg/L phenol can be completely degraded in the presence of 280 mg/L m-cresol. On the contrary, the phenol at low concentration (100 – 500 mg/L) became a sole carbon and energy source for *C. tropicalis* in the initial phase of biodegradation and accelerated the assimilation of m–cresol, resulting in the fact that m–cresol biodegradation velocity was higher than that without phenol. The capacity of *C. tropicalis* for m–cresol biodegradation was increased up to 320 mg/L in the presence of 60 – 100 mg/L phenol. The maximum m–cresol biodegradation velocity was obtained in the presence of initial phenol concentration of 80 mg/L. The initial m–cresol concentration of 60 mg/L completely inhibited the biodegradation of initial phenol concentration of 1800 mg/L. Authors also modeled the kinetics for dual substrate system.

Juang and Tsai (2006) studied the growth kinetics of *Pseudomonas putida* CCRC 14365 for biodegradation of single phenol and sodium salicylate and the mixture of these substrates in a batch reactor. Haldane model was used for single substrate growth kinetic study of both the substrates. It was reported that sodium salicylate supported the growth of *p. putida* and also induced the production of enzymes those were required for the

transformation of non-growth substrates as 4–chlorophenol and carbazole. They observed that *P. putida* cells adapted with sodium salicylate showed improved ability for degradation of sodium salicylate itself and the enhanced ability for phenol biodegradation. However, the rate of phenol biodegradation was higher in comparison to that of sodium salicylate. The substrate inhibition effect of sodium salicylate was higher on the microorganism while the substrate inhibition effect of phenol was not much significant. They described specific growth rate of the cells on dual substrates by an extended Haldane equation and it was found that the inhibitory interactions between two substrates were not purely competitive in nature. The magnitude of interaction constants suggested that the sodium salicylate inhibited the rate of phenol removal in a competitive and uncompetitive manner, much more than phenol inhibited the rate of sodium salicylate removal.

Jiang *et al.* (2007) studied phenol biodegradation in presence of 4-chlorophenol using *Candida tropicalis.* The fungus was able to degrade phenol concentration of 2000 mg/L within 66 h and 350 mg/L 4-chlorophenol in 55 h. In the dual substrate system study phenol concentration was taken in the range of 100 – 800 mg/L, keeping the 4-chlorophenol concentration constant at 350 mg/L. The phenol concentration from 100 – 600 mg/L enhanced the biodegradation of 4-chlorophenol which was achieved up to the concentration of 420 mg/L. 4-chlorophenol exerted strong inhibition to the biodegradation of phenol and no phenol biodegradation was observed beyond the phenol concentration of 800 mg/L in the presence of 350 mg/L 4-chlorophenol.

Bai *et al.* (2007) presented the kinetics of cell growth and biodegradation of phenol (10 – 1400 mg/L) and m–cresol (5 – 200 mg/L) in single and dual substrate systems using *Alcaligene faecalis*. In this batch biodegradation study, both the substrates were observed inhibitory to cell growth and to their own biodegradation after a certain initial substrate concentration. Rates of cell growth and biodegradation were higher on phenol than that on m–cresol alone due to the larger inhibitory effect of m–cresol. Authors used Haldane model for phenol and proposed modified Haldane model for m–cresol (due to the strong inhibition effect of m–cresol on microorganisms) to study the single substrate growth kinetics. They modeled specific substrate utilization rate in terms of specific biomass growth rate and the value of maintenance energy coefficient for phenol and m–cresol were

observed as 0.113 h^{-1} and 0.017 h^{-1} respectively while the true growth yield coefficient values were 1.32 g/g and 0.594 g/g for phenol and m–cresol respectively. In dual substrate system study, it was found that due to the presence of interaction between phenol and m–cresol the behavior of cell growth and substrate degradation differ greatly from the single substrate system. The overall cell growth rates on the dual substrate was higher than those of m–cresol alone and smaller than those of phenol alone. The total cell growth was the co-action of phenol and m–cresol. They modeled the Kinetics of overall cell growth on phenol in the presence of m–cresol and compared their model with the sum kinetic model given by Yoon et al. (1977).

Saravanan *et al.* (2008b) developed internal loop airlift bioreactor (ILALR) to study phenol and m–cresol biodegradation as single and dual substrate systems using mixed culture predominantly *Pseudomonas sp.* isolated from sewage treatment plant, under batch and fed batch operating conditions. The culture could degrade a maximum initial concentration of 600 mg/L phenol in 47 h and 400 mg/L m–cresol in 36 h completely. The inhibitory effect of phenol was observed early at lower initial phenol concentration of 200 mg/L in the batch shake flask study while the inhibition effect of phenol took place at initial phenol concentration 300 mg/L when biodegradation was carried out in ILALR. Authors reported that the ILALR system failed to degrade m–cresol concentration higher than 400 mg/L whereas in shake flask study it degraded a maximum m–cresol concentration of 900 mg/L. Both shake flask and ILALR studies showed similar substrate inhibition at initial m–cresol concentration of 200 mg/L. To estimate the bio-kinetic parameters in single substrate biodegradation study, the experimental data of specific growth rate and substrate concentration were fitted to Haldane model. In comparison to the batch shake flask study, estimated values of kinetic parameters were found to be high except the value of inhibition constant. The higher growth rate denoted the good potential of the ILALR system in degrading phenol using *Pseudomonas sp.* In case of m–cresol biodegradation though the system had a good geometry for mixing and dissolved oxygen but the toxicity of the m–cresol affected the system scale-up performance.

Batch biodegradation dual substrate system studies of both the substrates were carried out using phenol and m–cresol in the concentration range of 100 to 300 mg/L and phenol was found to be degraded preferentially in comparison to m–cresol. The reason is that phenol is less inhibitory carbon source in comparison to m–cresol and therefore it is easily consumed by the microorganisms. Low phenol concentrations between 100 to 200 mg/L, enhanced the biodegradation of m–cresol whereas in presence of higher phenol concentrations the mixed culture could not degrade both the substrates due to the combined concentration effect. Microbial growth and substrate degradation pattern of the culture in the presence of both phenol and m–cresol in the medium differed quite largely from that of the single substrate study, due to the availability of more carbon source in the multi-substrate system. Culture took more time to grow when m–cresol was present in the medium (together with phenol). However, the biomass output was also high. Sum kinetic model proposed by Yoon *et al.* (1977) was used to predict its variations due to various combinations of the concentrations of phenol and m–cresol, for evaluation of the interaction between phenol and m–cresol on the growth of the culture, and the relative effects of the two substrates on their individual degradation. From the results, it was concluded that phenol exhibited stronger inhibition on m–cresol degradation than m–cresol on phenol.

Single substrate biodegradation studies carried out in fed batch ILALR system resulted into the complete biodegradation of both the substrates phenol and m–cresol in 26 h and 36 h respectively up to the initial concentration of 600 mg/L. In comparison to simple batch mode, the degradation time was very less in the fed batch mode even with higher concentrations. It was observed that under simple batch operation, the reactor could not degrade m–cresol beyond initial concentration of 400 mg/L. This shortcoming was overcome in the study by employing fed batch operation at high feed concentration of m–cresol 10 to 600 mg/L.

The dual substrate biodegradation of phenol and m–cresol in fed batch ILALR system using the initial concentration of 300 mg/L for both the substrates resulted into the complete degradation of both the substrates in 10.3 h, phenol was degraded by the culture preferentially and a short lag period of 3 h was observed in case of m–cresol.

Yemendzhiev *et al.* (2008) focused on the growth behaviour and biodegradation potential of *Aspergillus awamori* for a mixture of two substrates phenol and p–cresol. The single substrate growth kinetics was studied using Haldane model. Authors used SKIP model to find out substrate interactions during dual substrate biodegradation. Authors checked the activity of hydroxylase enzyme for hydroquinone, p–methicatechol in comparison to its activity for phenol and p–cresol as substrates. They observed it better for hydroquinone, p-methicatechol in comparison to phenol and p–cresol.

Wang *et al.* (2009) used a fungal strain *Candida albicans* PDY-07 to study anaerobic biodegradation of phenol and m–cresol as single and dual substrate in batch cultures. The strain was able to degrade phenol upto the concentration of 1800 mg/L. Effect of various initial m–cresol concentrations on the biomass growth was studied and Haldane growth kinetic model was used to describe the behaviour of cell growth on m–cresol. Authors observed substrate interaction during dual substrate biodegradation of phenol and m–cresol. Both the substrates interfered in the biodegradation process of each other but the presence of lower phenol concentrations resulted into the biodegradation of m–cresol in shorter durations. Maximum m–cresol biodegradation rate was observed in the presence of initial phenol concentration of 200 mg/L. To study the substrate interaction between two substrates, a sum kinetic model was used and the values of kinetic parameters were determined.

Jiang *et al.* (2010) investigated the biodegradation potential of mutant strain CTM2 of wild type *Candida tropicalis* for phenol and m–cresol as single and dual substrate. The mutant strain was obtained by the treatment of wild type *Candida tropicalis* with He, Ne irradiation. The mutant strain showed its enhanced biodegradation ability and degraded 2600 mg/L, 300 mg/L of phenol and m–cresol respectively. Haldane growth kinetic model was used for single substrate growth kinetic studies. For dual substrate biodegradation studies phenol was used up to the initial concentration of 500 mg/L and the strain showed complete biodegradation of 300 mg/L m–cresol in presence of 350 mg/L phenol within 46 h. The inhibition by m–cresol to phenol biodegradation was much higher than inhibition by phenol. Addition of low initial concentrations of phenol increased the rate of m–cresol biodegradation.

Yao *et al.* (2011) focused on the biodegradation of N-heterocyclic compounds in single and dual substrate biodegradation systems with m–cresol. Authors studied the substrate interactions of m–cresol and pyridine as single and dual substrates, using a bacterial strain *Lysinibacillus cresolivorans*. Biomass growth and substrate biodegradation kinetics were studied for initial m–cresol and pyridine concentrations upto 1200 mg/L, 150 mg/L respectively. Haldane kinetic model was used to describe the single substrate kinetics. In dual substrate biodegradation system m–cresol inhibited the utilization of pyridine much more than the pyridine inhibited the utilization of m–cresol. As a growth substrate, m–cresol was more easily utilized to synthesize the new cells. This study concludes that *L. cresolivorans* may be a potential source for degradation of m–cresol in industrial wastewater containing pyridine.

Cokgor *et al.* (2011) evaluated the biodegradation kinetics of peptone and 2,6-dihydroxybenzoic acid (2, 6-DHBA), using a dual microbial culture. The study was done in a laboratory scale sequencing batch reactor and peptone mixture feeding was continuously supplemented with 2,6-DHBA. Experimental data were derived from three bench reactors: first one was supplemented with peptone mixture only, second was fed with 2,6-dihydroxybenzoic acid (2, 6-DHBA), and the third reactor had both the substrates. A mechanistic model was developed and used to study the biodegradation characteristics and kinetics for both the substrates; peptone mixture and 2,6-DHBA. The study offered new perspective for evaluation of biodegradation of xenobiotics with two component biomass models in real systems. It supports the development of a dual microbial community through acclimation, with the selective growth of a second group of microorganisms specifically capable of metabolizing 2,6-DHBA as an organic carbon source.

2.3 STUDIES ON MAINTENANCE ENERGY REQUIRMENTS IN BIODEGRADATION

In this section the literature pertaining to the studies on the estimation of maintenance energy expenditure has been summarized.

Pirt (1982) focused on the maintenance energy requirements and their quantification for the growth of microorganisms. Pirt (1965) gave the basic idea about maintenance energy and reported that if the maintenance energy requirement was zero then the growth yield would have its maximum value. Author indicated the possibility of deriving maintenance energy from the maximum specific growth rate. Afterwards in this study, the author proposed a maintenance energy model based on the idea that there are two components of maintenance energy; one is independent of specific growth rate and remains constant during the culture growth while the other one decreases linearly with the increase in specific growth rate and becomes zero at the maximum specific growth rate.

Hess *et al.* (1996) proposed a biodegradation kinetic model with the incorporation of maintenance energy requirement (for maintenance activity of biomass during the biodegradation). The new model was tested using experimental data from the biodegradation of 2-4 dinitrophenol and pentachlorophenol by microorganisms indigenous to soil and by bacteria inoculated to soil respectively. The model described long term carbon dioxide respiration data well in comparison to the models without consideration of maintenance energy requirment. The carbon dioxide respiration takes place in the case of low substrate concentrations.

Seker *et al.* (1997) investigated the phenol biodegradation by *Pseudomonas putida*, in a continuously fed stirred-tank reactor, in aerobic condition at a dilution range varying between 0.0174 – 0.278 h^{-1} to cover the inhibition region of phenol and wide range of dissolved oxygen. Non linear analysis was done to study dual substrate growth kinetics. Monod kinetic model for oxygen and Haldane model were used to study cell growth kinetics for phenol. Authors constructed a dual substrate kinetic model for microbial growth on phenol and oxygen, through nonlinear analysis of fermentation data. The constructed model was used to calculate maintenance factor for both oxygen and phenol and saturation coefficient for oxygen was also calculated successfully using the constructed model. The results obtained from model agreed well with experimental observations of the study.

Low and Chase (1999) focused on the substrate consumption by microorganisms for the maintenance and biomass synthesis. The study was conducted with a purpose to reduce the biomass production during wastewater treatment, so that the wastewater process could be engineered to divert microorganisms from utilization of consumed substrate for biomass synthesis to energy requiring functions associated with non-growth activities. Authors studied the effect of biomass concentration on biomass synthesis. The experimentation was done in a chemostat where *pseudomonas putida* was grown aerobically. The biomass was concentrated in the reactor and a portion of it was recycled to increase biomass concentration. Authors observed that the substrate removal efficiency of biomass remained constant at each biomass concentration. As the biomass concentration increased, there was a decrease in biomass production. But the maintenance energy and true growth yield coefficients remained constant with the biomass recycled each time.

Minkevich *et al.* (2002) worked on the observed biomass growth yield and specific growth rate of the ethanol – utilizing yeast *Candida valida.* They studied over a wide range of concentrations of two substrates; ethanol and zinc. Ethanol as a substrate had advantage since its consumption was not coupled with product formation of anaerobic fermentation pathways. In all experiments the culture was grown continuously, using bistat and pH-auxostat. A mathematical model was also developed for statistical treatment of the data on energy expenditure for cell maintenance. This model contains a minimal set of physiological parameters necessary for both qualitative conformity of the data and for sufficient accuracy of fitting. Authors reported that the model can be expanded to the cases of more substrates and transient processes. The concept of the effect of inhibiting substrate concentration on cell maintenance is useful for a description of microbial populations in an environment containing toxic substances.

Peter van Bodegom (2007) presented a review paper on the quantification of maintenance energy in microbial cultures. Author analyzed the sensitivity of maintenance energy requirements on underlying components and indicated that the overall maintenance energy requirements depended nonlinearly on relative death rates, relative growth rates, growth yield, and endogenous metabolism. The review of studies on maintenance energy showed that growth-dependent adaptations of maintenance energy varied with time. A

conceptual model that explicitly described the various non-growth components also showed a strong dependence of overall maintenance energy requirements on the relative growth rate. Apart from relative growth rates, overall maintenance depended in a nonlinear way on the combination of relative death rates, physiological maintenance, and growth yield. This analysis emphasized that the components underlying maintenance energy requirements should be considered and distinguished explicitly.

2.4 MOTIVATION FOR THE PRESENT RESEARCH WORK

In this chapter a review of research work carried out for the treatment of phenolic compounds using biodegradation technology has been presented. In these studies, the microorganisms such as bacteria, fungus and algae have been used, as pure or mixed culture for the biodegradation of phenolic compounds. Most of the studies have been reported on the biodegradation of phenol, a few of them are on the biodegradation of phenol derivatives, namely p–cresol, m–cresol, p-nitrophenol etc. using bacterial strains for the treatment of wastewater contaminated with the phenolic compounds. Although there are studies available on the use of pure culture of fungal strains for the biodegradation of phenolic compounds but they are focused on metabolic pathway detection. There is a lack of studies based on the growth and biodegradation kinetics of phenolic compounds using fungal strains in single and dual substrate systems, and the quantification of maintenance energy required during the process of biodegradation has also not been reported.

To the best of our knowledge there is not a single study on the biodegradation dynamics and maintenance energy estimation for the biodegradation of phenolic compounds using filamentous fungus *Gliomastix indicus*. In view of the above, in the present research work the dynamics of biodegradation with the quantification of maintenance energy requirements for three phenolic compounds, namely phenol, resorcinol and p–cresol using *Gliomastix indicus* MTCC 3869 has been planned in two systems: single substrate biodegradation system and dual substrate biodegradation system. These batch culture studies have been carried out to fulfill the objectives of present research work.

2.5 CONCLUDING REMARKS

Biodegradation technique is an economical and ecofriendly way for the treatment of wastewater contaminated with phenolic compounds in the present scenario. Though a review on the studies available on biodegradation kinetics has been presented in summarized form, it provides a good deal of information on the biodegradation of phenolic compounds. This review work describes the growth and biodegradation kinetics of phenolic compounds as single and dual substrate biodegradation system and the estimation of maintenance energy requirement during the process of biodegradation.

CHAPTER III

MATERIALS AND METHODS

3.0 INTRODUCTION

The present research work concerns with the biological removal of phenol, resorcinol, and p–cresol from the aqueous solution by fungal strain *G. indicus* in single substrate and dual substrate systems. This Chapter has been devoted to the experimental procedure adopted to carry out the proposed research studies. The experiments have been performed in aqueous solution at fixed temperature and pH values using batch culture conditions. Besides, the mathematical models of maintenance energy requirement and to describe the degradation dynamics in single and dual substrate systems are to be simulated. The required computational techniques for these simulations and parameter estimation have been presented in brief at the end of the Chapter. The procedure to take experimental data has been described. Also the materials used the preparation of growth medium and inoculum development of fungal strain *G. indicus* at different concentrations of phenol, resorcinol, and p–cresol in single and dual substrate systems have been presented. The analytical procedures for the determination of biomass and substrate concentrations have been given.

3.1 EXPERIMENTAL STUDIES

3.1.1 Materials

3.1.1.1 Growth Medium

The growth and survival of microorganisms require culture medium either solid or liquid. For this purpose a culture medium is selected, considering the nutritional requirements of the microorganisms. There are the three types of culture media; natural medium, semi-synthetic medium and synthetic medium. Generally the chemical composition of natural medium is unknown because it contains natural products such as

yeast, peptone. Synthetic medium contains required nutrients of known concentration depending upon the nutritional requirement of microorganisms. Medium with partially known composition is called semi-synthetic medium such as potato dextrose agar, where the potato is a natural product and the nutrients composition in it is unknown [Dubey and Maheshwari (2002)]. In the present research work for the biodegradation kinetic studies semi-synthetic and synthetic media have been used. Potato dextrose agar medium has been used for revival and further maintenance of the fungal culture. The composition of this medium is given in Table 3.1.

Table 3.1: Composition of potato dextrose agar medium

Medium Components	Concentration (g/L)
Potatoes	200
Dextrose	20
Agar	15

For experimental studies a liquid synthetic medium known as modified czapeck medium has been used. The medium was prepared into Part A and Part B, to avoid the precipitation due to the presence of ferrous salt during autoclaving. The compositions of Part A and Part B are given in Table 3.2. At the time of medium preparation for fungal culture the two parts were mixed in 9:1 ratio at room temperature. The pH of both the media was set at 6 using 1N NaOH solution.

Table 3.2: Composition of modified czapeck medium

Components of Part A	Concentration (mg/L)
NH_4NO_3	3
$MgSO_4 \cdot 7H_2O$	0.5
KCl	0.5
Components of Part B	**Concentration (mg/L)**
K_2HPO_4	1
$FeSO_4 \cdot 7H_2O$	0.01

3.1.1.2 Microorganism

The pure culture of fungus *Gliomastix indicus* MTCC 3869 was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India.

3.1.1.3 Chemicals

All the chemicals used in the experimentation including phenol, resorcinol and p–cresol were of AR grade with more than 99% purity. These chemicals were from HiMedia Laboratories Pvt. Ltd. Mumbai, Loba Chemie Pvt. Ltd. Mumbai, Ranbaxy Fine Chemicals Ltd. New Delhi, Reidel Chemicals, Hapur.

3.1.1.4 Sterility

The glasswares used for experimentation were washed with distilled water, dried in the oven at 75 °C temperature and cotton plugged. The culture media and conical flasks with cotton plugs were then sterilized by autoclaving them at 15 psi pressure, 121 °C temperature for 15 minutes. After the autoclaving, medium preparation and inoculation steps were done under aseptic conditions of U. V. chamber over the flame of a spirit lamp.

3.1.2 Methods

3.1.2.1 Maintenance of Culture

The fungus was maintained over potato dextrose agar (PDA) medium by the serial transfers at temperature 28 °C, after each fifteen days gap. The composition of PDA medium has been given in the Table 3.1. The fast growing colonies of the fungus covered 80 mm diameter petri plate within 48 h. Initially two petri plates with fungal culture were transferred to refrigerator, to be used as stock culture which was used to initiate the process of acclimatization.

3.1.2.2 Acclimatization and Inoculum Development

Acclimatization experiments were conducted for familiarization of fungal strain to phenolic environment. During acclimatization phase, production of some new enzymes was induced into the microorganism. These enzymes took place in the biochemical reactions involved in biodegradation process. This process is very

important when dealing with toxic substrates like phenol, resorcinol and p–cresol. In the present research work, the three phenolic compounds phenol, resorcinol and p–cresol were degraded using fungus *G. indicus* strain MTCC 3869 at high concentration of phenol up to 1000 mg/L, resorcinol up to 1300 mg/L, and p–cresol up to 700 mg/L. To acclimatize the fungus, 2% glucose was added for the appropriate growth of the fungus in modified czapeck medium containing substrate (phenol/resorcinol/p–cresol). The composition of the medium has been given in the Table 3.2. Cultures were acclimatized, by exposing them to the toxic substrate in a series of conical flasks (250 mL) with working volume of 100 mL. Glucose concentration was decreased gradually along with increasing concentration of the toxic substrate into the medium for a period of 2 months. The developed inoculum was used for all batch culture experiments during the exponential growth phase of the culture. pH 6 and the temperature of 28 °C in the incubator were maintained during the whole experimentation.

3.1.2.3 Experiments

In this study, the biodegradation of the three substrates, as single substrate was studied in the range of 10 to 1000 mg/L, 10 to 1300 mg/L, and 10 to 700 mg/L for phenol, resorcinol, and p–cresol respectively. For each concentration of the substrate, fourteen flasks of 250 mL capacity with 50 mL working volume were used and kept in BOD incubator-cum-orbital shaker at 28 °C and 150 rpm. Inoculation step was done in aseptic conditions of UV chamber and 5 % V/V inoculum was taken. The lag phase was found to be completed within 19, 15, 24 h for phenol, resorcinol and p–cresol respectively. For the dual substrate system biodegradation study of phenol with p–cresol, and phenol with resorcinol, phenol was taken in combinations with resorcinol and p–cresol separately. These combinations are shown in Table 3.3. The biomass growth and degradation of the three substrates were observed till there was no further change of the concentration of substrates in the medium. Each batch experimental run was repeated twice under identical conditions and the values were averaged to get true experimental value.

Table 3.3: Combinations of phenol with p–cresol and resorcinol in dual substrate system

Concentration of phenol (mg/L)	Concentration of p–cresol (mg/L)
100	300
200	200
300	100
100	300
200	200
300	100

3.1.2.4 Determination of Substrate and Biomass Concentrations

During experimentation, samples were taken from experimental flasks at subsequent time intervals and were subjected to centrifugation at 8000 rpm for 15 minute at 25 °C. The supernatant was separated out for analysis of substrate concentration by high performance liquid chromatography (HPLC), using a Waters system (USA), with a Symmetry® C18, 5 µm (250 mm×4.6 mm, Waters, Ireland) column. The calibration curves for the three substrates (1 - 10 mg/L) were drawn by plotting peak area versus substrate concentration. The curve was linear within this substrate concentration range with $R^2 = 0.99$ for the three substrates. Peak area for each point was calculated as an average value of five injections. Elution was performed with 400/300 (v/v) methanol/water at a flow rate of 1.0 mL min^{-1}, and detection was realized with a photodiode array detector (Waters 2998) at 271, 274, 277 nm for phenol, resorcinol and p–cresol respectively while in dual substrate biodegradation systems detection was realized at 275 nm [Saravanan *et al.* (2008b)]. The retention time for phenol, resorcinol and p–cresol were 3.35, 2.53 and 4.54 minute respectively.

For the analysis of biomass growth, oven dry method was used in this experiment. After centrifugation, biomass was found in the form of a pellet on the bottom of the centrifuge tube, and was washed with distilled water using centrifugation again. After washing, the biomass pellet was taken out on filter paper in a petri plate and was kept in the oven at 80 °C for 24 h [Shuler and Kargi (2003)]. After water

evaporation from the biomass pellet, dry biomass was weighed using a chemical balance for the analysis of biomass growth.

3.2 COMPUTATIONAL TECHNIQUES

The expressions for specific growth rate and specific biodegradation rate cannot be expressed mathematically as a linear combination of the model parameters. Thus, the growth and degradation kinetic data are generally modelled by a nonlinear function of model parameters. The nonlinear least square regression analysis is used for the prediction of values of unknown model parameters which are estimated from the experimental data. The least square method estimates the values of the parameters that minimizes the sum of squared residuals resulting in the best fitting of the experimental data. For minimization of a nonlinear function iterative procedure is used. Therefore, the initial guess of parameters and the solution convergence criteria are required to be specified before starting the computations. The iterations are terminated when the solution satisfies the convergence criteria.

In the single substrate degradation system the growth and degradation kinetic model parameters are estimated by using curve fitting tool in MATLAB which performs nonlinear least square regression technique. The numerical optimization algorithm is naturally associated with this technique. Here, Levenberg-Marquardt algorithm (LM algorithm) is applied to perform the nonlinear least square regression analysis. Therefore, a reasonable initial guess value of the parameters is given to initiate the computations. The solution by LM algorithm can be found even if guess values are far away from the final value. Owing to this advantage, LM algorithm is applied for the estimation of growth and degradation kinetic model parameters for dual substrate systems. Therefore, the required computations are performed using this optimization tool in MATLAB 7.2.

In case of single substrate and dual substrate systems the biodegradation dynamics is described by a set of nonlinear ordinary differential equations (ODE). Since all required boundary conditions are given at $t = 0$, the sets of ODEs constitute the initial value problem. Further, the number of boundary conditions and dependent variables in ODEs are equal to that of differential equations. Therefore, one and only one unique solution is obtained. The set of ODEs is widely solved with great accuracy

by Runge – Kutta class of methods. Among various order methods of this class, mostly a combination of fourth- and fifth-order Runge – Kutta methods are applied to solve the set of ODEs efficiently. The solution algorithm following Runge – Kutta class of methods is built-in MATLAB. In the present study the sets of ODEs have been solved by using ODE solver tool "ode 45" of MATLAB 7.2.

3.3 CONCLUDING REMARKS

The experimental methodology to determine the growth and degradation data for phenol, resorcinol, and p–cresol in single and dual substrate systems has been described. In addition to this, computational techniques to simulate growth and degradation kinetic models, maintenance energy requirement model, and degradation dynamics have been presented.

CHAPTER IV

BIODEGRADATION IN SINGLE SUBSTRATE SYSTEMS

4.0 INTRODUCTION

The biodegradation studies on phenol, resorcinol, and p–cresol as single substrate in wastewater using pure or mixed culture has been presented in Table 2.1 of Chapter II. In the present study, the ability of new filamentous fungus *G. indicus* to utilize phenol, resorcinol, and p–cresol as sole carbon and energy source, is demonstrated. The batch experiments are conducted to investigate the time dependent biomass and substrate concentration data at various initial concentrations of phenol, resorcinol, and p–cresol. The knowledge of growth and biodegradation kinetics, the growth yield, and biodegradation dynamics is essential for the successful design of large scale bioprocess systems, to improve the removal efficiency and for the optimization of operating conditions [Monteiro *et al.* (2000)]. In the first section of the chapter, the key concepts of growth and degradation kinetics, growth yields, maintenance energy expenditure have been outlined for the sake of general understanding of the biodegradation process. The growth kinetic models for phenol, resorcinol, and p–cresol which relate the specific growth rate of substrate concentration, have been investigated by fitting the experimental data to available kinetic models. In conjunction to this, the variation in observed growth yield and maintenance energy expenditure with substrate concentration has been modelled for phenol, resorcinol, and p–cresol in single substrate system. The new kinetic models for specific substrate utilization rates of phenol, resorcinol, and p–cresol are proposed. In the last, the chapter includes the simulation of mathematical model to describe the biodegradation dynamics of single substrates for the sake of completeness.

4.1 KINETIC MODELLING

In the present study, biodegradation of phenol, resorcinol, and p–cresol has been carried out in shake flask experiments at fixed values of initial pH, temperature,

and shaking rate (rpm) of incubator cum shaker. The flask necks were sealed with cotton plugs. Therefore, it may be assumed that the oxygen concentration provided by the aeration is sufficient, and is not limited. As a result, biomass growth and phenol biodegradation rates are considered to be limited by the substrate concentration only. In the exponential growth phase, since nutrients are available in large amounts, growth rate is considered as independent of nutrient concentration and depends only on the biomass concentration that was added to the medium as inoculum. In batch culture experiments, during exponential growth phase, if death rate of biomass is negligibly small in comparison to growth rate, the biomass growth rate is expressed as

$$\frac{dX}{dt} = \mu_g X \tag{4.1}$$

The integration of Eq. (4.1) with boundary condition ($X = X_o$ at $t = 0$) yields,

$$X = X_o e^{\mu_g t} \tag{4.2}$$

The mass balance on biomass in terms of biomass growth yield gives,

$$\frac{dX}{dt} = \left(Y_{X/S}\right)_T \left(-\frac{dS}{dt}\right) \tag{4.3}$$

Eq. (4.3) on integration with boundary condition ($X = X_o$ at $S = S_o$) keeping biomass growth yield constant, gives

$$X - X_o = \left(Y_{X/S}\right)_T (S_o - S) \tag{4.4}$$

Rate of substrate consumption $\left(-\frac{dS}{dt}\right)$, analogous to biomass growth rate (Eq. (4.1)) as a function of biomass concentration is:

$$-\frac{dS}{dt} = q_S X \tag{4.5}$$

The rate of substrate consumption as a function of biomass concentration using true growth yield and maintenance coefficient is expressed as

$$\frac{dS}{dt} = -\left(\frac{dX}{dt} \frac{1}{\left(Y_{X/S}\right)_T} + m_S X \right) \tag{4.6}$$

m_S gives the rate of substrate consumption for cell maintenance and $\left(Y_{X/S}\right)_T$ is true growth yield which is the maximum growth yield. For the survival of cells and to overcome the growth inhibition effect of substrate, a significantly higher amount of maintenance energy is required in comparison to other cultures where energy providing substrate is non-toxic substance like glucose, fructose, molasses etc [Shuler and Kargi (2003)]. Therefore, during the biodegradation kinetic studies of a microorganism, estimation of maintenance energy is a crucial step.

During the exponential growth phase, the energy is required for the growth and multiplication of cells in addition to the minimum constant amount of maintenance energy. As the substrate concentration is increased in the medium, this portion of maintenance energy keeps on decreasing along with the increasing specific growth rate $\left(\mu_g\right)$, till the specific growth rate achieves its maximum value. At maximum specific growth rate it becomes zero. In view of this, there are two components of maintenance energy, one is constant and is required during the whole cultivation period, starting from the lag phase to the death phase, and the other component is growth dependent [Pirt (1975), Kovarova *et al.* (1996), Neijssel and Tempest (1976)]. Thus,

$$m_S = m_1 + m_2$$

where m_1 is constant component of energy and m_2 is the growth dependent component of energy.

Pirt (1982) gave the following equation for the growth dependent component of maintenance coefficient:

$$m_2 = k\left(1 - \frac{\mu_g}{\mu_{g\max}}\right) \tag{4.7}$$

where k is a positive quantity that depends on the substrate-microorganism system. For the same concept, Neijssel and Tempest (1976) have suggested that the growth

dependent component of maintenance energy is proportional to the specific growth rate (μ_g) and can be written as

$m_2 = k\mu_g$, where k is constant and greater than zero.

The experimental observations show that the dependence of maintenance energy expenditure for the growth on the specific growth rate only is not possible. Maintenance energy expenditure into the cells, is affected by the temperature and the salt concentrations in the medium [Pirt (1975), Pirt (1982), Mainzer and Hempfling (1976), Wallace and Holms (1986)]. But Tsai and Lee (1990) have reported that the environmental conditions do not affect the maintenance energy requirement of the cells. However, the experimental observations prove that the maintenance energy expenditure varies from one to the other substrate-microorganism system along with the culture conditions such as temperature, pH, salt concentrations and substrate toxicity in the nutrient medium. Minkevich *et al.* (2000) have reported that in the medium, there are limited mineral and substrate concentrations and therefore, the experimental data deviate from the straight line models given by Pirt (1982). This observation indicates that Eq. (4.7) given by Pirt is not always applicable. In the present study, there was no condition of minerals and the substrate concentration limitation into the medium and all the experimental data were taken during the exponential growth phase only. Therefore, Pirt's Eq. (4.7) has been used for further study.

The relation between specific degradation rate (q_S) and specific growth rate (μ_g) that describes the minimum substrate consumption for the cell maintenance is as follows [Pirt (1975), Parks (2005), Bodegom (2007)]:

$$q_S = m_1 + \frac{\mu_g}{\left(Y_{X/S}\right)_T} \tag{4.8}$$

In above equation $\left(Y_{X/S}\right)_T$ is true or maximum growth yield coefficient which is defined on the basis of mass of the substrate actually utilized for the biomass production. When the substrate is utilized in more than one process, the observed growth yield $\left(\left(Y_{X/S}\right)_o\right)$ is different from the true growth yield. The observed growth yield is defined on the basis

of total mass of substrate consumed. The observed growth yield is not necessarily constant throughout the batch culture experiment as it depends on the substrate concentration and the growth rate significantly while true growth yield remains constant. Besides, the observed growth yield coefficient is evaluated on the basis of averaged values of biomass growth and substrate utilization for entire incubation period. The specific degradation rate (q_S) can also be written as,

$$q_S = \frac{\mu_g}{\left(Y_{X/S}\right)_o} \tag{4.9}$$

From Eq. (4.8) and (4.9) one gets,

$$\frac{1}{\left(Y_{X/S}\right)_o} = \frac{1}{\left(Y_{X/S}\right)_T} + \frac{m_1}{\mu_g} \tag{4.10}$$

Eq. (4.10) clearly shows that the observed growth yield is not constant, it varies with specific growth rate, and incorporates the maintenance energy expenditure. However, the estimation of maintenance energy consumption by Eq. (4.10) does not include the maintenance energy expenditure at the time of cell growth, cell multiplication and endogenous metabolism at stationary phase, while the specific growth rate is assumed to be the net relative growth rate. On incorporating the growth dependent component of maintenance energy from Eq. (4.7) into Eq. (4.8), one gets,

$$q_S = \frac{\mu_g}{\left(Y_{X/S}\right)_T} + m_1 + k\left(1 - \frac{\mu_g}{\mu_{g\max}}\right) \tag{4.11}$$

Since $\left(Y_{X/S}\right)_T$, k, $\mu_{g\max}$ and m_1 are constants, Eq. (4.11) can be reduced to

$$q_S = A\mu_g + B \tag{4.12}$$

where $A = \left(\frac{1}{\left(Y_{X/S}\right)_T} - \frac{k}{\mu_{g\max}}\right)$ and $B = \left(k + m_1\right)$

Further, Pirt (1987) has reported that the Eq. (4.11) is not applicable during the condition of very low specific growth rates, caused by the formation of dormant cells in

the medium. In the current study, since there is no dormant cell formation observed during the experimentation, Eq. (4.11) is used for the estimation of the maintenance energy expenditure during biodegradation of all three substrates phenol, resorcinol, and p–cresol.

If the substrate utilization for the maintenance energy expenditure is neglected, the Eq. (4.11) is reduced to,

$$q_S = \frac{\mu_g}{\left(Y_{X/S}\right)_T} \tag{4.13}$$

Kargi and Eker (2005) have suggested to define the substrate degradation rate on the basis of initial rates. The proposed substrate degradation rate is written as,

$$\left(\frac{dS}{dt}\right)_o = -q_{So} X_o \tag{4.14}$$

where $\left(\frac{dS}{dt}\right)_o$ and q_{So} are the rates of substrate degradation (mg/L.h) and specific rates of substrate degradation (h^{-1}) respectively, defined on the basis of initial biomass and substrate concentrations, X_o and S_o.

The doubling time (τ_d) is the time required to double the cell concentration. For the exponential growth phase, it can be calculated by setting $X = X_o/2$ in Eq. (4.2) as follows:

$$\tau_d = \frac{0.693}{\mu_g} \tag{4.15}$$

For the exponential growth phase, doubling time based on the cell mass and doubling time based on the cell number are equal. This is due to the fact that the average cell

composition and size do not change with the time in this phase. For deceleration growth phase, both are unequal due to change in the cell composition and size.

In order to develop kinetic model to represent the specific growth rate (μ_g) on phenol, resorcinol, and p–cresol, two approaches are encountered in the literature. One approach considers substrate as non-inhibitory compound and other considers substrate as inhibitory compound. In the case of non-inhibitory substrate, the growth kinetics is represented widely by Monod kinetic model [Kumar *et al.* (2005), Lucas *et al.* (2005), Tanyolac and Beyenal (1998), Kumaran and Paruchuri (1997)] as given below.

$$\mu_g = \frac{\mu_{g\max} S}{K_S + S} \tag{4.16}$$

where $\mu_{g\max}$ is maximum specific growth rate (h^{-1}) and K_S is saturation constant. This equation is similar to Michaelis – Menten kinetics for single enzyme reaction system with sufficiently low enzyme activity. The Monod equation describes substrate limited growth only when growth is slow and population density is low without substrate inhibition [Shuler and Kargi (2003)]. On similar situation, various other kinetic models have been proposed in the literature. Few of them including Monod equations, are reviewed and discussed by Shuler and Kargi (2003) in their excellent book. Out of these models, inspite of being empirical, Monod kinetic model has widespread applicability for representing the growth kinetics of non-inhibitory substrates. The experimental studies on biodegradation of, phenol, resorcinol, and p–cresol show that at low concentration of substrate, there is no substrate inhibition. Therefore, in the present study Monod kinetic model may be fitted to the experimental data at very low concentration of phenol, resorcinol, and p–cresol. The Eq. (4.16) can be transformed into the linear form to find out the kinetic parameters K_S and $\mu_{g\max}$ as follows:

$$\frac{1}{\mu_g} = \frac{K_S}{\mu_{g\max}} \frac{1}{S} + \frac{1}{\mu_{g\max}} \tag{4.17}$$

Once the kinetic parameters are estimated, the biomass growth rate can be calculated by substituting Eq. (4.17) in Eq. (4.1).

The second approach represents the biomass growth kinetics for the substrate inhibition condition. The inhibitory nature of phenol, resorcinol, and p–cresol at high concentration is well known. Therefore, the Monod model has been modified by including substrate inhibition because biomass growth is generally observed to be affected by the substrate inhibition at high substrate concentration [Juang and Tsai (2006), Yan *et al.* (2006), Kumar *et al.* (2005), Maeda *et al.* (2005), Yan *et al.* (2005), Acuna – Arguelles *et al.* (2003), Monteiro *et al.* (2000), Wang and Loh (1999), Tanyolac and Beyenal (1998), Kumaran and Paruchuri (1997), Yang and Humphrey (1975)]. With this view, in order to represent the growth kinetics, single substrate inhibition models are searched in the literature. Various proposed single substrate inhibition models have been reviewed by Edwards (1970). Out of these models five models are selected as listed in Table 4.1.

Table 4.1: Single substrate growth kinetic models [Edwards (1970)]

S. No.	Growth kinetic model	Mathematical equation
1	Andrews and Noack	$\mu_g = \frac{\mu_{g\max} S}{S + K_S + \frac{S^2}{K_i}}$
2	Haldane	$\mu_g = \frac{\mu_{g\max} S}{S + K_S + \frac{S^2}{K_i} + \frac{SK_S}{K_i}}$
3	Yano/ Edward	$\mu_g = \frac{\mu_{g\max} S}{S + K_S + \frac{S^2}{K_i}\left(1 + \frac{S}{K}\right)}$
4	Webb	$\mu_g = \frac{\mu_{g\max} S\left(1 + \frac{S}{K}\right)}{S + K_S + \frac{S^2}{K_i}}$
5	Aiba	$\mu_g = \frac{\mu_{g\max} S \exp^{(-S/K_i)}}{S + K_S}$

These models are either 3 parameter models or 4 parameter models. These substrate inhibition models are capable of predicting the maximum substrate concentration above which biomass growth gets inhibited. All five growth kinetic

models listed in Table 4.1 are fitted to the experimental data to evaluate the model parameters and check the suitability of model for present biodegradation study. The most suitable kinetic model is substituted in Eq. (4.1) to get biomass growth rate.

