TOXICITY AND TREATABILITY OF CHLOROPHENOLS UNDER AEROBIC AND ANOXIC CONDITIONS

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ABSTRACT

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Toxicity and treatability of chlorophenols (2-CP, 4-CP and 2,4-DCP) were investigated both under aerobic and anoxic conditions using batch and aerobic fed-batch reactors. Toxicity and treatability of 2-CP was studied in aerobic batch reactors using only unacclimated culture due to observed low toxic effect on unacclimated activated sludge culture, whereas, toxicity and treatability of 4-CP and 2,4-DCP were studied using both acclimated and unacclimated cultures. Results showed that 2,4-DCP is the most toxic, and 2-CP is the least toxic compound among the examined chlorophenols. It was observed that there was a remarkable decrease in the toxic effects of chlorophenols on the microorganisms when acclimated to chlorophenols. Aerobic batch reactor experiments showed that although no removal of chlorophenol even at low concentrations was observed when unacclimated culture was used, complete removal of 4-CP and 2,4-DCP were

observed even at the concentrations of 300 and 150 mg/L, respectively with acclimated culture.

During the acclimation of culture to 4-CP and 2,4-DCP in aerobic fed-batch reactors, removals of those were also followed at two different sludge retention time (SRT) values, namely, 8 and 15 days. Concentration of chlorophenols in the influents of reactors was increased in stepwise small increments to allow acclimitization of microorganisms. Results showed that 4-CP and 2,4-DCP could be degraded completely even at the concentrations of 135 and 75 mg/L, respectively.

In addition to treatability studies in the presence of peptone as readily degradable substrate, treatability of 4-CP and 2,4-DCP as sole carbon and energy source were studied in aerobic batch and sequencing batch reactors (SBR). In batch reactors, 4-CP and 2,4-DCP could be used as sole organic carbon source by the chlorophenol acclimated microorganisms up to concentrations of 200 and 51 mg/L, respectively. Aerobic SBRs showed excellent performance in the removal of 4-CP and 2,4-DCP and complete removal could be achieved up to concentrations of 547 and 157 mg/L, respectively.

Anoxic toxicity studies showed that chlorophenols even at low concentrations could adversely affect the nitrate uptake rate and chemical oxygen demand (COD) removal efficiency under anoxic conditions and it was observed that chlorophenols are more toxic under anoxic conditions compared to aerobic conditions.

Keywords: Chlorophenols, Activated Sludge, Toxicity, Biodegradation

ÖZ

KLOROFENOLLERİN AEROBİK VE ANOKSİK KOŞULLAR ALTINDA TOKSİSİTESİ VE ARITILABİLİRLİĞİ

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Klorofenollerin (2-CP, 4-CP ve 2,4-DCP) toksisitesi ve arıtılabilirliliği hem aerobik, hem de anoksik koşullar altında kesikli ve aerobik kesikli-beslemeli reaktörler kullanılarak araştırılmıştır. 2-CP'ün toksisitesi ve arıtılabilirliliği aklime edilmemiş kültür üzerine bulunan düşük toksik etkiden dolayı, aerobik kesikli reaktörlerde sadece aklime edilmemiş kültürle çalışılmasına rağmen; 4-CP ve 2,4-DCP'nin toksisite ve arıtılabilirlikleri hem aklime edilmiş, hem de aklime edilmemiş kültürle çalışılmıştır. Sonuçlar, incelenen klorofenoller içinde 2,4-DCP'nin en toksik ve 2-CP'ün en az toksik madde olduğunu göstermiştir. Mikroorganizmalar klorofenollere aklime edildiklerinde klorofenollerin toksik etkilerinde önemli bir azalma olduğu gözlenmiştir. Aerobik kesikli reaktör deneyleri aklime edilmemiş kültür kullanıldığında, düşük konsantrasyonlarda klorofenol arıtımı gözlenmemesine rağmen, aklime edilmiş kültürle 4-CP ve 2,4-

DCP'nin, sırasıyla 300 mg/L ve 150 mg/L konsantrasyonlarında bile tamamen giderildiği gözlenmiştir.

Aerobik kesikli-beslemeli reaktörlerde kültürin 4-CP ve 2,4-DCP'ye aklimasyonu süresince iki farklı çamur bekletme zamanında, 8 ve 15 gün, arıtımları da izlenmiştir. Reaktörlerin girişlerinde klorofenol konsantrasyonları küçük artışlarla kademeli olarak arttırılmıştır. Sonuçlar, 4-CP ve 2,4-DCP'nin sırasıyla 135 ve 75 mg/L konsantrasyonlarda dahi tamamen arıtılabileğini göstermiştir.

Kolay ayrışabilir besi maddesi olarak peptonun varlığında sürdürülen arıtılabilirlik çalışmalarına ilaveten, 4-CP ve 2,4-DCP'nin tek karbon ve enerji kaynağı olarak arıtılabilirlikleri aerobik kesikli ve ardışık kesikli reaktörler (AKR) kullanılarak da çalışılmıştır. Kesikli reaktörlerde, 4-CP ve 2,4-DCP tek organik karbon kaynağı olarak klorofenollere aklime edilmiş mikroorganizmalar tarafından sırasıyla 200 ve 51 mg/L'ye kadar kullanılabilmiştir. Aerobik AKR'ler 4-CP ve 2,4-DCP arıtımında çok iyi performans göstermiş ve sırasıyla 547 ve 157 mg/L'ye kadar tam arıtım başarılmıştır.

Anoksik toksisite çalışmaları, klorofenollerin düşük konsantrasyonlarda dahi nitrat kullanım hızını ve KOİ (Kimyasal oksijen ihtiyacı) giderim verimini anoksik koşullar altında olumsuz olarak etkilediğini göstermiş ve klorofenollerin anoksik koşullar altında, aerobik koşullara kıyasla daha toksik olduğunu göstermiştir.

Anahtar Kelimeler: Klorofenoller, Aktif Çamur, Toksisite, Biyolojik parçalanma

To Yücehan and Şenay

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CHAPTER 1

INTRODUCTION

1.1 General

The development of industrial and agricultural human activities requires molecules not necessarily available in the nature. This lead to the synthesis of new organic compounds known as xenobiotics (Lora *et al.*, 2000). Chlorophenols are one of the types of these chemicals and widely used as biocides for wood preservation. In addition to chlorophenol manufacturing and utilization, chlorination of phenolic compounds may take place in bleaching of pulp, water disinfection, or combustion of organic waste leading to water and soil contamination (Puhakka *et al.*, 1992). Ground water contamination figures may vary from 150 μg/L (Valo *et al.*, 1990) to 100-200 mg/L (Ettala *et al.*, 1992). Chlorophenols are recalcitrant to biodegradation and therefore persistent in nature and their discharge to environment is of great concern because of their toxicity and suspected carcinogenicity (Keith *et al.*, 1979). Also, they are included in the U.S. Environmental Protection Agency Pollutant List (Zhang and Wiegel, 1989).

In literature, there are several studies about the aerobic and anaerobic treatability of chlorophenols and different results have been reported depending on the type of chlorophenols, electron acceptors, co-substrates, reactor configuration and concentration of chlorophenols (Table 1.1). As seen from Table 1.1, degradation at

high concentrations have been investigated in advanced types of reactors, such as, fluidized bed reactors, up-flow aerobic and anaerobic filters; there is lack of information on the treatment of chlorophenols at high concentrations using activated sludge process, which is one of the most commonly used system in treatment of municipal and industrial wastewaters.

Also, chlorophenols, when received by biological treatment processes, exert certain toxic effects. Understanding of these effects could, in fact, help greatly understanding their removal within the treatment processes. The toxic effects of chlorophenols on the activated sludge have been studied in terms of respirometric oxygen uptake (OUR) (Chan *et al.,* 1999; Kong *et al.,* 1993 and 1996) and sludge activity (Vallecilo A. *et al.,* 2000). On the other hand, there are some evidence in the literature that oxygen consumption may not be correlated with the microbial growth (Chang *et al.,* 1993; Neijsel, 1976). Also, generally unacclimated culture has been used in the determination of toxicity of chlorophenols. Therefore, toxicity values determined using respirometer may not reflect the real situation and could be one of the reasons of having different toxicity values with different methods (Table 1.2). Moreover, toxicity studies should include the effects of chlorophenols not only on OUR, but also on some other parameters, such as maximum specific growth rate (μ_m), chemical oxygen demand (COD) removal, yield coefficient (Y) and sludge activity (SA) on the both acclimated and unacclimated cultures, in order to understand the situation in all aspects.

In literature, in addition to aerobic and anaerobic treatability studies on chlorophenols, there are some studies conducted under anoxic conditions (Table 1.1). In these studies, however, not sufficient information is available on the toxic effect of chlorophenols on the nitrate uptake rate and COD removal efficiency when those enter a wastewater treatment plant in which denitrification unit is used to remove nitrate. Stringent requirements on the discharge quality as well as a need for protection of receiving waters required that nutrients should be removed from wastewaters (Chui *et al.,*2001). Therefore, there is a need for understanding effects

of chlorophenols on the nitrogen uptake and COD removal efficiency as well as treatability of chlorophenols under anoxic conditions.

Therefore, a necessity arises to investigate the toxicity and treatability of chlorophenols using both acclimated and unacclimated cultures to evaluate the effect of acclimation on the treatment and toxicity of chlorophenols under aerobic conditions. As explained above, treatability of chlorophenols at high concentrations in the presence and absence of a readily degradable substrate is to be studied using activated sludge culture.

1.2. Objective & Scope

The purposes of this study are

- To investigate the toxicity and treatability of some selected chlorophenols, under aerobic and anoxic conditions.

- To investigate whether a readily degradable substrate is obligatory or only speeds up the treatment of chlorophenols.

For the purposes stated above, toxicity and biodegradability of 2-Chlorophenol (2-CP), 4-Chlorophenol (4-CP) and 2,4-Dichlorophenol (2,4-DCP) were determined using batch and fed-batch reactors under aerobic conditions. Effect of acclimation to 4-CP and 2,4-DCP was also sought. Fed-batch reactors were operated at two different solid retention time (SRT), namely, 8 and 15 days (d) in order to obtain chlorophenol acclimated culture to be used during the batch experiments with acclimated culture. Removal of chlorophenols in fed-batch reactors in the presence of peptone as readily degradable substrate was also followed. Moreover, treatability of 4-CP and 2,4-DCP when present as sole carbon and energy source was investigated in sequencing batch reactors (SBR) to observe any effect of reactor

configuration. In addition to studies under aerobic conditions, batch experiments with 4-CP and 2,4-DCP were repeated with unacclimated culture under anoxic conditions in order to investigate the anoxic treatability of chlorophenols and to observe the effect of chlorophenols on the nitrate uptake rate of denitrifying microorganisms.

Table 1.1. Summary of Studies on Treatabity of Chlorophenols

Type of Chlorophenol	Concentration	Removal Efficiency (%)	Type of Reactor	Reference
2,4,6-TCP as sole carbon and energy source	20 mg/L TOC	82-83	Aerobic Fluidized Bed Reactors with OLR of 260-430 $g/m^3/d$	
2,3,4,6-TeCP and PCP as sole organic carbon and energy source	30 mg/L TOC	99.3 for TeCP 74.5 for PCP	Aerobic Continuous Flow Fluidized Bed Reactor with OLR of 230-400 $g/m^3/d$	Puhakka and Jarvinen 1992
Simulated CP contaminated ground water	15±0.3 mg/L 2,4,6-TCP; 26.3±1.9 mg/L 2,3,4,6-TeCP; 3.8±0.6 mg/L PCP	> 99	Aerobic Continuous Flow Fluidized Bed Reactor with OLR of 217 $g/m^3/d$	
2,3,4,6-TeCP 2,4,6-TCP PCP	40 mg/L 40 mg/L 43 mg/L	99.6 98.4 83.5	Aerobic Continuous Flow Fluidized Bed Reactor with OLR of 350-480 mg/L/d	Puhakka *et al.*, 1994

Table 1.1 continued

4-CP	36	>99.9		
2,4-DCP	45	>99.9		
2,6-DCP	45	>99.9		
2,5-DCP	29	2		
3,5-DCP	45, 23	1		
2,4,6-TCP	54	>99		
2,3,4,6-TeCP	64	>99.3	Aerobic Fluidized Bed Treatment	Puhakka *et al.*, 1995
PCP	58	>99.5		
Mixtures of				
2,4-DCP	9.1	>99		
2,6-DCP	8.5	>99		
2,4,6-TCP	11.2	>99		
PCP	14.9	83		
Mixtures of				
2,4,6-TCP	15.1	99.5		
2,3,4,6-TeCP	26.3	99.6		
PCP	3.8	92.5		
4-CP as sole carbon and energy source	35.7 mg/L	85-88	Oxic Fluidized Bed Reactor wit OLR of 0.17-0.86 g/L/d	
2,4-DP as sole carbon and energy source	45.3 mg/L	80-83	Oxic Fluidized Bed Reactor with OLR of 0.22-1.09 g/L/d	Shieh *et al.*, 1990

Table 1.1 continued

Mixtures of CPs	50 mg/L	76-99.5 depending on the percent of dilution	Activated sludge Process	Mc Allister *et al.*, 1993
Mixtures of CPs	4-120µg/L	71	Activated Sludge Process	Ettala *et al.*, 1992
Contaminated ground water	7-11 mg/L 2,4,6-TCP 32-36 mg/L 2,3,4,6-TeCP and 1.8-2.3 mg/L PCP	99.9 at CP loading of 740 mg/L/d 80 at Cp loading rate of 2130 mg/L/d	Aerobic Fluidized Bed	Jarvinen *et al.*, 1994
CP Contaminated Ground water with 2,3,4,6-TeCP; 2,4,6-TCP and PCP	3-130 mg/L	100	Immobilized Rhodococci Polyurethane Carrier With OLR of 74-426 mg/L/d	Valo *et al.*, 1990
2,4,6-TCP	1544.6 µmol/L	100	Activated Sludge Process with OLR of 11.4-mg COD/L/h	Lora *et al.*, 2000

Table 1.1 continued

2,4,6-TCP acetic acid as readily degradable substrate	25 mg/L 100 mg/L TCP	>90 >85	Activated Sludge Process	Vallecillo *et al.*, 2000
2,4-DCP in the presence of phenol as primary substrate	480 mg/L COD (162 mg/L phenol and 140 mg/L 2,4-DCP)	85.9-97 COD 92-99.3 for 2,4-DCP	Sequencing Batch Reactor with and without activated carbon	Ha *et al.*, 2000
	560 mg/L COD (175 mg/L Phenol and 152 mg/L 2,4-DCP)	83.6-96.8 COD 83.9-98.5 for 2,4-DCP		
Kraft mill effluent containing mixtures of CPs	4-120 ppb	Below the detection limit	Aerobic Treatment Lagoons	Cespedes *et al.*, 1996
Bleached kraft mill effluent containing mixtures of CPs	155-270 µg/L	75	Facultative Stabilization Basin	Hall *et al.*, 1994
		70	Aerobic Stabilization Basin	
		40	Activated Sludge Process	

Table 1.1 Continued

PCP Sugar as a readily degradable substrate	40 mg/L PCP	1 mg/L in the effluent (97.5 removal)	Activated Sludge Process with addition of PCP degrading culture	Edgehill and Finn (1983)
Mixtures of 2-CP, 3-CP and 4-CP	3.3 mg/L of each three monochlorophenol	>99	Non-aerated Fixed Bed Bioreactor H_2O_2 as O_2 source	Schollhorn *et al.*, 1994
2-CP	520 mg/L	97- 58 depending on influent flow rate	Packed Bed Reactor with a Silica Based Porous Support For the Fungus	Lewandowski *et al.*, 1990
2-CP 3-CP 4-CP Phenol as primary substrate	64 mg/L 64 mg/L 64 mg/L	13-28 100 100	Batch Reactors inoculated with yeast, *Rhodotorula Glutinis*	Katayama-Hiyama *et al.*, 1994
4-CP sodium glutamate and phenol as energy and carbon source	200 mg/L	100	Batch Reactors inoculated with *Pseudomonas putida*	Wang *et al.*, 2000

Table 1.1 continued

2-CP	10 mg/L			
3-CP	12 mg/L			
4-CP	11 mg/L	100	Batch Reactors in which sulfate was used as electron acceptor	Liu *et al.*, 1996
2,5-DCP	18 mg/L			
3,5-DCP	23 mg/L			
4-CP	10 mg/L TOC	80		
2,4-DCP	10-20 mg/L TOC	No removal	Anoxic Fluidized Bed Reactor	Puhakka *et al.*, 1992
PCP	20 mg/L TOC	No removal	(nitrate was used as electron acceptor)	
(CPs were used as sole organic carbon source)				

Table 1.1 continued

PCP TCP Trichloroguaiacol	7.1 mg/L 7.1 mg/L 1 mg/L	99 99 95	Anaerobic Fluidized Bed Reactor	Puhakka *et al.*, 1994
PCP 3-CP PCP 2,6-DCP PCP PCP	3.2-8.1 10-20 0.1 82-16 4.5 40-60	35 100-90 95 >90 99 99	Up-flow Anaerobic Sludge Blanket Reactor	Christiansen *et al.*, 1995
2,4,6-TCP	94.9 µM	100	Anaerobic-Aerobic Sequential Batch Reactors	Kafkewitz *et al.*, 1992
2,4,6-TCP 2,4,6-TCP	40-150 µM 66 µM	100/ dechlorination 100	Anaerobic Serum Bottles Two-stage Continuous Anaerobic-aerobic Process	Armenante *et al.*, 1999
2,4-DCP	10,20,40,70 mg/L	98 of DCP dechlorination 70-98 removal in aerobic reactor	Sequential Anaerobic-Aerobic Biofilm Reactor with tire chips as packing materials	Shin *et al.*, 1999

Table 1.2. Summary of Studies on The Toxicity of Chlorophenols

Type of Chlorophenol	Toxicity Value (IC_{50}, mg/L)	Method Applied	Reference
3,5-DCP	11.7	RODTOX (Rapid Oxygen Demand and TOXicity Tester)	Kong *et al.*, 1993
	1.81	Microtox	
Phenol	623.5	Respirometer	Chan *et al.*, 1999
3,5-DCP	48.9	Respirometer	
	2.9	Microtox 15 min.	
	7	BOD (biological oxygen Demand)	
		ISO (A)	
	10	ISO (B)	
	6	OECD	
	20		
PCP	31.2	Respirometer	
	15	BOD	
	21	ISO (A)	
	32	ISO (B)	
	25	OECD	
2-CP	380	OECD	
	118.2	Respirometer	

Table 1.2 continued

PCP	7.5 mg/L caused 67.4% and 58.7% inhibition at 1st and 2nd feed, respectively	% toxicity on Sludge Activity	Vallecillo *et al.*, 2000
2,4,6-TCP	350 mg/L caused 45.3% and 38.8% inhibition at 1st and 2nd feed, respectively.		
2,4-DCP	25 mg/L caused 48.6% and 49.7 % inhibition at 1st and 2nd feed, respectively.		
4-CP	300 mg/L caused 42.7 % and 37.3 % inhibition at 1st and 2nd feed, respectively.		
Phenol	750 mg/L caused 54.8 % and 42.8 % inhibition at 1st and 2nd feed, respectively.		

1.3. Literature Review

Chlorophenols constitute a series of 19 compounds consisting of mono-, di, tri-, tetrachlorophenol (TeCP), and pentachlorophenol (PCP). Chlorophenols with less than three chlorines seem to be of limited use today, PCP and the lower chlorophenols have been used as biocides to control bacteria, fungi, algae, mollusks, insects, slime, and other biota. The use of polychlorinated phenols has been banned or restricted in several countries since the late 1980s, but because of past practices, chlorophenols are widespread in the environment today (Hale *et al.*, 1994).

Properties of some selected compounds, namely, 2-CP, 4-CP and 2,4-DCP are given in Table A.1 (Appendix A).

Potential environmental sources of chlorinated phenols include:

- Direct soil applications as biocides;
- Leaching or vaporizing from treated wood items;
- Synthesis during routine chlorination process of drinking water and wastewater at treatment plants, since both water sources can contain aromatic compounds of natural origin;
- Synthesis during production of bleached pulp in which chlorine is used;
- Releases from factories into air and water;
- Incineration of waste materials and burning of fresh lignocellulosic biomass, e. g. forest fires.

A considerable amount of information is available regarding the stability of chlorinated phenols in the environment. The following generalization can be made:

- Chlophenols are much more environmentally stable than the parent unsubstituted phenol.

- As the number of chlorine substituents increases, the rate of aerobic decomposition decreases, whereas, opposite is generally true for anaerobic decomposition.
- Compounds containing a meta-chlorine (i.e. 3-chloro- or 2,4,5-trichlorophenol) are more persistent under aerobic conditions than compounds lacking a chlorine substituent in meta positions to the hydroxyl group.

Chlorinated phenols may be removed from water body via:

- Volatilization,
- Photodegradation,
- Adsorption onto suspended or bottom sediments, and
- Microbial degradation. (Hale *et al.*, 1994).

It is apparent from the studies on contaminated wood-preserving sites that polychlorinated phenols are persistent in soil and ground water. Self-cleaning systems are slow, and the contamination may thus persist for decades. While degradation of chlorophenols may be slow in the environment, it is, however, possible to isolate microbes capable of utilizing PCP and other types of chlorinated phenols as a source of carbon and energy. In addition to aerobic isolates, obligate anaerobic bacteria that reductively dechlorinate chlorophenols have been isolated.

Because chlorophenols are among the most widely used industrial organic compounds and listed as priority pollutants by the U.S. Environmental Protection Agency (U.S.EPA), reductive dechlorination and toxicity of chlorinated aromatic compounds are of great importance both in natural environment and bioremediation technologies (Jin and Bhattacharya, 1997).

Bacteria use different strategies to degrade chlorophenols:

- Mono- and dichlorophenols are usually degraded aerobically by hydroxylation to chlorocatecols and by a spontaneous dechlorination after ortho-cleavage of the chlorocatecols.
- Thrichloro- and polychlorophenols are degraded aerobically via parahydroquinones, which are subsequently dechlorinated before ring cleavage.
- All chlorophenols (mono- to PCP) are degraded under anaerobic conditions by a variety of microbial communities; degradation is initiated by reductive dechlorination followed by ring cleavage (Hale *et al.,* 1994).

1.3.1. Toxicity Studies for Chlorophenols

With the increased world-wide industrialization, it becomes increasingly important to assess the environmental impact of toxic substances discharged to the environment. Nowadays, activated sludge process is among the most widely used biological wastewater treatment systems in the world. The heterogeneous microbial community present in the sludge allows the system to be flexible, with regard to considerable fluctuations in the incoming wastewater composition. However, this capacity is limited. When the concentration of certain compounds increases, the inhibition threshold concentration may be reached and this can adversely affect the organic and nutrient removal function. Therefore, it is important to screen and monitor on-line the potential toxicity of the influents to the microorganisms prior to their introduction into the biodegradation process (Kong *et al.,* 1993). There are several methods to determine the toxicity of substance. Some of these methods are respiration rate, ATP content, enzymatic activity, bacterial luminescence and substrate uptake rate (Chan *et al.,* 1999).

Kong *et al.* (1993) used RODTOX (Rapid Oxygen Demand and TOXicity tester), an activated sludge-based respirographic biosensor, to find toxicity of some

chemicals on activated sludge and compared this method with Microtox test. The IC_{50} (the concentration that exhibits 50 % inhibition) values using RODTOX and MICROTOX for 3,5-DCP was found as 11.7 and 1.81 mg/L, respectively. This result shows that there is a big difference between IC_{50} values found in two different methods. On the other hand, Chan *et al.* (1999) used respirometer to determine the toxicity of phenolic compounds to activated sludge microorganisms. Results showed that phenol is less toxic than other substituted phenolic compounds and phenol with bromine solution is less toxic than phenol with nitro and chlorine substitution. Moreover, the inhibitory effects of the chlorophenols increase with the degree of chlorination. Chan *et al.* pointed different findings for these types chlorophenol in literature.

Kong *et al.* (1996) used ARIKA (Automated Respiration Inhibition Kinetics Analysis) for simultaneous determination of the inhibitory effect of a toxicant on the degradation of multiple biogenic substrates (C and N) within the period of a working day. Effect of 3,5-Dichloropehnol (3,5-DCP) on C oxidation was reported that at low 3,5-DCP concentrations, μ_m increases with toxicant concentration. They claimed that this may be due to uncoupler effect (increase in oxygen consumption without generation of additional ATP). At higher 3,5-DCP concentrations, μ_m decreases while K_s increases with increasing toxicant concentration. In this study, it was stated that the nitrification process is more sensitive to 3,5-DCP than C-oxidation.

In another study, the toxicity of chlorophenols was determined by comparing the sludge activity (mg COD removed by g of biomass and time) of reactors with different chlorophenol concentration to that of reactor without chlorophenol (Vallecillo *et al.,* 2000). Study showed that after second feed toxicity did not decrease at high rates, which could be due to insufficient acclimation periods. This behavior was different than that was observed under anaerobic conditions in which an important increase on the elimination rate was observed at second feed, however toxicity of chlorophenols was higher under anaerobic conditions (Valecillo *et al.,*

1999). From the obtained results it was concluded that under aerobic conditions, percentage of inhibition increased with increased toxic compound concentration and toxicity increased with the number of chlorine atoms, except for 2,4-DCP, which was more toxic than 2,4,6-TCP; PCP was the most toxic of tested compounds. For PCP, inhibition was higher than 90 % for a concentration of 12.5 mg/L; however, for 4-CP, this inhibition value was obtained at 500 mg/L concentration.

The toxic effects of chlorophenols on the activated sludge have been studied in terms of respirometric oxygen uptake (OUR) (Chan *et. al.,* 1999; Kong *et al.,* 1993 and 1996). On the other hand, there are some evidence in the literature that oxygen consumption may not be correlated with the microbial growth (Chang *et al.,* 1993; Neijsel,1976). Therefore, toxicity values determined using respirometer may not reflect the real situation and could be one of the reasons for having different toxicity values with different methods. In these studies, unacclimated sludge has been used and effect of biomass acclimation on the toxicity of chlorophenols is not well known. Vallecilo *et al.* stated that three is no effect of acclimation on the toxicity of chlorophenols as toxicity values are the same after second feed.

1.3.2. Aerobic and Anoxic Treatment of Chlorophenols

Puhakka and Jarvinen (1992) studied the degradation of polychlorinated phenols in continuous-flow fluidized-bed reactors using pure oxygen for aeration and celite carrier for cell immobilization. High dilution rates and chlorophenols as the only source of carbon and energy were used for maintenance of the mixed biofilm culture. No supplemental carbon or energy source was required for continuous operation and maintenance of the mixed chlorophenol degrading culture and the possibility of o-methylation would be expected to be minimal. Initial concentration of 2,4,6-TCP was set to 20 mg TOC/L. Inorganic chloride (ICI) releases indicated 100 % dechlorination of 2,4,6-TCP in all pseudo-steady-state experiments. TOC removals were 82-83 % for 2,4,6-TCP. The effluent 2,4,6-TCP concentration was

0.1 mg/L or less. These results demonstrated that 2,4,6-TCP was readily biodegradable by the immobilized aerobic microbes. After then the reactor was switched to 2,3,4,6-TeCP solution containing 11 % PCP as impurity. The feed concentration was set at 20 mg TOC/L and fed at 5 h HRT. Then concentration of TeCP was increased to 30 mg TOC/L while HRT remained at 5 h. These feed patterns corresponded to loading rates between 230 and 400 g chlorophenol/m^3/d and mean TOC removals were observed to be 99.3 % for 2,3,4,6-TeCP and 74.5 % for PCP. In this study, treatment of simulated ground water contaminated with chlorophenolic compounds was also investigated and results showed that the mean chlorophenols removals were higher than 90 % when the reactor was fed with a mixture of 15±0.3 mg/L 2,4,6-TCP, 26.3±1.9 mg/L 2,3,4,6-TeCP and 3.8±0.6 mg/L PCP at chlorophenol loading rate of 217g/m^3/d and HRT of 5 h for 5 weeks.

In another study, Puhakka *et al.* (1994) investigated the aerobic and anaerobic biotransformation of several chlorinated, methylated monoaromatic compounds in batch and continuous-flow fluidized bed reactors. They reported that under aerobic condition PCP, 2,3,4,6-TeCP, 2,4,6-TCP, 4,5-DCC (dichlorocatechol) were completely degraded in batch reactors within 8 d. However, methoxylated chlorophenols (dichloroguaiacol (DCG), trichloroguaiacol (TCG), trichlorosyringol (TCS) were recalcitrant towards aerobic degradation, whereas 5,6-dichlorovanilin was partially degraded in aerobic mixture. The addition of tryptone yeast extract did not affect the removal of chlorophenols. Under anaerobic conditions, these compounds were dechlorinated and transformed into aerobically degradable forms. It was postulated that O-methylation products resulting in aerobic conditions may resist to aerobic microbial attack and have a high potential for bioaccumulation. The high rate operation (HRT 5 h) of aerobic fluidized bed reactor resulted in the mean removal of 99.6 % for 2,3,4,6-TeCP, 98.4 % for 2,4,6-TCP and 83.5 % for PCP over a period of 235 d. Initial chlorophenols concentrations in aerobic fluidized bed reactor were 40 mg/L for 2,3,4,6-TeCP, and 2,4,6-TCP, 43 mg/L for PCP.

Similarly, in a study carried with various chlorophenols, Puhakka *et al.* (1995) showed that chlorophenols can be degraded in an aerobic fluidized bed reactor with 1-5 h HRTs and results are summarized in Table 1.1.

The ability of immobilized cells grow under oxic and fluidized-bed conditions to degrade 4-chorophenol and 2,4-dichlorophenol was investigated by Shieh *et al.* (1990). The pseudo-steady-state performance of chlorinated phenol degradation was evaluated under different dilute rate conditions, with chlorophenols as the sole organic carbon sources. Good TOC removal was observed in both cases: 85-88 % for 4-CP and 80-83 % for 2,4-DCP (initial TOC concentration was 20 mg/L). This TOC removal performance remained unchanged despite a five-fold increase in dilution rate. Results indicated that, with feed 4-CP and 2,4-DCP concentrations at 35.7 and 45.3 mg/L, respectively, good and stable removal performance is achievable at empty bed hydraulic retention times as low as 1 h.

Contrary to findings of Puhakka *et al.* (1995); McAllister *et al.* (1993) showed that, dilution of a leachate including 50 mg/L 2,4-DCP and some other phenolic compounds is necessary to achieve a good removal efficiency using activated sludge process. Similarly, Mc Allister *et al.* (1993) emphasized the importance of inlet concentrations of chlorophenols on the efficiency of treatment. They stated that an increase in the influent TOC concentration from 280 mg/L to 378 mg/L resulted in a decrease in the removal efficiency from 99.5 % to 76 %.

Activated sludge treatment of ground water contaminated with chlorophenols at low concentrations (4-120 μg/L) was investigated by Ettala *et al.* (1992). They reported that high removal was achieved with steady dosing of chlorophenols at high SRTs (8-10 d). According to the mass balance, 71 % of chlorophenols was removed and 68 % was biodegraded. A chlorophenols uptake rate of 29 mg/kg MLVSS.d was achieved. However, doubling the CP feeding decreased percent CP removal. Right after doubling the dosage, the CP uptake rate also decreased which can be partly explained by the lower temperature in the process. Therefore, increase

of the CP loading should be preferred in summer. However, McAllister *et al.* (1993) achieved good chlorophenols removal efficiencies at relatively higher concentrations using activated sludge process.

In contrary to reported partial treatment efficiencies of chlorophenols using activated sludge processes and deterioration in treatment system at low temperatures when activated sludge process was used (Ettala *et al.,* 1992), Jarvinen *et al.* (1994) reported that chlorophenols could be degraded even at low temperatures in mg/L levels using aerobic fluidized bed reactor. In this study, degradation of chlorinated phenolic compounds was investigated at different temperatures and HRTs. Concentrations of chlorophenols in contaminated ground water were as follows: 7-11 for 2,4,6-TCP; 32-36 mg/L for 2,3,4,6-TeCP and 1.8-2.3 mg/L for PCP. Experiments were carried out in four aerobic fluidized bed reactors with different carrier materials. Temperatures of reactors was gradually decreased even at ambient temperature of ground water and results demonstrated that fluidized bed treatment of CP-contaminated ground water produced close to drinking water quality effluent at ambient ground water temperature and CP loading rate as high as 740 mg CP/ L/d (HRT = 1.4 h). Fluidized bed treatment resulted in 80 % CP removal at loading rate of 2130 mg CP/L/d and 0.44 h HRT. At the temperature below 7 ^{o}C, suspended solid concentration slightly increased but did not affect the CP degradation. This study showed that aerobic fluidized bed reactor can be used to treat ground water contaminated with chlorophenolic compounds at the ambient temperatures of ground water, thus, avoids the heating expenses.

Similar to Jarvinen *et al.* (1994), Valo *et al.* (1990) reported that biodegradation of chlorophenols from simulated contaminated groundwater by immobilized bacteria could be achieved. The chlorophenols (3-130 mg/L) used in this study were 2,3,4,6-TeCP, 2,4,6-TCP and PCP, in a ratio of 8:1:1 by weight. Chlorophenols-mineralizing *Rhodococci* was immobilized on a polyurethane carrier. The filter was fed at 4 ^{0}C to see if the chlorophenols were adsorbed and degraded also at ground water temperature. Four periods of 1 d cold operation at 4^{o}C (74-426 mg

chlorophenols /L/d) was followed by 5-16 d at 25°C. Filter did not discharged organic chloride at 4 °C, but it started to release chloride immediately when the temperature was raised to 25°C. Although Jarvinen *et al.* (1994) reported degradation of chlorophenols even at the ambient temperatures of ground water, Valo *et. al* reported that chlorophenols were absorbed from cold water onto a polyurethane bed material and then biodegraded at 25°C while recycling one bed volume of water in the biofilter. The polyurethane immobilized *Rhodococci* during a persistent cooling (4 °C) and heating (25 °C) cycle did not lower the chlorophenols degrading activity. Chlorophenols degradation started as soon as the temperature was raised from 4°C to 25 °C. The biofilter with *Rhodococci* remained active for over 4 months. The recirculating water became acid as a result of dechlorination at 25°C after each cold adsorption period. The chlorophenols concentration of the column effluent was 1/1000-1/10000 of the influent products.

Vallecillo *et al.* (2000) studied the aerobic biodegradability and toxicity of some chlorophenolic compounds (PCP, 2,4,6-TCP, 2,4-DCP and 4-CP) in batch reactors and the behavior of a continuous activated sludge reactor feeding with 2,4,6-TCP and acetic acid in order to determine the feasibility of this treatment for degradation of such recalcitrant compounds. Activated sludge treatment of 2,4,6-TCP was studied at different CP and COD concentrations. At 1500 mg/L COD and 25 mg/L TCP concentration, COD removal was higher than 95 % and TCP removal was 90 % operating with an hydraulic retention time of 1.25 d. Operating with the same HRT, removal higher than 85 % was reached for TCP concentration of 100 mg/L. They reported that TCP removal was dependent on COD concentration and TCP removal increased strongly when COD influent was changed from 600 to 1000 mg/L.

In most of the studies a readily degradable substrate was used as a primary substrate. However, Lora *et al.* (2000) investigated the adaptation of activated sludge microorganisms to 2,4,6-TCP when phenol was used as an energy supplier co-substrate and progressively replaced by TCP. Continuous activated sludge

reactor with 1 d HRT and volumetric COD loading rate of 11 mg COD/L/.h was operated. Inoculum used in this study was not acclimated to phenol, however, required lag period was short, 6 d for phenol removal and 26 d for COD removal. During acclimation period conversion of TCP to another chlorinated compounds was observed and after acclimation, these intermediates were completely removed. So, it was stated that adaptation of reactor to TCP was a multi-step phenomenon; initially TCP was converted to other chlorinated compounds with every little effect on phenol and COD removals. After this period, dehalogenation of these chlorinated organic compounds was observed. During TCP adaptation period a biomass loss was observed which allowed the F/M to increase. When only TCP was fed to reactor (1544.6 μmol/L), biodegradation decreased to 6.2 % due to this shock loading. During this shock period, resulted in loss of biomass because of energy starvation and substrate accumulation within the reactor. The return to a feeding composed of 50 % of phenol and 50 % TCP increased the removal efficiency of TCP to 100 %. After progressively increase of TCP ratio in feed solution to 100 %, TCP removal efficiency of 100 % was attained. Then, it was understood that 100 % removal of TCP could be achieved by a good management of acclimation period.

Similarly, Ha *et al.* (2000) studied COD removal of phenolic wastewater in the presence of phenol as primary substrate. They used biological activated carbon-sequencing batch reactor in the presence of 2,4-DCP and at different SRT (3, 5, 8 d) values and reactors with and without activated carbon were compared to each other in view of COD and 2,4-DCP removal efficiencies. Two different COD concentration, 480 mg/L (162 mg/L phenol and 140 mg/L 2,4-DCP) and 560 mg/L (175 mg/L phenol and 152 mg/L 2,4-DCP), were used in the study. COD removal efficiency of reactors was between 84 and 97 %. When COD increased from 480 to 560 mg/L, an increased in effluent COD was observed, but systems operated for comparatively longer SRTs showed less increase than for shorter SRTs. It was observed that addition of activated carbon to biological system decreased fluctuation of COD removal efficiency. Different SRTs have resulted in different biodegradation rates. The highest specific removal rate was observed at the 3 d SRT

that was the shortest SRT in this study. Effect of addition of activated carbon was clearer at short SRTs than longer SRTs.

Also, pulp and paper industry effluents can contain several types of chlorophenols and due to different types of chlorophenols and sometimes lack of a readily degradable substrate, it is difficult to degrade these types of wastewaters. Cespedes *et al.* (1996) studied the removal of chlorinated phenols during aerobic treatment of effluents from kraft pulps bleached with chlorine-based chemicals, with or without hemicelluloses. Chlorine bleached kraft mill effluents without hemicellulose was treated by using two aerobic treatment lagoons reactors connected in series. BOD removal was between the 77.5 and 85 % and there is no additional BOD removal in second reactor. The influent chlorophenols concentration was about 4-200 ppb and the effluent concentration of chlorophenols was below the detection limit.

