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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

BIOLOGICAL TREATMENT AND TOXICITY REMOVAL FROM WASTEWATERS CONTAINING CHLORINATED AROMATIC COMPOUNDS IN ROTATING PERFORATED TUBES AND BRUSH BIOFILM REACTORS

by

Serkan EKER

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BIOLOGICAL TREATMENT AND TOXICITY REMOVAL FROM WASTEWATERS CONTAINING CHLORINATED AROMATIC COMPOUNDS IN ROTATING PERFORATED TUBES AND BRUSH BIOFILM REACTORS

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Serkan EKER

Ph.D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"BIOLOGICAL TREATMENT AND TOXICITY REMOVAL FROM WASTEWATERS CONTAINING CHLORINATED AROMATIC COMPOUNDS IN ROTATING PERFORATED TUBES AND BRUSH BIOFILM REACTORS"** completed by **SERKAN EKER** under supervision of **PROF. DR. FİKRET KARGI** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

Prof. Dr. Fikret KARGI
Supervisor

Assoc. Prof. Dr. İlgi K. KAPDAN
Committee Member

Assoc. Prof. Dr. Nuri AZBAR
Committee Member

Jury member

Jury member

Prof. Dr. Cahit HELVACI
Director
Graduate School of Natural and Applied Sciences

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To the memory of my father, Özcan EKER

Serkan EKER

BIOLOGICAL TREATMENT AND TOXICITY REMOVAL FROM WASTEWATERS CONTAINING CHLORINATED AROMATIC COMPOUNDS IN ROTATING PERFORATED TUBES AND BRUSH BIOFILM REACTORS

ABSTRACT

Rotating perforated tubes and rotating brush biofilm reactors were used for 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP), COD and toxicity removals from synthetic wastewater. Box-Wilson and Box-Behnken statistical experiment design methods were used to evaluate the experimental results and determine the optimum operating conditions maximizing chlorophenol, COD and toxicity removals. Toxicity of wastewater was analyzed by dehydrogenase enzyme activity known as resazurin assay method.

Both reactors were found to be very effective in removing chlorophenols over a large range of operating conditions. Chlorophenols and their degradation intermediates were the major toxic compounds causing low COD and chlorophenol removals. Nearly complete removal of chlorophenols required high biofilm surface area (high A/Q ratio) and high feed COD contents yielding high biomass densities. Percent chlorophenol and toxicity removals increased with increasing feed COD and A/Q ratio and with decreasing chlorophenol concentrations for both reactors. Percent COD removal increased with increasing feed COD up to a certain concentration and decreased with further increases in the feed COD. High A/Q ratio and low feed chlorophenol concentrations yielded high COD removals. To avoid inhibition of high chlorophenol concentrations on the biofilm microorganisms and to obtain high COD, chlorophenol and toxicity removals, the system should be operated at high A/Q ratio and feed COD.

RTBR seemed to be more effective for removal of COD and chlorophenols when operated under the same A/Q ratio due to formation of thicker and denser biofilms on the tube surfaces. Similar trends were observed for percent toxicity removals.

RTBR performed better than RBBR at high chlorophenol concentrations yielding high COD, chlorophenol and toxicity removals.

Keywords: Biological treatment, Box-Behnken experimental design, Box-Wilson experimental design, chemical oxygen demand (COD), chlorophenols, 4-chlorophenol, dichlorophenol, operating conditions, phenolic compounds, rotating perforated tubes biofilm reactor, rotating brush biofilm reactor, toxicity removal, trichlorophenol, wastewater treatment

KLORLU AROMATİK BİLEŞİK İÇEREN ATIKSULARIN DÖNEN DELİKLİ BORULAR BİYOFİLM VE DÖNEN FIRÇA BİYOFİLM REAKTÖRLERİ İLE ARITIMI VE TOKSİSİTE GİDERİMİ

ÖZ

Sentetik atıksudan 4-klorofenol, 2,4-diklorofenol, 2,4,6-triklorofenol, KOİ ve toksisite giderimi için dönen delikli boru biyofilm reaktörü ve dönen fırça biyofilm reaktörü kullanıldı. Deney sonuçlarının değerlendirilmesinde ve KOİ, klorofenol ve toksisite giderimlerini maksimum yapan optimum işletme şartlarının bulunmasında Box-Wilson ve Box-Behnken istatiksel deney tasarımları kullanıldı. Atıksuyun toksisitesinin belirlenmesi "Resazurin Assay" metodu olarak adlandırılan dehidrojenaz enzim aktivitesinin izlemesi ile saptandı.

Her iki reaktorün de geniş işletme aralıklarında klorofenol gideriminde etkili olduğu görülmüştür. Klorofenoller ve parçalanma ürünlerinin toksik olması düşük KOİ ve klorofenol giderime neden olmaktadır. Klorofenollerin tam giderimi için yüksek biyofilm oluşumunu sağlayan yüksek KOİ ve yüksek biyofilm yüzey alanı (A/Q oranı) gerekmektedir. Her iki reaktörde de klorofenol ve toksisite giderim verimi, giriş KOİ konsantrasyonunun ve A/Q oranının artmasıyla artmakta, klorofenol konsantrasyonu ile azalmaktadır. KOİ giderim verimi, belli giriş KOİ konsantrasyon değerine kadar artmakta ve daha yüksek KOİ konsantrasyon değerlerinde düşmektedir. Yüksek A/Q ve düşük giriş klorofenol konsantrasyonları yüksek KOİ verimleri sağlamaktadır. Yüksek KOİ, klorofenol ve toksisite verimleri sağlayabilmek ve biyofilm organizmaları üzerinde klorofenol inhibisyonu engelleyebilmek için reaktör yüksek A/Q oranı ve yüksek KOİ konsantrasyonlarında işletilmelidir.

Aynı işletme koşullarında, borular üzerinde yüksek ve yoğun biyofilm oluşmasından dolayı dönen delikli boru biyofilm reaktörü KOİ ve klorofenol gideriminde dönen fırca reaktorunden daha etkili olmuştur. Toksisite giderimi içinde benzer eğilimler gözlenmiştir.

Yüksek klorofenol konsantrasyonlarında, dönen delikli boru biyofilm reaktörü KOİ, klorofenol ve toksisite giderme verimlerinde dönen fırça biyofilm reaktörüne göre daha yüksek performans göstermiştir.

Anahtar sözcükler: Atıksu arıtımı, biyolojik arıtma, Box-Behnken deney tasarımı, Box-Wilson deney tasarımı, dönen delikli boru biyofilm reaktörü, dönen fırça biyofilm reaktörü, fenolik bileşikler, işletme şartları, kimyasal oksijen ihtiyacı (KOİ), 4-klorofenol, 2,4-diklorofenol, 2,4,6-triklorofenol, toksisite giderimi,

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CHAPTER ONE
INTRODUCTION

1.1 The Problem Statement

Chlorinated aromatic compounds such as chlorophenols are among the most important and versatile industrial organics which are widely used as insecticides, herbicides, and fungicides as well as preservatives for wood, glue, paint, vegetable fibers and leather. Chlorophenols are present in paper pulp mills and contaminated soils around wood-preserving industries, groundwater environments due to leaching from contaminated soils, and surface water due to surface runoff or direct industrial waste discharges. (Li D.Y., *et al,* 1991; Annachhatre A.P. and Gheewala S.H. 1996; Wang C.C., *et al.,* 2000)

Dichlorophenols are mainly found as intermediates formed during the production of 2,4-dichlorophenoxyacetic acid, which is a herbicide in the pesticide industry. This compound is also used in a feedstock mixture to produce the wood preservative, pentachlorophenol. Other past commercial uses for 2,4-DCP include moth proofing and as an antiseptic. Currently, 2,4-DCP is still in use, and some of the industries are producing this compound (Kiefer M.C., *et al.,* 1998).

The major uses of 2,4,6-TCP are as an antiseptic and pesticide. Its use also includes preserving wood, leather and glue, and preventing the build-up of mildew on fabric. In addition, 2,4,6-TCP is used as an intermediate to produce other chemicals. This compound is used as a feedstock in the production of 2,3,4,6-tetrachlorophenol and pentachlorophenol. Generally, 2,4,6-TCP is presently not in use (or limited), but it is produced as a by-product (Kiefer M.C., *et al.,* 1998).

During their manufacture or use, these chemicals are often discharged into the environment. Chlorophenols are highly toxic and their discharge into environment must be regulated. Most of the chlorophenols cause toxic effects on plants and animals.

Environmental regulations are based on identification and control of special substances in effluents. However, some complex chemicals like chlorinated aromatic compounds in wastewater need additional control measures to ensure an appropriate level of environmental protection. One of those control parameters is the toxicity measurement for industrial effluents, which are potentially toxic. Therefore, such wastewaters should be treated effectively to reduce toxicity as well as COD and chlorophenols.

1.2 Physiochemical Properties of Chlorinated Compounds

Three compounds of interest in the chlorophenol family are 4-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol. Synonyms frequently encountered include, 4-CP, 2,4-DCP or DCP and 2,4,6-TCP or TCP, respectively. Phenolic compounds are synthetically produced chemicals. Physically, the structures are composed of a benzene ring with varying numbers of chlorine substituents and one hydroxyl group on the ring (Kiefer M.C., *et al.*, 1998).

The solubility of 2,4-DCP in water is approximately 4.5 g/l. This is a significant drop off from 4-chlorophenol's solubility (20 g/l), but subsequently represents the trend in this chemical family as the number of chlorine substitutions increase. The solubility drops to 0.434 g/l in 2,4,6-TCP and 0.014 g/l pentachlorophenol (Kiefer M.C., *et al.*, 1998).

The chemical oxygen demand (COD) and the theoretical amount of chloride released from the degradation of each chlorophenol were calculated from the following oxidation reaction:

2- or 4-CP: $C_6H_5Ocl + 6.5^o_2 \rightarrow 6CO_2 + 2H_2O + HCl$

DCP: $C_6H_4Ocl_2 + 6^o_2 \rightarrow 6CO_2 + H_2O + 2HCl$

TCP: $C_6H_3Ocl_3 + 5.5O_2 \rightarrow 6CO_2 + 3HCl$

Thus, the COD of 2CP, 4CP, DCP and TCP are estimated to be 1.62, 1.62, 1.18, and 0.89 mg O_2 mg chlorophenol^{-1}, respectively. The theoretical amount of chloride

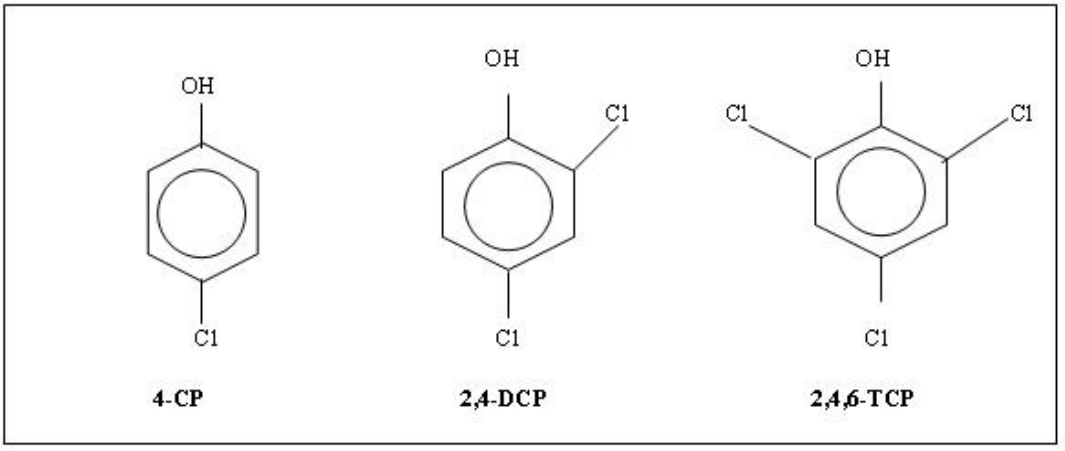
released iss 0.276, 0.276, 0.435 and 0.538 mg Cl mg chlorophenol^{-1}, respectively (Ziloue H. et al., 2006).

The physiochemical properties and structures of those compounds are presented in Table 1.1 and 1.2 (Kiefer M.C., *et al.*, 1998; Ziloue H. et al., 2006). Toxicity of chlorophenol was determined by using resazurin assay method by us. Unacclimated mixed culture were used in the IC_{50} of chlorophenol experiments.

Table 1.1Physiochemical Properties of 4-CP, 2,4-DCP and 2,4,6-TCP

	4-CP	**2,4-DCP**	**2,4,6-TCP**
Chemical formula	C_6H_4ClOH	$C_6H_3Cl_2OH$	$C_6H_2C_3OH$
Molecular weight (g/mol)	128.56	163	197.5
Color of solid	White to pink	White	Yellow
Melting point ©	43.2-43.7	45	69.5
Boiling point ©	220	210	246
Solubility (g l^{-1})	20	4.5	0.434
COD (mg l^{-1})	1.62	1.18	0.89
Toxicity (IC_{50}) (mg l^{-1}) *mixed culture	490	250	38

Table 1.2 Chemical Structures of 4-CP, 2,4-DCP and 2,4,6-TCP



1.3 Toxicity of Chlorinated Compounds

Chlorinated aromatic compounds are toxic to the environment and any kind of life forms with varying level of toxicity depending on the chemical structure and the number of chlorine groups. Chlorophenols are not readily biodegradable and are toxic to most types of microorganisms even at low concentrations. Phenol can be growth inhibitory to even those species, which have the metabolic capacity of using it as the growth substrate. Chlorophenols can be toxic at relatively low concentrations of 5-25 mg/l. In a study comparing 50% toxicity levels of chlorophenols, PCP was found to be the most toxic. Toxicity decreased as the number of chlorine substituents decreased.

1.4 Treatment Methods of Chlorinated Compounds

Physical, chemical and biological methods such as activated carbon adsorption, chemical oxidation and aerobic/anaerobic biological degradation are used for removal of chlorinated aromatics from wastewater. Different treatment systems, aerobic as well as anaerobic are employed in industry.

The literature contains limited information on the biological treatment systems used for removal of toxic organic wastes. The most common biological wastewater treatment method is the activated sludge process, which can be used after some modifications for removal of chlorinated compounds from wastewater.

There are limited number of reports available on treatment of chlorinated compounds by using continuous biofilm reactors. Biofilm processes, in which the microorganisms are attached to an inert support material, offer a number of advantages over conventional activated sludge system for treatment of toxic waste waters.

- Biofilm systems are more resistant to shock loadings and process fluctuations
- Biofilms are better protected against toxic or inhibitory compounds than free suspension cultures and may degrade these compounds at higher rates

- Biomass concentration can be 5-10 times higher than that of the conventional activated sludge system
- Biofilm reactors provide high degradation rates and smaller reactor volumes
- Biotic and abiotic phases are separated. Sludge settling and recycle are eliminated in biofilm reactors.

New investigations focus on development of new treatment technologies such as fixed-film systems to treat wastewaters containing chlorophenols for complete mineralization of chlorinated compounds.

1.5 Biodegradation of Chlorinated Compounds

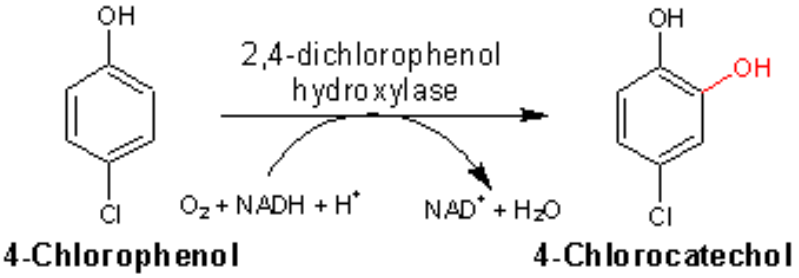
The biodegradability of aromatic compounds depends on the number, type and position of substituents on the aromatic ring. Chlorophenols are not readily biodegradable and the rate of biodegradation decreases with increasing number of chlorine substituents on the aromatic ring. Several chlorinated compounds can be removed under aerobic and anaerobic conditions. Two main strategies can be differentiated: (1) the halogen substituents are removed as an initial step in degradation via reductive, hydrolytic or oxic mechanisms (2) dehalogenation occurs after cleavage of the aromatic ring from an aliphatic intermediate. A critical step in the degradation of chlorinated compounds is the cleavage of the halogen-carbon bond. (Haggblom M.M, 1992; Annachhatre A.P. and Gheewala S.H. 1996)

The degree of degradation varies from compound to compound. Some are apparently resistant to microbial attack. Others may be partially broken down to non-biodegradable intermediates or transformed to possibly more toxic by-products. Complete biodegradation will result in the mineralization of the compound to carbon dioxide or methane, and in the case of halo-aromatics with release of the halogen substituents as halide. In aerobic conditions, oxygen is both the terminal electron acceptor and frequently is a reactant in the initial reactions. In the absence of the oxygen, nitrate, sulfate, carbonate may function as alternate electron acceptors. The

absence or presence of electron acceptors may affect the biodegradability of a compound. Degradation of chlorinated compounds under anaerobic conditions is less well understood. (Haggblom M.M, 1992; Annachhatre A.P. and Gheewala S.H. 1996)

Many aerobic bacteria and fungi such as *Pseudomonas, Alcaligenes, Arthrobacter, Nocardia, Rhodococcus, Mycobaterium, Achromobacter* and *Bacillus* are capable of using aromatic compounds as the sole carbon and energy source. One of the most extensively investigated organisms is *Pseudomonas sp.* The mechanisms of aerobic degradation differ amongst chlorophenols depending on the degree of chlorination. There is a clear division of the bacterial isolates in two groups: (1) strains that degrade mono and di-chlorophenols, but do not attack more chlorinated phenols, (2) strains that degrade pentachlorophenol and other highly toxic chlorinated phenols, but do not degrade mono and di-chlorophenols. Mono and dichlorinated phenols are degraded by bacteria through chlorinated catechols. Generally chlorophenols are oxidized to chlorocatechols by a phenol hydroxylase (phenol monooxygenase) and then degraded by a ring cleavage. Dehalogenation takes place as a fortuitous reaction only after cleavage of the aromatic ring. (Haggblom M.M, 1992)

Figure 1.1 and 1.2 depict degradation pathways of 4-chlorophenol and 2,4-dichlorophenol. These pathways were contributed by Brian Hill, Dartmouth College. It was updated by Eva Young and Dong Jun Oh, University of Minnesota. (Young Eva, 2008)



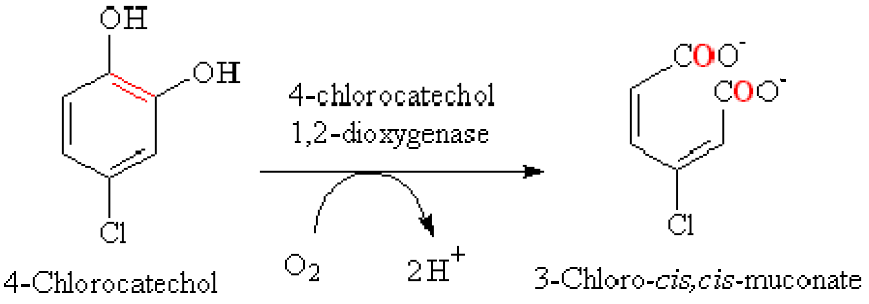
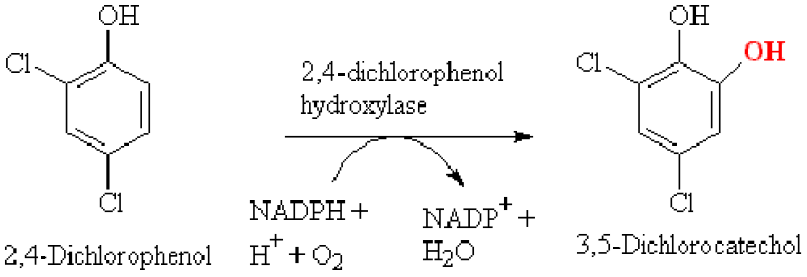
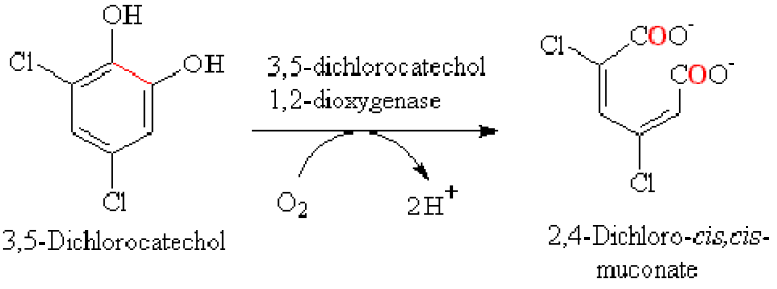
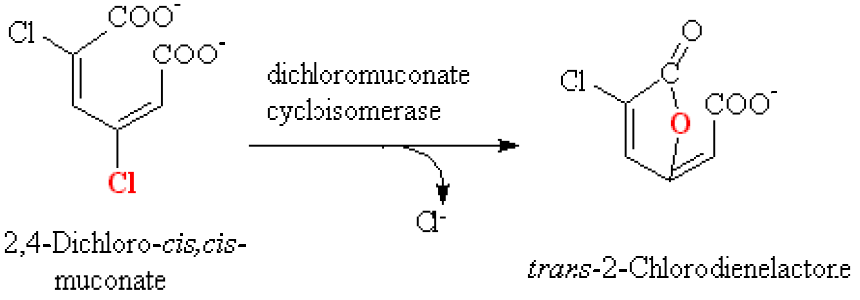
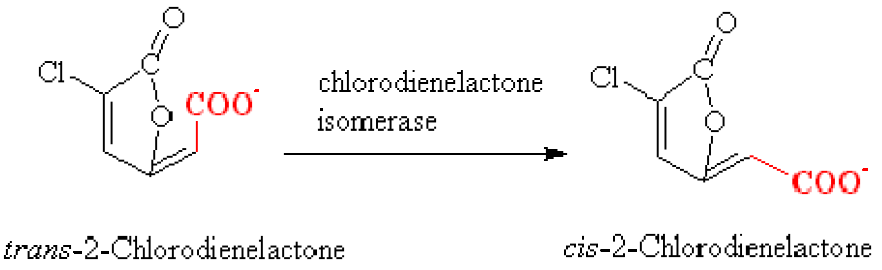


Figure 1.1 Degradation pathway of 4-chlorophenol (via ortho)

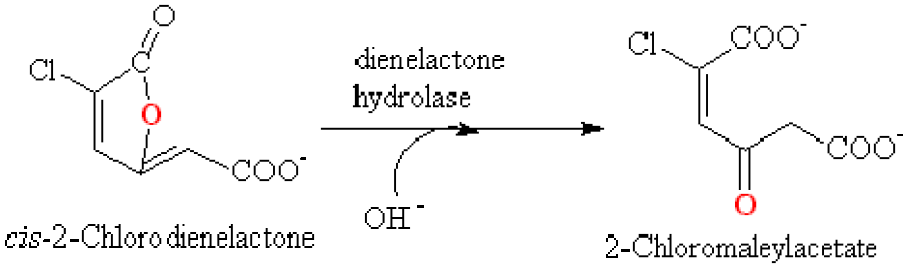




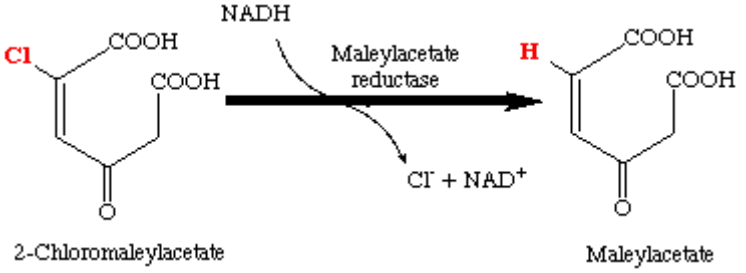




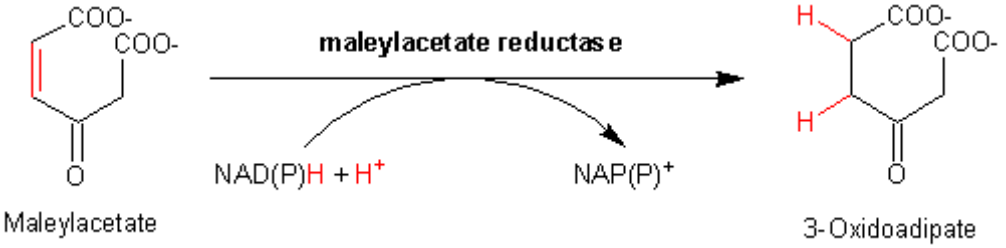
trans-2-Chlorodienelactone *cis*-2-Chlorodienelactone



cis-2-Chlorodienelactone 2-Chloromaleylacetate



2-Chloromaleylacetate Maleylacetate



Maleylacetate 3-Oxidoadipate

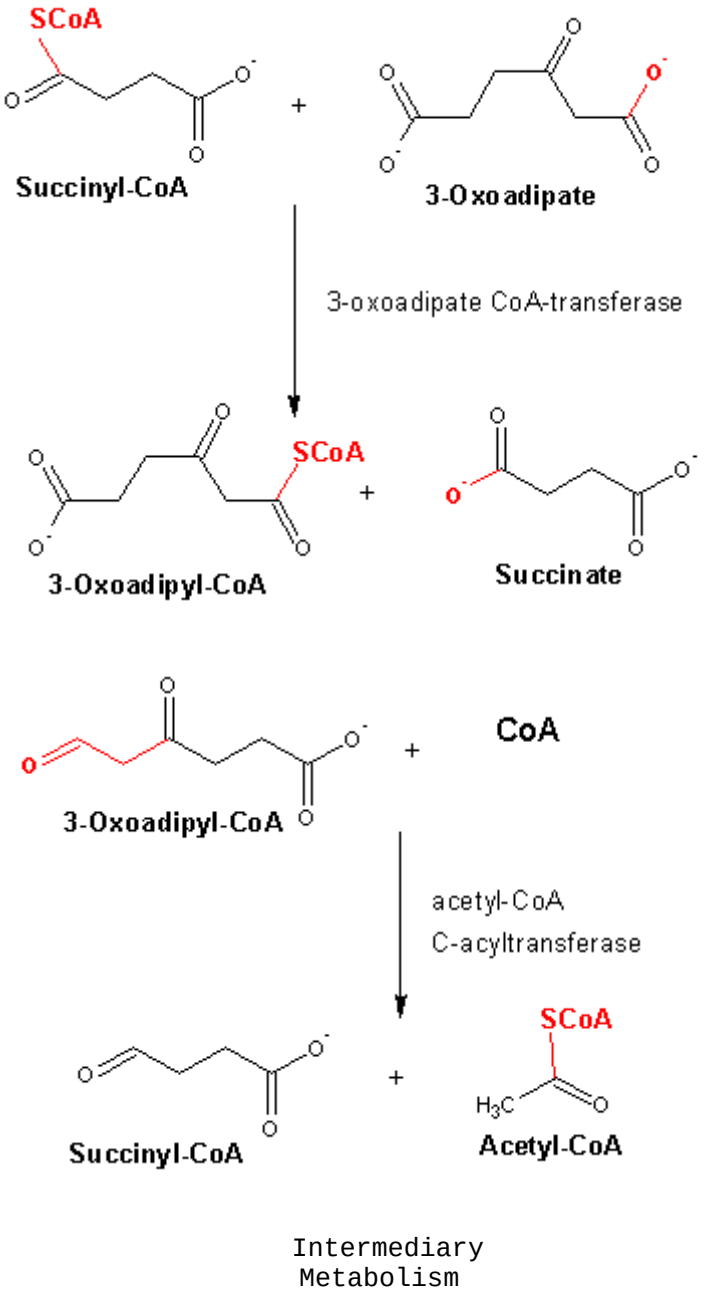


Figure 1.2 Degradation pathway of 2,4-dichlorophenol

1.6 Objectives and Scope of this Study

The objective of this thesis is to use newly developed RTBR and RBBR for removal of chlorophenols from synthetic wastewater and to investigate the effects of operating parameters on COD, chlorophenol and toxicity removals. The focus in

wastewater treatment has recently shifted to removal of toxic compounds. Despite the xenobiotic nature of these compounds, many are partially degradable by acclimated microorganisms.

The selected chlorophenols for this study are 4-chlorophenol (4CP), 2,4-dichlorophenol (DCP) and 2,4,6-trichlorophenol (TCP) as toxic substances.

Objectives of the proposed study can be summarized as follows:

- To develop and use new biofilm reactors namely RTBR and RBBR for biological treatment of chlorophenol containing wastewaters
- To investigate the effects of important operating variables such as the feed COD, chlorophenol concentrations and the A/Q ratio on percent COD, chlorophenol and toxicity removals for different chlorophenol compounds
- To determine toxicity levels of wastewater and percent inhibition of bacteria by the resazurin reduction method during treatment.
- To use statistical experiment design and response surface methodology (RSM) to evaluate the system performance and to determine the optimal operating conditions maximizing COD, chlorophenol and toxicity removals.
- To compare the performances of RTBR and RBBR for removal of 4CP, DCP and TCP for a large range of operating conditions.
- To develop a mathematical model describing the rate of COD removal as function of COD and chlorophenol concentrations and to determine the model constants by using the experimental data

CHAPTER TWO
LITERATURE REVIEW

Different physical, chemical and biological methods such as activated carbon adsorption, chemical oxidation and aerobic/anaerobic biological degradation were used for removal of chlorophenols from wastewater (Radwan K.H. and Ramanujam T.K., 1996; Swaminathan G. and Ramanujam T.K., 1999; Armenante, P.M., *et al*., 1999; Shin H.S., *et al*., 1999; Atuanya E.I., *et al.*, 2000; Jung M.V., *et al.*, 2001; Bali U. and Sengul F., 2002; Zhiqiang H., *et al*., 2005; Mayer J.G., et al., 2008; Bajaj M. *et al*., 2008). Adsorption and ion exchange methods are usually used to concentrate the chlorophenols on the solid phase, which require further treatment, by chemical or biological oxidation for complete mineralization. Chemical oxidation methods are fast, but expensive which may result in formation of undesirable by products. Biodegradation of chlorophenols by aerobic or anaerobic treatment methods are more specific and relatively inexpensive (Annachatre A.P. and Gheewala S.H, 1996; Armenante, P.M., *et al*., 1999; Atuanya E.I., *et al.*, 2000; Bali U. and Sengul F., 2002).

Most of the investigations on biodegradation of chlorophenols were focused on suspended pure culture studies using different bacteria and fungi (Li D.Y., *et al*., 1991; Dapaah S.Y. and Hill G.A., 1992; Hill G.A., *et al.*, 1996; Yee D.C. and Wood T.K., 1997; Steinle P., *et al*., 1998; Fahr K., *et al*., 1999; Kim M.H. and Hao O.J., 1999; Wang S.J. and Loh K.C., 1999; Wang C.C., *et al.*, 2000(a); Farrell A. and Quity B., 2002; Kargi F. and Eker S. 2004, 2005; Jiang Y. *et al.*,2008; Sahinkaya E. *et al* 2009). However, mixed microbial communities provide better rates of biodegradation as compared to pure cultures. Complete mineralization of chlorinated compounds can be obtained by using mixed acclimated microorganisms (Jiehua Y. and Owen W., 1994, 1996).

Recent investigations on biodegradation of chlorophenols focused on the use of immobilized cells or biofilm reactors (Shieh et al., 1990; Radwan and Ramanujam, 1996; Shin *et al.*, 1999; Swaminathan and Ramanujan, 1998; Alemzadeh I., *et al*.,

2002; Kim *et al*, 2002; Sahinkaya E. and Dilek B.D., 2006; Ziloue H. et al., 2006; Nicolella C. *et al*., 2009).

Aerobic organisms were reported to be more effective in biodegradation of chlorinated phenols as compared to anaerobic organisms. However, biological degradation of chlorophenols was improved by using anaerobic and aerobic systems in combination (Armenante P.M., *et al*., 1999; Atuanya E.I., *et al.,* 2000). Armenante P.M. (1999) reported that 2,4,6-TCP was almost completely degraded in a two-stage anaerobic–aerobic process.

Sponza D.T. and Uluköy A. investigated the treatability of 2,4-dichlorophenol (DCP) in an anaerobic/aerobic sequential reactor system when molasses was used as carbon source. 2,4-DCP removal efficiency decreased from 99 to 78.7% when the initial 2,4-DCP concentration and 2,4-DCP loading rates were increased from 5 to 120 mg l^{-1} and from 0.006 to 0.144 g l^{-1}d. The maximum COD removal efficiency was achieved as 77% at a 2,4-DCP loading rate of 0.042 g l^{-1}d.

Aerobic treatment of chlorophenols require short period time as compared to anaerobic treatment. Liu D. and Pacepavicius G.(1990) investigated aerobic and anaerobic biodegradation of 18-chlorophenols using a pentachlorophenol-degrading bacterial culture. The results showed that lag time increased with increasing number of chlorine groups.

Addition of growth substrates such as glucose into the medium was shown to improve the extent of biodegradation of chlorinated aromatic compounds (Wang S.J. and Loh K.C., 1999; Tay *et al.*, 2001). Usually, a carbohydrate substrate was used as the primary metabolite and the chlorophenols were the cometabolite in biodegradation of chlorophenols (Hill G.A., *et al.,* 1996; Kim M.H.and Hao O.J., 1999; Wang and Loh, 1999). On the contrary, Yang C.F., et al. (2005), reported that no significant amounts of 2,4-DCP were removed during incubation in the presence of supplementary carbon sources. Microorganisms were inhibited by toxicity of DCP of 75 mg l^{-1}.

Usually the bacteria are acclimated to chlorophenols, if not the bacteria require an adaption period for degradation of the compounds (Annachhatre A.P.and Gheewala S.H., 1996; Bali U. and Sengul F., 2002; Sahinkaya and Dilek, 2002; Snyder C.J.P., *et al.*, 2006).

High concentrations of chlorophenols are usually inhibitory for microorganisms. (Kargi F. and Eker S. 2004, 2005) However, adaptation of microorganisms to chlorophenols was found to improve biodegradation capability of the organisms and alleviate inhibition effects to some extent (Annachhatre A.P.and Gheewala S.H., 1996; Sahinkaya and Dilek., 2005).

Annachhatre A.P.and Gheewala S.H. (1996) also reported that aerobic processes are sensitive to high chlorophenol concentrations and also to chlorophenol shock loads. Preadaptation of sludge can improve sludge activity by reducing the time required for complete degradation of chlorophenols and provide protection from shock loadings.

Acclimation period of the mixed culture takes a long time as compared to pure cultures. After acclimation period, mixed culture can use the chlorophenols as sole carbon source to support the metabolic activities (Swaminathan G. and Ramanujam T.K.,1999).

There are a limited number of reports avaliable on treatment of chlorinated compounds by using biofilm reactors. 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. (Radwan K.H. and Ramanujam T.K. 1996; Kargi F. and Eker S. 2001, 2002, 2005; Ziloue H. et al., 2006). Packed column, rotating biodisc, fludized bed and UASB reactors were used for aerobic and anaerobic treatment of chlorophenol containing wastewaters. (Shieh B.W.K., *et al*., 1990; Radwan, K.H. and Ramanujam T.K.,1996; Swaminathan G. and Ramanujam T.K.,1999; Shin H.S., *et al*., 1999; ; Alemzadeh I., *et al*., 2002; Kim J.H., *et al.*, 2002; Ziloue H. et al., 2006). However, it is difficult to control some parameters such as the biofilm thickness, dissolved oxygen concentration, pH, and

redox potential in biofilm reactors due to heterogenous nature of such reactors. (Kargi F. and Eker S., 2001, 2002)

Most of the literature studies on 2,4-DCP biodegradation considered 2,4-DCP concentrations lower than 200 mg l^{-1} with 2,4-DCP removals less than 80% (Yee D.C. and Wood T.K., 1997; Steinle P., *et al*., 1998; Wang C.C., *et al*., 2000(a); Sahinkaya E and Dilek F.B., 2002).

Petroleum refinery wastwaters containing 2.65 mg l^{-1} phenol was treated with a 95.5% efficiency using 4-stage RBC (Congram G.E., 1976) Radwan K.H.*et al*, (1997) reported that 2,4-DCP of 200 mg l^{-1} was removed by a modified RBC and percent removal was 99.2%.

Radwan K.H. and Ramanujam T.K. (1996) reported the staging in the RBC system design is very important especially at higher organic loading and if high effluent treatment quality is required. The most significant factors affecting on the substrate removal were organic loading followed by hydraulic loading rate, hydraulic retention time and influent substrate concentration.

Biodegradation of toxic organic compounds such as 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, pentachlorophneol, 2-nitrophenol, di-ethyl phthalate and di-n-butyl phthalate by using RBC were studied and treated by Tokuz R.Y. (1991). 2,4-dichlorophneol concentration range in the influent wastewater was 7 to 14 mg l^{-1}.

Using a multi-stage RBC, Swaminathan G. and Ramanujam T.K. (1999) reported that most of the 2,4-dichlorophenol into the RBC was utilized in the first stage of the system. For a desired effluent concentration at the end, a multistage RBC system was reported to be essential.