For the prediction of specific degradation rate (q_S), the Haldane model for μ_g has often been applied directly in Eq. (4.13) where maintenance energy requirement has been neglected [Shuler and Kargi (2003), Saez *et al.* (2003), Jiang *et al.* (2007), Hao *et al.* (2002)] and biomass growth yield has been assumed to be constant. As a result, same parametric values obtained for Haldane growth model, have been used for estimating the values of q_S at different operating conditions. However, the sufficient maintenance requirement results into the variation of observed biomass growth yield with the substrate concentration [Hao *et al.* (2002), Minkevich *et al.* (2000)]. Similar findings have also been observed experimentally in the present study. This fact has been explained mathematically by proposing substrate degradation kinetic models analogous to biomass growth kinetic models with different kinetic parameters [Saravanan *et al.* (2008a)]. These models relate specific degradation rate (q_S) to the substrate concentration. Identically, kinetic models for q_{So} are proposed. These models are abbreviated to M1, M2, M3, M4, M5, M6, M7, M8 and are listed in Table 4.2.

In order to study the dynamics of phenol degradation, three mathematical models have been considered. For clarity of solution description, these models are represented by model - a, model - b and model - c. The set of mathematical equations in each model is given in Table 4.3. Model - a includes the variation of observed biomass growth yield and maintenance energy coefficient in defining specific degradation rate (q_S). Model - b is based on the initial rates as described above. Where as in model - c, the correlation for q_S shows negligible maintenance energy with a constant biomass growth yield coefficient which has often been used for the prediction of phenol degradation rate. Thus, the degradation dynamics has been studied by considering two mathematical expressions for q_S represented by Eq. (4.12) and Eq. (4.13), and one for q_{So} along with growth and degradation rate equations.

Table 4.2: Single substrate degradation kinetic models

Models based upon actual degradation rates			
S. No.	**Degradation kinetic model**	**Mathematical equation**	**Analogous growth kinetic model**
1	M1	$q_S = \frac{q_{S\max} S}{S + K_S^{'} + \frac{S^2}{K_i^{'}}}$	Andrews and Noack
2	M2	$q_S = \frac{q_{S\max} S}{S + K_S^{'} + \frac{S^2}{K_i^{'}} + \frac{SK_S^{'}}{K_i^{'}}}$	Haldane
3	M3	$q_S = \frac{q_{S\max} S}{S + K_S^{'} + \frac{S^2}{K_i^{'}}\left(1 + \frac{S}{K^{'}}\right)}$	Yano/Edward
4	M4	$q_S = \frac{q_{S\max} S\left(1 + \frac{S}{K^{'}}\right)}{S + K_S^{'} + \frac{S^2}{K_i^{'}}}$	Webb
Models based upon initial degradation rates			
S. No.	**Degradation kinetic model**	**Mathematical equation**	**Analogous growth kinetic model**
5	M5	$q_{So} = \frac{q_{So\max} S_o}{S_o + K_S^{''} + \frac{S_o^2}{K_i^{''}}}$	Andrews and Noack
6	M6	$q_{So} = \frac{q_{So\max} S_o}{S_o + K_S^{''} + \frac{S_o^2}{K_i^{''}} + \frac{S_o K_S^{''}}{K_i^{''}}}$	Haldane
7	M7	$q_{So} = \frac{q_{So\max} S_o}{S_o + K_S^{''} + \frac{S_o^2}{K_i^{''}}\left(1 + \frac{S_o}{K^{''}}\right)}$	Yano/Edward
8	M8	$q_{So} = \frac{q_{So\max} S_o\left(1 + \frac{S_o}{K^{''}}\right)}{S_o + K_S^{''} + \frac{S_o^2}{K_i^{''}}}$	Webb

Table 4.3: Set of dynamic model equations used for the prediction of substrate degradation profiles with time

S. No.	Model	Set of Equations
1	Model – a	(i) $\frac{dX}{dt} = \mu_g X$
		(ii) $\frac{dS}{dt} = -q_S X$
		(iii) $\mu_g = \frac{\mu_{g\max} S}{S + K_S + \left(\frac{S^2}{K_i}\right)\left(1 + \frac{S}{K}\right)}$ For resorcinol
		or
		$\mu_g = \frac{\mu_{g\max} S}{S + K_S + \frac{S^2}{K_i}}$ For phenol and p–cresol
		(iv) $q_S = \left(\frac{1}{(Y_{X/S})_T} - \frac{k}{\mu_{g\max}}\right)\mu_g + k + m_1$
2	Model – b	(i) $\frac{dS}{dt} = -q_{So} X_o$
		(ii) $q_{So} = \frac{q_{So\max} S_o \left(1 + \frac{S_o}{K''}\right)}{S_o + K''_S + \frac{S_o^2}{K''_i}}$ For phenol
3	Model – c	(i) $\frac{dX}{dt} = \mu_g X$
		(ii) $\frac{dS}{dt} = -q_S X$
		(iii) $\mu_g = \frac{\mu_{g\max} S}{S + K_S + \left(\frac{S^2}{K_i}\right)\left(1 + \frac{S}{K}\right)}$ For resorcinol
		$\mu_g = \frac{\mu_{g\max} S}{S + K_S + \frac{S^2}{K_i}}$ For phenol and p–cresol
		(iv) $q_S = \frac{\mu_g}{(Y_{X/S})_T}$

4.2 RESULTS AND DISCUSSION

4.2.1 Growth Kinetics

The kinetic model is described by observing the biomass growth rate with time at different initial concentrations of phenol in batch experiments. In the present study, the batch culture experiments were conducted into the mineral medium containing phenol as sole carbon source at initial concentrations varying from 10 to 1000 mg/L, keeping the temperature and pH values constant at 28 °C and 6 respectively. The biomass and phenol concentrations were measured at different time intervals till phenol was consumed to a large extent by the procedure mentioned in the Chapter III, section 3.2.1.4. Figure 4.1 depicts the time required for complete phenol biodegradation at different initial concentrations by *G. indicus*. It is observed that the complete biodegradation at initial phenol concentration of 100 mg/L is achieved after about 10 h. The duration of complete biodegradation at initial phenol concentration more than 100 mg/L, is not in accordance with the step increase of initial phenol concentration as it has been observed in the case of phenol concentrations less than 100 mg/L. In higher concentration range (> 400 mg/L), the time of complete biodegradation varies more or less linearly with the increase in initial phenol concentration but with lower biodegradation rates as compared to the biodegradation rate at the initial concentrations less than 100 mg/L. This reduction in biodegradation rate may be attributed to the fact that the phenol is toxic in nature and exhibits inhibitory effect on the growth of *G. indicus* at higher concentrations.

The concentration of fungal biomass at low phenol concentrations is shown in Fig. 4.2 and the biomass concentration at higher phenol concentrations is shown in Fig. 4.3. It is clear from Fig. 4.3 that the growth rate decreases with the increase in the initial concentration of phenol. Initially at lower concentrations the reduction in growth rate is not much higher (Fig. 4.2). These results demonstrate that phenol inhibits the growth of *G. indicus* at higher initial concentrations. The value of specific growth rate (μ_g) is calculated by using Eq. (4.2) at different initial concentrations of phenol keeping initial concentration of biomass constant at 15 mg/L. Dependency of specific growth rate on initial concentration of phenol is shown in Fig. 4.4.

It can be seen that specific growth rate (μ_g) tends to increase with the increase in phenol concentration, reaches to a peak value and finally decreases. The maximum specific growth rate is achieved at initial phenol concentration of about 70 mg/L. The decline trend of μ_g beyond 70 mg/L indicates that the phenol is inhibitory type of substrate, and the inhibition effect predominates at concentrations higher than 70 mg/L. Further increase in phenol concentration exhibits strong substrate inhibition on biomass growth and on its biodegradation resulting in very low value of specific growth rate. The doubling time (τ_d) values at various initial concentrations (Fig. 4.4) indicate the time required for doubling the biomass concentration. It is calculated by using Eq. (4.15). It decreases with the increase in phenol concentration up to 70 mg/L due to non-inhibitory effect of phenol on biomass growth indicating the higher biomass growth and biodegradation rates. At higher substrate concentrations (>70 mg/L), the substrate inhibition reduces the growth rate and consequently increases the doubling time.

In order to assess the growth behaviour of *G. indicus* to degrade phenol, four single substrate inhibition growth kinetic models, namely Andrews and Noack, Haldane, Yano, and Webb, are selected from the literature as listed in Table 4.1. These models have been used on the premise that they consist only three or four parameters. A close look on these kinetic models reveals that at very low substrate concentrations, the kinetic models may be reduced to Monod kinetic model. All the four growth kinetic models have been fitted to the experimental data. In this study, the parameters of different growth models have been estimated iteratively by non-linear least square regression technique using MATLAB 7.2. This software utilizes the curve fitting tool box for minimizing the sum of square of residuals. Figure 4.5 compares the predictions of all four kinetic models with the experimental results. This figure shows that all models except Webb model show close predictions to experimental values under the initial substrate concentration ranging from 10 to 1000 mg/L. The predictions by the remaining three models do not differ widely at all concentrations. Therefore, one should discriminate among these models by statistical analysis. The goodness of the fit of the experimental data to the proposed kinetic models is generally evaluated by correlation coefficient R^2 and per cent standard deviation $(\Delta\mu_g\%)$ between the experimental and predicted values of each model.

The per cent standard deviation has been calculated by the following equation:

$$\Delta\mu_g\% = \left(\frac{\sqrt{\Sigma\left[\left(\mu_{g.\exp} - \mu_{g.pred}\right)/\mu_{g.pred}\right]^2}}{N} \right) 100 \qquad (4.18)$$

where $\mu_{g.\exp}$ is the specific growth rate based on the experimental data and $\mu_{g.pred}$ is the corresponding predicted specific growth rate according to the model under study with the best fitted kinetic constants, N is the number of measurements. It is clear that lower the value of per cent standard deviation, the better is the fit of the experimental data. The estimated values of kinetic parameters of various models along with R^2 and per cent standard deviation are mentioned in the Table 4.4. The comparison of four kinetic models has essentially been done to derive the kinetic constants for the phenol biodegradation by *G. indicus*.

Table 4.4: Estimated values of kinetic parameters of biomass growth models for phenol

S. No.	Model	Estimated values of growth kinetic parameters				R^2	Per cent Standard deviation ($\Delta\mu_g\%$)
		μ_{gmax} (h^{-1})	K_S (mg/L)	K_i (mg/L)	K (mg/L)		
1	Haldane	0.485	53.56	53.59	-	0.97	2.75
2	Yano	0.262	36.08	128.5	784.6	0.99	2.16
3	Andrews and Noack	0.462	78.29	44.49	-	0.98	2.10
4	Webb	0.240	29.88	114.9	9510	0.95	6.42

From Table 4.4, it may be concluded that the Haldane, Yano, and Andrews and Noack models give comparable predictions with high R^2 values (> 0.97) and low values of per cent standard deviation. The predictions by Webb model differ significantly. Although, Haldane, Yano, and Andrews and Noack models describe the growth kinetics with equal goodness, it is desirable to use models with less number of parameters. Therefore, the results in the Table 4.4 lead to the suggestion that Andrews and Noack model can be successfully used to describe the growth kinetics of *G. indicus* for the phenol biodegradation as it contains three parameters only.

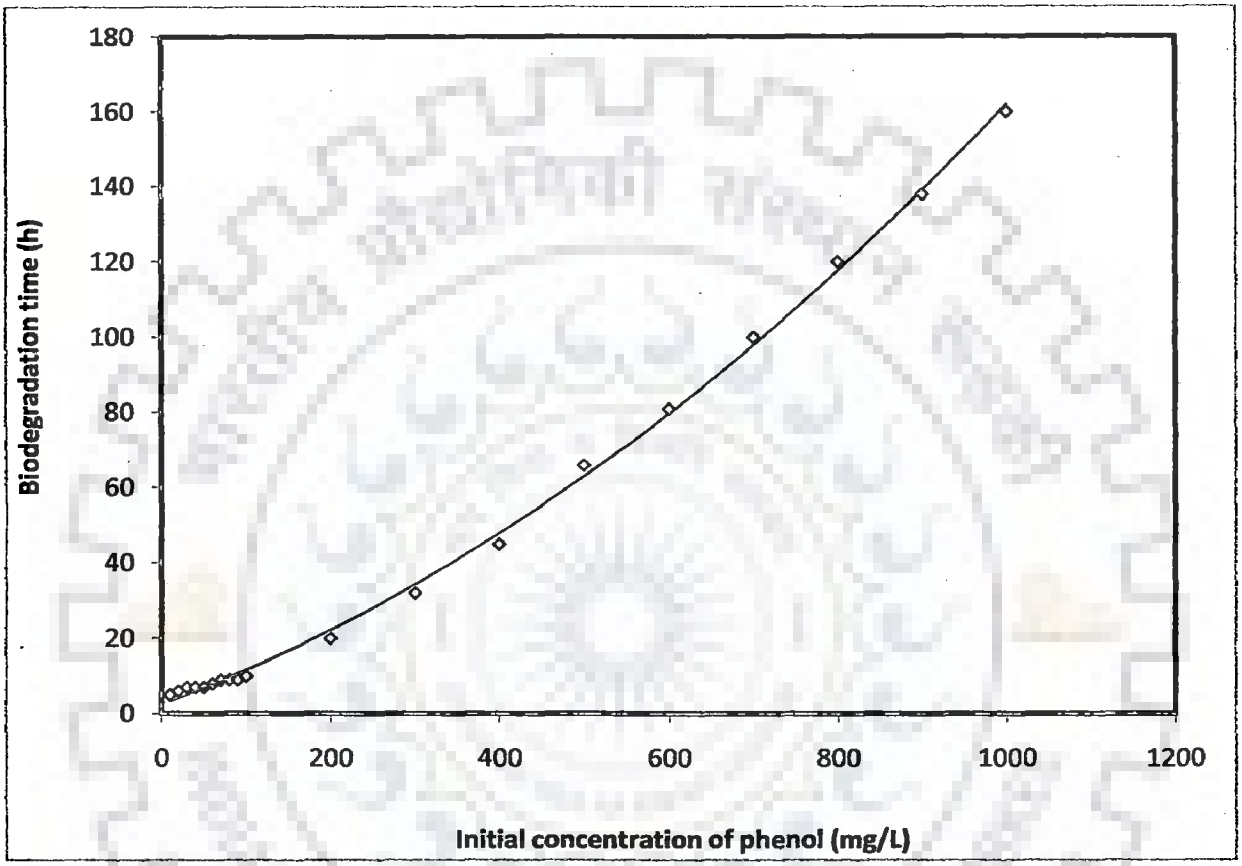

Figure 4.1: Time for complete biodegradation of phenol at various initial concentrations

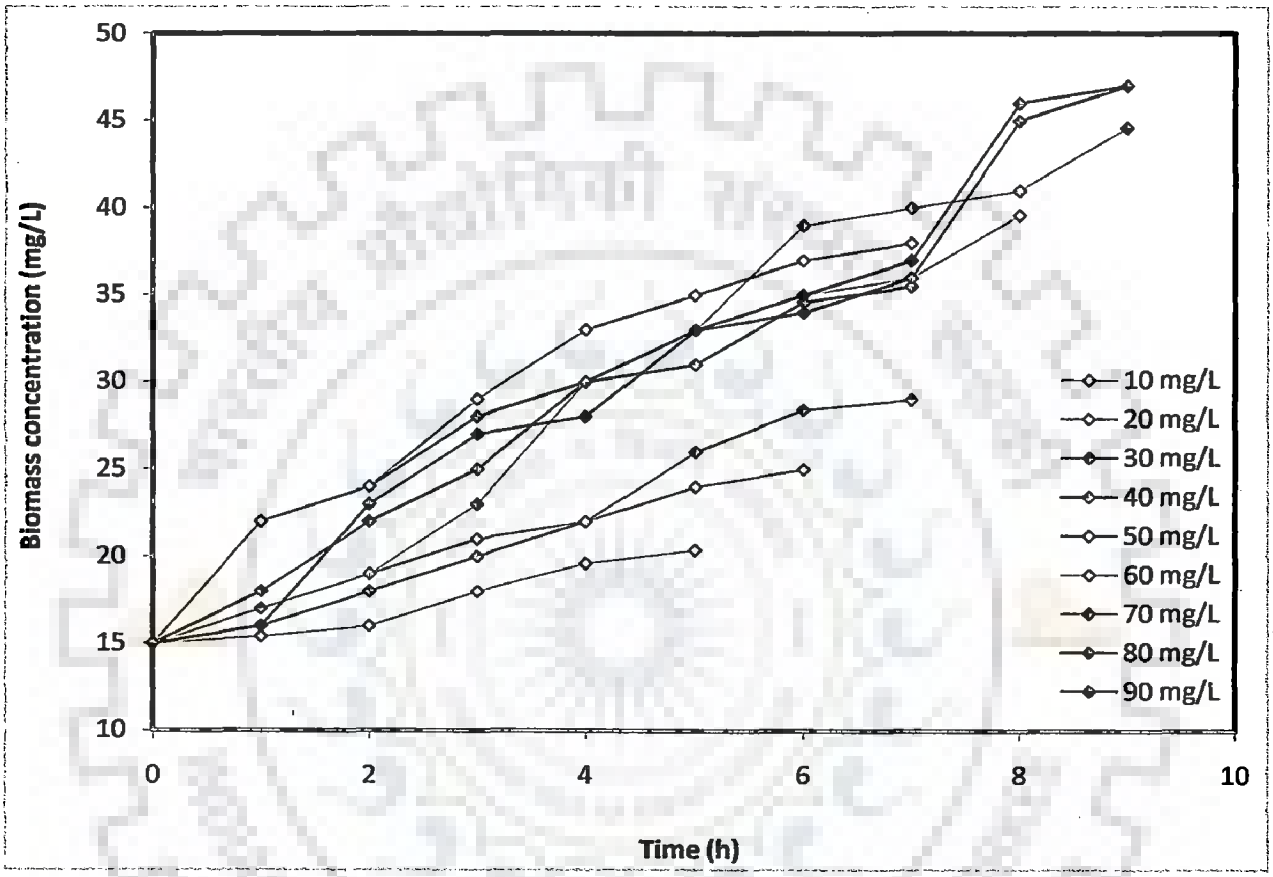

Figure 4.2: Biomass concentration in the mineral medium containing phenol at lower initial concentrations

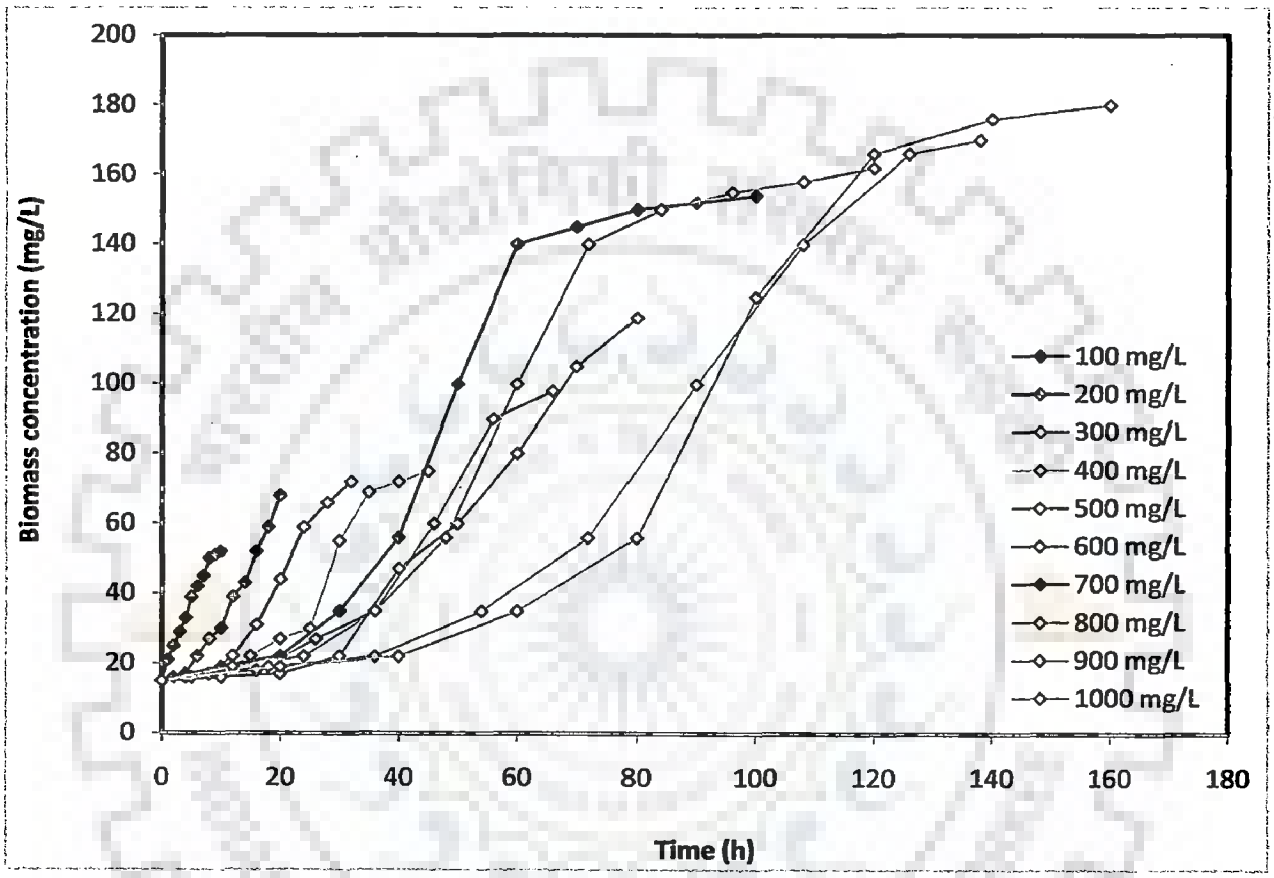

Figure 4.3: Biomass concentration in the mineral medium containing phenol at higher initial concentrations

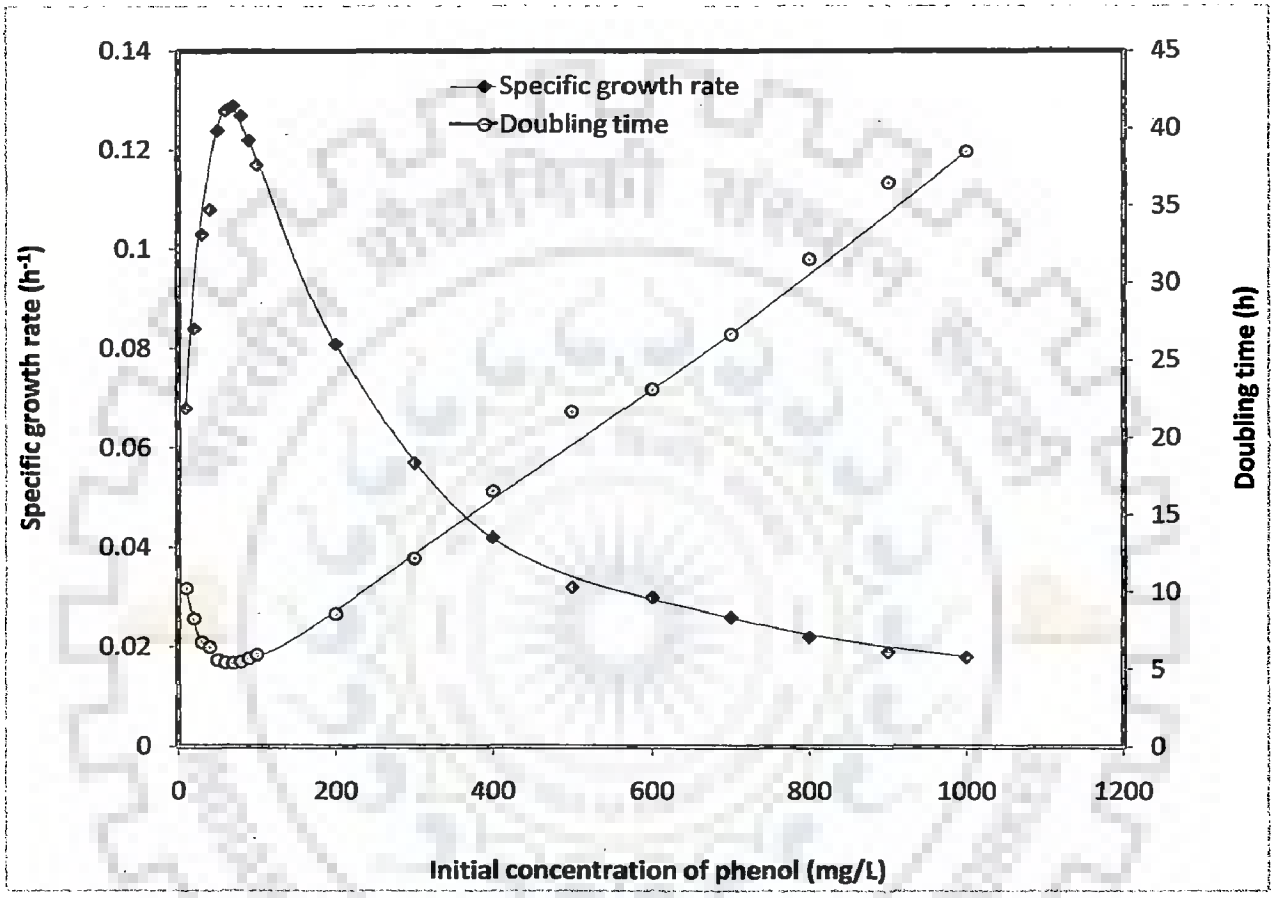

Figure 4.4: Variation in specific growth rate and doubling time with initial phenol concentration

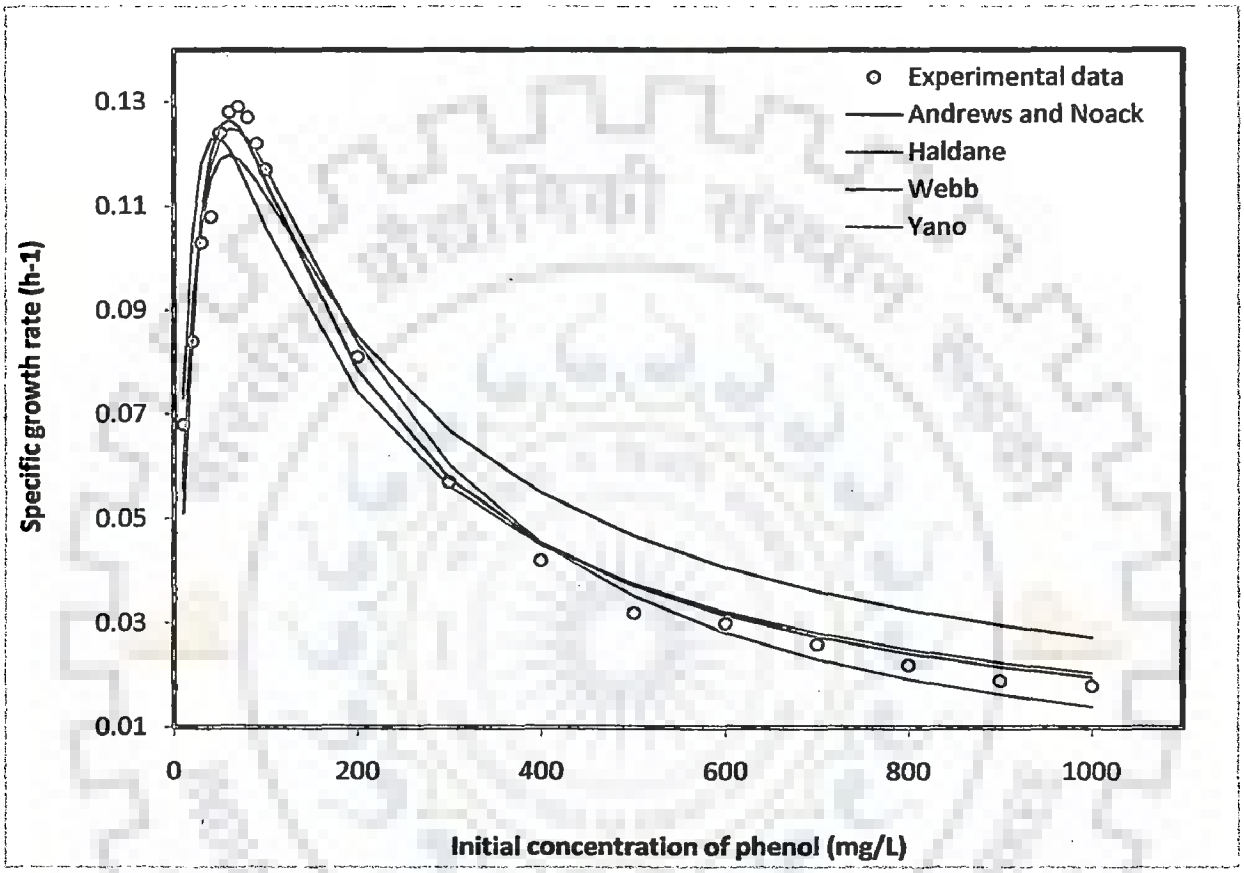

Figure 4.5: Comparison between kinetic model predictions and experimentally determined specific growth rates at different initial concentrations of phenol

Hence, in the present biodegradation study Andrews and Noack kinetic model has been selected for further discussion of results. The exact comparison of kinetic parameters and thereby the degradation efficiency with the results available in the literature is difficult due to different cell density, medium components and other environmental factors [Juang and Tsai (2006), Shen *et al.* (2009)]. However, the literature on phenol degradation using fungal strain has been summarized in Table 4.5 for the comparison of kinetic model parameters estimated in the present study. The $\mu_{g\max}$ value reported in this work (0.462 h^{-1}) is much larger than those estimated in the literature. The large value of $\mu_{g\max}$ in this study indicates that the *G. indicus* degrades the phenol more rapidly in non-inhibition conditions. The kinetic constant K_S is half saturation constant. Its magnitude indicates the affinity of biomass to the substrate. Its small values indicate the high affinity due to which maximum growth rate can be attained in short time duration. Table 4.5 shows that K_S values in the literature are in the range of 1.01 to 11.7 mg/L. The K_S value obtained in the present study is much larger than those reported in the literature. The magnitude of kinetic parameter K_i indicates the inhibition tendency (resistance) and the degree of substrate toxicity to the microorganism in the medium. Larger K_i value reveals that the substrate is less toxic to the microorganism. In the present study (Table 4.5), the value of K_i is 44.49 mg/L which falls in the range reported in the literature except those reported in the study on *Aspergillus awamori*. The lower value of K_i indicates that the phenol offers high inhibition to *G. indicus* growth. In view of aforementioned results, it may be concluded that the growth rate of *G. indicus* on phenol is comparable with the growth rate of other popular fungal strains in the literature at prevailing operating conditions.

Table 4.5: Comparison of kinetic model parameters for the biodegradation of phenol using fungal strains

S. No.	Microbial strain	Concentration range (mg/L)	Model	Kinetic parameters			Experimental conditions	Reference
				μ_{gmax} (h^{-1})	K_S (mg/L)	K_i (mg/L)		
1	*Alcaligenes faecalis*	10 - 1400	Haldane	0.15	2.22	145.40	pH - 7.2 temperature - 30°C	Bai *et al.* (2007)
2	*Candida tropicalis*	2000	Haldane	0.48	11.70	208.0	pH - 6 temperature - 30°C	Yan *et al.* (2006)
3	*Aspergillus awamori* NRRL 3112	300 - 1000	Haldane	0.23	1.01	1.02	pH - 5.5 temperature - 30°C	Stoilova *et al.* (2006)
4	*Gliomastix indicus* MTCC 3869	10 - 1000	Andrews and Noack	0.462	78.29	44.49	pH - 6 temperature - 28°C	Present study

Fungal strain *G. indicus* was cultured in the medium containing either resorcinol or p–cresol as the sole energy and carbon source at temperature of 28 °C and pH of 6. The initial concentration of resorcinol was varied from 10 to 1300 mg/L while the initial concentration of p–cresol was varied in the range of 10 to 700 mg/L. The biomass concentration for both the substrates was measured at different time intervals as mentioned in the section 3.2.1.4 of Chapter III. Figure 4.6 and 4.7 illustrate the time required for the complete degradation of resorcinol and p–cresol at different initial concentrations respectively. Both figures clearly show that at low initial concentrations of substrate, the biodegradation rate is high and it decelerates with increase in the initial concentration of the substrate. The biodegradation time for resorcinol at initial concentration of 700 mg/L and 1300 mg/L was observed as 69 h and 183 h respectively. While complete biodegradation of p–cresol at initial concentration of 700 mg/L was achieved in 122 h, which is much higher than that for resorcinol at initial concentration of 700 mg/L. The time variation trend of both the substrates is similar to that of the phenol as shown in Fig. 4.1. The reduction in degradation rate at higher concentrations indicates that the resorcinol and p–cresol are inhibitory type substrates.

Figures 4.8 and 4.9 show the fungal biomass growth in lower and higher initial concentration range of resorcinol respectively. Similarly, Figs. 4.10 and 4.11 demonstrate the biomass growth in case of p–cresol at its lower and higher concentrations respectively. These figures clearly indicate that at one initial concentration of substrate, the time required for biomass growth for p–cresol is higher than the time required for biomass growth in case of resorcinol. For instance, the biomass concentration of 70 mg/L is achieved in 27 h for resorcinol (Fig. 4.9) while it takes 86 h to achieve the same concentration of biomass in case of p–cresol (Fig. 4.11), at the initial substrate concentration of 700 mg/L. In the case of phenol at initial concentration of 700 mg/L, biomass growth took 42 h to attain biomass concentration of 70 mg/L (Fig. 4.3). These observations conclude that p–cresol is more inhibitory substrate as compared to resorcinol and phenol.

The dependency of specific growth rate and doubling time on the initial substrate concentration is shown in Figs. 4.12 and 4.13 for resorcinol and p–cresol respectively.

The values of specific growth rate (μ_g) have been calculated at different initial concentrations of resorcinol and p–cresol using Eq. (4.2), keeping initial concentration of biomass constant at 15 mg/L. It can be seen that the specific growth rate (μ_g) increases with the initial substrate concentration, reaches to its maximum value and finally decreases. The maximum specific growth rate is achieved at the initial concentration of about 90 mg/L and 50 mg/L for resorcinol and p–cresol respectively. The decline trend of μ_g beyond the inhibitory concentrations of 90 mg/L and 50 mg/L indicates that both the resorcinol and p–cresol are inhibitory type substrates. The doubling time changes accordingly with the change in specific growth rate. In order to assess the specific growth rate of *G. indicus* for resorcinol and p–cresol, five single substrate inhibition growth kinetic models have been selected from the literature as listed in Table 4.1. In this study, parameters of different growth models have been estimated iteratively by non-linear least square technique using MATLAB 7.2 based on Windows XP. This software utilizes the curve fitting tool box for minimizing the sum of square of residuals. Estimated specific growth rate values by the selected models for entire experimental data range have been plotted against initial substrate concentrations in Figs. 4.14, 4.15 for resorcinol and p–cresol respectively. Figure 4.14 illustrates that the experimental values of μ_g are not close to the predicted values of μ_g by the models of Webb and Aiba. The same conclusion can be drawn by observing the Fig. 4.15. This figure shows that the predicted values by Haldane and Aiba models are not close to the experimental values of μ_g.

The estimated values of kinetic parameters involved in different models along with R^2 and per cent standard deviation (Eq. (4.18)) are mentioned in Tables 4.6 and 4.7 for resorcinol and p–cresol respectively. For the resorcinol, Haldane, Andrews and Noack models describe the growth kinetics with equal goodness while the predictions of Webb and Aiba models differ slightly. In the case of p–cresol, the predictions by Haldane, Webb, Yano and Aiba models differ widely from the experimental data. Hence, on the basis of correlation coefficient and the per cent standard deviation values in the present biodegradation study, Yano model for the resorcinol, and Andrews and Noack model for the p–cresol have been selected for further discussion of results.