Hall *et al.* (1994) compared chlorophenolic compounds removal efficiencies in bleached kraft mill effluents by using activated sludge, facultative stabilization basin and aerated stabilization basin treatment systems. Effects of operation variables such as SRT, HRT and temperature on the removal of chlorinated phenolic compounds were also investigated. In this study, wastewater concentrations of COD and total chlorophenolic compounds were 730-980 mg/L and 155-270 μg/L, respectively. Results showed that average chlorophenols removal efficiency of facultative stabilization basin, aerobic stabilization basin and activated sludge system were 75, 70 and 40 %, respectively. The substantial difference in average performance between the activated sludge and aerobic stabilization basin process suggests that under aerobic conditions, HRT is a controlling variable for chlorinated phenolic removal when SRT is the same for activated sludge and aerobic stabilization basin processes. Since both the activated sludge and aerobic stabilization basin systems were operated at the same temperatures and SRTs, the enhanced removal associated with aerobic stabilization basin must be due to longer HRT provided.

Controlled addition of specific microbial culture to treatment systems (biosuplementation, bioaugmentation) for stimulation of the degradation of specific organic compounds (SOCs) is a relatively new discipline in environmental engineering. The success for implementation of bioaugmentation as an integrated treatment process will depend on the competitiveness and activity of such specific cultures when exposed to environmental conditions common for wastewater treatment process (Jacobsen *et al.*, 1996).

Jacobsen *et al.* (1996) studied the growth and biodegradation kinetics of *Mysobacterium chlorophenolicus comb nov.* in a series of batch experiment and fate of PCP in bioaugmented activated sludge reactor. Batch studies showed that there was stimulating influence on the PCP degradation rates from primary substrate, but below 25 mg COD/L, there was no effect on PCP degradation rate. The maximum PCP degradation rate of the pure culture was found as 25.10^{-9} μg PCP/cell/d with a half saturation constant of 885 μg PCP/L. In continuous reactors it was found that reactor without *Mysobacterium chlorophenolicus comb nov.* showed no biodegradation of PCP, however reactors with *Mysobacterium chlorophenolicus comb nov.* could degrade PCP. It was found that presence of activated sludge microorganisms may be advantageous for affectivity of *M. chlooorophenolicus* culture. During the continuous experiments it was shown that the *M. chlooorophenolicus* culture was not very competitive under the operating conditions in the fermenter chemostat leading to contamination of culture. This indicated that the sterile fermentation techniques may be necessary for the use of this strain for bioaugmentation purposes.

Similar to Jacobsen *et al,* Edgehill and Finn (1983) showed that addition of PCP degrading culture to an activated sludge treatment system stimulated the degradation of PCP. Edgehill and Finn (1983) investigated the activated sludge treatment of PCP with a waste containing some sugars, and 40 mg/L. PCP, the laboratory-activated sludge required about seven d for acclimation. However, the direct addition of a 10 % inoculum from a batch culture of PCP-metabolizing

Arthrobacter to the mixed liquor caused progressive decline in the effluent concentration of PCP. After only two d, the level of PCP in the clarifier effluent reached a steady state concentration of about 1 mg/L.

Some researchers stated that the nitrifying bacteria were able to dehalogenate many halogenated compounds in pulp and paper wastewaters (Nevaleinen *et al.,* 1993). Contrary to these findings, Altınbaş *et al.* (1999) reported that nitrifying culture is more sensitive to bleaching effluent compared to aerobic culture. Altınbaş *et al.* (1999) studied the degradation of bleaching effluent containing some chlorinated organics in sequential activated sludge and nitrification system. They reported that removal of TOC, AOX and released ICI under increasing loading feed was found to be increased in the first stage of the experimental system. Effluent of activated sludge reactor containing undegraded organics was given to nitrifying system. On the other hand, removal of AOX and TOC, released ICI decreased with increasing loading in nitrifying system. Interestingly, removal of NH_4^+ remained stable during the study. These results showed that performance of nitrifying bacteria is very sensitive to bleaching effluent.

Schollhorn *et al.* (1994) investigated the degradation of three monochlorophenol (2-CP, 3-CP and 4-CP) in non-aerated fixed bed bioreactor at different concentration and hydraulic loading rates. During the start-up, reactor was inoculated with an enrichment of culture able to mineralize all three isomers of monochlorophenols and with a pure culture, *Alcaligenes* sp. A7-2, able to mineralize 2-CP and 4-CP. The reactor was filled with a medium containing 3.3 mg/L of each of three monochlorophenol. 1 % solution of H_2O_2 was added into rector to maintain an effluent concentration of oxygen above 1 mg/L. When addition of oxygen as H_2O_2 solution was stopped and only oxygen source from feed was used, only 50 % of monochlorophenol can be degraded. The order of efficiency of degradation under oxygen limiting conditions was 3-CP > 4-CP > 2-CP. When oxygen was supplied to the reactor in the form of H_2O_2 to maintain oxygen concentration at the effluent above 1mg/L, nearly complete degradation was observed. The order of efficiency of

degradation under oxygen saturated conditions was 4-CP > 3-CP > 2-CP. They postulated the different elimination rates of monochlorophenols under oxygen saturated and oxygen-limiting condition may be due to different concentration of available oxygen concentrations in each conditions or a change in the biofilm populations.

Lewandowski *et al.* (1990) reported that a packed-bed reactor employing a silica based porous support for the fungus, and a well mixed reactor employing alginate beads as the immobilizing medium were very effective in degrading 2-CP at inlet concentrations up to 520 ppm. This study also showed that immobilizing the fungus on a support is important. For example, if the batch reactor is compared with the well-mixed reactor containing alginate-immobilized fungus, the biodegradation rate constant increases by a factor of 40.

Katayama-Hiramaya *et al.* (1994) studied the degradation of phenol and mono-chlorophenol by yeast *Rhodotorula Glutinits*. They found out that the strain degraded 5 mM of phenol and utilized phenol as sole organic carbon source. 3-CP and 4-CP were well degraded and degradability of 3-CP and 4-CP was increased by the addition of phenol or CPs to the medium at the cell cultivation. A solution containing 0.5 mM (64 mg/L) of 2-, 3- or 4-CP was incubated with phenol-grown or CP-grown cells of strain (0.1 g , dry weight). After 24 hours of incubation it was observed that biodegradability of 2-CP was low at 13-28 %. However, 3-CP and 4-CP were completely degraded including the elimination of stoichiometric amount of chloride ion. It was found out that phenol is an excellent carbon source, comparable to glucose or even acetate. 3-CP and 4-CP were degraded but not utilized as sole organic carbon source at the same conditions, suggesting the co-metabolical degradation of CPs in the strain. The order of biodegradability of CPs was 2CP< 3-CP< 4-CP. These order suggest that the substrate specificity of phenolhyroxylase in the yeast may be related to the position of CI group in a CP molecule.

Toxic compounds (both growth and nongrowth substrates) and easily biodegradable substrates are often found to coexist. Consequently, substrate interactions among these multible compounds are often quite complicated and may result in the sequential (diauxie) or simultaneous utilization. In general, concurrent utilization of mixed substrate is much more desirable than the sequential utilization in biological treatment of wastes due to its higher removal efficiency. In the case of cometabolism, cell growth on mixed growth substrates may not necessarily fall into one of the two distinctive behaviors of diauxie or simultaneous utilization (Wang *et al.*, 2000). A conventional carbon source, like sodium glutamate, can enhance the transformation rate of 4-CP significantly; this was achieved only when phenol concentrations were higher than or at least equal to the concentration of 4-CP. In this cometabolism system, cell growth on phenol and sodium glutamate in the presence of 4-CP (nongrowth substrate) by *P. putida* exhibited a new growth pattern characterized by two exponential growth phases separated by an intermediate lag phase under some conditions. When sodium glutamate was used as sole growth substrate, 200 mg/L of 4-CP was very toxic to cell. When phenol only was used as growth substrate no significant removal of 4-CP was observed. However in the presence of both phenol and sodium glutamate as growth substrate, high removal efficiency was observed. This shows that the presence of sodium glutamate attenuated the toxicity of 4-CP, concomitantly enhancing both utilization rate of phenol and transformation rate of 4-CP. This study also showed that in the presence of readily degradable substrate, phenol supplementation is necessary to remove high concentration of 4-CP (Wang *et al.*, 2000). However, phenol is a toxic compound and its addition for in-situ treatment of 4-CP should be reduced or completely avoided.

In literature, there are several studies about the degradation of chlorophenols under aerobic and anaerobic conditions. However, under reducing conditions other than methanogenic conditions, less information on the fate of halogenated aromatic compounds is available (Haggblom *et al.*, 1993). This is unfortunate because anoxic reactors have become an important component of many wastewater treatment

systems in recent years. Because electron transport in anoxic environment is very similar in many respects to electron transport in aerobic environments, there is a tendency for engineers to assume that biodegradation potential of the two environments is similar. As in the anaerobic environments, many aromatics previously thought to be non-biodegradable in the absence of molecular oxygen have been shown to be degradable in anoxic environments, although unique reactions are required for the initial steps. The differences in biodegradability in various environments can be very important for process selection since use of multiple environments may be the key to successful destruction of some compounds (Grady, 1990).

Schollhorn *et al.* (1994) showed that no consumption of NO_3^- as an electron acceptor at high feed concentration of monochlorophenols could be observed. This finding also is in agreement with the observations by Schwien and Schmidt (1982) for *Alcaligenes* sp. A7-2. However, Puhakka *et al.* (1992), Hu and Shieh (1987) and Nguyen and Shieh (1995) showed that phenol and mono chlorophenols can be used as carbon source under anoxic conditions.

Nguyen and Shieh (1995) used fluidized bed reactors both for oxic and anoxic conditions. Under oxic conditions, carbon oxidation/nitrification reactors were operated at steady state under seven different total organic loading rates, from 0.057 up to 0.207 mg TOC/mg biomass-day. It was observed that di-isopropylamine, monoethylamine and phenol were in fact degradable under oxic conditions. At all loading rates these compounds degraded higher than 90 %. The corresponding nitrification rate achieved under these conditions was approximately 50 %. Under anoxic conditions, these compounds could be removed >85 % at loading rates less than 0.05 mg/mg-day, while at higher loading rates TOC removal decreased to 60 %. The average ratio of mg TOC removed to mg NO_3^-N removed was observed 1.26. These results showed that di-isopropylamine, monoethylamine and phenol could be utilized as organic carbon source under anoxic conditions. Nevertheless,

microbial activity under anoxic conditions was diminished when compared to that under oxic conditions.

Liu *et al.* (1996) investigated the dechlorination of chlorophenols in anoxic estuarine sediments in the presence of sulfate in batch reactors. 2-CP (10 mg/L) was dechlorinated within 10 d by nonadapted sediment culture. On subsequent additions of 2-CP, the dechlorination rate increased. 3-CP (12 mg/L) was dechlorinated in 31 d in nonadapted sediment cultures. On a subsequent addition of 3-CP into the sediment cultures, the dechlorination rate of 3-CP did not increase; this second addition also required 31 d. However, the dechlorination rate of 3-CP increased with the third addition. 4-CP (11 mg/L) required 90 d for dechlorination in the nonadapted sediment cultures. On a subsequent addition of 4-CP into the sediment cultures, the dechlorination rate of 4-CP increased. All the isomers of these three chlorophenols were dechlorinated to phenol during the first two to three substrate additions. The relative dechlorination rates of CPs were 2-CP >3-CP > 4-CP. However, Haggblom and Young (1990) reported different relative rates of monochlorophenols under sulfate reducing condition (4-CP > 3-CP > 2-CP).

Puhakka *et al.* (1992) investigated chlorophenol degradation under oxic and anoxic conditions in fluidized bed reactor. The degradation of 2,4,6-TCP, 2,3,4,6-TeCP and PCP as sole organic compounds was evaluated in oxic fluidized bed reactors originally inoculated with activated sludge used for 4-CP and 2,4-DCP degradation experiments for a period of 15 months. The pseudo-steady-state reactor operation at hydraulic retention time of 5 h was used for chlorophenol degradation. The initial concentration of PCP was 74 mg/L and degradation of PCP under these conditions was negligible. After three months of operation with PCP the feed was switched back to 2,4-DCP (20 mg TOC/L). Within 3 d, the reactor performance fully recovered as indicated by chlorine release from 2,4-DCP. This shows that PCP, although being recalcitrant towards degradation, caused no irreversible effects on immobilized aerobic microbial culture. After PCP, reactor was fed with 2,4,6-TCP (54 mg/L). Then the reactor feed was switched to 2,3,4,6-TeCP. TOC removals for

2,4,6-TCP and 2,3,4,6-TeCP were 82 and 71 % respectively. In addition, GC/MS results from selected effluent samples showed over 99 % removal both of 2,4,6-TCP and of 2,3,4,6-TeCP. In addition to aerobic chlorophenol degradation, anoxic degradation of chlorophenols was also studied. One anoxic reactor was inoculated with activated sludge originating from a pilot-scale unit treating simulated, municipal wastewater. The reactor was fed with 4-CP (10 mg/L TOC) as sole carbon source and NO_3-N concentration was 50 mg/L. the GS/MS results showed 80 % 4-CP removal and no intermediate degradation products. However, the length of enrichment periods, the removal rates and efficiencies were not comparable to those obtained in aerobic fluidized-bed systems. The mean 2.2 mg TOC removal per mg chloride release agree well with the theoretical TOC/Cl ratio of 2.0. Simultaneous nitrate removal was 12 % and TOC/NO_3^-N ratio was 1.5. In anoxic reactors fed with 2,4-DCP (10-20 mg TOC/L) or PCP (20 mg TOC/L) together with potassium nitrate, no removal of these chlorophenolic compounds were observed. When reactor with 2,4-DCP was switched to aerobic mode of operation complete degradation of 2,4-DCP was observed. This showed that an aerobic microbial culture was developed during the anoxic mode of operation. Similar finding was observed by Valo *et al.* (1985) showing that an aerobic culture maintained PCP degradation activity at an extremely low oxygen concentration (pO_2 0.0002 atm).

In contrast to findings of Puhakka *et. al.*, Hu and Shieh (1987) reported the 2,4-DCP removal in a denitrifying biofilm in which TOC/NO_3^-N ratio was 0.8-1.5.

1.3.3. Anaerobic Treatment of Chlorophenols

In addition to aerobic removal of chlorophenols, anaerobic treatment systems can be used to degrade chlorophenols. Aerobic microorganisms can metabolize mono- and di-chlorinated substances, but aerobic attack becomes less effective with more highly chlorinated compounds. On the other hand, the rate of dechlorinations under

anaerobic conditions is actually greatest for more heavily chlorinated compounds, but progressively slower as the chlorophenol molecules become less chlorinated. In particular, although monochlorophenols and more heavily chlorinated phenols can be degraded under anaerobic conditions, anaerobic cultures capable of dechlorinating more heavily chlorinated compounds have a limited ability to degrade monochlorophenols (Armenante *et al.*, 1999).

Efficiency of thermophilic anaerobic process (55 ^{0}C) to remove chlorinated phenolic compounds from bleached kraft mill effluent (BKME) was studied by Lepisto and Rintala (1994). In this study four different types of anaerobic processes: an upflow anaerobic sludge blanket (UASP) reactor; an UASB reactor enriched with sulfate; an UASB reactor with recirculation and a fixed bed reactor with recirculation at total influent chlorophenols concentration varying between 75.3 and 1010 µg/L. 2,4-DCP accumulation was observed in the study, however 2,4-DCP was completely removed when loading rate of reactors was increased. Contrary to AOX removal (AOX removal during the study varied between 19 and 67 % depending on loading rate and reactor type), the COD reduction efficiency in the four reactors decreased as the concentration of the influent COD increased (COD removal efficiency showed variation between 32 and 72 %).

Jin and Bhattacharya (1997) investigated the toxicity and treatability of 12 chlorophenolic compounds consisting of mono-, di- and trichlorophenolic compounds in anaerobic propionate enrichment culture in batch reactors. It was observed that trichlorophenols was the most toxic among the examined mono and dichlorophenolic compounds and 2,3,5-TCP was the most toxic among the examined trichlorophenolic compounds. The toxicity of selected chlorophenols to the propionate-fed culture decreased in the following order: 2,4,5-TCP > 2,3,5-TCP > 2,4,6-TCP> 2,3,6-TCP > 2,3-DCP > 2,6-DCP, 3-CP, 4-CP, 2-CP, 2,5-DCP, 3,4-DCP, 2,4-DCP. Most chlorophenols were dechlorinated reductively to less chlorinated compounds and/or phenol. Dechlorination at the ortho position was observed most frequently for TCPs. During the degradation studies in batch

experiments, it was observed that 2-CP degraded faster than 3-CP, which degraded more rapidly than 4-CP. Phenol also was detected during the degradation of 2,3-DCP; 2,6-DCP; 3,5-DCP; 2-CP; 3-CP; and 4-CP. The overall removal of selected chlorophenols in anaerobic propionate enrichment systems were in the following order: 2,3,5-TCP > 2,3,6-TCP > 3,5-DCP > 2,4-DCP > 2-CP > 3,4-DCP, 2,3-DCP, 2,5-DCP > 3-CP > 2,4,5-TCP > 2,6-DCP > 2,4,6-TCP > 4-CP.

Puhakka *et al.* (1994) studied continuous removal of chlorophenols in the anaerobic fluidized-bed reactor at influent concentration of 7.1 mg/L PCP and 2,4,6-TCP and 1.0 mg/L TCG. During steady operation at 24 h HRT, the mean TOC removal was 92 % with simultaneous mean methane production of 250 ml/g COD added. 99 % removals for TCP and PCP and over 95 % removal of TCG were achieved in this study. This study demonstrated that sequential anaerobic-aerobic treatment of pulping effluents, which is often proposed for overall waste management economy, was an attractive combination for mineralization of chlorinated monoaromatics as well.

Fang *et al.* (1999) investigated the efficiency of treating wastewater containing medium-strength of phenolic pollutants and nitrate in both continuous and batch reactors, the influence of a carbonhydrate co-substrate and interaction between methanogens and denitrifiers. Continuous experiments was conducted in UASB reactor at different COD / NO_3-N concentration containing 200 mg/L phenol and 100 mg/L m-cresol. Results showed that denitrifiers out-competed methanogens for substrates for carbon and electron supplies. At the COD / NO_3-N ratio of 3.34, all electrons were utilized by denitrification, as evidenced by the cease of methane production. The fraction of electron flow to methogenesis increased with the COD / NO_3-N ratio. It was shown that denitrifiers had superior m-cresol degrading capability than methanogens. When COD / NO_3-N ratio was 3.34 or lower, denitrifiers were able to degrade nearly 100 % of phenol and m-cresol with 1 day of hydraulic retention even in the absence of additional carbon source. Batch studies

showed that degradation of m-cresol was enhanced by the presence of either sucrose or phenol as cosubstrate.

1.3.4. Sequential Anaerobic and Aerobic Biological Treatment of Chlorophenols

Aerobically, mono- and di-chlorinated compounds are metabolized by means of an oxygen requiring enzymatic attack upon the aromatic rind, followed by halogen loss from a nonaromatic intermediate (Reineke and Knackmuss, 1988). This type of reaction is less often observed with more heavily halogenated compounds. In the absence of oxygen, the fate of halogenated compounds is the opposite of that observed in aerobic environment. Reductive dehalogenation without attack on the aromatic ring is the most described mode of attack. The rate of dehalogenation is the greatest for heavily halogenated compounds but is much slower for the mono- or di-halogenated compounds. Anaerobic dehalogenation can convert mono- and di-halogenated compounds to their non-halogenated aromatic parent compounds, however, the rate of these final dehalogenation is likely to be considerably slower than aerobic dehalogenation of these compounds. These facts immediately suggest the use sequential anaerobic and aerobic reactors for the treatment of more halogenated aromatic compounds. (Kafkewitz *et al.,* 1992). Kafkewitz *et al*, used both aerobic and anaerobic experiments in batch reactors. The initial attempt to develop dehalogenating consortia utilized defined media containing 1 g/L sodium acetate with either 2,4-DCP, 2,6-DCP, 3,5-DCP, 2-CP, or 4-CP (each at 20 mg/L) added and incubated at 30 °C for several months. No degradation of any target compounds was observed. However, when defined media was replaced by sterile, diluted digester fluid containing 20 mg/L of the halogenated target compound, significant dehalogenation of a number of target compounds was observed. After acclimation to 2,4,6-TCP, complete dehalogenation of 2,4,6-TCP was observed, however, 4-CP was the quantitatively significant product of the anaerobic enrichments. In order to remove dehalogenation byproduct produced under anaerobic conditions, 4-CP, aerobic step was necessary. The anaerobic enrichments

were transferred to aerobic conditions without any additional inoculation. This suggest that activity occurs under anaerobic conditions is due to facultative organisms. The aerobic organisms were tested in sterilized and clarified anaerobic enrichment culture fluid. Several of these organisms could be adapted to degrade 4-CP; the most common effective microorganisms were *Pseudomonas putida*, *Pseudomonas glathei*, and *Pseudomonas pseudoflava.* In the absence of pH adjustment little or no degradation could be demonstrated. However, addition of phosphate buffer greatly stimulated aerobic mineralization of 4-CP and phenol. In aerobic step, residual 2,4,6-TCP in anaerobic step was also mineralized.

Armenante *et al.* (1999) studied a two-stage anaerobic-aerobic process (including both batch and continuous reactors) to completely degrade 2,4,6-TCP. Anaerobic batch experiments in serum bottles showed that lag time for TCP degradation was about 40 h. After lag period, 2,4,6-TCP was degraded to 2,4-DCP, which was degraded to 4-CP. During the entire degradation process the sum of the concentration of all chlorophenolic compounds remained practically constant (initial concentration was 135 μM, final concentration was 140 μM) indicating that aromatic ring in the chlorophenolic compound molecules was not attacked, and the reductive dehalogenation was the only one involved. In the degradation experiments in anaerobic serum bottles, it was found that when the concentration of TCP was between 40 and 150 μM no inhibition or toxicity effect was observed. However, dehalogenation was totally inhibited when the concentration of 2,4,6-TCP was 908 μM. Anaerobic batch studies of dechlorination of 4-CP showed that reduction of 4-CP under anaerobic conditions is possible although much more slowly than that of more chlorinated phenolic compounds (82 d was required in anaerobic batch experiment). Effect of pH on aerobic process is important due to chancing pH during anaerobic dehalogenation process as reported by Kafkewitz *et al.* Aerobic batch studies showed that 4-CP degradation occurred only when pH was between 7.0 and 7.5. Degradation studies showed that 2,4,6-TCP (initial concentration was 66 μM) can be successfully removed in two-stage continuous anaerobic-aerobic process in which anaerobic microorganisms carry out the initial

dechlorination step, and aerobic organisms produce complete degradation of the dechlorination products produced in anaerobic step.

As carrier material in aerobic or anaerobic reactors different materials can be used. Shin *et al.* (1999) used tire chips as packing material for sequential anaerobic and aerobic biofilm reactors to remove persistent chlorinated hydrocarbons. In this study, 2,4-DCP was added at concentration of 10, 20, 40 and 70 mg/L, total influent COD was 1000 mg/L. Adsorption experiments for 4-CP and 2,4-DCP in batch reactors showed that maximum adsorption capacity of 2,4-DCP was only 0.3% of granular activated carbon. For 4-CP, maximum adsorption capacity was only 0.04% of granular activated carbon because 4-CP has greater polarity than DCP. Therefore, it was concluded that removal of 2,4-DCP and 4-CP through adsorption could be neglected. Biomass attachment tests showed that tire chips has not inhibitory effect on biofilm and can be used as carrier in anaerobic and aerobic reactors. 2,4-DCP was completely degraded in anaerobic reactor but 4-CP was produced as intermediate product in anaerobic reactor. 4-CP could not be removed in anaerobic reactor and it was further removed in aerobic reactor. In aerobic reactor removal efficiencies of 4-CP were 98 and 70% at concentrations of 0.16mM and 0.31mM respectively. This study has showed that wasted tires, which are difficult to dispose of or treat can be effectively used as a medium for biofilm reactors.

1.4. Theoretical Background

1.4.1. Quantitative Description of Growth

The qualitative observation of whether or not growth occurs in a culture is useful for many purposes. The further information obtained when the growth is measured quantitatively is often presented in the form of a graph of biomass against time. The data may be rendered more meaningful and concise if it is analysed in terms of the various growth parameters: specific growth rate or doubling time of the biomass, growth yield, metabolic quotients for substrate utilization and product formation, substrate affinity and maximum biomass.

1.4.1.1. Specific Growth Rate

The requirements for growth of biomass in a culture are:

- A viable inoculum
- An energy source
- Macro and micro nutrients
- Absence of inhibitors which prevent growth
- Suitable physicochemical conditions.

If all requirements for growth are satisfied, then the rate of growth (dx/dt) is proportional to the amount of biomass (X) in the culture. Hence,

$$dx = \mu x.dt \quad \text{.....1.1}$$

$$dx / dt = \mu x \quad \text{.....1.2}$$

The parameter μ, which represents the rate of growth per unit amount of biomass, is termed the *specific growth rate* and has the dimension of reciprocal time (1/t).

1.4.1.2. Yield Coefficient

The growth yield is defined as:

$$\Delta X / \Delta S = Y \quad \text{.....1.3}$$

Where ΔX is the increase in biomass consequent on utilization of the amount of substrate. More rigorously the growth yield is expressed by

$$Y = dx / ds \quad \text{.....1.4}$$

It should be noted that if x and s are the biomass and substrate concentration respectively, then $Y = -dx / ds$, the negative sign being introduced because x and s vary in opposite senses.

1.4.1.3. Metabolic Quotient

The rate of consumption of a substrate in a culture at a particular moment is given by

$$ds / dt = -qx \quad \text{.....1.5}$$

Where, x is the biomass and q is known as a metabolic quotient, or specific metabolic rate. The substrate consumed for growth in the small time interval, dt is described by;

$$ds/dt = \mu x/Y \quad \text{.....1.6}$$

Comparison of Eqn. 1.5 and 1.6 shows that

$$q = \mu/Y \quad \text{.....1.7}$$

The occurrence of constant exponential growth in batch cultures shows that growth rate may be unaffected by substrate concentration over a wide range; that is the growth process shows zero order kinetics. We might expect that substrate consumption would follow enzyme kinetic so that if S is the substrate concentration and q is the metabolic quotient;

$$q = q_m s/(s + K_s) \quad \text{.....1.8}$$

Where K_s, called the *saturation constant*, is equivalent to a Michaelis-Menten constant and q_m is the maximum value of q obtained when $s >> K_s$.
As previously noted that $q_m = \mu_m / Y$, then it follows that

$$\mu = \mu_m s/(s + K_s) \quad \text{.....1.9}$$

This equation is known as Monod equation.

The K_s value is inversely related the specific growth rate and is taken to represent the affinity of the organism to the substrate.

1.4.2. Batch Growth Kinetics

The different growth phases, which may occur in a culture is depicted in Figure 1.1. The kinetics as well as the cause for this course of events are related to properties of the microbial population and environmental conditions in the system. Among the

important environmental conditions are pH; temperature; dissolved oxygen concentration; amount and type of carbon source, nitrogen, phosphorus, and other nutrients; presence of inhibitory substances. These phases reflect changes in the biomass and in its environment. A lag phase may be considered a time of adjustment to a new environment. Cells obtained from sewage, soil, or a stock culture medium, wherein the environment was different from that into which they are now placed, can be expected to undergo a period of adjustment prior to assuming their maximum rate of growth (Gaudy,1980).

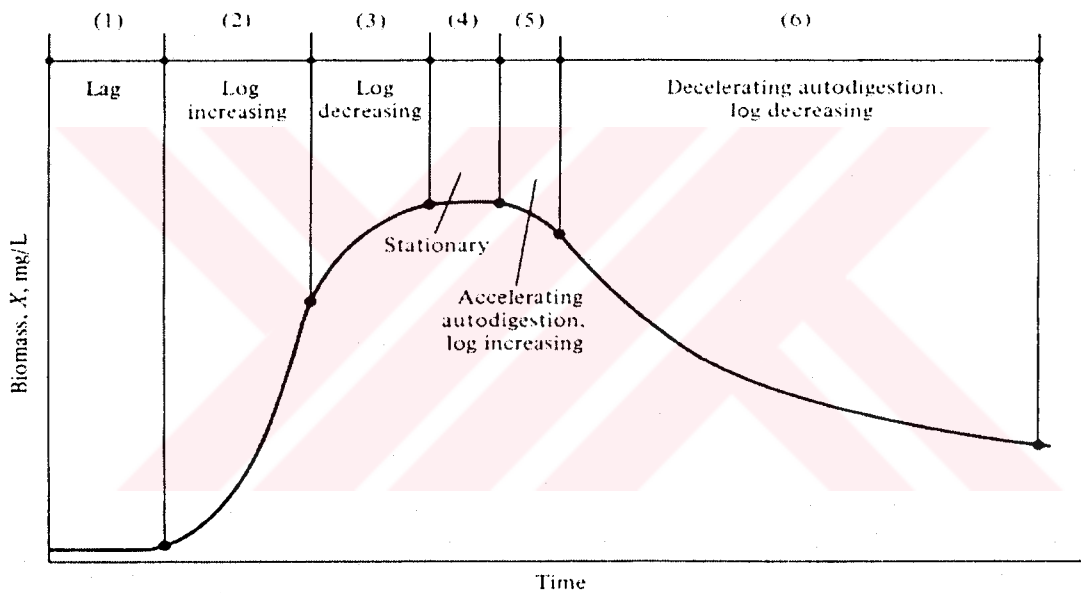


Figure 1.1. Typical growth curve in batch system

After the lag period growth occurs at the maximum rate and finally ceases due to lack of nutrient, or accumulation of an inhibitory product or some change in growth environment. After the biomass reaches its maximum, there may be a stationary phase where the amount of biomass remains constant but, sooner or later, the

biomass declines in amount as a result of maintenance metabolism or autolysis. The duration of exponentional growth phase depends partly on the initial concentration of growth limiting substrate. The period when nutrient limitation significantly affects the growth rate is limited to a small fraction of the last generation. This behavior is typical of most simple cultures and meaning that they are subject to an extreme type of nutritional regime, that is growth with excess substrate followed by sudden starvation. Consequently the periods of growth at rates less than the maximum rate are long enough to adjust its structure to that which is optimal for the growth rate. This limitation can, to some extent, be overcome fully by means of chemostat culture.

1.4.3. Energy and Carbon Source Requirements

For heterotrophic microorganisms it has been shown that the substrate acts both as a carbon and energy source. For these organisms it is necessary to distinguish between that fraction of the substrate, which is channeled into the synthesis function and that fraction of the substrate, which is channeled into the energy function and subsequently oxidized to provide energy for all cellular functions. Such a distinction can be made by performing a substrate balance for substrate utilized during an increment of time, Δt.

Total Substrate = Utilized	Substrate Utilized + For Synthesis	Substrate Oxidized for Energy	.....1.10

This relationship is shown in Eq.1.11, 1.12 and Figure 1.2.

Respiration

$(ed)_{red} + (ea)_{ox} \rightarrow (ed)_{ox} + (ea)_{red}$ 1.11

Energy

Synthesis

$(ed)_{red} + NH_4 \rightarrow C_5H_7NO_2$ (biomass).....1.12

(Org. C-source)

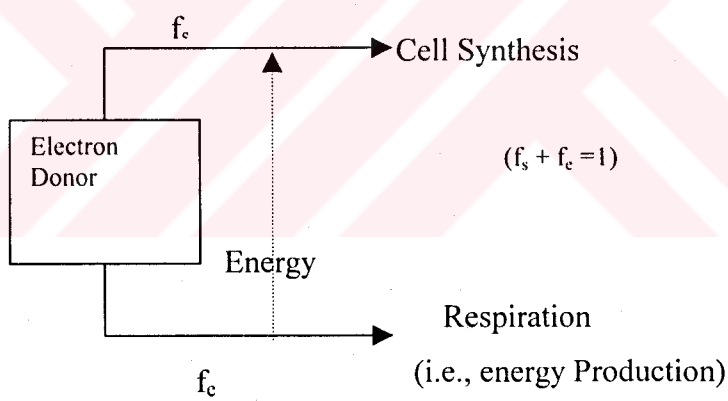


Figure 1.2. Energy flow Diagram Between Respiration and Cell Synthesis

As can be noted $f_s+f_e=1$ because a portion of energy is used for energy production and other part is used for cell synthesis. f_s and f_e are a function of both energetics and SRT.

Pirt (1975) has proposed that microorganisms require energy for growth (synthesis) as well as for maintenance functions such as turnover of cell materials, active transport, mobility, and so on.

1.4.4. Bioaugmentation and Cometabolism

The two major approaches to bioremediation of hazardous/oily wastes and environmental pollutants are microbial inoculation (seeding) and the bioaugmentation of naturally occurring microbial activities. In the most cases *bioaugmentation* consists of environmental modification to eliminate some limiting factors that is restricting the rates of microbial growth and metabolism of a polluting substance. For this approach to work the pollutant must not be recalcitrant. This means that microorganisms must have the genetic and physiological capability to degrade the substance. The most common factors controlled to stimulate biodegradative activities by bioaugmentation are nutrient concentrations (generally nitrogen and phosphorus), molecular oxygen concentration, redox potential, and moisture levels. Additionally, cosubstrate can be provided as growth-supporting substances (Atlas, 1993).

Co-metabolism (co-oxidation or co-transformation) refers to the ability of microorganisms to metabolize a compound which cannot be used as an independent source of energy or growth. Co-metabolism is thus a fortuitous process in which microorganisms while growing at the expense of one substrate also have the capacity to transform another compound without driving any benefit from its metabolism. Thus, microorganisms would need another substrate as a carbon and energy source on which to grow. This special type of metabolism, the incidental metabolism of a nongrowth substrate, has been named as co-metabolism. A

nongrowth substrate in this case will be defined as an organic compound that cannot serve as energy source, or a significant source of nutrients for the degrading culture. In co-metabolism, organisms uses one substrate as primary energy source and metabolize another compound utilizing the enzymes which are synthesized to degrade the primary substrate. A significant portion of total biodegradative activity towards xenobiotic compounds may involve co-metabolism. During degradation of aromatics a case of fortuitous metabolism is observed. That is, enzymes catabolizing aromatics also catabolize chlorinated aromatics in the same manner, probably due to relatively unspecific nature of these enzymes. Chlorinated products are no further degraded and tend to accumulate in the medium till another microorganisms capable of oxidizing take over. An example is the degradation of DDT, which is reportedly mineralized directly by only one organism, a fungus; other organisms studied appear to cometabolize only the compound, resulting in numerous transformation products that subsequently can be used by other organisms. The organism derive their energy and carbon from primary substrate but non from the xenobiotic, which acts as a secondary substrate. The reaction involved in co-metabolism includes dehalogenation, introduction of hydroxyl groups, ring cleavage, and oxidation of methyl group.

1.4.5. Diauxie Growth

Biological treatment of wastewater and industrial effluents often involves utilization and transformation of mixed substrates. Toxic compounds (both growth and nongrowth substrates) and nontoxic, easily degradable compounds are often found to coexist. Consequently, substrate interactions among these multible compounds are often quite complicated and may result in sequential (diauxie) or simultaneous utilization (Wang *et al*, 2000).

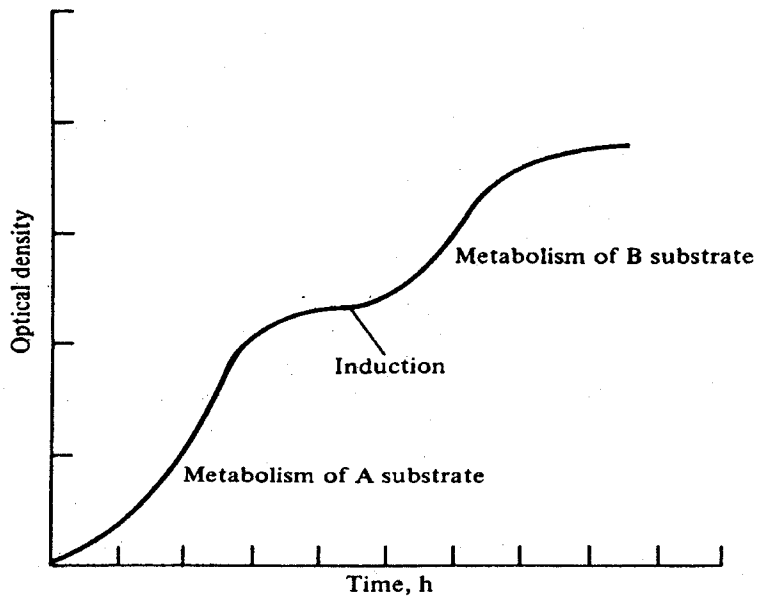


Figure 1.3 Typical diauxie growth pattern

Figure 1.3 shows a typical diauxie growth pattern. As can be seen from this figure, there are two distinct phases of growth (diauxie) as the result of sequential use of the two compounds. The pause between growth cycles was a period of adaptation to the second substrate. When the medium contained two substrates that the organism had the genetic capability to metabolize, the A substrate would be utilized completely before use of the B substrate began. The lag before use of the B substrate implied that the enzymes for its use were not made as long as the A substrate was present.

In certain mixes there was concurrent removal of both substrates, whereas in others there was sequential substrate removal. The blockage of metabolism of one compound by presence of other was not always so complete as to lead to sequential removal; certain combinations led to only partial blockage. Also, there are variations in the degree of blockage caused by various experimental (i.e., environmental) conditions. This sequential growth pattern also can be observed in a complex wastewater (Gaudy, 1980).

While a major part of the research on diauxie phenomena is concerned with cell growth on binary mixtures of sugars, reports on occurrence of diauxie in the biodegradation of hazardous compounds are relatively limited. Diaxue growth pattern was also reported in the degradation of mixed chlorinated phenols (Menke and Rehm, 1992). In contrast to observed diauxic growth patterns, concurrent utilization of multiple substrates in natural ecosystems and in waste-treatment systems is commonly observed. In general, the concurrent utilization of mixed substrates is much more desirable than the sequential utilization in biological treatment of wastes since in the former, enhanced removal rates and degradation efficiencies of mixtures of substrates are usually accomplished resulting from higher cell mass density. On the contrary, in diauxic growth, degradation of one growth substrate is severally inhibited by the presence of other; consequently a long lag or acclimation phase is required for the utilization of the second substrate (Wang *et al*, 2000).