The position, rather than the number of chlorine atoms, is more important in determining the biodegradation efficiency of chlorophenols. Usually biodegradability decreases and toxicity level increases with increasing number of chlorine groups (Chaudhry G.R. and Chapalamadugu S., 1991; Annachhatre A.P.and Gheewala S.H., 1996).

Different biological tests were used for toxicity assessment of individual chemicals or complex effluents (Liu D., 1986; Brouwer, H., 1991; Strotmann U.J.,*et al.*, 1993; Farre M. and Barcelo D., 2003). One of the toxicity assessment method used is ‘Resazurin Assay’, which is relatively simple, inexpensive and rapid method for assessment of the toxicity of chemical compounds and water samples (Liu D., 1986; Brouwer, H., 1991; Strotmann U.J.,*et al.*, 1993). Basic principle of the method is the measurement of percent inhibition on dehydrogenase activity of bacteria in the presence of toxic compounds. Toxicity values obtained with the Resazurin assay are comparable to those obtained with the more commonly used biological methods such as Daphnia magna, and Microtox TM (Farre M. and Barcelo D., 2003)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Experimental System

3.1.1 Rotating perforated tubes biofilm reactor

Figure 3.1 depicts a schematic diagram of the rotating perforated tubes biofilm reactor (RTBR). The experimental system consisted of a feed reservoir, wastewater tank containing battery of rotating tubes, driving motor, shaft and wastewater pump. The discs containing the battery of tubes were rotated by using a motor and a shaft passing through the central hole on the discs. Feed reservoir was placed in a deep refrigerator to keep the temperature below 5 ^{o}C in order to avoid any decomposition.

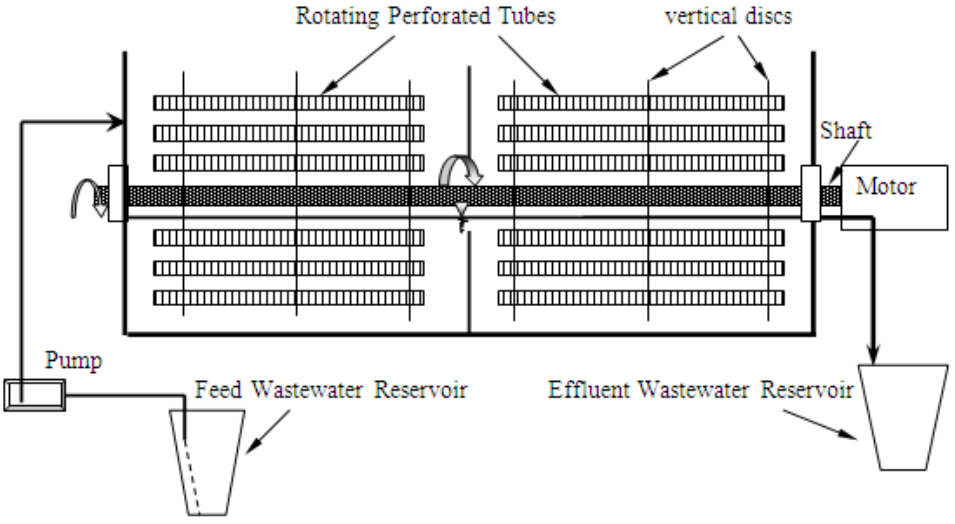


Figure 3.1 Schematic diagram of the rotating perforated tubes biofilm reactor (RTBR) used in experimental studies

The rotating tube system had two sections mounted on the same shaft each having 25 perforated tubes (total of 50 tubes) of length L= 25 cm made of PVC. Outer and inner diameter of the tubes were D_o = 2.1 cm and D_i = 1.3 cm resulting in a total

surface area of A = 1.34 m^2. Each tube had twenty holes of 0.5 cm in diameter located diagonally and 1 cm apart on their surfaces, which allowed air passage to the inner surface of the tubes. The outer surface of the tubes was corrugated to facilitate biofilm formation. The tubes were located on the peripheral area of the discs and were completely immersed in the wastewater tank. Organisms grow in form of biofilm on the outer and inner surfaces of the tubes. Total liquid volume in the tank was V = 9 L for 2,4-dichlorophenol removal experiments and V=12 L for the other experiments. Therefore, the biofilm area per unit wastewater volume in the tank was a = 149 m^2 m^{-3} and 112 m^2 m^{-3}, respectively for DCP and for 4CP, TCP removals..

3.1.2 Rotating brush biofilm reactor

Figure 3.2 depicts a schematic diagram of the rotating brush biofilm reactor (RBBR). The experimental system consisted of a feed reservoir, wastewater tank containing a battery of brushes, driving motor, shaft and wastewater pump. The vertical discs containing the battery of brushes were rotated by using a motor and a shaft passing through the central hole on the discs. Rods containing brushes were mounted on the discs through the holes on disc surfaces. The system was placed in a stainless steel reactor in the shape of half a barrel with dimensions of 60 cm length, 30 cm width (or diameter) and 20 cm depth open to atmosphere. The feed reservoir was placed in a deep refrigerator to keep the temperature below 5 ^oC to avoid any decomposition. The system had two sections mounted on the same shaft each having 12 brush rods with total of 24 brush rods of length L= 25 cm and diameter of D_o = 2.1 cm. Each brush rod contained 4200 bristles of length 0.9 cm and diameter of 0.6 mm yielding a total surface area of A = 2.11 m^2 including the brush and rod surface areas on 24 tubes. Part of the brushes was completely immersed in the wastewater tank during rotation and part of them was in direct contact with air. Organisms grow in form of biofilm on the surfaces of the brush structures and rod surfaces. Total liquid volume in the tank was V_L = 12 litre. Therefore, the biofilm area per unit wastewater volume in the tank was a = 176 m^2 m^{-3}. Biofilm surface area in RBBR (2.11 m^2) was considerably higher than that of the RTBR (1.34 m^2).

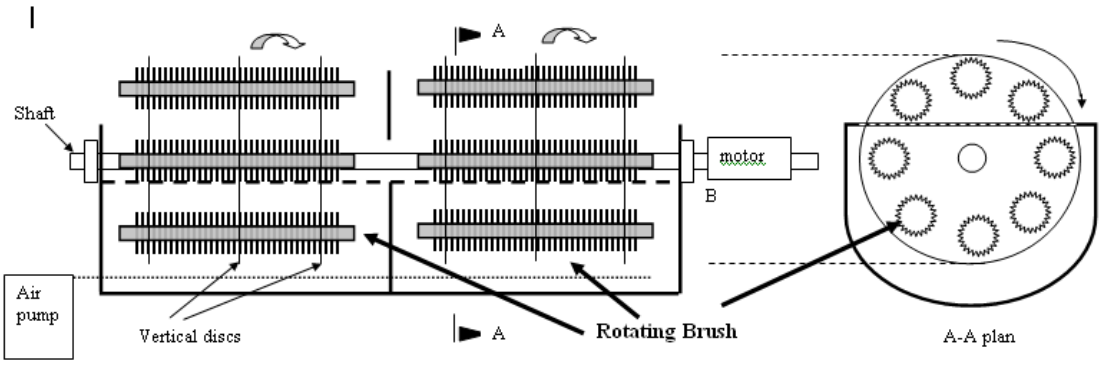


Figure 3.2 Schematic diagram of the rotating perforated tubes biofilm reactor (RTBR) used in experimental studies (RBBR)

3.2 Organisms

The activated sludge culture was obtained from Cigli municipal wastewater treatment plant in Izmir, Turkey. The sludge was cultivated in a growth media containing diluted molasses, urea, and K_2HPO_4 with a COD/N/P ratio of 100/8/2 using an incubator shaker at 200 rpm and 25 ^oC. Acclimation period was carried out using a gyratory incubator shaker and 500 ml Erlenmeyer flasks charged with 190 ml nutrient medium. Different amounts of 2,4-DCP were added into flasks and the initial pH was adjusted to 7. Flasks were inoculated with 10 ml culture and were incubated on the shaker at 30 ^oC. The pH was controlled at 7 by addition of sterile 0.1M NaOH everyday.

In one set of experiments for DCP removal using RTBR, the activated sludge culture was supplemented with the *Pseudomonas putida*. Pure culture of *Pseudomonas putida* (DSM 6978) capable of degrading DCP was obtained from Deutsche Sammlung von Mikroorganismen un Zellkulturen (DSMZ) GmbH, Braunschweig, Germany. The acclimated activated sludge and *Pseudomonas* culture were mixed (1/1, v/v) before using for inoculation of the RTBR for the DCP removal. The other experiments were carried out by using the activated sludge culture without addition of *Pseudomonas putida*.

Table 3.1 shows biomass concentration on the tube or brush surfaces (X_f) in form of biofilm and the suspended biomass concentration (X_s) in the tank. Wastewater in the tank was aerated gently in order to keep the suspended organisms active. Biofilm organisms were aerated by direct contact of air with the biofilm during rotation of the tubes.

Table 3.1 Biomass concentrations in biofilm reactors for all experiments

Biomass concentration		4-CP		2,4-DCP		2,4,6-TCP	
	Unit	RBBR	RTBR	RBBR	RTBR	RBBR	RTBR
X_f	mg m^{-2}	57000	49285	42000	55000	65000	50000
X_s	mg l^{-1}	2800	2019	3000	1500	3000	3000
X_f	mg m^{-2}	--	--	--	55000(*)	--	--
X_s	mg l^{-1}	--	--	--	1500(*)	--	--

(*): kinetic parameters estimation experiment by using RTBR

3.3 Experimental Procedure

Experiments were started batch wise. About 10 litre of the synthetic wastewater was placed in the treatment tank containing the battery of rotating tubes or brush tubes and was inoculated with the acclimated sludge culture. The system was operated batch wise for nearly two weeks by changing the wastewater media in every three days until a biofilm thickness of 1-1.5 mm was developed on the surfaces of the tubes. Continuous operation was started after biofilm development. Feed wastewater was fed to the reactor with a desired flow rate and removed with the same rate. Biofilm thickness was controlled around 1.5 mm by adjusting the feeding regime. The liquid phase in the tank was aerated and aeration was supplied to the biofilm by direct contact of the tubes or brush with air during rotation. The shaft was rotated with a constant speed of 12 rpm. pH was nearly 6.9 in the feed wastewater which increased to pH>8.0 in the tank because of ammonia released from biodegradation of urea. pH of the aeration tank content was adjusted manually to pH 7.5 by addition of dilute sulfuric acid several times a day. Temperature and pH were approximately $T = 25 \pm 2\ ^oC$ and $pH = 7.5 \pm 0.3$ during operation.

3.4 Analytical Methods

Samples were withdrawn from the first and the second stages everyday for analysis and centrifuged at 8000 rpm (7000 g) for 20 minutes to remove biomass from the liquid phase. Clear supernatants were analyzed for chlorophenol contents (4-CP, DCP and TCP). 4-aminoantipyrene colorimetric method developed for determination of phenol and derivatives in form of phenol index was used for analysis chlorophenols as specified in the Standard Methods (Greenberg A.E.,*et al*., 1989). Chlorophenol compounds and by-products were also analyzed by using an Agilent HPLC 1100 (Zorbax C18, Methanol:Water-50:50, 280nm, 1ml). Chemical oxygen demand (COD) was determined using the dichromate reflux method according to the Standard Methods (Greenberg A.E.,*et al*., 1989). Biomass concentrations were determined by filtering the samples through 0.45µ milipore filter and drying in an oven at 105 ^{o}C until constant weight (Greenberg A.E.,*et al*., 1989).

Resazurin reduction method was used to determine the toxicity of the feed and effluent wastewater (Liu D., 1986 - Farre M. and Barcelo D., 2003). The test organisms (washed activated sludge) to be subjected to the toxic feed and effluent wastewater were cultivated on nutrient broth before using for determination of the toxicity of wastewater samples. The test cultures were transferred every day to new medium to keep the sludge age constant during the course of toxicity measurements. In the presence of active bacterial culture with dehydrogenase enzyme activity, resazurin changes color from blue to pink forming the reduced compound resorufin. Inactive bacteria do not cause any change in the color of resazurin and the color remains blue. Therefore, the color of the resazurin solution is an indicator of bacterial activity. A visible spectrometer was used to determine the color at 610 nm. Percent toxicity removal was calculated by using the following equation:

$$E = 1 - (TOX_e / TOX_o) \qquad \text{(Eqn 1)}$$

where TOX_e and TOX_o are the toxicities of the effluent and the feed wastewaters which were determined with respect to the test organisms dehydrogenase activity unexposed to chlorophenols.

3.5 Wastewater Composition

Synthetic wastewater used throughout studies was composed of diluted molasses, urea, KH_2PO_4 and $MgSO_4$ resulting in COD/N/P ratio of 100/8/1.5 in the feed wastewater. $MgSO_4$ concentration in the feed was 50 mg l^{-1} in all experiments. COD and chlorophenol concentrations in the feed wastewater were adjusted to desired levels specified by the Box-Wilson and Box-Behnken experimental design methods.

3.6 Box- Wilson Statistical Experiment Design

Box-Wilson statistical experiment design and response surface methodology (RSM) were used for 4-chlorophenol (4-CP) and 2,4-dichlorophenol (2,4-DCP) removal using both the rotating perforated tubes (RTBR) and rotating brush biofilm reactors (RBBR). Box–Wilson statistical experimental design method was used to determine the effects of operating parameters such as A/Q ratio, feed COD and chlorophenol concentrations on percent COD, chlorophenol and toxicity removals. The same operating conditions (feed COD, chlorophenol contents and the A/Q ratio) were used for both reactors treating the same chlorophenol compound.

During the all statistical design of the experiments, three important operating parameters; feed chlorophenol concentration (X_1), feed COD_o (X_2) concentrations and A/Q ratio (X_3) were considered as independent variables. Experimental conditions of the Box-Wilson design are presented in section 4.1.

3.7 Box-Behnken Statistical Experiment Design

Box-Behnken statistical experiment design was used for 2,4,6- trichlorophenol (2,4,6-TCP) removal by using rotating perforated tubes (RTBR) and rotating brush biofilm reactors (RBBR). Box–Behnken statistical experiment design method was used to determine the effects of operating parameters such as A/Q ratio, feed COD and 2,4,6-TCP concentrations on percent COD, 2,4,6-TCP and toxicity removals. The same operating conditions were used for both reactors treating TCP containing wastewater. Three important operating parameters, 2,4,6-TCP (X_1), COD (X_2), and

A/Q ratio (X_3) were considered as independent variables. Experimental conditions of the Box-Behnken design are presented in section 4.2.

3.8 Kinetic Modeling and Parameter Estimation using RTBR

Variables were changed one at a time in a set of experiments and the data were used in estimation of the mathematical model constants. In experiments with variable feed DCP concentrations, 2,4-DCP concentration in the feed wastewater was changed between 0 and 400 mg l^{-1} while the feed COD_o = 5000 ± 200 mg l^{-1} and A/Q = 93 m^2.d m^{-3} (HRT=15 hours) were constant. In variable A/Q ratio experiments, A/Q ratio was changed between 31 and 217 m^2.d.m^{-3} (HRT = 5-35 hours) while the feed DCP and COD were constant at 100 ± 5 mg l^{-1} and 5000 ± 200 mg l^{-1}, respectively.

CHAPTER FOUR
THEORETICAL BACKGROUND

4.1 Box-Wilson Statistical Experimental Design

Box–Wilson statistical experiment design was used to determine the effects of major operating parameters on COD, chlorophenols and toxicity removals. Three important operating parameters; chlorophenol concentration in the feed wastewater (X_i), COD_o (X_{i+1}) and A/Q ratio (X_{i+2}) were chosen as independent variables. X_i, X_{i+1} and X_{i+2} were varied between low and high values. The experiments consisted of six axial (A), eight factorial (F) and a centre (C) point. The centre point was repeated three times to estimate the experimental error.

Design was codded as -1, -k, 0, +k, +1 and k values were defined by eqn 2.

$$\pm k = \text{center point} \pm [(\text{max}-\text{min})/2\sqrt{p}] \quad \text{(Eqn 2)}$$

(p=number of variables)

The performance of the system was described by the following response function:

$$Y=b_o+\underbrace{\Sigma b_i * X_i}_{\text{Linear}}+\underbrace{\Sigma b_{ij} * X_i * X_j}_{\text{interaction}}+\underbrace{\Sigma b_{ii} * X_i^2}_{\text{squared}} \qquad i,j=1,2,3\ldots..n \quad \text{(Eqn 3)}$$

The coefficients of the following response function were determined by using the experimental data and the Statistica 5.0 computer program for regression analysis.

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2 \quad \text{(Eqn 4)}$$

where Y is the predicted response function (percent COD, chlorophenol or toxicity removal), b_0 is the offset term. Box-Wilson design was used for 4-chlorophenol and 2,4-dichlorophenol removal experiments while Box-Behnken method was utilized for TCP removal.

In 4-chlorophenol removal experiments, feed 4-CP concentration (X_1) was varied between 0 and 1,000 mg l^{-1} while the feed COD concentration (X_2) was between 2,000 and 6,000 mg l^{-1} for both reactors. Similarly, in 2-4-DCP experiments, the feed 2,4-DCP concentration (X_1) was between 50 and 500 mg l^{-1} while feed COD concentration (X_2) was varied between 2,000 and 6,000 mg l^{-1} for both reactors. On the other hand, the A/Q ratio for both reactors were different because of different surface area and the wastewater volumes in different reactors. The A/Q ratios (X_3) were between 47-186 $m^2d\ m^{-3}$ and 73-293 $m^2d\ m^{-3}$ resulting in HRT values between 10 and 40 hours for RTBR and RBBR, respectively for the 4-CP removal experiments. For DCP removal experiments, the A/Q ratios (X_3) were between 62-248 $m^2d\ m^{-3}$ and 73-293 $m^2d\ m^{-3}$ for RTBR and RBBR, respectively.

Feed chlorophenol concentration (X_1), COD (X_2), A/Q ratio (X_3) were considered as independent variables. The axial, factorial and center levels of each variable designated as as -1, -k, 0, +k and +1, respectively are presented in Table 4.1

Experimental points of 4-chlorophenol and 2,4-dichlorophenol removal experiments for Box–Wilson statistical design are presented in Table 4-2 and Table 4-3, respetively. The experiments consisted of six axial (A), eight factorial (F) and a centre (C) point. The centre point was repeated three times to estimate the experimental error. Experiments were conducted until the system reached the steady state when the last three days measurements of chlorinated compounds, COD and toxicity were almost the same.

Response functions describing variations of dependent variables (percent chlorophenol, COD and toxicity removals) with the independent variables (X_i) can be expressed by Eqn 3 and the performance of the system was described by Eqn 4.

The response function coefficients were determined by using the experimental data and the Statistica 5.0 computer program for regression. The response functions for COD, 4-CP, 2,4-DCP and toxicity removals were approximated by the standard quadratic polynomial equation as presented in Eqn 4.

Table 4.1 The levels of independent variables in Box-Wilson statistical experimental design for 4-CP and 2,4-DCP removals in RTBR and RBBR.

Variable		Coded variable level				
4-CP experiments		Low level		Center level		High level
		-1	-k	0	+k	+1
4- chlorophenol (mgl^{-1})	X_1	0	211	500	789	1000
Chemical oxygen demand, COD ($mg\ l^{-1}$)	X_2	2000	2844	4000	5156	6000
A/Q ratio ($m^2\ d\ m^{-3}$) for RBBR	X_3	73	117	183	249	293
A/Q ratio ($m^2\ d\ m^{-3}$) for RTBR	X_3	47	74	116	158	186
Variable		Coded variable level				
2,4-DCP experiments		Low level		Center level		High level
		-1	-k	0	+k	+1
2,4- dichlorophenol (mgl^{-1})	X_1	50	145	275	405	500
Chemical oxygen demand, COD ($mg\ l^{-1}$)	X_2	2000	2844	4000	5156	6000
A/Q ratio ($m^2 d\ m^{-3}$) for RBBR	X_3	73	117	183	249	293
A/Q ratio ($m^2 d\ m^{-3}$) for RTBR	X_3	62	101	155	209	248

Table 4.2 Experimental data points used in Box-Wilson statistical design for 4-CP removal using RBBR and RTBR

Run	Experimental Data Points				
	$4\text{-}CP_o$	COD_o	HRT	A/Q (RBBR)	A/Q (RTBR)
	$mg\ l^{-1}$	$mg\ l^{-1}$	h	$m^2\ d\ m^{-3}$	$m^2\ d\ m^{-3}$
A1	0	4000	25	183	116
A2	1000	4000	25	183	116
A3	500	2000	25	183	116
A4	500	6000	25	183	116
A5	500	4000	10	73	47
A6	500	4000	40	293	186
F1	789	5156	34	249	158
F2	789	5156	16	117	74
F3	789	2844	34	249	158
F4	789	2844	16	117	74
F5	211	5156	34	249	158
F6	211	5156	16	117	74
F7	211	2844	34	249	158
F8	211	2844	16	117	74
C	500	4000	25	183	116

Table 4.3 Experimental data points used in Box-Wilson statistical design for 2,4-DCP removal using RBBR and RTBR

Run	Experimental Data Points				
	2,4-DCPp_o	COD_o	HRT	A/Q (RBBR)	A/Q (RTBR)
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	m^2 d m^{-3}
A1	50	4000	25	183	155
A2	500	4000	25	183	155
A3	275	2000	25	183	155
A4	275	6000	25	183	155
A5	275	4000	10	73	62
A6	275	4000	40	293	248
F1	405	5156	34	249	209
F2	405	5156	16	117	101
F3	405	2844	34	249	209
F4	405	2844	16	117	101
F5	145	5156	34	249	209
F6	145	5156	16	117	101
F7	145	2844	34	249	209
F8	145	2844	16	117	101
C	275	4000	25	183	155

4.2 Box-Behnken Statistical Experimental Design

The Box-Behnken design is an independent, rotatable quadratic design with no embedded factorial or fractional factorial points where the variable combinations are at the midpoints of the edges of the variable space and at the center. Among all statistical experiment designs, Box-Behnken design requires fewer runs than the others do. Box-Benken statiscal design provides significant model approach without assuming a quadratic response function. On the other hand, Box-Wilson design accepts the quadratic polynominal model without any significance testing. To compare the two different approaches, Box-Behnken design was used in 4,6-TCP removal experiments.

Three important operating parameters; chlorophenol concentration in the feed wastewater (X_i), COD_o (X_{i+1}) and A/Q ratio (X_{i+2}) were chosen as independent

variables. X_i, X_{i+1} and X_{i+2} were designed between low, mid and high values. The low, center and high levels of each variable codded as -1, 0, and +1.

The performance of the system was described by the following response function:

$$Y=b_o+\overbrace{\sum b_i*X_i}+\overbrace{\sum b_{ij}*X_i*X_j}+\overbrace{\sum b_{ii}*X_i^2} \qquad i,j=1,2,3\ldots..n \qquad \text{(Eqn 5)}$$

Linear interaction squared

The coefficients of the following response function were determined by using the experimental data and the Statistica 5.0 and State-Ease design expert 7.0 computer program for regression analysis.

$$Y=b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{11}X_{12}+b_{22}X_{22}+b_{33}X_{32} \quad \text{(Eqn 6)}$$

where Y is the predicted response function (percent COD, phenolic compounds or toxicity removal), b_0 is the offset term. 2,4,6-trichlorophenol experiments was designed by Box-Behnken statistical experimental design.

Three important operating parameters; 2,4,6-TCP_o (X_1) and feed COD_o (X_2) concentrations and A/Q ratio (X_3) were considered as independent variables. Feed 2,4,6-TCP concentration (X_1) was between 0 and 400 mg l^{-1} while the feed COD concentration (X_2) was varied between 1,000 and 4,000 mg l^{-1} for both reactors. Third independent variable (A/Q) was different because of different biofilm surface araes for both reactors. The A/Q ratio (X_3) was between 37 and 256 m^2 d m^{-3} resulting in HRT values between 5 and 35 h for RBBR. Similarly, A/Q ratio was was between 23 and 163 m^2 d m^{-3} resulting in HRT values between 5 and 35 h for the RTBR.

The low, center and high levels of each variable designated as -1, 0, and +1, respectively are presented in Table 4.4.

Table 4.4 The levels of independent variables in Box-Behnken statistical experiment design for 2,4,6-trichlorophenol removal in both reactors.

Variable	Symbol	Coded variable level		
2,4,6-TCP experiments		Low level	Center level	High level
		-1	0	+1
2,4,6- trichlorophenol ,TCP (mgl^{-1})	X_1	0	200	400
Chemical oxygen demand, COD ($mg\ l^{-1}$)	X_2	1000	2500	4000
A/Q ratio ($m^2\ d\ m^{-3}$) for RTBR	X_3	23	93	163
A/Q ratio ($m^2\ d\ m^{-3}$) for RBBR	X_3	37	146.5	256

Response functions describing variations of dependent variables (percent COD, 2,4,6-TCP and toxicity removals) with the independent variables (X_i) can be written as Eqn 5 and the performance of the system was described by Eqn 6.

Experimental data points used in Box-Behnken statistical design are presented in

Table 4.5 The response function coefficients were determined by using the experimental data and the Stat-Ease Design Expert 7.0 computer program. Experimental data was used for determination of the response function coefficients for each independent variable by iteration. Different response functions were used to correlate the experimental data and the most suitable one was determined by using the analysis of variance (ANOVA) program. ANOVA tests for all response functions indicated that the quadratic model provided the best fit to the experimental data with the lowest standard deviation, the highest correlation coefficient and the lowest p-value. The response functions for 2,4,6-TCP, COD and toxicity removals were approximated by Eqn 6.

Table 4.5 Experimental data points used in Box-Behnken statistical design in the order of increasing feed 2,4,6-TCP concentrations for RTBR and RBBR.

Run	Experimental Data Points				
	2,4,6-TCP_o	COD_o	HRT	A/Q (RBBR)	A/Q (RTBR)
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	m^2 d m^{-3}
1	1000	0	20	147	93
2	2500	0	35	256	163
3	4000	0	20	147	93
4	2500	0	5	37	23
5	1000	200	35	256	163
6	4000	200	35	256	163
7	2500	200	20	147	93
8	2500	200	20	147	93
9	2500	200	20	147	93
10	2500	200	20	147	93
11	2500	200	20	147	93
12	2500	400	35	256	163
13	1000	400	20	147	93
14	4000	400	20	147	93
15	1000	200	5	37	23
16	4000	200	5	37	23
17	2500	400	5	37	23

4.3 Kinetic Modeling and Parameter Estimation

By assuming completely mixed liquid phase in the RTBR or RBBR, a COD balance around the reactor yields the following equation:

$$Q(S_o - S) = \left(\frac{k_f X_f S}{K_s + S} A_f + \frac{k_s X_s S}{K_s + S} V_L\right)\left(\frac{K_{PC}}{K_{PC} + PC}\right) \qquad \text{(Eqn 7)}$$

(PC: phenolic compounds)

where Q is the flow rate of wastewater (l h^{-1}); S_o and S are the feed and effluent COD concentrations (mg l^{-1}); k_f and k_s are the specific COD removal rate constants for the biofilm and the suspended organisms, respectively (h^{-1}); X_f is the biomass concentration per unit biofilm surface (mg m^{-2}); Xs is the suspended biomass concentration in the liquid phase (mgl^{-1}); K_s is the saturation constant for COD removal (mg l^{-1}) which was assumed to be the same for both the biofilm and the suspended cells; A_f is the total biofilm surface area (m^2); V_L is the wastewater volume in the reactor (l); PC is the chlorinated compounds concentrations used in experiments in the reactor and the effluent (mg l^{-1}); K_{PC} is the chlorinated compounds inhibition constant for COD removal (mg l^{-1}).

The first and second terms on the right hand side of the Eqn 7 are the rate of COD removal by the biofilm and suspended organisms, respectively. The third term represents inhibition caused by the presence of phenolic compounds in the reactor. Monod type saturation kinetics were assumed for COD removal and the phenolic compounds inhibition was assumed to be non-competitive affecting only the maximum rates of COD removals

Eqn 7 can be rearranged as

$$R_s = \frac{Q(S_o - S)}{A_f} = \left(R_{mf} + \frac{R_{ms}}{a_f}\right)\left(\frac{S}{K_s + S}\right)\left(\frac{K_{PC}}{K_{PC} + PC}\right) \qquad \text{(Eqn 8)}$$

or

$$R_s = \frac{Q(S_o - S)}{A_f} = R_m\left(\frac{S}{K_s + S}\right)\left(\frac{K_{PC}}{K_{PC} + PC}\right) \qquad \text{(Eqn 9)}$$

where R_s is the rate of COD removal per unit biofilm surface area (mg COD.m^{-2}. h^{-1}); R_{mf} is the maximum rate of COD removal by the biofilm organisms (= k_f X_f, mg COD m^{-2} h^{-1}); R_{ms} is the maximum rate of COD removal by the suspended organisms (= k_s X_s, mg COD l^{-1} h^{-1}); a_f is the biofilm surface area per unit wastewater volume in

the reactor (m^2 biofilm. l^{-1} liquid) and R_m is the maximum rate of COD removal by both the biofilm and the suspended organisms per unit surface area of biofilm which is equal to $R_{mf} + R_{ms} / a_f$ (mg COD m^{-2} h^{-1}).

Statistica 5 program was used for iterative determination of the kinetic constants using the experimental data obtained by changing one variable at a time.

CHAPTER FIVE
RESULTS AND DISCUSSION

5.1 Biological Treatment of 4-Chlorophenol Containing Synthetic Wastewater

5.1.1 Removal of 4-chlorophenol by using Rotating Perforated Tubes Biofilm Reactor (RTBR)

Box-Wilson statistical experiment design was used to determine the effects of important operating variables on percent COD, 4-chlorophenol (4-CP) and toxicity removals. Three important operating parameters; feed 4-CP (X_1) and COD (X_2) concentrations in the feed wastewater and A/Q ratio (X_3) were chosen as independent variables. Coefficients of the response functions for each independent variable were determined by using the experimental data. The experimental data were correlated with the response functions by using the Statistica-5 regression and MsExcel-Solver program. The estimated coefficients of the response functions are presented in Table 5-1. The response functions with the determined coefficients were used in calculating the predicted values of percent COD, 4-CP and toxicity removals. Collected samples from each stage were analyzed. Most of the feed 4-CP, COD and toxicity were removed in the first stage, but the data from the second stage were used for estimation of coefficients. The benefit of the second stage was only further removal of COD and chlorophenols. The differences were less than 5% between the first and the second stages. A comparison of the experimental and predicted values for percent removals of COD, 4-CP and toxicity are presented in Table 5.2.

Table 5.1 Coefficients of the response functions for COD, 4-CP and toxicity removals in RTBR

	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{11}	b_{22}	b_{33}
Y_{COD} R^2=0.96	84.01	-6.47 $*10^{-2}$	1.58 $*10^{-3}$	2.82 $*10^{-1}$	-4.42 $*10^{-6}$	4.36 $*10^{-4}$	3.41 $*10^{-5}$	1.4 $*10^{-5}$	-5.89 $*10^{-7}$	-2.01 $*10^{-3}$
$Y_{4\text{-}CP}$ R^2=0.97	-5.91	-1.04 $*10^{-1}$	2.79 $*10^{-3}$	1.66	6.92 $*10^{-7}$	6.85 $*10^{-4}$	-2.18 $*10^{-5}$	5.18 $*10^{-7}$	-1.41 $*10^{-9}$	-6.32 $*10^{-3}$
$Y_{TOXICITY}$ R^2=0.89	-3.08	-5.03 $*10^{-2}$	-2.38 $*10^{-3}$	1.58	9.34 $*10^{-6}$	1.76 $*10^{-4}$	2.33 $*10^{-5}$	-2.25 $*10^{-5}$	-5.97 $*10^{-7}$	-5.75 $*10^{-3}$

Table 5.2 Comparison of experimental and predicted percent COD, 4-CP and toxicity removals in RTBR

	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{4\text{-}CP(exp)}$	$E_{4\text{-}CP(pred)}$	$E_{TOX(exp)}$	$E_{TOX(pred)}$
A1	98	100	-	-	-	-
A2	87	84	88	80	85	78
A3	91	90	95	91	98	90
A4	84	85	96	93	98	91
A5	66	68	33	30	49	35
A6	93	92	97	92	98	95
F1	91	92	95	100	95	100
F2	64	65	45	49	51	58
F3	93	96	97	100	88	91
F4	73	74	40	46	44	54
F5	97	95	97	97	96	98
F6	92	88	78	78	55	64
F7	93	92	96	98	97	100
F8	93	92	75	74	67	72
C(avg)	91	90	93	92	93	93

Percent COD removal efficiencies varied between 66 and 98%, while percent 4-CP removals were between 33 and 97%. Percent toxicity removals varied between 44 and 98%. Predicted and experimental values of COD, 4-CP and toxicity removals were in good agreement as presented in Table 5-2. Projections of the response functions on certain planes of constant COD, 4-CP and A/Q were drawn and presented in Figure 5.1 and 5.5.

Variations of percent COD removal with the feed COD (including COD content of 4-CP) and 4-CP concentrations at constant A/Q ratio of 116 $m^2\ d\ m^{-3}$ are depicted in Figure 5.1. Percent COD removal decreased steadily with increasing feed 4-CP content from 211 to 1000 mg l^{-1} for the feed COD concentrations between 2,000 and 6,000 mg l^{-1}, due to toxic effects of high 4-CP concentrations. At low 4-CP concentrations below 500 mg l^{-1}, percent COD removal was not affected from the variations in the feed COD since 4-CP inhibition on the microorganisms was not significant. However, at high feed 4-CP concentrations above 780 mg l^{-1}, percent COD removal decreased with increasing feed COD due to low biomass concentration in the system at high 4-CP contents. In order to obtain more than 90% COD removal,

the feed 4-CP concentration should be below 500 mg l^{-1} for all feed COD contents at an A/Q ratio of 116 m^2 d m^{-3}.

Figure 5.2 depicts variation of percent COD removal with the feed 4-CP content at different A/Q ratios at a constant feed COD of 6,000 mg l^{-1}. Increases in A/Q ratio from 47 to 186 m^2 d m^{-3} resulted in increases in percent COD removal because of high biofilm surface area and biomass content of the system. Percent COD removal decreased with increasing feed 4-CP because of toxic effects of high 4-CP contents on the microorganisms. However, toxic effects of 4-CP was overcome by increasing the A/Q ratio. At a high A/Q ratio of 186 m^2 d m^{-3} percent COD removal was not affected from increases in the feed 4-CP due to high biomass content of the system. The system should be operated at high A/Q ratios above 160 m^2 d m^{-3} at high feed 4-CP concentrations to obtain high percent COD removals. The effects of A/Q ratio, feed 4-CP and COD concentrations on 4-CP removal performance of the RTBR were also investigated.