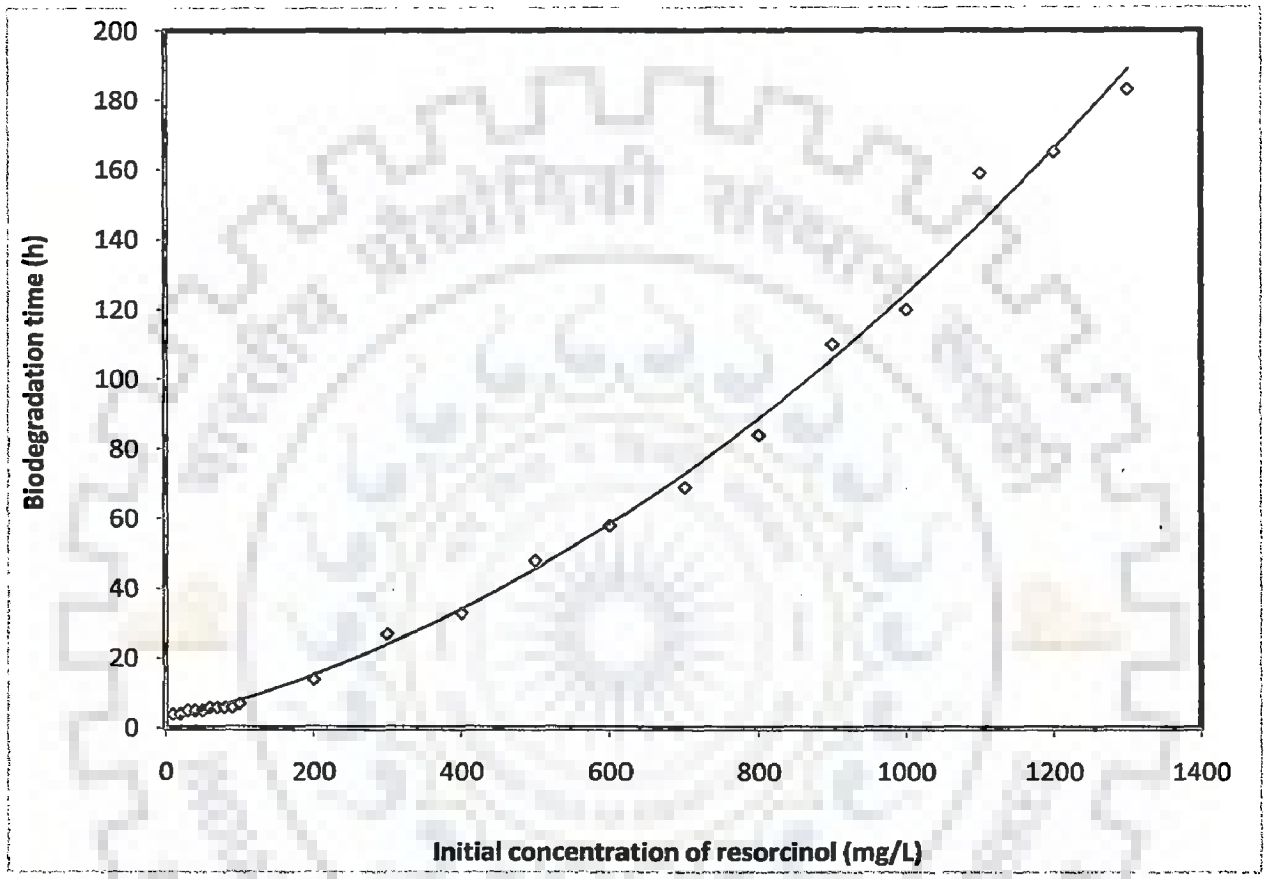

Figure 4.6: Time for complete biodegradation of resorcinol at various initial concentrations

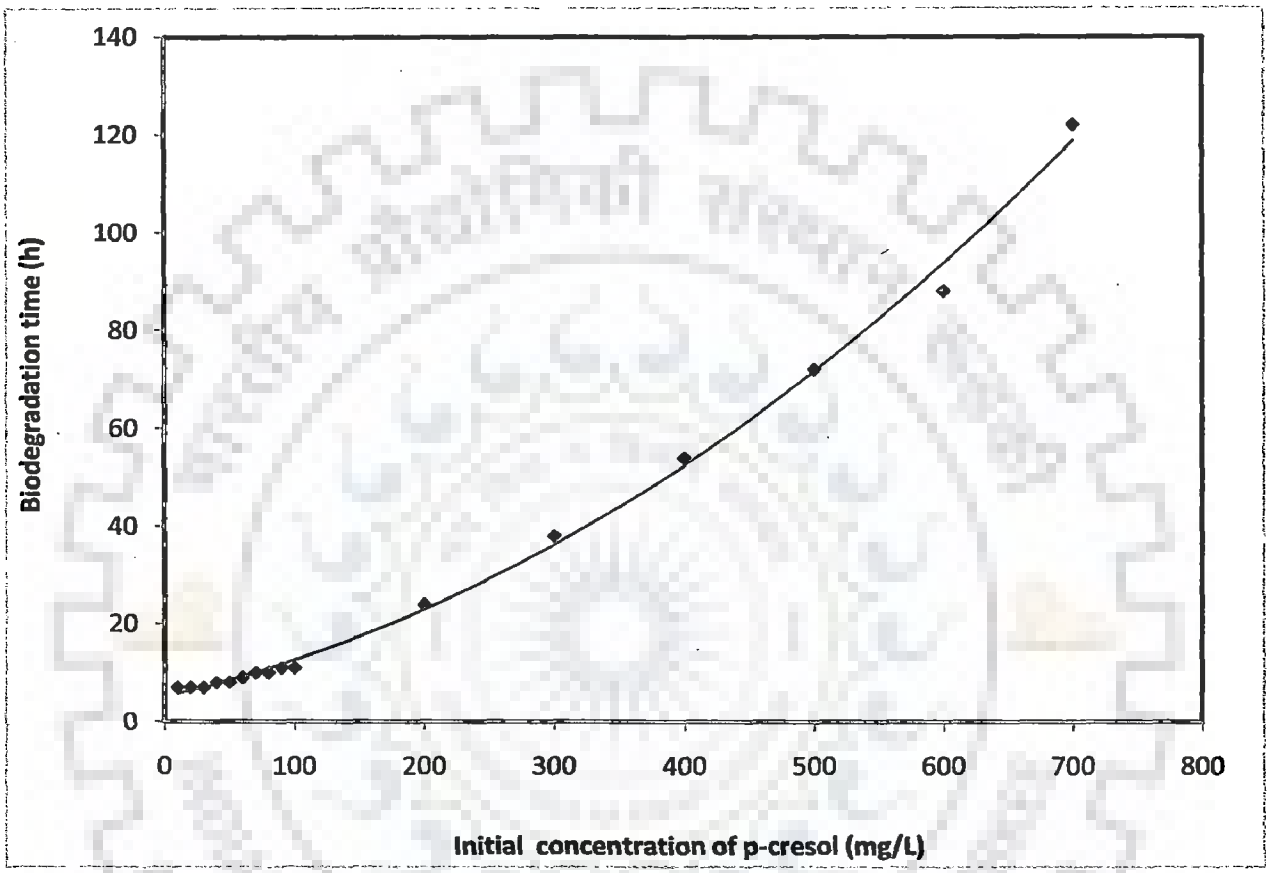

Figure 4.7: Time for complete biodegradation of p–cresol at various initial concentrations

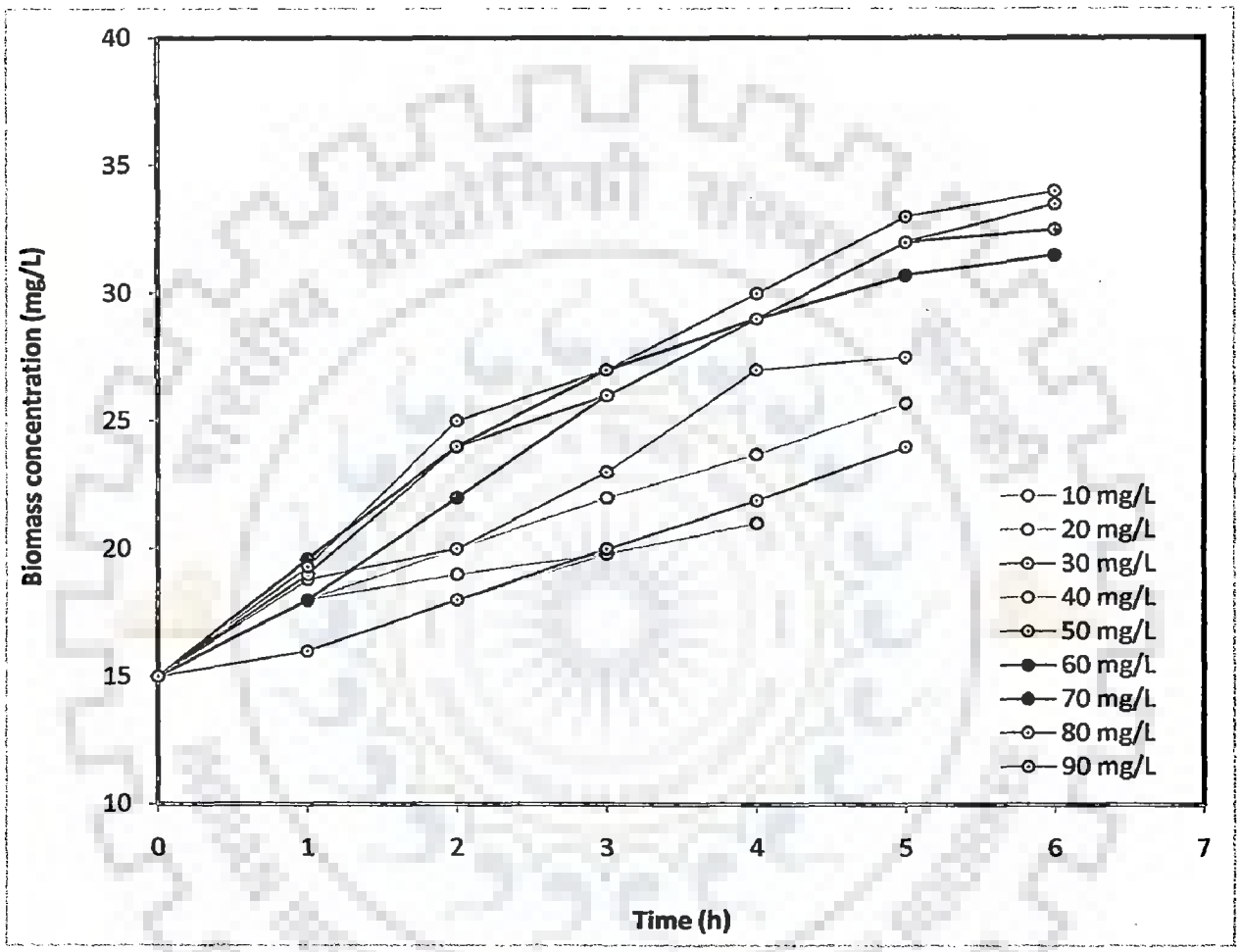

Figure 4.8: Biomass concentration in the mineral medium containing resorcinol at lower initial concentrations

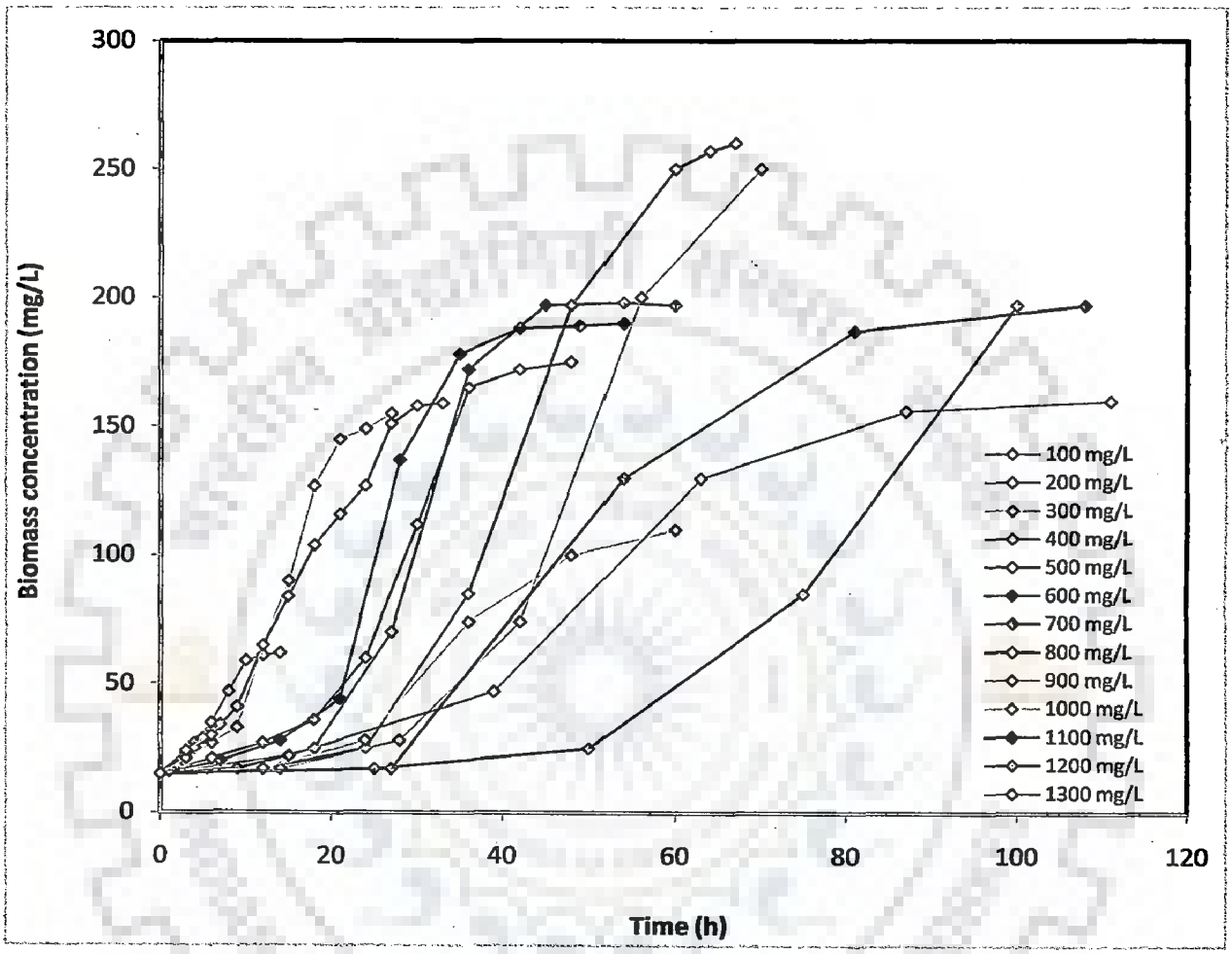

Figure 4.9: Biomass growth of *G. indicus* in the mineral medium containing resorcinol at higher initial concentrations

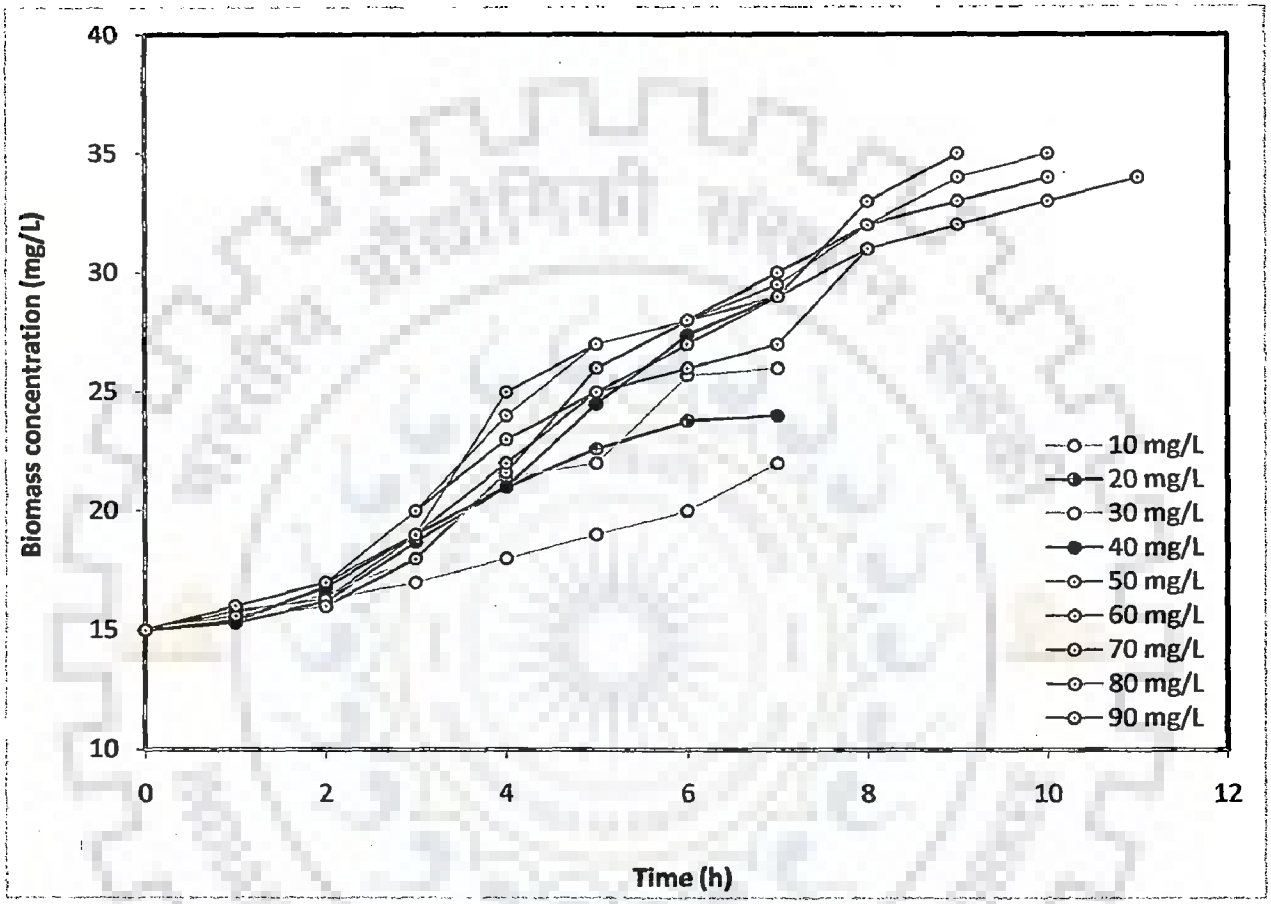

Figure 4.10: Biomass concentration in the mineral medium containing p–cresol at lower initial concentrations

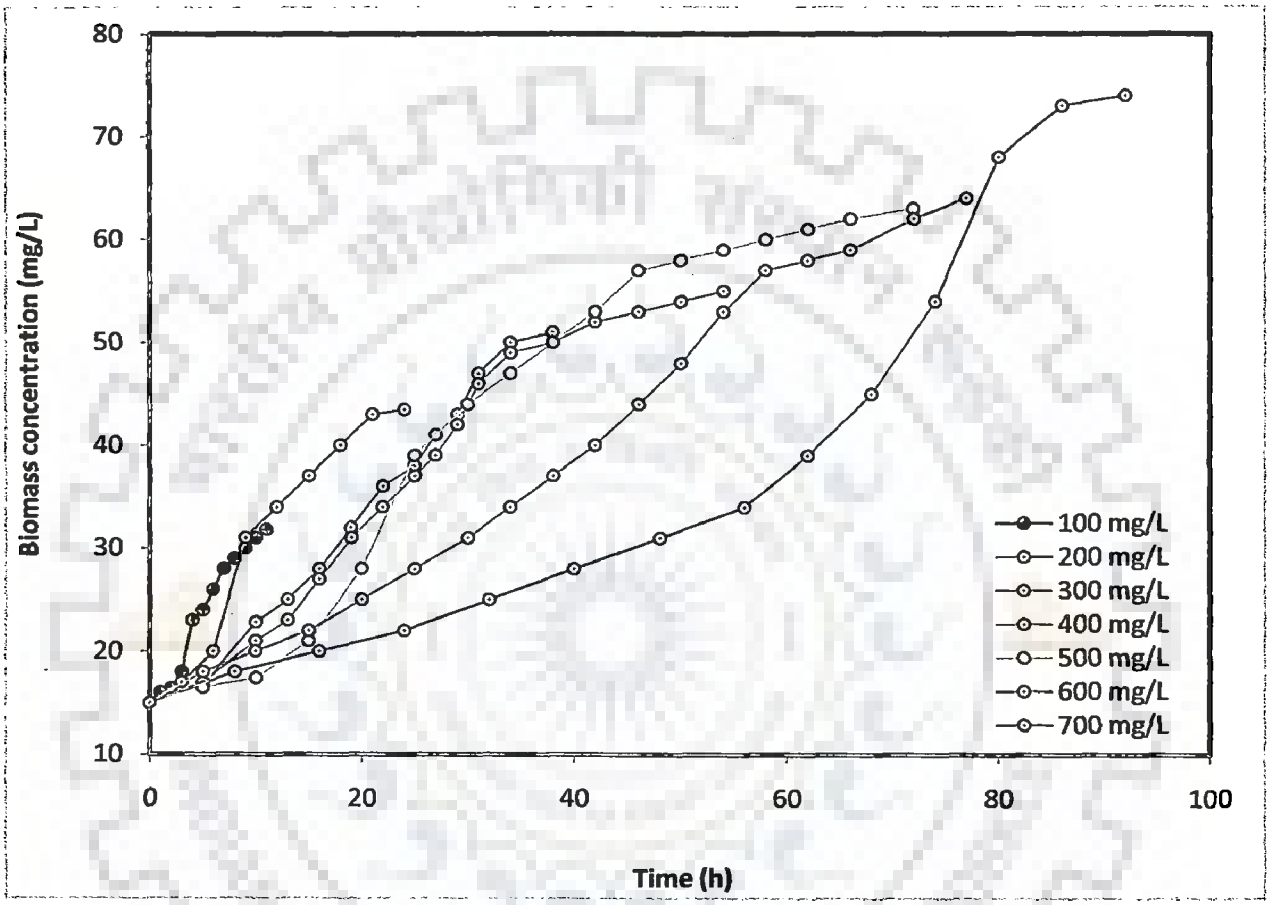

Figure 4.11: Biomass concentration in the mineral medium containing p–cresol at higher initial concentrations

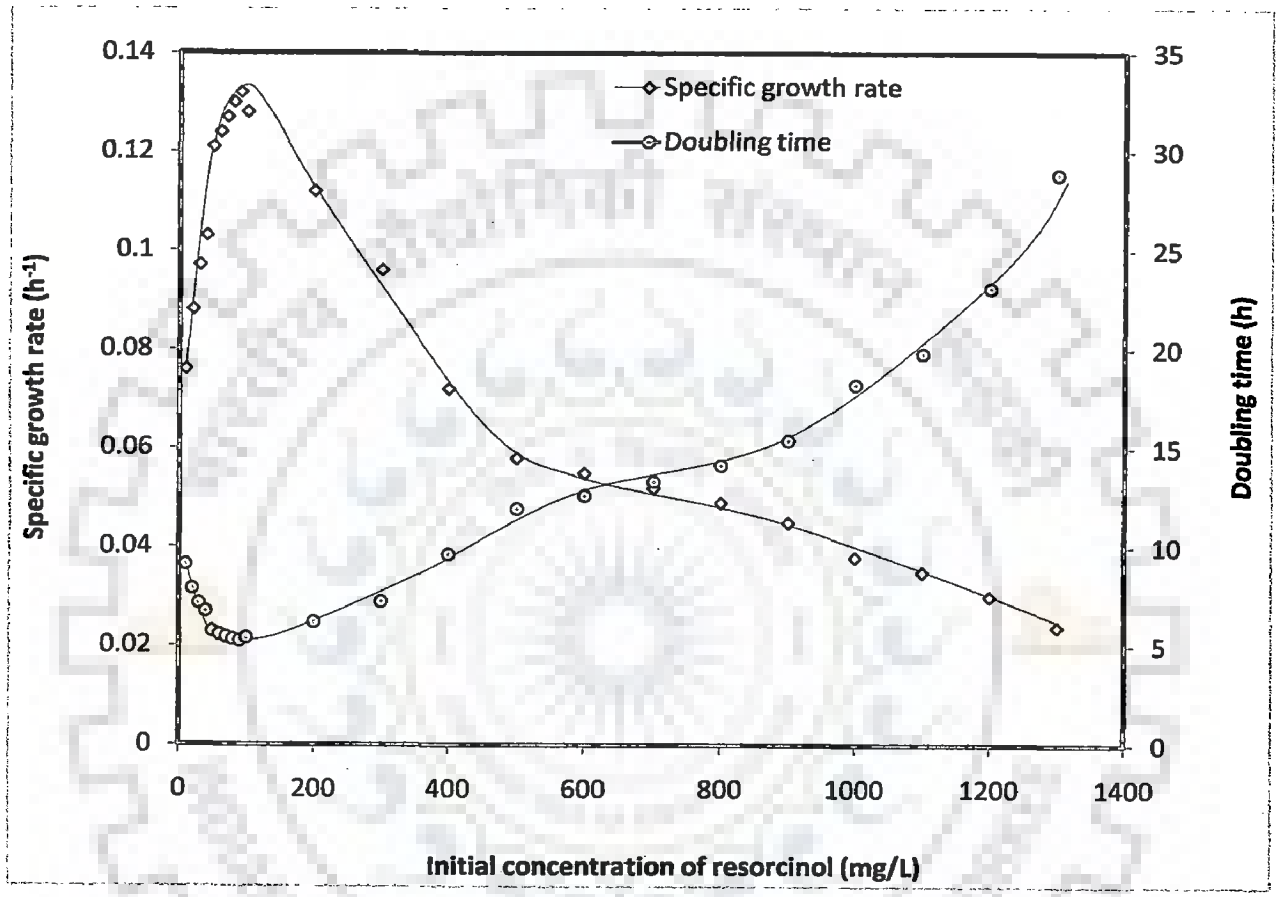

Figure 4.12: Variation in specific growth rate and doubling time with initial concentration of resorcinol

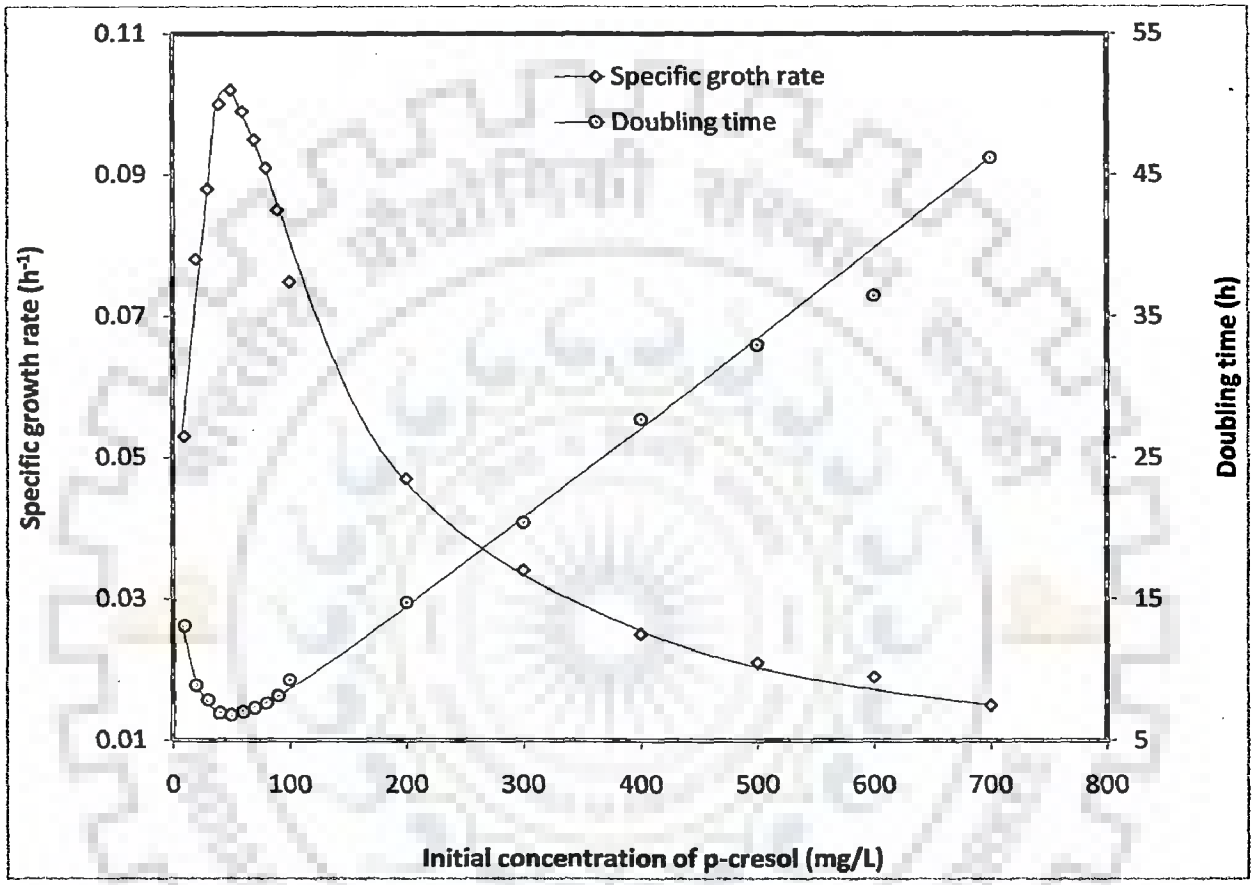

Figure 4.13: Variation in specific growth rate and doubling time with initial concentration of p–cresol

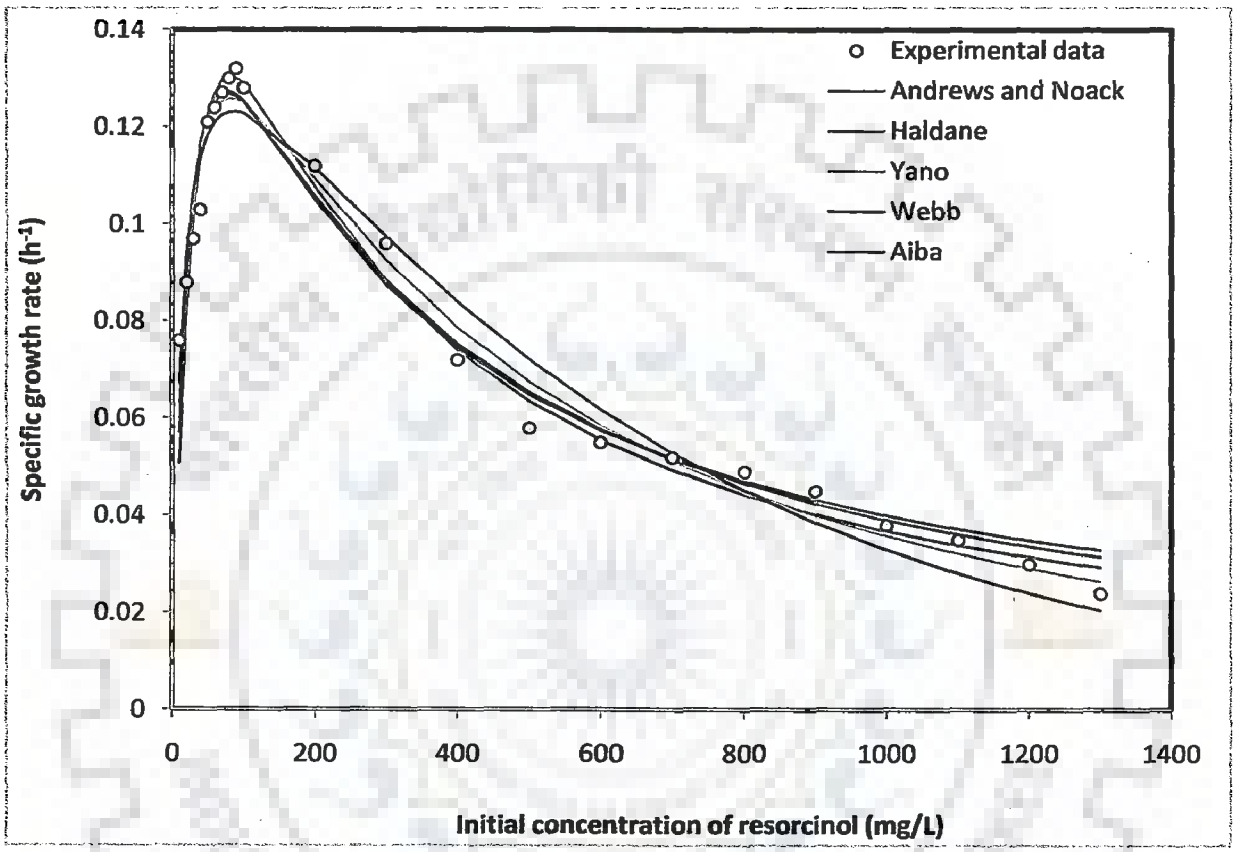

Figure 4.14: Comparison between kinetic model predictions and experimentally determined specific growth rates at different initial concentrations of resorcinol

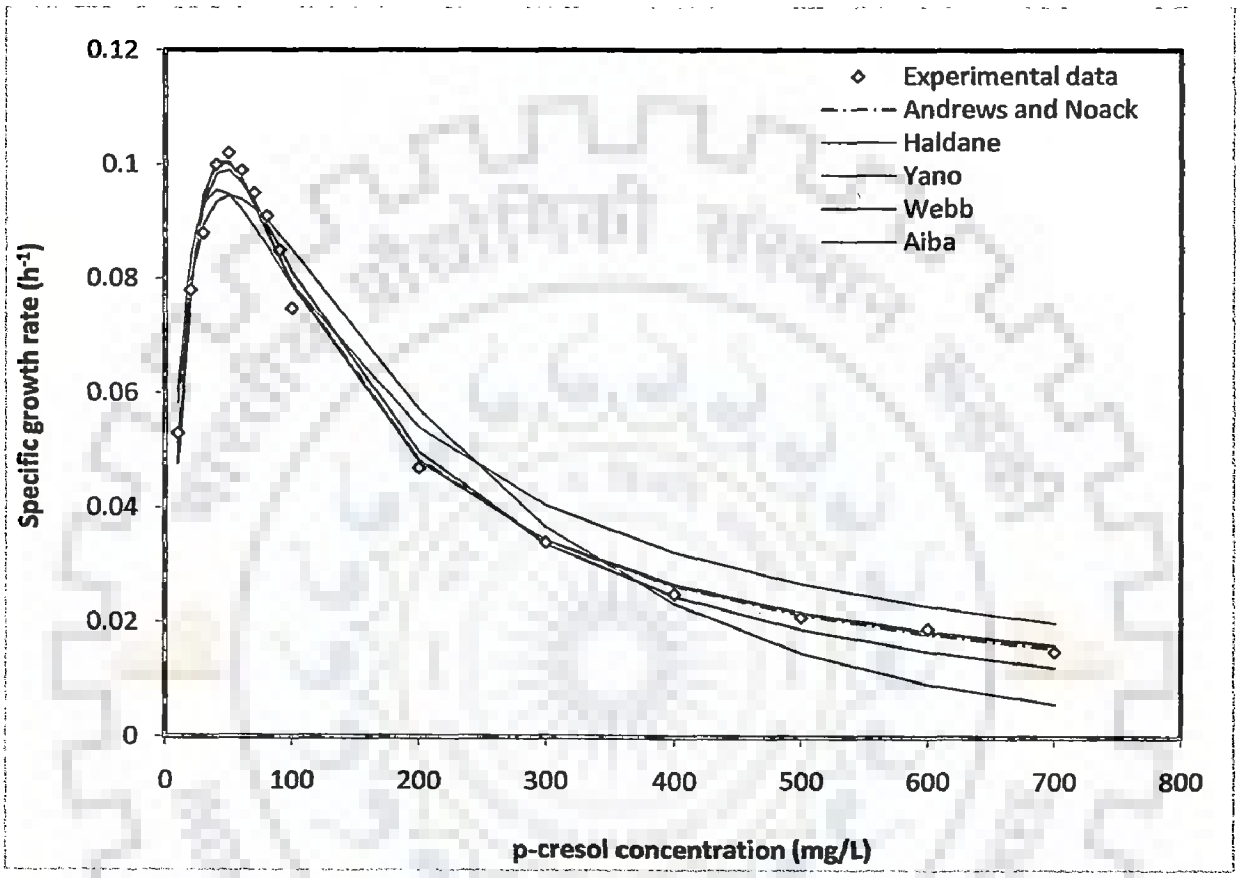

Figure 4.15: Comparison between kinetic model predictions and experimentally determined specific growth rates at different initial concentrations of p–cresol

Table 4.6: Estimated values of kinetic parameters of biomass growth models for resorcinol

S. No.	Model	Estimated value of growth kinetic parameters				R^2	Per cent Standard deviation ($\Delta\mu_g$%)
		μ_{gmax} (h^{-1})	K_S (mg/L)	K_i (mg/L)	K (mg/L)		
1	Haldane	0.640	100.0	67.54	-	0.963	2.17
2	Yano	0.185	19.83	376.0	1790.0	0.974	1.73
3	Andrews and Noack	0.147	12.17	9920.0	-	0.967	2.19
4	Webb	0.223	28.56	195.80	9944.0	0.966	2.47
5	Aiba	0.164	14.08	627.90	-	0.963	2.46

Table 4.7: Estimated values of kinetic parameters of biomass growth models for p–cresol

S. No.	Model	Estimated value of growth kinetic parameters				R^2	Per cent Standard deviation ($\Delta\mu_g$%)
		μ_{gmax} (h^{-1})	K_S (mg/L)	K_i (mg/L)	K (mg/L)		
1	Haldane	0.382	41.04	41.15	-	0.966	4.07
2	Yano	0.279	43.12	54.63	997.8	0.992	2.05
3	Andrews and Noack	0.512	91.87	21.99	-	0.993	1.03
4	Webb	0.577	105.8	18.75	9914.0	0.993	1.13
5	Aiba	0.157	15.7	214.0	-	0.962	5.68

4.2.2 Observed Biomass Growth yield coefficient

The batch experimental data of biomass growth and substrate concentrations have been taken at different time intervals during the bidegradation of phenol, resorcinol, and p–cresol at their various initial concentrations. These data have been used to determine the observed biomass growth yield coefficient $\left(Y_{X/S}\right)_o$ in case of phenol, resorcinol, and p–cresol as single substrate, using Eq. (4.4). The plot of $\left(X - X_o\right)$ versus $\left(S - S_o\right)$ for the exponential growth phase gives a straight line at each initial concentration of the substrate. The slope of the straight line has been estimated by the linear regression using MATLAB 7.2. The similar procedure has been followed for all initial substrate concentrations varying from 10 to 1000 mg/L (phenol), 10 to 1300 mg/L (resorcinol), and 10 to 700 mg/L (p–cresol). Figures 4.16, 4.17, and 4.18 show the variation of $\left(Y_{X/S}\right)_o$ with initial concentration of phenol, resorcinol, and p–cresol respectively. These profiles indicate that observed biomass growth yield is not constant but varies with the initial substrate concentrations. At lower initial concentration of substrate the observed biomass growth yield increases, attains a maximum value and then decreases with the increase in initial concentration of the substrate. The maximum value of observed biomass growth yield coefficient $\left(Y_{X/S}\right)_o$ is 0.437 g/g, obtained at initial phenol concentration of 70 mg/L. Likewise, the maximum value of $\left(Y_{X/S}\right)_o$ is 0.443 g/g at initial resorcinol concentration of 90 mg/L and then it decreases with the increase in initial concentration up to 1300 mg/L. In case of p–cresol, maximum observed biomass growth yield coefficient value is 0.31 g/g at the initial concentration of 50 mg/L, and beyond this initial concentration, the observed growth yield starts to decrease with the increase in initial p–cresol concentration. It is noticeable that the similar trend has been observed in case of the specific growth rate profiles with the initial concentrations of phenol, resorcinol, and p–cresol as shown in Figures 4.4, 4.12 and 4.13 respectively. The decreasing trend of specific growth rate beyond inhibitory initial substrate concentration results in the reduction in observed biomass growth yield coefficient value indicating that the substrate inhibition reduces the specific growth rate as well as observed biomass growth yield.

