12-2013

Biodegradation of Cresols Using Pseudomonas Putida Immobilized in Poly Vinyl Alcohol (PVA) Gel

Riham Mohamed Sorkatti Abu Bakr

UAEU College of Engineering

جامعة الإمارات العربية المتحدة
United Arab Emirates University

United Arab Emirates University

College of Engineering

Department of Chemical and Petroleum Engineering

Biodegradation of Cresols Using *Pseudomonas Putida* Immobilized in Poly Vinyl Alcohol (PVA) gel

Riham Mohamed Sorkatti Abu Bakr

This thesis is submitted in partial fulfillment of the requirements for the Master of Chemical Engineering degree

Under the direction of Professor Muftah Hassan El-Naas

December 2013

Declaration of Original Work

I, Riham Mohamed Sorkatti Abu Bakr, the undersigned, a graduate student at the United Arab Emirates University (UAEU) and the author of the thesis/dissertation titled " Biodegradation of Cresols Using *Pseudomonas Putida* Immobilized in Poly Vinyl Alcohol (PVA) gel", hereby solemnly declare that this thesis/dissertation is an original work done and prepared by me under the guidance of Prof. **Muftah Hasan El-Naas**, in the College of Engineering at UAEU. This work has not been previously formed as the basis for the award of any degree, diploma or similar title at this or any other university. The materials borrowed from other sources and included in my thesis/dissertation have been properly acknowledged.

Student's Signature.....Date...6/2/2014

Declaration of Copyright License

I, Riham Mohamed Sorkatti Abu Bakr , the undersigned, a graduate student at the United Arab Emirates University (UAEU) and the author of the thesis/dissertation titled " Biodegradation of Cresols Using *Pseudomonas Putida* Immobilized in Poly Vinyl Alcohol (PVA) gel", whose copyright is declared on the title of this thesis, grant access to UAEU the right to distribute my thesis to the users of UAEU Library, and to make partial or single copies only for such users or in case of a request from the library of any other university, or other education institution, on its behalf or for one of its users.

I further grant permission to UAEU to keep or make a digital copy for use in its circulating collection and without, changing the content to translate the thesis, if technically possible, to any medium or format for the purpose of the preservation of the digital work.

I further agree that permission for multiple copying of this work for scholarly purposes may be granted by either the author or the Dean of the College of Graduate Studies.

Student's Signature.....Date...6/2/2014

Signature Pages

Thesis Examination Committee:

1) Advisor: Prof. Muftah Hassan El-Naas

   Title: Professor

   Department: Chemical and Petroleum Engineering

   Institution: College of Engineering, UAE University

   Signature _____ Date 6/2/2014

2) Member: Dr. Sulaiman Al Zuhair

   Title: Associate Professor

   Department: Chemical and Petroleum Engineering

   Institution: College of Engineering, UAE University

   Signature _____ Date 6/2/2014

3) External Examiner: Prof. Christian Kennes

   Title: Professor

   Department: Chemical Engineering, School of Engineering

   Institution: University of Santiago de Compostela, Spain

   Signature C.O. Sulaiman Al-Zuhair Date 6/2/2014

Accepted by

Master's Program Director Dr. Sulaiman Al-Zuhair

Signature:_____ Date: 6/2/2014

Dean of the College Prof. Amr El-Dieb

Signature:_____ Date: 17/3/2014

Copy____of____

ABSTRACT

The characteristics and viability of polyvinyl alcohol (PVA) as a support material for biomass immobilization and utilization for the biodegradation of cresols have been evaluated. PVA gel pellets were prepared by repeated freezing-thawing method using different PVA compositions. The porous structures of the PVA pellets were examined using a Compound Microscope and SEM and revealed that the matrix structure and pore size distribution were affected by PVA composition. Mechanical properties of the PVA gel were also characterized to evaluate the PVA integrity as a matrix for immobilizing microbial cells and found to be dependent on the PVA mass composition. The prepared pellets were utilized for the immobilization of *Pseudomonas putida* and biodegradation of phenol over a long period of time. The results revealed that the capabilities of the biomass to degrade phenol increased with time and were depended on the PVA mass content and porous structure.

Batch and continuous experiments were also carried out to evaluate the biodegradation of cresols (*o*-, *p*- and *m-cresol*) using *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel in spouted bed bioreactor (SBBR). The effect of initial substrate concentration, temperature, pH and volume fraction of PVA particles on the biodegradation of each cresol isomer was evaluated. Continuous experiments were also carried to study the effect of other operating parameters such as air flow rate and residence time on the biodegradation efficiency. The biodegradation capabilities of *P.putida* were found to be highly affected by operating parameters and the dependency varied for each cresol isomer. In addition, the potential of *P.putida* in the biodegradation of binary and ternary

mixtures of cresols was examined in the continuous process and compared with single component biodegradation. Batch experimental data for each system were used to evaluate the kinetics parameters in order to utilize them in modeling the continuous biodegradation process. The model predictions were in very good agreement with experimental results.

ACKNOWLEDGMENTS

Sincere thanks are expressed to my advisor Prof. Muftah El-Naas for his guidance, support and constructive feedback throughout my thesis study. I am grateful for the time he has taken to guide and support my efforts.

My thanks extended to my parents and sister who helped me along the way. I am sure they suspected it was endless. Also, special thanks are extended to all members of the Chemical and Petroleum Engineering Department at the UAE University for assisting me during my studies and research.

Finally, I would like to acknowledge the financial support provided by the Japan Cooperation Centre Petroleum (JCCP) as part of the JCCP Project C and the technical support of the JX Nippon Research Institute Co., Ltd (JX-NRI). I would also like to thank Manal Abu Al-Haija, Sami Abdulla and Abd-El Satar Nour El-Deen for their help with the experimental work.

DEDICATION

This thesis is dedicated to my parents, sisters, uncles and friends who have supported me all the way since I began my postgraduate study. I would like to extend this dedication to my thesis advisor whose motivation and encouragement was a great source of knowledge and experience.

Table of Contents

LIST OF PUBLICATIONS

1. Surkatti, R. and M. H. El-Naas, Experimental evaluation and modeling of the biological treatment of wastewater contaminated by *p-cresol* using *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel, *Journal of Water Process Engineering*, under review.

2. El-Naas M. H., A-H. I. Mourad, and R. Surkatti, Evaluation of the Characteristics of Polyvinyl alcohol (PVA) as Matrices for the Immobilization of *Pseudomonas Putida*, *International Biodeterioration & Biodegradation* 85 (2013) 413-420.

3. Surkatti R.,M. H. El-Naas and A-H Mourad, Evaluation of the Mechanical Properties of Poly Vinyl Alcohol (PVA) Gel for Biomass Immobilization, *The World Congress of Chemical Engineering, WCCE 9*, August 2013, Seoul, Korea.

4. El-Naas, M. H. and R. Surkatti, Evaluation of the Biodegradation of *P*-cresol in a Spouted Bed Bio-Reactor (SBBR), *The 15th International Biotechnology Symposium*, Daegu, Korea, September 2012.

5. Surkatti, R. and M. H. El-Naas, Biodegradation of *O-cresol* by *Pseudomonas Putida* Immobilized in Polyvinyl alcohol (PVA), *The 1st International Conference on Desalination and Environment*, Abu Dhabi, UAE, November 2011.

6. El-Naas, M. H., A. I. Mourad, S. Al-Zuhair, and R. Surkatti, Characterization of Polyvinyl alcohol (PVA) Gel as Support for Microbial Immobilization , *Journal of Biotechnology*,150, Supplement 1 (2010).

LIST OF TABLES

LIST OF FIGURES

LIST OF NOMENCLATURE

F_s	volumetric flow rate (l/hr)
K_I	substrate inhibition constant (mg/l)
K_s	substrate affinity constant (mg/l)
q_{max}	maximum degradation rate (h^{-1})
q_s	specific consumption rate (h^{-1})
$-r_s$	rate of removal of substrate (mg/l. h)
r_x	microorganism growth rate (mg/l. h)
S	substrate concentration (mg/l)
S_o	initial substrate concentration (mg/l)
T	time (h)
V	reactor volume (l),
X	biomass concentration (mg/l)
Y	cell mass yield (g/g)
μ	Specific growth rate (h^{-1})

CHAPTER ONE

Introduction &Literature Review

1.1. Introduction

Phenol and its methylated derivatives, cresol isomers (*o-*, *m-* and *p-cresol*), are common pollutants from various industrial effluents including oil refineries, pharmaceutical, petrochemical, dying varnish, coal conversion and paper production plants [1-4]. Hence, it is important to reduce phenols concentration in industrial effluents to tolerant level prior to being discharged into the environment. The treatment alternatives such as solvent extraction [5], activated carbon adsorption [6], chemical oxidation [7] and ion exchange [8] have their drawbacks, such as high cost and hazardous by-products formation. Biological treatment has gained considerable interest in removing organic pollutants. Biodegradation is versatile, environmental friendly, inexpensive and has an ability to mineralize the organic pollutants [9-15]. Several aerobic and anaerobic microorganism have shown

Acinetobacter sp.[11, 16], *Peacilomyces variotii* [17], *Panarochaete chrysosporium* [18-20], *Burkholderia vietnamiensis* [21], *Ralstonia eutropha* [22] and *Pseudomonas putida* [23].

Pseudomonas putida is a member of the fluorescent pseudomonad group, which grows optimally at room temperature. *P.putida* is a kind of soil bacteria thatare able to colonize in several habitats, including soil, freshwater, and the surfaces of living organisms [24, 25]. The genus *Pseudomonas* contains the most versatile organisms with the ability to mineralize various complex aromatic compounds [26], therefore it has great potential for different biotechnological applications, particularly in the areas of bioremediation and biocatalysts [27].

1.1. Introduction

Phenol and its methylated derivatives, cresol isomers (*o-*, *m-* and *p-cresol*), are common pollutants from various industrial effluents including oil refineries, pharmaceutical, petrochemical, dying varnish, coal conversion and paper production plants [1-4]. Hence, it is important to reduce phenols concentration in industrial effluents to tolerant level prior to being discharged into the environment. The treatment alternatives such as solvent extraction [5], activated carbon adsorption [6], chemical oxidation [7] and ion exchange [8] have their drawbacks, such as high cost and hazardous by-products formation. Biological treatment has gained considerable interest in removing organic pollutants. Biodegradation is versatile, environmental friendly, inexpensive and has an ability to mineralize the organic pollutants [9-15]. Several aerobic and anaerobic microorganism have shown high performance in the biodegradation of organic compounds including; *Acinetobacter* sp.[11, 16], *Peacilomyces variotii* [17], *Panarochaete chrysosporium* [18-20], *Burkholderia vietnamiensis* [21], *Ralstonia eutropha* [22] and *Pseudomonas putida* [23].

Pseudomonas putida is a member of the fluorescent pseudomonad group, which grows optimally at room temperature. *P.putida* is a kind of soil bacteria thatare able to colonize in several habitats, including soil, freshwater, and the surfaces of living organisms [24, 25]. The genus *Pseudomonas* contains the most versatile organisms with the ability to mineralize various complex aromatic compounds [26], therefore it has great potential for different biotechnological applications, particularly in the areas of bioremediation and biocatalysts [27].

Recently, biomass immobilization is a new technology that has received increasing interest in the area of wastewater biotreatment [28, 29]. Compared with free cells, immobilized cells have several advantages, such as increasing mechanical strength, reducing reactor volume and providing stable treatment. Moreover, immobilization of biomass improve the biomass performance due to the increasing the biodegradation rate through a higher cell loading, easily controlled, protecting microorganisms from harsh environmental conditions and wash out from the reactors [30-32]. Among the gel carriers, polyvinyl alcohol (PVA) is a promising synthetic polymer to be used as bacterial immobilization matrix; PVA is inexpensive, non-toxic, non-carcinogenic, and water-soluble. It is a white soluble high-molecular compound, not soluble in ordinary organic solvents. PVA has relatively good tensile strength, impact strength, and wear resistance. It also has excellent electrical insulation and more durable than ca-alginate [31, 33, 35]. Several cross-linking methods have been proposed in order to improve chemical stability and mechanical properties such as PVA-boric acid and PVA-Ca methods (chemical cross-linking), however, these cross linking agents are difficult to eliminate after preparation. Other cross linking techniques using iterative freezing thawing technique (physical cross linking), which does not involve the presence of cross linking reagents, is more favorable for cells immobilization. In addition to their nontoxic nature, these freeze/thawed gels have demonstrated enhanced mechanical properties and highly porous structure [35, 36].

1.2. Literature Review

Due to their wide use in different industrial fields, phenolic compounds such as cresols constitute are an important class of pollutants that can cause adverse effect on the aquatic ecosystem. Several techniques have been evaluated in the literature in order to develop effective approaches to reduce the concentration of these toxic compounds in waste stream. This literature review focuses on the effect of cresols on human life, current removal technologies, biodegradation process and other treatment alternatives.

1.2.1. Cresols

Cresols are phenol derivatives also known as methylphenols or hydroxytoluenes; they enter the environment through three isomeric forms (*ortho*, *meta*, and *para* or *o*, *m*, and *p*). They are polar molecules that are water soluble and have frequently been found in groundwater [37]. They are known to be efficiently stripped from gas phase by rain and fog, ending up in aquatic systems [38, 39]. Moreover, they have been detected in sediment, soil, surface, mainly near point sources [40-43]. Cresols have been used extensively in a number of industrial applications, including disinfectants production, resin, surfactants, dye intermediates, photographic development and pesticides manufacturing, as well as in the area of pharmaceutical, food and motor fuels [44]. *o-Cresol* is used as precursors of dye intermediates, synthesis of epoxy resins, pharmaceuticals and adducts for various synthetic uses [46]. *P-cresol* has a range of uses including, fumigants, explosives, and photographic development [45]; where *m-cresol* forms a basis of fragrance and flavor substances, disinfectants, preservation and

explosives [46]. The concentration of these compounds in agro-industrial wastewaters can ranged from 0.05 to 10 g/l, depending on the type and origin of the effluent [47].

High distribution of cresols in the environment indicates the extensive exposure to humans. However, levels of exposure depend on the lifestyle, occupation and location. Many government agencies recommend discharge limits for hazardous contaminants in wastewater released from industries. Legislations in the UAE limit the total phenols in industrial water discharged to the marine environment to 0.1 mg/l, compared to that set by USEPA of 0.168 mg/l. According to the Agency of Toxic Substances Registry (ATSDR), cresols are potentially carcinogenic and highly toxic. United State Environmental Protection Agency (USEPA) has classified cresols within group C: possible human carcinogenic; this same agency has recommended that cresols cause, even at low concentration, adverse effects on the nervous system, cardiovascular system, lungs, kidney and liver, as well as respiratory irritations in animals and humans. In humans, cresols or their metabolites are detected in tissues and urine following inhalation, dermal, or accidental and intentional oral exposure, in addition to causing corrosive damage at the site of contact [48-50].

1.2.2. Removal Technologies

Benzene derivatives are less reactive and more stable than single-chain compounds due to the large amount of energy required to break a part of the cyclic structure [51]. Therefore, efforts are now being made to destroy phenolic compounds using environmental friendly techniques, without external resources of energy. Several physical, chemical and biological technologies are available and have shown to be effective in treating a variety

of phenolic pollutants in the aqueous streams [46, 50, 52, 53]. Although the removal of phenolic components in general, and phenol in particular have been well documented in the literature [6, 54], few investigations were reported about the removal of cresols from wastewater stream by physical and chemical technologies such as chemical oxidation [7], photocatalytic degradation [55] photoelectro-Fenton degradation [56] and ozone oxidation [57].

Fenton process has been applied for treating wastewater containing cresols in batch reactors [58]. Hadjar et al. [59] reported that *p-cresol* can be removed using novel technique of carbon/diatomite composites prepared from diatomite and a carbon precursor. In recent years, electrochemical degradation has gained much attention in cresols removal; Groenen-Serrano et al. [47] reported the successful removal of cresols (*o-*,*m-* and *p-cresol*) via electrochemical interaction using flow filter-press reactor. Chu et al. [60] also studied the electrochemical degradation of *m-cresol* using porous carbon-nanotube. Suarez Ojeda et al. [61] have successfully reported the combined use of two stages of physical and biological treatment of wastewater for high strength *o-cresol*. The process consist of Catalyst Wet Air Oxidation (CWAO) with activated carbon (AC), followed by aerobic biological treatment using activated sludge.

1.2.3. Biodegradation process

The last few decades has shown that the use of biological treatment for the removal of organic pollutants from wastewater stream has been very effective. Generally, the biodegradation of phenolic compounds in wastewater has been widely studied using different microorganisms [10, 11, 13], including bacteria [30, 62, 63], fungus [17, 18], and yeast [64]. In spite of the toxic properties of cresols and the presence of a methyl

group in cresol molecules, which inhibits their degradability (cresol-degradation), a number of microorganisms can utilize cresol as a sole source of carbon and energy required for their growth and survival, under aerobic and anaerobic conditions [65-69].

Perron et al. [39] studied the degradation of relatively high phenol and cresols *(o-,p-* and *m-cresol)* concentration at low temperature in suspended- carrier biofilm process using two aerobic stages, with fungus followed by bacteria. The biodegradation of *o-cresol* using immobilized *Pseudomonas Putida* in batch recirculation bioreactor system was performed, and the kinetics parameters of *o-cresol* aerobic biodegradation were investigated using Haldane inhibition model by Pazarlioglu et al. [49].

P-cresol biodegradation has been studied by number of researchers as a pure compound and in mixtures [69]. Arya et al. [66] reported that the biodegradation of *p-cresol* and resorcinol can take place using filamentous fungus *Gliomastix indicus* for *p-cresol* concentration up to 700 mg/L in the batch culture experiments at 28 °C and pH of 6. The effect of initial substrate concentration on maintenance energy and the substrate degradation profile have been modeled for both compounds. Additional work by Kumar et al. [65] examined the biodegradation kinetics of *p-cresol* as a single compound using pure culture, *Gliomastix indicus*. The effect of pH on the growth rate and the nitrogen source effect were examined. In addition, the initial concentration of *p-cresol* ranging from 10-700 mg/l was tested using mixed culture in batch reactor [70]. A Sequencing Batch Reactor (SBR) was used by Basheer et al. [45] for *p-cresol* biodegradation as a pure component. The study was performed at initial concentrations up to 800 mg/L and the kinetics analysis was carried out according to Haldane model.

According to literature *m-cresol* does not provide necessary energy to sustain the growth of microbial culture in maintaining high process efficiency [3]. Therefore, there is a need for the presence of simple alternative carbon source, such as glucose to support the growth of microorganisms [3]. The Biodegradation of mixture of phenol and *m-cresol* has been examined by many researchers using several types of microorganisms [4, 12, 42, 64, 71, 72]. The biodegradation of mixed substrate of *m-cresol* and pyridine was studied using *Lysinibacillus cresolivorance*, as well as, *cresolivorans* and was found to exhibit a great potential in degrading *m-cresol* as a single component; furthermore, the kinetics and characterization of *m-cresol* has also been investigated [73, 74].

1.2.3.1. Factors that affect the biodegradation process

Despite the high efficiency and the low cost of the biodegradation process for the removal of toxic compounds from wastewaters, the method still has some restrictions. The rate of biodegradation depends, not only on the properties of the substrate and the presence of microorganisms able to degrade the toxic compound, but also on environmental conditions such as oxygen content, temperature, pH, availability of nutrients and the presence of other substances [75-77]. Each of these parameters should be optimized to obtain a high degradation performance. Substrate concentration is one of the most important parameters that affect the biodegradation process. The inhibition of substrate may take place, due to the formation of intermediates and extracellular products. The presence of these components may decrease the biomass yield and increase the maintenance energy to overcome the inhibition effect [78-80]. Substrate inhibition is

a feature of phenol metabolism for different microorganisms, depending on the concentration level of phenol [10, 17, 81].

Operating conditions can also affect the efficiency of the biodegradation process. Enzymes are greatly stabilized by weak hydrogen bonds, and thus highly affected by the variation of pH [82]. It is well known that many microorganisms obtain their maximum growth rate at a pH range of 6.5 to 7.5 [65]. Temperature is another important factor that affects the biodegradation process. An increase in temperature results in more kinetics energy for the enzymes molecules and reactants, thus enhancing the rate of reaction due to increasing the frequency of collision. At high temperature sufficient energy is gained to overcome the weak interactions holding the globular protein together, and deactivation follows [82, 83]. According to Yong Lu et al. [18], the biodegradation of phenolic compounds was highest at temperature ranging from 28 to 37°C. However the biodegradation of phenol and *p-cresol* was extensively inhibited at 40°C.

Single compounds are rarely present, while mixtures of complex substrates that are not always easily degradable are often confronted. Biodegradation of mixed substrate is more complicated than that for single compound. The biodegradable process of mixed substrate can be slow or inefficient, particularly when the substrates are recalcitrant to microbial biodegradation. More recently, detrimental interactions were shown for different classes of organic pollutants present in mixtures; it was suggested that they interfered with enzymatic induction thereby repressing the mechanism for catabolism [84]. However, when external source of carbon are introduced as energy source, it helps in the synthesis of the enzymes involved in the metabolic breakdown of recalcitrant compounds [4, 85].

1.2.3.2. Pseudomonas putida

Several aerobic and anaerobic microorganisms have shown high performance in the biodegradation of organic compounds. Aerobic microorganisms are more efficient for degrading toxic compounds such as cresols, because they grow faster than anaerobes and usually transform organic compounds to inorganic compounds (CO_2, H_2O) [17, 67]. Many aerobic bacteria are capable of using aromatic compounds as the sole source of carbon and energy [80, 86]. *Pseudomonas putida* has been studied widely by many researchers for the degradation of phenols in several types of batch and continuous bioreactors, at different conditions [87, 88]. These include: degradation of 2,4-dichlorophenol and *4-Cl-m-cresol* [89]; kinetics biodegradation of phenol and catachol [90]; degradation of phenol and sodium salicylate mixture by suspended *P.putida* [10]; biodegradation of phenolic industrial wastewater in fluidized bed bioreactor[63]; degradation of propargite [23]; degradation of phenol and TCE [30] and biodegradation of phenol in trickle bed reactor [91].

1.2.3.3. Cresols biodegradation mechanism

In almost all cases, the important pathway for cresol biodegradation using *Pseudomonas* species is initiated by the formation of *3-methylcatechol* for *o-cresol* and *4-methylcatechol* in *p-cresol*; mixture of *3-methyl* and *4-methylcatechol* was found to be the first intermediate from *m-cresol* [92]. The resulting catechol derivatives molecule can be degraded by two alternative pathways depending on the responsible microorganisms. In the *ortho* pathway, the aromatic ring is cleaved between the catechol hydroxyls by a catechol *1,2-oxygenase*, or *2,3-oxygenase* in the meta pathway. The appearance of the

yellow color in the mixture indicates meta pathway for the phenols biodegradation mechanism [93].

It was proposed by Hopper and Taylor [93] that the biodegradation of *m-cresol* biodegradation is metabolized by the hydroxylation to methyl- substitute catechol and ring-fission by meta cleavage, when the microorganisms grow by *m-cresol*. Whereas *p-cresol*, with *4-methylecatechol* as an intermediate, is degraded by meta cleavage pathway when the biomass grows by phenol or any other cresol isomers. Another pathway for *p-cresol* biodegradation, initiated by the oxidation to the *4-hydroxybenzoate* and *protocatechuate*, was proposed which indicated *ortho* cleavage [92]. The biodegradation pathways of the (*o-*, *p-* and *m-cresol*) are shown in Figures 1.1.

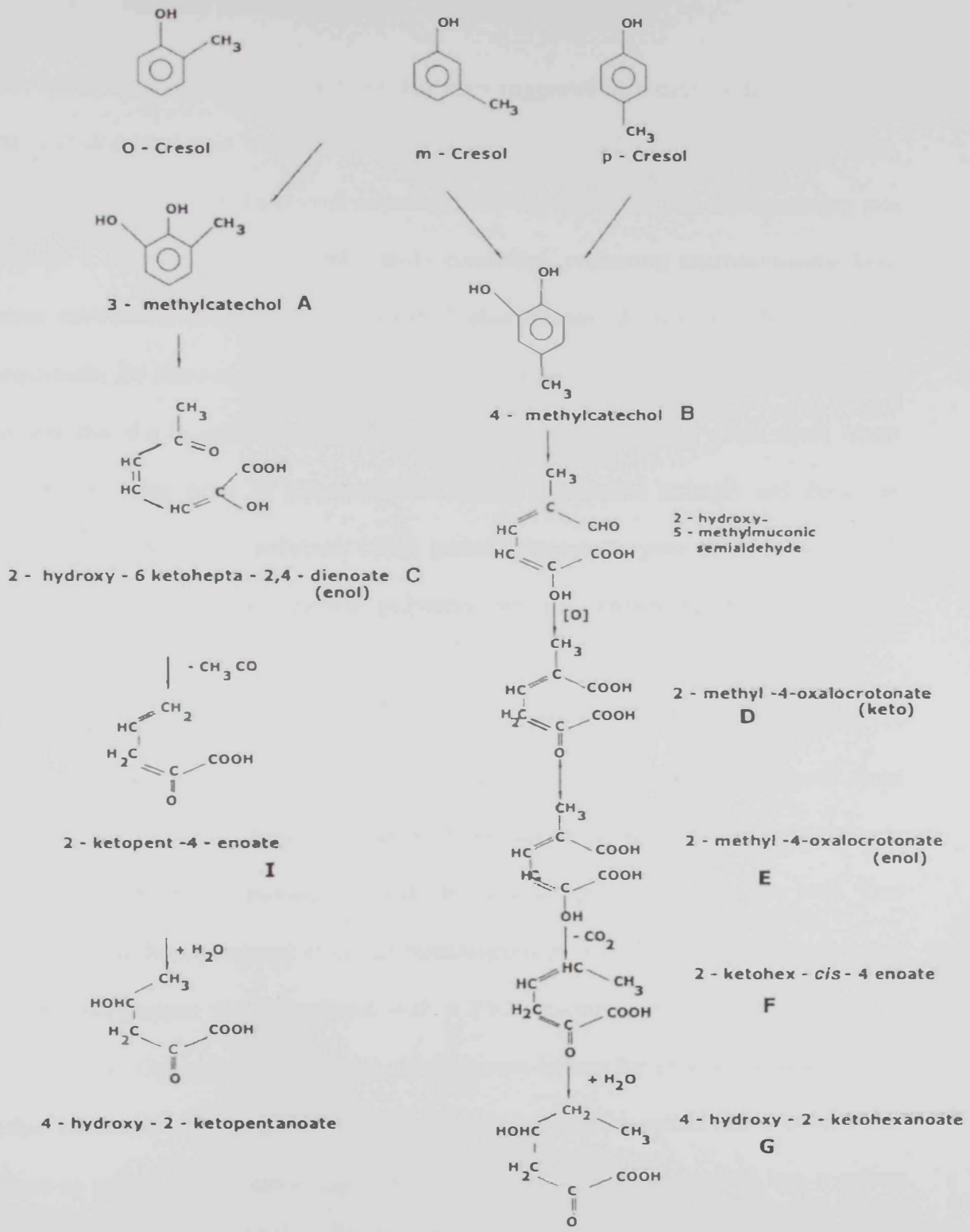


Figure 1.1: Pathways for the biodegradation of *o-*, *p-* and *m-cresol* by *Pseudomonas Putida* [92]

1.2.3.4. Biomass Immobilization

The immobilization of microorganisms has been suggested as a strategy for maintaining efficient degradation in wastewater treatment systems. When compared with free cells, the immobilized cells have several advantages such as increasing the biodegradation rate through a higher cell loading [94], easily controlled, protecting microorganisms from harsh environmental conditions, allowing higher biomass density providing a greater opportunity for reuse and recovery and reducing reactor volume [30, 31, 95]. It has been proved that the immobilization of enzymes on synthetic polymer gives much better reusability; these types of polymers have strong mechanical strength and durability compared to the natural polymers which generally possesses poor mechanical strength and durability. However, natural polymers are not known to be toxic to the microorganisms [96, 97].

Many gel matrices have been proposed as possible carriers for immobilization of biomass. The use of polyvinyl alcohol (PVA) as an immobilization carrier started about 20 years ago [98]. It is a polymer of great interest due to its desirable characterization in the area of biosensors, biocatalysts and biomedical applications [32, 99, 100]. Two methods have been proposed to entrap microorganisms using PVA; in both techniques, the microorganisms are first mixed with a PVA viscous solution, then the PVA is polymerized. One technique is the chemically cross-linking by adding chemical and the other by iterative freezing-thawing. Microorganisms can be immobilized using PVA, alone or mixed with alginate, into spherical gel beads through dripping into a gelatin solution [21, 34]. This chemical technique has been used in Baker's yeast immobilization using four PVA-alginate compositions. In order to investigate the enzymes activity and

comparing with the free enzymes, PVA and calcium alginate were prepared by a freezing and thawing cycle process and used for activated sludge immobilization. The characterization and evaluation of some physical properties for the PVA-Ca alginate system was investigated by (Ca-alginate) [101]. It was proposed that the strain *Burkholderia vietnamiensis* C09V to be immobilized using on PVA–alginate–kaolin gel beads (chemical cross-linking) as a biomaterial to improve the degradation of crystal violet from aqueous solution [21]. *Bacillus subtilis* cells entrapped in polyvinyl alcohol PVA-cryogel bed reactor, generated by using PVA–alginate beads, efficiently removed DMF from industrial effluents, even in the presence of certain organic solvents [102].

Other cell immobilization techniques involve the preparation of PVA gel by physical cross-linking through the freezing/thawing method. In the field of wastewater treatment, El Naas et al. [75] used the PVA freezing-thawing technique at -20°C to immobilize *P.putida*. Dang-sheng et al. [103] also applied the freezing-thawing technique at -20°C for 10 h and thawing at room temperature for 2 h. Polyvinyl alcohol (PVA) was also used as a gel matrix to immobilize *Acinetobacter sp.* strain PDI 2by repeated freezing and thawing [11].

Previous research showed that physical parameters such as concentration and number of freezing-thawing cycles can highly affect the prepared polymer, and the prepared polymer becomes harder by the increasing the freezing-thawing cycles [104].

Mechanical properties become important as polymer technology moves from laboratory into process development. The prediction and control of mechanical properties in gels is of great importance in assessing the applicability of gels. Many researchers have focusedonstudying the mechanical properties of PVA gels. Gauthier et al. [105] have

studied the degree of cross-linking and mechanical properties of cross-linked PVA beads cross-linked with epichlorohydrin (PVA-EP) use in solid-phase organic synthesis. Jeremie et al. [106] have characterized the mechanical properties of polyvinyl alcohol (PVA) cryogel in order to investigate its utility for intravascular elastography. They have shown that PVA cryogel becomes harder with increasing number of freeze-thaw cycles. Kristi et al. [107] studied the dependence of hydrogel mechanical properties on various parameters that are usually examined in material selection process. The results showed that the mechanical properties are highly dependent on the polymer structure, especially the cross-linking density and the degree of swelling. It has been reported by Stammen et al. [108] that the critical barrier to Poly(vinyl alcohol) (PVA) hydrogels used as biomaterials (load-bearing tissue replacements) is the lack of sufficient mechanical properties. Compressive failure of the hydrogels was found to occur between 45 and 60% strain, depending on water content.

1.3. Aims of the Study

The overall objective of this thesis is to evaluate the biodegradation of cresols using *P. putida* immobilized in PVA Gel. This is divided into the following main objectives:

- Characterize PVA gel prepared by the freezing-thawing technique as support material for biomass immobilization, and study the performance of the prepared PVA gel in phenol biodegradation for long periods of time.

- Investigate the effect of several parameters such as initial substrate concentration, operating temperature, pH and PVA volume fraction on the batch biodegradation of *o-,p-* and *m-cresol*. Moreover, study the effect of initial concentration, air flow rate, liquid flow rate and residence time on the continuous biodegradation process.
- Investigate the performance of immobilized bacteria in the biodegradation of binary and ternary mixture of cresols in the continuous mode, and compare it with the biodegradation of single component. Finally, use Monod and Haldane kinetics to model the continuous biodegradation process using data generated from the batch experimental work.

CHAPTER TWO

Materials &Analytical Methods

2.1. Materials

A special strain of the bacterium *P. putida* was obtained from Cleveland Biotech. LTD, UK in a cereal from (AMNITE P300). The cereal contains a consortium of microorganisms, with *P. putida* as the dominate strain. Analytical grade phenol and cresols (*o-* , *p-* and *m- cresol*) was purchased from BDH Chemicals, UK. Synthetic phenol and cresols solutions were prepared for the desired concentration in distilled water before each experimental run. The solutions were always kept in a brown flask to avoid light oxidation of the phenol. All other chemicals and PVA powder were of analytical grade and were also obtained from BDH, UK.

2.2. Analytical Techniques

2.2.1. Microscopic Analysis

Cuts of PVA pellets were prepared as thin sections using microtome sectioning machine (Leica RM2255). PVA pellets were cut into thin sections (~20 µm) in a the microtome using stainless steel microtome blade. Images were obtained using Compound Microscope (Leica DM6000), for the microscopic analysis, adjustments of the diaphragms and filters as well as adjustments of luminous intensity to the magnification and contrast methods can be carried out and reproduced automatically. The transmitted-light illumination system is based on a commercially available 12 V, 100 W halogen bulb, the luminous intensity of which is automatically adjusted to the pre-set value based on the density of the specimen and the light stream in the object. The different transmitted-light methods generally require different condensers and/or condenser heads.

2.2.2. Analysis of Cresols

Phenol and cresols concentrations in the biomass free samples were determined quantitatively using Chrompack Gas Chromatograph, Model CP9001. Analysis has been accomplished by GC with flame ionization detection (GC/FID) Model CP9001. Samples were filtered through a filter syringe with (0.45μm pore size). The GC was equipped with a capillary column (Stabilwax, 30m, 0.25 mm ID) and a flame ionization detector which was set at 250 °C. A sample of 1 μl was filtered through syringe filter with pore size of 0.45 μm and injected into the GC. The temperature program started at 100 °C and increased at a rate of 20 °C min^{-1} to 180 °C. The accuracy of the analyzer was checked to be within ±0.5 mg/l. Measurements for each phenol and cresols samples were carried out in duplicates and a standard solution was used to recheck the accuracy of the GC after every 4 h of continuous operation.

All experimental results reported in this thesis were based on averaging results of repeated experimental runs (duplicates), with the standard deviation ranging from 2 to 5% of the reported average. It must be mentioned that *m*-and *p-cresol,* could not be separated properly in the analysis due to the overlapping between two picks.

2.3. *Experimental Procedure*

2.3.1. Preparation of bacterial cereal

Bacterial suspension was prepared as reported by El Naas et al. [75]. 100 g of the bacterial cereal was mixed with 1000 ml of 0.22 % hexametaphosphate solution buffered by Na_2CO_3 to a pH of 8. The mixture was homogenized in a blender for about 1h, decanted and kept in the refrigerator at 4°C for 24h. Bacteria slurry was prepared by four

consecutive steps of low speed centrifugation at 6000 rpm for 15 min. The supernatants were collected and centrifuged again at 10,000 rpm for 20 min. The precipitated amount from the three centrifugations (which contains the harvested bacteria cells) were collected, suspended as slurry in distilled water and kept in the refrigerator for subsequent immobilization.