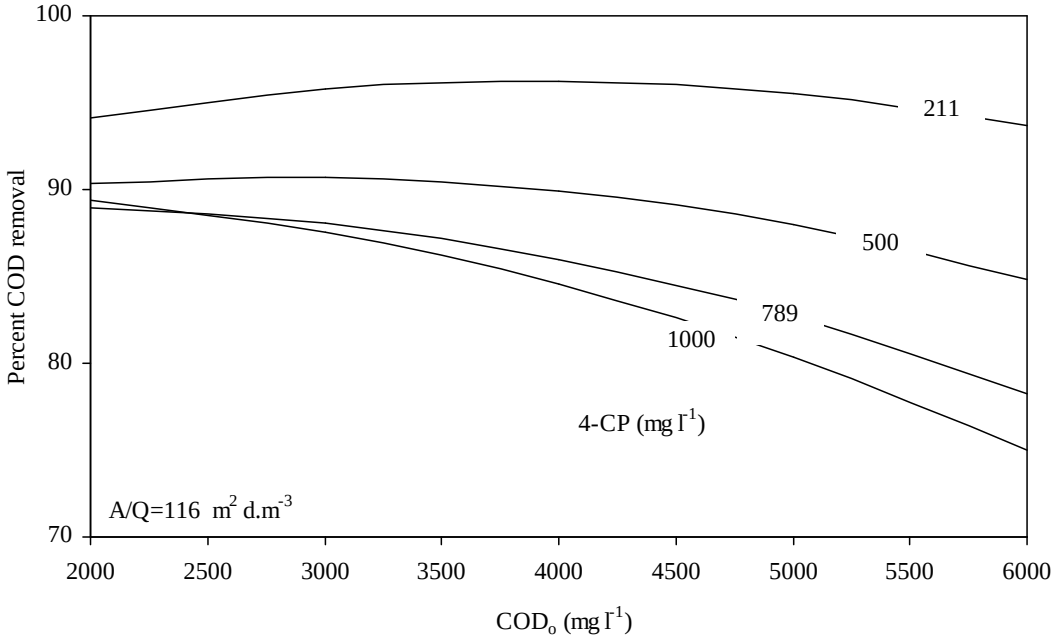


Figure 5.1 Variation of percent COD removal with the feed COD at different feed 4-CP concentrations

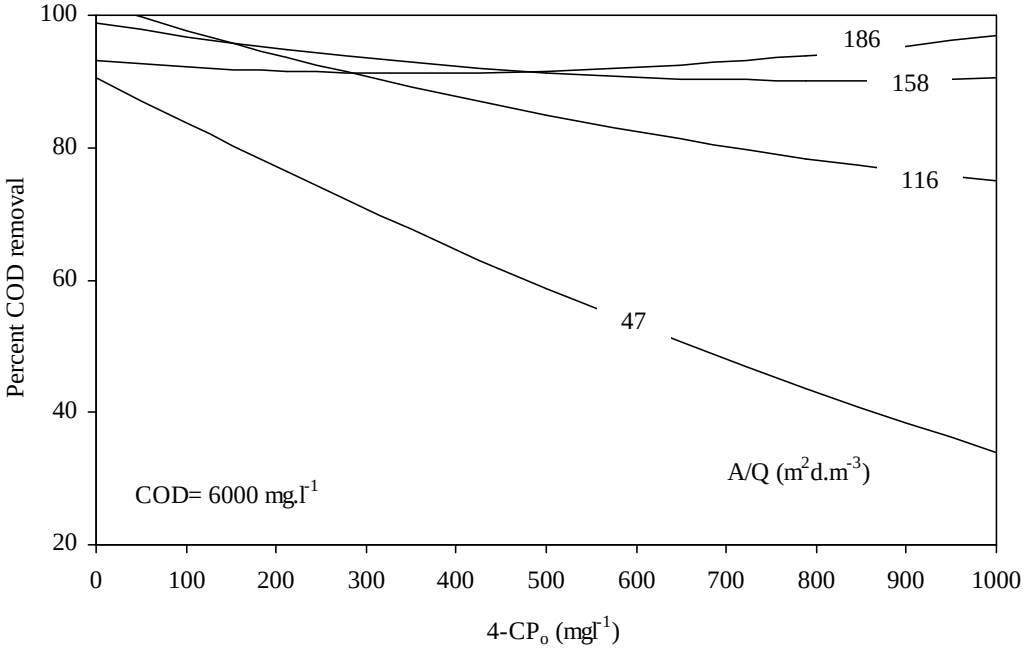


Figure 5.2 Variation of percent COD removal with the feed 4-CP at different A/Q ratios

Variation of percent 4-CP removal with the feed 4-CP concentration at different A/Q ratios at a constant feed COD of 6,000 mg l^{-1} is depicted in Figure 5.3. Percent 4-CP removal increased with increasing A/Q ratio as a result of increasing biofilm surface area yielding high biomass contents up to the A/Q ratio of 158 m^2 d m^{-3}. Further increases in A/Q ratio above 158 m^2 d m^{-3} caused decreases in percent 4-CP removal probably as a result of low feed flow rates at high A/Q ratios and insufficient COD loads to support high concentrations of biomass. Increases in the feed 4-CP contents resulted in decreases in percent 4-CP removal at low A/Q ratios below 116 m^2 d m^{-3} due to low biomass contents affected from 4-CP inhibitions. However, at high A/Q ratios above 158 m^2 d m^{-3}, 4-CP removal increased with the increasing feed 4-CP since 4-CP was used as carbon source by the dense biofilm organisms at low feed flow rates. At the feed COD and 4-CP concentrations of 6000 mg l^{-1} and 1000 mg l^{-1}, the system should be operated at an A/Q ratio of above 158 m^2 d m^{-3} in order to obtain more than 95% 4-CP removal.

Figure 5.4 depicts variation of percent 4-CP removal with the feed 4-CP and COD concentrations at a constant A/Q ratio of 116 m^2 d m^{-3}. Percent 4-CP removal decreased with increasing feed 4-CP content due to toxic effects of high 4-CP contents on the microorganisms. Increasing feed 4-CP content from 211 to 1000 mg l^{-1} resulted in a decrease in 4-CP removal efficiency from 99% to 83% at a feed COD of 6,000 mg l^{-1}. Increases in the feed COD resulted in increases in percent 4-CP removal. An increase in the feed COD from 2,000 mg l^{-1} to 6,000 mg l^{-1} resulted in an increase in percent 4-CP removal, from 78 to 83% when the feed 4-CP was 1000 mg l^{-1} at A/Q = 116 m^2 d m^{-3}. This is probably due to formation of high-density biofilm at high feed COD contents. Nearly complete removal of 4-CP required feed COD of 6000 mg l^{-1} and A/Q ratio larger than 116 m^2 d m^{-3} for the feed 4-CP of 211 mg l^{-1}.

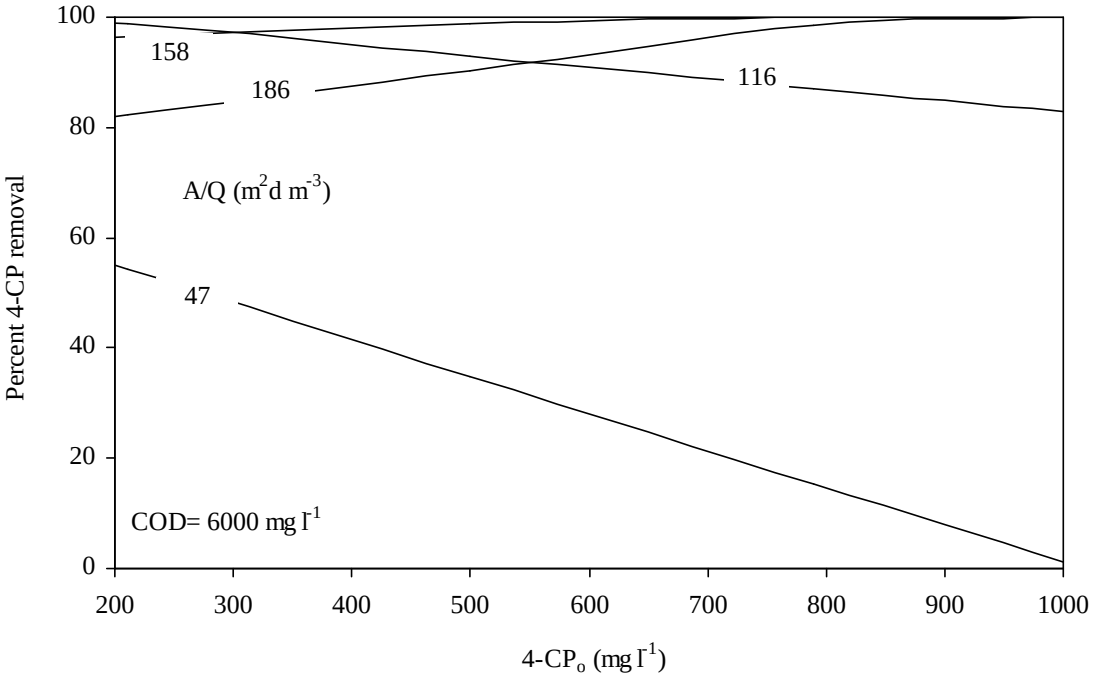


Figure 5.3 Variation of percent 4-CP removal with the feed 4-CP at different A/Q ratios

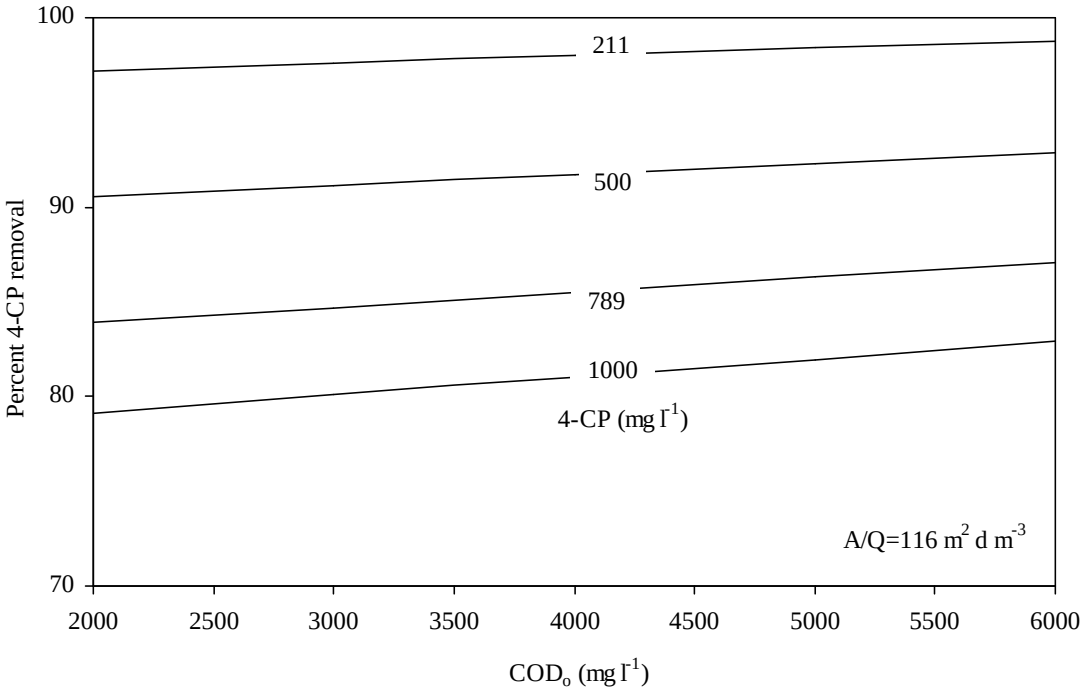


Figure 5.4 Variation of percent 4-CP removal with the feed 4-CP at different feed COD concentrations

Variations of percent toxicity removal with the feed 4-CP content at different feed COD content and constant A/Q ratio of 186 m^2 d m^{-3} are depicted in Figure 5.5. Percent toxicity removals depict similar behavior as that of the 4-CP removal since 4-CP or its degradation intermediates are the major toxic compounds in the system. At low feed COD contents (< 4000 mg l^{-1}) yielding low biomass content in the system, percent toxicity removal decreased with increasing feed 4-CP due to toxic effects of high 4-CP contents. However, at high feed COD contents above 5000 mg l^{-1}, percent toxicity removal increased with increasing feed 4-CP content due to high biomass concentration and effective degradation of 4-CP. At high feed 4-CP contents above 400 mg l^{-1}, increases in the feed COD resulted in considerable increases in percent toxicity removal due to high-density biomass formation at high feed COD contents. At low feed 4-CP contents below 400 mg l^{-1}, percent toxicity removal at low feed COD contents were higher than those at high feed CODs, due to effective biodegradation of 4-CP at low feed COD and 4-CP contents yielding low toxicity effluents. In order to obtain more than 95% toxicity removal at the feed 4-CP of 1000 mg l^{-1}, the feed COD must be 6000 mg l^{-1} and the A/Q = 186 m^2 d m^{-3}. High feed

COD and biofilm surface area (high A/Q ratio) are required for effective removal of toxicity and 4-CP at high feed 4-CP contents.

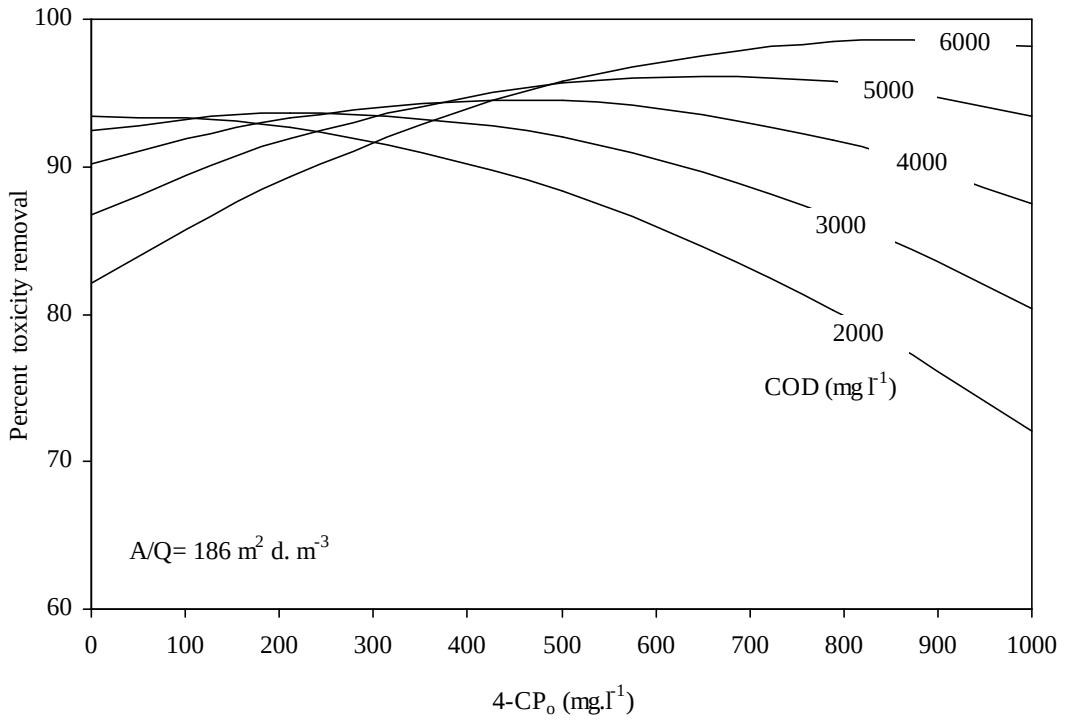


Figure 5.5 Variation of percent toxicity removal with the feed 4-CP content at different feed COD concentrations

5.1.2 Removal of 4-chlorophenol by using Rotating Brush Biofilm Reactor (RBBR)

Box–Wilson statistical experiment design was used to determine the effects of operating parameters such as A/Q ratio, feed COD_o and 4-chlorophenol ($4\text{-}CP_o$) concentrations on percent COD, 4-CP and toxicity removals. Three important operating parameters; $4\text{-}CP_o$ (X_1) and COD_o (X_2) concentrations in the feed wastewater and A/Q ratio (X_3) were chosen as independent variables. $4\text{-}CP_o$ concentration (X_1) was varied between 0 and 1000 mg l^{-1} while the feed COD_o concentration (X_2) was between 2,000 and 6,000 mg l^{-1}. The A/Q ratio (X_3) was varied between 73 and 293 m^2 d m^{-3}, resulting in hydraulic residence times between 10 and 40 h.

The experimental data were correlated with the response functions by using the Statistica-5 regression and MsExcel-Solver program. Similarly, most of the 4-chlorophenol was removed in the first stage. The contribution of the last stage was less than 10% COD and 4-CP removal. The data obtained from the second stage were used for estimation of the response function coefficients.. The estimated coefficients of the response functions are presented in Table 5.3. The response functions with the determined coefficients were used in calculating the predicted values of percent COD, 4-CP and toxicity removals. A comparison of the experimental and predicted values for percent removals of COD, 4-CP and toxicity are presented in Table 5.4. Predicted and experimental values of COD, 4-CP and toxicity removals were in good agreement as shown in Table 5.4 indicating the accuracy of the predictions by the response functions.

Table 5.3 Coefficients of the response functions for COD, 4-CP and toxicity removals in RBBR

	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{11}	b_{22}	b_{33}
Y_{COD} R^2=0.93	46.95	-5.82 $*10^{-2}$	1.66 $*10^{-2}$	2.83 $*10^{-1}$	-2.51 $*10^{-6}$	3.06 $*10^{-4}$	1.76 $*10^{-5}$	-3.66 $*10^{-6}$	-2.58 $*10^{-6}$	-1.05 $*10^{-3}$
$Y_{4\text{-}CP}$ R^2=0.96	-50.89	-8.15 $*10^{-2}$	1.42 $*10^{-2}$	1.27	-4 $*10^{-6}$	5.11 $*10^{-4}$	-2.26 $*10^{-5}$	-1.7 $*10^{-5}$	-9.7 $*10^{-7}$	-3.11 $*10^{-3}$
$Y_{TOXICITY}$ R^2=0.94	-61.48	-9.88 $*10^{-2}$	1.47 $*10^{-2}$	1.31	-1.6 $*10^{-6}$	5.33 $*10^{-4}$	-2.1 $*10^{-5}$	-7.9 $*10^{-7}$	-1.2 $*10^{-6}$	-3.28 $*10^{-3}$

Table 5.4 Comparison of experimental and predicted percent COD, 4-CP and toxicity removals in RBBR

	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{4\text{-}CP(exp)}$	$E_{4\text{-}CP(pred)}$	$E_{TOX(exp)}$	$E_{TOX(pred)}$
A1	97	-	-	-	-	-
A2	90	86	93	83	89	83
A3	93	90	98	93	90	83
A4	76	80	96	93	90	83
A5	67	69	30	26	23	16
A6	96	95	96	92	88	79
F1	95	94	98	100	93	96
F2	65	65	40	47	36	42
F3	93	98	99	100	94	100
F4	70	72	39	44	36	39
F5	96	94	96	97	75	82
F6	92	87	81	79	63	67
F7	94	94	98	97	79	84

F8	92	91	71	72	55	62
C (avg)	94	94	97	97	88	88

Projections of the response functions on certain planes of constant COD, 4-CP and A/Q are presented in Figure 5.6 and 5-10.

Variations of percent COD removal with the feed COD (including COD content of 4-CP) and 4-CP concentrations at constant A/Q ratio of 167 m^2 d m^{-3} are depicted in Figure 5.6. Percent COD removal decreased with increasing feed 4-CP concentration from 211 mg l^{-1} to 1000 mg l^{-1} for all feed COD concentrations, due to toxic effects of high 4-CP contents on the microorganisms. Percent COD removal increased slightly up to feed COD of 4,000 mg l^{-1}, and decreased with increasing feed COD above 4,000 mgl^{-1}, due to adverse effects of high COD loading rates on the organisms (substrate inhibition). Nearly complete COD removal was obtained at a feed COD of 3800 mg l^{-1} and A/Q ratio of 167 m^2 d m^{-3} when the feed 4- CP concentration was lower than 122 mg l^{-1}.

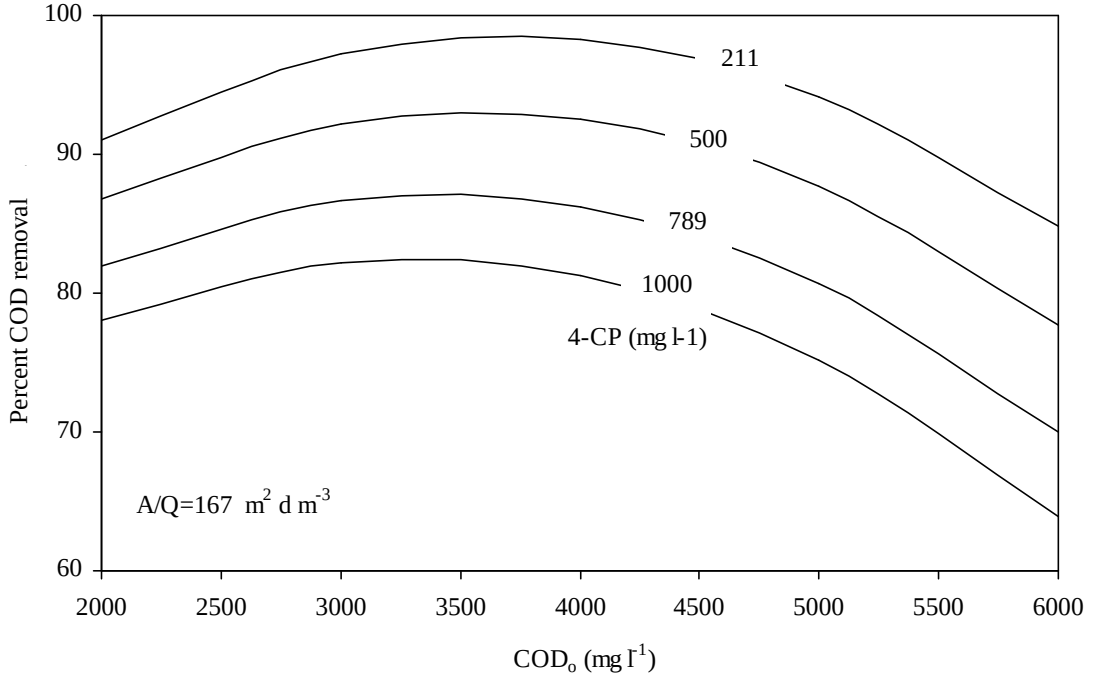


Figure 5.6 Variation of percent COD removal with feed COD concentration at different feed 4-CP concentrations

Figure 5.7 shows variations of percent COD removal with feed 4-CP concentration at different A/Q ratios and constant feed COD of 6,000 mg l^{-1}. Percent COD removal increased with increasing A/Q ratio and reached a maximum level at A/Q ratio of around 249 m^2 d m^{-3}. Percent COD removal was almost constant around 86% for the whole feed 4-CP concentrations at constant A/Q ratio of 249 m^2 d m^{-3}. Further increases in A/Q ratio did not cause significant changes in percent COD removal due to presence of sufficient biomass on the surfaces of brush. Percent COD removal decreased with increasing feed 4-CP concentrations as a result of 4-CP inhibition on the organisms for A/Q ratios between 73 and 183 m^2 d m^{-3}. At high A/Q ratio (low feed flow rates) of 293 m^2 d m^{-3} percent COD removal slightly increased with increasing feed 4-CP concentration due to low 4-CP loading rates at low feed flow rates yielding low level of 4-CP inhibitions.

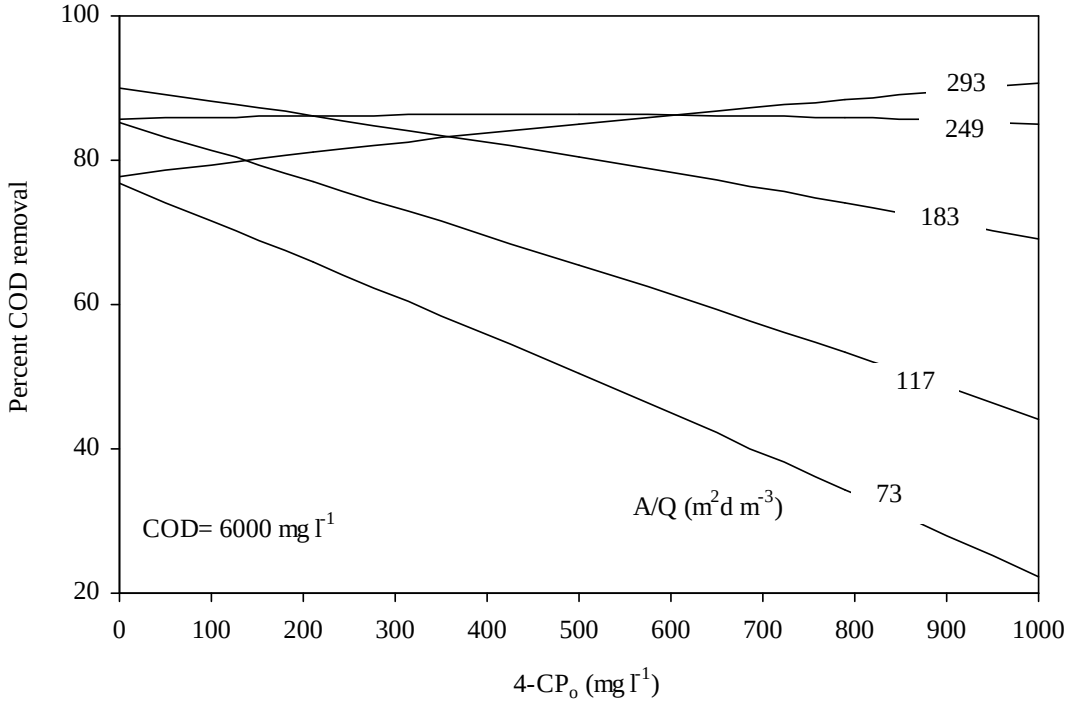


Figure 5.7 Variation of percent COD removal with feed 4-CP concentration at different A/Q ratios

Variations of percent 4-CP removal with the feed COD content at different feed 4-CP concentrations and constant A/Q ratio of 117 m^2 d m^{-3} are depicted Figure 5.8. Percent 4-CP removal decreased with increasing feed 4-CP concentration due to

toxic effects of high 4-CP contents on the organisms. Percent 4-CP removal increased from 32% to 78% when feed 4-CP concentration decreased from 1000 mg.l^{-1} to 211 mg l^{-1} at the feed COD concentration of 4,000 mg l^{-1}. Percent 4-CP removal increased with increasing feed COD concentration for the feed 4-CP contents below 500 mg l^{-1} due to negligible 4-CP inhibitions and high biomass concentrations at high feed COD contents.

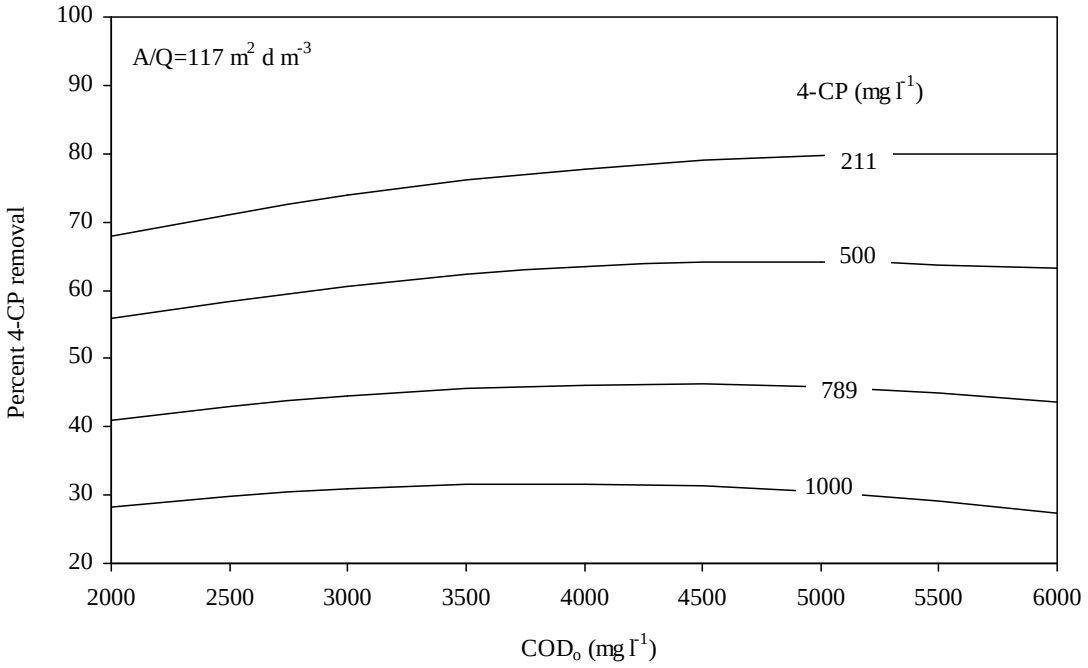


Figure 5.8 Variation of percent 4-CP removal with feed COD concentration at different feed 4-CP concentrations

Figure 5.9 depicts variations of percent 4-CP removal with the feed 4-CP concentration at different A/Q ratios and constant feed COD of 6,000 mg l^{-1}. Increases in feed 4-CP concentrations resulted in decreases in 4-CP removals at low A/Q ratios up to 183 m^2 d m^{-3} due to toxic effects of 4-CP on the organisms and low biomass concentrations at low A/Q ratios. At high A/Q ratios above 249 m^2 d m^{-3} (low feed flow rates) percent 4-CP removals increased with increasing feed 4-CP contents probably due to low 4-CP loading rates (Q. 4-CP_o) at low feed flow rates or high A/Q ratios. Again, increases in A/Q ratio at constant feed 4-CP concentrations resulted in increases in percent 4-CP removals up to A/Q of 249 m^2 d m^{-3} due to larger biofilm surface area per unit 4-CP loading rates at high A/Q values or low feed

flow rates. Nearly complete 4-CP removal was obtained for feed 4-CP concentrations below 205 mg l^{-1} at a feed COD of 5170 mg l^{-1} and A/Q ratio of nearly 170 m^2 d m^{-3}.

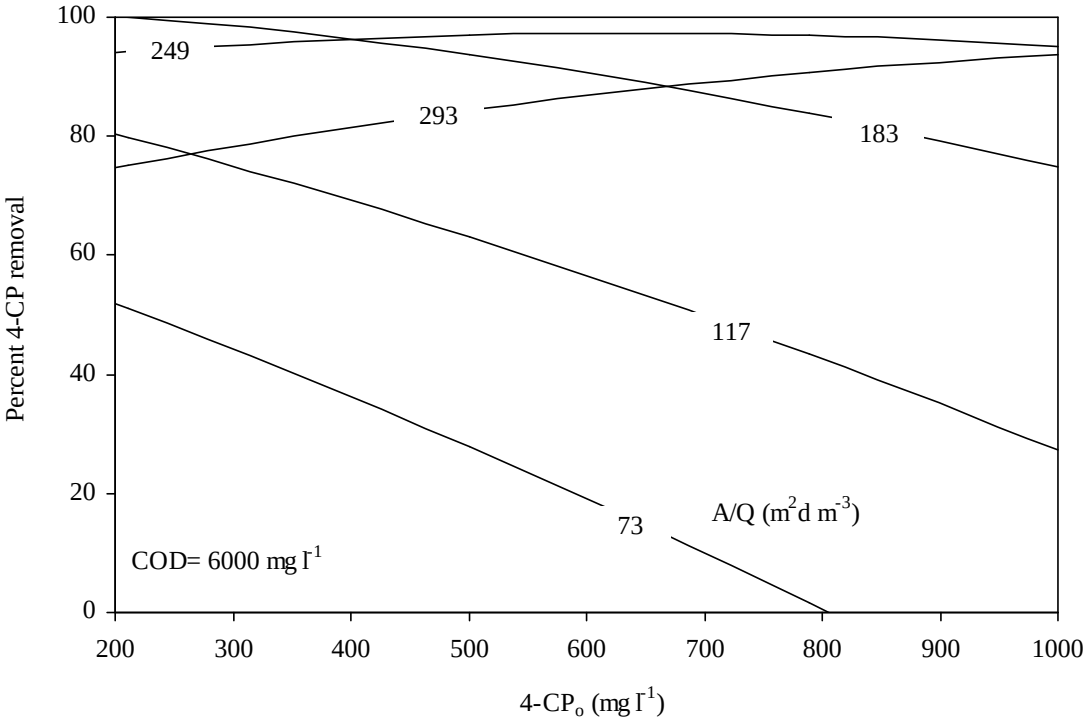


Figure 5.9 Variations of percent 4-CP removal with feed 4-CP concentration at different A/Q ratio

Variations of percent toxicity removal with feed 4-CP concentration at different feed COD concentrations and constant A/Q ratio of 183 m^2 d m^{-3} are depicted in Figure 5.10. Percent toxicity removal decreased from 90% to 83% with increasing feed 4-CP content at a feed COD of 4,000 mg l^{-1} due to toxic effects of high 4-CP contents on the organisms. Percent toxicity removal increased with increasing feed COD up to 4,000 mg l^{-1} at constant feed 4-CP content probably due to high biomass concentrations at high feed COD contents (or high COD loading rates) while the inhibition caused by the 4-CP was unchanged. Decreases in percent toxicity removal with increasing feed COD contents above 4000 mg l^{-1} is probably due to preferential utilization of COD compounds present in molasses (sucrose) rather than 4-CP which resulted in high 4-CP contents in the reactor causing inhibitions. The optimum A/Q ratio was found to be 183 m^2 d m^{-3} for maximum percent toxicity removal. The system should be operated at optimum A/Q ratio (183 m^2 d m^{-3}) in order to obtain nearly complete toxicity removal when the feed COD is around 4,000 mgl^{-1}.

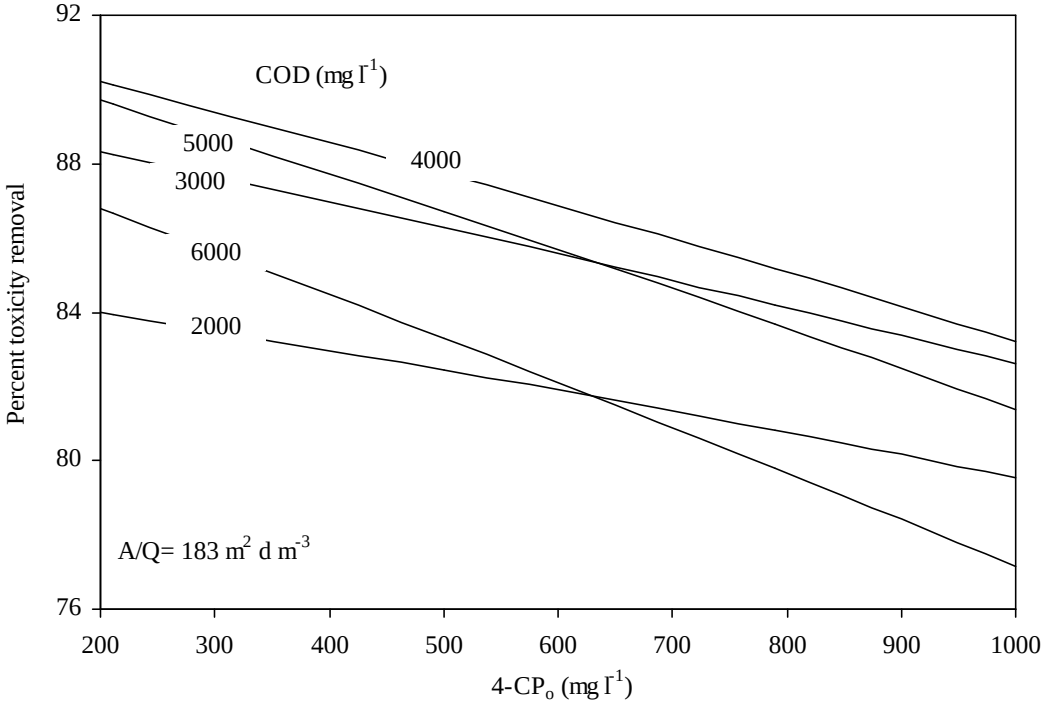


Figure 5.10 Variation of percent toxicity removal with feed 4-CP concentrations at different feed COD concentrations

In order to test the reliability of the response function predictions, three experiments different from the Box-Wilson experimental points were carried out. The results are compared with the Box-Wilson response function predictions in Table 5.5. The first two experiments were within the range of independent variables; the third one was outside of the range. Response function predictions were in good agreement with the experimental data indicating the reliability of the response function predictions within and the outside the range of experimental points.

Table 5.5 Comparison of the experimental and predicted percent 4-CP, COD and toxicity removals for different experimental points.

$4\text{-}CP_o$ (mg l^{-1})	COD_o (mg l^{-1})	A/Q (m^2 d. m^{-3})	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{4\text{-}CP(exp)}$	$E_{4\text{-}CP(pred)}$	$E_{TOX(exp)}$	$E_{TOX(pred)}$
350	3450	147	89	93	85	86	74	76
511	2555	147	84	87	77	78	70	69
1255	6367	330	96	93	97	86	99	100

5.1.3 Comparison of performance of RTBR and RBBR for 4-chlorophenol removal

One of the major objectives of this study is to compare the performances of the newly developed biofilm reactors namely 'rotating tubes biofilm reactor (RTBR)' and the 'rotating brush biofilm reactor (RBBR)' for biological treatment of chlorophenols containing synthetic wastewater under the same experimental conditions.

As compared to our previous study on biological treatment of 4-CP containing wastewater using a rotating brush biofilm reactor (RBBR), the 4-CP and COD removal performance of the RTBR was better especially at high feed 4-CP contents due to formation of denser and thicker biofilm on the rotating tube surfaces.

The results of the RBBR study were compared with the results of the RTBR in Table 5.6 at the lowest, medium and the highest levels of the operating parameters. COD, 4-CP removals and toxicity removals are much better in the RTBR than RBBR due to thicker and denser biofilm formation in RTBR.

Table 5.6 Comparison of COD, 4-CP and toxicity removal efficiencies of rotating brush (RBBR) and rotating tubes biofilm (RTBR) reactors.

$4\text{-}CP_0$	COD_0	A/Q	E_{COD} (%)		$E_{4\text{-}CP}$ (%)		$E_{Toxicity}$ (%)	
(mg l^{-1})	(mg l^{-1})	(m^2 d m^{-3})	RBBR	RTBR	RBBR	RTBR	RBBR	RTBR
0	2000	100	91	100	-	-	-	-
500	4000	150	90	93	84	100	75	100
1000	6000	200	74	99	83	100	85	94

Figure 5.11 depicts a comparison of percent COD removal of RTBR and RBBR at different A/Q ratios and feed 4-CP. COD removal efficiency increased with increasing A/Q ratio for both biofilm reactors. However, with the further increases in A/Q ratio, percent COD removal in RTBR decreased due to lack of food to support of highly dense biofilm organisms. These trends were more significant in the RTBR as compared to RBBR. Percent COD removals with the RTBR were considerably higher than those of RBBR due to formation of denser biofilm on the tube surfaces.

Figure 5.12 depicts a comparison of 4-CP removal performances of RTBR and RBBR at different feed 4-CP and A/Q ratio at a constant COD concentration.