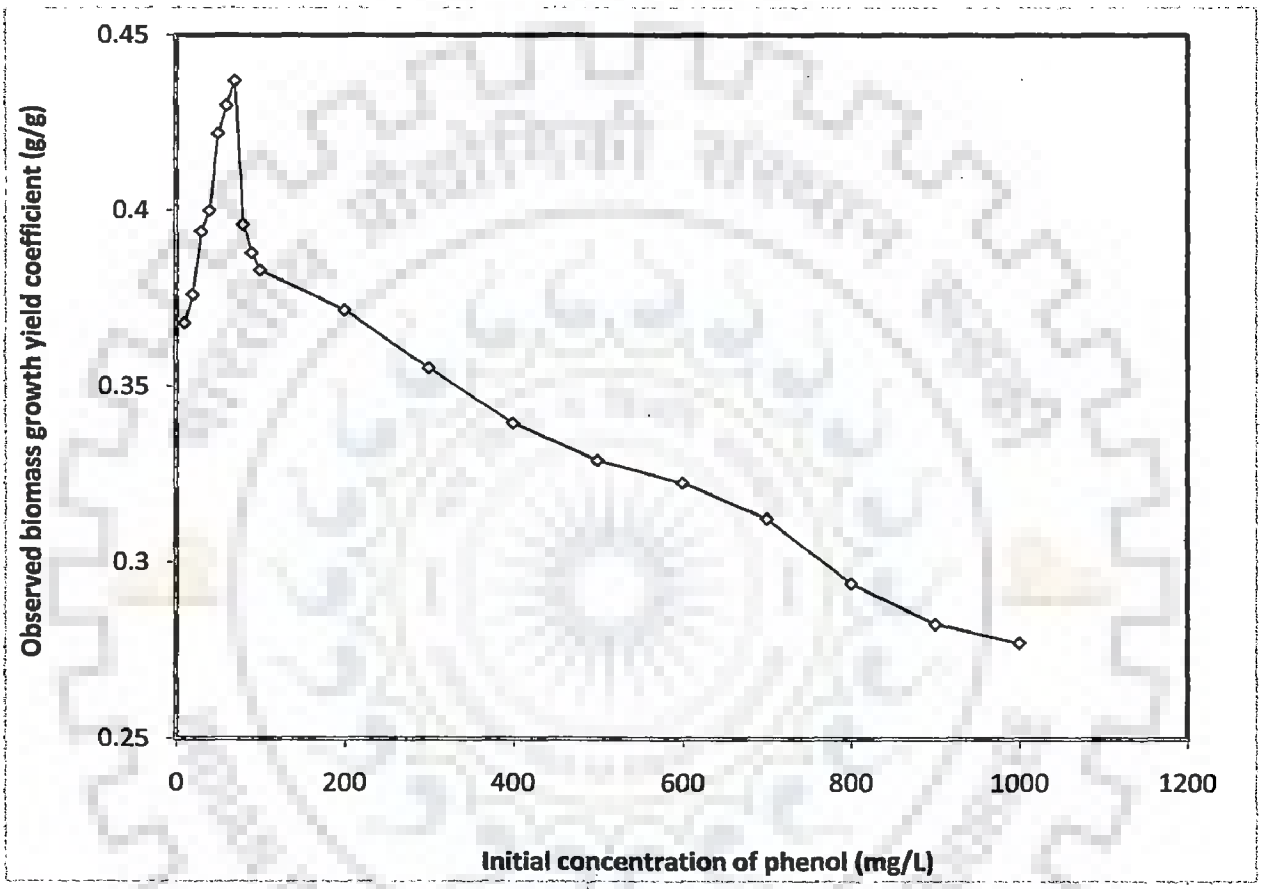

Figure 4.16: Variation of observed biomass growth yield with initial concentration of phenol

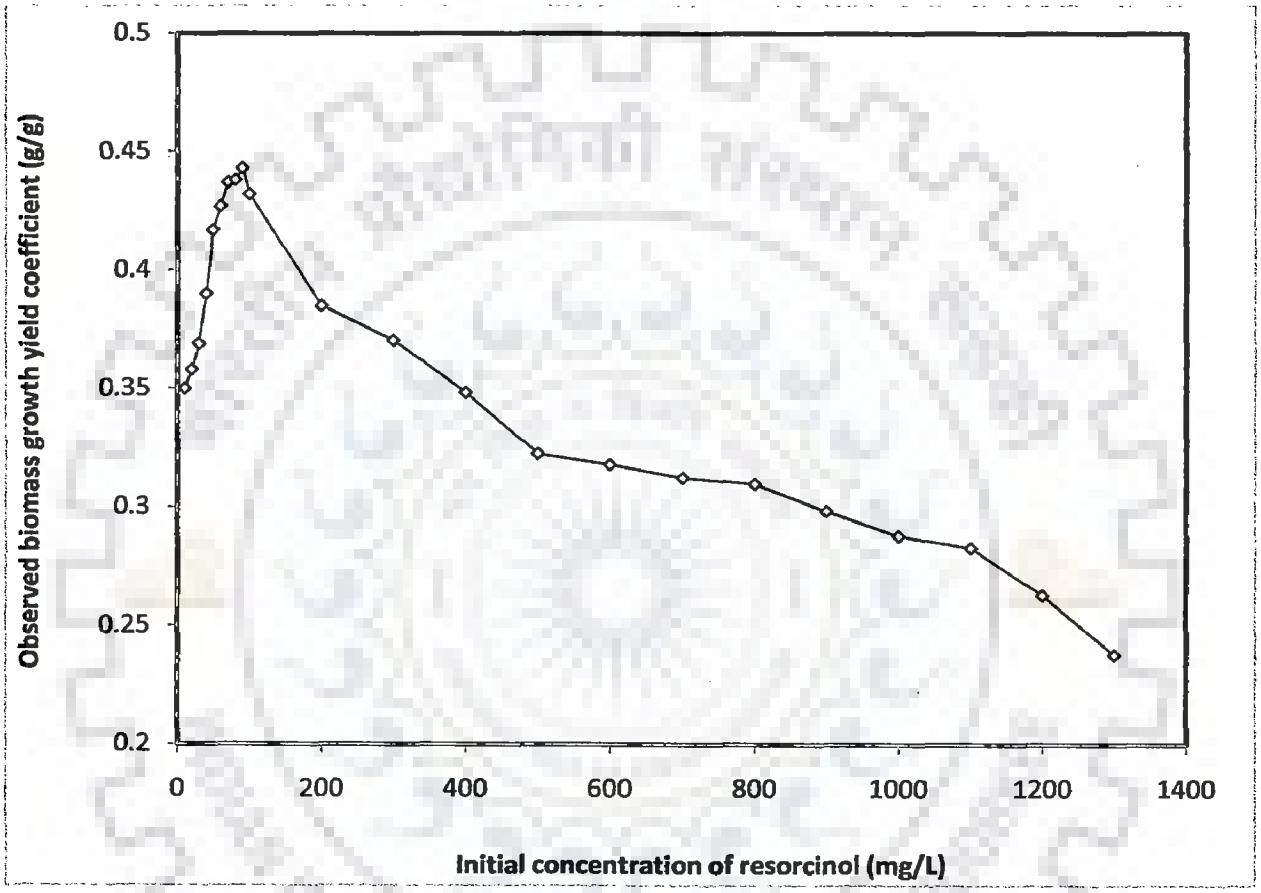

Figure 4.17: Variation of observed biomass growth yield with initial concentration of resorcinol

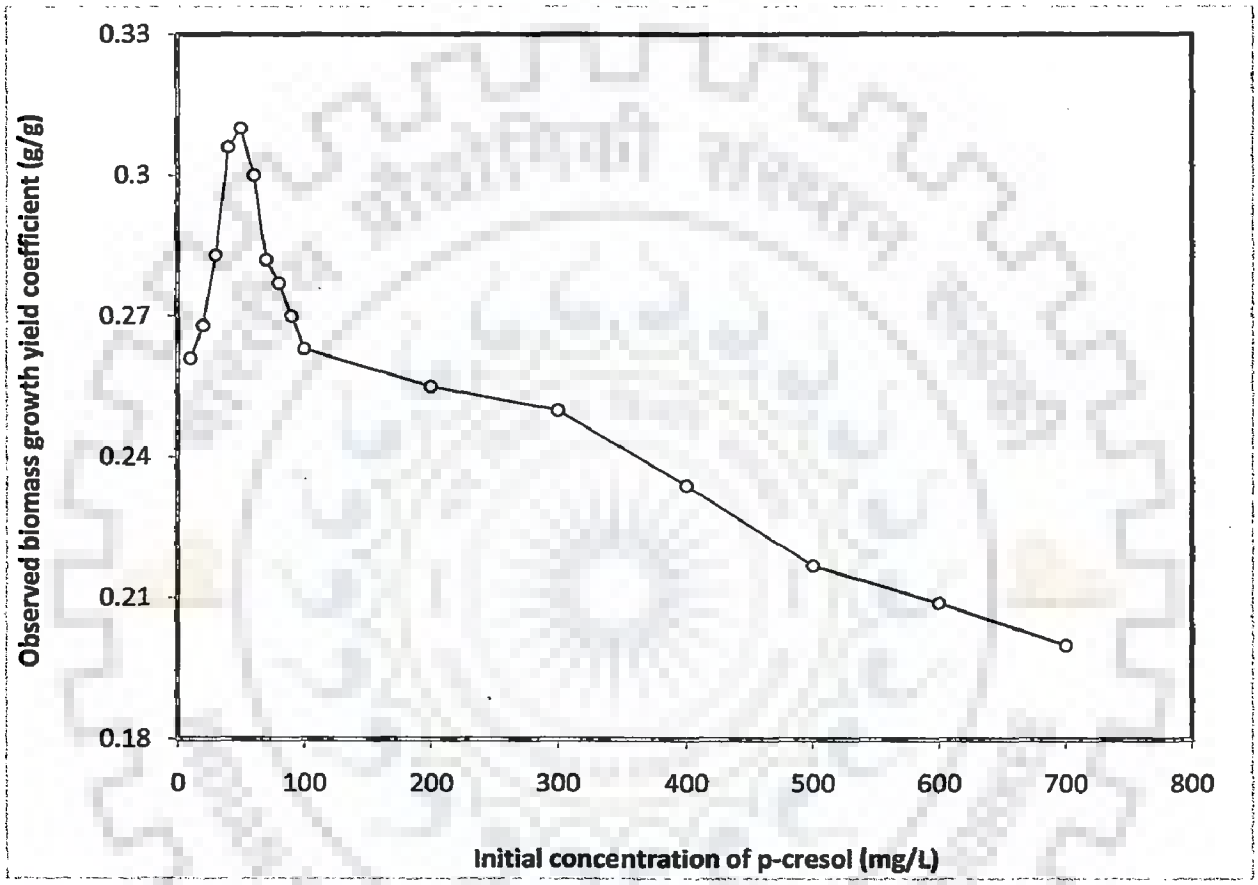

Figure 4.18: Variation of observed biomass growth yield with initial concentration of p–cresol

4.2.3 Biodegradation Kinetics

In order to describe the biodegradation kinetics of phenol, resorcinol, and p–cresol in single substrate system using fungus *G. indicus*, batch experiments were conducted on various initial concentrations of phenol (10 to 1000 mg/L), resorcinol (10 to 1300 mg/L) and p–cresol (10 to 700 mg/L) keeping temperature 28 °C and pH value 6. The variation in the substrate concentration and biomass concentration with time was monitored using the procedure mentioned in the section 3.1.2.4 of Chapter III. The measurement of concentrations of biomass and substrate were followed till the substrate was consumed to a large extent. The values of specific degradation rate (q_S) have been calculated at different initial substrate concentrations by using specific growth rate (μ_g) and observed biomass growth yield coefficient $(Y_{X/S})_o$, at their respective initial concentration of substrate according to Eq. (4.9). Values of initial specific degradation rate (q_{So}) are determined using initial substrate degradation rates as

$$q_{So} = -\frac{1}{X_o}\frac{\Delta S}{\Delta t} \tag{4.19}$$

where ΔS is $(S_t - S_o)$ and Δt is total time required to reduce the substrate concentration from S_o to S_t.

Figures 4.19 and 4.20 show the variation of phenol concentration with time at lower initial concentrations (10 to 90 mg/L) and at higher initial concentrations (100 to 1000 mg/L) respectively. These degradation trends indicate that the inhibition at high initial concentrations of phenol reduces the biodegradation rate and thereby increases the biodegradation time to consume it completely. Figures 4.21 and 4.22 depict the variation of specific degradation rate (q_S) and initial specific degradation rate (q_{So}) with initial phenol concentrations respectively. In both the figures, it can be seen that the specific degradation rate increases with the initial phenol concentration up to 70 mg/L and then decreases with the higher initial phenol concentrations.

Since the experimental data presented in Figs. 4.21 and 4.22 represent a typical substrate inhibition kinetics, the kinetic models M1, M2, M3 and M4 have been

selected to describe q_S and models M5, M6, M7 and M8 have been selected to describe q_{So} as discussed in section 4.1. These models are listed in the Table 4.2. In order to estimate the model parameters of eight substrate degradation models, a nonlinear least square regression analysis of experimental data was carried out in MATLAB 7.2 based on Windows XP. The estimated values of parameters are summarized in Table 4.8. The selection of best fitted model has been done on the basis of correlation coefficient R^2 and per cent standard deviation $\Delta q_S\%$. The values of correlation coefficient (R^2) for degradation model parameters are also given in the Table 4.8. Per cent standard deviation $(\Delta q_S\%)$ between experimental and predicted values for each model is calculated by Eq. (4.20).

$$\Delta q_S\% = \left(\frac{\sqrt{\Sigma\left[\left(q_{S.\exp} - q_{S.pred}\right)/q_{S.pred}\right]^2}}{N} \right) 100 \qquad (4.20)$$

where $q_{S.\exp}$ is experimental specific degradation rate and $q_{S.pred}$ is the corresponding predicted specific degradation rate according to the model under study with the best fitted kinetic constants, N is the number of measurements.

Figures 4.23 and 4.24 provide the comparison of different models vis - a - vis experimental results of q_S and q_{So} respectively. It is observed from the Table 4.8 that values of correlation coefficient R^2 are very close to each other in four models M1, M2, M3 and M4 (>0.97) and in models M5, M6, M7 and M8 (>0.95). Thus it is very difficult to decide best model to represent the experimental data on the basis of correlation coefficient R^2. Better criteria to test the goodness of the fit of degradation data is by per cent standard deviation. Table 4.8 shows the per cent standard deviation for all eight models. In case of q_S the per cent standard deviations are minimum and very close for models M1 and M2. In case of q_{So} the minimum value of per cent standard deviation is for model M8. It is noteworthy that the per cent standard deviation values are highest for models M3 and M7 in the two categories.

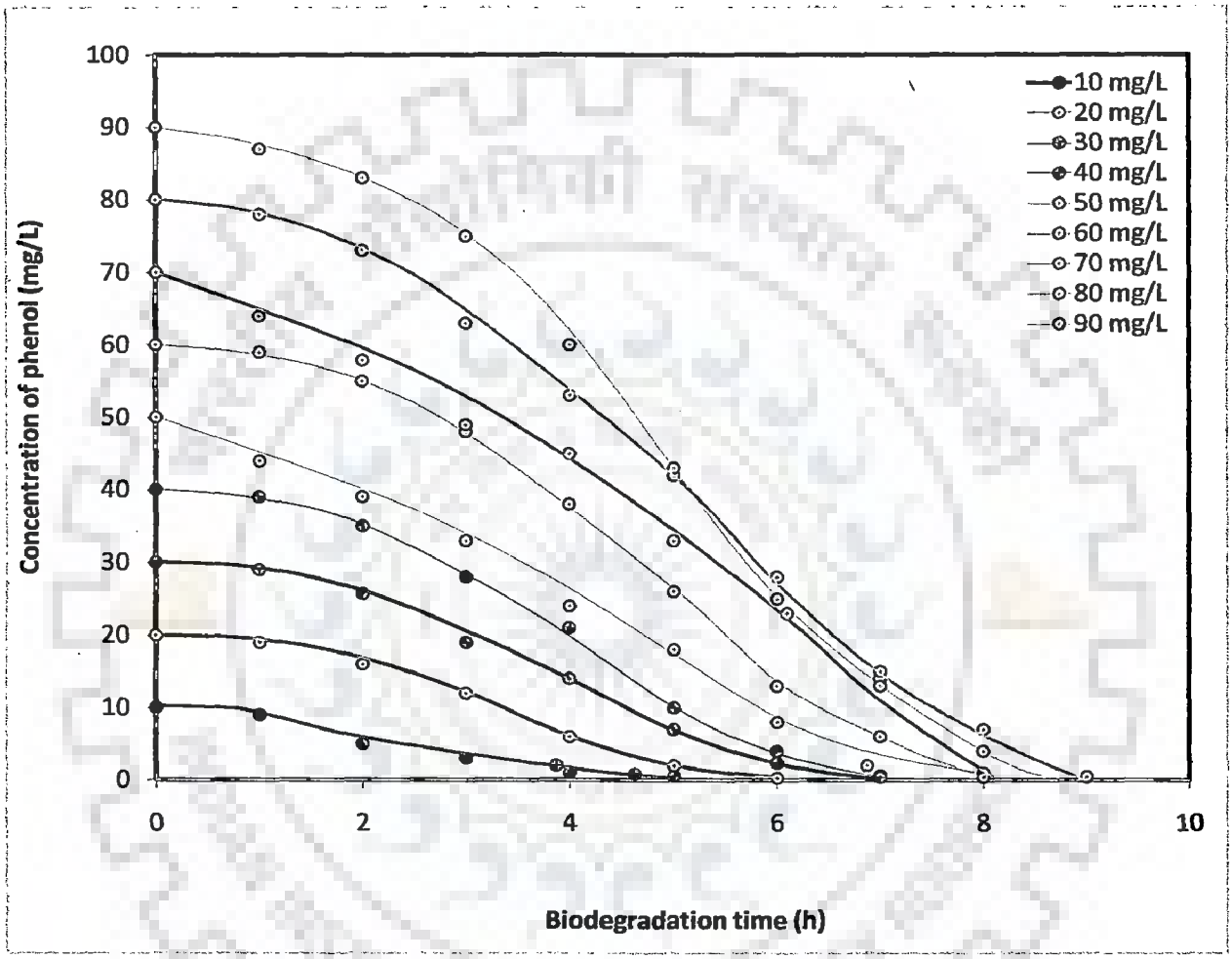

Figure 4.19: Biodegradation behaviour of phenol in the range of lower initial concentrations

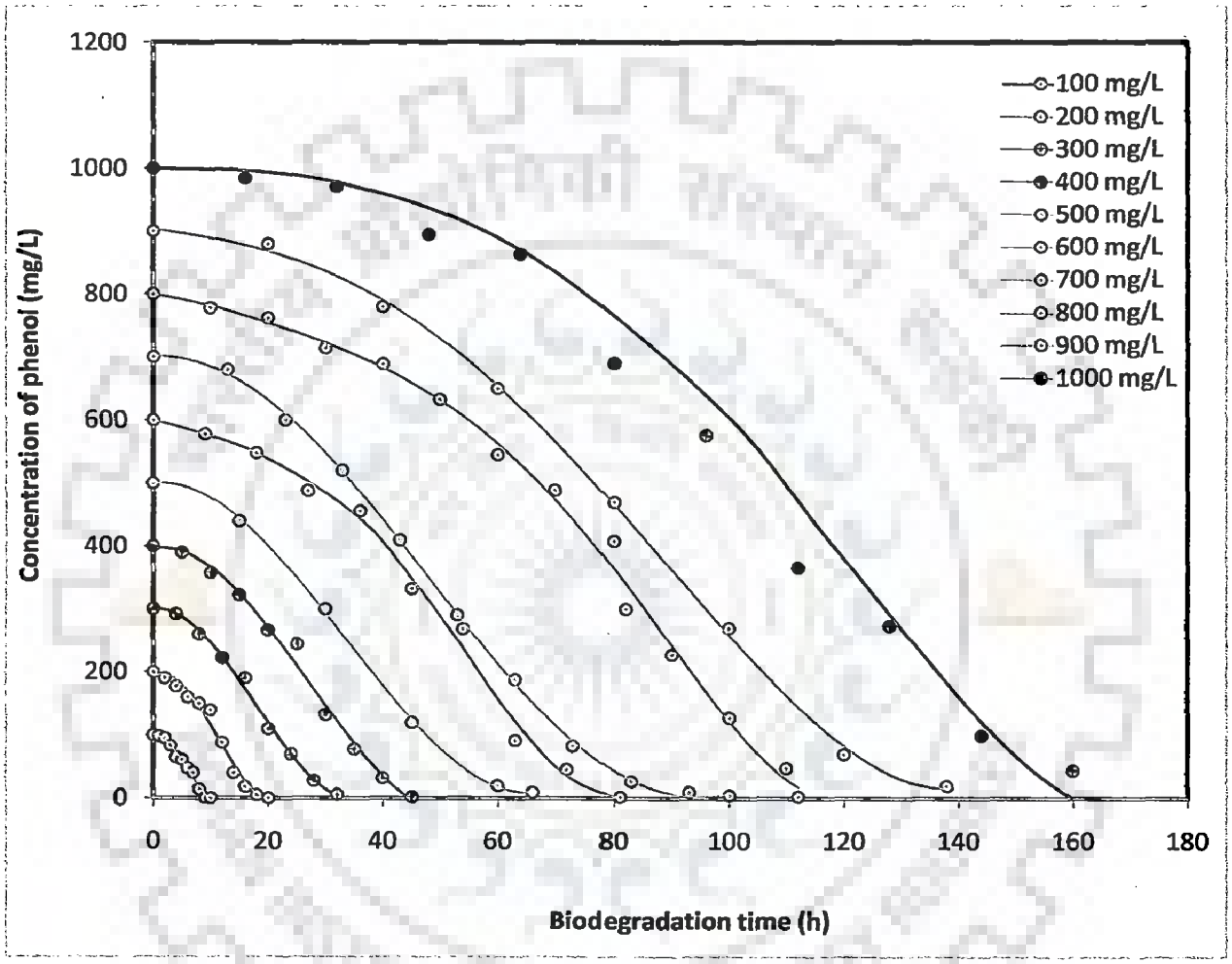

Figure 4.20: Biodegradation behaviour of phenol in the range of higher initial concentrations

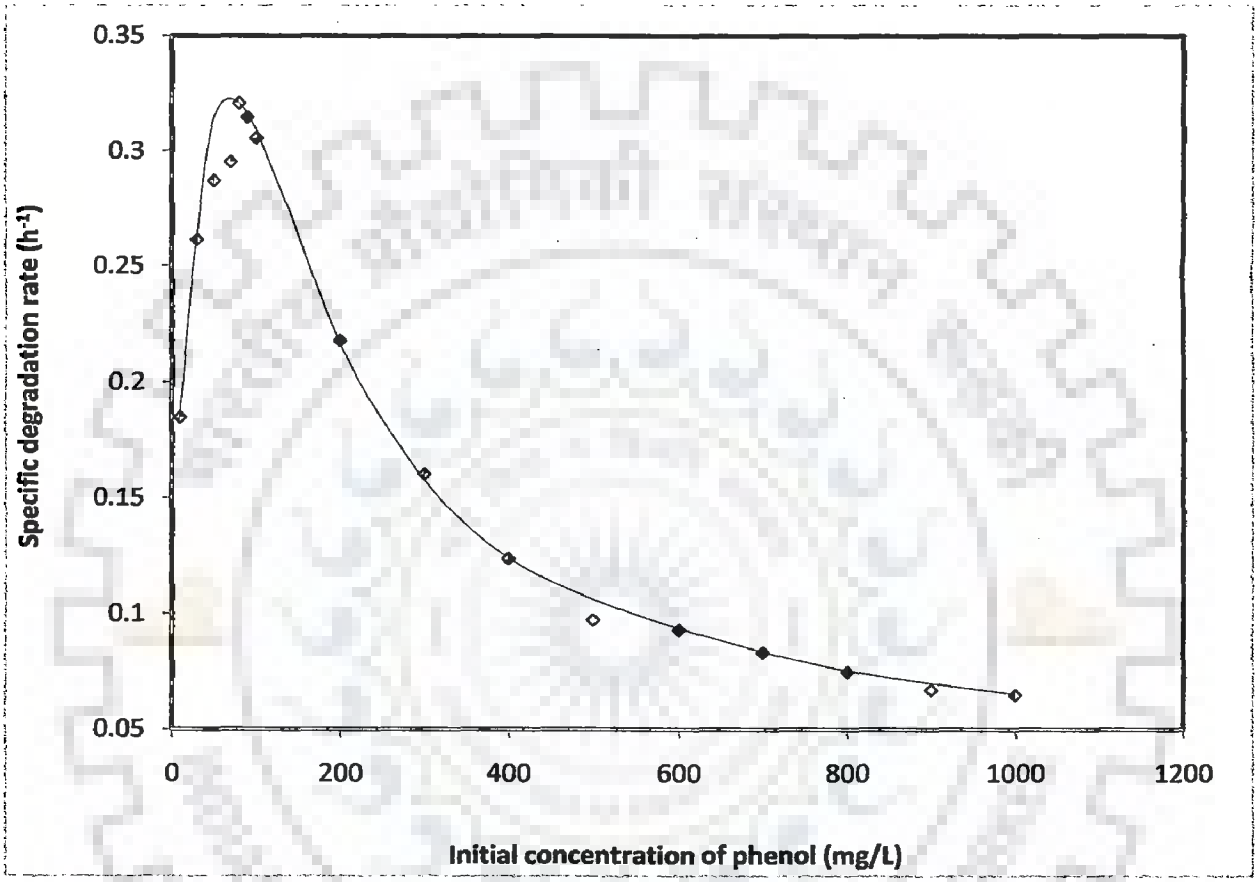

Figure 4.21: Experimentally determined specific degradation rates at various initial concentrations of phenol

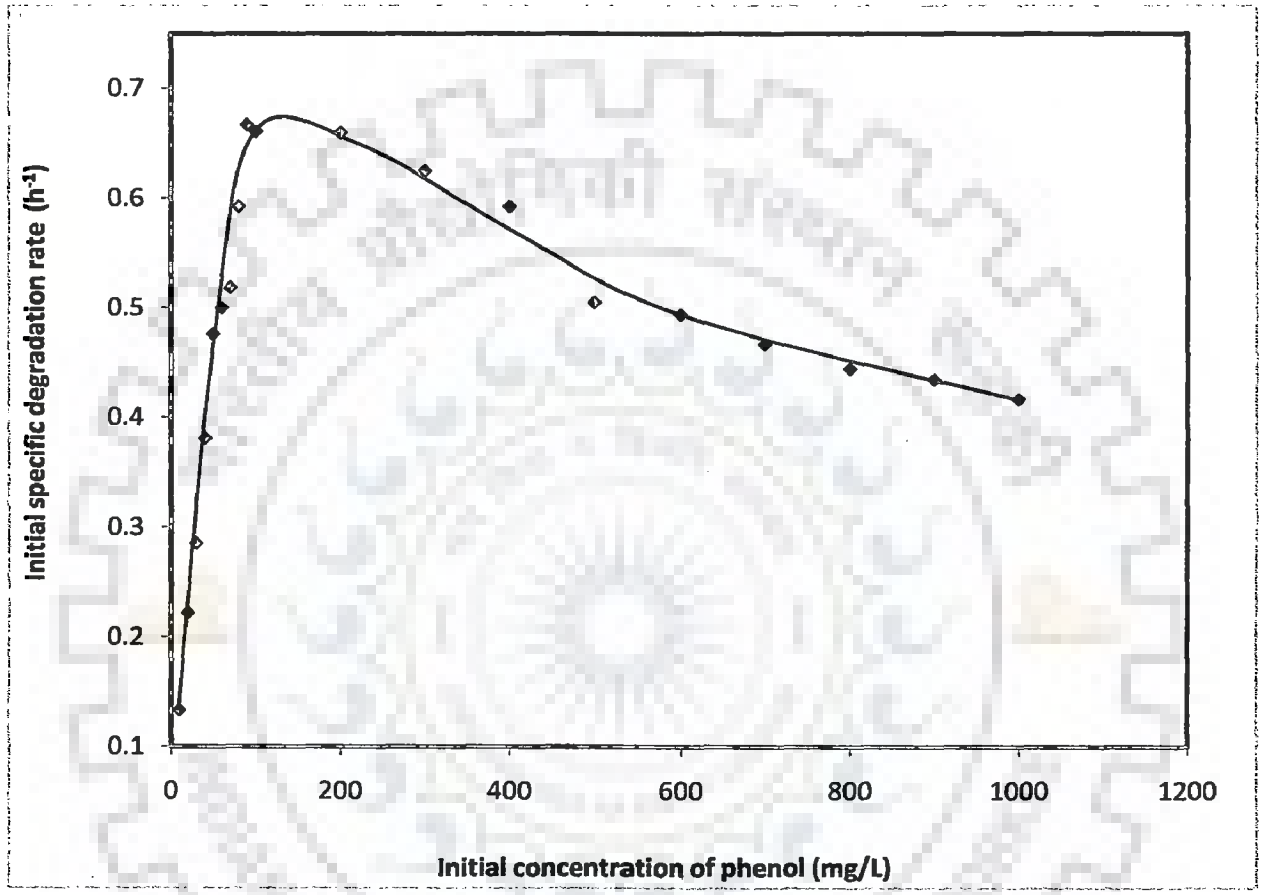

Figure 4.22: Experimentally determined initial specific degradation rates at various different initial concentrations of phenol

Although the remarkable similarity is observed in the results of three models (M1, M2, M4) for q_S, the three parameter model M1 has been selected to represent the specific degradation rate (q_S) and four parameter model M8 has been selected to represent the initial specific degradation rate (q_{So}). The slight difference in the values may be due to the nonlinear fitting procedure. Estimated values of kinetic constants $q_{S\max}$, $K_S^{'}$ and $K_i^{'}$ of M1, for specific degradation rate are 0.631 h^{-1}, 30.04 mg/L and 111.9 mg/L respectively. Kinetic constants for initial specific degradation rate (model M8) are $q_{So\max}$ = 3.268 h^{-1}, $K_S^{''}$ = 290.4 mg/L, $K_i^{''}$ = 50.41 mg/L and $K^{''}$ = 589.9 mg/L. Therefore, the specific degradation rates q_S and q_{So} can be mathematically expressed according to models M1 and M8 respectively by following equations:

$$q_S = \frac{0.631S}{30.04 + S + \frac{S^2}{111.9}} \tag{4.21}$$

$$q_{So} = \frac{3.268\left(1 + \frac{S}{589.9}\right)}{S + 290.4 + \frac{S^2}{50.41}} \tag{4.22}$$

$K_S^{'}$ and $K_S^{''}$ indicate the affinity to degradation, $q_{S\max}$ and $q_{So\max}$ indicate the rate of degradation and $K_i^{'}$, $K_i^{''}$, $K^{'}$, $K^{''}$ are indicators of the degree of substrate inhibition. In case of inhibitory substrates, it is not possible to observe an actual $q_{S\max}$.

Table 4.8: Estimated values of biodegradation kinetic model parameters for phenol

S. No.	Model	Estimated values of degradation Kinetic model parameters								R^2	Per cent Standard deviation (Δq_S%)
		$q_{S_{max}}$ (h^{-1})	$q_{So_{max}}$ (h^{-1})	K_S (mg/L)	K_S'' (mg/L)	K_i' (mg/L)	K_i'' (mg/L)	K' (mg/L)	K'' (mg/L)		
1	M 1	0.631	-	30.04	-	111.9	-	-	-	0.976	1.931
2	M 2	1.177	-	54.83	-	60.64	-	-	-	0.976	1.931
3	M 3	0.495	-	19.70	-	221.6	-	1000.0	-	0.979	2.698
4	M 4	0.659	-	32.34	-	100.1	-	9973.0	-	0.975	2.021
5	M 5	-	1.315	-	87.35	-	413.1	-	-	0.957	1.514
6	M 6	-	1.888	-	125.4	-	287.8	-	-	0.957	1.514
7	M 7	-	1.307	-	86.52	-	420.4	-	9919.0	0.957	1.719
8	M 8	-	3.268	-	290.4	-	50.41	-	589.9	0.983	1.232

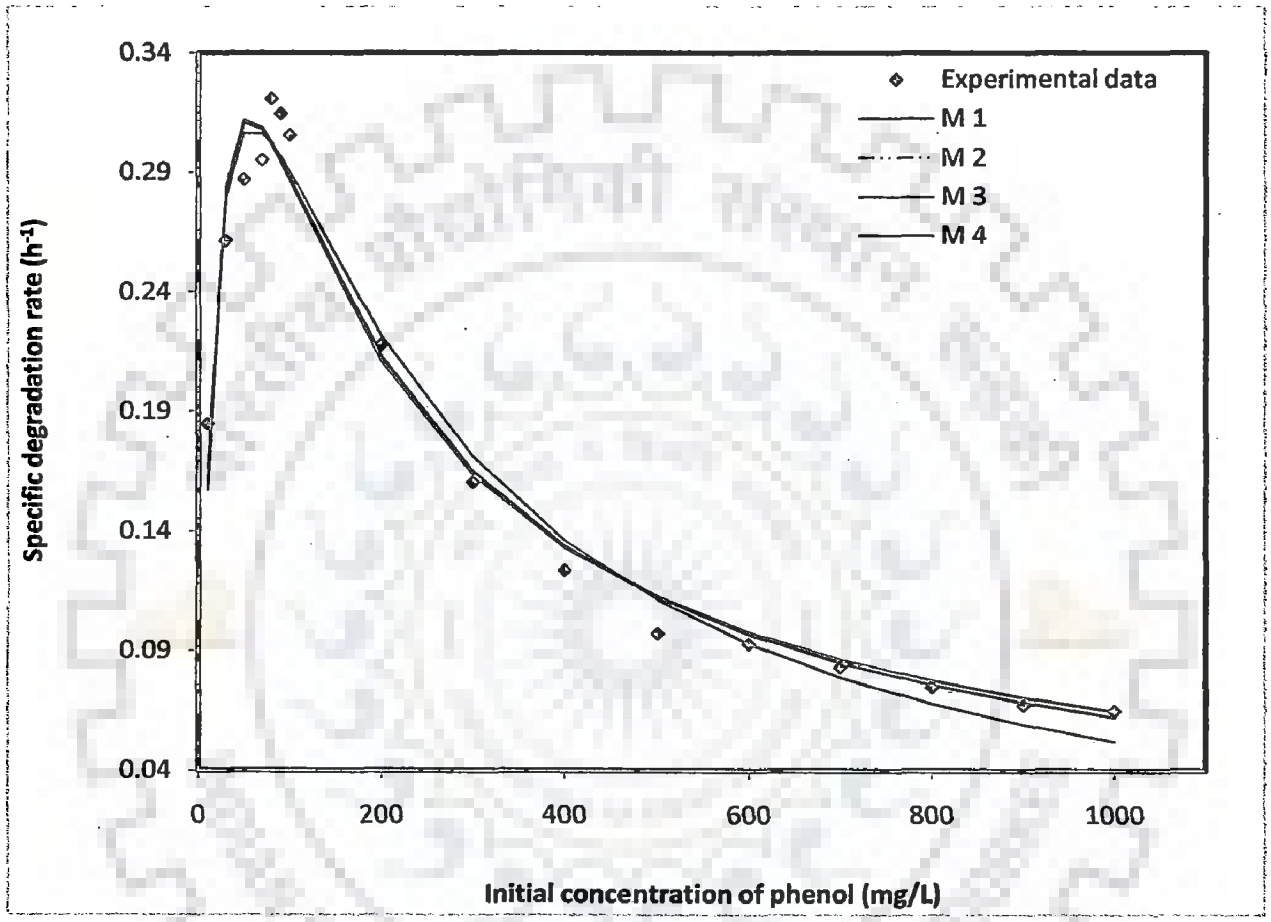

Figure 4.23: Comparison between kinetic model predictions and experimentally determined specific degradation rates at different initial concentrations of phenol

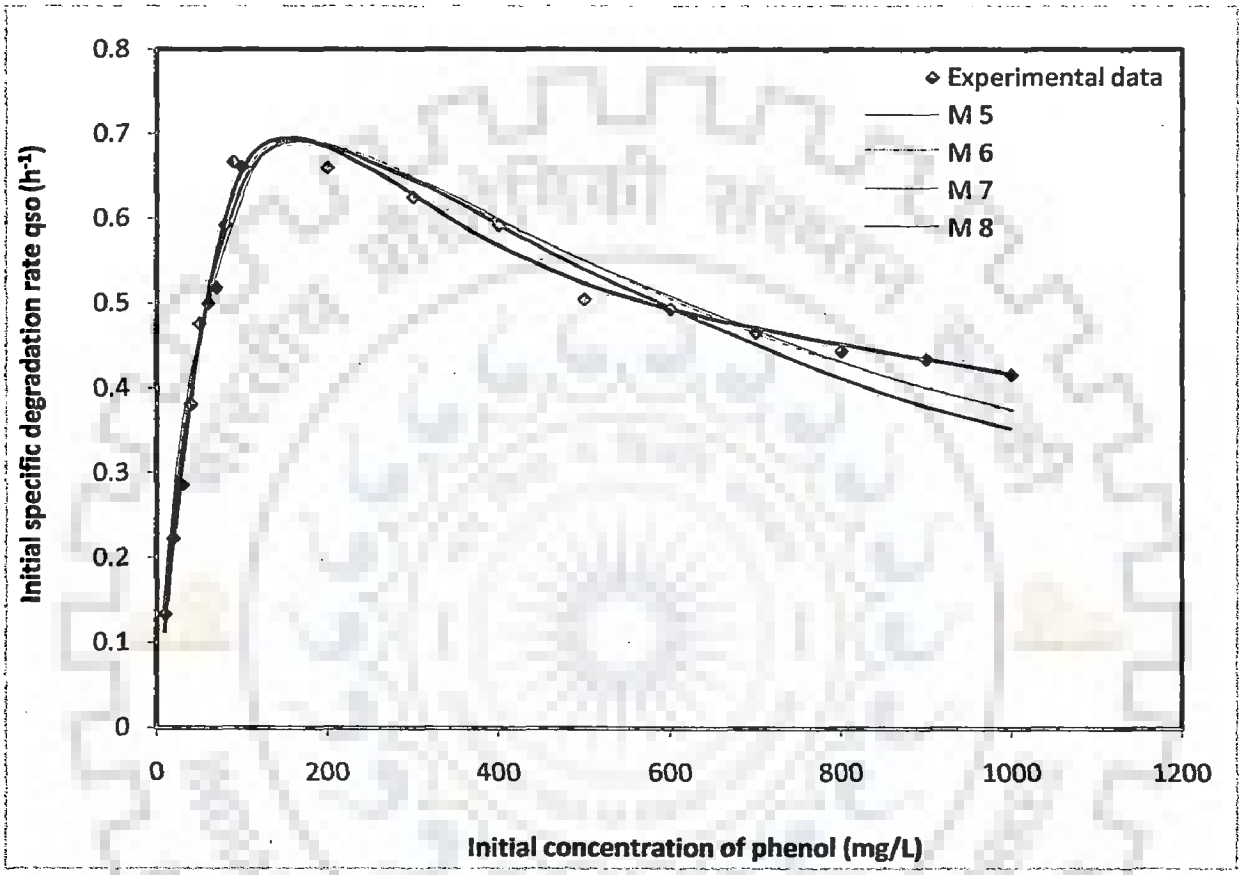

Figure 4.24: Comparison between kinetic model predictions and experimentally determined initial specific degradation rates at different initial concentrations of phenol

The value of S_o^* at which q_S attains its maximum value $q_{S\max}^*$, can be obtained by differentiating Eq. (4.21) with respect to S and equating it to zero. The value of S_o^* along with the values of parameters $q_{S\max}$, K_S', and K_i', is substituted in Eq. (4.21) to get corresponding value of $q_{S\max}^*$. Thus, the values of S_o^* and $q_{S\max}^*$ can be determined by the following equations:

$$S_o^* = \sqrt{K_S' K_i'} \tag{4.23}$$

$$q_{S\max}^* = \frac{q_{S\max}}{2\left(\sqrt{\frac{K_S'}{K_i'}}\right)+1} \tag{4.24}$$

Values of $q_{S\max}^*$ and S_o^* are computed as 0.31 h^{-1} and 58 mg/L respectively, using Eqs. (4.23) and (4.24). Eq. (4.24) reflects that at larger $\frac{K_S'}{K_i'}$ value, the smaller $q_{S\max}^*$ value will be relative to $q_{S\max}$, and thus greater will be the degree of inhibition. Similar calculations are performed on selected specific growth kinetic model by Andrews and Noack for phenol (Table 4.4). The Eq. (4.25) is written according to model and S_o^* and values are calculated by replacing K_S', K_i' and $q_{S\max}$ by K_S, K_i and $\mu_{g\max}$ in Eqs. (4.23) and (4.24)

$$\mu_g = \frac{0.462S}{78.29 + S + \frac{S^2}{44.49}} \tag{4.25}$$

Finally computed values of $\mu_{g\max}^*$ and S_o^* are 0.126 h^{-1} and 59.01 mg/L respectively. Here also, degree of inhibition is determined by $\frac{K_S}{K_i}$ ratio as $\mu_{g\max}^*$ becomes closer to $\mu_{g\max}$ at low value of $\frac{K_S}{K_i}$. In view of above results, it can be concluded that if substrate is inhibitory, it is not possible to observe an actual $q_{S\max}$ and $\mu_{g\max}$.

Figures 4.25 and 4.26 illustrate the resorcinol concentration profiles with time at low initial concentrations (10 to 90 mg/L) and at high initial concentrations (100 to

1300 mg/L) respectively. The rate of biodegradation of resorcinol decelerates as initial concentration is increased, indicating that high concentration of resorcinol inhibits its degradation. Similarly Figs. 4.27 and 4.28 show the p–cresol degradation profiles with time at low initial concentrations (10 to 90 mg/L) and at higher initial concentrations respectively. A close look of Figs. 4.19, 4.20 and Figs. 4.25 to 4.28, shows that the time required for degradation of p–cresol at one concentration is higher than the time required for degradation of phenol and resorcinol. Likewise, the degradation of phenol takes more time in comparison to the resorcinol at the same concentration. For instance, at the initial concentration of 70 mg/L, the complete degradation for phenol, resorcinol, and p–cresol is observed at 8.5 h, 6 h, and 11 h respectively. At initial concentration of 600 mg/L, the complete degradation time is 80 h, 55 h, and 90 h for phenol, resorcinol, and p–cresol respectively. These findings support the previous conclusion that the minimum inhibition is imposed by resorcinol and the maximum by p–cresol, in phenol – resorcinol – p–cresol series.

Figures 4.29 and 4.30 represent the variation of specific degradation rate with initial concentrations of resorcinol and p–cresol respectively. For resorcinol, the maximum value of specific degradation rate has been achieved at the initial concentration of 90 mg/L at which specific growth rate was found to be maximum. In the case of p–cresol, maximum value of specific degradation rate is found at the concentration of 70 mg/L while the maximum specific growth rate value was found at the initial concentration of 50 mg/L. Similar trend has also been reported by Minkevich *et al.* (2000) in their biodegradation studies on ethanol – *Candida valida* system. They have emphasized that the specific degradation rate q_s continues to increase with the substrate concentration due to the increase of cell maintenance rate whereas the observed growth yield coefficient continues to decrease which in turn decreases the specific growth rate.