2.3.2. Preparation of PVA gel

2.3.2.1.Variation of freezing-thawing time

Homogenous PVA solutions were prepared by dissolving 50, 100 and 150 g of PVA powder in warm distilled water (70-80 °C), to prepare 1 L of solution having PVA contents of 5, 10, and 15 mass %. Heating and mixing by a glass rod continued during the entire dissolving process to form well-mixed homogenous solutions. The prepared PVA solutions were then allowed to cool at room temperature before adding 10 ml of the previously prepared bacterial suspension. The PVA-biomass solutions were then well stirred for 10-15 min to insure homogeneity and then poured into molds and kept in a freezer at about -4 °C. PVA matrix was cross-linked by repetitive freezing-thawing method (physical cross linking). Several thawing periods were examined in the cross-linking step. The prepared gels were thawed for different periods of time (3, 6, 9, 12, 18 and24 h)at room temperature. The samples were then frozen at -4 °C for 24 h .The freezing-thawing process was repeated 5 times, and PVA particles were rinsed with distilled water to remove any uncross-linking.

2.3.2.2.Variation of PVA mass content

Homogenous PVA solutions were prepared by dissolving 50, 100, 150 and 200 g of PVA powder in warm distilled water (70- 80 °C), to prepare 1 L of solution having PVA contents of 5, 10, 15 and 20 mass % following the same procedure described in the previous section. The only difference here is that the freezing and thawing periods were fixed at 24 h and 3h, respectively.

2.3.2.3.Mechanical testing of PVA pellets

Unlike the majority of polymers, swollen hydrogels are extremely weak materials, which can exhibit poor mechanical strength. This weakness and the requirement that the sample does not reduce in size during testing need special care. In the current work, a specific experimental procedure for testing PVA has been followed. After preparing the viscous PVA solution, as described in Section 2.3.2.2, the solution was poured (casted) in a plastic mold with six rectangular spaces. Each space has nominal dimensions of 5.5 mm width, 12 mm depth and 100 mm length. The PVA molds were kept in the freezer at about -4 °C and PVA matrices were cross-linked by freezing-thawing method. After completing the freezing-thawing process with 5 cycles, the samples were allowed to thaw at room temperature for 30 min before performing the tensile tests. In most of uniaxial tensile testing standard, dumbbell-shaped and dogbone samples are usually used. Dumbbell-shaped specimens were used by several investigators[109-113]. Dogbone shaped specimen was used by Mourad et al.[114] to test diedrawn polymeric samples and by Aneth el al. [107] to test PVA/Gelatin samples. In these studies an abrasive paper was placed between the hydrogel sample and the grip surface to prevent slippage during loading. Some difficulties have been encountered in preparing and testing the standard

dumbbell-shaped and dogbone-shaped samples of PVA gel. Therefore, specimens with rectangular cross section have been used. The specimens have been prepared by molding technique with nominal dimensions of 5.5 mm×12 mm×100 mm. Specimens were prepared with relatively extended length to ensure good gripping. Special fixture has been prepared to install the specimen in the gripping system of the tensile testing machine. The fixture consists mainly of special type of soft tissues and rubber bands. The tissue papers and rubber bands were carefully wound around the two ends of the specimen. Then the two ends were placed between two rubber pieces before installing the specimen into the gripping system of the machine. These procedures of fixation eliminate both slippage and specimen damage during loading. The tensile tests were conducted on a universal material testing machine (MTS) of 100 KN load cell. All samples were tested at room temperature and at a constant overhead speed of 25 mm/min until the sample reached ultimate failure.

When testing PVA gel samples, it was expected that the samples would be subjected to water loss which could essentially influence the mechanical performance. In particular, as the temperature increased, water loss could become more prominent and lead to increase in moduli with temperature which were in reality the result of water loss and subsequent changes in the PVA gel structure. Therefore, all tests were conducted at a relatively high overhead speed of 25 mm/min to minimize the time of the test and in turn water loss. This also helped to avoid coating the hydrogel samples with any material such as petroleum gel or silicone vacuum grease that might affect the measured properties.

2.3.3. Acclimatization

Cuts of PVA 5, 10 , 15 1nd 20 mass% containing biomass were placed in 300 ml bubble column reactors with 200mg/l of glucose (as an easy biodegradation source of carbon) in addition to other essential mineral nutrients with continuous aeration. Nutrient mineral medium solution was prepared by dissolving in 1 L of distilled water 825 mg of mineral salt mixture consisting of: 299.58 mg $MgSO_4.7H_2O$, 249.65 mg K_2HPO_4, 149.80 mg $CaCl_2.2H_2O$, 119.83 mg $(NH_4)_2CO_3$, 3.50 mg $FeSO_4.7H_2O$, 1.30 mg $ZnSO_4.7H_2O$, 1.30 mg $MnCl_2.4H_2O$, 0.018 mg $CuSO_4.5H_2O$, 0.015 mg $CoCl_2.6H_2O$ and 0.013 mg a2$MoO_4.2H_2O$. To precisely determine the added small masses, larger amounts were first dissolved in 100 ml of distilled water. A 5 ml sample was then added to the rest of the salts and diluted to a total volume of 1 L [115].

The bacteria acclimatization to phenol was achieved by gradually increasing the concentration of phenol and decreasing the concentration of glucose. Once the maximum concentration of phenol is reached (200 mg/l) and the concentration of glucose dropped to zero, the immobilized bacteria is considered fully acclimatized to phenol concentration up to 200 mg/l [75].

2.3.4. Spouted Bed Bioreactor (SBBR)

The Spouted Bed Bioreactor was made of Plexiglas with a total volume of 300 ml and fitted with a surrounding jacket for temperature control. Air was continuously introduced at a specified flow rate into the reactor to enhance mixing and at the same time provide excess oxygen to maintain aerobic conditions [115]. The temperature of the reactor content was controlled to the desired value by the surrounding jacket. The SBBR is

characterized by a systematic intense mixing due to the cyclic motion of particles within the bed, that is generated by a single air jet injected through an orifice in the bottom of the reactor [88]. Schematic diagrams of the Spouted Bed Bioreactor for batch and continuous systems are shown in Figs. 2.1 and 2.2, respectively.

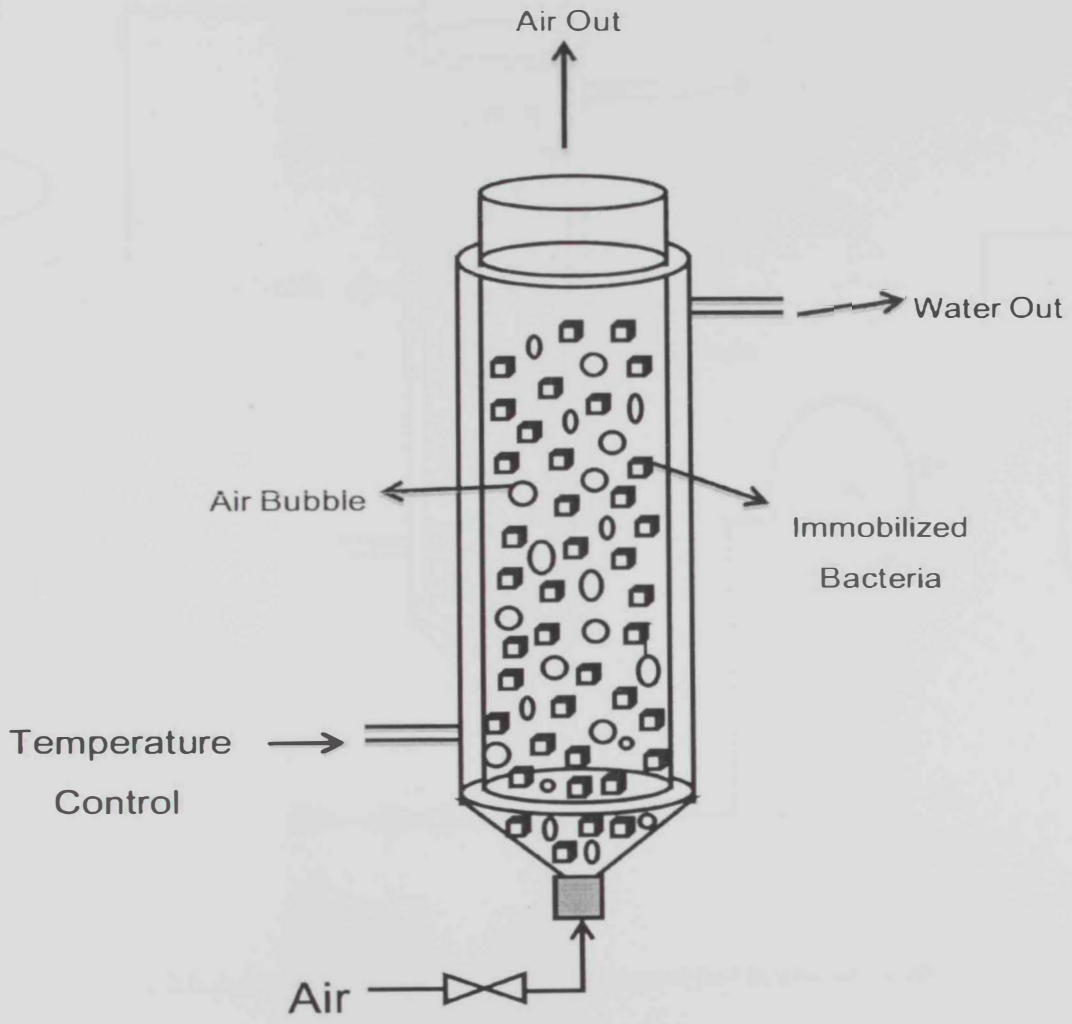


Figure 2.1: A schematic diagram of the batch Spouted Bed Bioreactor (SBBR)

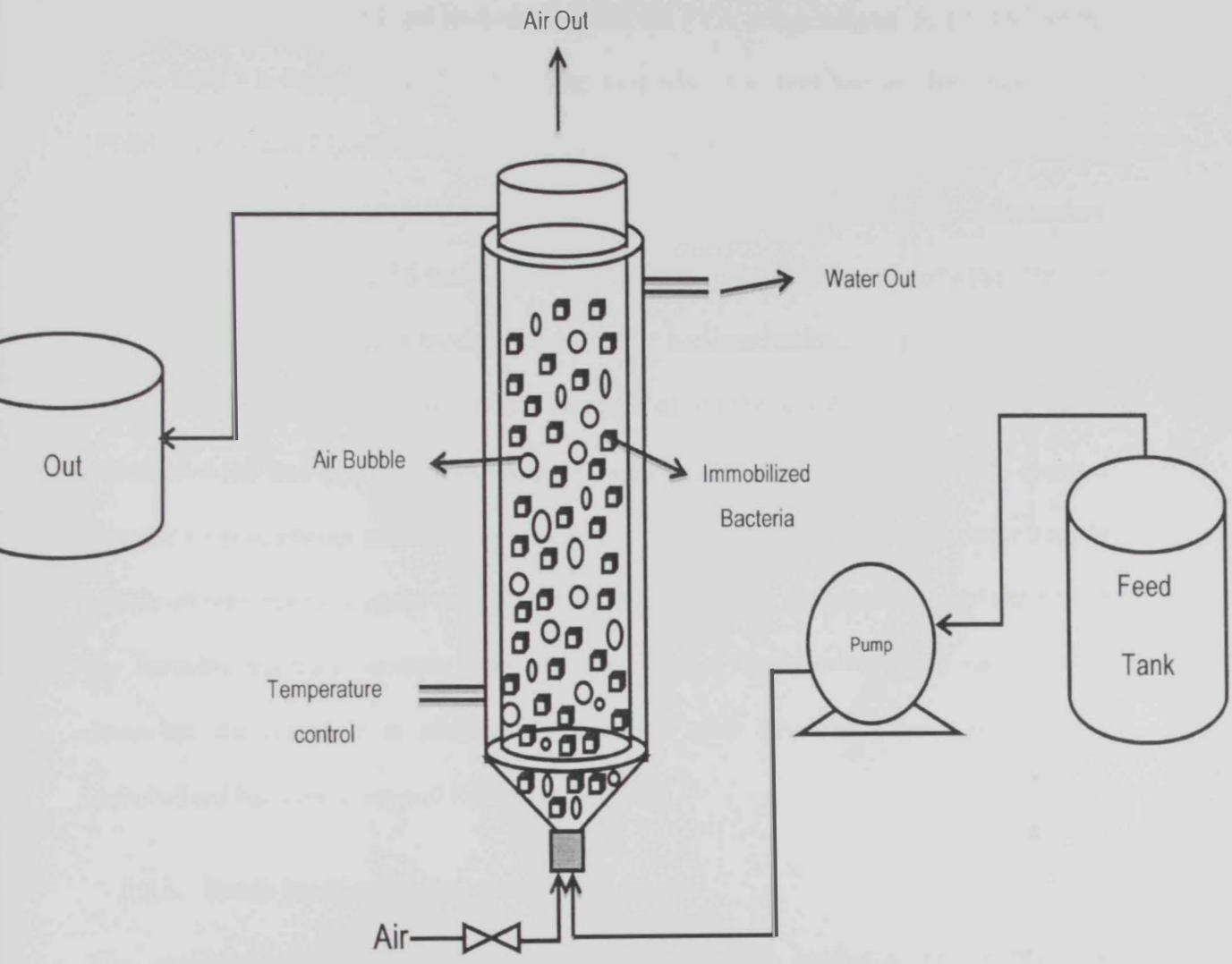


Figure 2.2: A schematic diagram of continuous Spouted Bed Bioreactor (SBBR)

2.3.5. Biodegradation performance of immobilized bacteria in several PVA mass%

The acclimatized immobilized bacteria in different PVA compositions, 5, 10, 15 and 20 mass% with 3 h thawing and 24 h freezing were placed in four spouted bed bioreactors (SBBR) with phenol initial concentration of 200 mg/l and mineral nutrients. Experiments were carried out at optimum conditions as reported by El-Naas et al.[75]; temperature was adjusted at 30°C, pH 7.8 and 0.003 l/min air flow rate. In order to study the effect of PVA mass% on the phenol biodegradation after acclimatization, all PVA pellets were kept in bubble column reactors with 200 mg/l of synthetic solution with the mineral nutrients. After one month, the experiments were carried again in four (SBBR) systems then the drop in phenol concentration with time was analyzed. PVA pellets were kept in bubble column reactors again with phenol solution, in order to assess the applicability of the immobilized bacteria over long periods of time; experiments were ran again to determine the decrease in phenol concentration after two and three months using immobilized bacteria in several PVA compositions.

2.3.6. Batch biodegradation process for cresols

The acclimatized immobilized bacteria were adapted gradually on o-cresol to concentration up to 200 mg/l over a period of 7 days by placing the PVA particles (5 mass%) in batch Spouted Bed Bioreactor (SBBR). *O-cresol* solution were prepared by dissolving *o-cresol* powder in the solution of menial nutrients prepared as Sec 2.3.3. Batch experiments were then carried out to study the effect of initial concentration, temperature, pH and PVA vol.% on the biodegradation process.

All experimental results reported in the next sections were based on averaging results of repeated experimental runs (duplicates).

2.3.7. Continuous biodegradation process of cresols

Experiments were conducted to study the continuous biodegradation process. Synthetic solution of *o-cresol* or *p-cresol* with different initial concentrations were continuously fed to the bioreactor using a peristaltic pump (Watson Marlow, Model 323) for a period of 4h. The reactor temperature and pH were kept constant at 8 and 35°C, which are the optimum conditions obtained in the batch study. In all experiments, the volume fraction of the PVA pellets was kept at 30 % of the total operating volume.

CHAPTER THREE

Results & Discussion

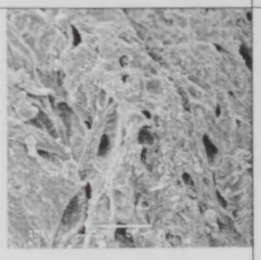
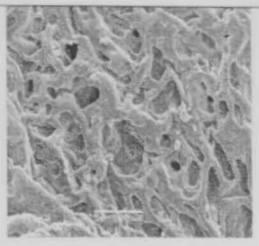
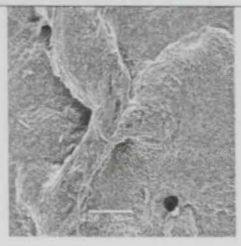
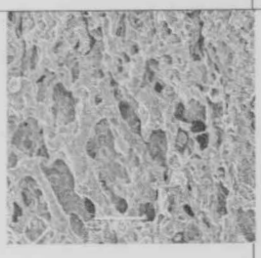
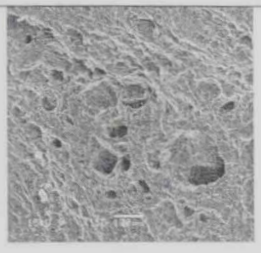
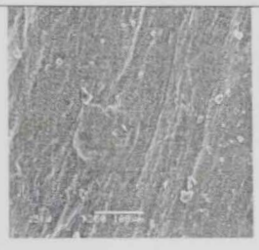
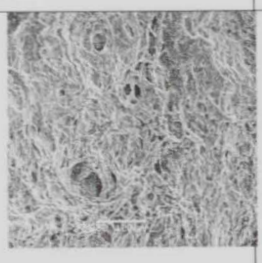
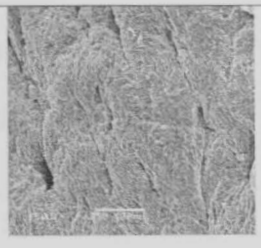
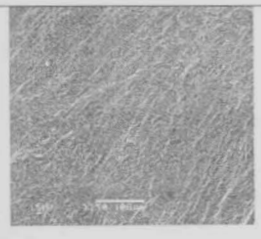
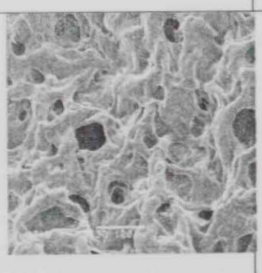
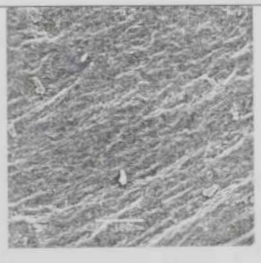
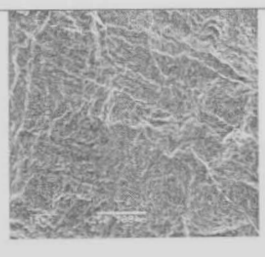
3.1. Evaluation of PVA gel as an immobilization matrices

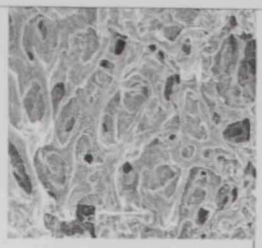
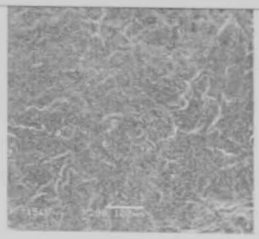
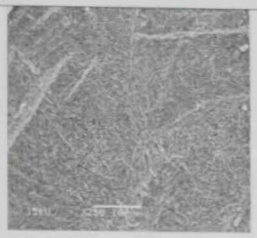
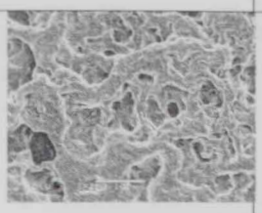
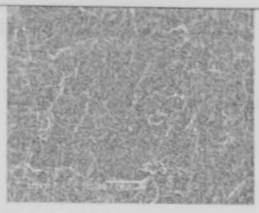
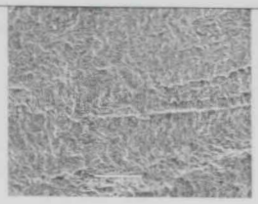
3.1.1. Effect of PVA composition and thawing time on pores structure

PVA gel prepared by the iterative freezing-thawing technique is well known for its good mechanical properties and porous structure [35, 36, 103, 116]. In the freezing-thawing cycles, fine crystallites are formed due to the slow heat treatment. The crystallites serve as physical cross-links by making an insoluble three-dimensional network [117, 118]. Phase separation, crystal formation and hydrogen bonding may take place during the solidification of PVA solution; the phase separation leads to the formation of PVA-rich and PVA-poor regions. In PVA rich region, crystallization and hydrogen bonding may occur due to the specific properties of PVA. The connectivity and spreading of this region all over the gel cause extending in the network structure, which will result in porous formation [118].

The structure of the prepared polymer is highly affected by the preparation conditions. In order to investigate the effect of thawing time and PVA composition on the porous structure of the PVA gel, several mass contents (5, 10, and 15 %) were prepared by thawing for (3 to 24 h) at room temperature. PVA samples were frozen at -4°C for 24 h, and freezing-thawing cycles were repeated 5 times. The porous structure of freeze dried PVA samples were studied and compared using Scanning Electric Microscope (SEM). Table 3.1 shows the microscopic analysis for PVA gel prepared by varying PVA composition and thawing time.

Table 3.1: SEM images of several PVA compositions at different thawing hours.

PVA % / Thaw	5%	10%	15%
3h			
6h			
9h			
12h			

18h			
24h			

It can be noticed that the samples with PVA content of 5 mass % have good porous structure for all thawing periods; furthermore, the distribution and size of pores are highly dependent on the thawing time for this composition. In contrast, samples with PVA content of 10 % show good porous structure at low thawing periods (from 3 to 9), and the pore distribution decrease by increasing thawing time. Diminished porosity was observed for PVA 15 mass% at all thawing periods. When comparing the porous structure of 5 and 10 mass % at the same thawing time, the 10 % shows better porosity in 3 h thawing than the 5 mass %; whereas in 6 h thawing, no significant difference in the pores distribution is observed in both pellets. These results highlight the significant effect of preparation conditions on the cross-linking and the porous network of the prepared gel. The porous structure of the prepared polymer is affected by thawing time, freezing time and the PVA content. Variation in these conditions may affect the crystal formation, which directly affects the pores structure within the polymer [35, 36].

3.1.2. Effect of PVA concentration on porosity (F-T: 24-3h)

The porous structure of the biomass carrier plays an important role in the biodegradation process, since it allows substrate and oxygen diffusion into the internal surface and hence improves the growth rate of the biomass within the pellets. In order to investigate the porous structure of the PVA gel pellets at different PVA mass contents ranging from 5 to 20%, the gel pellets were cut into thin sections in a microtome sectioning machine and then examined using a Compound Microscope (Leica DM 6000) by the (Bright Field) contrast method. Microscopic images for different samples of PVA mass content (5, 10, 15 and 20%) were tested and an example of each PVA mass% is presented in Figures 3.1 (a)-(d) using 63× magnification. The images clearly show highly porous structure for all PVA mass contents except 15% and the pores seem to be distributed with varying sizes across the PVA pellet, which indicates that PVA matrices have suitable structure for biomass immobilization. The matrices with 10 mass % PVA had the highest porosity comparing with other pellets and showed a stable microstructure with evenly distributed pores as presented in Figures 3.1(b); the pore size is smaller than that in both 5 and 20 mass %, which seemed to be varying in size and unevenly distributed over the displayed surface. Figure 3.1(d) clearly indicates that the porous structure of the prepared polymer is not density dependent and may depend more on the crystal formation [119, 120], which is related to the concentration of the PVA in the solution, freezing time and thawing time [121]. Pellets with 10 mass % PVA seemed to have reached high crystallinity in the 24 h freezing and 3 h thawing as observed in the network structure.

The PVA matrices with 15 mass % (Figure 3.1c) appears to have diminished porosity due to the inappropriate freezing-thawing time for crystal formation, where the freezing

thawing process could not achieve the optimum conditions for crystallization which consequently affected the pore formation and porosity.

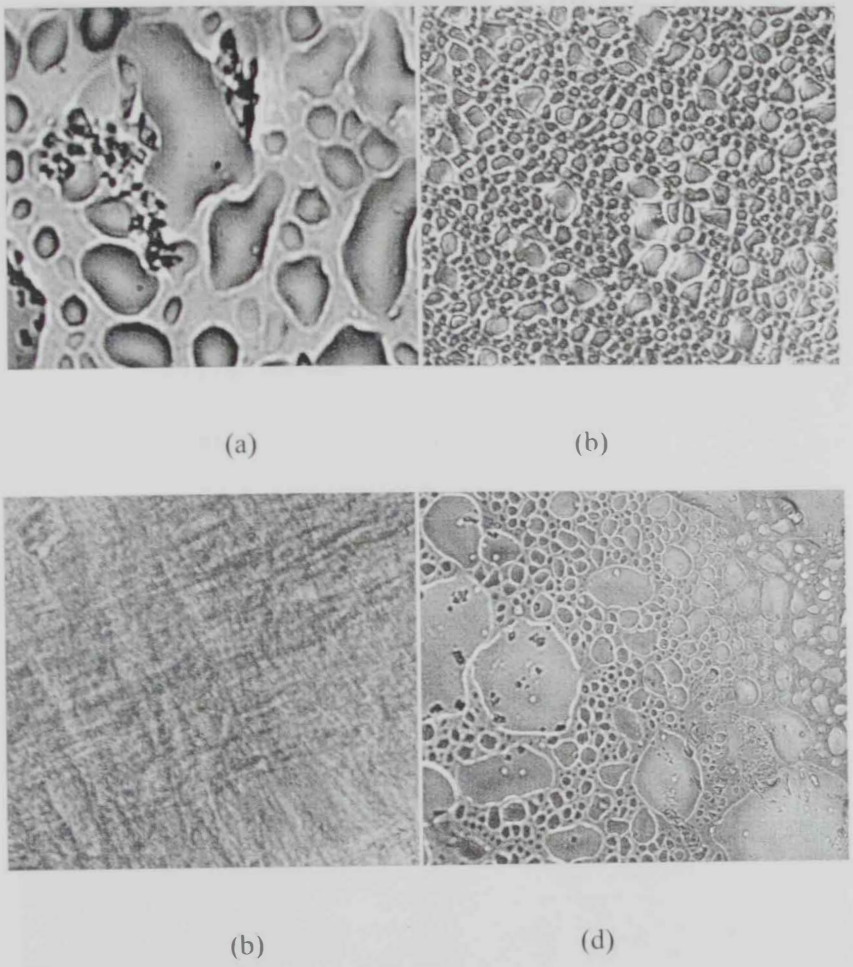
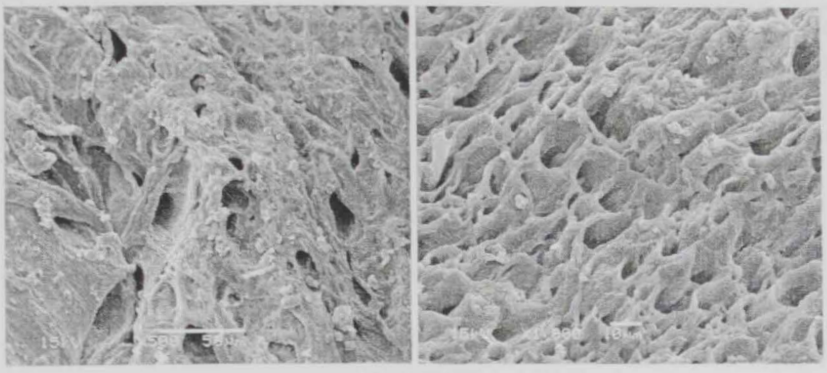
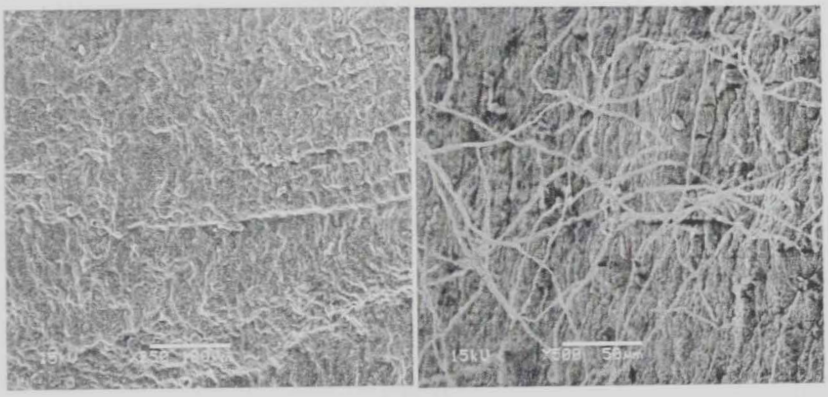


Figure 3.1: Compound Microscope images of PVA; (a) 5 mass% ; (b) 10 mass%; (c) 15 mass%; (d) 20 mass% ;(freeing-thawing; 24h-3h)

The porous structures for pellets with different PVA mass contents were also examined using SEM for freeze-dried samples and presented in Figure 3.2 (a)-(d). The SEM images show three-dimensional networks for the cross-linked polymers, with pore size and pore distribution similar to those observed by the Compound microscope. PVA 20 mass%.



(a) (b)



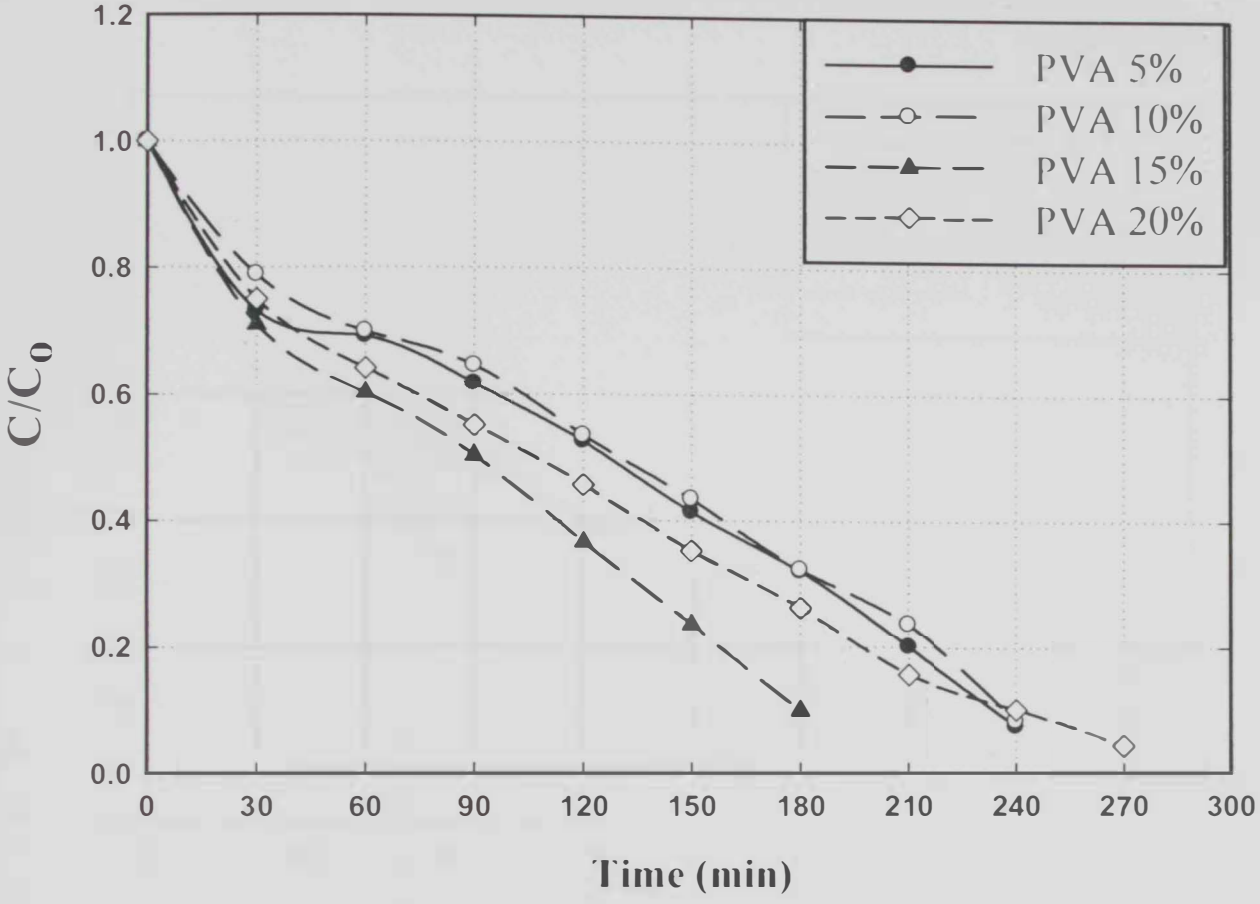
(c) (d)

Figure 3.2: SEM images of PVA; (a) 5 mass% ; (b) 10 mass%; (c) 15 mass%; (d) 20 mass%; (freezing-thawing ; 24h-3h)

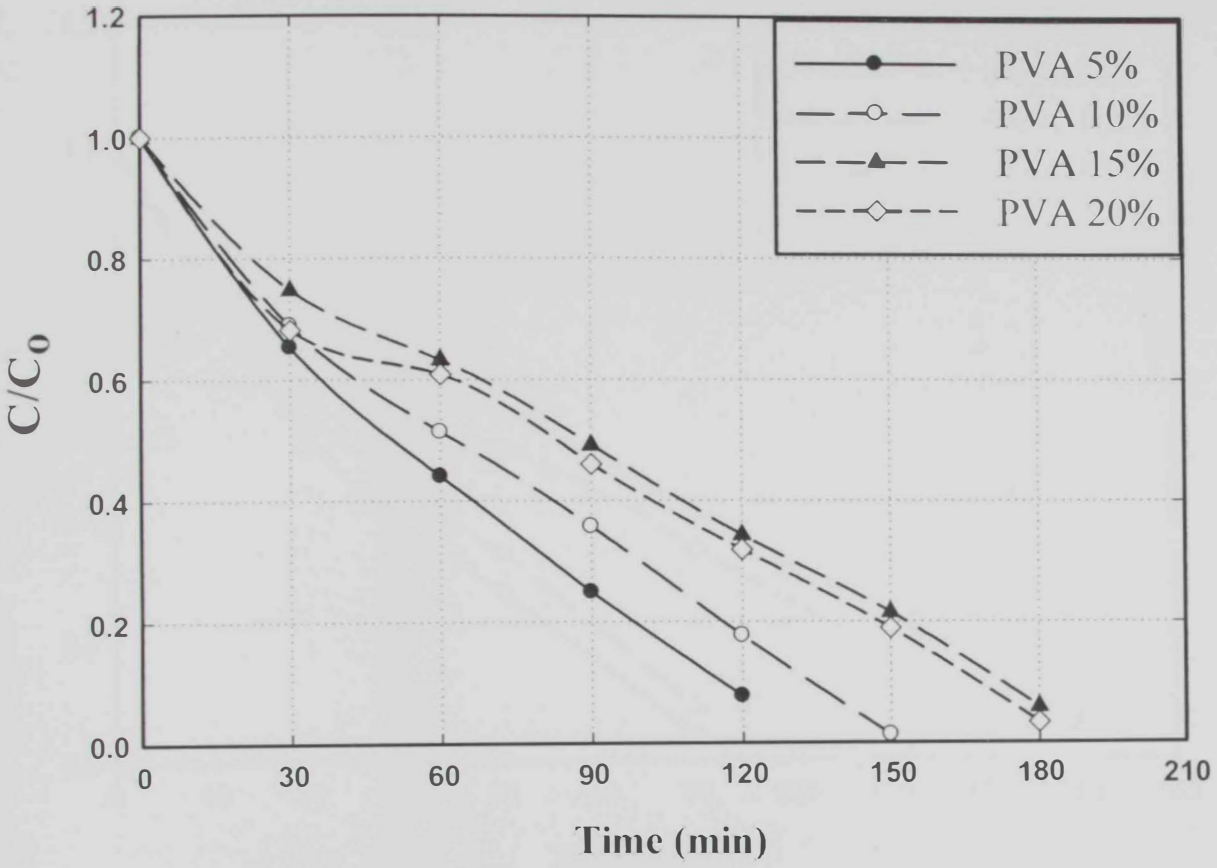
3.1.3. Biodegradation rate

The biodegradation capabilities of *P.putida* immobilized in PVA pellets with different mass contents were assessed over long periods of time ranging from one to three months. Batch experiments were carried out for the biodegradation of phenol in four SBBR systems using bacteria immobilized in 5, 10, 15 and 20 mass % PVA for a period of one, two and three months after acclimatization. All experiments were carried at reactor temperature of 30°C and pH of 7; the results presented in this section are the average of two to three runs. Plots for the dimensionless concentration as a function of time for the biodegradation of 200 mg/l of phenol are shown in Figures 3.3 a to d, for experiments carried out immediately after acclimatization, one, two and then after three months. The biodegradation rates were calculated from the slopes of the best fitted straight line for concentration versus time plots and summarized in Figure 3.4.

