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Aerobic Biodegradation of 2, 4 Dichlorophenol In A Spouted Bed Bio-Reactor (Sbbr)

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AEROBIC BIODEGRADATION OF 2, 4 DICHLOROPHENOL IN A SPOUTED BED BIO-REACTOR (SBBR)

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This dissertation is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Under the direction of Professor Muftah El-Naas

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Declaration of Original Work

I, Taghreed Tahseen Al-Khalid, the undersigned, a graduate student at the United Arab Emirates University (UAEU) and the author of the dissertation entitled "Aerobic Biodegradation of 2, 4 Dichlorophenol in a Spouted Bed Bio-Reactor (SBBR)", hereby solemnly declare that this dissertation is an original research 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 academic degree, diploma or similar title at this or any other university. The materials borrowed from other sources and included in my dissertation have been properly acknowledged.

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ABSTRACT

Chlorinated phenolic compounds represent a major class of hazardous pollutants commonly encountered in wastewater generated by the petroleum and petrochemical industries. In this regard, biological treatment, as an economical and green technology, has been shown to be one of the most promising approaches for the removal of many organic water pollutants such as chlorophenols. Under aerobic conditions, bacteria utilize chlorophenols as a source of carbon and energy. This study aimed at developing an integrated system for biodegradation of 2, 4 dichlorophenol (DCP) in a specially designed spouted bed bioreactor (SBBR), characterized by systematic intense mixing, resulting in enhanced biodegradation rates. The system utilizes microbial immobilization of an effective commercial bacterial consortium, consisting mainly of *Pseudomonas putida*, in order to retain the biomass within the reactor. The bacteria were immobilized in polyvinyl alcohol (PVA) gel particles and used in the SBBR to remove DCP from the wastewater.

The role of glucose, which has often been included as a carbon source during initial biomass acclimatization, was investigated and found not to be significant enough to justify its inclusion in the acclimatization process. In addition, the effects of several operating parameters were investigated in the batch mode operation, then modeled and optimized for maximum degradation rate by response surface methodology. The process was further tested in a continuous mode in order to evaluate the SBBR hydrodynamics in terms of stability and sustainability to shock loads. Total removal of the contaminant was achieved in the batch process at every initial concentration up to 200 mg/l, whereas a combined 80% removal at a throughput of 1400 g/m^3day was obtained during

continuous operation. Additionally, the process was mathematically modeled using a dynamic modeling approach in order to assess reaction and mass transfer limitations. The results supported the use of the PVA immobilization technique as the most effective decontamination process. Finally, the process was evaluated for the treatment of refinery wastewater and was proven to be very effective.

Keywords: Spouted bed bioreactor; Aerobic biodegradation; Immobilization; Polyvinyl alcohol (PVA) gel; Process modeling

ملخص

تمثل مركبات كلوريدات الفينول فئة كبيرة من الملوثات الخطرة التي توجد عادة في المياه العادمة الناتجة عن البترول والصناعات البتروكيماوية. في هذا الصدد، أثبتت المعالجة البيولوجية أنها واحدة من أكثر الأساليب الواعدة والناجحة لإزالة الكثير من ملوثات المياه العضوية مثل الكلوروفينولات.

تقوم البكتيريا تحت ظروف من التهوية الجيدة بتحويل الكلوروفينولات لمصدر للكربون والطاقة. لقد هدفت الدراسة إلى تطوير نظام متكامل للتحليل والتحويل العضوي لمركب 2، 4 ثنائي كلوروفينول في مفاعل انبثاق حيوي خاص صمم خصيصا لهذا الغرض، حيث يتميز بكفاءة عالية في خلط مكونات التفاعل، وهذا يؤدى إلى ارتفاع معدلات التحلل البيولوجي. في هذا النظام تم توظيف أحدث التقنيات المستخدمة في تثبيت البكتيريا الفاعلة القادرة على التعامل مع المركبات الهيدروكربونية، داخل مكعبات مسامية من مادة بوليفينيل الكحول، التي تمتاز بدرجة مسامية عالية وقدرة علي المحافظة علي البكتريا بداخلها بشكل دائم وتسمح للمياة الملوثة والمراد معالجتها بالتغلغل داخل مساماتها لتعالجها البكتريا.

استهلت الدراسة ببحث دور الجلوكوز في تأقلم المادة الحيوية مع الوسط العضوي وتبين أن ليس له تأثير مهم بما يبرر اعتماده في خطوات تأقلم البكتيريا مع المادة العضوية الملوثة. إضافة إلى ذلك تم بحث تأثير عدد من العوامل المؤثرة في عملية الانحلال الحيوي، ومن ثم نمذجتها بما يؤدي لإيجاد أفضل الظروف الموائمة لعملية التحليل العضوي لمادة ثنائي الكلوروفينول. لاحقا، تم اختبار عملية الانحلال تحت نظام التشغيل المستمر، وهو المعتمد في عمليات الصناعة، وذلك لفحص فاعلية النظام تحت تأثير عدة عوامل والتأكد من قدرته على تحمل التغيرات المفاجئة أثناء التشغيل واستدامته. لقد أثبت النظام فاعليته بالتمكن من التخلص من مادة ثنائي الكلوروفينول بنسبة 100% في التشغيل المحدود وتحقيق إنتاجية عالية في التشغيل المستمر. ومن أجل تحديد دور كل من عملية انتقال المادة وسرعة التفاعل في عملية الانحلال، تم إخضاع النظام لمزيد من النمذجة الديناميكية لتقييم التأثيرات الخاصة بهذه العوامل، وكانت النتائج في صالح استخدام طريقة تثبيت المادة الحيوية في مكعبات البوليفينيل الكحولي. أخيرا، تم تقييم هذه الطريقة لمعالجة عينات من المياه العادمة لمصافي تكرير النفط وأثبتت أنها فعالة جدا.

الكلمات الرئيسية: مفاعل الانبثاق الحيوي؛ تحلل حيوي هوائي؛ تثبيت البكتيريا؛ البوليفينيل الكحولي الهلامي؛ نمذجة العمليات

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DEDICATION

To my parents, my children and with special thanks and gratitude

to my husband

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[1] Al-Khalid, T. and M. El-Naas, Evaluating the Performance of Spouted Bed Bio-Reactor (SBBR) during Aerobic Biodegradation of 2, 4 Dichlorophenol (DCP), *Wastewater and Biosolids Treatment and Reuse: Bridging Modeling and Experimental Studies*, June 8-14, 2014, Otranto, Italy.

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NOMENCLATURE

AFR	Air flow rate (l/min)
a_{p}	External surface area of non-spherical particle (cm^2)
BCR	Bubble column reactor
b_i, b_{ii}, b_{ij}	Coefficients of in RSM of linear effect, square effect and interaction effect, respectively
C, C_o	Concentration, initial concentration (mg/l)
COD	Chemical oxygen demand (mg O_2/l)
CSTR	Continuous stirred tank reactor
D	Dilution rate (h^{-1})
DC	Degradation capacity (mg/l.h)
DCP	Dichlorophenol
D_{e}	Effective diffusivity (diffusion coefficient cm^2/s)
Di	Internal diameter of SBBR (cm)
F	Volumetric flow rate (ml/min)
FBR	Fluidized bed reactor
HRT	Hydraulic residence (or retention) time (min)
k_{d}	Decay coefficient (h^{-1}) in Eq. (1.3)
K_{i}	Substrate inhibition constant (mg/l)
K_{s}	Half saturation coefficient (mg/l)
K_{ow}	Octanol/water partitioning constant
LFR	Liquid flow rate = F
M	Mass (mg)
OLR	Organic loading rate (mg/l.h)
PBR	Packed bed reactor
pKa	Ground state acidity constant
q_{s}	Specific consumption rate (mg/g.l.h)
q	Degradation rate (mg/l.h)
q_{max}	maximum degradation rate (mg/l.h)
r	Radial position in the pellet (cm)
r_S	Rate of removal of substrate S
R	Radius of pellet (cm)
R^2	Coefficient of determination
RRW	Real refinery wastewater

RSM	Response surface methodology
RTD	Residence time distribution
S	Substrate concentration (mg/l) in Eq. (2.11.4)
S_i, S_e	Influent and effluent substrate concentration (mg/l), respectively, in Eq. (4.4.4)
SBBR	Spouted bed bioreactor
SBR	Sequencing batch reactor
t	Time (h)
t_m	Mean residence time (h)
TOC	Total organic carbon (mg C/l)
V	Reactor volume (ml)
V_L	Liquid volume in the reactor (ml)
V_p	Volume of non-spherical particle (cm^3)
v	Actual specific biodegradation rate (mg/g.l.h) in Eq. (4.5.9)
x_i	Input variable in RSM model (uncoded)
x_0	Actual parameter value at the center point in RSM model
X_i	Input coded variable in RSM model in Eq. (2.10.2)
X	Biomass concentration (mg/l) in Eq. (2.11.1)
Y	Response in RSM model in Eq. (2.10.2)
Y	Cell mass yield (g/g) in Eq. (2.11.2)

GREEK LETTERS

Δ	Step change value in RSM model
μ	Specific growth rate (h^{-1})
τ	Reactor space time (h)
$\emptyset$	Thiele modulus
η	Effectiveness factor

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

1. INTRODUCTION

1.1 OVERVIEW

Contamination of soil, air and water, is one of the major problems facing the industrialized world today, and respect of the environment is the cornerstone of sustainable development. Meanwhile, the pressures of an ever-increasing population and industrial development have led to the addition of an array of man-made chemicals to the environment, leading to significant deterioration in environmental quality. A major class of these chemicals is chlorinated phenolic compounds, most of which are hazardous pollutants that are highly toxic even at low concentrations. Petroleum refineries are a major source of these compounds in wastewaters. In view of the fact that environmental legislation in the UAE is very stringent, the management of wastewater containing high concentrations of phenols represents a major economic and environmental challenge to the oil industry. Emphasis has been placed on green technology, and in this regard, biological treatment has proved to be the most promising and most economic approach for the removal of many organic water pollutants such as chlorophenols. This study aims at developing an integrated system for biodegradation of chlorophenols, considering 2, 4 Dichlorophenol (2, 4 DCP) as a model contaminant. The system utilizes a state of the art technique for the immobilization of an effective commercial bacterial consortium, in a specially designed spouted bed bioreactor that promises to be effective in industrial processes for the treatment of real oil refinery wastewater.

1.2 PROBLEM

The chemical and petroleum industries generate a wide variety of highly toxic organic pollutants, which have led to a cumulative hazardous effect on the environment. Effluents from these industries often contain aromatic organic compounds that are resistant to natural degradation and therefore persist in the environment. This makes them capable of long range transportation and bioaccumulation in human and animal tissue. Organic pollutants represent a group of chemicals that can be seriously hazardous to human health [1-3].

Contamination of soil, surface water and underground water by aromatic organic pollutants such as phenol and its derivatives has caused great concern worldwide. Phenol and phenolic compounds are among the most prevalent forms of chemical pollutants in industrial wastewater, generated mainly from oil refineries, coal conversion plants, petrochemicals, polymeric resins, pharmaceuticals, smelting and related metallurgical operations [4-6]. Phenols are well known for their high toxicity to human life, aquatic life and others [7-9]. Ingestion of 1 gram of phenol is reported to be lethal to humans [10]. Phenols are considered to be among the most hazardous contaminants, and they are certainly among the most difficult to remove [11]. They are not amenable to conventional treatment processes and, in the presence of chlorine, can react to produce chlorophenols, which are carcinogenic and even more resistant to degradation than phenol itself [12, 13]. The US Environmental Protection Agency (EPA) calls for lowering phenol content in wastewater to less than 1 mg/l [14].

Chlorophenols are xenobiotic contaminants that are often found in waste discharges of many industries including petrochemicals, oil refineries, plastics, pesticides, biocides, wood preservers, pulp and insulation materials [15-17]. Some

of them can be formed during the chlorination of wastewater and drinking water and as a result of the breakdown of pesticides and chlorinated aromatic compounds [16, 18, 19]. Due to their high toxicity, strong odor, persistence in the environment and suspected carcinogenicity to living organisms, chlorophenols pose a serious ecological problem as environmental pollutants [15, 16, 18-20]. They can cause liver and kidney damage, cardiac toxicity including a weak pulse, cardiac depression and reduced blood pressure [7, 10]. Long-term exposure has been reported to cause chronic fatigue or neuropsychiatric problems along with skin infections, chronic respiratory symptoms and impaired fertility [21]. Among various chlorophenols, 2 chlorophenol (2 CP) and 2, 4 DCP were included in the EPA (USA) list of priority pollutants. Besides, 2 CP, 4 chlorophenol (4 CP) and 2, 4 DCP are the most significant chlorinated phenols formed as by-products of water chlorination [13, 22, 23]. Most countries specify the maximum allowable concentration of total phenols in effluent streams to be less than 1 mg/l [24]. However, higher concentrations were frequently found in contaminated environments, where the reported levels of chlorophenols ranged from 150 µg/l to 200 mg/l and even more [25]. Environmental legislation in the UAE limits the total phenols in industrial water discharged to the marine environment to 0.1 mg/l [26].

Although concerted efforts have been made to replace fossil fuels, crude oil remains an important raw material and the ever-increasing global energy demand is expected to soar by 44% over the next two decades and oil will account for 32% of the world's energy supply by 2030 [27]. A large volume of water is used in refinery processes, especially during distillation, hydro-treating, desalination and cooling systems, which makes petroleum refineries the main

source of phenolic compounds in wastewaters [13, 28, 29]. It has been reported that the volume of petroleum refinery effluents generated during processing is 0.4–1.6 times the amount of the crude oil processed. Thus, based on the current yield of 84 million barrels per day (mbpd) of crude oil, a total of 33.6 mbpd of effluent is generated globally [27]. The data clearly indicate that effluents from the oil industry will continue to be produced and discharged into the world's main water bodies [27]. Due to its complex composition, this water has to be properly treated to fit the standard limits for final disposal as established by environmental agencies, or to be reused in oil/gas exploitation [12].

Because of increasing social and political concern on the environment, the research field for water purification has been growing extensively in the last few decades, as water quality control and regulations against hazardous pollutants have become stricter in many countries [12, 30]. The management of wastewater containing high concentrations of phenols represents a major economic and environmental challenge to most industries.

1.3 OBJECTIVE

The aim of this study is to investigate the aerobic biodegradation of 2, 4 DCP in synthetic and real wastewater by a special commercial bacterial consortium, in which *Pseudomonas putida* is dominant, utilizing an advanced technique of biomass immobilization in a polyvinyl alcohol (PVA) gel matrix. The study has been carried out under batch and continuous operation conditions, in a specially designed spouted bed bioreactor (SBBR) that has proved to be very effective. This integrated approach is believed to achieve synergistically enhanced removal rates and to treat the contaminated effluents from petroleum refineries in an ecologically friendly manner. Although the capabilities of *P. putida* to degrade

aromatic hydrocarbons are very well established, the development of an effective approach that may be used for industrial applications is still lacking. To the best of the author's knowledge, there are no reports in the extant literature on the biodegradation of 2, 4 DCP in moving bed reactors with cells immobilized in PVA gel, applied to oil refinery wastewater.

1.4 SCOPE OF THE STUDY

After an exploratory investigation of chlorophenol biodegradation, the role of glucose in biomass acclimatization was investigated in addition to the effect of several operating parameters, thereby modeled by response surface methodology (RSM) and optimized in the batch operation for maximum degradation rate. The batch process was also tested using two different SBBR geometries. The performance of the SBBR in the continuous mode was evaluated and compared to that of the packed bed reactor (PBR) and was further subjected to dynamic modeling to assess the reaction and mass transfer limitations. Finally, the process was evaluated for the treatment of real refinery wastewater.

Following this introduction, a literature review is presented in Chapter Two. Chapter Three is devoted to materials and methods, with a description of the experimental set-up and procedure. Chapter Four presents the results and an interpretive discussion of the data in the following sections: Exploratory investigation; the role of glucose in biomass acclimatization; optimization of biodegradation of 2, 4 DCP in SBBR; continuous biodegradation of 2, 4 DCP in SBBR; modeling of the biodegradation of 2, 4 DCP; and batch treatment of real refinery wastewater in SBBR. Finally, conclusions and recommendations are summarized in Chapter Five.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

2. LITERATURE REVIEW

Several technologies are available for the treatment of industrial wastewater polluted with chlorophenols. Phenolic compounds can be economically recovered from water at concentrations above 2000–4000 mg/l. Below this level, removal via biological, physico-chemical and chemical processes is recommended [31, 32].

2.1 WHY IS BIODEGRADATION FAVOURABLE?

Biodegradation, which is sometimes referred to as catabolism, is defined as the breakdown of organic chemicals by living organisms into metabolic by-products with lower toxicity. This process is catalyzed by enzymes, where an initial chemical is converted stepwise through various intermediates into end products. The conversion of a chemical to completely oxidized end products is called mineralization [33]. Removal of chlorophenolic compounds from wastewater is a challenging task for process engineers involved in making plant operations, in line with health, safety and environmental management compliance, which is a must for sustainable development [34].

Efficient treatment methods for the removal of chlorophenols such as activated carbon adsorption, ion exchange, liquid–liquid extraction and chemical oxidation are available. It has been reported that the adsorption technique using activated carbon is the most efficient with a high adsorption capacity and the advantage that activated carbon can be regenerated and reused for several cycles [35]. Yuan and Keane [36] considered catalytic hydrodechlorination as a most progressive method for detoxifying chlorinated organic waste and treated 2,4

DCP and 2 CP as model reactants. This technique has also been investigated by others [37, 38]. Electrochemical methods, such as electrocoagulation have gained great attention in recent years for wastewater treatment and have been studied for the treatment of petroleum refinery wastewater [13, 14, 28, 39] and industrial wastewater [4]. These methods offer many distinctive advantages such as energy efficiency, safety and environmental compatibility as they have few or no harmful effects on the environment because these techniques do not involve the use of harmful reagents [13, 14]. Photocatalytic degradation techniques have also been widely used in wastewater treatment [20, 27].

However, most of these physico-chemical methods suffer from serious drawbacks such as high cost. In addition, most of these techniques do not degrade the contaminant, but rather transfer it to another phase, which results in the formation of hazardous by-products (secondary pollution) [14, 35, 40]. On the other hand, biodegradation is considered a more environmental friendly and cost effective alternative. Moreover, compared with physico-chemical methods, the biodegradation of chlorophenols is universally preferred, because of the possibility of complete mineralization [2, 11], which results in the complete conversion of a compound into its inorganic mineral constituents [1]. Biological transformation by microorganisms has become one of the key options to deal with environmental pollution problems caused by these compounds [22]. To this end, it is worth mentioning that biological processes cannot treat a high concentration of phenolics in industrial wastewater due to the biorefractory nature of these contaminants [4, 6, 41]. In this regard, integrated methods combining photochemical or electrochemical pretreatment and biological processes are well documented [6, 30, 42]. The degradation of different chlorophenols via sequential

biological and advanced oxidative processes has been investigated [43]. In addition, the synergistic combination of adsorption and biodegradation processes has long been recognized by many researchers [40, 42, 44-46].

2.2 STRUCTURE AND PROPERTIES OF CHLOROPHENOLS

Phenol is an aromatic hydrocarbon containing a hydroxyl group (OH) attached to the benzene ring: it is a basic structural unit for a variety of synthetic organic compounds. Of the four halogenated classes of phenols (fluoro, chloro, bromo, and iodo), the chlorophenols have the highest industrial value and are well understood with regard to their distribution and reactions in the environment [47]. However, they are the most problematic in terms of biodegradation [48]. The chemical structures of phenol and some commonly studied chlorinated aromatic compounds are given in Fig. 2.2.1.

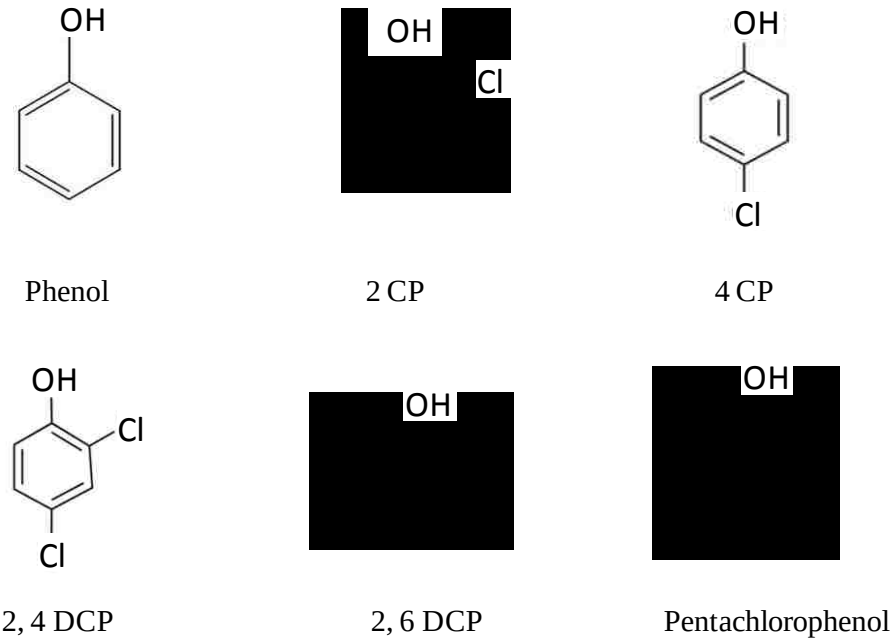


Fig. 2.2.1 Chemical structure of phenol and common chlorophenols [49]

Phenolic compounds have high stability due to the difficulty of cleaving the benzene ring. However, several microorganisms can tolerate phenols and use them as carbon and energy sources [50, 51]. In the case of chlorophenol, the presence of chloride in the ring makes it more challenging to degrade biologically. The chlorine atom in the ring could inhibit the enzyme activity of ring cleavage, and thus enhance the resistance to biodegradation of chlorophenols [15, 48]. The position and the number of chlorine groups on the aromatic ring have a profound effect on the biodegradability of chlorophenols. Usually, biodegradability decreases and toxicity levels increase with an increasing number of chlorine groups [52-54]. Additionally, the *meta*-position of the halogen on the phenol ring needs more energy than the *ortho*- and the *para*-ones [48, 49, 55].

Chlorophenols, especially mono-chlorophenols can easily migrate within different aqueous environments and contaminate groundwater and bioaccumulate in aquatic organisms because of their relatively high solubility in water [18, 43, 56]. It may be possible to predict the biodegradability of chlorophenols by using octanol/water partitioning constants (K_{ow}) [57]. Values of (K_{ow}) for chlorophenols increase with chlorination, indicating an affinity for the higher chlorophenols to bioaccumulate [22]. The key to understanding the environmental photochemistry of phenols is ground state acidity constant (p*K*a), which decreases with electron-withdrawing substituents (e.g., the halogens) positioned on the aromatic ring. This can have important effects on the environmental fate of halophenols. For example, the p*K*a of phenol 9.95 decreases to 9.38 for 4 CP, 7.68 for 2, 4 DCP and to 4.74 for pentachlorophenol [22, 47]. Solubility, volatility and hydrophilicity of chlorophenols decrease with an increase in the degree of chlorination [58].

2.3 MICROORGANISMS IN CHLOROPHENOL BIODEGRADATION

A large number of microorganisms including bacteria, fungi and algae are capable of degrading chlorophenols. However, a major drawback in biological treatment is the inhibition of microorganisms at high substrate concentrations [10]. Depending on microbial abilities to grow in specific conditions, organic material can be degraded aerobically or anaerobically [15]. Although both aerobic and anaerobic microorganisms are able to degrade chlorophenols, aerobic processes are typically preferred. Aerobic microorganisms are more efficient for degrading toxic compounds because they grow faster and usually achieve complete mineralization of toxic organic compounds to inorganic compounds (CO_2, H_2O) [59-61]. For these reasons, there is limited interest in the utilization of anaerobic bacteria for the degradation of chlorophenols. However, there have been several studies in this regard [62-68]. This study focuses on the aerobic biodegradation of 2, 4 DCP.

2.3.1 Bacteria

A large number of chlorophenol degrading bacteria have been isolated and characterized at the physiological and genetic level. The microorganisms used are usually aerobes, including *Pseudomonas* sp., *Alcaligenes* sp., *Azotobacter* sp., *Rhodococcus* sp. and *Cryptococcus* sp. [60, 69]. Pure and mixed cultures of the *Pseudomonas* genus are the most commonly utilized biomass for the biodegradation of chlorophenols [58, 70]. Specifically, *Pseudomonas putida*, has commonly been used for the biodegradation of phenols due to its high removal efficiency [71, 72]. Responses of *P. putida* to chemical stresses have indicated that its cells could use diverse protective mechanisms for survival in various extreme environments. These studies could help in synthesizing new bacterial

strains with enhanced degradation capability and improved tolerance to toxic pollutants [73].

Although, the *Pseudomonas* genus has been widely used to treat chlorophenols, considerable attention has been recently directed towards new and more efficient microorganisms. *Ralstonia pickettii*, formerly known as *Burkholderia pickettii*, has several advantages over other candidate strains such as *P. putida*, in that it is only weakly pathogenic with no phytopathogenic or animal pathogenic incidents being reported. *R. pickettii* strain LD1 can metabolize mono-chlorophenols, which represent an important challenge as they may be formed during the chlorination of wastewater [74]. This aerobic bacterium, was able to totally degrade 2 CP (initial concentration: 195 mg/l), 3 CP (initial concentration: 73.5 mg/l) and 4 CP (initial concentration: 97 mg/l) within 30, 30 and 40 h of incubation, respectively, under batch conditions. The rates at which mono-chlorophenols degraded were found to depend on the type of chlorophenol: 2 CP > 4 CP > 3 CP (when used as sole carbon sources) [16]. Al-Zuhair and El-Naas [75] isolated an indigenous mixed bacterial culture, identified mainly as *R. pickettii*, from oil sludge samples at a local petroleum refinery. Its efficiency for phenol removal was found to be comparable to that of a commercially available mixed culture consisting mainly of *P. putida.*

2.3.2 Fungi

Fungi have a significant role in the recycling of aromatic compounds in the biosphere. They are capable of consuming a wide variety of carbon sources via enzymatic mechanisms, thus providing the possibility of metabolizing chlorophenols [70]. The most abundant fungi in polluted environments are yeasts. Some yeasts such as *Candida tropicalis*, *Fusarium flocciferium* and *Trichosporon*

cutaneum are capable of utilizing phenols as their major carbon and energy sources [55]. Moreover, there are studies attesting to the ability of strains from *Penicillium*, *Aspergillus*, *Graphium and Phanerochaete* genera to disintegrate chlorophenolic compounds [8, 70]. Rubilar et al. [76] analyzed the degradation of chlorophenols by white rot fungi, which are a group of organisms very suitable for the removal of chlorinated phenolic compounds. They are robust organisms that are tolerant to the presence of high concentrations of various pollutants, even with a low bioavailability and this ability is mainly due to their very powerful extracellular oxidative enzymatic system [77]. Li et al. [78] reported the first study on biodegradation of 4 CP by *Fusarium* sp. HJ01.

2.3.3 Algae

Only recently there has been more interest in investigating the capabilities of algae for chlorophenol biodegradation. While some algae have low tolerance to the acute toxicity of chlorophenols, both cyanobacteria and eukaryotic microalgae (e.g., *Chlorella* sp., *Scenedesmu*s sp., *Selenastrum capricornutum*, *Tetraselmis marina*, *Ochromonas danica*, *Lyngbya gracilis*, *Nostoc punctiforme*, *Oscillatoria animalis*, and *Phormidium foveolamm*) are capable of biotransforming phenolic compounds [79]. Papazi and Kotzabasis [48] reported that the biodegradation of phenolic compounds by microalgae seems to be mostly a bioenergetic process, which means that it needs additional energy sources that can be exogenously supplied as carbon and light. Klekner and Kosaric [80] conducted a study on the degradation of binary phenolic mixtures by *Chlorella*, in which phenol might have been expected to induce the proper metabolic pathway of other phenol derivatives. Other studies have been reported on biodegradation of phenols by algae [81, 82].

2.4 MECHANISM OF CHLOROPHENOL BIODEGRADATION

Metabolic processes are governed by the action of enzymes, which are specific to each type of reaction, thus microbial metabolism is a process of energy conversion sustained by specific reactions, providing the ultimate source of energy [55]. The aerobic biodegradation process requires the presence of molecular oxygen to initiate enzymatic attack on the aromatic ring. A typical pathway for metabolizing phenols is to hydroxylate the ring using the enzyme phenol hydroxylase, form catechol and then open the ring through *ortho*- (also termed β-ketoadipate pathway) or *meta*- oxidation [79, 83]. Phenol hydroxylase represents the first enzyme in the metabolic pathway of phenol degradation.

Both *ortho*- and *meta*- pathways are distinguishable by measuring their characteristic enzyme activities. In the *ortho*- pathway, the aromatic ring is cleaved by the enzyme catechol 1, 2 dioxygenase (C12O). In the *meta*- pathway, the ring is cleaved by the enzyme catechol 2, 3 dioxygenase (C23O). Thus, the ring is opened and then degraded [1, 55, 84]. The ring cleavage can occur in two different orientations and this difference in the cleavage site is used to classify catechol dioxygenases into two groups: the intradiol (such as C12O) and extradiol (such as C23O) cleaving enzymes [85]. The genes of ring cleavage dioxygenases may serve as good targets for monitoring the biodegrading biomass, thus providing a rapid method for monitoring the microbial community during the treatment process [86].

The key enzymes in the microbial degradation of chlorophenols are the oxygenases and dioxygenases [22]. According to the literature describing chlorophenol biodegradation pathways, anaerobic degradation of chlorophenols includes reductive dehalogenation of the first step, with the preferential

replacement of chlorine by hydrogen from *ortho*- position. The aerobic degradation of chlorophenols may occur either through the *ortho*- pathway or the *meta*- pathway, following their transformation to chlorocatechols [23, 51, 87]. Thus, catechol is a central intermediate in the aerobic degradation pathways of various aromatic compounds. Fava et al. [16] also suggested the chlorocatechol pathway in their investigation of aerobic degradation of different mono-chlorophenols by a *Pseudomonas pickettii* strain. However, aerobic biodegradation of mono and dichlorophenols follows mainly the *ortho*- cleavage pathway after the formation of chlorocatechol, because the *meta*- cleavage produces toxic products from chlorocatechol [87, 88]. Conversion of chlorocatechol as a rule does not proceed via *meta*- cleavage of the aromatic ring, as the catechol dioxygenase enzyme is inactivated by the accumulation of an intermediate, which is toxic for cells. *Meta*- cleavage of chloroaromatics generally results in the formation of dead- end metabolites. In general, the *ortho*-cleavage pathway is required for the complete degradation of mono and di-chlorophenols [51]. Only rarely have *meta*- cleavage pathways been observed for chlorocatechols [89]. In a study by Farrell and Quilty [51], *P. putida* CP1 was added to a commercial mixed culture that degraded 2 CP. The augmented mixed culture degraded 2 CP using an *ortho*- cleavage pathway, whereas previous degradation by the mixed culture occurred using the *meta*- cleavage pathway. A proposed *ortho*- cleavage pathway for aerobic biodegradation of 2, 4 DCP is depicted in Fig. 2.4.1. Discussion of biodegradation pathways and mechanisms can be found in the literature [1, 49, 55, 90-95].

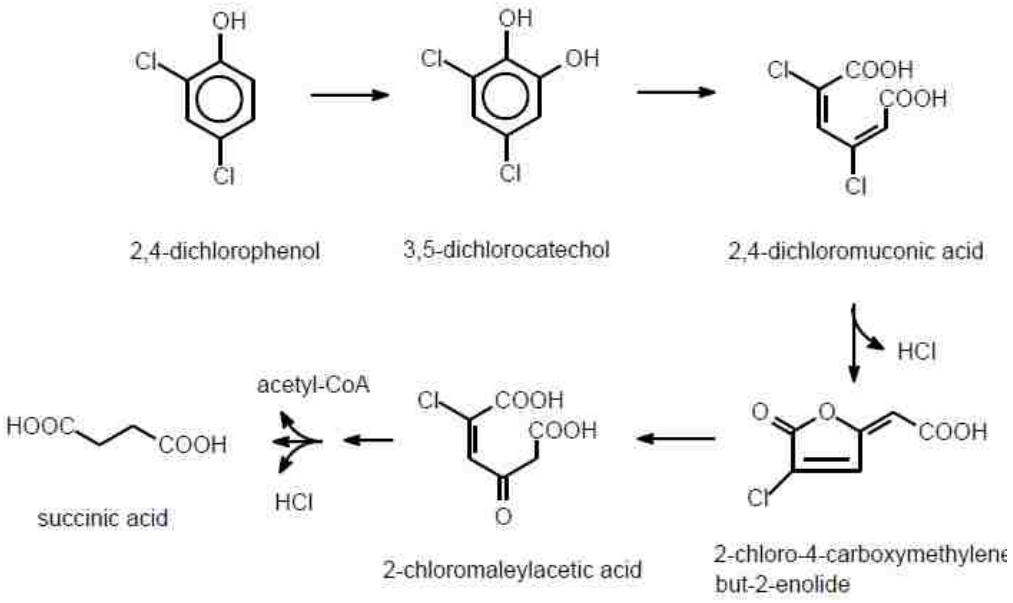


Fig. 2.4.1 Proposed *ortho*- pathway of the aerobic degradation of 2, 4 DCP by bacteria [96]

2.5 FACTORS AFFECTING BIODEGRADATION OF CHLOROPHENOLS

Biodegradation is a multifaceted process, which involves a combination of many biotic and abiotic factors [92]. There are many factors that can affect the degradation ability or metabolism of microorganisms by either preventing or stimulating the growth of the organisms. These factors may include pH, temperature, oxygen content and availability (aeration and agitation), bioavailability (availability of the contaminants to microbes), bacterial abundance and substrate concentration [1, 11, 55, 92]. Each of these factors should be optimized for the selected organism to achieve the maximum degradation of the chosen organic compound.

2.5.1 Effect of pH

Alkalinity and pH have important roles to play in the biological treatment of wastewater. Extreme pH values of the medium (less than 3 or greater than 9) as

well as sudden changes in pH in which the microbe is present can inhibit its growth [55]. In aerobic biological oxidation, pH in the range of 6.0–9.0 is suitable [97]. Consequently, laboratory studies on chlorophenol degradation are usually carried out at or near neutral pH values (pH = 7.0). However, Alva and Peyton [98] reported that a haloalkaliphilic bacterium, *Halomonas campisalis*, is able to thrive in both saline and alkaline environments such as soda lakes. The bacterium degraded phenol and catechol in alkaline (pH values of 8-11) and saline environments (0-150 g/l NaCl). This is especially significant for an industrial effluent of great environmental concern, such as petroleum refinery spent caustic, alkaline and saline waste streams that contains phenol as well as other aromatic compounds [26, 98].

2.5.2 Effect of Temperature

Each organism has a certain temperature range for growth. Conventionally, these organisms can be divided into psychrophilic and psychrotrophic organisms. Psychrophiles are organisms that have minimum, optimum and maximum growth temperatures of ≤ 0, ≤ 15, ≤ 20 °C, respectively. The corresponding temperatures for psychrotrophs are 0–5, > 15 and > 20 °C. It is evident that the most interesting organisms, are often the psychrotrophs, since they are also active at temperatures above 20 °C [99]. Therefore, the temperature-dependent performance of biological processes may be strongly influenced by their content of psychrotrophic bacteria. Most studies on chlorophenol biodegradation have been carried out in temperature range 25 – 30 °C. Li et al. [99] reported that there has been little focus on microorganisms that can function at low temperatures. Therefore, it is necessary to find a kind of organism that can adapt to significant daily and seasonal fluctuations in environmental temperatures,

with a focus on cold-adapted microorganisms in environments where they are needed. The bacterial strain *P. putida* LY1, a psychrotrophic microorganism, was shown to grow on phenol as the sole carbon and energy source and survive well in a wide range of temperatures [99]. On the other hand, only a few studies have addressed biodegradation of phenols by thermophilic or hyperthermophilic microorganisms although many phenolic effluents are hot. Such microorganisms can be robust biocatalysts with potential applications [100]. A strain of *P. putida* has successfully been used to degrade phenol at a low temperature of 10 °C, while a bacterium *Bacillus stearothermophilus* has also been used to effectively degrade phenol at 50 °C [55]. Several studies have been documented in this regard [100-102].

As a result, temperature significantly affects the critical process and design parameters, such as the critical dilution rate, which corresponds to the limit above which biomass washout occurs and, therefore, failure of the biological treatment process [101]. According to Onysko et al. [101], there is a lack of sufficient information about the change in microbial kinetics as a function of temperature. The authors attempted to model the temperature dependence of cell growth and phenol biodegradation kinetics of the psychrotrophic bacterium *P. putida* Q5 in both batch and continuous cultures in the range of 10–25 °C.

2.5.3 Substrate Concentration

High concentrations of chlorophenols are usually inhibitory to microorganisms growth. However, pre-adaptation or acclimatization of microorganisms to chlorophenols improves their biodegradation capability and alleviates the inhibition effect to some extent [103]. Sometimes, due to substrate inhibition, combined processes have been used to treat highly loaded wastewaters,

such as the sequential UV–biological degradation of a mixture of chlorophenols [104] and sequential anaerobic–aerobic reactors [23, 105]. According to Wang et al. [106], little information on bacteria with a high tolerance to phenols and high metabolizing activity is available. Therefore, there still exits the need to isolate new phenols-degrading bacteria that can grow even with high concentrations of phenols.

2.5.4 Natural Carbon Sources

The ability of microbial communities to degrade pollutants is affected by the presence of naturally occurring carbon sources. In general, adaptation to variations in the concentration of nutrients such as glucose, yeast extract and $(NH_4)_2SO_4$ enhances the ability to degrade phenols. However, it was shown that different chlorophenols may give different responses to the presence of biogenic substrate [19, 107]. The rate of degradation was also proved to be strongly dependent on the composition of the medium affecting the degradation efficiency. Shourian et al. [9] found that phenol-containing minimal medium supplemented with mannitol and casein showed the highest degradation rate among all the carbon and nitrogen nutrients tested. The highest inhibitory effects were attained by the addition of sucrose and glycerol. In another study by Stehlickova et al. [108], higher biodegradation rates and more intensive growth were observed during cultivation in the presence of potassium humate (humic substance) in comparison to cultivation without its addition. This effect of humic substances was also confirmed by Hahn et al. [109], reporting reduction of 2,4 DCP toxicity to *P. putida* after oxidative incubation with humic substances.

2.6 MIXED SUBSTRATES AND MIXED CULTURES

Although a wastewater treatment plant generally receives influent containing a mixture of recalcitrant organic chemicals and biogenic substrates, so far, most studies have focused on the degradation of a single type of chlorophenols [107]. Microorganisms in nature grow mostly on mixtures of substrates and growth may not be controlled by a single nutrient but by two or more nutrients or substrates simultaneously. When mixed substrates are used, substrate competition and crossed inhibition could severely affect the microbial growth and biodegradation rates [110]. The degradation of chlorophenol can be performed by pure or mixed cultures. It is likely that an application of the mixed culture permits faster degradation than a pure culture [111]. Biodegradation of mixed substrates by pure cultures can be a slow and inefficient process, particularly, when substrates are recalcitrant to biodegradation or inhibit cell growth. Many pure-culture studies have shown that toxic intermediates accumulate during biodegradation, because a single organism may not have the ability to completely mineralize the xenobiotics [94, 112]. Therefore, the treatment of chlorophenols using mixed cultures would be more informative and practical. The main advantage of the microbial consortium formed by mixed culture is this synergistic effect of all of the species present in the flocs [60]. Several studies addressed substrate interactions in different mixtures of phenolic compounds [113-116]. Many other studies have been carried out on the use of mixed cultures for mixed substrates [21, 53, 110, 117, 118]. Microbial consortiums showed better overall removal efficiencies than single strains.

2.6.1 Co-metabolism

Biodegradation can also result from co-metabolism in which case the chlorinated aromatics are transformed while microorganisms are utilizing other substrates to gain energy for synthesis of the enzymes involved in the metabolic breakdown of recalcitrant compounds. This could result in increased removal rates for these compounds [110, 119, 120]. This is an important example of the influence of substrate interaction during the biodegradation process. Co-metabolism is defined as the degradation of a non-growth compound only in the presence of another organic material that serves as the primary growth substrate. This phenomenon has been attributed to the production of broad-specificity enzymes, where both the primary substrate and the other compound compete for the same enzyme [121]. Phenol has been claimed as a good growth substrate in the biodegradation of chlorophenolic compounds because of its similar chemical structure and lower toxicity [103].

2.6.2 Diauxy

Another form of substrate interaction is diauxy. Diauxic growth implies the inhibition of the consumption of one growth substrate by the presence of another, which requires a larger acclimation period for the utilization of the second substrate. In contrast to diauxic growth patterns, concurrent utilization of multiple substrates in natural ecosystems and in wastewater treatment systems is commonly observed [103]. Stoilova et al. [70] reported the diauxy phenomenon in a study that investigated the ability of fungus *Aspergillus awamori* NRRL 3112 to degrade binary mixtures of phenol, catechol, 2,4 DCP and 2,6-dimethoxyphenol. The main finding was that for all combinations containing DCP, diauxy was observed. The assimilation of the second phenolic derivative was suppressed until

DCP had been completely degraded. In other mixtures, no diauxy was observed and both substrates were metabolized simultaneously.

2.6.3 Substrate Interaction: Closed versus Open System

The interactions of chemical compounds with a diversity of microbial species involved in the biodegradation process are different in batch or continuous systems. In the first one (closed system) the relatively high substrate concentration at the beginning of the batch process could inhibit cellular growth, thus affecting the substrate biodegradation rate. In a steady state continuous system (open system), low substrate levels are maintained in the reaction mixture, therefore, catabolitic repression and growth inhibiting effects are smaller than those observed in the batch system: thus, mixed substrates can be simultaneously degraded [110]. On the other hand, continuous reactors require low dilution rates to avoid instability or low conversion [122, 123]. The biodegradation of mixed chlorophenols by a microbial consortium has been studied in a steady state continuous operation [110].

2.7 CONTAMINATED SITES AND INDUSTRIAL EFFLUENTS

Shourian et al. [9] reported that many pollution problems resulting from the release of aromatic chemicals occur in rivers, lakes, groundwater, and process effluents from industry. Accordingly, environmental bacterial strains isolated from contaminated sites are expected to play a vital role in the bioremediation of contaminated areas. In their study, *Pseudomonas* sp. SA01 was isolated from pharmaceutical wastewater. Chrzanowski et al. [124] investigated the biodegradation performance of a bacterial consortium, isolated from crude-oil contaminated soil, in degrading a commercial diesel fuel employed as a model hydrocarbon-rich effluent, which was also co-contaminated with a mixture of

phenol and chlorophenols. Other researchers have utilized microbial biomasses isolated from phenol-contaminated soil [2, 125], wastewater plants [50], effluents from coke processing units [126] and municipal gasworks [127].

It has been reported [25, 56] that the majority of studies on the biodegradation of chlorophenols have been conducted only under laboratory conditions and scaling up to field conditions is not a simple process. More research is needed to understand the fundamental mechanism of biodegradation under field conditions. A study on the scale up of 2,4 DCP removal from synthetic wastewater, using a stirred tank reactor was conducted by Angelini et al. [25]. The study helped to design a method for the continuous and safe treatment of phenolic compounds in waste effluents on a large scale. Moreover, it has been remarked that studies on biodegradation of phenols have primarily been approached by simulating industrial waste effluents with synthetic model solutions [49]. El-Naas et al. [42] developed a novel integrated three-step system combining an electrochemical process, a biological treatment with a spouted bed bioreactor (SBBR) and an adsorption fixed bed column packed with granular activated carbon for the treatment of contaminated refinery wastewater. Agarry et al. [128] treated refinery wastewater in their study, which investigated the phenol-biodegrading potential of two indigenous *Pseudomonas* species. Only a limited amount of work has focused on biodegradation of phenols in oil refinery wastewater [128-130].