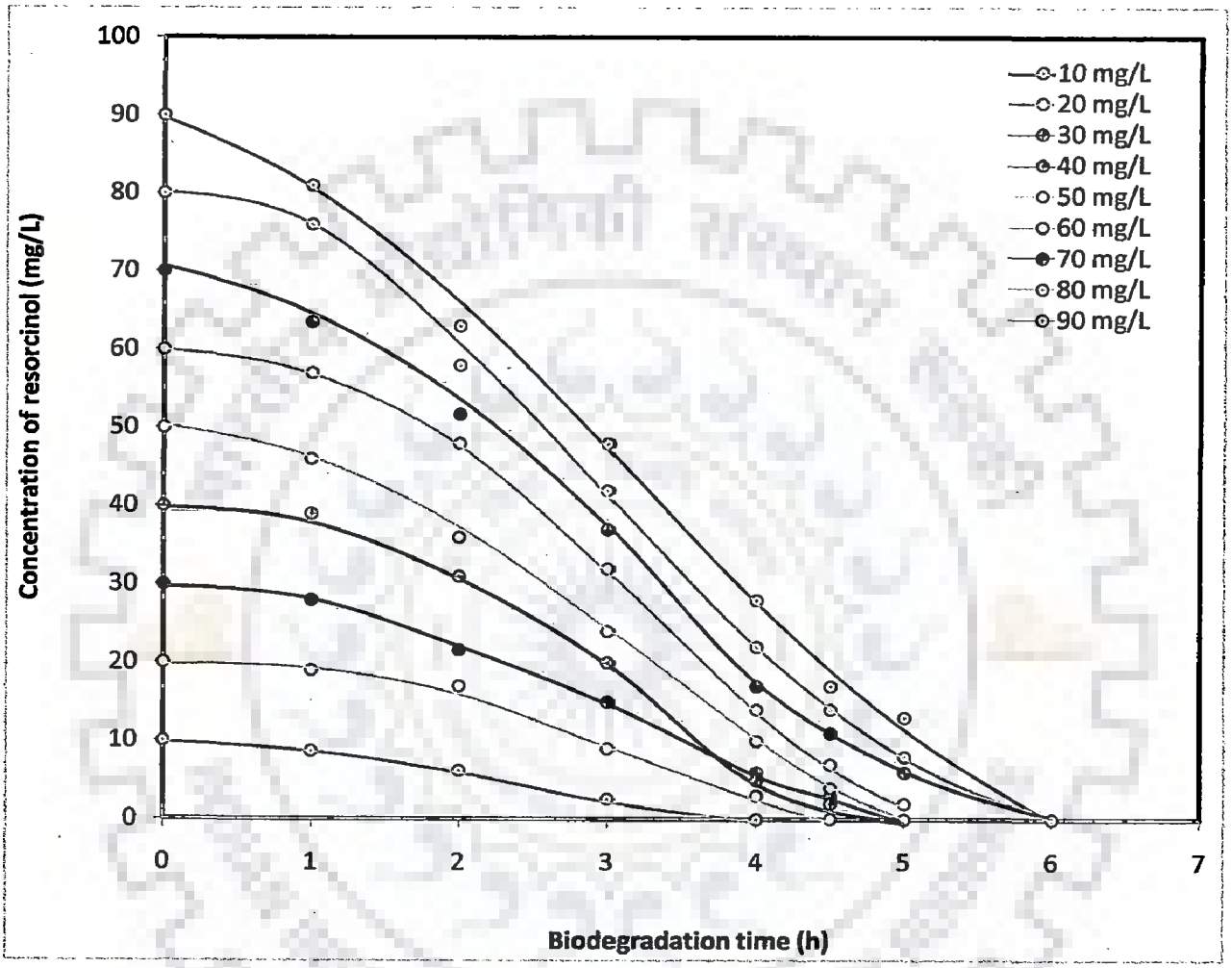

Figure 4.25: Biodegradation of resorcinol in lower initial concentration range

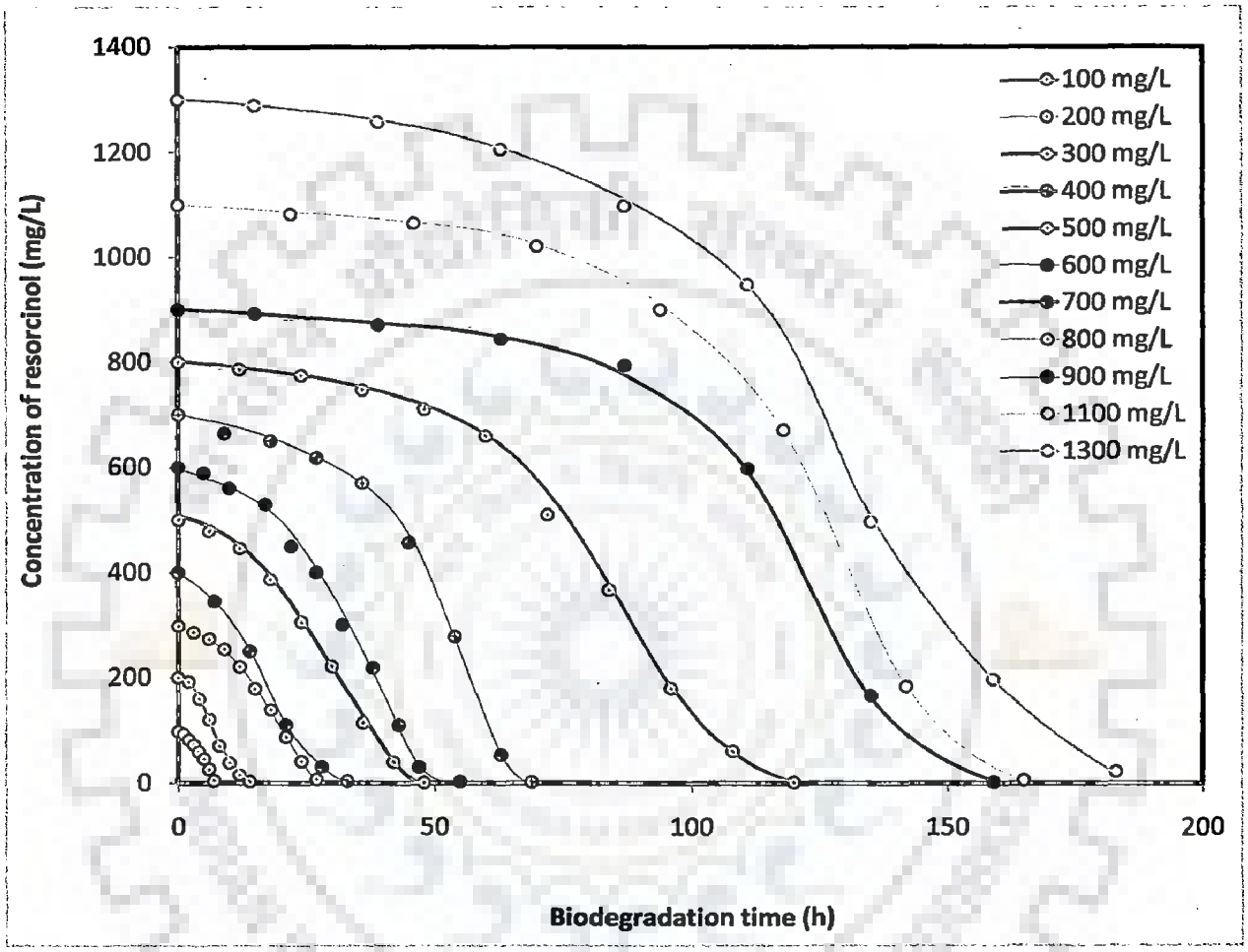

Figure 4.26: Biodegradation of resorcinol in higher initial concentration range

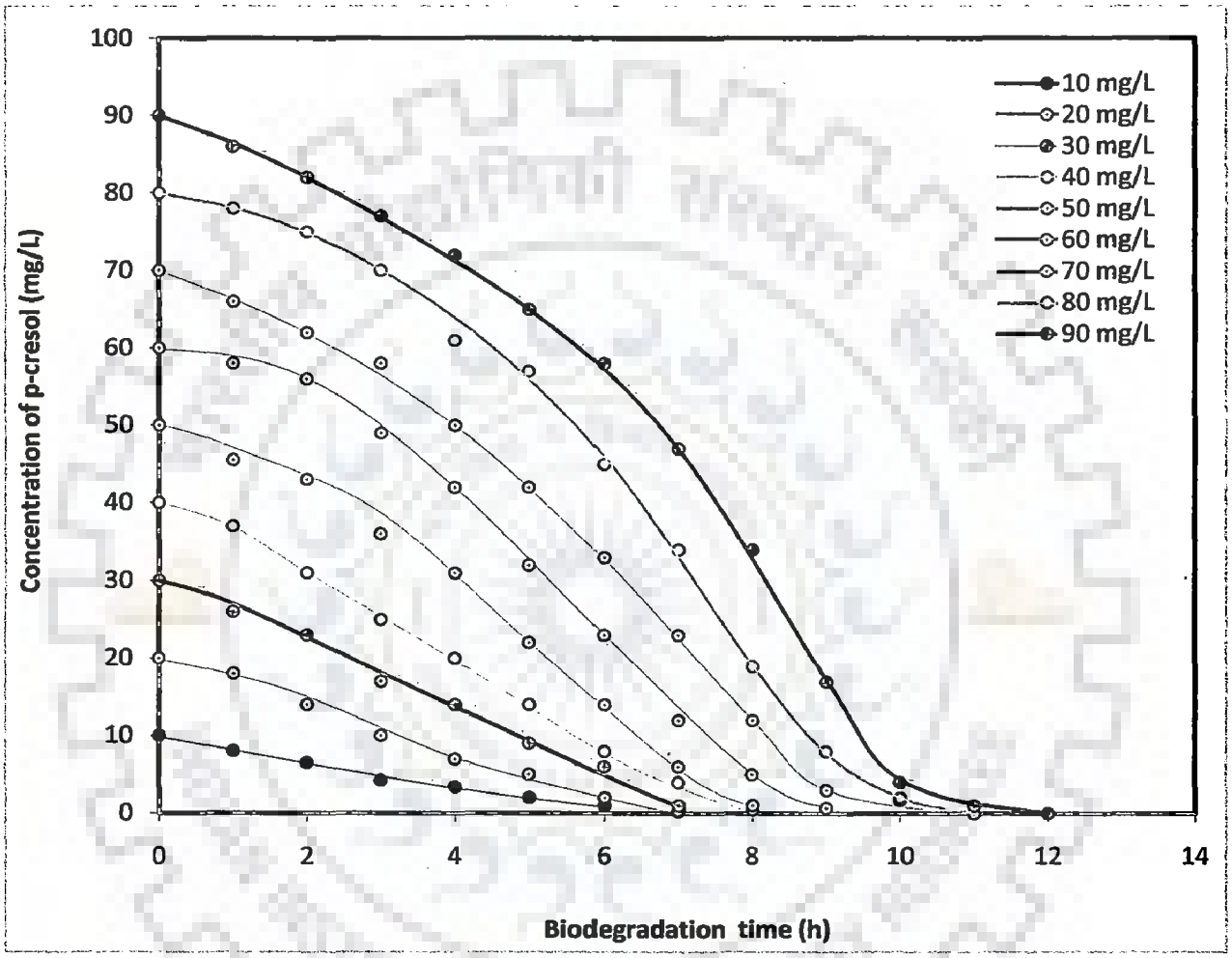

Figure 4.27: Biodegradation of p–cresol in lower initial concentration range

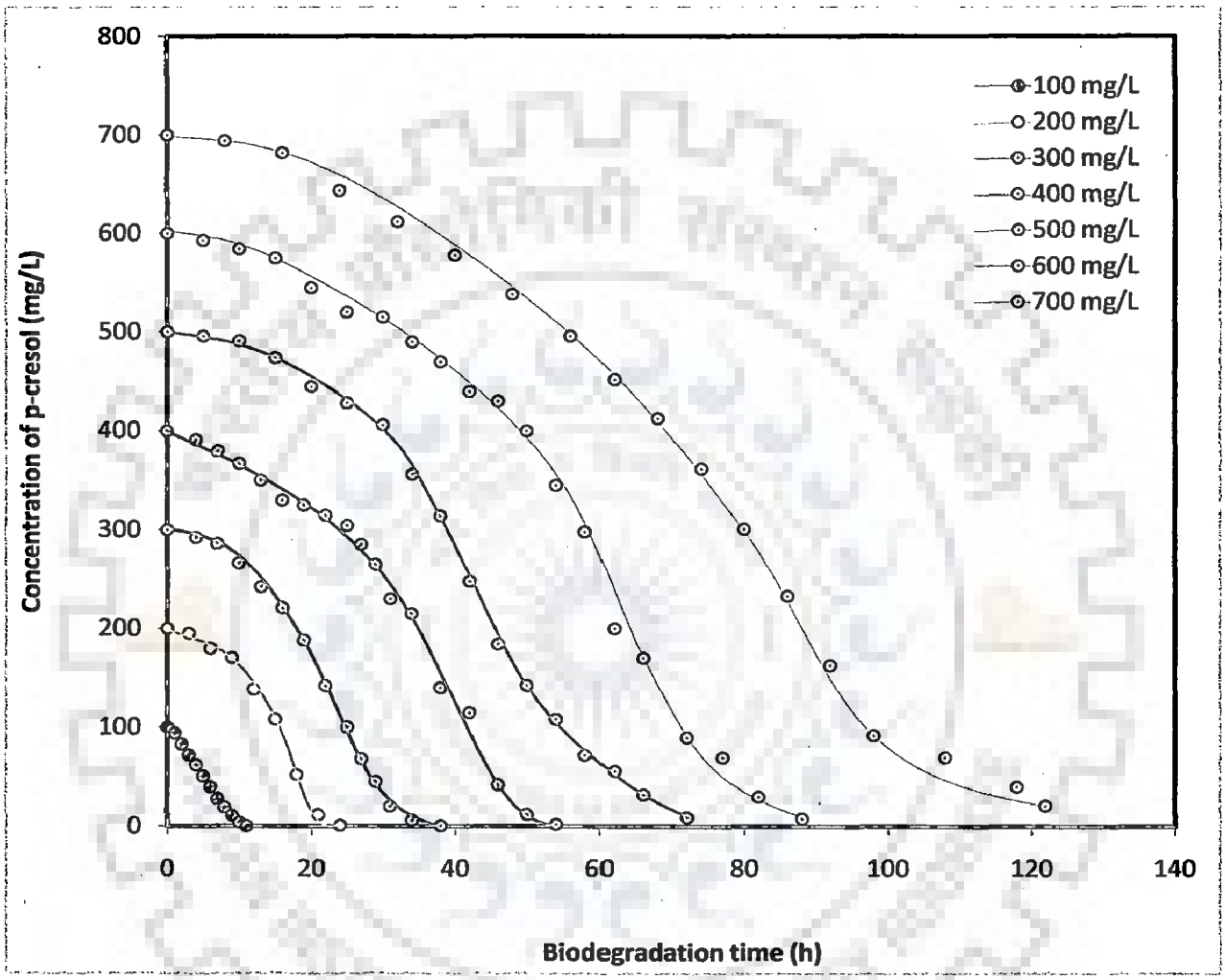

Figure 4.28: Biodegradation of p–cresol in higher initial concentration range

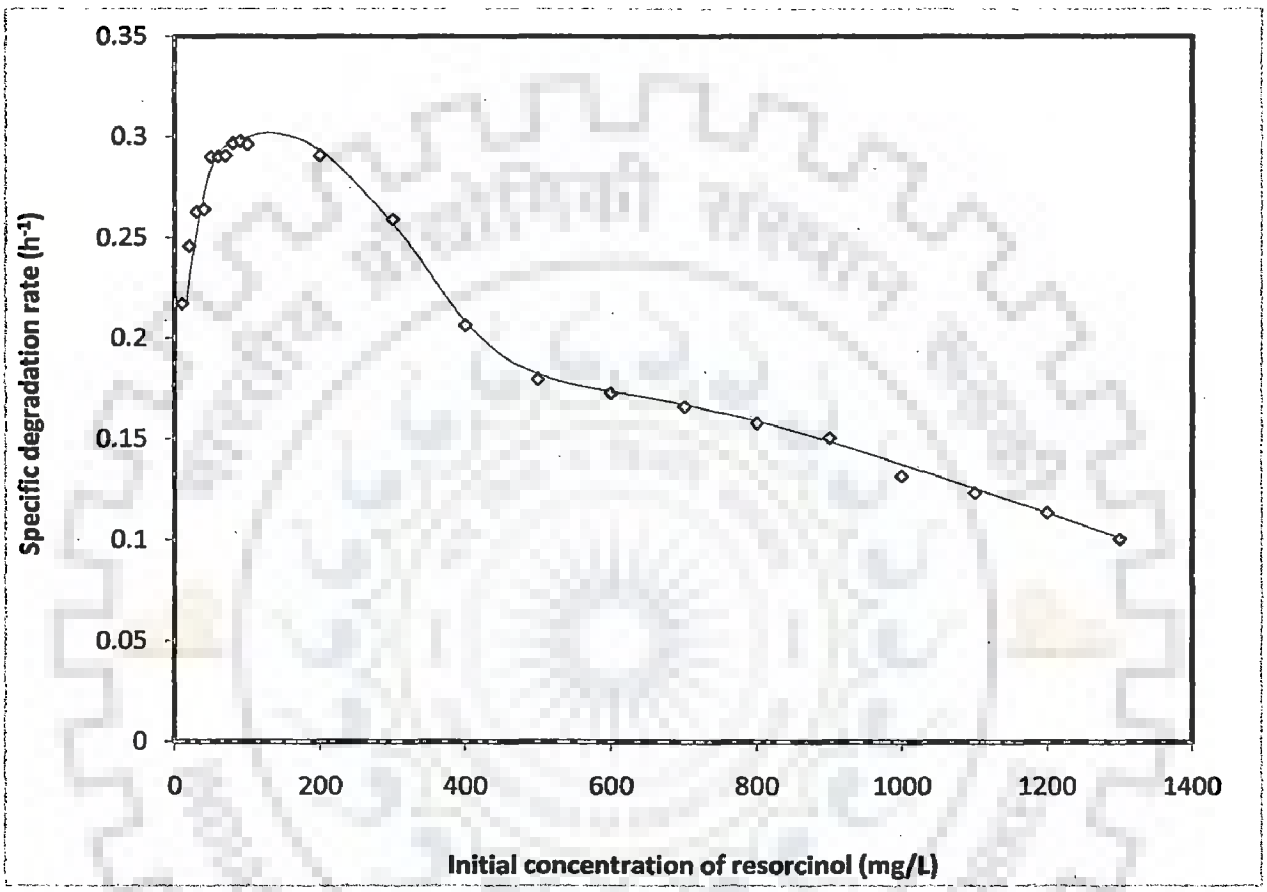

Figure 4.29: Experimentally determined specific degradation rates at different initial concentrations of resorcinol

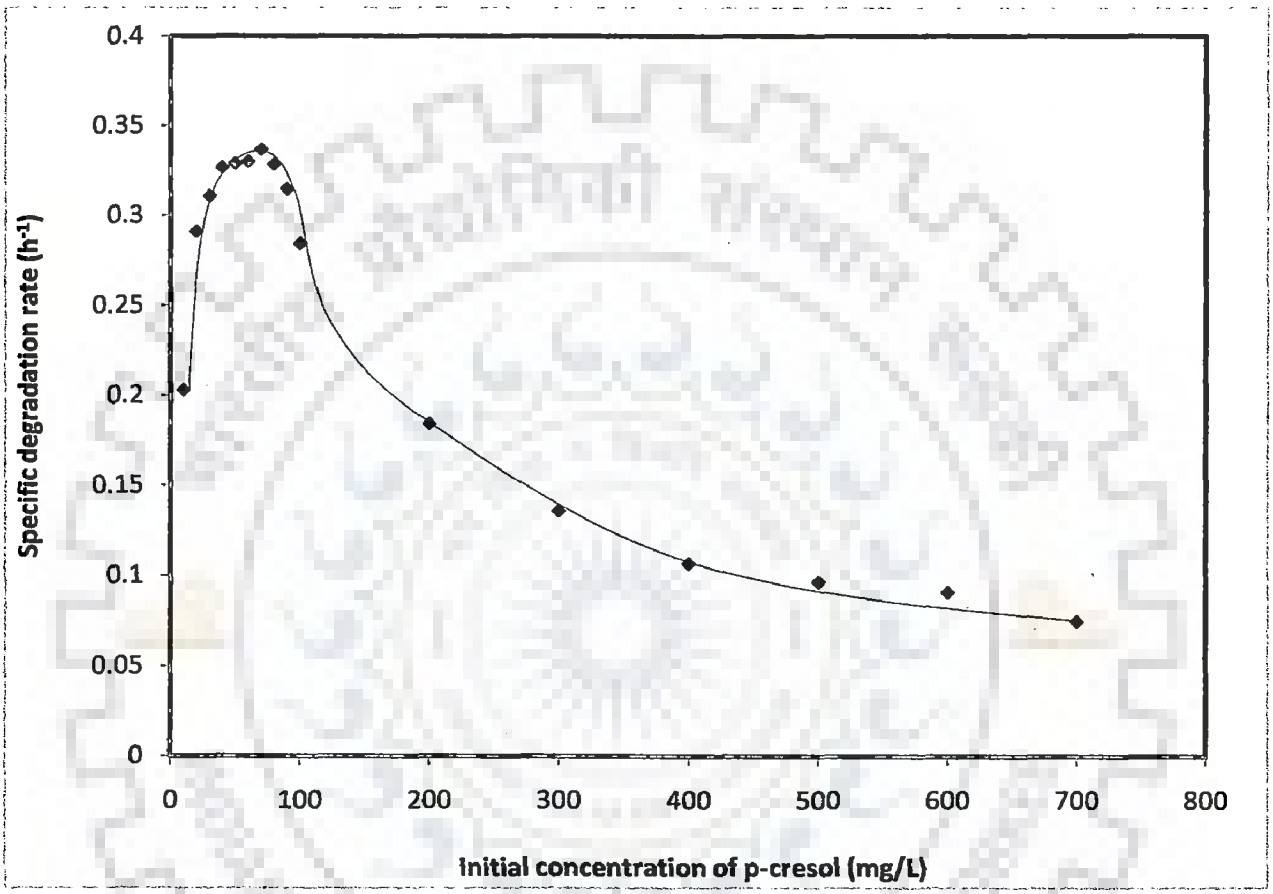

Figure 4.30: Experimentally determined specific degradation rates at different initial concentrations of p–cresol

In order to model the specific biodegradation rate, five models (M1, M2, M3, M4 and M5) analogous to single substrate growth kinetic models, have been proposed and are listed in Table 4.2. The parameters of these models have been estimated using a nonlinear least square regression analysis of experimental data, on MATLAB 7.2 based on Windows XP. Figure 4.31 shows the comparison between experimental and model predictions of specific degradation rates for resorcinol while Fig. 4.32 shows specific degradation rates for p–cresol. The values of kinetic constants for specific degradation rate of resorcinol and p–cresol are presented in the Tables 4.9 and 4.10 respectively. Predictions of models M1, M2, M3, and M5 show good agreement with the experimentally determined specific degradation rates of resorcinol with correlation coefficient R^2 value ≥ 0.97. In case of p–cresol, model M1, M2 and M3 are found to fit the experimental specific degradation rate data with the correlation coefficient (R^2) value > 0.98. Therefore, for the selection of the best fit model to the experimental specific degradation rate data, per cent standard deviation ($\Delta q_S\%$) values have been estimated by applying Eq. (4.20). The per cent standard deviation value is minimum for models M3 and M1 for specific degradation rate data of resorcinol and p–cresol respectively. Therefore, the model M3 for resorcinol and the M1 for p–cresol have been selected for further biodegradation kinetic studies. Model M3 is a four parameter model.

Table 4.9: Estimated values of kinetic parameters for resorcinol degradation

S. No.	Model	Estimated values of resorcinol degradation kinetic model parameters				R^2	Per cent standard deviation ($\Delta q_S\%$)
		$q_{S_{max}}$ (h^{-1})	K'_S (mg/L)	K'_i (mg/L)	K' (mg/L)		
1	M1	0.366	8.48	582.6	-	0.970	1.23
2	M2	0.37	8.60	573.9	-	0.970	1.15
3	M3	0.347	7.07	853.5	2386.0	0.976	1.04
4	M4	0.372	8.93	498.5	1000.0	0.967	1.27
5	M5	0.338	6.22	1040.0		0.972	1.22

Table 4.10: Estimated values of kinetic parameters for p–cresol degradation

S. No.	Model	Estimated values of p–cresol degradation kinetic model parameters				R^2	Per cent standard deviation (Δq_S%)
		$q_{S_{max}}$ (h^{-1})	K_S' (mg/L)	K_i' (mg/L)	K' (mg/L)		
1	M1	0.759	27.88	73.42	-	0.989	1.30
2	M2	1.344	44.14	44.36	-	0.987	1.43
3	M3	0.594	18.64	132.2	1000.0	0.987	2.22
4	M4	1.168	51.43	32.38	1000.0	0.979	1.91

The specific degradation rates for resorcinol and p–cresol can now be mathematically expressed by Eqs. (4.26) and (4.27) respectively as given below:

$$q_S = \frac{0.347S}{S + 7.07 + \left(\frac{S^2}{853.5}\right)\left(1 + \frac{S}{2386}\right)} \quad \text{For resorcinol} \qquad (4.26)$$

$$q_S = \frac{0.759S}{27.88 + S + \frac{S^2}{73.42}} \quad \text{For p–cresol} \qquad (4.27)$$

K_S' is saturation constant that indicates the substrate affinity to biomass while K_i' and K' are constants indicating the degree of substrate inhibition. During experimental study, resorcinol and p–cresol have been observed as inhibitory substrates. Therefore, it is not possible to observe an actual degradation rate. The value of initial substrate concentration $\left(S_o^*\right)$ at which degradation rate $\left(q_S\right)$ attains its maximum value $q_{S\max}^*$, can be obtained by differentiating Eqs. (4.26) and (4.27) with respect to S and equating them to zero. The value of S_o^* along with the values of parameters $q_{S\max}$, K_S', K_i', and K' is substituted in Eqs. (4.26) and (4.27) to get corresponding values of $q_{S\max}^*$. The values of S_o^* and $q_{S\max}^*$ are determined by using Eqs. (4.23) and (4.24) for p–cresol.

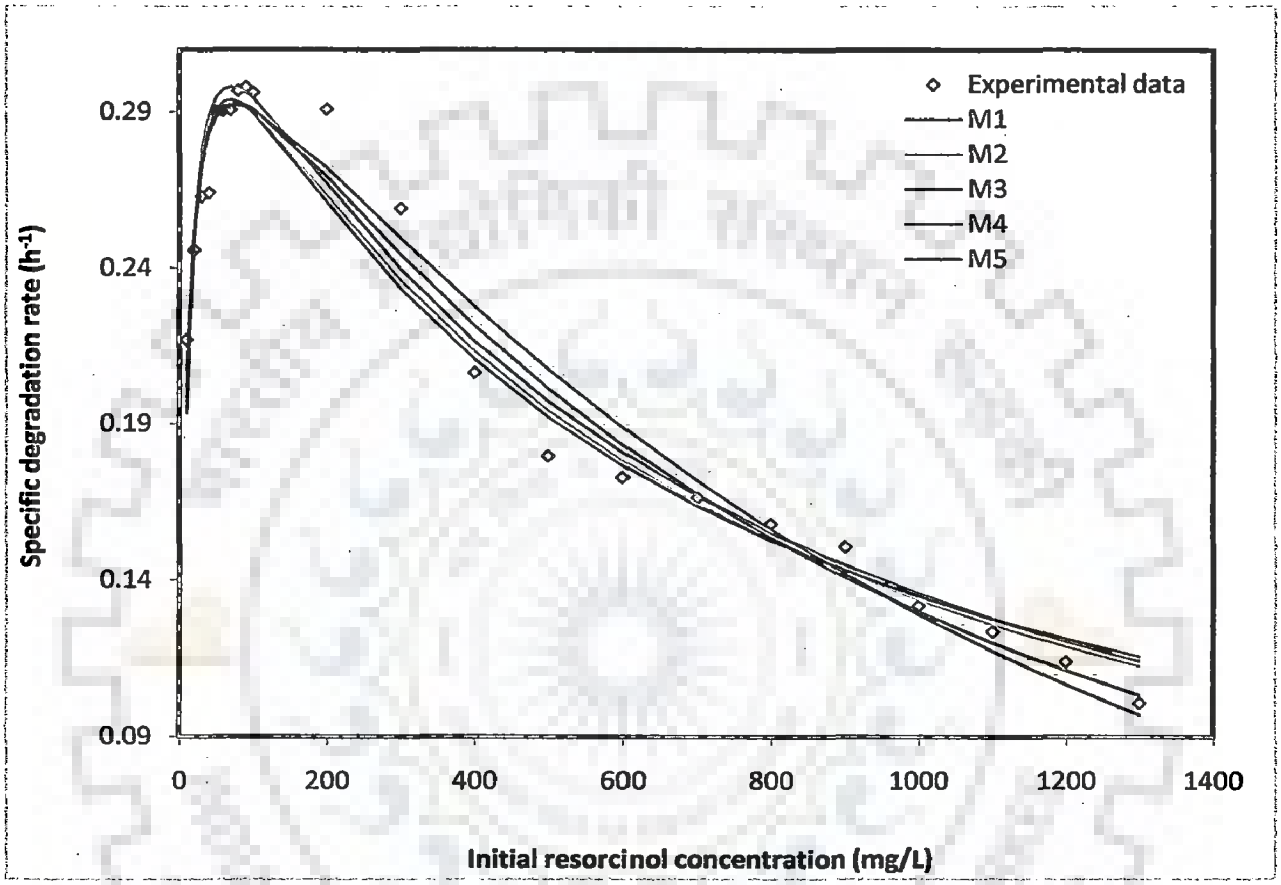

Figure 4.31: Comparison between kinetic model predictions and experimentally determined specific degradation rates at different initial concentrations of resorcinol

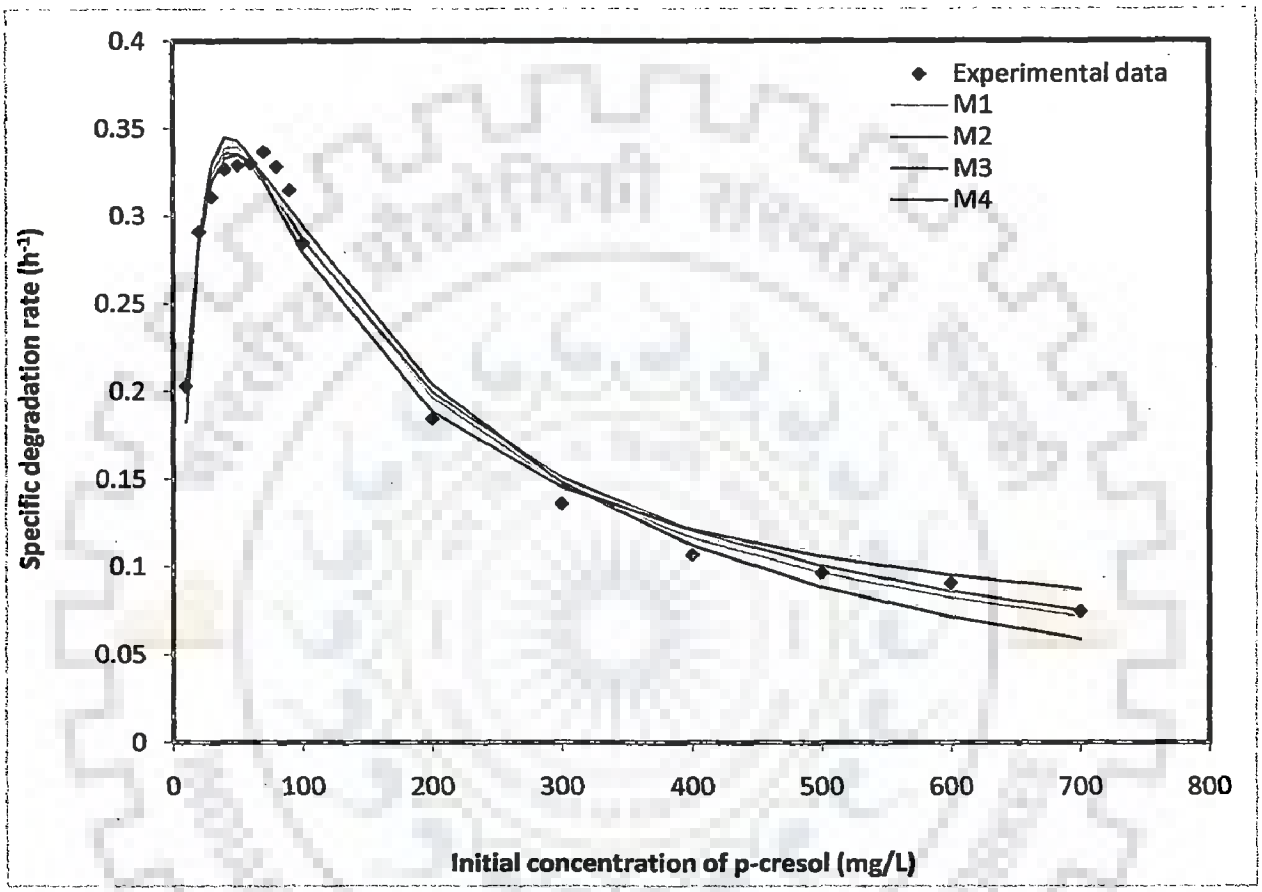

Figure 4.32: Comparison between kinetic model predictions and experimentally determined specific degradation rates at different initial concentrations of p–cresol

Values of $q^*_{S\max}$ and S^*_o are computed as 0.292 h^{-1} and 75.4 mg/L for resorcinol, and as 0.339 h^{-1} and 45.24 mg/L for p–cresol. Eq. (4.24) reflects that at larger $\frac{K_S}{K_i}$ value, the smaller $q^*_{S\max}$ value will be achieved relative to $q_{S\max}$, indicating higher degree of inhibition.

Similar calculations are performed on the best fitted specific growth kinetic models (section 4.2.1), for resorcinol and p–cresol to get S^*_o and $\mu^*_{g\max}$ values. The Yano model for resorcinol and Andrews and Noack model for p–cresol are written as follows:

$$\mu_g = \frac{0.185S}{S+19.83+\left(\frac{S^2}{376}\right)\left(1+\frac{S}{1790}\right)} \qquad \text{For resorcinol} \qquad (4.28)$$

$$\mu_g = \frac{0.512S}{S+91.87+\left(\frac{S^2}{21.99}\right)} \qquad \text{For p–cresol} \qquad (4.29)$$

Finally computed values of S^*_o and $\mu^*_{g\max}$ are 82.7 mg/L and 0.115 h^{-1} for resorcinol, and 44.94 mg/L and 0.101 h^{-1} for p–cresol. Here also, degree of inhibition is determined by $\frac{K_S}{K_i}$ ratio. $\mu^*_{g\max}$ becomes closer to $\mu_{g\max}$ at lower value of $\frac{K_S}{K_i}$.

The substrate concentration S^* at maximum values of q_S and μ_g can be obtained by setting $\frac{dq_S}{dS}\left(and\frac{d\mu_g}{dS}\right)$ equal to zero. In one case it is possible to obtain explit expression for the substrate concentration S^*. However, in the other case, it is in principle possible to obtain explicit analytical expression for substrate concentration S^* as the resulting polynomial equation is of third degree. But the expression is very complex and cumbersome. In this case, resulting polynomial equation has been solved numerically by using MATLAB software. This method is also computationally efficient. Once the substrate concentration S^* is obtained by solving $\frac{dq_S}{dS}\left(and\frac{d\mu_g}{dS}\right)=0$

by either analytically or numerically, the maximum values of q_S and μ_g can be obtained by substituting the substrate concentration S^* in their respective equation.

4.2.4 Maintenance Energy Expenditure

For the estimation of maintenance energy coefficient values at each initial concentration of substrate Eq. (4.12) has been used. The values of specific degradation rate (q_S) at different initial concentrations have been plotted against specific growth rate (μ_g) values at respective initial substrate concentrations. The slope and intercept of this plot give the values of constants A and B of Eq. (4.12). The mathematical expressions for A and B are as follows.

$$A = \left(\frac{1}{\left(Y_{X/S}\right)_T} - \frac{k}{\mu_{g\max}} \right) \tag{4.30}$$

$$B = \left(k + m_1\right) \tag{4.31}$$

The values of k and m_1 have been calculated solving the Eqs. (4.30) and (4.31) at fixed values of $\left(Y_{X/S}\right)_T$ and $\mu_{g\max}$ for a substrate simultaneously. Thus, the following equation is used to estimate the maintenance energy expenditure at each initial concentration of substrate during biodegradation and growth of biomass

$$m_S = m_1 + k\left(1 - \frac{\mu_g}{\mu_{g\max}}\right) \tag{4.32}$$

The true growth yield coefficient $\left(Y_{X/S}\right)_T$ is the maximum value of observed growth yield coefficient estimated from Figs. 4.16, 4.17 and 4.18 for degradation of phenol, resorcinol, and p–cresol respectively. The values of $\left(Y_{X/S}\right)_T$ for phenol, resorcinol, and p–cresol are 0.437, 0.443, 0.310 g/g respectively.

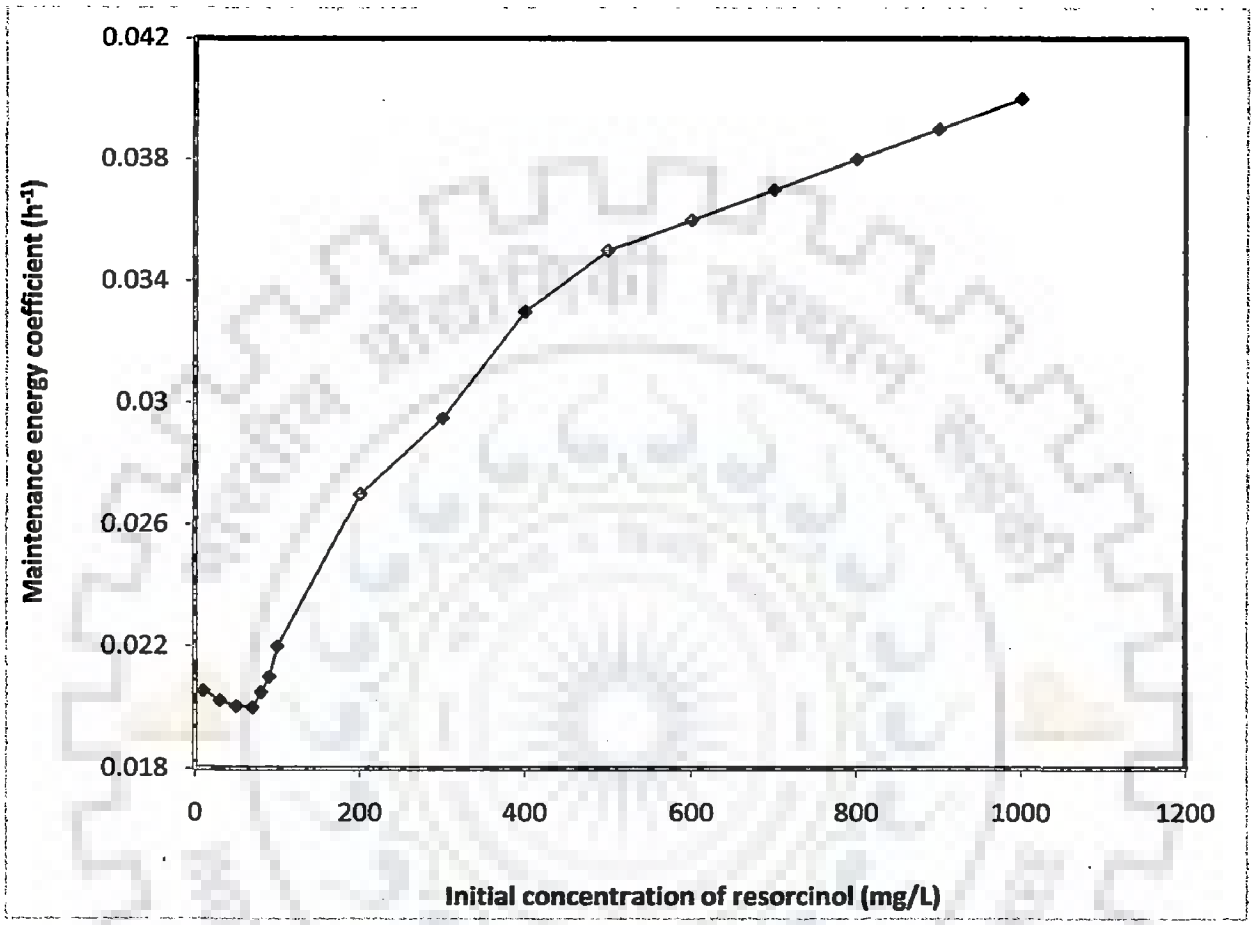

Figure 4.33: Variation in maintenance energy coefficient with initial concentrations of phenol

The values of $\mu_{g\max}$ are 0.462, 0.185, and 0.512 h^{-1} for phenol, resorcinol, and p–cresol respectively. The values of $\mu_{g\max}$ and $\left(Y_{X/S}\right)_T$ are substituted in Eqs. (4.30) and (4.31) to get the values of k and m_1. Finally m_S has been estimated by Eq. (4.32) for phenol, resorcinol, and p–cresol at their various initial concentrations. The variation in m_S with initial substrate concentration is shown in Figs. 4.33 to 4.35 during the biodegradation of phenol, resorcinol, and p–cresol respectively. As the initial substrate concentration is enhanced in the medium gradually, beyond the inhibitory initial concentration of substrate, the observed growth yield value tends to decrease while there is a simultaneous increase in the required maintenance energy value at each initial concentration of substrate, along with the increasing inhibition effect. For phenol, the maintenance energy coefficients are found in the range of 0.020 – 0.04 h^{-1}. In Figure 4.33 the value of maintenance energy coefficient m_S is 0.020 h^{-1} at initial phenol concentration of 70 mg/L that is the minimum required maintenance energy, and 0.04 h^{-1} at initial concentration of 1000 mg/L which is the maximum value of maintenance energy. Thus, after estimating the k – values, the maintenance energy model equations can be restated as follows:

$$m_S = 0.020 + 0.0212\left(1 - \frac{\mu_g}{0.129}\right) \tag{4.33}$$

Eq. (4.33) represents maintenance energy requirement of cells with variation in specific growth rate during phenol biodegradation. The maximum specific growth rate value was found at initial concentration of 90 mg/L for resorcinol (Fig. 4.12), and at 50 mg/L for p–cresol (Fig. 4.13). Therefore, the minimum value of maintenance energy coefficients falls at 90 mg/L for resorcinol (Fig. 4.34) and at 50 mg/L for p–cresol (Fig. 4.35). Maintenance energy coefficients are found to be in the range of 0.0135 – 0.0572 h^{-1} for resorcinol and 0.0229 – 0.0324 h^{-1} for p–cresol. In Fig. 4.34, the minimum required value of maintenance energy coefficient m_S is 0.0135 h^{-1} at initial resorcinol concentration of 90 mg/L and maximum required value of 0.0573 h^{-1} at initial concentration of 1300 mg/L. Similarly, it is clear from the Fig. 4.35 that for the p–cresol the minimum value of maintenance energy coefficient is 0.0229 h^{-1} at inhibitory initial concentration of 50 mg/L, while the value of maximum maintenance energy coefficient is 0.0324 h^{-1} at initial p–cresol concentration of 700 mg/L. Thus, after

estimating the values of k, the model equation to represent maintenance energy expenditure Eq. (4.32) can be restated as follows:

$$m_S = 0.0135 + 0.054\left(1 - \frac{\mu_g}{0.132}\right) \qquad \text{For resorcinol} \qquad (4.34)$$

$$m_S = 0.0229 + 0.011\left(1 - \frac{\mu_g}{0.102}\right) \qquad \text{For p–cresol} \qquad (4.35)$$

Eqs. (4.34) and (4.35) represent maintenance energy requirement for cells during resorcinol and p–cresol biodegradation respectively. The decreasing trend of specific growth rate and increasing maintenance energy coefficient (m_S) beyond the inhibitory initial substrate concentration results into reduction in the observed growth yield.