CHAPTER 2

MATERIALS AND METHODS

In this chapter, chemicals, inocula, experimental set-ups and analytical methods are described.

2.1. Chemicals

Chlorophenols used in the study (2-CP, 4-CP and 2,4-DCP), the chemicals used for growth medium and methanol used for HPLC analysis were obtained from Merck Chemical Co., Germany.

Powder pillows for NO_3-N measurement were obtained from Hach Co.,USA.

2.2. Cultures and Medium

Activated sludge culture used as inocula for fed-batch reactors were obtained from activated sludge unit of Greater Municipality of Ankara Domestic Wastewater Treatment Plant. Culture obtained from the fed-batch reactor not receiving chlorophenol and operated at 8 d of SRT (base-line reactor) was used as initial inocula for the batch experiments conducted with unacclimated culture.

Other fed-batch reactors (SRT = 8 d and 15 d) receiving 4-CP and 2,4-DCP at stepwise small increments (from 0 – 130 mg/L 4-CP and 0-75 mg/L 2,4-DCP) were operated in order to obtain chlorophenol acclimated cultures to be used as inocula for the batch reactors conducted with acclimated cultures.

Initial inocula for the anoxic batch experiments were obtained from a fed-batch reactor operated at 8 d of SRT. The reactor was operated with 6 h under anoxic and 16 h aerobic cycles.

Synthetic wastewater used throughout the experiments is given in Table 2.1. The peptone was used both carbon and nitrogen source. The phosphate salts were added to the synthetic medium to provide both buffer and as the source of phosphorus to the microorganisms. The cultures were carbon limited in all the experiments and proteous-peptone (Oxoid) was present as readily degradable carbon source.

Table 2.1. Composition of the Synthetic Wastewater (Dilek *et al.*, 1998)

Constituents	Concentration (mg l^{-1})
Proteous-Peptone	407 (COD= 500 mg l^{-1})[1]
NaCl	407.4
Na_2SO_4	44.6
K_2HPO_4	44.6
$MgCl_2.6H_2O$	3.7
$FeCl_2.2H_2O$	3.7
$CaCl_2.2H_2O$	3.7
$MnSO_4$	0.057
$ZnSO_4$	0.046
$CoSO_4$	0.049
$CuSO_4$	0.076

2.3. Experimental Procedure

Toxicity and treatability studies of chlorophenols were conducted under both aerobic and anoxic conditions.

2.3.1. Toxicity and Treatability Studies Under Aerobic Conditions

Under aerobic conditions, toxicity and treatability studies were conducted using both batch and fed-batch reactors. Batch experiments for 4-CP and 2,4-DCP were carried out using both acclimated and unacclimated cultures, whereas, only unacclimated culture was used for the toxicity and treatability determination of 2-CP.

2.3.1.1. Batch Experiments Under Aerobic Conditions

2.3.1.1.1. Batch Experiments Under Aerobic Conditions Using Unacclimated Culture

The batch reactors inoculated with unacclimated culture was operated for the determination of toxicity and treatability of 2-CP, 4-CP and 2,4-DCP.

Experiments were conducted in 500 ml Erlenmeyer flasks stoppered with cotton plugs. The working liquid volume was 250 mL.

A stock solution of chlorophenols was used to adjust different concentrations of chlorophenols in reactors (30-300 mg/L for 2-CP; 57-274 mg/L for 4-CP and 22-100 mg/L for 2,4-DPC). Stock solution of 2,4-DCP was prepared in 0.01 N NaOH solution to have complete solubilization. In addition to reactors receiving chlorophenols at different concentrations, a base-line reactor that is devoid of chlorophenol was provided for both acclimated and unacclimated cultures. In order to find out amount of chlorophenol removed via evaporation, control rectors

without biomass were operated at the same conditions. All experiments were carried out in an orbital shaking incubator at a shaking rate of 200 rpm at the constant temperature of 25 °C. Samples taken from the reactors at various time intervals during incubation were analyzed for biomass as optical density (OD), COD and chlorophenol concentrations. The mixed liquor volatile suspended solids (MLVSS) measurements were also carried out at the end of exponential growth phase to calculate Y and SA.

Also, batch experiments using effluents of fed-batch reactors receiving chlorophenols at the initial concentration of 130 mg/L 4-CP and 75 mg/L 2,4-DCP were carried out to find out the toxicity of 4-CP and 2,4-DCP after treatment. In this set of experiment, peptone and other required inorganic compounds (Table 2.1) were added to batch reactors not to limit the growth of biomass.

In another set of experiment, chlorophenols were used as a sole organic carbon source. In this set of experiments, reactors were fed with chlorophenols and other required minerals not to limit the growth of biomass. In this set of experiment, NH_4Cl at the concentration of 200 mg/L was added to the reactors to supply about 52 mg/L N, which is equal to amount of nitrogen coming from pepton when it was added as carbon and nitrogen source.

2.3.1.1.2. Batch experiments Under Aerobic Conditions Using Acclimated Culture

In addition to experiments with unacclimated culture, batch reactors inoculated with acclimated culture was also operated at the same experimental conditions to understand the effect of acclimation on the toxicity removal of chlorophenols. The same synthetic wastewater (Table 2.1) used in experiments with unacclimated culture was used. Reactors were operated at various concentrations of 4-CP (130-390 mg/L) and 2,4-DCP (76-200 mg/L).

4-CP and 2,4-DCP acclimated cultures were obtained from fed-batch reactors (SRT= 8 d) receiving 130 mg/L 4-CP and 75 mg/L 2,4-DCP, respectively.

Also, batch experiments in which chlorophenols were used as sole organic carbon source were conducted to examine the treatability of chlorophenols in the absence of a readily degradable substrate. Inorganic matters required for biomass were added not to limit the growth of cultures as in the case of experiments conducted with unacclimated culture and NH_4Cl was used as nitrogen source. Treatability of 4-CP was examined using both 4-CP and 2,4-DCP acclimated cultures at various concentrations of 4-CP (50-200 mg/L 4-CP for reactors inoculated with 4-CP acclimated culture and 138 mg/L 4-CP for reactor inoculated with 2,4-DCP acclimated culture). Similarly, treatability of 2,4-DCP when present as sole organic carbon source was studied both with 4-CP and 2,4-DCP acclimated cultures at different initial concentrations of 2,4-DCP (51 and 76.6 mg/L 2,4-DCP for reactors inoculated with 2,4-DCP acclimated culture, and 77 mg/L 2,4-DCP for reactor inoculated with 4-CP acclimated culture). All of these reactors were operated at the same conditions with those receiving peptone as explained above. Samples from the reactors were taken for the analysis of chlorophenols at different time intervals.

2.3.1.2. Aerobic Fed-batch Reactors

Treatability of 4-CP and 2,4-DCP was investigated in aerobic fed-batch reactors at two different SRT values, namely 8 and 15 d. In addition to reactors used for treatability of chlorophenols, another reactor not receiving chlorophenol (control reactor) was operated. So, during the study five aerobic fed-batch reactors were operated.

Total volume of each fed-batch reactor was 2.5 L and working volume was 2 L. Reactors were fed once a day with the same growth media used for batch experiments (Table 2.1). Hence, reactors were operated in fill and draw mode. In order to maintain intended SRT values, excess sludge (250 mL/day for SRT 8 days;

133 mL/day for SRT 15 days) was drawn from the mixed liquor of reactors once a day. Reactors were kept in a water-bath in order to maintain temperature around 25°C. Oxygen was supplied to reactors using air pumps to keep dissolved oxygen level above 2 mg/L. A schematic representation of reactors is given in Figure 2.1.

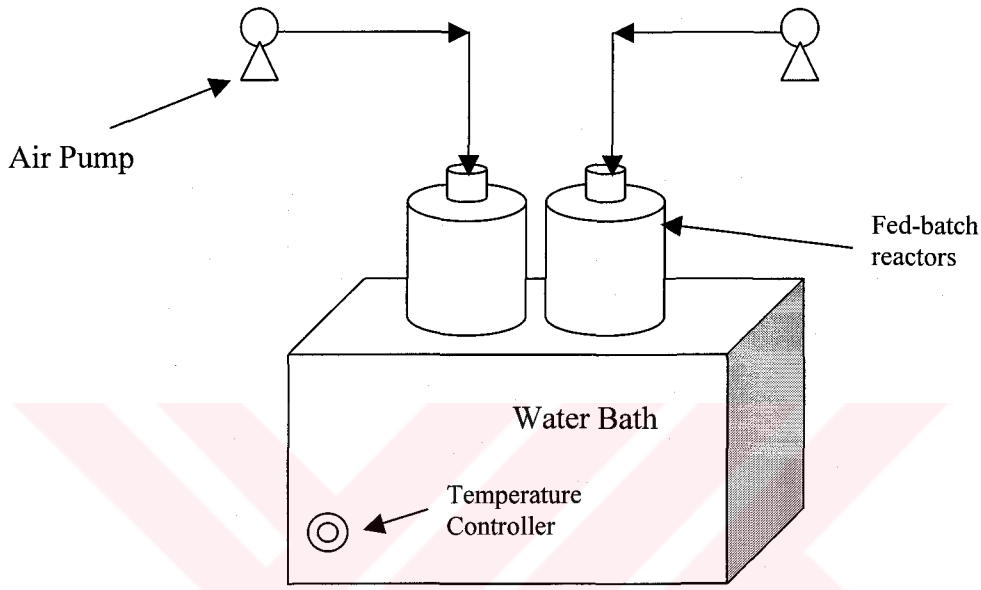


Figure 2.1. Experimental setup of aerobic fed-batch reactors

Activated sludge sample taken from Greater Municipality of Ankara Domestic Wastewater Treatment Plant was used as inocula for the fed-batch reactors. About 1 month was required for the fed-batch reactors to reach steady-state conditions. MLVSS and effluent COD concentrations were monitored to ensure steady state conditions. After reaching steady-state conditions, reactors were fed with chlorophenol in stepwise small increments.

At each feeding concentration of chlorophenol, MLVSS and effluent COD concentrations were monitored to ensure that reactors were in steady-state

conditions. After reaching steady-state conditions, samples were taken from the reactors for the analysis of MLVSS, influent and effluent COD and chlorophenol concentrations. Table 2.2 shows the influent chlorophenol concentrations applied throughout the study.

In addition to steady-state experiment conditions, shock loadings were also examined. After increasing chlorophenol concentrations, following 1st, 2nd and 3rd days, samples were taken from the effluent of reactors for the analysis of chlorophenols.

Table 2.2. Influent Concentrations of 4-CP and 2,4-DCP

Days (for reactors fed with 4-CP)	Influent 4-CP mg/L	Days (for reactors fed with 2,4-DCP)	Influent 2,4-DCP (mg/L)
1-23	0	1-23	0
23	10	23	6
49	31	49	9
59	50	65	20
86	78.4	86	40
104	100.5	104	50
152	134.5	152	74.48

2.3.1.3. Sequencing Batch Reactor Experiments

Fed-batch reactors operated at the 8 days of SRT were switched to sequencing batch reactors (SBR) mode. In this set of experiments, chlorophenols (4-CP and 2,4-DCP) were fed to the reactors as sole carbon and energy source. NH_4Cl was used as nitrogen source as in the case of batch reactors in which chlorophenols were used as sole carbon and energy source. A schematic representation of reactors is

given in Figure 2.2. Feed solution was pumped to reactors by a Watson Marlow 505 ID pump for each reactor. Flow rate of 4-CP and 2,4-DCP feeds were 4 and 3.38 mL / min, respectively.

The SBRs of about 2 L working volume were run with a total cycle time of 24 h. Filling and settling times were 8 and 1 h, respectively. During the operation, temperature was maintained about at 25 °C in a water-bath. Aeration was applied during both filling and reaction cycles to keep oxygen concentration above 2 mg/L and mix to the content of reactor. Reactors were operated for 24 d and only on the Day 13, 250 mL of excess sludge was drawn off from each completely mixed reactor.

2.3.2. Toxicity and Treatability of Chlorophenols Under Anoxic Conditions

In addition to aerobic toxicity and treatability studies, the effect of chlorophenols concentrations on the nitrate uptake rate, as well as on the COD and chlorophenols removal efficiencies under anoxic conditions were also examined in batch reactors. Activated sludge to be used as initial inocula for the batch reactors (initial MLVSS was 1000-1200 mg/L) were obtained from a fed-batch reactor operated under simultaneous anoxic (6 h) and aerobic (15 h) conditions in order to simulate a plant treating nitrogen using aerobic and anoxic environments. For the fed-batch reactor, initial inocula were taken from Greater Municipality of Ankara Domestic Wastewater Treatment Plant as for the other aerobic fed-batch reactors. Feed solution used for the anoxic fed-batch reactor was the same as used the aerobic reactor, with the exception of NO_3^- addition to maintain COD/NO_3^- ratio around 5. During the anoxic cycle of the reactor, N_2 gas was passed through the reactor about 1-2 h following feeding to avoid penetration of oxygen. Reactor was mixed with magnetic stirrer in the anoxic cycle. After passing N_2 gas through the reactor, reactor was tightly stoppered to avoid the penetration of oxygen. After anoxic stage, reactor was aerated and mixed by using air pumps, similar to other aerobic reactors. During the anoxic cycle of reactor, reactor was operated at room temperature (about

20°C), whereas, in the aerobic cycle, reactor was operated in a water-bath to keep temperature around 25°C. It was not possible to carry the anoxic experiments at 25 °C due to absence of mixing facility in water-bath system.

At different concentrations of chlorophenols (20-50 mg/L 4-CP and 10-27 mg/L 2,4-DCP) and in the presence of peptone as a readily degradable substrate, effect of chlorophenols on the removal of COD and NO_3^- uptake rate was examined in batch reactors. In addition to reactors receiving chlorophenols at different concentrations, reactor devoid of chlorophenol (base-line reactor) was also operated. Total volume of each batch reactor was 300 mL with the 250 mL of working volume. N_2 gas was passed through the reactors for the first 6-8 h to mix reactors and avoid the penetration of oxygen, then reactors were tightly stoppered and mixing operation was carried out in a shaker at about 200 rpm. Samples were taken from the batch reactors at different time intervals for the analysis of NO_3-N, COD and chlorophenol concentrations. At the end of experiments, samples were also taken for the analysis of MLVSS.

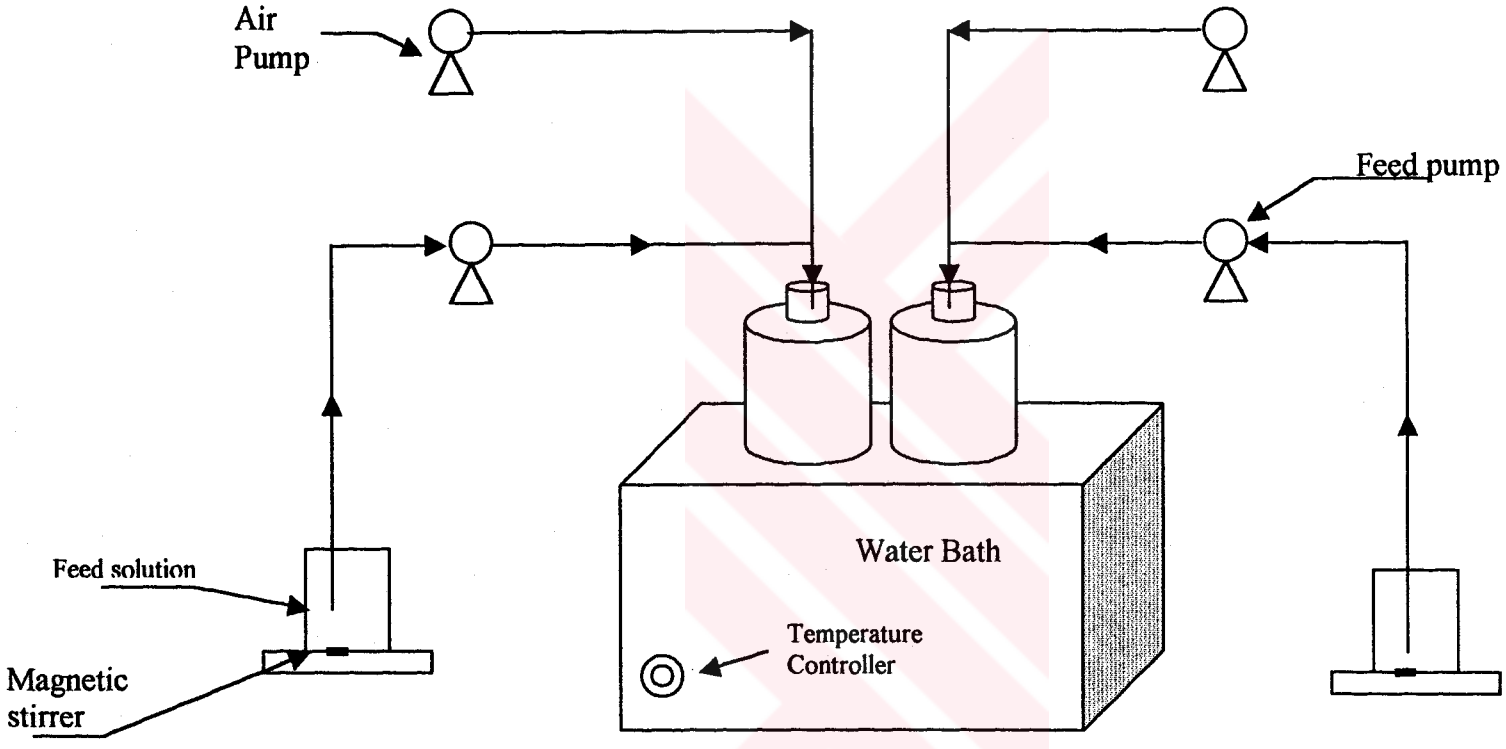


Figure 2.2. Experimental set-up of aerobic SBRs

2.4. Analytical Methods

The MLSS concentrations were determined by passing the samples through 0.45μm pore size of filters and drying at 105°C for 1 h. MLVSS concentrations were determined by ignition of filter at 600 °C for 30 min. OD was measured using Baush and Lamb Spectronic 20 spectrophotometer at 550 nm. MLSS and MLVSS concentrations were then followed using the calibration curves (Figures A1 and A2 (Appendix A)).

Samples were centrifugated prior to COD analysis and COD measurements were carried out according to the EPA approved reactor digestion method as given in Hach Water Analysis Handbook (1988). In this method, range of COD analyses is between 0-1500 mg/L. After 2 h digestion, COD values of samples were directly read using Hach Spectrophotometer (Model No 45600-02, Cole Parmer Insrument Co., USA).

For NO_3-N measurements, Nitra Ver 5 Nitrate Reagent Powder Pillows (HACH Company Cat. No. 14034-99) for high range NO_3-N measurements (0-30 mg/L NO_3-N) were used. Powder pillows were added to centrifugated samples and NO_3-N concentration were read at 500 nm using Hach Spectrophotometer (Model No 45600-02, Cole Parmer Insrument Co., USA). Actual NO_3-N concentrations were found out using calibration curve given in Figure A 3 (Appendix A).

Chlorophenol concentrations in batch reactors inoculated with unacclimated culture were measured using Direct Photometric Method (*Standard Methods,* 1995). In this method phenolic compounds react with 4-aminoantipyrine in the presence of potassium ferricyanide to form a colored antipyrine dye. Samples were centrifugated prior to chlorophenol analysis. Absorbance of colored samples was measured at 500 nm. Calibration curves used to determine the concentrations of

2-CP, 4-CP and 2,4-DCP were given in Figs A 4, A 5 and A 6 (Appendix A), respectively.

4-CP and 2,4-DCP concentrations during the batch experiments with acclimated cultures and fed-batch reactors were measured using High Performance Liquid Chromatography (HPLC) (Shimadzu, LC-10AT vp) which is equipped with Nucleosil C18 column (4.6 mm ×250 mm), LC-10Atvp solvent delivery module, an SCL-10Avp system controller, a SPD-10Avp UV-VIS detector set at 280 nm. Retention times for 4-CP and 2,4-DCP were 6.67 and 10.8 minutes, respectively. Solvent used in the analyses was methanol (60 %), pure water (38 %) and acetic acid (2 %) at the flow rate of 1ml /min. Injection volume of sample was 20μL. Calibration curves for the measurement of 4-CP and 2,4-DCP are given in Figures A 7 and A 8, respectively.

In addition, influent and effluent samples of fed-batch reactors were also tracked using UV / VIS spectrophotometer (Varian, Cary 100 conc.). Centrifugated samples were scanned from 190 to 500 nm.

CHAPTER 3

RESULTS AND DISCUSSION

In this chapter, the results of aerobic batch experiments with acclimated and unacclimated culture, batch experiments under anoxic conditions, aerobic fed-batch reactors and SBRs experiments are presented and discussed.

3.1. Batch Experiments Under Aerobic Conditions

In the presence of a readily degradable substrate (peptone) and 2-CP, 4-CP and 2,4-DCP at various concentrations, batch experiments were conducted to determine the toxic effect of chlorophenols on unacclimated culture. Effects of chlorophenols on % COD removal efficiency, SA and Y values of unacclimated activated sludge microorganisms were sought. Experiments were carried out both for reactors receiving chlorophenol at different concentrations and reactor devoid of chlorophenol (base-line reactor). Kinetic parameters determined for base-line study were compared to reactors receiving chlorophenols of various concentrations. IC_{50} values (concentration of chlorophenol at which the value of examined kinetic parameter decrease to half of its original value) were determined both for acclimated and unacclimated culture.

During incubation of batch reactors, samples were taken from the reactors for measurements of biomass, COD and chlorophenol concentrations at various time intervals.

Also, batch experiments with unacclimated culture, where 4-CP and 2,4-DCP serve as sole organic carbon source were carried out to determine whether these compounds can be used as sole organic carbon source and effect of a readily degradable substrate on the overall performance of chlorophenol removal.

After acclimation of culture to 4-CP and 2,4-DCP, as in the case of unacclimated culture, batch experiments were carried out with acclimated culture to understand the effect of acclimation on the toxicity of chlorophenols.

3.1.1. Base-Line Study

Biomass concentrations measured as OD and organic matter concentrations measured as COD at various time intervals for the base-line reactor inoculated with unacclimated culture are given in Figure 3.1. This figure shows that stationary phase was reached within 10 h with 4 h of lag period. The COD removal efficiency was found to be 64 %. Y and SA values were found to be 0.468 mg MLVSS / mg COD and 0.203 mg COD/mg MLVSS.h, respectively.

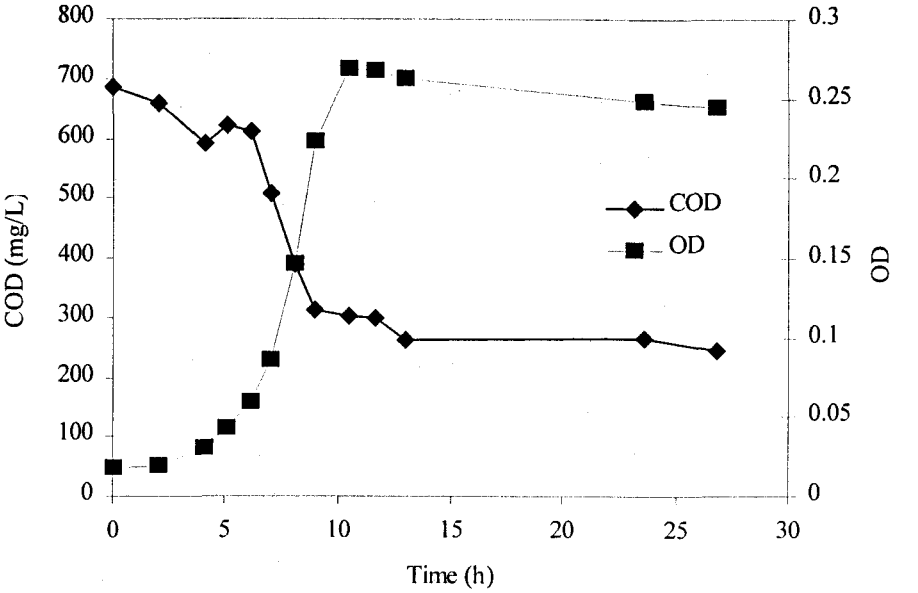


Figure 3.1. OD and COD values with time for base-line reactor

The value of μ_m was determined by plotting ln OD against time for the exponential phase of growth. As seen from Figure 3.2, μ_m for base-line reactor was determined as 0.3671 h^{-1}.

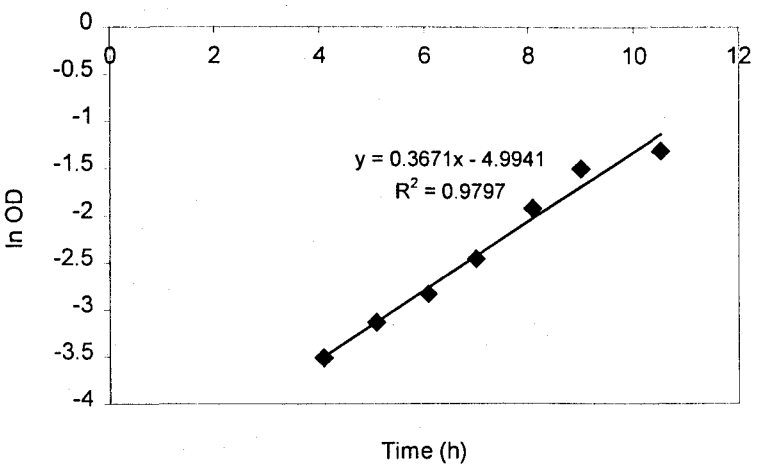


Figure 3.2. ln OD with time for base-line reactor.

3.1.2. Batch Experiments with Unacclimated Culture

In this section, results of batch experiments conducted with unacclimated culture in the presence of peptone as a readily degradable substrate and chlorophenols at different concentrations are presented.

3.1.2.1. Effect of 2-CP on Unacclimated Culture

Effects of 2-CP on the values of μ_m, Y, SA and the removal efficiencies of COD and chlorophenol were examined in batch reactors. Varying concentrations of 2-CP (30, 70, 120 and 300 mg/L) and peptone (about 500 mg/L COD) were added into batch reactors inoculated with unacclimated culture taken from fed-batch reactor operated at 8 days of SRT. Samples were drawn from reactors for the measurement of biomass, COD and 2-CP concentrations at various time intervals. Time course variations of these parameters are presented in Figures B.1-B.4 (Appendix B). In order to find out the amount of chlorophenol removed by volatilization, blank reactors without biomass seed were operated at the same operational conditions.

Lag period for reactor receiving 30 mg/L 2-CP was observed as 4 h (Figure B.1), while it was 7 and 10 h for the concentration of 70 and 300 mg/L 2-CP (Figures B.2 and B4), respectively. These results show that longer lag periods at higher concentrations of 2-CP were required due to toxic effect of 2-CP. Based on the data presented in Figures B.1-B.4, the biochemical constants, μ_m and Y were calculated for each 2-CP concentration studied. The results obtained are presented in Table 3.1. This table shows that μ_m values decreased with increasing concentrations of 2-CP. The percent inhibitions on μ_m values at different concentrations of 2-CP were calculated assuming that there is no inhibition on base-line reactor. Figure 3.3 shows that % inhibition of 2-CP increased with increasing concentration of 2-CP. It can also be depicted from this figure that IC_{50} value for 2-CP is 230 mg/L.

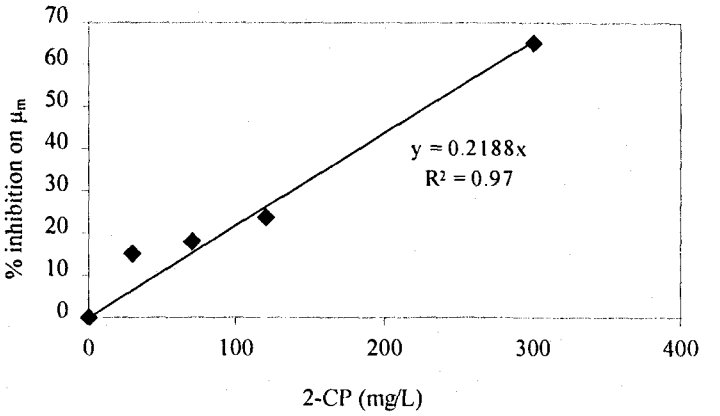


Figure 3.3. % Inhibition of 2-CP on μ_m

Table 3.1. Results of Reactors Receiving Different Concentrations of 2-CP

2-CP (mg/L)	% COD removal	Yield (mg MLVSS / mg COD)	μ_m (h^{-1})
0 (base-line)	64	0.468	0.367
30	64	0.414	0.311
70	57	0.307	0.305
120	59	0.418	0.279
300	35	0.359	0.128

Effect of a toxic compound on the % COD removal efficiency is one of the most important considerations because major aim of treatment is to remove organic matter from wastewater. Table 3.1 shows that % COD removal efficiency decreased with increasing 2-CP concentration. The percent inhibitions on the % COD removal efficiencies can be seen in Figure 3.4. As shown, COD removal efficiency at 230 mg/L 2-CP was inhibited by 32 %, whereas, at the same 2-CP concentration, μ_m

value of the culture decreased by 50 %. Therefore, it can be said that μ_m is more sensitive parameter to 2-CP compared to % COD removal efficiency.

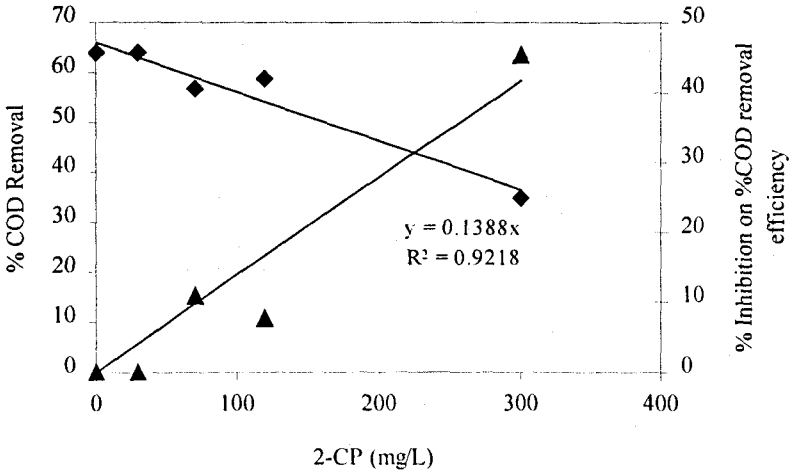


Figure 3.4. % COD Removal at different 2-CP Concentrations
(♦, % COD removal; ▲, % inhibition on COD removal efficiency)

Figure 3.5 presents the Y values at different 2-CP concentrations. As can be seen from this figure, the value of Y first decreased sharply with the addition of 2-CP, however, at higher concentrations, there was relatively less variation in Y values with 2-CP concentration.

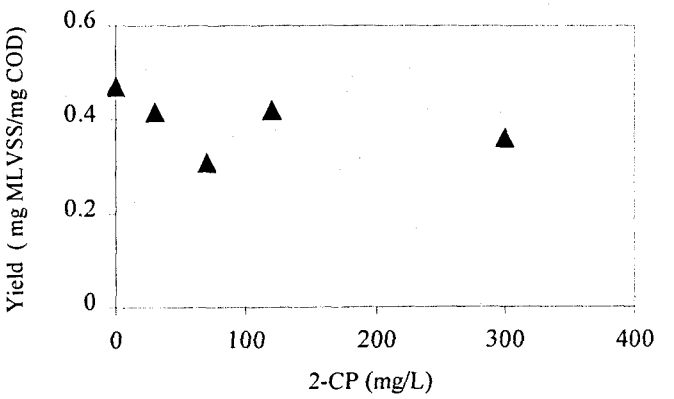


Figure 3.5. Y values at different 2-CP concentrations

It can be seen from Figure 3.6 that SA values increased up to concentration of 70 mg/L and beyond this point, it decreased as the 2-CP concentration increases. These results revealed that COD removal per unit biomass increased up to concentration of 70 mg/L 2-CP, however, it then decreased at higher concentrations of 2-CP due to toxic effect of 2-CP.

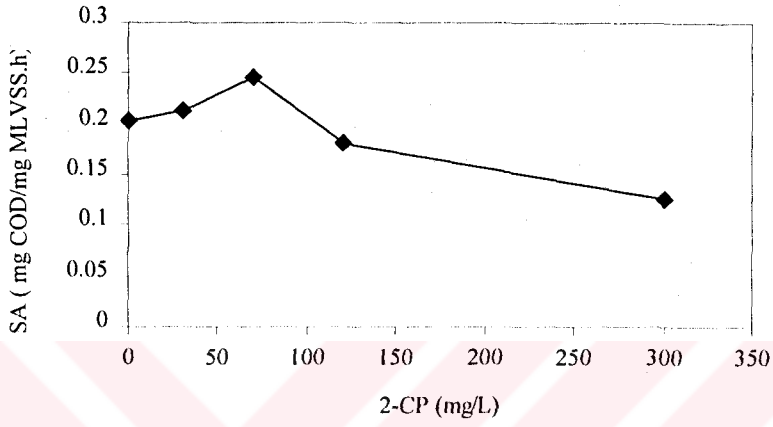


Figure 3.6. SA values at different 2-CP concentrations

No removal of 2-CP was observed at all concentrations examined (Figures B.5.1-3) and the data for blank reactors indicated that volatilization of 2-CP was negligible.

3.1.2.2. Effect of 4-CP on Unacclimated Culture

The time course variations of biomass as OD and substrate concentration as COD at different concentrations of 4-CP (57, 112, 155 and 274 mg/L) are given in Figures B.6-9.

The lag phase for reactor receiving 57 mg/L 4-CP was observed to be 5 h (Figure B.6), which is equal to that for the base-line reactor. This shows that at the concentration of 57 mg/L, there was no adverse effect on the growth of biomass. However, at the concentration of 112 mg/L, the lag phase increased to 7h; and at higher concentrations of 4-CP, a lag phase of 10 h was observed. Figures B.6-9

indicates a slight effect of increasing 4-CP concentration on the growth of unacclimated culture.

The values of μ_m at different 4-CP concentrations were calculated using the data corresponding to the exponential phases of growths (Figures B.6-9). Calculated μ_m values were then plotted against 4-CP concentrations and Figure 3.7 was obtained. As can be seen, μ_m values for the reactors receiving various concentrations of 4-CP decreased with increasing concentrations of 4-CP. IC_{50} value for 4-CP on the basis of μ_m appeared to be 130 mg/L which is comparably smaller than that for 2-CP (230 mg/L). This finding revealed that 4-CP is more toxic to unacclimated sludge biomass than 2-CP on the basis of μ_m.

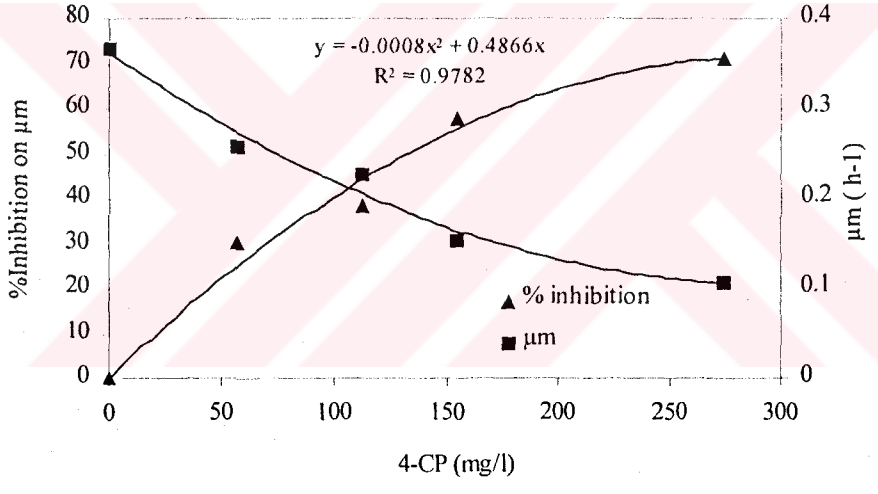


Figure 3.7 % Inhibition of 4-CP at various concentrations on μ_m

The percent COD removal efficiencies and toxic effects of 4-CP on % COD removal are shown in Figure 3.8. The percent COD removal efficiency was not affected from 4-CP till the 57 mg/L, however, beyond this point a sharp decrease in the % COD removal efficiency was observed. The % COD removal efficiency decreased from a base-line value of 64 % to 35 % at the concentration of 155 mg/L

4-CP. Further increase in 4-CP concentration resulted in almost no change in % COD removal.

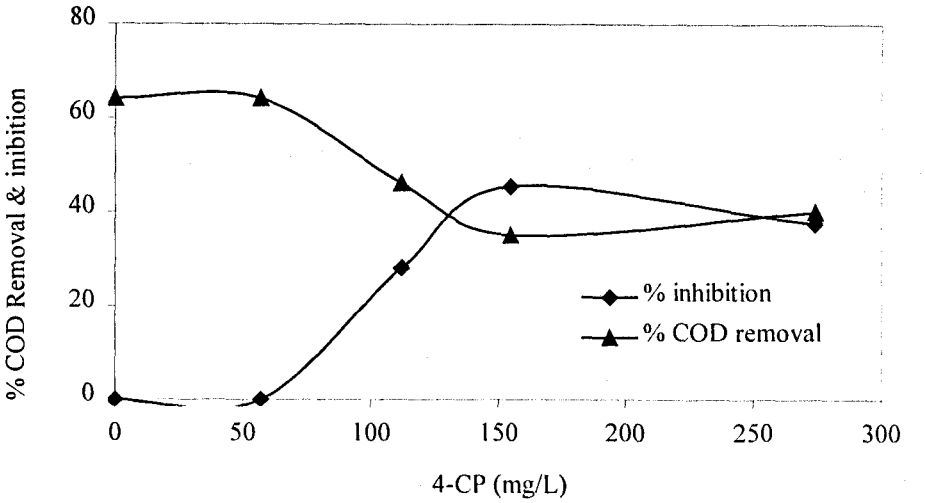


Figure 3.8. % COD Removal at various 4-CP Concentrations

Using the data presented in Figures B.6-9, the values of Y were calculated and the effect of 4-CP concentration on the Y values was sought. It was observed that the Y values increased slightly with addition of 4-CP up to a concentration of 155 mg/L and a sharp decrease was then observed with an increase in 4-CP concentration to 274 mg/L (Figure 3.9). Therefore, it can be said that biomass production per unit of COD increased up to concentration of 155 mg/L, whereas, further this point a higher portion of COD was used for maintenance rather than biomass production due to toxic effect of 4-CP at 274 mg/L.