2.8 IMMOBILIZATION AND BIOFILM REACTORS

Most of the investigations on biodegradation of chlorophenols focused on suspended pure culture studies using different bacteria and fungi. Recent investigations on biodegradation of chlorophenols focused on the use of

immobilized cells or biofilm reactors with high performance, such as fluidized bed bioreactors, fixed bed biofilm reactors, trickling bioreactors, microporous membranes and combined anaerobic–aerobic bioreactors, in which microbial cells were attached or immobilized on the carriers [53, 54, 69, 129]. Suspended culture systems such as conventional activated sludge processes usually fail to remove high concentrations of chlorophenols from wastewater due to the toxic nature of these compounds. Two of the major problems encountered with this approach are the inability to settle the sludge, and the excess formation of scum-foam [129]. It has been reported that the use of free bacterial cells for wastewater treatment in activated sludge processes creates problems such as solid waste disposal, while immobilized microorganisms are capable of effective treatment with little sludge formation [2, 106].

2.8.1 Advantages of Immobilization

To enhance biodegradability, bacterial activity is increased through immobilization by various technologies, including bead entrapment, carrier binding, adsorption, encapsulation, cell coating, and film attachment [69]. Immobilization will greatly reduce the damage to microbial mass by chlorophenols and minimize the loss of microbial cells [118]. It is an important and effective technique that is usually employed to serve several purposes, including the protection of the biomass from the toxicity of chlorophenols at high concentrations as well as the ease of separation and reutilization of the biomass [2, 106].

Biofilm reactors are more resistant to high concentrations of chlorophenols because of high biomass concentrations and diffusion barriers within the biofilm for the toxic compounds. Therefore, biofilm systems usually result in better

reactor performance as compared to suspended growth systems and usually produce high removal efficiencies [53]. They are also simpler to operate and more energy efficient [129]. On the other hand, control of biofilm thickness, dissolved oxygen concentration and pH in biofilm reactors is difficult [107]. The immobilization process affects bioreactor performance. The main advantage of immobilization is the retention of active biomass on support particles, giving shorter hydraulic retention times and no biomass washout in continuous operation [131, 132]. Another advantage is the flexibility of reactor design and the improved thermal and operational stability. Since the biomass can be reused, a large volume of wastewater can be continuously treated using a defined quantity of immobilized cells [133]. This configuration has also been found to have a relatively higher tolerance to toxic and organic shock loads [27].

2.8.2 Relevant Studies on Biofilm Reactors

Information on aerobic chlorophenol degradation in bioreactor systems is still limited, probably due to the toxicity or inhibition of chlorophenols to microorganisms. A few studies reported the experimental results of employing immobilized biomass strategies to overcome the acute toxicity effect of chlorophenols [15, 107].

Sahinkaya and Dilek [107] reported that in a suspended growth reactor 2,4 DCP removal efficiency decreased sharply for concentrations above 150 mg/l. Therefore, biological degradation of chlorophenols in suspended growth bioreactors is difficult, especially when the feed contains more than one chlorophenol. Their study aimed at assessing the applicability of a two-stage rotating biological contactor (RBC) based bioprocess for the bioremediation of a mixture of 4 CP and 2, 4 DCP at high loading rates with varying biogenic

substrate concentrations. A similar study [19] was carried out in a sequencing batch reactor (SBR), which is thought to be an attractive alternative to conventional biological wastewater treatment systems, mainly because of its simplicity, flexibility of operation and cost effectiveness for small-scale treatment facilities [19]. In another study [69], the performance of an airlift inner-loop bioreactor, with honeycomb ceramic as a carrier, was investigated to degrade 2,4 DCP alone or mixed with phenol under fed-batch and continuous operation. Gallego et al. [117] investigated biodegradation of a mixture of 2 CP, phenol and *m*-cresol, by pure and mixed indigenous cultures in both a batch microfermentor and a continuous flow fluidized bed reactor (FBR) filled with granular activated carbon.

Kim et al. [61] conducted a comparative study on the biodegradation of a mixture of phenol and chlorophenols with a defined mixed culture in a continuous stirred tank reactor (CSTR) and PBR. The degradation capacity of the PBR was higher than that of the CSTR, but the PBR was unsuitable for oxygen-sensitive microorganisms. The main advantage of a CSTR is the simplicity of its operation and the adjustability of its retention time. On the other hand, it is vulnerable to shock and washout and it takes a long time to recover from perturbation. A PBR can recover from shock quickly because active microorganisms are held on a stationary surface, thus they can be resistant to washout and shock. A severe disadvantage of a PBR, however, is that it produces excess biomass resulting in severe flow stoppages [61].

Several studies on the use of immobilized cells in different bioreactor types are documented. Microbial strains immobilized on polymer matrices have been reported as being suitable for the degradation of phenols [134]. Quan et al.

[135] used a mixed culture immobilized in PVA gel beads and supplemented to SBR to treat synthetic wastewater containing 2,4 DCP. The gel beads were cross-linked at room temperature. SBR is operated in a periodic mode, where conditions can be changed periodically, which is useful to facilitate the survival and growth of the cells and reserve their activity. As a synthetic polymer, PVA has better mechanical properties and it is more durable than Ca-alginate, which is biodegradable and can be subject to abrasion [11, 71, 136].

The effectiveness of this method has also been investigated by El-Naas et al. [11] in a study to assess the biodegradation of phenol by *P. putida* immobilized in PVA gel matrix in a bubble column reactor (BCR). This study emphasized the importance of the phenol uptake per mass of PVA, which is a significant economic consideration in designing an industrial process for wastewater treatment. Subsequent studies were performed in the batch and continuous modes in a SBBR [71, 136], which is characterized by systematic intense mixing due the cyclic motion of particles within the bed generated by a single air jet injected through an orifice in the bottom of the reactor. The immobilization technique involved cross-linking in the gel structure by repeated cycles of freezing-thawing to increase the mechanical strength of the polymer. Al-Zuhair and El-Naas [137] evaluated the biodegradation of phenol at high concentrations using *P. putida* immobilized in PVA gel matrices, employing two immobilization techniques and two reactors, namely BCR and SBBR. Both reactor configurations and both immobilization techniques proved to be effective. Biodegradation of phenol by immobilization of other bacterial strains in a PVA gel was also studied by Liu et al. [2] and Wang et al. [106], utilizing the same technique of cyclic freezing-thawing. Jianlong et al. [138] also evaluated two

different PVA immobilizing techniques in the microbial degradation of quinoline by *B. pickettii*.

2.8.3 Aerobic Granulation

Attention in recent years has focused on developing aerobic granules in SBRs [139, 140]. Aerobic granulation is a novel environmental biotechnique, which involves cell-to-cell adhesion. Unlike biofilm, aerobic granules are formed through self-immobilization of microorganisms (especially bacteria) without any carrier material under aerobic or alternative aerobic–anaerobic conditions [141]. Under high shear force conditions, microorganisms would promote the formation of compact granules to protect cells from the chemical toxicity at high substrate concentrations [140]. This was confirmed by a laser scanning microscopic test, which revealed that live cells are principally distributed throughout the surface layer of the granule, with an extracellular polymeric substance layer covering them to protect the cells from phenol toxicity [139, 142]. Aerobic granulation offers better effluent quality and higher treatment efficiency, with granules having a dense and strong microbial structure, good settling ability, high biomass retention and better tolerance to higher organic loading rates. It is a novel wastewater treatment technology that can quickly decontaminate highly contaminated wastewater. Aerobic granules yield a very high biomass concentration (up to 15,000 mg/l) and have the ability to degrade high-strength wastewater (up to 15 kg COD/m^3/day) [142]. Utilization of immobilized cells or biofilm reactors was reported to improve chlorophenol removal due to high biomass retention [140, 143].

2.8.4 Immobilized Enzymes

Enzymes are versatile catalysts with a growing number of applications in biotechnology. These enzymes are commonly used in biochemical processes such as in the food industry, pharmaceuticals, biomedical engineering, chemical manufacturing and analytical processes. Moreover, there has been a growing recognition of the direct application of destructive enzymes obtained from degrading microorganisms for the catalytic treatment or remediation of contaminated water, soils and sediments [144]. The use of enzymes in industrial processes is usually linked to a reduced consumption of energy as well as chemicals and thus beneficial for the environment [145]. In biodegradation processes, free enzymes often undergo deactivation as a result of sudden changes in the surrounding environment such as in the pH, temperature and chemical and hydrodynamic shocks. These shortcomings can be overcome by immobilization. There are many benefits of enzyme immobilization, including enhanced stability against abrupt changes in environmental factors, extended lifetime, increased reusability and easy handling. A few recent studies which targeted the removal of phenolic pollutants by enzymatic treatment are documented [144, 146-151]. These studies have focused on introducing novel, practical and inexpensive immobilization methods, utilizing various enzymes, such as laccases, peroxidases, tyrosinases and dioxygenases.

2.9 OPERATION MODES AND REACTOR TYPES

Biological reactors involve a variety of geometries and hydraulic systems. Batch or semi-batch reactors are often used for laboratory studies, for anaerobic digestion, and for the manufacture of pharmaceuticals. Flow reactors are often employed for aerobic treatment of municipal and industrial wastes [152]. Batch

operation is usually associated with very low initial substrate concentration, which affects the productivity of the process. On the other hand, continuous reactors require low dilution rates to avoid instability or low conversion. Processes involving immobilized cells often encounter problems with nutrient and oxygen transfer [123].

In flow reactors, the two extremes of mixing are represented by well-stirred (stirred tank reactor) and plug flow reactors. Intermediate degrees of mixing are often described by well-stirred reactors in series or by plug flow reactors with axial dispersion. More complex mixing models can be devised but their use is constrained by the limitations of the system. The overall model of the reactor is obtained by combining the equations for the hydraulic regime and the kinetics of the reactions [152]. An advantage of a well-mixed reactor is the uniformity of oxygen consumption rates by the microorganisms. With a plug flow reactor, the oxygen demand is usually greatest at the inlet region where the substrate concentration is high. Thus, the design of aerators for a plug flow reactor is constrained by the ability to provide more oxygen transfer in the inlet region. A plug flow reactor generally produces a higher conversion of substrate in a given volume than a well-mixed reactor. If no biomass enters a plug flow reactor, no reaction can occur and the reactor washes out. On the other hand, the influent to a well-stirred reactor is mixed with vessel fluid containing biomass so that the reaction can be sustained even in the absence of biomass in the feed stream. At the end, the two types of reactors are idealized models that are difficult to achieve in large scale biological reactors. The advantage of a stirred tank reactor is that operating it and adjusting its retention time is simple [153]. The use

of stirred tank reactor has been documented in the batch mode [154, 155] and continuous mode [101, 156].

The treatment of wastewater containing phenols has been focusing on employing and exploring new types of bioreactors targeting practical utilization and efficient long-term performance [71]. In addition to the conventional biofilm reactors mentioned in Section 2.8, there are other types of novel reactors, which include the use of hollow fiber membrane contactors [157-159], microbial fuel cells [160-162], rotating rope bioreactors [163], pulsed plate bioreactors [164-166], two phase partitioning bioreactors [167-169] and foam emulsion bioreactors [170]. However, most of these reactors have difficulty in long-term operation and scaling up, which limit their practical application in any industrial process [71].

Hybrid bioreactors, which combine a biofilm reactor with an aerated tank (suspended culture) have also been reported. A combination of the two types of growth in one system has created interest. It incorporates the advantages of a biofilm system that is stable and capable of handling shock loads in a combined attached and suspended growth system that offers simplicity of operation and economic advantages [145, 171, 172].

2.10 RESPONSE SURFACE METHODOLOGY (RSM)

RSM is the most widely used statistical technique for bioprocess optimization [173]. It is a graphical statistical modeling technique used to evaluate the relationship between a set of controllable experimental factors and observed results [174], where the interactions among the possible influencing parameters can be evaluated with a limited number of experiments [173]. This optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, and

predicting the response and checking the validity of the model [173, 175]. The relationship between the response and the experimental factors is:

$$Y = f(x_1, x_2, x_3, x_4 \ldots x_k) \quad (2.10.1)$$

For statistical calculations the variable x_i is coded X_i according to: $X_i = (x_i - x_0)/\Delta x$ where x_0 is the actual value at the center point and Δx is the step change value. The mathematical relationship of the response of these variables can be approximated by the quadratic (second degree) polynomial equation:

$$Y = b_0 + \Sigma\, b_i X_i + \Sigma\, b_{ii} X_i^2 + \Sigma\, b_{ij} X_i X_j \quad (2.10.2)$$

Y is the predicted response, b_0 the offset term, b_i the linear effect, b_{ii} the square effect, and b_{ij} the interaction effect. The low, middle, and high levels of each variable (equally spaced) are designated -1, 0, and +1, respectively. Once the mathematical model is verified, it can predict the response at any point in the field conditions.

The use of statistical experimental design in the optimization of biotreatment processes has been well documented [134, 175-182]. However, few studies were reported to optimize influencing factors and their interactions to improve phenols removal efficiency [176]. Kusic et al. [20] applied the Box-Behnken experimental design inherent in the RSM to investigate the feasibility of a UV/ferrioxalate system for the degradation of 4 CP as a model wastewater pollutant. Kargi and Eker [53] used a Box-Wilson design to optimize the removal of 2, 4 DCP from synthetic wastewater in a rotating perforated tube biofilm reactor with activated sludge culture supplemented with *P. putida*. Bhattacharya and Banerjee [174] and Bhattacharya et al. [183] employed RSM for the study of enzyme mediated biodegradation of 2,4 DCP. Dey and Mukherjee [171] used RSM with a Central Composite Design for process optimization of a hybrid

reactor comprising of a trickling biofilm filter and aeration tank for removal of a mixture of phenol and *m*-cresol, in terms of pollutants concentrations and residence time.

2.11 BIODEGRADATION KINETICS AND MASS TRANSFER

Knowledge on the biodegradation kinetics of toxic compounds is necessary for improvement in process control and removal efficiency [60, 184]. Modeling any biodegradation process involves relating the specific growth rate of the biomass to the consumption rate of the substrate [11]. A variety of kinetic models have been used to describe the dynamics of microbial growth on phenols (Table 2.11.1 [49]).

Based on material balance, the rate of biomass growth and the rate of substrate utilization (both in mg/l.h) can be represented by the following Eqs. (2.11.1) and (2.11.2), respectively:

$$\frac{\mathrm{dX}}{\mathrm{dt}} = \mu \mathrm{X} - k_{\mathrm{d}} \mathrm{X} = \mu_{\mathrm{net}} \mathrm{X} \qquad \text{or} \qquad \frac{\mathrm{dlnX}}{\mathrm{dt}} = \mu_{\mathrm{net}} \tag{2.11.1}$$

$$\frac{\mathrm{d}S}{\mathrm{dt}} = -\frac{\mu \mathrm{X}}{\mathrm{Y}}, \tag{2.11.2}$$

where Y is the cell mass yield (g/g) = dX/d*S*; X is the biomass concentration (mg/l); *S* is the substrate concentration (mg/l); k_{d} is the decay coefficient (h^{-1}); μ is the specific growth rate (h^{-1}) [5, 185]. Two of the most widely used models for the biodegradation of phenols are the Monod Model (Table 2.11.1, Eq. (2.11.3)) and the Haldane Model (Table 2.11.1, Eq. (2.11.4)), where K_s (mg/l) is the half saturation coefficient (it shows the microorganism's affinity to the substrate) and K_i is the substrate inhibition constant (mg/l). These two models are used to describe the specific growth rate dependence on substrate concentration. The first considers the phenolic substance as a non-inhibitory compound and, therefore,

neglects the inhibitory effect; whereas the second takes into consideration the inhibitory effect of phenolic material [11]. However, the Haldane Model is the most widely used model that accounts for substrate inhibition because of its wide applicability and mathematical simplicity for representing the cell growth kinetics of the inhibitory substrate. It has fewer parameters and it is easily used for representing continuous biological reactors [5, 11, 185]. The Haldane equation represents a modification of the original Monod equation to account for inhibitory effects when a substrate (or substrate biotransformation intermediate) is toxic to the degrading population. The effects of this self-inhibition are incorporated into the expression with an inhibition term, S^2/K_i in the denominator. A larger K_i value indicates that the culture is less sensitive to substrate inhibition. It should be noted that when K_i is very large the Haldane equation simplifies to the Monod equation [153]. For mixed-substrate kinetics, models were tested that incorporated both substrate and intermediate inhibition (competitive and non-competitive) [155].

Sahinkaya and Dilek [60] pointed out that the primary reason for wastewater treatment is to remove organic impurities and not to cultivate biomass; therefore, the maximum removal rate should be determined rather than specific growth rate. In the literature, however, no sound kinetic models have been suggested for inhibitory effects on rate and extent of degradation over a large range of DCP concentrations [59, 60, 69, 186]. The emphasis was on conducting kinetic studies with acclimated mixed cultures, as mixed culture is responsible for substrate utilization in real full scale applications. Batch degradation experiments clearly demonstrated the inhibitory effect of 2, 4 DCP on its own degradation at high concentrations (80-109 mg/l). Kargi and Eker [59, 186] used different strains of pure *P. putida* in batch shake flasks to develop a

kinetic model describing the biodegradation of DCP for a large range of initial DCP concentrations between 30 and 300 mg/l [59] and between 50 and 750 mg/l [186].

Although the immobilization of biomass by entrapment has several advantages, its major drawback is nutrient or product diffusion limitation because of the resistance of the protective structure. Diffusion limitation is a general problem that often decreases the efficiency of degradation [72, 123, 187]. The biomass is not easily accessible to pollutants since the majority of sites will lie within the bead. So a good support material for biomass immobilization should be rigid, chemically inert, and inexpensive. It should bind the cells firmly and should have a high loading capacity and a loose structure for fewer diffusion limitations [187]. However, a study by El-Naas et al. [188] showed that PVA gel pellets of immobilized *P. putida*, prepared by iterative freezing-thawing method, have relatively good porous structure, which enhanced the biodegradation rate of phenol, as well as high mechanical strength and durability. De Queiroz et al. [189] demonstrated the feasibility of preparing mechanically more stable Alginate-PVA microspheres for use in bioreactors. Alginate is a natural polymer that is biodegradable but subject to abrasion, whereas PVA is a synthetic hydrogel with better mechanical properties and lower mass transfer resistance. The authors proposed the hybridization of both polymers to form microspheres of porous structure with lower resistance to mass transfer, for enzyme and cell immobilization. The porous structure of the carrier can decrease diffusion limitation for both; substrate phenols and their products. This issue has been of special significance in developing novel techniques for enzyme immobilization [149].

Also, knowledge of external mass transfer coefficients for the transfer of substrate from the bulk phase to the surface of the biofilm is essential in the design and modeling of bioreactors. Vinod and Reddy [190] developed a diffusion-reaction model for phenol biodegradation with *P. putida* immobilized on solid particles in a fluidized bed bioreactor, assuming that cell growth and limiting substrate utilization kinetics obtained from suspended cell culture can be applied equally well to the immobilized microorganism. A dimensionless correlation was developed for the mass transfer coefficient in terms of Sherwood, Reynolds and Schmidt numbers. On the other hand, when a microorganism is attached to a porous carrier matrix the internal mass-transfer limitations considerably affect the intrinsic kinetics. Banerjee et al. [191] proposed a mathematical model that accounted for internal and external mass transfer limitations. El-Naas et al. [71] also proved that the mass transfer and therefore the accessibility of the biomass to phenol was enhanced by decreasing the PVA particle size, as biodegradation of phenol was carried out in a spouted bed bioreactor that is characterized by intense mixing, and thus the external mass transfer resistance was ignored and only the intraparticle diffusion resistance was assumed [71, 136].

Table 2.11.1 Biodegradation kinetic models

Name	Equation		Example References
Monod	$\mu = \frac{\mu_{max} S}{K_s + S}$	(2.11.3)	[5, 11, 192, 193]
Haldane	$\mu = \frac{\mu_{max} S}{K_s + S + \left(\frac{S^2}{K_i}\right)}$	(2.11.4)	[5, 10, 153, 184, 185, 193]
Linearized Haldane	$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{S}{K_i \mu_{max}}$	(2.11.5)	[5, 158]
Han - Levenspiel	$\mu = \frac{\mu_{max}\left[1-\frac{S}{S_m}\right]^n}{K_s + S - \left[1-\frac{S}{S_m}\right]^m}$, m, n: constants	(2.11.6)	[192, 193]
Yano	$\mu = \frac{\mu_{max} S}{S + K_s + \left(\frac{S^2}{K_i}\right)\left[1+\left(\frac{S}{K}\right)\right]}$, K is a constant	(2.11.7)	[101, 112, 194, 195]
Edwards	$\mu = \mu_{max}\left[\exp\left(\frac{-S}{K_i}\right) - \exp\left(\frac{-S}{K_s}\right)\right]$	(2.111.8)	[101, 112, 194, 195]
Wang-Loh[a]	$-\frac{dS}{X dt} = \frac{R_{max} S}{k_s + S + f(i)}$	(2.11.9)	[196]
	$f(i) = \frac{(S_0 - S)^2}{K_p}$	(2.11.9a)	
	$X = X_0 e^{\mu t}$	(2.11.9b)	
	$\mu = \frac{\mu_{max} S_0}{K_s + S_0 + \left(\frac{{S_0}^2}{K_i}\right)}$	(2.11.9c)	
Monod: sum kinetics Binary mixture, no interaction	$\mu = \frac{\mu_{max,1} S_1}{K_{s,1} + S_1} + \frac{\mu_{max,2} S_2}{K_{s,2} + S_2}$	(2.11.10)	[155]

[a] R_{max} is the maximum specific consumption rate of substrate (mg/mg.h), K_s is the saturation constant for substrate consumption (mg/l).
X_o and S_o are initial biomass and substrate concentration, respectively (mg/l).
f(i) represents the functional relationship of the effect of metabolic intermediates on phenol degradation, K_p is a proportionality constant.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

3. MATERIALS AND METHODS

This chapter starts by addressing the generalities adopted throughout this study and then gives specific and detailed descriptions of the procedures and tools pertinent to the different stages of the work.

3.1 REAGENTS

Analytical grade phenol was purchased from BDH Chemicals (England). 2, 4 DCP was obtained from Sigma-Aldrich (Germany) with purity greater than 99%. It has the appearance of colorless needle-like crystals, white or pale yellow solid. 2, 4 DCP has a density of 1.38 g/cm^3, a boiling point of 210 °C, a melting point of 42 °C and a water solubility of 4.5 g/l at 20 °C. The synthetic phenol and 2, 4 DCP solutions were prepared at the required concentrations by adding a predetermined amount of the organic substance to the nutrient solution, which had 825 mg/l of the essential mineral nutrients with concentrations shown in Table 3.1.1. The solid 2, 4 DCP was dissolved by heating to 35 °C. The prepared solutions were always kept in dark containers in closed cabinets to avoid the light oxidation of phenol or 2, 4 DCP. All other chemicals and the PVA powder were of analytical grade and were obtained from BDH Chemicals (England).

Table 3.1.1 Composition of nutrient mineral medium

Component	Concentration, mg/l
$MgSO_4.7H_2O$	300
K_2HPO_4	250
$CaCl_2.2H_2O$	150
$(NH_4)_2CO_3$	120
$FeSO_4.7H_2O$	3.5
$ZnSO_4.7H_2O$	1.3
$MnCl_2.H_2O$	0.13
$CuSO_4.5H_2O$	0.018
$CoCl_2.6H_2O$	0.015
$Na_2MnO_4.2H_2O$	0.013
Total	824.98

3.2 PREPARATION OF MICROBIAL CULTURE

A special strain of the bacterium *P. putida* was obtained in cereal form (AMNITE P300) from Cleveland Biotech, Ltd. (England). The cereal contained a consortium of 10 strains belonging predominantly to the *Pseudomonas* genera, with *P. putida* as the dominating strain. According to the supplier, the product is most effective when operating conditions are close to the optimum: pH: 7.0 ranging from 6.5-8.5; temperature: 25 °C ranging from 8-40 °C; dissolved oxygen (mg/l): 2 in a range > 1.

Amnite P300 is a granular powder (Fig. 3.2.1), which can rapidly degrade a wide variety of complex organic compounds. This product is homogeneous, safe and non-pathogenic.

Fig. 3.2.1 AMNITE P300 bacterial cereal form

Bacterial cells were extracted from the cereal, through consecutive centrifugation steps using IEC CL31R Multispeed Centrifuge, Thermo Electron Cooperation (USA) as in the procedure described by El-Naas et al. [11] . A 100 g portion of the cereal was mixed in a 1000 ml solution of 0.22% sodium hexametaphosphate, buffered with Na_2CO_3, to a pH of 8.5. The mixture was homogenized in a blender for about one hour, decanted and kept in the refrigerator at 4 °C for 24 hours. Bacterial slurry was prepared in four consecutive steps of low speed centrifugation at 6000 rpm for 15 minutes. The supernatants were collected and centrifuged again at 10,000 rpm for 20 minutes. The biomass attached to the walls of the tubes was re-suspended as slurry in distilled water and kept in the refrigerator for subsequent immobilization. This preparation yields an average of 30 ml of bacterial suspension.

3.3 IMMOBILIZATION

Polyvinyl alcohol (PVA) gel was used for immobilizing the bacteria cells. A homogenous 10 wt% PVA viscous solution was prepared by mixing 100 g of PVA powder with 900 ml of distilled water at about 70–80 °C. The 10% mixture is known to result in a good quality polymer matrix with high porosity [11, 71].

The mixture was allowed to cool to room temperature before adding 10 ml of the bacterial suspension prepared in Section 3.2 and then it was stirred well for 10–15 minutes to ensure the homogeneity of the solution. The solution was poured into special molds and kept in a freezer at −20 °C for 24 hours, then transferred to room temperature and allowed to thaw for 3-4 hours. The freezing–thawing process was repeated 5 times. This improves the cross-linking in the polymerized PVA gel structure. The frozen molds were cut into 1 cm^3 cubes, washed with distilled water to remove any uncross-linked chains and sent for acclimatization. The PVA gel particles of immobilized bacteria are shown in Fig. 3.3.1.

Fig. 3.3.1 PVA gel particles of immobilized bacteria

3.4 BIOMASS ACCLIMATIZATION

3.4.1 Method A

According to this method, a portion of 90 ml of the immobilized bacteria, prepared as in Section 3.3, was suspended in solution in a measuring cylinder, to a total volume of 300 ml (30% PVA). The solution contained 1000 mg/l of glucose as an easily biodegradable source of organic carbon in addition to 825 mg/l of other essential mineral nutrients with concentrations as shown in Table 3.1.1. The bacteria were then slowly acclimatized to substrate (phenol or 2, 4 DCP)

concentrations by increasing the substrate concentration from zero to 50, 100, 150, up to 200 mg/l over a period of 5 days. At the same time, the glucose concentration was gradually reduced from 1000 mg/l to zero (1000, 750, 500, 250, 0 mg/l). A water bath (WiseBath Model WSB-30, Korea) was used to maintain the temperature at 30 °C during the acclimatization process.

3.4.2 Method B

In this method, the acclimatization was carried out without the use of glucose. It is believed to be more stimulating for the bacteria to remove glucose from the acclimatization process, and so the bacteria were acclimatized directly to low concentrations of phenol or 2, 4 DCP, followed by a gradual increase to the targeted maximum. A portion of 90 ml of the immobilized bacteria was suspended in a measuring cylinder to a total volume of 300 ml in solution containing 25 mg/l of phenol or 2, 4 DCP as a source of organic carbon dissolved in the nutrient solution. The bacteria were then slowly acclimatized to substrate concentrations by increasing the substrate concentration from 25 to 50, 100, 150, up to 200 mg/l over a period of 5 days; the PVA particles were washed with distilled water after each step. Again, the water bath (WiseBath) was used to maintain the temperature at 30 °C during acclimatization.

3.5 ANALYTICAL TECHNIQUES

3.5.1 Spectrophotometric Analysis

Two types of spectrophotometers were used. The first is a Shimadzu UV Spectrophotometer that gives direct measurement of substance concentration based on established calibration curves using standard solutions. The second is a HACH LANGE Spectrophotometer that depends on single use-reagent kits; also based on established calibration curves of the substance concentrations.

3.5.1.1 Shimadzu UV Spectrophotometer

Phenol and 2, 4 DCP concentrations in the biomass-free samples were quantitatively determined using a Shimadzu UV Spectrophotometer, Model UV-1800 (Japan) at 270 and 243 nm, respectively. Measurements were made after filtering the samples through a 0.45 µm GHP Acrodisc filter. It is worth noting that the prepared solutions were always checked for the adsorption effect on the filter and it was found to be negligible. Residual concentration was determined against a calibration curve of standard solutions of known concentrations and a standard solution was used to recheck the accuracy of the spectrophotometer every day. This equipment was also used for qualitative analysis of treated real refinery wastewater (RRW).

3.5.1.2 HACH LANGE Spectrophotometer

Concentration of total phenols in the biomass-free samples was quantitatively determined at 510 nm using a HACH, LANGE DR 5000 spectrophotometer (Germany). Measurements were made after filtering the samples through a 0.45 µm GHP Acrodisc filter. Residual concentration was determined against a calibration curve of standard solutions of known concentrations. The kits used are HACH LANGE GMBH: LCK 346, 5-200 mg/l.

The same equipment, supplemented by a heater HACH: DRB 200 (Digital Reactor Block) was also used for measuring chemical oxygen demand (COD) of the treated RRW. Measurements were made after filtering the samples through a 0.45 µm GHP Acrodisc filter. The COD was determined against a calibration curve of standard solutions of known COD. The kits used were HACH LANGE LCK 514 100-2000 m/l O_2.

3.5.2 Gas Chromatograph

The concentration of 2, 4 DCP in the biomass-free samples was determined using Shimadzu Gas Chromatograph, Model GC 2014 (Japan) and equipped with a capillary column and electron capture detector (ECD). The injector and detector temperatures were 275 and 300 °C, respectively. The column temperature program started at 120 °C and increased at a rate of 25 °C min^{-1} to 220 °C. Measurements were made after filtering a one-µl sample through a 0.45 µm GHP Acrodisc filter, then injecting it into the GC. Residual concentration was determined against a calibration curve of standard solutions of known concentrations and a standard solution was used to recheck the accuracy of the GC after every 4 hours of continuous operation. Although UV spectrophotometers were used for preliminary experiments, the ECD GC was used for determining the concentration of DCP for most of the study due to its accuracy and reproducibility.

During the treatment of RRW, the concentrations of phenol and cresols were determined using a Shimadzu Gas Chromatograph (Model GC 2010, Japan) equipped with a capillary column and a flame ionization detector (FID). The detector temperature was 250 °C. The column temperature program started at 100 °C and increased at a rate of 20 °C min^{-1} to180 °C.

3.5.3 Total Organic Carbon (TOC)

TOC measurements were done for the treated RRW using Shimadzu Total Organic Carbon Analyzer TOC-VCSH (Japan). Measurements were done after filtering the samples through a 0.45 µm GHP Acrodisc filter.

3.5.4 Measurement of pH

A portable pH meter, Sartorius PT 10 (Germany) with an accuracy of ±0.005 and a readability of ±0.01, was used for pH measurements.

3.5.5 Residence Time Distribution (RTD)

HACH: HQ14d (Germany) was used for electric conductivity (µs/cm) measurement after injection of 5 ml of 1N NaOH at the inlet point for the SBBR. The reactor was filled with blank PVA particles with a volume of 305 ml and about 730 ml of distilled water with an air flow rate (AFR) of 3 l/min and a temperature of 30 °C. Distilled water was continuously pumped at a flow rate of 10.4 ml/min.

3.5.6 Bacterial Cell Count

The cell concentration (cells ml^{-1}) at any given cultivation time was calculated from a pre-prepared calibration curve between optical density at 680 nm and cell concentration determined using a Neubauer Hemocytometer (PZO, Poland), placed on a Nikon, Eclipse LV100 (Poland) microscope.

3.5.7 Statistical Analysis and Reproducibility

All experimental results reported in the study were based on averaging results of repeated experimental runs (duplicates or triplicates), with the standard deviation ranging from 2 to 5% of the reported average. RSM was used to optimize the parameters in addition to an ANOVA procedure.

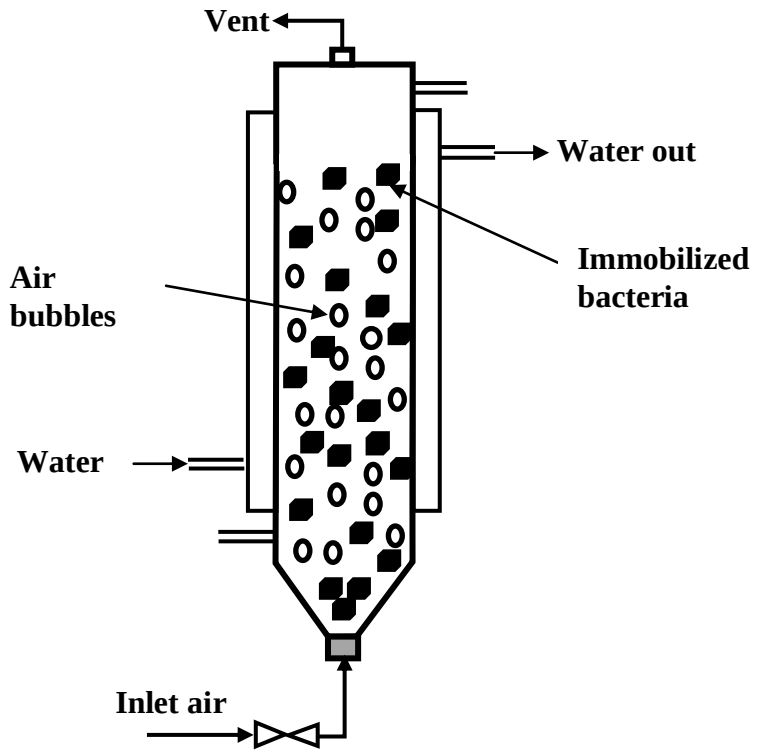
3.6 REACTORS

This study was carried out utilizing three types of reactors. In early stages, as described in Sections 3.7.1 and 3.7.2, a bubble column reactor (BCR) was used, which is a measuring cylinder immersed in a water bath (WiseBath) for temperature control. However, most of the experimental work was carried out in a

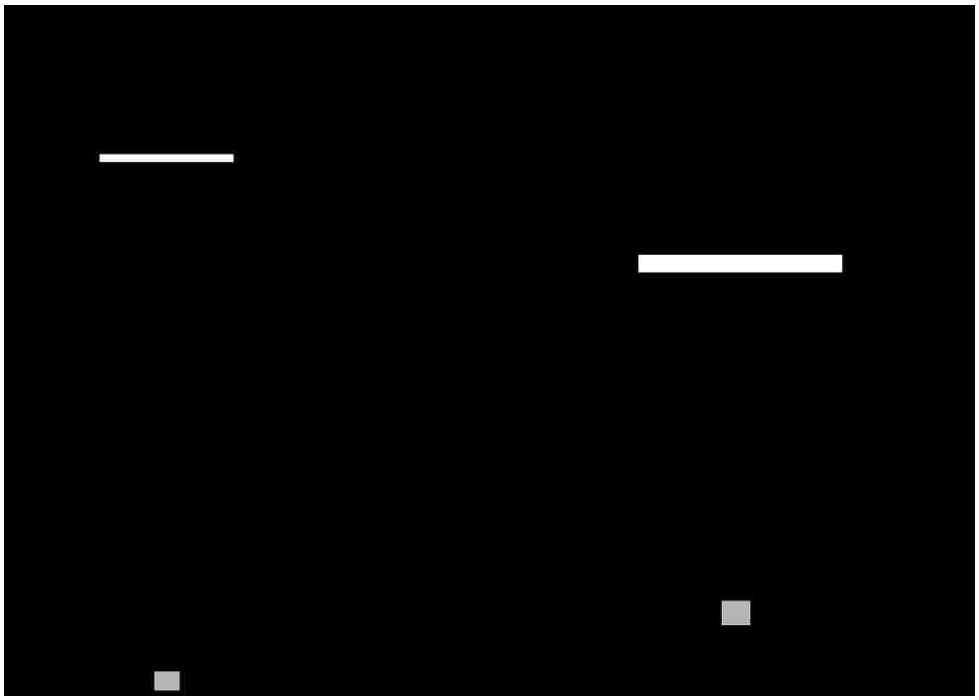
spouted bed bioreactor (SBBR). For comparative purposes, part of the work was performed in a packed bed reactor (PBR). The following are descriptions of the SBBR and the PBR.

3.6.1 Spouted Bed Bioreactor

The SBBR is characterized by a systematic intense mixing due the cyclic motion of particles within the bed, which is generated by a single air jet injected through an orifice in the bottom of the reactor [71, 136, 137, 188]. This reactor configuration is known to provide better mixing than conventional bubble column bioreactors [137]. The spouted bed bioreactor was made of Plexiglas with a total volume of 1.1 liters and is equipped with a surrounding jacket for temperature control. The temperature of the reactor system was controlled by circulating water in the reactor jacket from a water bath (Julabo, Model F34-ED/9116634) set at the desired value. Air was continuously introduced at a pre-specified flow rate into the reactor to enhance mixing and at the same time provide excess oxygen to sustain aerobic conditions. A schematic diagram of the SBBR is shown in Fig. 3.6.1 (a). Another SBBR with different geometrical ratios was used to assess the effect of reactor configuration, in terms of diameter/height ratio, on the biodegradation rate. For the sake of simplicity, the two reactors are referred to as ‘long’ and ‘short’, respectively. The dimensions of both reactors are shown in Fig. 3.6.1 (b).



(a)



Long Short

(b)

Fig. 3.6.1 A schematic diagram of the spouted bed bioreactor (a) with dimensions in cm of long and short SBBR (b)

3.6.2 Packed Bed Bioreactor

The PBR was made of Plexiglas with a total volume of 400 ml, an internal diameter of 3.6 cm and a bed height of 35.5 cm. The reactor is equipped with a surrounding jacket for temperature control. The temperature of the reactor system was controlled by circulating water in the reactor jacket from a water bath (Julabo) set at the desired value. Air was continuously introduced at a pre-specified flow rate into the reactor to enhance mixing and at the same time provide excess oxygen to sustain aerobic conditions.

3.7 EXPERIMENTAL PROCEDURE

The following is a detailed description of the experimental procedure and tools related to the different stages of the study, they are presented in the next chapter on results and discussion in the same sequence.

3.7.1 Exploratory Investigation

3.7.1.1 Acclimatization of *P. putida* to 2 Chlorophenol (2 CP)

As with Method A in Section 3.4.1 (glucose inclusion) with the substrate being 2 CP.

3.7.1.2 Biodegradation of 2 CP or 2, 4 DCP by *P. putida* or *R. pickettii*

After acclimatization, and before stating the runs, 40 ml of PVA gel cubes were soaked into the substrate solution at a required concentration for 10 minutes, then the soaking solution was decanted and the fresh solution added. This step is needed to reduce the dilution effect in the reactor due to the water contained in the PVA gel particles. All biodegradation experiments using the culture were performed in 250 ml BCR containing a total volume of 135 ml. The BCR was initially filled with a standard mineral medium, prepared according to Table 3.1.1, which contained the required concentration of the substrate and 30 vol% PVA gel

cubes with immobilized bacteria. The BCR was immersed in a water bath (WiseBath) to control the temperature. Air was continuously introduced into the reactor at a flow rate designed to induce good mixing and at the same time provide excess oxygen to sustain aerobic conditions. The initial pH of the reactor content ranged from 8.1-8.5. Samples were withdrawn at regular time intervals and analyzed for residual 2 CP or 2, 4 DCP concentrations using a HACH, LANGE DR 5000 spectrophotometer (Section 3.5.1.2).

3.7.1.3 Extraction and Isolation of Bacterial Strains from Refinery Soil Samples

Standard nutrient agar medium and a selective mineral agar medium were prepared separately using 170 ml of standard nutrient solution of a composition as given in Table 3.1.1 and a selective mineral medium solution with 5 g of powdered agar. Both agar media were spread on Petri dishes and sterilized in an autoclave (Hirayama HG-50, Japan) for 3 hours. After cooling to room temperature, samples of the crude bacterial suspension, which had been extracted from the soil according to the method in Section 3.2, were spread on the standard nutrient dishes and incubated until bacterial colonies were observed. All bacterial colonies found on the nutrient agar plates were inoculated into sterilized selective mineral plates. The dominant colonies of the selective mineral medium were then inoculated into sterilized flasks containing a selective medium and left to grow in an incubator shaker at 35 °C and 150 rpm, until considerable turbidity was observed. The isolated bacteria were then sent for identification.

3.7.2 The Role of Glucose in Biomass Acclimatization

3.7.2.1 Acclimatization to Phenol or 2, 4 DCP

Two portions of PVA-immobilized *P. putida* were acclimatized to phenol according to Method A and B in Section 3.4. Acclimatization to 2, 4 DCP was done in the same way. The portions acclimatized according to Methods A and B are referred to as Bacteria A and Bacteria B, respectively.

3.7.2.2 Biodegradation of Phenol or 2, 4 DCP by *P. putida* in BCR

The same procedure as in Section 3.7.1.2 was followed. However, the immobilized culture volume was 90 ml of PVA gel cubes. Batch experiments were performed in 500 ml measuring cylinder containing a total volume of 300 ml of standard mineral medium, which contained the required concentration of the substrate and 30 vol% PVA gel cubes with immobilized bacteria. The study was performed in a batch bubble column reactor in two phases. In the first phase, *P. putida* was acclimatized to phenol, and the study carried out with phenol as a substrate and then continued with 2, 4 DCP. In the second phase, the study was carried out with freshly prepared PVA-immobilized bacteria cells that were directly acclimatized to 2, 4 DCP.

3.7.3 Optimization of Biodegradation of 2, 4 DCP in SBBR

3.7.3.1 Acclimatization to 2, 4 DCP

Freshly prepared PVA gel particles with immobilized bacteria were prepared. The frozen molds were divided into three portions with the specified sizes of 1, 0.5 and 0.25 cm^3 cubes, each of about 350 ml in volume. The bacteria were adapted directly to 2, 4 DCP according to Method B in Section 3.4.2. Each portion of the three different sizes of the immobilized bacteria was suspended in a glass bottle to a total volume of about 1100 ml in nutrient solution containing the

predetermined concentration of DCP, for at least 24 hours, before moving to the next acclimatization step.

3.7.3.2 Batch Removal of 2, 4 DCP in SBBR

The same procedure as in Section 3.7.1.2 was followed, with a working volume (about 1 liter) in the reactor. When required, the pH was adjusted by adding a few drops of either HCl or NaOH according to the desired value. Samples were withdrawn at regular time intervals and analyzed for residual 2, 4 DCP concentrations using a Gas Chromatograph, Model GC 2014 with ECD (Section 3.5.2).