Percent 4-CP removals with the RTBR were considerably higher than those of RBBR due to formation of denser biofilm on the tube surfaces. At a feed 4-CP concentration of 211 mg l^{-1}, percent 4-CP removal with the RTBR was above 92% whereas 4-CP removal was around 67% with the RBBR at A/Q ratio of 100 $m^2 d\ m^{-3}$. At the highest feed 4-CP concentration of 1000 mg l^{-1}, percent 4-CP removals were nearly 67% and 14% for the RTBR and RBBR, respectively. Apparently, RBBR was more affected from 4CP inhibitions due to thinner biofilm formation resulting in low biomass concentrations.

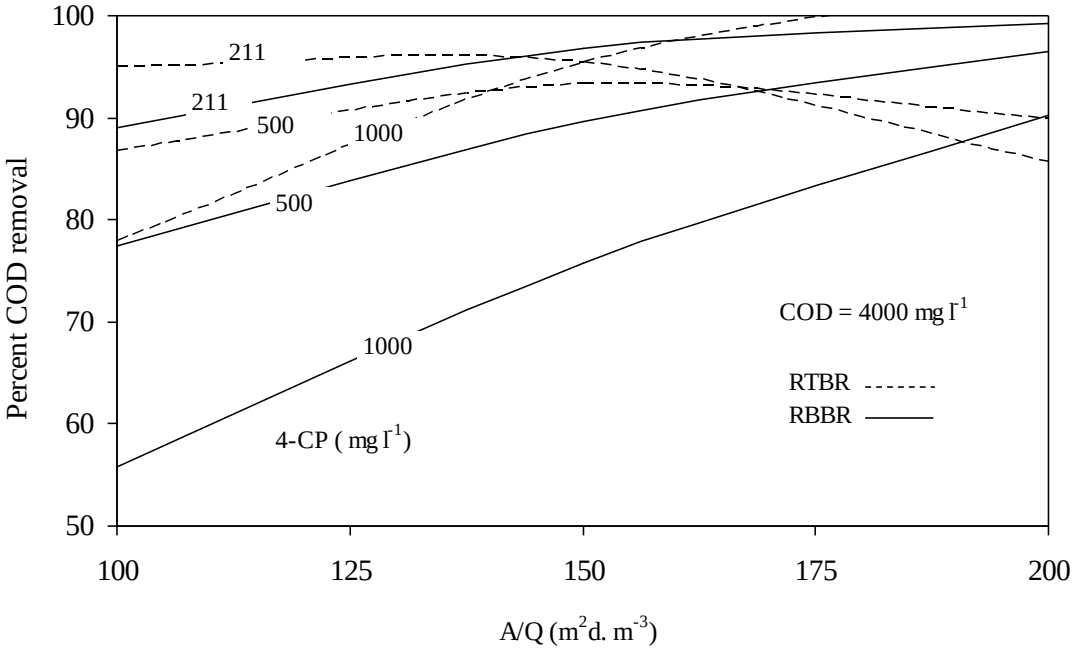


Figure 5.11 Comparison of RTBR and RBBR for percent COD removal with different 4-CP concentrations

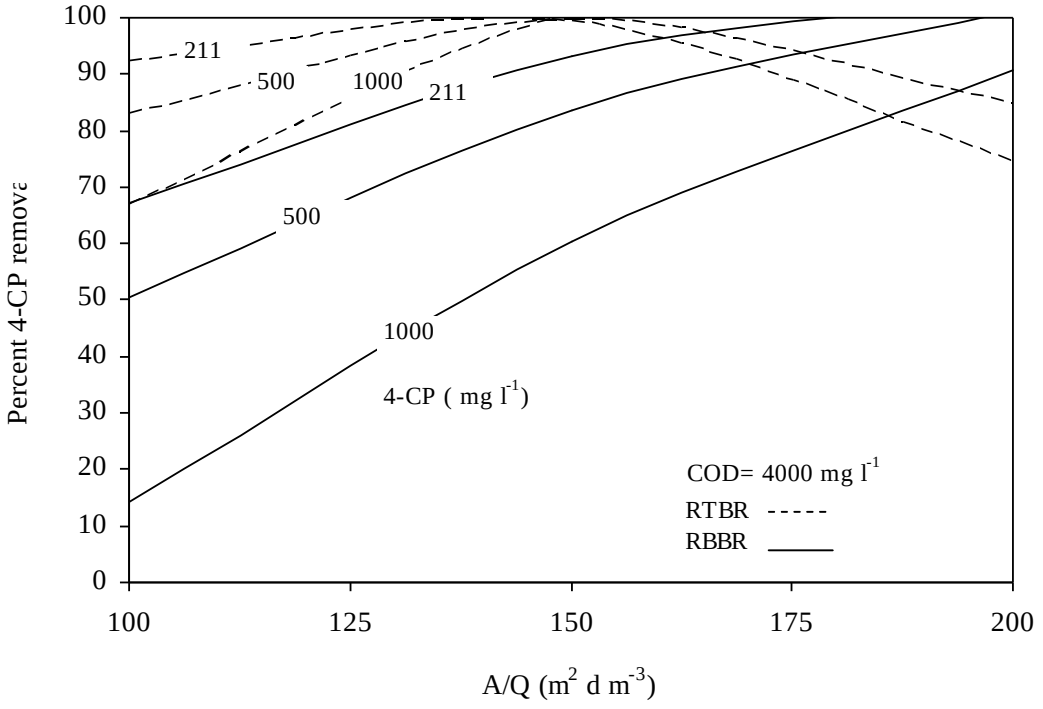


Figure 5.12 Comparison of RTBR and RBBR for percent 4-CP removal with different 4-CP concentrations

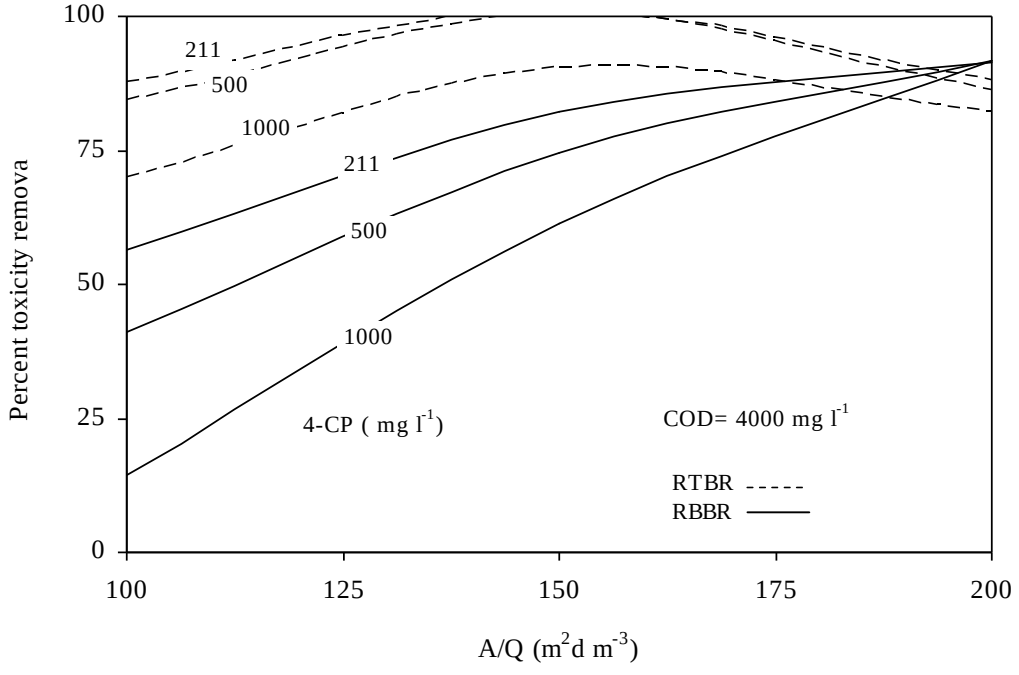


Figure 5.13 Comparison of RTBR and RBBR for percent toxicity removal with different 4-CP concentrations

Figure 5.13 depicts a comparison of toxicity removal performances of RTBR and RBBR at different feed 4-CP and A/Q ratio at a constant COD concentration. Percent toxicity removals with the RTBR were higher as compared to those of RBBR due to highly dense biofilm formation in RTBR. At a feed 4-CP concentration of 211 mg l^{-1}, percent toxicity removal with the RTBR was above 88% whereas toxicity removal was around 56% with the RBBR at A/Q ratio of 100 m^2 d m^{-3}. At the highest feed 4-CP concentration of 1000 mg l^{-1}, percent toxicity removals were nearly 70% and 14% for the RTBR and RBBR, respectively.

The results clearly indicated that the RTBR performed better than the RBBR. Despite the fact that the surface area with the RBBR was larger, the total amount of biomass in the RTBR was higher due to formation of thicker and denser biofilms on the tube surfaces. The differences between performances of both reactors become more significant in favor of RTBR at low A/Q ratios and high 4CP concentrations.

As compared to literature reports our studies covered a larger range of 4-CP concentration and reports much higher COD, 4CP and toxicity removals. In literature studies initial 4-CP contents were less than 300 mg l^{-1}. (Hill G.A., *et al*., 1996; Wang S.J., and Loh K.C. 1999; Wang C.C., *et al*.,2000a; Bali U. and Sengul F. 2002; Sahinkaya E. and Filiz B.D 2005; Zilouei H, Guieysse B. and Mattiasson B., 2006) while 1000 mg l^{-1} 4CP concentrations were used in or experiments. Also, most of the literature on the subject matter reports batch data with pure culture without any toxicity measurements. The results of the RTBR and RBBR experiments showed that 4-CP, COD and the toxicity were removed effectively even at high feed 4CP contents by using high A/Q ratios and a feed COD of above 4000 mg l^{-1}.

5.2 Biological Treatment of 2,4-Dichlorophenol Containing Wastewater

5.2.1 Removal of 2,4-dichlorophenol by using Rotating Perforated Tubes Biofilm Reactor (RTBR)

Biological degradation and removal of 2,4-dichlorophenol (2,4-DCP) from synthetic wastewater was investigated by using the rotating perforated tubes biofilm reactor (RTBR). *Pseudomonas putida* culture was added to the activated sludge

inoculation in this set of experiments. Thereore, the biofilm contained both the *P. putida* and the activated sludge microorganisms in this set of experiments. This was the major difference between this and the other set of experiments.The effects of important operating variables on percent COD, 2,4- DCP and toxicity removals were investigated by using a Box-Wilson statistical experiment design. 2,4-dichlorophenol (DCP) concentration in the feed wastewater (X_1), COD_o (X_2) and A/Q ratio (X_3) were chosen as independent variables. 2,4-DCP concentration (X_1) was changed between 50 and 500 mg l^{-1} while the feed COD (X_2) was between 2,000 and 6,000 mg l^{-1}. The A/Q ratio (X_3) was varied between 62 and 248 $m^2d.m^{-3}$, resulting in hydraulic residence times between 10 and 40 h. The results of second stage were used for estimation. Experimental data were used for determination of the response function coefficients for each independent variable. A Statistica 5.0 program was used for regression analysis. The estimated coefficients of the response functions are presented in Table 5.7.

Table 5.7 Coefficients of the response functions for COD, 2,4-DCP and toxicity removals in RTBR

	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{11}	b_{22}	b_{33}
Y_{COD} R^2=0.85	81.98	-1.7 $*10^{-2}$	-3.4 $*10^{-3}$	2.31 $*10^{-1}$	-1.7 $*10^{-6}$	-7.8 $*10^{-6}$	9.99 $*10^{-6}$	2.68 $*10^{-5}$	2.7 $*10^{-7}$	-7.5 $*10^{-4}$
$Y_{2,4\text{-DCP}}$ R^2=0.64	106.4	-7.1 $*10^{-2}$	-1.53 $*10^{-2}$	3.2 $*10^{-1}$	8.77 $*10^{-8}$	-5.9 $*10^{-5}$	4.14 $*10^{-6}$	1.4 $*10^{-4}$	1.75 $*10^{-6}$	-8.3 $*10^{-4}$
$Y_{TOXICITY}$ R^2=0.71	-36.3	3.34 $*10^{-1}$	-3.86 $*10^{-3}$	6.73 $*10^{-1}$	5.21 $*10^{-5}$	-4.2 $*10^{-4}$	-1.4 $*10^{-4}$	-8.5 $*10^{-4}$	1.4 $*10^{-6}$	6.67 $*10^{-4}$

The response functions with the determined coefficients were used in calculating the predicted values of percent COD, 2,4-DCP and toxicity removals.

Table 5.8 presents a comparison of the experimental and predicted values for percent removals of COD, 2,4-DCP and toxicity. COD removal efficiencies varied between 79 and 94, while percent 2,4-DCP removals were between 72 and 100. Percent toxicity removals varied between 11 and 94. Predicted and experimental values of COD, 2,4-DCP and toxicity removals were in good agreement as shown in Table 5.8.

Table 5.8 Observed and predicted COD, 2,4-DCP and toxicity removal efficiencies in RTBR

	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{2,4\text{-}DCP(exp)}$	$E_{2,4\text{-}DCP(pred)}$	$E_{TOX(exp)}$	$E_{TOX(pred)}$
A1	94	96	99	100	33	23
A2	91	91	100	100	11	30
A3	92	93	100	100	87	78
A4	93	93	99	100	51	74
A5	79	82	72	82	53	59
A6	91	89	99	94	85	91
F1	90	92	99	100	78	68
F2	88	86	97	93	94	70
F3	91	91	98	100	83	72
F4	89	88	100	95	34	36
F5	95	95	98	100	65	51
F6	91	90	99	92	39	41
F7	93	93	100	100	63	80
F8	93	90	98	94	41	44
C (avg)	92	92	95	95	71	70

Projections of the response functions on certain planes of constant COD, 2,4-DCP and A/Q are presented in Figure 5.14 to 5-19.

Variations of percent COD removals with the feed COD and 2,4-DCP concentrations at constant A/Q ratio of 200 $m^2.d.m^{-3}$ are depicted in Figure 5.14. Percent COD removal decreased steadily with increasing feed 2,4-DCP content from 50 mg l^{-1} to 500 mg l^{-1} for the feed COD concentrations between 2,000 mg l^{-1} and 6,000 mg l^{-1}, due to increasing toxic effects of 2,4-DCP at high DCP contents. Percent COD removal slightly decreased with increasing feed COD up to 4,000 mg l^{-1}, due to adverse effects of increasing COD loading rates on the organisms at constant A/Q ratio. Further increases in the feed COD resulted in increases in percent COD removal, because of lower 2,4-DCP/COD ratios in the feed resulting in lower 2,4-DCP toxicity.

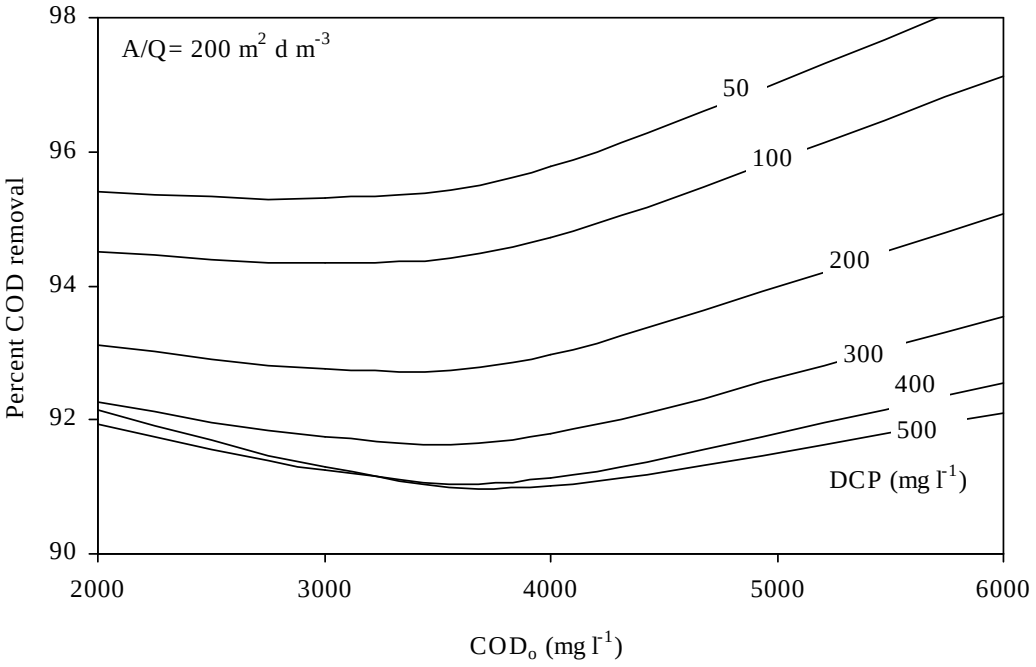


Figure 5.14 Variation of percent COD removal with the feed COD at different feed 2,4-DCP concentrations

Figure 5.15 depicts variation of percent COD removal with the feed 2,4-DCP content at different A/Q ratios and constant feed COD of 4,000 mg l^{-1}. Percent COD removal decreased steadily with increasing feed 2,4-DCP as a result of increasing toxic effects of 2,4-DCP on the organisms. Increases in A/Q ratio from 60 to 200 m^2 d m^{-3} resulted in increases in percent COD removal because of increasing biofilm surface area and biomass concentration. However, further increases in A/Q ratio caused decreases in percent COD removal because of low feed flow rates and insufficient supply of COD to support high biomass concentrations at high A/Q ratios. The optimal A/Q ratio was around 200 m^2 d m^{-3} for the experimental conditions shown in Figure 5.15. The system should be operated at low feed COD contents of 2,000 mg l^{-1}, high A/Q ratios (200 m^2.d.m^{-3}) and low feed 2,4-DCP contents (50 mg l^{-1}) in order to obtain high percent COD removals.

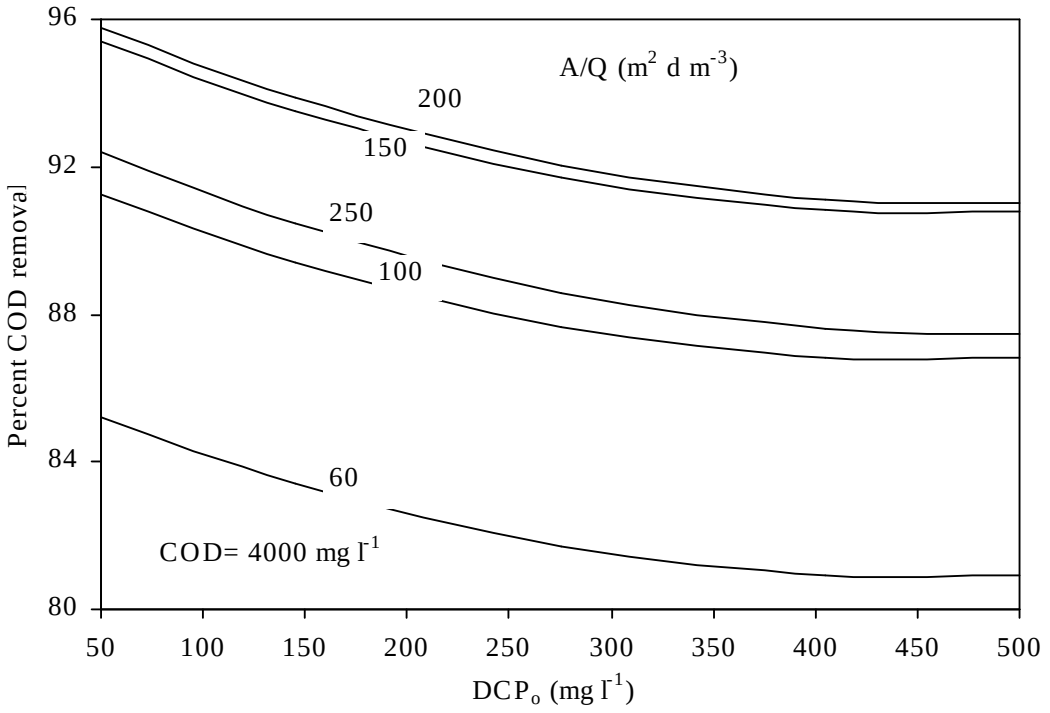


Figure 5.15 Variation of percent COD removal with the feed 2,4-DCP at different A/Q ratios

Variation of percent 2,4-DCP removal with the feed 2,4-DCP concentration at different A/Q ratios and constant feed COD of 4,000 mg l^{-1} is depicted in Figure 5.16. Percent DCP removal increased with increasing A/Q ratio as a result of increasing biofilm surface area yielding high biomass concentrations upto 200 m^2 d m^{-3}. Further increases in A/Q ratio caused decreases in percent 2,4-DCP removal probably as a result of low feed flow rates and insufficient COD loads to support high concentrations of biomass. Increasing feed 2,4-DCP contents resulted in decreases in percent 2,4-DCP removal up to nearly 300 mg l^{-1} because of toxic effects of high 2,4-DCP concentrations. Further increases in feed 2,4-DCP (>300 mg l^{-1}) resulted in increases in percent 2,4-DCP removal probably because of stimulation and domination of 2,4-DCP degrading organism *Pseudomonas putida* at high 2,4-DCP concentrations.

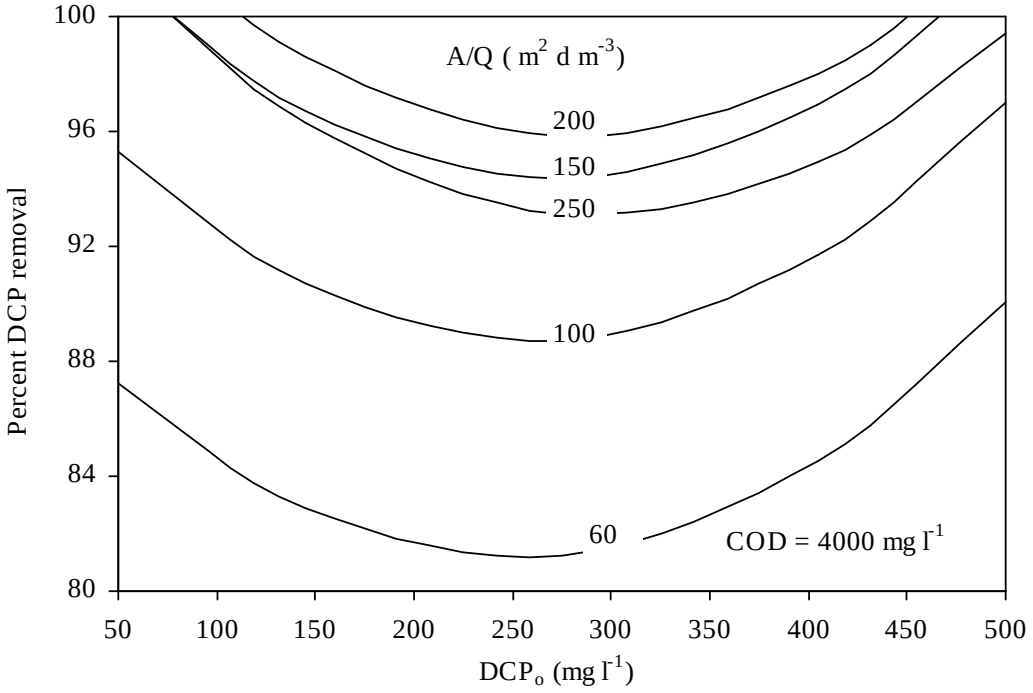


Figure 5.16 Variation of percent 2,4-DCP removal with the feed 2,4-DCP at different A/Q ratios

Figure 5.17 depicts variation of percent 2,4-DCP removal with A/Q ratio at different feed COD concentrations at a constant feed 2,4-DCP of 300 mg l^{-1}. Percent 2,4-DCP removal increased with increasing A/Q ratio as a result of increasing biofilm surface area and reached a maximum level at A/Q ratio of around 200 m^2 d m^{-3}. Increasing A/Q ratio from 60 to 200 m^2 d m^{-3} resulted in an increase in 2,4-DCP removal efficiency from 82% to 95% at a feed COD of 4,000 mg l^{-1}. Further increases in A/Q ratio resulted in decreases in percent COD removal probably due to insufficient COD loading rates (low flow rates) to support high biomass concentrations at high A/Q ratios. 2,4-DCP removal efficiency was maximum at low feed COD content of 2,000 mg l^{-1} because of essential degradation of 2,4-DCP at low COD contents. Increases in the feed COD from 2,000 mg l^{-1} to 4,000 mg l^{-1} resulted in decreases in percent 2,4-DCP removal, probably because of preferential biodegradation of carbonaceous COD compounds over 2,4-DCP. Further increases in the feed COD above 4,000 mg l^{-1} resulted in higher percent 2,4-DCP removals

probably because of high biomass concentrations at high feed COD contents and also because of low feed 2,4-DCP/COD ratios obtained at high feed COD levels.

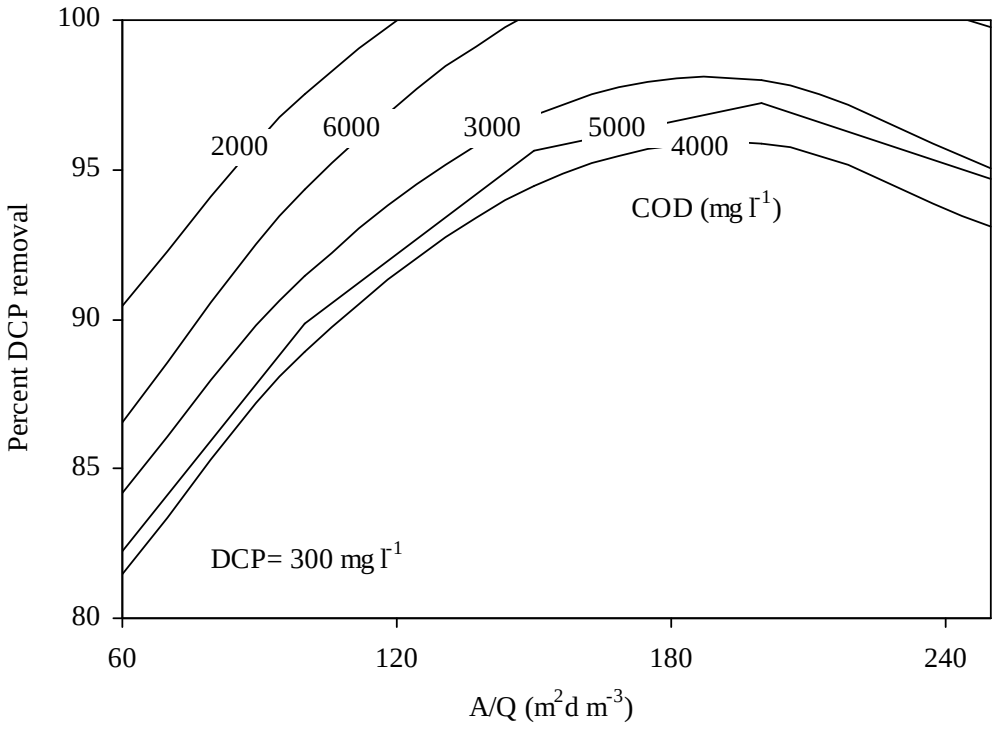


Figure 5.17 Variation of percent 2,4-DCP removal with A/Q ratio at different feed COD concentrations

Removal of toxicity from the wastewater containing different concentrations of 2,4-DCP is another important aspect of this study. Variations of percent toxicity removal from the synthetic wastewater with the feed 2,4-DCP content at different A/Q ratios and constant feed COD of 4,000 mg l^{-1} are depicted in Figure 5.18. Percent toxicity removal increased with increasing A/Q ratio at constant feed COD and 2,4-DCP, because of high biofilm surface area and therefore, high biomass concentrations at high A/Q ratios. Percent toxicity removal increased with increasing feed 2,4-DCP content up to nearly 250 mg l^{-1} because of substrate (2,4-DCP) limitations at low feed 2,4-DCP contents. The decreases in percent 2,4-DCP removals for the feed 2,4-DCP contents above 250 mg l^{-1} may be because of formation of more toxic end products from biodegradation of 2,4-DCP. The system

should be operated at high A/Q ratio (250 m^2 d. m^{-3}) and nearly 250 mg l^{-1} feed 2,4-DCP concentration in order to obtain maximum percent toxicity removal when the feed COD is 4,000 mg l^{-1}.

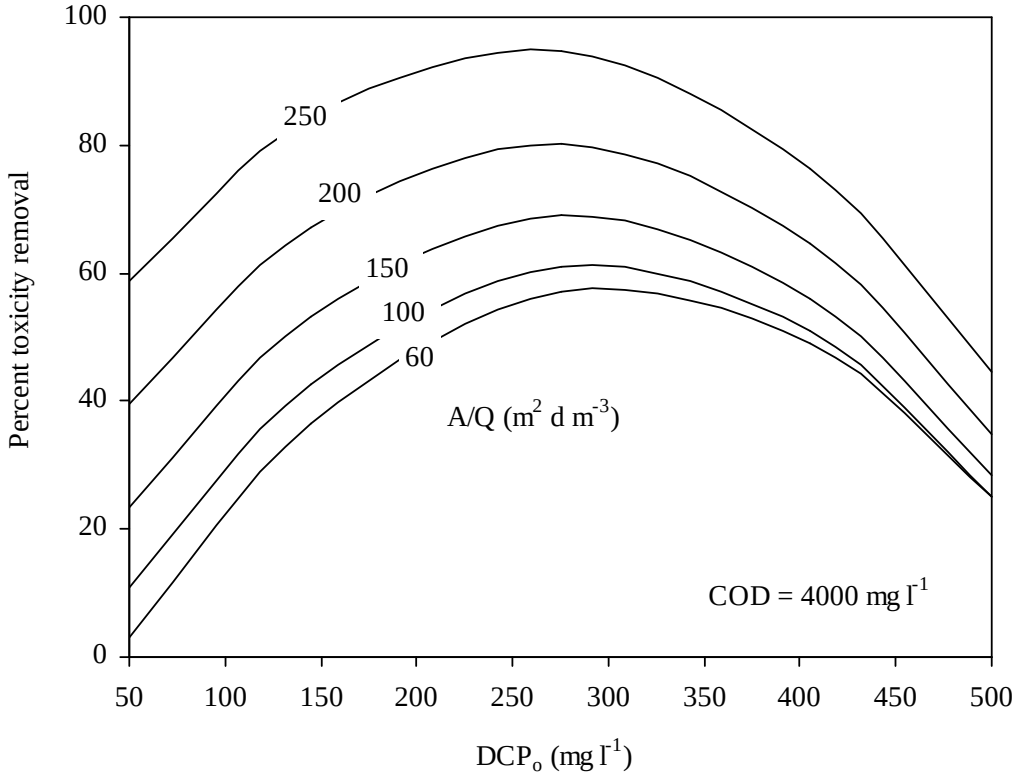


Figure 5.18 Variation of percent toxicity removal with the feed 2,4-DCP content at different A/Q ratios

Variations of percent toxicity removal with A/Q ratio at different feed COD contents and constant feed 2,4-DCP of 300 mg l^{-1} are depicted in Figure 5.19. At low feed COD contents below 4,000 mg l^{-1}, percent toxicity removal increased with increasing A/Q ratio as a result of increasing biofilm surface area and essential degradation of 2,4-DCP at low feed COD contents. However, at high feed COD contents above 5,000 mg l^{-1}, percent toxicity removal decreased with increasing A/Q ratio, probably because of preferential degradation of carbonaceous COD compounds over 2,4-DCP resulting in lower 2,4-DCP and percent toxicity removals. High A/Q ratio of 200 m^2 d m^{-3} and low COD content of 2,000 mg l^{-1} resulted in nearly complete toxicity removal when the feed 2,4-DCP was 300 mg l^{-1}.

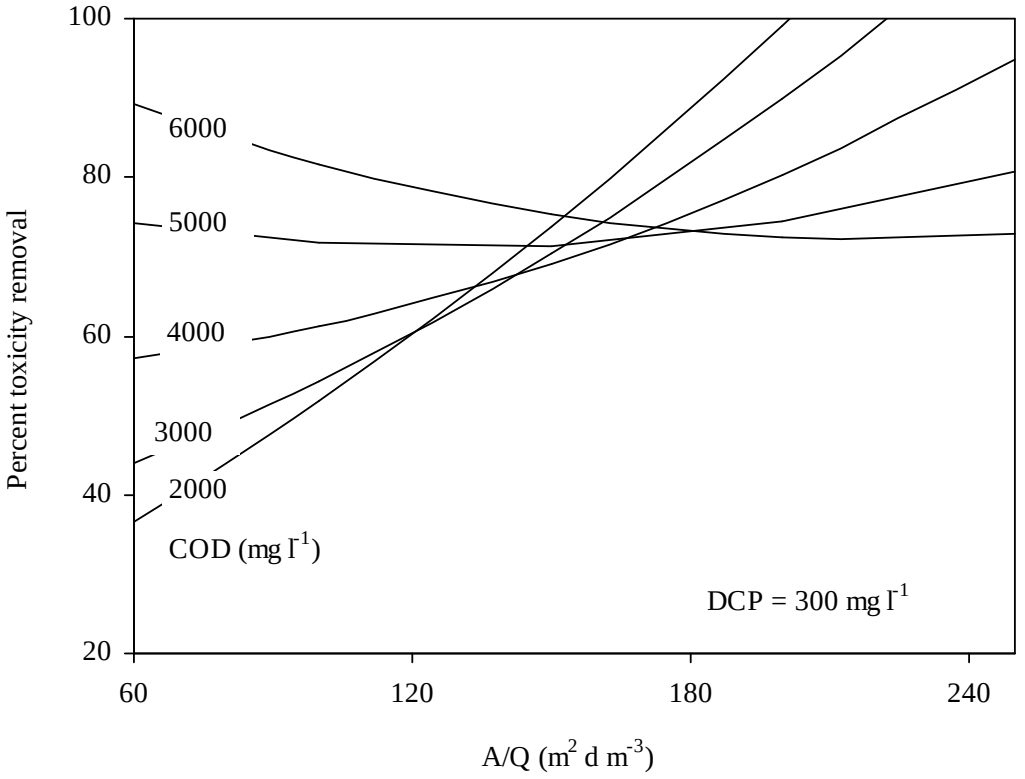


Figure 5.19 Variation of percent toxicity removal with A/Q ratio at different feed COD concentrations

5.2.2 Removal of 2,4-dichlorophenol by using Rotating Brush Biofilm Reactor (RBBR)

Experiments were conducted for 2,4-dichlorophenol (2,4-DCP) removal with RBBR under the same operating conditions as that of the RTBR. However, the bacterial culture did not include *P.putida* in this set of experiments. A Box–Wilson statistical experiment design method was used to investigate the effects of the operating parameters on percent COD, 2,4- DCP and toxicity removals.

COD, 2,4-DCP and toxicity removals from the synthetic wastewater were investigated at variable feed 2,4-DCP (50-500 mg l^{-1}), COD (2000-6000 mg l^{-1}), and the A/Q ratio (73-293 $m^2 d\ m^{-3}$). Three important operating parameters, feed 2,4-DCP_o (X_1) and COD_o (X_2) concentrations and A/Q ratio (X_3) were coded as independent variables.

Experimental data was used for determination of the response function coefficients for each independent variable by iteration. The data from the second stage were used for estimation of the coefficients. The difference in percent COD and DCP removals were less than 3% between the first and the second stage. However, second stage was beneficial especially for percent toxicity removal at high A/Q ratios and high feed 2,4-DCP contents. The estimated coefficients of the response functions are presented in Table 5.9. Predicted values of the response functions using the estimated coefficients are compared with the experimental results in Table 5.10. Response function predictions were in good agreement with the experimental data.

Table 5.9 Coefficients of the response functions for COD, 2,4-DCP and toxicity removals in RBBR.

	b_0	b_1	b_2	b_3	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
Y_{COD} R^2=0.94	77.86	8.96 $*10^{-3}$	5.4 $*10^{-4}$	1.38 $*10^{-1}$	-6.25 $*10^{-5}$	1.57 $*10^{-7}$	-4.8 $*10^{-4}$	-3.9 $*10^{-6}$	1.92 $*10^{-4}$	-1.21 $*10^{-6}$
$Y_{2,4\text{-}CP}$ R^2=0.90	96.64	-5.35 $*10^{-2}$	-5.27 $*10^{-3}$	1.74 $*10^{-1}$	6.68 $*10^{-5}$	5.87 $*10^{-7}$	-4.18 $*10^{-4}$	2.87 $*10^{-6}$	1.52 $*10^{-5}$	1.59 $*10^{-7}$
$Y_{TOXICITY}$ R^2=0.59	76.73	-5.61 $*10^{-2}$	-4.73 $*10^{-3}$	3.8 $*10^{-1}$	2.4 $*10^{-5}$	-2.99 $*10^{-7}$	-1.08 $*10^{-3}$	1.4 $*10^{-5}$	-1.11 $*10^{-4}$	2.19 $*10^{-7}$

Table 5.10 Observed and predicted COD, 2,4-DCP and toxicity removal efficiencies in RBBR.