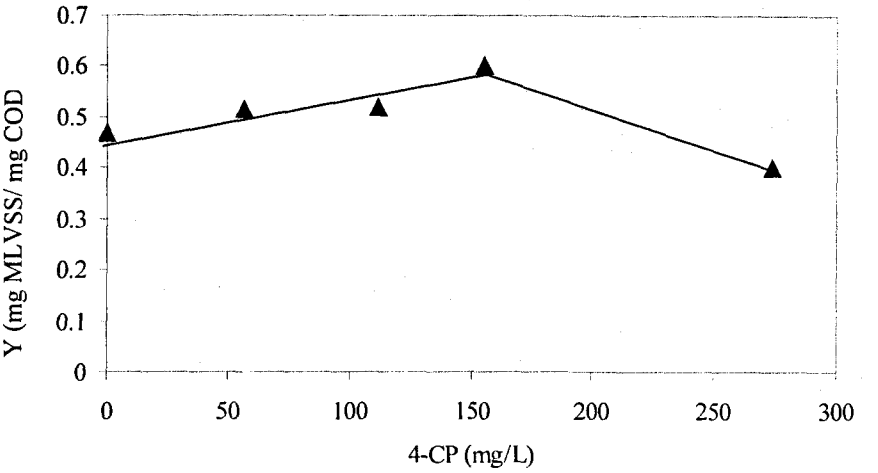


Figure 3.9. Y at various concentration of 4-CP

Effect of 4-CP on the values of SA values can be seen from Figure 3.10. As can be seen from this figure, the SA values decreased up to 112 mg/L of 4-CP, and a slight increase was then observed when 4-CP concentration was increased to 274 mg/L. This increase is, in fact, an expected trend considering almost the same % COD removal values attained and decreasing Y values when 4-CP concentration was increased from 155 to 274 mg/L.

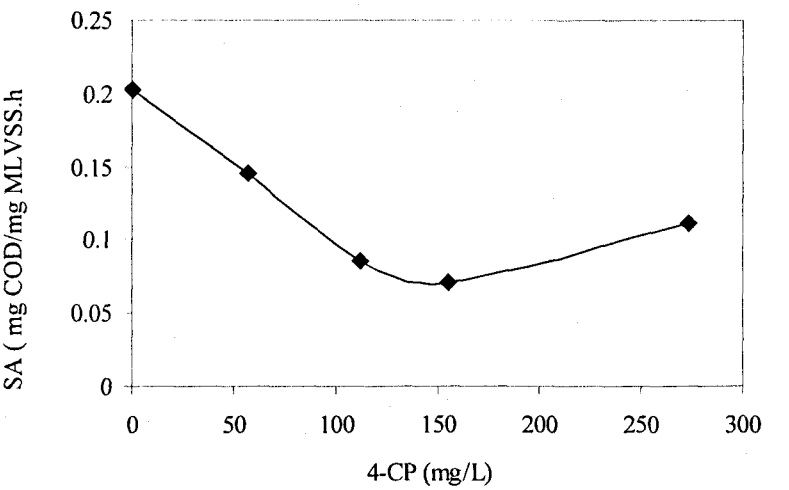


Figure 3.10. SA values at different 4-CP concentrations

Biological removal of 4-CP was also followed and results presented in Figures B.10.1-10.4 were obtained. As seen, no detectable biological removal of 4-CP was observed throughout the experiments with unacclimated culture. Data for control reactors revealed that volatilization of 4-CP was also negligible.

3.1.2.3. Effect of 2,4-DCP on Unacclimated Culture

The same experiments conducted for 2- and 4-CP were also carried out for 2,4-DCP. Variations of OD and COD values with time are given in Figure B.11-14 (Appendix B) for reactors receiving various concentration of 2,4-DCP (22, 47.5, 77 and 100 mg/L) and observed kinetic constants at different concentrations of 2,4-DCP are given in Figures 3.11-14.

The lag periods of the microorganisms at the concentration of 22 and 77 mg/L 2,4-DCP were observed to be 5 and 10h (Figs B.11 and B.13), respectively, whereas, almost no growth was observed within 27 h when concentration of 2,4-DCP was increased to 100 mg/L (Figure B.14). On the other hand, even at the concentration of 300 mg/L 2-CP and 274 mg/L 4-CP, only 10 h lag period was observed. This shows that even at low concentrations of 2,4-DCP, growth of unacclimated culture was affected adversely and toxic effect of 2,4-DCP on the basis of biomass growth was much higher than those of examined mono-chlorophenols (2-CP and 4-CP).

The values of μ_m at different 2,4-DCP concentrations were calculated using the data corresponding to the exponential phases of growth curves. The percent inhibitions were calculated on the basis of depression on μ_m value of base-line study. Figure 3.11 depicts μ_m and % inhibition on the values of μ_m at various concentrations of 2,4-DCP. Figure 3.11 shows that there is a good correlation between 2,4-DCP concentrations and % inhibition values and IC_{50} value for 2,4-DCP on the basis of μ_m was determined as 72 mg/L.

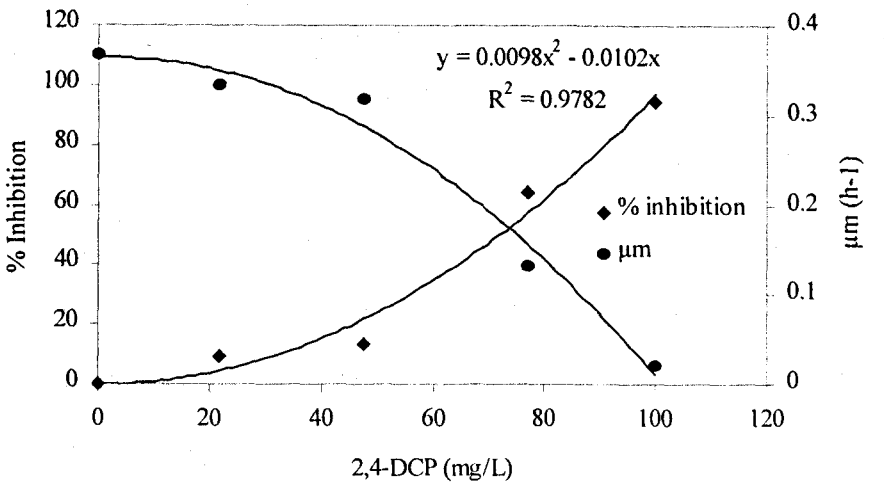


Figure 3.11. % Inhibition of 2,4-DCP at various concentrations on μ_m

The percent COD removal efficiencies at various 2,4-DCP concentrations are presented in Figure 3.12. It can be seen from this figure that at the concentrations of 22 and 47.5 mg/L 2,4-DCP the % COD removal efficiencies decreased from a base-line value of 64 % to 44 and 36 %, respectively. However, in case of 4-CP the % COD removal efficiency was not affected adversely at the concentration of 57 mg/L 4-CP, and in case of 2-CP the % COD removal efficiency decreased to 35 % at the concentration of 300 mg/L 2-CP. Then it can be inferred that even at the low concentrations of 2,4-DCP, the % COD removal efficiency was adversely affected. The percent inhibitions of 2,4-DCP at various concentrations on the basis of % COD removal efficiency can also be seen in Figure 3.12. As can be seen from this figure, the % inhibition on the % COD removal efficiency increased with increasing concentrations of 2,4-DCP up to 77 mg/L, however, at the concentration of 100 mg/L 2,4-DCP, observed % inhibition on the COD removal efficiency was similar to that at the concentration of 77 mg/L 2,4-DCP. Although, almost no biomass growth was observed (Figure B.14), 28 % COD removal efficiency was observed at the concentration of 100 mg/L. The reason of this may be speculated as that the biomass channeled COD into the energy for maintenance but not for growth. The value of IC_{50} on the basis of COD removal efficiency was found to be 60 mg/L, which is slightly lower than that observed on the basis of μ_m. Although μ_m

decreased with increasing concentrations of 2,4-DCP, the % COD removal efficiency reached a plateau level after 75 mg/L 2,4-DCP.

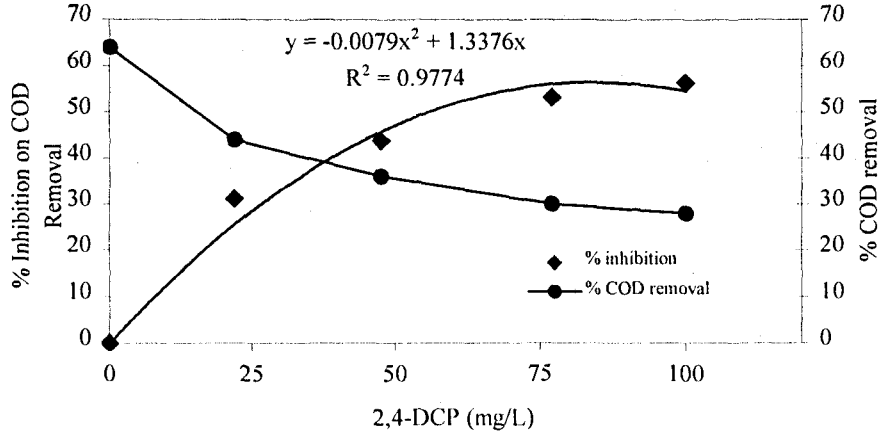


Figure 3.12. % COD Removal at various 2,4-DCP Concentrations

Using the data presented in Figures B.11-14, the values of Y were calculated. The variations of Y with 2,4-DCP concentrations are presented in Figure 3.13. This Figure shows that the Y values up to concentration of 77 mg/L 2,4-DCP surprisingly increased to very high level and the value of Y at the concentration of 100 mg/L 2,4-DCP was so small that almost complete inhibition can be assumed. The reason of increased Y values may be due to the fact that energy gained from utilized substrate was channeled into biomass growth rather than maintenance up to concentration of 77 mg/L 2,4-DCP, whereas, opposite was observed at 100 mg/L 2,4-DCP.

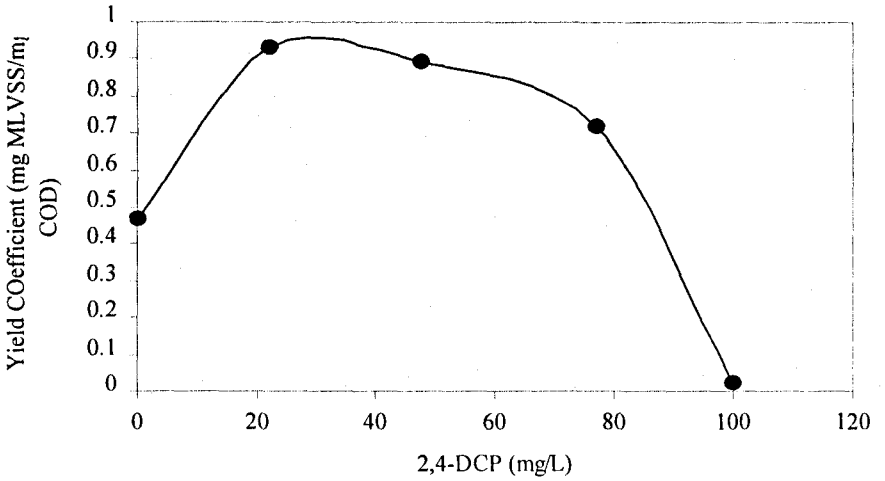


Figure 3.13. Y at various 2,4-DCP concentrations.

The values of % inhibition on SA increased linearly with increasing concentration of 2,4-DCP and the value of IC_{50} on the basis of SA was found as 47 mg/L (Figure 3.14), which is smaller than observed IC_{50} values on the basis of the values of % COD removal efficiency and μ_m. This shows that the SA is more sensitive parameter to 2,4-DCP compared to μ_m and % COD removal efficiency. Decreased SA values with 2,4-DCP concentrations also confirmed channeling of energy mainly for maintenance. As presented above, three different IC_{50} values were observed on the basis of different kinetic parameters.

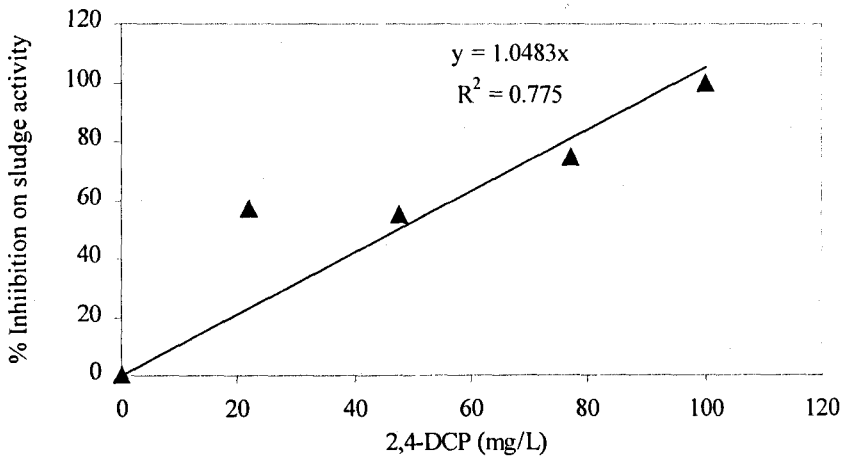


Figure 3.14. % Inhibition on SA at various Concentrations of 2,4-DCP

Figures B.15.1-4 revealed that no removal of 2,4-DCP throughout study was observed and blank reactors showed that physical loss of 2,4-DCP was negligible.

IC_{50} values found on the basis of μ_m for 2-CP, 4-CP and 2,4-DCP are given in Table.3.2.

Table 3.2. Observed IC_{50} values on the basis of μ_m for Unacclimated Activated Sludge Biomass

Compound	Observed IC_{50} Values (mg/L)
2-CP	230
4-CP	130
2,4-DCP	72

As seen from this table, toxicity of examined chlorophenols on the basis of μ_m value is following the order of 2,4-DCP > 4-CP > 2-CP. It can then be inferred that both the number of chlorine and its position is important in toxicity determination of chlorophenols.

3.1.2.4. Experiments with Unacclimated Culture for which Chlorophenols Serve as Sole Organic Carbon Source

In this set of experiments, batch reactors were operated in the presence of the chlorophenols serving as sole organic carbon source. 4-CP (26 and 130 mg/L) and 2,4-DCP (25 and 50 mg/L) were added into the growth media as both energy and carbon source. Other inorganic matters and nitrogen in the form of NH_4CI was added not to limit the growth of biomass.

As stated earlier, control reactors showed that physical removal of 4-CP and 2,4-DCP was negligible. It was observed that unacclimated biomass is able to use 4-CP as a sole organic carbon source (Figures 3.15, 3.16). It was also shown that 4-CP removal within 5 days was nearly complete and beyond this point no significant removal of chlorophenol was observed. Chlorophenol removal at the concentration of 26 mg/L 4-CP started without lag phase, however, chlorophenol removal at the concentration of 130 mg/L of 4-CP started after 1 day. 4-CP removals at initial concentrations of 26 and 130 mg/L within 18 days were determined to be 44 and 30 %, respectively. Hence, it can be stated that % removal of 4-CP decreases with increasing concentration of 4-CP.

It was interesting to observe that 4-CP could be removed when it exists alone, but not together with peptone. It was attributed to the easier biodegradation of peptone as compared to 4-CP and hence preference of peptone by the microorganisms, as expected.

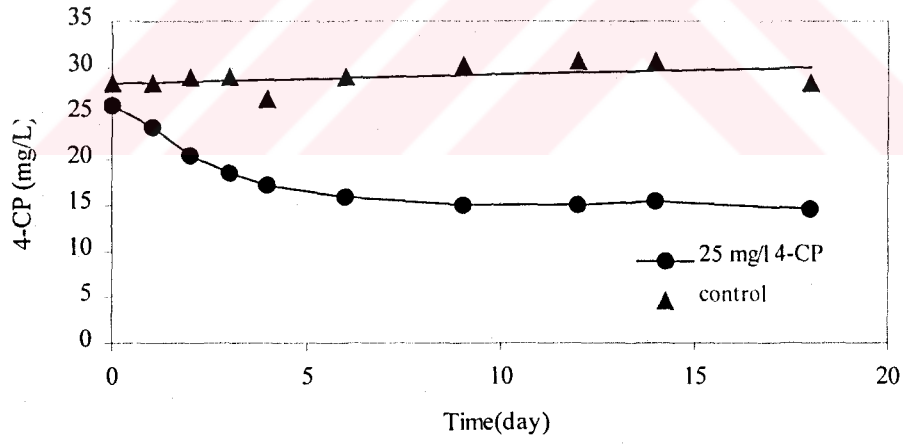


Figure 3.15. Concentration of 4-CP with time for reactor where 4-CP was used as sole organic carbon source (25 mg/L 4-CP)

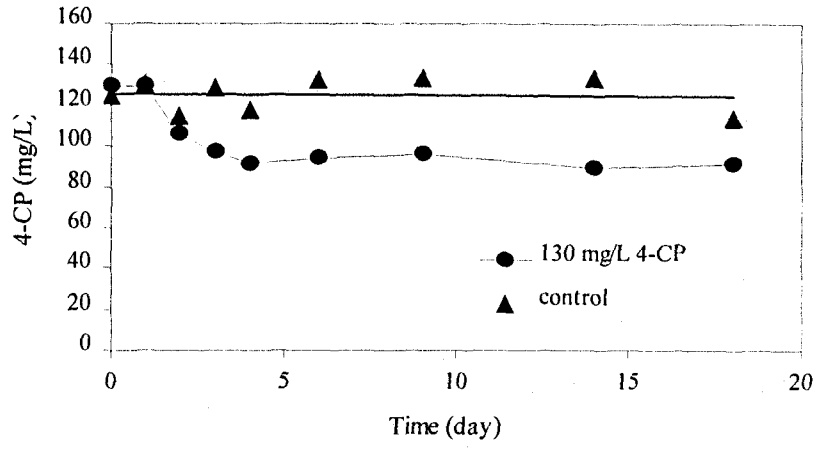


Figure 3.16. Concentration of 4-CP with time for reactor where 4-CP was used as sole organic carbon source (130 mg/L of 4-CP)

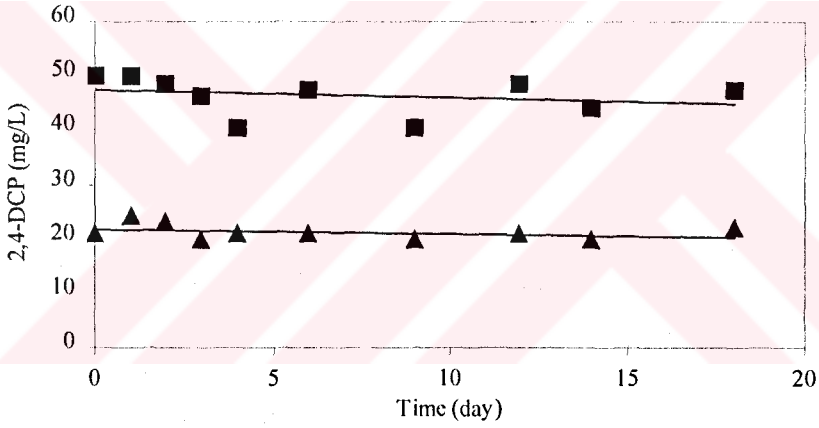


Figure 3.17. Concentration of 2,4-DCP with time for reactors where 2,4-DCP was used as sole organic carbon source (▲, 25 mg/L; ■, 50 mg/L)

However, unlike 4-CP, no removal of 2,4-DCP by unacclimated culture of microorganisms was observed when present as a sole organic carbon source at the initial concentrations of 25 and 50 mg/L 2,4-DCP (Figure 3.17).

3.1.3. Batch Experiments with Acclimated Culture

Results of batch experiments conducted with culture acclimated to 4-CP and 2,4-DCP are presented in this section. Acclimated culture experiments were not carried out for 2-CP due to observed low toxicity on unacclimated culture. Base-line reactors were operated at the same conditions with other reactors, except the addition of chlorophenols to growth medium. Figures B.16-1 and 16.2 show the time course variations of biomass as OD and substrate concentrations as COD for base-line reactors inoculated with 4-CP and 2,4-DCP acclimated culture, respectively.

3.1.3.1. Effect of 4-CP on Acclimated Culture

Batch experiments were conducted with 4-CP acclimated culture in the presence of peptone as readily biodegradable substrate and various concentrations of 4-CP (130, 200, 300 and 390 mg/L). Culture was acclimated to 130 mg/L, which was the IC_{50} level for unacclimated culture on the basis of μ_m. As stated in Section 2.3.1.2, acclimation was realized in fed-batch reactors and during the acclimation, stepwise increments of 4-CP concentration were carried out. Time course variations of biomass as OD and substrate concentrations as COD were followed for reactors at various concentrations of 4-CP (Figures B.17-20).

It was observed that the lag phase varying from 5-10 h depending on the 4-CP concentrations for unacclimated culture (Figures B.6-9) disappeared when culture was acclimated to 4-CP (Figures B.17-20). Batch growth studies showed that at the concentrations of 130 and 200 mg/L of 4-CP, stationary phase was reached within 24 h, however, at the concentration of 300 mg/L of 4-CP, stationary phase was reached within 2 days for acclimated culture due to toxic effect of 4-CP at high concentrations.

The values of μ_m, % COD removal and SA at various 4-CP concentrations are presented in Table 3.3 for acclimated culture. The values of these parameters obtained for unacclimated culture are also included in this table for the purpose of comparison.

As can be seen from Table 3.3, the μ_m values decreased with increasing concentrations of 4-CP for both acclimated and unacclimated cultures. The μ_m value at the concentration of 300 mg/L 4-CP decreased from a base-line value of 0.131 to 0.042 h^{-1} when acclimated culture was used, whereas, the μ_m value at the concentration of 274 mg/L 4-CP decreased from a base-line value of 0.367 to 0.106 h^{-1} when unacclimated culture was used. As can be seen from these results the μ_m values of acclimated culture were smaller than those of unacclimated culture. The reason of this can be attributed to the fact that new culture was generated following acclimation and the new culture had low growth rate compared to the mixed unacclimated activated sludge culture.

Table 3.3. Results of Batch Experiments at various concentrations of 4-CP inoculated with 4-CP Acclimated and Unacclimated Culture

	4-CP ($mg\ l^{-1}$)	% COD removal	SA ($mg\ COD(mg\ MLVSS.h)^{-1}$)	μ_m (h^{-1})
4-CP Acclimated culture	0	62	0.094	0.131
	130	74	0.0677	0.073
	200	67	0.0631	0.070
	300	72	0.0508	0.042
	390	24	0.0344	0.020
Unacclimated Culture	0	64	0.203	0.367
	57	64	0.145	0.256
	112	46	0.085	0.226
	155	35	0.071	0.154
	274	40	0.112	0.106

The percent inhibitions observed on the μ_m values of acclimated culture is shown in Figure 3.18. IC_{50} value on the basis of μ_m was found to be 218 mg/L with 4-CP acclimated culture (Figure 3.18), whereas, it was 130 mg/L when unacclimated culture was used (Figure 3.7). These results show that after acclimation the toxicity of 4-CP on the μ_m values decreased by % 68.

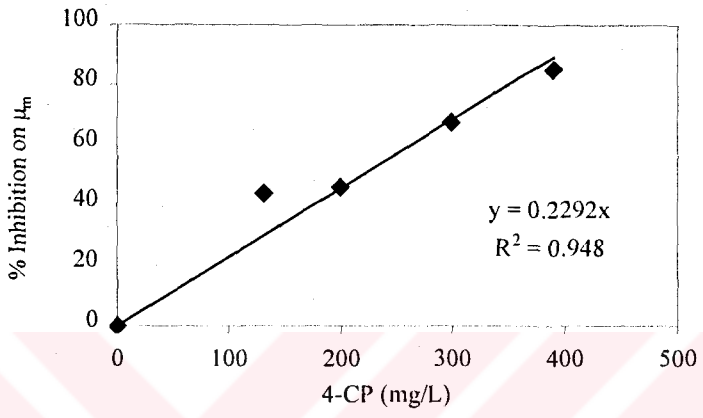


Figure 3.18. % Inhibition of 4-CP at various concentrations on μ_m values

Table 3.3 shows that the percent COD removal efficiencies remained constant up to the concentration of 300 mg/L 4-CP and observed % COD removals at the concentrations of 130, 200 and 300 mg/L of 4-CP were slightly higher than that observed for base-line reactor (62 %) when acclimated culture was used. However, the COD removal efficiency decreased from a base-line value of 64 to 35 % at the concentration of 155 mg/L 4-CP when reactors were inoculated with unacclimated culture. These results show that culture acclimated to 130 mg/L of 4-CP was not affected adversely from 4-CP up to concentration of 300 mg/L, whereas a remarkable decrease was observed at higher concentration of 4-CP on the basis of COD removal efficiency. Although culture was acclimated to 130 mg/L 4-CP, they could tolerate 2.3 times higher concentration of 4-CP on the basis of % COD removal efficiency. These results revealed that acclimation of culture established a microbial consortium with improved ability to tolerate toxic effect of 4-CP on the basis of % COD removal.

Effect of 4-CP concentration on the Y values of acclimated culture is shown in Figure 3.19. This figure shows that the value of Y for base-line study was determined as 0.96 mg MLVSS / mg COD, whereas, for reactors receiving 4-CP at various concentrations, it was found to show variation between 0.40-0.60 mg MLVSS / mg COD for acclimated culture. Although addition of 4-CP caused a sharp decrease, Y values for reactors receiving 4-CP of various concentrations remained constant even at high 4-CP concentrations for acclimated culture. However, the values of Y had increased slightly with addition of 4-CP up to the concentration of 155 mg/L and a sharp decrease had been observed at the concentration of 274 mg/L of 4-CP for unacclimated culture (Figure 3.9).

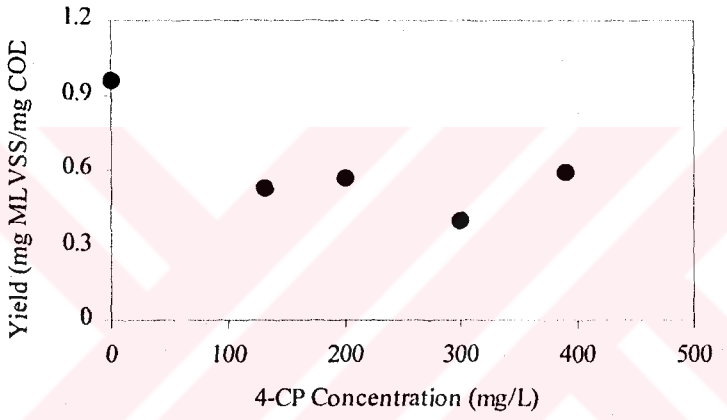


Figure 3.19. Effect of 4-CPat various concentrations on Y

Table 3.3 shows that the SA values decreased with increasing concentration of 4-CP for acclimated culture and IC_{50} value on the basis of SA was found as 307 mg/L (Figure 3.20), whereas, at the concentration of 112 mg/L 4-CP, 55 % inhibition on the SA had been observed for unacclimated culture (Figure 3.10). This shows that IC_{50} value on the basis of SA increased about three times with acclimation, whereas IC_{50} on the basis of μ_m increased about 1.7 times with acclimation. However, Vallecillo *et al.* (2000) reported similar toxicity values on the basis of SA for both acclimated and unacclimated culture.

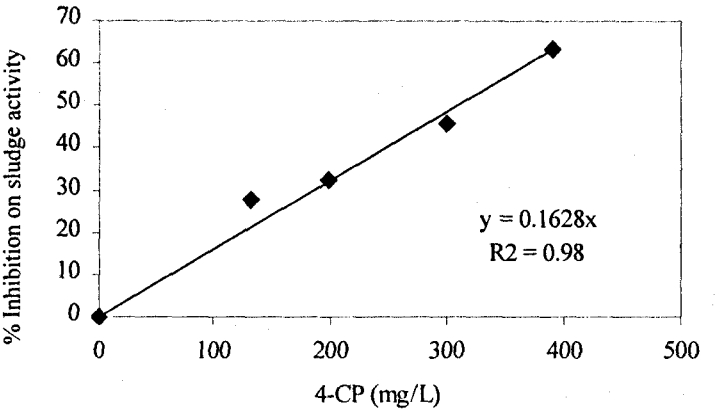


Figure 3.20. % Inhibition of 4-CPat various concentrations on SA

Figure 3.21 depicts time course variation of 4-CP for reactors receiving different concentrations of 4-CP with acclimated culture. As seen from the figure that 100 % 4-CP removal is possible at the initial 4-CP concentration of 130 mg/L within 1 day when acclimated culture was used as inocula. About 80 % removal was observed in reactor receiving 200 mg/L of 4-CP within 1 day and complete removal was achieved within 2 days. Although 100 % of 130 mg/L and 80 % of 200 mg/L of 4-CP could be degraded within 1 day, no remarkable degradation of 4-CP at the concentration of 300 mg/L was observed within 1 day. However, following the 1^{st} day of incubation, 4-CP removal rate increased sharply and 100 % removal of 4-CP was achieved at the end of 2^{nd} day. This was attributed to the fact that culture was acclimated to 130 mg/L 4-CP in fed-batch reactors; therefore, longer time was required for adaptation to the higher 4-CP concentrations. 4-CP degradation was not observed within 76 h at the concentration of 390 mg/L 4-CP. This shows that culture acclimated to 130 mg/L 4-CP could degrade 4-CP up to 300 mg/L. In order to remove 4-CP higher than 300 mg/L, culture should be acclimated to higher concentrations of 4-CP. Although effective removal of 4-CP (Figure 3.21) was observed up to 300 mg/L 4-CP using acclimated culture, no removal of 4-CP had been observed even at 57 mg/L 4-CP with unacclimated culture (Figures B.10.1-4).

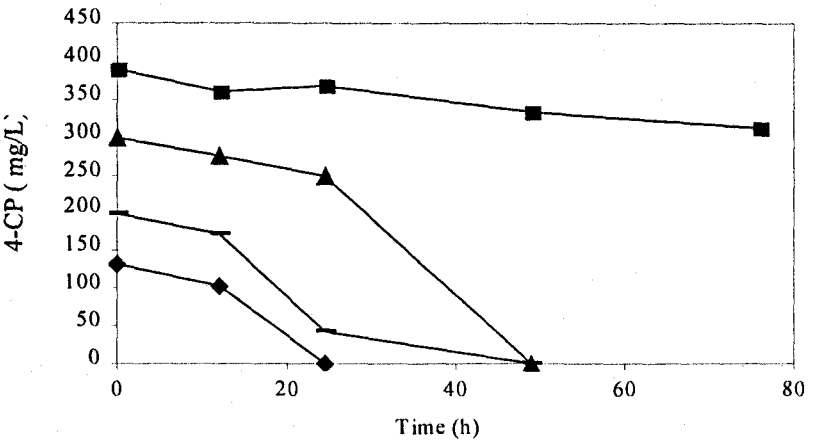


Figure 3.21. 4-CP Concentrations with Time for Batch Reactors Receiving Different Concentrations of 4-CP (♦, 130 mg/L; -, 200 mg/L; ▲, 300 mg/L; ■, 390 mg/L)

Figure 3.22 depicts the HPLC results of influent and 2nd day effluent samples of batch reactor receiving 200 mg/L 4-CP. In this figure, HPLC result for base-line reactor is also given for the purpose of comparison. As seen from the figure, almost complete removal of 4-CP within 2 day was achieved (>99.8%) and comparison of HPLC result of effluent sample with those of influent and base-line reactor revealed that no intermediate compound was produced due to removal of 4-CP.

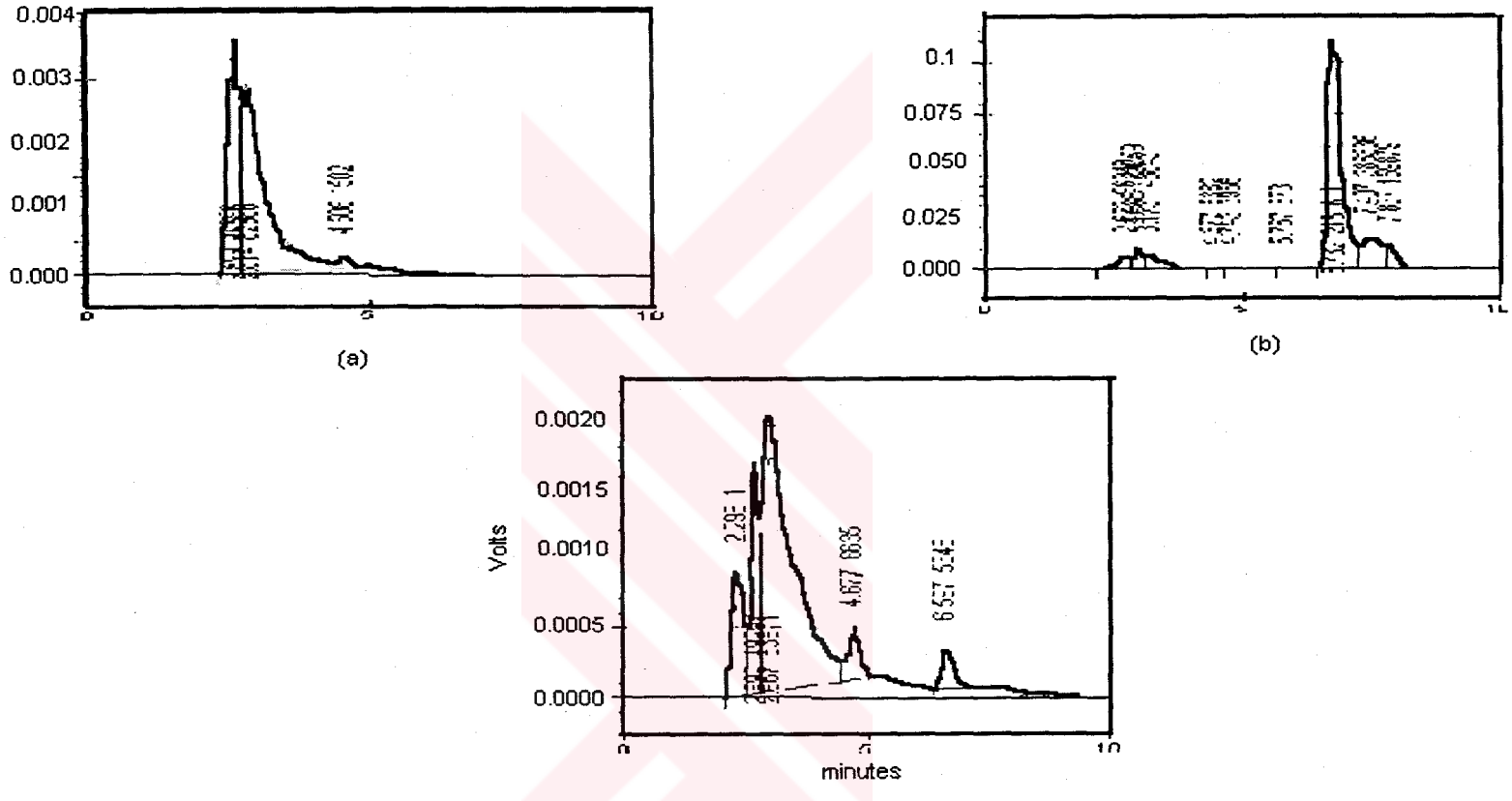


Figure 3.22. HPLC results for (a) base-line (b)influent of reactor receiving 200 mg/L (c) 2nd day effluent of reactor receiving 200 mg/L 4-CP

3.1.3.2. Effect of 2,4-DCP on Acclimated Culture

Batch experiments were conducted with 2,4-DCP acclimated culture in the presence of peptone (500 mg COD/L) as readily biodegradable substrate and 2,4-DCP at various concentrations (76.6, 101.2, 148.7 and 195 mg/L). Time course variations of OD and COD concentration of reactors receiving various concentrations of 2,4-DCP are given in Figure B.21-24 (Appendix B).

In this set of experiments culture was acclimated to 75 mg/L 2,4-DCP at which μ_m value of unacclimated culture decreased to about half of its original value (Section 3.1.2.3.).

As discussed in Section 3.1.2.3, a lag phase between 5-13 h depending on the concentration of 2,4-DCP (22-77 mg/L) (Figure B.11-13) was experienced and almost no growth had been observed within 27 h at the concentration of 100 mg/L 2,4-DCP (Figure B.14). However, when culture acclimated to 75 mg/L 2,4-DCP was used as inocula, lag phase was not observed even at the concentration of 195 mg/L 2,4-DCP (Figure B.21-24). These results revealed that although growth of unacclimated culture was almost completely inhibited at 100 mg/L 2,4-DCP, no adverse effect on acclimated culture even at 195 mg/L 2,4-DCP was observed.

Effect of 2,4-DCP on the values of μ_m, Y, SA, and % COD removal efficiencies are given in Table 3.4 for both acclimated and unacclimated culture, in a comparative manner. As can be seen from this table, μ_m of unacclimated culture decreased with increasing concentration of 2,4-DCP and the value for μ_m could not be determined at 100 mg/L 2,4-DCP due to the nearly complete inhibition of growth. However, when acclimated culture was used, μ_m increased to 0.17 h^{-1} from a base-line value of 0.11 h^{-1} with the addition of 76.6 mg/L 2,4-DCP. Beyond this point, the μ_m decreased with increasing concentration of 2,4-DCP and became almost equal to the base-line value at the concentration of 195 mg/L. As can be seen from Table 3.4, the μ_m values of acclimated culture are smaller than those observed for

unacclimated culture. This shows that culture having ability to degrade 2,4-DCP consist of slow growing microorganisms as in the case of 4-CP.