3.7.3.3 Control Experiments

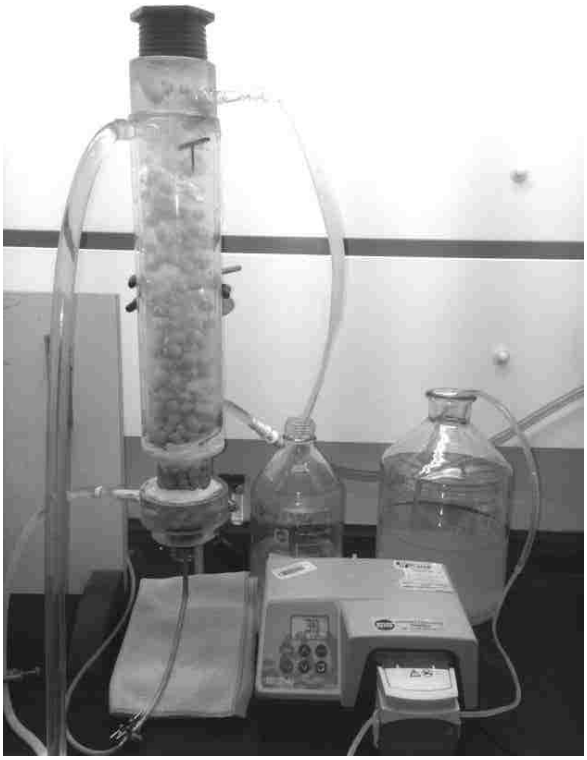
Several control experiments were carried out to evaluate the contribution of different abiotic factors to the removal of 2, 4 DCP. All experiments were performed in the SBBR, under the same operating conditions of DCP degradation. Only the effect of adsorption on the blank PVA gel cubes (bacteria-free) was tested in closed bottles in an orbital shaker bath (Lab Companion: SI-300) at 30 °C with a speed of 100 rpm.

3.7.4 Continuous Biodegradation of 2, 4 DCP

3.7.4.1 Continuous Biodegradation in SBBR

The portion of immobilized *P. putida* in PVA gel particles of size 1 cm^3 that were used for the optimization study in Section 3.7.3 was utilized again for the experiments on continuous biodegradation in the SBBR, in a ratio of 30 vol% of the working volume. A peristaltic pump (Watson Marlow, Model 323 Bredel Pumps, England) was used. All experiments were carried out at a temperature of 30 °C, with inlet AFR of 3 l/min. Samples were withdrawn at regular time intervals, and analyzed for residual 2, 4 DCP concentrations using the ECD GC

(Section 3.5.2). A photograph and a schematic diagram of the system are shown in Fig. 3.7.1.



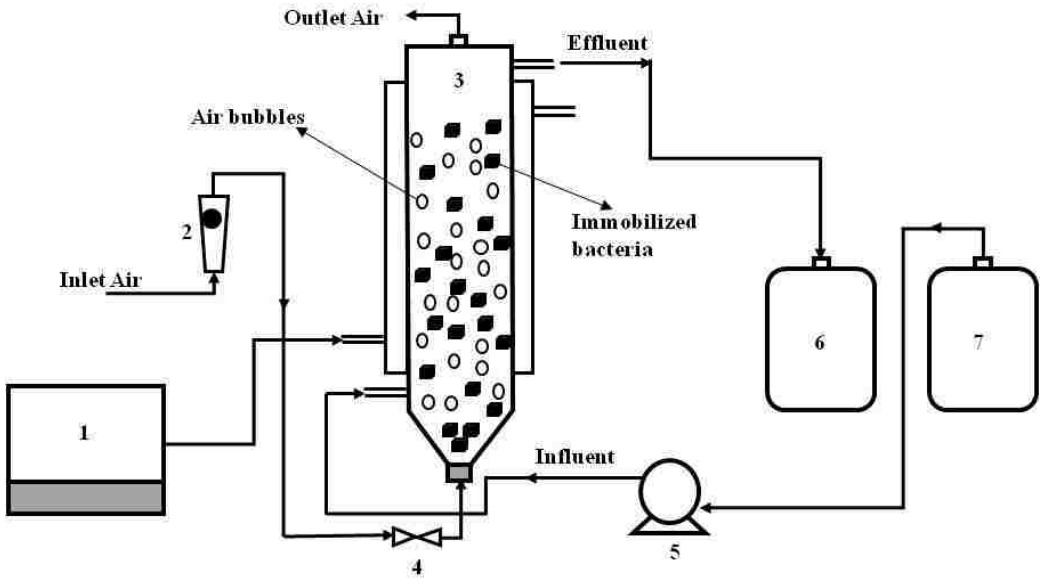


Fig. 3.7.1 A photograph and a schematic diagram of the SBBR in continuous operation: (1) refrigerated /heating circulator; (2) flowmeter; (3) SBBR; (4) valve; (5) peristaltic pump; (6) effluent; (7) feed

3.7.4.2 Continuous Biodegradation in Packed Bed Bioreactor

The packed bed reactor was packed with the same PVA particles of immobilized *P. putida*, which were used in the previous section. The same procedure as in Section 3.7.4.1 was followed. A photograph of the system is shown in Fig. 3.7.2.

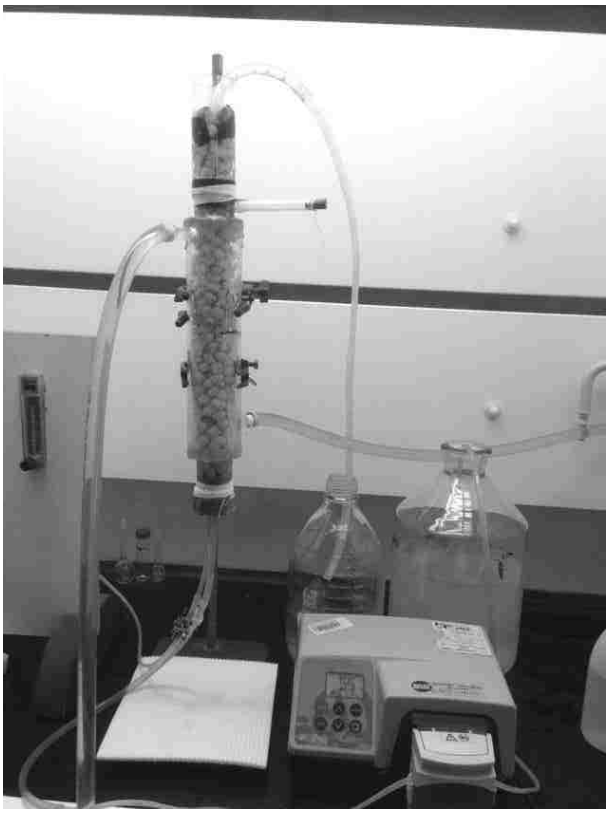


Fig. 3.7.2 A photograph of the PBR in continuous operation

3.7.5 Batch Biodegradation of 2, 4 DCP by Free Bacteria

To compare the kinetics of 2, 4 DCP biodegradation with free and immobilized *P. putida*, fresh bacteria were extracted from 20 g of the commercial cereal to prepare about 8.5 ml of bacterial suspension, as explained in Section 3.2. Half of this quantity was added to a 1 liter measuring cylinder containing 750 ml of nutrient solution with 25 mg/l 2, 4 DCP. This quantity of bacterial suspension

is approximately equivalent to the amount of immobilized bacteria in 300 ml of PVA gel cubes, which is the standard PVA volume used in the 1.1 liter SBBR. The acclimatization of the free bacteria continued according to Method B in Section 3.4.2, keeping the solution and free bacteria in the measuring cylinder and compensating for the nutrient and DCP by adding a concentrated nutrient solution and a concentrated DCP once it is depleted to the required concentration for the acclimatization step.

At the end of the acclimatization, the same solution with bacterial suspension was transferred to the SBBR to evaluate 2, 4 DCP biodegradation by the free suspended bacteria, with a need to replenish the nutrients and DCP to the desired concentrations by adding concentrated solutions to a total of 750 ml of solution in the measuring cylinder.

3.7.6 Batch Treatment of RRW in the SBBR

Real refinery wastewater (RRW) samples were obtained from a local petroleum refinery and were preserved in dark, plastic containers.

3.7.6.1 Acclimatization of Immobilized Bacteria to RRW

The immobilized *P. putida* were acclimatized in the SBBR to the RRW according to Method B (no glucose inclusion). The RRW was diluted to 10% of its initial concentration. The temperature in the reactor was controlled at 30 °C and the inlet AFR was kept constant at 3 l/min. The acclimatization continued in a stepwise manner with a gradual increase in the ratio of RRW from 10% to 20%, 30% and 40% over a period of one weak.

3.7.6.2 Degradation of Phenolics in RRW in the SBBR

The PVA gel particles with acclimatized bacteria were added in the SBBR to a medium composed of: 680 ml of RRW and 48 ml of concentrated nutrient

solution to give the standard nutrients concentration of 825 mg/l. The procedure followed was according to the normal batch treatment in the SBBR (Section 3.7.3.2), at 30 °C, with an inlet AFR of 3 l/min and without any pH adjustment. Samples were withdrawn at regular time intervals, and analyzed for residual 2, 4 DCP concentrations using the Gas Chromatograph, Model GC 2014 with ECD (Section 3.5.2). They were also analyzed for phenol and cresols using the Gas Chromatograph, Model GC 2010 with FID. Moreover, the samples were analyzed for total phenols by the spectrophotometer using the reagent kits HACH LANGE GMBH: LCK 346 5-200 mg/l, as well as COD using reagent kits LCK 514 100-2000 m/l O_2 (Section 3.5.1.2) and TOC.

CHAPTER FOUR

RESULTS AND DISCUSSION

CHAPTER FOUR

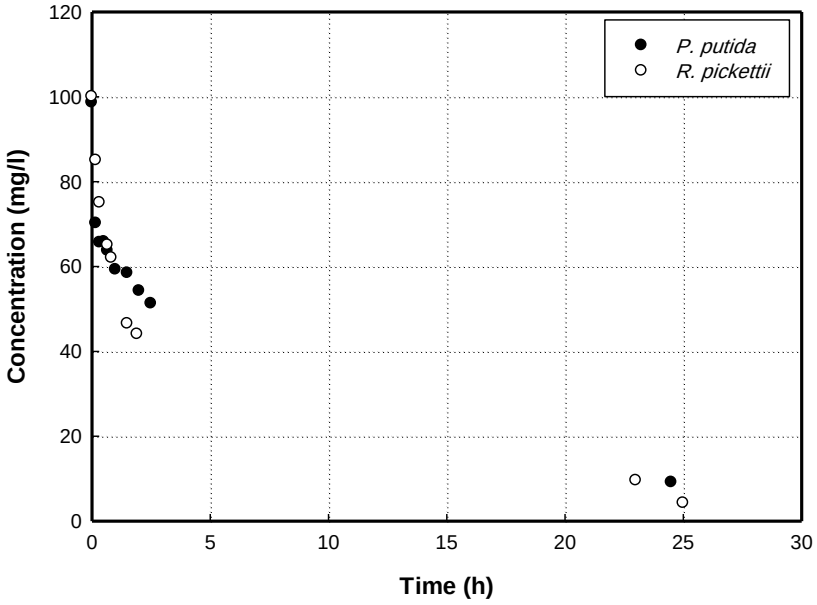
4. RESULTS AND DISCUSSION

4.1 EXPLORATORY INVESTIGATION

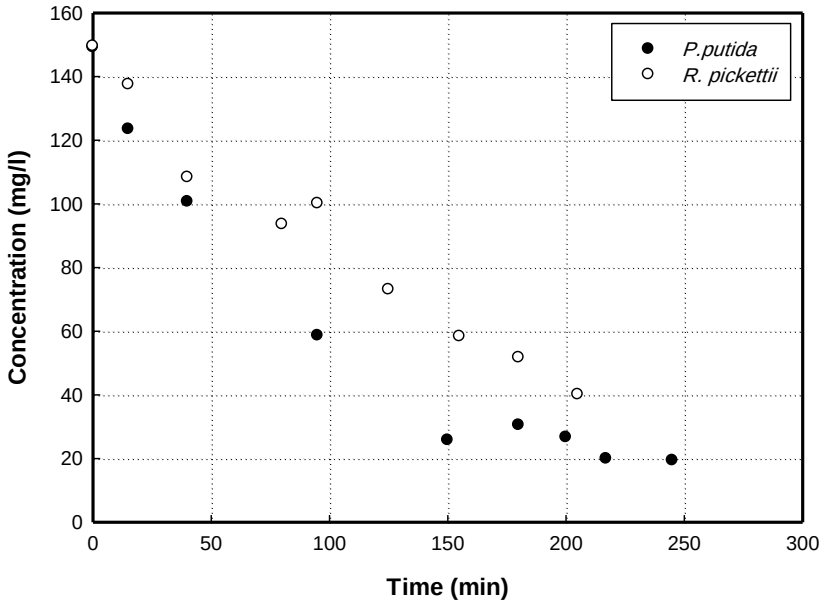
This part of the thesis presents results on the preliminary experimentation with an indigenous bacterium isolated from oil-sludge samples from a local refinery in the United Arab Emirates, which has been identified by the Netherlands Culture Collection of Bacteria (NCCB) to consist mainly of *Ralstonia pickettii* [75]. This strain was found to be capable of utilizing phenol as the sole carbon source and was immobilized and acclimatized to phenol [75]. In the present exploratory investigation, the effectiveness of *R. pickettii* for the removal of 2 CP and 2, 4 DCP was evaluated in a bubble column reactor (BCR) and compared to that of a commercially available consortium, in which *P. putida* is predominant.

4.1.1 Biodegradation of 2 Chlorophenol

A proof-of-concept was performed to test the efficiency of *R. pickettii*, which was previously acclimatized to phenol and the commercial consortium that was acclimatized to 2 CP; both immobilized in PVA gel matrix for biodegradation of 2 CP; with an initial 2 CP concentration (C_o) of 100 and 150 mg/l, at a temperature (T) of 30 °C. The results are shown in Fig. 4.1.1. More than 90% removal was achieved within about 24 hours. The biodegradation ability of the indigenous bacteria, *R. pickettii*, isolated from refinery sludge, was comparable to that of the commercially available consortium.



(a)



(b)

Fig. 4.1.1 Exploratory investigation: variation of 2 CP concentration with time in BCR: C_o = 100 mg/l (a) and 150 mg/l (b); T = 30 °C

4.1.2 Biodegradation of 2, 4 DCP

The degrading capability of *R. pickettii* for 2, 4 DCP was tested and compared to that of the commerial consortium at an initial DCP concentration of 25 mg/l and a temperature of 30 °C, as shown in Fig. 4.1.2. Experiments were then performed with an initial DCP concentration of 50 mg/l at room temperature (22 °C), 30 and 33-35 °C, as shown in Figs. 4.1.3, 4.1.4 and 4.1.5, respectively. The plots are dimensionless DCP concentrations (C/C_o) as a function of time. The performance of *R. pickettii* was comparable to that of *P. putida*, or even better. The degradation efficiency was higher at C_o of 50 mg/l and improved with increasing temperature. In spite of the fact that 2, 4 DCP is not as easily biodegradable as 2 CP, the preliminary results suggest that *R. pickettii* is a promising biomass that can be extracted from the soil of local refineries in the UAE.

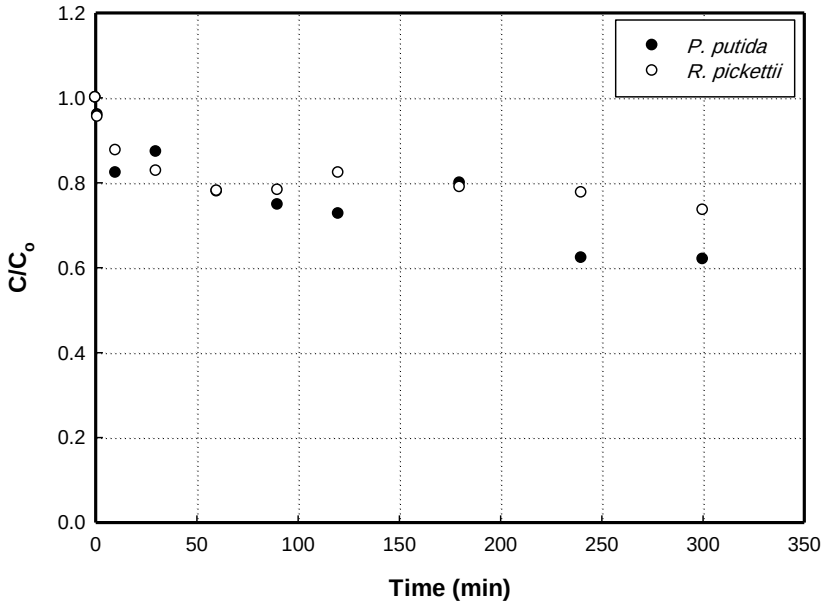


Fig. 4.1.2 Exploratory investigation: dimensionless 2, 4 DCP concentration versus time in BCR: C_o = 25 mg/l; T = 30 °C

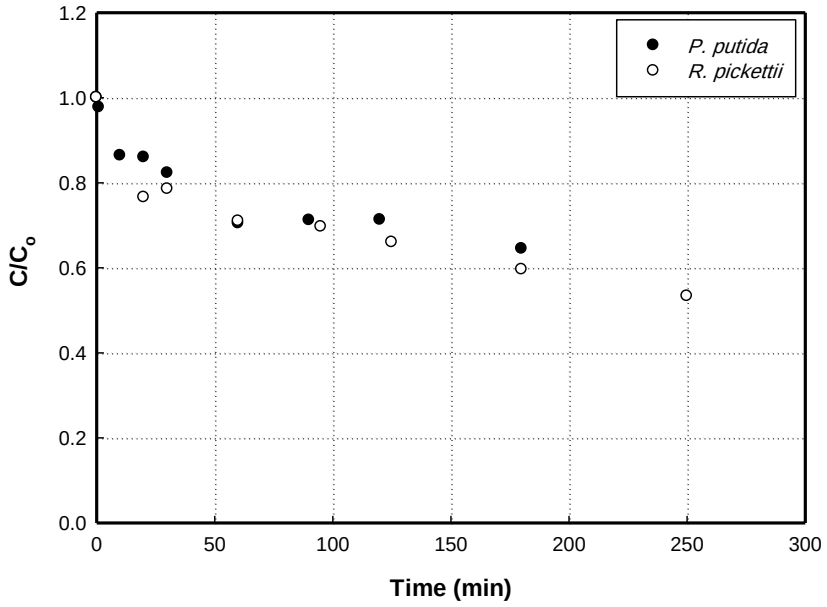


Fig. 4.1.3 Exploratory investigation: dimensionless 2, 4 DCP concentration versus time in BCR: C_o = 50 mg/l; T = 22 °C

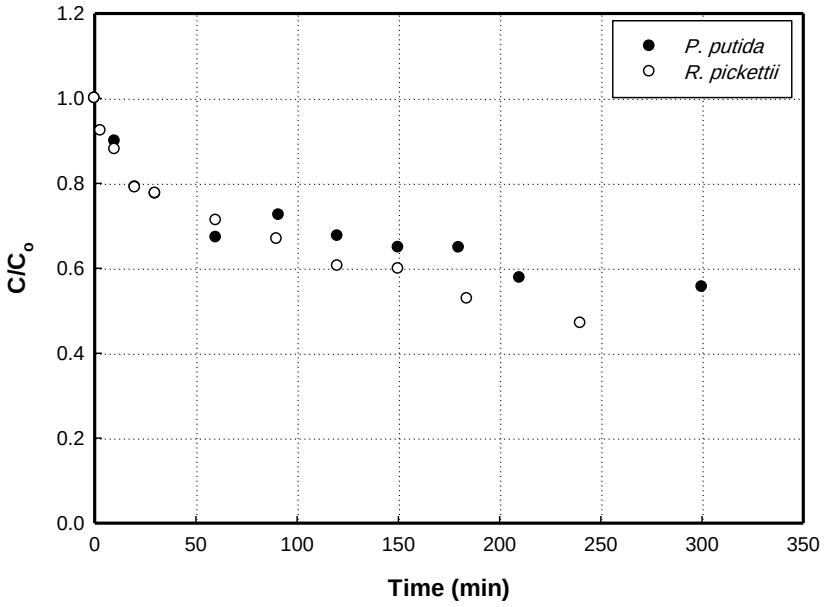


Fig. 4.1.4 Exploratory investigation: dimensionless 2, 4 DCP concentration versus time in BCR: C_o = 50 mg/l; T = 30 °C

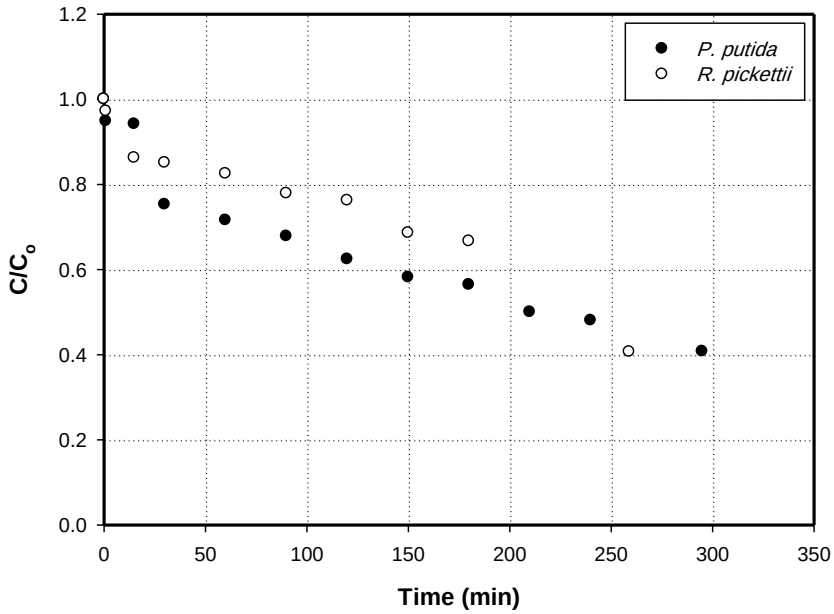


Fig. 4.1.5 Exploratory investigation: dimensionless 2, 4 CP concentration versus time in BCR: C_o = 50 mg/l; T = 33-35 °C

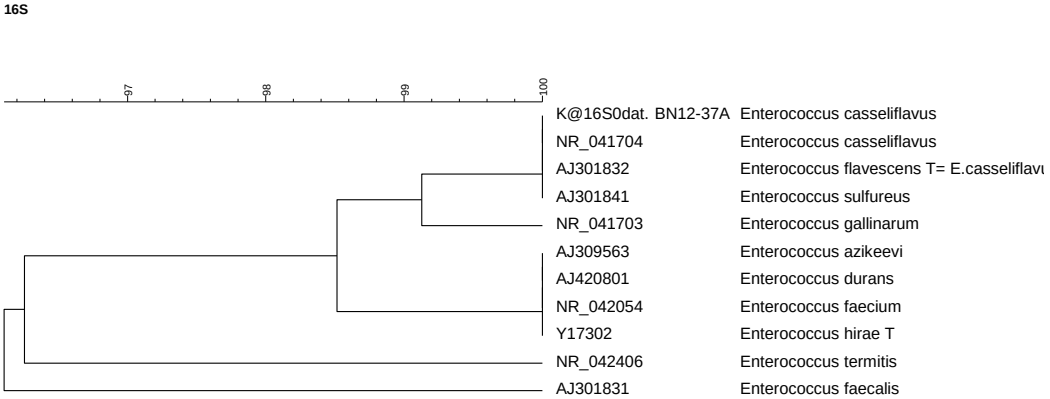
4.1.3 Extraction of Bacterial Strains from Soil Samples

An attempt was made to isolate *R. pickettii* from soil samples obtained from a local refinery, by consecutive growth and enrichment on selective media of phenol (S1), 2 CP (S2) and 2, 4 DCP (S3). A sample from the enriched bacterial strain of each selective media was then sent to NCCB for identification. The strains identified, as reported by the NCCB, are listed in Table 4.1.1. The phylogenetic analysis is described by Fig. 4.1.7. It is apparent that the strain *Pseudomonas stutzeri* was detected in each of the three samples, S1, S2 and S3, but the strain *R. pickettii* was not identified in any of them. It is worth noting here that soil samples from different locations and in different time intervals may differ greatly in their bacterial content. At the early stages of the exploratory investigation, the point behind extracting bacterial strains from local soil samples was the intention of carrying out the study with a potent indigenous microorganism, such as *R. pichettii* or *P. putida*. However, rather sophisticated logistics were associated with

identification due to having to send the samples abroad, which made it rather difficult to isolate and identify samples. For this reason, the study focused on the commercial biomass *P. putida*. This area of research into potent indigenous bacterial strains remains a research topic of high priority.

Table 4.1.1 Isolated bacterial strains by the NCCB, grown on different selective media

Selective medium	Isolated bacterial strains
S1 (phenol)	BN12-35A *Pseudomonas stutzeri* BN12-35B *Stenotrophomonas* species
S2 (2 CP)	BN12-36A1 *Stenotrophomonas acidaminiphila* BN12-36A2 *Pseudomonas stutzeri*
S3 (2, 4 DCP)	BN12-37A *Enterococcus casseliflavus* BN12-37B *Stenotrophomonas acidaminiphila* BN12-37C1 *Pseudomonas stutzeri*



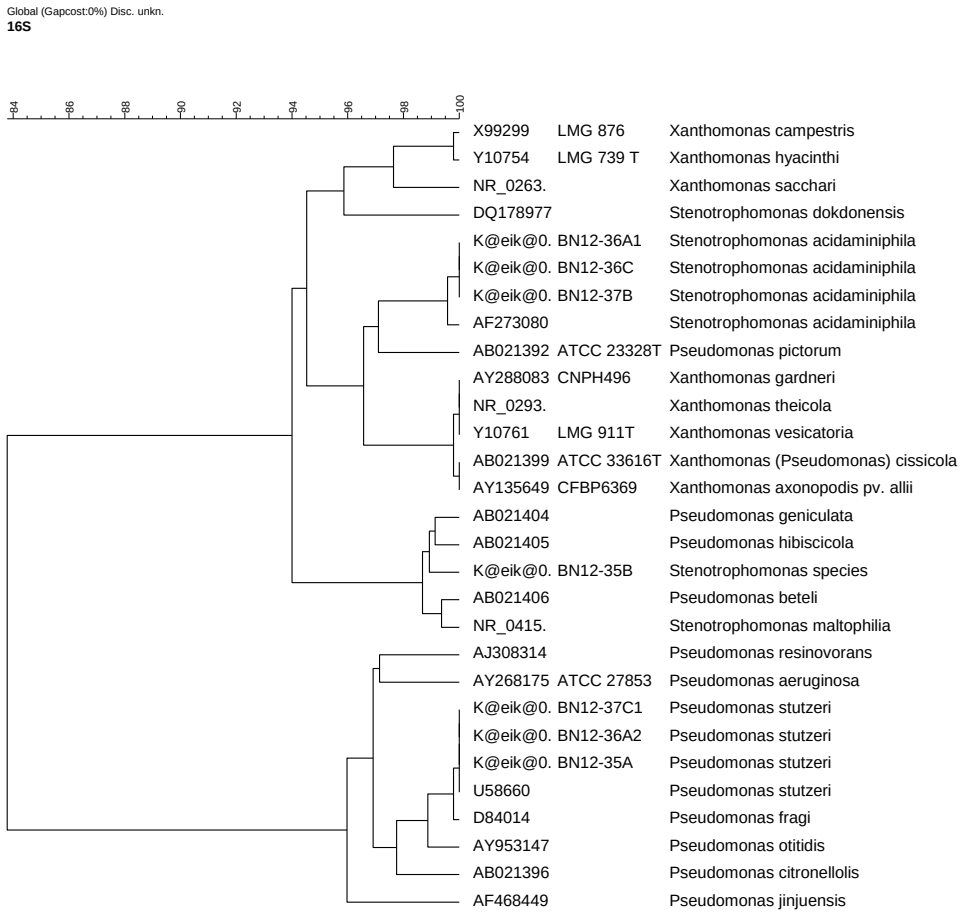


Fig. 4.1.6 Neighbor-joining phylogenetic tree of the bacterial strains grown on S1 (phenol), S2 (2 CP) and S3 (2, 4 DCP) (NCCB The Netherlands Culture Collection of Bacteria)

4.2 THE ROLE OF GLUCOSE IN BIOMASS ACCLIMATIZATION

During biological treatment, the bacteria must first be acclimatized to the phenolic compound, which is known to be toxic to microorganisms. It has been reported that phenol is inhibitory to bacterial growth at concentrations above 0.05 g/l [71, 131]. Therefore, in order to obtain efficient biodegradation, microbial acclimatization (adaptation) to phenol or its derivatives is necessary [11, 49, 71, 131, 140]. The effect of lag times at the beginning of the process is especially recognized when the biomass cells are not pre-adapted to the contaminant medium. The lag time increases with increased initial concentrations of a phenolic compound [49]. This process has often been carried out by utilizing glucose as an easily biodegradable source of carbon [5, 109, 131, 158, 177, 192, 193, 197-199]. Based on the same concept, Contreras et al. [200] used cheese whey instead of glucose; whereas Tziotzios et al. [201] used sodium acetate as an initial carbon source. In studying the biodegradation of a mixture of chlorophenols, Sahinkaya and Dilek [19, 107] replaced glucose with peptone in the acclimation process. In other previous work [11, 71, 136, 188], the bacterial cells were activated by gradually increasing the concentration of glucose over a period of a few days. The activation of the bacteria was confirmed through microscopic analysis and an observed reduction in the glucose concentration [11]. Once activated, the bacterial cells were then slowly acclimatized to phenol by a gradual increase in the phenol concentration accompanied with a gradual reduction in the glucose concentration. One may suggest that it is more stimulating for the bacteria to remove glucose from the acclimatization process and start directly with low concentrations of phenol, or its derivative, followed by a gradual increase to the targeted maximum. The aim of this part of the present study, therefore, was to assess the justification

for the addition of glucose during biomass acclimatization for the biodegradation of phenol and 2, 4 DCP by PVA-immobilized *P. putida* in a bubble column bioreactor. The study was conducted in two parts in a batch bubble column reactor. In the first part, *P. putida* was acclimatized to phenol and then biodegradation experiments were carried out with phenol followed by 2, 4 DCP as a substrate. In the second part, the study was carried out with freshly prepared and PVA-immobilized bacterial cells that were directly adapted to 2, 4 DCP. Only 2, 4 DCP was used as a substrate in this part. All experiments were performed in a bubble column reactor (BCR).

4.2.1 Acclimatization to Phenol

4.2.1.1 Biodegradation of phenol

The experimental results for the batch biodegradation of phenol at different initial concentrations are shown in Figs. 4.2.1, 4.2.2 and 4.2.3, for three different sets of initial concentrations. It is clear that degradation by the bacteria acclimatized without glucose (Method B) was faster for every concentration. It is noticeable that the reduction in phenol concentration is practically linear in relation to time, and therefore, the biodegradation rates were calculated from the slopes of the best fitted straight line for concentration versus time. Also, the plots indicate that at high initial concentrations the rate of phenol removal keeps constant for a longer time compared to that for low initial phenol concentrations. The same trend was noticed by El-Naas et al. [71] and was attributed to mass transfer limitations at low phenol concentrations, with the bacteria within the PVA particles having limited access to phenol. It has been reported that the biodegradation rate can change from first-order toward zero-order over time, with the contaminants at first being rapidly consumed but slowing as intraparticle

diffusion began to limit the replenishment of contaminant concentrations in the bulk aqueous phase. Then, at low substrate concentrations, namely if the substrate concentration S is much less than the half velocity constant ($S << K_s$), first order kinetics prevail, or if $S >> K_s$, the degradation follows zero-order kinetics [202]. It is believed that this specifically applies for reactions with a lesser inhibition effect. The biodegradation rate increases when increasing the initial concentration of phenol, reaching a maximum of 40 mg/l.h at 75-100 mg/l for the glucose-acclimatized bacteria (Method A) and 47 mg/l.h at 100 mg/l for the no-glucose-acclimatized bacteria (Method B), as shown in Fig. 4.2.4. The maximum rate for the group B is comparable to the result of previous work by El-Naas et al. [11], which was obtained by glucose-acclimatized bacteria at 75 mg/l. The degradation rate changes slightly in the range of 100-150 mg/l for both bacteria and then continues to increase. It can be observed that substrate inhibition was not encountered in the range of substrate concentrations studied, mainly due to the immobilization of the bacteria within the PVA matrix that shielded them from direct contact with the high phenol concentration. As depicted in Fig. 4.2.4, this is more noticeable in the case of the no-glucose acclimatized bacteria, which emphasizes its better performance.

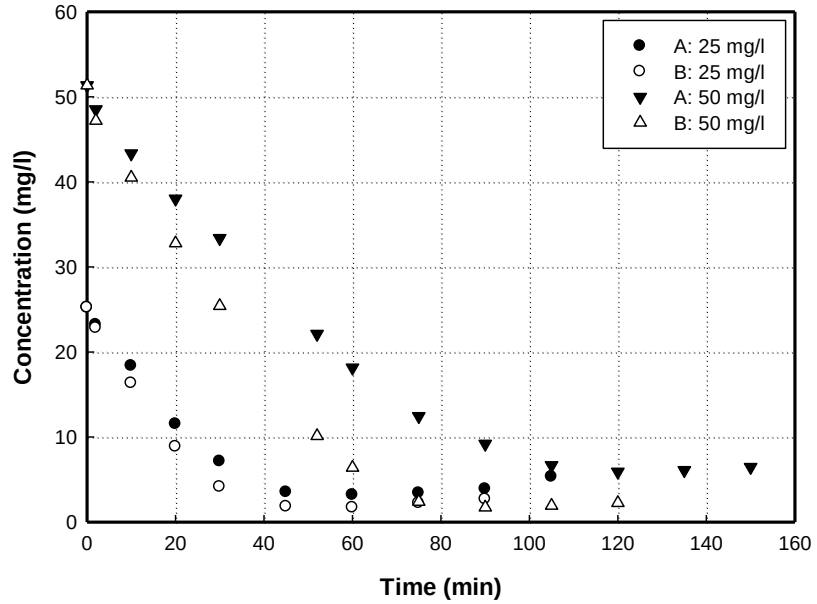


Fig. 4.2.1 Effect of acclimatization method to phenol: variation of phenol concentration with time, C_o = 25, 50 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

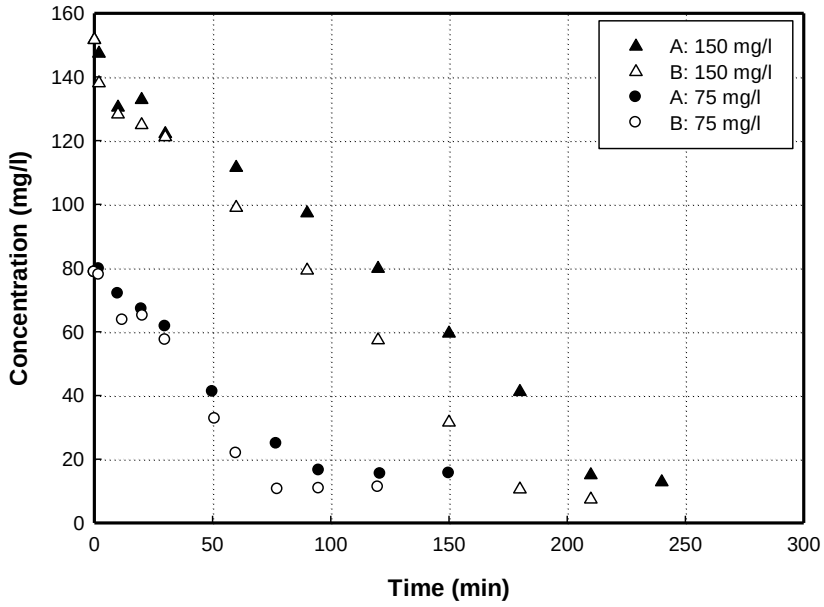


Fig. 4.2.2 Effect of acclimatization method to phenol: variation of phenol concentration with time, C_o = 75, 150 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

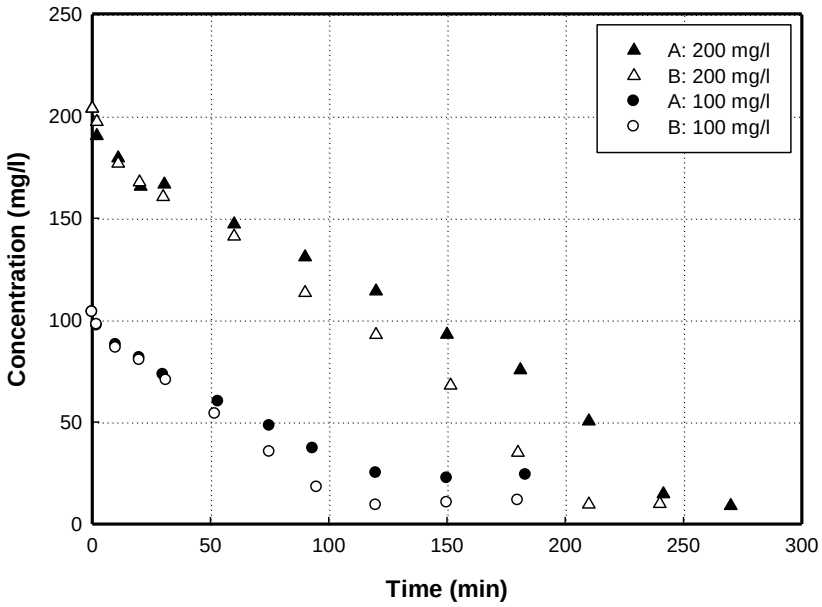


Fig. 4.2.3 Effect of acclimatization method to phenol: variation of phenol concentration with time, C_o = 100, 200 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

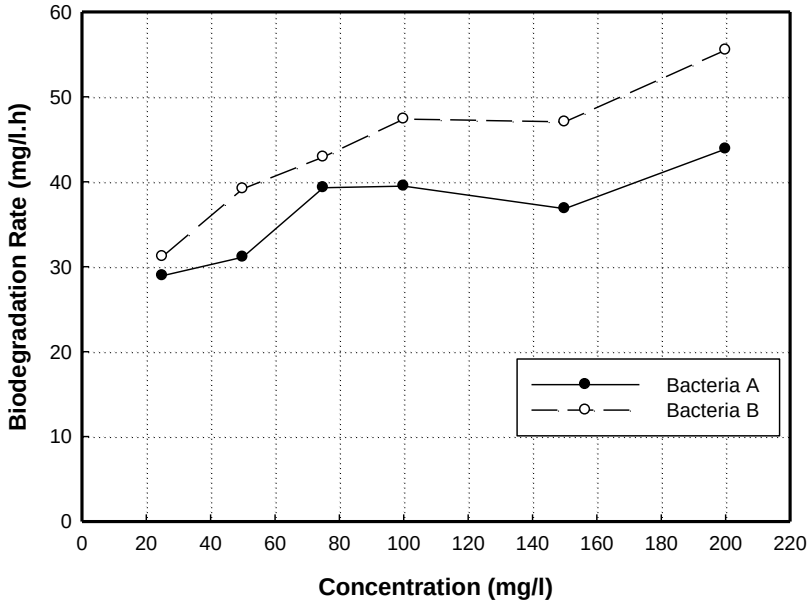


Fig. 4.2.4 Effect of acclimatization method to phenol: biodegradation rate at different initial phenol concentrations: A refers to Method A (with glucose), B refers to Method B (without glucose)

4.2.1.2 Biodegradation of 2, 4 DCP

Experimentation was continued with the same bacteria as were used for phenol biodegradation, for initial 2, 4 DCP concentrations of 25 to 100 mg/l. For comparison purposes, the results of the four concentrations are shown in Fig. 4.2.5. It is noticeable that the reduction in 2, 4 DCP concentration is much lower than that of phenol, mainly because 2, 4 DCP is much less biodegradable and the bacteria have not been acclimatized to this substrate. As depicted in Fig. 4.2.5, the performance of the portions A and B are almost identical. The biodegradation rate increases when increasing the initial concentration of 2, 4 DCP as shown in Fig. 4.2.6.

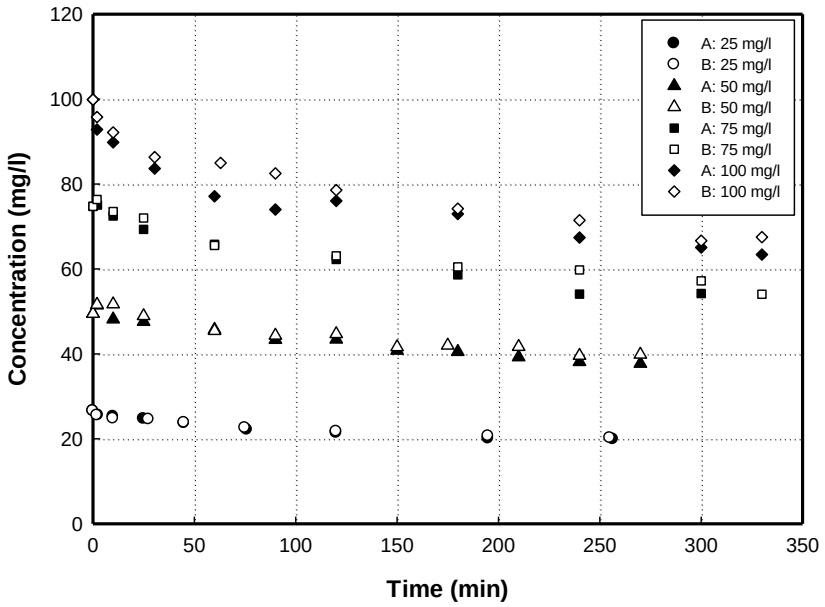


Fig. 4.2.5 Effect of acclimatization method to phenol: variation of 2, 4 DCP concentration with time, C_o = 25-100 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

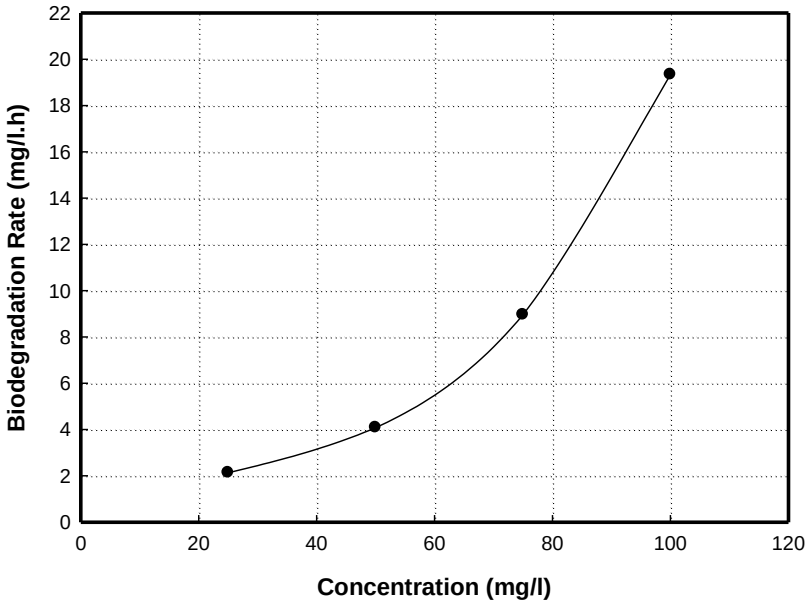


Fig. 4.2.6 Effect of acclimatization method to phenol: biodegradation rate at different initial DCP concentrations by either bacteria A or B: A refers to Method A (with glucose), B refers to Method B (without glucose)

4.2.2 Acclimatization to 2, 4 DCP

Freshly prepared immobilized bacteria, directly acclimatized to 2, 4 DCP up to 200 mg/l as explained in Section 3.4, were used for the biodegradation of 2, 4 DCP with initial concentrations of 25, 50, 75 and 100 mg/l. The acclimatization of the two portions (the one with glucose A; and the no-glucose B) followed the same procedure as for phenol in Section 4.2.1 but starting with a 2, 4 DCP concentration of 25 mg/l for the two portions A and B, followed by a gradual increase of 2, 4 DCP concentration up to 200 mg/l (25, 50, 100, 150, 200 mg/l). During the acclimatization process, the concentration was monitored along with time and the results for initial concentration of 25, 50, 150 and 200 mg/l are shown in Figs. 4.2.7 and 4.2.8. Although the performance of the glucose-acclimatized bacteria (Method A) is slightly better at low initial concentrations, they are quite close at the high concentrations. This is represented by the

biodegradation rate shown in Fig. 4.2.9. The lines in the figure are connections between the experimental data and are shown to highlight the trend. This behaviour clearly shows the substrate inhibition effect, which is best described by the Haldane Model (Eq. (2.11.4) in Table 2.11.1).