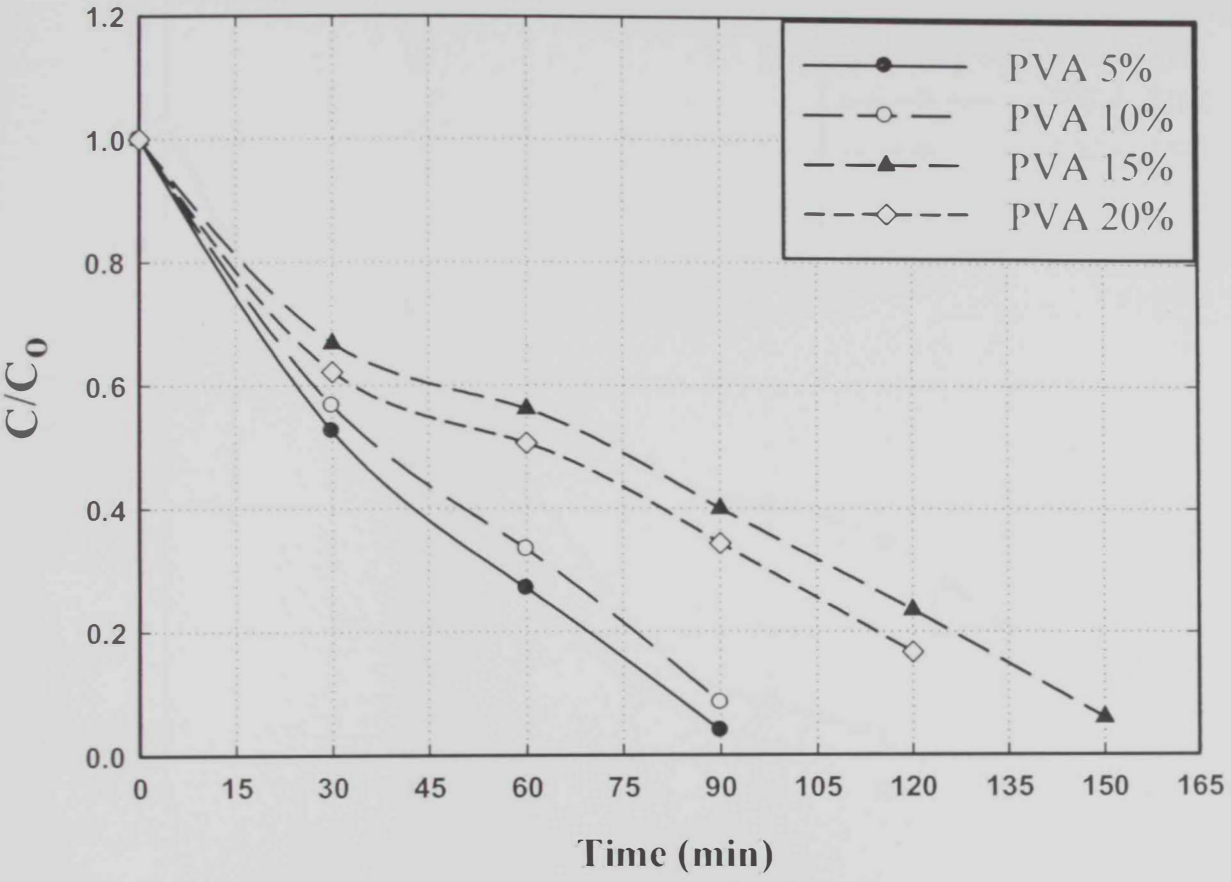
Directly after acclimatization (Figure 3.3a), the biodegradation rate seemed to be similar for the different PVA contents with the 15 % showing slightly better rate compared to the other three. This could be attributed to the possibility that for this PVA matrix, more biomass was available at the outer surface due to the poor cross-linking and diminished porosity as mentioned in the previous section. However, the biodegradation rate seemed to drastically improve with time and the rate of improvement varied and depended on the PVA content as shown in Figures 3.3 (b-d). The biodegradation process is expected to improve with time as the number of biomass cells within the PVA matrices increases significantly and can only be limited by the porous structure of the PVA pellet.



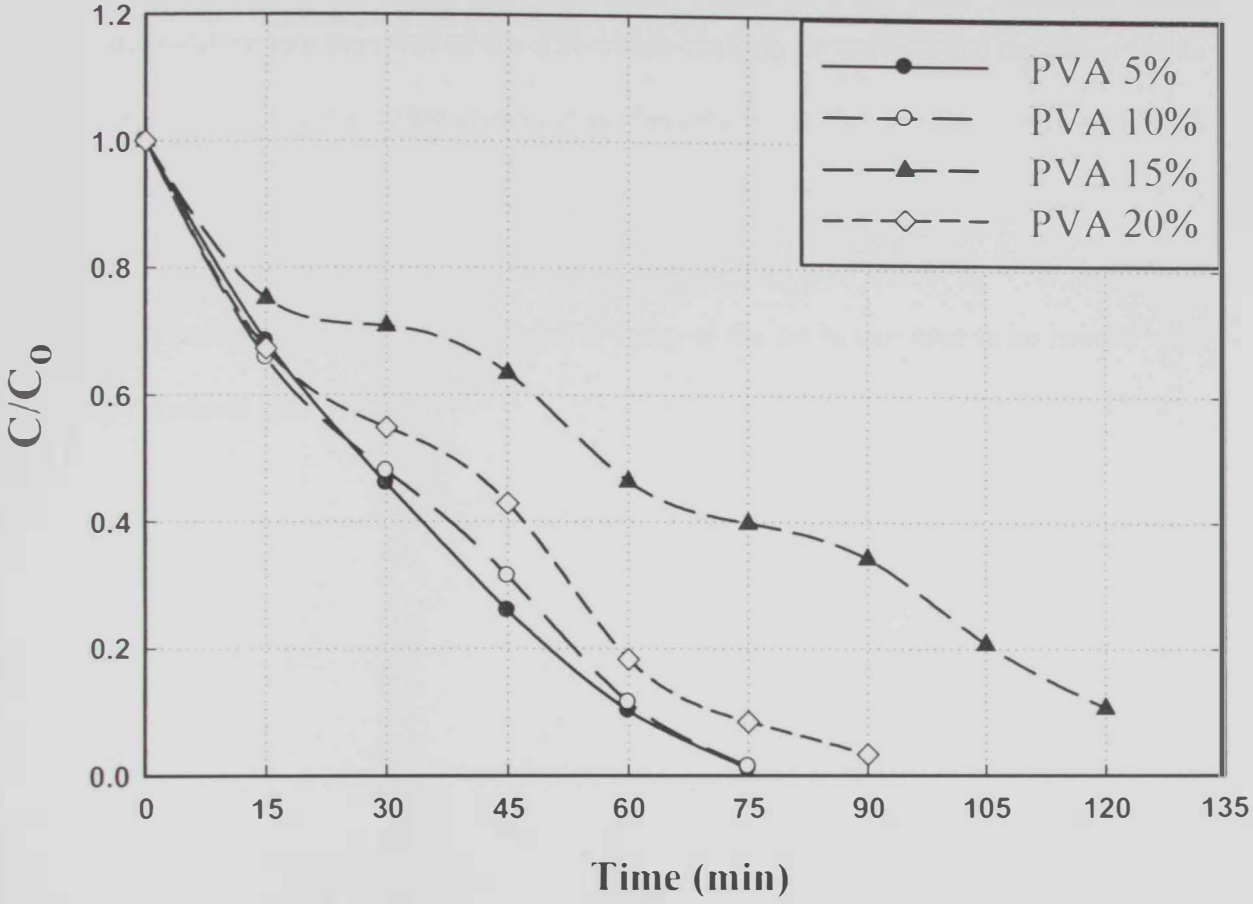
(a) Directly after acclimatization



(b) After one month.



(c) After two months



(d) After three months

Figure 3.3: Variation of phenol concentration for several PVA pellets for the biodegradation process after different periods: initial phenol concentrations= 200 mg/l: T= 30°C; pH= 7.5: (a) After acclimatization: (b) After one month: (c) After two months; (d) After three months

This is clearly reflected in the results presented in Figure 3.4, as porous pellets (5 and 10 %) showed better biodegradation rate after three months than those that are less porous. The least porous matrix (15 %) exhibited the least biodegradation rate after three months as shown in the figure. Although the SEM and Compound microscope analysis revealed that the 10 % PVA pellets had the most porous structure, it showed slightly lower biodegradation rate than that of the 5 % which can only be attributed to the pores size in PVA mass% that enhance the growth of the bacteria inside the polymer, moreover PVA 5 mass% may has better mixing and hence better mass transfer for the lighter pellets. This was observed experimentally as the 5 % pellets showed better movement and better mixing inside the reactor as compared to those of the 10 % that tend to be heavier with more hindered movement.

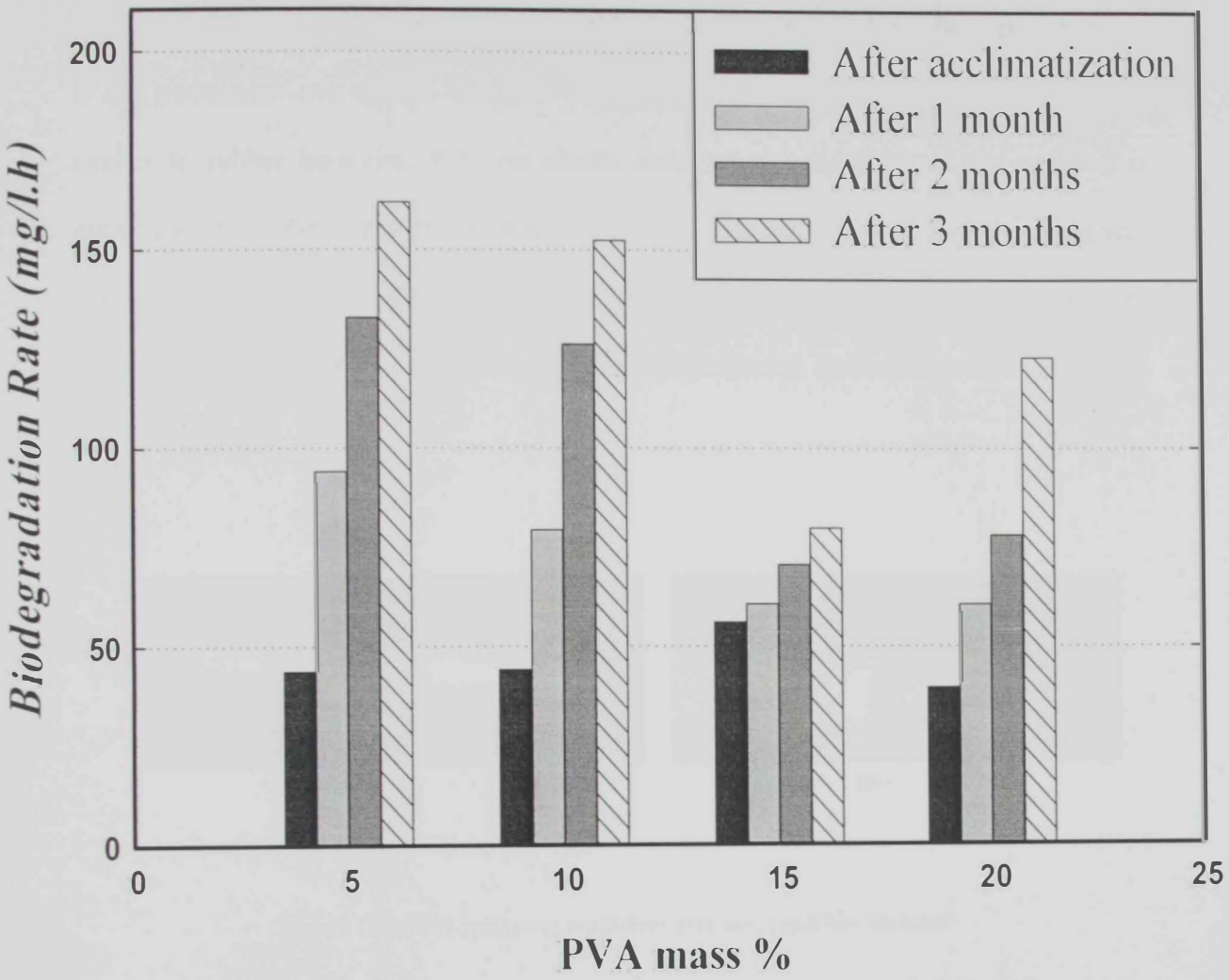
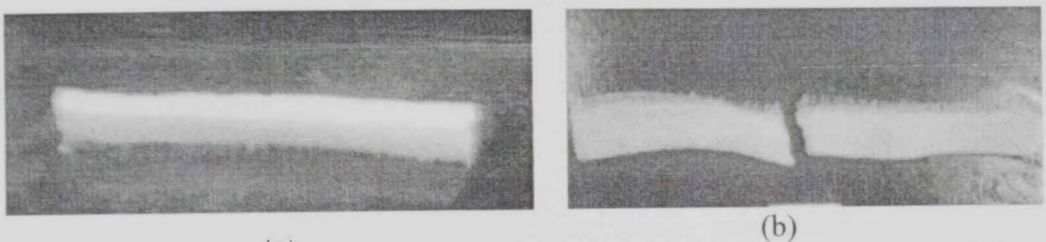


Figure 3.4: Comparison of phenol biodegradation rate for different PVA pellets after several periods of time

3.1.4. Mechanical Behavior

Mechanical tests were carried out to evaluate the tensile properties of PVA gel (fracture strength, percent elongation and Modulus of elasticity). Although the material exhibits large strain at fracture and rubbery behavior, it is not a ductile material because the strain is not permanent and almost the total elongation is recovered after fracture in away similar to rubber behavior. It is an elastic material and elasticity of the samples is ascertained from the stress-strain profiles. No local deformation or necking/yielding was observed while testing as shown in Figures 3.5a and 3.5b. The fracture surface is smooth flat and perpendicular to load line in almost all specimens as the cleavage behavior in brittle materials.



(a) (b)

Figure 3.5: PVA specimen. (a) Before fracture; (b) After fractured

Tensile tests were conducted on samples with different PVA contents (5 %, 10 %, 15 %, 20% and 25 %). Three samples were tested for each PVA % and the curves demonstrated a good level of repeatability. The average values of the properties have been reported. Representative stress-strain curves for the different PVA contents are presented in Figure 3.6.The stress varies with the strain following almost a linear relationship. The behavior

shows that the PVA gels respond to stresses with nearly instantaneous and fully reversible deformation. This may be attributed to the fact that the gels are cross-linked networks (as rubber) with a large free volume that allows them to respond to external loads with a rapid rearrangement of the polymer segments. When a gel is in the region of rubber-like behavior, its mechanical behavior is dependent primarily on the structural design of the polymer network. At low enough temperature, these gels may lose their rubber elastic properties and demonstrate viscoelastic behavior. Similar observation has been reported by Kristi et al. [107].

Figure 3.7 demonstrates the variation of the maximum fracture strength (fracture load) with PVA contents. The material eventually offers increasing resistance to the load, and the curve turns markedly upward as PVA concentration increases. The variation follows almost an exponential function. This improvement in the mechanical properties (fracture strength, percent elongation, and stiffness) with increasing PVA concentration is due to the combined effect of density and/or porosity and cross-linking. It has been reported by Ma and Xiong [103] that the increase in PVA mass % may produce remarkable increase in the cross-linking. This in turns leads to observed improvement in the properties. In addition, porosity plays an important role in the variation in these properties. PVA matrices that have high porous structure and low density such as PVA 5 % and 10 % showed less mechanical properties than others. As has been mentioned before in Section 3.2, the porosity of the produced PVA gel depends on the process parameters such as PVA mass %, freezing and the thawing cycles [122].

PVA mass % of 15 % produced a gel with less porosity as presented in Figures 5 and 6, this may explain the remarkable increase in the fracture stress (see Figure 3.7), when PVA mass % increased from 10 % to 15. The slope of the curves increases with PVA concentration which is an indicator for the apparent improvement in the stiffness (modulus of elasticity) of the material. This behavior is shown in Figure 3.8. Such improvement is also dependent on the combined effect of the process parameters. These observations have also been reported by Gupta et al. [122].

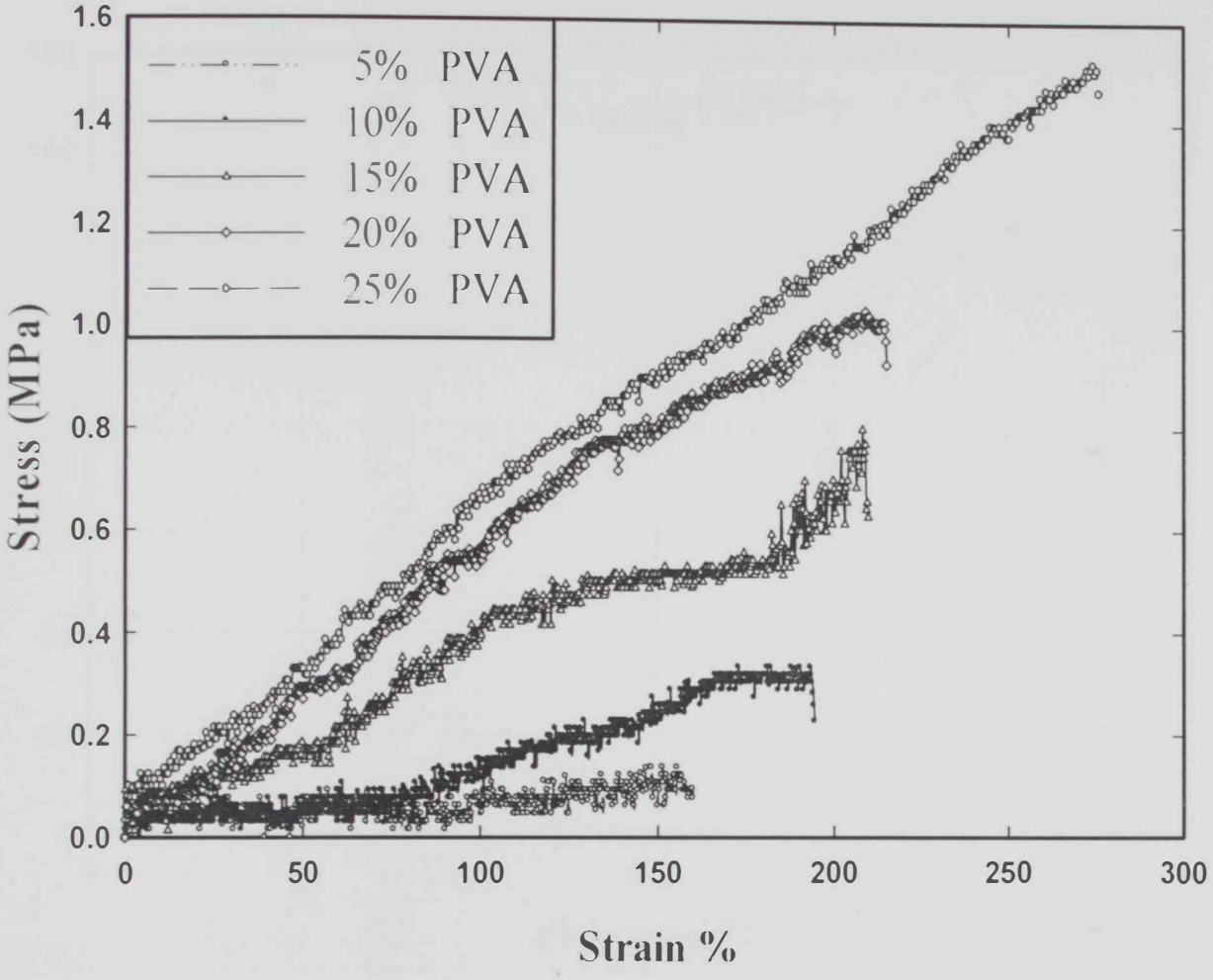


Figure 3.6: Variation of Stress with Strain % for different PVA mass %

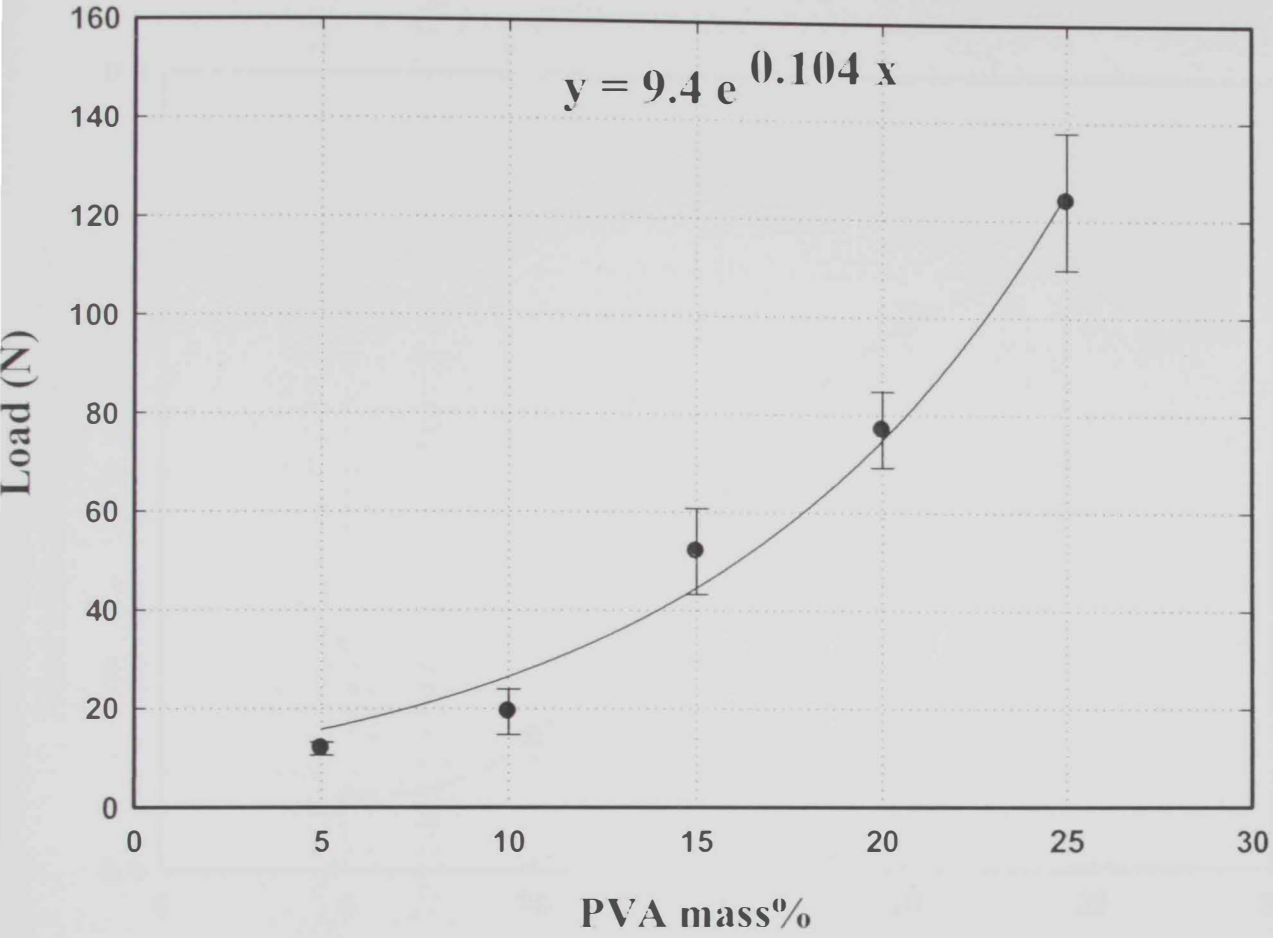


Figure 3.7: Effect of PVA mass% on the fracture load

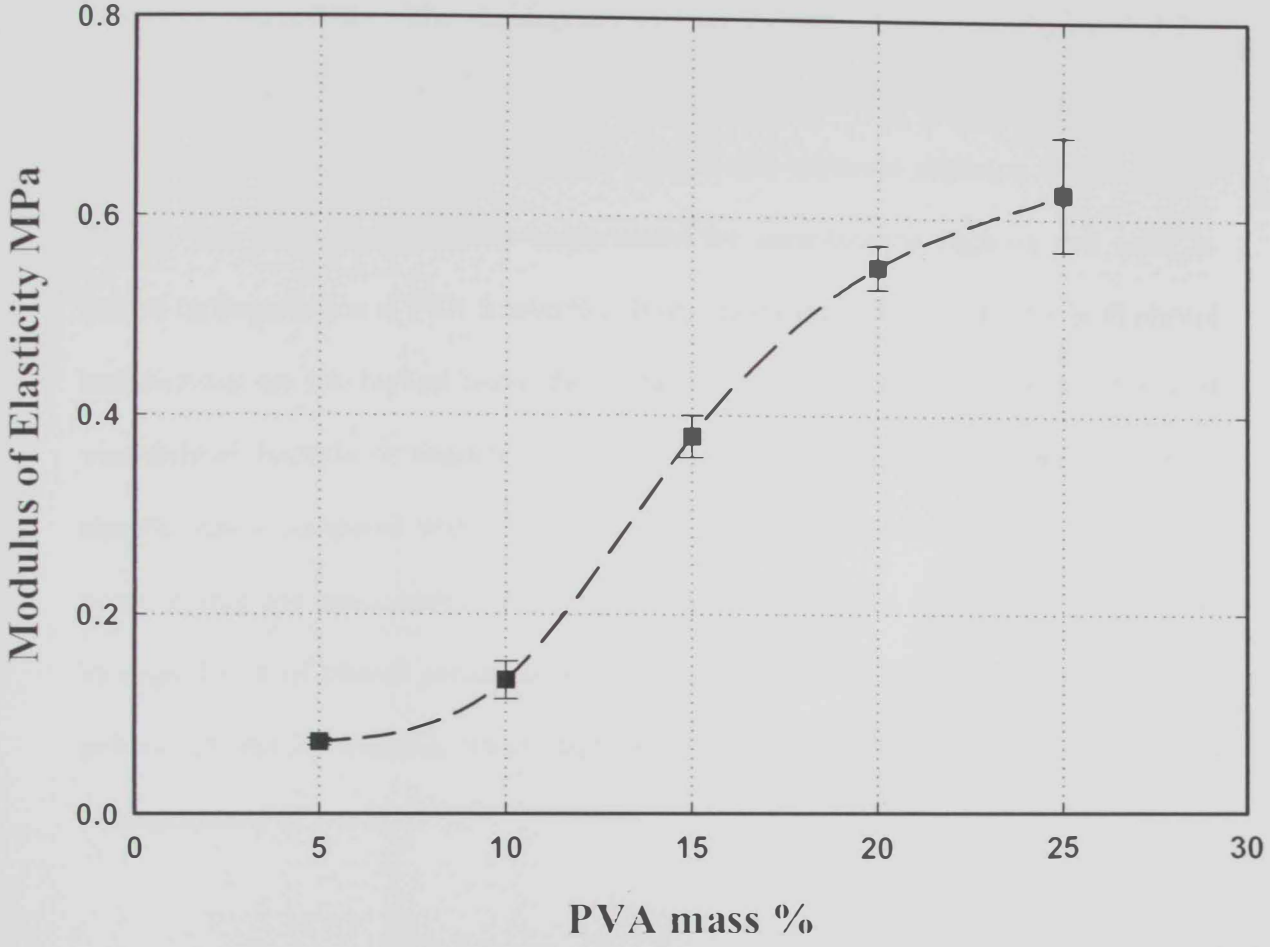


Figure 3.8: Variation of modulus of elasticity with PVA mass %

3.1.5. A comparison between phenol and *o-cresol* biodegradation

In order to compare the degradation performance of the immobilized bacteria in several PVA pellets, batch experiments were carried out simultaneously in four SBBR systems using PVA mass contents of 5, 10, 15 and 20 % after three months. The initial substrate concentration, temperature pH, and air flow rate were adjusted at 200 mg/l, 30°C, 7.8 and 3 l/min, respectively. The biodegradation results are shown in Figure 3.9.The biodegradation rate is the lowest using PVA 15 mass%, due to the absence of the porous structure which resulted in reducing the oxygen and substrate diffusion. These results suggest that the acceptable PVA composition for immobilizing bacteria and using in cresols biodegradation is PVA 5 mass%. Clearly the biodegradation rates for both phenol and *o-cresol* are the highest using the 5 mass %. It can be noticed that the ability of immobilized bacteria to degrade *o-cresol* and phenol is higher using both 5 and 10 mass% pellets compared with 15 and 20 PVA mass%. This could be attributed to the better mixing and hence better mass transfer associated with the lighter PVA pellets. The biodegradation of phenol seems to be better than that of *o-cresol* when using heavier pellets (15 and 20 mass%), which highlights the importance of mass transfer for the biodegradation of *o-cresol*.

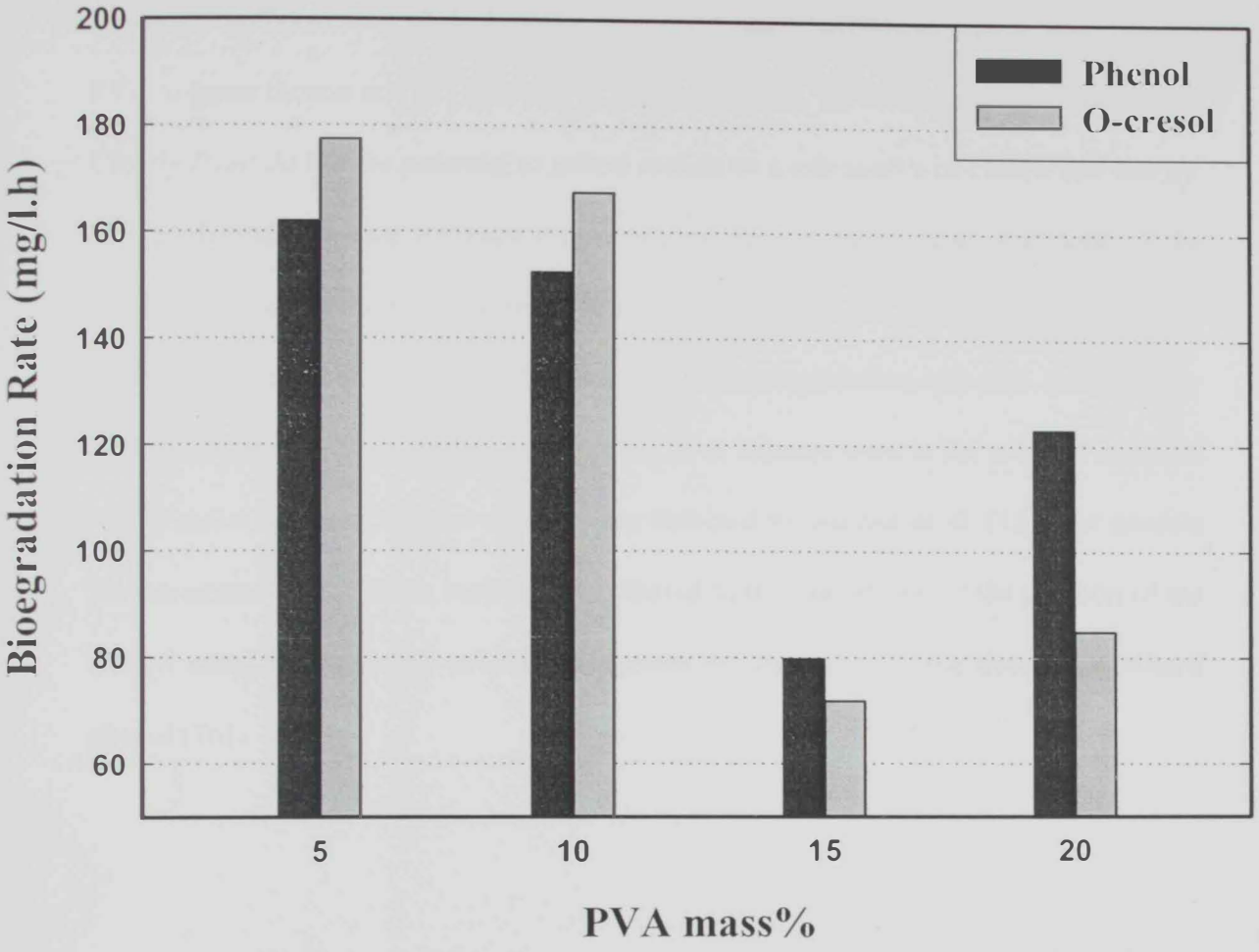


Figure 3.9: Biodegradation rate of phenol and *o-cresol* using *P.putida* immobilized in several PVA mass %

3.2. Biodegradation of cresols using P.putida immobilized in PVA 5 mass %

In this part of the study, the capability of *P.putida* to mineralize the three cresol isomers (*o*-, *p*- and *m-cresol*) was evaluated in a batch SBBR. The experiments were carried out for single components, while keeping the initial substrate concentration, temperature, PVA volume faction and pH constant at 200 mg/l, 30°C, 30 vol.%, and 7.8,respectively. Clearly *P.putida* has the potential to utilize cresols as a sole source of carbon and energy. The biodegradation rate for each cresol isomer was calculated from the slope of the reduction in concentration with time. As shown in Figure 3.10, *o-cresol* seems to be the most favorable cresol isomer for *P.putida*, and the biodegradation rate of *p*- and *m-cresol* slightly differ. The biodegradation rate of the three isomers were in the order of *o-cresol* > *p-cresol*> *m-cresol*; similar results were obtained by Ahmed et al. [123] for another *Pseudomonas* strain. These results are attributed to the importance of the position of the methyl substitute; *o*- and *p*-substituted phenols are more degradable than *m*-substituted phenol [76].