The COD removal efficiency increased from a base-line value of 52 % to 71 % with the addition of 76.6 mg/L 2,4-DCP, whereas, it decreased slightly when 2,4-DCP concentration was increased from 76.6 to 148.7 mg/L when acclimated culture was used as inocula (Table 3.4). A further increase in 2,4-DCP caused COD removal efficiency to drop to its base-line value. It can then be stated that addition of 2,4-DCP up to 148.7 mg/L stimulated both maximum growth rate and COD removal efficiency of acclimated culture. In order to achieve high COD removal efficiency in the presence of 2,4-DCP at concentrations higher than 195 mg/L, biomass should be acclimated to higher concentrations. Another important point that should be noted is that although culture was acclimated to 75 mg/L of 2,4-DCP, the COD removal efficiency and μ_m values were not adversely affected even at the concentration of 195 mg/L of 2,4-DCP. In the case of unacclimated culture, the % COD removal value at the concentration of 22 mg/L had been observed as 44 % and it had decreased to 30 % when the concentration was increased to 77 mg/L of 2,4-DCP (Figure 3.12). However, COD removal, as noted above, remained at about 70 % up to concentration of 148.7 mg/L of 2,4-DCP when acclimated culture was used. These results show that although 2,4-DCP possesses high toxic effect on the % COD removal efficiency of the unacclimated culture, no adverse effect of 2,4-DCP was observed up to a concentration of 195 mg/L 2,4-DCP on the % COD removal efficiency of the culture acclimated to 75 mg/L 2,4-DCP.

Figure 3.23 presents % inhibitions on the Y values of 2,4-DCP acclimated culture at various concentrations. As can be seen from this figure, the values of Y decreased linearly with increasing concentration of 2,4-DCP within the stated range and IC_{50} on the basis of Y was observed to be 170 mg/L for acclimated culture. However, the Y values observed for unacclimated culture had been higher than those for acclimated culture. This can be attributed to the new culture developed upon acclimation. Higher Y values observed in the case of unacclimated culture might

indicate that unacclimated culture used a higher portion of COD for growth whereas acclimated ones use a higher portion of COD for energy requirements.

Effect of increasing concentrations of 2,4-DCP on SA values of acclimated culture is shown in Figure 3.24. As can be seen from this figure, SA values increased linearly with increasing concentration of 2,4-DCP. This shows that substrate removal per unit of biomass increased with increasing concentration of 2,4-DCP, however, opposite had been observed for unacclimated culture (Table 3.4).

Table 3.4. Results of Batch Experiments for Both Acclimated and Unacclimated Culture Receiving 2,4-DCP at Different Concentrations

	2,4-DCP (mg/L)	%COD Removal	SA mg COD / (mg MLVSS.h)	Y (mg MLVSS / (mg COD)	μ_m (h^{-1})
Unacclimated Culture	0	64	0.203	0.47	0.367
	22	44	0.088	0.93	0.332
	47.5	36	0.092	0.89	0.318
	77	30	0.052	0.72	0.131
	100	28	-	0*	0*
Acclimated Culture	0	52	0.102	0.74	0.11
	76.6	71	0.13	0.61	0.17
	101.2	67	0.147	0.60	0.17
	148.7	68	0.161	0.34	0.11
	195	54	0.169	0.30	0.10

* No growth was observed within 30 h

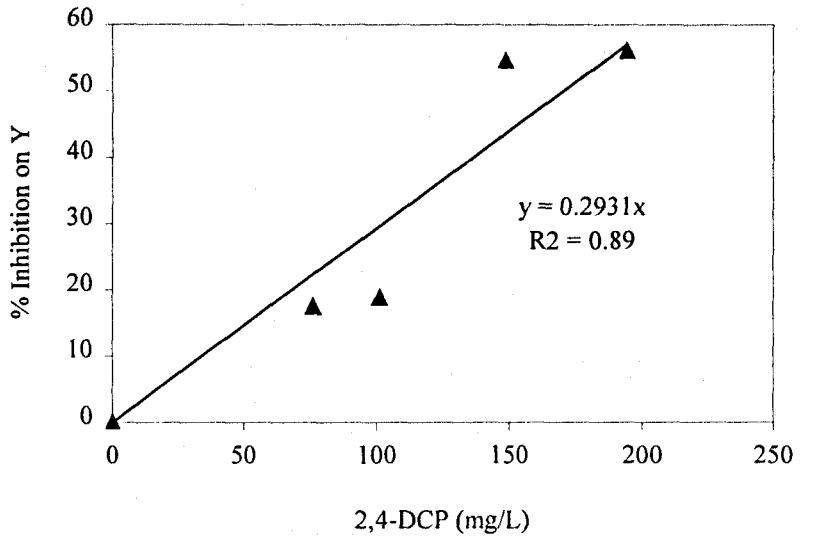


Figure 3.23. Effect of 2,4-DCP Concentrations on Y

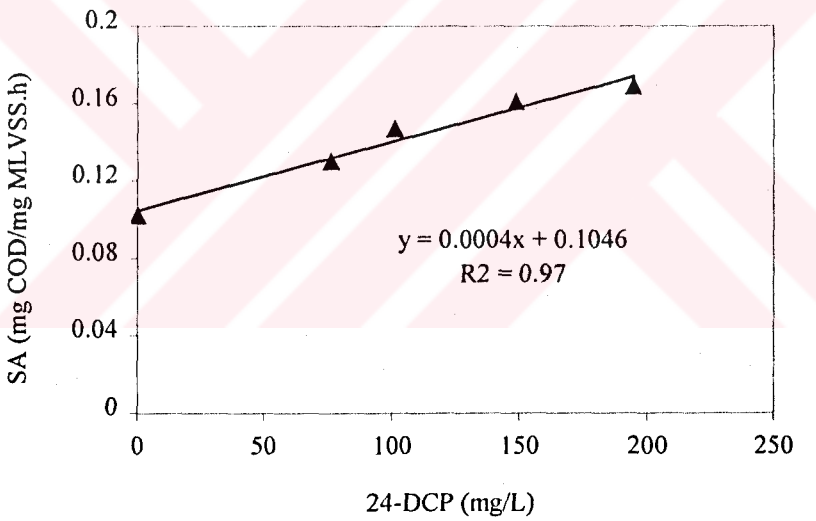


Figure 3.24. Effect of 2,4-DCP Concentration on SA

Removal of 2,4-DCP was also followed and the results presented in Figure 3.25 were obtained. As can be depicted from this figure, almost complete removal of 2,4-DCP was observed at the initial concentrations of 76.6 and 101.2 mg/L within 4 days and over the 92 % removal was observed within 6 days at the initial

concentration of about 150 mg/L. However, only 43 % chlorophenol removal was achieved within 145 h when initial 2,4-DCP concentration was increased to 195 mg/L (Figure 3 25). Then, it can be stated that culture acclimated to 75 mg/L 2,4-DCP can remove 2,4-DCP efficiently up to the initial concentration of about 148.7 mg/L. So, the positive effect of acclimation on the microorganisms was appreciably high as 2,4-DCP removal was not even possible when unacclimated culture was used (see Section 3.1.2.2). HPLC results of influent and 6th day effluent of reactor receiving 150 mg/L 2,4-DCP are given in Figure 3.26. As noted above, 92 % removal of 2,4-DCP was achieved in the period of 6 days, and two peaks which were not observed in the influent, appeared at 4.38 and 4.68 min. with the areas of 17196 and 39243, respectively. Although these peaks could not be identified, the similar peak at 4.508 (Figure 3.22) was observed in the influent line of base-line reactor. The observation of such a peak in the feed solution of base-line reactor reveals that some chlorinated compound may come from tap water since tap water had been used in the preparation of feed solution.

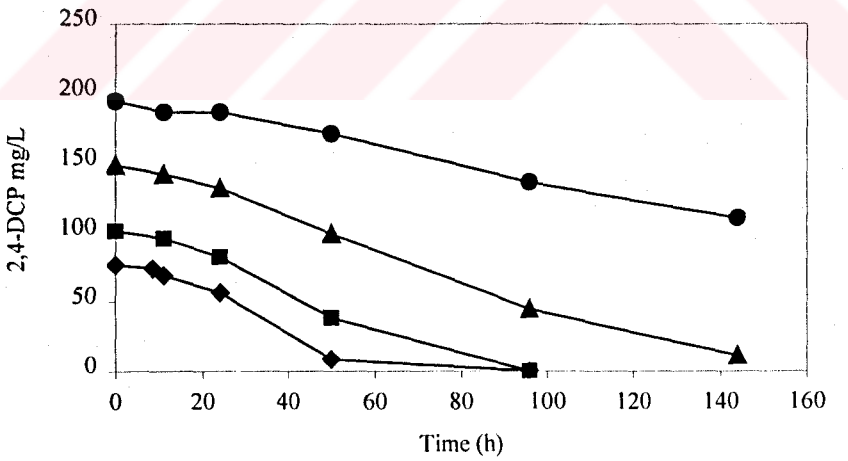
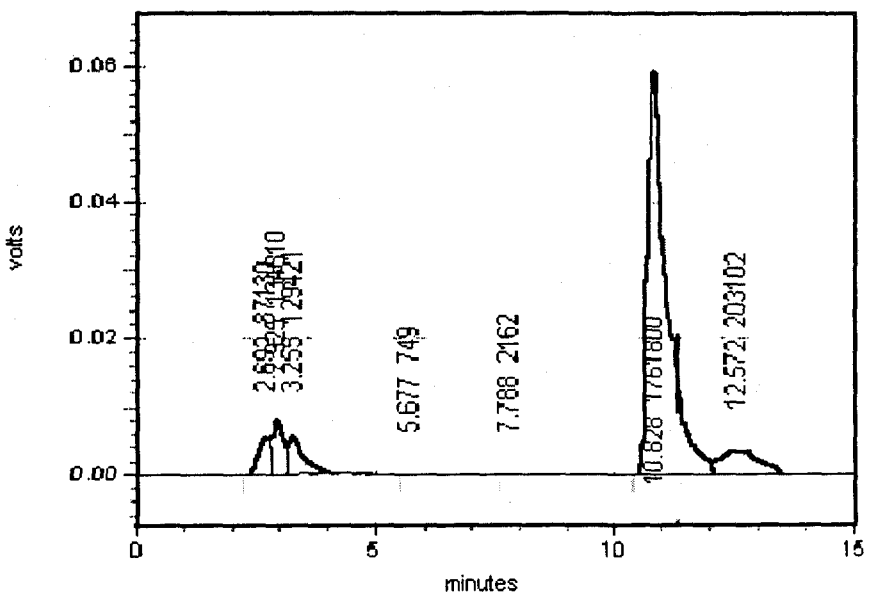
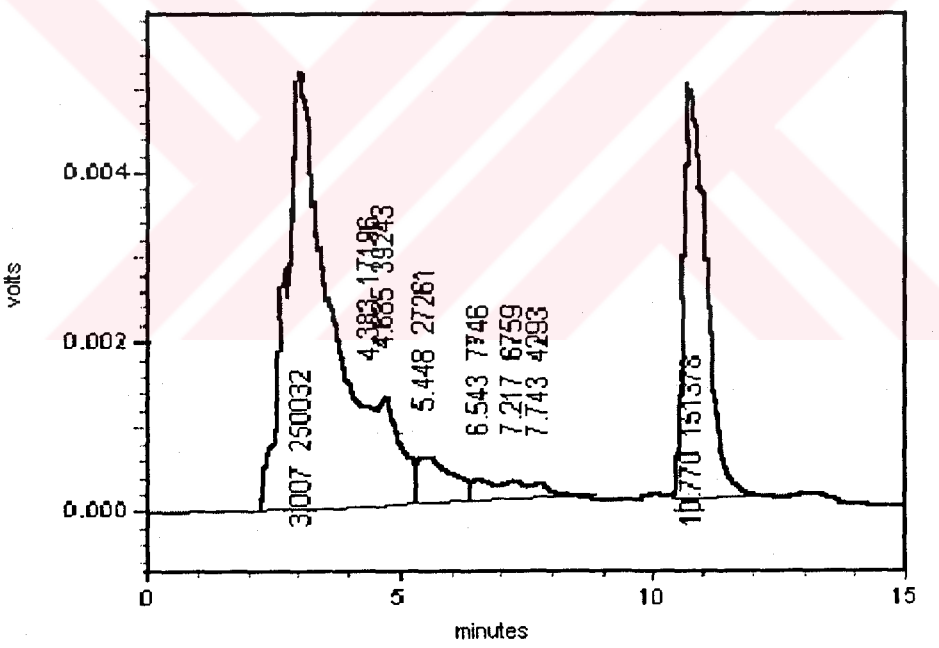


Figure 3.25 Concentration of 2,4-DCP with time (♦, 76.6 mg/L 2,4-DCP; ■, 101.2 mg/L 2,4-DCP; ▲, 148.7 mg/L 2,4-DCP; ●, 195 mg/L 2,4-DCP)



(a)



(b)

Figure 3.26. HPLC results for reactor receiving 148.7 mg/L 2,4-DCP (a) influent (b) reactor effluent after 6 days incubation

3.1.3.3. Experiments with Acclimated Culture in which Chlorophenols Serve as Sole Organic Carbon Source

In this set of experiments chlorophenols were added as a the sole organic carbon source to find out whether a readily degradable substrate is obligatory for acclimated culture to achieve chlorophenol removal or presence of a readily degradable substrate only speeds up the removal of chlorophenols.

3.1.3.3.1. Experiments with Acclimated Culture in which 4-CP Serve as Sole Organic Carbon Source

In this set of experiments, 4-CP was added as sole energy and organic carbon source at different concentrations (50, 140, 200 mg/L) to the growth medium inoculated with acclimated culture. In order to examine the effect of acclimation to a similar compound on 4-CP removal, removal of 138 mg/L of 4-CP was examined using 2,4-DCP acclimated culture as inocula.

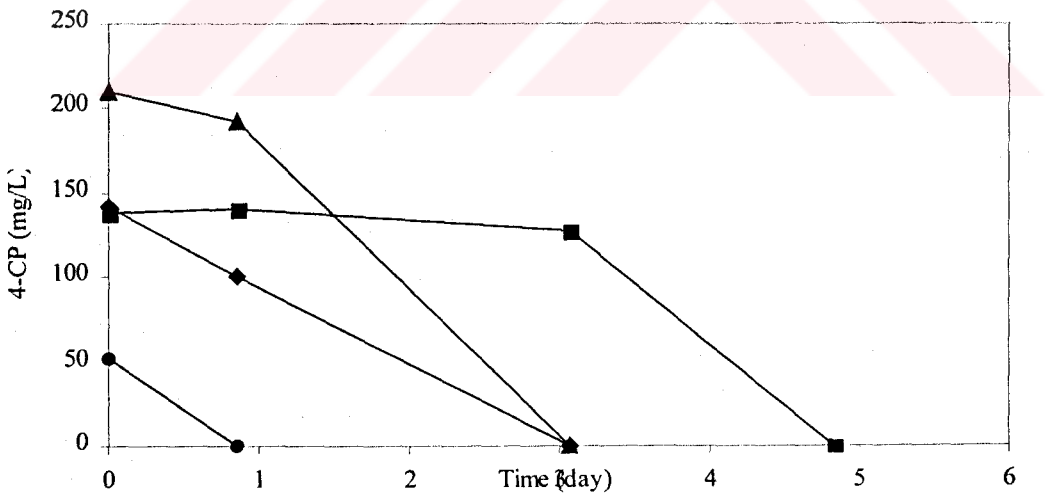
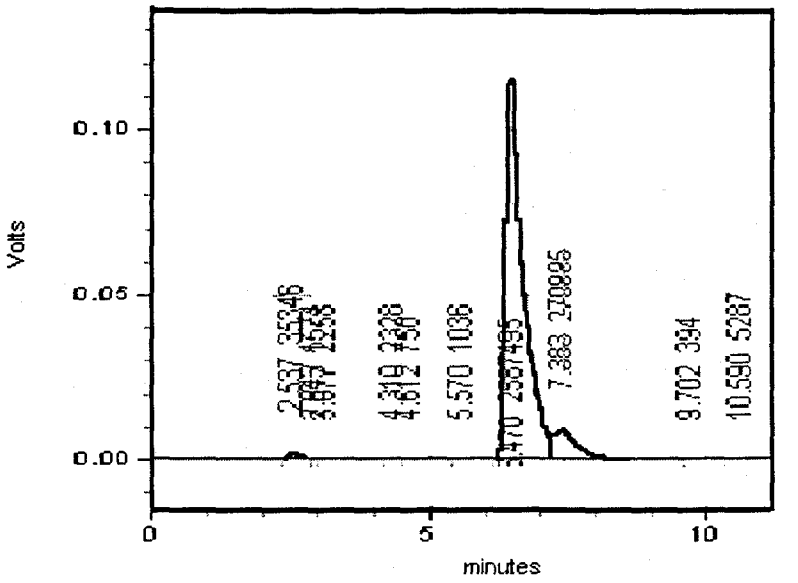
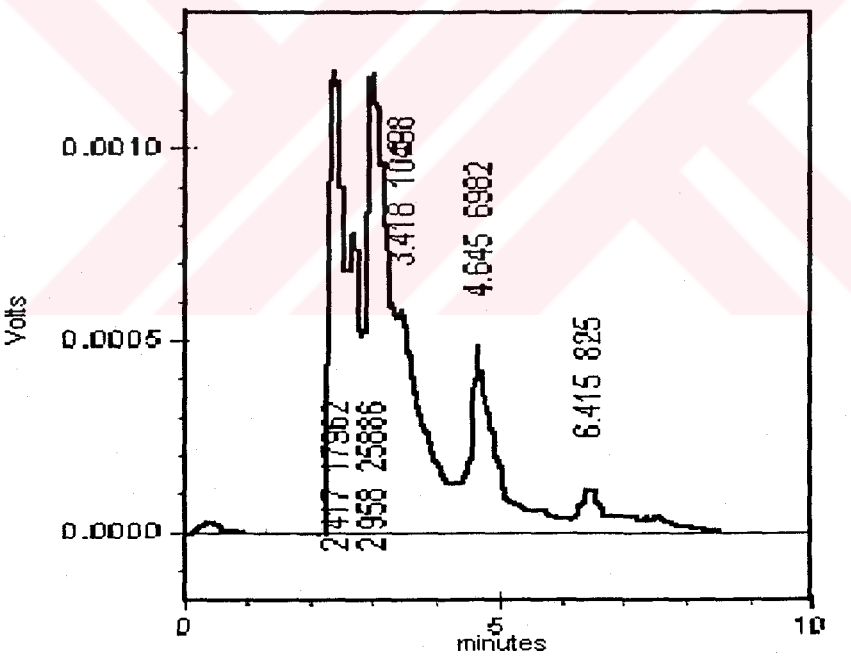


Figure 3.27. 4-CP Concentrations with Time for Reactor in which 4-CP Serves as Sole Organic Carbon Source (• , 50 mg/L; ♦ 140 mg/L; ▲, 200 mg/L; ■, 138 mg/L inoculated with 2,4-DCP acclimated culture)

Figure 3.27 shows that 4-CP at the concentrations of 50 and 200 mg/L could be completely removed within 1 and 3 days, respectively. Figure 3.28 shows the HPLC results for reactor receiving 200 mg/L 4-CP as sole carbon and energy source. As seen from the HPLC results, almost complete removal of 4-CP was achieved (>99.9 %); the peak having the area of 750 (detention time of 4.612 min.) in the influent appeared in the effluent with the area of 6982 (detention time was 4.645). Although this peak could not be identified, the same peak was also detected in the HPLC result of base-line reactor with the area of 1502 (detention time was 4.508 min) (Figure 3.22) as in the case of 2,4-DCP removal at high concentrations as explained Section 3.1.3.2. As noted previously, feed solution of base-line study was prepared using the tap water; therefore, it can be stated that intermediate coming from the degradation of 4-CP is also present in tap water. The presence of such a compound in tap water may be produced during the chlorination process in water treatment plant. Possibility of chlorophenol production during the chlorination of water was also reported by Puhakka *et al.* (1992) and Hale and Wiegel (1994). Comparison of the peak area in the effluent of reactor receiving 200 mg/L 4-CP and that of base-line reactor showed that concentration of the compound in effluent of the reactor treating 4-CP was about 4.6 times higher that in base-line reactor. Although complete removal of 4-CP in a short time period was observed with acclimated culture, experiments with unacclimated culture showed that 26 mg/L of 4-CP could be removed about 44 % within 9 days and no further degradation was observed following further 9 days (Figure 3 15). As seen from Figure 3.27, 140 mg/L of 4-CP was removed within 3 days without any lag phase and removal rate was constant throughout the experiment. However, as discussed in section 3.1.2.4, 30 % removal of 130 mg/L of 4-CP with unacclimated culture had been observed within 5 days and 1day lag period was required to start degradation (Figure 3.16).



(a)



(b)

Figure 3.28. HPLC results for reactor receiving 200 mg/L 4-CP (a) influent (b) reactor effluent after 3 days incubation

Although 140 mg/L of 4-CP could be removed within 3 days when 4-CP was added as a sole organic carbon source (Figure 3.27), 130 mg/L of 4-CP could be removed within 1 day in the presence of a readily degradable substrate (Figure 3.21). This indicates that although presence of a readily degradable substrate is not obligatory to remove 4-CP, its presence only speeds up the 4-CP removal. So, it can be stated that 4-CP was used as secondary substrate

When culture acclimated to 2,4-DCP was used to remove 4-CP, it was observed that 138 mg/L of 4-CP could be removed completely within about 5 days, although about 3 days lag period was observed (Figure 3.27). These results indicated that culture acclimated to another compound, which is analog of the interest compound, can use the compound even as sole organic carbon source. Although better results were observed with culture acclimated to 4-CP, it should be noted that 2,4-DCP acclimated culture could use 4-CP as sole organic carbon source better than unacclimated culture (comparison of Figure 3.27 and Figures 3.15-3.16).

Further, these removals were achieved in the absence of any supplementary substrates. On the other hand, Wang *et al.* (2000) stated phenol supplementation is necessary to degrade 4-CP by *P. putida.* However, it is not certain if 4-CP contaminated sites always contain such specific growth substrates such as phenol or if concentration is high enough. Since phenol is also a toxic pollutant, its addition for transformation of 4-CP should be minimized if it cannot be completely avoided (Wang *et al.* 2000).

From the foregoing discussion, it can be inferred that acclimation is necessary to remove toxic compounds and after acclimation a new culture having ability to remove chlorophenols efficiently can be generated. The presence of readily degradable substrate appeared to speed up the removal of 4-CP.

3.1.3.3.2. Experiments with Acclimated Culture in which 2,4-DCP Serve as Sole Organic Carbon Source

2,4-DCP was added as the sole carbon source to the growth medium at concentrations of 51 and 78.6 mg/L. In order to examine the effect of acclimation to similar compounds on the basis of 2,4-DCP removal, 2,4-DCP at the concentration of 77 mg/L was introduced as the sole organic carbon source to the media in which 4-CP acclimated culture was used as inocula.

Although 2,4-DCP could not be removed even at low concentrations with unacclimated culture (Figures B.15.1-15.4, Appendix B), Figure 3.29 showed that 51 mg/L of 2,4-DCP could be removed completely within 7 days when acclimated culture was used. Also, 40 % removal of 78.6 mg/L of 2,4-DCP could be achieved within 10 days. However, as discussed in section 3.1.3.2, comparably higher concentration of 2,4-DCP (150 mg/L) was possible to be removed completely in the presence of readily degradable substrate. Then, it can be stated that a readily degradable substrate is required to remove 2,4-DCP at high concentrations; hence, 2,4-DCP is removed by cometabolism.

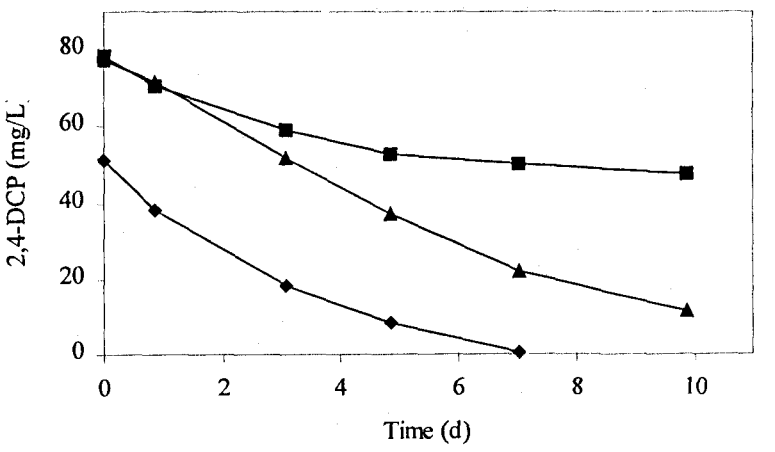
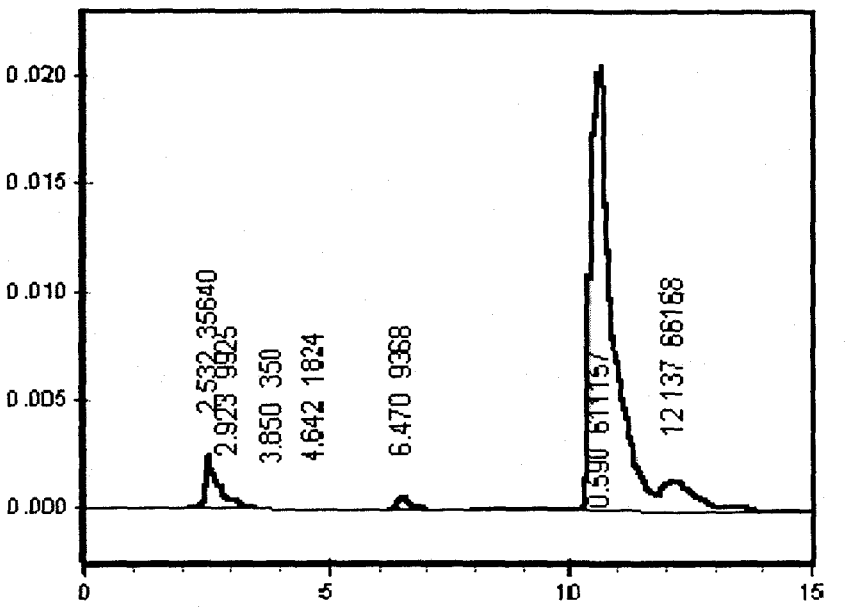


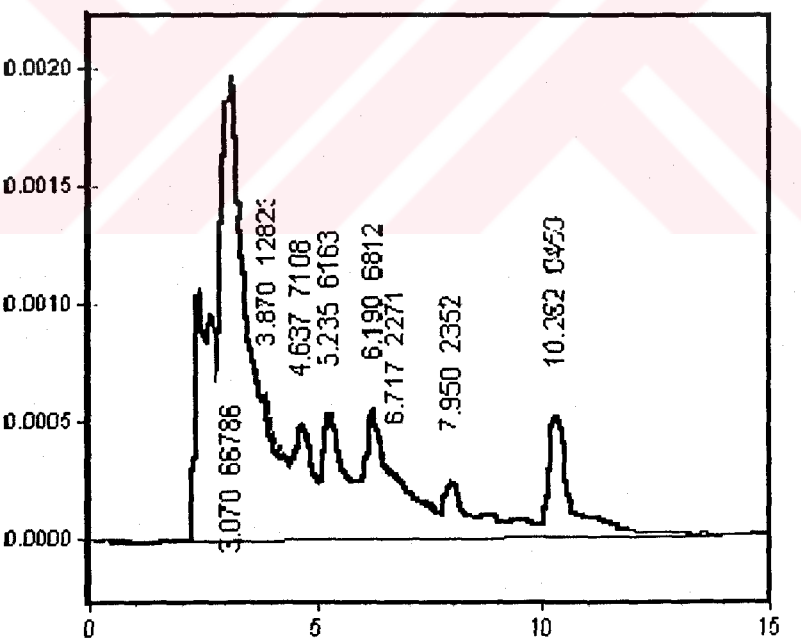
Figure 3.29. 2,4-DCP concentrations with time for reactors receiving various concentrations of 2,4-DCP (♦, 51 mg/L; ■, 78.6 mg/L; ▲, 77 mg/L inoculated with 4-CP acclimated culture)

Treatability of 2,4-DCP as a sole organic compound using 4-CP acclimated culture was also studied. Results showed that 85 % removal was achieved within 10 days (Figure 3.29). However, as stated above, only 40 % of 78.6 mg/L 2,4-DCP was removed within the same days when 2,4-DCP acclimated culture was used. So, interestingly, 4-CP acclimated culture could use 2,4-DCP as a sole organic compound better than 2,4-DCP acclimated culture. As noted previously (section 3.1.3.3.1), 4-CP could be removed effectively in the absence of a readily degradable substrate and 4-CP acclimated culture gained ability to remove a toxic compound without using a readily degradable substrate. Therefore, this may be the reason of better ability of 4-CP acclimated culture in removing 2,4-DCP when present as a sole organic compound.

Figure 3.30 shows the HPLC results of influent and effluent of reactor receiving 51 mg/L 2,4-DCP as sole carbon and energy source. The figure shows that as in the case of 4-CP, the peak in influent with the area of 1824 (4.642 min. of detention time) appeared in the effluent with the area of 7108 (detention time of 4.637 min.). These results show that the same compound from the degradation of 4-CP and 2,4-DCP was produced and it could not be identified.



(a)



(b)

Figure 3.30. HPLC results for reactor receiving 51 mg/L 2,4-DCP as sole carbon and energy source (a) influent (b) reactor effluent after 7 days incubation

3.2. Batch Experiments with Unacclimated Culture Under Anoxic Conditions

In this part of study, batch experiments under anoxic conditions were conducted with unacclimated culture in the presence of peptone (500-600 mg/L) as readily degradable substrate and chlorophenols (either 4-CP or 2,4-DCP) at different concentrations. Variations of COD, NO_3-N and chlorophenol concentration with time were monitored for each chlorophenol of various concentrations.

The inhibition of denitrification was determined by comparing NO_3-N uptake rate at the accelerated phase and the overall % COD removal efficiency of base-line reactor with those of reactors receiving various concentrations of chlorophenol.

Result of experiment for base-line study is given in Figure 3.31. It is clear that there are two different rates for denitrification and COD degradation process. Similar trends were also observed for the reactors receiving different type and concentrations of chlorophenols. As seen from Figure 3.31, the first denitrification rate is much higher than the second one and all the inhibition calculations were based on the denitrification rate observed at the first phase (NO_3-N uptake rate at the accelerated phase) for base-line and other reactors receiving chlorophenol. Another important point is that in the determination of denitrification rate, total biomass concentration was used, however, Drysdale *et al.* (2001) reported that denitrification cannot be totally attributable to just the heterotrophic organisms or even all the heterotrophic organisms, with other microorganisms contributing. However, in this study, this is of no importance in the anoxic toxicity determination since for all experimental studies, the same sludge was used for both base-line and other reactors and toxicity was determined by comparing results of base-line reactor with those of the reactors receiving chlorophenols. For the determination of inhibition, overall COD removal efficiency was considered. Figure 3.31 shows that COD and NO_3-N removal were nearly completed in 5-6th h and no significant removal was observed after this point.

Nitrate uptake rate for base-line reactor was found as 15.53 mg NO_3-N / g MLVSS. h and the %COD removal efficiency was observed to be 61 %.

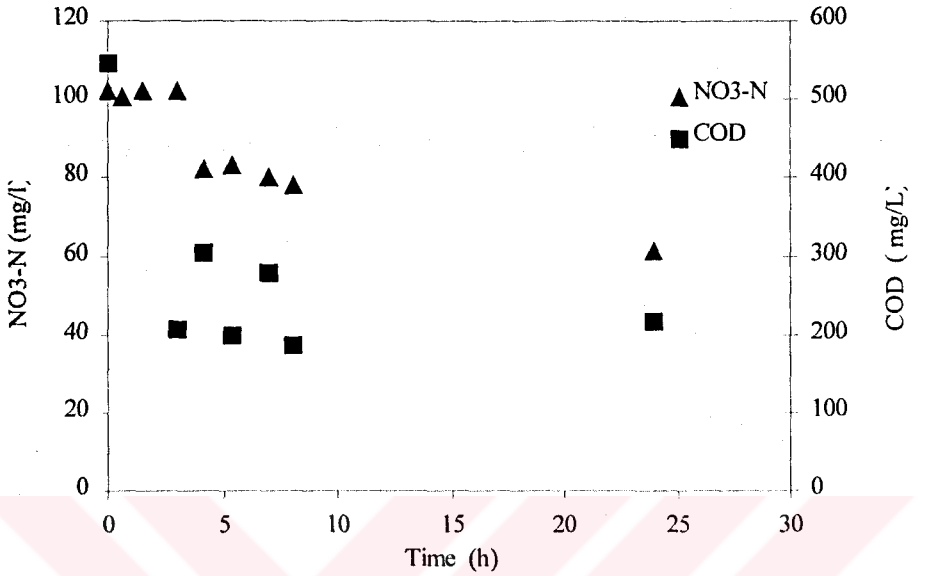


Figure 3.31. NO_3-N and COD concentrations with time for base-line reactor

3.2.1. Effect of 4-CP on Unacclimated Culture Under Anoxic Conditions

In this set of experiment, toxicity and treatability of 4-CP under anoxic conditions were studied using unacclimated activated sludge culture. It was aimed to investigate not only the treatment of chlorophenols under anoxic conditions but also effects of chlorophenols on the performance of denitrification units of the wastewater treatment plants. Hence, NO_3^- was provided as to serve as an electron acceptor to the microorganisms.

Batch experiments, as in the case of base-line study, were carried out and time course variations of COD and NO_3-N for different concentrations (20, 33, 38, 50 mg/L) of 4-CP were given in Figures C.1-4 (Appendix C).

In Figure 3.32, the values of NO_3-N uptake rate and the % inhibition of denitrification on the basis of NO_3-N uptake rate is shown as a function of 4-CP concentration. According to this figure, increased 4-CP concentration led to noticeable inhibition of denitrification and the inhibition increased linearly as a function of 4-CP concentration. IC_{50} value was found to be 24 mg/L 4-CP. Therefore; it can be concluded that even at very low 4-CP concentrations, NO_3-N uptake rate could be adversely affected. The inhibition effect of 4-CP can also be expressed as depression on the % NO_3-N removal efficiency. Although, 24 mg/L 4-CP caused 50 % decrease of NO_3-N uptake rate of accelerated phase, Figure 3.33 shows that overall NO_3-N removal was not affected at 20 mg/L and further increase in the concentration of 4-CP to 33 mg/L caused sharp decrease of NO_3-N removal from a base-line value of 40 to 23 %.

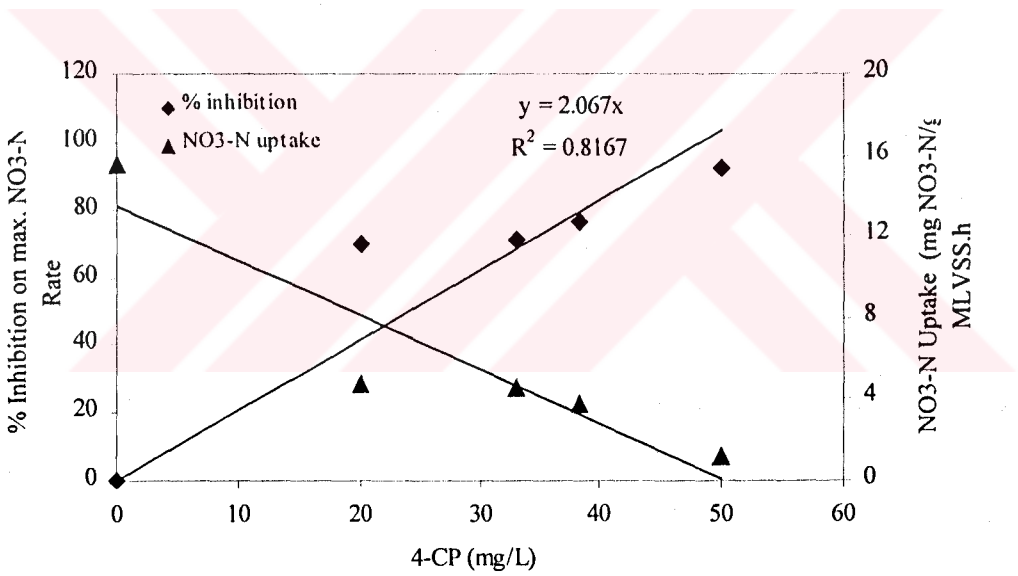


Figure 3.32. NO_3-N uptake rates and % Inhibition at various 4-CP concentrations

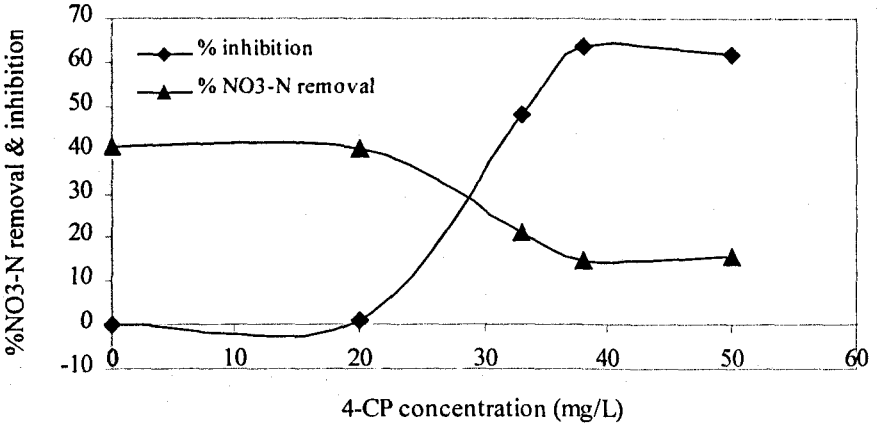


Figure 3.33. % NO_3-N removals and % inhibitions at various 4-CP concentrations

In addition to NO_3-N removal rate, the % inhibition of 4-CP was also determined on the basis of % COD removal efficiency. Figure 3.34 shows the % inhibition exerted by 4-CP at different concentrations on the basis of % COD removal efficiency. As can be seen from this figure, 4-CP adversely affected the % COD removal efficiency even at low concentrations and IC_{50} value based on the % COD removal was found as 70 mg/L. However, experiments conducted with unacclimated aerobic culture had shown that IC_{50} value based on % COD removal efficiency was not reached even at 274 mg/L of 4-CP. Therefore, it can be said that anoxic culture is more sensitive to 4-CP than aerobic culture based on the % COD removal efficiency. The IC_{50} value based on the % COD removal efficiency under anoxic conditions was about three times higher than that based on the NO_3-N uptake rate (24 mg/L). This shows that the NO_3-N uptake rate was more sensitive to 4-CP than the % COD removal efficiency.