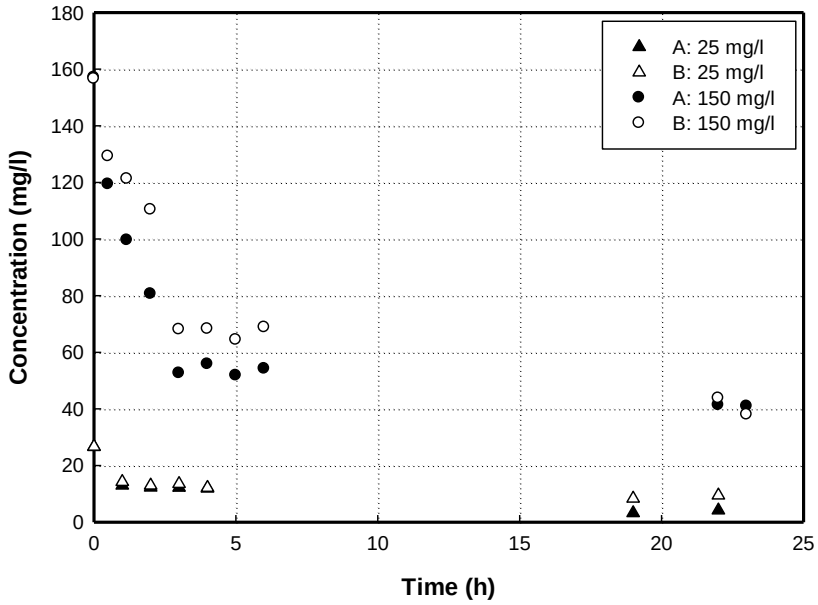


Fig. 4.2.7 Effect of acclimatization method to 2, 4 DCP: variation of 2, 4 DCP concentration with time during acclimatization, C_o = 25, 150 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

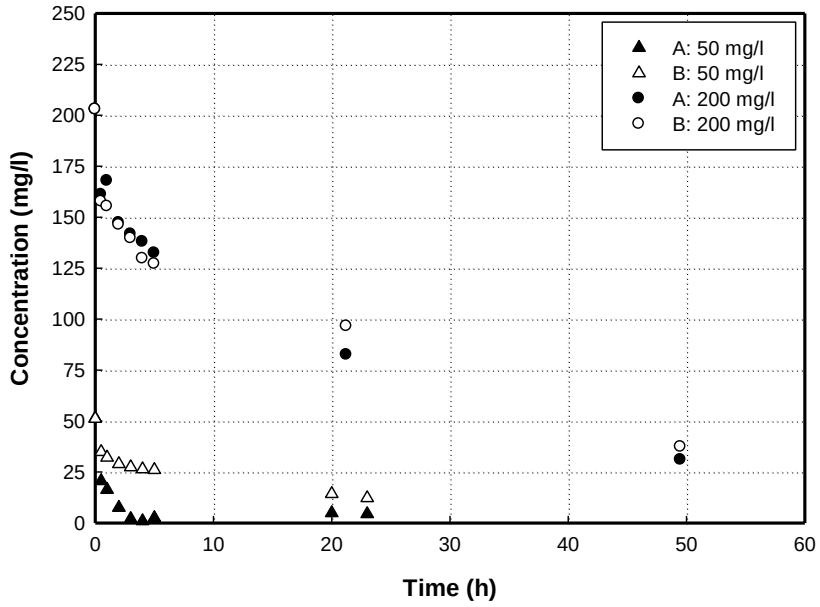


Fig. 4.2.8 Effect of acclimatization method to 2, 4 DCP: variation of 2, 4 DCP concentration with time during acclimatization, C_o = 50, 200 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

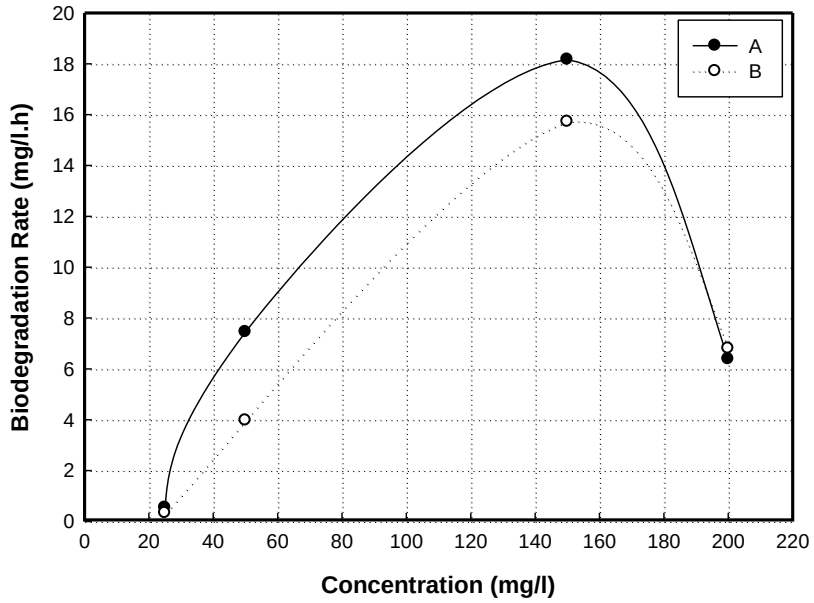


Fig. 4.2.9 Effect of acclimatization method to 2, 4 DCP: biodegradation rate during acclimatization at different 2, 4 DCP initial concentrations: A refers to Method A (with glucose), B refers to Method B (without glucose)

4.2.2.1 Biodegradation of 2, 4 DCP

The results of 2, 4 DCP biodegradation with initial concentration of 25, 50, 75 and 100 mg/l are shown in Figs. 4.2.10 and 4.2.11. It is noticeable that the degradation rate for the no-glucose bacterial portion (Method B) is slightly lower than the rate for the glucose-acclimatized portion (Method A). This is opposite to that observed in Section 4.2.1 for phenol biodegradation. This can be justified by the fact that 2, 4 DCP is not as easily biodegradable as phenol, and the addition of glucose in the acclimatization process may enhance the performance of the bacteria. The biodegradation rates are depicted in Fig. 4.2.12. It is evident that the biodegradation rate reached a maximum at an initial 2, 4 DCP concentration of about 75 mg/l and that both acclimatization schemes, A and B, had almost the same biodegradation rate, with a substrate inhibition effect, which is best described by the Haldane Model (lines are connections between experimental data). From these results presented in Figs. 4.2.10 to 4.2.12, it can be concluded that although the biodegradation rate is slightly higher for the glucose-acclimatized bacteria, the difference is not significant enough to justify glucose-based acclimatization.

It is noticeable that the biodegradation rate in this part reached a maximum at an initial 2, 4 DCP concentration of about 75 mg/l, whereas it continued to increase in the first part (Section 4.2.1.2), with the phenol-acclimatized bacteria, further beyond this concentration (Fig. 4.2.6). This is believed to be due to the fact that the phenol-acclimatized bacteria had not been fully acclimatized to 2, 4 DCP. This is also confirmed by the continuously increasing rate up to an initial concentration of 150 mg/l in the acclimatization process at the beginning of this part of study (Fig. 4.2.9).

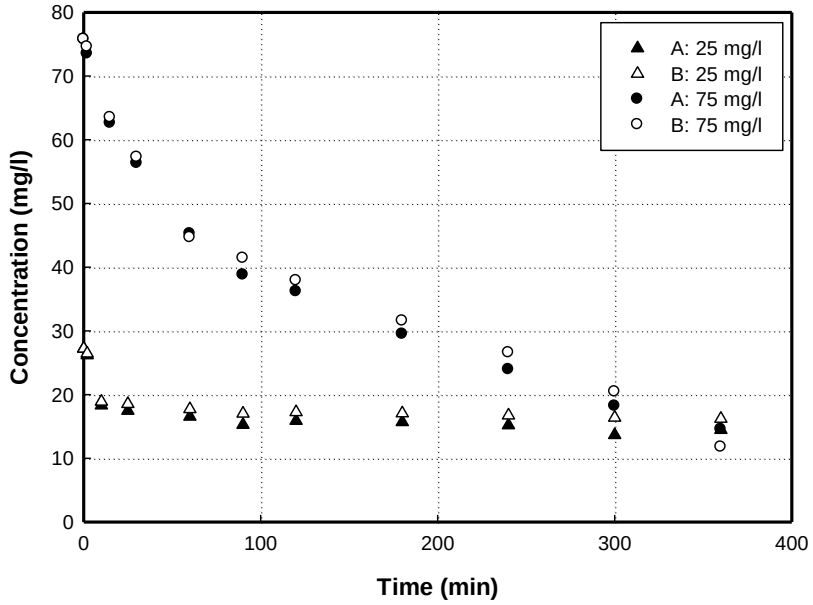


Fig. 4.2.10 Effect of acclimatization method to 2, 4 DCP: variation of 2, 4 DCP concentration with time, C_o = 25, 75 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

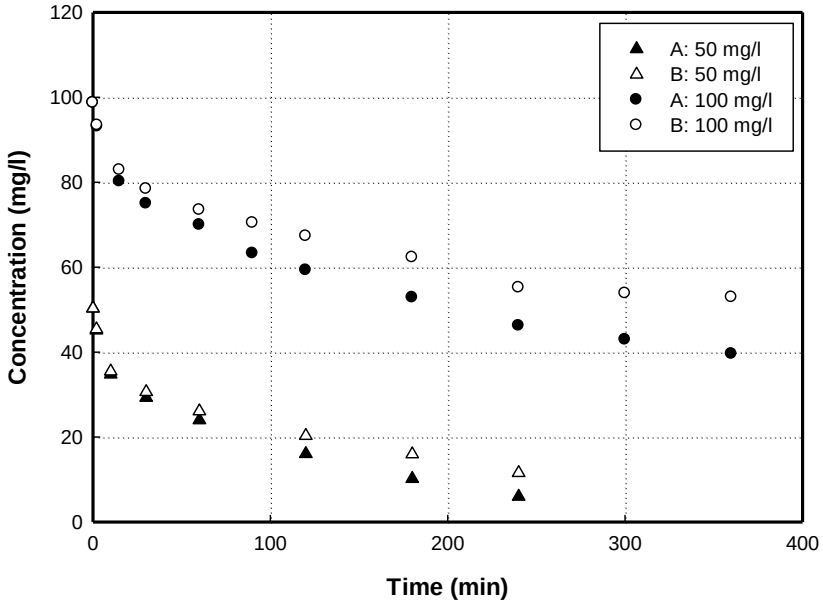


Fig. 4.2.11 Effect of acclimatization method to 2, 4 DCP: variation of 2, 4 DCP concentration with time, C_o = 50, 100 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

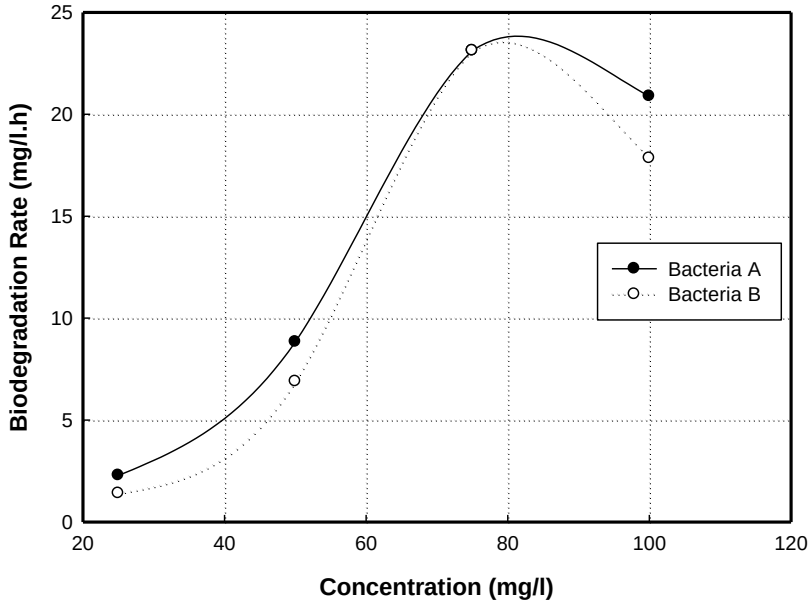


Fig. 4.2.12 Effect of acclimatization method to 2, 4 DCP: biodegradation rate at different 2, 4 DCP initial concentrations: A refers to Method A (with glucose), B refers to Method B (without glucose)

To this end, it is noteworthy that in the course of this study on the role of glucose in biomass acclimatization, a transient phenomenon was noticed involving a suppression or decrease in the degradation rate when repeating an experiment before achieving a complete removal of the organic substrate from the previous run [203]. This phenomenon is discussed in the subsequent Section 4.2.2.2.

Few runs were performed using a spouted bed bioreactor (SBBR). It was found that there is a continuous improvement in the performance of both portions of bacteria as shown by repeated runs in Figs. 4.2.13 and 4.2.14 for portions A and B, respectively; whereas the averages are represented by Fig. 4.2.15. From the averages, it is clear that the biodegradation rate for portion B has become closer to that of portion A.

To sum up, the rates achieved by both portions A and B were either very close or slightly better for the glucose-acclimatized bacteria, but not significant enough to justify the inclusion of glucose in the acclimatization process. The absence of glucose from the acclimatization process either stimulates the biodegradation capabilities of *P. putida* or has no significant effect on its performance during biodegradation.

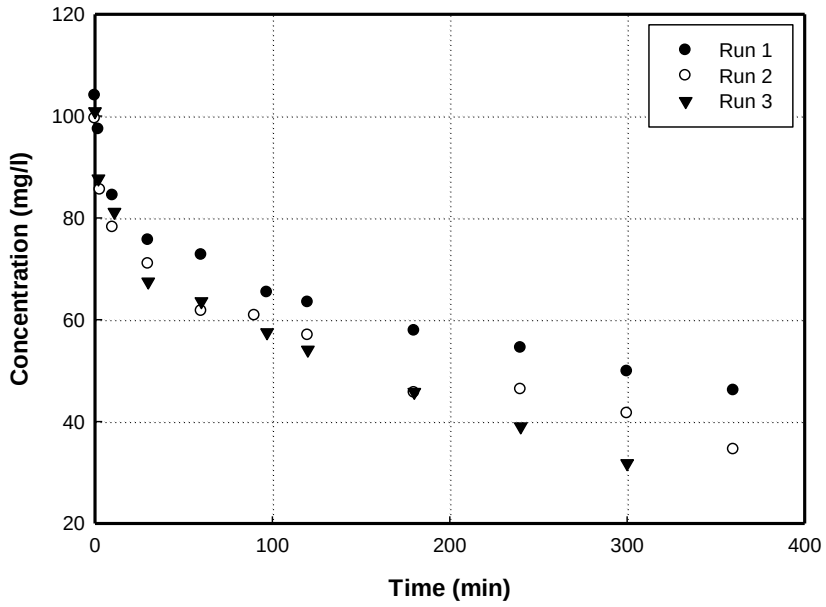


Fig. 4.2.13 Effect of acclimatization method to 2, 4 DCP: variation of 2, 4 DCP concentration with time in SBBR, $C_{o=}$ 100 mg/l: Bacteria A (with glucose)

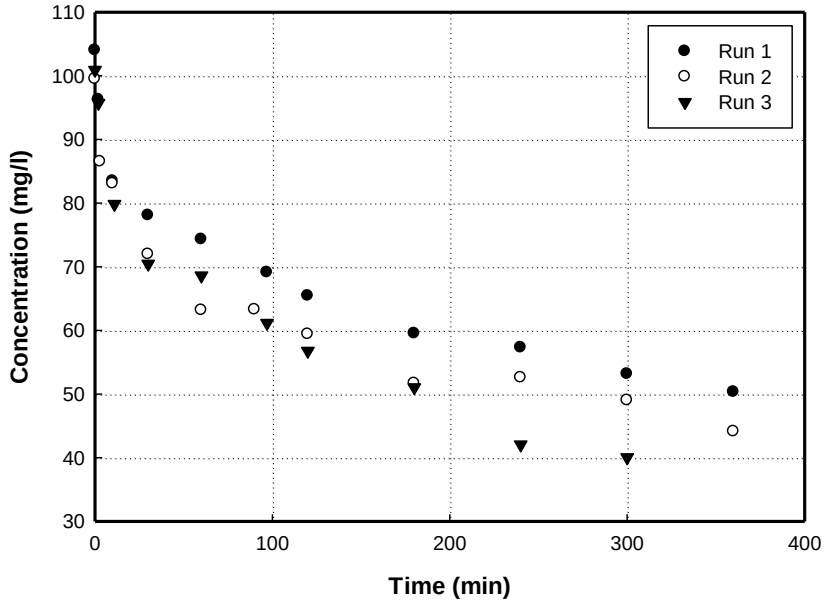


Fig. 4.2.14 Effect of acclimatization method to 2, 4 DCP: variation of 2, 4 DCP concentration with time in SBBR, C_o = 100 mg/l: Bacteria B (without glucose)

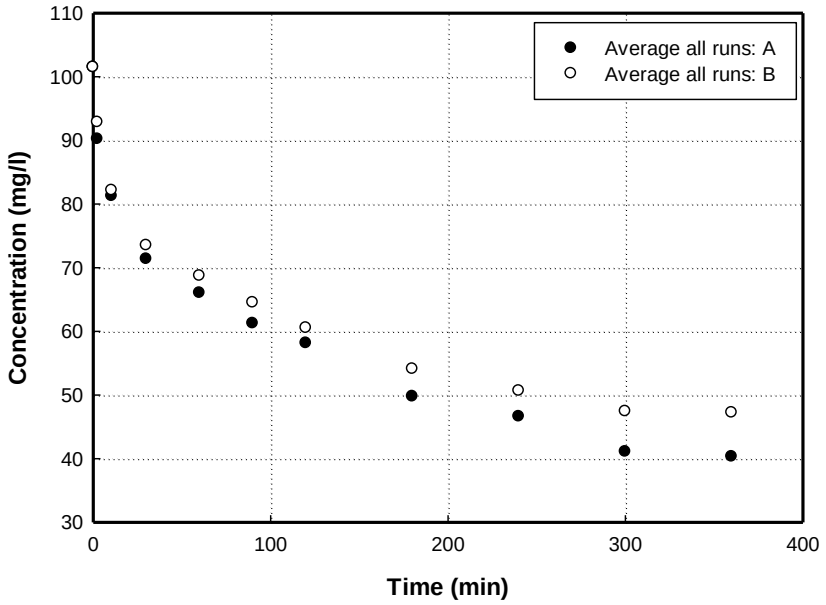


Fig. 4.2.15 Effect of acclimatization method to 2, 4 DCP: variation of 2, 4 DCP concentration with time in SBBR, C_o = 100 mg/l: A refers to Method A (with glucose), B refers to Method B (without glucose)

4.2.2.2 Transient Suppression in Biodegradation of 2, 4 DCP

The phenomenon of suppression in the degradation rate, when running an experiment before achieving a previous complete removal of substrate, is noticed was depicted by Runs 1A and 1B in Fig. 4.2.16, which were carried out directly after the last step of the acclimatization process at 200 mg/l, that ended with about 25 mg/l and did not reach a zero concentration. For a concentration of 50 mg/l, several runs were carried out as shown in Fig. 4.2.17 and 4.2.18 for bacterial portions A and B, respectively. A repeat of Run 1 (Run 1R) was performed on the same day as performing Run 1, after about half an hour of terminating the original Run 1. The same procedures applied for Run 2R. It is obvious that: 1. Repeating an experiment before achieving a complete removal of substrate slows the degradation rate, as depicted by Runs 1R and 2R, which were performed on the same day as performing Runs 1 and 2, respectively. 2. The degradation rate was continuously improving upon repeating the experiment after complete degradation, as shown by Runs 1, 2 and 3. For an initial substrate concentration of 100 mg/l, the same phenomenon of decrease in the degradation rate, when repeating an experiment before achieving a complete removal of substrate, applies also, as shown by Runs AR and BR in Fig. 4.2.19, for bacterial portions A and B, respectively. This phenomenon could be attributed to the effect of carbon starvation on cells growth and biodegradation capabilities. Shourian et al. [9] found that carbon starved cells had higher growth rates and higher biodegradation rates of phenol in comparison with non-starved cells in the exponential growth phase. They reported that the rate of phenol degradation is influenced by the energetic status or growth phase of the cells. The same findings were reported by Khleifat [204] for the degradation of phenol by *Ewingella americana*. According

to that study, starvation increased the cells ability to utilize the organic substrate and the nutrients, and decreased the lag time. Khleifat [204] explained that the quantity of the enzymes is the same but it is the time of early or late expression of catabolic genes that makes the difference. Additionally, a study by Varma and Gaikwad [205] for biodegradation of phenol by the yeast *Candida tropicalis* NCMI 3556 revealed that grown cells (already exiting the exponential phase) could degrade 100% of 2000 mg/l phenol whereas only 32% could be degraded when phenol was added to a medium with growing cells (still in the exponential phase). More elaborate studies should be directed at this issue. However, in subsequent experiments, this issue has been taken care of and the complete removal of substrate has been confirmed.

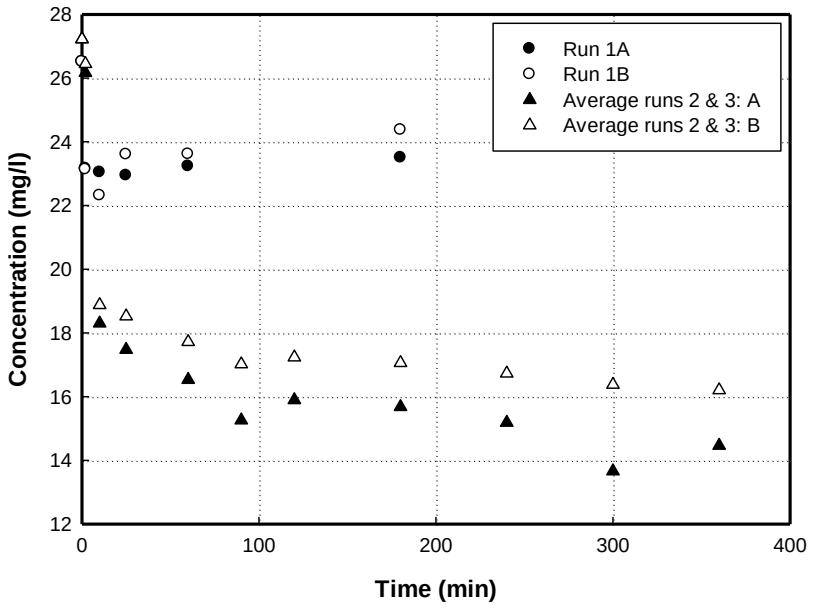


Fig. 4.2.16 Suppression effect in Runs 1A and 1B, C_o = 25 mg/l: A refers to acclimatization Method A (with glucose), B refers to acclimatization Method B (without glucose)

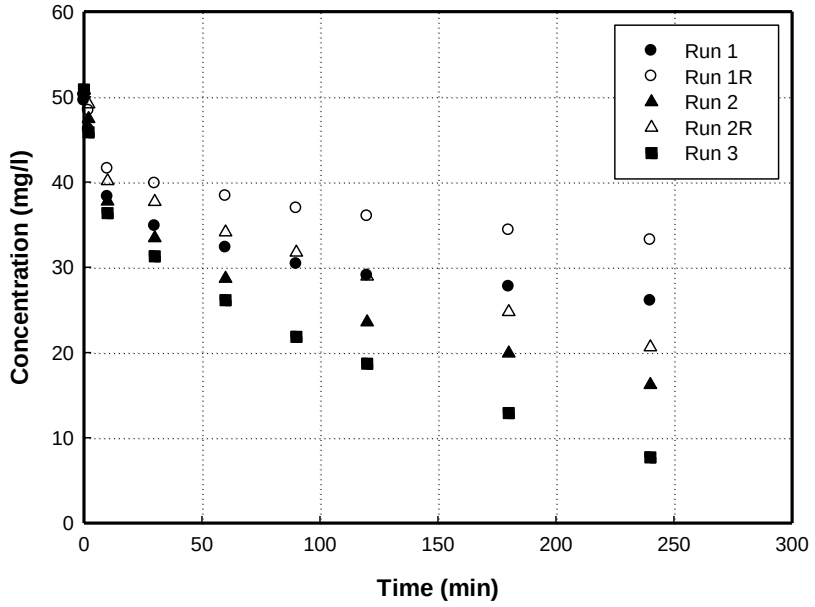


Fig. 4.2.17 Suppression effect in Runs 1R and 2R, C_o = 50 mg/l: Bacteria A (glucose-acclimatized)

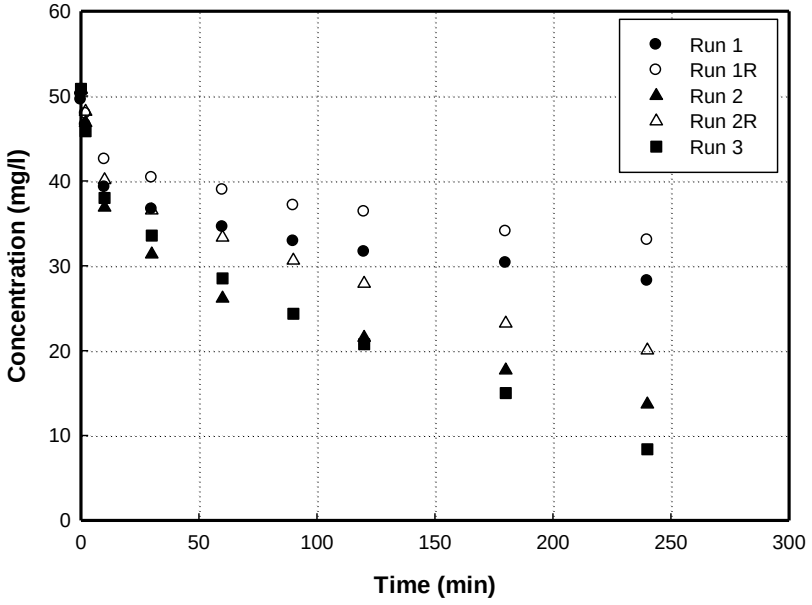


Fig. 4.2.18 Suppression effect in Runs 1R and 2R, C_o = 50 mg/l: Bacteria B (no glucose-acclimatized)

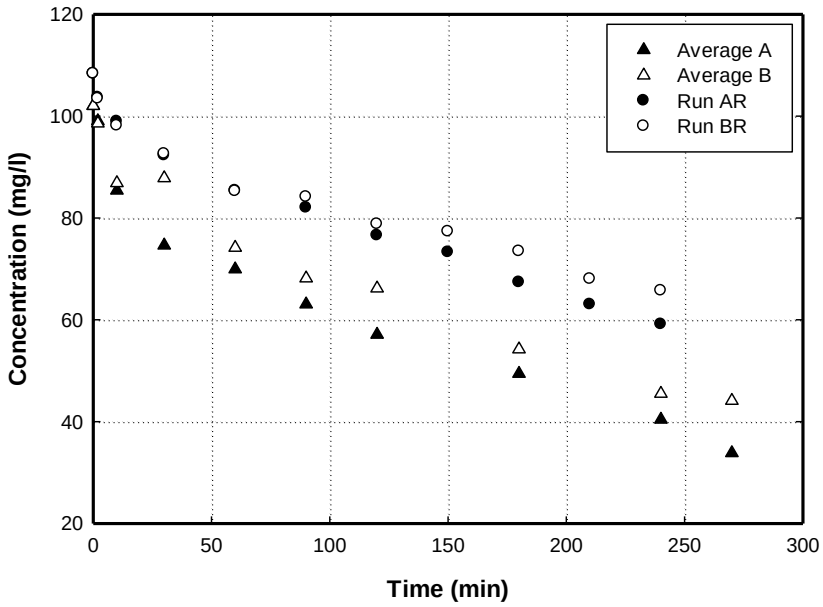


Fig. 4.2.19 Suppression effect in Runs AR and BR, C_o = 100 mg/l: A refers to acclimatization Method A (with glucose); B refers to acclimatization Method B (without glucose)

4.3 OPTIMIZATION OF BIODEGRADATION OF 2, 4 DCP IN SBBR

Since the biodegradation of chlorophenols is strongly influenced by many factors, such as pH, temperature, initial substrate concentration and others, it is crucial to search for the key influencing factors. Optimization of the biodegradation conditions is a prerequisite to large-scale applications of microbial biodegradation processes [206]. In recent years, optimization techniques have been employed to address the important problems of contaminated site management [183]. To carry out such work using conventional techniques such as the "one-factor-at-a-time" method is extremely laborious and time-consuming. Moreover, such methods do not detect interactions between two or more factors and might lead to misinterpretations of the results [176, 178, 206]. In contrast, the statistical design of experiments can optimize the parameters affecting the biodegradation process and save a lot of time and money, decreasing considerably the number of trials needed to investigate a multi-variable phenomenon [179, 206]. The present study focused on the optimization of process parameters of 2, 4 DCP aerobic biodegradation by the commercial strain of *P. putida* immobilized in PVA gel matrix, using a planned design of experiments by response surface methodology (RSM). The classic single-parameter selection technique was initially used to approach the optimum range for each of the main factors. The response surface methodology with Box–Behnken design was subsequently applied to determine the effects of significant parameters, and their interactions, in the removal of 2, 4 DCP and to identify the optimal conditions for its degradation based on the degradation rates achieved from batch experiments in the SBBR. The goal was to develop an effective quadratic regression model that is capable of predicting the rate in terms of the main independent variables,

namely temperature, initial pH and initial concentration. Moreover, experiments were carried out for a reactor configuration of different diameter/length ratio with the other factors fixed at their values which gave the best 2, 4 DCP removals.

4.3.1 Single Factor Batch Experiments for Determining the Optimal Range of Different Factors

This part of the study was based on a classic single-parameter selection technique to approach the optimum range for each of the main factors (temperature T, initial pH value, initial DCP concentration C_o, size of immobilized particles, and inlet air flow rate AFR). These factors were found critical in the biodegradation processes in many previous studies [11, 55, 71, 134, 136, 174, 180, 181, 183, 207]. The first three factors were subsequently optimized using response surface methodology.

Experiments were carried out with different initial 2, 4 DCP concentrations (25, 50, 100, 150, 200 mg/l), temperatures (22, 25, 30, 35, 40 °C) and initial pH (5, 6, 7, 8, 9, 10). Three levels were considered for PVA particle size (0.25, 0.5, 1 cm^3) and five levels of air flow rate (1, 2, 3, 4, 5 l/min) for a total working reactor volume of 1000 ml. In each experiment, one factor was changed, with the other factors remaining constant. At the end of this study, the PVA particle size and the inlet air flow rate were fixed while the range of temperature, pH and initial DCP concentration were chosen for further optimization by RSM.

4.3.1.1 Confirmation of Biodegradation

Since biodegradation experiments are usually carried out at around 30 °C with continuous aeration, both stripping of 2, 4 DCP and adsorption during this process may make some contribution to the overall biodegradation rate. Fifteen

experimental runs were carried out to evaluate the contribution of stripping and adsorption on bacteria-free PVA in comparison to the overall biodegradation rate. Details of the above runs are shown in Table 4.3.1. Nutrients were added to the DCP solution in some runs to evaluate the unlikely but possible contribution of external bacteria to the biodegradation of DCP. These control tests were performed under identical operating conditions to those employed in the biodegradation experiments. Unless otherwise indicated, the initial DCP concentration was 75 mg/l and the initial pH was about 8.2, which is the original pH value of the solution without any adjustment. Also air was introduced at an AFR of 3 l/min, unless indicated there is no aeration. Whenever involved, the ratio of PVA was 30 vol%. The biodegradation rates for the different runs are presented in Table 4.3.1. It is apparent that the contributions of stripping and adsorption as well as the contribution of other types of bacteria to the reduction in DCP concentration seem to be negligible compared to that of the biodegradation by immobilized *P. putida* in PVA, which confirms that the 2, 4 DCP is predominantly removed by biodegradation. It is noteworthy to compare runs 1 and 2, 4 and 5, for the rate of reduction due to stripping and adsorption, respectively. In both cases, it is noticeable that the rate of DCP reduction in the nutrient medium is lower than that in distilled H_2O. This may be explained by the relation between the pH of the medium and the ground state acidity constant (p*K*a). For 2, 4 DCP the p*K*a is 7.68 [22], which implies that 2, 4 DCP will be mainly in molecular form if the pH of the medium is less than p*K*a [45]. Martí et al. [208] felt that if the pH is lower than p*K*a, then the compound will be mostly in non-ionized, less soluble and more volatile form. On the other hand, if the pH is higher than p*K*a value, it will be mostly ionized, more soluble and less volatile. Based on this

concept, and in view of the fact that the pH of the medium is about 6.3 and 8.2 in the case of nutrient absence and presence, respectively, the lower degradation rate in the case of nutrient presence can be justified.

Table 4.3.1 Experimental runs to evaluate the contribution of different factors to the biodegradation rate of 2, 4 DCP

Run	Conditions	Rate of DCP reduction, mg/(l.h): 3h, 4h	Rate of DCP reduction, mg/(l.h): 24 h
1	DCP only at 30 °C	4.83	1.72
2	DCP and 825 mg/l nutrient at 30 °C	0.94	0.66
3	DCP 50 mg/l and 825 mg/l nutrient at 35 °C	0.0	
4	DCP and PVA (blank) closed cap (no aeration) at 30 °C	7.76	1.03
5	DCP and 825 mg/l nutrient and PVA (blank) closed cap (no aeration) at 30 °C	4.49, 3.38	0.83
6	DCP and 825 mg/l nutrient and used PVA (blank) closed cap (no aeration) at 30 °C	1.68	0.26
7	DCP, PVA (blank) and 825 mg/l nutrient at 30 °C	6.37, 5.01	2.03
8	DCP, used PVA (blank) from Run 7, and 825 mg/l nutrient at 30 °C	4.43, 3.65	
9	DCP, used PVA (blank) from Run 8, and 825 mg/l nutrient at 30 °C	2.61	1.24
10	DCP and 825 mg/l nutrient at 40 °C	2.54	0.24
11	DCP and 825 mg/l nutrient at 30 °C, pH 5.0	4.42	1.57
12	DCP 50 mg/l and 825 mg/l nutrient at 30 °C, pH 5.*0*	2.86	1.31
13	DCP and 825 mg/l nutrient at 30 °C, pH 9.0	2.5	0.81
14	DCP, 825 mg/l nutrient and immobilized bacteria in PVA at 30 °C	37.5 (until 100% removal)	
15	DCP, 825 mg/l nutrient and immobilized bacteria in PVA at 30 °C, after 9 months of preparation	69.72 (until 100% removal)	

The adsorption of DCP by PVA is significant only the first time and expected to be negligible for continuous runs, as the adsorption sites get saturated. This can be realized by comparing Run 5 with Run 6, and Runs 7, 8 and 9, in

which the adsorption effect decreased upon repeated use of PVA particles. Run 9 is the most representative case for assessing the removal of DCP without bacteria.

4.3.1.2 Effect of PVA Particle Size

Experiments were carried out to investigate the effect of PVA particle size of 1.0, 0.5 and 0.25 cm^3 for a range of DCP initial concentrations (5, 10, 25, 50 and 100 mg/l). The pH was kept at the initial value without any adjustment in the range of 8.2-8.4, while the temperature and AFR were fixed at 30 °C and 3 l/min, respectively. It was expected that reducing the particle size would improve mixing inside the reactor and enhance mass transfer by lowering diffusion limitations and increasing the surface area, which makes the biomass more accessible to the substrate [71, 134]. This was observed to be more pronounced at low initial concentrations (10, 25 and 50 mg/l) rather than high concentration of 100 mg/l as shown by Figs. 4.3.1 and 4.3.2. This may be justified by the fact that PVA particles are highly porous and therefore the resistance to mass transfer is negligible when coupled with high bulk concentrations of the substrate [11]. Fig. 4.3.3 presents a comparison of the three particle sizes at a low initial DCP concentration of about 11 mg/l, for an inlet AFR of 1 l/min (a) and 5 l/min (b). According to El-Naas et al. [71], the enhancement of the biodegradation rate was more pronounced for high air flow rates due to the combined effect of good mixing and reduced particle size, which resulted in improving the mass transfer inside the reactor. However, this is not significant in the results in Fig. 4.3.3. This could be attributed to the fact that these experiments were carried out directly after acclimatization, and it is suspected that there was a loss of cells due to the cutting process, which could still not be compensated at the early stages of the

process. This is depicted by the degradation by the 0.5 cm^3 particles, which seemed to be the slowest in most of the runs.

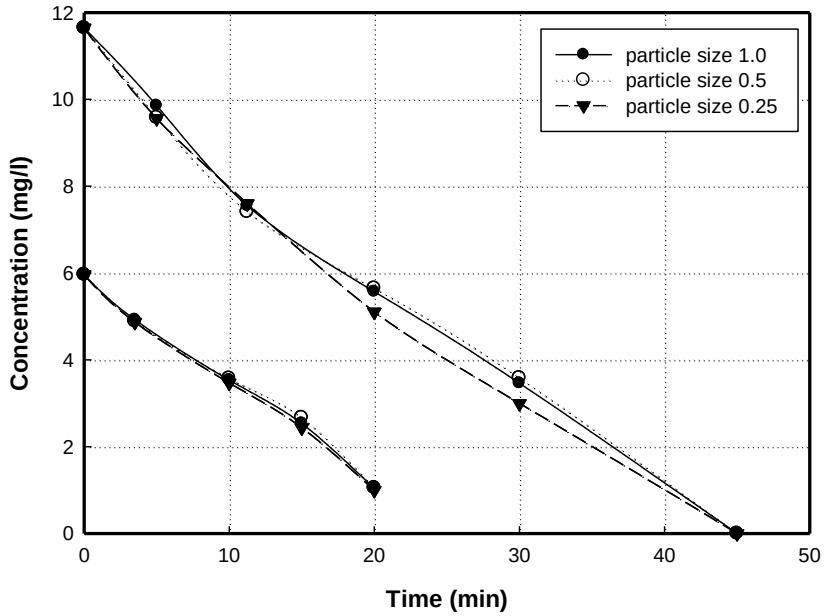


Fig. 4.3.1 Effect of PVA particle size at low initial DCP concentration: T = 30 °C; pH = 8.2-8.4; AFR = 3 l/min

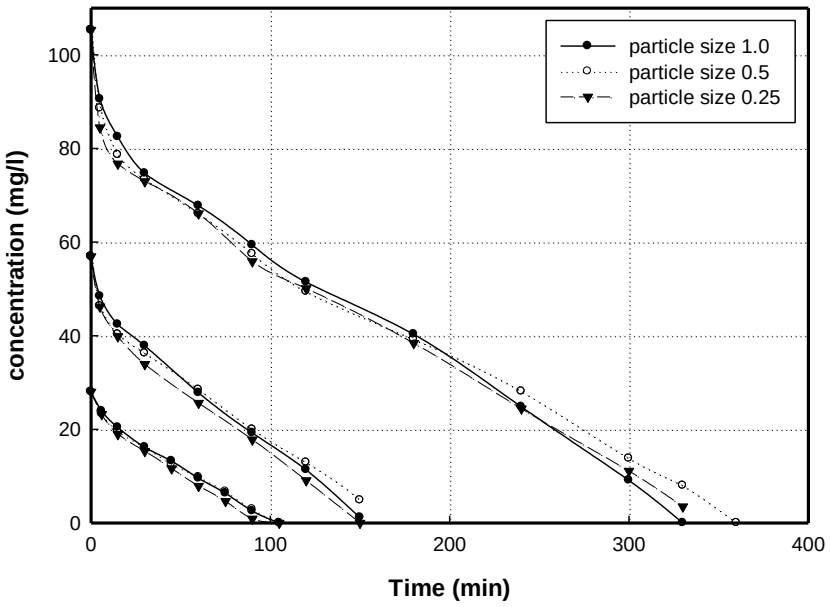
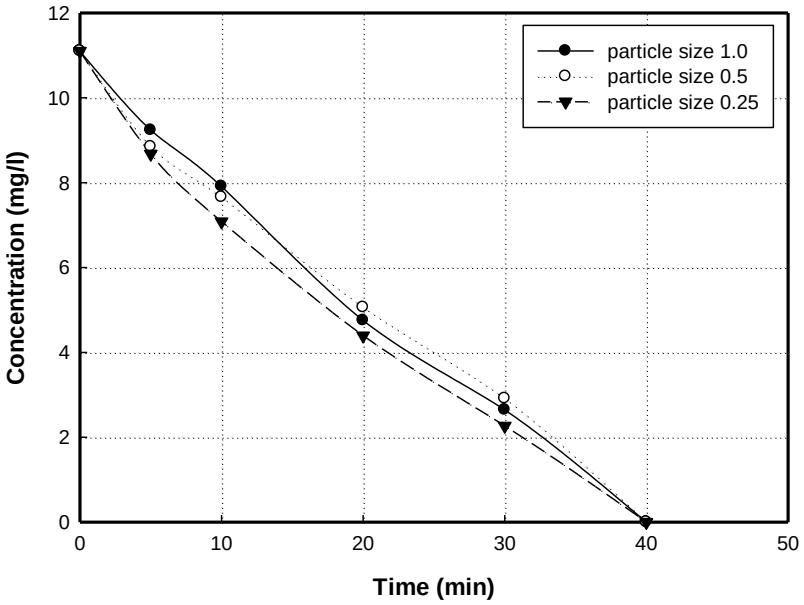
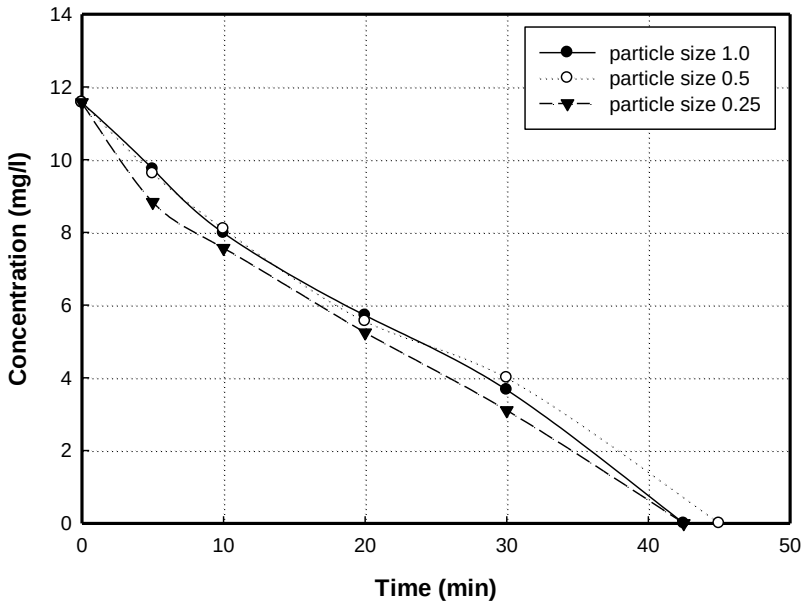


Fig. 4.3.2 Effect of PVA particle size at high initial DCP concentration: T = 30 °C; pH = 8.2-8.4; AFR = 3 l/min



(a)



(b)

Fig. 4.3.3 Effect of PVA particle size at AFR of 1 l/min (a) and 5 l/min (b): C_o = 11 mg/l; T = 30 °C; pH = 8.2-8.4

Based on the above and in view of the technical difficulty in using the 0.25 cm^3 particles due to the attrition effect, it was decided to conduct all subsequent experiments with *P. putida* immobilized in PVA gel particles of 1 cm^3 (for simplicity, particle size in the figures will be designated as 1.0, 0.5, 0.25).