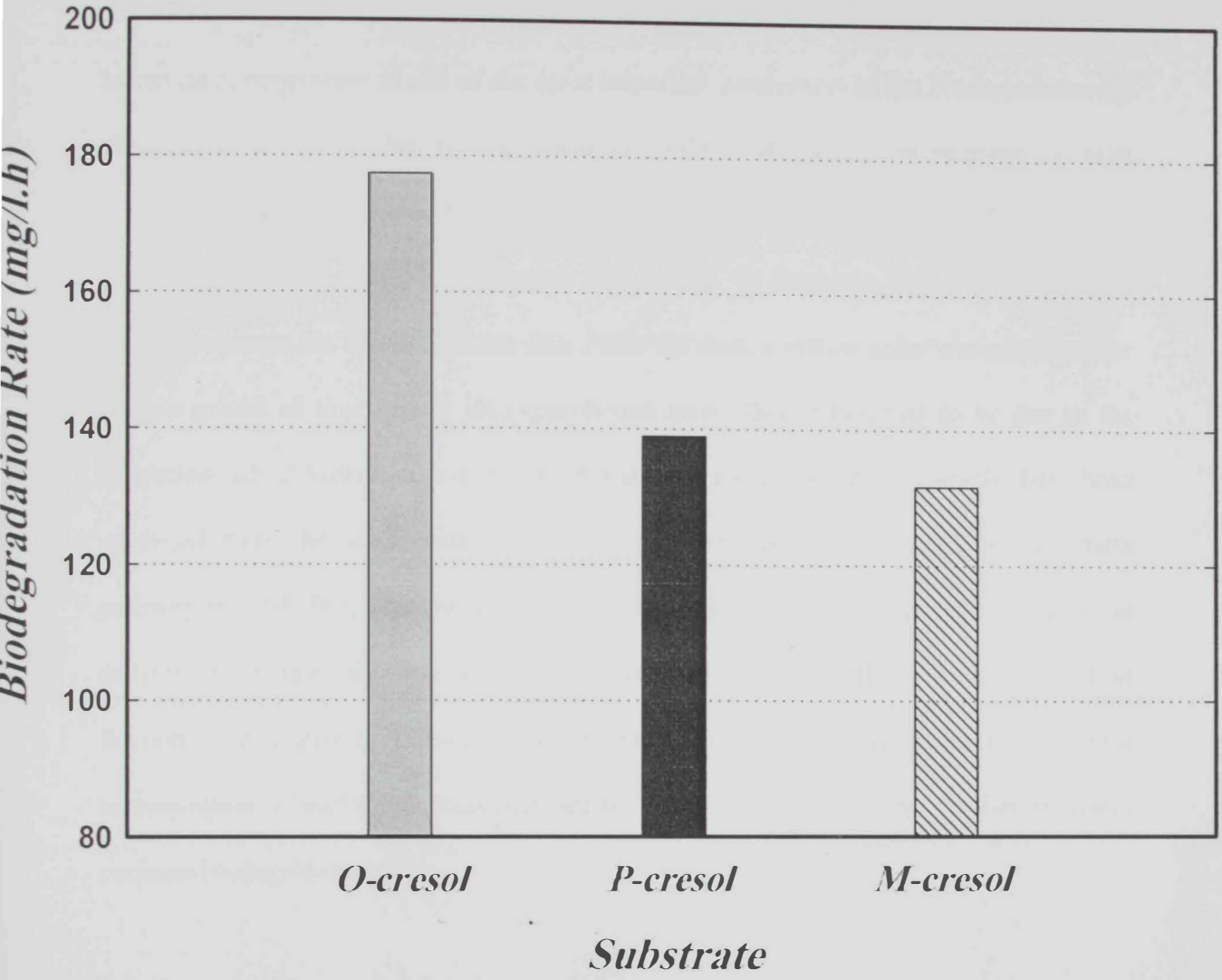


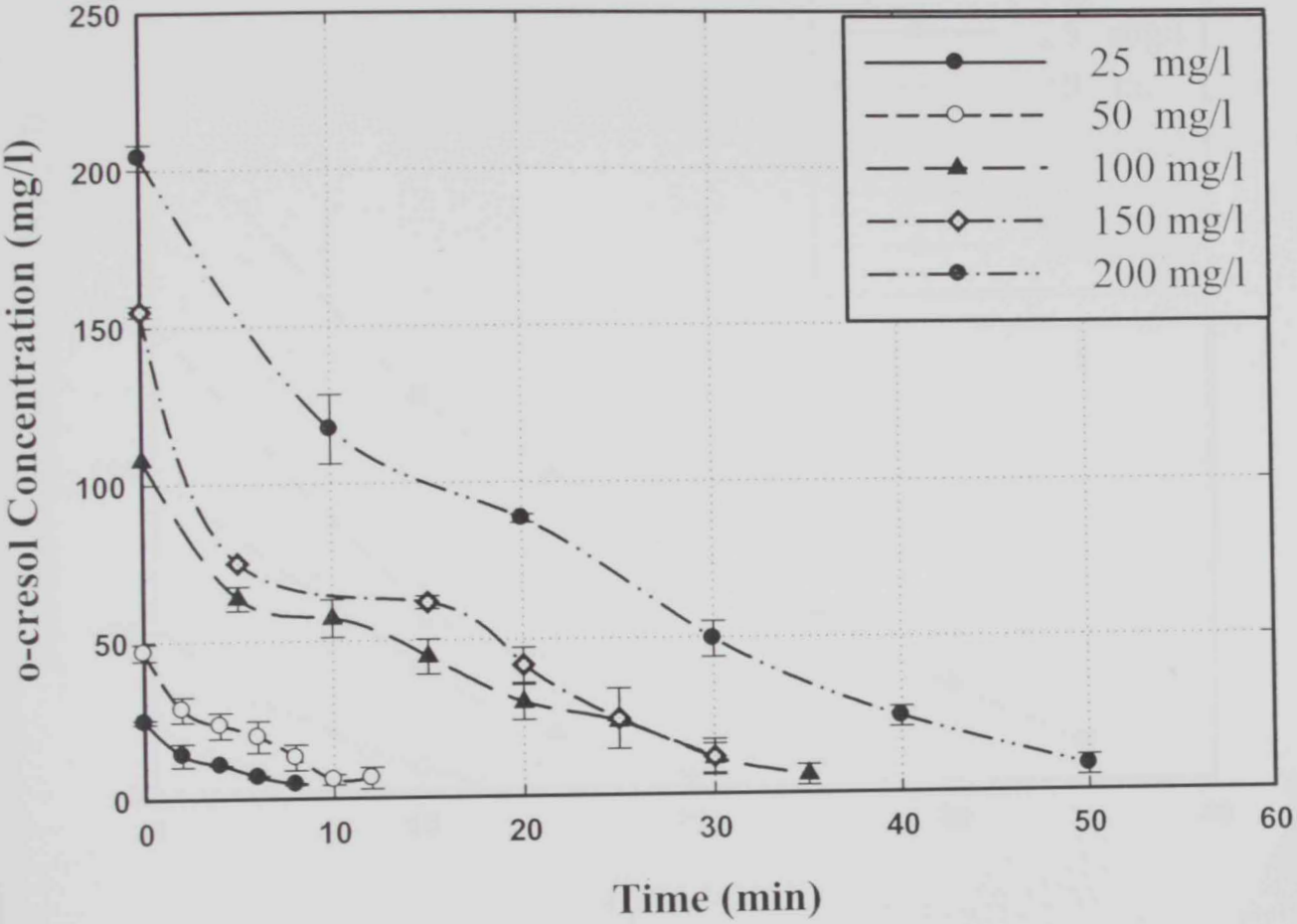
Figure 3.10: Biodegradation rate for different cresol isomers

3.3. *Biodegradation of Single component*

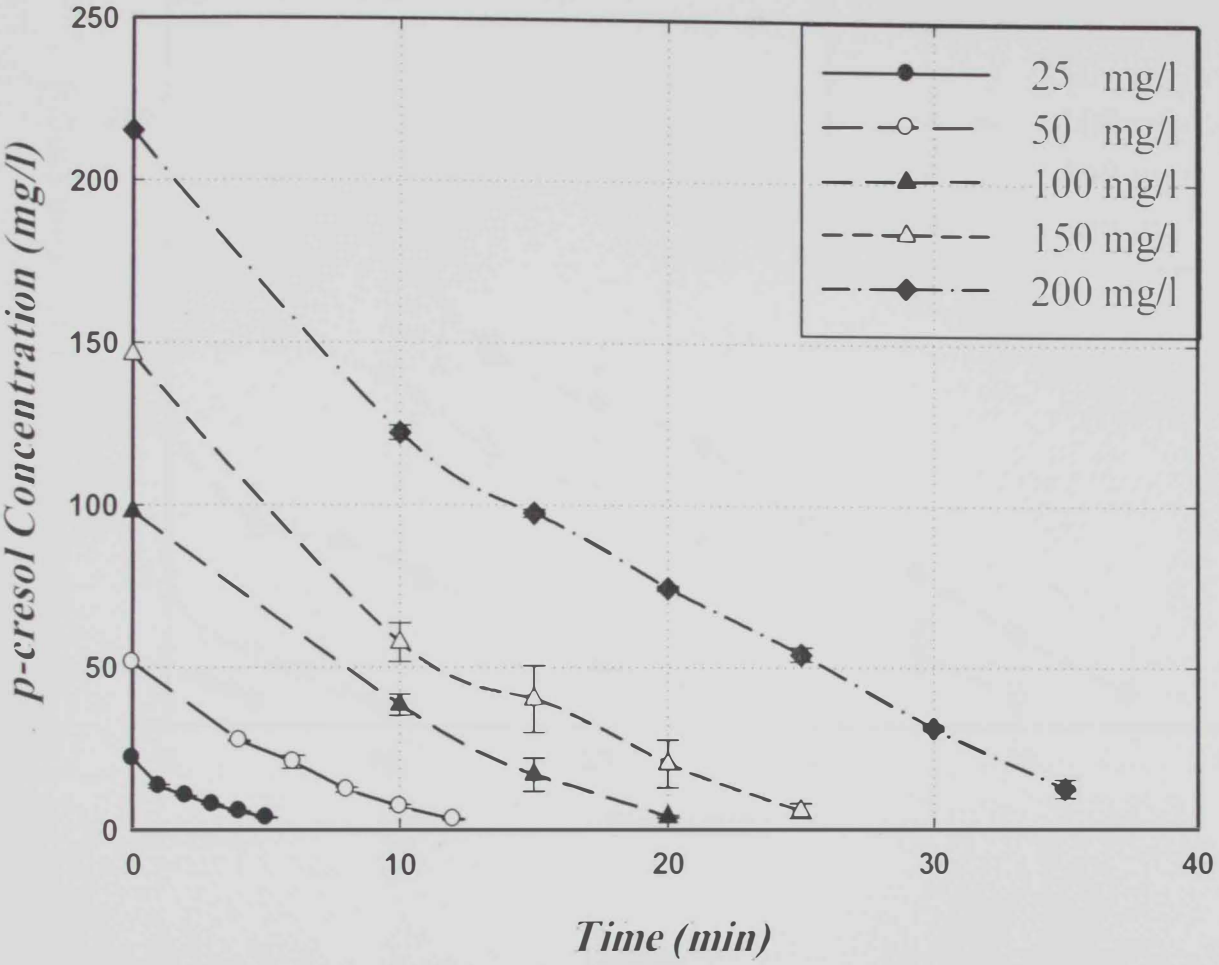
3.3.1. Batch Biodegradation of cresols (*o-*, *p-* and *m-cresol*)

3.3.1.1.Effect of initial concentration

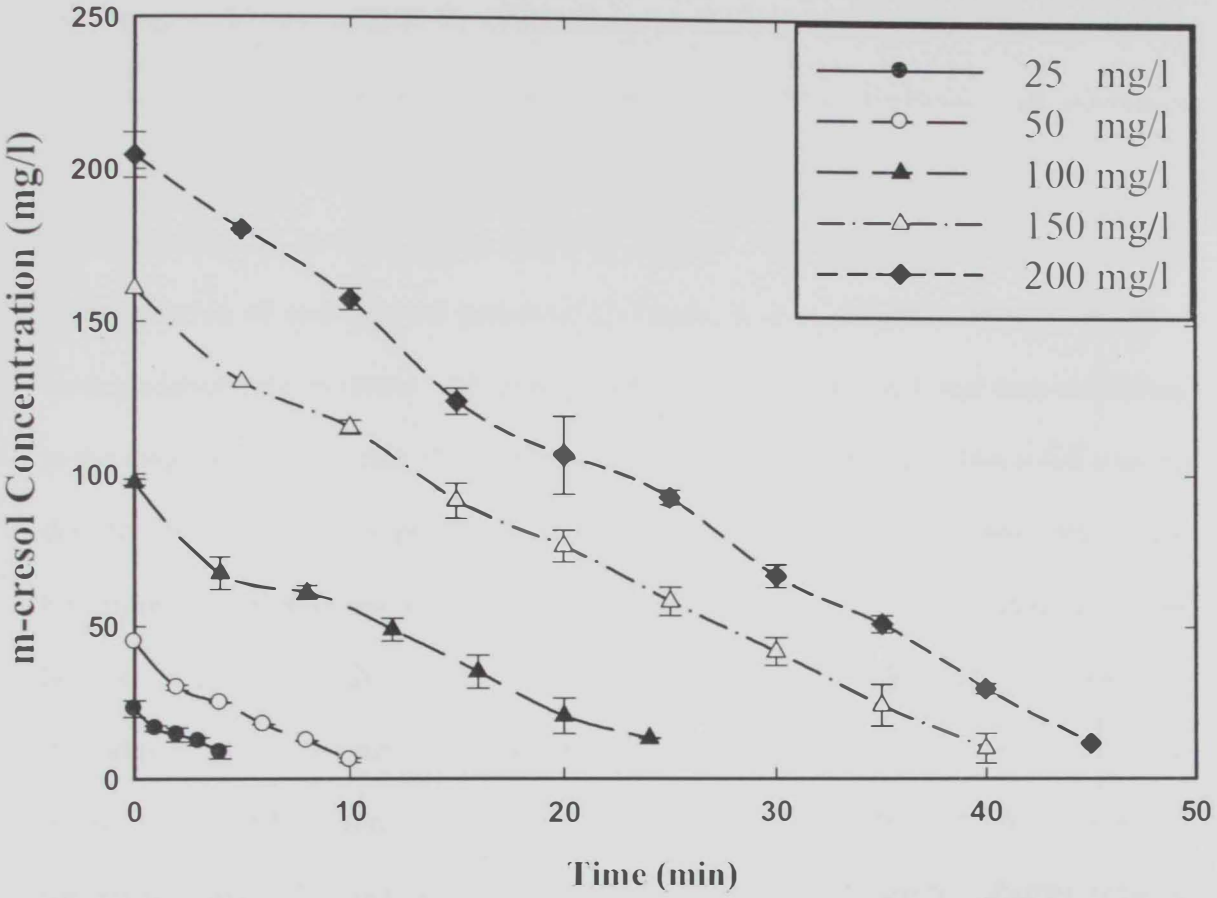
Substrate concentration is one of the most important parameters in the biodegradation of phenolic compounds, due to the inhibitory effect of these contaminants at high concentrations. Batch experiments were carried out at various initial cresols concentrations (25, 50, 100, 150 and 200 mg/l), to evaluate the effect of initial concentration on the biodegradation rate. From the start, a yellow color was observed for a short period of time during all experimental runs. This is believed to be due to the formation of *2-hydroxymuconicsemialdehyde*and its homologues, which has been produced from the metabolizing of *3-methylcatechol*and *4-methylecatechol* by meta pathway [92, 93, 96]. The concentration of each cresol isomer (*o-*, *p-* and *m-cresol*) at different time intervals was measured quantitatively by GC (FID) as mentioned in Section 2.2.2. Figure 3.11(a)–(c) shows the experimental data of the reduction in *cresol* concentration. Clearly the reduction seems to be linear with time, which indicates constant biodegradation rates.


250
200
150
100
50
0
0
10
20
30
40
50
60
o-cresol Concentration (mg/l)
Time (min)
25 mg/l
50 mg/l
100 mg/l
150 mg/l
200 mg/l

(a) O-cresol


250
200
150
100
50
0
0
10
20
30
40
p-cresol Concentration (mg/l)
Time (min)
25 mg/l
50 mg/l
100 mg/l
150 mg/l
200 mg/l

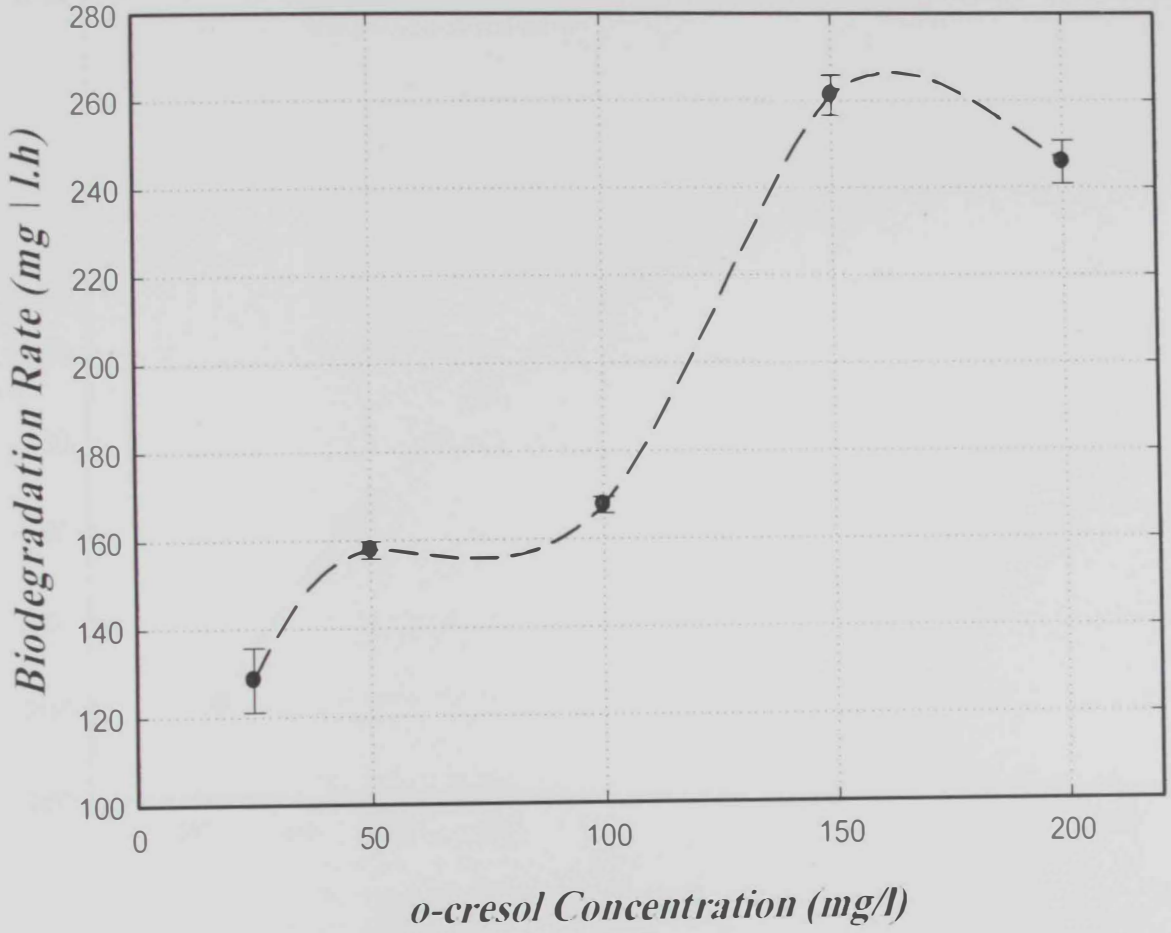
(b) *P-cresol*



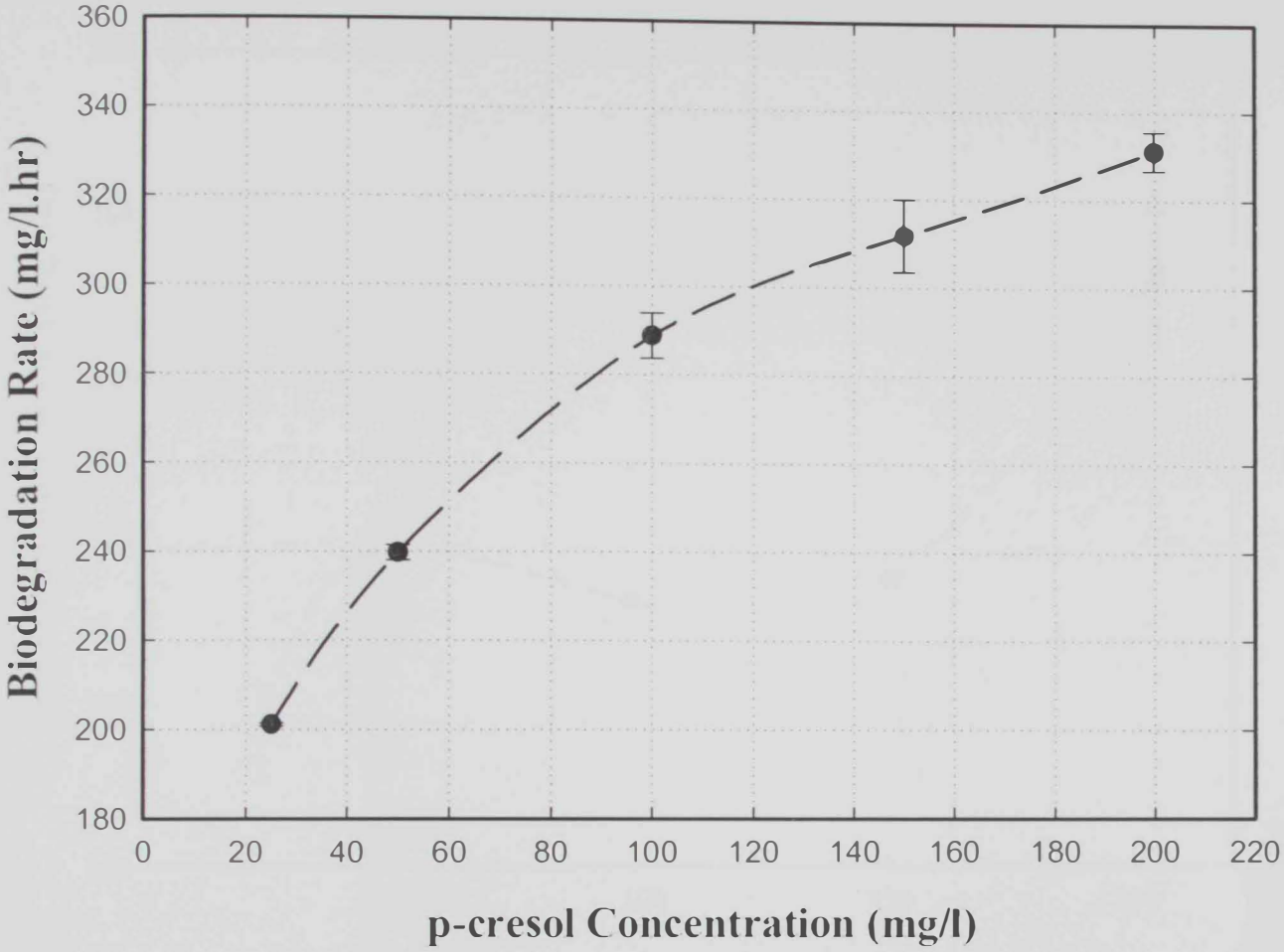
(c) *M-cresol*

Figure 3.11: Variation of initial cresol concentration with time; PVA volume 30%; *T*=30◦C; (a) *o-cresol*; (b) *p-cresol*; (c) *m-cresol*

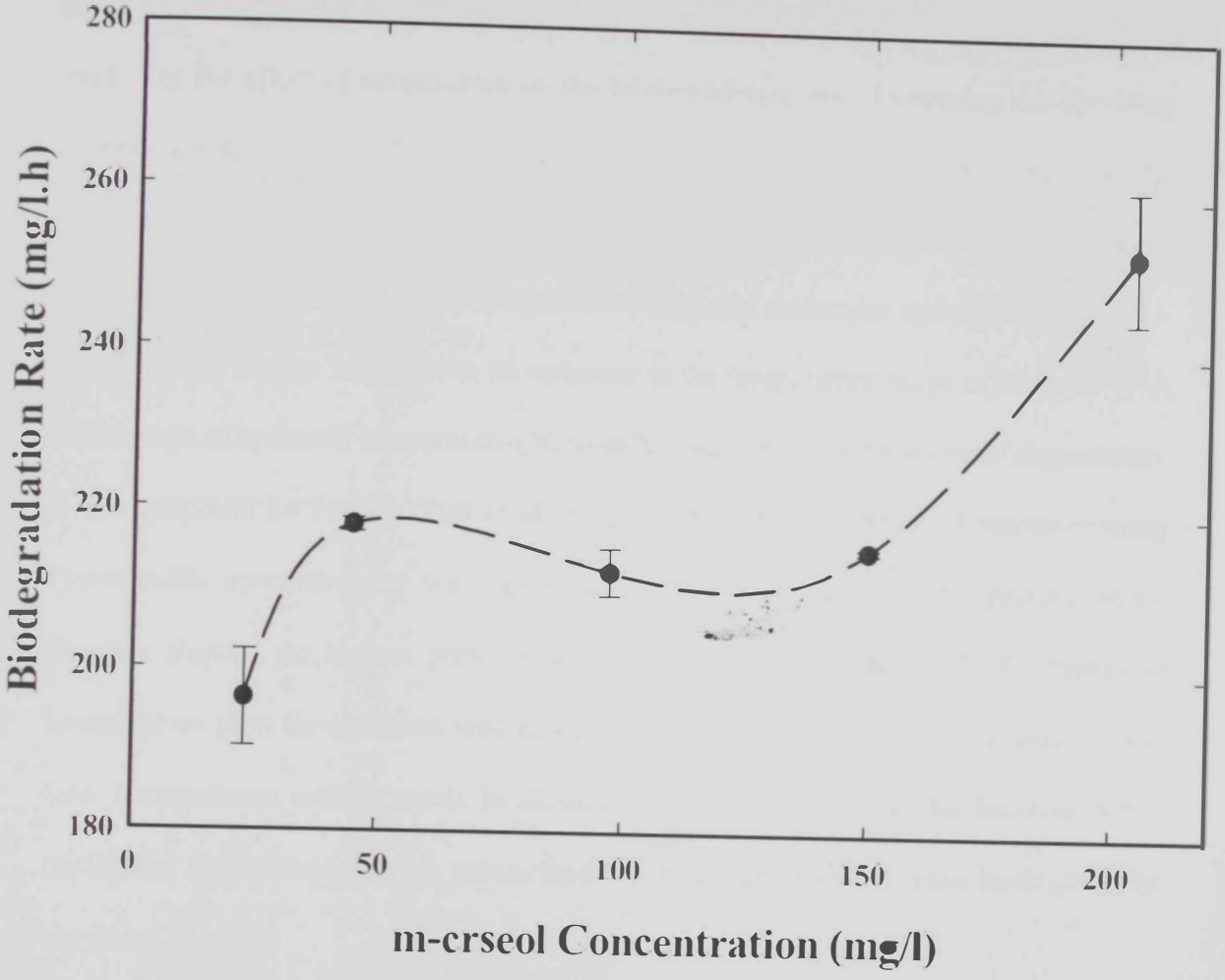
At high substrate concentration (150 mg/l), *o-cresol* is toxic to the microbial activity and inhibited the biodegradation process (Figure 3.12 a). Previous study recommended that the substrate inhibition is a feature of *o-cresol* degradation using *P.putida* and the inhibition effect was observed at initial concentration of 200 mg/l [49, 124]. In contrast, the biomass did not seem to be inhibited by *p-cresol* as indicated by the exponential increase of the biodegradation rate shown in Figure 3.12 b. However, the inhibitory effect of *p-cresol* has been reported at 400 mg/l by Basheer et al. [45] during the biodegradation of *p-cresol* using activated sludge. The experimental results for the biodegradation of *m-cresol* are presented in Figure 3.12 c, and show that the *m-cresol* biodegradation rate increases with concentration from 25 to 50 mg/l, and then stabilizing in the range between 50 and 150 mg/l before rising again at 200 mg/l. This result may be due to the ability of *P.putida* in degrading meta position of cresol, where the biodegradation of *m-cresol* is much difficult than other methyl substitute as discussed in Section 3.2. Therefore the biodegradation rate is stabilized at lower concentrations, and increases by raising the initial concentration. The lack of inhibitory effect in *p-cresol* and *m-cresol* even at high concentrations of 200 mg/l could be attributed to the presence of the immobilizing PVA gel, which acts as a protective shelter against substrate toxicity [11].


Biodegradation Rate (mg / l.h)
280
260
240
220
200
180
160
140
120
100
0
50
100
150
200
o-cresol Concentration (mg/l)

(a) *O-cresol*


Biodegradation Rate (mg/l.hr)
360
340
320
300
280
260
240
220
200
180
0
20
40
60
80
100
120
140
160
180
200
220
p-cresol Concentration (mg/l)

(b) *P-cresol*

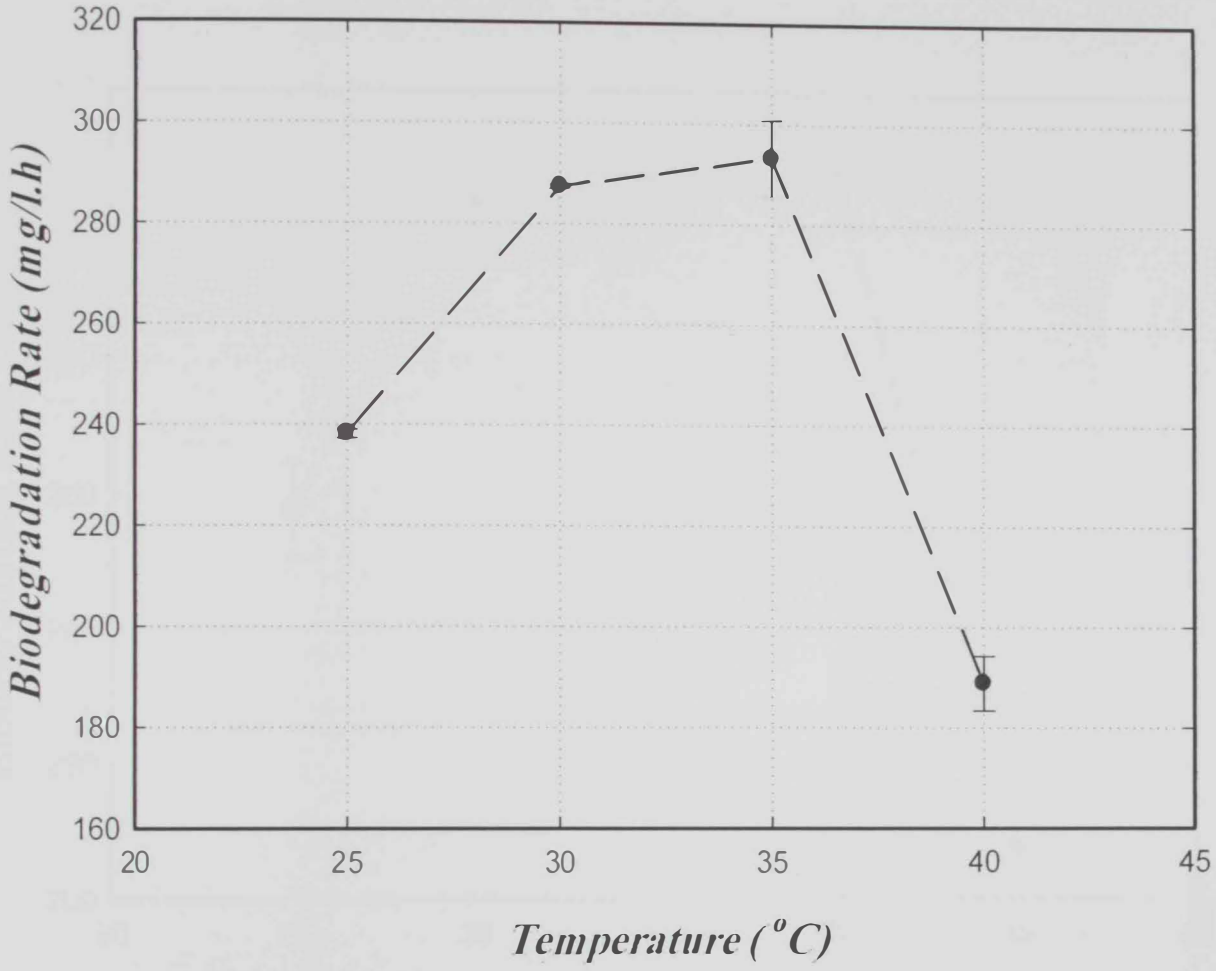


(c) M-cresol

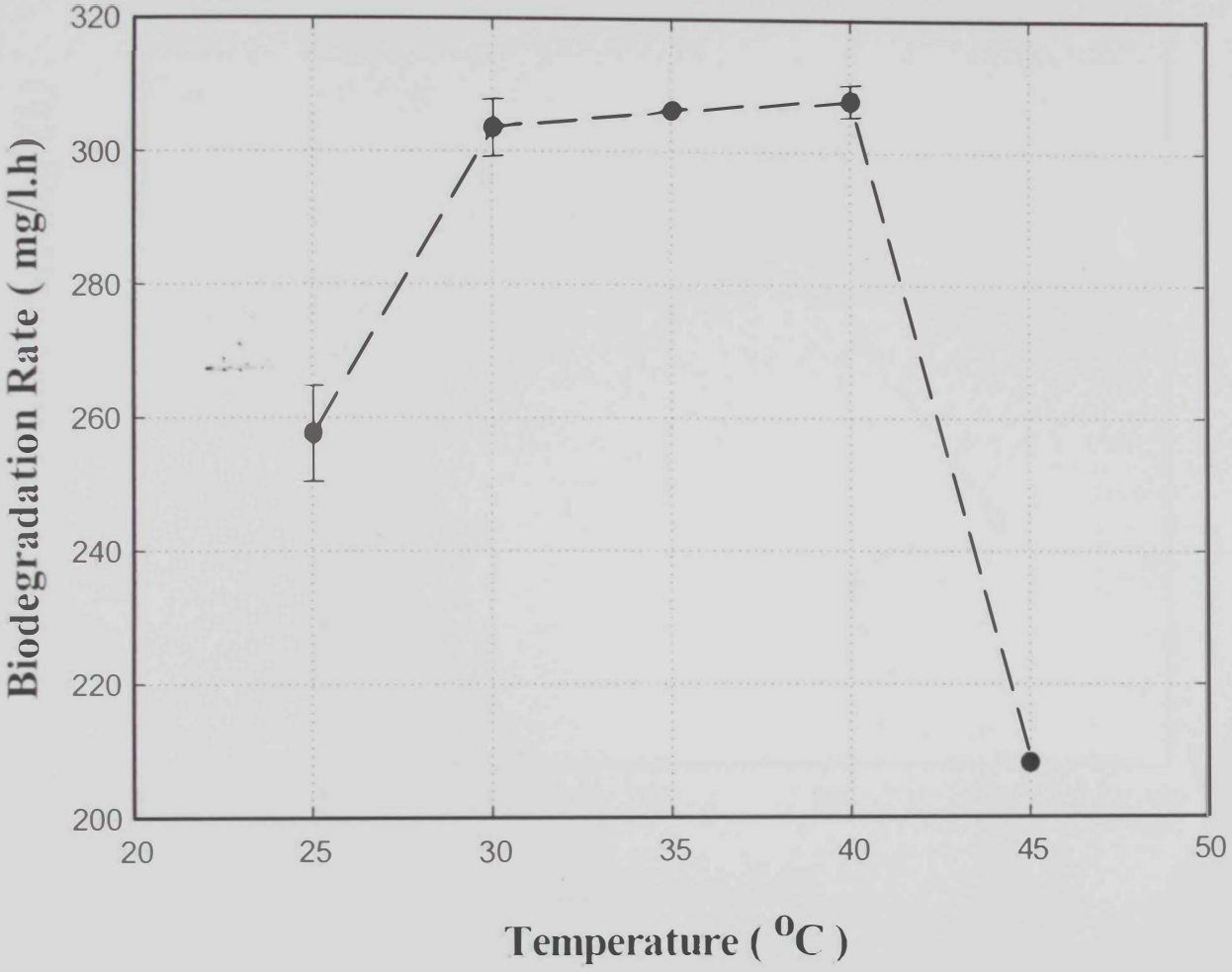
Figure 3.12: Biodegradation rate at different cresol concentrations; PVA volume= 30% of the total volume ;*T*=30°C; (a) *o-cresol*; (b) *p-cresol*; (c) *m-cresol*