This study concludes that the substrate inhibition reduces the specific growth rate as well as biomass growth yield due to the increase in the value of maintenance energy coefficient for phenol (or resorcinol or p–cresol) - *G. indicus* system. In single substrate biodegradation studies, it has been observed that the growth rate of *G. indicus* in resorcinol is higher than in phenol, which in turn is higher than p–cresol. As due to their inhibitory nature in the order resorcinol<phenol<p-cresol. As a consequence, the growth component of the maintenance energy expenditure is the highest in the resorcinol, while it is the lowest in case of p–cresol.

4.2.5 Computed Profiles of Substrate Degradation

The substrate degradation profiles with time have been computed by simulating the mathematical models as described in section 4.1. For this purpose, three mathematical models have been proposed. These models are sets of ordinary differential equations and algebraic equations. The models differ in the way of defining specific degradation rate (q_S). Keeping this in view, the models are named as model – a, model – b and model – c. The mathematical equations for each model are given in Table 4.3. To obtain degradation profiles with time, the three sets of model equations corresponding to the selected growth and degradation kinetic models for phenol, resorcinol, and p–cresol have been solved by ordinary differential equation solver tool of MATLAB 7.2.

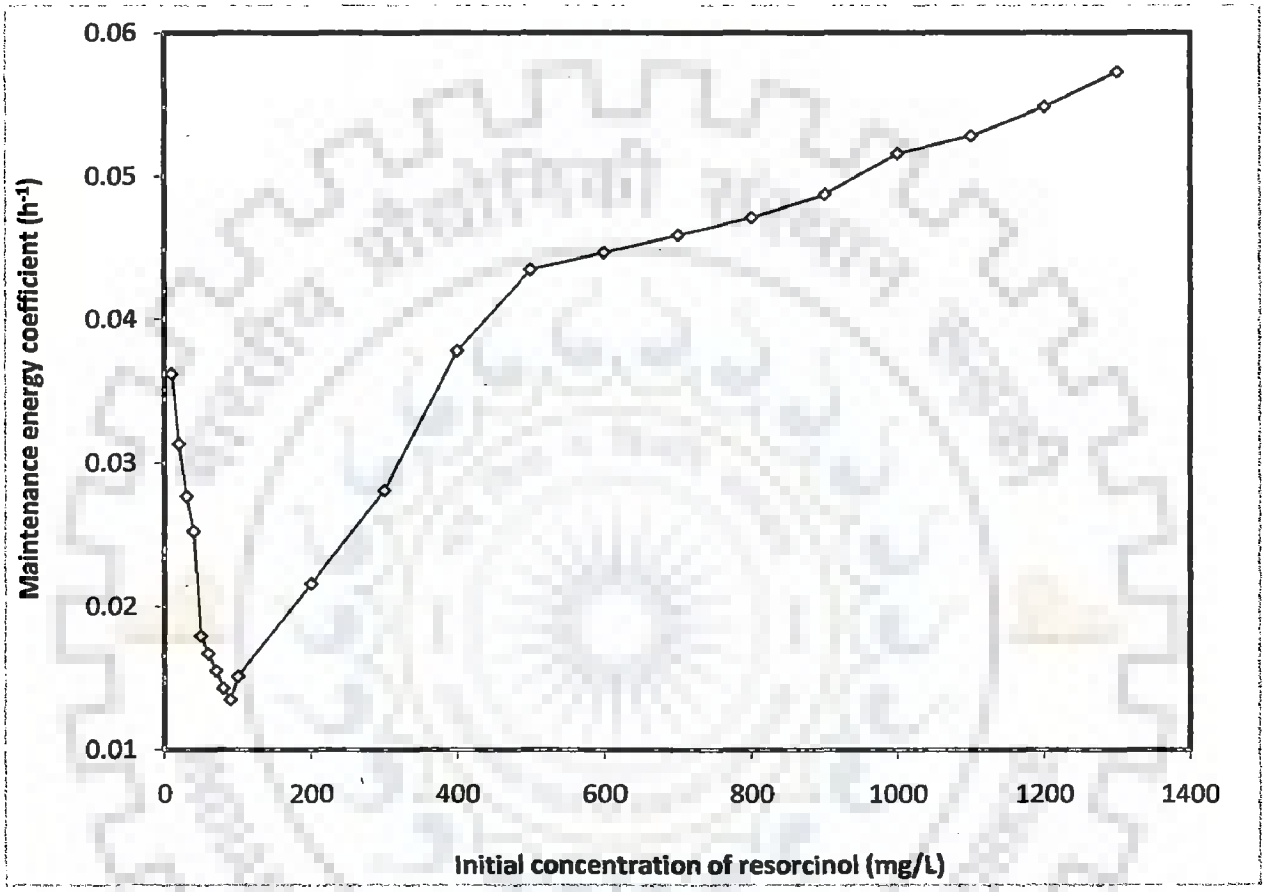

Figure 4.34: Variation in maintenance energy coefficient with initial concentration of resorcinol

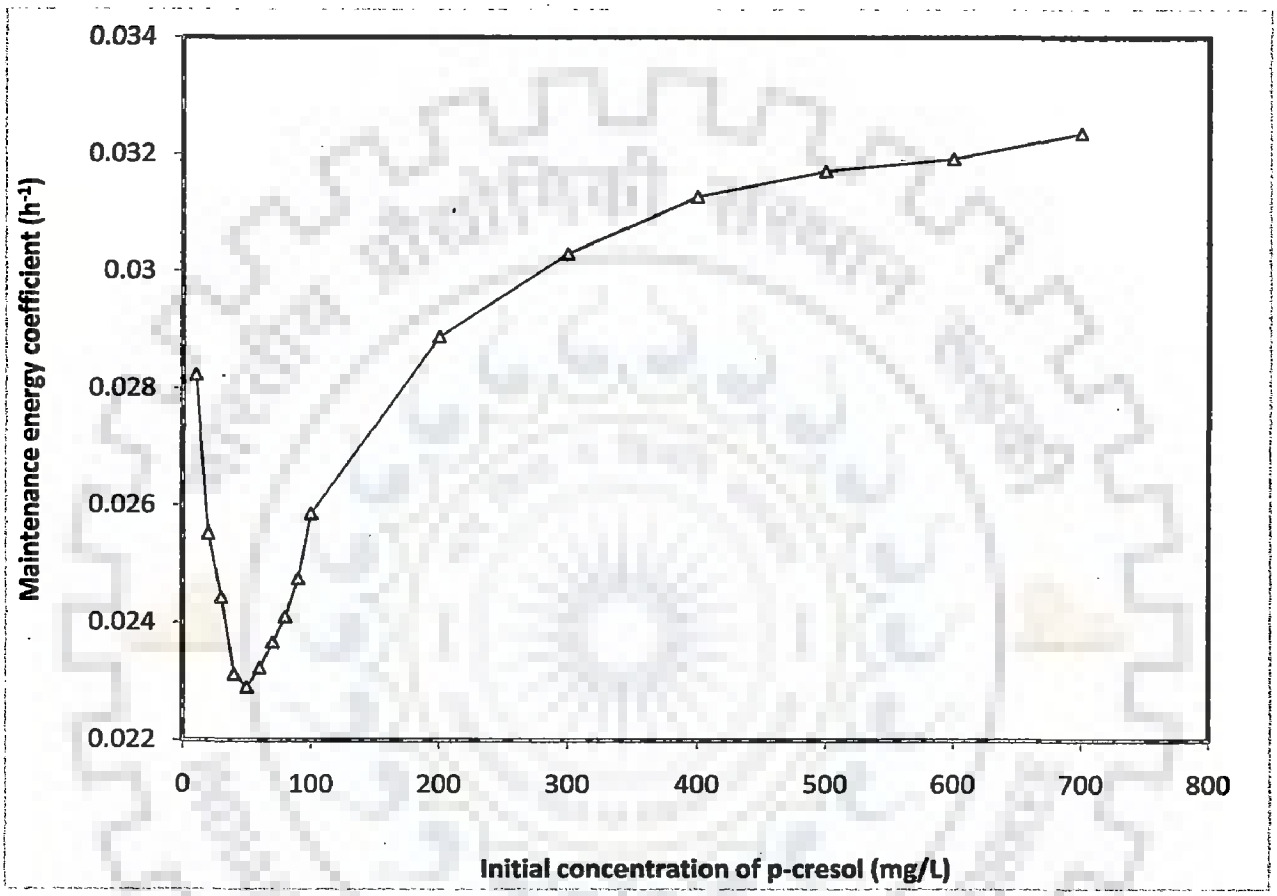

Figure 4.35: Variation in maintenance energy coefficient with initial concentration of p–cresol

The simulations have been performed for different initial phenol concentrations in the range of 10 - 1000 mg/L. Simulation and experimental results are presented in Figs. 4.36 and 4.37 for comparison. It can be seen that the simulation predictions by model - a corroborates the experimental data very well in the full initial phenol concentration range of 10 - 1000 mg/L. Predictions by model – b show the poor agreement with the experimental data. This may be due to the presentation of Eq. (4.14) on the basis of initial biomass concentration. Therefore, it is evident that even on getting lowest value (1.232) of per cent standard deviation (M8), model - b cannot be considered a good model to predict degradation profiles. The predictions by model - c are in a better agreement with the experimental data in lower phenol concentration range (Fig. 4.36) but in higher concentration range this model predicts lower degradation time than the experimentally observed degradation time (Fig. 4.37). The reason may be that model - c does not take into account the effect of initial phenol concentration on biomass growth yield due to variable maintenance energy expenditure. Incorporation of this effect makes the model - a better than model - c. Thus, the results obtained here indicate that the proposed model - a is quite useful and powerful to fit and predict the phenol degradation over the entire range of phenol concentration (10 -1000 mg/L).

The model simulation results on phenol clearly indicate that model – b is inadequate to describe the degradation profiles with time. Therefore, in case of resorcinol and p–cresol, only two models, model – a and model – c, have been considered for simulations. To obtain degradation profiles with time, two sets of model equations corresponding to the selected growth and degradation kinetic models for resorcinol and p–cresol have been solved by ordinary differential equation solver tool of MATLAB 7.2. The solution is represented in the Figs. 4.38 and 4.39 for resorcinol, and Figs. 4.40 and 4.41 for p–cresol. The simulations have been performed for different initial substrate concentrations in the range of 10 to 1300 mg/L and 10 to 700 mg/L for resorcinol and p–cresol respectively. It can be seen that the simulation predictions by model - a are in good agreement with the experimental data in the full initial substrate concentration range for both the substrates, resorcinol and p–cresol. Model - a involves variable observed growth yield and maintenance energy coefficient. Predictions by the model - c do not fit to the experimental data of resorcinol properly. However, predictions of model - c are close to the experimental biodegradation data of p– cresol

at lower concentrations only (Fig. 4.40). The reason may be that the model - c does not consider maintenance energy expenditure and variation in growth yield as is shown in the expression of q_S in the model. Thus, these results indicate that the proposed model - a is quite adequate and useful to predict the substrate degradation over the entire range of substrate concentration 10 to 1300 mg/L and 10 to 700 mg/L for resorcinol and p–cresol respectively.

4.3 CONCLUDING REMARKS

The batch biodegradation studies have been carried out on phenol, resorcinol, and p–cresol using filamentous fungus *G. indicus* in single substrate system. The models to represent growth and biodegradation kinetics have been selected by fitting the experimental data to various available and proposed kinetic models. The variation in observed growth yield with the initial substrate concentration has been investigated. The maintenance energy expenditure has been modelled and its variation with the specific growth rate has been incorporated to describe the substrate degradation rate. The biodegradation dynamics has been modelled with and without the inclusion of maintenance energy expenditure and variation in observed growth yield. From the model simulations, concluded that the incorporation of maintenance energy expenditure and thereby variation in the observed growth yield is necessary to describe the biodegradation profiles with time for phenol, resorcinol, and p–cresol.

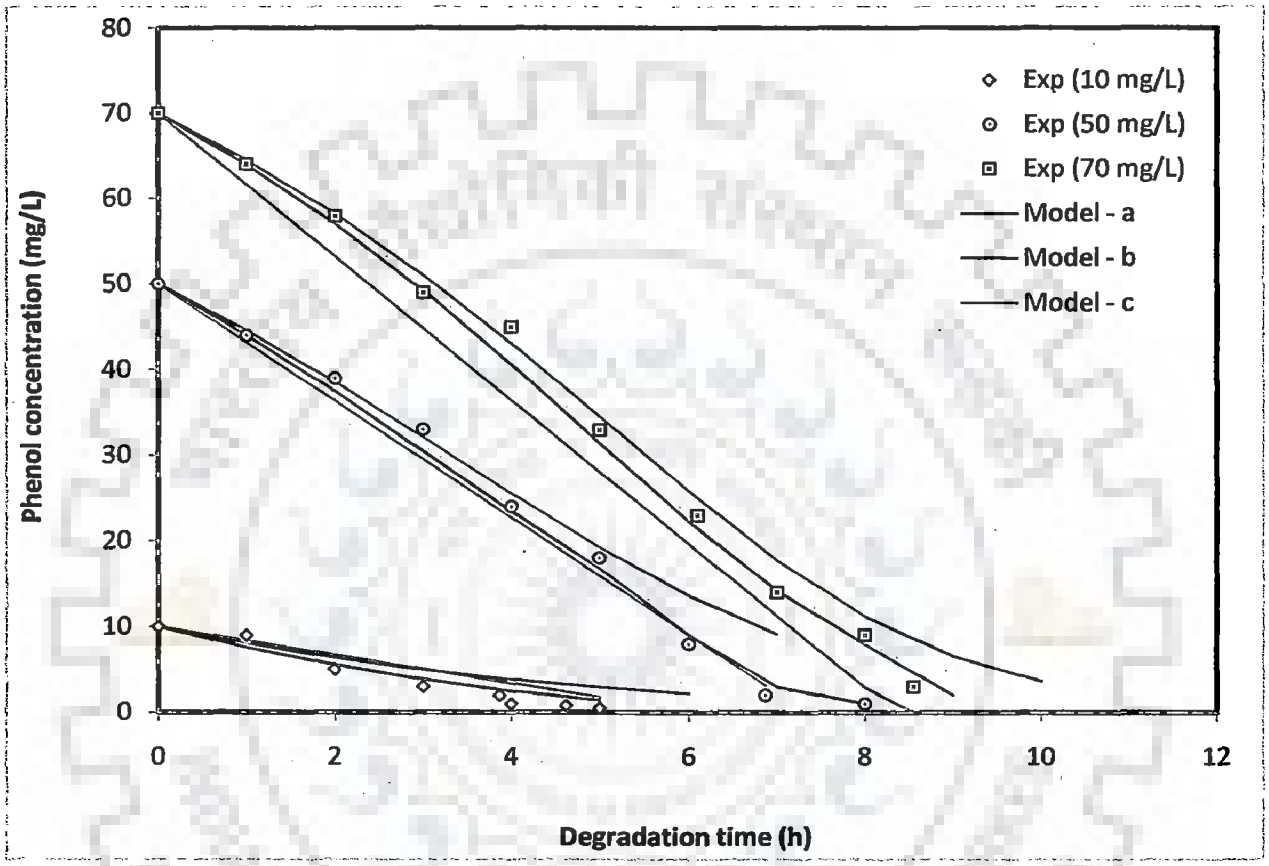

Figure 4.36: Phenol degradation profiles by the models in lower range of initial concentration

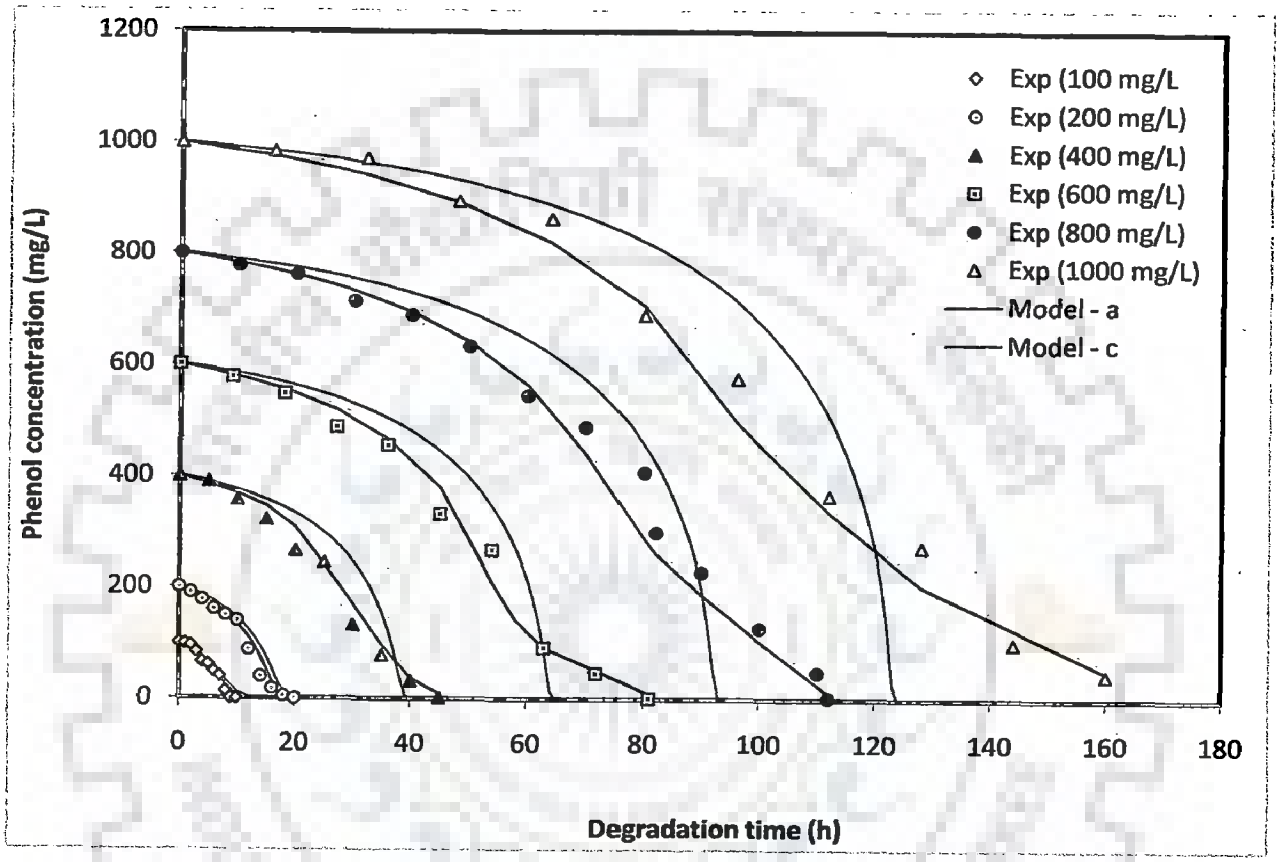

Figure 4.37: Phenol degradation profiles by the models in higher range of initial concentration

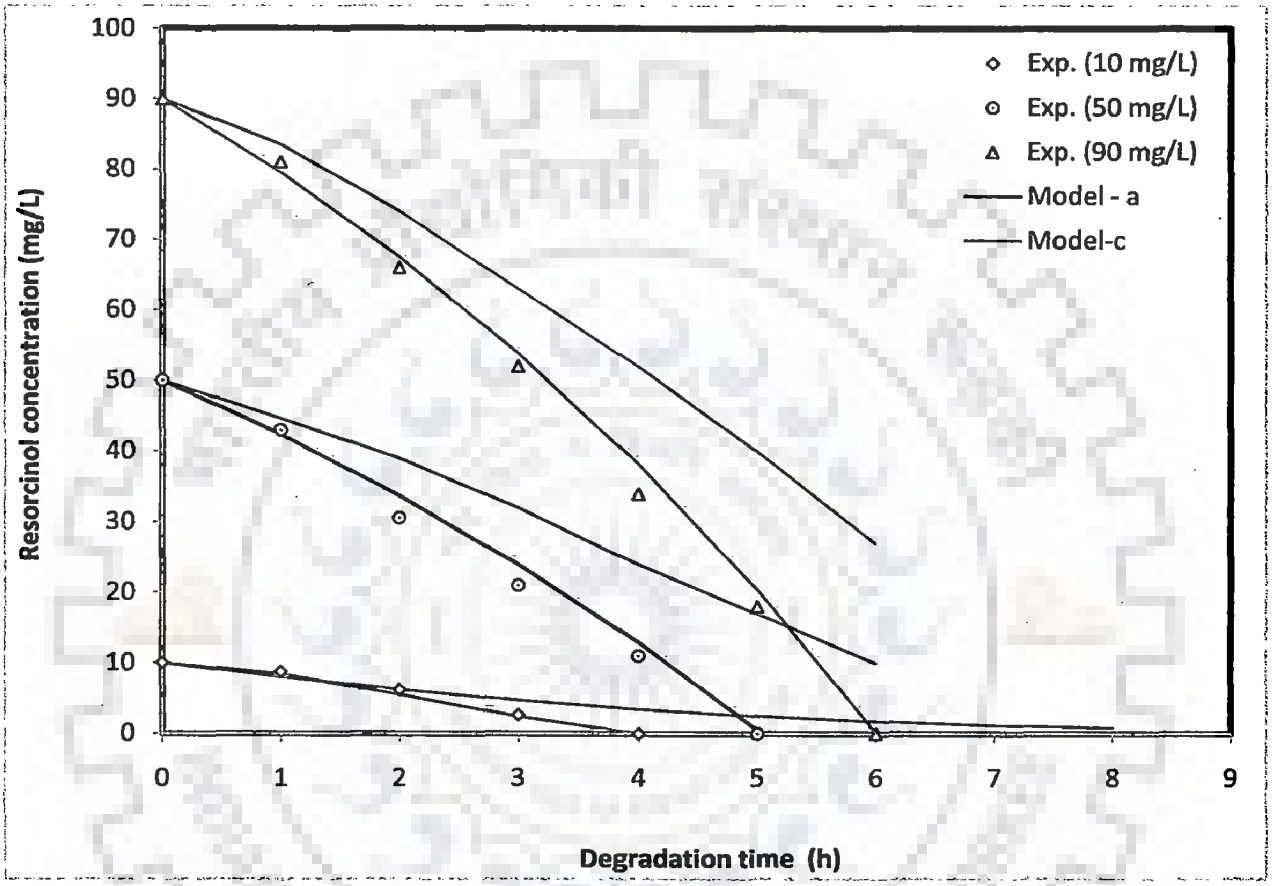

Figure 4.38: Resorcinol degradation profiles by the models in lower initial concentration range

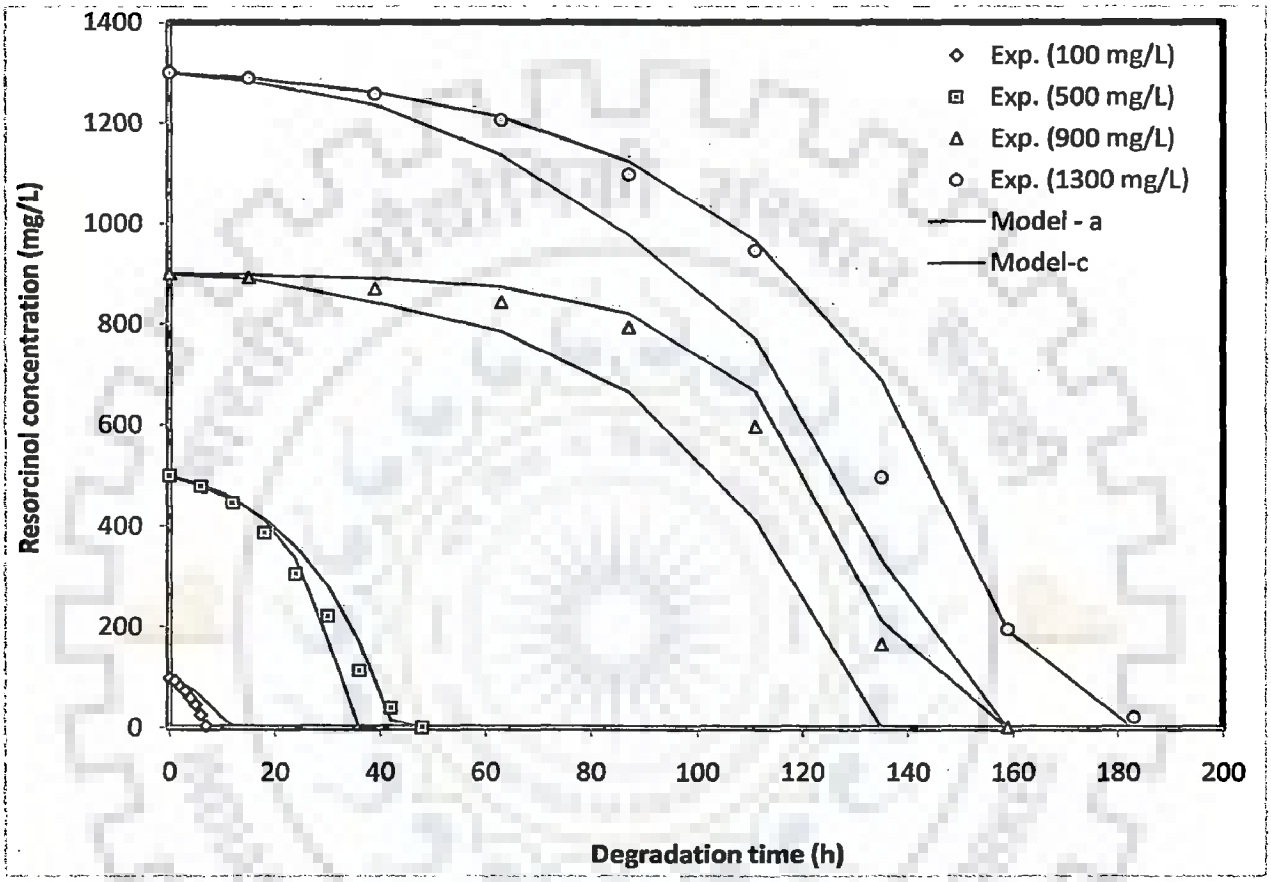

Figure 4.39: Resorcinol degradation profiles by the models in higher initial concentration range

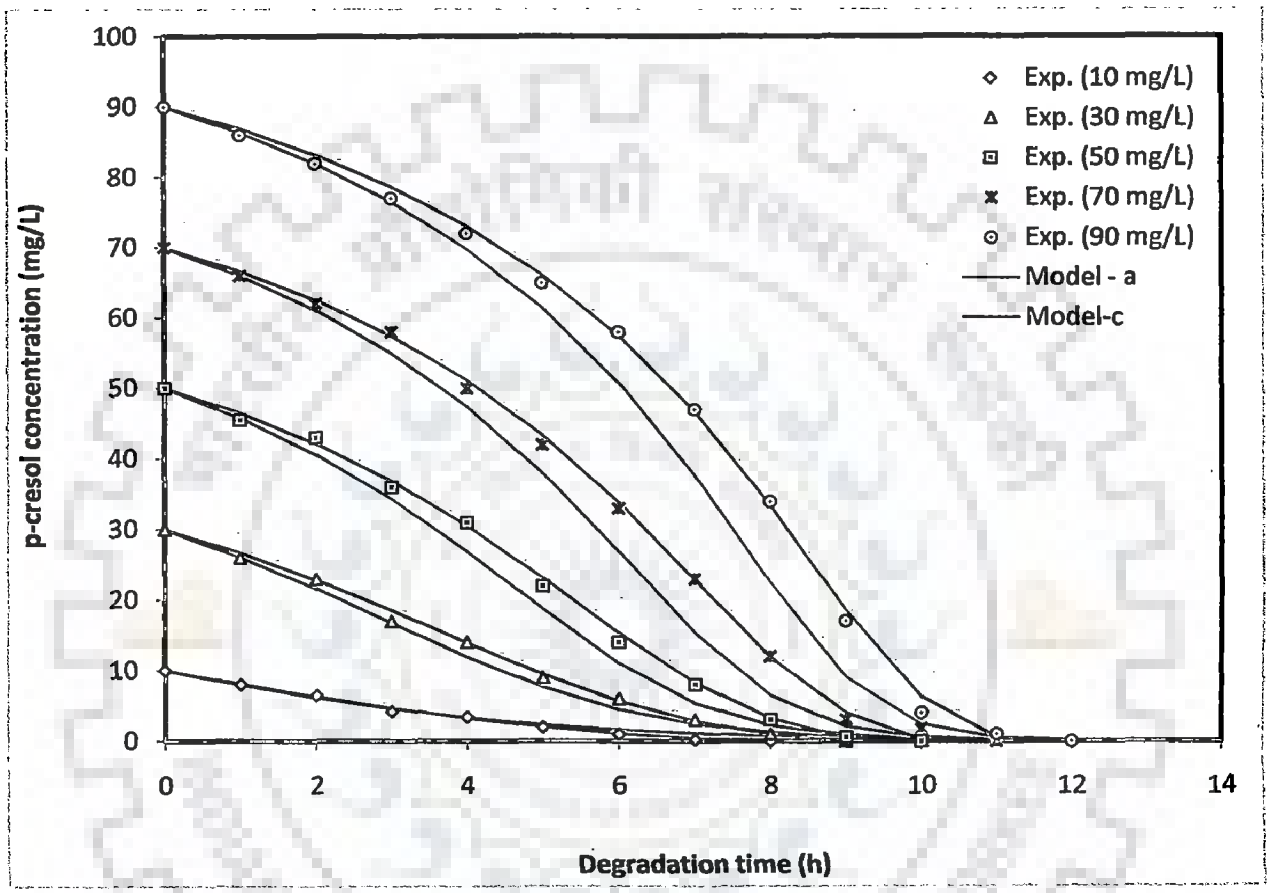

Figure 4.40: p–cresol degradation profiles by the models in lower initial concentration range

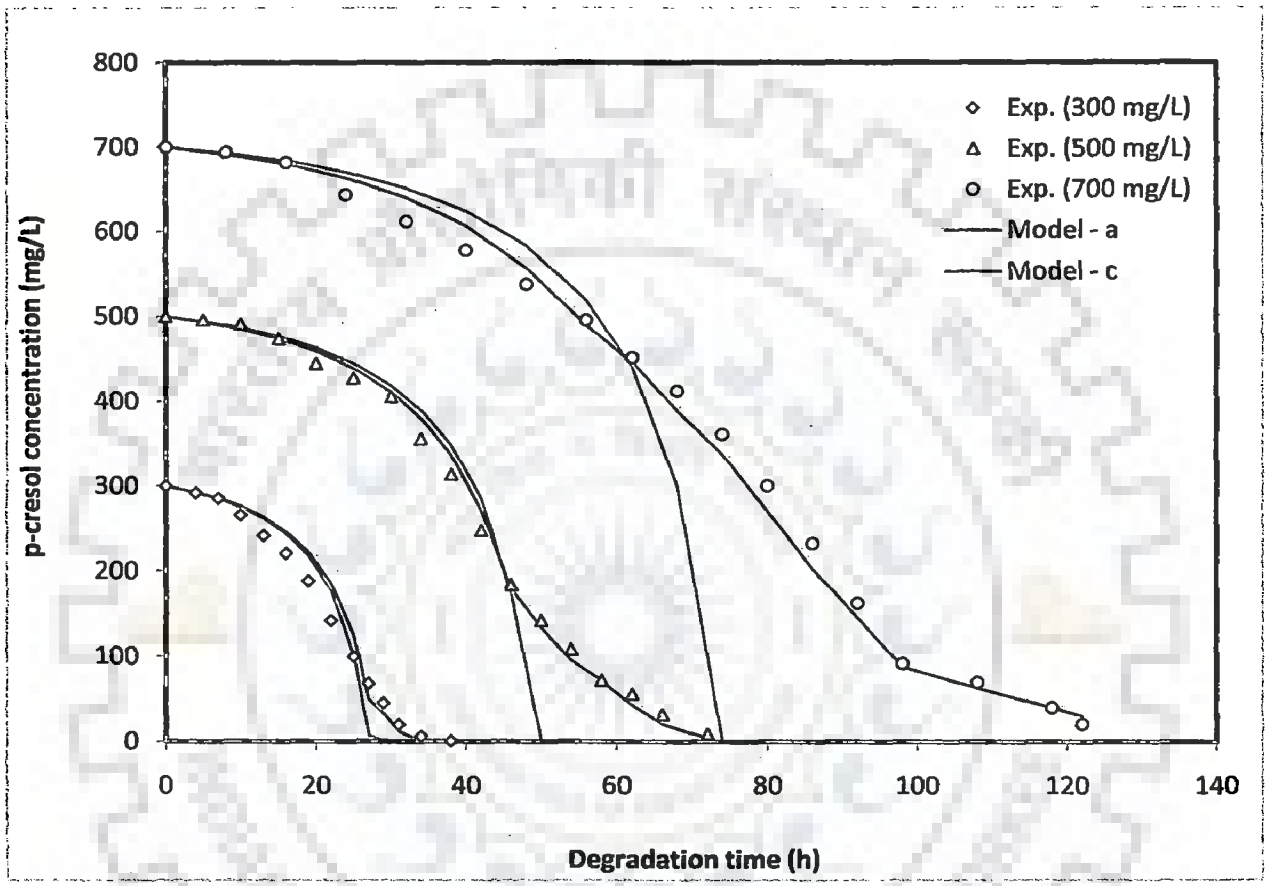

Figure 4.41: p–cresol degradation profiles by the models in higher initial concentration range

CHAPTER V

BIODEGRADATION IN DUAL SUBSTRATE SYSTEMS

5.0 INTRODUCTION

The biodegradation of more than one growth limiting substrates is a complex process due to the substrate interactions that can alter the degradation rates relative to the rate with pure substrate. In the present Chapter, the biodegradation of phenol in presence of resorcinol and p–cresol in two dual substrate systems, viz. phenol – resorcinol and phenol – p–cresol, has been discussed. The Chapter starts with the development of mathematical models for biomass growth and substrate consumption rates with the selection of suitable growth and degradation kinetic models. The kinetic parameters including interaction parameters have been estimated to describe the growth and degradation kinetic models for both dual substrate degradation systems. Further, the degradation profiles of phenol, resorcinol and p–cresol with time in dual substrate system have been modelled by incorporating specifically the variation in maintenance energy expenditure with substrate concentrations. The degradation dynamic models have been simulated to validate the model predictions with the experimental observations.

5.1 KINETIC MODELLING

5.1.1 Growth Kinetics

In multiple substrate system, the behaviour of biomass growth and thereby the substrate degradation may differ greatly from the single substrate degradation system due to the presence of substrate interactions between two substrates. The substrate interactions make the process more complex to model the growth and substrate degradation kinetics. For multiple mixed substrates, the single substrate growth model needs to be modified to represent the enzymatic effects of each substrate on the utilization of other substrates [Yoon *et al.* (1977)]. Different specific growth rate

models during the degradation of multiple interacting substrates have been developed analogous to enzyme inhibition kinetics. The analogy between enzyme kinetics and biomass growth kinetics has been made due to the identical patterns of the inhibition to biomass growth and enzyme activity [Littlejohns and Daugulis (2008), Shuler and Kargi (2003)].

Various types of substrate interaction patterns including competitive, non-competitive and uncompetitive inhibition of growth are observed in different dual substrate biodegradation systems [Shuler and Kargi (2003)]. Wang and Loh (2001) investigated the co-metabolism of phenol and 4-chlorophenol in the presence of sodium glutamate. Kar *et al.* (1997) investigated the biodegradation of phenol in the presence of o–cresol and p–cresol using *Arthrobacter* species. They observed strong competitive inhibition of p–cresol to phenol biodegradation and vice versa. The presence of o–cresol enhanced the biodegradation of phenol while phenol had null effect on o–cresol biodegradation. Hofrichter *et al.* (1995) presented a study on the co-metabolic degradation of o–cresol and 2, 6-dimethylphenol in dual substrate biodegradation system using pure culture of fungus *Penicillium frequentans* Bi 7/2. Alvarez and Vogel (1991) reported the involvement of three phenomena, namely induction, inhibition, and co-metabolism, during the substrate interaction in the biodegradation of a mixture of benzene, toluene and p–xylene by *Pseudomonas* sp. strain CFS-215. The authors compared the changes in lag period and in pseudo-zero order biodegradation rates to evaluate these interactions. Meyer *et al.* (1984) reported diauxic type degradation of benzene in the presence of phenol.