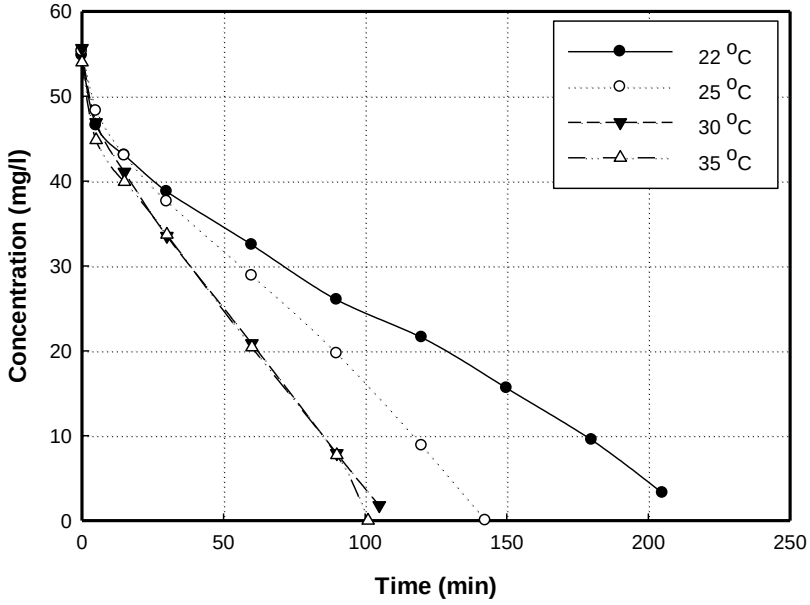
4.3.1.3 Effect of Temperature

The high dependence of enzymatic activity and rate of metabolic rate on temperature makes it an important parameter [180]. Experiments were carried out to assess the effect of temperature in a range from 22 °C (room temperature) to 40 °C in two phases as shown in Figs. 4.3.4 (a and b). The results for the first phase (Fig. 4.3.4 (a)) indicated that the degradation rate increases with temperature up to 30 °C but does not change at 35 °C. However, a strange phenomenon took place in the repeated run at 35 °C, represented by a suppression of the degradation rate and accompanied by the appearance of a peculiar peak in the GC chromatogram, which grew to a maximum before it started to diminish towards the end of the biodegradation. It took the biomass about one week of daily operations to overcome this incidental effect. Once the previous biodegradation ability was restored, the second phase started in order to cover the whole temperature range. Since biodegradation capacity changes due to biomass growth in the PVA matrices it was essential to conduct all the experiments for one factor sequentially within a short period of time. Thus all the experiments at different temperatures were repeated as shown in Fig. 4.3.4 (b). It is clear that performance had improved as represented by shorter degradation times at every temperature. The trend in Fig. 4.3.4 (a) is reiterated by an increase in biodegradation rates with temperature up to a maximum at 30 °C, which does not change significantly at 35 °C (there was only one run at 35 °C). At 40 °C the degradation rate decreased,

and again there was suppression in the biodegradation rate, which was even more pronounced than the previous one and believed to be due to the higher production of metabolites beyond 30 °C. According to a preliminary GC-MS identification, the possibility of an odd peak would be due to an aromatic material, namely dimethyl phthalate, or vitamin A aldehyde, which could be the bacterial enzyme released in those conditions. However, it has been pointed out by several studies [3, 88, 155, 173, 199] that the formation of dead end metabolic intermediates or the toxic effect of the substrate on the cells is often associated with a yellowish to brownish colour change, resulting from involving the *meta*-pathway for degradation, which is related to incomplete mineralization [87, 88]. Such a colour change, however, has not been detected in the present study. Rigas et al. [179] reported in their study on biodegradation of lindane that no significant new peaks were observed in the chromatograms obtained by the GC, which was considered evidence for fast intermediate reaction rates in the pathway of biotransformation and high mineralization of the pollutant. It is clear that this intermediate detection and characterization is worth further investigation.

El-Naas et al. [11] believed that sudden exposure to temperatures higher than 35 °C may have a detrimental effect on the bacterial enzymes that are usually responsible for benzene ring cleavage, which is a key step in the biodegradation process. A decrease in the rate of degradation may be due to a decrease in the effective reactivity of the multi-enzyme complex system in the cell [180]. A similar observation was reported by others [134]. Additionally, extreme temperatures lower the water solubility of oxygen resulting in insufficient levels to support a higher reaction rate at elevated temperatures [207]. However, from the same Fig. 4.3.4, it can be observed that the fastest degradation was at

temperatures of 30-35 °C. An early investigation with the immobilized bacteria as used in Section 4.2.1 that had been acclimatized to phenol and later used in the SBBR for the removal of 2, 4 DCP, revealed the same temperature effect, as depicted in Figs. 4.3.5 and 4.3.6 by the bacteria termed as ‘old’, whereas the freshly prepared bacteria used in this section is termed as ‘new’. The higher degradation rates achieved by the old bacteria may be justified by the fact that the phenol-acclimatized bacteria had been used for a longer time, thus was more adapted to 2, 4 DCP.



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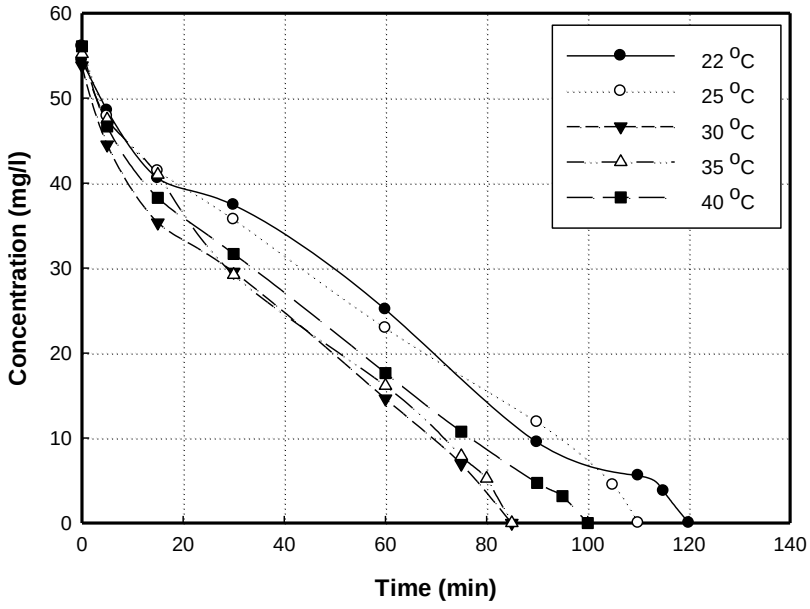


(b)

Fig. 4.3.4 Effect of temperature: experiments of phase 1 (a) and phase 2 (b): C_o = 55 mg/l; pH = 8.2-8.4; AFR = 3 l/min

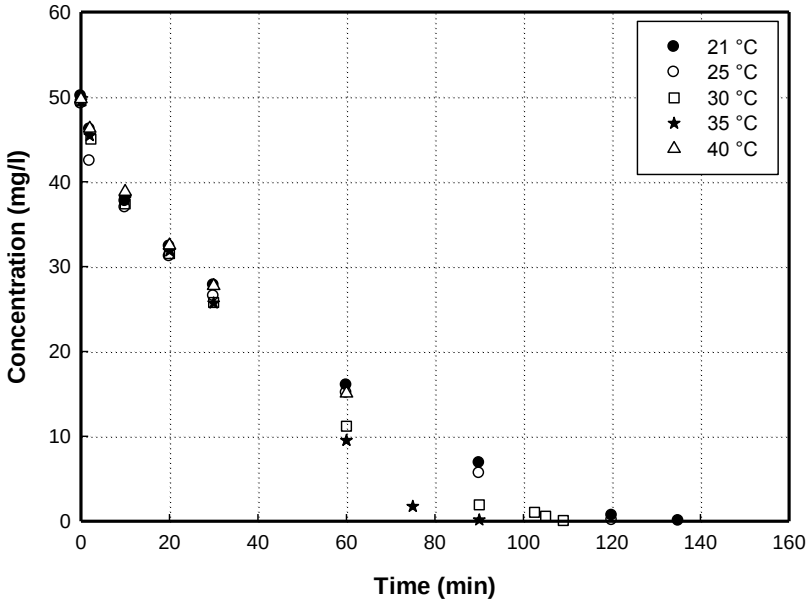


Fig. 4.3.5 Effect of temperature by old bacteria: C_o = 50 mg/l; pH = 8.0-8.2; AFR = 3 l/min

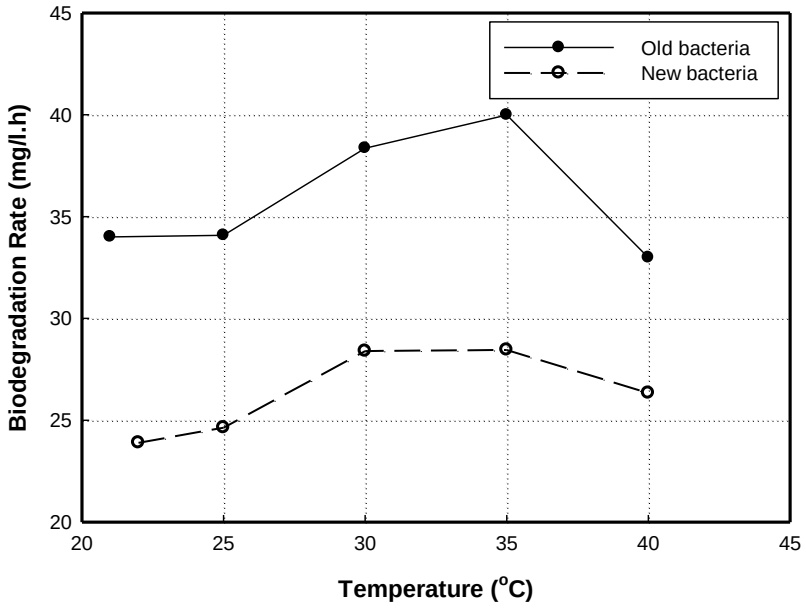


Fig. 4.3.6 Effect of temperature on DCP degradation rate by old and new bacteria: C_o = 50-55 mg/l; pH = 8.0-8.4; AFR = 3 l/min

4.3.1.4 Effect of Initial pH

The initial pH of the culture medium plays an important role in microbial growth and enzyme activity and is one of the most important parameters taken into consideration in the development of biotreatment processes [181, 206]. The effect of the initial pH (from 5.0 to 10.0) on the DCP degradation rate was investigated. As shown in Fig. 4.3.7, at each initial pH of the culture medium the DCP was completely used within 100 minutes. However, the maximum degradation rate was observed when the initial pH was 5.0 and the lowest rate when it was 10.0, whereas the degradation times were very similar in the 7.0-9.0 range. Therefore, the optimum pH value occurred at 5.0. This agrees with other studies that utilized either enzymes or whole cells for DCP biodegradation [207].

When following the pH values along with the biodegradation process, it was clear that the pH followed a similar trend to that of the reduction of DCP concentration. This may be attributed to the formation of organic acids from the intermediates formed during the degradation process. Sheeja and Murugesan [134] reported that the subsequent degradation of these acids causes the pH to rise but the final pH remains less than the initial set pH value: this was evident in the experiments in the current study. The decrease in pH may be also due to HCl formation from the biodegradation of chlorophenols [19]. El-Naas et al. [11] mentioned that a decrease in pH is caused by production of CO_2 resulting in the formation of carbonic acid. However, this decrease in pH value was confirmed in cases where the initial pH was higher than 7.0. The greater the initial pH the greater the decrease in its value. On the contrary, when the initial pH was less than or equal to 7.0, the pH increased during the reaction time. The lower the

initial pH the higher the increase in its value. In both cases, the final pH would be in the range of 6.5-8.0, which is the optimum range for the bacteria to thrive.

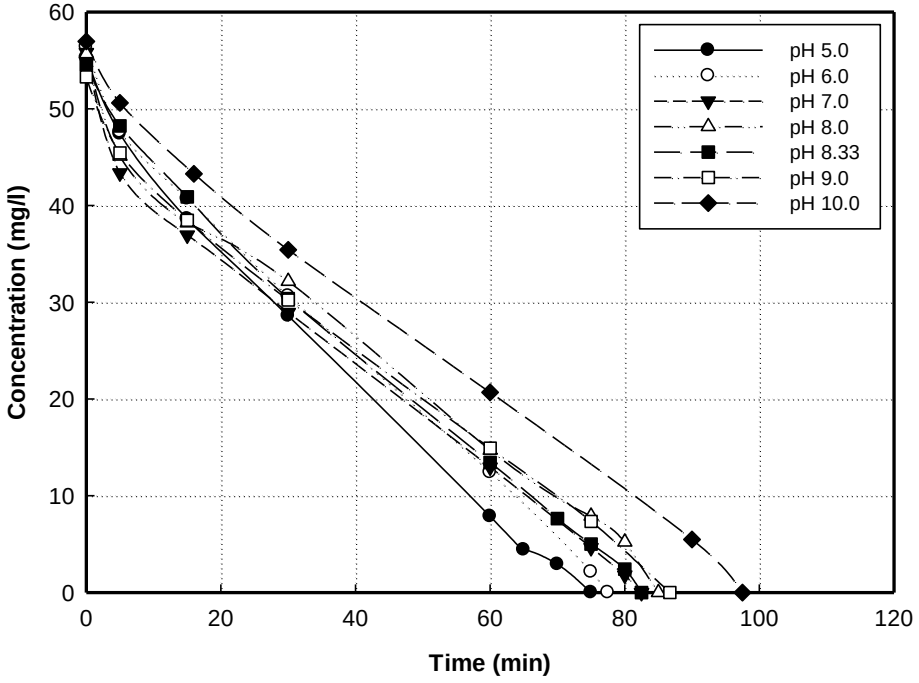


Fig. 4.3.7 Effect of initial pH on DCP degradation: T = 30 °C; C_o = 55 mg/l; AFR = 3 l/min

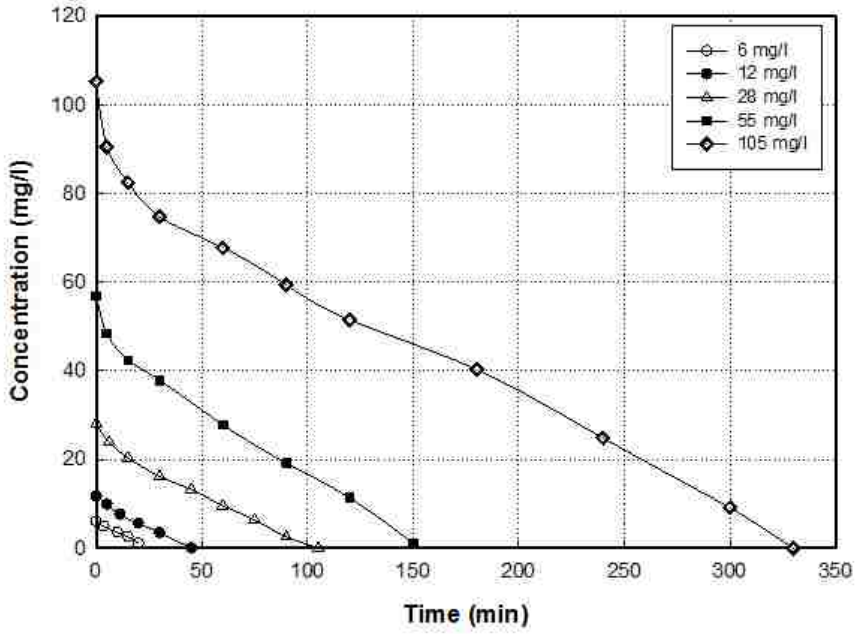
4.3.1.5 Effect of Initial DCP Concentration

Initial substrate concentration plays an important role in the biodegradation process, as some hydrocarbon contaminants, including DCP, are known to have inhibitory effects on the activity of the biomass. Experiments were carried out in the range of 25-200 mg/l as shown in Fig. 4.3.8 (b). Fig. 4.3.8 (a) presents results from the previous group of experiments, which were performed during the study of particle size effect (for particle size 1.0). Complete degradation of DCP was observed at every initial concentration, which means that the inhibition effect was not significant, mainly due to the immobilization of the bacteria within the PVA matrix that protects it from direct contact with the high contaminant concentration. It was noticed that the reduction in DCP concentration

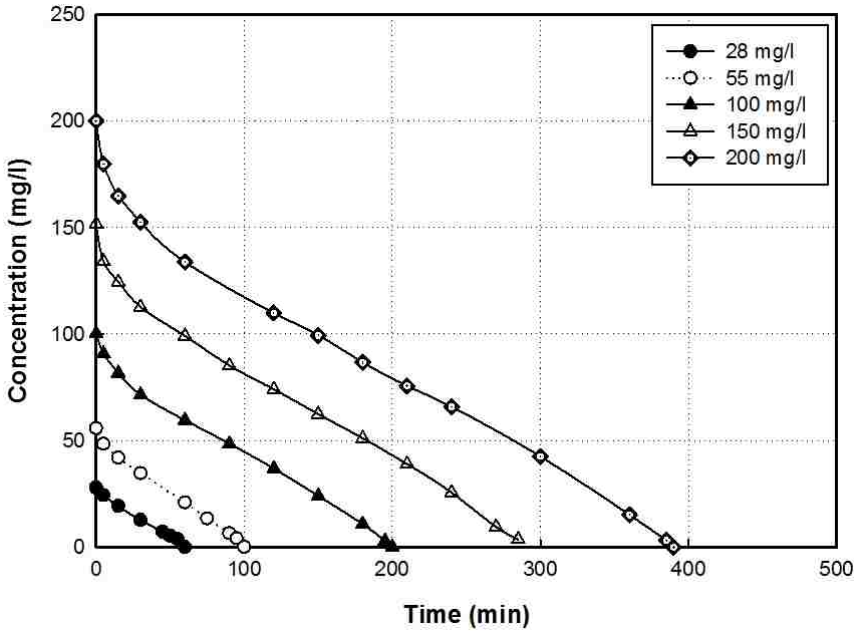
is practically linear in relation to time, and therefore, the degradation rate is constant and follows zero-order kinetics for all initial concentrations of DCP. A similar trend was observed by El-Naas et al. [11] for the biodegradation of phenol. Biodegradation rates for the degradation data in Figs. 4.3.8 (a) and (b) are presented in Figs. 4.3.9 (a) and (b), respectively. In both figures the degradation rate reached a maximum at an initial substrate concentration of 55 mg/l, then decreased with the decrease being less pronounced beyond an initial concentration of 100 mg/l (Fig. 4.3.9 (b)). This can be explained by a minor inhibitory effect toward the cells. On the other hand, the lower biodegradation rate at low DCP concentration could be attributed to mass transfer control, where less DCP is accessible to the biomass. It is worth mentioning that the rates in the experiments of the more recent study in Fig. 4.3.9 (b) are superior to those of the previous study in Fig. 4.3.9 (a), which indicates the increase in the activity of the biomass as a result of cell growth over a time span of almost two months between the two studies. This continued improvement in the performance of the biomass upon repeated use has been highlighted by others [72, 188, 209, 210] and is also noticed by referring to raw 15 in Table 3.3.1, which shows almost double the biodegradation rate from 37 to 70 mg/l.h, over a time lapse of 3-4 months. The biodegradation rates were calculated from the slopes of the best fitted straight line, ignoring the initial segment of fast drop in DCP concentration as it is thought to be influenced by the dilution effect of water release from the PVA gel particles (water accounts for about 90% of the gel mass). For comparison purposes the global rate, which accounts for the whole degradation curve, was also calculated.

Referring to Fig. 4.2.12 for the biodegradation rates of 2, 4 DCP obtained during that experimental stage, it can be noticed that the rate then reached a

maximum at an initial DCP concentration of 75 mg/l, whereas the maximum is 55 mg/l in Fig. 4.3.9. It is believed that the maximum may be close to 70-75 mg/l, but the experimental range of 50-75 mg/l was not investigated in the present data set (Fig. 4.3.8 and Fig. 4.3.9). The lines are connections between the experimental data highlighting the trend. This behaviour clearly shows the substrate inhibition effect, which is best described by the Haldane Model (Eq. (2.11.4): Table 2.11.1) and will be discussed later in the section on the modeling of the biodegradation of 2, 4 DCP.

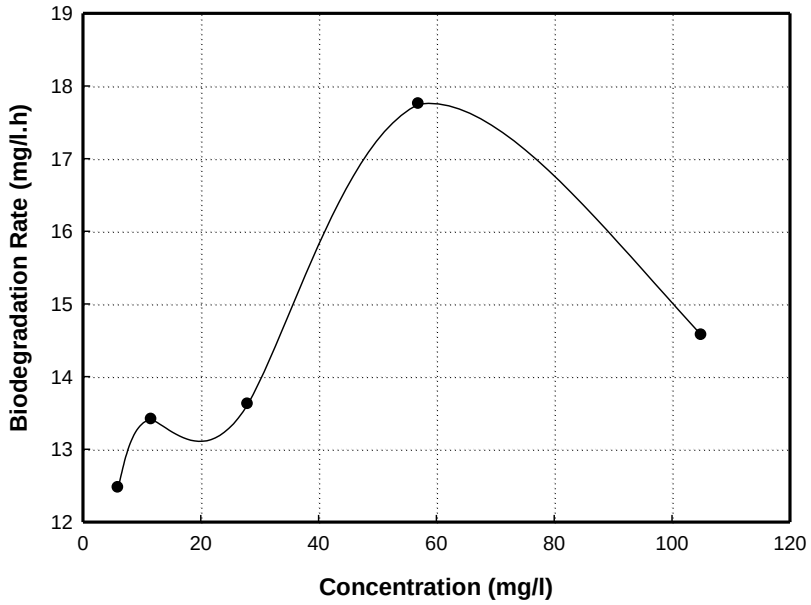


(a)



(b)

Fig. 4.3.8 Effect of initial DCP concentration: previous set (a) and recent set (b): T = 30 °C; pH = 8.2-8.4; AFR = 3 l/min



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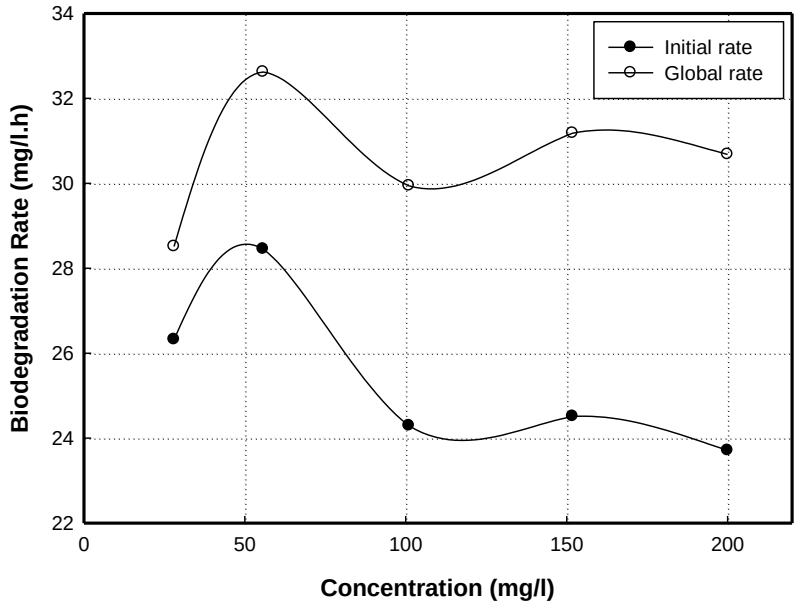


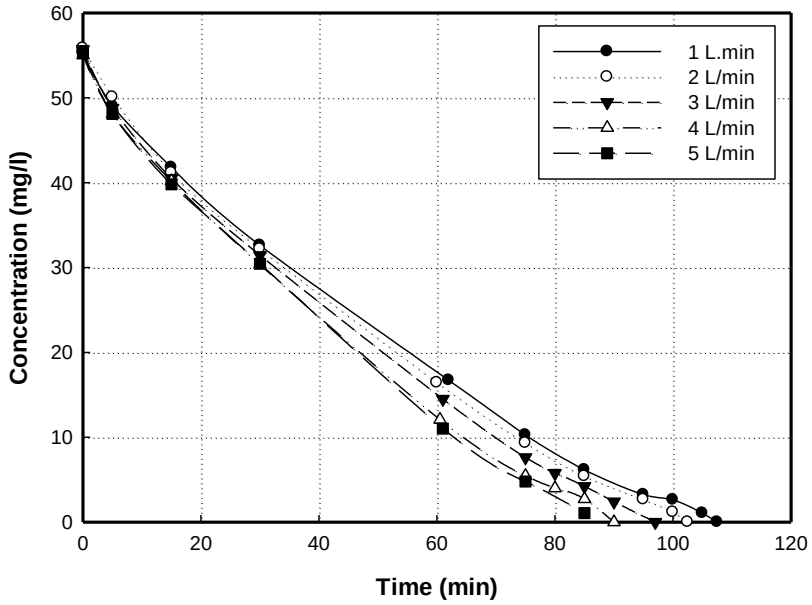
(b)

Fig. 4.3.9 Effect of initial DCP concentration on biodegradation rate: previous set (a) and recent set (b): T = 30 °C; pH = 8.2-8.4; AFR = 3 l/min

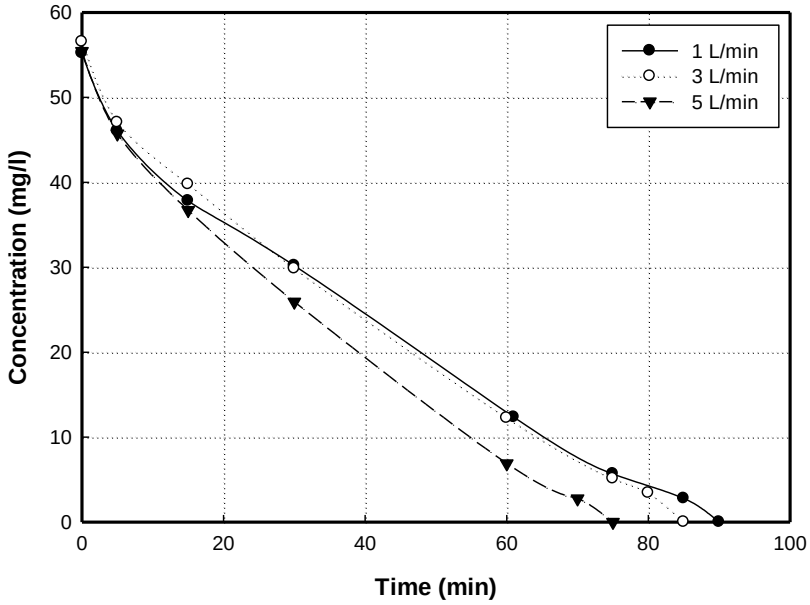
4.3.1.6 Effect of Air Flow Rate in SBBR

Air flow rate (AFR) affects the biodegradation process in an SBBR via two main factors: mixing and aeration, which provides the necessary oxygen [71]. To determine the effect of AFR, two groups of experiments were carried out at an initial concentration of 55 mg/l using different air flow rates: one group was conducted without an adjustment of the initial pH (original pH of 8.3) as shown in Fig. 4.3.10 (a), whereas the other group was conducted with the initial pH adjusted to 5.0 as illustrated by Fig. 4.3.10 (b). Both figures show an increase in the degradation rate with increasing AFR being more pronounced at an initial pH value of 5.0. This is mainly due to the reduction in external mass transfer resistance resulting from increased agitation, and the increase in the amount of dissolved oxygen. These effects are combined with a stripping effect that is more influential at lower pH values (refer to Section 4.3.1.1 for the relation between pH and the p*K*a constant).

In their study, El-Naas et al. [71] proved that at an AFR higher than 1 l/min, the main factor for enhancing continuous biodegradation of phenol, in an SBBR at an initial concentration of 30 mg/l, is the availability of sufficient oxygen rather than the mixing. This highlights the importance of the mixing effect for smaller particle sizes or very low initial substrate concentrations. It was reported that high AFR may produce excessive foaming or air bubble coalescence [71, 180], which could adversely affect the biodegradation process; however this effect was not observed in this study. To avoid these adverse effects at a high AFR, which may also cause slugging and attrition of the PVA particles, and to minimize the role of stripping, it was decided to conduct all the following experiments with an AFR of 3.0 l/min.



(a)



(b)

Fig. 4.3.10 Effect of inlet AFR at initial pH 8.3 (a) and 5.0 (b): T = 30 °C; C_o = 55 mg/l

4.3.2 Response Surface Methodology

The objective of the RSM was to determine the optimum operating conditions for the given process. The effects of the main parameters, namely temperature, pH and initial DCP concentration, and their interactions were studied for further optimization of DCP biodegradation employing the Box-Behnken factorial design. The design with three factors and three levels, including three replicates at the centre point, was used for fitting a second order response and analyzing the main and interactive effects of these parameters on DCP degradation using Minitab 16 Statistical Software. The optimum ranges for the three factors obtained in the single factor experiments were used as reference levels. The range and the levels of the process variables under study are given in Table 4.3.2: temperature (25, 30 and 35 °C), pH (5, 7 and 9) and initial DCP concentration (25, 75 and 125 mg/l), which served as critical variables A, B, and C, respectively. The independent variables along with their levels are shown in Table 4.3.2. The design was applied to the three parameters at three levels. The experimental conditions and the corresponding results obtained, expressed as a global biodegradation rate, are summarized in Table 4.3.3.

Table 4.3.2 Experimental design range and coded levels of the design factors

Factors	Coded values		
	-1	0	1
A: Temperature, °C	25	30	35
B: Initial pH value	5	7	9
C: Initial DCP concentration, mg/l	25	75	125

Table 4.3.3 The Box-Behnken experimental design along with the actual and predicted responses

Run order	A (T)	B (pH)	C (C_o)	Global degradation rate, mg/(l.h)	
				Experimental	Predicted
1	0	1	1	34.85	33.30
2	-1	0	1	28.41	29.47
3	1	-1	0	41.71	41.22
4	0	0	0	37.02	37.69
5	-1	1	0	29.70	30.19
6	1	1	0	34.74	34.86
7	1	0	1	33.07	34.50
8	0	1	-1	27.24	28.18
9	0	0	0	37.70	37.69
10	0	0	0	38.35	37.69
11	0	-1	1	37.16	36.22
12	-1	-1	0	36.40	36.28
13	-1	0	-1	29.30	27.87
14	1	0	-1	33.52	32.46
15	0	-1	-1	36.15	37.70

4.3.2.1 Regression Model

The experimental data were fitted to a second order polynomial regression model containing 3 linear, 3 quadratic and 3 interaction terms. By applying multiple regression analysis to the experimental data, the following second order polynomial equation was found to adequately represent the global rate of DCP degradation. The mathematical expressions are given below in terms of coded factors:

$$Y = 37.69 + 2.40A - 3.11B + 0.91C - 2.41A^2 + 0.36B^2 - 4.20C^2 - 0.07AB + 0.11AC + 1.65BC \quad (4.3.1)$$

and in terms of uncoded factors:

$$Y = -50.90 + 6.29T - 3.86pH + 0.14C_o - 0.1T^2 + 0.09pH^2 - 0.002C_o^2 - 0.01TpH + 0.0004TC_o + 0.017pHC_o \quad (4.3.2)$$

where A, B and C are the independent factors (temperature, pH and initial DCP concentration) in the Box-Behnken design, and *Y* is the response represented by the global degradation rate of DCP. Positive values for A and C in Eq. (4.3.1) indicated that the DCP degradation rate increased as temperature and initial DCP concentration increased, whereas it increased as pH decreased. This was reflected in the negative coefficient of B. Results for the first-order terms were as expected since the data collected from the biodegradation experiments exhibited the same effects.

To assess how well the model represents the data, the statistical significance of the regression model of 2, 4 DCP biodegradation was analyzed by an analysis of variance (ANOVA) of the quadratic equation (Table 4.3.4). The regression coefficients and the interaction between independent factors were considered as statistically significant for *p*-values below 0.05. The analysis of variance of the regression model demonstrates that the model is highly significant (Table 4.3.4), as indicated by a coefficient of determination (R^2) value of 0.9403 and a low failure probability (p = 0.014). Moreover, the value of the R^2 is a measure of the goodness of fit of the model and indicates that 94.03% of the total variations in the response can be explained by the model. The value of the adjusted R^2 at 0.8328 is also high enough to agree for the significance of the model, thus confirming the model's adequacy. In addition, the *p*-value of 0.091 for the lack-of-fit of the model indicates that it is insignificant. The coefficients of the regression model represented by Eq. (4.3.1) are listed in Table 4.3.5; they contain three linear, three quadratic, three interaction terms and one block term.

The significance of each coefficient was determined by student's *t*-test and *p*-values, which are listed in Table 4.3.5. The larger the magnitude of the *t*-value and smaller the *p*-value, the more significant is the corresponding coefficient. This implies that the first order main effects of A (temperature) and B (pH) and the second order effects of A and C (initial DCP concentration) are highly significant as is evident from their respective *p*-values < 0.05. The quadratic effect of C was more pronounced than its linear effect. All the interaction effects had *p*-values > 0.05, making them insignificant. This observation can be attributed to the fact that some of the parameters under study did not have a profound effect on each other for the biodegradation of 2, 4 DCP.

Table 4.3.4 Analysis of variance (ANOVA) for the regression model

Source	Degree of freedom	Sum of squares	Adjusted sum of squares	*F*-value	*p*-value
Model	9	225.244	225.244	8.75	0.014
Linear	3	130.288	130.288	15.18	0.006
A	1	46.224	46.224	16.16	0.010
B	1	77.439	77.439	27.07	0.003
C	1	6.625	6.625	2.32	0.189
Square	3	84.000	84.000	9.79	0.016
A^2	1	17.089	21.512	7.52	0.041
B^2	1	1.740	0.482	0.17	0.699
C^2	1	65.171	65.171	22.78	0.005
Interaction	3	10.957	10.957	1.28	0.378
A*B	1	0.018	0.018	0.01	0.939
A*C	1	0.048	0.048	0.02	0.902
B*C	1	10.89	10.89	3.81	0.109
Residual Error	5	14.305	14.305		
Lack-of-fit	3	13.342	13.421	10.11	0.091
Pure error	2	0.885	0.885		
Total	14	239.549			
R^2 = 94.03%		Adjusted R^2 = 83.28%			

Table 4.3.5 Coefficients for the quadratic regression model

Term	Value	Standard error	*t*-value	*p*-value
Constant	37.69	0.9766	38.594	0.000
Temp: A	2.4038	0.5980	4.019	0.010
pH: B	-3.1113	0.5980	-5.203	0.003
DCP Conc: C	0.9100	0.5980	1.522	0.189
Temp*Temp: A^2	-2.4137	0.8803	-2.742	0.41
pH*pH: B^2	0.3612	0.8803	0.410	0.699
DCP Conc*DCP Conc: C^2	-4.2013	0.8803	-4.773	0.005
Temp*pH: AB	-0.0675	0.8457	-0.080	0.939
Temp*DCP Conc: AC	0.1100	0.8457	0.130	0.902
pH*DCP Conc: BC	1.6500	0.8457	1.951	0.109

The model's adequacy was further verified by plotting the normal probability and parity plots (see Figs. 4.3.11 and 4.3.12, respectively). The residual analysis shows that there was no evidence of outliers as all the standardized residuals fell within the range of -2 to +2, and they were randomly distributed around zero. This indicates a high degree of correlation between the observed values and the predicted values. The parity plot showed a satisfactory correlation between the experimental and predicted values (obtained from Eq. (4.3.1)) for the rate of DCP degradation. The points cluster very closely around the diagonal line indicating low discrepancies between the experimental and predicted values.

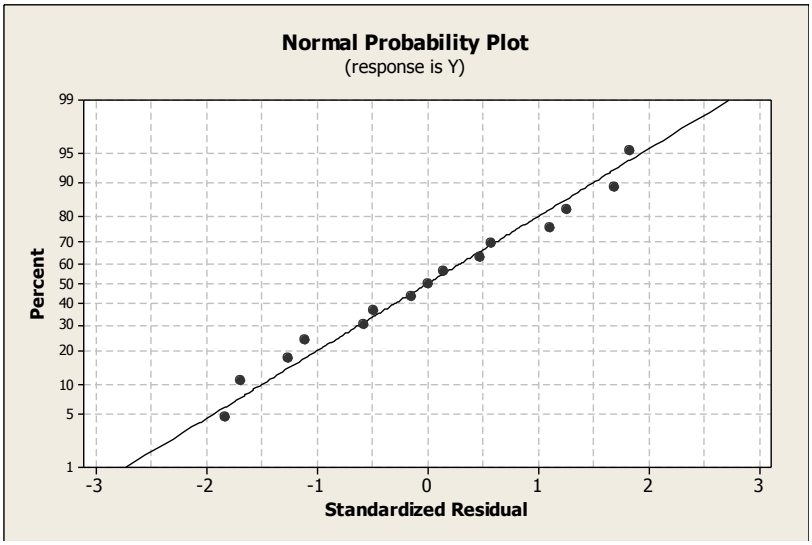


Fig. 4.3.11 Normal probability plot of standardized residuals

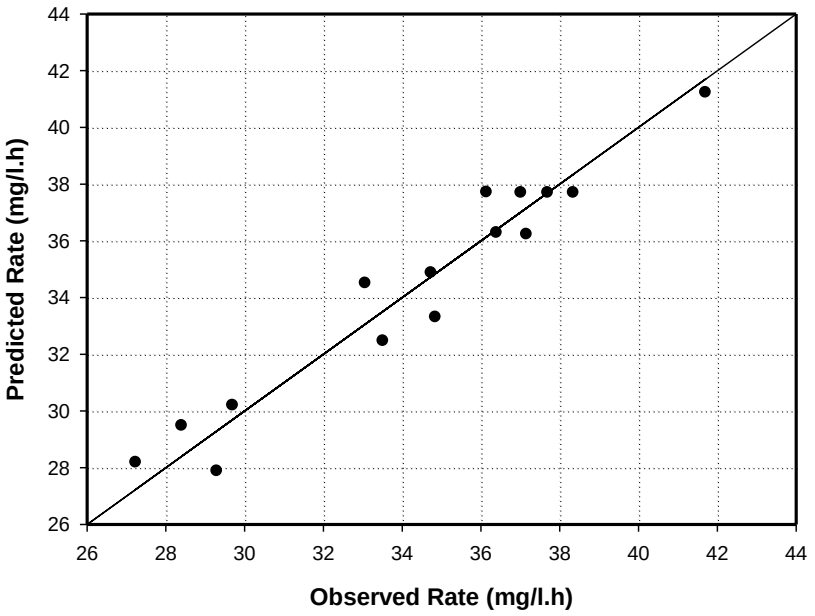


Fig. 4.3.12 Parity plot: predicted versus observed rate

4.3.2.2 Model Optimization for Maximum Degradation Rate

The contour plots and their respective three dimensional surface plots provide a visual interpretation of the interaction between two variables and facilitate the location of optimum experimental conditions [176]. Figs. 4.3.13 and 4.3.14 represent the contour and surface plots, respectively, for the optimization of DCP degradation. The shape of the contour plots provides a measure for the significance of the mutual interaction between the variables. An elliptical contour plot indicates a significant interaction between variables [176, 180]. Also, the more the curvature in the surface plots, the more significant the interaction. This is depicted by the elliptical nature of the contour plots and their respective curved surface plots for the mutual interactions AB, AC and BC. Figs. 4.3.13 and 4.3.14 suggest the optimum range of the process variables. Using a response optimizer by Minitab, the stationary point presenting maximum aerobic biodegradation rate, inside the experimentation region, had the following critical values: temperature 32.58 °C (0.5152), pH 5.0 (-1) and initial DCP concentration 70.46 mg/l (-0.0909). Under these conditions the predicted aerobic global biodegradation rate was 41.82 mg/ l.h.