3.3.1.2.Effect of temperature

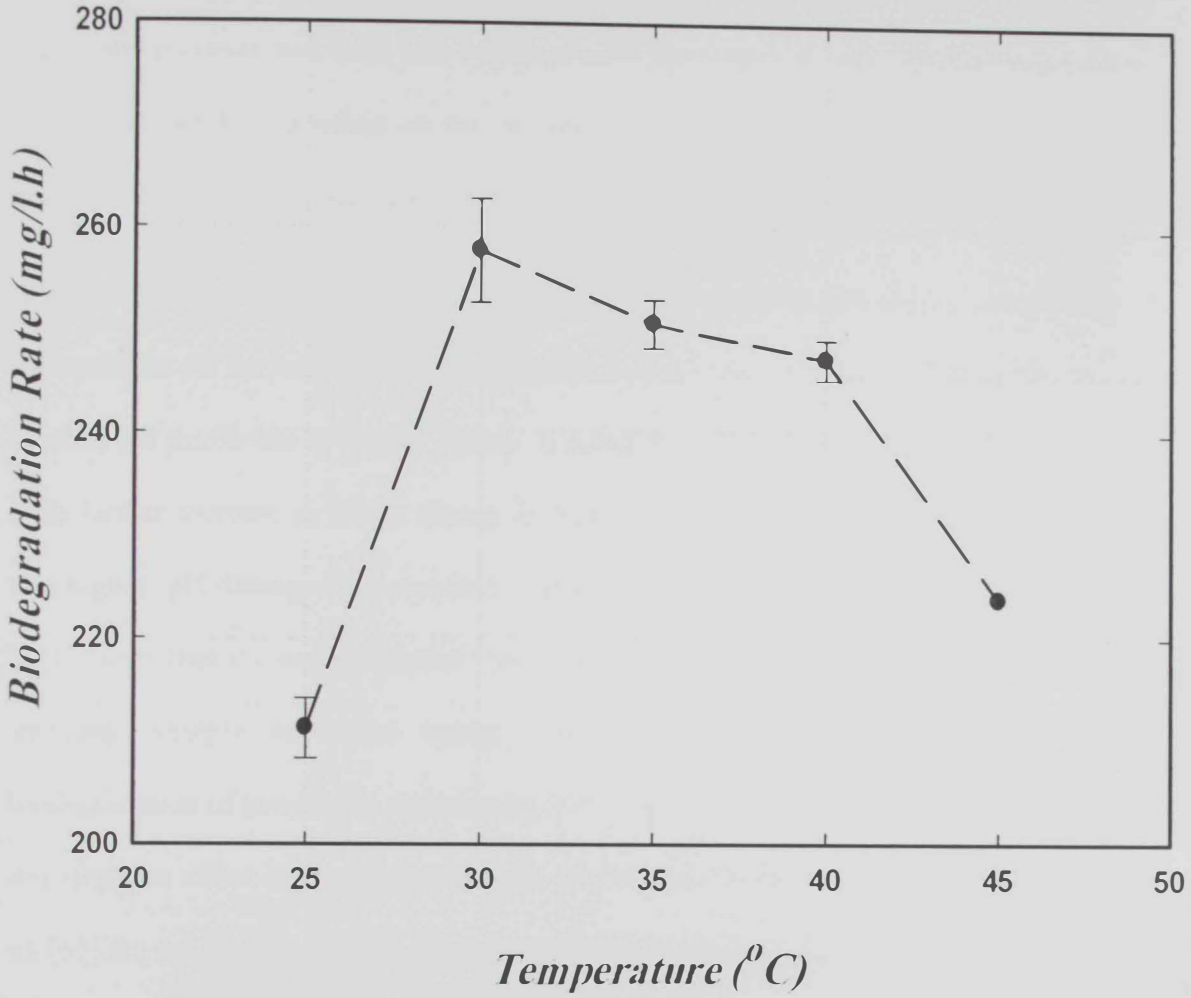
Experiments were carried out at temperatures ranging from 25 to 45°C to study the effect of temperature on cresols removal. *O-cresol* initial concentration was kept constant at 150 mg/l, and the initial concentrations of *p-* and *m-cresol* were kept at 200 mg/l. The initial pH solution was adjusted to be fixed at 7. Figure 3.13 presents the experimental results for the effect of temperature on the biodegradation rate. Increasing the operating temperature from 25 to 30°C seems to enhance the capability of *P.putida* for the biodegradation of all cresol isomers. As mentioned earlier, an increase in the temperature basically results in more kinetics energy of the enzymes molecules and reactants [77]. *O-cresol* biodegradation rate reaches an optimum in the temperature range of 30 to 35°C. A wider range of optimum temperature (30 to 40°C) was observed for *p-cresol* degradation. It was proposed by Haiyan You et al. [74] that the biodegradation of *m-cresol* using *Lysinibacillu scresolivorans* was optimized at 35°C; where as in the present study, *P.putida* showed the highest performance in *m-cresol* degradation at 30°C. Higher or lower values than the optimum tend to have negative effect on the biodegradation rate. Low temperatures usually result in slowing down the activity of the bacteria, while raising the temperature to high values leads to deactivation of the main biodegradation enzymes [83, 125].


320
300
280
260
240
220
200
180
160
20
25
30
35
40
45
Biodegradation Rate (mg/l.h)
Temperature (°C)

(a) *O-cresol*


320
300
280
260
240
220
200
Biodegradation Rate (mg/l.h)
20
25
30
35
40
45
50
Temperature (°C)

(b) *P-cresol*



(c) *M-cresol*

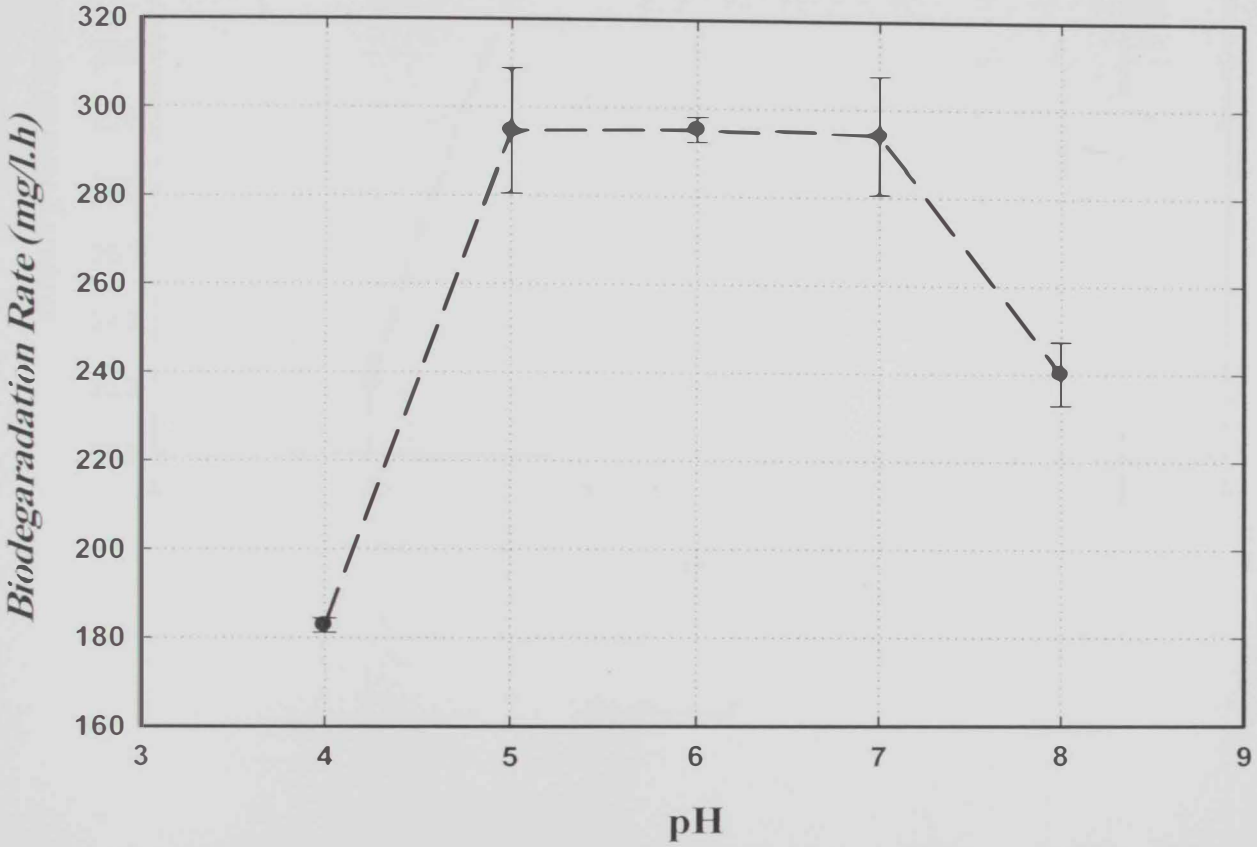
Figure 3.13: Variation of biodegradation rate for all cresols isomers with temperature. Initial pH 7.8; PVA volume= 30% of total volume

3.3.1.3.Effect of pH

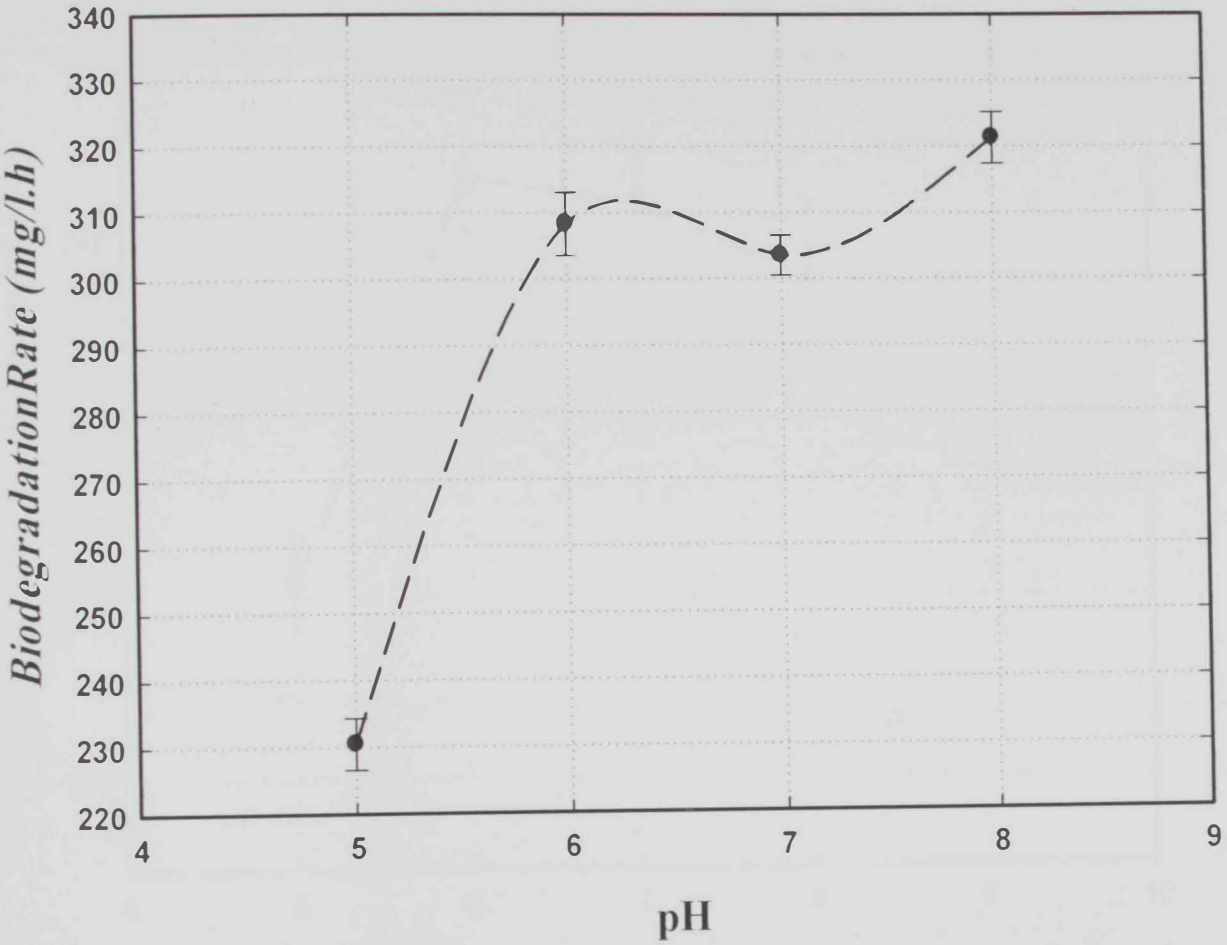
Being proteins, enzymes are stabilized by weak hydrogen bonds and are generally affected by the variation of pH [82]. In order to study the effect of initial pH on the biodegradation of cresols, experiments were conducted at pH ranging from 4 to 8 for *o-cresol* and *p-cresol*, and from 4 to 9 for*m-cresol*. The initial pH was adjusted using drops of HCl or NaOH depending on the desired pH value. The initial concentrations of *o-cresol* were kept at 150 mg/l and for both *p-* and *m-cresol* at 200 mg/l.

It is believed that most organisms cannot tolerate pH levels below 4.0 or above 9.5, and the optimum pH for most microorganisms lies in this range. *O-cresol* biodegradation rate reached the maximum at the pH values ranging from 5 to 7, and then declined sharply with further increase in pH as shown in Figure 3.14 a. Severe environment with lower and higher pH damaged the enzymes, affecting the degradability of the bacteria. Figure 3.14 shows that the biodegradation rate of *p-cresol* is diminished at pH 5, and the rate increases sharply at higher values. The experimental results indicate that the biodegradation of *p-cresol* is optimized at pH 8, and it can be varied from 6 to 8 without any negative effect on the biodegradation rate. Similar results were reported by Singh et al. [65] for the biodegradation of *p-cresol* using *Gliomastix indius*.

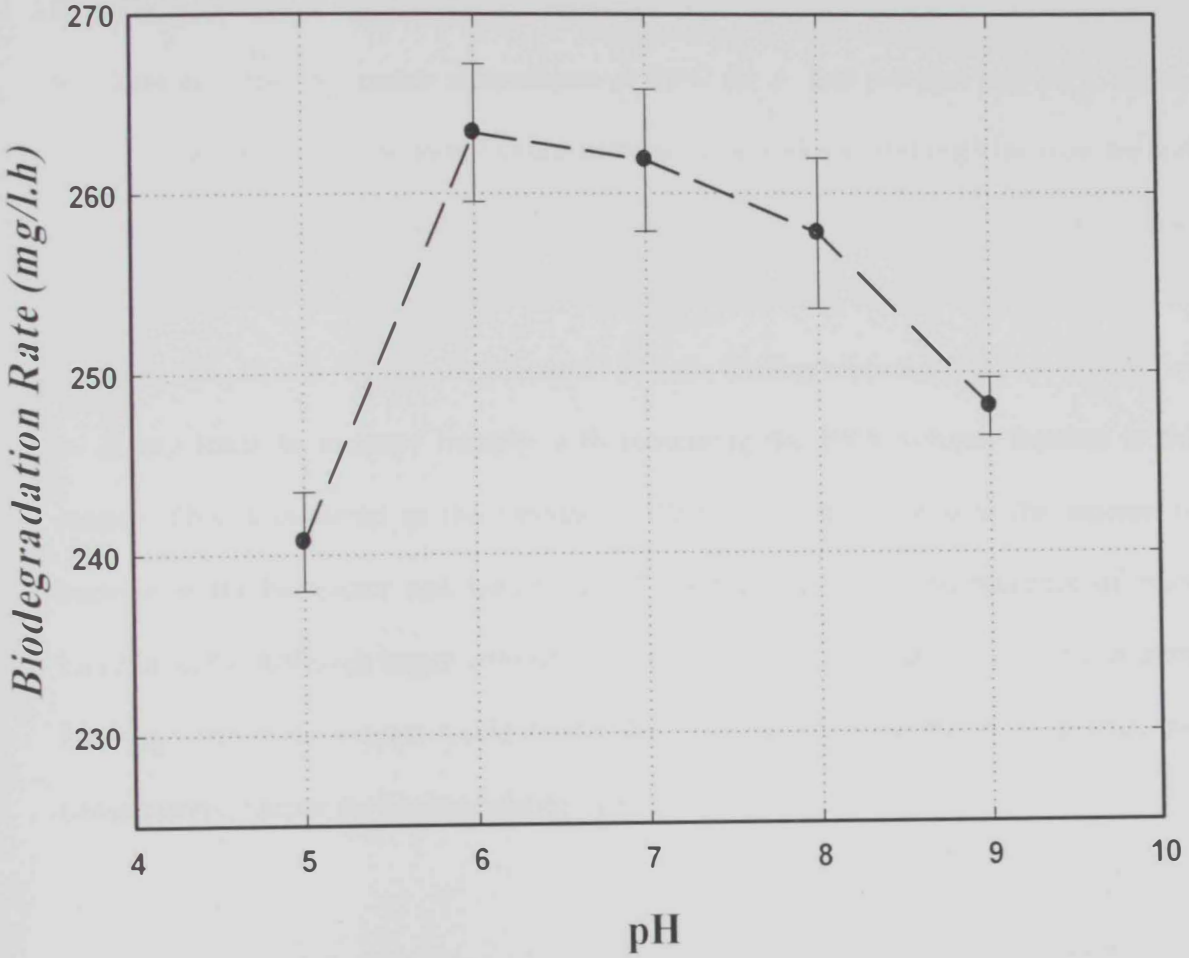
The ability of the *P.putida* to degrade *m*-cresol reached the maximum in the range of pH of 6 (Figure 3.14 c);the rate decreases slightly at pH 7 and drops again at pH 8. In a similar study, Haiyan Yao et al. [73] reported that the biodegradation of *m-cresol* using *Lysinibacillus cresolivorance* was optimum at pH of 7. These results support the statement that the growth rate of most of the organisms usually reaches the maximum in the pH range of 6.5 to 7.5 [66].



(a) *O-cresol*


340
330
320
310
300
290
280
270
260
250
240
230
220
Biodegradation Rate (mg/l.h)
4
5
6
7
8
9
pH

(b) *P-cresol*



(c)*M-cresol*

Figure 3.14: Effect of the initial solution pH on the biodegradation rate of phenol; PVA volume = 30% of the total volume; (a) *o-cresol*; (b) *p-cresol*; (c) *m-cresol*

3.3.1.4.Effect of PVA volume fraction

The amount of PVA pellets, which contains the active biomass, is one of the most important factors that can affect the biodegradation process. Experiments were conducted to study the effect of PVA volume fraction on the biodegradation rate. The solution pH was kept at 7 and the reactor temperature at 35°C for *o-* and *p-cresol* and 30°C for *m-cresol* biodegradation. The initial concentrations were 150 and 200 mg/l for *o-cresol* and both *p-* and *m-cresol*, respectively. The total operating volume of the bioreactor was maintained at 300 ml. Figure 3.15 shows the biodegradation rate for three PVA volume fractions (20, 30 and 40%). It is noticeable that the biodegradation rate of all cresols *(o-, p- & m-)* tends to increase linearly with increasing the PVA volume fraction in the reactor. This is expected as the amount of PVA is directly related to the amount of bacteria in the bioreactor and hence more PVA particles mean the presence of more bacterial cells. Although larger volume fractions were not tested, it is expected that more PVA particles in the reactor would hinder the movement and mixing of the pellets, and consequently, reduce the biodegradation rate.

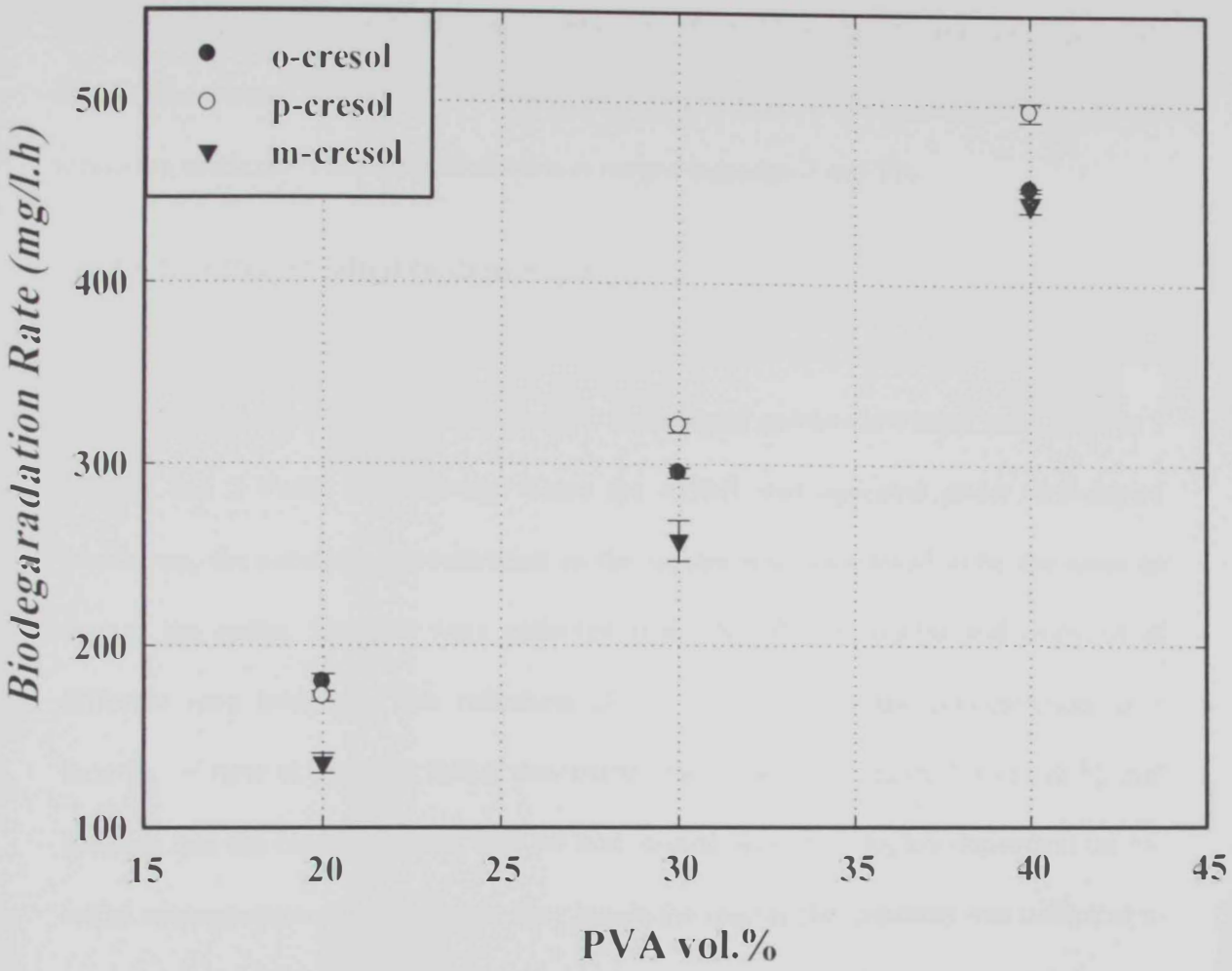


Figure 3.15: Effect of PVA vol.% on cresols biodegradation rate

3.3.2. Continuous Biodegradation of cresol

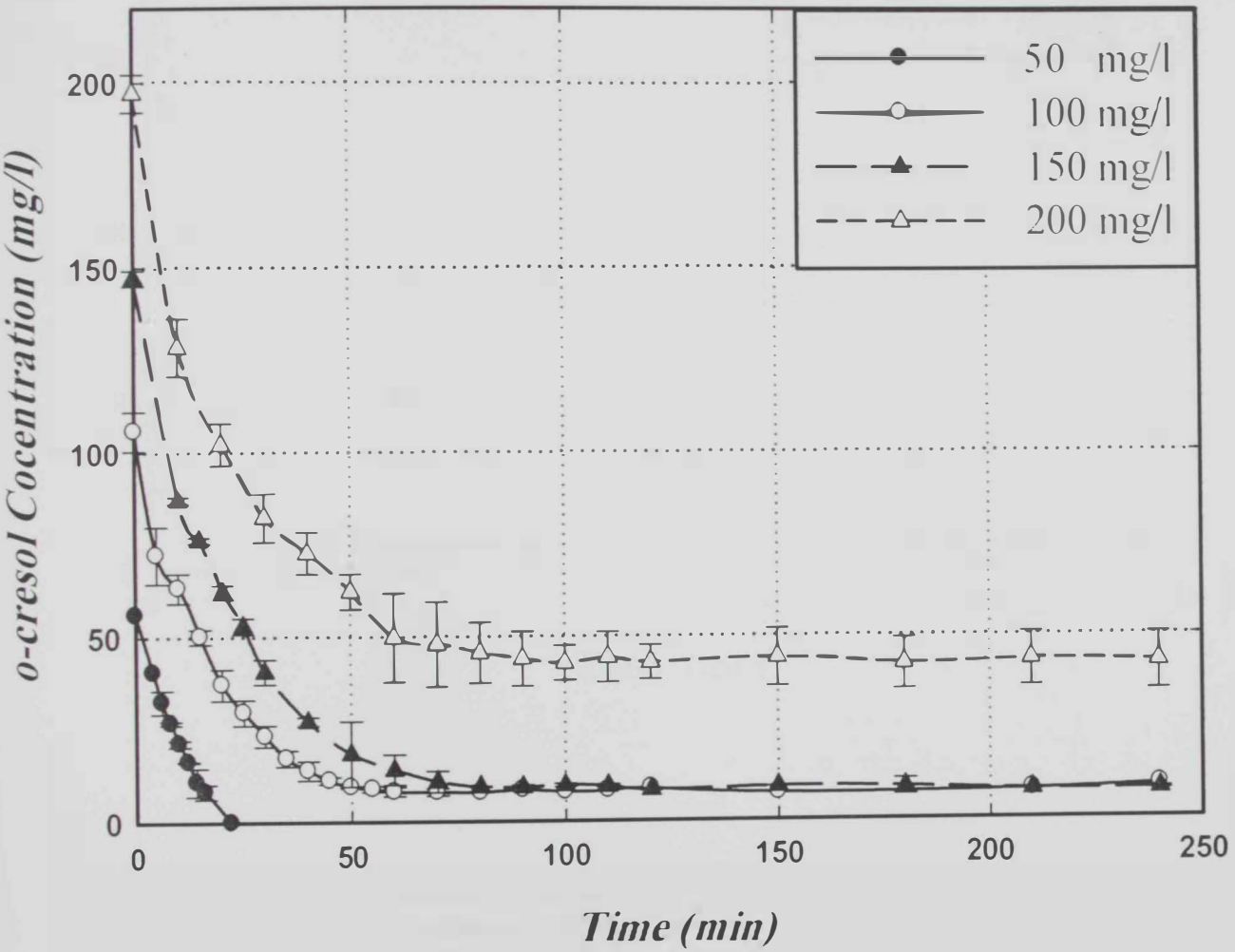
Although the batch experimental work provided essential data regarding the effect of certain operating parameters on the biodegradation rate, continuous operation is vital in assessing the potential industrial application of the biodegradation process. Again, all experiments were carried out in duplicates and average values are reported in the following sections. The standard deviation ranged between 2 and 5%.

3.3.2.1.Effect of initial concentration

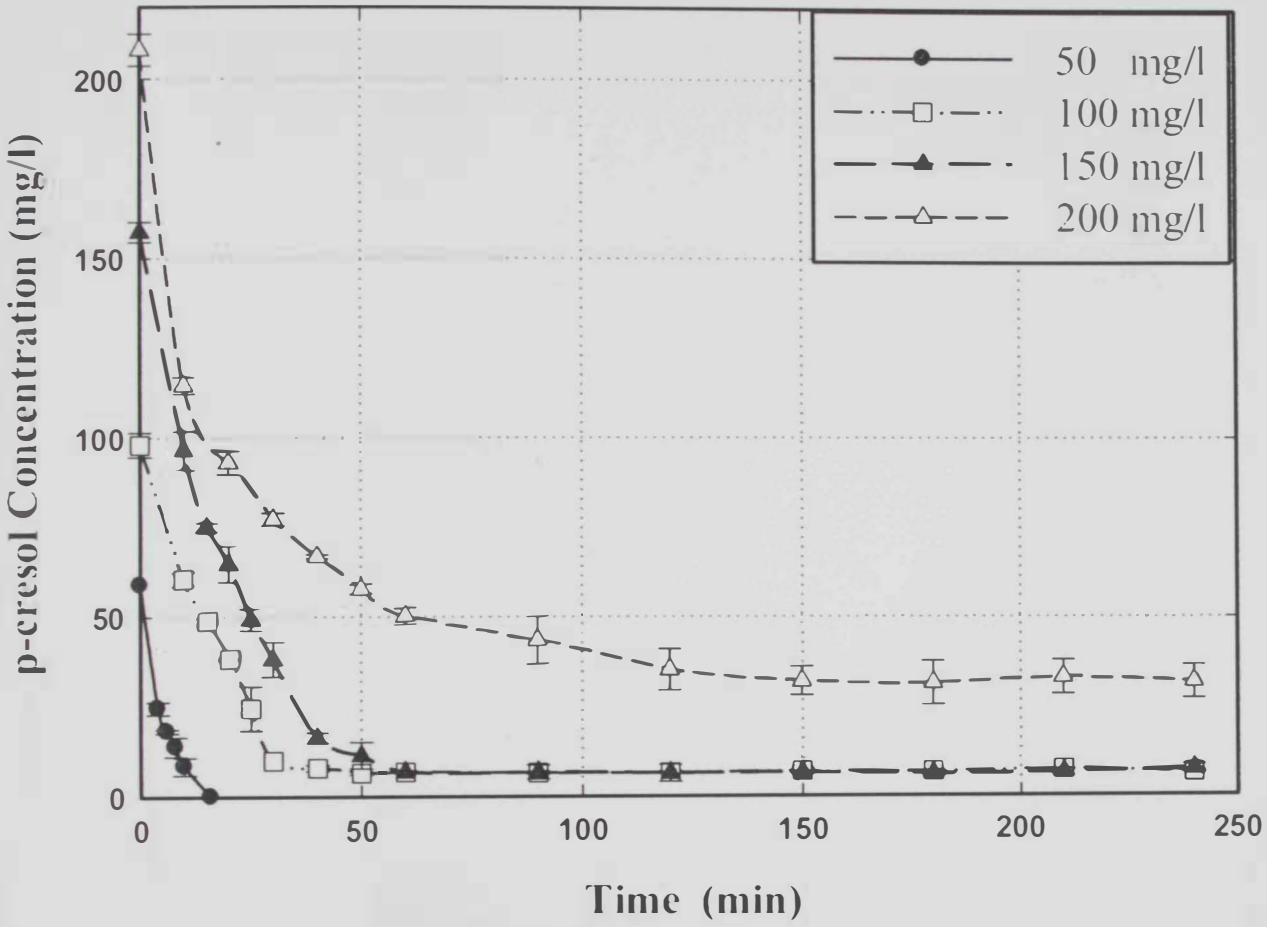
The effect of the initial concentration of *o-cresol* and *p-cresol* was studied at four initial concentrations: 50, 100, 150 and 200 mg/l. Both liquid and air flow rates were fixed at 5 ml/min and 2 l/min, respectively. Since the SBBR was operated under well-mixed conditions, the substrate concentration in the reactor was considered to be the same as that of the outlet. Samples were collected from the effluent stream and analyzed at different time intervals. The reduction of *o-cresol* and *p-cresol* concentration as a function of time at different initial concentrations is shown in Figure 3.16 (a & b), and indicate that the biodegradation rate for both cresol isomers is highly dependent on the initial concentration. The residence time inside the reactor (60 minutes) was sufficient to completely consume the substrates at low concentration (50 mg/l).

However, at higher concentrations, substrates were not completely degraded, but steady state was achieved within one residence time. It must be mentioned here that during the startup of the biodegradation process, sharp reduction in cresol concentration was observed, followed by a period of slow reduction before stabilizing. A faster diffusion step due to the physiochemical interactions between the organic chemicals and microbial

cell walls is believed to have resulted in the first stage of the biodegradation process [126, 127]. During this phase, the removal by the biodegradation is less significant. However, in the second phase, the entire exposed surface of the PVA pellets gets saturated with the substrate or reach closed to saturation, then the removal by adsorption becomes less significant. In this phase, the contribution of biodegradation becomes significant.