In the present study, the experimental data, obtained from the single substrate batch experiments, indicate that the phenol, resorcinol, and p–cresol are toxic substrates and exert substrate inhibition on biomass growth [Chapter IV]. The dual substrate batch experimental data for phenol – resorcinol and phenol – p–cresol systems indicate the occurrence of cross inhibition between phenol and resorcinol, and between phenol and p–cresol. In the literature, various sequences of reactions analogous to enzymatic reactions have been proposed to model the growth kinetics [Bai *et al.* (2007), Abuhamed *et al.* (2004), Wang and Loh (2001), Reardon *et al.* (2000), Loh and Yu (2000), Wang and Loh (1999), Wang *et al.* (1996), Yoon *et al.* (1977)]. In the present situation, one possible sequence of reactions based on the

enzymatic reactions for the mixed substrate system consisting of substrates S_1 and S_2, is as follows [Bai *et al.* (2007), Wang and Loh (2001), Yoon *et al.* (1977)];

$$X + S_1 \underset{k_{1b}}{\overset{k_1}{\longleftrightarrow}} X_1 \tag{5.1}$$

$$X_1 \xrightarrow{k_2} 2X \tag{5.2}$$

$$X_1 + S_1 \underset{k_{3b}}{\overset{k_3}{\longleftrightarrow}} \overline{X_1 S_1} \tag{5.3}$$

$$X_1 + S_2 \underset{k_{4b}}{\overset{k_4}{\longleftrightarrow}} \overline{X_1 S_2} \tag{5.4}$$

$$\overline{X_1 S_1} + S_2 \underset{k_{5b}}{\overset{k_5}{\longleftrightarrow}} \overline{X_1 S_1 S_2} \tag{5.5}$$

$$\overline{X_1 S_2} + S_1 \underset{k_{6b}}{\overset{k_6}{\longleftrightarrow}} \overline{X_1 S_2 S_1} \tag{5.6}$$

$$X + S_2 \underset{k_{7b}}{\overset{k_7}{\longleftrightarrow}} X_2 \tag{5.7}$$

$$X_2 \xrightarrow{k_8} 2X \tag{5.8}$$

$$X_2 + S_2 \underset{k_{9b}}{\overset{k_9}{\longleftrightarrow}} \overline{X_2 S_2} \tag{5.9}$$

$$X_2 + S_1 \underset{k_{10b}}{\overset{k_{10}}{\longleftrightarrow}} \overline{X_2 S_1} \tag{5.10}$$

$$\overline{X_2S_2} + S_1 \underset{k_{11b}}{\overset{k_{11}}{\longleftrightarrow}} \overline{X_2S_2S_1} \tag{5.11}$$

$$\overline{X_2S_1} + S_2 \underset{k_{12b}}{\overset{k_{12}}{\longleftrightarrow}} \overline{X_2S_1S_2} \tag{5.12}$$

In Eqs. (5.1) – (5.12) X_1 and X_2 are different intermediate states of the microorganisms in which substrates S_1 and S_2 are consumed respectively but no biomass growth has occurred. Other intermediates are $\overline{X_1S_1}$, $\overline{X_1S_2}$, $\overline{X_1S_1S_2}$, $\overline{X_2S_2}$, $\overline{X_2S_1}$, and $\overline{X_2S_1S_2}$ complexes. In most of the dual substrate systems during two substrate reactions, it appears that a ternary intermediate complex $\overline{X_1S_1S_2}$ and $\overline{X_2S_1S_2}$ may be formed with both substrates [Bailey and Ollis (1986)]. These facts are incorporated in Eqs. (5.5), (5.6), (5.11), and (5.12). Eqs. (5.2) and (5.8) are assumed to be irreversible first order reactions, whereas remaining Eqs. (5.1), (5.3) – (5.7), (5.9) – (5.12) are assumed to be reversible, and are with respect to each of the reactants and products. Eqs. (5.1) – (5.3) represent substrate inhibition of S_1 and Eqs. (5.7) – (5.9) represent substrate inhibition by substrate S_2. Eqs. (5.4) – (5.6) and (5.10) – (5.12) represent the cross inhibition between substrates S_1 and S_2.

The active intermediates may follow two reaction pathways. In one pathway, the active intermediate may be deactivated which is just the reverse reaction of their formation (reversible reaction). In the alternative pathway, the active intermediate decomposes spontaneously, to form stable products (irreversible reaction) [Fogler (1999)]. It is very difficult to measure the concentration of active intermediates because they are highly reactive and very short lived. Consequently, the evaluation of reaction rate laws in their present form becomes quite difficult. Besides, in most of the instances it is not possible to eliminate the concentration of active intermediates in the differential form of the mass balance equation, to obtain the solution. However, an approximate solution may be obtained, exploring the pseudo-steady state hypothesis (PSSH) method. In pseudo-steady state approximation, the rate of formation of active intermediates is assumed to be equal to its rate of disappearance. As a result, the net

rate of formation of active intermediates is zero. Thus, the concentration of active intermediates can be expressed in terms of the concentrations of biomass and substrate. The approximation by PSSH is applicable due to two conditions of intermediates: they have very short life time because of their high reactivity and are present in low concentrations. Further, it is known that the net rate of formation of any reaction species involved in many simultaneous reactions is the sum of the rates of formation of that reaction species in each reaction. On this basis, the net rate of formation of i^{th} reaction species occurring in N different reactions can be generalized as,

$$r_i = \sum_{j=1}^{N} r_{ij} \qquad ; \quad j = 1 \rightarrow N \tag{5.13}$$

In the above series of reaction steps Eqs. (5.1) – (5.12), each reaction is elementary in which the reaction orders and stoichiometry coefficients are identical. Thus, on applying Eq. (5.13) and PSSH, the net rate of formation of the active intermediates in Eqs. (5.1) – (5.12), can be expressed as follows:

$$\frac{d[X_1]}{dt} = k_1[X][S_1] - k_{1b}[X_1] - k_2[X_1] - k_3[X_1][S_1] + k_{3b}[X_1S_1] - k_4[X_1][S_2] + k_{4b}[X_1S_2] = 0 \tag{5.14}$$

$$\frac{d[X_2]}{dt} = k_7[X][S_2] - k_{7b}[X_2] - k_8[X_2] - k_9[X_2][S_2] + k_{9b}[X_2S_2] - k_{10}[X_2][S_1] + k_{10b}[X_2S_1] = 0 \tag{5.15}$$

$$\frac{d[X_1S_1]}{dt} = k_3[X_1][S_1] - k_{3b}[X_1S_1] - k_5[X_1S_1][S_2] + k_{5b}[X_1S_1S_2] = 0 \tag{5.16}$$

$$\frac{d[X_1S_2]}{dt} = k_4[X_1][S_2] - k_{4b}[X_1S_2] - k_6[X_1S_2][S_1] + k_{6b}[X_1S_2S_1] = 0 \tag{5.17}$$

$$\frac{d[X_1S_1S_2]}{dt} = k_5[X_1S_1][S_2] - k_{5b}[X_1S_1S_2] + k_6[X_1S_2][S_1] - k_{6b}[X_1S_1S_2] = 0 \tag{5.18}$$

$$\frac{d[X_2S_1]}{dt} = k_{10}[X_2][S_1] - k_{10b}[X_2S_1] - k_{12}[X_2S_1][S_2] + k_{12b}[X_2S_1S_2] = 0 \tag{5.19}$$

$$\frac{d[X_2S_2]}{dt} = k_9[X_2][S_2] - k_{9b}[X_2S_2] - k_{11}[X_2S_2][S_1] + k_{11b}[X_2S_1S_2] = 0 \quad (5.20)$$

$$\frac{d[X_2S_1S_2]}{dt} = k_{11}[X_2S_2][S_1] - k_{11b}[X_2S_1S_2] + k_{12}[X_2S_1][S_2] - k_{12b}[X_2S_1S_2] = 0 \quad (5.21)$$

The Eqs. (5.14) – (5.21) are solved simultaneously to obtain the concentrations of intermediates in terms of biomass and substrate concentrations. The final expressions are as follows:

$$[X_1] = \frac{[X][S_1]}{K_{S1}} \quad (5.22)$$

$$[X_1S_1] = \frac{[X][S_1]^2}{K_{S1}K_{i1}} \quad (5.23)$$

$$[X_1S_2] = \frac{[X][S_1][S_2]}{K_{S1}K_{11}} \quad (5.24)$$

$$[X_1S_1S_2] = \frac{[X][S_1]^2[S_2]}{K_{S1}K_{i1}K_{21}} \quad (5.25)$$

$$[X_1S_2S_1] = \frac{[X][S_1]^2[S_2]}{K_{S1}K_{11}K_{31}} \quad (5.26)$$

$$[X_2] = \frac{[X][S_2]}{K_{S2}} \quad (5.27)$$

$$[X_2S_2] = \frac{[X][S_2]^2}{K_{S2}K_{i2}} \quad (5.28)$$

$$[X_2S_1] = \frac{[X][S_2][S_1]}{K_{S2}K_{12}} \quad (5.29)$$

$$[X_2S_2S_1] = \frac{[X][S_2]^2[S_1]}{K_{S2}K_{i2}K_{22}} \quad (5.30)$$

$$[X_2S_1S_2]=\frac{[X][S_2]^2[S_1]}{K_{S2}K_{12}K_{32}} \tag{5.31}$$

In above equations,

$$\frac{1}{K_{S1}}=\frac{k_1}{k_{1b}+k_2} \quad , \quad \frac{1}{K_{S2}}=\frac{k_7}{k_{7b}+k_8} \quad , \quad \frac{1}{K_{i1}}=\frac{k_3}{k_{3b}}$$

$$\frac{1}{K_{11}}=\frac{k_4}{k_{4b}} \quad , \quad \frac{1}{K_{21}}=\frac{k_5}{k_{5b}} \quad , \quad \frac{1}{K_{31}}=\frac{k_6}{k_{6b}}$$

$$\frac{1}{K_{i2}}=\frac{k_9}{k_{9b}} \quad , \quad \frac{1}{K_{12}}=\frac{k_{10}}{k_{10b}} \quad , \quad \frac{1}{K_{22}}=\frac{k_{11}}{k_{11b}} \quad , \quad \frac{1}{K_{32}}=\frac{k_{12}}{k_{12b}}$$

The total biomass growth rate can be represented as

$$\frac{d[X_T]}{dt}=\frac{d}{dt}\begin{bmatrix}[X]+[X_1]+[X_2]+[X_1S_1]+[X_1S_2]+[X_1S_1S_2]+[X_1S_2S_1]\\+[X_2S_1]+[X_2S_2]+[X_2S_1S_2]+[X_2S_2S_1]\end{bmatrix} \tag{5.32}$$

For the sake of simplicity, square bracket representing concentration has been removed in all the following equations. Therefore, variables X_T, X, X_1, X_2, S_1, and S_2 themselves represent the concentration.

By Eqs. (5.14) – (5.21)

$$\frac{dX_T}{dt}=\frac{dX}{dt}=\mu_g X_T \tag{5.33}$$

From Eqs. (5.2) and (5.8)

$$\frac{dX}{dt}=k_2X_1+k_8X_2 \tag{5.34}$$

On combining Eqs. (5.33) and (5.34),

$$\mu_g=\frac{k_2X_1+k_8X_2}{X_T} \tag{5.35}$$

The substitution of various values from Eqs. (5.22) – (5.31) in Eq. (5.35), gives,

$$\mu_g = \frac{\mu_{g\max 1} S_1}{D_1} + \frac{\mu_{g\max 2} S_2}{D_2} \tag{5.36}$$

where,

$$D_1 = K_{S1} + S_1 + \frac{S_1^2}{K_{i1}} + \frac{S_1 S_2}{K_{S1}} + \frac{S_1^2 S_2}{K_{41}} + fS_2 + f\frac{S_2^2}{K_{i2}} + \frac{f}{K_{42}} S_2^2 S_1 \tag{5.37}$$

$$D_2 = K_{S2} + S_2 + \frac{S_2^2}{K_{i2}} + \frac{S_1 S_2}{K_{S2}} + \frac{S_2^2 S_1}{K_{42}} + \frac{S_1}{f} + \frac{S_1^2}{fK_{i1}} + \frac{S_1^2 S_2}{fK_{41}} \tag{5.38}$$

$$f = \frac{K_{S1}}{K_{S2}} \quad , \quad \frac{1}{K_{41}} = \left(\frac{1}{K_{i1} K_{21}} + \frac{1}{K_{11} K_{31}} \right) \quad , \quad \frac{1}{K_{42}} = \left(\frac{1}{K_{i2} K_{22}} + \frac{1}{K_{12} K_{32}} \right)$$

$$k_2 = \mu_{g\max 1} \quad , \quad k_8 = \mu_{g\max 2}$$

$$\frac{1}{K_{S1}} = \frac{f}{K_{12}} + \frac{1}{K_{11}} \quad , \quad \frac{1}{K_{52}} = \frac{1}{K_{12}} + \frac{1}{fK_{11}}$$

The values of $\mu_{g\max 1}$, K_{S1}, K_{i1} and $\mu_{g\max 2}$, K_{S2}, and K_{i2} can be obtained separately from the kinetics of individual biomass growth on phenol, resorcinol, and p–cresol as sole energy and carbon source. The other parameters including ' f ' can be determined as independent parameter by fitting the experimental data [Littlejohns and Daugulis (2008), Shuler and Kargi (2003), Saez and Rittmann (1993), Bailey and Ollis (1986), Yoon *et al.* (1977)]. The Eq. (5.36) indicates that by imposing PSSH on the formation of cellular intermediates, the resultant specific growth kinetic model for dual substrate system can be expressed as

$$\mu_g = \mu_{g1} + \mu_{g2} \tag{5.39}$$

μ_{g1} and μ_{g2} represent specific growth rates of the biomass on S_1 and S_2 respectively. The relationship of μ_{g1} and μ_{g2} indicate that μ_{g1} and μ_{g2} depend on both substrates

due to inhibition and interaction effects of each substrate on the other. Similar type of expressions have been proposed by many authors [Abuhamed *et al.* (2004), Wang and Loh (2001), Reardon *et al.* (2000), Loh and Yu (2000), Wang *et al.* (1996)].

There may exist various types of inhibition interaction in dual substrate systems which may be classified as competitive, non-competitive, and uncompetitive inhibition [Littlejohns and Daugulis (2008), Shuler and Kargi (2003), Saez and Rittmann (1993), Bailey and Ollis (1986), Yoon *et al.* (1977)]. In competitive inhibition both substrates combine with one group of enzyme and one substrate affects the enzyme affinity for other substrate. Thus the value of maximum possible reaction velocity is not affected while the substrate affinity is altered. The kinetic model incorporating purely competitive inhibition is

$$\mu_g = \frac{\mu_{g\max 1} S_1}{K_{S1} + S_1 + \frac{K_{S1} S_2}{K_{S2}}} + \frac{\mu_{g\max 2} S_2}{K_{S2} + S_2 + \frac{K_{S2} S_1}{K_{S1}}} \tag{5.40}$$

In non-competitive inhibition, both substrates simultaneously bind to the enzyme and form nonreactive ternary complex. The binding of either of the substrates does not influence the affinity of other substrate to form complex with the enzyme. This inhibition decreases the maximum possible reaction velocity. On increasing the substrate concentration to any level cannot increase the reaction rate as possible with the uninhibited biomass growth rate. The specific biomass growth model based on this type of interaction is,

$$\mu_g = \frac{\mu_{g\max 1} S_1}{\left(K_{S1} + S_1\right)\left(1 + \frac{S_2}{K_{S2}}\right)} + \frac{\mu_{g\max 2} S_2}{\left(K_{S2} + S_2\right)\left(1 + \frac{S_1}{K_{S1}}\right)} \tag{5.41}$$

The uncompetitive inhibition is special case of non-competitive inhibition where one substrate can bind to only a substrate enzyme complex and not to the free enzyme [Saez and Rittmann (1993), Bailey and Ollis (1986)]. The specific growth model can be represented as

$$\mu_g = \frac{\mu_{g\max 1} S_1}{K_{S1} + S_1\left(1 + \frac{S_2}{K_{S2}}\right)} + \frac{\mu_{g\max 2} S_2}{K_{S2} + S_2\left(1 + \frac{S_1}{K_{S1}}\right)} \tag{5.42}$$

The research efforts on dual substrate biodegradation by many authors [Littlejohns and Daugulis (2008), Juang and Tsai (2006), Yan *et al.* (2006), Saez and Rittmann (1993)] reported that the interaction between two substrates could not be described by categorising it under competitive, non-competitive, and uncompetitive inhibition. Comparison of Eq. (5.36) with Eqs. (5.40) – (5.42) clearly supports the literature findings. Furthermore, in the study by Yan *et al.* (2006) on phenol and m–cresol, the values of coefficients of $S_1^2 S_2$, $S_2^2 S_1$, S_1^2 and S_2^2 in Eq. (5.36) are found to be negligibly small. Finally, an alternative model that takes into account the unspecified type of substrate inhibition has been formulated. This model consists of the interaction parameters that are treated as unknown.

According to this description, Juang and Tsai (2006) expressed specific growth rate in dual substrate system as follows:

$$\mu_g = \frac{\mu_{g\max 1} S_1}{K_{S1} + S_1 + \frac{S_1^2}{K_{i1}} + I_{a,1} S_2 + I_{b,1} S_1 S_2} + \frac{\mu_{g\max 2} S_2}{K_{S2} + S_2 + \frac{S_2^2}{K_{i2}} + I_{a,2} S_1 + I_{b,2} S_1 S_2} \tag{5.43}$$

where $I_{a,1}$ represents inhibition to the degradation of S_1 in the presence of S_2. $I_{a,2}$ represents inhibition to the degradation of S_2 in the presence of S_1. While $I_{b,1}$ shows inhibition to the degradation of S_1 due to the presence of both the S_1 and S_2. Similarly $I_{b,2}$ shows the inhibition to the degradation of S_2 due to the presence of both the substrates. These interaction parameters take into account all interactions jointly as considered in the Eq. (5.36). The values of kinetic constants $\mu_{g\max}$, K_S, K_i are the same as in the single substrate biodegradation system (1 and 2 on suffix refer to S_1 and S_2 respectively). There is a concept of four substrate interaction patterns for dual substrate system applied to various studies [Reardon *et al.* (2000), Wang *et al.* (1996)]. The conditions corresponding to these interaction patterns are applied to Eq. (5.43) to get final results to represent and discuss the growth kinetics.

Pattern 1:

The conditions $I_{b,1} = 0$, $I_{b,2} = 0$, $I_{a,1} \neq 0$, $I_{a,2} \neq 0$ represent the competitive cross inhibition between the two substrates.

Pattern 2:

The conditions $I_{b,1} \neq 0$, $I_{b,2} \neq 0$, $I_{a,1} = 0$, $I_{a,2} = 0$ represent the uncompetitive cross inhibition.

Pattern 3:

The conditions $I_{b,1} = 0$, $I_{b,2} = 0$, but either $I_{a,1} \neq 0$, $I_{a,2} = 0$ or $I_{a,1} = 0$, $I_{a,2} \neq 0$ indicate the competitive partial inhibition.

Pattern 4:

The conditions $I_{a,1} = 0$, $I_{a,2} = 0$, but either $I_{b,1} \neq 0$, $I_{b,2} = 0$ or $I_{b,1} = 0$, $I_{b,2} \neq 0$ show the uncompetitive partial inhibition between the two substrates.

5.1.2 Biodegradation Kinetics

In dual substrate biodegradation system, the consumption rates of S_1 and S_2 can be described by using Eqs. (5.44) and (5.45) respectively

$$\frac{dS_1}{dt} = -q_{S1} X_T \tag{5.44}$$

$$\frac{dS_2}{dt} = -q_{S2} X_T \tag{5.45}$$

The specific degradation rates of substrates S_1 and S_2 can be represented by Eqs. (5.46) and (5.47) respectively

$$q_{S1} = \frac{\mu_{g1}}{\left(Y_{X/S}\right)_{T1}} \tag{5.46}$$

$$q_{S2} = \frac{\mu_{g2}}{\left(Y_{X/S}\right)_{T2}} \tag{5.47}$$

Here $\left(Y_{X/S}\right)_{T1}$ and $\left(Y_{X/S}\right)_{T2}$ are the maximum growth yield coefficients with respect to substrates S_1 and S_2 respectively. The Eqs. (5.46) and (5.47) hold true when maintenance requirements are negligible. In biodegradation, a part of substrate is used by the biomass to form new biomass cells and another part is used to perform metabolic activities irrespective of growth. These non-growth metabolic activities are performed by consumption of energy, termed as maintenance energy expenditure and are described by maintenance energy coefficient m_S. Pirt (1982) initially defined maintenance energy coefficient as the minimum substrate consumption to maintain the cell activity whereas Jetten *et al.* (1990) introduced maintenance as minimum amount of substrate above which growth occurred. The maintenance includes in general following non-growth components [Bodegom (2007), Russell and Cook (1995), Stouthamer *et al.* (1990), Chesbro (1988), Mason *et al.* (1986), Tempest and Neijssel (1984), Mandelstam (1958)]:

(i) Shifts in metabolic pathways
(ii) Cell motility
(iii) Changes in stored polymeric carbon
(iv) Energy spilling reactions
(v) Control of water and mineral salt levels in the cell termed as osmoregulation
(vi) Extra cellular loses of compounds not involved in osmoregulation
(vii) Synthesis and internal turnover of macromolecular compounds such as enzymes and RNA
(viii) Defence against oxygen stress

These non-growth components represent various measures to quantify the maintenance energy required in biodegradation process. Out of above eight components, the osmoregulation, cell motility, and synthesis of enzymes and RNA are included in the physiological maintenance. This maintenance dominates the non-growth consumption of energy under starvation conditions of the cells of biomass [Russell and Cook (1995)].

It is analysed by many researchers that apart from biomass growth, some measure of maintenance energy is needed to provide proper description of substrate degradation dynamics in dual substrate system [Jiang *et al.* (2010), Bai *et al.* (2007), Yan *et al.* (2006)]. Maintenance energy was considered as a constant quantity specific to substrate – microorganism system in various kinetic modelling studies [Jiang *et al.* (2010), Bai *et al.* (2007), Yan *et al.* (2006)]. The maintenance energy coefficient m_{si} can be incorporated in defining specific degradation rate q_{Si} for given substrate 'i' in dual substrate system as follows:

$$q_{Si} = m_{Si} + \frac{\mu_{gi}}{\left(Y_{X/S}\right)_{Ti}} \tag{5.48}$$

In this approach, maintenance denotes extra substrate consumption not used for growth purposes. Neijssel and Tempest (1976), and Hempfling and Mainzer (1975) reported the measurements of the maintenance energy and found the variation in it. Pirt (1982) described the maintenance as growth rate dependent parameter and postulated a modification to this theory by considering maintenance dependent on the specific growth rate and by including a portion that decreases with the increasing specific growth rate. Accordingly, the Eq. (5.48) is rewritten as

$$q_{Si} = \frac{\mu_{gi}}{\left(Y_{X/S}\right)_{Ti}} + m_{1i} + k_i\left(1 - \frac{\mu_{gi}}{\mu_{g\max i}}\right) \tag{5.49}$$

where m_{1i} denotes the constant maintenance energy coefficient and the third term of the equation denotes the growth rate dependent maintenance energy coefficient for substrate i in dual substrate system.

The specific substrate degradation rate for substrate 'i' can also be defined in terms of observed growth yield coefficient $\left(Y_{X/S}\right)_{oi}$ as follows:

$$q_{Si} = \frac{\mu_{gi}}{\left(Y_{X/S}\right)_{oi}} \tag{5.50}$$

Here, it is important to distinguish $\left(Y_{X/S}\right)_{oi}$ from $\left(Y_{X/S}\right)_{Ti}$. $\left(Y_{X/S}\right)_{Ti}$ considers consumption of substrate for biomass growth only, while $\left(Y_{X/S}\right)_{oi}$ is the yield corrected for maintenance. It implies that $\left(Y_{X/S}\right)_{Ti}$ is supposed to be higher and less variable with substrate concentration than $\left(Y_{X/S}\right)_{oi}$. Experimentally it is found that for the single substrate systems, $\left(Y_{X/S}\right)_{oi}$ decreases with the decreasing growth rates. Therefore, relating specific degradation rate with growth rate in terms of $\left(Y_{X/S}\right)_{Ti}$ (Eqs. (5.46) and (5.47)) is incorrect [Bodegom (2007)]. On comparing Eq. (5.50) with Eq. (5.49), it is clear that $\left(Y_{X/S}\right)_{oi}$ incorporates the maintenance energy expenditure. However, Bodegom (2007) suggested that the maintenance parameter might be estimated separately.

Pirt (1982) defined maintenance energy coefficient and thereby the specific degradation rate as a linear function of specific growth rate as is clearly shown in Eq. (5.49). This type of linear relationship between q_{Si} and μ_{gi} is applicable to single substrate system as described in Chapter IV. In dual substrate system, the linear relationship between q_{Si} and μ_{gi} has been used in empirical formulation [Jiang *et al.* (2010), Bai *et al.* (2007), Yan *et al.* (2006), Minkevich *et al.* (2000)]. In these formulations $\left(Y_{X/S}\right)_{Ti}$ has been derived empirically as the maximum yield after correcting for constant maintenance energy expenditure.

To the best of our knowledge, there is no other biodegradation study available in the literature which quantifies the variation in maintenance energy expenditure when higher concentrations of the substrates are present in the mixture, and biomass growth follows inhibition kinetics. The work presented here, is a reasonable starting point for the development and validation of mathematical model to describe the maintenance energy expenditure and thereby the specific degradation rate for mixtures of two homologous substrates.

The sensitivity analysis of maintenance has been done by Bodegom (2007). This analysis indicates the importance of various non-growth components and emphasizes that overall maintenance depends nonlinearly on relative growth rate,

relative death rate, growth yield and endogenous metabolism. The maintenance is a dynamic process and ideally maintenance description should incorporate the dynamics of each non-growth component. There is no constant relation between these non-growth parameters. The simple combinations of these parameters can not be made due to partial overlapping of these parameters. The conceptual analysis on various non-growth components by Bodegom (2007) shows strong dependence of overall maintenance on growth rate. This overall maintenance depends on the growth rate in a non linear way. Further, the analysis on growth yield in case of dual substrate system indicates that it is difficult to find out the growth yield for substrate 'i' present in the mixture because the biomass only represents the total growth and its decomposition in two parts corresponding to two substrates, is extremely difficult. Therefore, instead of Eq. (5.50), Eq. (5.48) is considered to describe specific degradation rate for dual substrate system where $\left(Y_{X/S}\right)_{Ti}$ may be assumed to be a constant parameter, not measured experimentally but estimated empirically by fitting the experimental data to empirical mathematical model.

In view of above discussion, to generate a non-linear function of μ_{gi}, it is convenient to express maintenance energy coefficient m_{Si} for substrate 'i' in the following second degree polynomial form

$$m_{Si} = A_{1i} + A_{2i}\mu_{gi} + A_{3i}\mu_{gi}^2 \tag{5.51}$$

With substitution of Eq. (5.51) in the model Eq. (5.48), the specific degradation rate of substrate 'i' in dual substrate system, can be transformed in the following equation:

$$q_{Si} = \frac{\mu_{gi}}{\left(Y_{X/S}\right)_{Ti}} + A_{1i} + A_{2i}\mu_{gi} + A_{3i}\mu_{gi}^2 \tag{5.52}$$

Where A_{1i}, A_{2i} and A_{3i} are constants of polynomial expression. On keeping $\left(Y_{X/S}\right)_{Ti}$ constant, Eq. (5.52) can be reduced to

$$q_{Si} = A_{1i} + A_{4i}\mu_{gi} + A_{3i}\mu_{gi}^2 \tag{5.53}$$

where $$A_{4i} = \left[\frac{1}{\left(Y_{X/S}\right)_{Ti}} + A_{2i} \right]$$

The application of Eq. (5.53) has been demonstrated by fitting the experimental data for degradation of each substrate in dual substrate system as discussed in the next section.

The biodegradation dynamics of both substrates in dual substrate system has been demonstrated by solving the set of model equations along with allied boundary conditions as listed in Table 5.1. The set of mathematical model equations (Table 5.1) consists of three ordinary differential equations (ODE) and four algebraic equations. All the required boundary conditions are available at initial stage, which is at $t = 0$. Here t is independent variable. The solution of these model equations provides the time dependent profiles of three variables, namely X_T, S_1 and S_2.

Table 5.1: Model equations for substrate degradation dynamics

Model Equations	Boundary conditions
$\frac{dX_T}{dt} = \left(\mu_{g1} + \mu_{g2}\right) X_T$	at $t = 0$
$\frac{dS_1}{dt} = -q_{S1} X_T$	$X_T = X_{To}$
$\frac{dS_2}{dt} = -q_{S2} X_T$	$S_1 = S_{1o}$
$q_{S1} = A_{11} + A_{41}\mu_{g1} + A_{31}\mu_{g1}^2$	$S_2 = S_{2o}$
$q_{S2} = A_{12} + A_{42}\mu_{g2} + A_{32}\mu_{g2}^2$	
$\mu_{g1} = \frac{\mu_{g\max 1} S_1}{K_{S1} + S_1 + \frac{S_1^2}{K_{i1}} + I_{a,1} S_2 + I_{b,1} S_1 S_2}$	
$\mu_{g2} = \frac{\mu_{g\max 2} S_2}{K_{S2} + S_2 + \frac{S_2^2}{K_{i2}} + I_{a,2} S_1 + I_{b,2} S_1 S_2}$	

5.2 RESULTS AND DISCUSSION

5.2.1 Growth Kinetic Study

A series of batch biodegradation experiments were conducted to study the biomass growth rate and interaction between the two substrates in two dual substrate degradation systems. Eq. (5.43) (given in section 5.1.1) has been used for the purpose.

Phenol – p–Cresol System

In the single substrate biodegradation studies on phenol and p–cresol, it has been observed that both are toxic substrates. They cause inhibition to biomass growth and self biodegradation at the initial concentrations higher than 70 mg/L for phenol, and 50 mg/L for p–cresol. In the dual substrate biodegradation system of phenol with p–cresol, the growth kinetics of fungal biomass and substrate interaction between phenol and p–cresol has been studied at the initial concentrations higher than the inhibitory concentrations of both the substrates. The biodegradation of Phenol and p–cresol has been studied in three combinations of concentrations; 100 mg/L phenol – 300 mg/L p–cresol, 200 mg/L phenol – 200 mg/L p–cresol, and 300 mg/L phenol – 100 mg/L p–cresol. Figures 5.1, 5.2, and 5.3 show the biodegradation trend of phenol and p–cresol with the biomass growth curve for the combinations of phenol and p–cresol. The values of interaction parameters have been estimated by Levenberg-Marquardt nonlinear regression program. Eq. (5.43) describes four type of substrate inhibition during dual substrate degradation. The equation was solved applying the four conditions given for the four patterns of substrate interaction in the dual substrate system. The conditions given for pattern 1 are found applicable to experimental data of phenol – p–cresol system. The estimated values of interaction parameters $I_{a,1}$ and $I_{a,2}$ show the involvement of competitive cross inhibition in phenol – p–cresol system as the substrate interaction. Values of interaction parameters ($I_{a,1}$= 0.044, $I_{a,2}$= 1.17) imply that phenol inhibits p–cresol degradation more than the p–cresol causes inhibition to phenol degradation in the medium. Experimental study reveals that phenol is consumed preferentially by fungus *G. indicus* in phenol – p–cresol system. Specific growth rate of fungal biomass in phenol – p–cresol system can be expressed as given below.

$$\mu_g = \frac{0.462S_1}{78.29 + S_1 + \frac{S_1^2}{44.29} + 0.044S_2} + \frac{0.512S_1}{91.87 + S_2 + \frac{S_2^2}{21.99} + 1.17S_1} \quad (5.54)$$

Phenol – Resorcinol System

To study the effect of the presence of resorcinol on phenol biodegradation, phenol and resorcinol have been taken in combinations of 100 mg/L phenol – 300 mg/L resorcinol, 200 mg/L phenol – 200 mg/L resorcinol, and 300 mg/L phenol – 100 mg/L resorcinol. The results of single substrate biodegradation study show that resorcinol is an inhibitory substrate like phenol. It causes substrate inhibition effect in the medium at an initial concentration of 90 mg/L. Hence, the biodegradation of resorcinol in presence of phenol has been studied at the concentrations higher than 90 mg/L. Biodegradation behaviour of phenol with resorcinol and biomass concentration has been demonstrated in Figs. 5.4, 5.5 and 5.6. To estimate the values of interaction parameters, Levenberg-Marquardt nonlinear regression program has been used.

The analysis of Eq. (5.43) for phenol – resorcinol system by Levenberg-Marquardt nonlinear regression program, applying the four conditions showed that the competitive cross inhibition takes place during the biodegradation of phenol with resorcinol. The values of interaction parameters have been obtained as $I_{a,1} = 1.09$, $I_{a,2} = 0.052$ for phenol – resorcinol degradation system, which indicate that resorcinol has stronger inhibition effect on phenol degradation in comparison to the inhibition caused by phenol to resorcinol degradation. During the experimental study it has been observed that the fungus consumed resorcinol in preference to phenol. The specific growth rate of biomass in phenol – resorcinol system is expressed as follows:

$$\mu_g = \frac{0.462S_1}{78.29 + S_1 + \frac{S_1^2}{44.29} + 1.09S_2} + \frac{0.185S_2}{19.83 + S_2 + \frac{S_2^2}{376}\left(1 + \frac{S_2}{1790}\right) + 0.052S_1} \quad (5.55)$$

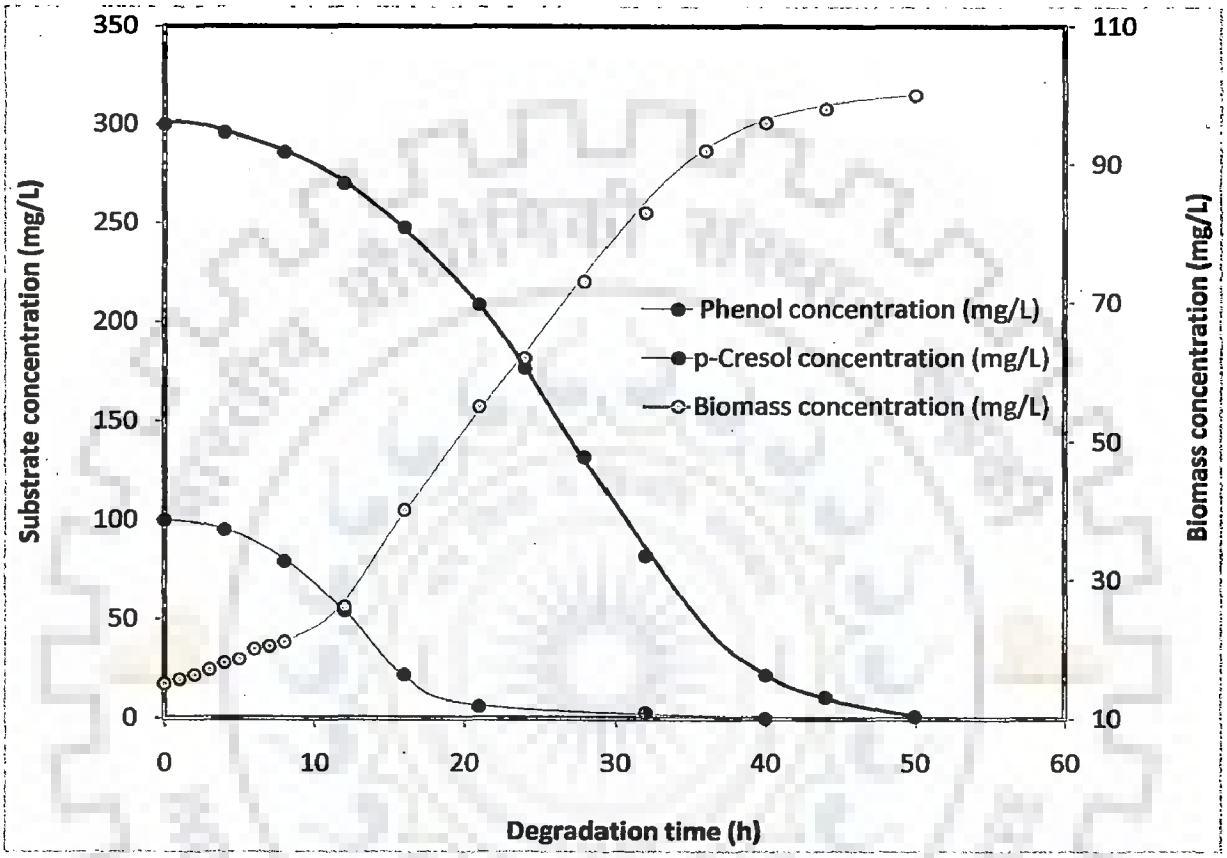

Fig. 5.1: Biomass growth and biodegradation behaviour of phenol (100 mg/L) and p–cresol (300 mg/L) in dual substrate system

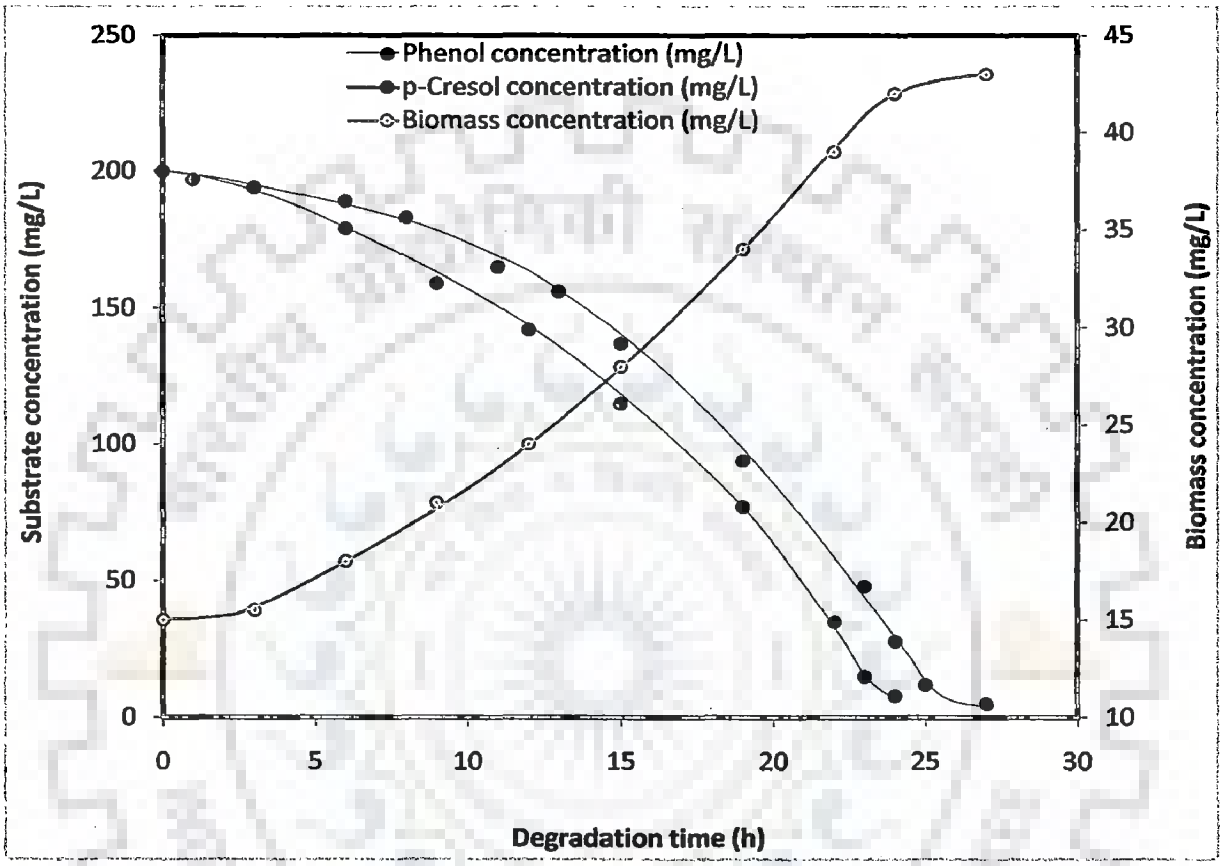

Fig. 5.2: Biomass growth and biodegradation behaviour of phenol (200 mg/L) and p–cresol (200 mg/L) in dual substrate system