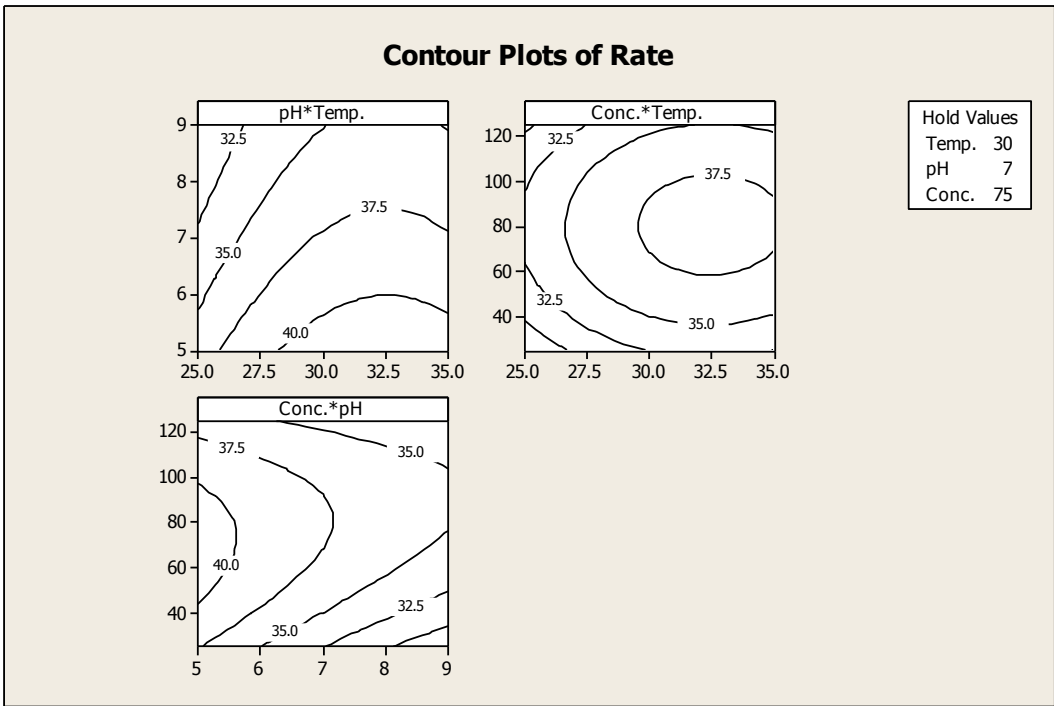


Fig. 4.3.13 Contour plots of response versus the different interactions of independent variables

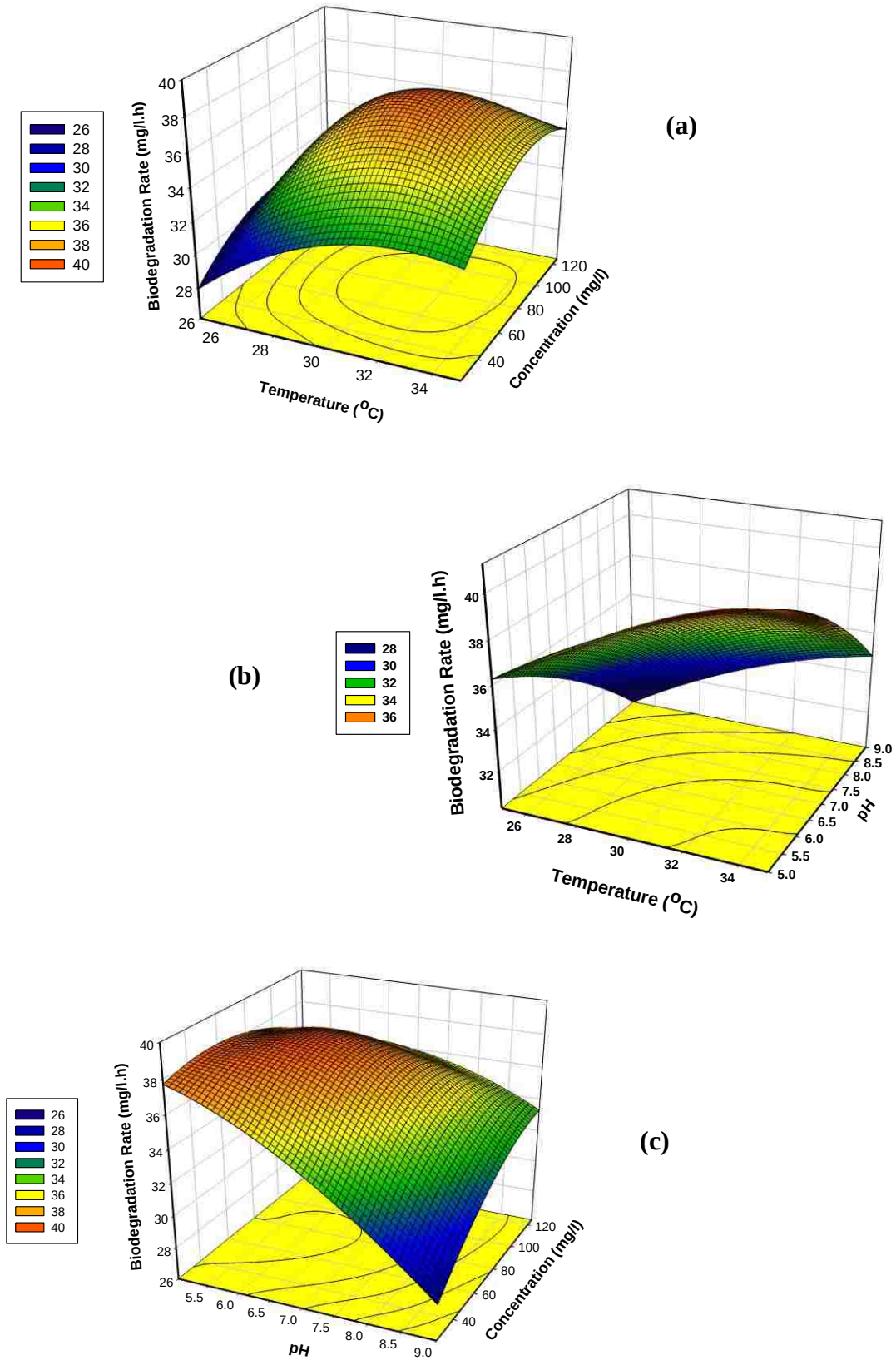


Fig. 4.3.14 Response surface plots of response versus the different interactions of independent variables (pH, initial concentration and temperature were held constant at their respective center levels in (a), (b) and (c), respectively)

4.3.2.3 Validation of the Model

For a model to be declared practically fit, it is important to validate the model to ensure that it gives sufficient approximation of the results obtained in the field [183]. The suitability of the model, as represented by Eq. (4.3.1) and (4.3.2), for predicting the optimum response was tested by performing batch biodegradation experiments using predicted optimum conditions. Also, other selected conditions were tested to compare the experimental degradation rates with those predicted by the model. The validation experiments were replicated. Table 4.3.6 summarizes the results of the experimental and predicted responses at the 95% confidence interval. It shows the closeness of the model to the experimental results, confirming that the model can be considered quite reliable for predicting the aerobic biodegradation of DCP by *P. putida*. Recalling Section 4.3.1.3 on temperature's effect, the observation was that on carrying out the replicated runs at 35 °C there was a suppression of the degradation rate, accompanied by the appearance of a peculiar peak in the chromatogram: it is worth noticing that the bacterial activity was at risk even at a temperature of 33 °C. Replicated rates at this temperature could be achieved only when the suppression effect had been reduced. Thus, although the RSM predicted an optimum operating temperature of 32.5 °C, the sustainability of the bacterial activity is doubtful at this temperature. Bioprocesses are rather sensitive in terms of achieving reproducibility, which imposes some limitations on the applicability of the model.

Table 4.3.6 Validation of the RSM regression model

T, °C	Initial pH	C_o, mg/l	Experimental rate, mg/l.h	Predicted rate, mg/l.h	95% Confidence interval
30	9.0	53.67	35.38	33.08	30.27, 35.90
30	8.4	28	27.67	30.06	27.12, 32.95
32.5	5.0	71.17	40.05	41.82*	38.96, 44.69
27	6.0	81.18	33.91	36.94	34.63, 39.26
27	6.0	41	31.96	34.93	32.57, 37.55
30	8.3	55.17	34.37	34.39	32.04, 36.70
30	5.0	54.37	42.87	40.75	37.94, 43.56
30	5.0	75	39.4	41.16	38.43, 43.90

*Degradation rate at the optimum conditions

For the sake of comparison, the degradation rates from other reported batch studies are shown in Table 4.3.7. Generally, partial removal of chlorophenols in the range of 40-60% has often been reported [90]. It is obvious that the rates obtained in this study are better than those reported in the extant literature.

Table 4.3.7 Degradation rates of 2, 4 DCP from batch studies in the literature

Initial DCP concentration (mg/l)	Degradation rate (mg/l.h)	% Removal	Ref.
1000 (fungi), free	8.3	100	[8]
500 (fungi), free	5.21	100	[70]
113	28.75	100	[143]
120 (free)	0.48	40	[41]
80, free	2,67	100	[60]
75	37.5 70	100 100	This work: 4 months; 9 months after preparation

4.3.3 Effect of Reactor Geometry on Batch Degradation of 2, 4 DCP in SBBR

The degradation of 2, 4 DCP was evaluated in another reactor of almost the same volume of about 1.1 liters but with a different Di/H ratio; where of Di is the internal diameter and H is the height of the reactor. For this reactor the ratio Di/H is 0.42, compared to 0.13 for the previous reactor (refer to Section 3.6 for the dimensions of both reactors). For the sake of simplicity, the reactors are designated as long and short for the previous reactor and the reactor used in this part of the study, respectively.

The reference baseline for operating conditions of zero coded values (Table 4.3.2) was selected for the experiments. Figs. 4.3.15 and 4.3.16 describe the degradation trend in relation to time for different inlet AFR, for both the long and short reactors, respectively.

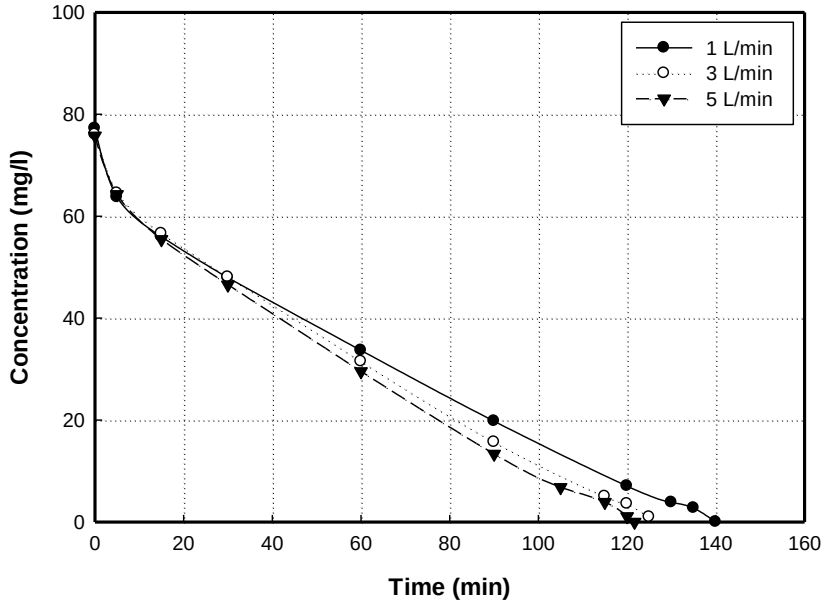


Fig. 4.3.15 Effect of inlet AFR at initial pH 7.0: T = 30 °C; C_o = 75 mg/l for long reactor

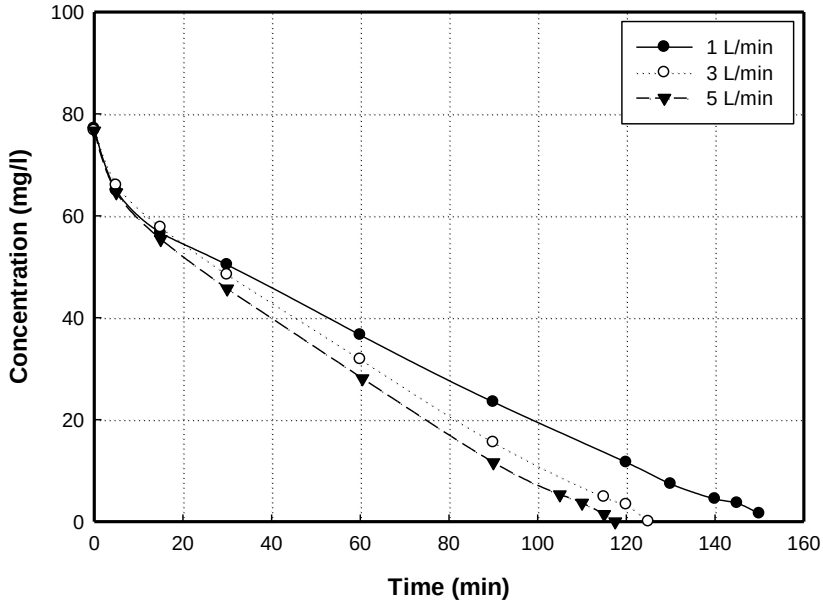


Fig. 4.3.16 Effect of inlet AFR at initial pH 7.0: T = 30 °C; C_o = 75 mg/l for short reactor

Comparing the degradation times in both figures, there is an increase in the degradation rate consistent with an increase in the AFR. This is mainly due to the reduction in external mass transfer. Stripping of DCP could make a contribution to the improvement of the degradation rate; however, it is expected to have a negligible effect at pH ≥ 7.0. It appears from Figs. 4.3.15 and 4.3.16 that there is not much difference in the performance of the two reactors, except for the superiority of the long reactor at a low AFR of 1 l/min. This may be attributed to the fact that a large portion of the PVA particles were settling on the wide base in the short reactor, forming a dead zone of poor mixing as illustrated by the snapshot in Fig. 4.3.17, and compared to the good mixing regime in the same reactor at an AFR of 3 l/min, which is depicted in snapshot Fig. 4.3.18.

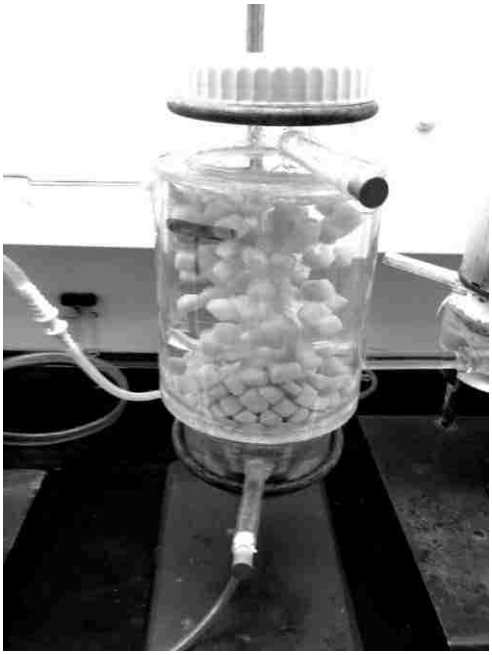


Fig. 4.3.17 Mixing regime in the short reactor at AFR of 1 l/min: pH 7.0; T = 30 °C; C_o = 75 mg/l

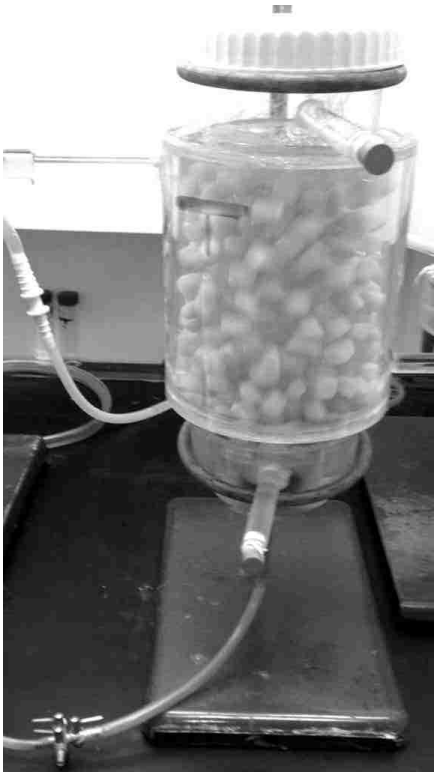


Fig. 4.3.18 Mixing regime in the short reactor at AFR of 3 l/min: pH 7.0; T = 30 °C; C_o = 75 mg/l

It was anticipated that the difference in the effect of reactor geometry, or Di/H ratio, on the degradation rate would be more pronounced at low initial substrate concentrations, so batch experiments were carried out in both reactors with initial DCP concentrations of 55 mg/l and AFR of 3 l/min. The results are shown in Fig. 4.3.19. Again, the degradation time in the two reactors is identical, which emphasizes the negligible difference between the two reactors at a normal AFR. In all the above runs, it was obvious that the short reactor was more difficult in terms of temperature control, required a longer time for transient heating and required a greater heating load to achieve the same temperature inside the reactor. A discussion based on the residence time distribution is presented in the next section on the continuous biodegradation of 2, 4 DCP.

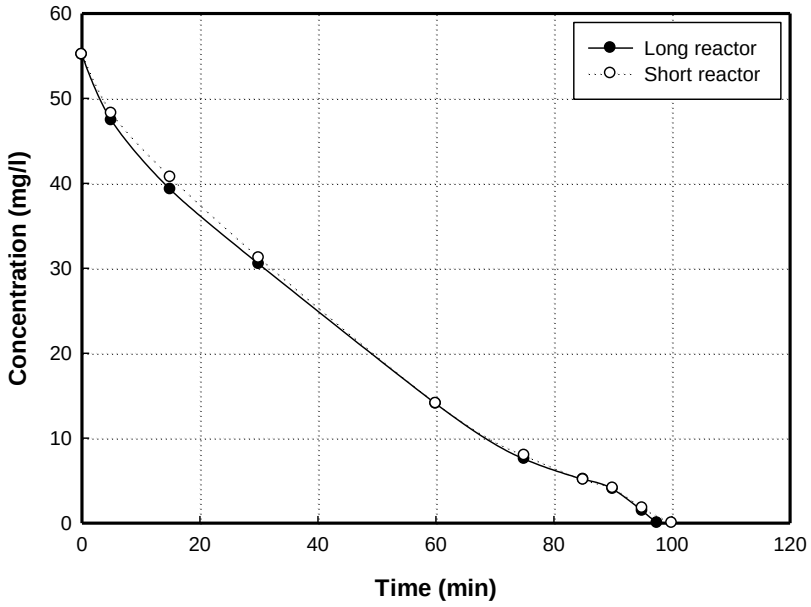


Fig. 4.3.19 Degradation of DCP in long and short reactors: AFR = 3 l/min; initial pH = 8.2; T = 30 °C; C_o = 55 mg/l

4.4 CONTINUOUS BIODEGRADATION OF 2, 4 DCP

In view of the many benefits of immobilization, it has often been reported to be particularly useful in continuous processes and suitable for large scale industrial application [211, 212]. In many cases, the process is moved to a continuous mode when the acclimatization step in a batch reactor is finished [158]. When considering the practical applications of engineered systems for wastewater treatment, it is advantageous to investigate the continuous operation of the bioreactor system under different operating conditions. This is not a straightforward procedure as continuous biological wastewater treatment operations often suffer from problems such as oxygen limitation, low degradation rates and biofouling from uncontrolled biomass growth [213]. In light of these facts, utilizing the full potential of a continuous operation in a laboratory scale SBBR would be better in a more industry-like setting, which can provide valuable information for the full-scale application of this system.

The objective of this part of the study was to investigate the performance of the SBBR for DCP degradation under continuous operation conditions. The various issues investigated were the hydrodynamics of the reactor in terms of the hydraulic residence time (HRT), the initial concentration of DCP in the feed and the pH of medium and degradation capacities. The bioreactor was thereafter evaluated in terms of stability and tolerance to organic and hydraulic shock loads, resulting from concentration and flow rate fluctuations. Furthermore, the SBBR performance was also compared to that of a packed bed reactor to ascertain the main characteristics of each reactor.

4.4.1 Reactor Hydrodynamics: Residence Time Distribution (RTD)

The mean residence time t_m in the reactor was determined by the tracer injection technique outlined in Section 3.5.5. The conductivity responses to the NaOH injections are presented in Fig. 4.4.1 for an average flow rate F of 10.4 ml/min. Special care was taken in synchronizing the pulse input with the initiation of data accumulation for RTD analysis and in calibrating the pump to ensure that the reactor space time ($\tau = V / F$), where V is reactor volume (ml) and F is the volumetric flow rate (ml/min), was accurately measured.

In Fig. 4.4.1, the results of three runs are presented. As freshly prepared blank PVA gel particles were used, it is highly probable that adsorption of the tracer on the PVA particles took place, and consequently was released during the analysis in accordance with the adsorption equilibrium between the PVA and the fluid medium in the reactor. This would result in prolonged residence time and a greater spread of the RTD. This trend is clearly noticed for Run 1 in which it was expected that the adsorption would be more pronounced. It then decreased in subsequent runs as more adsorption sites were occupied. All of these curves are smooth and show a fast increase at the beginning followed by an exponential decrease, as expected for a continuous stirred tank reactor (CSTR), which is the nearest model to that of the SBBR hydrodynamics. A delay in the increase from time zero and the roughness of the increases would indicate irregular and ineffective mixing behavior as compared to the ideal mixing curve that should increase abruptly with an instantaneous rise at time zero, and decay exponentially thereafter [214]. Considering the ideal behavior of the response, it can be deduced that the last run (Run 3) was the closest to the ideal situation.

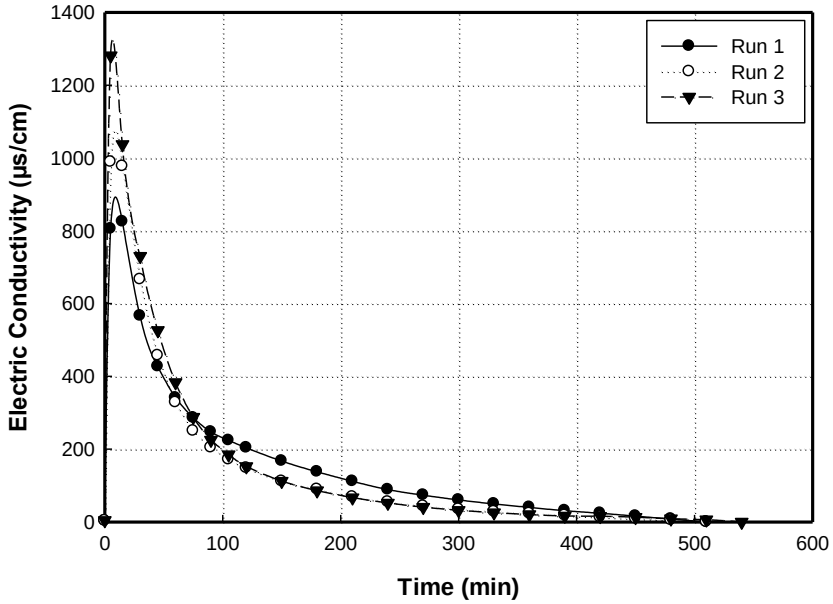


Fig. 4.4.1 Long SBBR conductivity response to a pulse tracer input: F = 10.4 ml/min; T = 30 °C; AFR = 3 l/min

The RTD was obtained from the experimental data in a typical manner [215] as follows:

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \tag{4.4.1}$$

where $C(t)$ represents the concentration of the tracer. The mean residence time (t_m) was calculated by integrating

$$t_m = \int_0^\infty tE(t)dt \tag{4.4.2}$$

The variance, or square, of the standard deviation of the RTD is calculated using

$$\sigma^2 = \int_0^\infty (t - t_m)^2 E(t)dt \tag{4.4.3}$$

The magnitude of this 2nd moment is an indication of the spread of the RTD. For an ideal CSTR, $t_m = \tau$ and the value of σ/t_m should be 1.0, which means that for an ideal CSTR the standard deviation of the RTD is as large as the mean itself [214, 215].

A previous Section (4.3.3) indicated that the batch degradation of 2, 4 DCP had been investigated in another reactor of the same volume but with different geometry, termed the short reactor, and it would be advantageous to relate the results obtained then to the RTD of both reactors (short and long). The RTD was also obtained for the short reactor in the same manner. The results of the two runs are shown in Fig. 4.4.2. For the sake of comparison, the average of Runs 2 and 3 of the long SBBR is also displayed. It is obvious that Runs 1 and 2 for the short SBBR are almost identical, which is perhaps due to the absence of the adsorption effect after repeated use of the PVA particles. The response curves are smooth with an abrupt instantaneous rise followed by an exponential decrease. However, the curves of the short SBBR have a wider spread, which could mean a greater deviation from an ideal CSTR.

The hydrodynamic behavior of both reactors was determined by the mean and the variance of the RTD, as given by Eqs. (4.4.1), (4.4.2) and (4.4.3). The solution was reached via numerical methods of integration [215]. The results are listed in Table 4.4.1 as averages of the duplicate runs. The volume V_L is the liquid volume accounting for about 70% of the moving bed. It was found that a total volume of 1040 ml inside each reactor is required to induce an overflow in continuous operation with an AFR of 3 l/min. The shorter mean residence time of the long reactor is closer to the space time, which makes the reactor closer to an ideal CSTR. The smaller standard deviation σ explains a narrower distribution of the response curve. The longer residence time of the short reactor explains its relatively poor mixing characteristics at lower ARF, as was demonstrated in Section 4.3.3.

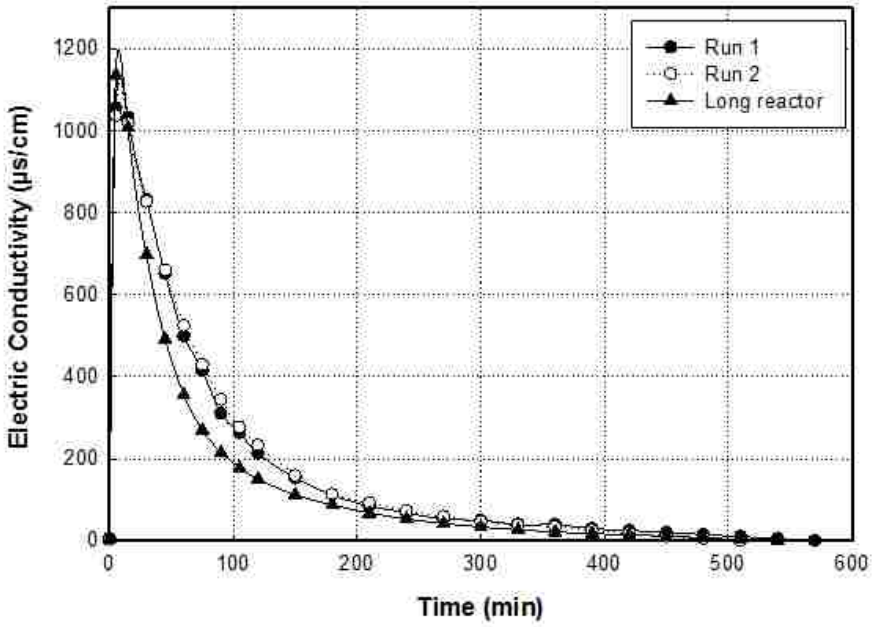


Fig. 4.4.2 Short SBBR conductivity response to a pulse tracer input: F = 10.4 ml/min; T = 30 °C; AFR = 3 l/min

Table 4.4.1 RTD measurements for long and short SBBRs

Parameter	Long reactor	Short reactor
Mean residence time $t_{m,}$ min	84.76	93.94
Standard deviation σ, min	95.1	98.7
Reactor volume V, ml	1100	1080
Liquid volume V_L , ml	735	735
Space time τ, min	71	71

Since the mean residence time has already been estimated, it is used in the calculations where HRT appears in an equation.

4.4.2 Degradation Capacity: Effect of pH, HRT and Initial Concentration

It was intended to carry out the continuous biodegradation experiments at the optimum operating conditions, as determined by RSM. These conditions for maximum aerobic biodegradation rates inside the experimentation region had the

following critical values: temperature 32.5 °C, pH 5.0 and initial DCP concentration was 70.46 mg/l. However, it is worth recalling from Section 4.3.2 that a risky situation was often encountered whenever the temperature exceeded 32 °C, accompanied by the appearance of a peculiar peak in the GC chromatogram and sometimes a suppression of the degradation rate. Replicated rates above this temperature could be achieved only when the suppression effect had been reduced. Thus, considering the risk and realizing that the normal operating temperature of 30 °C is very close to 32.5 °C, it was decided to continue the experiments at 30 °C. As for the initial 2, 4 DCP concentration, the standard operating concentration was 75 mg/l, which is very close to the optimum of 70.5 mg/l.

4.4.2.1 Effect of pH

The results of continuous operation at three initial pH values are shown in Fig. 4.4.3. The value of pH 8.2 is the original value of the solution without any adjustment. It is obvious that there was no significant difference in the investigated range. Considering that pH adjustment is tedious and time consuming pretreatment, it was more practical to continue subsequent experiments with the original pH value of the solution without any adjustment.

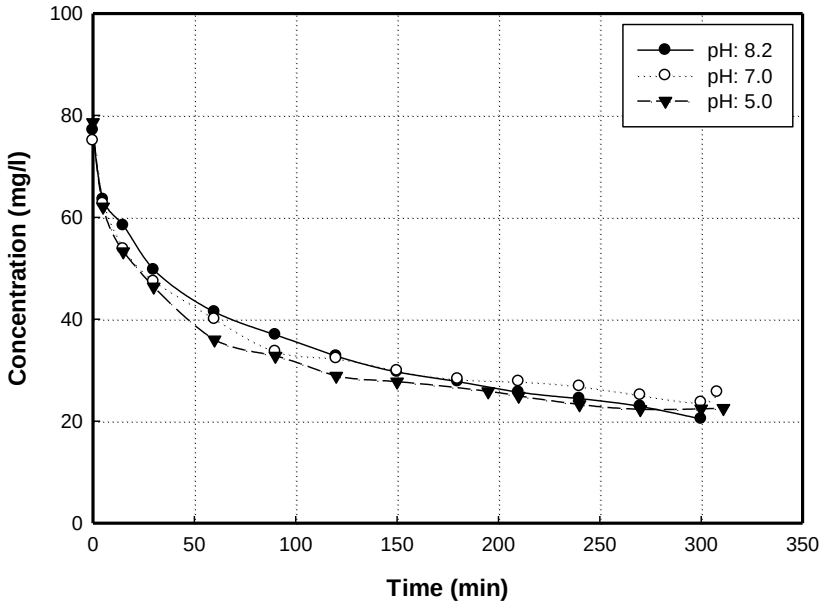


Fig. 4.4.3 Effect of initial pH in continuous operation: T = 30 °C; C_o = 75 mg/l; *F* = 10.4 ml/min; AFR = 3 l/min

4.4.2.2 Effect of Hydraulic Residence Time (HRT)

The effect of liquid flow rate (LFR) was studied for four different LFR, namely 3.3, 5.3, 10.4 and 15 ml/min, starting with an initial substrate concentration of 75 mg/l. Since the SBBR is characterized by systematic intense mixing, the substrate concentration in the reactor was expected to be the same as that of the outlet. The concentration profiles are depicted in Fig. 4.4.4, which shows that the initial biodegradation rate decreased with increasing LFR. This is expected, since increasing the LFR, or in other words decreasing the HRT, gives the immobilized biomass less contact time with the substrate and nutrients, which results in a lower biodegradation rate. The mean residence time for the LFR of 10.4 ml/min was estimated to be 1.42 hours (84.76 min: Table 4.4.1). Therefore it was estimated to be 4.45, 2.79 and 0.98 hours for the LFR of 3.3, 5.3 and 15 ml/min, respectively. A plot of the initial biodegradation rates, calculated for the transient time before reaching a steady state (S. S.), is shown as a function of the

mean residence time in Fig. 4.4.5. A near perfect straight line fits quite well with a high coefficient of determination, R^2, of 0.998. The results in terms of 2, 4 DCP steady state concentration in the effluent, and therefore, the percent removal of 2, 4 DCP are illustrated by Fig. 4.4.6.

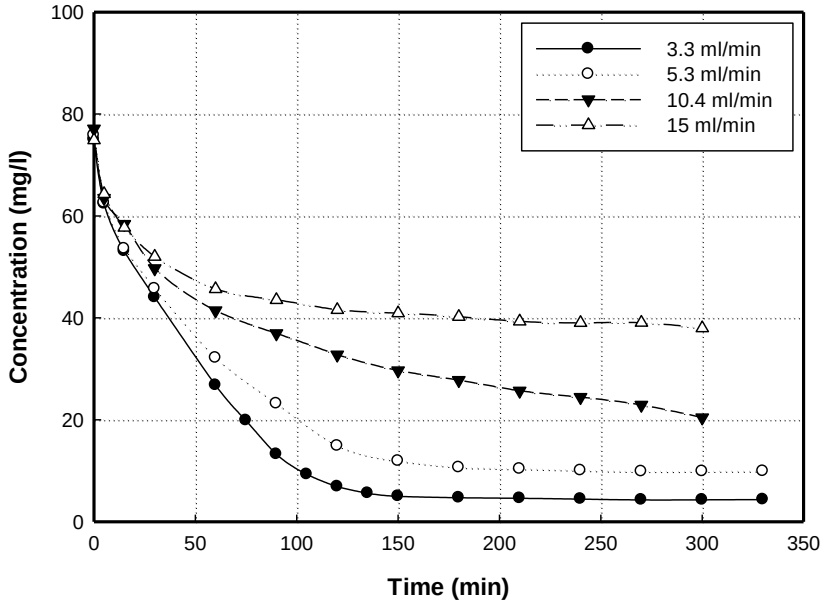


Fig. 4.4.4 Variation of DCP concentration with time for different liquid flow rates: T = 30 °C; C_o = 75 mg/l; initial pH = 8.2; AFR = 3 l/min

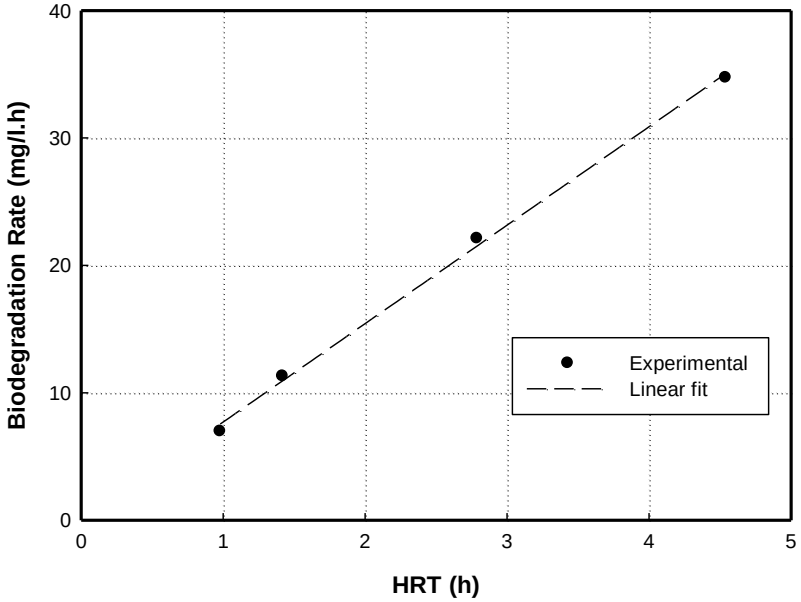


Fig. 4.4.5 Initial biodegradation rate of 2, 4 DCP versus mean residence time: T = 30 °C; C_o = 75 mg/l; initial pH = 8.2; AFR = 3 l/min

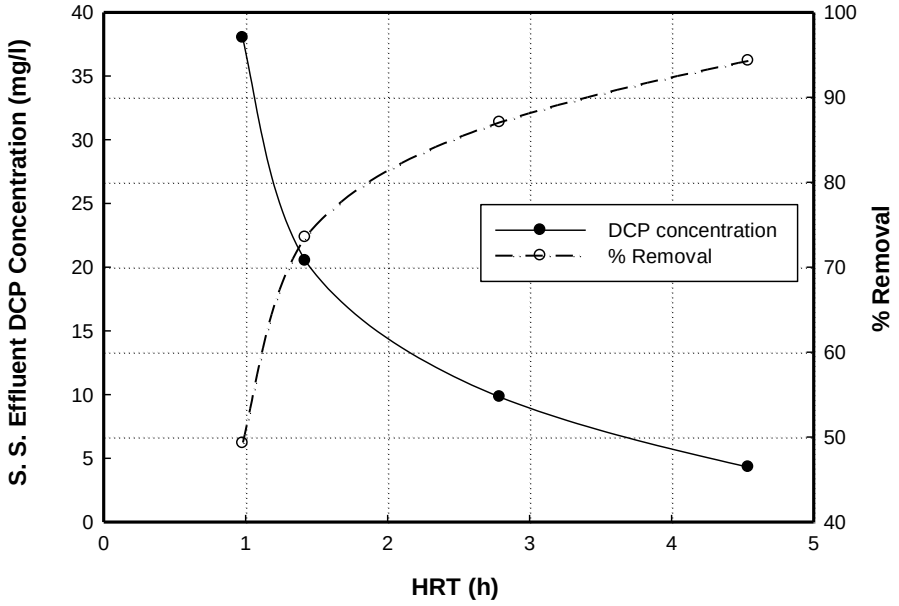


Fig. 4.4.6 Effect of liquid flow rate on steady state effluent concentration of DCP and percent removal: T = 30 °C; C_o = 75 mg/l; initial pH = 8.2; AFR = 3 l/min

In fact increasing the liquid flow rate may have two opposing effects. On one hand, the higher flow velocity around the PVA pellets may improve external mass transfer, especially for low substrate concentrations. On the other hand, the

reduced residence time decreases the contact time between the immobilized bacteria and the substrate. The former effect does not seem probable in the present case, since a relatively high initial 2, 4 DCP concentration of 75 mg/l is considered. What seems to be the dominating effect is the observed reduction in biodegradation efficiency as a result of reduced contact time between the immobilized biomass and the substrate.

At steady state, an important measure that has often been employed to assess the performance of a bioreactor is the elimination or degradation capacity (*DC*), which is a measure of the hydraulic throughput. It is simply defined by the degradation rate from a substrate material balance as:

$$DC = (S_i - S_e)D \tag{4.4.4}$$

where S_i and S_e are influent and effluent substrate concentrations, respectively; and D is the dilution rate (1/HRT). Another criterion often used in continuous mode operations is the organic loading rate (OLR) of the feed, defined as:

$$OLR = \frac{S_i F}{V} = \frac{S_i}{HRT} = S_i D \tag{4.4.5}$$

and is related to the amount of material that can be processed over a given period of time and therefore, for a given throughput, the reactor volume and associated capital and operating costs are minimized when HRT is made as small as possible. An increase in the LFR implies an increase in dilution rates and consequently a decrease in HRT. A plot of the effect of the liquid flow rate on the degradation capacity as well as the percent removal, when the initial DCP concentration was kept at 75 mg/l, is shown in Fig. 4.4.7. The degradation capacity increased to maximum at an LFR of about 11 ml/min and then started decreasing, whereas the percent removal decreased continually. This trend has also been described by others [213, 216]. Li and Loh [213] pointed out that there

exists an optimum degradation capacity with respect to hydraulic retention time because of the tradeoff in the amount of substrate degraded against the increase in feed rates. As the LFR increased, the substrate OLR increased and the steady state amount of substrate that was degraded decreased. The tradeoff between these two parameters resulted in optimal degradation capacity. This means that continuous operation of an SBBR system can be conducted at either the optimal DCP degradation capacity (in this case an LFR of 11 ml/min) or the highest feed rate permissible for a predetermined minimum amount of DCP degradation, for example 5.3 ml/min feed rate for a minimum 85% degradation of the 75 mg/l 2, 4 DCP feed (Fig. 4.4.7). This is in contrast to a batch operation, in which a bioreactor is operated until the desired substrate percent removal is achieved.

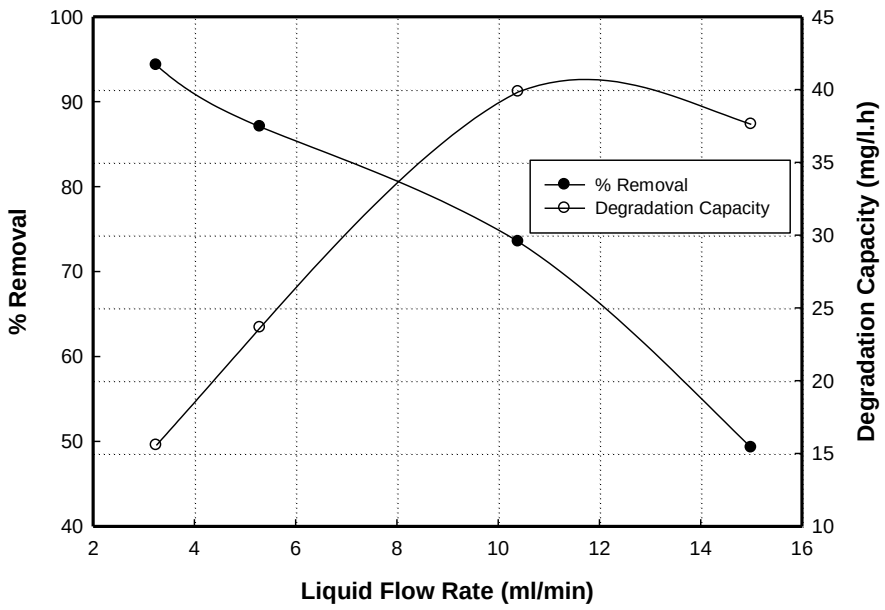


Fig. 4.4.7 Effect of liquid flow rate on degradation capacity and percent removal: T = 30 °C; C_o = 75 mg/l; initial pH = 8.2; AFR = 3 l/min

4.4.2.3 Effect of Influent Concentration of 2, 4 DCP

The effect of initial 2, 4 DCP concentration on the SBBR performance was studied at four initial concentrations of 25, 50, 75 and 100 mg/l. Both liquid and air flow rates were fixed at 5.3 ml/min and 3 l/min, respectively. The reduction of 2, 4 DCP concentration as a function of time at the different initial concentrations is shown in Fig. 4.4.8, and indicates that the initial biodegradation rate in the transient stages is highly dependent on the initial concentration. The time courses of the three initial concentrations of 25, 50 and 75 mg/l reached a steady state within one HRT (2.79 h=167 min), whereas it took the solution of 100 mg/l about 1.5 HRT (approximately 4 hours) to reach a steady state. The overall percent removal is plotted as a function of the initial concentration in Fig. 4.4.9. The figure also shows a plot of the initial biodegradation rates, calculated for the transient time before reaching a steady state. In every case the percent removal reached more than 88%, with minor fluctuations in the percent removal and the biodegradation rate: both were almost constant.

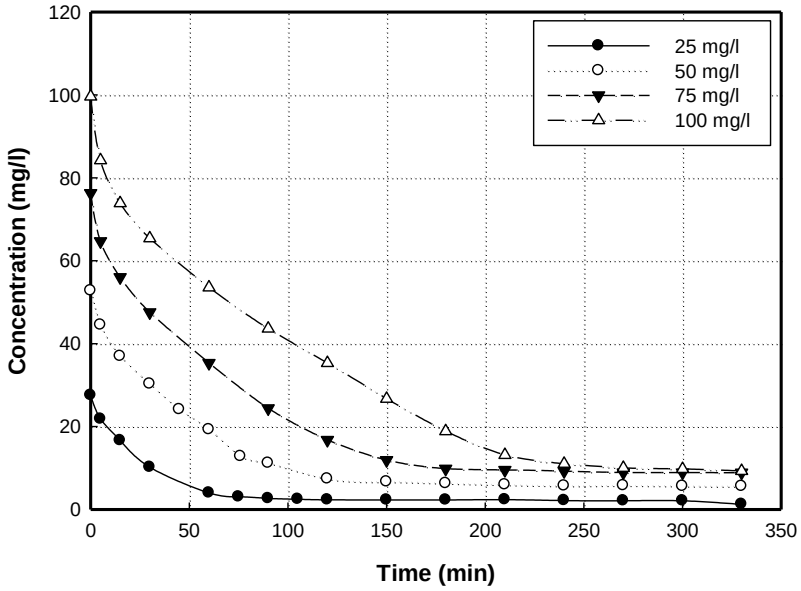


Fig. 4.4.8 Variation of concentration with time for different initial DCP concentrations in continuous operation: T = 30 °C; LFR = 5.3 ml/min; initial pH = 8-8.5; AFR = 3 l/min

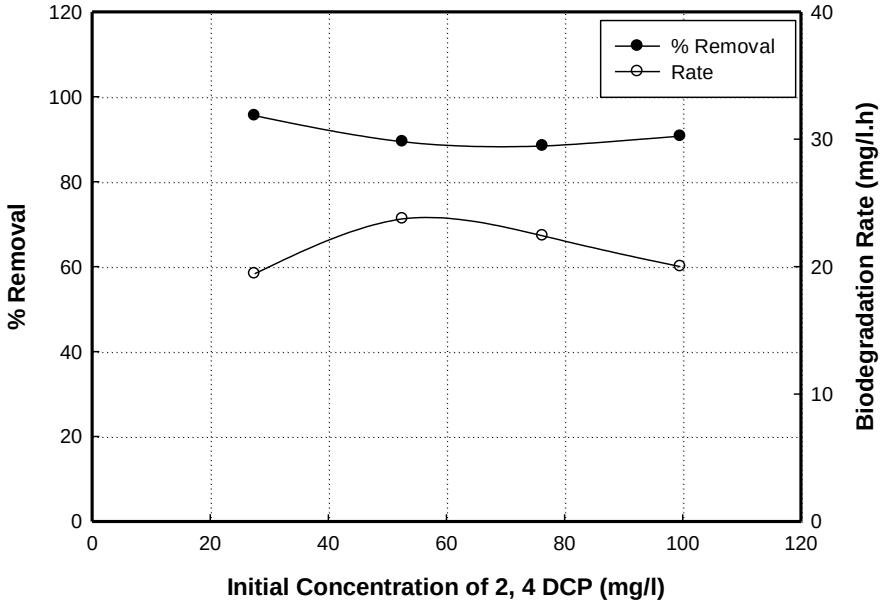


Fig. 4.4.9 Effect of initial DCP concentration on percent removal and initial degradation rate in continuous operation: T = 30 °C; LFR = 5.3 ml/min; initial pH = 8-8.5; AFR = 3 l/min

Referring to Section 4.4.2.2 regarding organic loading rate (ORL) and degradation capacity DC, the OLR and DC values from the combined sets of experiments (those on the effect of LFR for an initial concentration of 75 mg/l; and those on the effect of initial concentration with an LFR of 5.3 ml/min) were calculated by Eqs. (4.4.4) and (4.4.5). The results are shown in Table 4.4.2. For the sake of comparison the percent removal for each case is also included.