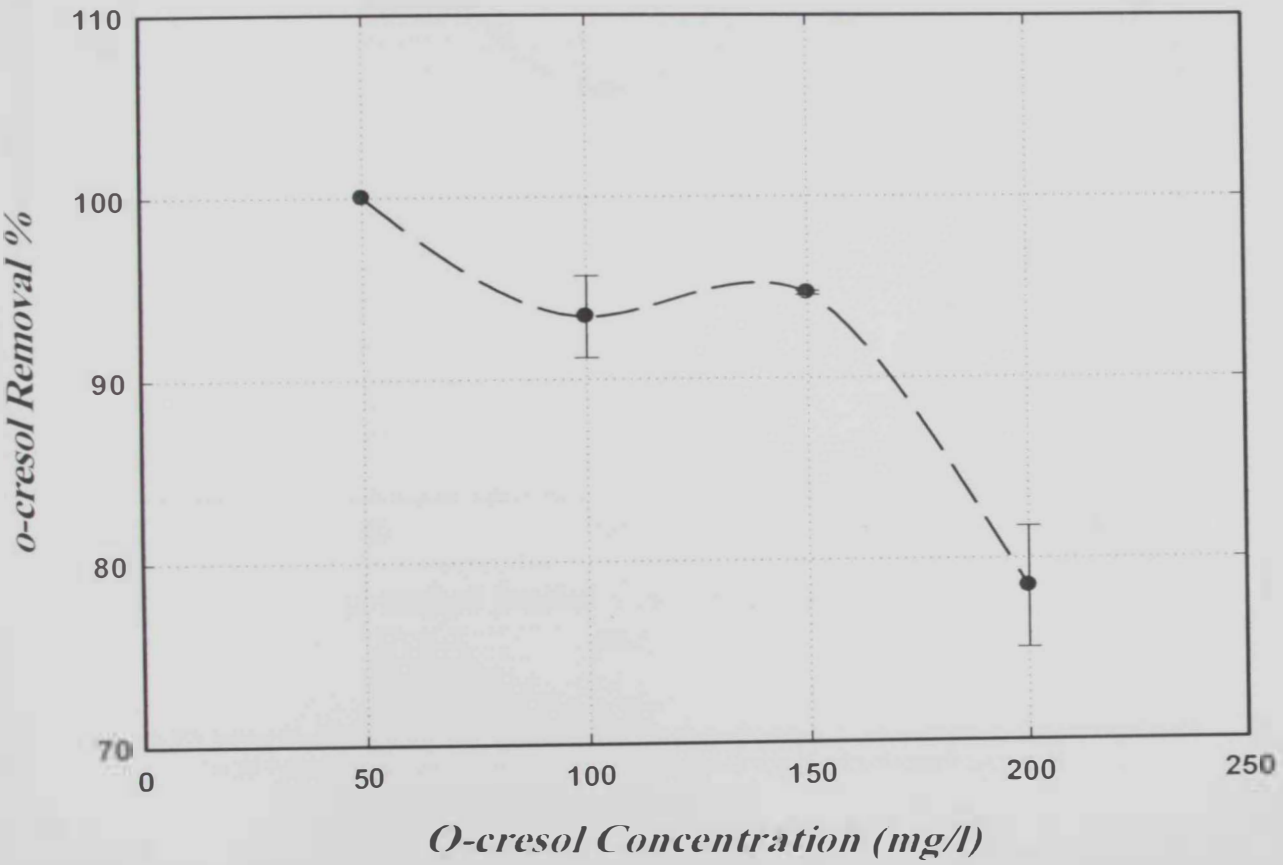
(a) *O-cresol*



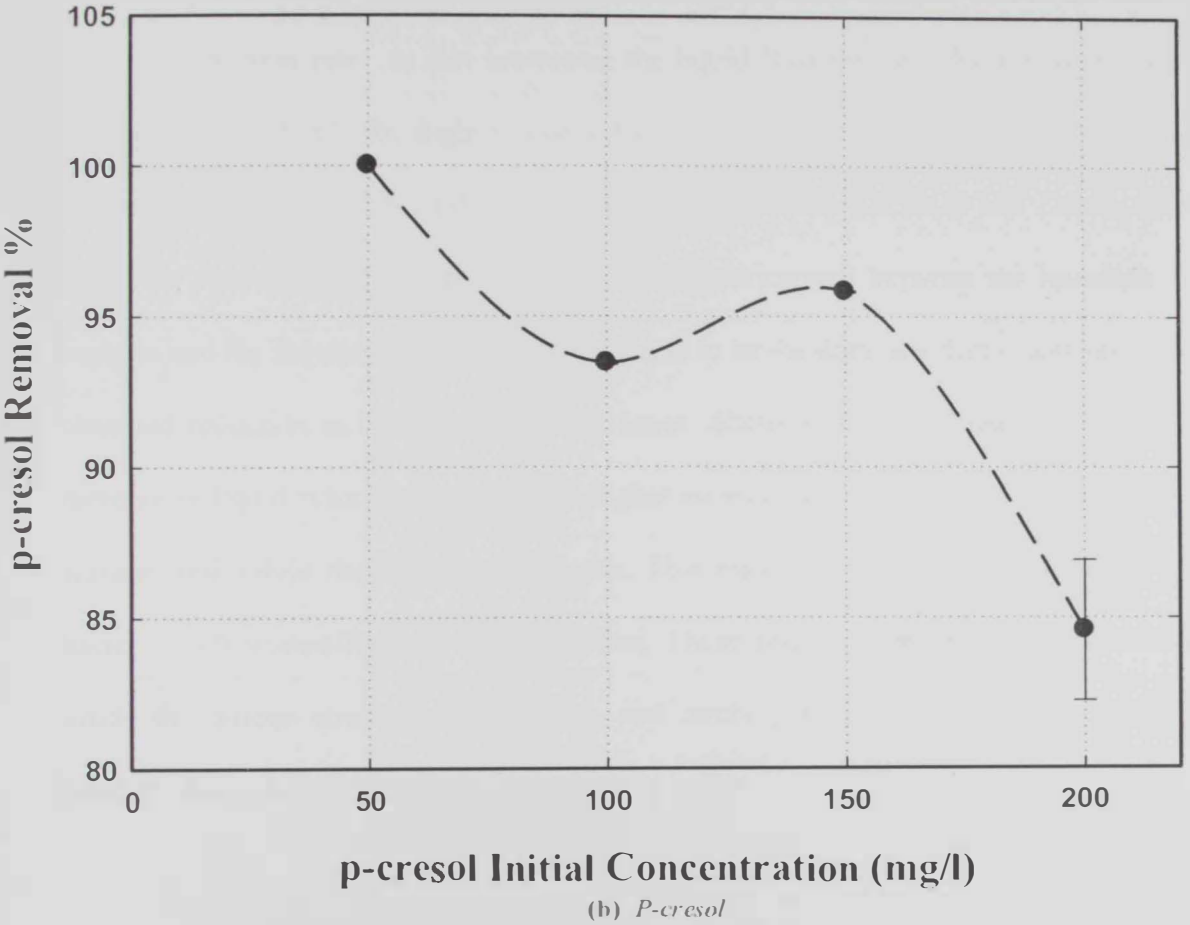
(a) *P-cresol*

Figure3.16: *Cresol* concentration as a function of time for different initial p-cresol concentrations. Reactor temperature = 35 °C; air flow rate 2 l/min; liquid flow rate = 5 ml/min; (a) *o-cresol*; (b) *p-cresol*

The overall percent removal is plotted as a function of initial concentration in Figure 3.17(a & b). In cases of *o-cresol*, the percent removal at high concentration (200 mg/l) reached 79%, compared with that for *p-cresol* where the maximum removal percentage at the same concentration reached 85%. This could be attributed to the absence of the inhibition effect on the biodegradation process of *p-cresol* as concluded from the batch study.



(a) *O-cresol*

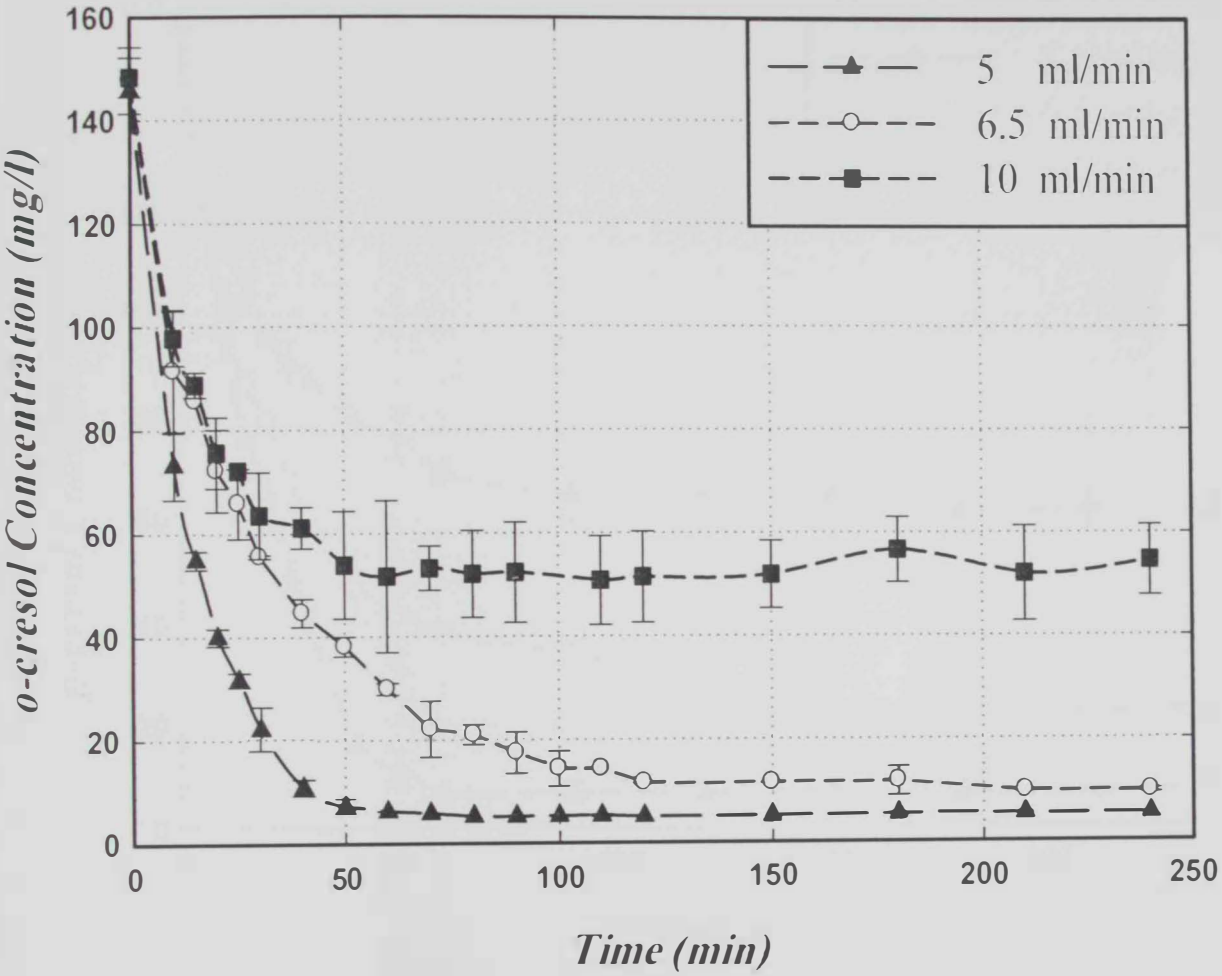


(b) *P-cresol*

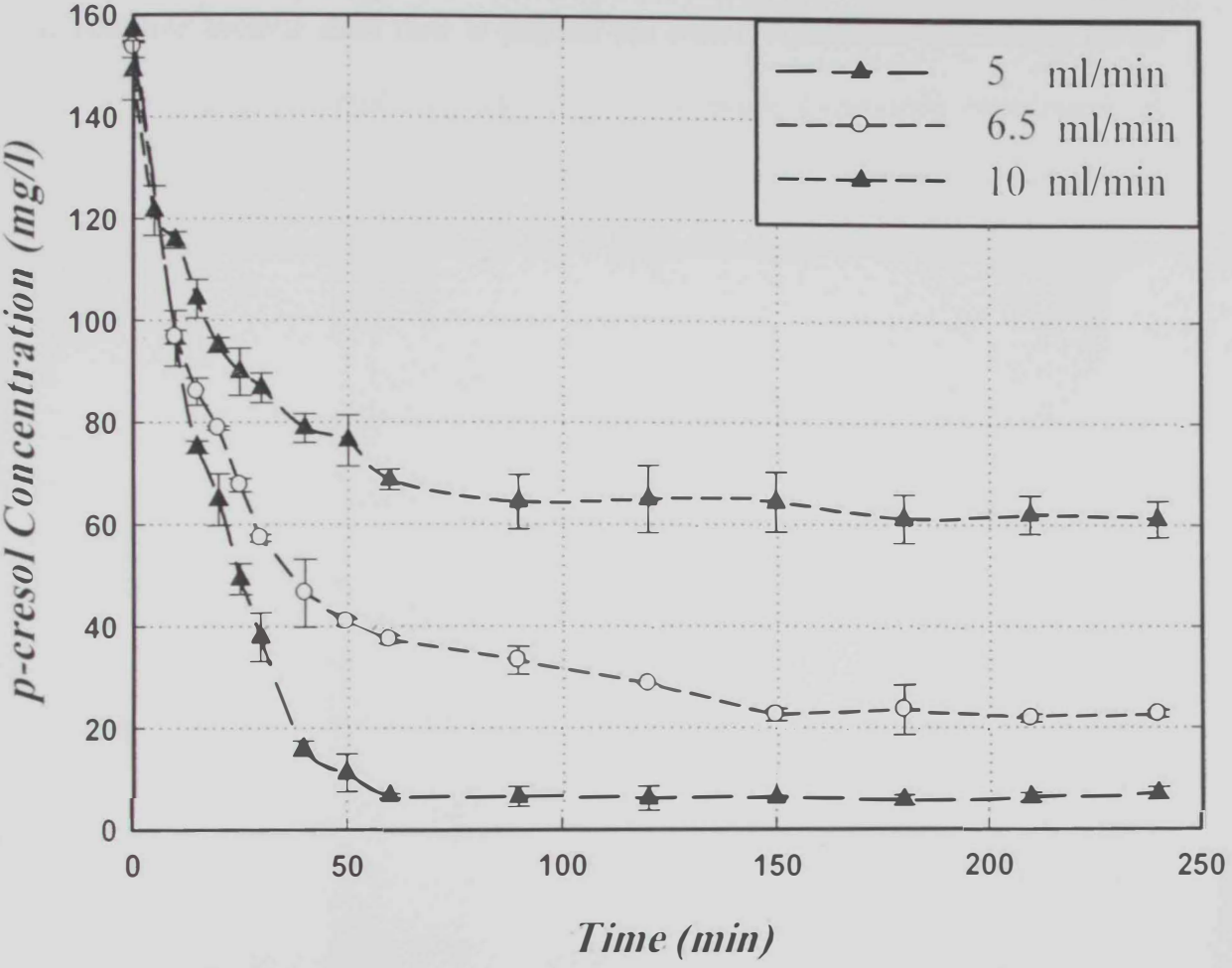
Figure3.17: Initial biodegradation rate of *cresol* as a function of initial concentration. Reactor temperature = 35 ° C; air flow rate 2 l/min; liquid flow rate = 5 ml/min; (a) *o-cresol; p-cresol*

3.3.2.2.Effect of liquid flow rate

The effect of liquid flow rate on the biodegradation efficiency was evaluated for three different flow rates (5, 6.5 and 10 ml/min), while keeping *cresol* initial concentration and airflow rate constant at 150 mg/l and 2 l/min. It is often anticipated that increasing the liquid flow rate would reduce the residence time inside the bioreactor and hence reduce the biodegradation rate. In fact increasing the liquid flow rate may have two opposing factors. On one hand, the higher flow velocity around the PVA pellets may improve external mass transfer, especially for low concentrations of cresol (*o*- & *p*-). On the other hand, the reduced residence time will decrease the contact between the immobilized bacteria and the substrate [128]. The latter seems to be the dominate factor and hence the observed reduction in biodegradation efficiency. Shetty et al. [129] suggested that the increase in liquid velocity may result in higher erosion of the biomass from the biofilm surface, and reduce the biodegradation rate. This may be valid for biofilms but not for bacterial cell immobilized in PVA particles. These cells are usually very well nested inside the porous structure of the PVA and rarely get stripped away in significant numbers due to high liquid flow.


160
140
120
100
80
60
40
20
0
0
50
100
150
200
250
o-cresol Concentration (mg/l)
Time (min)
5 ml/min
6.5 ml/min
10 ml/min

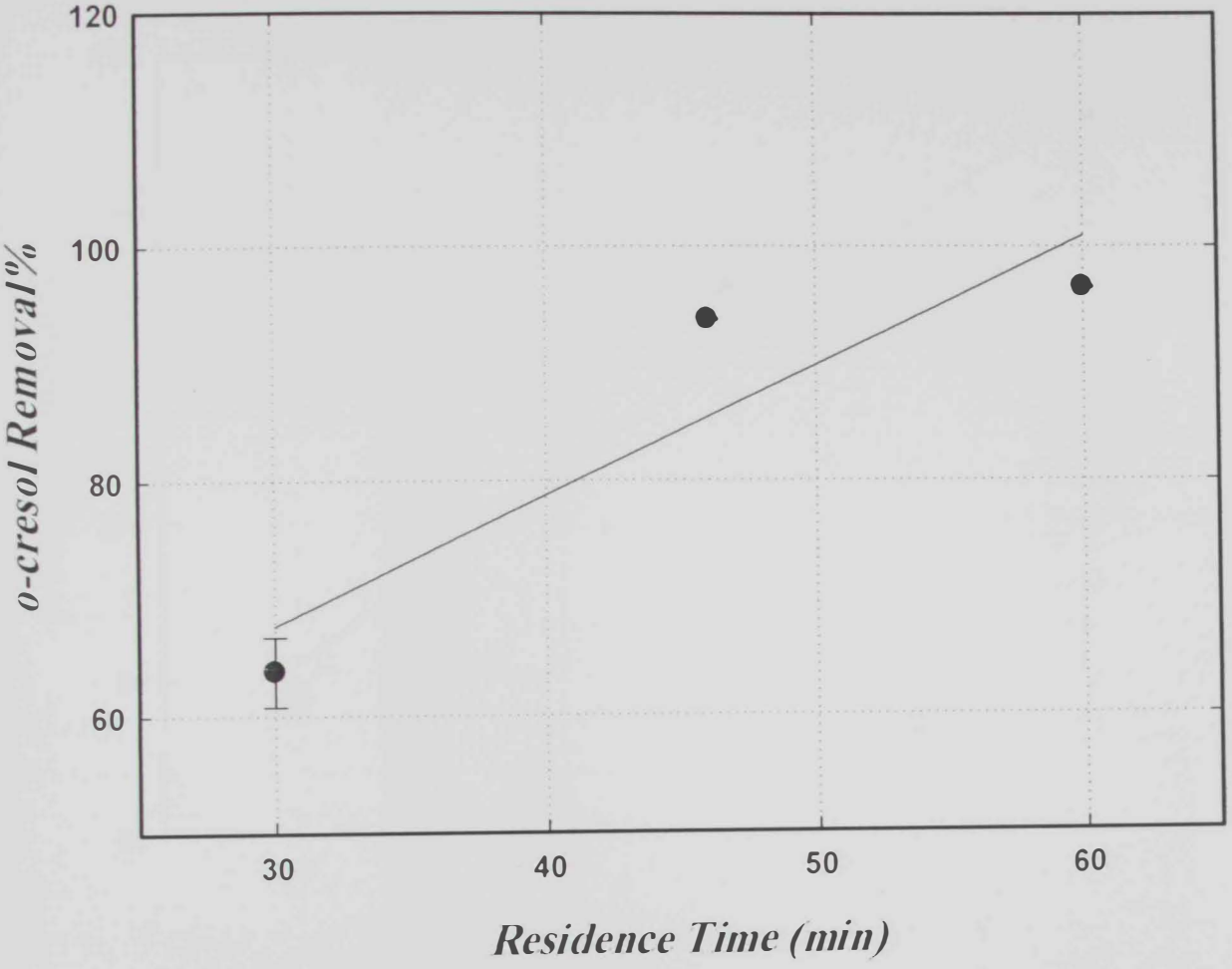
(a) *O-cresol*



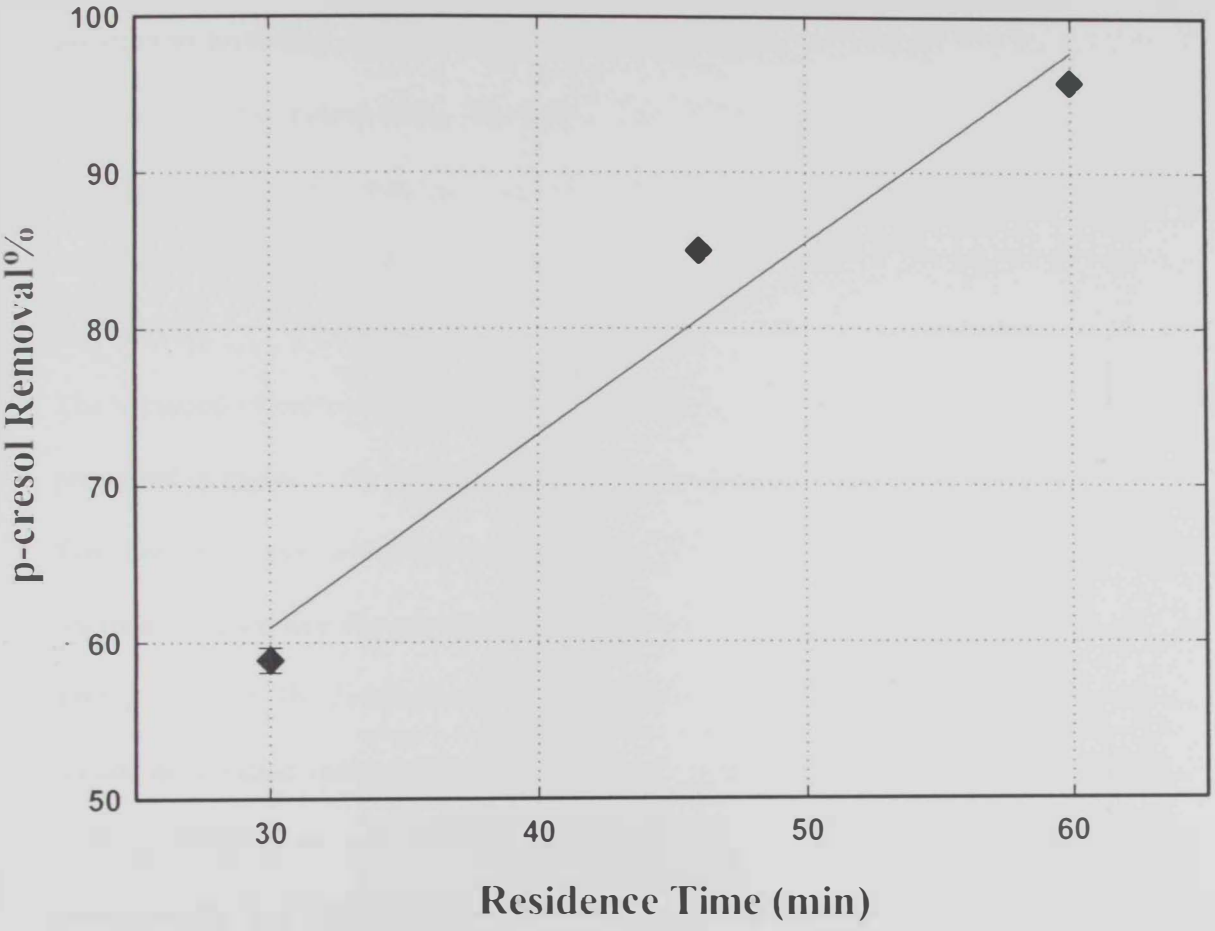
(b)*P-cresol*

Figure 3.18: Concentration of *cresol* in the reactor as a function of time for different liquid flow rates (LF). Initial *cresol* concentration = 150 mg/l; reactor temperature = 35 °C; air flow rate 2 l/min; (a) *o-cresol*; (b) *p-cresol*

The residence time for the three liquid flow rates (2.5, 5 and 6.5 l/min) were estimated to be 60, 46 and 30 min, respectively. A plot of the substrate removal as a function of residence time is shown in Figure 3.19 (a &b). Obviously, the removal % increases linearly with increasing the residence time. In both cases, longer residence time gives the immobilized bacteria more time to degrade the *cresol*. *P.putida* is well known for its ability to degrade *o-cresol* more than *p-cresol* in single components experiments, as concluded from the biodegradation results for all cresol isomers; therefore lower residence time tends to highly affect *p-cresol* degradation more than *o-cresol* degradation



(a) *O-cresol*



(b) *P-cresol*

Figure 3.19: Biodegradation rate of *cresol* as a function of residence time. Initial *cresol* concentration = 150 mg/l; reactor temperature = 35°C; air flow rate 2 l/min; (a) *o-cresol*; (b) *p-cresol*

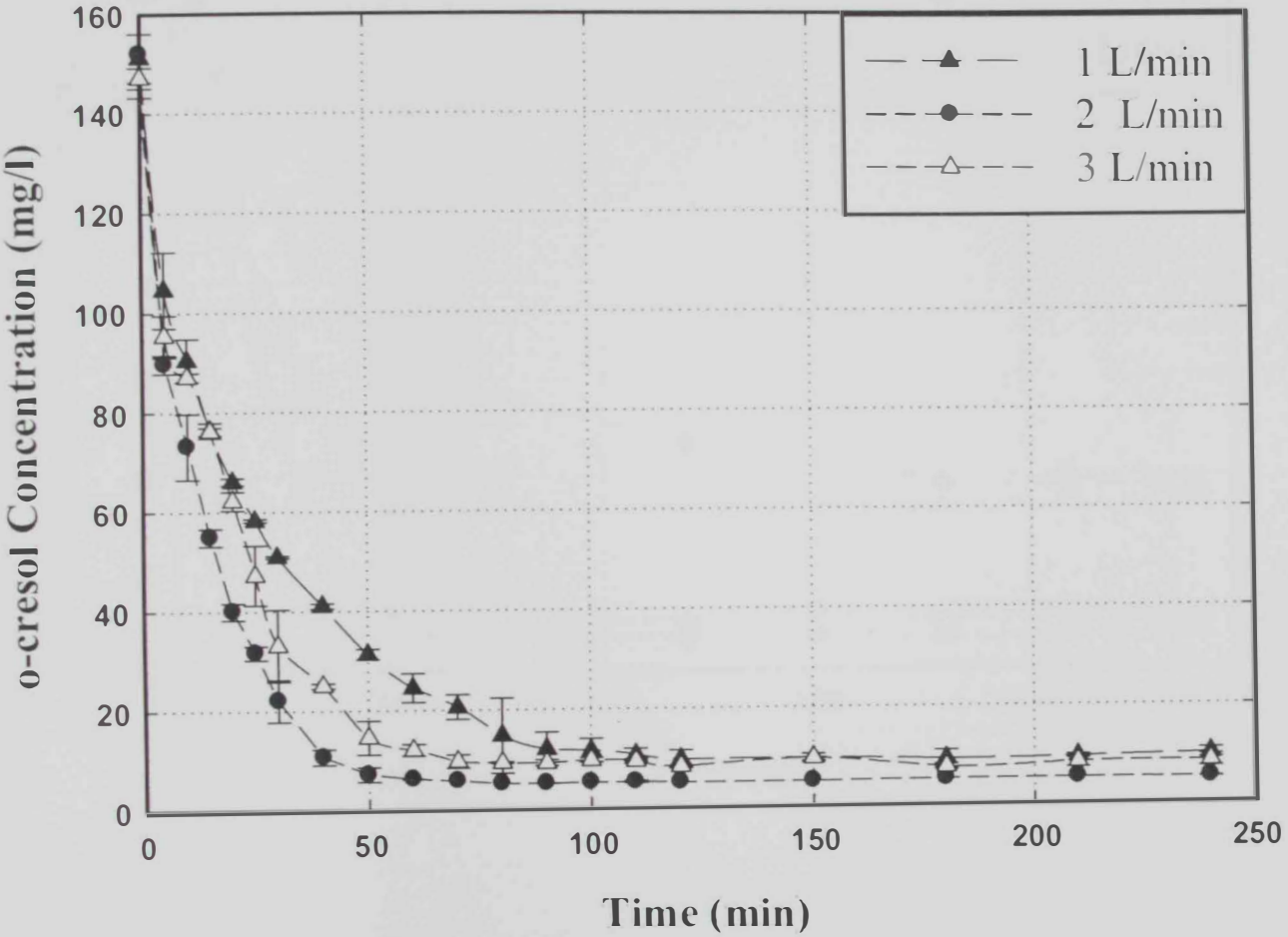
3.3.2.3.Effect of Air flow rate

Aerobic biodegradation processes require systems which ensure the adequate oxygen supply for maintaining the growth of the microorganisms. Air flow rate plays an important role in the biodegradation process using aerobic bacteria such as *P.putida*. In addition to providing enough oxygen for the biodegradation process, it enhances mixing and particles movement in the bioreactor. The effect of air flow rate on the continuous biodegradation process was investigated for three different values: 1, 2 and 3 l/min. The initial substrate concentration and liquid flow rate were fixed at 150 mg/l and 5 ml/min, respectively.

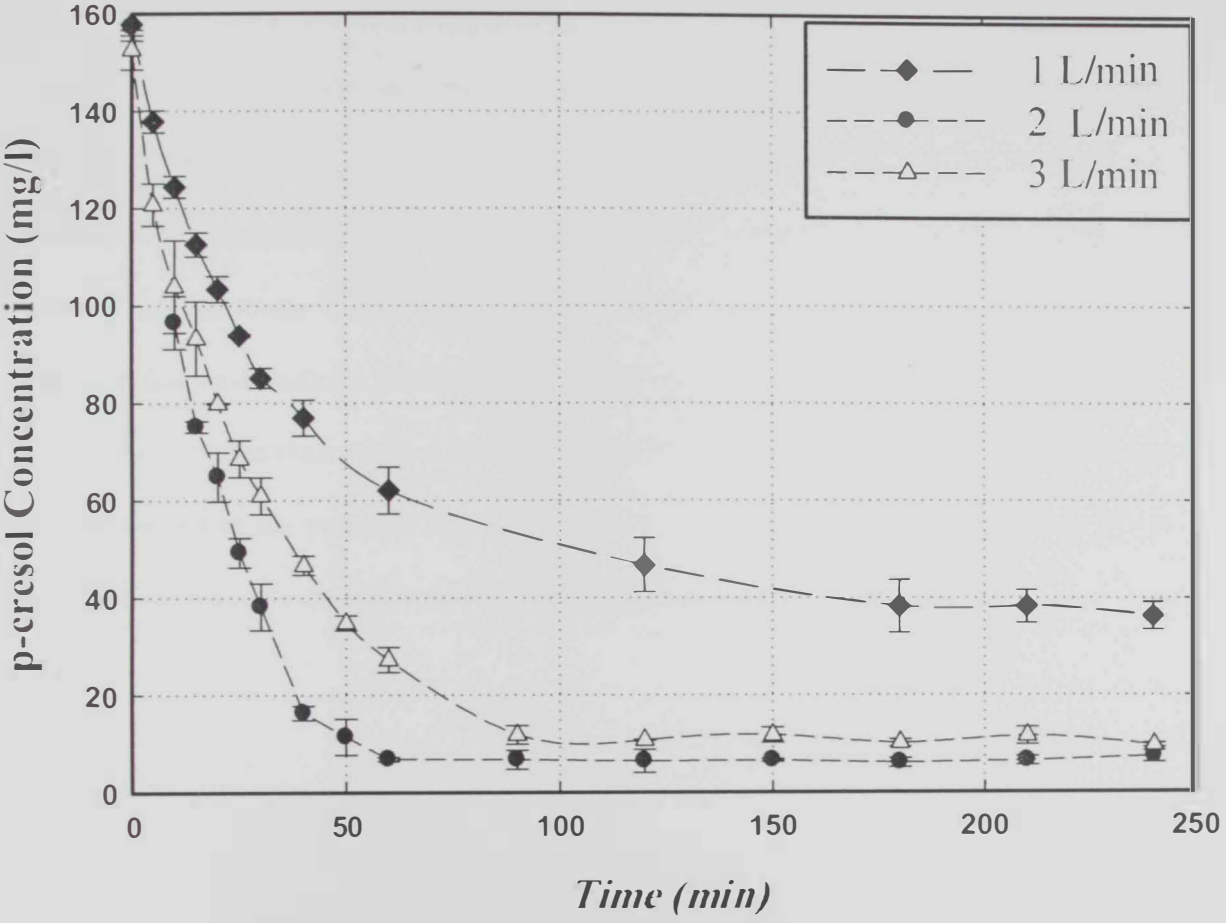
The variation of *o-cresol* and *p-cresol* concentrations with time for the three flow rate is presented in Figure 3.20. It seems that both biodegradation processes are optimized at air flow rate of 2 l/min, which can provide sufficient amount of oxygen for the immobilized bacteria to complete the aerobic biodegradation process. At a lower air flow rate (1 l/min), however, the distribution of PVA pellets was not uniform through the bioreactor. Visual observation indicated that this flow rate could not provide enough air lift to give good spouting of the particles and consequently, most of particles were settling at the bottom of the bed with negligible movement. Although higher flow rate (3 l/min) may provide more than enough oxygen for the aerobic biodegradation, it does not seem to provide the smooth mixing needed for good mass transfer.

Based on visual observation, high flow rates lead to slugging making the small spouted bed bioreactor lose its systematic cyclic motion and good particle movement. Similar observations were reported by Salehi et al. [86] and El-Naas et al. [128], who noted that after a certain air flow rate the biodegradation rate could no longer be improved. It must

be noted here that the *p-cresol* biodegradation process tends to be more affected by lowering the air flow rate, compared to that for *o-cresol*. This may be attributed to the deficient amount of oxygen for metabolizing *p-cresol* at this air flow rate; where the oxygen content is sufficient to completely mineralize *o-cresol*.



(a) *O-cresol*



(b) *P-cresol*

Figure 3.20: Concentration of *cresol* as a function of time for different air flow rates. Reactor temperature = 35°C; liquid flow rate = 5ml/min: (a) *o-cresol*; (b) *p-cresol*.

3.4. Continuous biodegradation of binary mixture (o- & p-cresol)

In this part of the study, the biodegradation process on binary mixture of cresols were carried out in continuous mode, using initial concentrations of 100 mg/l, air flow rate of 2 l/min, reactor temperature of 35°C and pH of 7. The solution of *o- p-cresol* binary mixture was continuously fed with liquid flow rate of 5 ml/min at a ratio of 1:1. Experiments were also conducted for *o-* and *p-cresol* biodegradation as a single substrate with initial concentration of 100 mg/l for each cresol. As shown in Figure 3.21,the biodegradation of *p-cresol* in the mixture is not affected by the presence of *o-cresol*, while the biodegradation of *o-cresol* in the mixture is lower than that when *o-cresol* is alone. *P.putida* seems to prefer other cresol isomers (*p- and m-cresol*) over *o-cresol*. It is believed that co-metabolic products may results in more resistance in the biodegradation, or even more toxic than original component producing dead-end product [85]. This result can be related to the presence of co-substrate in *o-cresol* biodegradation process, where co-substrate partially transformed without producing energy for cell growth.