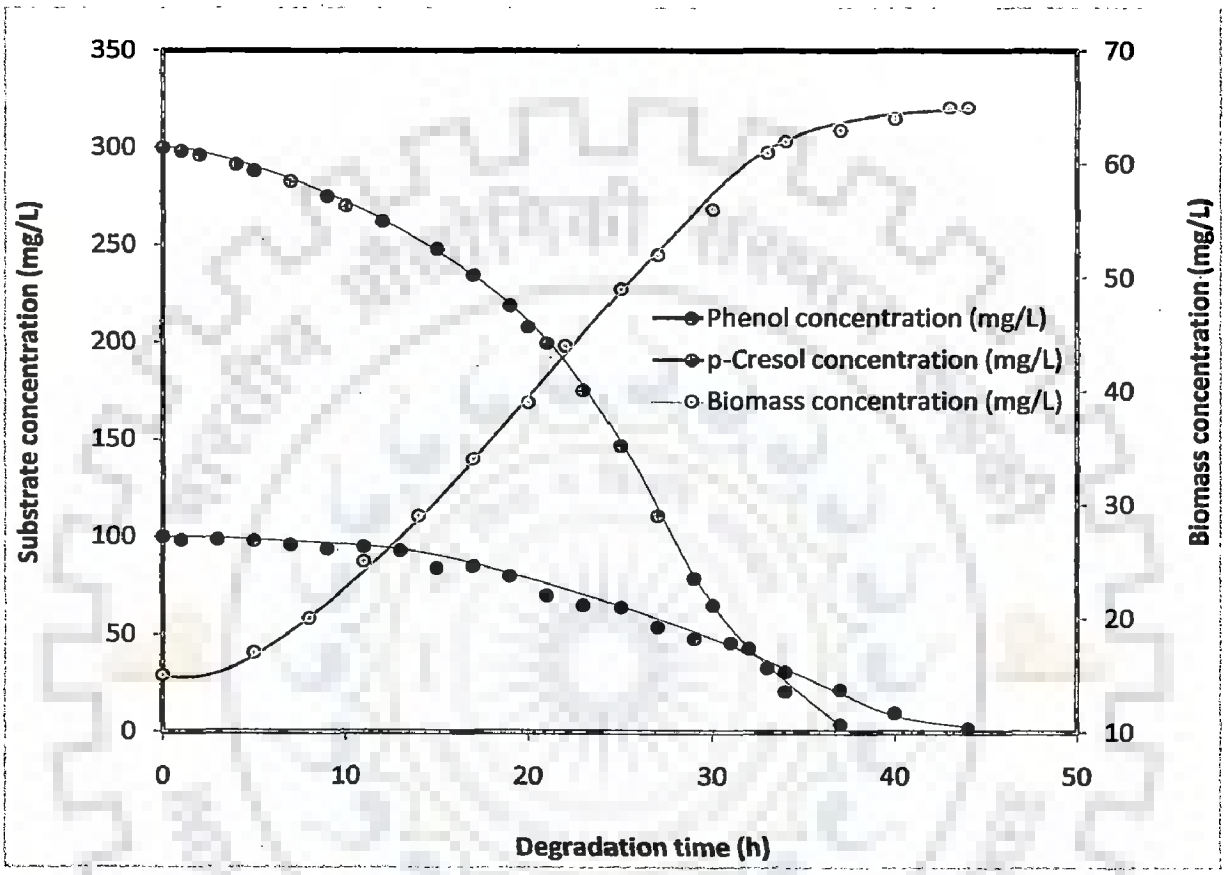

Fig. 5.3: Biomass growth and biodegradation behaviour of phenol (300 mg/L) and p–cresol (100 mg/L) in dual substrate system

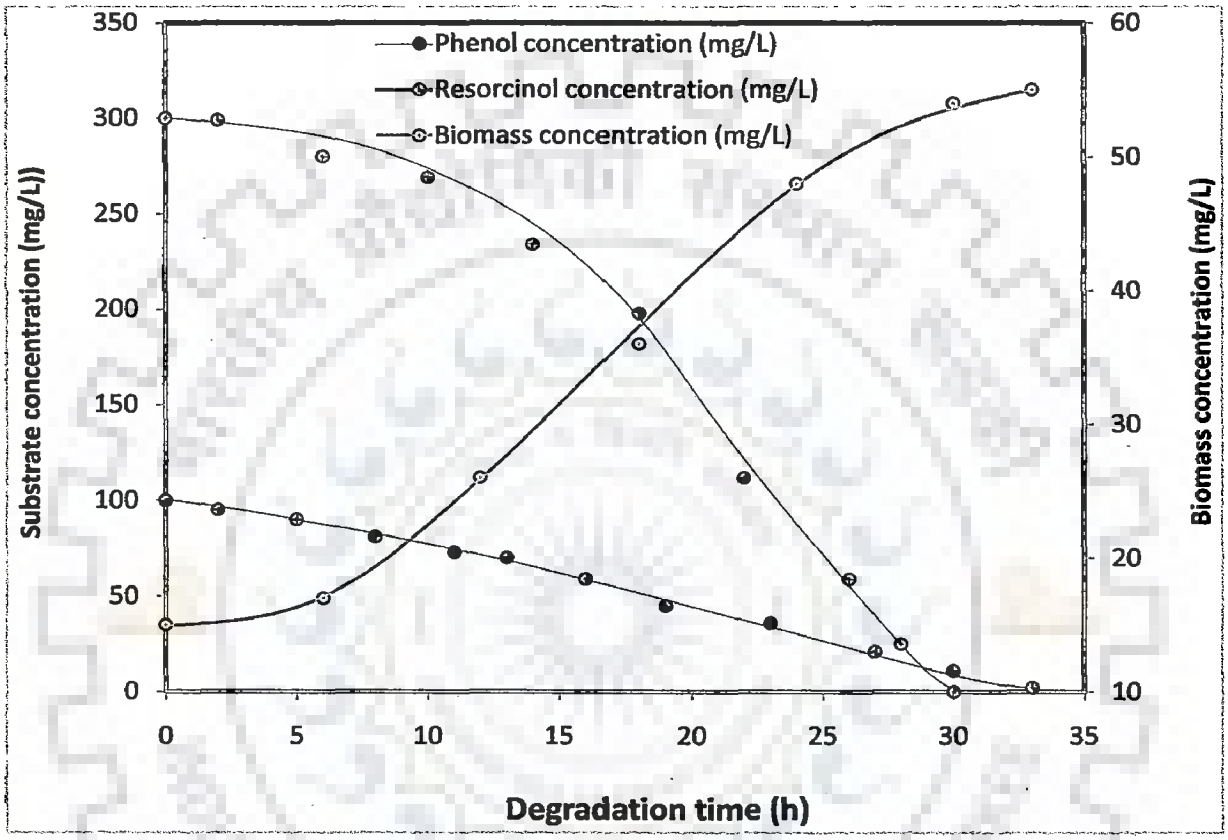

Fig. 5.4: Biomass growth and biodegradation behaviour of phenol (100 mg/L) and resorcinol (300 mg/L) in dual substrate system

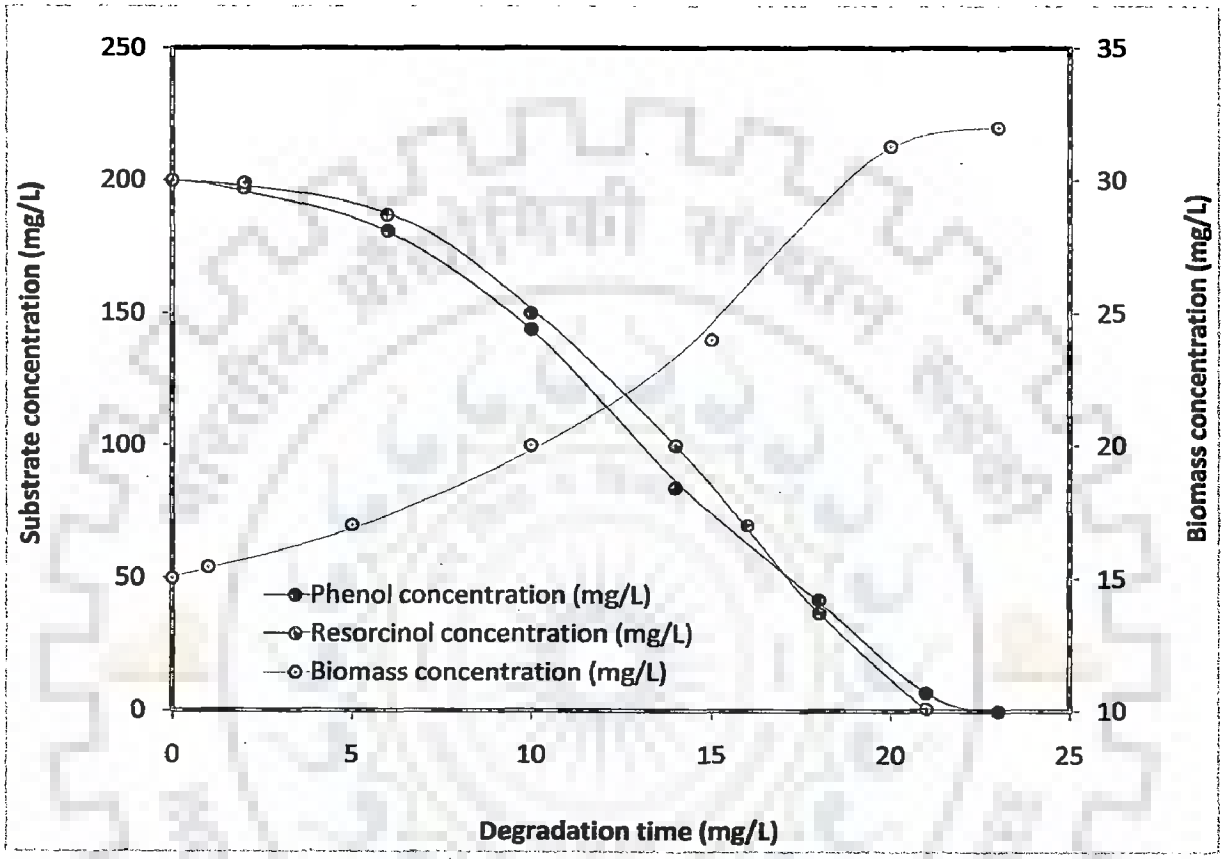

Fig. 5.5: Biomass growth and biodegradation behaviour of phenol (200 mg/L) and resorcinol (200 mg/L) in dual substrate system

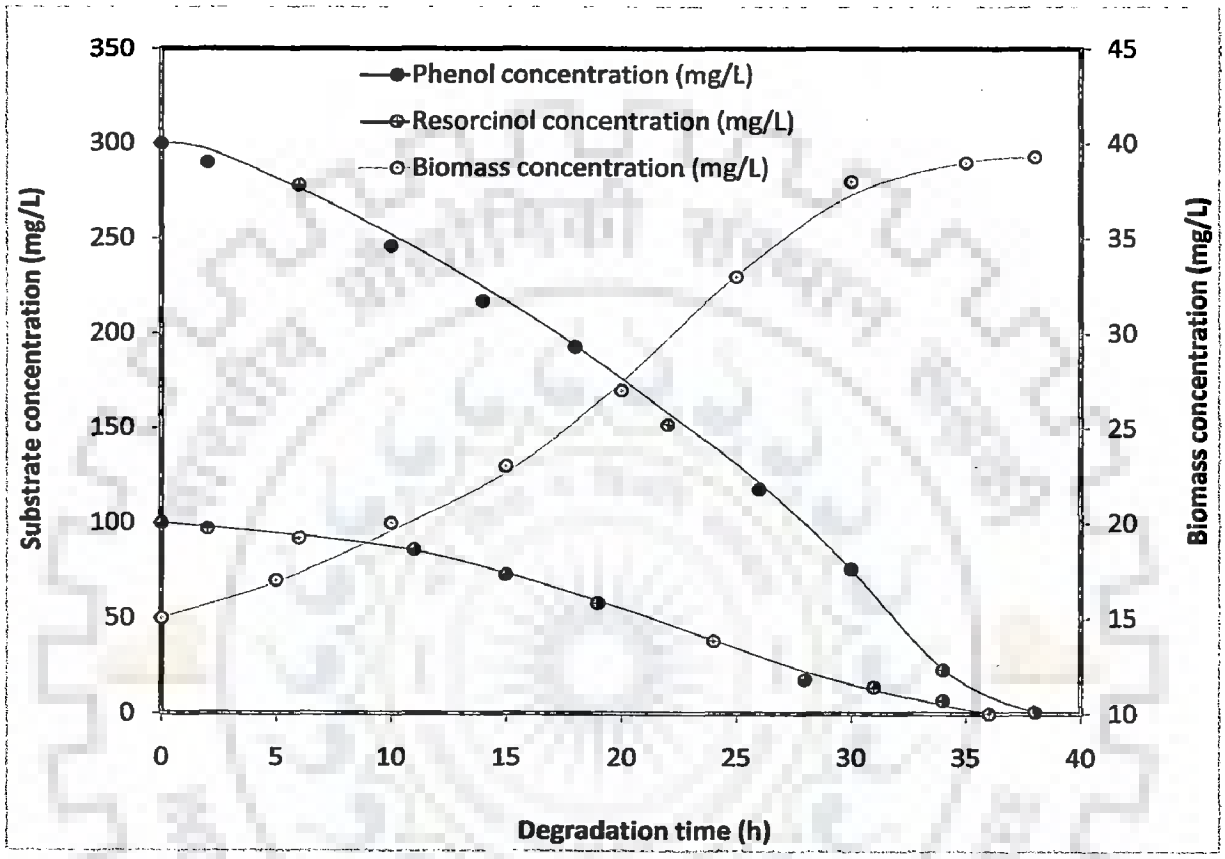

Fig. 5.6: Biomass growth and biodegradation behaviour of phenol (300 mg/L) and resorcinol (100 mg/L) in dual substrate system

The expressions of Eq. (5.54) and (5.55) are similar to the SKIP model or Sum kinetic model equation as proposed and discussed by Yoon *et al.* (1977). This study on phenol, resorcinol, and p–cresol in dual substrate system indicates the ascending order of toxicity of these substrates for G. *indicus* as resorcinol>phenol>p–cresol. Phenol, resorcinol, and p–cresol are homologous substrates, therefore, competitive cross inhibition is likely to take place [Littlejohns and Daugulis (2008)]. The results of dual substrate degradation study are supported by the reported findings of Saravanan *et al.* (2008b) and Wang *et al.* (2009), where the less toxic substrate causes stronger inhibition to the degradation of more toxic substrate in the dual substrate degradation system. A few studies on different dual substrate degradation systems with the estimated values of their interaction parameters have been summarized in Table 5.2 along with the values of interaction parameters obtained for the two dual substrate systems in the present study. All the model parameters are corresponding to SKIP model.

5.2.2 Degradation Kinetic Study

In order to study the biodegradation kinetics of phenol, resorcinol, and p–cresol in the dual substrate systems using fungus *G. indicus*, batch experiments were conducted on various combinations of phenol with p–cresol and resorcinol, at temperature of 28 °C and pH of 6. The substrate and biomass concentrations with time were monitored using the method given in section 3.1.2.4 of Chapter III. The concentrations of biomass and substrates in the medium were measured till the substrates were consumed to the large extent. The experimental values of specific degradation rates (q_{S1}) and (q_{S2}) are calculated using Eqs. (5.46) and (5.47) for phenol, resorcinol, and p–cresol in the two dual substrate systems; phenol – p–cresol and phenol – resorcinol. A mathematical model Eq. (5.53) has been proposed to study the variation of specific degradation rates of the substrates with specific growth rate of biomass at for every combination of phenol with p–cresol and resorcinol. For each substrate in dual substrate system, the constants involved in Eq. (5.53) have been estimated by using the curve fitting tool in MATLAB 7.2.

Table 5.2 The estimated values of interaction parameters of SKIP model for different dual substrate systems

Dual substrate system	Microorganism	Interaction parameter values		Type of substrate inhibition	References
		$I_{a,1}$ (–)	$I_{a,2}$ (L/mg)		
m-Cresol – Pyridine	*Lysinibacillus cresolivorans*	0.11	0.76	Competitive inhibition	Yao *et al.* (2011)
Phenol – m-Cresol	*Candida albicans* PDY-07	2.91	1.79	Competitive inhibition	Wang *et al.* (2009)
Phenol – m-Cresol	*Alcaligenes faecalis*	4.82	4.12	Competitive inhibition	Bai *et al.* (2007)
Phenol – m-Cresol	Mixed culture	3.9	9.9	Competitive inhibition	Saravanan *et al.* (2008b)
Benzene - Toluene Benzene - Phenol Toluene - Phenol	*Pseudomonas putida* F1	5.16 1.08 1.03	0.49 0.27 0.14	Competitive inhibition	Abuhamed *et al.* (2004)
Phenol – p-Cresol	*Aspergillus awamori*	8.6	0.3	Competitive inhibition	Yemendzhiev *et al.* (2008)
Phenol – Sodium salicylate	*Pseudomonas putida*	0.277	0.126	Competitive + uncompetitive inhibition	Juang and Tsai (2006)
Phenol – p-Cresol Phenol – Resorcinol	*Gliomastix indicus* MTCC 3869	0.044 1.09	1.17 0.052	Competitive inhibition	Present study

Thus, the specific degradation rates for each substrate can be expressed as follows:

phenol – p–cresol system

$$q_{S1} = 0.038 + 1.071\mu_{g1} - 2.460\mu_{g1}^2 \qquad (R^2 = 1) \quad \text{For phenol} \qquad (5.56)$$

$$q_{S2} = 0.046 - 2.175\mu_{g2} + 62.56\mu_{g2}^2 \qquad (R^2 = 1) \quad \text{For p–cresol} \qquad (5.57)$$

phenol – resorcinol system

$$q_{S1} = 3.369 - 108.2\mu_{g1} + 886.7\mu_{g1}^2 \qquad (R^2 = 1) \quad \text{For phenol} \qquad (5.58)$$

$$q_{S2} = 2.520 - 51.34\mu_{g2} + 274.9\mu_{g2}^2 \qquad (R^2 = 1) \quad \text{For resorcinol} \qquad (5.59)$$

From the values of regression coefficient ($R^2 = 1$) in above expressions, we conclude that the simulated model predictions are well consistent with the experimental data. These results justify the idea of inclusion of maintenance energy expenditure varying nonlinearly with the growth rate, to quantify the specific degradation rate in dual substrate system. Thus, it is suggested that the Eq. (5.53) may be very well adopted for the assessment of specific degradation rate for the substrate in dual substrate system.

5.2.3 Computed Substrate Degradation Profiles in Dual Substrate Systems

In the present study, the substrate degradation profiles with time have been computed for the two dual substrate degradation systems under study; phenol – p–cresol and phenol – resorcinol. For this purpose, the model equations have been mentioned in the Table 5.1 along with boundary conditions. During the exponential growth phase of batch culture, the substrate consumption in lag phase is negligibly small in comparison to the high substrate concentration in the mixture. Therefore, the initial substrate concentration of both the substrates in dual substrate system can be used as initial boundary conditions required to solve the model equations. The model equations have been solved simultaneously using ordinary differential equation solver tool "ode 45" of MATLAB 7.2 for the both dual substrate systems. The simulated results are discussed below.

Phenol – p–Cresol System

The figure 5.7 shows the comparison of the model predictions and the experimentally obtained degradation data on phenol and p–cresol in dual substrate system at the total initial substrate concentration of 400 mg/L. The model corroborates the experimental data of phenol and p–cresol degradation well for each combination of concentrations of the two substrates. In a combination of 100 mg/L phenol and 300 mg/L p–cresol, the observed biodegradation times of phenol and p–cresol are 33 h and 50 h respectively. In the single substrate degradation system, it takes 10 h for the 100 mg/L phenol and 38 h for 300 mg/L p–cresol. Since observed rate of degradation of either substrate in dual substrate system is slower than the degradation rate of either substrate alone in single substrate system, a competitive type of cross inhibition between phenol and p–cresol has thus been observed [Kar *et al.* (1997)]. Therefore, the presence of p–cresol in phenol – p–cresol system decreases the phenol degradation rate. On increasing the phenol concentration in combination with p–cresol the biodegradation time of both the substrates is further increased due to the competitive cross inhibition caused by both of them for each other. These observations support the findings of growth kinetic studies on phenol – p–cresol system (section 5.2) carried out to test the four substrate interaction patterns (Eq. 5.54).

Phenol – Resorcinol System

The figure 5.8 shows the experimental and model predictions for time profiles of phenol and resorcinol in phenol – resorcinol system, at the total initial substrate concentration of 400 mg/L. The simulated values of the model show good agreement with experimental data of phenol and resorcinol degradation for each of the combination of concentrations of phenol and resorcinol. This figure shows that it takes less time for resorcinol degradation in comparison to phenol. The biodegradation time for 100 mg/L resorcinol in combination with 300 mg/L phenol has been observed 36 h while it took 38 h for the degradation of 300 mg/L phenol. Biodegradation of 100 mg/L and 300 mg/L phenol occurs in 7 and 32 h respectively, in single substrate degradation system. When resorcinol is present with phenol in the medium it causes inhibition to phenol degradation.

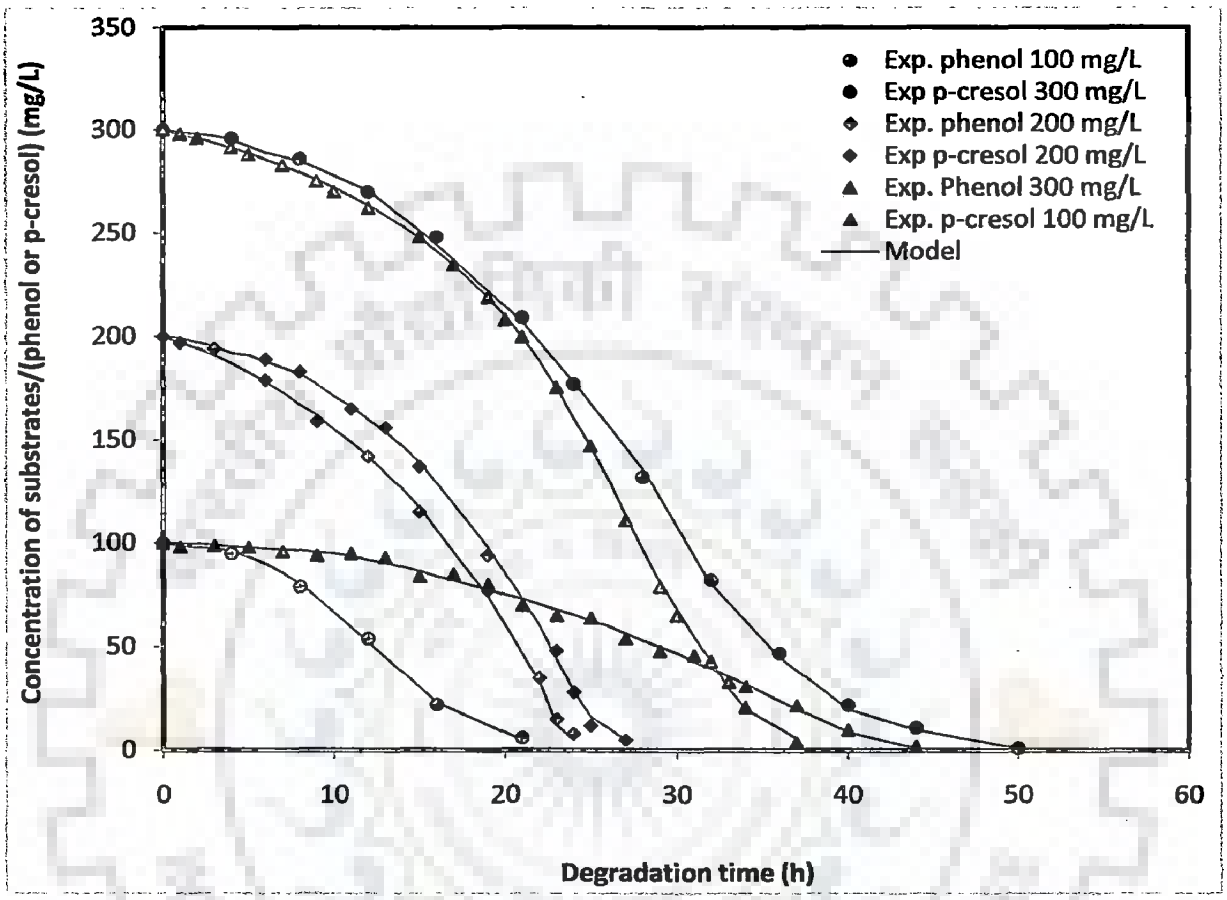

Fig. 5.7: Phenol and p–Cresol degradation profiles predicted by the model with experimental data at their different combinations

Since the biodegradation time for both the substrates in dual substrate system is larger than the biodegradation time for these substrates in single substrate system, the competitive cross inhibition is expected to occur between phenol and resorcinol. This fact is supported by the growth kinetic studies on phenol and resorcinol described by the Eq. (5.55) with condition $I_{b,1} = I_{b,2} = 0$.

5.3 CONCLUDING REMARKS

Biodegradation of phenol, resorcinol, and p–cresol has been carried out in dual substrate system; phenol – p–cresol, and phenol – resorcinol, using *Gliomastix indicus*. The fungus degraded phenol as well as p–cresol and resorcinol in the dual substrate systems efficiently. The presence of one substrate has inhibited the biodegradation kinetics of the other. For the analysis of specific growth rate and the type of substrate interaction involved in the two dual substrate systems a kinetic model has been proposed. A specific degradation rate model has also been proposed, for the three substrates in the dual substrate systems. This model incorporates the variation of biomass growth yield and the maintenance energy expenditure with substrate consumption. Further, the biodegradation dynamics of phenol, resorcinol, and p–cresol in the dual substrate systems has been modelled. The simulated values of the model agree well with the experimental data on the substrate degradation in dual substrate system.

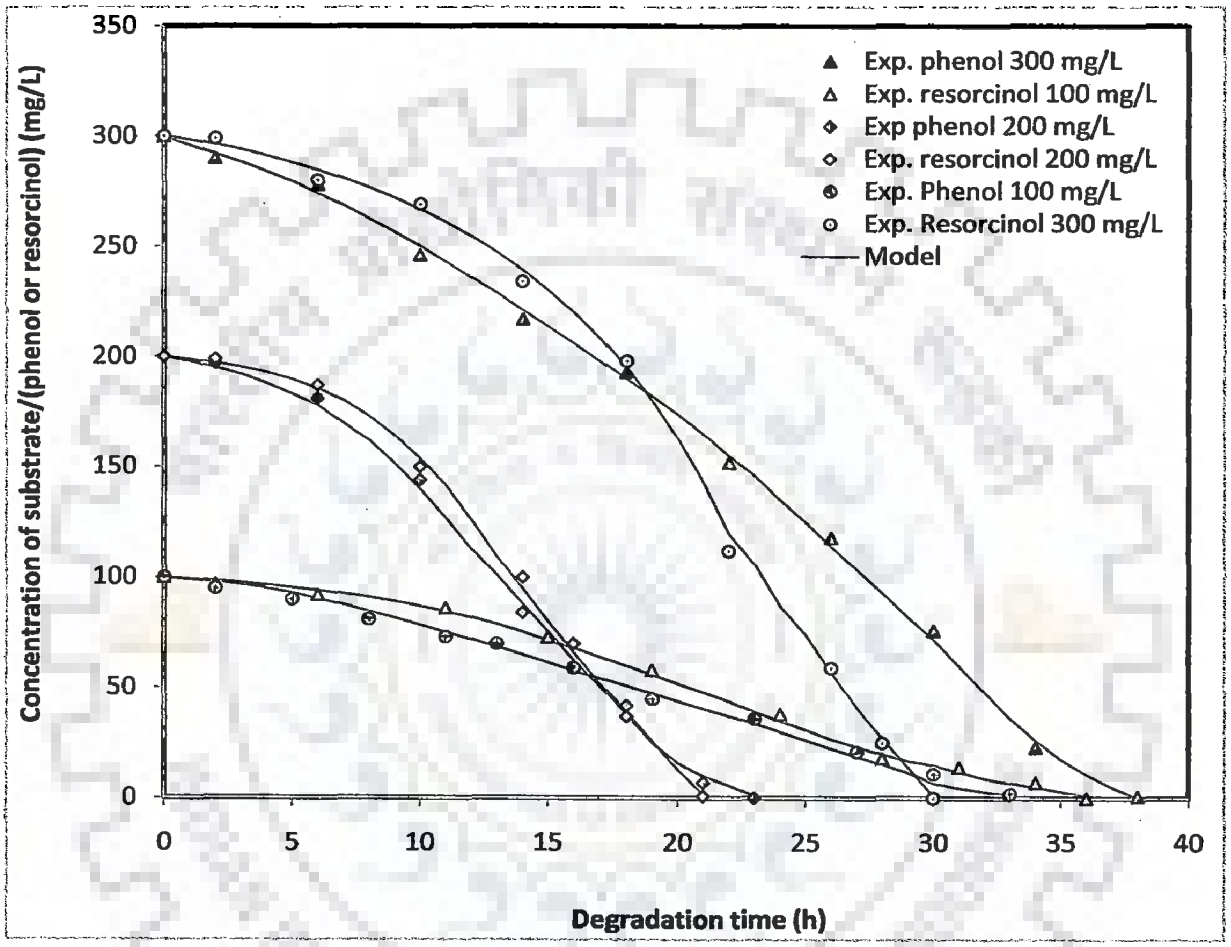

Fig. 5.8: Phenol and resorcinol degradation profiles predicted by the model with experimental data at their different combinations

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.0 INTRODUCTION

The present research program is concerned with the removal of phenol, resorcinol, and p–cresol from the simulated aqueous solution (considered as wastewater) in single and dual substrate systems by the process of biodegradation using filamentous fungus *Gliomastix indicus* MTCC 3869. The results of biodegradation studies have been summarized separately related to single substrate degradation and dual substrate degradation studies. According to the objectives mentioned in the section 1.5 of Chapter I, the conclusions have summarized compiled section wise as given below.

6.1 SINGLE SUBSTRATE BIODEGRADATION STUDIES

(i) The batch aerobic biodegradation experiments were conducted on phenol, resorcinol, and p–cresol separately as single energy and carbon source to the fungal strain. The fungal strain was acclimatized at 1000 mg/L of phenol, at 1300 mg/L of resorcinol, and at 700 mg/L of p–cresol on separate petri plates. The corresponding lag phases were 19 h for phenol, 15 h for resorcinol, and 24 h for p–cresol.

(ii) The batch experiments were conducted at fixed inoculums concentration of 15 mg/L and different substrate concentrations to study the growth and degradation kinetics. The initial concentrations of the substrates varied between 10 to 1000 mg/L for phenol, 10 to 1300 mg/L for resorcinol, and 10 to 700 mg/L for p–cresol. The biomass and substrate concentrations were measured. Phenol, resorcinol, and p–cresol were biodegraded with maximum rate at 70 mg/L, 90 mg/L, and 50 mg/L respectively.

(iii) Five growth kinetic models were fitted to the experimentally observed data of specific growth rate (μ_g) for phenol, resorcinol, and p–cresol. The kinetic parameters

were determined using non-linear curve fitting tool of MATLAB 7.2. The model of Andrews and Noack was found to be the most suitable kinetic model for entire initial concentration range of phenol and p–cresol. The model of Yano provided the best results for resorcinol. The resulting mathematical model equations to represent specific growth rate of biomass in presence of phenol, resorcinol, and p–cresol were as follows:

For phenol

$$\mu_g = \frac{0.462S}{78.29 + S + \frac{S^2}{44.49}} \tag{4.25}$$

For resorcinol

$$\mu_g = \frac{0.185S}{S + 19.83 + \left(\frac{S^2}{376}\right)\left(1 + \frac{S}{1790}\right)} \tag{4.28}$$

For p–cresol

$$\mu_g = \frac{0.512S}{S + 91.87 + \left(\frac{S^2}{21.99}\right)} \tag{4.29}$$

(iv) The variation of maintenance energy expenditure with the specific growth rate (μ_g) was modelled using Pirt's equation. The parameters of this equation were estimated by using least square regression analysis by using the experimental data on specific degradation rate and specific growth rate corresponding to phenol, resorcinol, and p–cresol biodegradation. The model equations to describe maintenance energy coefficient for each substrate are as follows:

For phenol

$$m_S = 0.020 + 0.0212\left(1 - \frac{\mu_g}{0.129}\right) \tag{4.33}$$

For resorcinol

$$m_S = 0.0135 + 0.054\left(1 - \frac{\mu_g}{0.132}\right) \tag{4.34}$$

For p–cresol

$$m_S = 0.0229 + 0.011\left(1 - \frac{\mu_g}{0.102}\right) \tag{4.35}$$

(v) Five kinetic models to represent specific degradation rate were proposed. These models were analogous to five growth models. The model parameters were estimated by using a non-linear least square regression analysis of experimental data on MATLAB 7.2 based on Windows XP. The degradation models analogous to Andrews and Noack, Yano, and Andrews and Noack described well the kinetics for the degradation of phenol, resorcinol, and p–cresol respectively. The degradation kinetic models were as follows

For phenol

$$q_S = \frac{0.631S}{30.04 + S + \frac{S^2}{111.9}} \tag{4.21}$$

For resorcinol

$$q_S = \frac{0.347S}{S + 7.07 + \left(\frac{S^2}{853.5}\right)\left(1 + \frac{S}{2386}\right)} \tag{4.26}$$

For p–cresol

$$q_S = \frac{0.759S}{27.88 + S + \frac{S^2}{73.42}} \tag{4.27}$$

(vi) At each initial substrate concentration, the observed growth yield coefficient was determined by linearizing specific growth rate with specific degradation rate for phenol, resorcinol, and p–cresol. From the trends of observed growth yield coefficient

profiles as a function of initial substrate concentration, it was concluded that the observed growth yield was not constant but varied with the initial substrate concentration similar to the variation of specific growth rate. Maximum observed biomass growth yield $\left(\left(Y_{X/S}\right)_T\right)$ was 0.437 g/g at 70 mg/L for phenol, 0.443 g/g at 90 mg/L for resorcinol, and 0.31 g/g at 50 mg/L for p–cresol.

(vii) Three mathematical models were proposed to simulate the phenol, resorcinol, and p–cresol degradation profiles with time. Out of these three, model – a incorporated the variation of observed growth yield and maintenance energy expenditure with initial substrate concentration to describe the substrate degradation rate. The model equations were solved using ODE solver tool of MATLAB 7.2. The simulated results by model – a agreed well with the experimental results for entire concentration range of phenol, resorcinol, and p–cresol. This model is given in Table 4.3 and is reproduced below for the sake of completeness.

Model – a

(i) $$\frac{dX}{dt} = \mu_g X$$

(ii) $$\frac{dS}{dt} = -q_S X$$

(iii) $$\mu_g = \frac{\mu_{g\max} S}{S + K_S + \left(\frac{S^2}{K_i}\right)\left(1 + \frac{S}{K}\right)}$$ For resorcinol

or

$$\mu_g = \frac{\mu_{g\max} S}{S + K_S + \frac{S^2}{K_i}}$$ For phenol and p–cresol

(iv) $$q_S = \left(\frac{1}{\left(Y_{X/S}\right)_T} - \frac{k}{\mu_{g\max}}\right)\mu_g + k + m_1$$

The model – b (given in Table 4.3), where maintenance energy expenditure was neglected, could predict the close results to the experimental degradation data at lower substrate concentrations only. The model – c (given in Table 4.3), where specific degradation rate (q_S) was defined on the basis of initial rates, failed to yield a satisfactory performance to describe the degradation profiles at any concentration range of phenol, resorcinol, and p–cresol. Thus, the inclusion of substrate inhibition effect on maintenance energy expenditure and observed growth yield seems to be necessary for the description of degradation rate.

6.2 DUAL SUBSTRATE BIODEGRADATION STUDIES

(i) The batch biodegradation experiments were conducted on two dual substrate systems; phenol – p–cresol, and phenol – resorcinol. The fungal strain was acclimatized at different combinations of phenol with p–cresol, and resorcinol at initial concentrations higher than 90 mg/L.

(ii) The batch experiments were conducted in three combinations of concentrations of phenol and p–cresol; 100 mg/L phenol with 300 mg/L p–cresol, 200 mg/L phenol with 200 mg/L p–cresol, 300 mg/L phenol with 100 mg/L p–cresol. The biomass growth and substrate concentration were estimated to study the growth kinetics. The model to describe specific growth rate was derived in the form of SKIP model. The model parameters were estimated by Levenberg-Marquardt nonlinear regression program. Four types of substrate inhibition were tested. Competitive cross inhibition was found to be applicable true. The specific growth rate in phenol – p–cresol system was expressed as given below

$$\mu_g = \frac{0.462S_1}{78.29 + S_1 + \frac{S_1^2}{44.29} + 0.044S_2} + \frac{0.512S_1}{91.87 + S_2 + \frac{S_2^2}{21.99} + 1.17S_1} \qquad (5.54)$$

From the values of interaction parameters $I_{a,1} = 0.044, I_{b,1} = 1.17$, it was concluded that the phenol inhibited p–cresol degradation more than p–cresol inhibited phenol degradation.

The batch degradation experiments were also conducted on phenol – resorcinol system at three combinations; 100 mg/L phenol with 300 mg/L resorcinol, 200 mg/L

phenol with 200 mg/L resorcinol, 300 mg/L phenol with 100 mg/L resorcinol. The growth kinetic model parameters were similarly estimated by Levenberg-Marquardt nonlinear regression program. The resultant specific growth rate expression was as follows:

$$\mu_g = \frac{0.462S_1}{78.29 + S_1 + \frac{S_1^2}{44.29} + 1.09S_2} + \frac{0.185S_2}{19.83 + S_2 + \frac{S_2^2}{376}\left(1 + \frac{S_2}{1790}\right) + 0.052S_1} \tag{5.55}$$

In phenol – resorcinol system also competitive cross inhibition type substrate interaction was applicable. From the values of interaction parameters; $I_{a,1} = 1.09, I_{b,1} = 0.052$ for phenol – resorcinol degradation system, it was concluded that the resorcinol inhibited degradation of phenol more strongly than the phenol inhibited resorcinol degradation. As a result, fungal strain consumed more resorcinol in preference to phenol.

(iii) A conceptual model to describe the variation of maintenance energy expenditure with the specific growth rate in dual substrate system was proposed. For one substrate, this model equation relates the specific degradation rate with the specific growth rate by a quadratic polynomial.

The batch experiments were conducted and specific degradation rate (q_{Si}) values were estimated for each substrate 'i' in two dual substrate systems: phenol – p–cresol, and phenol – resorcinol. The model parameters were computed by curve fitting tool of MATLAB 7.2. The specific degradation rate for each substrate in two dual substrate systems were expressed as follows

phenol – p–cresol system

For phenol:

$$q_{S1} = 0.038 + 1.071\mu_{g1} - 2.460\mu_{g1}^2 \tag{5.56}$$

For p–cresol:

$$q_{S2} = 0.046 - 2.175\mu_{g2} + 62.56\mu_{g2}^2 \quad (5.57)$$

phenol – resorcinol system

For phenol:

$$q_{S1} = 3.369 - 108.2\mu_{g1} + 886.7\mu_{g1}^2 \quad (5.58)$$

For resorcinol:

$$q_{S2} = 2.520 - 51.34\mu_{g2} + 274.9\mu_{g2}^2 \quad (5.59)$$

The values of regression coefficient (R^2 = 1) indicated that the proposed model agreed very well with experimental data. Thus, it was concluded that wide variation in specific degradation rate values from the experimental values can be rectified by incorporating maintenance energy expenditure to model the specific degradation rate.

(iv) The substrate degradation profiles with time were computed for both the dual substrate systems, phenol – p–cresol, and phenol – resorcinol, by solving mathematical equations by ODE solver tool of MATLAB 7.2. The simulated results provided an excellent prediction of substrate degradation rate in dual substrate system.

In dual substrate systems, the dynamic modelling studies by Juang and Tsai (2006) could predict the time profiles only for the substrates present in an equimolar mixture or for the substrate initially present in a large amount. In the present study, the proposed dynamic modelling study is applicable for any combination of substrate concentrations in dual substrate systems.

The modelling and experimental studies on biodegradation of phenol, resorcinol, and p–cresol in single and dual substrate systems revealed that the filamentous fungus *G. indicus* has potential to be used in wastewater treatment and also for the bioremediation of soil contaminated with resorcinol and p-cresol. Further, the proposed modelling study would be useful for the optimal design and operation of aerobic biological treatment units. For designing a bioreactor unit to treat industrial wastewaters, a complete knowledge of interaction patterns and quantification of

interaction parameters are very important. The results and conclusions presented in the present research are of practical significance for safe and stable design of biodegradation unit to provide more efficient degradation of pollutants in wastewater.

6.3 RECOMMENDATIONS FOR FUTURE WORK

(i) As the present research work pertains to biodegradation studies of phenol, resorcinol, p–cresol containing wastewaters in single and dual substrate batch biodegradation systems, the biodegradation studies in continuous systems such as fixed or fluidized bed reactors are desirable.

(ii) In this research work, basic constitutive relationships such as growth kinetics, the degradation dynamics with the effect of variation in biomass growth yield and expenditure of substrate in the form of maintenance energy have been obtained for the biodegradation of phenol, resorcinol, and p–cresol by *Gliomastix indicus*. Hence, it is suggested that the modelling and simulation of fixed or fluidized bed reactors for biodegradation can be performed. Experimental results may be obtained and utilized to validate the developed models.

(iii) In this research work, biodegradation of phenol, resorcinol, and p–cresol in the single substrate degradation system has been done separately, and the biodegradation studies of these substrates have been conducted in the dual substrate systems as well. However, the efficiency of biodegradation can be further enhanced by using the bio-activated carbon (BAC), where *G. indicus* shall be immobilized on PAC (Powered Activated Carbon). Thus, studies on the BAC with *G. indicus* for the treatment of phenolic wastewaters are desirable.

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