Table 4.4.2 Effect of LFR and initial concentration of 2, 4 DCP on degradation capacity and percent removal

Varying parameter		Effluent C (S_e) at S. S., mg/l	OLR, mg/l.h	DC, mg/l.h	% Removal
LFR, ml/min:	3.3	4.35	16.50	15.56	94.2
	5.3	9.88	27.14	23.64	87.0
	10.4	20.50	54.27	39.84	73.5
	15	38.00	76.37	37.61	49.3
C_o, mg/l:	27.53	1.30	9.86	9.41	95.5
	52.76	5.57	18.89	16.89	89.4
	76.30	8.85	27.32	24.15	88.4
	99.60	9.30	35.65	32.32	90.7

The results in Table 4.4.2 are depicted in Fig. 4.4.10. The combined effects of LFR and initial 2, 4 DCP concentration are combined in one factor, the organic loading rate, which is directly related to the degradation capacity. The aforementioned discussion on organic loading rate and degradation capacity in Section 4.4.2.2 is manifest again in another way. For example, consider the results obtained at an LFR of 5.3 ml/min; when 2, 4 DCP concentration was increased from 27 to 100 mg/l; the degradation capacity increased from 9.41 to 32.32 mg/l.h. It is believed that increasing the DCP concentration enhances the mass transfer from the bulk fluid to the biomass, leading to two major effects on the cells. On one hand, the larger mass transfer of DCP allows more cells to grow,

and so generates higher cell density. On the other hand, the higher DCP concentration in the PVA particles exerts higher substrate inhibition on the cells [213]. These two effects negate each other at the 2, 4 DCP concentration range investigated (25-100 mg/l). The percent removal maintained a high value, exceeding 85% with an OLR of about 35 mg/l.h, and then it started decreasing, whereas the degradation capacity continued to increase up to an OLR of about 55 mg/l.h. The results presented in this investigation demonstrate that operating conditions can be compromised based on either the desired degradation efficiency (percent removal) or the desired degradation capacity.

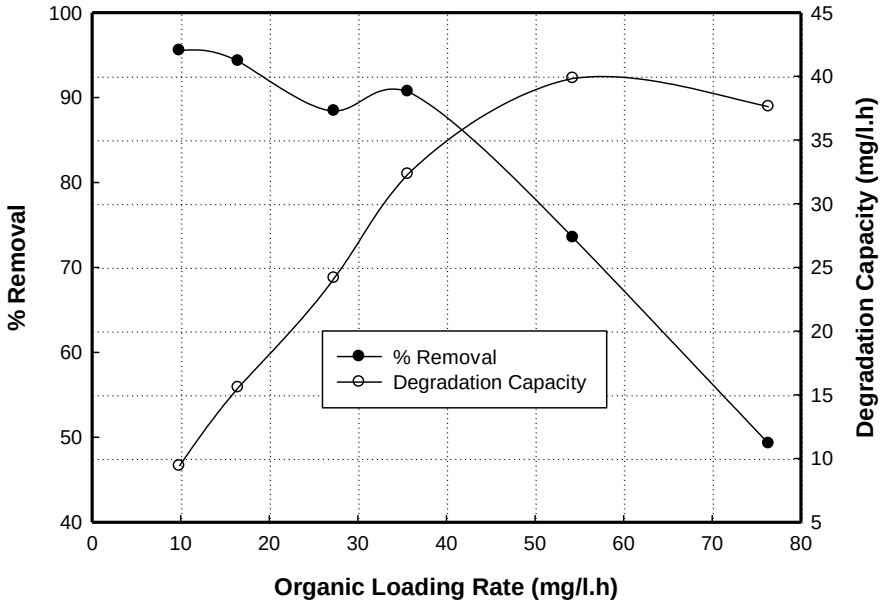


Fig. 4.4.10 Effect of organic loading rate on degradation capacity and percent removal: T = 30 °C; initial pH = 8-8.5AFR = 3 l/min

4.4.3 Response to Shock Loading

The performance of the SBBR was evaluated in terms of stability and tolerance to organic and hydraulic shock loads, resulting from concentration and flow rate fluctuations. In order to study the capability of the system to withstand sudden increases in 2, 4 DCP concentration, the system was operated at an HRT of 2.79 hours, resulting from an LFR of 5.3 ml/min, with an influent DCP concentration of 75 mg/l. After 3.5 hours, the system was exposed to a sudden organic load shock by increasing the influent concentration of 2, 4 DCP to 100 mg/l, which was maintained for 2.5 hours, then the normal influent concentration of 75 mg/l was resumed. The response of the system to such an organic shock load is illustrated in Fig. 4.4.11. It clearly shows that the SBBR was able to respond to the shock in a stable manner and exhibited a fast recovery after the shock load had ceased.

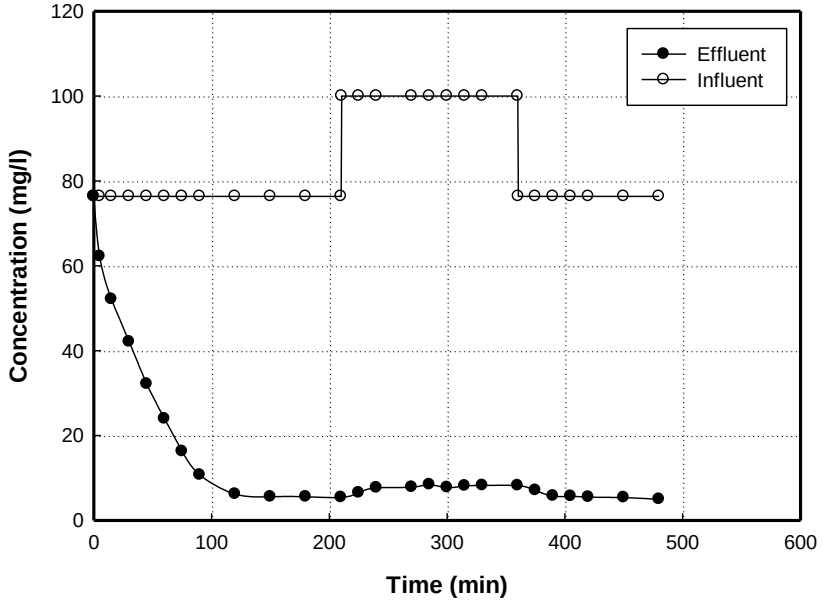


Fig. 4.4.11 Response of SBBR to organic shock loading: T = 30 °C; LFR = 5.3 ml/min; initial pH = 8.2; AFR = 3 l/min

Similarly, the ability of the SBBR system to handle a hydraulic shock load was examined at a constant DCP influent concentration of 75 mg/l, while suddenly decreasing the HRT from 2.79 hours to 1.42 hours, resulting from LFR of 5.3 and 10.4 ml/min, respectively. The shock was imposed after 3.5 hours and persisted for 2.5 hours, then it was stopped and the normal HRT of 2.79 hours was resumed. The response behavior is shown in Fig. 4.4.12. The system again demonstrated stability in its response to the shock load and could sustain high hydraulic loading.

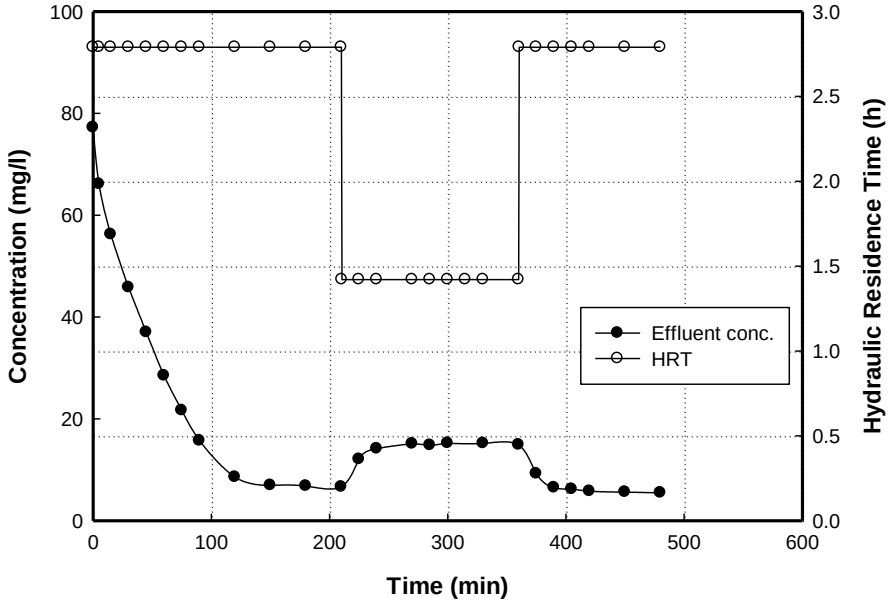


Fig. 4.4.12 Response of SBBR to hydraulic shock loading: T = 30 °C; C_o = 75 mg/l; initial pH = 8.2; AFR = 3 l/min

Shock loads of phenolic wastewater have been reported to be responsible for severe disruptions in conventional industrial biological treatments and may result in the complete failure of the treatment process [130, 131]. Systems with immobilized cells are known to be more stable to shock loads [111, 129]. A robust industrial application should be able to cope with organic shock loading

rates and hydraulic fluctuations. In this regard, the SBBR has proven to be highly stable and efficient in responding to shock loads.

4.4.4 Biodegradation of 2, 4 DCP in a Packed Bed Bioreactor (PBR)

To compare the performance of SBBR to that of a packed bed reactor, experiments were conducted to investigate the degradation 2, 4 DCP in the packed bed reactor described in Section 3.6.2 and according to the procedure outlined in Section 3.7.4.2. The comparison would be more valid for a high LFR, so an LFR of 15 ml/min was kept constant in every experiment, the flow was upwards in a flooded mode operation. The influent DCP concentration was 75 mg/l. The time courses of 2, 4 DCP concentration reduction are presented for three runs in Fig. 4.4.13, together with that of the SBBR at the same LFR, for the sake of comparison.

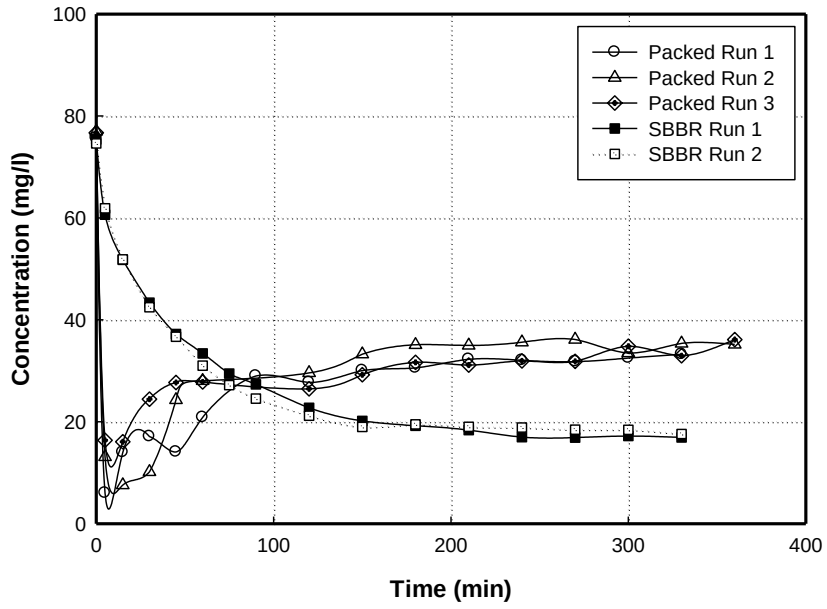


Fig. 4.4.13 Biodegradation of 2, 4 DCP in PBR; T = 30 °C; C_o = 75 mg/l; LFR = 15 ml/min; initial pH = 8.2; AFR = 3 l/min

The superiority of the SBBR performance is pronounced in terms of stability and a higher percent removal. The instability of the PBR is manifest by the fluctuating profiles of the three runs, especially in the unsteady state region, whereas the SBBR showed higher consistency in the two runs shown. Such a difference in the behavior of two reactors is expected in light of the better mixing characteristics of the SBBR and the mass and heat transfer limitations of the PBR. Several studies highlighted the favourable mass transfer provided by fluidization, not to mention better contact between the phases and a lower pressure drop provided by spouted beds [71, 199, 217]. On the other hand, packed bed reactors were reported to have problems with the formation of dead zones, channeling [164, 215] and poor temperature control [49]. In this context, it was noticed during experimentation that although the PBR volume is less than half the volume of the SBBR, a greater heating load was required by the PBR to keep the same 30 °C temperature inside the reactor. Moreover, oxygen limitation was also reported [72].

After investigating the biodegradation of 2, 4 DCP in an SBBR and a PBR in continuous mode operation, it is advantageous to compare the degradation capacities (DC) with those reported in the literature. Referring to Table 4.4.2, the maximum DC for the SBBR was 37.6 mg/l/h achieved at an LFR of 15 ml/min, with a percent removal of 49.3%. The values in the table belong to experiments performed during that time range. After a time span of 45 days of running the biodegradation experiments in a continuous mode, the DC of the SBBR at the same LFR of 15 ml/min (HRT 0.98 hours) improved significantly to 58.9 mg/l.h (1414 g/m^3day) and the percent removal increased to 77% (Fig. 4.4.13). The percent removal for the PBR had an average of 53.3%, however considering the

small volume of the PBR (~ 400 ml), the DC was calculated by Eq. (4.4.4) to be 90 mg/l.h (2160 g/m^3day), where the dilution rate *D* is substituted by (LFR/reactor volume). Different studies on 2, 4 DCP biodegradation report conflicting values for the DC, depending on the volume. Safont et al. [199] pointed out that when calculating OLR and DC, different results may be obtained depending on the volume. Quan et al. [69] did not consider the volume in their reported values, which were about 1.25 mg/h for an initial concentration of about 30 mg/l (compared to 51.9 and 36 mg/h, for SBBR and PBR, respectively). Safont et al. [199] reported a value of 2.79 kg/m^3d in the treatment of phenol in a draft tube SBBR, which was considered high enough for successful treatment. Comparing this value of phenol to the degradation capacities obtained in this study by the SBBR and PBR for DCP, which is a much more difficult substance for biodegradation than phenol; and expecting the activity of the biomass to improve upon continuous operation, the values are promising for industrial application. Other reported values from the extant literature are listed in Table 4.4.3.

Table 4.4.3 Degradation capacities and percent removals of DCP from different studies of continuous operation

Initial DCP concentration (mg/l)	Degradation capacity (g/m^3.d)	% Removal	Ref.
91.5	35.2	95.6	[218]
30	45-120	100	[23]
50	37.5	100	[53]
	113.3	78.7	[96]
	93-155	52-86	[96]
120		87	[96]
110	1202	> 98	[107]
75	775-1414	77-90	This work (SBBR)

4.5 MODELING OF THE BIODEGRADATION OF 2, 4 DCP

Mathematical modeling can be helpful in understanding the behavior of bioprocesses and predicting the component concentrations of the system [10]. Modeling any biodegradation process involves relating the specific growth rate of the biomass to the consumption rate of the substrate. However, in heterogeneous reactions involving solid phase catalysts, concentration gradients occur within phase boundary layers around gas bubbles and solids. More pronounced gradients are found inside solid biocatalysts such as pellets, biofilms and immobilized cells. In all of these systems, the rate of the reaction depends on the rate of mass transfer outside or within the solid catalyst [122].

This section presents results on kinetic modeling of batch biodegradation of 2, 4 DCP in SBBR, followed by dynamic modeling and simulation in the continuous mode operation. It also presents degradation data of DCP by free suspended *P. putida* during the acclimatization step and during batch treatment in the SBBR to arrive at a predictive analysis for the diffusion limitations in immobilized systems based on intrinsic kinetics of degradation by the free cells.

4.5.1 Determination of Kinetic Parameters

In Section 2.11, based on material balance, the rate of biomass growth and the rate of substrate utilization (both in mg/l.h) was represented by Eqs. (2.11.1) and (2.11.2), respectively:

$$\frac{\mathrm{dX}}{\mathrm{dt}} = \mu \mathrm{X} - k_{\mathrm{d}} \mathrm{X} = \mu_{\mathrm{net}} \mathrm{X} \qquad \text{or} \qquad \frac{\mathrm{dlnX}}{\mathrm{dt}} = \mu_{\mathrm{net}} \tag{2.11.1}$$

$$\frac{\mathrm{d}S}{\mathrm{dt}} = -\frac{\mu \mathrm{X}}{\mathrm{Y}} \tag{2.11.2}$$

where Y is the cell mass yield (g/g) = dX/d*S*; X is the biomass concentration (mg/l); *S* is the substrate concentration (mg/l); k_{d} is the endogenous decay coefficient (h^{-1}); μ is the specific growth rate (h^{-1}) [5, 185]. During the

exponential growth phase, k_d may be neglected [158]. The specific consumption rate of the substrate (q_s) is expressed as follows:

$$q_s = \frac{dS}{Xdt} = \frac{\mu}{Y} \quad (4.5.1)$$

A variety of kinetic models have been used to describe the dynamics of microbial growth on phenols (Table 2.11.1). The Haldane Model (Eq. (2.11.4)), which incorporates the inhibitory effect of toxic substrates, has been the most widely used. This model can be used to predict variations in the biodegradation rate with initial substrate concentrations, utilizing the relation in Eq. (2.11.4) and assuming that Y is constant over the concentration range. This assumption is valid if the substrate concentration is much higher than K_s (i.e. S [redacted] K_s) [11]. Then, the model form for the degradation rate q (mg/l.h) is:

$$q = \frac{q_{max} S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \quad (4.5.2)$$

where q_{max} is the maximum degradation rate (mg/l.h), K_s is the half saturation coefficient (mg/l) and K_i is the substrate inhibition constant (mg/l). Eq. (4.5.2) was used with the experimental data presented in Section 4.3.1.5 and illustrated in Fig. 4.3.8 (b). The degradation rate q was determined from a plot of the substrate concentration S versus time for each initial concentration S_o, as explained in Section 4.3.1.5, with results shown in Fig. 4.3.9 (b). From the values of q and S_o, the values of kinetic parameters were obtained by a non-linear regression using SigmaPlot software (SPSS 11.0). Two sets of kinetic parameters were obtained based on Fig. 4.3.9 (b): one is based on the normal rate and the other is based on the global rate. The Haldane Model fitted the data adequately as shown in Fig. 4.5.1 with coefficients of determination, R^2, of 0.991 and 0.994 for the rate and

global rate, respectively. The kinetic parameters are listed in Table 4.5.1 including values obtained by other researchers for the biodegradation of 2, 4 DCP.

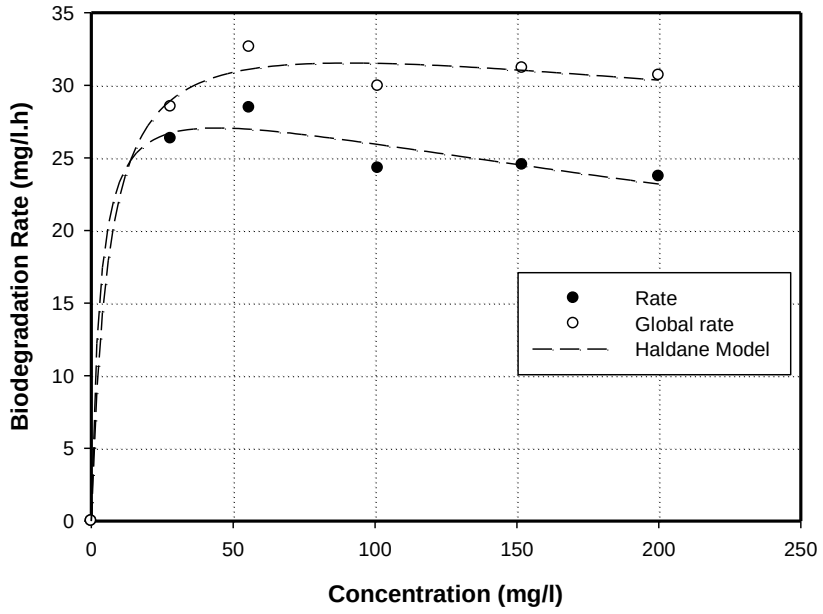


Fig. 4.5.1 Kinetic fitting of 2, 4 DCP degradation using Haldane Model

Table 4.5.1 Kinetic parameters (Haldane Model) of 2, 4 DCP biodegradation

Microorganism	q_{max}	Concentration (mg/l)	K_s (mg/l)	K_i (mg/l)	Ref.
P. putida	30.77 mg/l.h	25-200	3.03	641.62	This study
	Global rate: 35.69 mg/l.h		5.93	1364.5	
Aerobic granular sludge	358.4 mg/ g MLVSS/h	13-230	175.2	4.6	[143]
Mixed culture	112 mg/ g MLVSS/h	12.5-108.8	13	44.46	[60]
Aerobic granules	478 mg/ g MLVSS/h	50-300	587	19.1	[15]
Mixed culture in GAC (granulated activated carbon)	0.33 mg/g MLSS/h	100	3	360 $\pm$ 42	[45]

Substrate inhibition results in lower degradation rates and slower biomass growth rates due to an increase in energy expenditure for maintaining the cells [219]. A larger K_i value indicates that the culture is less sensitive to substrate inhibition, whereas an increase in the value of K_s indicates an increase in the biodegradation time, as it is related to slower biomass growth. It has been reported that a quantitative analysis proves that an increase in K_s value from 1 to 10 g/m^3, then the time requirement for a 100% reduction level is increased from 20,000 to 50,000 s. Thus, K_s plays a very important role in the dynamics of microbial substrate degradation [7]. The half saturation coefficient influences the growth kinetics in low concentration regions [5]. With reference to the Table 4.5.1, the low K_s and high K_i values obtained in this study are obvious.

It has been reported in the literature that for a wide range of phenols concentrations the Haldane equation simulates a phenolic substrate degradation profile only when different sets of model parameters are used [10]. Different Haldane kinetic parameter values were obtained even when the same microbial strain was studied. It is evident that there is a considerable lack of consistency in the Haldane parameters for specified combinations of an organism and a substrate and there are different reasons for this variability. The inadequacy of the Haldane equation has been attributed to the inhibition of metabolic intermediates of phenols degradation [8, 10, 55, 196]. Monterio et al. [184] and Contreras et al. [200] explained the variation of kinetic parameters by the different inoculum's history, with regard to acclimatization; the different microorganisms and media that were used; the different initial substrate concentrations and the mode of growth cultivation (batch versus continuous cultivation) and environmental factors such as pH and temperature. However, Bajaj et al. [193] compared the

Han-Levenspiel Model (Eq. (2.11.6), Table 2.11.1) and the Haldane Model and concluded that the former is based on the effect of a product that may be formed during degradation, whereas the latter is based on the effect of a substrate on a culture growth. Therefore, they pointed out that for studies with well acclimatized biomass, in which there seemed to be no accumulation of metabolic product during the degradation process, the Haldane Model would better represent the degradation process. This applies to the present study, as no accumulation of intermediates, as detected by color change in the reaction medium, was noticed during the experimentation.

On the other hand, Wang and Loh [196] found that the Haldane equation was not sufficient for modeling phenol degradation, especially for high initial phenol concentrations, although it could correlate specific growth rates with initial substrate concentrations very well. Nuhoglu and Yalcin [10] argued that the degree of inhibition is determined by K_s/K_i ratio, and not just by K_i alone. The larger the value of K_s/K_i, the smaller the value of $\mu*$ (μ value that corresponds to critical substrate concentration S^* where $d\mu/dS$ = 0.0) is relative to μ_{max}, and thus, the greater the degree of inhibition, as evident from the following Eq. (4.5.3). The value S^* is the critical substrate concentration beyond which the substrate removal rate drops.

$$\mu = \frac{\mu_{max} S}{K_s + S + \left(\frac{S^2}{K_i}\right)} \tag{2.11.4}$$

$$\mu^* = \frac{\mu_{max}}{2\left(\sqrt{K_s/K_i}\right) + 1} \tag{4.5.3}$$

For this study, the K_s/K_i values were 4.72 x (10^{-3}) and 4.35 x (10^{-3}), based on a normal rate and a global rate, respectively. These values show very low degrees of inhibition compared to the values in other studies. Wang and Loh

[196] pointed out that particularly for $S_0 \gg \sqrt{K_s K_i}$, which is the critical substrate concentration S^*, the Haldane Model will erroneously model substrate consumption, especially when the substrate has been consumed to a significant extent. According to Onysko [101], the $\mu*$ value is a key factor in predicting washout in steady state conditions in continuous bioprocesses; using a Haldane-based prediction would result in lower predicted critical dilution rates. Several other studies emphasized the importance of $\mu*$ and K_s/K_i values [143, 153, 210].

Apparently, there exists a need for better understanding of substrate inhibition kinetic models. In particular, the gradual lessening of substrate inhibition as the substrate concentration decreases warrants further investigation into substrate inhibition kinetic models. However, a more structured model would require greater effort in terms of estimating an increased number of parameters and the identification of suitable variables [220].

4.5.2 Dynamic Modeling of DCP Degradation in Continuous Operation

The global biodegradation rate was calculated based on the specific consumption rate, where 2, 4 DCP is the limiting substrate, 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:

$$\frac{dM_A}{dt} = F(S_{Ao} - S_A) - r_A V \qquad (4.5.4)$$

where M_A is the mass (mg) of substrate A in the reactor, t is the time (h), F is the volumetric flow rate (l/h), S_A is the substrate concentration at a certain time, $-r_A$ is the rate of removal of substrate A (mg/l.h) and V is the reactor volume (l).

Dividing the equation by V;

$$\frac{dS}{dt} = \frac{F}{V}(S_o - S) - r_S \qquad (4.5.5)$$

The substrate uptake rate is given by:

$$-r_s = q \tag{4.5.6}$$

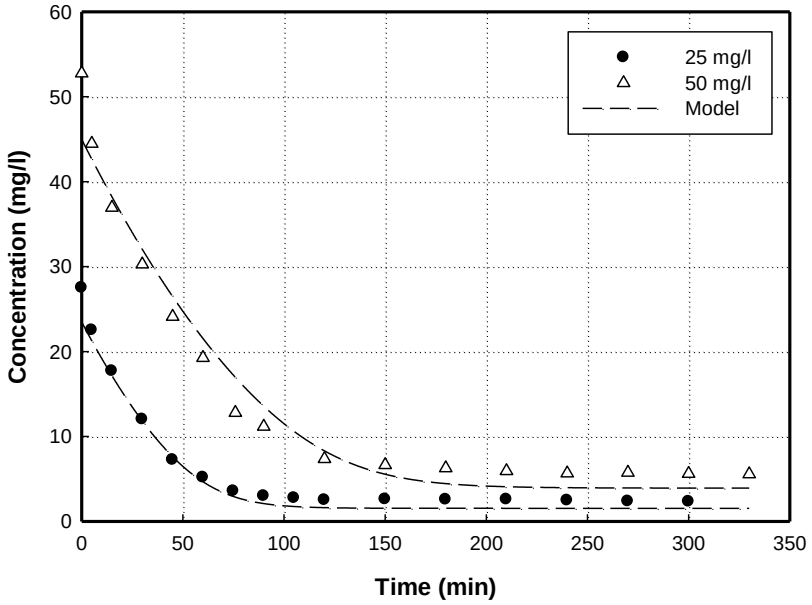
Assuming constant yield in the bioreactor as mentioned in Section 4.5.1, the substrate consumption can be described as follows:

$$-r_s = \frac{q_{\max} S}{K_S + S + \left(\frac{S^2}{K_i}\right)} \tag{4.5.7}$$

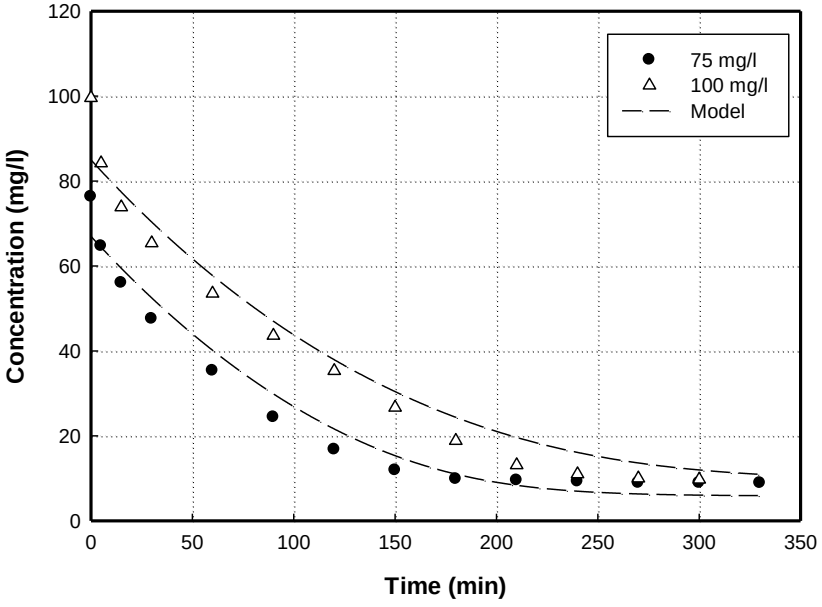
Combining Eqs. (4.5.5) and (4.5.7), the change of 2, 4 DCP concentration in the reactor can be evaluated as:

$$\frac{dS}{dt} = \frac{F}{V}(S_o - S) - \frac{q_{\max}\ S}{k_s + S + \left(\frac{S^2}{K_i}\right)} \tag{4.5.8}$$

The model was applied to the experimental data presented in Section 4.4.2.3, for which the 2, 4 DCP degradation profiles were displayed in Fig. 4.4.8. The variation of the substrate concentration with time, as given by Eq. (4.5.8), was evaluated using (E-Z Solve). The model predictions are compared with the experimental data in Fig. 4.5.2 (a) for the initial DCP concentrations of 25 and 50 mg/l and Fig. 4.5.2 (b) for the initial DCP concentrations of 75 and 100 mg/l. The figure shows very good agreement between the model predictions and the experimental data, except for the initial fast drop resulting from water release from PVA particles. This agreement, especially at the lower influent concentrations, confirms the validity of the proposed model for simulating the continuous biodegradation of 2, 4 DCP in SBBR. The disagreement at the higher initial concentrations is expected as it is known that the Haldane Model is inadequate for describing the degradation of phenols at high initial concentrations, although it can correlate the specific degradation rates with initial substrate concentrations very well.



(a)



(b)

Fig. 4.5.2 Variation of DCP concentration with time in continuous operation: experimental and simulated concentration for the data in Fig. 4.4.8: (a) (S_o=25 and 50 mg/l), (b) (S_o=75 and 100 mg/l)

4.5.3 Degradation of DCP by Free Bacteria and Mass Transfer Limitations

Because concentrations vary in solid catalysts, local rates of reaction also vary depending on the position within the particle. This local rate of reaction is known as the true rate or intrinsic rate. Kinetic equations for heterogeneous reactions are developed with mass transfer constraints. The rate of disappearance of substrate from the bulk liquid must equal the overall rate of conversion by reaction; this is the so-called observed rate. In heterogeneous systems, rates of reaction and substrate mass transfer are interrelated. Rate of mass transfer depends on the concentration gradient established within the solid catalyst; this in turn depends on the rate of substrate uptake by the reaction. On the other hand, the rate of the reaction depends on the availability of the substrate, which in turn depends on the rate of mass transfer [122]. One approach to assess intrinsic kinetics is to carry out the biodegradation process using free biomass, with the same mass and under the same conditions as with immobilized cells.

4.5.3.1 Acclimatization of Free Cells and Batch Degradation of 2, 4 DCP

Based on the above discussion, free cells of *P. putida* were acclimatized to 2, 4 DCP up to 200 mg/l. The degradation profiles at different initial concentrations are shown in Fig. 4.5.3. The biodegradation rates were calculated from the slopes of the plots and compared to those obtained by immobilized cells during acclimatization (Fig. 4.5.4). Compared to free cells, immobilized cells exhibited a lower degradation rate when DCP concentrations were below 110 mg/l. However, when DCP concentrations were over 110 mg/l, immobilized cells performed with better degradation rates. The toxicity of DCP at high concentrations could inhibit the growth of free cells and result in a lower degradation rate of 2, 4 DCP, whereas the carrier material for the immobilized

cells provided a protective shelter against the toxicity of 2, 4 DCP. This trend with immobilized cells has also been reported by other researchers [2, 106].

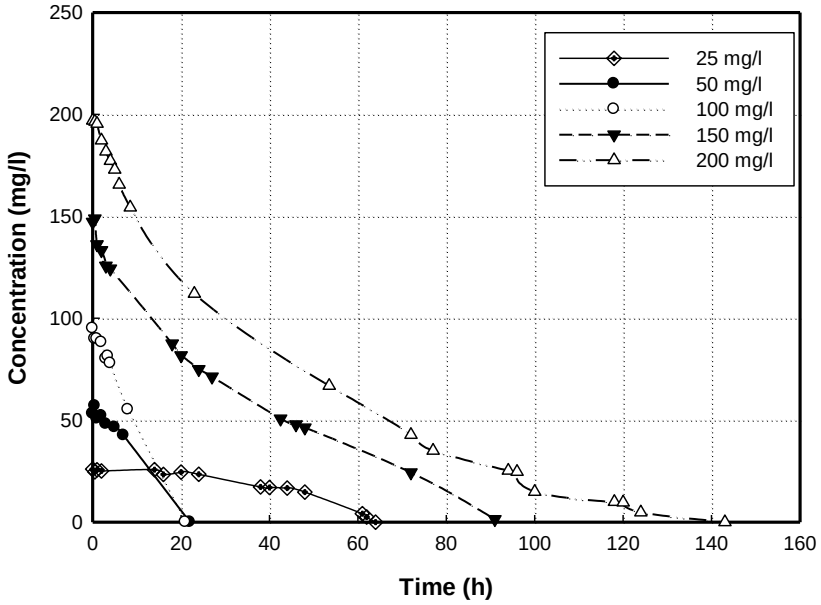


Fig. 4.5.3 Degradation profiles during acclimatization of free cells: T = 30 °C

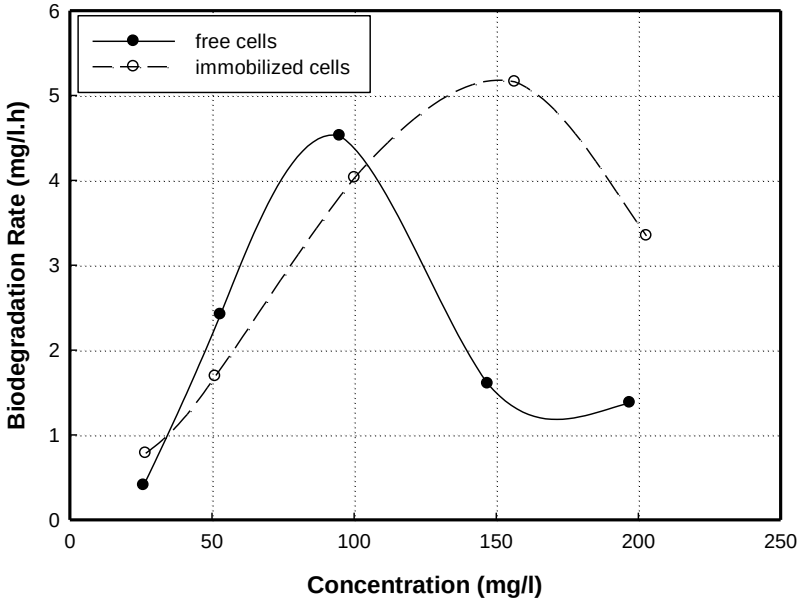


Fig. 4.5.4 Biodegradation rates by free and immobilized bacteria during acclimatization: T = 30 °C

At the conclusion of the acclimatization stage, two runs were conducted for biodegradation of 2, 4 DCP in the SBBR by free cells under normal operating conditions. The degradation rates obtained from these runs had to be compared to those obtained from two other runs performed using the immobilized cells, at the end of their acclimatization to DCP. The results are shown in Fig. 4.5.5. To confirm the growth of the free cells, a cell count analysis was made giving a density of 48.32 x (10^4) and 328.9 x (10^4) cells/ml, for bacterial suspension at the start of acclimatization and for the same suspension after running the two experiments, respectively. Discussion of these results is presented in the next section.

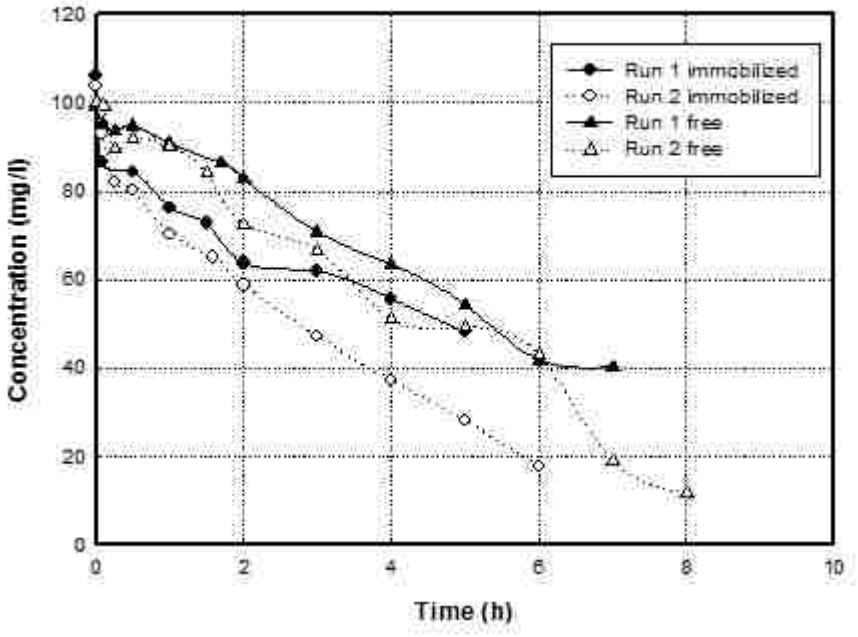


Fig. 4.5.5 Biodegradation of 2, 4 DCP by free and immobilized bacteria: C_o = 100 mg/l; T = 30 °C; initial pH = 8.0; AFR = 3 l/min

4.5.3.1 Kinetic Model in the Immobilized Cell System

One of the major objectives in analyzing heterogeneous reactions is to determine the relative influences of mass transfer and reaction on observed

reaction rates. If a reaction proceeds rapidly, it is likely that mass transfer will be too slow to supply substrate at the required rate. The observed rate would be significantly affected and the reaction would be a diffusion-limited reaction [122]. When cells are entrapped in a microporous matrix such as PVA gel particles, the matrix may cause internal mass transfer resistance to the substrate. During the biodegradation process, 2, 4 DCP, being a substrate for these cells, diffuses from the bulk liquid to the surface of the PVA gel pellets and then into the pores of the pellets. The cells entrapped inside the pellets consume DCP leading to simultaneous diffusion and reaction. The overall rate (observed rate) would depend primarily on the effective diffusivity within the microporous matrix of the PVA. With the following mathematical manipulations, a simplified method is used to estimate the effective diffusivity of 2, 4 DCP within the PVA pellets in a batch test and then compare it to its diffusivity in water (the case with free cells), to assess the extent of internal diffusion resistance [3, 72, 221, 222]. In the model, the following basic assumptions are applied:

1. The substrate concentrations at the particle surface are the same as those in the bulk of the liquid. In other words, the liquid phase mass transfer resistance is negligible [223]. This is justified because the SBBR is characterized by intense mixing; El Naas, et al. [136] reported that the external mass transfer can thus be ignored and uniform concentration across the height of the reactor can be assumed.
2. All PVA pellets are isothermal and spherical with the same radius.
3. Entrapped cells are uniformly distributed in the pellets.
4. The influence of free cells released from pellets into the bulk liquid is negligible.

5. Substrate concentration varies in only one direction. For a sphere, it is assumed that it varies only in the radial direction.
6. The degradation kinetics are expressed by first order kinetics (most of the studies which approached kinetics modeling in immobilized cells considered this assumption, with the justification that it is correct for degradation at lower phenol concentrations [3, 72, 222, 223]).

On the basis of these assumptions, Eq. (4.5.9) states that the diffusion rate of 2, 4 DCP is equal to the reaction rate in a steady state:

$$\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr} = \frac{\rho_p}{D_e}v = \frac{\rho_p}{D_e}kC \qquad (4.5.9)$$

where C is the DCP concentration within the pellet (mg/l), r is the radial position within the pellet (cm), ρ_p is the density of dried cells (g/cm^3), D_e is the effective diffusivity (also called diffusion coefficient) of 2, 4 DCP within the pellet (cm^2/s), and k is the first order degradation rate constant l/(g dried organism.h), v is the actual biodegradation rate.

Solving Eq. (4.5.9) with boundary conditions dC/dr = 0 at r = 0 and C = C_s at r = R, the distribution of DCP concentration within the pellet can be obtained

$$\frac{C}{C_s} = \frac{r}{R}\frac{\sinh(3\emptyset r/R)}{\sinh(3\emptyset)} \qquad (4.5.10)$$

where R is the radius of the pellet (cm), C_s is the DCP concentration at the pellet surface (mg/l), and $\emptyset$ is the Thiele modulus defined as:

$$\emptyset = \frac{R}{3}\sqrt{\frac{k\prime}{D_e}} \qquad (4.5.11)$$

where k' is the rate constant ($k' = k\rho_p$). In the presence of diffusion limitation, the rate of substrate consumption is expressed in terms of the effectiveness factor. The effectiveness factor (η) is defined as the ratio of the reaction rate with

diffusion limitation (actual or observed rate v) to the reaction rate with no diffusion limitation (or the rate evaluated at outer surface conditions v_s):

$$v = \eta v_s \tag{4.5.12}$$

Assuming the actual degradation rate v is equal to the rate of diffusion at r = R

$$v = 4\pi R^2 \left(-\frac{dC}{dr}\right)_{r=R} \tag{4.5.13}$$

At the outer surface, the degradation rate is expressed as:

$$v_s = 4\pi r R^3 k' \tag{4.5.14}$$

Referring to Eq. (4.5.10) for differentiation and substituting in Eq. (4.5.12), then

$$\eta = \frac{1}{\phi}\left[\frac{1}{\tanh 3\phi} - \frac{1}{3\phi}\right] \tag{4.5.15}$$

The relative importance of diffusion and degradation reaction processes is evident from Eq. (4.5.15). For small values of ∅, η is close to 1 and the internal mass transfer has less effect on the rate and the degradation reaction step controls the overall rate. These conditions mean that the rate at the center of the pellet is the same as the rate at the surface and the volume of pellet is fully effective. On the contrary, for large values of ∅, the internal diffusion has a large effect on the rate. That is, the relatively slow diffusion in the pellet indicates that the substrate has been degraded at the outer periphery before it diffuses into the pellet [72, 215].