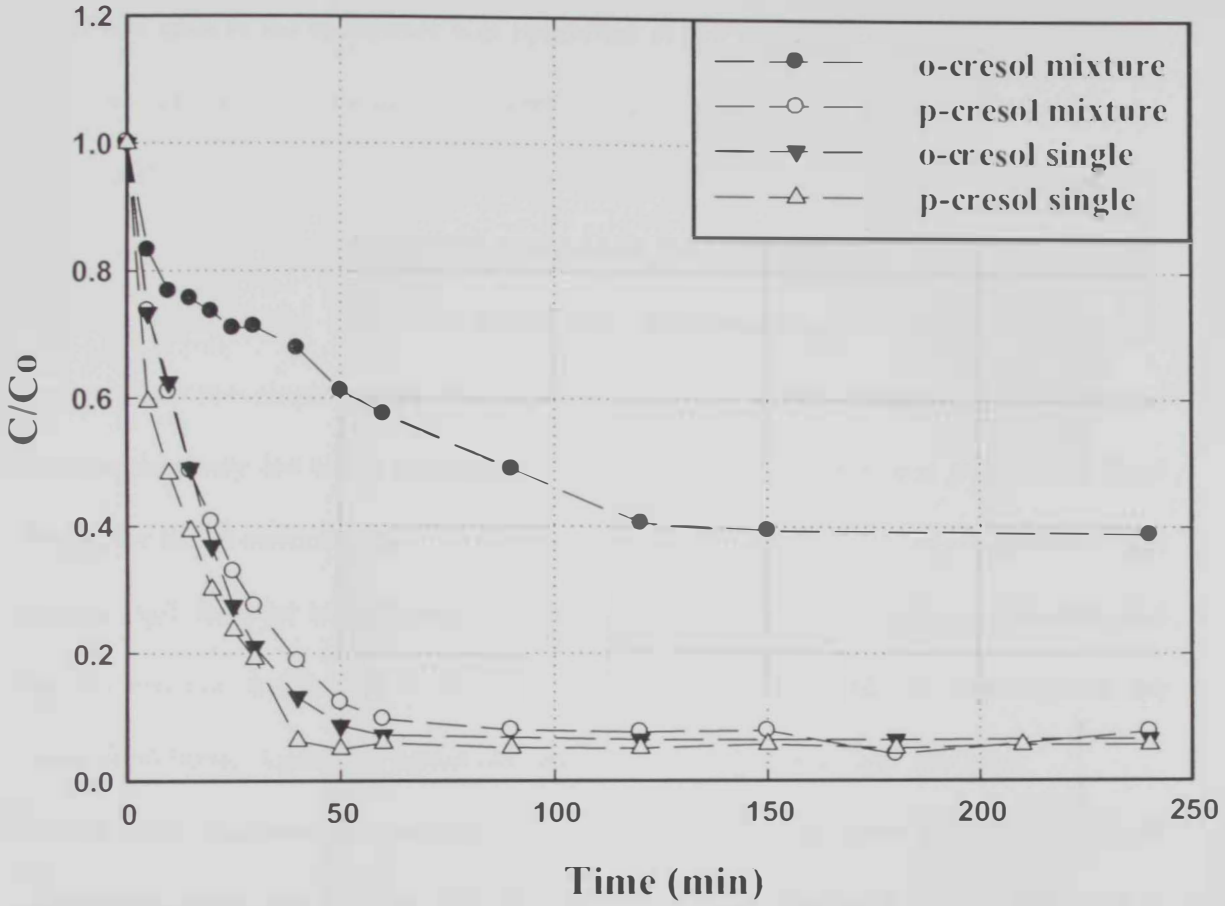


Figure 3.21: Compression between continuous biodegradation for cresols (*o-*, and *p-cresol*) as single component and in the binary mixture

3.5. Continuous biodegradation for ternary mixture (o-, p- & m-cresol)

Little information is available in the literature on the biodegradation of ternary mixtures of cresols. Therefore, continuous experiments were carried out to evaluate the biodegradation of mixture of the three cresol isomers (*o-*, *p-* and *m-cresol*). The main residence time in the bioreactor was optimized at (60 minutes), while the air flow rate, solution pH and temperature were kept constant at 2 l/min, 7 and 35°C, respectively. Some difficulties were faced in the separation of *p-cresol* and *m-cresol* in the analysis due to the similar properties of both compounds, which produced overlapping in the GC analysis, as discussed in Section 2.2.2. Thus, the ternary mixture was divided in the analysis into two single groups, *o-cresol* and *p-,m-cresol*. The synthetic cresols solution was continuously fed to the bioreactor at equal ratio of *o-cresol* and *p-,m*-cresol for 4 hours, the initial concentration of each component (*o-cresol*) and (*p-, m-cresol*) were kept at 100 mg/l. In order to compare the biodegradation process for single components and ternary mixture, another set of experiments for single compound was conducted at the same conditions. Again, the initial concentration of each isomer was 100 mg/l.

Figure 3.22 illustrates the reduction of the concentration of each isomer as a single component and in the mixture. The degradation of *p-,m-cresol* as a single component is almost the same as in the ternary mixture. It is interesting to notice that the biodegradation of *o-cresol* is highly affected by the presence of *p-,m-cresol*. The decrease in the removal of *o-cresol* in the presence of *p-* and *m-cresol* may be attributed to the competitive inhibition and the toxicity of other substances as reported for BTEX mixtures [130], or due to the formation of the toxic intermediates as in the case of the 3-chlophenol degradation [131].

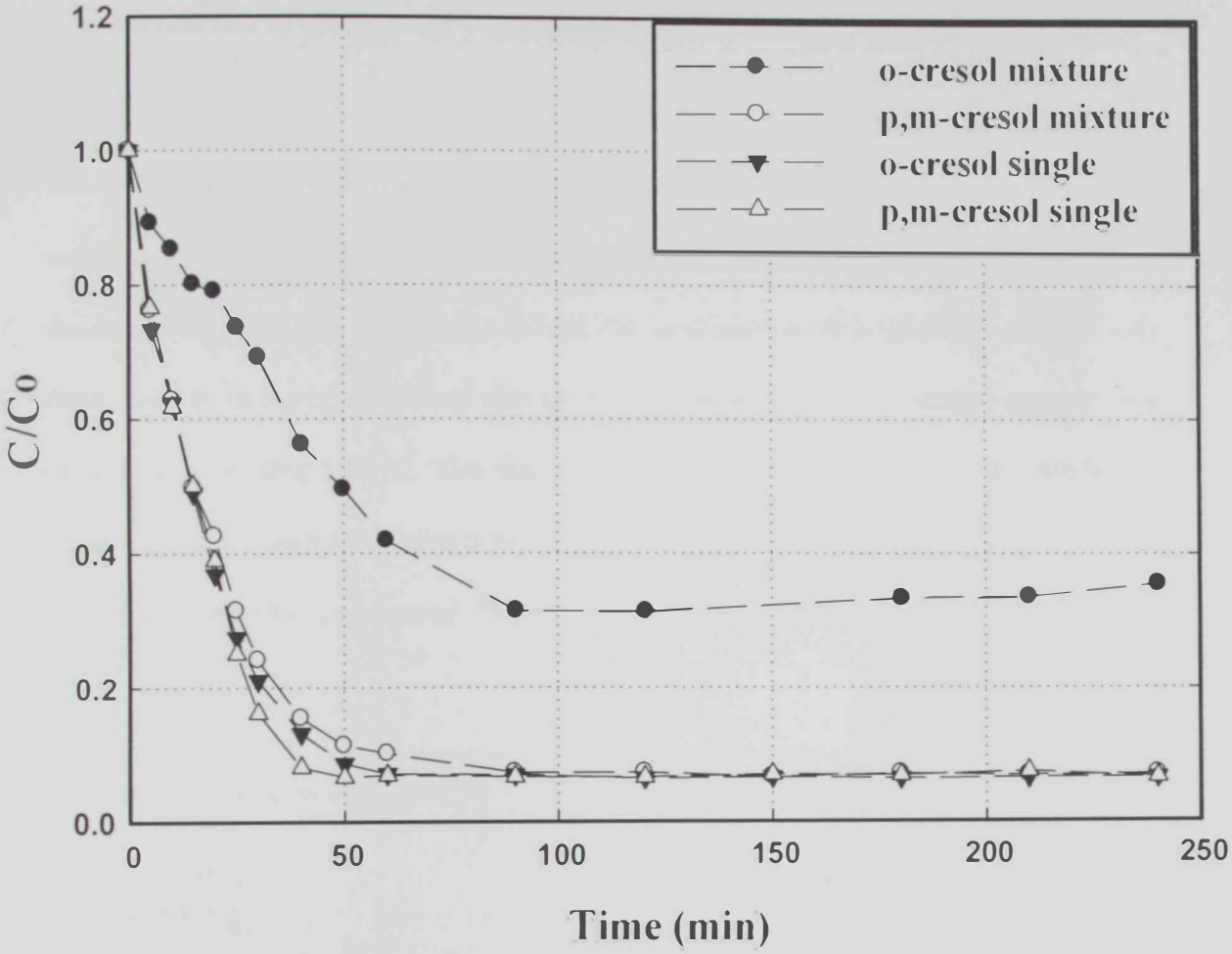


Figure 3.22: Comparison between continuous biodegradation for cresols (*o-, p- and m-cresol*) as single component and in the ternary mixture

3.6. Modeling of the biodegradation process

Modeling of the biodegradation process involves relating the specific growth rate of the biomass to the consumption rate of the substrate. Based on material balance, the specific consumption rate of the substrate is expressed as in Equation (1):

$$q_S = \frac{dS}{Xdt} = \frac{\mu}{Y} \quad (1)$$

Where, q_S is the specific consumption rate (h^{-1}); X is the biomass concentration (mg/l); S is the substrate concentration (mg/l); Y is the cell mass yield (g/g); In order to represent the degradation kinetics of *o-* and *p-cresol*, two available models (Haldane and Monod) were used to fit the experimental data obtained from the batch degradation experiments (described in Section 3.3.1.1). The first model considers o- or *p-cresol* as non-inhibitory compound and neglects the inhibitory effect, while the second one takes into account the inhibitory effect of *o-* or *p-cresol*. The Monod model is expressed as:

$$q_S = \frac{q_{max}\, S}{K_S + S_o} \quad (2)$$

The Haldane model is expressed by:

$$q_S = \frac{q_{max} S}{K_S + S_o + \frac{S_o^2}{K_I}} \quad (3)$$

Where, S_o is the initial substrate concentration (mg/l), q_{max} is the maximum consumption rate (h^{-1}), K_S is the half saturation coefficient (mg/l) and K_I is the substrate inhibition constant (mg/l). These two models can be used to predict the variations of the biodegradation rate (q) with initial *p-cresol* concentrations, utilizing the relation in Eq. (1) and assuming that Y is constant over the concentration range. This assumption is valid if the substance concentration is much higher than K's (S>>Ks) [75]. The degradation rate

q_s was determined from a plot of the substrate concentration S versus time for each initial substance concentration S_o. From the values of q and S_o the values of kinetics parameters for each model were obtained, using SigmaPlot non-linear regression which uses the Marquardt-Levenberg algorithm.

Both models are fitted to *o-cresol* biodegradation process with high accuracy, and the kinetics represent in Table 3.2 .However, Haldane model has R^2 closer to 1, as well as the value of K_I is accepted, which indicated the presence of the inhibition effect in *o-cresol* biodegradation. Figure 3.23 shows the comparison of experimental data and fitted models.

Table 3.2: Monod and Haldane kinetics parameters for *o-cresol*.

Model	q_{max} (h^{-1})	K_s (mg/l)	K_I (mg/l)	R^2
Monod	307.96	39.49	-	0.944
Haldane	369.9	54.04	1087.22	0.946

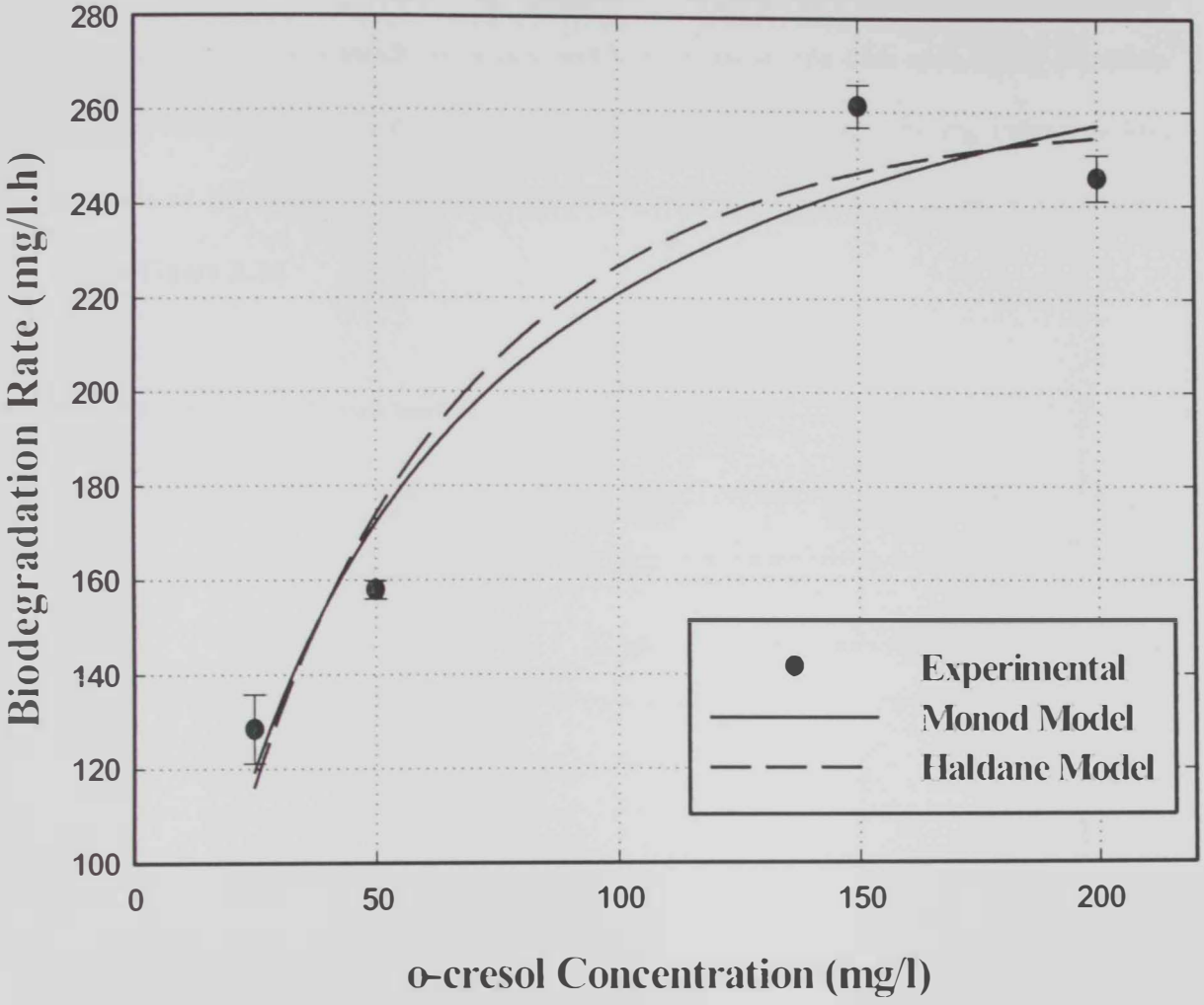


Figure 3.23: Comparison of the experimental data with fitted Monod model for *o-cresol*

P-cresol biodegradation process was also expressed by Monod and Haldane models, and the parameters values for both models are presented in Table 3.3. Although both models give acceptable fit for the data with the same R^2 value (0.975), The Monod non-inhibitory model clearly gives a much more acceptable fit between the data and model equation. Large K_I value was obtained in Haldane Model, which indicates that the culture is less sensitive to the substrate inhibition. Monod model is compared with the experimental data in Figure 3.24.

Table 3.3: Monod and Haldane kinetics parameters for *p-cresol*

Model	q_{max} (h^{-1})	K_s (mg/l)	K_I (mg/l)	R^2
Monod	357.9	21.64	-	0.9748
Haldane	357.9	21.64	1.903×10^{-8}	0.9748

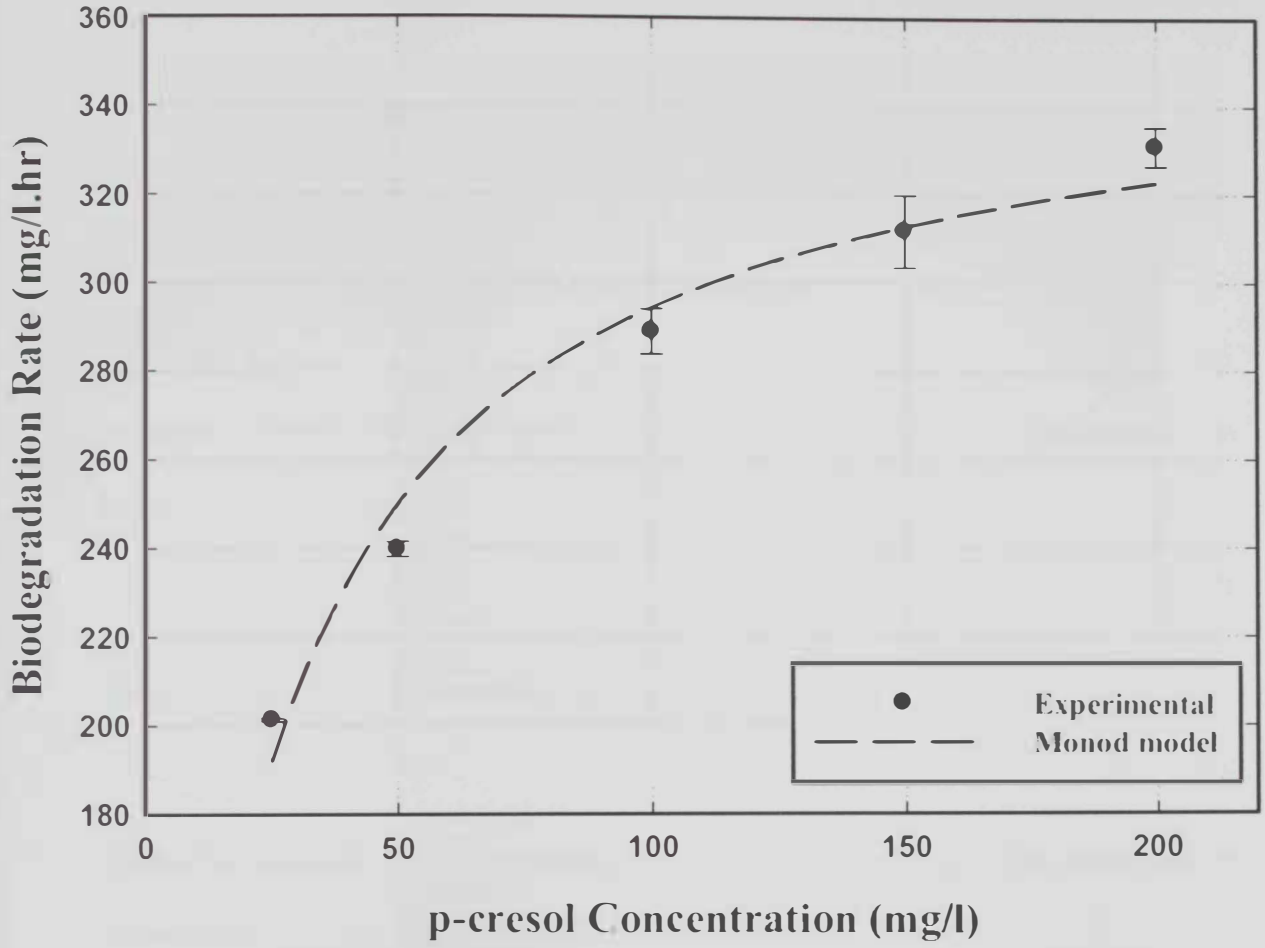


Figure 3.24: Comparison of the experimental data with fitted Haldane model for *p-cresol*

The global biodegradation rate was calculated based on the specific consumption rate, where *o-cresol* and *p-cresol* is the limiting substrate in each system, while oxygen and other nutrients are in excess. Assuming perfect mixing in the bioreactor, the mass balance of the continuous flow reactor can be expressed as follows:

$$\frac{dN_A}{dt} = F_A(C_{Ao} - C_A) - r_A v \quad (4)$$

Dividing the equation by V:

$$\frac{dS}{dt} = \frac{F_S}{V}(S_o - S) - r_S \quad (5)$$

The substrate uptake rate according to Eq. (1) is given by:

$$-r_s = yr_x = q_s \quad (6)$$

Assuming constant yield in the bioreactor as mentioned above, *o-cresol* consumption can be described as follows

$$-r_s = \frac{q_{max}\ S}{k_s + S + \frac{S^2}{k_i}} \quad (7)$$

Where for p-cresol the consumption can be expressed as

$$-r_s = \frac{q_{max}\ S}{k_s + S} \quad (8)$$

Combining equations 5 and 7, the change of *o-cresol* concentration in the reactor can be evaluated as:

$$\frac{dS}{dt} = \frac{F_S}{V}(S_o - S) - \frac{q_{max}\ S}{k_s + S} \quad (9)$$

P-cresol concentration in the reactor can be evaluated by the combination of equations 5 and 8 as follow:

$$\frac{dS}{dt} = \frac{F_S}{V}(S_o - S) - \frac{q_{max}\ S}{k_s + S + \frac{S^2}{k_i}} \quad (10)$$

Where S_o is the input substrate concentration in (mg/l), S is the concentration at desired time mg/l, $(-r_s)$ is the rate of removal of substrate (mg/l.h), r_x is the microorganism growth rate (mg/l. h), x the microorganism concentration (mg/l), V is the reactor volume (l), t is the time (h) and F_s is the volumetric flow rate (l/h). The model was applied for different *o-cresol and p-cresol* concentrations in the effluent given by Equations (9) and (10) for *o-cresol* and *p-cresol*, respectively. The variation of the substrate concentration with time was evaluated using (E-Z Solve). The model prediction and the experimental data are shown in Figures 3.25 and 3.26.The Figures show very good agreement between the model predications and the experimental data and confirm the validity of the proposed model for simulating the continuous biodegradation of *o-cresol* and *p-cresol* in SBBR.

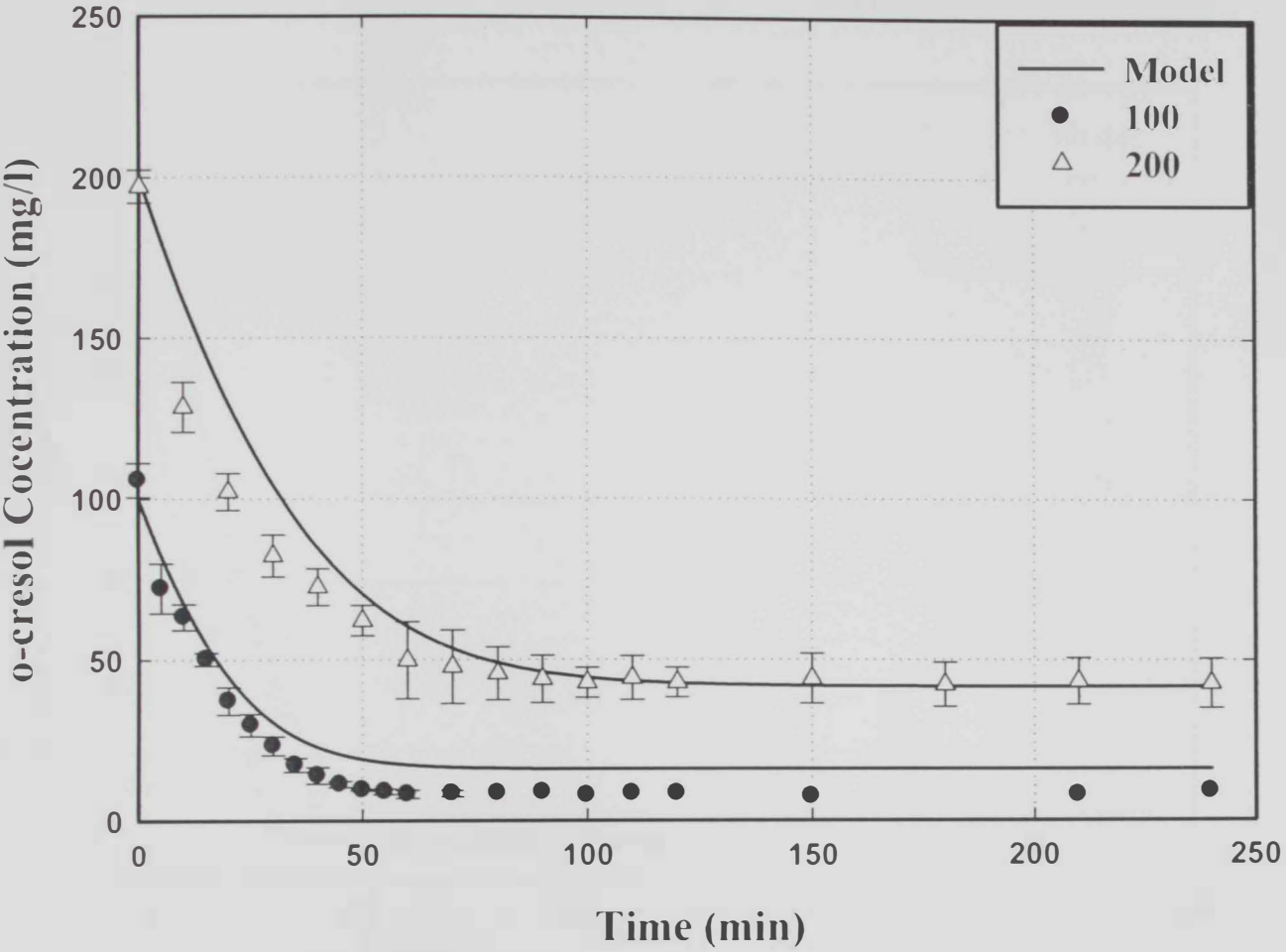


Figure 3.25: Comparison of the continuous experimental data with fitted model (S_0=100 and 200 mg/l)

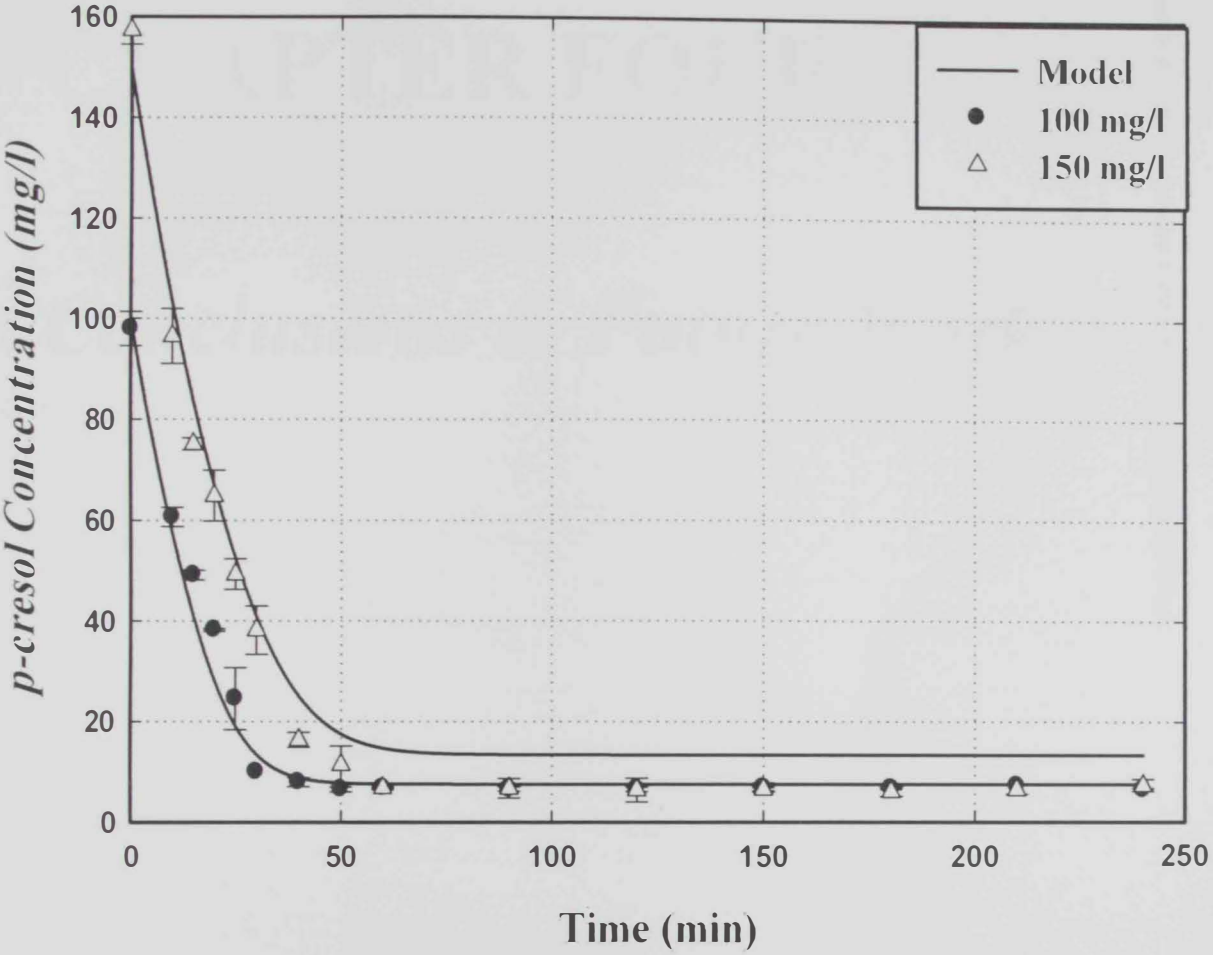


Figure 3.26: Comparison of the continuous experimental data with fitted model (S_0=100 and 150 mg/l)

CHAPTER FOUR

Conclusions & Future Work

4.1.Conclusions

PVA gel was prepared by freezing-thawing technique to be used as an immobilization matrix. The porous structures were examined for several PVA mass% and thawing periods using compound microscope and SEM, and revealed dependency on the PVA mass content and thawing time. The mechanical properties for the PVA gel pellets with different mass content were evaluated; tensile testing indicated that the PVA gels possess a rubbery, elastic nature, fibril network structure. Both fracture stress, percent elongation and modulus of elasticity increase with PVA content. The phenol degradability of *P. putida* immobilized in the PVA pellets was found to drastically improve with time and depend on the porous structure of the pellets.

The biodegradation of cresols using immobilized bacteria immobilized in PVA 5 mass% was performed in order to compare the degradability of the three cresols isomer. Results indicated the biodegradation of *o-*, *p-* and *m-cresol* was in the order of *o-cresol* >*p-cresol* >*m-cresol*. Batch biodegradation for each cresol isomer (*o-*, *p-* and *m-cresol*) was studied at different operating parameters such as initial concentration, temperature, pH and PVA volume fraction. *O-cresol* biodegradation process is optimized at temperature of 35°C, pH ranging from 6 to 8. The biodegradation rate was increased with the initial concentration and the inhibition effect was observed at 150 mg/l. The optimal condition for *p-cresol* biodegradation process is pH of 8, temperature ranging from 30 to of 40°C. The rate was increased exponentially with the initial concentration as well. *M-cresol* biodegradation rate was

the maximum at temperature and pH of 30°C and 6, respectively. In addition, the biodegradation performance was increased with the PVA volume fraction for all cresol isomers.

Continuous biodegradation results indicated that *P.putida* has high potential in degrading *o-cresol* and *p-cresol* as a single component, for concentration up to 200 mg/l and the removal percentage was more than 79 and 85% for both cresols, respectively. The biodegradation efficiency was affected by other operating parameters such as air flow rate and residence time, with optimal air flow rate of 2 l/min. In addition to these parameters the biodegradation of cresols in binary or ternary mixtures revealed that the biodegradation of *o-cresol* was inhibited by the presence of *p-cresol* or *m-cresol* in the solution.

The biodegradation of *o-cresol* was successfully described as inhibitory substrate by Haldane model, while the biodegradation of *p-cresol* was successfully described by Monod model. The obtained kinetics parameters for *o-* , and *p-cresol* were utilized for modeling the continuous biodegradation process and showed very good fit to the experimental results.

4.2.Recommendations for future work

The immobilization of the biomass in the present work has been investigated using PVA gel as a support material. Further investigations on other types of polymers are required in order to improve the immobilization technique. Further studies could be carried out to examine the degradation capability of *P.putida* for other phenol derivatives and aromatic compounds as toxic pollutants presents in the wastewater.

The present work focused mainly on the biodegradation of cresols using *P.putida* as a type of microorganisms; further studies should be carried out by other strains of bacteria or fungus that has a potential to use cresols as a sole source of carbon and energy.

Only preliminary studies on the biodegradation of cresols in SBBR have been carried out. Extended investigations on the optimization and kinetic study should be performed for better performance of the SBBR. Mathematical modeling of the biodegradation of cresols mixture in continuous SBBR should be carried out for better understanding of the system performance.

Bibliography

[1] K.-L. Ho, Y.-Y. Chen, and D.-J. Lee, "Functional consortia for cresol-degrading activated sludges: Toxicity-to-extinction approach," *Bioresource Technology*, vol. 101, no. 23, pp. 9000-9005, 2010.

[2] D. Wojcieszyńska, K. Hupert-Kocurek, I. Greń, and U. Guzik, "High activity catechol 2,3-dioxygenase from the cresols – Degrading Stenotrophomonas maltophilia strain KB2," *International Biodeterioration & Biodegradation*, vol. 65, no. 6, pp. 853-858, 2011.

[3] k. Pakshirajan, D. Chugh, and P. Saravanan, "Feasibility of m-cresol degradation using an indigenous mixed microbial culture with glucose as co-substrate," vol. 10, pp. 303-308, 2008.

[4] G.-M. Zhou, and H. H. P. Fang, "Co-degradation of phenol and m-cresol in a UASB reactor," *Bioresource Technology*, vol. 61, no. 1, pp. 47-52, 7//, 1997.

[5] R.-S. Juang, H.-C. Kao, and K.-J. Tseng, "Kinetics of phenol removal from saline solutions by solvent extraction coupled with degradation in a two-phase partitioning bioreactor," *Separation and Purification Technology*, vol. 71, no. 3, pp. 285-292, 2010.

[6] M. H. El-Naas, S. Al-Zuhair, and M. A. Alhaija, "Removal of phenol from petroleum refinery wastewater through adsorption on date-pit activated carbon," *Chemical Engineering Journal*, vol. 162, no. 3, pp. 997-1005, 9/1/, 2010.

[7] Y. Zheng, D. O. Hill, and C. H. Kuo, "Destruction of cresols by chemical oxidation," *Journal of Hazardous Materials*, vol. 34, no. 2, pp. 245-260, 1993.

[8] M. Caetano, C. Valderrama, A. Farran, and J. L. Cortina, "Phenol removal from aqueous solution by adsorption and ion exchange mechanisms onto polymeric resins," *Journal of Colloid and Interface Science*, vol. 338, no. 2, pp. 402-409, 2009.

[9] O. Tepe, and A. Y. Dursun, "Combined effects of external mass transfer and biodegradation rates on removal of phenol by immobilized Ralstonia eutropha in a packed bed reactor," *Journal of Hazardous Materials*, vol. 151, no. 1, pp. 9-16, 2008.

[10] S.-Y. Tsai, and R.-S. Juang, "Biodegradation of phenol and sodium salicylate mixtures by suspended Pseudomonas putida CCRC 14365," *Journal of Hazardous Materials*, vol. 138, no. 1, pp. 125-132, 2006.