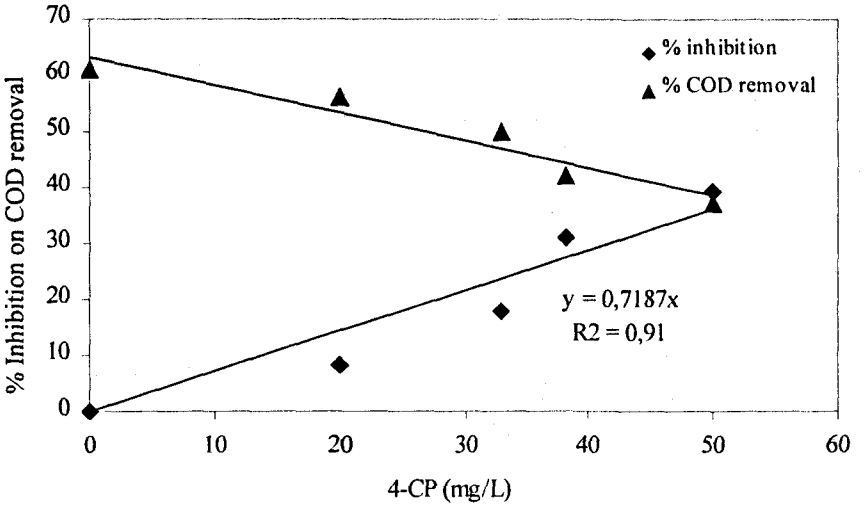


Figure 3.34. Effect of 4-CP concentrations on % COD removal

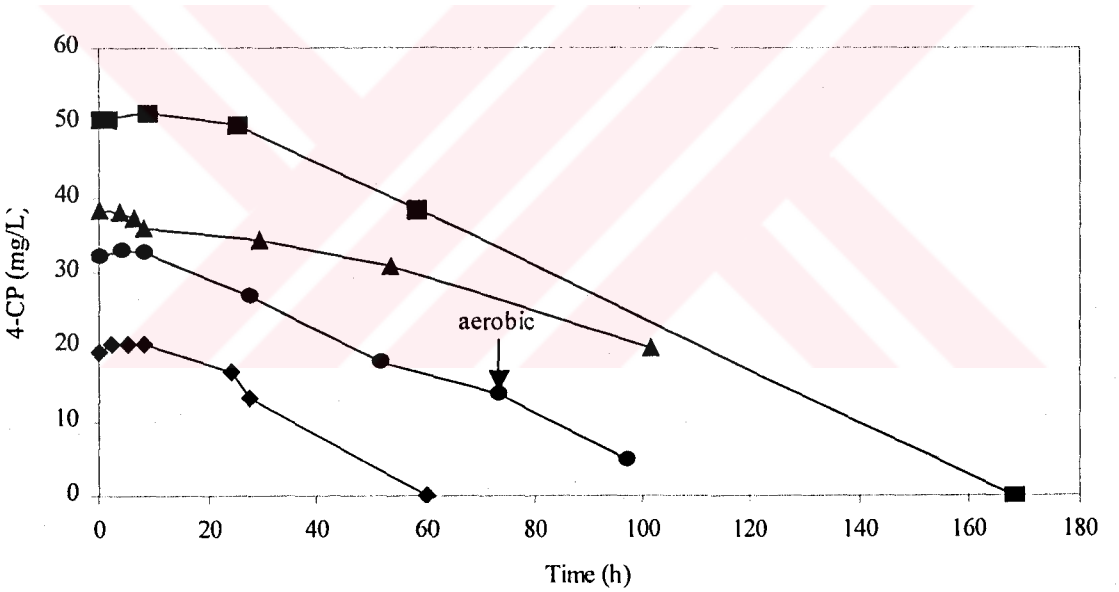


Figure 3.35. Chlorophenol concentration with time (♦,20 mg/L; •, 32.4 mg/L; ▲, 38 mg/L; ■, 50.5 mg/L 4-CP)

In Figure 3.35, time course variation of 4-CP concentration for various initial concentrations are presented. This figure indicates that at the first 8-10 h, no removal of 4-CP was observed at the concentrations of 20 and 33 mg/L. When 4-

CP concentration was further increased to 38 and 50 mg/L, degradation of 4-CP was not observed until 24 h of incubation. These results show that achieving the degradation of 4-CP under anoxic conditions is a difficult task even at low concentrations. Another important point should be noted that in the first 4-6 h of incubation, COD removal was nearly completed for all sets of experiments (Figures C.1-4) and degradation of 4-CP started further this point. This is an expected result since 4-CP is a toxic compound and unacclimated microorganisms preferred peptone rather than 4-CP. Figure 3.35 shows that when the initial concentration of 4-CP was set to 20 mg/L, almost complete degradation was observed within 60 h, on the other hand, when chlorophenol concentration was increased to 33 mg/L, only 58 % of 4-CP could be removed within 73 h. In order to understand effect of aerobic treatment following anoxic treatment process on the removal of chlorophenols, anoxic conditions was shifted to aerobic conditions after about 75 h of anoxic incubation of reactor receiving 33 mg/L 4-CP. Figure 3.35 shows that anoxic microorganisms could adapt easily to aerobic conditions, whereas, no difference in the removal rate of chlorophenol was observed. Hence, aerobic conditions could not enhance the degradation of 4-CP after anoxic conditions. Another explanation may be that aerobic microbial culture had developed during the anoxic environment and they carried the 4-CP degradation under anoxic conditions. Conversely, Valo *et al.* (1985) and Puhakka *et al.* (1992) reported similar findings. They reported that aerobic microorganisms could degrade PCP at extremely low oxygen concentrations (pO_2 = 0.0002 atm).

3.2.2. Effect of 2,4-DCP on Unacclimated Culture Under Anoxic Conditions

As in the case of 4-CP, in order to examine the toxic effects of 2,4-DCP on the denitrification, batch experiments were performed at different concentrations of 2,4-DCP using unacclimated bacteria as seed. Time course variations of NO_3-N and COD values at various concentrations of 2,4-DCP (10, 18, 27 mg/L) are given in Figures C.5-7 (Appendix C).

As can be seen from Figures C.5-7, during the first 8 h, NO_3-N concentration increased regardless of 2,4-DCP concentrations. The reason of this unexpected increase can be attributed to the fact that interference of 2,4-DCP to NO_3-N measurement with modified cadmium reduction method. Due to the difficulty in the measurement of NO_3-N in the presence of 2,4-DCP, toxicity determination of 2,4-DCP on the basis of NO_3-N uptake rate would be meaningless.

The % COD removal efficiencies for the reactors receiving 10, 18, 27 mg/L 2,4-DCP were 60, 59 and 41 %, respectively. As seen from Figure 3.34, the % COD removal efficiency of base-line reactor had been found to be 61 %. These results show that the % COD removal efficiency was not adversely affected till 18 mg/L 2,4-DCP, whereas, when 2,4-DCP concentration was further increased to 27 mg/L, about 33 % decrease of the % COD removal efficiency was observed compared to COD removal efficiency of base-line reactor.

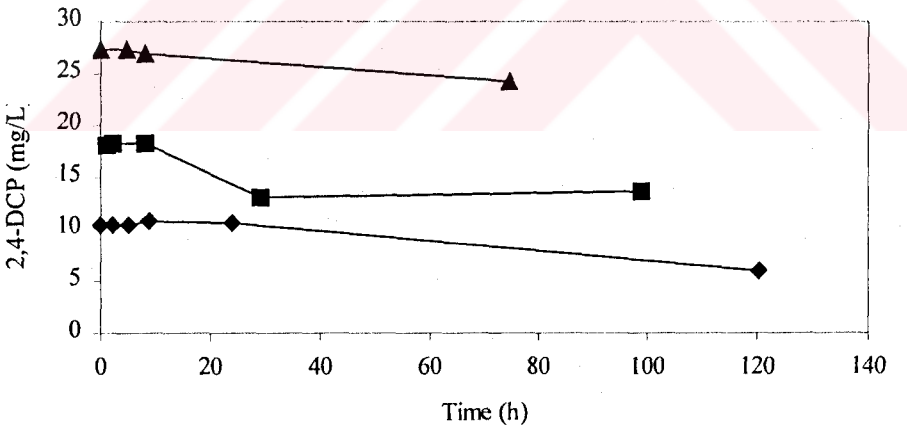


Figure 3.36. 2,4-DCP variations with time (♦, 10 mg/L; ■, 18 mg/L; ▲,27 mg/L 2,4-DCP)

Variations of 2,4-DCP concentrations as a function of time are demonstrated in Figure 3.36. This figure shows that the respective 2,4-DCP removals at the concentration of 10, 18 and 27 mg/L were 40 % within 120 h, 25 % within 100 h and 11 % within 75 h. Based on these results, it can be said that the removal efficiency of 2,4-DCP decreased as the 2,4-DCP concentrations increases. When Figure 3.35 is compared with Figure 3.36, it is clear that 4-CP is much more degradable under anoxic conditions. Similar result was also reported by Puhakka *et al.* (1992). They reported that 4-CP could be used as electron donor, however, 2,4-DCP and PCP cannot be used as electron donor under anoxic conditions. Contrary to these findings, Hu and Shieh (1987) reported that 2,4-DCP removal could be achieved in a denitrifying biofilm in which TOC/NO_3^-N ratio was 0.8-1.5.

3.3. Aerobic Fed-Batch Reactor Experiments

In this set of experiments, the treatability of 4-CP and 2,4-DCP was investigated in fed-batch reactors operated at two different SRTs, namely 8 and 15 d. Chlorophenol concentrations in the influent line were increased gradually with stepwise small increments to ensure the acclimation of microorganisms. Samples were taken for the analyses of COD, MLVSS and chlorophenol after steady-state conditions were reached. In addition to steady state experiments, shock loadings were also examined.

3.3.1. Fed-Batch Experiments for the Treatment of 4-CP

Table 3.5 and 3.6 represent the results obtained for reactors operated at 15 and 8 d of SRT, respectively. As can be seen from these tables, 4-CP could be removed completely at all examined concentrations (10-134.5 mg/L). It is worth to point out because none of the literature study did report the removal of 4-CP using activated sludge process at such high concentrations (Table 1.1). Although Wang *et al.*

(2000) reported that 4-CP could be removed completely in batch reactors at the concentration of 200 mg/L; phenol supplementation in addition to a readily degradable carbon source was necessary to induce enzymes required for 4-CP removal. However, phenol is a toxic compound and its addition for in-situ treatment of 4-CP should be reduced or completely avoided.

In our study without supplementation of phenol (only supplying peptone as readily degradable substrate), 4-CP could be removed completely up to a concentration of 135 mg/L in fed-batch reactors and complete removal of 4-CP in batch reactors had been achieved up to 300 mg/L (Figure 3.21) using culture acclimated to 130 mg/L 4-CP. Therefore, it can be postulated that 4-CP can be removed at high concentrations using activated sludge process as long as a proper acclimation procedure is followed.

HPLC results for fed-batch reactors receiving 4-CP at the concentration of 135 mg/L show that complete removal of 4-CP was observed for the both reactors operated at 8 and 15 d of SRT (Figure 3.37). In addition to peak of chlorophenol itself, another peak with the area of 647 at 4.293 min, was observed in the influent and this peak with the area of 2643 at 4.617 min was observed in the effluent of reactor operated at 8 d of SRT. The peak observed at the effluent of the reactor is the same observed in the batch reactors receiving 4-CP (as discussed in Section 3.1.3.3.1.). A comparison of the peak area of 1502 encountered in the base-line study (Figure 3.22) with that for the effluent of fed-batch reactor operated at 8 d of SRT reveals that the concentration of this intermediate is not high to cause adverse effects on the biological life of receiving body. As an important point it should be noted that concentration of the compound in feed solutions prepared at different times were different from each other due to fluctuations in the concentration of the compound in tap water.

Table 3.5. Results of Fed-Batch Reactor Experiments for Different 4-CP Concentrations (SRT=15 d)

Days	4-CP (mg/L)	COD inf. (mg/L)	% COD removal	% 4-CP removal	MLVSS (mg/L)
	0	565	70	100	2200
23	10	705	64	100	
49	31	693	56	100	2280
59	50	742	66	100	1920
86	78.4	741	58	100	2165
104	100.5	788	62	100	2253
152	134.5	888	69	100	2480

Table 3.6. Results of Fed-Batch Reactor Experiments for Different 4-CP Concentrations (SRT=8 d)

Days	4-CP (mg/L)	COD inf. (mg/L)	% COD removal	% 4-CP removal	MLVSS (mg/L)
	0	606	60	100	1393
23	10	677	63	100	
49	31	693	56	100	1347
59	50	742	62	100	1092
86	78.4	741	58	100	1477
104	100.5	788	61	100	1660
152	134.5	888	68	100	1600

As can be seen from the Table 3.5 and 3.6, no adverse effect of 4-CP on the % COD removal efficiency was observed. Further, no remarkable difference was observed between the results obtained for 8 and 15 d of SRT.

In order to find out any possible intermediate product, which could not be determined by HPLC, UV scans was carried out for influent and effluent of reactor receiving 130 mg/L of 4-CP. In addition to the reactor receiving 4-CP, UV scan was also carried out for the feed solution of base-line reactor for the purpose of comparison. From Figure 3.38, it can be concluded that, as in the case of HPLC, 4-CP was completely removed and no significant intermediate was detected in UV scans. A peak observed in UV scan of base-line reactor at about 280 nm was due to peptone.

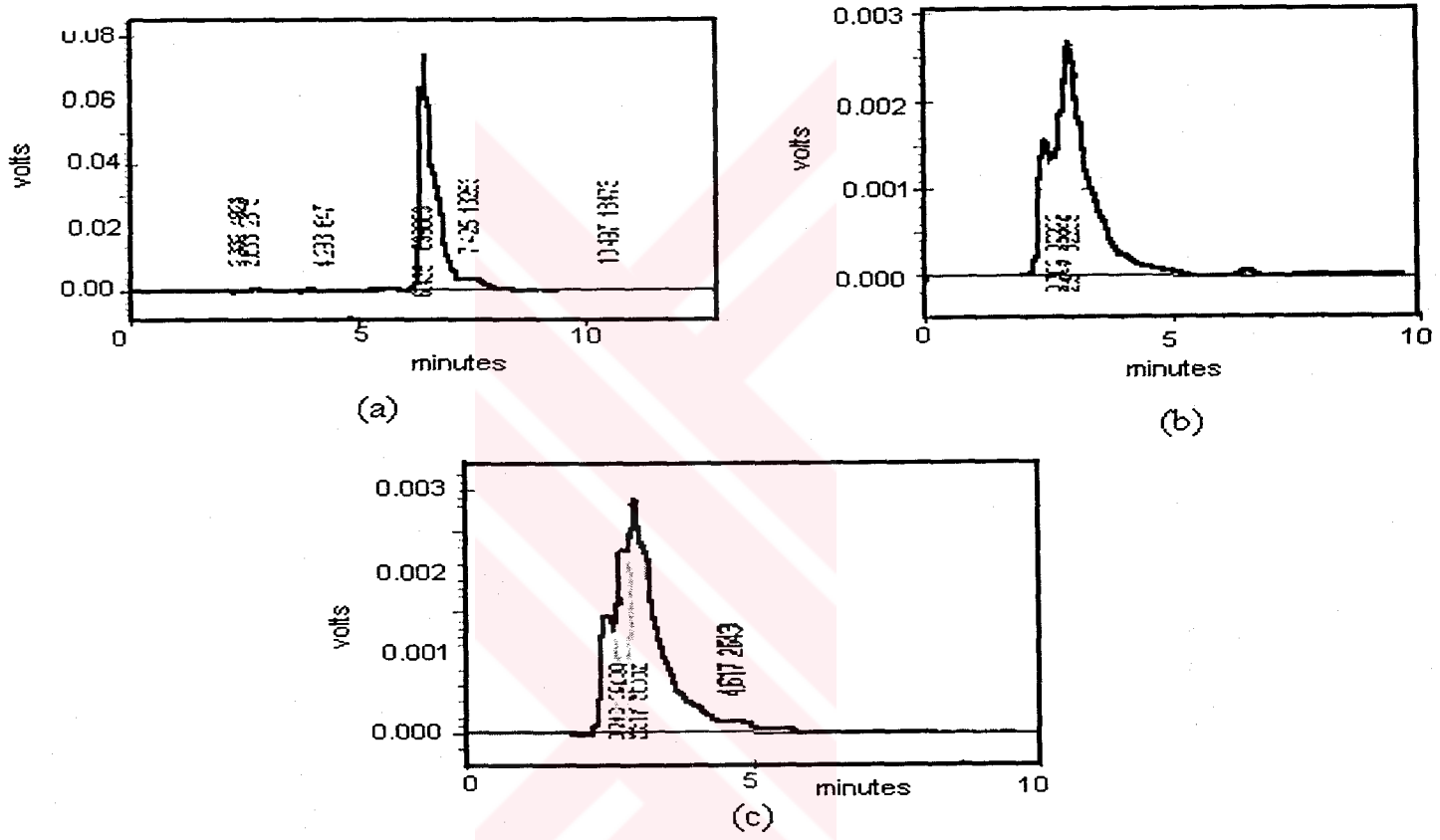
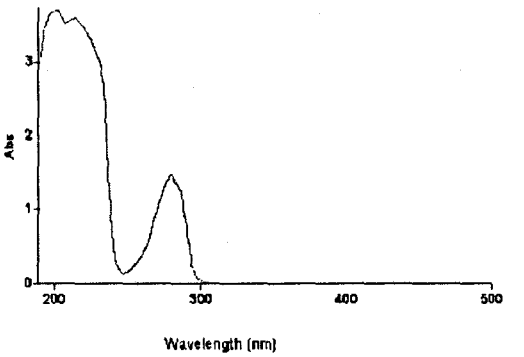
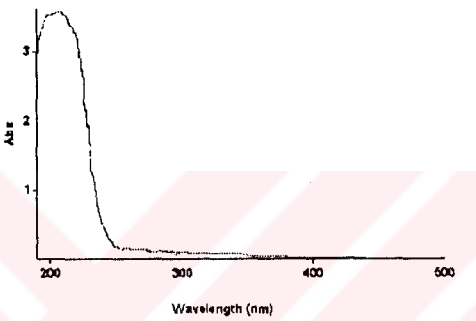


Figure 3.37. HPLC results for semi-continuous reactors receiving 135 mg/L 4-CP (a) influent (b) Effluent for reactor operated at 15 d of SRT (c) Effluent for reactor operated at 8 d of SRT



(a)



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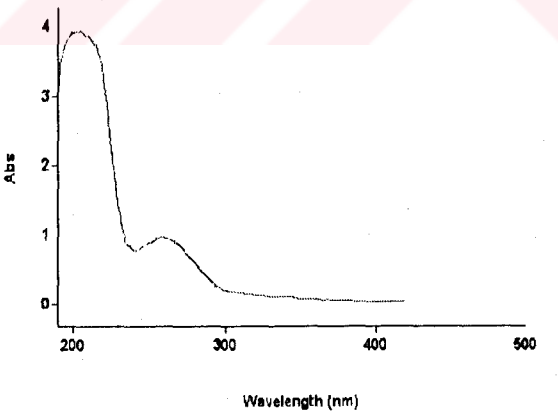


(c)

Figure 3.38. UV scans (a) influent for reactor receiving 130 mg/L 4-CP and operated at 8 d of SRT (b) effluent of reactor receiving 130 mg/L 4-CP (c) influent of base-line reactor

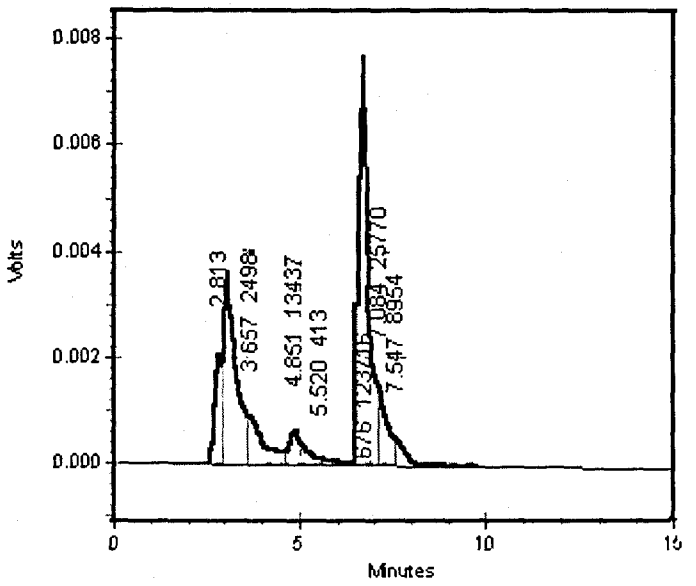
3.3.1.1. Shock Loadings of 4-CP

In order to understand the effects of shock loadings on 4-CP removal efficiency, concentration of 4-CP was increased suddenly from 30 mg/L to 50 mg/L in both reactors (i.e. 8 and 15 d SRT). Following shock loading, samples were taken from each reactor in the 1st and 3rd d following the shock loading, for measurement of 4-CP. Similarly, following the steady state conditions reached at 50 mg/L 4-CP, 4-CP concentration was increased to 75 mg/L. Results obtained are presented in Table 3.7. As can be seen from this, in the 1st day following shock loading of 4-CP, removal attained was about 70 and 100 % for the SRT values of 8 and 15 d, respectively. Measurements carried out on the samples of 3rd day proved that 4-CP was completely removed in both reactors. It can then be stated that reactors operated at higher SRT values showed higher tolerance to shock loadings compared to reactors operated at lower SRT values. Similar findings were also reported by Ettala M. *et al.* (1992) and Ha *et al.* (2000).

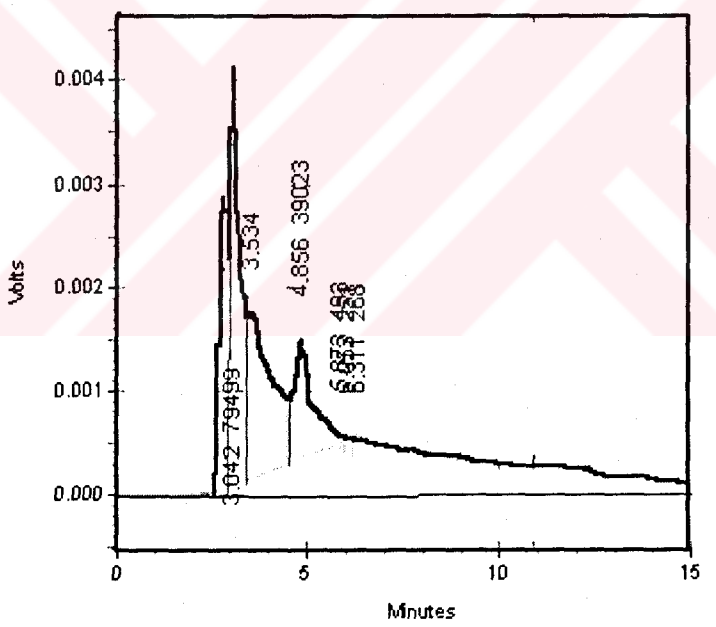
Table 3.7. The Percent 4-CP removals at shock loadings

SRT of Reactor (d)	Influent 4-CP (mg/L)	% 4-CP Removal •
8	50	77
15	50	100
8	75	71.5
15	75	100

• samples were taken after 1 day of shock loading



(a)



(b)

Figure 3.39. HPLC result for sample taken at the 1st day following shock loading of 4-CP (concentration of 4-CP was increased from 30 mg/L to 50 mg/L) for reactor operated at (a) 8 d (b) 15 d of SRT.

However, although 4-CP was completely removed at the SRT of 15 d after 1st day of shock loading, HPLC results (Figure 3.39) revealed that 4-CP was first converted to another compound with the detention time of 4.856 min., which was then completely removed when steady state conditions were reached. At the 1st day following shock loading, color of reactor effluent turned to yellowish color possibly due to the intermediates of chlorophenol degradation. However, after full acclimation, this color disappeared and HPLC results also showed that intermediates coming from the degradation of 4-CP could be removed completely. Similar findings were also observed by Lora *et al.* (2000), who stated that during acclimation period conversion of TCP to another chlorinated compounds was observed; however following acclimation, these chlorinated organic compounds produced were removed.

3.3.2. Fed-Batch Experiments for the Treatment of 2,4-DCP

In fed-batch reactors, 2,4-DCP and COD removals were examined at two different SRT values, namely, 8 and 15 d. 2,4-DCP removal was studied up to 75 mg/L at which concentration, μ_m of unacclimated culture had decreased to about half of its original value, as discussed in section 3.1.2.3. At all examined concentrations, complete 2,4-DCP removals were observed after steady state conditions were reached. Table 3.8 and 3.9 shows the results obtained for reactors operated at 15 and 8 d of SRT, respectively.

As can be followed from Table 3.8 and 3.9, decrease in the % COD removal efficiencies in both reactors was observed. However, after full of acclimation, the % COD removal efficiencies increased back to 70 % again at the concentration of 20 mg/L 2,4-DCP. Then concentration of 2,4-DCP was increased to 50 mg/L, the % COD removal values decreased in both reactors, especially for the reactor operated at 8 d of SRT.

Table 3.8. Results of Fed-Batch Reactor Experiments for Different 2,4-CP Concentrations (SRT=15 d)

Days	2,4-DCP (mg/L)	COD inf. (mg/L)	% COD removal	MLVSS (mg/L)	% 2,4-DCP removal
	0	565	70	2200	100
23	6	766	67		100
49	9	649	49	2175	100
65	20	703	75	1725	100
86	40	665	58	2212	100
104	50	684	52	1770	100
152	74.48	769	72	2490	100

Table 3.9. Results of Fed-Batch Reactor Experiments for Different 2,4-CP Concentrations (SRT= 8 d)

Days	2,4-DCP (mg/L)	COD inf. (mg/L)	% COD removal	MLVSS (mg/L)	% 2,4-DCP removal
	0	606	60	1393	100
23	6	811	54		100
49	9	648.76	51	1352	100
65	20	703	73	944	100
86	40	665	54	1350	100
104	50	684	40	980	100
152	74.48	769	63	1670	100

HPLC results for the influent and effluent samples of reactors receiving 74.48 mg/L 2,4-DCP are given in Figure 3.40. At all the examined concentrations of 2,4-DCP, complete removal was observed. Although no intermediate was observed in the treatment of 2,4-DCP up to concentration of 74.48 mg/L, an intermediate having 4.642 min. of retention time was observed for reactor operated at 8 d of SRT. The intermediate observed at the effluent of the reactor had been also observed in the treatment of 4-CP as discussed previously. Although this intermediate could not be identified, it is clear that this compound is produced both in the treatment of 4-CP and 2,4-DCP and after a period of acclimation, it could be removed completely. It should be noted that although intermediate was observed in the effluents of both reactors, comparison of peak areas revealed that the intermediate concentration in the effluent of reactor operated at 8 d of SRT was 5.4 times higher than that in the effluent of reactor operated at 15 d of SRT. Hence, it can be stated that reactor operated at higher SRT values was more stable for the treatment of chlorophenols at high concentrations.

In an attempt to understand if any other intermediate product that cannot be detected by HPLC column, is present or not, UV scans were also carried out for the influent and effluents of the reactors. UV scans obtained are given in Figure 3.41 for the reactor receiving 75 mg/L 2,4-DCP and operated at 8 d of SRT. UV scans results showed that similar to HPLC analysis, complete removal of 2,4-DCP was observed, whereas, intermediate observed by HPLC analysis could not be detected in UV scans of effluent samples.

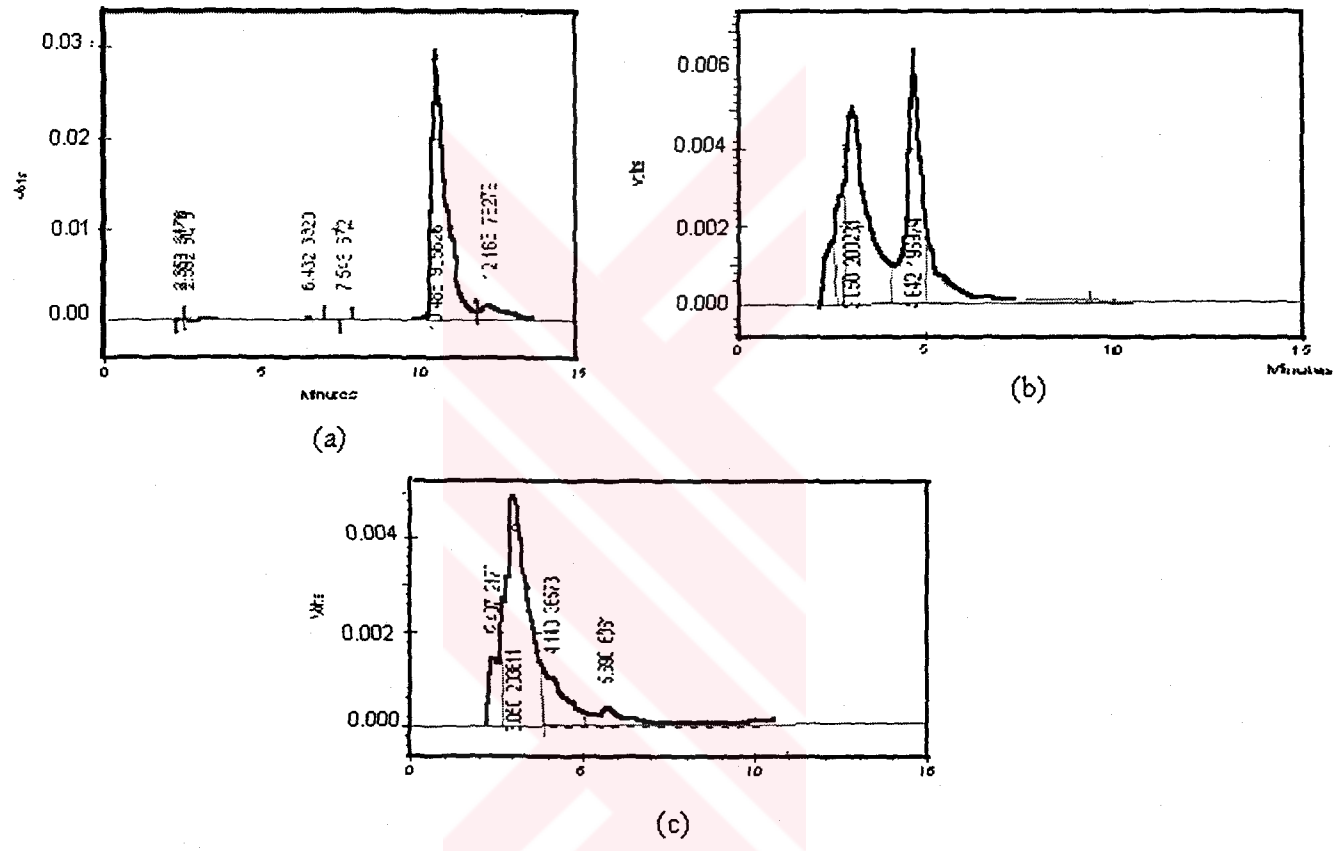
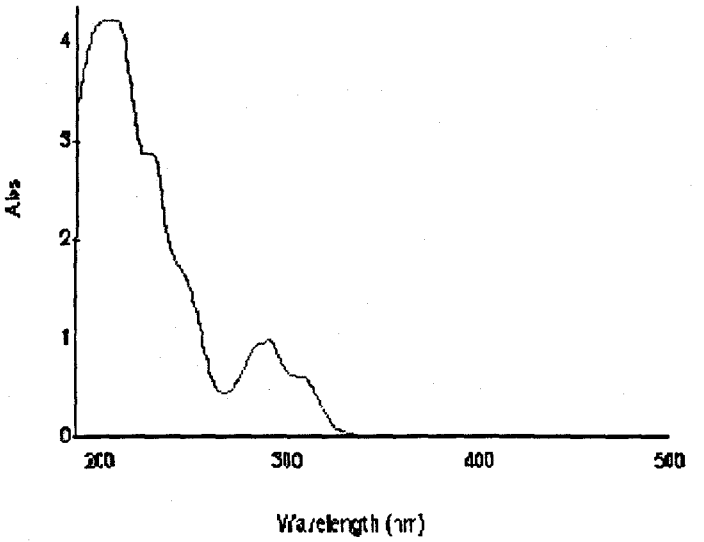
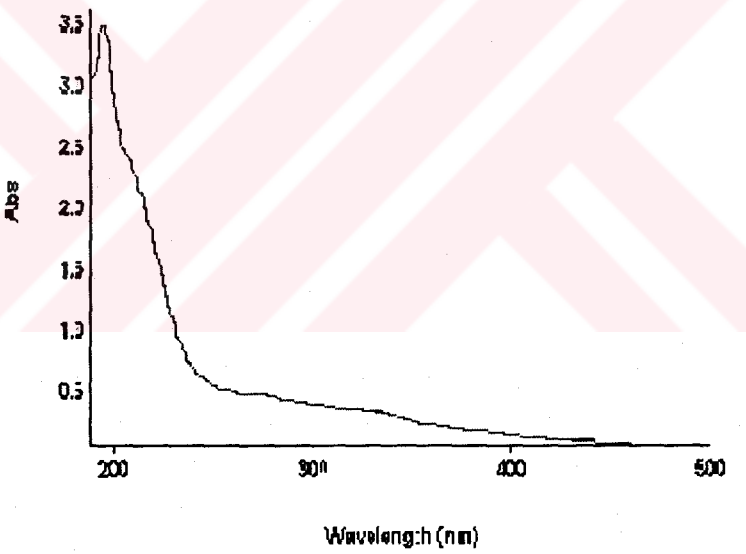


Figure 3.40. HPLC results for reactors receiving 74.48 mg/L 2,4-DCP (a) influent (b) effluent for reactor operated at 8 d of SRT (c) effluent for reactor operated at 15 d of SRT



(a)



(b)

Figure 3.41. UV scans for reactor receiving 75 mg/L 2,4-DCP and operated at 8 d of SRT (a) influent (b) effluent

3.3.3. Batch Experiments Conducted with Effluents of Fed-Batch Reactors

In order to investigate the toxicity of effluents from fed-batch reactors, batch toxicity experiments were conducted with effluents of fed-batch reactors fed with 130 mg/L 4-CP and 75 mg/L 2,4-DCP, respectively. All inorganic compounds required for growth and peptone as the source of readily degradable substrate were added to batch reactors not to limit the growth of biomass. Batch experiments were conducted using unacclimated culture to determine the toxicity of reactor effluents if discharged to a receiving body.

Time course variation of OD and COD values for reactors conducted with effluents of fed-batch reactors receiving 130 mg/L 4-CP and 75 mg/L 2,4-DCP are given in Figures B.25 and B.26 (Appendix B), respectively and results are summarized in Table 3.10.

Table 3.10. Results of Batch Experiments Conducted with Fed-Batch (SRT 8 d) Reactors Effluents Using Unacclimated Culture

Reactor Effluent of	% COD removal	% Inhibition on COD removal efficiency	μ_m (h^{-1})	% Inhibition on μ_m
130 mg/L 4-CP	44.5	30.4	0.303	17.5
75 mg/L 2,4-DCP	52.8	17.48	0.239	35

The percent inhibition values were calculated comparing the results of reactors receiving effluents of fed-batch reactors with those of base-line rector inoculated with unacclimated culture. As can be seen from Table 3.10, when μ_m value and COD removal efficiency of the reactor fed with effluent of fed-batch reactor

receiving 130 mg/L 4-CP was compared with those of the base-line reactor, the % inhibition on the COD removal efficiency and μ_m was found as 30.4 and 17.5, respectively, whereas, the % inhibition on the COD removal efficiency and the value of μ_m at the concentration of 130 mg/L (before treatment) had been found as 38 (Figure 3.8) and 50 % (Figure 3.7), respectively. These results show that there was a remarkable decrease in the % inhibition on the basis of μ_m after treatment; percent toxicity reduction was found to be 65 %, whereas, only 20 % reduction on the basis of COD removal efficiency was observed.

The percent inhibition caused by the effluents of fed-batch reactor receiving 75 mg/L 2,4-DCP on the COD removal efficiency and μ_m were found as 17.48 and 35 %, respectively, whereas, the % inhibition on the COD removal efficiency and μ_m prior to treatment of 2,4-DCP had been found as 55.88 (Figure 3.12) and % 54.36 (Figure 3.11), respectively. These results show that after treatment of 75 mg/L 2,4-DCP using fed-batch reactors, the percent reduction of toxicity on the basis of COD removal efficiency and μ_m were found as 68.7 and 36 %, respectively. Therefore, it can be said that there is remarkable decrease on the toxicity of 2,4-DCP following the treatment.

3.4. Aerobic Sequencing Batch Reactor Experiments

Fed-batch reactors (8 d of SRT) used for 4-CP and 2,4-DCP treatment were switched to SBR operation mode. Unlike fed-batch reactors, in this set of experiments, chlorophenols were fed to reactors as sole carbon and energy source. Other inorganic nutrients and nitrogen in the form of NH_4Cl were added to the growth medium not to limit the growth of biomass.