	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{2,4\text{-}DCP(exp)}$	$E_{2,4\text{-}CP(pred)}$	$E_{TOX(exp)}$	$E_{TOX(pred)}$
A1	93	92	100	100	100	100
A2	89	90	100	100	97	98
A3	93	94	99	99	96	96
A4	96	95	99	100	96	99
A5	86	87	87	89	70	81
A6	90	89	96	95	97	90
F1	92	92	99	99	99	99
F2	89	88	97	96	97	92
F3	93	93	97	98	86	91
F4	89	88	95	94	96	90
F5	91	92	99	99	97	99
F6	93	94	97	95	96	89
F7	89	90	99	99	97	99
F8	92	92	97	96	96	94
C (avg)	94	94	97	97	98	99

Variations of percent COD removal with the feed COD and 2,4-DCP concentrations at constant A/Q ratio of 70 m^2 d m^{-3} are depicted in Figure 5.20. Percent COD removal decreased with increasing feed 2,4-DCP concentration from 50 to 500 mg l^{-1} for all feed COD concentrations due to toxic effects of high 2,4-DCP concentrations. Percent COD removal increased with increasing feed COD at low feed 2,4-DCP contents (2,4-DCP< 300 mg l^{-1}) because of lack of 2,4-DCP inhibition, but remained almost constant for all feed COD's at high feed 2,4-DCP contents above 300 mg l^{-1}. The highest COD removal (93%) was obtained with the feed COD and 2,4-DCP of 6000 and 50 mg l^{-1}, respectively when A/Q ratio was 70 m^2 d m^{-3}.

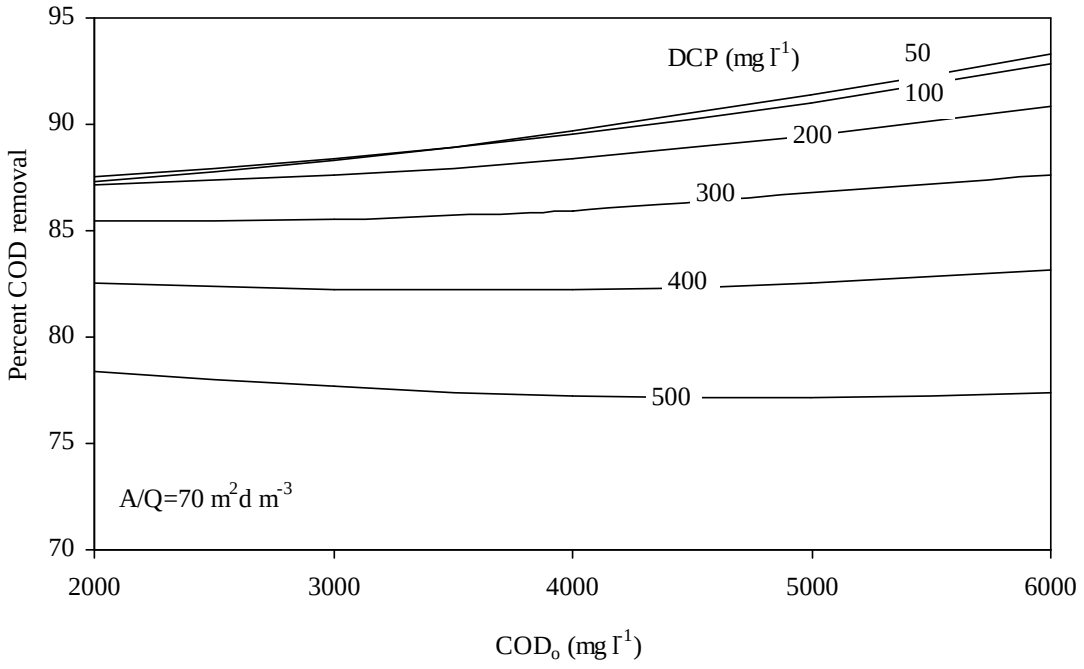


Figure 5.20 Variation of percent COD removal with the feed COD at different feed 2,4-DCP concentrations

Figure 5.21 depicts variations of percent COD removal with the A/Q ratio at different feed COD concentrations when the feed 2,4-DCP was 225 mg l^{-1}. COD removal increased with increasing feed COD due to formation of thicker biofilms at high feed COD contents and also due to substrate limitations at low feed CODs. Percent COD removal increased with increasing A/Q ratio and reached maximum level at A/Q ratio of around 200 m^2 d m^{-3} indicating biofilm surface area (or biomass

concentration) limitations at low A/Q ratios. Further increases in A/Q ratio resulted in decreases in percent COD removal probably because of low feed flow rates and insufficient COD loading rates to support high biomass concentrations. The optimal A/Q ratio was nearly 200 m^2 d m^{-3} below which COD removal was limited by biomass concentration (or biofilm surface area) and above which the limitation was due to insufficient substrate loading or low feed flow rates. The highest COD removal (97%) was obtained with the feed COD_o of 6000 mg l^{-1} and A/Q ratio of 200 m^2d m^{-3} when the feed 2,4-DCP was 225 mg l^{-1} yielding an optimal COD loading rate of 30 g COD $m^{-2}d^{-1}$.

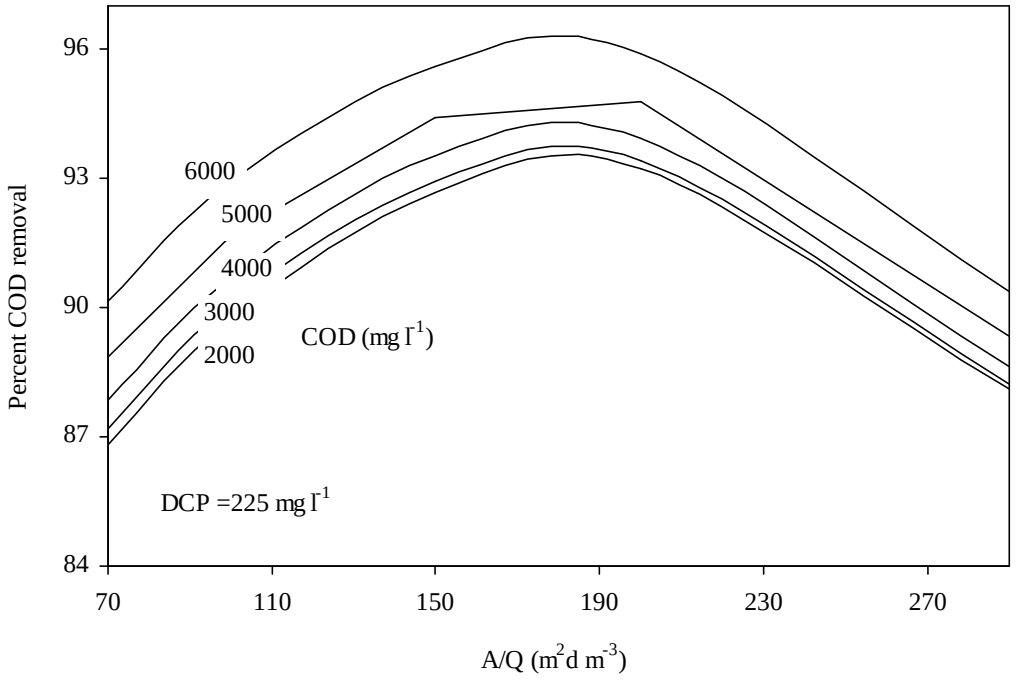


Figure 5.21 Variations of percent COD removal with the A/Q ratio at different feed COD concentrations

Variations of percent 2,4-DCP removal with the feed 2,4-DCP concentration at different A /Q ratios and constant feed COD of 4000 mg l^{-1} are depicted Figure 5.22. Percent 2,4-DCP removal increased with increasing A/Q ratio up to 200 m^2 d m^{-3} due to biofim area limitations at low A/Q ratios and then decreased with further increases in A/Q ratio due to insufficient substrate loading to support high biomass concentrations. The optimal A/Q ratio resulting in the highest 2,4-DCP removal was

nearly 200 m^2 d m^{-3} with the fed COD of 4000 mg l^{-1}. Percent 2,4-DCP removal decreased with increasing feed 2,4-DCP contents up to 300 mg l^{-1} due to toxic effects of high 2,4-DCP. Further increases in the feed 2,4-DCP above 300 mg l^{-1} resulted in increases in 2,4-DCP removal probably due to domination of 2,4-DCP degrading organisms or adaptation of the organisms since the experiments were performed in the order of increasing 2,4-DCP loading rates.

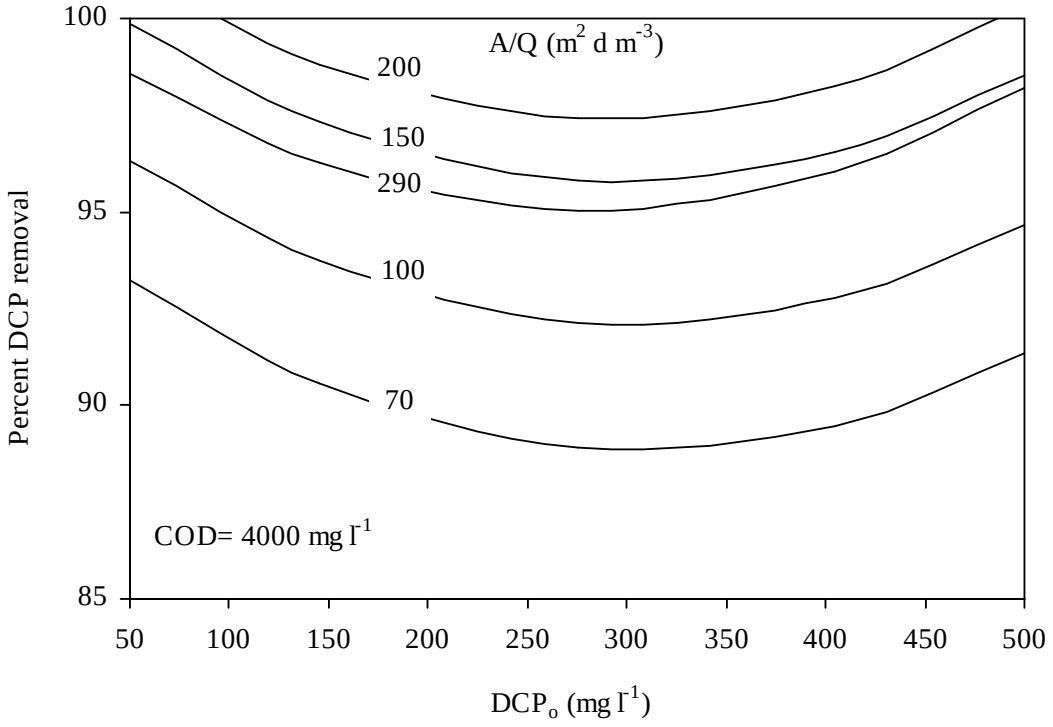


Figure 5.22 Variations of percent 2,4-DCP removal with the feed 2,4-DCP concentration at different A /Q ratios

Figure 5.23 depicts variation of percent 2,4-DCP removals with the A/Q ratio at different feed COD concentrations and a constant feed 2,4-DCP of 225 mg l^{-1}. Percent 2,4-DCP removal increased with increasing A/Q ratio up to 190-200 m^2 d m^{-3} due to biofilm surface area (or biomass concentration) limitations at low A/Q ratios. Further increases in A/Q ratio resulted in decreases in percent 2,4-DCP removal because of insufficient substrate loadings at low flow rates and high biomass concentrations. Increases in the fed COD from 2000 to 4000 mg l^{-1} resulted in

decreases in percent 2,4-DCP removal due to preferential utilization of COD compounds (mainly sucrose) present in dilute molasses instead of 2,4-DCP. Further increases in the feed COD above 4000 mg l^{-1} resulted in higher percent 2,4-DCP removals probably because of high biomass concentrations at high feed COD contents and also because of low feed 2,4-DCP/COD ratios obtained at high feed COD contents. The highest percent 2,4-DCP removal (100%) was obtained with the fed COD of 6000 mg l^{-1} and an A/Q ratio of 200 m^2 d m^{-3} with a feed 2,4-DCP of 225 mg l^{-1} yielding the optimal 2,4-DCP and COD loading rates of 30 gCOD m^{-2} d^{-1} and 1.2 g 2,4-DCP m^{-2} d^{-1} with an optimal feed 2,4-DCP/COD ratio of 3.8%.

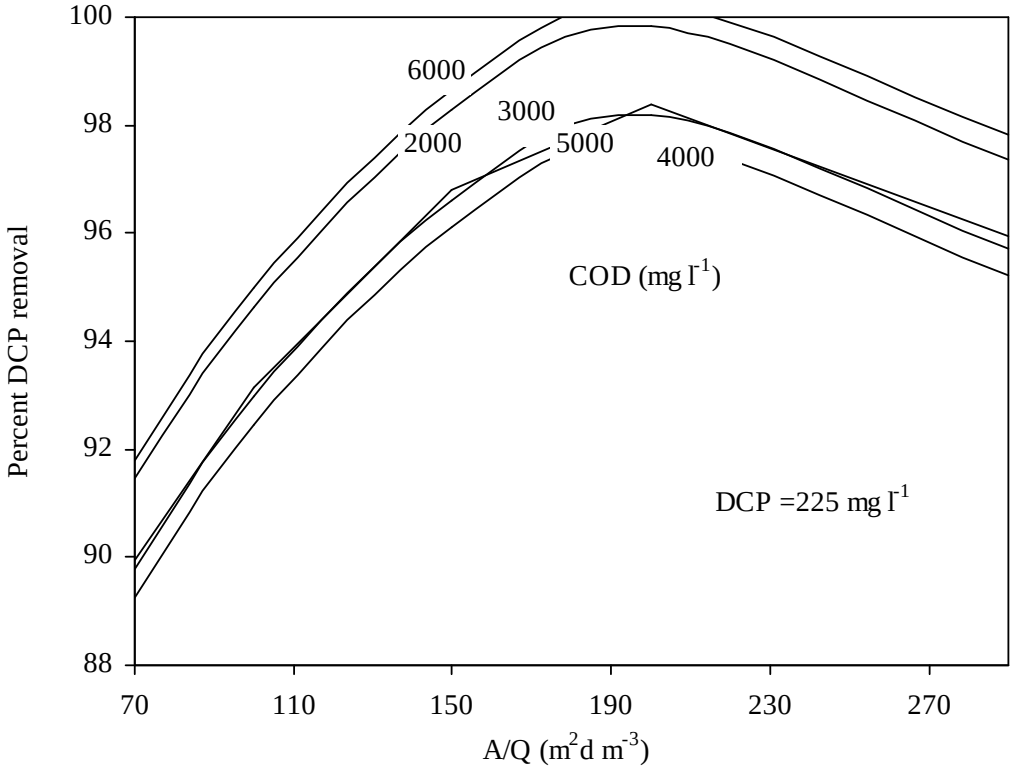


Figure 5.23 Variations of percent 2,4-DCP removal with the A/Q ratio at different feed COD concentrations

Variations of percent toxicity removal with the feed 2,4-DCP content at different A/Q ratios and a constant feed COD of 4000 mg l^{-1} are depicted in Figure 5.24. Percent toxicity removals increased with increasing A/Q ratio up to 200 m^2 d m^{-3} because of high biofilm surface area and therefore, high biomass concentrations at

high A/Q ratios. Similar to 2,4-DCP and COD removals further increases in A/Q ratio resulted in lower toxicity removals due to insufficient substrate loadings to support the biofilm organisms (i e., high A and low Q). Percent toxicity removal decreased with increasing feed 2,4-DCP at high A/Q ratios due to incomplete degradation of 2,4-DCP at high loading rates. However, 2,4-DCP removal was rather insensitive to the feed 2,4-DCP contents at low A/Q's below 150 m^2 d m^{-3} or high feed flow rates since the experiments were performed in the order of increasing 2,4-DCP loading rates or A/Q ratios allowing domination of 2,4-DCP degrading organisms or adaptation to 2,4-DCP. The system should be operated at A/Q ratio of 200 m^2 d. m^{-3} in order to obtain high toxicity removal when the feed COD is 4,000 mg l^{-1}.

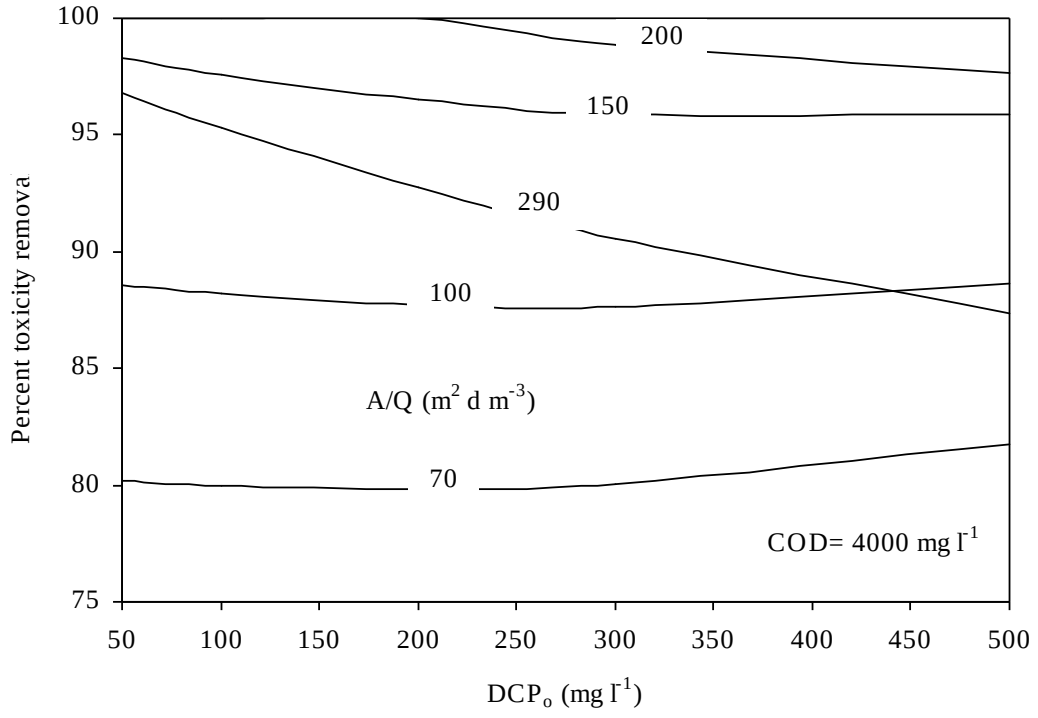


Figure 5.24 Variation of percent toxicity removal with the feed 2,4-DCP concentrations at different A/Q ratios

Variations of percent toxicity removal with A/Q ratio at different feed COD contents and constant feed 2,4-DCP of 225 mg l^{-1} are depicted in Figure 5.25. Similar to the 2,4-DCP removal, percent toxicity removal increased with increasing A/Q

ratio up to 200 m^2 d m^{-3} due to high biomass concentrations at high A/Q ratios. Further increases in A/Q ratio resulted in reduced percent 2,4-DCP removal because of insufficient substrate loadings at low flow rates. At high A/Q ratios above 200 m^2 d m^{-3} where the biofilm surface area or the biomass concentration is high, toxicity removal increased with increasing feed COD due to increasing COD loading rates (L_{COD} = Q COD_o) to support high biomass concentrations. A/Q ratio must be around 200 m^2 d m^{-3} when the feed COD is 6000 mg l^{-1} in order to maximize the toxicity removal.

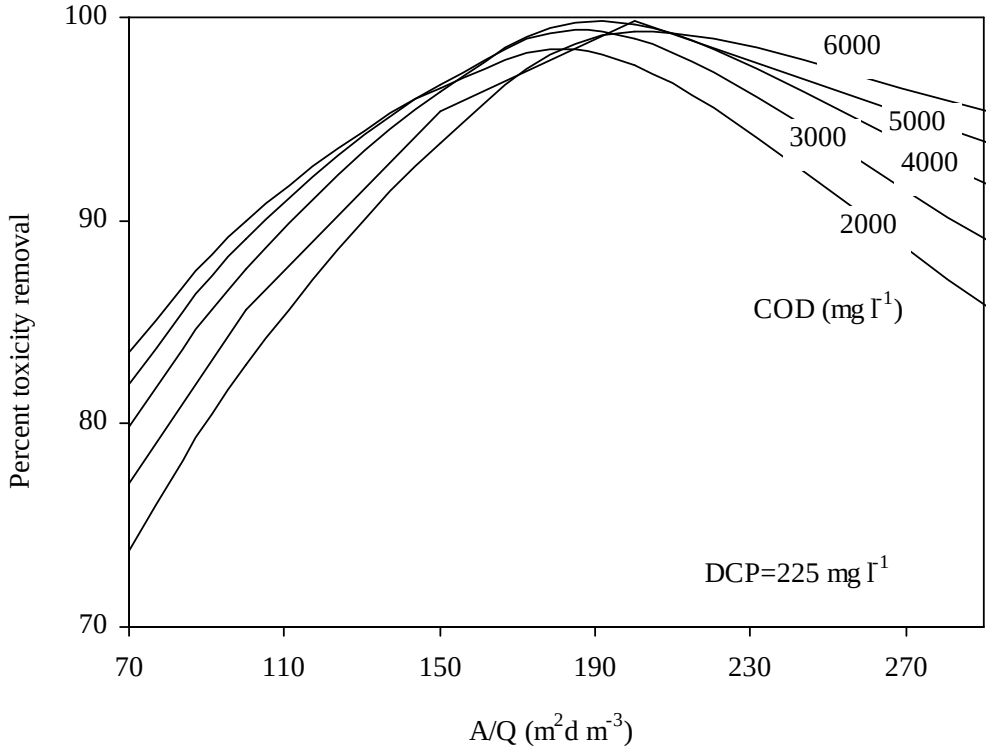


Figure 5.25 Variations of percent toxicity removal with the A/Q ratio at different feed COD concentrations

5.2.3 Comparison of RTBR and RBBR for removal of 2,4-dichlorophenol

The results of the RBBR study were compared with the RTBR in Table 5.11 at the lowest, medium and the highest levels of the operating parameters. Although the COD and 2,4-DCP removals are comparable for both reactors, toxicity removals are much better in the RBBR than RTBR. This is probably because of the difference in

composition of the microbial community in both reactors. Apparently, the organisms in the RBBR completely degraded 2,4-DCP resulting in high toxicity removals while the organisms in RTBR produced some intermediate products with some levels of toxicity.

Table 5.11 Comparison of COD, 2,4-DCP and toxicity removal efficiencies of rotating brush (RBBR) and rotating tubes biofilm (RTBR) reactors.

$2,4\text{-DCP}_0$	COD_0	A/Q	E_{COD} (%)		$E_{2,4\text{-DCP}}$ (%)		$E_{Toxicity}$ (%)	
(mg l^{-1})	(mg l^{-1})	(m^2 d m^{-3})	RBBR	RTBR	RBBR	RTBR	RBBR	RTBR
50	2000	70	87	89	96	98	89	10
225	4000	180	94	93	97	96	100	73
500	6000	290	89	85	100	100	100	45

Figure 5.26 depicts a comparison of percent COD removal of RTBR and RBBR at different A/Q ratio and feed 2,4-DCP. At low A/Q ratios and high 2,4-DCP concentrations (500 mg l^{-1}), percent COD removals with RTBR were significantly higher than the those of the RBBR. However, at high A/Q ratios such as 200 m^2 dm^{-3} percent COD removals were almost the same. The RTBR was more effective in COD removal at high 2,4-DCP loading rates. Due to formation of thicker biofilms the RTBR was less affected from DCP inhibitions. The differences in percent COD removals were negligible at low 2,4-DCP concentrations for all A/Q ratios.

Figure 5.27 depicts a comparison of 2,4-DCP removal performances of RTBR and RBBR at different feed 2,4-DCP and A/Q ratios at a constant feed COD concentration. A similar trend was observed for 2,4-DCP removals as that of the COD removals. At a feed 2,4-DCP concentration of 500 mg l^{-1}, percent 2,4-DCP removals with the RTBR were above 97% while the removals were around 95% with the RBBR at A/Q ratio of 100 m^2 d m^{-3}. The differences in percent DCP removals were negligible.

Figure 5.28 depicts a comparison of toxicity removal performances of RTBR and RBBR at different feed 2,4-DCP and A/Q ratios at a constant COD concentration. The percent toxicity removal obtained with RBBR was significantly better than the RTBR. These results clearly indicated that RBBR was more advantageous as compared to the RTBR for toxicity removal. Percent toxicity removal increased from 89 at A/Q ratio of 100 m^2 dm^{-3} to 98 at A/Q ratio of 200 m^2 dm^{-3} with RBBR for feed

2,4-DCP of 500 mg l^{-1}. However, toxicity removal of RTBR was changed from 25 to 35% under the same operating conditions. This is probably due to the differences in the composition of the microbial community.

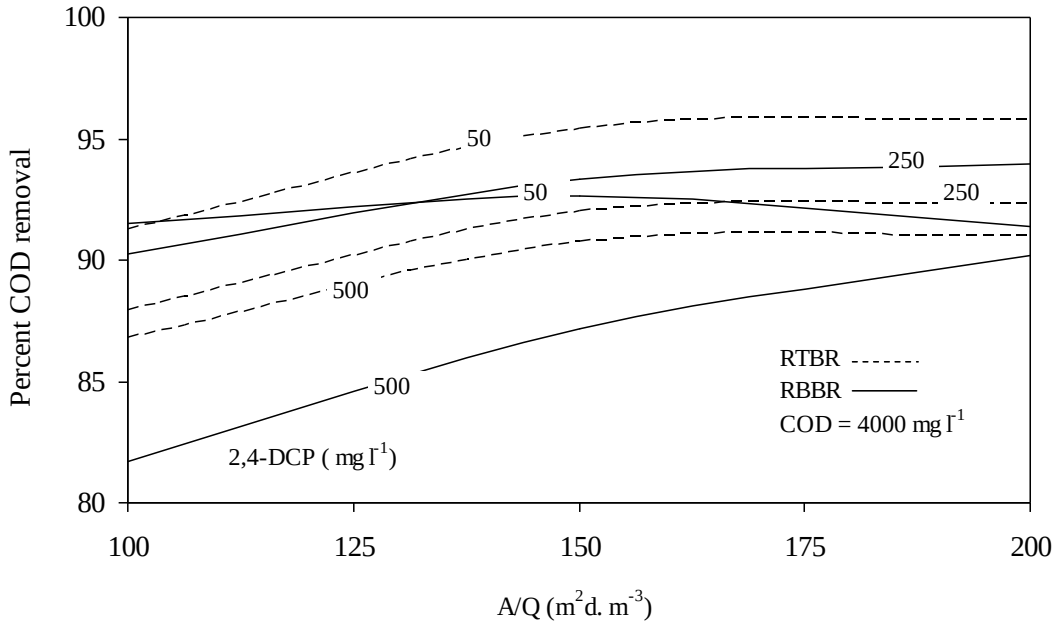


Figure 5.26 Comparison of RTBR and RBBR for percent COD removal at different 2,4-DCP concentrations

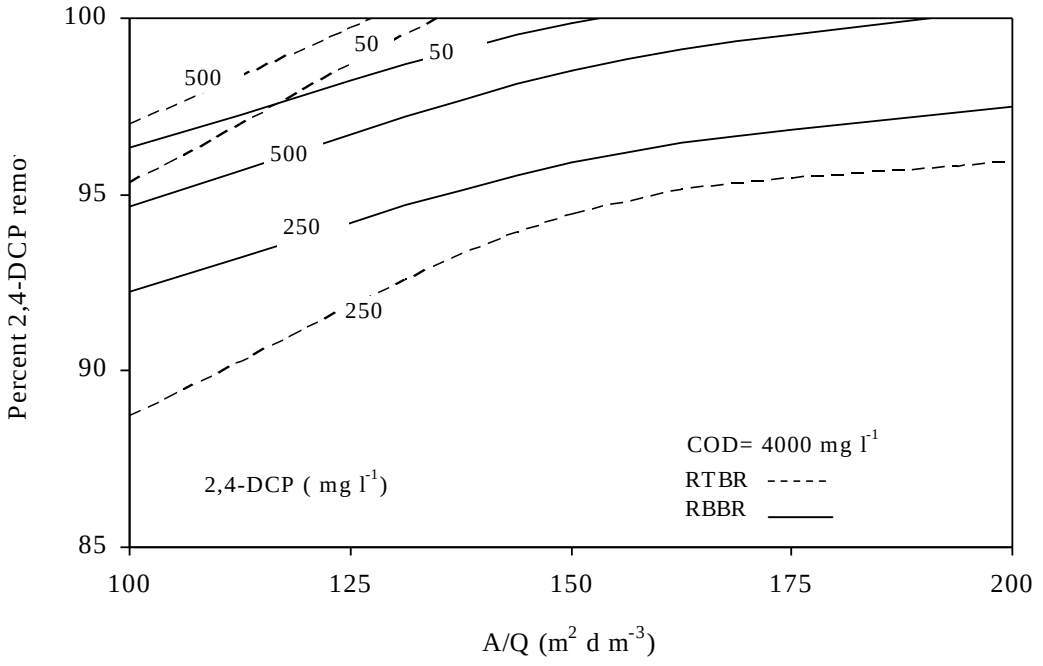


Figure 5.27 Comparison of RTBR and RBBR for percent 2,4-DCP removal at different 2,4-DCP concentrations

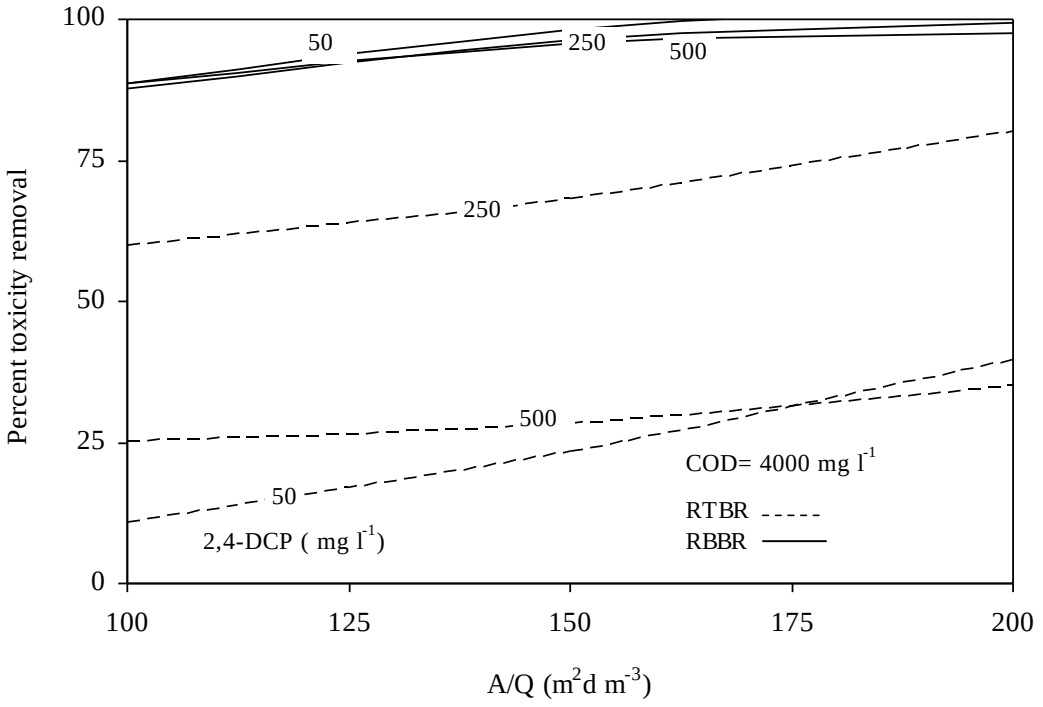


Figure 5.28 Comparison of RTBR and RBBR for percent toxicity removal at different 2,4-DCP concentrations

High concentrations of 2,4 DCP removals (500 mg l^{-1}) were achieved in our studies with the RTBR and the RBBR. Most of the literature studies on DCP removal used DCP concentrations lower than 200 mg l^{-1} with DCP removals less than 80% (Radwan K.H.*et al*, 1997; Yee and Wood, 1997; Steinle et al., 1998; Wang et al., 2000; Sahinkaya and Dilek, 2002). We were able to obtain almost complete DCP removals with a high feed DCP content of 500 mg l^{-1} due to high biomass concentrations in the RTBR and the RBBR. By using high A/Q ratios and suitable feed COD contents high degrees of COD, DCP and toxicity removals were obtained in our reactors as compared to the literature reports. Inclusion of *P.putida* in the activated sludge inoculum was not found to be beneficial. Further experiments were performed with the activated sludge inoculum without addition of *P. putida.*

5.3 Biological Treatment of 2,4,6-Trichlorophenol (TCP) Containing Wastewater

5.3.1 Removal of 2,4,6-trichlorophenol (TCP) by using Rotating Perforated Tubes Biofilm Reactor (RTBR)

All operating parameters and quadratic model were assumed significant during the previous studies using the Box-Wilson design. To ensure our approach, Box-Behnken statistical design was used in our studies on TCP removal. The differences between the models and significant factors were specified previously. Box–Behnken statistical experiment design was used to determine the effects of operating parameters such as A/Q ratio, feed COD and 2,4,6-TCP concentrations on percent 2,4,6-TCP, COD and toxicity removals since this method required fewer number of experiments. Three important operating parameters; feed 2,4,6-TCP_o (X_1), feed COD_o (X_1) concentrations and A/Q ratio (X_3) were considered as independent variables. Feed 2,4,6-TCP concentration (X_1) was between 0 and 400 mg l^{-1} while feed COD concentration (X_2) was varied between 1,000 and 4,000 mg l^{-1}. The A/Q ratio (X_3) was varied between 23 and 163 m^2 d m^{-3} resulting in HRT values between 5 and 35 h.

Experimental data were used for determination of the response function coefficients for each independent variable by iteration. The results collected from each stage were evaluated. Most of the TCP removal took place in the first stage. However, considerable TCP and toxicity removals were obtained in the second stage at high COD and TCP loading rates. The data from the second stage were used in determination of the response function coefficients. Different response functions were used to correlate the experimental data and the most suitable one was determined by using the analysis of variance (ANOVA) program. ANOVA tests for all response functions indicated that the quadratic model provided the best fit to the experimental data with the lowest standard deviation, the highest correlation coefficient and the lowest p-value.

The estimated coefficients of the response functions are presented in Table 5.12. Positive b_2 and b_3 values indicate positive effects of increases in the feed COD and A/Q ratio on percent COD, 2,4,6-TCP and toxicity removals; and negative b_1 values indicate adverse effects of increasing 2,4,6-TCP concentrations on the response functions. The predicted values of percent COD, 2,4,6-TCP and toxicity removals from the response functions with the estimated coefficients are compared with the experimental results in Table 5.13. Response function predictions were in good agreement with the experimental data with R^2 values larger than 0.97.

Table 5.12 Predicted response function coefficients for percent COD, 2,4,6-TCP and toxicity removals in RTBR.

	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{11}	b_{22}	b_{33}
Y_{COD} R^2=0.98	63.79	-2.72 $*10^{-2}$	1.83 $*10^{-2}$	9.58 $*10^{-2}$	-5 $*10^{-6}$	4.83 $*10^{-4}$	4.54 $*10^{-5}$	-4 $*10^{-5}$	-3.8 $*10^{-6}$	-1.2 $*10^{-3}$
Y_{TCP} R^2=0.98	12.95	-3.55 $*10^{-1}$	4.76 $*10^{-2}$	9.35 $*10^{-1}$	2.58 $*10^{-5}$	1.25 $*10^{-3}$	1.43 $*10^{-5}$	1.98 $*10^{-4}$	-9.4 $*10^{-6}$	-4.79 $*10^{-3}$
$Y_{TOXICITY}$ R^2=0.97	23.05	-4.35 $*10^{-1}$	4.63 $*10^{-2}$	8.18 $*10^{-1}$	1.67 $*10^{-5}$	1.54 $*10^{-3}$	1.67 $*10^{-5}$	2.9 $*10^{-4}$	-9.3 $*10^{-6}$	-4.51 $*10^{-3}$

Table 5.13 Comparison of the predicted and experimental percent COD, 2,4,6-TCP and toxicity removals in RTBR.

Run	$E_{COD\ (exp)}$	$E_{COD\ (pred)}$	$E_{2,4,6\text{-}TCP(exp)}$	$E_{2,4,6\text{-}TCP(pred)}$	$E_{TOX(exp)}$	$E_{TOX(pred)}$
1	79	81	-	-	-	-
2	90	88	-	-	-	-
3	91	91	-	-	-	-
4	90	90	-	-	-	-
5	77	77	64	62	54	55
6	92	94	95	86	75	72
7	93	94	93	95	71	83
8	93	94	97	95	87	83
9	95	94	99	95	98	83
10	95	94	95	95	80	83
11	95	94	93	95	80	83
12	97	97	95	100	89	91
13	80	80	49	45	38	36
14	86	84	80	82	58	59
15	77	75	10	19	9	12
16	73	73	35	37	23	23
17	70	72	25	21	3	2

(-): not applicable since feed 2,4,6-TCP was zero for those experiments.