[11] Y. Wang, Y. Tian, B. Han, H.-b. Zhao, J.-n. Bi, and B.-l. Cai, "Biodegradation of phenol by free and immobilized Acinetobacter sp. strain PD12," *Journal of Environmental Sciences*, vol. 19, no. 2, pp. 222-225, 2007.

[12] J. Bai, J.-P. Wen, H.-M. Li, and Y. Jiang, "Kinetic modeling of growth and biodegradation of phenol and m-cresol using Alcaligenes faecalis," *Process Biochemistry*, vol. 42, no. 4, pp. 510-517, 2007.

[13] H. Liu, Q. J. Yu, G. Wang, F. Ye, and Y. Cong, "Biodegradation of phenol at high concentration by a novel yeast Trichosporon montevideense PHE1," *Process Biochemistry*, vol. 46, no. 8, pp. 1678-1681, 2011.

[14] K. Lika, and I. A. Papadakis, "Modeling the biodegradation of phenolic compounds by microalgae," *Journal of Sea Research*, vol. 62, no. 2-3, pp. 135-146, 2009.

[15] P. Elavarasan, K. Kondamudi, and S. Upadhyayula, "Statistical optimization of process variables in batch alkylation of p-cresol with tert-butyl alcohol using ionic liquid catalyst by response surface methodology," *Chemical Engineering Journal*, vol. 155, no. 1–2, pp. 355-360, 2009.

[16] Y. J. Liu, A. N. Zhang, and X. C. Wang, "Biodegradation of phenol by using free and immobilized cells of Acinetobacter sp. XA05 and Sphingomonas sp. FG03," *Biochemical Engineering Journal*, vol. 44, no. 2-3, pp. 187-192, 2009.

[17] L. Wang, Y. Li, P. Yu, Z. Xie, Y. Luo, and Y. Lin, "Biodegradation of phenol at high concentration by a novel fungal strain Paecilomyces variotii JH6," *Journal of Hazardous Materials*, vol. 183, no. 1–3, pp. 366-371, 2010.

[18] Y. Lu, L. Yan, Y. Wang, S. Zhou, J. Fu, and J. Zhang, "Biodegradation of phenolic compounds from coking wastewater by immobilized white rot fungus *Phanerochaete chrysosporium*," *Journal of Hazardous Materials*, vol. 165, no. 1-3, pp. 1091-1097, 2009.

[19] C. Kennes and J.M. Lema, "Simultaneous biodegradation of p-cresol and phenol by the basydiomycete *Phanerochaete chrysosporium*," *Journal of Industerial Microbiology*, vol. 13, pp. 311-314, 1994.

[20] C. Kennes and J.M. Lema "Biodegradation of simulated creosotes (PAH and phenols) by *Phanerochaete Chrysosporium*," *Biotechnology*, vol. 6, pp.759-764, 1994.

[21] Y. Cheng, H. Lin, Z. Chen, M. Megharaj, and R. Naidu, "Biodegradation of crystal violet using Burkholderia vietnamiensis C09V immobilized on PVA–sodium alginate–kaolin gel beads," *Ecotoxicology and Environmental Safety*, vol. 83, pp. 108-114, 2012.

[22] A. Y. Dursun, and O. Tepe, "Internal mass transfer effect on biodegradation of phenol by Ca-alginate immobilized Ralstonia eutropha," *Journal of Hazardous Materials*, vol. 126, no. 1–3, pp. 105-111, 2005.

[23] S. Sarkar, S. Seenivasan, and R. P. S. Asir, "Biodegradation of propargite by Pseudomonas putida, isolated from tea rhizosphere," *Journal of Hazardous Materials*, vol. 174, no. 1-3, pp. 295-298, 2010.

[24] O. Bouallègue, R. Mzoughi, F. X. Weill, N. Mahdhaoui, Y. Ben Salem, H. Sboui, F. Grimont, and P. A. D. Grimont, "Outbreak of Pseudomonas putida bacteraemia in a neonatal intensive care unit," *Journal of Hospital Infection*, vol. 57, no. 1, pp. 88-91, 2004.

[25] C. Hee Jeong, and Y. Young Je, "Mathematical modelling and simulation of catechol production from benzoate using resting cells of Pseudomonas putida," *Process Biochemistry*, vol. 32, no. 5, pp. 423-432, 1997.

[26] A. Basu, D. Das, P. Bapat, P. P. Wangikar, and P. S. Phale, "Sequential utilization of substrates by Pseudomonas putida CSV86: Signatures of intermediate metabolites and online measurements," *Microbiological Research*, vol. 164, no. 4, pp. 429-437, 2009.

[27] K.-C. Loh, and B. Cao, "Paradigm in biodegradation using Pseudomonas putida—A review of proteomics studies," *Enzyme and Microbial Technology*, vol. 43, no. 1, pp. 1-12, 2008.

[28] C. Li, Y. Li, X. Cheng, L. Feng, C. Xi, and Y. Zhang, "Immobilization of Rhodococcus rhodochrous BX2 (an acetonitrile-degrading bacterium) with biofilm-forming bacteria for wastewater treatment," *Bioresource Technology*, vol. 131, no. 0, pp. 390-396, 2013.

[29] I.-S. Chang, C.-I. Kim, and B.-U. Nam, "The influence of poly-vinyl-alcohol (PVA) characteristics on the physical stability of encapsulated immobilization media for advanced wastewater treatment," *Process Biochemistry*, vol. 40, no. 9, pp. 3050-3054, 2005.

[30] Y.-M. Chen, T.-F. Lin, C. Huang, J.-C. Lin, and F.-M. Hsieh, "Degradation of phenol and TCE using suspended and chitosan-bead immobilized Pseudomonas putida," *Journal of Hazardous Materials*, vol. 148, no. 3, pp. 660-670, 2007.

[31] L.-s. Zhang, W.-z. Wu, and J.-l. Wang, "Immobilization of activated sludge using improved polyvinyl alcohol (PVA) gel," *Journal of Environmental Sciences*, vol. 19, no. 11, pp. 1293-1297, 2007.

[32] A. Magrí, M. B. Vanotti, and A. A. Szögi, "Anammox sludge immobilized in polyvinyl alcohol (PVA) cryogel carriers," *Bioresource Technology*, vol. 114, no. 0, pp. 231-240, 2012.

[33] Y. M. Awad, and N. S. Abuzaid, "The influence of residence time on the anodic oxidation of phenol," *Separation and Purification Technology*, vol. 18, no. 3, pp. 227-236, 2000.

[34] W. Yujian, Y. Xiaojuan, L. Hongyu, and T. Wei, "Immobilization of Acidithiobacillus ferrooxidans with complex of PVA and sodium alginate," *Polymer Degradation and Stability*, vol. 91, no. 10, pp. 2408-2414, 2006.

[35] C. M.Hassan, and N. A.Peppas, "Structure and Morphology of Freeze/Thawed PVA Hydrogels," *Macromolecules*, vol. 33, pp. 2472-2479, 2000.

[36] R. Hernández, A. Sarafian, D. López, and C. Mijangos, "Viscoelastic properties of poly(vinyl alcohol) hydrogels and ferrogels obtained through freezing-thawing cycles," *Polymer*, vol. 45, no. 16, pp. 5543-5549, 2004.

[37] C. Kennes, R. Mendez, and J. M. Lema, "Methanogenic degradation of p-cresol in batch and in continuous UASB reactors," *Water Research*, vol. 31, no. 7, pp. 1549-1554, 1997.

[38] Z. Xie, S. Le Calvé, V. Feigenbrugel, T. G. Preuß, R. Vinken, R. Ebinghaus, and W. Ruck, "Henry's law constants measurements of the nonylphenol isomer 4(3′,5′-dimethyl-3′-heptyl)-phenol, tertiary octylphenol and γ-hexachlorocyclohexane between 278 and 298 K," *Atmospheric Environment*, vol. 38, no. 29, pp. 4859-4868, 2004.

[39] N. Perron, and U. Welander, "Degradation of phenol and cresols at low temperatures using a suspended-carrier biofilm process," *Chemosphere*, vol. 55, no. 1, pp. 45-50, 2004.

[40] A. Scheyer, C. Graeff, S. Morville, P. Mirabel, and M. Millet, "Analysis of some organochlorine pesticides in an urban atmosphere (Strasbourg, east of France)," *Chemosphere*, vol. 58, no. 11, pp. 1517-1524, 2005.

[41] M. J. Spence, S. H. Bottrell, S. F. Thornton, and D. N. Lerner, "Isotopic modelling of the significance of bacterial sulphate reduction for phenol attenuation in a contaminated aquifer," *Journal of Contaminant Hydrology*, vol. 53, no. 3–4, pp. 285-304, 2001.

[42] Y. Jiang, X. Cai, D. Wu, and N. Ren, "Biodegradation of phenol and m-cresol by mutated Candida tropicalis," *Journal of Environmental Sciences*,vol. 22, no. 4, pp. 621-626, 2010.

[43] G. Vázquez-Rodríguez, C. B. Youssef, and J. Waissman-Vilanova, "Two-step modeling of the biodegradation of phenol by an acclimated activated sludge," *Chemical Engineering Journal*, vol. 117, no. 3, pp. 245-252, 2006.

[44] J. Zhou, W. Duan, J. Xu, and Y. Yang, "Experimental and Simulation Study on the Extraction of p-Cresol Using Centrifugal Extractors," *Chinese Journal of Chemical Engineering*, vol. 15, no. 2, pp. 209-214, 2007.

[45] F. Basheer, and I. H. Farooqi, "Biodegradation of p-cresol by aerobic granules in sequencing batch reactor," *Journal of Environmental Sciences*, vol. 24, no. 11, pp. 2012-2018, 2012.

[46] M. del Olmo, C. Díez, A. Molina, I. de Orbe, and J. L. Vílchez, "Resolution of phenol, o-cresol, m-cresol and p-cresol mixtures by excitation fluorescence using partial least-squares (PLS) multivariate calibration," *Analytica Chimica Acta*, vol. 335, no. 1–2, pp. 23-33, 1996.

[47] C. Flox, C. Arias, E. Brillas, A. Savall, and K. Groenen-Serrano, "Electrochemical incineration of cresols: A comparative study between PbO2 and boron-doped diamond anodes," *Chemosphere*, vol. 74, no. 10, pp. 1340-1347, 2009.

[48] M. Al Zarooni, and W. Elshorbagy, "Characterization and assessment of Al Ruwais refinery wastewater," *Journal of Hazardous Materials*, vol. 136, no. 3, pp. 398-405, 2006.

[49] N. Pazarlioglu, Y. Kaymaz, and A. Babaoglu, "Biodegradation kinetics of o-cresol by *Pseudomonas Putida* DSM 548 (pJP4) and o-cresol removal in a batch-recirculation bioreactor system.," *Electronic Journal of Biotechnology*, vol. 15, no. 1, 2011.

[50] J. M. Sanders, J. R. Bucher, J. C. Peckham, G. E. Kissling, M. R. Hejtmancik, and R. S. Chhabra, "Carcinogenesis studies of cresols in rats and mice," *Toxicology*, vol. 257, no. 1–2, pp. 33-39, 2009.

[51] Y. Han, Z. Li, X. Miao, and F. Zhang, "Statistical optimization of medium components to improve the chitinase activity of Streptomyces sp. Da11 associated with the South China Sea sponge Craniella australiensis," *Process Biochemistry*, vol. 43, no. 10, pp. 1088-1093, 2008.

[52] P. R. Gogate, "Treatment of wastewater streams containing phenolic compounds using hybrid techniques based on cavitation: A review of the current status and the way forward," *Ultrasonics Sonochemistry*, vol. 15, no. 1, pp. 1-15, 2008.

[53] T.-Y. Hsien, and Y.-H. Lin, "Biodegradation of phenolic wastewater in a fixed biofilm reactor," *Biochemical Engineering Journal*, vol. 27, no. 2, pp. 95-103, 12, 2005.

[54] B. K. Körbahti, and A. Tanyolaç, "Continuous electrochemical treatment of phenolic wastewater in a tubular reactor," *Water Research*, vol. 37, no. 7, pp. 1505-1514, 2003.

[55] A. Hatipoğlu, N. San, and Z. Çınar, "An experimental and theoretical investigation of the photocatalytic degradation of meta-cresol in TiO2 suspensions: a model for the product distribution," *Journal of Photochemistry and Photobiology A: Chemistry*, vol. 165, no. 1–3, pp. 119-129, 2004.

[56] C. Flox, P.-L. Cabot, F. Centellas, J. A. Garrido, R. M. Rodríguez, C. Arias, and E. Brillas, "Solar photoelectro-Fenton degradation of cresols using a flow reactor with a boron-doped diamond anode," *Applied Catalysis B: Environmental*, vol. 75, no. 1–2, pp. 17-28, 2007.

[57] M. C. Valsania, F. Fasano, S. D. Richardson, and M. Vincenti, "Investigation of the degradation of cresols in the treatments with ozone," *Water Research*, vol. 46, no. 8, pp. 2795-2804, 2012.

[58] V. Kavitha, and K. Palanivelu, "Destruction of cresols by Fenton oxidation process," *Water Research*, vol. 39, no. 13, pp. 3062-3072, 8//, 2005.

[59] H. Hadjar, B. Hamdi, and C. O. Ania, "Adsorption of p-cresol on novel diatomite/carbon composites," *Journal of Hazardous Materials*, vol. 188, no. 1–3, pp. 304-310, 2011.

[60] Y. Chu, D. Zhang, L. Liu, Y. Qian, and L. Li, "Electrochemical degradation of m-cresol using porous carbon-nanotube-containing cathode and Ti/SnO2–Sb2O5–IrO2 anode: Kinetics, byproducts and biodegradability," *Journal of Hazardous Materials*, vol. 252–253, no. 0, pp. 306-312, 2013.

[61] M. E. Suarez-Ojeda, A. Guisasola, J. A. Baeza, A. Fabregat, F. Stüber, A. Fortuny, J. Font, and J. Carrera, "Integrated catalytic wet air oxidation and aerobic biological treatment in a municipal WWTP of a high-strength o-cresol wastewater," *Chemosphere*, vol. 66, no. 11, pp. 2096-2105, 2007.

[62] N. K. Pazarlioglu, and A. Telefoncu, "Biodegradation of phenol by Pseudomonas putida immobilized on activated pumice particles," *Process Biochemistry*, vol. 40, no. 5, pp. 1807-1814, 2005.

[63] G. González, G. Herrera, M. T. García, and M. Peña, "Biodegradation of phenolic industrial wastewater in a fluidized bed bioreactor with immobilized cells of Pseudomonas putida," *Bioresource Technology*, vol. 80, no. 2, pp. 137-142, 2001.

[64] J. Yan, W. Jianping, B. Jing, W. Daoquan, and H. Zongding, "Phenol biodegradation by the yeast Candida tropicalis in the presence of m-cresol," *Biochemical Engineering Journal*, vol. 29, no. 3, pp. 227-234, 2006.

[65] R. K. Singh, S. Kumar, S. Kumar, and A. Kumar, "Biodegradation kinetic studies for the removal of p-cresol from wastewater using Gliomastix indicus MTCC 3869," *Biochemical Engineering Journal*, vol. 40, no. 2, pp. 293-303, 2008.

[66] D. Arya, S. Kumar, and S. Kumar, "Biodegradation dynamics and cell maintenance for the treatment of resorcinol and p-cresol by filamentous fungus Gliomastix indicus," *Journal of Hazardous Materials*, vol. 198, pp. 49-56, 2011.

[67] H. Ucun, E. Yildiz, and A. Nuhoglu, "Phenol biodegradation in a batch jet loop bioreactor (JLB): Kinetics study and pH variation," *Bioresource Technology*, vol. 101, no. 9, pp. 2965-2971, 2010.

[68] G. Tziotzios, M. Teliou, V. Kaltsouni, G. Lyberatos, and D. V. Vayenas, "Biological phenol removal using suspended growth and packed bed reactors," *Biochemical Engineering Journal*, vol. 26, no. 1, pp. 65-71, 2005.

[69] M. Claußen, and S. Schmidt, "Biodegradation of phenol and p-cresol by the hyphomycete Scedosporium apiospermum," *Research in Microbiology*, vol. 149, no. 6, pp. 399-406, 1998.

[70] A. y.-e. Hamitouche, Z. Bendjama, A. Amrane, F. Kaouah, D. Hamane, and R. Ikkene, "Biodegradation of P-Cresol by Mixed Culture in Batch Reactor – Effect of the Three Nitrogen Sources Used," *Procedia Engineering*, vol. 33, pp. 458-464, 2012.

[71] P. Saravanan, K. Pakshirajan, and P. Saha, "Biodegradation of phenol and m-cresol in a batch and fed batch operated internal loop airlift bioreactor by indigenous mixed microbial culture predominantly Pseudomonas sp," *Bioresource Technology*, vol. 99, no. 18, pp. 8553-8558, 2008.

[72] P. Saravanan, K. Pakshirajan, and P. Saha, "Kinetics of phenol and m-cresol biodegradation by an indigenous mixed microbial culture isolated from a sewage treatment plant," *Journal of Environmental Sciences*, vol. 20, no. 12, pp. 1508-1513, 2008.

[73] H. Yao, Y. Ren, C. Wei, and S. Yue, "Biodegradation characterisation and kinetics of *m*-cresol by *Lysinibacillus cresolivorans*," *Water SA*, vol. 37, 2011.

[74] H. Yao, Y. Ren, X. Deng, and C. Wei, "Dual substrates biodegradation kinetics of m-cresol and pyridine by Lysinibacillus cresolivorans," *Journal of Hazardous Materials*, vol. 186, no. 2–3, pp. 1136-1140, 2011.

[75] M. H. El-Naas, S. A. Al-Muhtaseb, and S. Makhlouf, "Biodegradation of phenol by Pseudomonas putida immobilized in polyvinyl alcohol (PVA) gel," *Journal of Hazardous Materials*, vol. 164, no. 2-3, pp. 720-725, 2009.

[76] T. Al-Khalid, and M. H. El-Naas, "Aerobic biodegradation of phenols: a comprehensive review," *Crit. Rev. Env. Sci. Technol.*, vol. 42, pp. 1631-1690, 2012.

[77] S. S. Bhattacharya, S. Karmakar, and R. Banerjee, "Optimization of laccase mediated biodegradation of 2,4-dichlorophenol using genetic algorithm," *Water Research*, vol. 43, no. 14, pp. 3503-3510, 2009.

[78] P. Allsopo, J., Y. Chisti, M. Moo-Young, and G. Sullivan, R., "Dynamic of phenol Degradation by *Pseudomonas Putida*," *Biotechnology and Bioengineering*, vol. 41, pp. 572-580, 1993.

[79] I. G. Minkevich, S. V. Andreyev, and V. K. Eroshin, "The effect of two inhibiting substrates on growth kinetics and cell maintenance of the yeast Candida valida," *Process Biochemistry*, vol. 36, no. 3, pp. 209-217, 2000.

[80] O. J. Hao, M. H. Kim, E. A. Seagren, and H. Kim, "Kinetics of phenol and chlorophenol utilization by Acinetobacter species," *Chemosphere*, vol. 46, no. 6, pp. 797-807, 2002.

[81] T.-P. Chung, H.-Y. Tseng, and R.-S. Juang, "Mass transfer effect and intermediate detection for phenol degradation in immobilized Pseudomonas putida systems," *Process Biochemistry*, vol. 38, no. 10, pp. 1497-1507, 2003.

[82] D. L. Nelson, and M. Cox, *Lehninger Principles of Biochemistry*, Third ed., New York: Macmillan Worth Publishers, 2000.

[83] J. Bailey, E., and D. Ollis, F., *Biochemical Engineering Fundamentals*, Seconed ed., Singapore: McGraw-Hill Book Co., 1986.

[84] G. Baggi, "Ecological implication of synergistic and antagonistic interactions among growth and non growth analogs present in mixture," *Annals of Microbiology*, vol. 50, pp. 103-115, 2000.

[85] A. Salmerón-Alcocer, N. Ruiz-Ordaz, C. Juárez-Ramírez, and J. Galíndez-Mayer, "Continuous biodegradation of single and mixed chlorophenols by a mixed microbial culture constituted by Burkholderia sp., Microbacterium phyllosphaerae, and Candida tropicalis," *Biochemical Engineering Journal*, vol. 37, no. 2, pp. 201-211, 2007.

[86] Z. Salehi, H. Yoshikawa, R. Mineta, and Y. Kawase, "Aerobic biodegradation of p-nitrophenol by acclimated waste activated sludge in a slurry bubble column," *Process Biochemistry*, vol. 46, no. 1, pp. 284-289, 2011.

[87] M. H. El-Naas, A.-H. I. Mourad, and R. Surkatti, "Evaluation of the characteristics of polyvinyl alcohol (PVA) as matrices for the immobilization of Pseudomonas putida," *International Biodeterioration & Biodegradation*, vol. 85, pp. 413-420, 2013.

[88] M. H. El-Naas, S. Al-Zuhair, and S. Makhlouf, "Batch degradation of phenol in a spouted bed bioreactor system," *Journal of Industrial and Engineering Chemistry*, vol. 16, no. 2, pp. 267-272, 2010.

[89] M. Ziagova, and M. Liakopoulou-Kyriakides, "Kinetics of 2,4-dichlorophenol and 4-Cl-m-cresol degradation by Pseudomonas sp. cultures in the presence of glucose," *Chemosphere*, vol. 68, no. 5, pp. 921-927, 2007.

[90] A. Kumar, S. Kumar, and S. Kumar, "Biodegradation kinetics of phenol and catechol using Pseudomonas putida MTCC 1194," *Biochemical Engineering Journal*, vol. 22, no. 2, pp. 151-159, 2005.

[91] C. S. A. Sá, and R. A. R. Boaventura, "Biodegradation of phenol by Pseudomonas putida DSM 548 in a trickling bed reactor," *Biochemical Engineering Journal*, vol. 9, no. 3, pp. 211-219, 2001.

[92] P. Y. Ahamad, A. Kunhi, and S. Divakar, "New metabolic pathway for o-cresol degradation by Pseudomonas sp. CP4 as evidenced by ^{1}HNMR spectroscopic studies," *World Journal of Microbiology and Biotechnology*, vol. 17, pp. 371-377, 2001.

[93] D. Hopper, J., and D. Taylor, G., "Pathway for the Degradation of m-cresol and p-cresol by Pseudomonas putida," *Journal of Biotechnology*, vol. 122, 1975.

[94] S. Sanjeev Kumar, M. S. Kumar, D. Siddavattam, and T. B. Karegoudar, "Generation of continuous packed bed reactor with PVA–alginate blend immobilized Ochrobactrum sp. DGVK1 cells for effective removal of N,N-dimethylformamide from industrial effluents," *Journal of Hazardous Materials*, vol. 199–200, no. 0, pp. 58-63, 2012.

[95] K. Tsekova, D. Todorova, and S. Ganeva, "Removal of heavy metals from industrial wastewater by free and immobilized cells of Aspergillus niger," *International Biodeterioration & Biodegradation*, vol. 64, no. 6, pp. 447-451, 2010.

[96] S. Masunaga, Y. Urushigawa, and Y. Yonezawa, "Biodegradation pathway of o-cresol by heterogeneous culture Phenol acclimated activated sludge," *Water Research*, vol. 20, no. 4, pp. 477-484, 1986.

[97] V. I. Lozinsky, and F. M. Plieva, "Poly(vinyl alcohol) cryogels employed as matrices for cell immobilization. 3. Overview of recent research and developments," *Enzyme and Microbial Technology*, vol. 23, no. 3-4, pp. 227-242, 1998.

[98] C. M. HASSAN, and N. A. PEPPAS, "Cellular PVA Hydrogels Produced by Freeze/Thawing," *Journal of Applied Polymer Science*, vol. 76, 2000.

[99] S. Jiang, S. Liu, and W. Feng, "PVA hydrogel properties for biomedical application," *Journal of the Mechanical Behavior of Biomedical Materials*, vol. 4, no. 7, pp. 1228-1233, 2011.

[100] J. Kumar, and S. F. D'Souza, "Preparation of PVA membrane for immobilization of GOD for glucose biosensor," *Talanta*, vol. 75, no. 1, pp. 183-188, 2008.

[101] A. Idris, N. A. M. Zain, and M. S. Suhaimi, "Immobilization of Baker's yeast invertase in PVA-alginate matrix using innovative immobilization technique," *Process Biochemistry*, vol. 43, no. 4, pp. 331-338, 2008.

[102] M. Szczesna-Antczak, and E. Galas, "Bacillus subtilis cells immobilised in PVA-cryogels," *Biomolecular Engineering*, vol. 17, no. 2, pp. 55-63, 2001.

[103] R.-y. Ma, and D.-s. Xiong, "Synthesis and properties of physically crosslinked poly (vinyl alcohol) hydrogels," *Journal of China University of Mining and Technology*, vol. 18, no. 2, pp. 271-274, 2008.

[104] J. Fromageau, "Characterization of PVA Cryogel for Intravascular Ultrasound Elasticity Imaging," D. V. Elisabeth Brusseau, ed., 2003.

[105] M. A. Gauthier, J. Luo, D. Calvet, C. Ni, X. X. Zhu, M. Garon, and M. D. Buschmann, "Degree of crosslinking and mechanical properties of crosslinked poly(vinyl alcohol) beads for use in solid-phase organic synthesis," *Polymer*, vol. 45, no. 24, pp. 8201-8210, 2004.

[106] F. Jeremie, B. Elisabeth, and V. Didier, "Characterization of PVA Cryogel for Intravascular Ultrasounds Elasticity Imaging," *IEEE Transactions on ultrasonics, ferroelectrics, and frequency contraol*, vol. 50, 2003.

[107] K. S. Anseth, C. N. Bowman, and L. Brannon-Peppas, "Mechanical properties of hydrogels and their experimental determination," *Biomaterials*, vol. 17, no. 17, pp. 1647-1657, 1996.

[108] J. A. Stammen, S. Williams, D. N. Ku, and R. E. Guldberg, "Mechanical properties of a novel PVA hydrogel in shear and unconfined compression," *Biomaterials*, vol. 22, no. 8, pp. 799-806, 2001.

[109] H. Fouad, A.-H. I. Mourad, and D. Barton, C . "Effect of pre-heat treatment on the static and dynamic thermo-mechanical properties of Ultra-High Molecular Weight Polyethylene" Polymer Testing," *Elsevier*, vol. 24, pp. 549-556, 2005.

[110] A.-H. I. Mourad, R. O. Akkad , A. A. Soliman, and T. M. Madkour, "Characterization of thermally treated and untreated polyethylene-polypropylene blends using DSC, TGA and IR techniques", Plastics, Rubber and Composites," *Macromolecular Engineering*, vol. 38, pp. 265-278, 2009.

[111] A.-H. I. Mourad, H. Fouad, and R. Elleithy, "Impact of some environmental conditions on the tensile, creep-recovery, relaxation, melting and crystallinity

behaviour of UHMWPE-GUR 410-medical grade," *Materials & Design*, vol. 30, no. 10, pp. 4112-4119, 2009.

[112] A.-H. I. Mourad, "Thermo-mechanical characteristics of thermally aged polyethylene/polypropylene blends," *Materials & Design*, vol. 31, no. 2, pp. 918-929, 2010.

[113] A. Dehbi, A.-H. I. Mourad , and A. Bouaza, "Degradation assessment of LDPE multilayer films used as a greenhouse cover: Natural and artificial aging impacts," *Applied polymer science*, vol. 124, pp. 2702-2716, 2012.

[114] A.-H. I. Mourad, N. Bekheet, A. Al-Butch, L. Abdel-Latif, D. Nafee, and D. C. Barton, "The effects of process parameters on the mechanical properties of die drawn polypropylene," *Polymer Testing*, vol. 24, pp. 169-180, 2005.

[115] S. Al-Zuhair, and M. El-Naas, "Immobilization of Pseudomonas putida in PVA gel particles for the biodegradation of phenol at high concentrations," *Biochemical Engineering Journal*, vol. 56, no. 1–2, pp. 46-50, 2011.

[116] P. J. Reséndiz-Hernández, O. S. Rodríguez-Fernández, and L. A. García-Cerda, "Synthesis of poly(vinyl alcohol)-magnetite ferrogel obtained by freezing-thawing technique," *Journal of Magnetism and Magnetic Materials*, vol. 320, no. 14, pp. 373- 376, 2008.

[117] A. S. Hickey, and N. A. Peppas, "Solute diffusion in poly(vinyl alcohol)/poly(acrylic acid) composite membranes prepared by freezing/thawing techniques," *Polymer*, vol. 38, no. 24, pp. 5931-5936, 1997.

[118] J. S. Gonzalez, and V. A. Alvarez, "The effect of the annealing on the poly(vinyl alcohol) obtained by freezing–thawing," *Thermochimica Acta*, vol. 521, no. 1–2, pp. 184-190, 2011.

[119] R. Ricciardi, G. Mangiapia, F. Lo Celso, L. Paduano, R. Triolo, F. Auriemma, C. De Rosa, and F. Lauprâtre, "Structural Organization of Poly(vinyl alcohol) Hydrogels Obtained by Freezing and thawing Techniqes," *Chem. Mater*, vol. 17, pp. 1183-1189, 2005.

[120] R. Ricciardi, F. Auriemma, and C. De Rosa, "Structure and Properties of Poly(vinyl alcohol) Hydrogels Obtained by Freeze/Thaw Techniqes," *Macromol. Symp*, pp. 49-63, 2005.

[121] C. M. Hassan, and N. A. Pappas, "Structure and Applications of Poly (vinyl alcohol) Hyrogels Produced by Conventional crosslinking or by Freezing/Thawing," *Advance in Polymer Science*, vol. 153, pp. 37-65, 2000.

[122] S. Gupta, S. Goswami, and A. Sinha, "A combined effect of freeze-thaw cycles and polymer concentration on the structure and mechanical properties of transparent PVA gels," *Biomedical Material.*, vol. 7, pp. 5006-5013, 2012.

[123] H. Farooqi, I., F. Basheer, and T. Ahmed, "Studies on Biodegradation of phenols and m-cresols by Upflow Anaerobic Sludge Blanket and Aerobic Sequential Batch Reactor.," *Global NEST Journal*, vol. 10, 2008.

[124] M. Maeda, A. Itoh, and Y. Kawase, "Kinetics for aerobic biological treatment of o-cresol containing wastewaters in a slurry bioreactor: biodegradation by utilizing waste activated sludge," *Biochemical Engineering Journal*, vol. 22, no. 2, pp. 97-103, 2005.

[125] I. de Ory, L. Enrique Romero, and D. Cantero, "Modeling the kinetics of growth of *Acetobacter aceti* in discontinuous culture: influence of the temperature of operation," *Applied Microbiology and Biotechnology*, vol. 49, no. 2, 1998.

[126] J. Wu, and H.-Q. Yu, "Biosorption of 2,4-dichlorophenol by immobilized white-rot fungus Phanerochaete chrysosporium from aqueous solutions," *Bioresource Technology*, vol. 98, no. 2, pp. 253-259, 2007.

[127] M. Tsezos, and J. P. Bell, "Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass," *Water Research*, vol. 23, no. 5, pp. 561-568, 1989.

[128] M. H. El-Naas, S. Al-Zuhair, and S. Makhlouf, "Continuous biodegradation of phenol in a spouted bed bioreactor (SBBR)," *Chemical Engineering Journal*, vol. 160, no. 2, pp. 565-570, 2010.

[129] K. Vidya Shetty, R. Ramanjaneyulu, and G. Srinikethan, "Biological phenol removal using immobilized cells in a pulsed plate bioreactor: Effect of dilution rate and influent phenol concentration," *Journal of Hazardous Materials*, vol. 149, no. 2, pp. 452-459, 2007.

[130] K. Reardon, F., D. Mosteller, C., and J. Bull Rogers, D., "Biodegradation kinetics of benzene, toluene , and phenol as single and mixed substrate for *Pseudomonas putida* F1," *Biotechnology and Bioengineering*, vol. 69, pp. 385-400, 2000.

[131] G. Klecka, M., and D. Gibson, T., "Inhibition of catechol 2,3-dioxygenase from Pseudomonas putida by 3-chlorophenol," *App. Environ. Microbiol*, vol. 41, pp. 1159-1165, 1981.

الخلاصة

تم تقييم مواصفات وجدوى استخدام بوليمر بولي فينيل الكحول في تثبيت البكتيريا المحللة للكريزولات، وقد تم تحضير البوليمر بطريقة تكرار التجمد والذوبان عدة مرات باستخدام تراكيب مختلفة من البوليمر. تم فحص البنية المسامية باستخدام مجهر المسح الالكتروني لتقييم توافق بولي فينيل الكحول في تثبيت البكتيريا لاستخدامها في معالجة المياه، وأثبتت النتائج أن حجم المسام وتوزيعها يتأثر كليا بتركيب البوليمر. وقد تمت دراسة الخواص الميكانيكية للبوليمرات ذات تراكيز مختلفة، وقد اتضح اعتماد هذه الخواص على تركيب البوليمر أيضا. تم اختبار البكتيريا المثبتة على أسطح البوليمرات ذات التركيزات المختلفة في إزالة الفينول من المياه على فترات زمنية طويلة. وقد كشفت النتائج أن تحفيز البكتيريا لإزالة مثل هذه المواد يزداد مع الوقت ويعتمد على البنية المسامية للبوليمر.

بعد دراسة خواص البوليمر كمادة مثبتة للبكتيريا تم استخدام البكتيريا المثبتة في إزالة أنواع مختلفة من الكريزولات الموجودة في المياه كل على حد، من خلال تجارب الدفعات والتجارب المستمرة في مفاعل بيولوجي، وقد تمت دراسة تأثير كل من تركيز الكريزولات في المحلول ودرجة الحرارة، إضافة إلى الأس الهيروجيني وكمية البكتيريا المثبتة في المفاعل في تجارب الدفعات. أما في التجارب المستمرة قد تمت دراسة تأثير معايير التشغيل الأخرى مثل التركيز وكل من معدل تدفق الهواء والسائل داخل المفاعل، وأثبتت نتائج الدراسة أن مقدرة البكتيريا علي تحليل الكريزولات تعتمد إلى حد كبير على معايير التشغيل وهذا الاعتماد يتغير مع نوع الكريزول. إلى جانب ذلك تمت تجربة البكتيريا في تحليل خليط ثنائي وثلاثي من الكريزولات في تجارب مستمرة، وقد تمت مقارنتها بتجارب الكريزولات الأحادية.

وأخيرا تم استخدام نتائج التجارب العملية لإيجاد قيم عوامل المتغيرات البيولوجية للكريزولات واستخدامها في محاكاة التجارب المستمرة، وقد اثبت نموذج المحاكاة توافق كبير بين النتائج العملية والمتوقعة.

جامعة الإمارات العربية المتحدة
United Arab Emirates University

UAEU College of Engineering

جامعة الإمارات العربية المتحدة

كلية الهندسة

قسم هندسة الكيمياء والبترول

التحلل البيولوجي للكريزولات باستخدام البكتيريا المثبتة على بوليمر بولي فينيل الكحول

رهام محمد سوركتي أبوبكر

رسالة مقدمة لاستكمال متطلبات الحصول على درجة الماجستير في الهندسة الكيميائية

قسم الهندسة الكيميائية والبترول

بإشراف أ. د. مفتاح حسن النعاس

ديسمبر 2013