As stated in Section 2.3.1.3, each aerobic SBR of 2 L working volume was run with one cycle per day. Settling and idle period was about 2 h; therefore, reaction period

was at least 22 h in each cycle. During the 24 d of operation, 250 mL of excess sludge was drawn off from reactors only on the Day 13.

Fed-batch batch reactors operated at the concentration of 135 mg/L 4-CP and 75 mg/L 2,4-DCP were switched to SBR mode of operation with the influent concentrations of 170 mg/L 4-CP and 80 mg/L 2,4-DCP respectively. When complete removal of 4-CP and 2,4-DCP were observed at the first day of SBR operation, concentration of 4-CP and 2,4-DCP were increased to 180 and 85 mg/L, respectively (Figures 3.42 and 3.43). As can be seen from Figures 3.42 and 3.43, reactors were operated at fixed 4-CP and 2,4-DCP concentrations for 8 d and no deterioration on the performance of reactors were observed. Then, concentrations of chlorophenols were increased further till the chlorophenol removal efficiency deteriorated.

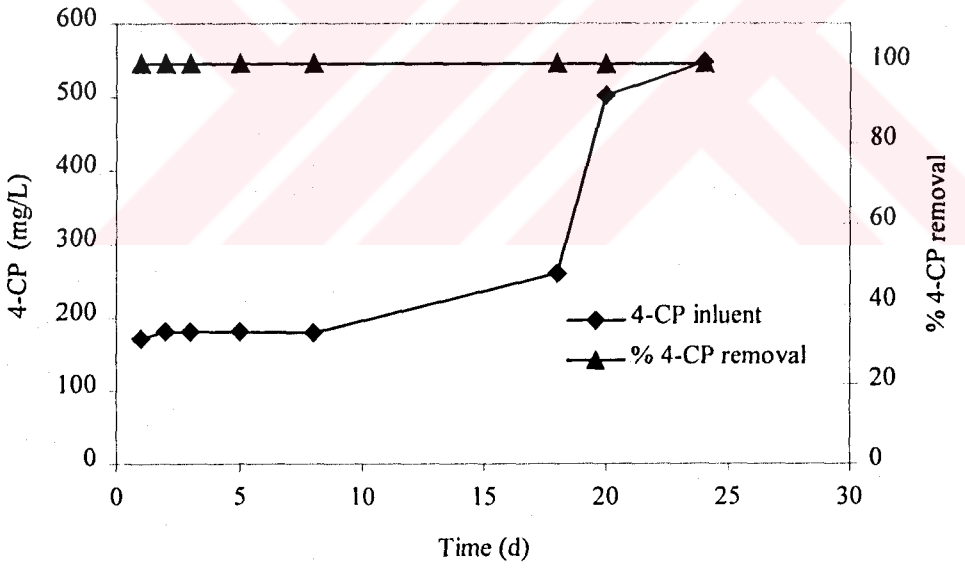


Figure 3.42. % 4-CP removal at different concentrations in SBR

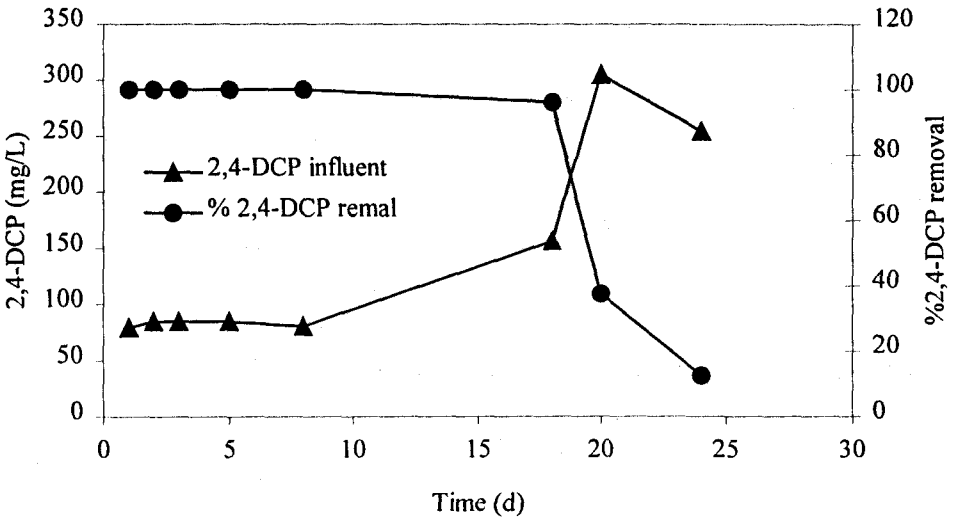


Figure 3.43. % 2,4-DCP removal at different concentrations in SBR

It can be seen from Figure 3.42, 4-CP concentration was further increased with small increments and 260 mg/L was reached on the Day 18 and complete removal was also observed at this concentration. Beyond this point, 4-CP concentration was increased to 502 and 547 mg/L (organic loading was 597 mg 4-CP/L/d) on the Days of 20 and 24, respectively and complete removals of 4-CP were also observed even at these sharp 4-CP concentration increases. These results revealed that complete removal of 4-CP as sole carbon and energy source could be achieved using SBR even at the concentration of 547 mg/L corresponding to 597 mg 4-CP/L/d of loading rate. As compared to literature findings, this is much higher concentration value even than those with fluidized bed treatment systems. In terms of organic loading rate, the same situation is valid as the highest organic loading rate studied in literature is smaller than 500 mg/L/d (Table 1.1).

As can be seen from the results of the HPLC analysis presented in Figure 3.44, although complete removal of 170 mg/L 4-CP was observed at the first day after feeding 4-CP as sole organic carbon source, an intermediate having 4.6 min. of retention time was observed. As discussed previously, the same compound was also observed in the treatment of 4-CP and 2,4-DCP when peptone was used as readily

degradable substrate in the fed-batch reactors (Sections 3.3.1.1 and 3.3.2). Although this intermediate could not be identified, HPLC results showed that 30 and 98 % removal of this intermediate compound was achieved on the Days 5 and 8, respectively. Hence, it can be stated that, although an intermediate coming from the transformation of 4-CP was produced, after acclimation of culture to this undefined intermediate, almost complete removal could be observed. However, at the influent concentration of 502 and 547 mg/L of 4-CP, HPLC results showed that the concentration of intermediate indicated a slight increase from 5824 to 6877 due to possibly shock loading of 4-CP (Figure 3.44).

As in the case of 4-CP, 2,4-DCP concentrations were increased in stepwise small increments and 157 mg/L 2,4-DCP was reached on the Day 18 (Figure 3.43). It can be seen from Figure 3.43, removal efficiency of 2,4-DCP decreased from 100 to 96 % when 2,4-DCP concentration was increased from 85 to 157 mg/L. When reactor was fed with 305 mg/L 2,4-DCP on Day the 20, reactor was not able to withstand with this sharp increase as removal efficiency of 2,4-DCP decreased sharply to 37 %. Then, 2,4-DCP concentration was decreased back to 255 mg/L on the Day 24 to see if the removal efficiency will be recovered, however, a decrease in the removal efficiency continued and fell down to 12 %. HPLC results are also given in Figure 3.45. From these results, it can be said that high 2,4-DCP removal can be achieved till the concentration of 157 mg/L corresponding to loading rate of 171 mg 2,4-DCP/L/d.

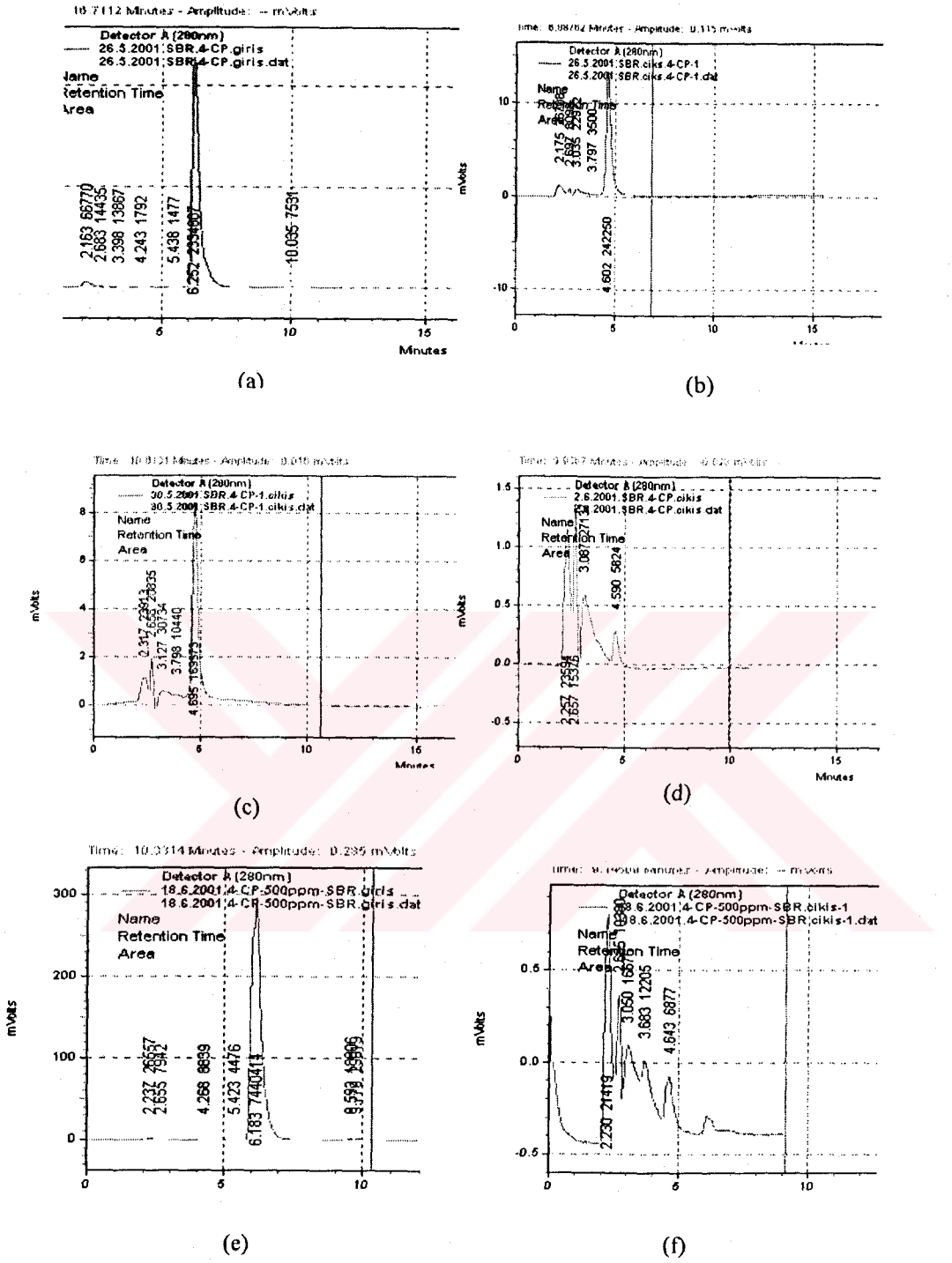


Figure 3.44. HPLC results for SBR receiving 4-CP (a) influent at 170 mg/L 4-CP (b) effluent on the Day 2 (c) effluent on the Day 5 (d) effluent on the Day 8 (e) influent at 547 mg/L 4-CP (f) effluent on the Day 24

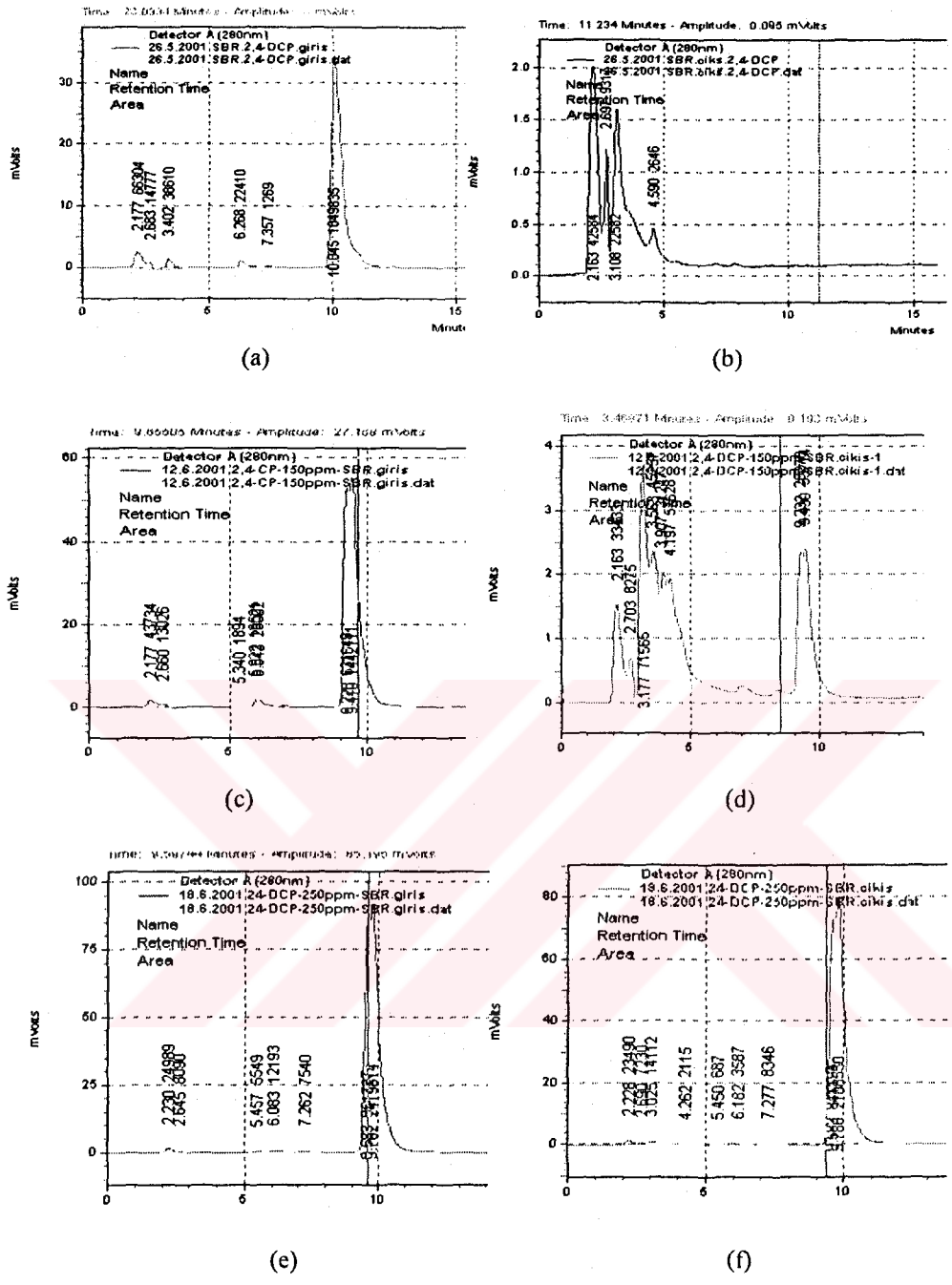


Figure 3.45. HPLC results for SBR receiving 2,4-DCP (a) influent at 80 mg/L 2,4-DCP (b) effluent on the Day 2 (c) influent on the Day 18 (157 mg/L 2,4-DCP) (d) effluent on the Day 18 (e) influent on the Day 24 (255 mg/L 2,4-DCP) (f) effluent on the Day 24

Variations in the concentration of MLVSS can be seen from Figure 3.46. This figure shows that MLVSS concentrations for reactors fed with 4-CP and 2,4-DCP varied between 1200-1800 mg/L and 793-1128 mg/L, respectively. For both reactors, the observed decrease in the MLVSS concentrations on the Day 18 was possibly due to increase in the concentrations of chlorophenols and sludge drawn off from the reactors on the Day 13. When concentration of 4-CP was increased to 547 mg/L, MLVSS concentration increased from 1225 to 1430 mg/L. However, for reactor fed with 2,4-DCP, decrease in the concentration of MLVSS from 948 to 793 mg/L was experienced when concentration was increased from 157 to 255 mg/L. MLVSS/MLSS ratio for reactors receiving 4-CP and 2,4-DCP varied between 83-88 % and 55-66 %, respectively, and steady decrease in the MLVSS/MLSS ratio was observed with increasing 2,4-DCP concentration.

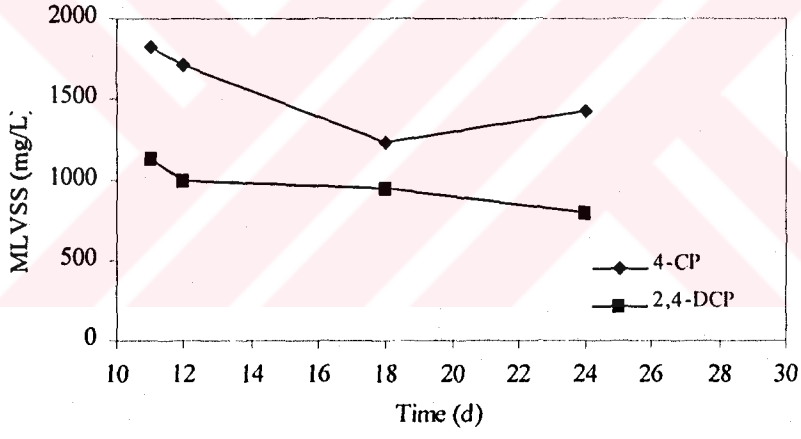


Figure 3.46. MLVSS concentrations at different influent concentrations of 4-CP and 2,4-DCP

As discussed in Section 3.1.3.1, although 4-CP removal had been observed up to concentration of 300 mg/L in batch reactors in the presence of peptone, as stated previously, complete removal of 4-CP as sole carbon and energy source at the

concentration of 547 mg/L (597 mg 4-CP/L/day of loading rate) was achieved in SBR (Figure 5.42). In batch reactors inoculated with acclimated culture, show that although 2,4-DCP removal could be possible up to concentration of 150 mg/L in the presence of readily degradable substrate (Figure 3.25), efficient removal could not be observed even at the concentration of 78.6 mg/L when it was introduced as sole carbon and energy source (Section 3.1.3.3.2). Comparisons of these results with those of SBR indicate that SBR is an excellent process for the removal of chlorophenols even when present as sole carbon and energy source.

CHAPTER 4

CONCLUSIONS

The following conclusions can be drawn from this study,

1. Among the examined chlorophenols, 2,4-DCP is the most and 2-CP is the least toxic compound on unacclimated activated sludge culture.

2. When 4-CP acclimated culture was used, toxicity of 4-CP decreased about 68 % on the basis of μ_m compared to unacclimated culture.

3. When unacclimated culture was used, the % COD removal efficiency decreased remarkably even at low concentrations of 4-CP and 2,4-DCP. However, there was no adverse effect of 4-CP and 2,4-DCP on the % COD removal efficiency even at the concentration of 300 mg/L and 150 mg/L, respectively, when culture is acclimated.

4. If culture is acclimated to a toxic compound at any concentration, higher concentrations of the compound can be tolerated on the basis of toxic removal efficiency, % COD removal efficiency and μ_m. Culture acclimated to 130 mg/L of 4-CP, can tolerate 2.3 times higher 4-CP concentration on the basis of COD removal efficiency. In the case of 2,4-DCP, acclimated culture could tolerate 2 times higher concentration of 2,4-DCP on the basis of % COD removal efficiency compared to unacclimated culture.

5. Y values of unacclimated culture increased surprisingly to very high values with addition of 2,4-DCP, however, Y values of acclimated culture decreased linearly with addition of 2,4-DCP and IC_{50} value of acclimated culture on the basis of Y was nearly 2.5 times higher than IC_{50} value of unacclimated culture on the basis of μ_m.

6. Although no removal was observed with unacclimated culture was used, complete removal of 300 mg/L 4-CP and 150 mg/L 2,4-DCP was observed in batch reactors in the presence of peptone with acclimated culture.

7. Although 4-CP and 2,4-DCP could be used as sole organic carbon source by acclimated culture, removal rates of 2,4-DCP and 4-CP were higher in the presence of a readily degradable substrate.

8. Culture acclimated to 4-CP could use 2,4-DCP better than 2,4-DCP acclimated culture. Although 3 d lag period was observed, 2,4-DCP acclimated culture could use 4-CP as sole organic carbon source.

9. In fed-batch reactors, complete removal of 4-CP and 2,4-DCP could be achieved even at the concentrations of about 130 mg/L and 75 mg/L, respectively.

10. Under anoxic conditions, chlorophenols are more toxic compared to aerobic conditions.

11. In SBRs, complete removal of 4-CP and 2,4-DCP as sole carbon and energy source could be achieved up to concentration of 547 and 157 mg/L, respectively. Although there are several studies on the treatment of 4-CP and 2,4-DCP, treatment of those as sole carbon and energy source at these high concentrations have not been reported.

CHAPTER 5

RECOMMENDATIONS

Recommendations for further research on the subject can be summarized as;

1. Similar studies should be done for polychlorophenols.

2. The combined effects of chlorophenols should be investigated in batch and continuous reactor experiments.

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APPENDIX A

CALIBRATION CURVES AND PROPERTIES OF STUDIED CHLOROPHENOLS

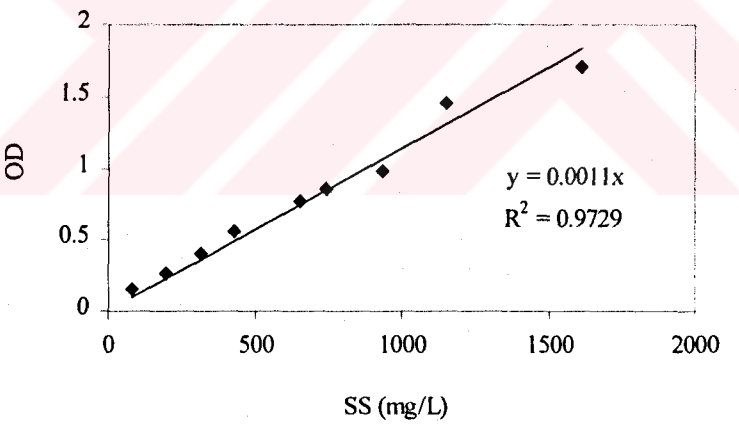

Figure A.1. Calibration curve for suspended solid (MLSS) measurement at 550 nm

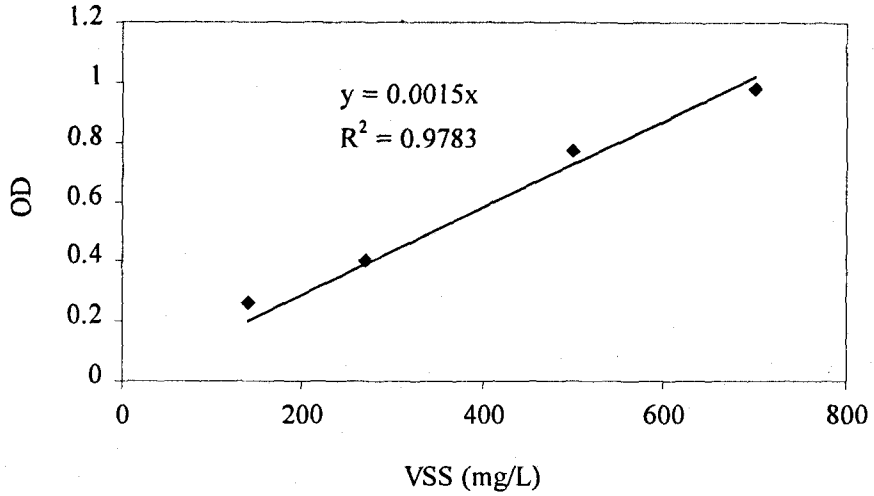

Figure A.2. Calibration curve for volatile suspended solid (MLVSS) measurement at 550 nm

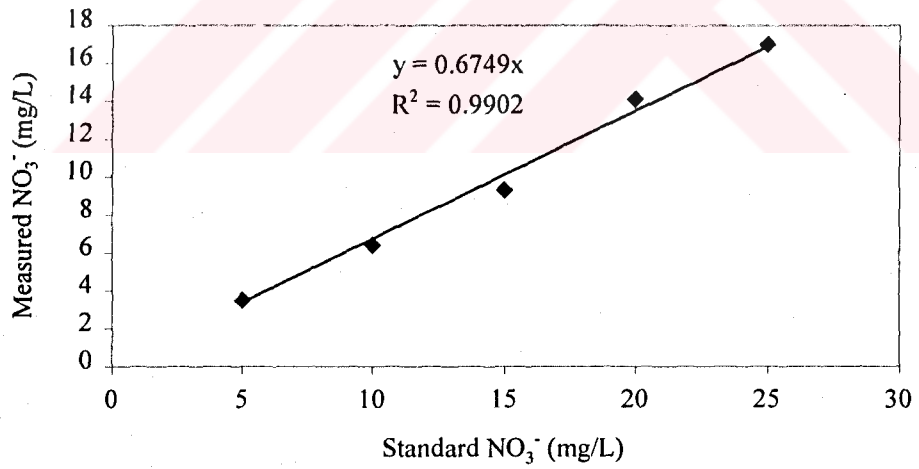

Figure A.3. Calibration curve for NO_3^- measurement

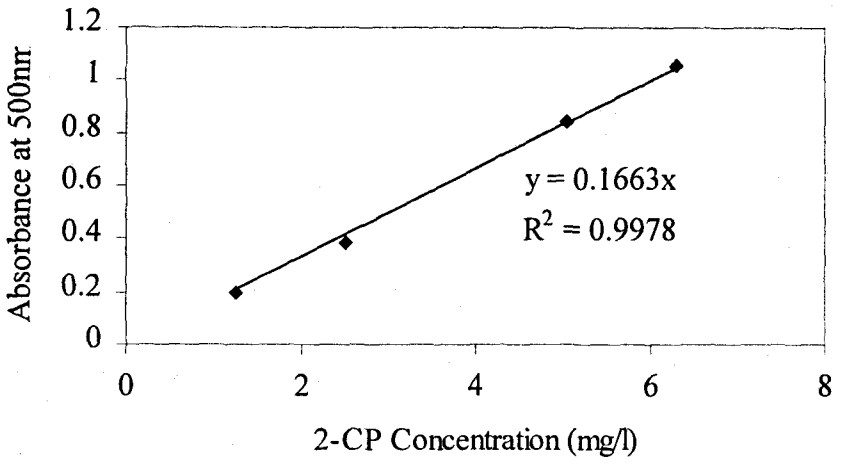

Figure A.4. 2-CP calibration curve for the direct photometric method

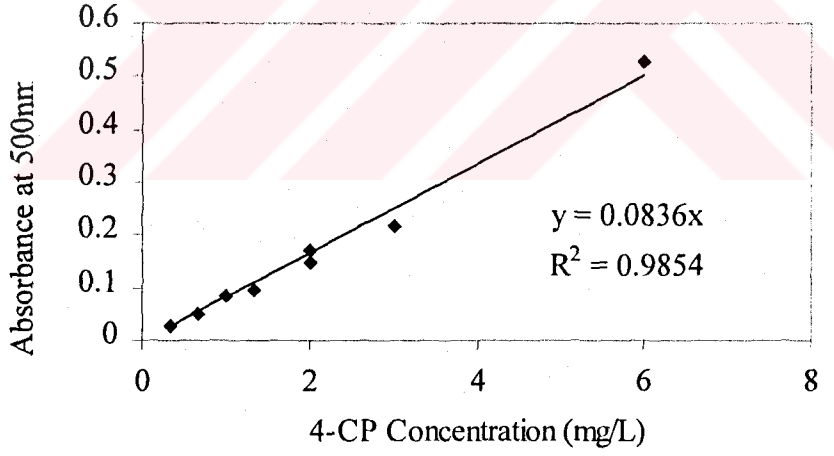

Figure A.5. 4-CP calibration curve for the direct photometric method

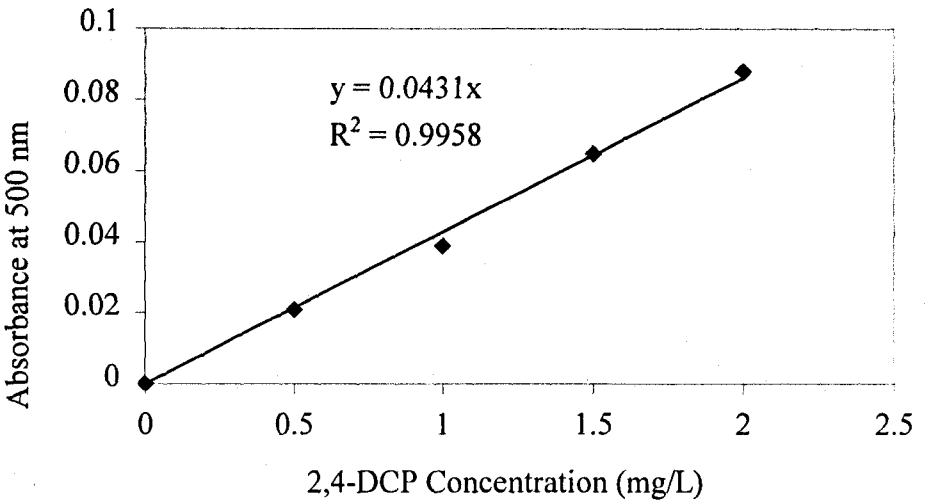

Figure A.6. 2,4-DCP calibration curve for the direct photometric method

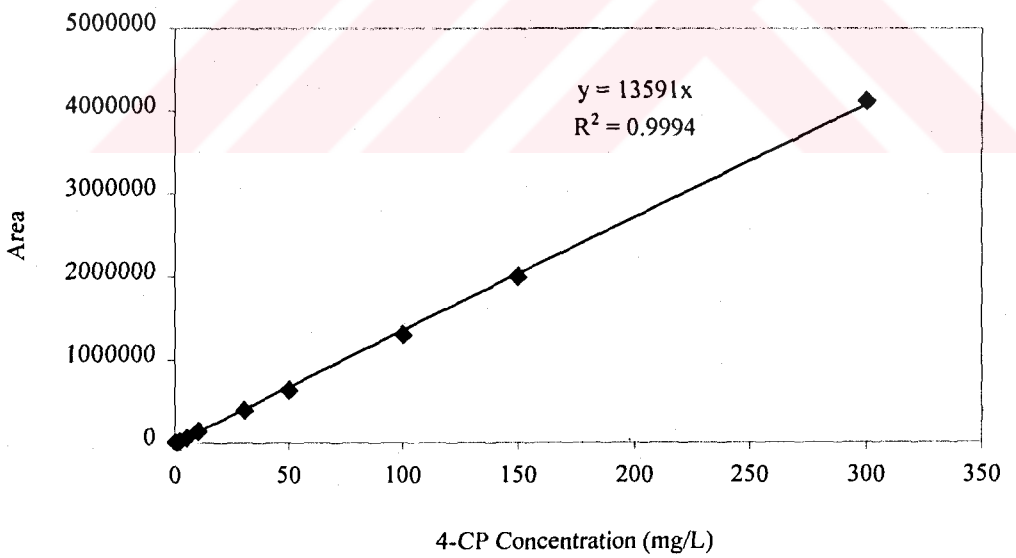

Figure A.7. 4-CP calibration curve for HPLC analysis

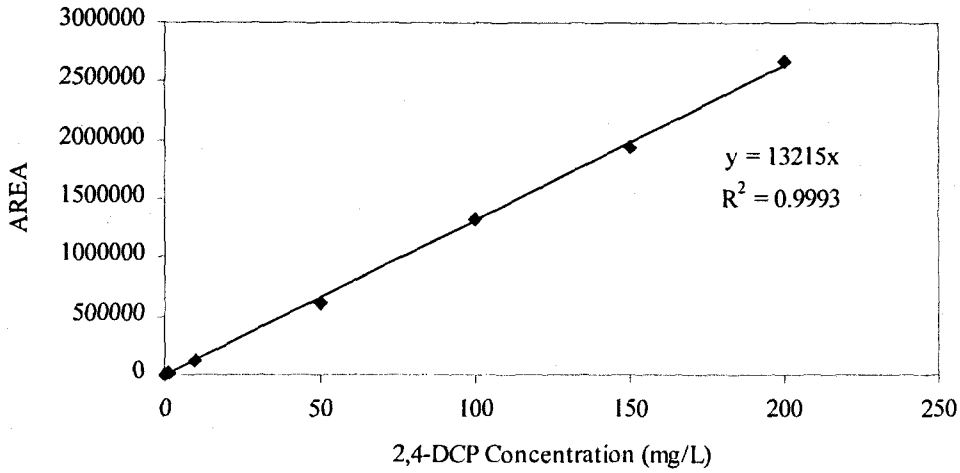

Figure A.8. 2,4-DCP calibration curve for HPLC analysis

Table A.1. Properties of Chlorophenols Used in the Study (Mackay *et al.*, 1995)

Compound Name	**2-hlorophenol**	**4-Chlorophenol**	**2,4-Dichlorophenol**
Molecular Fromula	$C_6H_4(OH)Cl$	ClC_6H_4OH	$C_6H_3Cl_2OH$
Molecular Weight	128.56	128.56	163.0
Meltin Point (C)	8.4	43.0	45.0
Boiling Point (C)	174.5	220	210
Dissociation Constant,pK_a	8.52	9.20	7.85
Water Solubility (g/m^3 at 25 C)	25 000	27 000	4500
Vapour Pressure (Pa at 25 C)	316	17.3	13
Henry's Law Constant ($pa.m^3/mol$)	1.065	0.0567	0.284
Octanol-water Coefficient, log Kow	2.20	2.39	3.08
Sorption Partition Coefficient, log Koc	3.60	1.85	2.59

APPENDIX B

RESULTS OF BATCH EXPERIMENTS UNDER AEROBIC CONDITIONS

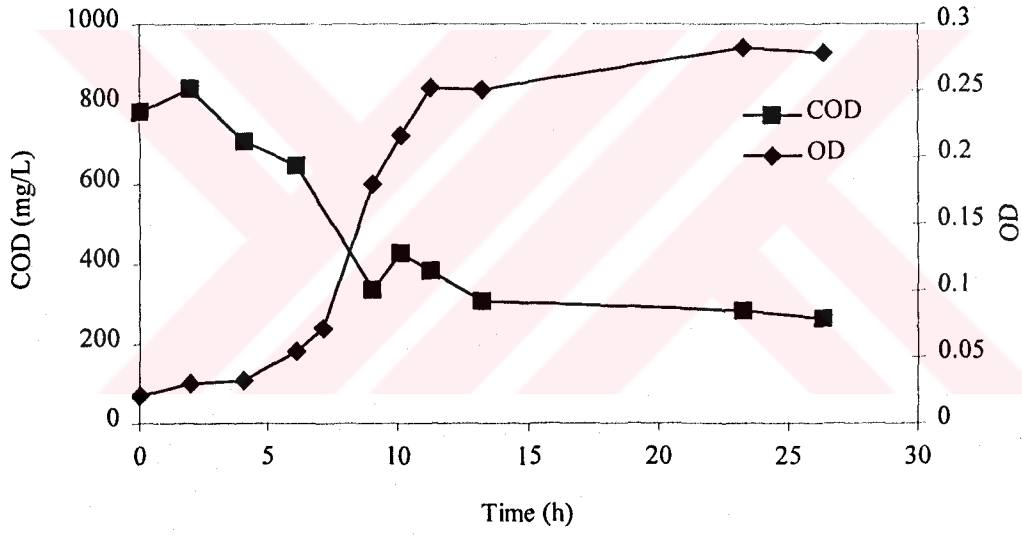

Figure B.1. Time course variations of OD and COD concentrations in the presence of 30 mg/L 2-CP for reactor inoculated with unacclimated culture

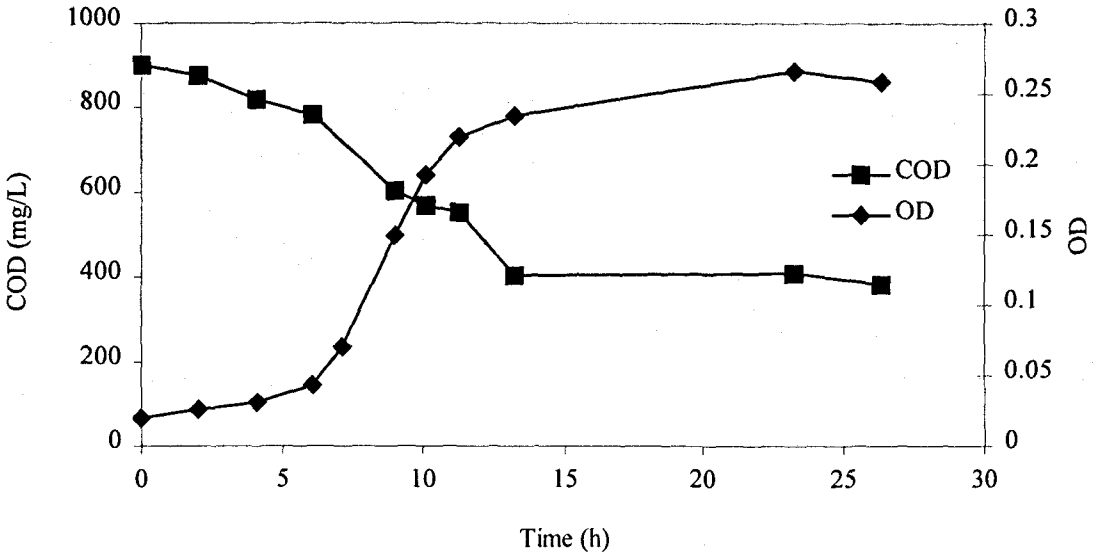

Figure B.2. Time course variations of OD and COD concentrations in the presence of 70 mg/L 2-CP for reactor inoculated with unacclimated culture