Variations of percent COD removal with A/Q ratio at different feed COD contents are depicted in Figure 5.29 at constant feed 2,4,6-TCP of 200 mg l^{-1}. Percent COD removal increased with increasing A/Q ratio due to high biofilm surface area or high active biomass concentrations at high A/Q ratios. At low feed COD contents such as 1000 and 2000 mg l^{-1} percent COD removal was maximum at an A/Q ratio of nearly 110 m^2 d m^{-3}. Further increases in the A/Q ratio reduced percent COD removal due to insufficient COD loading to support biofilm organisms at low flow rates or high A/Q ratios. The optimum A/Q ratio shifted to larger values for high feed COD contents yielding the optimum A/Q value of nearly 140 m^2 d m^{-3} for the fed COD of 3000 and 4000 mg l^{-1}. High COD loadings required larger A/Q ratios or larger biofilm areas for maximum COD removal. At constant A/Q ratio percent COD removal increased with increasing feed COD up to 3000 mg l^{-1} due to formation of thicker biofilms with the high feed COD contents and also due to COD limitations at

low feed COD contents. COD removal decreased for the feed COD of 4000 mg l^{-1} due to adverse effects of excess COD loading (ie thicker biofilm formation and diffusion limitations within the biofilm) at high feed COD contents. When the feed 2,4,6-TCP was 200 mg l^{-1}, the maximum percent COD removal (97 %) was obtained with the feed COD of nearly 3000 mg l^{-1} and A/Q ratio of 133 m^2 d m^{-3}.

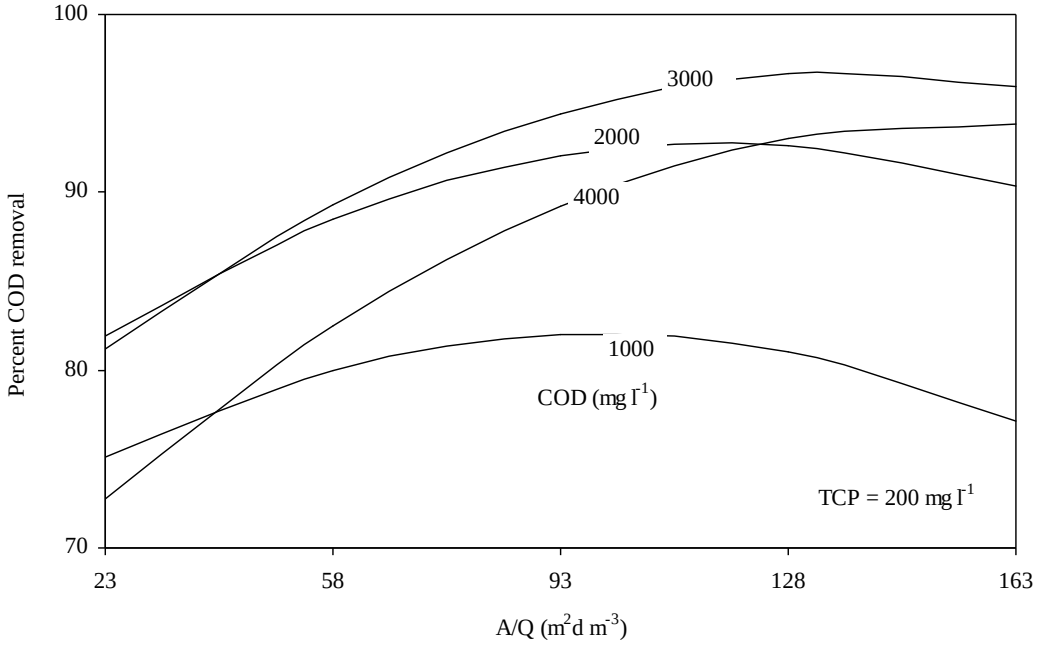


Figure 5.29 Variation of percent COD removal with A/Q ratio at different feed COD concentrations

Figure 5.30 depicts variation of percent COD removal with the feed 2,4,6-TCP content at different feed COD's at a constant A/Q ratio of 93 m^2d m^{-3}. Percent COD removal decreased with increasing feed 2,4,6-TCP due to toxic effects of 2,4,6-TCP contents on the microorganisms. This decrease was more pronounced at high feed COD contents. At low feed COD concentrations (< 2000 mg l^{-1}), COD removal was not affected from the feed 2,4,6-TCP probably due to effective biodegradation of 2,4,6-TCP at low feed COD levels. However, at high feed COD's the adverse effects of 2,4,6-TCP was more pronounced at high 2,4,6-TCP levels due to preferable use of sucrose present in molasses instead of 2,4,6-TCP biodegradation. At a constant feed 2,4,6-TCP, COD removal increased with increasing feed COD up to 3000 mg l^{-1} and then decreased due adverse effects of high COD loadings. Probably high feed CODs

resulted in formation of thick biofilms in which the substrate and DO availability were limited. The optimal feed COD was 3000 mg l^{-1} for all feed 2,4,6-TCP contents when A/Q was 93 m^2 d m^{-3}.

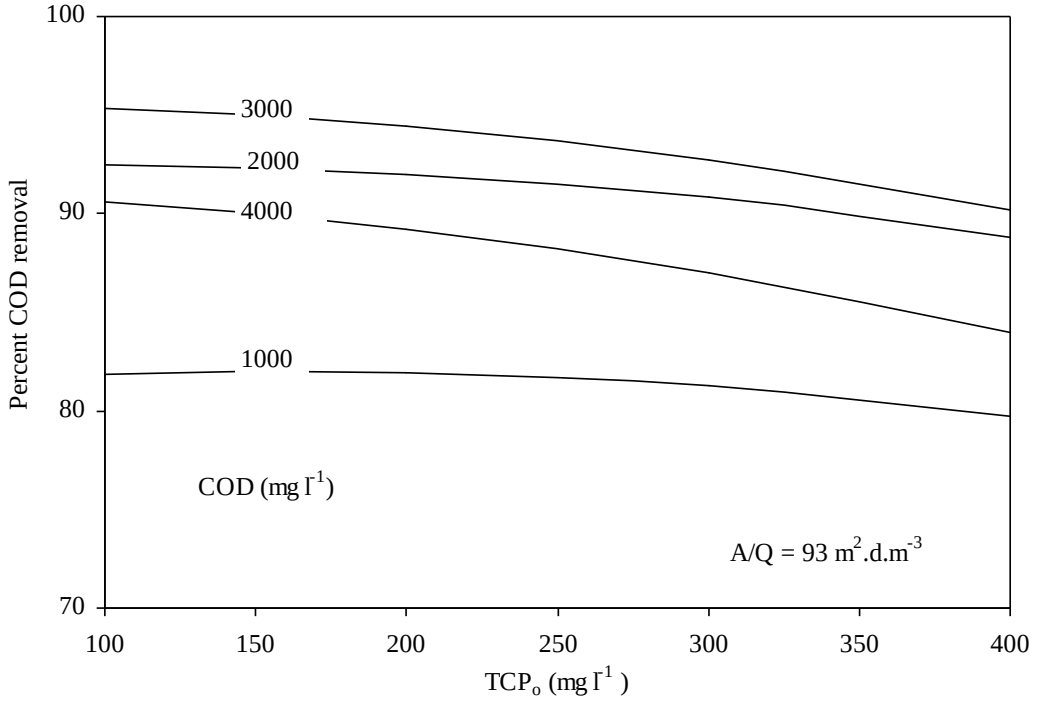


Figure 5.30 Variation of percent COD removal with the feed 2,4,6-TCP at different feed COD concentrations

Variation of percent 2,4,6-TCP removal with A/Q ratio at different feed COD's are depicted in Figure 5.31 at a constant feed 2,4,6-TCP of 200 mg l^{-1}. 2,4,6-TCP removal increased with A/Q ratio due to high biomass concentrations at high A/Q ratios since biomass concentration is proportional to the biofilm surface area (A). A/Q ratio of 120 m^2 d m^{-3} was sufficient for maximum 2,4,6-TCP removal at all feed COD contents tested. At constant A/Q ratio (constant biofilm surface area) percent 2,4,6-TCP removal increased with increasing feed COD up to 3000 mg l^{-1} due to COD limitations at low COD loadings. 2,4,6-TCP removal decreased with further increases in the feed COD to 4000 mg l^{-1}. This may be thick biofilm formation at high feed CODs and DO limitations within the biofilms. Biofilm organisms may have preferably degraded sucrose in molasses which resulted in lower percent 2,4,6-TCP removals at high COD loadings. The optimal feed COD and A/Q ratio were

approximately 3000 mg l^{-1} and 130 m^2 d m^{-3}, respectively for complete removal of 2,4,6-TCP when the feed 2,4,6-TCP was 200 mg l^{-1}.

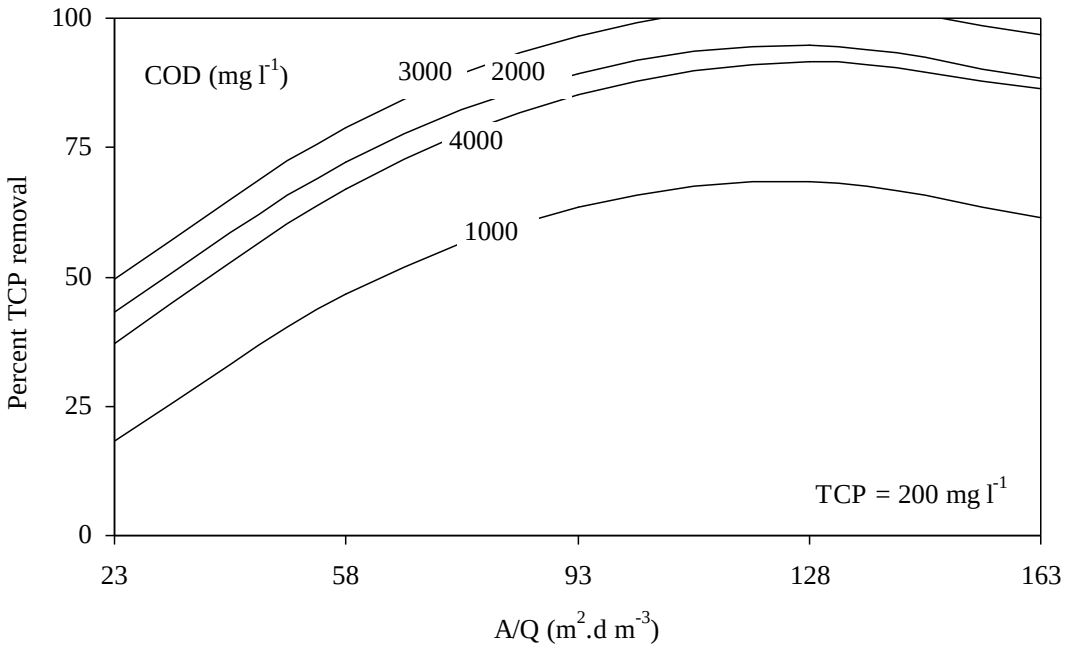


Figure 5.31 Variation of percent 2,4,6-TCP removal with A/Q ratio at different feed COD concentrations

Variations of percent 2,4,6-TCP removal with the feed 2,4,6-TCP at different feed COD contents and constant A/Q ratio of 93 m^2 d m^{-3} are depicted in Figure 5.32. Percent 2,4,6-TCP removal decreased with increasing feed 2,4,6-TCP due to toxic effects of high 2,4,6-TCP concentrations. Adverse effects of feed 2,4,6-TCP were more pronounced at low feed COD concentrations due to low active biomass concentrations at low COD loadings. As the feed COD increased the adverse effects of 2,4,6-TCP became less pronounced due to high active biomass concentrations at high COD loadings. 2,4,6-TCP removal also increased with increasing feed COD up to 3000 mg l^{-1} at a constant feed 2,4,6-TCP content due to formation of thicker biofilms at high feed CODs. Further increases in the feed COD above 3000 mg l^{-1} resulted in decreases in 2,4,6-TCP removal probably due to excessively thick biofilm formation and DO limitations in the thick biofilms. Preferable use of sucrose present in molasses over 2,4,6-TCP may be another reason for low TCP removals at high COD loadings. Feed COD should be around 3000 mg l^{-1} in order to maximize 2,4,6-

TCP removal (> 90%) for all feed 2,4,6-TCP contents for an A/Q value of 93 m^2 d m^{-3}.

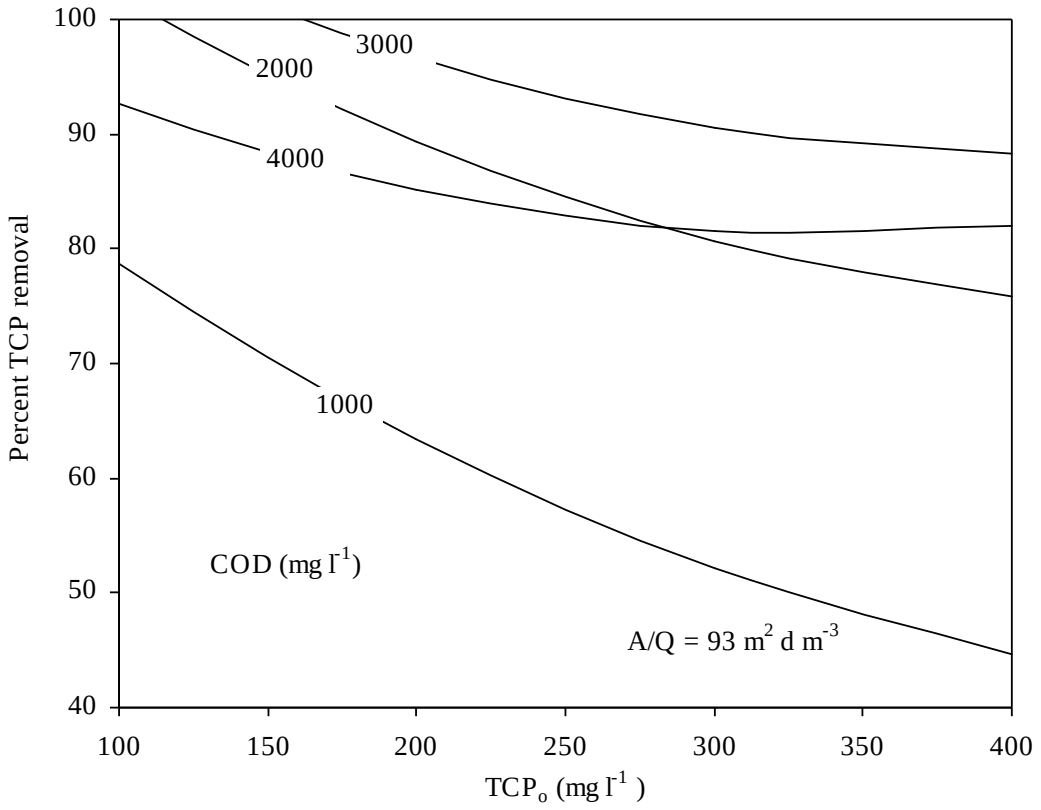


Figure 5.32 Variation of percent 2,4,6-TCP removal with the feed 2,4,6-TCP at different feed COD concentrations

Toxicity removals from the TCP containing wastewater depicted similar behavior to the 2,4,6-TCP removals since the major toxic compound in the medium was 2,4,6-TCP and/or its degradation products. Variations of percent toxicity removal with A/Q ratio at different feed COD's and constant 2,4,6-TCP of 200 mg l^{-1} is depicted in Figure 5.33 which shows the same trend as in Figure 5.31 (2,4,6-TCP removal). Percent toxicity removal increased with increasing A/Q ratio due to high concentrations of active biofilm organisms at high A/Q ratios since biomass concentration is proportional to the biofilm surface area (A). A/Q ratio of 120 m^2 d m^{-3} was sufficient for maximum toxicity removal at all feed COD contents tested. At constant A/Q ratio (constant biofilm surface area) percent toxicity removal increased with increasing feed COD up to 3000 mg l^{-1} due to increases in biomass

concentrations with the feed COD. Toxicity removal decreased with further increases in the feed COD to 4000 mg l^{-1}, probably due to formation of very thick biofilms and DO limitations within the biofilm. Preferable utilization of sucrose present in molasses instead of 2,4,6-TCP may be another reason for low toxicity removals at very high feed COD contents. The optimal feed COD and A/Q ratio were approximately 3000 mg l^{-1} and 130 m^2 d m^{-3}, respectively for complete removal of toxicity when the feed 2,4,6-TCP was 200 mg l^{-1}. Percent toxicity removals were lower than 2,4,6-TCP removals probably due to some toxic intermediate formation from 2,4,6-TCP biodegradation.

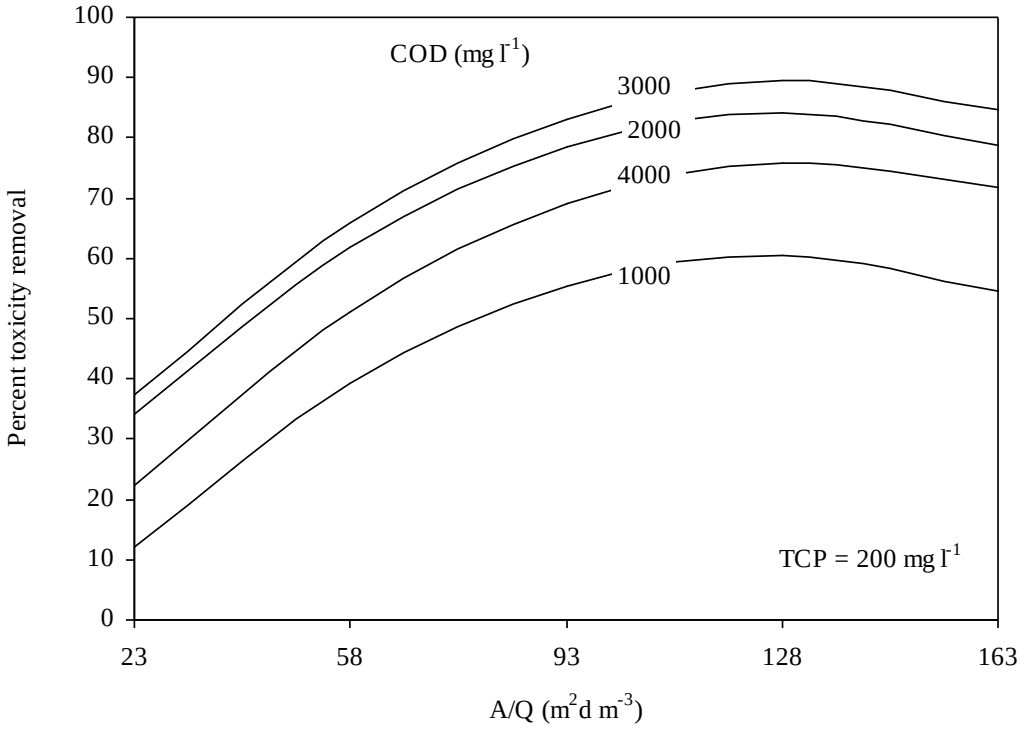


Figure 5.33 Variation of percent toxicity removal with the A/Q ratio at different feed COD concentrations

Figure 5.34 depicts variations of percent toxicity removal with the feed 2,4,6-TCP at different feed COD contents and a constant A/Q ratio of 93 m^2 d m^{-3}. The curves in Figure 5.34 depict the similar trends as in Figure 5.32 (2,4,6-TCP removal). Toxicity removal decreased with increasing feed 2,4,6-TCP due to toxic effects of high 2,4,6-TCP concentrations on the microorganisms. Reductions in percent toxicity removal with increases in the feed 2,4,6-TCP were more pronounced at low feed

COD contents due to low active biomass concentration at low COD loadings. Adverse effects of high feed 2,4,6-TCP contents were reduced by increasing the feed COD content yielding high active biomass concentrations. Percent toxicity removal at a constant feed 2,4,6-TCP increased with increasing feed COD up to 3000 mg l^{-1} due to insufficient biomass concentrations at low COD loadings. Further increases in the feed COD yielded lower toxicity removals probably due to formation of thick biofilms and DO/substrate limitations inside the thick biofilms. The system should be operated with a fed COD of 3000 mg l^{-1} at all feed 2,4,6-TCP contents to maximize toxicity removal when A/Q ratio was 93 m^2 d m^{-3}.

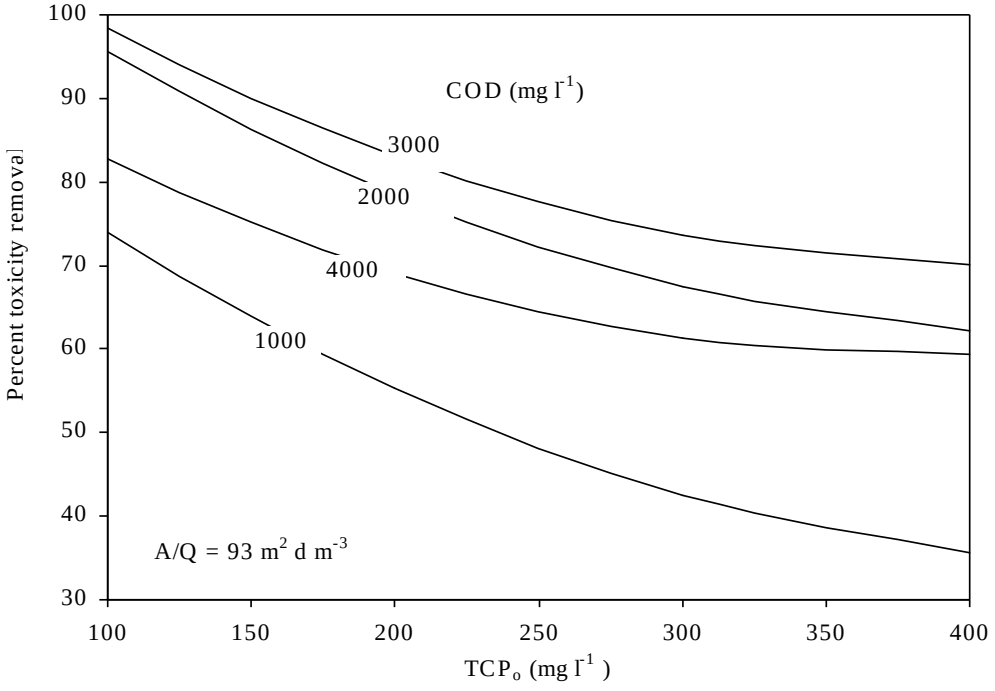


Figure 5.34 Variation of percent toxicity removal with the feed 2,4,6-TCP at different feed COD concentrations

Table 5.14 summarizes the optimum feed COD and A/Q ratios maximizing COD, 2,4,6-TCP and toxicity removals for different feed 2,4,6-TCP concentrations. Percent removal of TCP was fixed at 100% and maximum percent COD and toxicity removals were searched in determining the optimum conditions for desired feed TCP contents. For the feed 2,4,6-TCP of 100 mg l^{-1}, feed COD of 1950 mg l^{-1} and A/Q ratio of 104 m^2 d m^{-3} are required for maximum COD (92%), 2,4,6-TCP (100%) and toxicity (96%) removals. The feed COD and A/Q ratio of 2985 mg l^{-1} and 165 m^2 d

m^{-3} are required for the feed 2,4,6-TCP of 400 mg l^{-1} in order to maximize COD (99%), 2,4,6-TCP (100%) and toxicity (93%) removals. High feed 2,4,6-TCP contents required high A/Q ratio and high feed COD contents for high removal efficiencies.

Table 5.14 Optimum operating conditions for different feed 2,4,6-TCP concentrations as predicted from the response functions

$2,4,6\text{-}TCP_o$ (mg l^{-1})	COD_o (mg l^{-1})	A/Q (m^2 d m^{-3})	$E_{2,4,6\text{-}TCP}$ (%)	E_{COD} (%)	E_{TOX} (%)
100	1950	104	100	92	96
200	2936	133	100	97	90
300	2884	147	100	97	87
400	2985	165	100	99	93

5.3.2 Removal of 2,4,6-trichlorophenol by using Rotating Brush Biofilm Reactor (RBBR)

The same experimental conditions of the Box–Behnken statistical experiment design were used for TCP removal in RBBR as that of the RTBR. Three important operating parameters; feed 2,4,6-TCP (X_1) and feed COD_o (X_2) concentrations and A/Q ratio (X_3) were considered as independent variables. Feed 2,4,6-TCP concentration (X_1) was between 0 and 400 mg l^{-1} while the Feed COD concentration (X_2) was varied between 1,000 and 4,000 mg l^{-1}. The A/Q ratio (X_3) was varied between 37 and 256 m^2 d m^{-3} resulting in HRT values between 5 and 35 h.

Experimental data was used for determination of the response function coefficients for each independent variable by iteration. The second stage contribution to the COD and TCP removal was less than 5%. The second stage contributed to the mineralization of the by products of TCP degradation. However, at low A/Q ratios such as 37 m^2 d m^{-3} or high loading rates, the second stage contributed significantly to the percent COD and toxicity removals. At low loading rates only the first stage is sufficient for effective COD and TCP removals. Experimental data obtained in the second stage were used in estimating the coefficients of the response functions. Different response functions were used to correlate the experimental data and the most suitable one was determined by using the analysis of variance (ANOVA) program. ANOVA test for different response function models indicated that the

quadratic model provided the best fit to the experimental data with the lowest standard deviation, the highest correlation coefficient and the lowest p-value. The estimated coefficients of the response functions are presented in Table 5.15. Comparison of the experimental and predicted 2,4,6-TCP, COD and toxicity removals are presented in Table 5.16.

Table 5.15 Coefficients of the response functions for 2,4,6-TCP, COD and toxicity removals in RBBR

	b_0	b_1	b_2	b_3	b_{12}	b_{13}	b_{23}	b_{11}	b_{22}	b_{33}
Y_{COD} R^2=0.98	68.76	-3.73 $*10^{-2}$	1.61 $*10^{-2}$	9.04 $*10^{-2}$	-6.7 $*10^{-6}$	2.4 $*10^{-4}$	3.35 $*10^{-5}$	-2.3 $*10^{-5}$	-3.6 $*10^{-6}$	-5.4 $*10^{-4}$
Y_{TCP} R^2=0.98	29.93	-3.75 $*10^{-1}$	4.28 $*10^{-2}$	4.72 $*10^{-1}$	2.5 $*10^{-5}$	9.13 $*10^{-4}$	4.26 $*10^{-5}$	1.94 $*10^{-4}$	-9.4 $*10^{-6}$	-1.9 $*10^{-3}$
$Y_{TOXICITY}$ R^2=0.97	26.88	-4.25 $*10^{-1}$	4.17 $*10^{-2}$	5.33 $*10^{-1}$	2 $*10^{-5}$	1.02 $*10^{-3}$	1.52 $*10^{-5}$	2.77 $*10^{-4}$	-8.7 $*10^{-6}$	-1.83 $*10^{-3}$

Table 5.16 Comparison of experimental and predicted 2,4,6-TCP, COD and toxicity removals in RBBR

Run	$E_{COD (exp)}$	$E_{COD (pred)}$	$E_{2,4,6\text{-}TCP(exp)}$	$E_{2,4,6\text{-}TCP(pred)}$	$E_{TOX(exp)}$	$E_{TOX(pred)}$
1	91	88	-	-	-	-
2	98	96	-	-	-	-
3	95	96	-	-	-	-
4	88	92	-	-	-	-
5	75	80	55	55	66	62
6	95	96	95	90	84	80
7	96	96	95	94	86	87
8	95	96	92	94	83	87
9	97	96	99	94	98	87
10	95	96	92	94	83	87
11	95	96	92	94	83	87
12	99	95	98	100	96	100
13	82	81	46	43	44	43
14	78	81	76	78	68	67
15	78	77	19	24	11	15
16	76	71	31	31	19	23
17	68	70	18	16	7	4

(-): not applicable since feed 2,4,6-TCP was zero for those experiments.

Variations of percent COD removal with the feed COD and 2,4,6-TCP concentrations at constant A/Q ratio of 37 m^2 d m^{-3} are depicted in Figure 5.35. Percent COD removal decreased with increasing feed 2,4,6-TCP concentration from 0 mg l^{-1} to 400 mg l^{-1} for all feed COD concentrations, due to toxic effects of increasing feed 2,4,6-TCP concentrations. Percent COD removal reached a maximum value for all 2,4,6-TCP concentration when the feed COD concentration was around 2500 mg l^{-1}, but decreased with further increases in the feed COD due to adverse effects of high COD loading rates. COD loading rate determines the amount of bacteria in the system or the biofilm thickness. At low COD loadings, COD removal is low due to low biomass concentrations in the system in form of biofilm. At high COD loading rates, thick biofilm formation and DO/substrate limitations in the thick biofilm cause low COD removals. Therefore, there is an optimum COD loading rate or the feed COD content resulting in an optimum biofilm thickness.

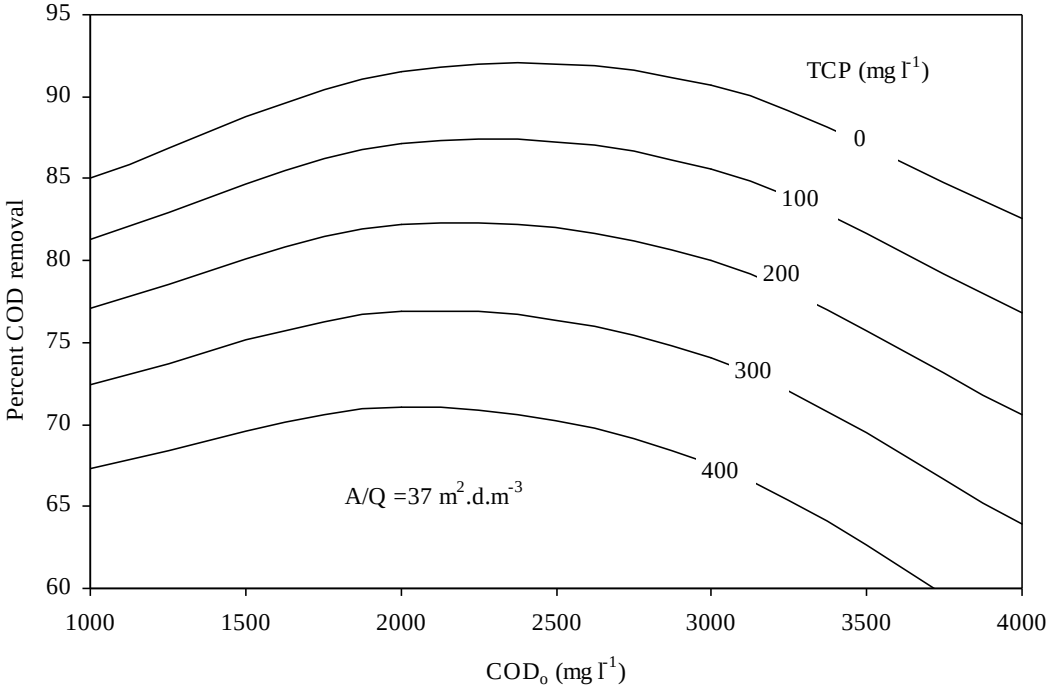


Figure 5.35 Variation of percent COD removal with feed COD concentration at different feed 2,4,6-TCP concentrations

Figure 5.36 shows variations of percent COD removal with feed 2,4,6-TCP concentration at different A/Q ratios and constant feed COD of 1,000 mg l^{-1}. Percent

COD removal increased with increasing A/Q ratio and reached a maximum level at A/Q ratio of around 222 m^2 d m^{-3}. Further increases in A/Q ratio caused decreases in percent COD removal probably because of low feed flow rates and insufficient COD loading rates to support microbial growth. Percent COD removal decreased with increasing feed 2,4,6-TCP concentrations as a result of toxic effects of high 2,4,6-TCP contents on the organisms. Percent COD removals increased with increasing A/Q ratio due to increased biofilm surface area up to A/Q ratio of 222 m^2 d $m^{-3.}$ Further increases in A/Q ratio resulted in lower percent COD removals due to low flow rates and insufficient COD loadings to support high concentrations of biofilm organisms. Low feed 2,4,6-TCP and high A/Q ratios (around 200 m^2 dm^{-3}) and nearly 2500 mg l^{-1} feed COD concentrations resulted in high COD removals.

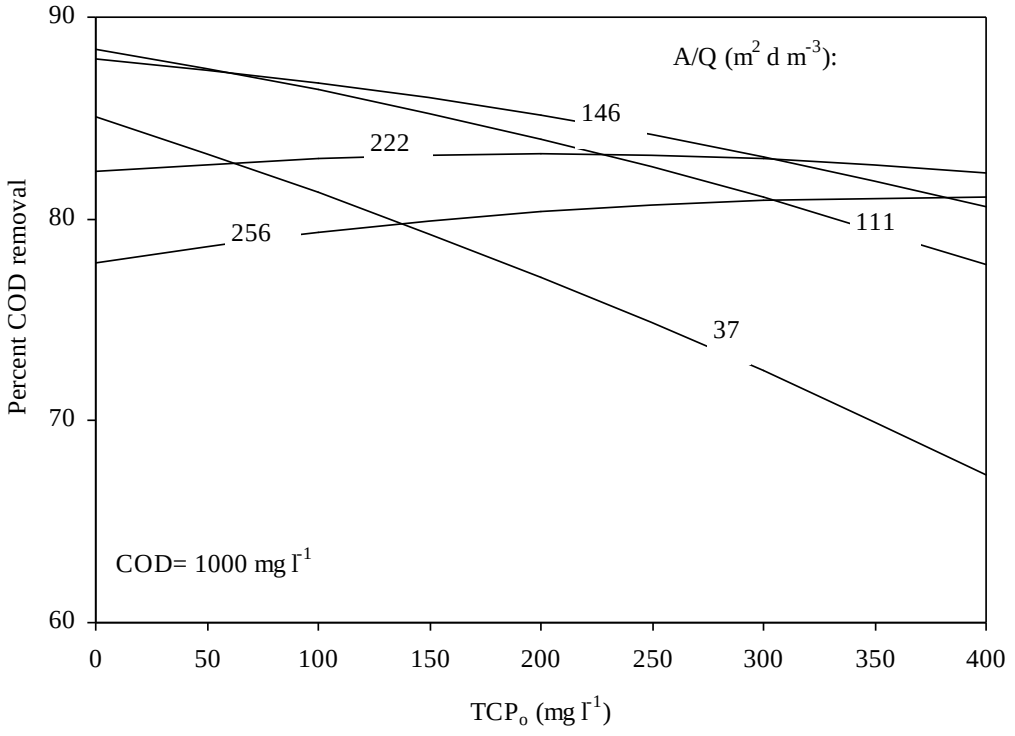


Figure 5.36 Variation of percent COD removal with feed 2,4,6-TCP concentration at different A/Q ratios

Variations of percent 2,4,6-TCP removal with the feed COD content at different feed 2,4,6-TCP concentrations and constant A/Q ratio of 37 m^2 d m^{-3} are depicted in Figure 5.37. Percent 2,4,6-TCP removal decreased with increasing feed 2,4,6-TCP

concentration due to toxic effects of high 2,4,6-TCP contents on the organisms. Percent 2,4,6-TCP removal increased with increasing feed COD up to feed COD of 2000-2500 mg l^{-1} due to low biomass concentrations and substrate limitations at low COD loadings. However, 2,4,6-TCP removals decreased with further increases in the feed COD above 2500 mg l^{-1} due to formation of thick biofilms and DO/substrate limitations inside the biofilm. Preferential utilization of COD compounds in molasses (mainly sucrose) rather than 2,4,6-TCP may be another reason for low percent TCP removals at high feed COD contents.