Referring back to Fig. 4.5.5 for the comparison of DCP degradation by free and immobilized cells, the following analysis was applied:

Run 1: the degradation rates of free and immobilized cells were found to be 9.06 and 7.8 mg/l.h, respectively. To account for sphericity, the radius $R_{nonsphere}$ was calculated from [224]

$$R_{\text{nonsphere}} = 3\left(\frac{V_p}{a_p}\right) \tag{4.5.15}$$

where V_p and a_p are the volume and external surface area of a single non-spherical pellet, respectively. For a 1 cm^3-cubic pellet, V_p and a_p are 1 cm^3 and 6 cm^2, respectively and $R_{nonsphere}$ is 0.50 cm. So η = 7.80/9.06 = 0.86; this gives $\emptyset$ value of 0.54, *k'* was obtained from ln (C_o/C) in Fig. 4.5.5 to be 0.272 h^{-1}. Substituting in Eq. (4.5.11), the value of D_e was calculated to be 7.2 x (10^{-6}) cm^2/s, which is comparable to the diffusivity of 2, 4 DCP in water at 25 °C , which equals 8.77 x (10^{-6}) cm^2/s [225]. The high value of η, the low value of $\emptyset$ and the comparable value of diffusivity in the PVA gel matrix to that in water indicate that the internal mass transfer resistance was small and that degradation of 2, 4 DCP by PVA immobilized cells in the SBBR is not limited by diffusion.

Run 2: the degradation rates of free and immobilized cells were almost identical in the range 10.7-11 mg/l.h, which mean that there was no internal or external resistance. This can be attributed to the improvement of the cells performance upon repeated use directly after acclimatization. Hsieh et al. [72] noted in their study on phenol degradation by *P. putida* immobilized in chitosan beads that the Thiele modulus decreased by a factor of 5 and D_e increased by a factor of 37 for repeatedly used immobilized cells. This indicated a shift in the mechanism for phenol degradation from diffusion limitations for the freshly prepared beads, towards reaction limitations for the twice used beads. Although these predictions are simple and limited in concept, they are useful in visualizing the process. A good gel polymer for immobilization must be porous and relatively soft to allow diffusion of reactants and products to and from the interior of the particle [122].

4.6 BATCH TREATMENT OF REAL REFINERY WASTEWATER IN SBBR

Petroleum refineries are a large consumers of water, especially for cooling systems, crude desalting, distillation, stripping and for flushing during maintenance and shut down [26]. The estimated average water consumption in processing a barrel of crude oil is 65–90 gallons (246–341 liters) [42]. Petroleum refinery wastewater is characterized by the presence of several aromatic hydrocarbons and also inorganic substances [130]. It is generally accepted that a light fraction of aliphatic, aromatic petroleum hydrocarbons, ammonia, sulfides, cyanides and halogenated organic substances contribute to high chemical oxygen demand (COD) [29, 42]. The quantity and characteristics of wastewater depends on the process configuration [28]. It has been reported that refineries generate polluted wastewater, containing COD levels of approximately 3600–5300 mg/l [42], phenol levels of 20–200 mg/l, benzene levels of 1–100 mg/l, heavy metal levels of 0.1–100 mg/l for chrome and 0.2–10 mg/l for lead, and other organic and inorganic pollutants [26, 28]. Phenolic compounds contribute to COD in industrial effluents ranging from 40% to 80% of the total [226]. Although studies on refinery wastewater treatment are well documented in the literature, most of these studies have been conducted by using model wastewater [28].

As the main objective of this work is the transfer of the results to industrial application, this section presents results on the batch treatment of real refinery wastewater (RRW) for the biological removal of phenolic compounds in a spouted bed bioreactor by PVA immobilized *P. putida*. Due to the diversity and complexity of refinery wastewater, phenols have been accepted as a suitable compound to indicate the biodegradation performance [130]. The terms ‘phenols’ or ‘total phenols’ or ‘phenolics’ in wastewater treatment technology are used

interchangeably either to denote simple phenol or a mixture of phenolic compounds in wastewater [226]. It has been reported that for the quantification of the performance of industrial wastewater treatment processes, most studies consider global variables such as COD and TOC (total organic carbon) as conventional parameters for the evaluation of the quality of wastewater [154, 227]. Accordingly, these variables were considered for monitoring the treatment process at this stage of the experiments. RRW samples were obtained from a local petroleum refinery and were preserved in dark, plastic containers. To ensure a stable composition, the feed container was refrigerated at 4 °C.

4.6.1 Characterization of RRW

The wastewater was dark greenish in color and had a pungent odor. Analyses of the samples are given as ranges of values in Table 4.6.1. It was noticed that *m*, and *p*-cresol peak in the GC chromatogram at the same retention time, so their concentration is given as a sum. The same observation applies for 2, 4 DCP and 2, 5 DCP.

Table 4.6.1 Characteristics of RRW samples as ranges of values

Parameter	Value
pH	8.4-8.8
COD (mg/l)	2500-2900
TOC (mg/l)	770-800
Total phenols (mg/l)	160-185
Phenol (mg/l)	35-40
o-Cresol (mg/l)	62-67
m, *p*-Cresol (mg/l)	37-47
2, 4 & 2, 5 DCP (mg/l)	28-32

4.6.2 Results from the Acclimatization Process

The percent removal of 2, 4 and 2, 5 DCP as well as the percent removal of total phenols, at the end of 24 hours of each treatment in the stepwise acclimatization of the immobilized bacteria are listed in Table 4.6.2. At the end of 24 hours, the color was no longer green.

Table 4.6.2 Removal of 2, 4 and 2, 5 DCP and total phenols during acclimatization of bacteria to RRW

%RRW in acclimatization step	% Removal of 2, 4 & 2, 5 DCP in 24 hours	% Removal of total phenols in 24 hours
10	53.38	Not available
20	51.32	Not available
30	53.91	67.46
40	49.06	78.73

The degradation of phenolics in the RRW medium during acclimatization in the SBBR was also verified by UV absorption spectroscopy. In general, it was found that different aromatic compounds showed absorbance maxima at wavelength 220-280 nm (refer to Fig. 4.6.1 for phenol and a mixture of cresols, and Fig. 4.6.2 for 2, 4 DCP and a mixture of chlorophenols). The UV absorption spectra for the 10%, 20%, 30% and 40% RRW, before and after 24 hours of acclimatization are shown in Figs. 4.6.3, 4.6.4, 4.6.5 and 4.6.6, respectively. The figures demonstrate that the microbial treatment during acclimatization removed the phenolic components from the acclimatization medium. The removal of phenolic compounds is also revealed in the chromatograms, as exemplified by Fig. 4.6.7 for 40% RRW at the start of the acclimatization experiment (a) and after 24 hours (b). The roughness in the chromatogram decreased appreciably, in terms of far fewer peaks and lesser heights or areas under the peaks. It should be noticed that 2, 4, 6 trichlorophenol (2, 4, 6 TCP) showed a rather high peak after

24 hours, however this peak accounts for less than 1 mg/l. This is because the developed GC method is highly sensitive to 2, 4, 6 TCP, and therefore, minor concentrations are represented by very high peaks. The first peak from the left is just background, which was detected even in a plain nutrient solution.

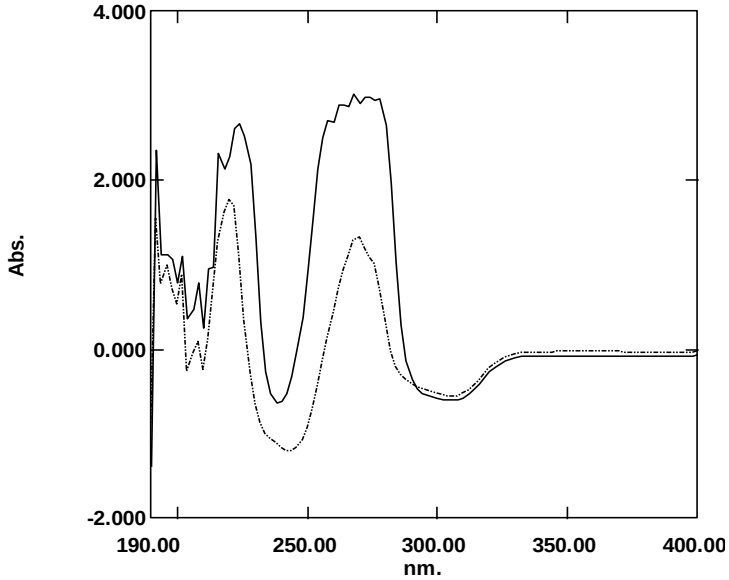


Fig. 4.6.1 UV absorption spectra for phenol (dotted line) and a mixture of cresols (solid line)

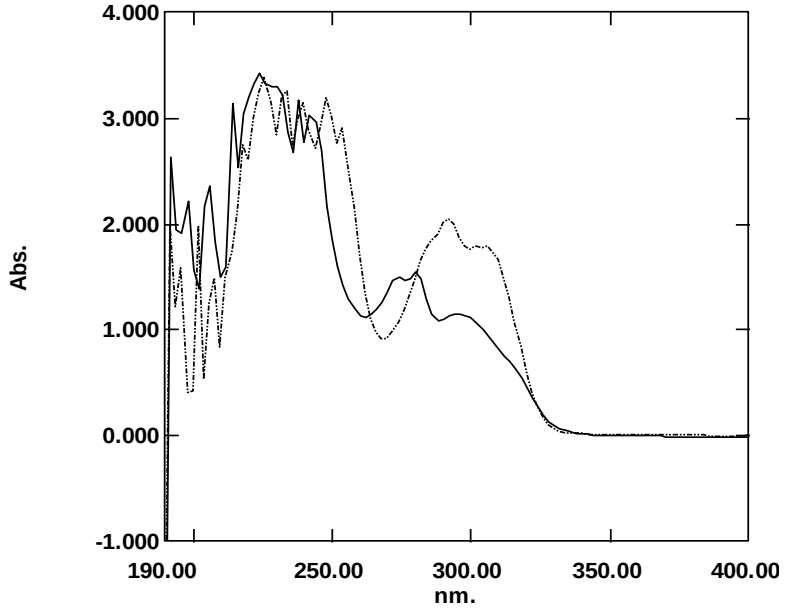


Fig. 4.6.2 UV absorption spectra for 2, 4 DCP (dotted line) and a mixture of CPs (solid line)

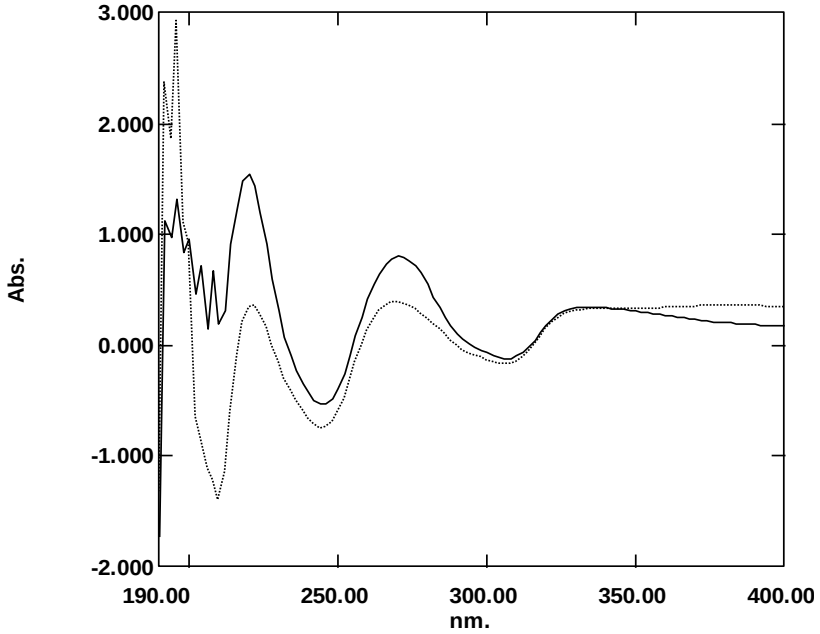


Fig. 4.6.3 UV absorption spectra for 10% RRW medium at the start of acclimatization (solid line) and after 24 hours (dotted line): T = 30 °C; AFR = 3 l/min

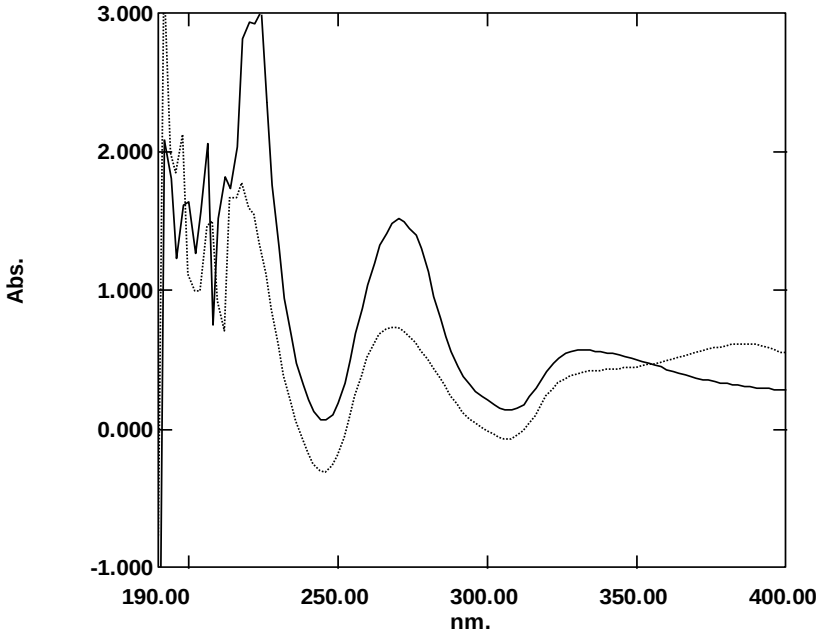


Fig. 4.6.4 UV absorption spectra for 20% RRW medium at the start of acclimatization (solid line) and after 24 hours (dotted line): T = 30 °C; AFR = 3 l/min

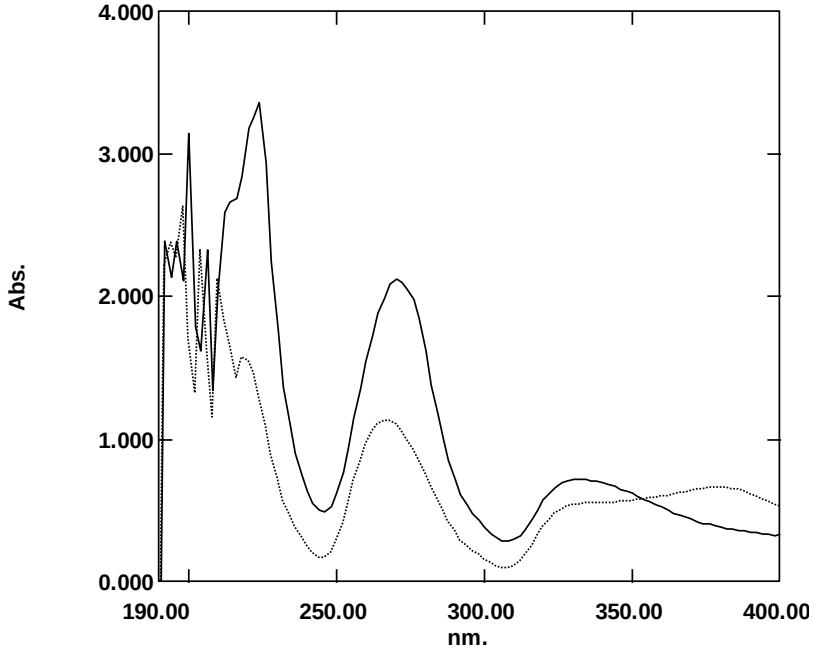


Fig. 4.6.5 UV absorption spectra for 30% RRW medium at the start of acclimatization (solid line) and after 24 hours (dotted line): T = 30 °C; AFR = 3 l/min

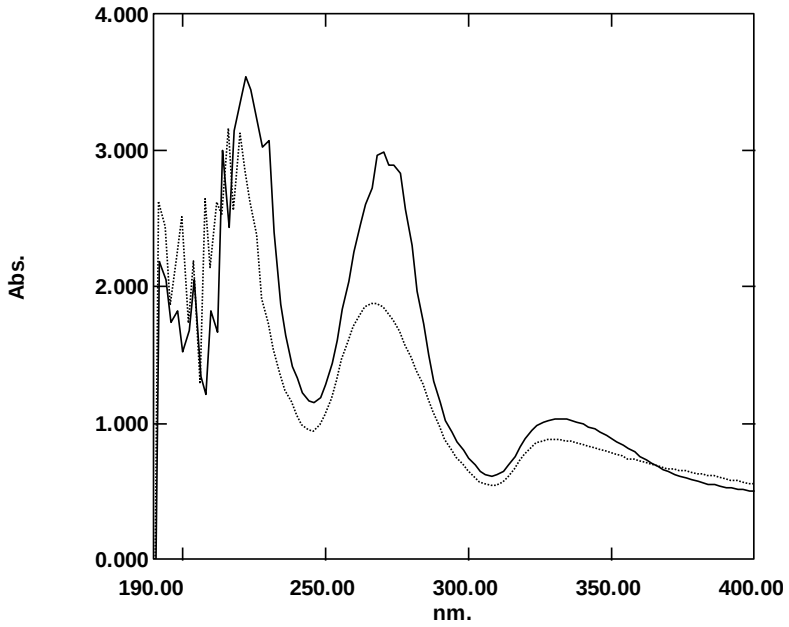
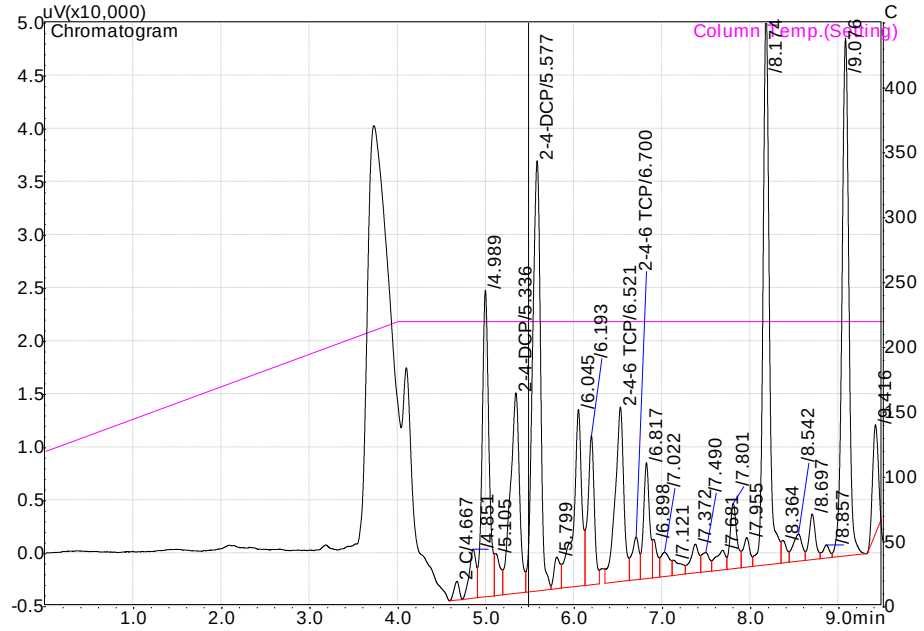
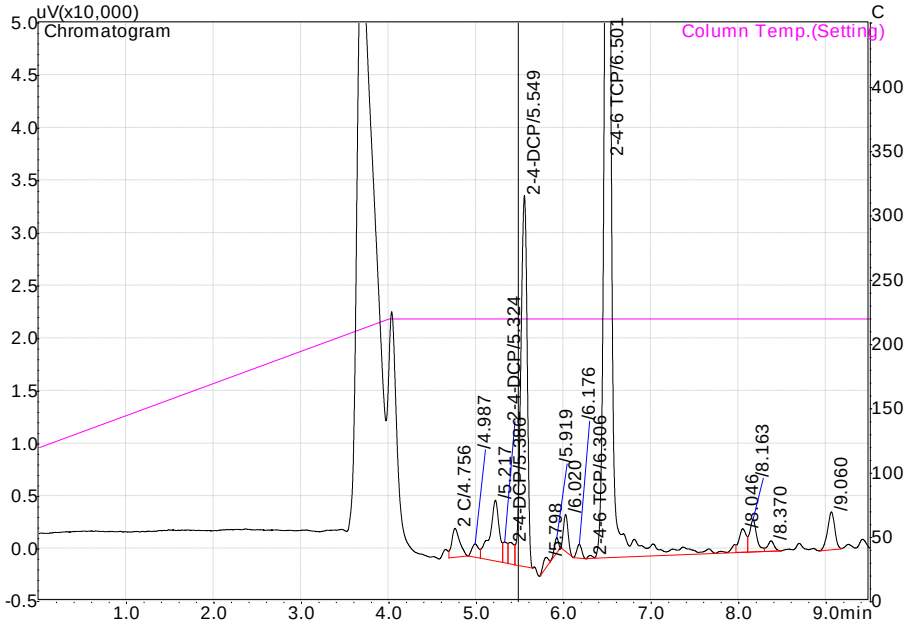


Fig. 4.6.6 UV absorption spectra for 40% RRW medium at the start of acclimatization (solid line) and after 24 hours (dotted line): T = 30 °C; AFR = 3 l/min



(a)



(b)

Fig. 4.6.7 Chromatogram of 40% RRW medium at the start of treatment (a) and after 24 hours of treatment (b)

4.6.3 Results from Batch Treatment of RRW in SBBR

At the end of the acclimatization process, up to 40% RRW, two batch runs were conducted in the SBBR with 100% RRW according to the procedure outlined in Section 3.7.6. The temperature was maintained at 30 °C, AFR at 3.0 l/min and the pH was kept at the original medium pH of about 8.4-8.8 without any adjustment. This would be a more realistic situation as the RRW can be highly alkaline. This aspect has also been emphasized by Karakaya et al. [228] in a study on the biodegradation of raw olive mill wastewater, in which a process novelty was highlighted when dealing with raw wastewater without dilution, sterilization, pH adjustment or any additional chemical/physical treatments. According to the authors, these are energy and time consuming steps, which limit the applicability of this biodegradation process to an industrial scale. The importance of this criterion in industrial processes was pointed out by others [44].

The 24 hours time courses for different phenolics degradation and corresponding percent reduction in concentrations are shown in Figs. 4.6.8 and 4.6.9, respectively. Total removals can be achieved for all cresols, whereas the percent removals for phenol and DCP were approximately 87% and 63%, respectively. Significant overall reduction in total phenols of almost 90% was observed. That is comparable to a total phenols reduction of 89% as obtained by Karakaya et al. [228] in 120 hours, during batch treatment of olive mill wastewater with an initial total phenols content of 622 mg/l. Other reported removals of total phenols from olive mill wastewaters fall in the range of 15-54% [228]. However, unlike controlled laboratory experiments with model synthetic wastewater, a causal relationship is difficult to establish in real applications. In this regard, laboratory treatments with simulated wastewater have been more

efficient than treatments of raw wastewater, where critical parameters are more difficult to control and the interference of other factors prevails. For example, it is documented that the presence of 2, 4 DCP imposes inhibition on the biodegradation of phenol [70, 80].

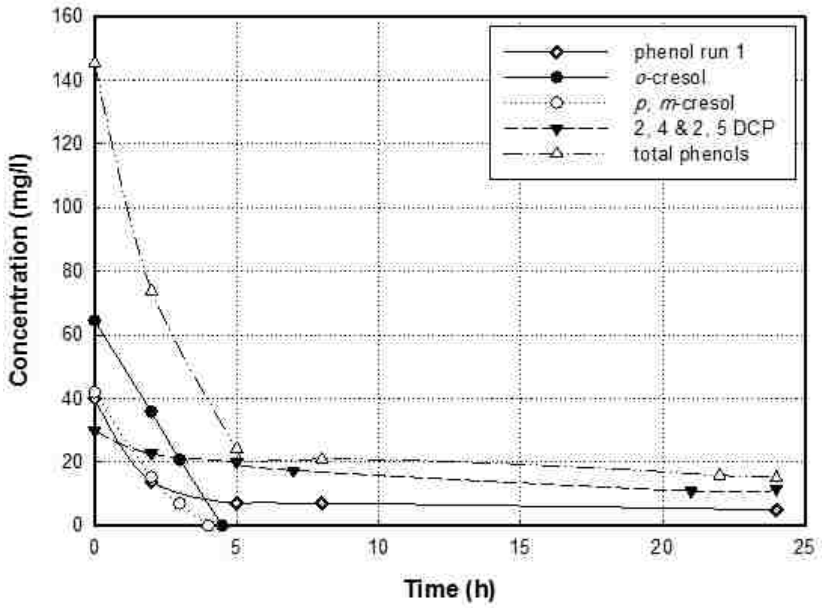


Fig. 4.6.8 Time course of degradation of different phenolic compounds during batch treatment of RRW: T = 30 °C; initial pH = 8.7; AFR = 3 l/min

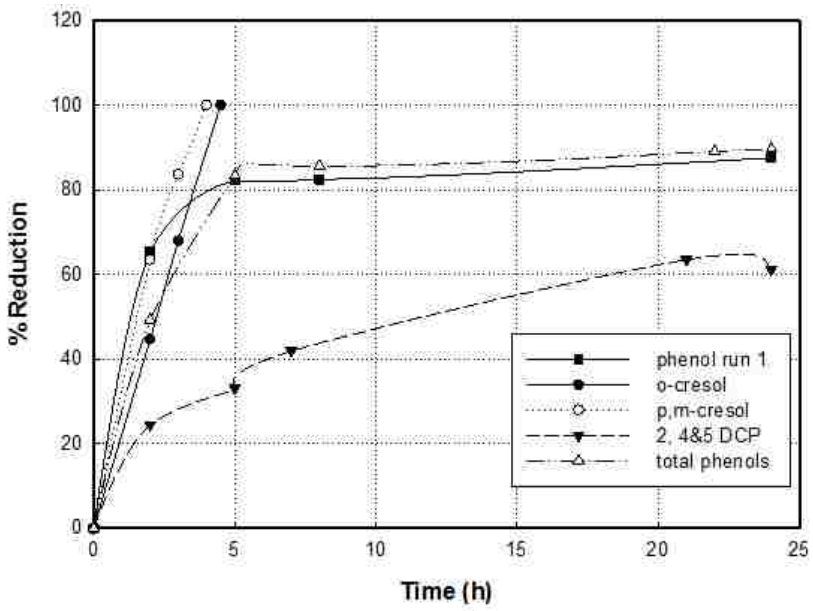


Fig. 4.6.9 Percent reduction in different phenolic compounds during batch treatment of RRW: T = 30 °C; initial pH = 8.7; AFR = 3 l/min

The behaviour of the TOC and COD values during the biodegradation process showed a trend very similar to that of total phenols, indicating that there was no intermediate enriched accumulation [131, 229]. Other non-biodegradable and inorganic substances contributed to the high COD values, whereas the presence of non phenolic organic compounds accounted for the high TOC values [230]. The ratio of COD to TOC was found to be around 3.0, which is consistent with the ratio reported in the literature for industrial wastewater [231]. The corresponding percent reductions in COD and TOC values, compared to the percent reduction in total phenols are shown in Fig. 4.6.10. Maximum percent reductions were 59% and 54% for COD and TOC, respectively.

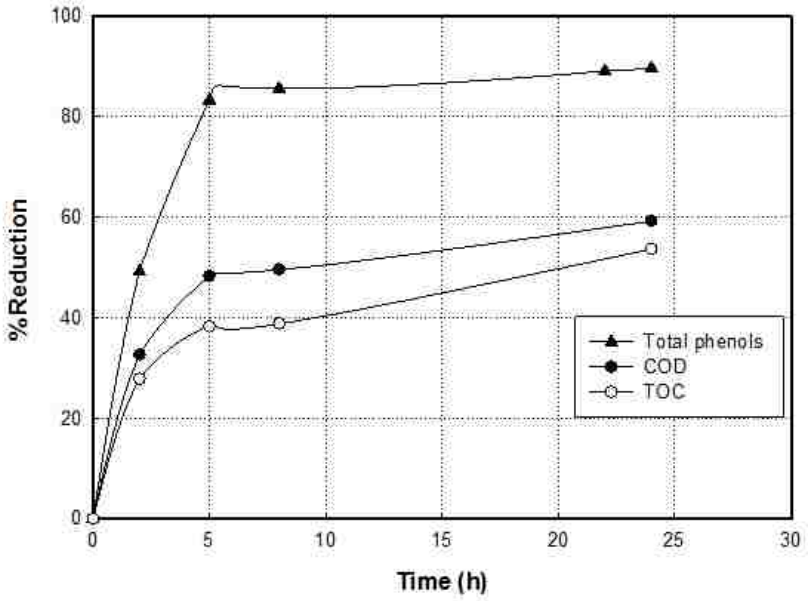


Fig. 4.6.10 Percent reduction in total phenols, COD and TOC during batch treatment of RRW: T = 30 °C; initial pH = 8.7; AFR = 3 l/min

The degradation of phenolics in the RRW medium during batch treatment in the SBBR was also verified by UV absorption spectroscopy. As illustrated by Fig. 4.6.11, the spectrum analysis of RRW in the wavelength range of 220-280 nm before and after the biological treatment demonstrate the differences attributed to the biomass activity. The removal of phenolic compounds is also revealed in the chromatograms, as exemplified by Fig. 4.6.12 for 100% RRW at the start of the treatment (a) and after 24 hours (b). The roughness in the chromatogram decreased appreciably, in terms of far fewer peaks and lesser heights or areas under the peaks.

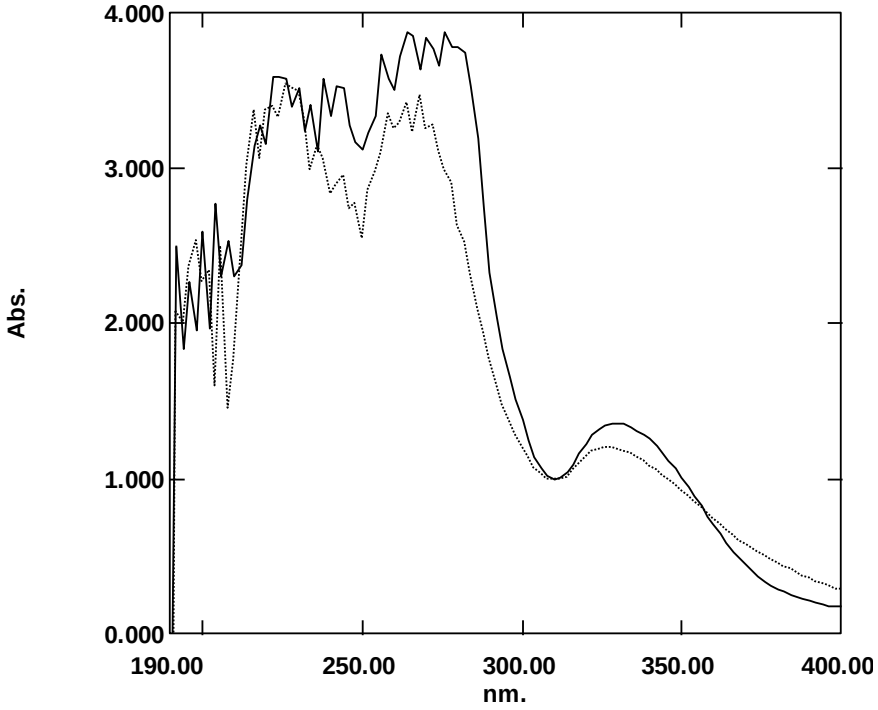
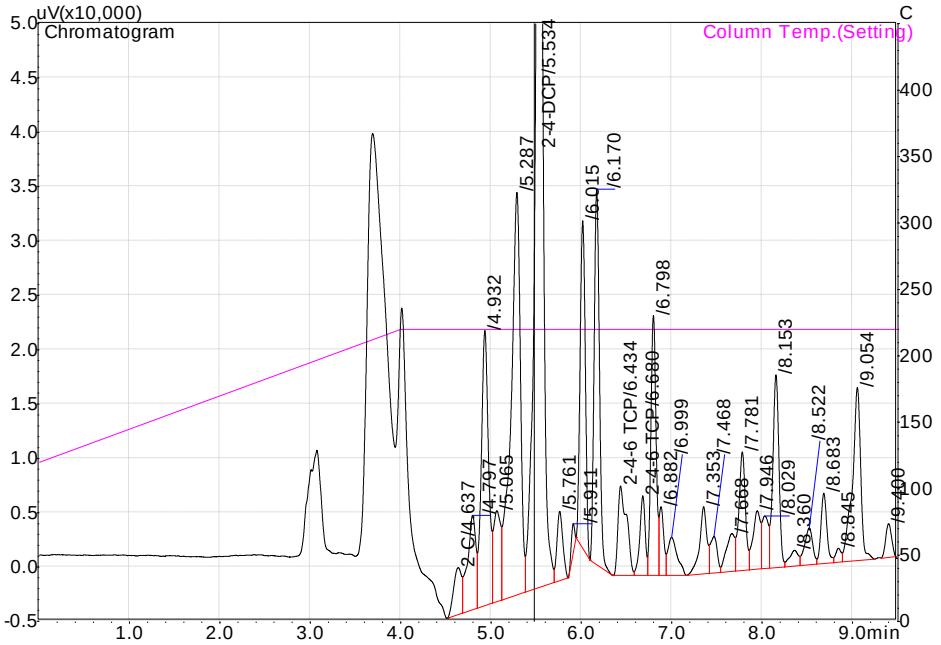
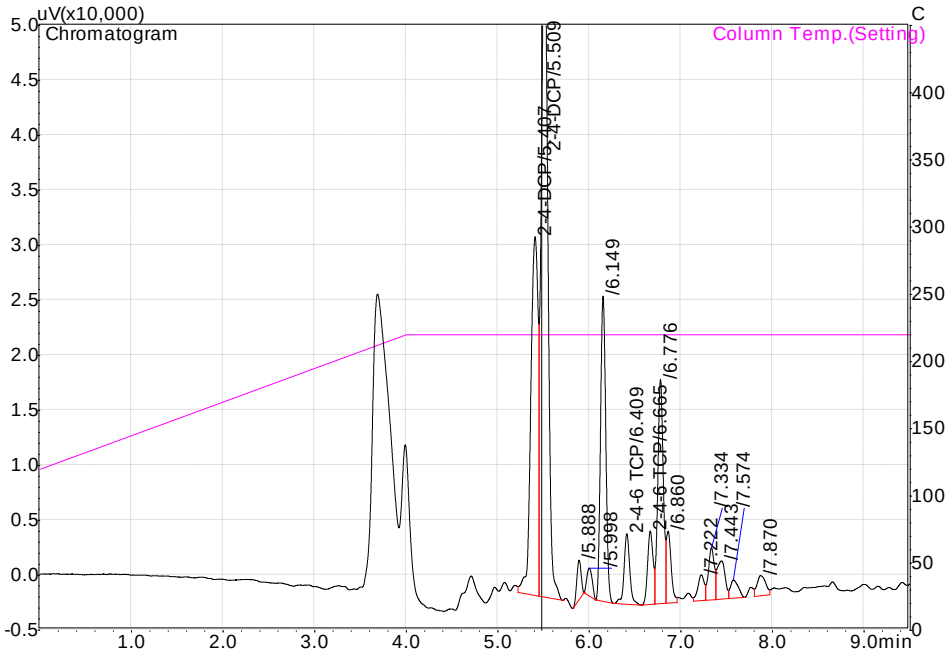


Fig. 4.6.11 UV absorption spectra for 100% RRW at the start of the treatment (solid line) and after 24 hours (dotted line): T = 30 °C; initial pH = 8.7; AFR = 3 l/min



(a)



(b)

Fig. 4.6.12 Chromatogram of 100% RRW medium at the start of treatment (a) and after 24 hours of treatment (b)

To sum, we can conclude with a brief assessment of the batch treatment of RRW in the SBBR in terms of the removal efficiencies achieved. It has been reported that biological processes are inadequate in treating industrial wastewater and cannot be used individually on a full scale [6, 42]. With this in mind, the emphasis has been on pretreatment processes and integrated schemes combining methods and technologies. El-Naas et al. [42] developed a three-step process for the treatment of petroleum refinery wastewater in an arrangement of electrocoagulation–biodegradation–adsorption and showed that the SBBR biodegradation unit would have achieved only 20-25% reduction in COD and phenols if it had been used individually, whereas almost complete reduction was achieved with this synergistic scheme. In view of the fact that the batch treatment of RRW in this study was conducted in only two runs, due to the time limitations of the study and the difficulty in securing large enough quantities of wastewater, the results are quite satisfactory. It was anticipated that the degradation capability of the immobilized biomass would have improved had it been given the chance to grow more and therefore achieve better removal of phenols and COD from RRW. The reductions achieved in phenols and COD are merely due to the biodegradation effect without any pre- or post-treatment. A comparison of the percent reduction in COD achieved in this study with those reported in the literature using different single and combined processes, for the batch and continuous treatment of several types of industrial wastewaters, is presented in Table 4.6.3

Table 4.6.3 Percent COD reductions using different methods for the treatment of different industrial wastewaters

Wastewater source	Treatment method	COD, mg/l	COD % reduction	Ref.
Batch study				
Olive mill wastewater	Biodegradation	100,000-200,000	51.3-70	[232]
Olive mill wastewater	Biodegradation	6500	56.91	[233]
Dyeing	Fenton oxidation – membrane bioreactor	1100-1300	77-80	[234]
Rayon industry	Electro-fenton and chemical precipitation	2400	88	[235]
Petroleum refinery	This work	2700	59	
Continuous study				
Coke	Fenton oxidation-biodegradation	2470	36.4	[236]
Petrochemical	Coagulation-biofilter	19440	95	[237]
Oil refinery	Biodegradation	510± 401.9	85-90	[129]
Industrial production of phenolic resins	Biodegradation	124,000	>95	[230]
Whey-enriched olive mill wastewater	Biodegradation	75,000	62	[238]
Petroleum refinery	Electrocoagulation-biodegradation-adsorption	4190	97	[42]

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

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5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The present study aimed at developing an integrated system for the biodegradation of chlorophenols, considering 2, 4 dichlorophenol as a model contaminant. The system utilized state of the art techniques of immobilization of an effective commercial bacterial consortium, consisting mainly of *Pseudomonas putida*, in a specially designed SBBR that promises to be effective for applications in industrial processes for real oil refinery wastewater. This integrated approach is believed to synergistically enhance the removal rates and treat the contaminated effluents from petroleum refineries in an ecologically friendly manner.

The study started by testing the validity of a routine practice that has often been commonly implemented in biomass acclimatization, which is the inclusion of glucose in biomass acclimatization. The results revealed that the biodegradation rates achieved by glucose-acclimatized bacteria and the no-glucose acclimatized bacteria were either very close or slightly better for the former, but not significantly enough to justify the inclusion of glucose in the acclimatization process. The absence of glucose from the acclimatization process either stimulated the biodegradation capabilities of *P. putida* or had no significant effect on its performance during biodegradation.

In the course of experimenting on the role of glucose in acclimatization, an important finding was the suppression in the degradation rate when running an experiment before achieving a complete removal of substrate. This phenomenon

could be attributed to the effect of starvation on the cells that enhanced the cells' ability to utilize the organic substrate and nutrients.

The bacteria were immobilized in Polyvinyl Alcohol (PVA) gel particles and used in the SBBR to remove DCP from synthetic wastewater in a batch process. Successful batch and continuous biodegradation of 2, 4 DCP was achieved in the SBBR under different operating conditions. In the batch process, 100% removal of 2, 4 DCP was achieved for all DCP initial concentrations, up to 200 mg/l, at comparable or superior removal rates to those reported in the literature. This is a material that is known for its recalcitrant nature in biodegradation, and for which removal efficiencies in the range of 40-60% have often been reported. *P. putida* was well adapted to the contaminant upon repeated use and its degradation activity almost doubled after four months of repeated use from 38 mg/l.h to 70 mg/l.h.

Although the biodegradation of 2, 4 DCP followed a Haldane-Inhibition Model, no significant inhibition was observed in every initial concentration, which means that the inhibition effect was not significant, mainly due to the immobilization of the bacteria within the PVA matrix, that protects it from direct contact with the contaminant.

Utilizing the full potential of the response surface methodology (RSM) for statistical optimization, and based on preliminary screening, the effects and interaction of the main parameters, namely temperature, pH and initial DCP concentration were determined through a regression model using a Box-Behnken experimental design. The point representing maximum aerobic biodegradation rate inside the experimentation region was determined as having the following critical values: temperature 32.58 °C, pH 5.0 and the initial DCP concentration

was 70.46 mg/l. Under these conditions, the predicted aerobic global biodegradation rate was 41.82 mg/ l.h.

Since the ultimate goal of a laboratory-scale process is its industrial application, the performance of the process in the continuous mode, which is an industry-like setting, was investigated. The various issues investigated were the hydrodynamics of the reactor in terms of the hydraulic residence time (HRT), the initial concentration of DCP in the feed and the degradation capacity. When tested for tolerance to shock loads, the SBBR showed stability and sustainability, as it could sustain a 100% increase in organic loading rate and recover quickly to its normal operating mode once the shock load had been removed. Compared to the packed bed reactor, the SBBR proved to be more stable and achieved higher removal efficiencies (77% versus 53% for the PBR) with a HRT of 1 hour and an initial DCP concentration of 75 mg/l.

A characteristic operating design factor for continuous reactors is the degradation capacity, which is related to the amount of material that can be processed over a given period of time and therefore, for a given throughput, the reactor volume and associated capital and operating costs are minimized when HRT is made as small as possible. In this study, the maximum degradation capacities obtained were 1414 and 2160 g/m^3d, by the SBBR and PBR, respectively, with corresponding removal efficiencies of 77% and 53%, respectively. For an economic application, a compromise should be made to arrive at an economical decision.

When subjected to further preliminary modeling, the dynamic behavior of 2, 4 DCP was adequately simulated based on a Haldane Model. Also, mass transfer limitations were assessed as insignificant at an initial DCP concentration

of 100 mg/l, which emphasizes the highly porous structure of the PVA gel that protects the biomass from the toxic effects of the contaminant, while at the same time remaining accessible to the substrate and nutrient transfer. The efficiency of the process in the treatment of real refinery wastewater was investigated and the results obtained favoured the process. 100% removal efficiencies could be achieved for all cresols; whereas the removal efficiencies for phenol and DCP were approximately 87% and 63%, respectively. Significant overall reduction in total phenols of almost 90% was observed.

5.2 RECOMMENDATIONS

With the aim of enhancing the performance of this laboratory-scale process, that promises to be efficient for the treatment of wastewater, the following recommendations are proposed:

- As indigenous microbial strains are expected to be more adapted to the local environmental conditions, the search for potent indigenous bacterial strains remains a high priority. In this regard, there exists a need to develop logistics associated with the isolation and identification of microorganisms, so that it is no longer a limiting factor in this area of research.
- To get a better understanding of the process of biodegradation, research should be directed to the area of the effect of different intermediates and metabolites during the degradation of contaminants. The technical complexity associated with this issue imposed constraints throughout this study on realizing some of the abnormal trends related to metabolic pathways and enzymatic activities.
- Evaluation of the process efficiency in the treatment of real refinery wastewater in continuous operation. This would be a more realistic approach, which could provide valuable information for real industrial applications.

- Work on the development of a rigorous and well-structured kinetic model that incorporates reaction and mass transfer limitations and, in turn, would help in approaching the process evaluation with in-depth analysis.
- As a few studies employed chloride measurements to confirm complete mineralization by comparing the measured chloride to the stoichiometric release of chloride, it would be advantageous to adopt this technique in an attempt to quantify incomplete mineralization.
- Carrying out research on starvation and its effect on cells degrading capacities.

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