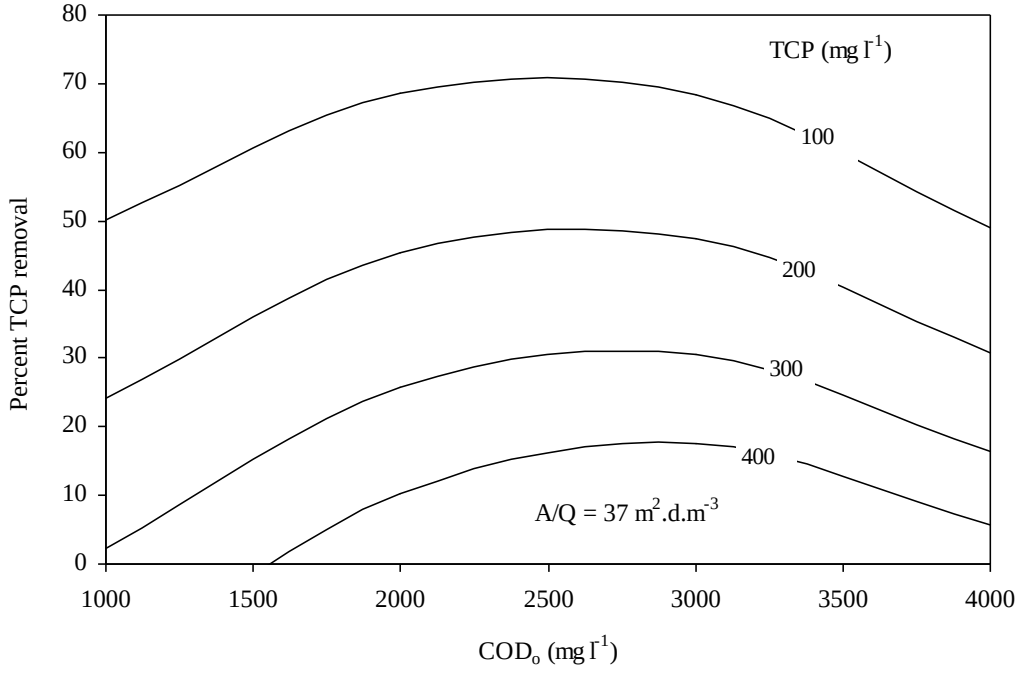


Figure 5.37 Variations of percent 2,4,6-TCP removal with the feed COD content at different feed 2,4,6-TCP concentrations

Figure 5.38 depicts variations of percent 2,4,6-TCP removal with feed 2,4,6-TCP concentration at different A/Q ratios and constant feed COD of 1,000 mg l^{-1}. Percent 2,4,6-TCP removals decreased with increasing feed 2,4,6-TCP concentrations due to toxic effects of 2,4,6-TCP on the organisms when A/Q ratio was lower than 222 m^2 d m^{-3} . At high A/Q ratios above 200 m^2 d m^{-3} due to high concentrations of biofilm organisms the feed 2,4,6-TCP content did not affect percent 2,4,6-TCP removals. In other words, 2,4,6-TCP inhibition on the organism was eliminated by operating the

system at high A/Q ratios or high biomass concentrations. Percent 2,4,6-TCP removals also increased with increasing A/Q ratios up to A/Q 146 m^2 d m^{-3} due to high concentrations of biofilm organisms at high A/Q ratios. However, further increases in A/Q ratio resulted in decreases in 2,4,6-TCP removals due to insufficient COD loadings (or low flow rates) to support microbial growth at high A/Q ratios. Complete 2,4,6-TCP removal for the feed 2,4,6-TCP of 320 mg l^{-1} can be obtained with an A/Q ratio of 256 m^2 d. m^{-3} and the feed COD content of 3650 mg l^{-1}.

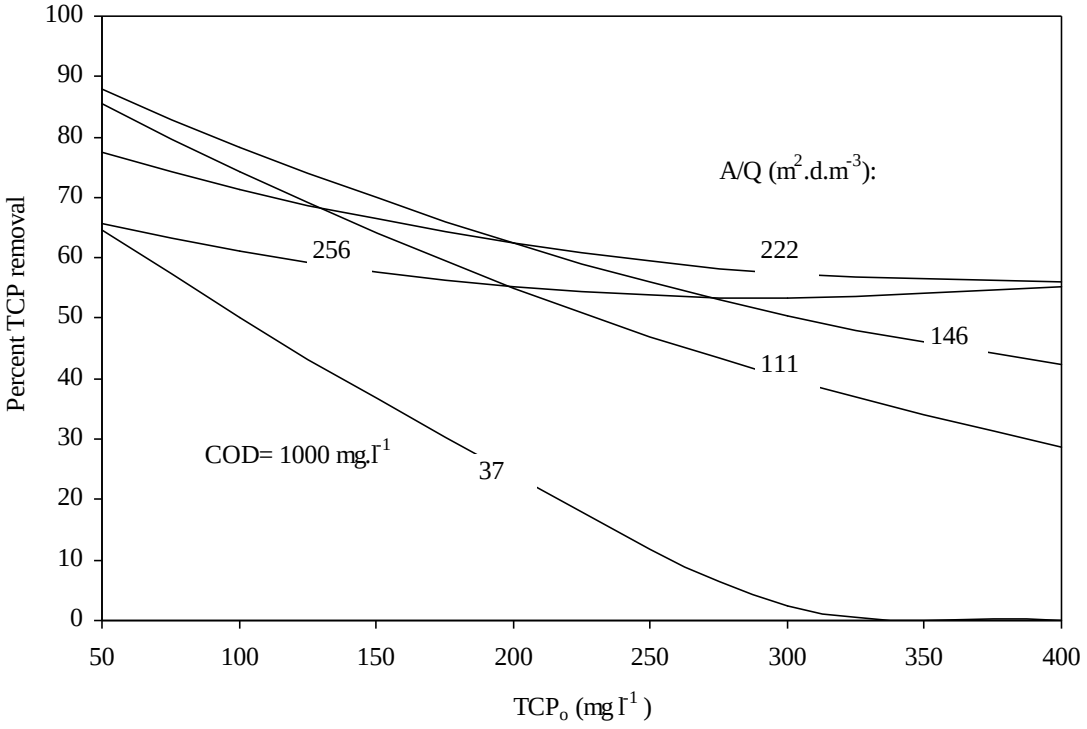


Figure 5.38 Variations of percent 2,4,6-TCP removal with feed 2,4,6-TCP concentration at different A/Q ratios

Removal of toxicity from the wastewater containing different concentrations of 2,4,6-TCP is another important aspect of this study which is closely related to 2,4,6-TCP removals. Variations of percent toxicity removal with feed 2,4,6-TCP concentration at different A/Q ratios and constant feed COD of 1000 mg l^{-1} are depicted in Figure 5.39. Percent toxicity removal decreased with increasing feed 2,4,6-TCP content due to toxic effects of high 2,4,6-TCP contents on the organisms. Percent toxicity removal increased with increasing A/Q ratio up to 222 m^2 d m^{-3} due to increasing concentrations of biofilm organisms. Further increases in A/Q ratio

resulted in decreases in 2,4,6-TCP removals due to low flow rates or COD loading rates to support microbial growth. The system should be operated at an A/Q ratio of 200-220 m^2 d m^{-3} for all feed 2,4,6-TCP concentrations to obtain high 2,4,6-TCP removals.

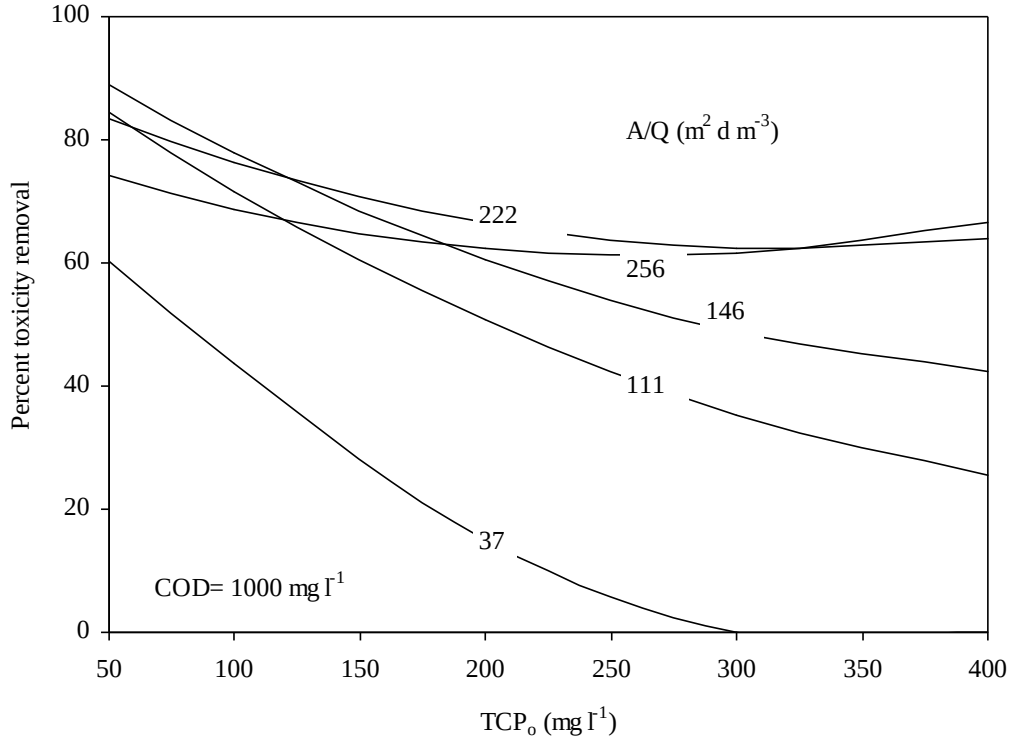


Figure 5.39 Variation of percent toxicity removal with feed 2,4,6-TCP concentrations at different feed A/Q ratios

Figure 5.40 depicts variations of percent toxicity removals with the feed COD concentration at different feed 2,4,6-TCP content and a constant A/Q ratio of 111 m^2 d m^{-3}. Percent toxicity removals decreased with increasing feed 2,4,6-TCP contents for all feed COD concentrations due to toxic effects of high 2,4,6-TCP contents on the organisms. Feed COD concentration also had considerable effect on toxicity removals, which increased up to feed COD of 2500 mg l^{-1} due to increasing biomass concentrations with the feed COD. However, feed COD contents above 2500 resulted in decreases in percent toxicity removals due to thick biofilm formation and DO/substrate unavailability within the thick biofilm. The biofilm microorganisms may have preferably utilized COD compounds in molasses (mainly sucrose) at high

COD loadings yielding high effluent TCP contents and toxicities or low percent toxicity removals.

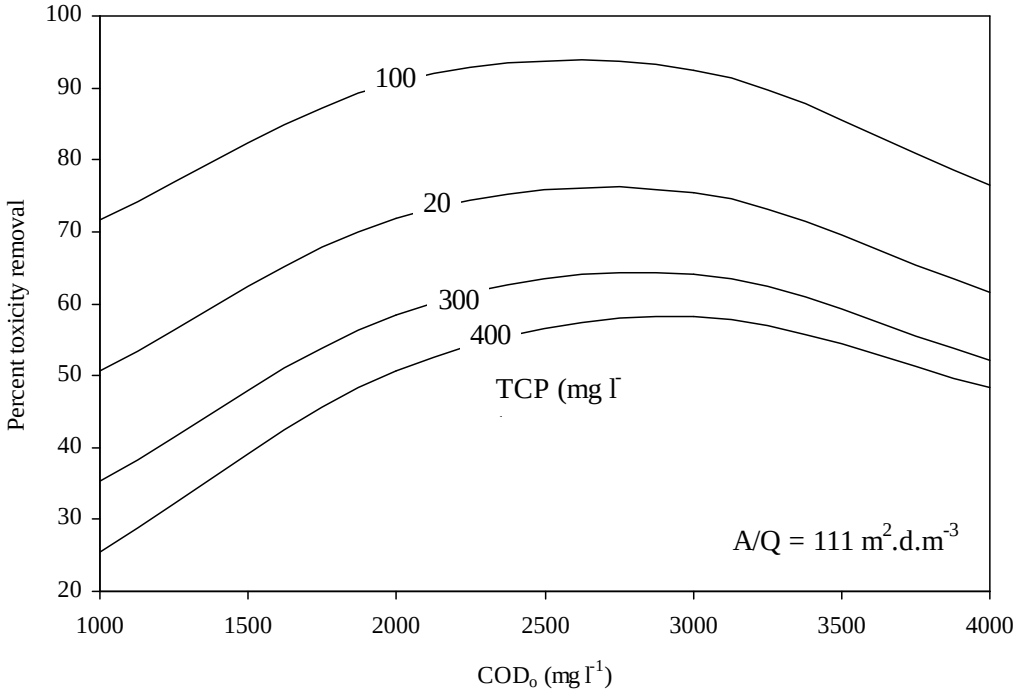


Figure 5.40 Variations of percent toxicity removal with the feed COD at different feed 2,4,6-TCP concentrations.

Due to highly toxic nature of 2,4,6-TCP, literature studies report less than 65% 2,4,6-TCP removals even with feed 2,4,6-TCP contents lower than 100 mg l^{-1} for aerobic treatment of 2,4,6-TCP containing wastewaters (Wang CC, *et al*.,2000(b); Correa J, *et al*.,2003). Combined anaerobic-aerobic treatment systems were reported to result in higher 2,4,6-TCP removals due to anaerobic degradation of 2,4,6-TCP (Armenante, P.M.,*et al*, 1999;Vallecillo A, *et al*, 1999; Atuanya EI, *et al*, 2000). However, the feed 2,4,6-TCP contents in those studies were lower than 100 mg l^{-1}. As compared to the literature studies on biological treatment of 2,4,6-TCP containing wastewaters, our study with the RBBR resulted in more than 90% COD, 2,4,6-TCP and toxicity removals from synthetic wastewater containing much higher 2,4,6-TCP concentrations (up to 400 mg l^{-1}) at much lower hydraulic residence times of 25 hours or A/Q ratio of 194 m^2 m^{-3}. The reason for the superior performance of the RBBR used is probably high biofilm surface area or high biomass concentrations and also effective

aeration and sufficiently high COD in the feed wastewater. Considering the fact that most of the removal takes place in the first stage, almost the same degree of removals can be obtained with nearly 12 h HRT or with an A/Q ratio of 100 m^2 m^{-3}

Table 5.17 summarizes the optimum feed COD and A/Q ratios maximizing COD, 2,4,6-TCP and toxicity removals for different feed 2,4,6-TCP concentrations. Percent removal of TCP was fixed at 100% and the maximum percent COD and toxicity removals were searched in determining the optimum operating conditions for desired feed TCP contents. For the feed 2,4,6-TCP of 100 mg l^{-1}, feed COD of 2106 mg l^{-1} and A/Q ratio of 215 m^2 d m^{-3} are required for maximum COD (96%), 2,4,6-TCP (100%) and toxicity (99%) removals. The feed COD and A/Q ratio of 2733 mg l^{-1} and 210 m^2 d m^{-3} are required for the feed 2,4,6-TCP of 400 mg l^{-1} in order to maximize COD (94%), 2,4,6-TCP (100%) and toxicity (97%) removals. High feed 2,4,6-TCP contents required high A/Q ratio and high feed COD contents for high removal efficiencies.

Table 5.17 Optimum operating conditions for different feed 2,4,6-TCP concentrations as predicted from the response functions

$2,4,6\text{-}TCP_o$ (mg l^{-1})	COD_o (mg l^{-1})	A/Q (m^2 d m^{-3})	$E_{2,4,6\text{-}TCP}$ (%)	E_{COD} (%)	E_{TOX} (%)
100	2106	215	100	96	99
200	2890	188	100	98	94
300	3544	224	100	96	91
400	2733	210	100	94	97

5.3.3 Comparison of performance of RTBR and RBBR for 2,4,6-trichlorophenol removal

As compared to our study on 2,4,6-TCP removal using the RBBR, the RTBR yielded higher COD and 2,4,6-TCP removals for the same A/Q ratio and the feed COD contents probably due to high biomass content of the RTBR. For the feed 2,4,6-TCP of 400 mg l^{-1}, a feed COD of 3000 mg l^{-1} and A/Q ratio of 256 m^2 d m^{-3} are required for maximum COD (96%), 2,4,6-TCP (100%) and toxicity (100%) removals in RBBR. However, the feed COD and A/Q requirements are 3000 mg l^{-1} and 165 m^2 d m^{-3} for the RTBR. Lower surface area requirements for the same degree

of removal in the RTBR is probably due to thicker biofilm formation on the rotating tube surfaces as compared to the brush surfaces.

The results of the RBBR study were compared with the results of the RTBR in Table 5.18 at the lowest, medium and the highest levels of the operating parameters. COD, TCP removals and toxicity removals are much better in the RTBR than RBBR. The performance of RTBR was better especially at high feed 2,4,6-TCP and COD contents due to formation of denser and thicker biofilm on the rotating tube surfaces.

Table 5.18 Comparison of COD, 2,4,6-TCP and toxicity removal efficiencies of rotating brush (RBBR) and rotating tubes biofilm (RTBR) reactors.

2,4,6-TCP$_0$	COD$_0$	A/Q	E_{COD} (%)		$E_{2,4,6\text{-}TCP}$ (%)		$E_{Toxicity}$ (%)	
(mg l^{-1})	(mg l^{-1})	(m^2 d m^{-3})	RBBR	RTBR	RBBR	RTBR	RBBR	RTBR
0	1000	50	86	82	-	-	-	-
200	2500	100	91	95	80	97	80	85
400	4000	150	82	94	80	100	80	82

Figure 5.41 depicts a comparison of percent COD removals obtained using RTBR and RBBR at different A/Q ratios and feed 2,4,6-TCPs. Percent COD removal decreased with increasing 2,4,6-TCP concentration for both reactors. However, percent COD removal with RTBR was significantly larger than that of the RBBR. At a feed 2,4,6-TCP concentration of 500 mg l^{-1}, percent COD removals with the RTBR were above 81%, whereas percent COD removals were around 73 % with the RBBR at A/Q ratio of 50 m^2 d m^{-3}.

Figure 5.42 depicts a comparison of 2,4,6-TCP removal performances of RTBR and RBBR at different feed 2,4,6-TCPs and A/Q ratios at a constant feed COD concentration. A similar trend was observed for the percent 2,4,6-TCP removal as that of the COD removal. At a feed 2,4,6-TCP concentration of 400 mg l^{-1}, percent 2,4,6-TCP removals with the RTBR were above 51% whereas TCP removals were around 26% with the RBBR at A/Q ratio of 50 m^2 d m^{-3}. This difference was more significant at high 2,4,6-TCP loading rates.

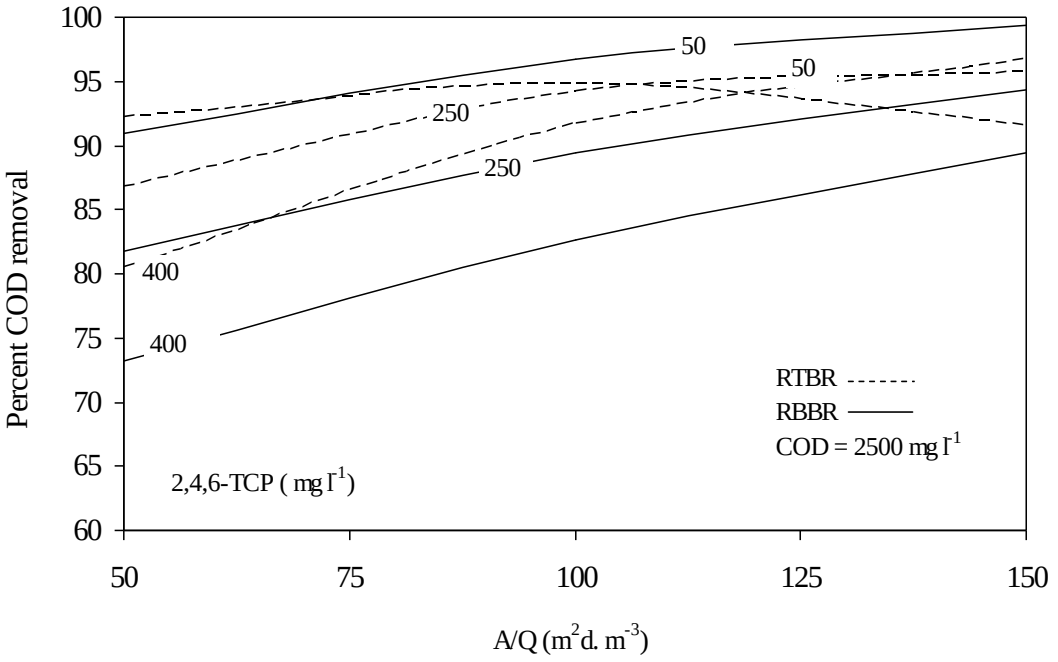


Figure 5.41 Comparison of RTBR and RBBR for percent COD removal at different 2,4,6-TCP concentrations

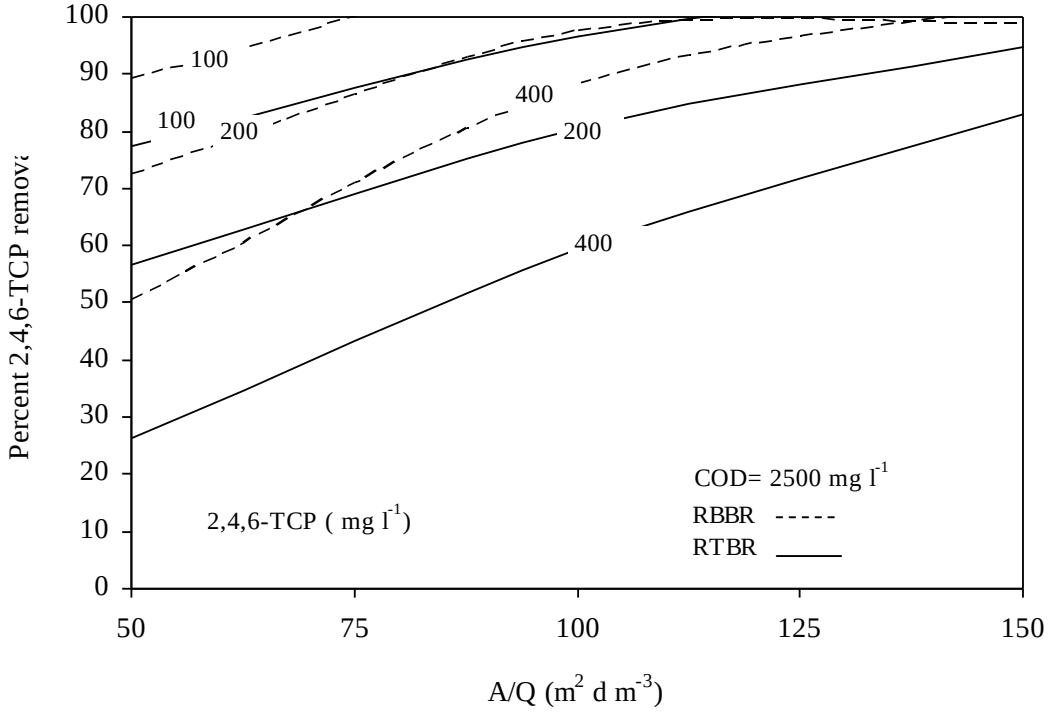


Figure 5.42 Comparison of RTBR and RBBR for percent 2,4,6-TCP removal at different 2,4,6-TCP concentrations

Figure 5.43 depicts a comparison of toxicity removal performances of RTBR and RBBR at different feed 2,4,6-TCPs and A/Q ratios at a constant feed COD concentration of 2500 mg l^{-1}. Percent toxicity removal increased from 33% at A/Q ratio of 50 m^2 dm^{-3} to 90% at A/Q ratio of 150 m^2 dm^{-3} with RTBR. However, increases in percent toxicity removal with the RBBR were from 15 to 76% for the same values of A/Q ratio. RTBR was clearly more advantageous as compared to RBBR in terms of TCP removal performances.

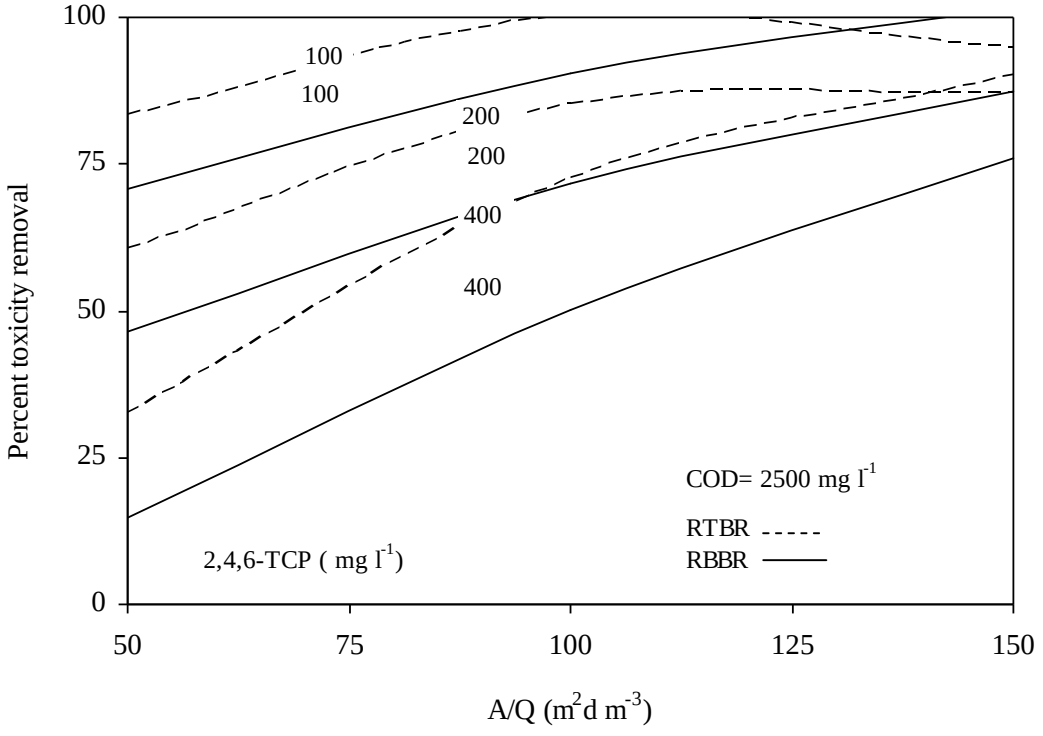


Figure 5.43 Comparison of RTBR and RBBR for percent toxicity removal at different 2,4,6-TCP concentrations

Biodegradation of 2,4,6-TCP of 400 mg l^{-1} was evaluated. Based on literature knowledge, 400 mgl^{-1} concentration has high inhibitory effect on microorganisms. There are not studies about COD removal with TCP degradation and its toxicity (Liu D. and Pacepavicius G., 1990; Li D.Y *et al.*, 1991; Correa J., *et al.*, 2003; Wang C.C., *et al*, 2000b; Snyder C.J.P., *et al.*, 2006). Therefore, the highest initial

concentration of TCP was tried to remove from wastewater by using RTBR and RBBR. The results showed that 2,4,6-TCP and its toxic content were successfully removed by using RTBR and RBBR reactors, which were developed by advisor. Due to the design of RBBR and RTBR, biofilm was more resistant to inhibitory effect of TCP. Therefore, high percent removal for TCP, COD and toxicity were obtained simultaneously.

5.4 Determination of Kinetic Constants for Treatment of DCP Containing wastewater using the RTBR

The experimental results obtained with the statistically designed experiments were not suitable for kinetic parameter estimation. The correlation coefficients for the regression analyses were lower than $R^2<0.60$. Therefore, model estimations for COD, and chlorophenol removals were not in good agreement with the experimental results. For this reason, another set of experiments were performed using the RTBR by varying one variable at a time in order to determine the model coefficients.

Two sets of experiments with variable A/Q ratio and feed DCP concentration (or feed DCP/COD ratio) were performed and COD, DCP and toxicity removals were quantified as functions of those independent variables using the RTBR.

5.4.1 Effects of A/Q ratio

A/Q ratio is an important operating parameter defining the available biofilm surface area per unit hydraulic loading. In variable A/Q experiments the flow rate of wastewater was changed to yield A/Q ratio between 31 and 217 m2.d m-3 (HRT = 5-35 hours) while feed COD and DCP contents were kept constant at 5000 ± 200 mg l-1 and 100 ± 5 mg l-1, respectively. Figure 5.44 depicts variation of percent COD removal and effluent COD concentration with the A/Q ratio Percent COD removal increased and effluent COD decreased with increasing A/Q ratio due to larger biofilm surface area and higher biomass concentrations at high A/Q ratios. Percent COD removal increased from 61% to 97% when A/Q ratio increased from 31 to 130 m2.d m-3. Further increases in A/Q ratio did not result in significant improvements

in percent COD removals and yielded a COD removal of 99% at an A/Q ratio of 217 m^2.d m^{-3}.

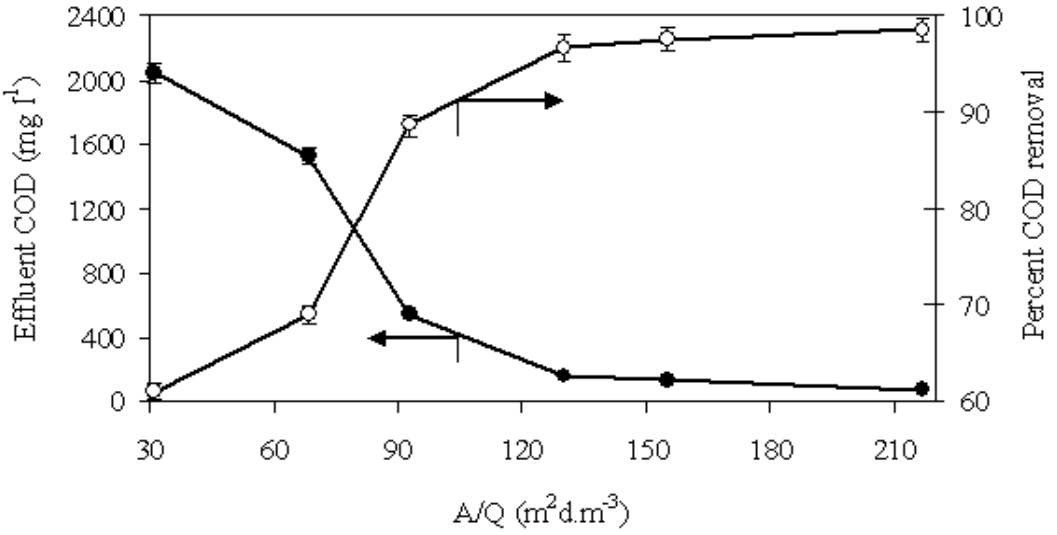


Figure 5.44. Variation of percent COD removal and effluent COD with A/Q ratio. COD_o = 5000 mg l^{-1}, DCP = 100 mg l^{-1}

Variation of percent DCP removal and the effluent DCP concentration with A/Q ratio is presented in Figure 5.45.Percent DCP removal increased sharply from 46% to 96% when A/Q ratio increased from 31 to 68 m^2.d m^{-3}. Increases in percent DCP removal for A/Q ratio larger than 68 m^2.d m^{-3} were minimal resulting in 98% and 100% DCP removals at A/Q ratios 130 and 217 m^2 d m^{-3}, respectively. Again an A/Q ratio of 130 m^2 d m^{-3} was found to be the most suitable A/Q ratio resulting in nearly complete DCP removal (98%). Effluent DCP concentrations decreased with increasing A/Q ratio resulting in 2 mg l^{-1} DCP concentration at A/Q ratio of 130 m^2 d m^{-3}.

Figure 5.46 depicts variation of percent toxicity removal and the effluent toxicity with the A/Q ratio. Toxicity of the wastewater is closely related with DCP content of wastewater. For this reason, variation of toxicity removal is quite similar to variation of DCP removal with the A/Q ratio. Similar to Figure 5.45, percent toxicity removal increased sharply from 74 to 94% when A/Q ratio increased from 31 to 68 m^2.d m^{-3}.

Further increases in A/Q ratio caused only marginal increases in percent toxicity removal resulting in 96% and 100% toxicity removals at A/Q ratios of 130 and 217 m^2 d m^{-3}. The effluent was completely detoxified for A/Q ratios above 130 m^2 d m^{-3}.

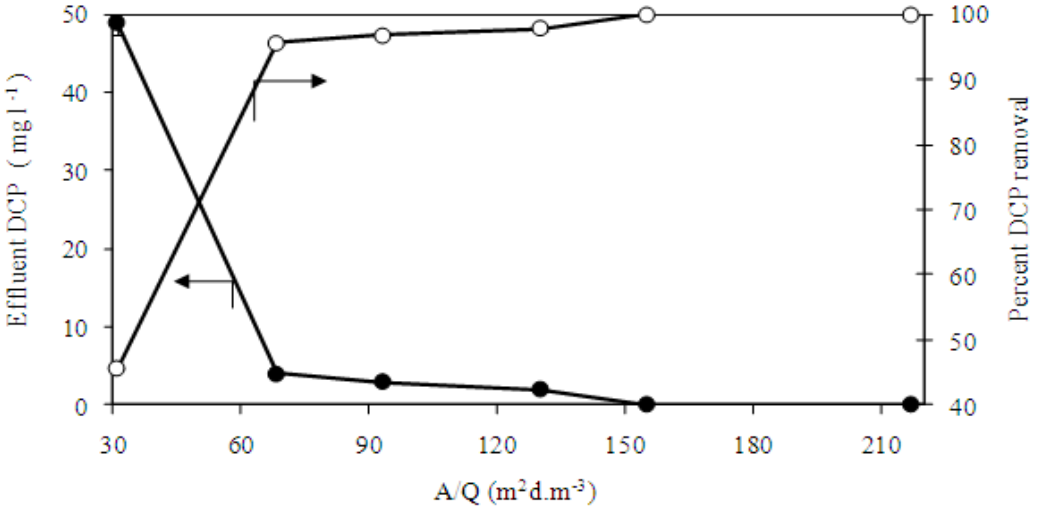


Figure 5.45 Variation of percent DCP removal and effluent DCP with A/Q ratio. COD_o = 5000 mg l^{-1}, DCP = 100 mg l^{-1}

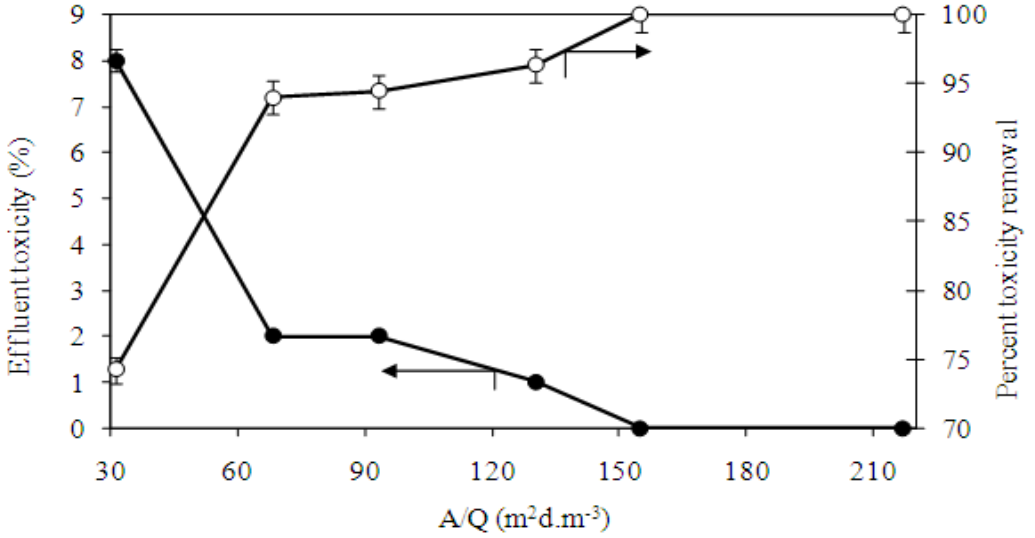


Figure 5.46 Variation of percent toxicity removal and the effluent toxicity with A/Q ratio. COD_o= 5000 mg l^{-1}, DCP = 100 mg l^{-1}

5.4.2 Effects of feed DCP concentration

A series of experiments with variable feed DCP concentration or feed DCP/COD ratio were carried out by changing the feed DCP between 50 and 400 mg l^{-1} while the feed COD and A/Q ratio were kept constant at 5000 ± 200 mg l^{-1} and 93 m^2 d m^{-3} (HRT = 15 hours), respectively. Feed DCP_o /COD_o ratio varied between 0.01 and 0.08 mg DCP/ mg COD in this set of experiments.

Variation of percent COD removal and the effluent COD with the feed DCP concentration and the feed DCP/COD ratio are depicted in

Figure 5.47. Percent COD removal decreased and the effluent COD increased with increasing feed DCP content. Percent COD removal decreased from 95% to 79% and further to 64% when the feed DCP increased from 50 to 193 mg l^{-1} and further to 393 mg l^{-1}, respectively. This is due to toxic effects of DCP on biofilm organisms.

Figure 5.48 depicts variation of percent DCP removal and the effluent DCP concentrations with the feed DCP and the DCP/COD ratio. Percent DCP removal was nearly complete (100%) resulting in almost zero DCP concentration in the aeration tank for the feed DCP contents up to 260 mg l^{-1}. Further increases in the feed DCP above 260 mg l^{-1} resulted in slightly lower percent DCP removals with 97% DCP removal for the feed DCP content of 393 mg l^{-1}. Apparently, DCP was effectively degraded for all feed DCP contents between 50 and 393 mg l^{-1} leaving negligible DCP in the aeration tank.

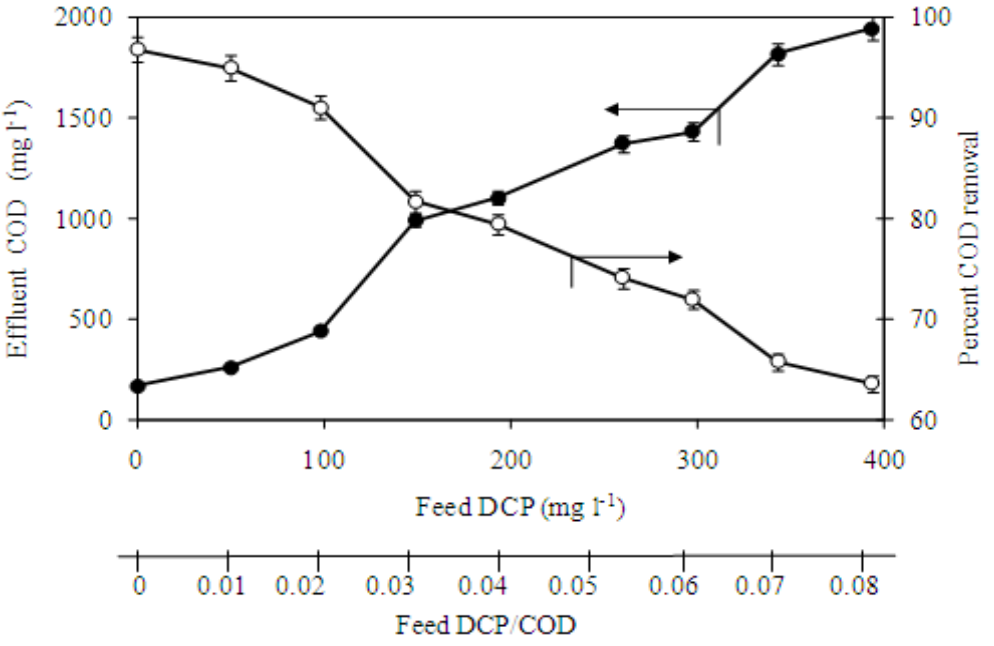


Figure 5.47 Variation of percent COD removal and effluent COD with the feed DCP and DCP/COD ratio. COD_o = 5000 mg l^{-1}, A/Q= 93 m^2 d m^{-3}

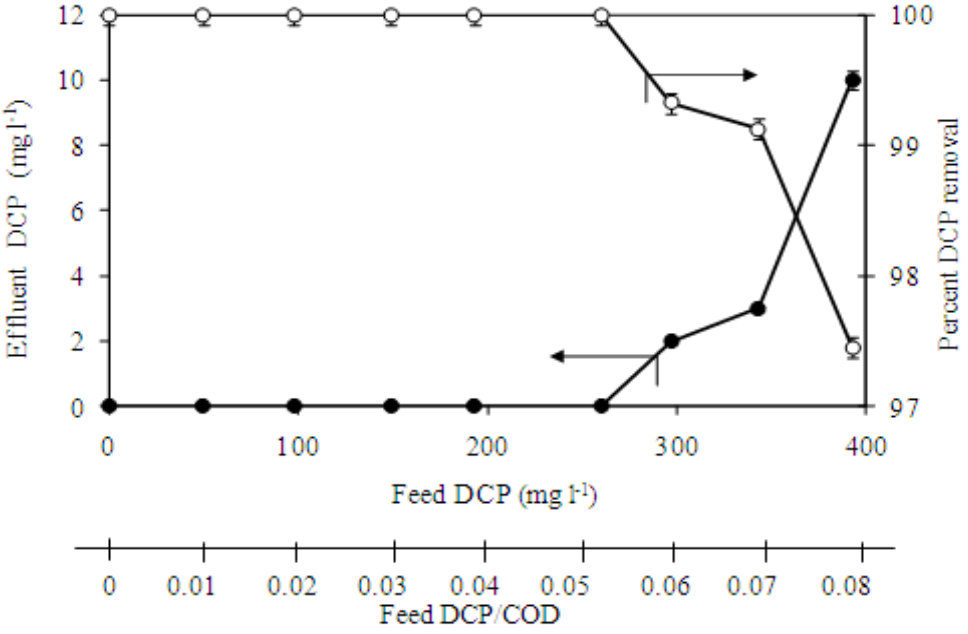


Figure 5.48Variation of percent DCP removal and effluent DCP with the feed DCP and DCP/COD ratio. COD_o = 5000 mg l^{-1}, A/Q= 93 m^2 d m^{-3}

Variation of percent toxicity removal and the effluent toxicity with the feed DCP concentrations are depicted in Figure 5.49. The trends in toxicity removals are quite similar to DCP removals as shown in Figure 5.48. Percent toxicity removal was 100% and the effluent toxicities were zero for the feed DCP content up to 300 mg l^{-1}, since DCP biodegradation was almost complete. Percent toxicity removal decreased to 95% when the feed DCP content increased to 393 mg l^{-1}. The results indicated that the biofilm reactor and the microbial culture was very effective in removing dichlorophenol and toxicity from the wastewater for a very wide range of feed DCP contents between 50 and 393 mg l^{-1}.

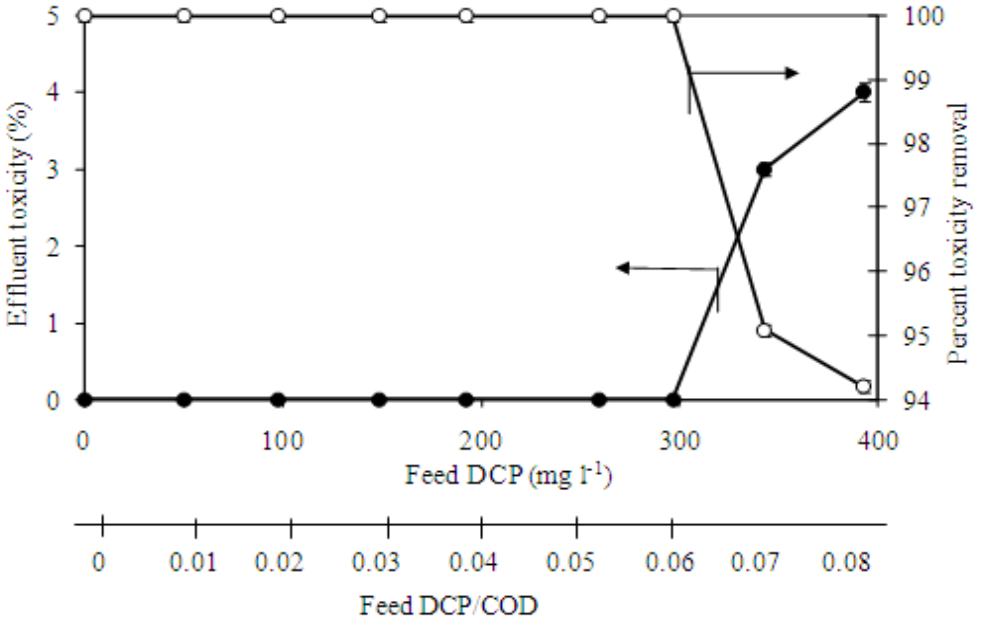


Figure 5.49 Variation of percent toxicity removal and effluent toxicity with the feed DCP and DCP/COD ratio. COD_o = 5000 mg l^{-1}, A/Q= 93 m^2 d m^{-3}

5.4.3 Kinetic modeling and parameter estimation

Table 5.19 summarizes the experimental data obtained with different A/Q ratios and the feed DCP contents. Experimental data with zero reactor DCP concentrations were considered for determination of the rate constants of eqn 8 where the DCP inhibition term was neglected due to no DCP content in the reactor. Statistica 5

program was used for iterative determination of the kinetic constants which were found to be

R_{mf} = 51 mg COD m^{-2} h^{-1},

R_{ms} = 343 mg COD l^{-1} h^{-1},

K_s = 81 mg COD l^{-1} (R^2 = 0.85),

yielding R_m = 2350 mg COD m^{-2} h^{-1}

By using the definitions of $R_{mf} = k_f X_f$ and $R_{ms} = k_s X_s$ and the average values of X_f = 55,000 mg biomass m^{-2} and X_s = 1500 mg biomass l^{-1}, the following values were found for the rate constants

$k_f = 9.27\ 10^{-4}\ h^{-1} = 0.022\ d^{-1}$, and $k_s = 0.229\ h^{-1} = 5.5\ d^{-1}$

Experimental data with non-zero DCP concentrations and the previously determined constants (R_{mf}, R_{ms} and K_s) were used to determine the DCP inhibition constant (K_{DCP}) by iteration using the Statistica 5 computer program. Following value was obtained for the inhibition constant.

K_{DCP} = 26 mg l^{-1} (R^2 = 0.86).

Therefore, eqn 9 takes the following form with the estimated constants

$$R_s = \frac{2350\ S}{81 + S}\left(\frac{26}{26 + DCP}\right)$$

where S is the COD concentration in the reactor (or effluent, mg l^{-1}); and Rs is the rate of COD removal (mg COD m^{-2} h^{-1}). Low K_{DCP} value (one-third of K_s) indicates strong DCP inhibition on the organisms.

The experimental and predicted R_s values are also presented in Table 5.19, which indicated that the model predictions were in good agreement with the experimental data. A similar model could be used for DCP removal if more data were available

with incomplete DCP removals (i.e., non-zero reactor DCP contents). Since DCP removal was almost complete in most of the experiments ($DCP_e = 0$), DCP inhibition on kinetics of DCP removal could not be quantified with high level of confidence.

Table 5.19 Experimental data used in mathematical modeling

COD_o (mg/L)	COD_e (mg/L)	DCP_o (mg/L)	DCP_e (mg/L)	Experimental $R_{s\,COD}$ ($mg/m^2.h$)	Predicted $R_{s\,COD}$ ($mg/m^2.h$)
4950	70	103	0	937	1089
5230	132	104	0	1370	1456
5130	260	50	0	2181	1792
4900	440	98	0	1997	1985
5400	990	149	0	1975	2172
4779	161	94	2	1477	1451
4770	542	95	3	1893	1832
4910	1525	93	4	2067	1933
5330	1939	393	10	1518	1627

CHAPTER SIX
CONCLUSIONS

Rotating perforated tubes biofilm reactor (RTBR) and rotating brush biofilm reactors (RBBR) were used for biological treatment of chlorophenol (4-CP, 2,4-DCP and 2,4,6-TCP) containing synthetic wastewater. Effects of major operating variables such as the feed COD, chlorophenol and also A/Q ratio on percent COD, chlorophenol and toxicity removals were investigated by using Box-Behnken and Box-Wilson statistical experiment designs and the response surface methodology (RSM). The experimental data were correlated by a response function and the coefficients were determined by regression analysis.

Feed COD content and A/Q ratio positively affected COD removal since increasing feed COD contents resulted in high biomass density and more effective COD removal by the RTBR. Increases in the feed 4-CP content adversely affected the COD removals because of toxic effects of 4-CP on the microorganisms. The most favorable conditions maximizing percent COD removal (100%) were COD_o = 4300 mg l^{-1}, and A/Q = 129 m^2 d m^{-3} for the feed 4-CP of 211 mg l^{-1}. Percent 4-CP removal also increased with increasing A/Q ratio and the feed COD content due to high biomass concentrations at high feed COD and A/Q ratios. High feed COD and high A/Q ratios were required for high 4-CP removals at high feed 4-CP contents. Percent toxicity removals from the feed wastewater also increased with increasing A/Q ratio because of high biofilm surface area at high A/Q ratios. The optimal operating conditions for RTBR (A/Q ratio and feed COD) varied depending on the feed 4-CP concentration. For the highest feed 4-CP concentration tested (1000 mg l^{-1}), the A/Q ratio of 186 m^2 d m^{-3} and the feed COD of 6000 mg l^{-1} are required for more than 95% COD, 4-CP and toxicity removals. Feed COD content of 6,000 mg l^{-1} and high A/Q ratio of 186 m^2 d m^{-3} resulted in nearly 99% toxicity removal.

The most favorable conditions maximizing COD removal efficiency for the RBBR was obtained with a feed COD of 3800 mgl^{-1} and A/Q ratio of nearly 167 m^2 d m^{-3} when the feed 4- CP concentration was lower than 122 mg l^{-1}. 4-CP contents lower than 205 mg l^{-1} and high A/Q ratios above 170 m^2 d m^{-3} were found to be more

favorable for high percent 4-CP and toxicity removals at feed COD of nearly 5170 mg l^{-1}. In order to obtain complete toxicity removal, the system should be operated at optimum A/Q ratio of nearly 183 m^2 d m^{-3}. Optimum conditions resulting in maximum COD, 4-CP and toxicity removals were found to be A/Q ratio of nearly 180 m^2 d m^{-3}, feed COD of nearly 4,000 mg l^{-1} and feed 4-CP of lower than 205 mg l^{-1}.

The RTBR was found to be more effective in 4-CP and toxicity removal as compared to RBBR because of formation of thicker and denser biofilms on the tube surfaces yielding high biomass content in the system. The RTBR was proven to be very effective in removing high concentrations of 4-CP with relatively low A/Q ratios due to thicker biofilm formation as compared to the RBBR.

A similar approach was used in biological treatment of DCP containing wastewater. Increasing feed DCP contents adversely affected the COD removals because of toxic effects of DCP on the microorganisms. Percent COD removals increased with increasing A/Q ratio upto 200 m^2 d m^{-3} for RTBR. The most favorable conditions maximizing percent COD removal (98%) were COD_o = 2000 mg l^{-1} , DCP_o =50 mg l^{-1} and A/Q = 200 m^2 d m^{-3}. Percent DCP removal also increased with increasing A/Q ratio upto 200 m^2 d m^{-3}. Decreasing feed COD contents caused increases in percent DCP removals due to essential use of DCP in the absence of sufficient carbonaceous compounds. Low feed COD and DCP contents and high A/Q ratios were found to be more favorable for high percent DCP removals. Feed DCP content of 50 mg l^{-1} and A/Q ratio of 200 m^2 d m^{-3} resulted in nearly complete removal of DCP. Percent toxicity removals from the feed wastewater also increased with increasing A/Q ratio because of high biofilm surface area at high A/Q ratios. Low feed COD content of 2000 mg $l^{-1}$and high A/Q ratio of 200 m^2 d m^{-3} resulted in maximum toxicity removal of nearly 100% in RTBR.

The optimum A/Q ratio for RBBR resulting in the highest COD (90%), DCP (100%) and toxicity (100%) removals with the highest feed COD (6000 mg l^{-1}) and DCP (500 mg l^{-1}) contents was nearly 210 m^2 d m^{-3}. Percent COD removals decreased with increasing feed DCP due to toxic effects of DCP on the organisms, but increased with increasing A/Q ratio due to high concentrations of biofilm

organisms at high A/Q ratios. Percent DCP and toxicity removals also increased with increasing A/Q ratio and decreasing feed DCP concentrations.

Most of the literature studies on 2,4-DCP biodegradation considered 2,4-DCP concentrations lower than 200 mg l^{-1}. We were able to obtain almost complete 2,4-DCP removals with a high feed 2,4-DCP content of 500 mg l^{-1} due to high biomass concentration obtained in the rotating tubes biofilm reactor. RTBR was shown to be more effective in COD and DCP removals as compared to the RBBR due to formation of highly dense biofilm on the tube surfaces providing high biomass concentrations in the reactor.

The same experimental conditions were used for TCP removal by using the RTBR and RBBR. TCP and COD removals increased with increasing A/Q ratio and decreasing TCP concentration in RTBR. High A/Q ratios (> 120 m^2 d m^{-3}) and feed COD concentrations (3000 mg l^{-1}) must be used in order to obtain high removal efficiencies at high feed TCP contents (> 200 mg L^{-1}). For the feed TCP content of 400 mg L^{-1}, the optimal operating conditions maximizing COD (99%), TCP(100%) and toxicity (93%) removals were COD_o of 3000 mg L^{-1} and A/Q ratio of nearly 165 m^2 d m^{-3} in the RTBR. Percent toxicity removals were always less than TCP removals indicating presence of other toxic compounds or formation of some toxic intermediates during TCP biodegradation.

Similar trends were observed with the RBBR for TCP removal as that of the RTBR. The optimal operating conditions maximizing COD, TCP and toxicity removals for RBBR were the feed COD of 4000 mg l^{-1} and A/Q ratio of nearly 194 m^2 d m^{-3} when feed TCP was below 400 mg l^{-1}. At he highest TCP concentration of 400 mg l^{-1}, 95 % COD, 100 % TCP and 99% toxicity removals were obtained with a feed COD of 3800 mg l^{-1} and A/Q ratio of 256 m^2 d m^{-3}. RBBR required larger biofilm surface area as compared to the RTBR for the same degree of COD and TCP removals due to formation of thinner biofilms on the brush surfaces as compared to thicker biofilms on the tube surfaces in the RTBR.

Operation at high A/Q ratios above 120 m^2 d m^{-3} and 190 m^2 d m^{-3} resulted in high biomass concentrations, eliminated TCP and toxicity from the effluent for

RTBR and RBBR, respectively. Percent COD removal with RTBR was significantly larger than that of the RBBR at the same A/Q ratio. At a feed 2,4,6-TCP concentration of 400 mg l^{-1}, percent 2,4,6-TCP removals with the RTBR were above 51% whereas TCP removals were around 26% with the RBBR at A/Q ratio of 50 m^2 d m^{-3}. At a feed 2,4,6-TCP concentration of 500 mg l^{-1}, percent COD removals with the RTBR were above 81% whereas percent COD removals were around 73 % with the RBBR at A/Q ratio of 50 m^2 d m^{-3}.

In general, both reactors were found to be very effective in removing chlorophenols over a large range of operating conditions. However, RTBR seemed to be more effective for removal of COD and chlorophenols when operated under the same A/Q ratio due to formation of thicker and denser biofilms on the tube surfaces. High chlorophenol concentrations in the feed caused inhibitions on the biofilm microorganisms. Therefore, the system should be operated at high A/Q ratio and feed COD contents (> 3000 mg l^{-1}) when the feed chlorophenol contents were high, in order to obtain high COD, chlorophenol and toxicity removals. High COD loadings also yielded high biomass contents in the reactors resulting in more effective chlorophenol and toxicity removals up to a certain COD loading which results in the optimum biofilm thickness. However, further increases above the optimum COD loadings yield unfavorably thick biofilms causing DO/substrate limitations within the thick biofilms and therefore reducing chlorophenol and COD removals. The optimal operating conditions vary depending on the feed chlorophenol concentrations, which can be determined by using the response functions with the determined coefficients and a proper optimization program.

A mathematical model was developed describing the COD and chlorophenol removals in the RTBR and the RBBR. Two sets of experiments with variable A/Q ratio and feed DCP concentration were performed to estimate the kinetic constants of the mathematical model by using the RTBR. Effects of important operating variables such as A/Q ratio and the feed DCP or feed DCP/COD ratio on COD, DCP and toxicity removals were investigated. Percent COD, DCP and toxicity removals increased with increasing A/Q ratio. An A/Q ratio of 130 m^2.d m^{-3} was found to be satisfactory for nearly complete removal of COD, DCP and toxicity when the feed

COD and DCP were 5000 ± 200 mg l^{-1} and 100 ± 5 mg l^{-1}, respectively. Percent DCP and toxicity removals were 100% up to feed DCP content of 300 mg l^{-1}, which decreased slightly to 97% for the feed DCP of 393 mg l^{-1} indicating that the system was very effective for DCP and toxicity removals within the feed DCP range tested. However, percent COD removal decreased with the feed DCP content almost linearly probably due to toxic effects of DCP on the microorganisms. Percent COD removal was above 90% for the feed DCP of 100 mg l^{-1}, which decreased to 64% for the feed DCP of 393 mg l^{-1}. The experimental data obtained with those experiments were used for determination of the kinetic constants by iteration. Good agreement between the model predictions and the experimental data indicated validity of the model for the system used.

There are rather limited number of literature reports available on treatment of chlorinated compounds by using continuous biofilm reactors where chlorophenol concentrations are lower than 200 mg l^{-1}. Furthermore, no data on toxicity removals were reported in the literature on chlorophenol degradations. Our studies covered a large range of chlorophenol concentrations, A/Q ratios and feed COD contents and reported considerable toxicity removals in the RTBR and the RBBR. Percent chlorophenol removals obtained in our study are considerably higher than those reported in literature since highly dense and well-aerated biofilm reactors were used in this study as compared to literature studies. High removal efficiencies obtained with the RTBR and RBBR over a large range of chlorophenol loading rates indicated the fact that the reactors were resistant to shock loadings.

Recommendations for future studies

Some of the recommendations for the future studies using the same reactors are summarized below:

- The performance of the reactors can be investigated by using industrial wastewater containing different chlorophenols.
- Multistage systems can be used to improve the performance of the system for chlorophenol removal
- Step feeding can be applied in order to improve the performance of the reactors
- A mixture of chlorophenols may be used in the feed wastewater and the removals may be investigated
- Special bacterial cultures capable of effective degradation of chlorophenols may be supplemented to improve the system performance
- Microbiological analysis of the biofilm cultures and the degradation intermediates may be investigated to provide better understanding of the chlorophenol degradation
- The reactors may be used for treatment of wastewaters containing other toxic compounds such as benzene-toluene-xylene (BTX)
- The reactors may be used for nitrification and denitrification of toxic compound containing wastewaters
- Nutrient removal (COD, N, P) from high N and P containing wastewaters may be studied using the RTBR and the RBBR

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APPENDICES

RAW EXPERIMENTAL DATA AND FIGURES

A.1 Raw Data for 4-Chlorophenol Removal Experiments

A.1.1 Raw Data for removal of 4-chlorophenol by using Rotating Perforated Tubes Biofilm Reactor

Table A.1 Raw data for Box-Wilson experimental points and 4-CP, COD and toxicity removal by using RTBR

Run	Experimental Data Points				4-Chlorophenol					COD					Toxicity				
	$4\text{-}CP_o$	COD_o	HRT	A/Q	$4\text{-}CP_o$	$4\text{-}CP_{e1}$	$4\text{-}CP_{e2}$	E_1	E_2	COD_o	COD_{e1}	COD_{e2}	E_1	E_2	TOX_o	TOX_{e1}	TOX_{e2}	E_1	E_2
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%
A1	0	4000	25	116	0	0	0	-	-	4210	90	70	98	98	0	0	0	-	-
A2	1000	4000	25	116	1061	137	129	87	88	4110	633	540	85	87	114	24	17	79	85
A3	500	2000	25	116	487	25	22	95	95	1920	219	169	89	91	61	4	1	93	98
A4	500	6000	25	116	488	22	20	95	96	5900	1130	959	81	84	44	3	1	93	98
A5	500	4000	10	47	510	351	344	31	33	4100	1430	1390	65	66	57	33	29	42	49
A6	500	4000	40	186	491	19	16	96	97	4120	650	270	84	93	54	4	1	93	98
F1	789	5156	34	158	801	48	38	94	95	5210	545	462	90	91	63	7	3	89	95
F2	789	5156	16	74	790	436	433	45	45	5065	2000	1830	61	64	68	37	33	46	51
F3	789	2844	34	158	796	34	22	96	97	2770	288	202	90	93	69	13	8	81	88
F4	789	2844	16	74	780	489	471	37	40	2965	880	801	70	73	77	49	43	36	44
F5	211	5156	34	158	200	11	7	95	97	5200	210	149	96	97	26	5	1	81	96
F6	211	5156	16	74	210	55	47	74	78	5177	520	389	90	92	29	13	13	55	55
F7	211	2844	34	158	198	9	8	95	96	2780	319	200	89	93	32	1	1	97	97
F8	211	2844	16	74	217	55	55	75	75	2800	660	198	76	93	27	9	9	67	67
C	500	4000	25	116	505	45	33	91	93	3990	543	346	86	91	46	6	3	87	93
C	500	4000	25	116	511	39	20	92	96	3870	305	290	92	93	49	5	4	90	92
C	500	4000	25	116	509	24	20	95	96	3800	290	245	92	94	50	5	2	90	96
C	500	4000	25	116	490	30	24	94	95	4090	291	260	93	94	50	4	4	92	92

A.1.2 Raw Data for removal of 4-chlorophenol by using Rotating Brush Biofilm Reactor

Table A.2 Raw data for Box-Wilson experimental points and 4-CP, COD and toxicity removal results by using RBBR

Run	Experimental Data Points				4-Chlorophenol					COD					Toxicity				
	$4\text{-}CP_o$	COD_o	HRT	A/Q	$4\text{-}CP_o$	$4\text{-}CP_{e1}$	$4\text{-}CP_{e2}$	E_1	E_2	COD_o	COD_{e1}	COD_{e2}	E_1	E_2	TOX_o	TOX_{e1}	TOX_{e2}	E_1	E_2
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%
A1	0	4000	25	183	0	0	0	-	-	3930	110	110	97	97	0	0	0	-	-
A2	1000	4000	25	183	989	75	71	92	93	4000	461	398	88	90	99	11	11	88	89
A3	500	2000	25	183	502	11	11	98	98	2141	193	151	91	93	52	5	5	90	90
A4	500	6000	25	183	500	21	20	96	96	6070	1637	1434	73	76	48	5	5	90	90
A5	500	4000	10	73	505	365	355	28	30	3984	1400	1330	65	67	51	39	39	22	23
A6	500	4000	40	293	488	15	18	97	96	3980	695	151	83	96	49	6	6	88	88
F1	789	5156	34	249	774	16	15	98	98	5100	399	275	92	95	76	6	6	93	93
F2	789	5156	16	117	769	465	458	40	40	5415	2176	1911	60	65	76	50	48	34	36
F3	789	2844	34	249	770	8	8	99	99	2930	250	195	91	93	78	5	5	94	94
F4	789	2844	16	117	800	501	488	37	39	2881	987	854	66	70	81	53	52	34	36
F5	211	5156	34	249	206	8	8	96	96	5070	200	200	96	96	20	5	5	75	75
F6	211	5156	16	117	226	42	42	81	81	5000	531	404	89	92	22	8	8	63	63
F7	211	2844	34	249	210	4	4	98	98	2900	290	160	90	94	22	5	5	80	79
F8	211	2844	16	117	228	67	67	71	71	2827	700	240	75	92	24	11	11	55	55
C	500	4000	25	183	496	27	25	95	95	4200	994	365	76	91	50	7	7	87	87
C	500	4000	25	183	522	33	10	94	98	4000	225	149	94	96	52	7	5	86	89
C	500	4000	25	183	522	27	11	95	98	4000	233	155	94	96	52	7	5	87	89
C	500	4000	25	183	522	38	10	93	98	4000	254	148	94	96	52	8	5	85	89

A.2 Raw Data for 2,4-Dichlorophenol Removal Experiments

A.2.1 Raw Data for removal of 2,4-dichlorophenol by using Rotating Perforated Tubes Biofilm Reactor

Table A.3 Raw data for Box-Wilson experimental points and 2,4-DCP, COD and toxicity removal by using RTBR

Run	Experimental Data Points				2,4-Dichlorophenol					COD					Toxicity				
	DCPo	COD_o	HRT	A/Q	DCPo	DCPe1	DCPe2	E_1	E_2	COD_o	COD_{e1}	COD_{e2}	E_1	E_2	TOX_o	TOX_{e1}	TOX_{e2}	E_1	E_2
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%
A1	50	4000	25	155	55	-	1	-	99	4306	-	258	-	94	23	-	15	-	33
A2	500	4000	25	155	495	-	0	-	100	3978	-	358	-	91	98	-	87	-	11
A3	275	2000	25	155	232	-	0	-	100	2080	-	166	-	92	47	-	6	-	87
A4	275	6000	25	155	264	-	3	-	99	6020	-	421	-	93	76	-	37	-	51
A5	275	4000	10	62	266	-	74	-	72	4130	-	867	-	79	79	-	37	-	53
A6	275	4000	40	248	237	-	2	-	99	4200	-	378	-	91	73	-	11	-	85
F1	405	5156	34	209	400	-	4	-	99	5251	-	525	-	90	60	-	13	-	78
F2	405	5156	16	101	395	-	12	-	97	5160	-	619	-	88	50	-	3	-	94
F3	405	2844	34	209	395	-	8	-	98	2900	-	261	-	91	73	-	12	-	83
F4	405	2844	16	101	405	-	0	-	100	2785	-	306	-	89	100	-	66	-	34
F5	145	5156	34	209	130	-	3	-	98	5155	-	258	-	95	35	-	12	-	65
F6	145	5156	16	101	134	-	1	-	99	5027	-	452	-	91	31	-	19	-	39
F7	145	2844	34	209	132	-	0	-	100	3200	-	224	-	93	43	-	16	-	63
F8	145	2844	16	101	143	-	3	-	98	2815	-	197	-	93	46	-	27	-	41
C	275	4000	25	155	281	-	14	-	95	4242	-	339	-	92	74	-	21	-	71

A.2.2 Raw Data for removal of 2,4-dichlorophenol by using Rotating Brush Biofilm Reactor

Table A.4 Raw data for Box-Wilson experimental points and 2,4-DCP, COD and toxicity removal results by using RBBR

Run	Experimental Data Points				2,4-Dichlorophenol					COD					Toxicity				
	DCP_o	COD_o	HRT	A/Q	DCP_o	DCP_{e1}	DCP_{e2}	E_1	E_2	COD_o	COD_{e1}	COD_{e2}	E_1	E_2	TOX_o	TOX_{e1}	TOX_{e2}	E_1	E_2
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%
A1	50	4000	25	183	50	1	1	99	100	4059	556	284	86	93	15	1	0	93	100
A2	500	4000	25	183	491	9	1	98	100	4157	557	457	87	89	80	9	2	89	97
A3	275	2000	25	183	271	7	3	97	99	1968	197	138	90	93	56	10	2	82	96
A4	275	6000	25	183	288	7	3	97	99	5930	440	237	93	96	50	6	2	89	96
A5	275	4000	10	73	290	57	38	80	87	4130	841	578	80	86	58	23	18	61	70
A6	275	4000	40	293	275	1	11	100	96	3907	439	391	89	90	48	1	1	97	97
F1	405	5156	34	249	401	7	4	98	99	5206	562	416	89	92	71	9	1	87	99
F2	405	5156	16	117	395	18	12	95	97	5294	637	582	88	89	68	1	2	99	97
F3	405	2844	34	249	400	17	12	96	97	2930	302	205	90	93	71	12	10	83	86
F4	405	2844	16	117	390	25	20	94	95	2960	415	326	86	89	65	5	3	92	96
F5	145	5156	34	249	143	1	1	99	99	5100	479	459	91	91	30	1	1	97	97
F6	145	5156	16	117	153	3	5	98	97	4965	497	348	90	93	35	1	1	97	96
F7	145	2844	34	249	140	1	1	99	99	2877	368	316	87	89	36	1	1	97	97
F8	145	2844	16	117	139	5	4	97	97	3000	281	240	91	92	34	1	1	96	96
C	275	4000	25	183	260	13	8	95	97	4110	400	247	90	94	53	4	1	92	98

A.3 Raw Data for 2,4,6-Trichlorophenol Removal Experiments

A.3.1 Raw Data for removal of 2,4,6-trichlorophenol by using Rotating Perforated Tubes Biofilm Reactor

Table A.5 Raw data for Box-Wilson experimental points and 2,4,6-TCP, COD and toxicity removal by using RTBR

Run	Experimental Data Points				2,4,6-Trichlorophenol					COD					Toxicity				
	TCP_o	COD_o	HRT	A/Q	TCP_o	TCP_{e1}	TCP_{e2}	E_1	E_2	COD_o	COD_{e1}	COD_{e2}	E_1	E_2	TOX_o	TOX_{e1}	TOX_{e2}	E_1	E_2
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%
1	0	4000	20	93	0	0	0	-	-	3945	510	355	87	91	0	0	0	-	-
2	200	2500	20	93	230	26	17	89	93	2486	313	180	87	93	34	16	10	53	71
3	0	2500	35	163	0	0	0	-	-	2720	267	260	90	90	0	0	0	-	-
4	400	4000	20	93	400	93	82	77	80	4600	989	663	79	86	71	35	30	51	58
5	200	4000	35	163	202	16	10	92	95	4090	486	310	88	92	44	13	11	70	75
6	200	2500	20	93	191	21	10	89	95	2290	161	111	93	95	50	13	10	74	80
7	200	1000	5	23	195	190	175	3	10	1170	401	266	66	77	51	51	46,6	0	9
8	200	4000	5	23	222	166	144	25	35	4100	1565	1110	62	73	52	44	40	15	23
9	0	2500	5	23	0	0	0	-	-	2870	311	287	89	90	0	0	0	-	-
10	400	2500	5	23	404	331	303	18	25	2651	1010	790	62	70	72	70	70	3	3
11	0	1000	20	93	0	0	0	-	-	980	253	203	74	79	0	0	0	-	-
12	400	1000	20	93	400	222	206	45	49	1000	334	197	67	80	77	48	48	38	38
13	200	2500	20	93	200	5	3	98	99	2730	210	130	92	95	50	1	1	98	98
14	400	2500	35	163	400	25	19	94	95	2600	127	76	95	97	80	9	9	89	89
15	200	1000	35	163	211	88	77	58	64	1165	309	267	73	77	50	27	23	46	54
16	200	2500	20	93	186	10	6	95	97	2600	280	177	89	93	45	6	6	87	87
17	200	2500	20	93	195	22	14	89	93	2390	176	118	93	95	49	11	10	78	80

A.3.2 Raw Data for removal of 2,4,6-trichlorophenol by using Rotating Brush Biofilm Reactor

Table A.6 Raw data for Box-Wilson experimental points and 2,4,6-TCP, COD and toxicity removal results by using RBBR

Run	Experimental Data Points				2,4,6-Trichlorophenol					COD					Toxicity				
	TCP_o	COD_o	HRT	A/Q	TCP_o	TCP_{e1}	TCP_{e2}	E_1	E_2	COD_o	COD_{e1}	COD_{e2}	E_1	E_2	TOX_o	TOX_{e1}	TOX_{e2}	E_1	E_2
	mg l^{-1}	mg l^{-1}	h	m^2 d m^{-3}	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%	mg l^{-1}	mg l^{-1}	mg l^{-1}	%	%
1	0	4000	20	147	0	0	0	-	-	3920	190	190	95	95	0	0	0	-	-
2	200	2500	20	147	183	22	14	88	92	2486	287	127	88	95	29	5	5	83	83
3	0	2500	35	256	0	0	0	-	-	2500	69	59	97	98	0	0	0	-	-
4	400	4000	20	147	387	103	94	73	76	4290	1480	935	66	78	69	31	22	55	68
5	200	4000	35	256	190	14	9	93	95	3986	370	197	91	95	37	7	6	81	84
6	200	2500	20	147	201	12	10	94	95	2510	157	99	94	96	44	6	6	86	86
7	200	1000	5	37	210	189	171	10	19	1399	466	312	67	78	44	42	39	5	11
8	200	4000	5	37	216	167	149	23	31	4160	1520	1016	63	76	42	40	34	5	19
9	0	2500	5	37	0	0	0	-	-	2610	320	321	88	88	0	0	0	-	-
10	400	2500	5	37	388	330	319	15	18	2557	901	810	65	68	71	68	66	4	7
11	0	1000	20	147	0	0	0	-	-	950	88	88	91	91	0	0	0	-	-
12	400	1000	20	147	426	251	229	41	46	1100	300	201	73	82	79	46	44	42	44
13	200	2500	20	147	204	4	2	98	99	2428	132	80	95	97	45	1	1	98	98
14	400	2500	35	256	400	10	9	98	98	2600	45	28	98	99	76	3	3	96	96
15	200	1000	35	256	194	87	87	55	55	970	276	241	72	75	41	15	14	63	66
16	200	2500	20	147	191	19	16	90	92	2452	173	133	93	95	36	6	6	83	83
17	200	2500	20	147	204	23	17	89	92	2410	188	116	92	95	40	7	7	83	83

A.4 Raw Data for 2,4-Dichlorophenol Removal Experiments for determination of kinetic constants using the RTBR

Table A.7 Raw data for 2,4-DCP, COD and toxicity removal results by using RTBR

Run			2,4-Dichlorophenol					COD					Toxicity				
	HRT (h)	A/Q	DCP_o	DCP_{e1}	DCP_{e2}	E_1	E_2	COD_o	COD_{e1}	COD_{e2}	E_1	E_2	TOX_o	TOX_{e1}	TOX_{e2}	E_1	E_2
1	35	217	103	-	0.0	-	100	4950	-	70	-	99	32	-	0.0	-	100
2	25	155	104	-	0.0	-	100	5230	-	132	-	97	35	-	0.0	-	100
3	21	130	94	-	2.0	-	98	4779	-	161	-	97	27	-	1.0	-	96
4	15	93	95	-	3.0	-	97	4770	-	542	-	89	36	-	2.0	-	94
5	11	68	93	-	4.0	-	96	4910	-	1525	-	69	33	-	2.0	-	94
6	5	31	90	-	49.0	-	46	5246	-	2046	-	61	31	-	8.0	-	74
8	15	93	0	0.0	0.0	100	100	5055	311	167	94	97	0	0	0.0	100	100
9	15	93	50	1.5	0.0	97	100	5130	790	260	85	95	11	0.0	0.0	100	100
10	15	93	98	2.0	0.0	98	100	4900	810	440	83	91	28	0.0	0.0	100	100
11	15	93	149	3.0	0.0	98	100	5400	1370	990	75	82	36	3.0	0.0	92	100
12	15	93	193	2.0	0.0	99	100	5340	1900	1100	64	79	41	7.0	0.0	83	100
13	15	93	260	3.0	0.0	99	100	5290	2200	1370	58	74	54	9.0	0.0	83	100
14	15	93	297	5.0	2.0	98	99	5100	2330	1430	54	72	55	8.0	0.0	85	100
15	15	93	343	7.0	3.0	98	99	5300	1990	1815	62	66	61	12.0	3.0	80	95
16	15	93	393	16.0	10.0	96	97	5330	2100	1939	61	64	69	16.0	4.0	77	94