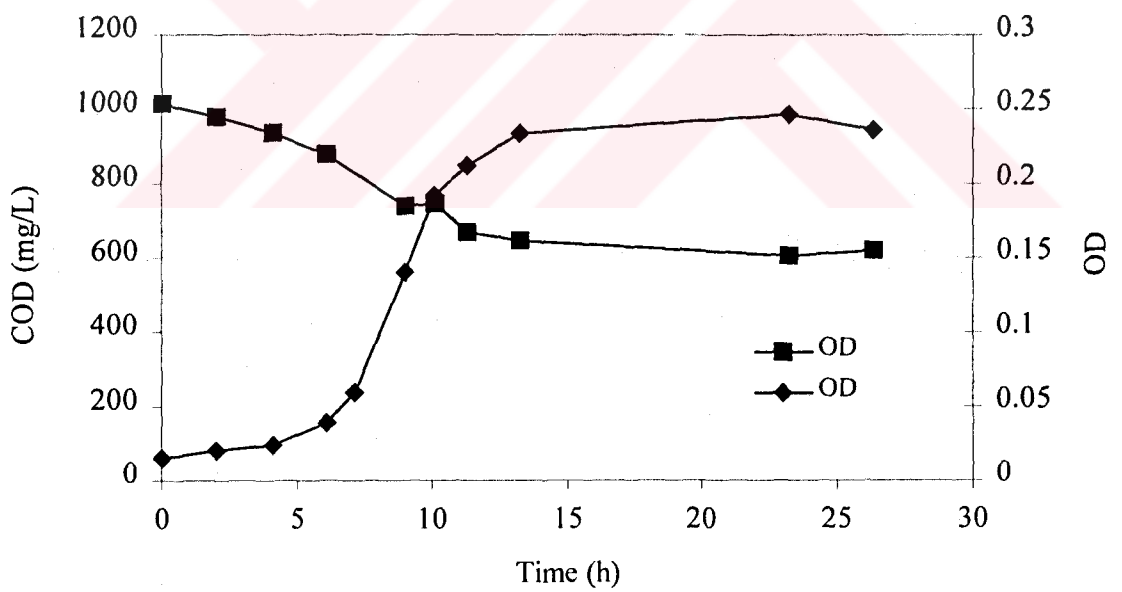

Figure B.3. Time course variations of OD and COD concentrations in the presence of 120 mg/L 2-CP for reactor inoculated with unacclimated culture

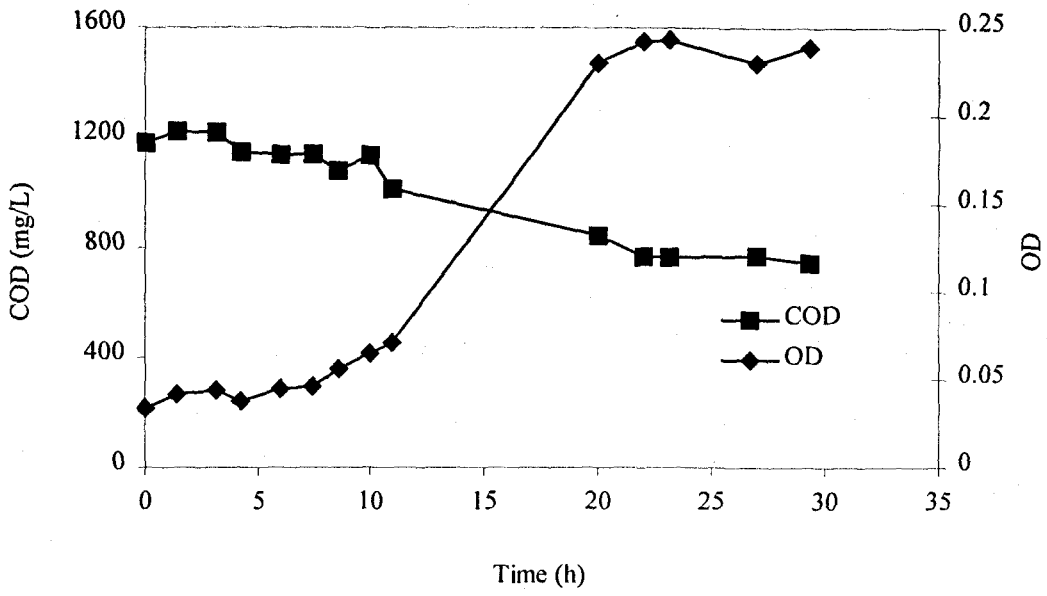

Figure B.4. Time course variations of OD and COD concentrations in the presence of 300 mg/L 2-CP for reactor inoculated with unacclimated culture

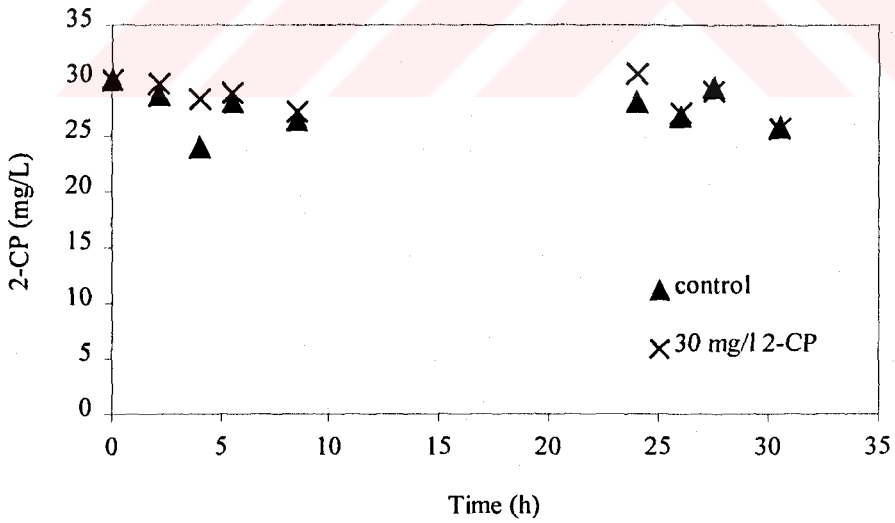

Figure B.5.1. Time course variation of 2-CP at the initial concentration of 30 mg/L

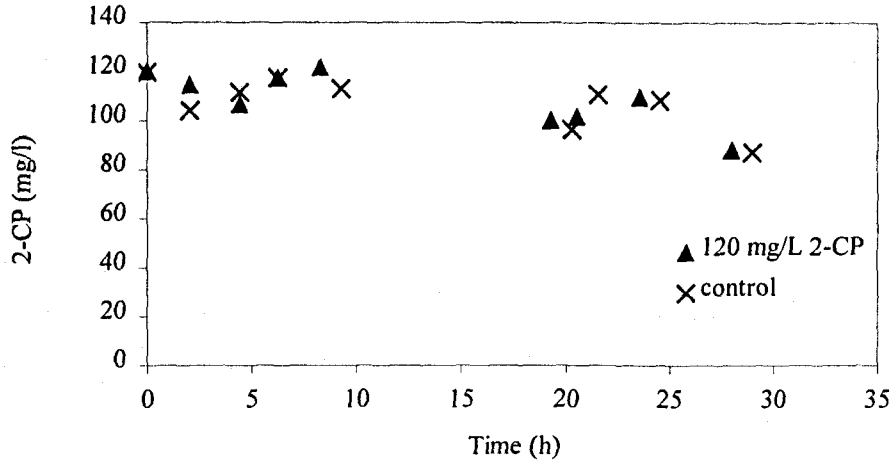

Figure B.5.2. Time course variation of 2-CP at the initial concentration of 120 mg/L

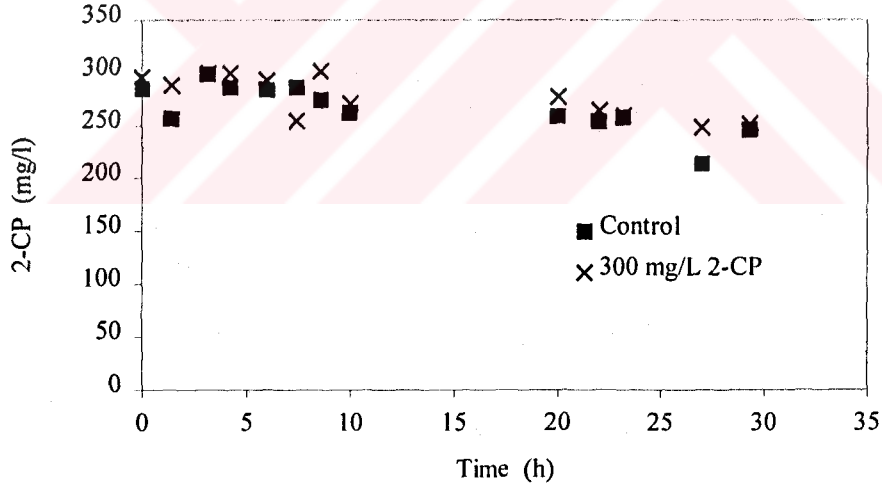

Figure B.5.3. Time course variation of 2-CP at the initial concentration of 300 mg/L

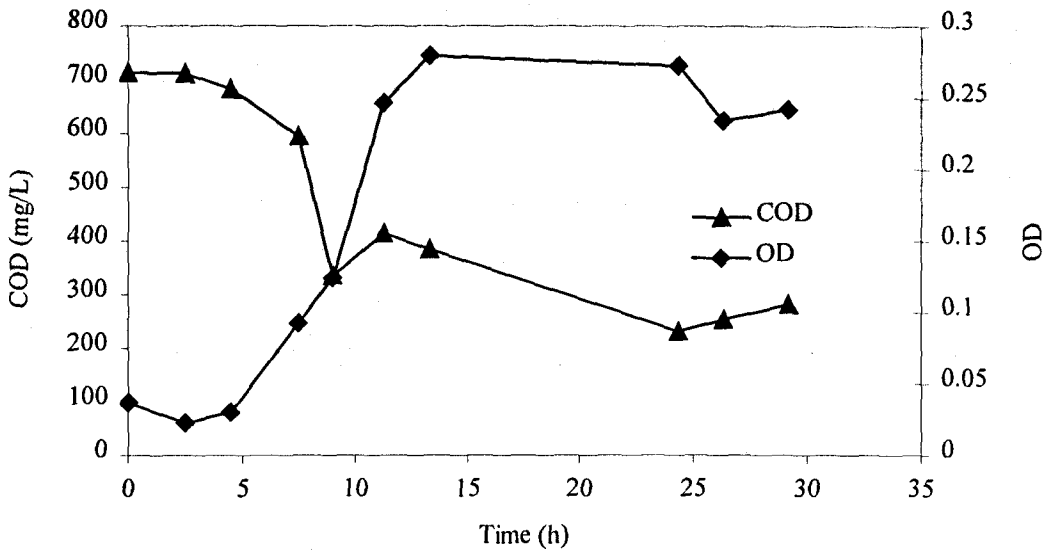

Figure B.6. Time course variations of OD and COD concentrations in the presence of 57 mg/L 4-CP for reactor inoculated with unacclimated culture

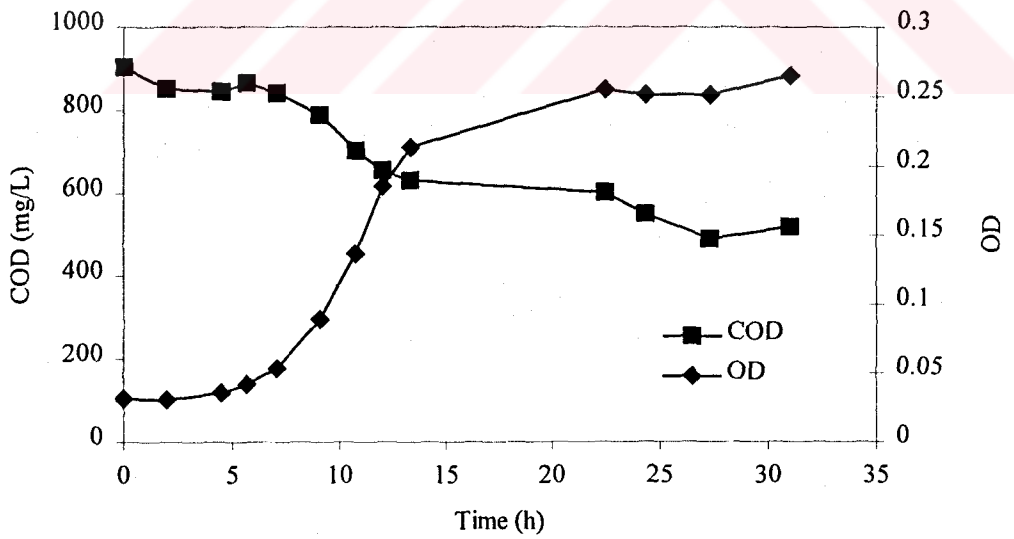

Figure B.7. Time course variations of OD and COD concentrations in the presence of 112 mg/L 4-CP for reactor inoculated with unacclimated culture

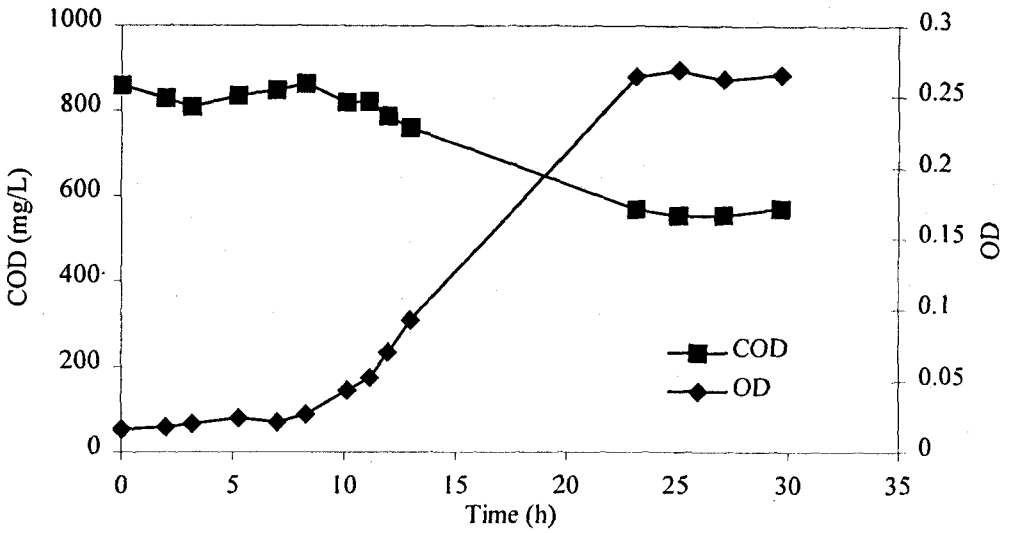

Figure B.8. Time course variations of OD and COD concentrations in the presence of 155 mg/L 4-CP for reactor inoculated with unacclimated culture

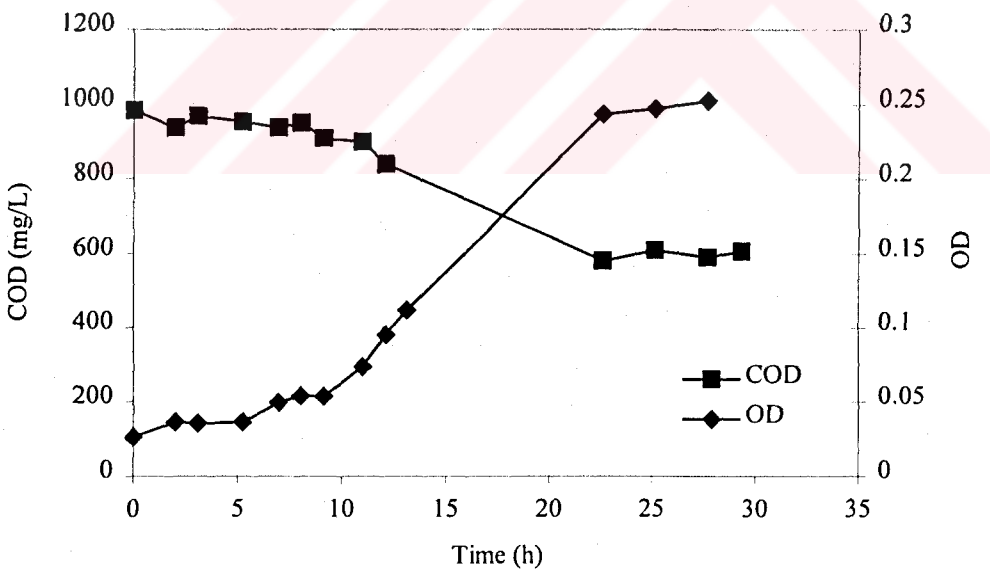

Figure B.9. Time course variations of OD and COD concentrations in the presence of 274 mg/L 4-CP for reactor inoculated with unacclimated culture

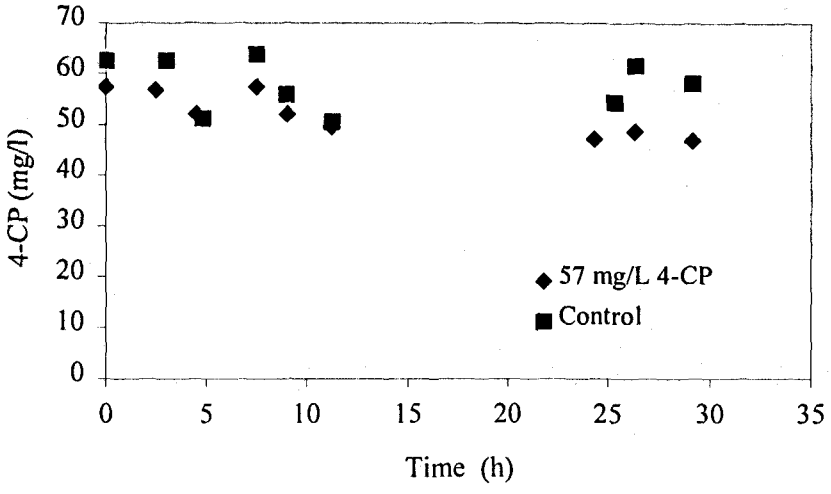

Figure B.10.1. Time course variation of 4-CP at the initial concentration of 57 mg/L

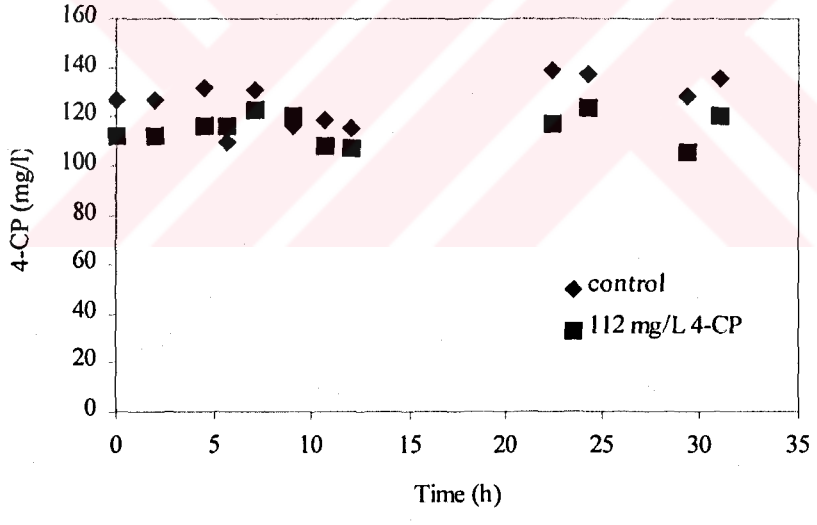

Figure B.10.2. Time course variation of 4-CP at the initial concentration of 112 mg/L

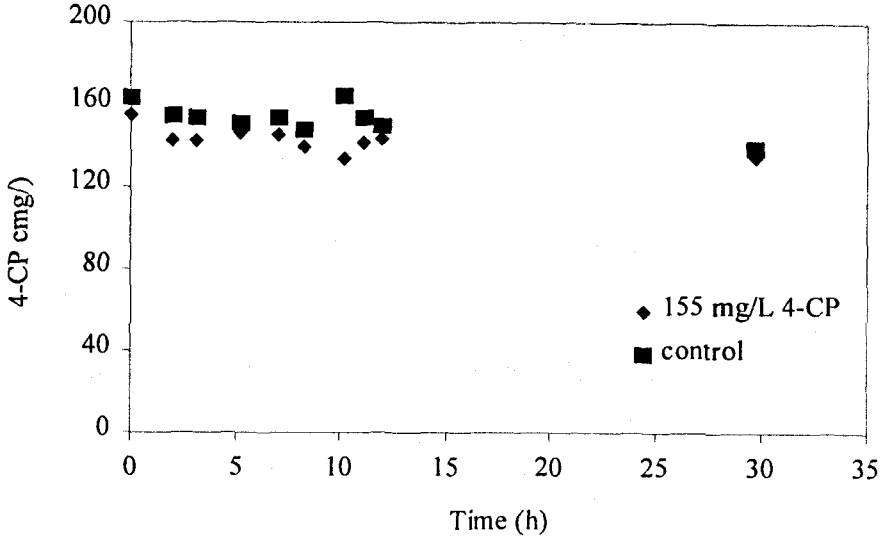

Figure B.10.3 Time course variation of 4-CP at the initial concentration of 155 mg/L

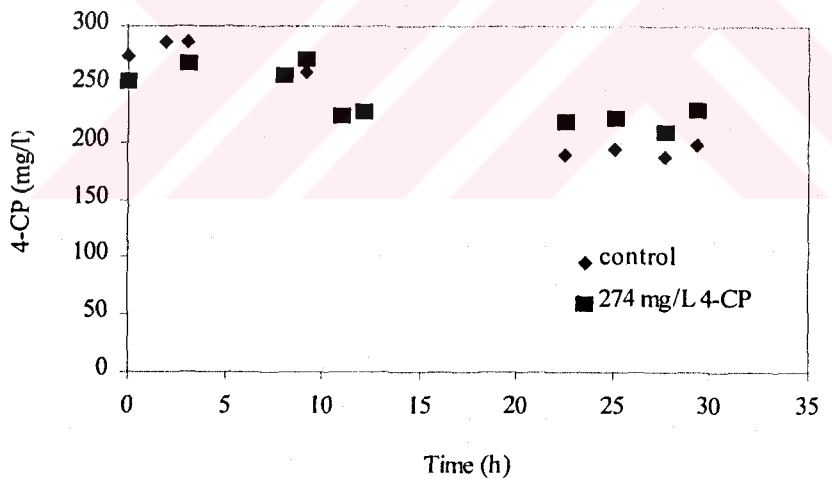

Figure B.10.4. Time course variation of 4-CP at the initial concentration of 274 mg/L

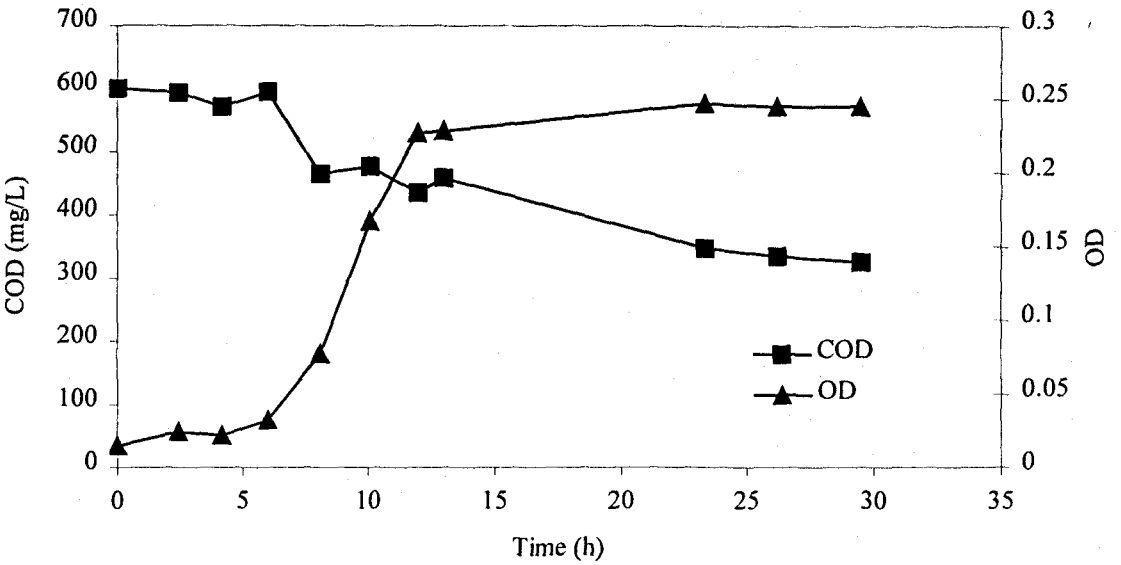

Figure B.11. Time course variations of OD and COD concentrations in the presence of 22 mg/L 2,4-DCP for reactor inoculated with unacclimated culture

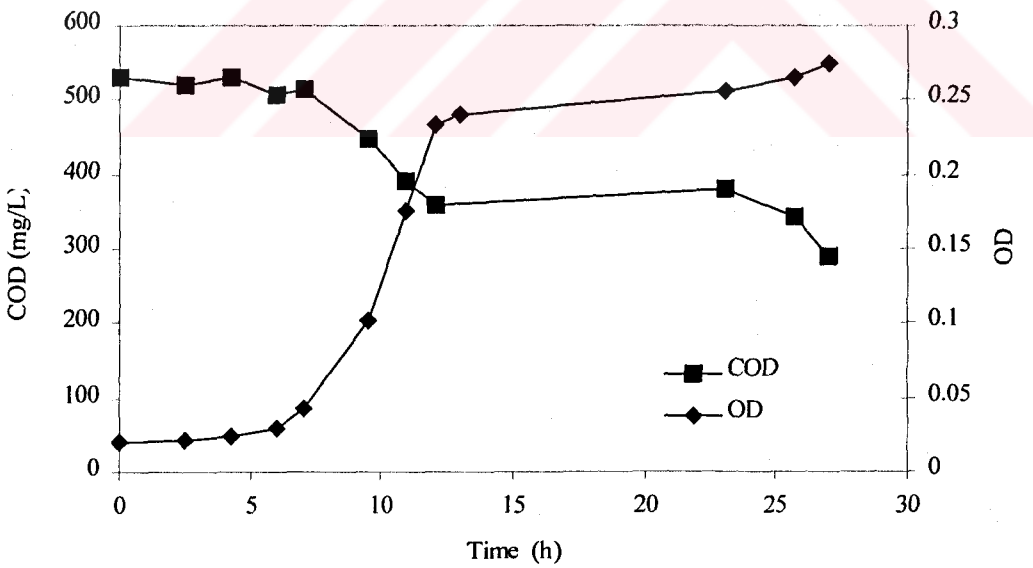

Figure B.12. Time course variations of OD and COD concentrations in the presence of 47.5 mg/L 2,4-DCP for reactor inoculated with unacclimated culture

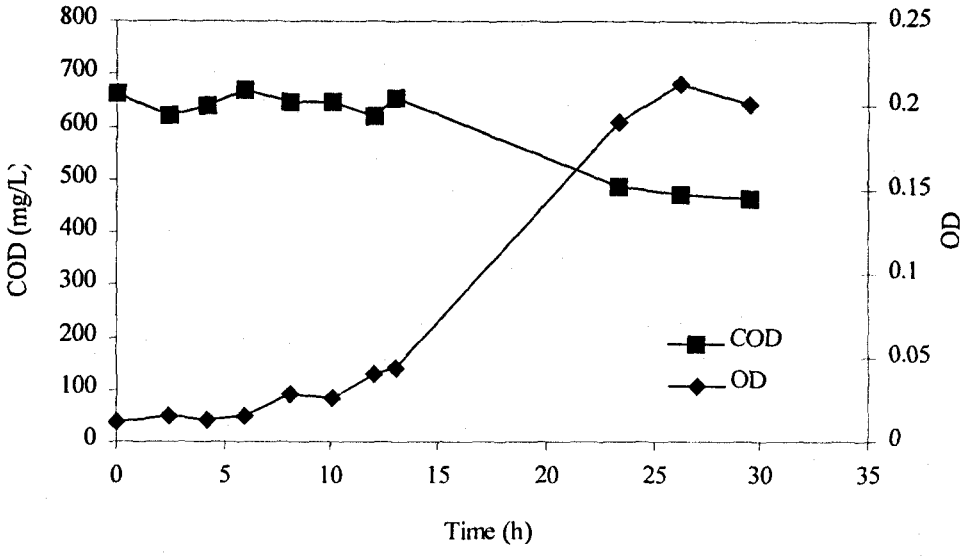

Figure B.13. Time course variations of OD and COD concentrations in the presence of 77 mg/L 2,4-DCP for reactor inoculated with unacclimated culture

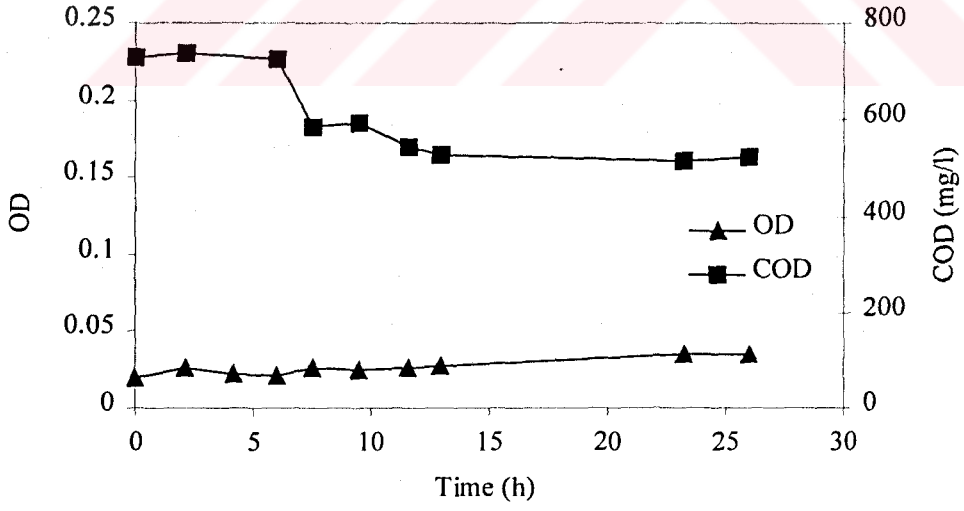

Figure B.14. Time course variations of OD and COD concentrations in the presence of 100 mg/L 2,4-DCP for reactor inoculated with unacclimated culture

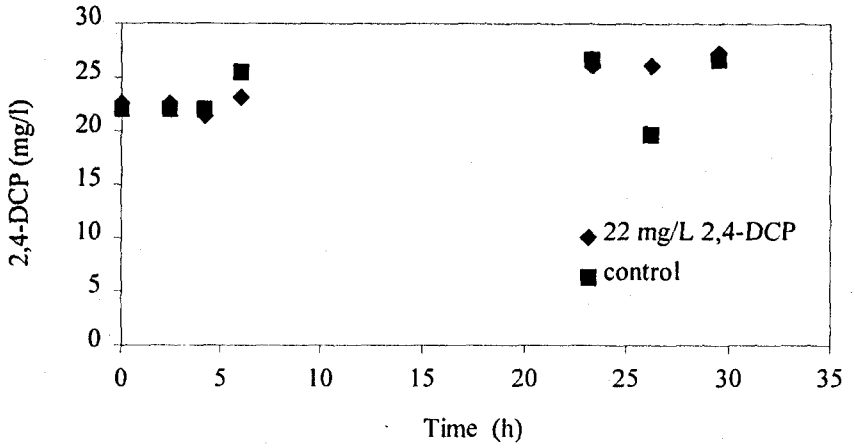

Figure B.15.1. Time course variation of 2,4-DCP at the initial concentration of 22 mg/L

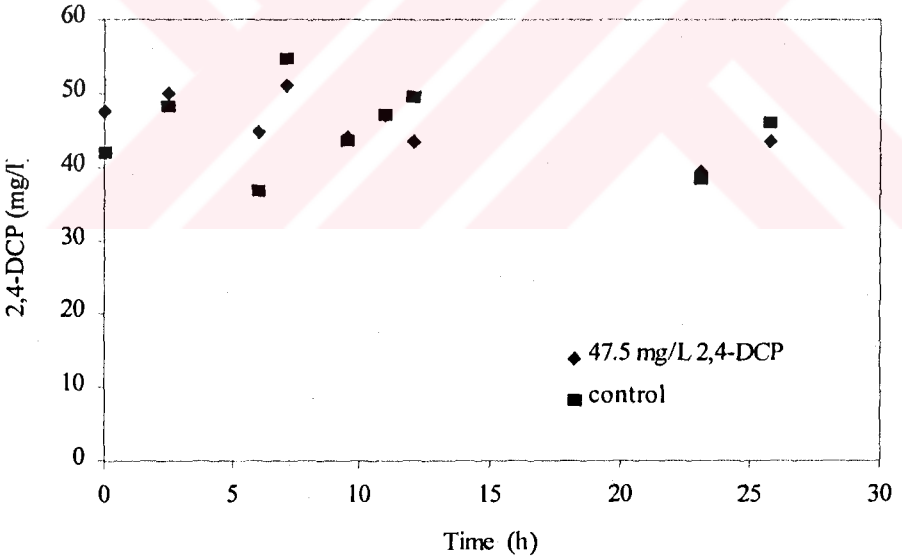

Figure B.15.2. Time course variation of 2,4-DCP at the initial concentration of 47.5 mg/L

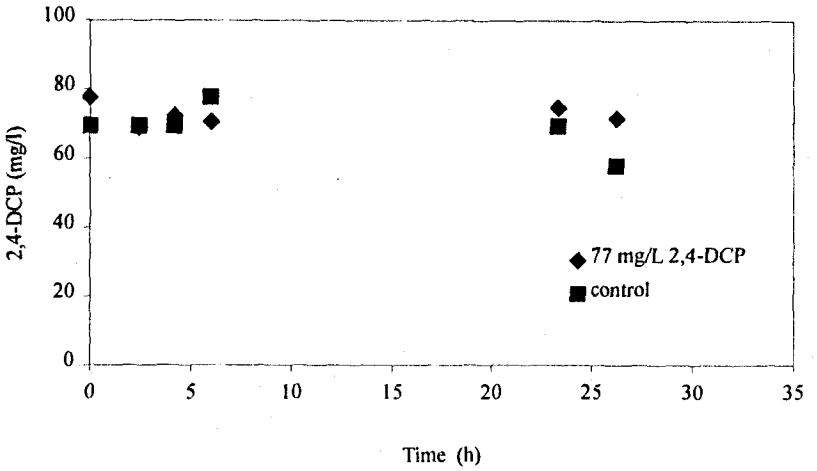

Figure B.15.3. Time course variation of 2,4-DCP at the initial concentration of 77 mg/L

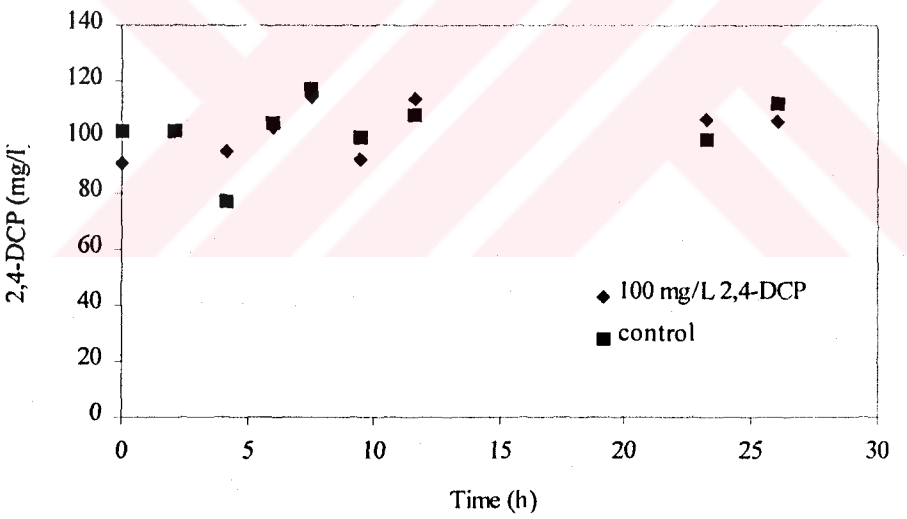

Figure B.15.4. Time course variation of 2,4-DCP at the initial concentration of 100 mg/L

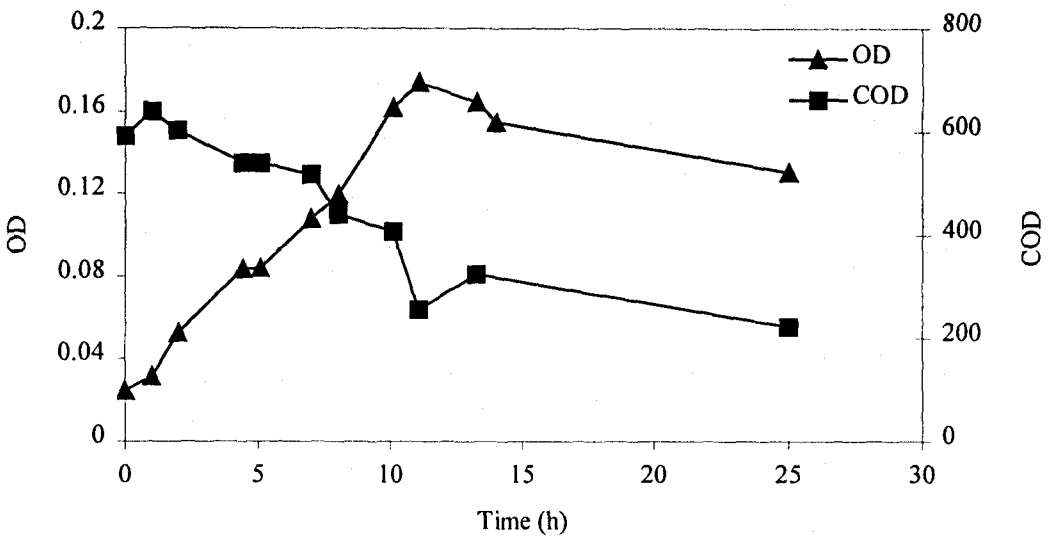

Figure B.16.1. Time course variations of OD and COD concentrations for base-line reactor inoculated with 4-CP acclimated culture

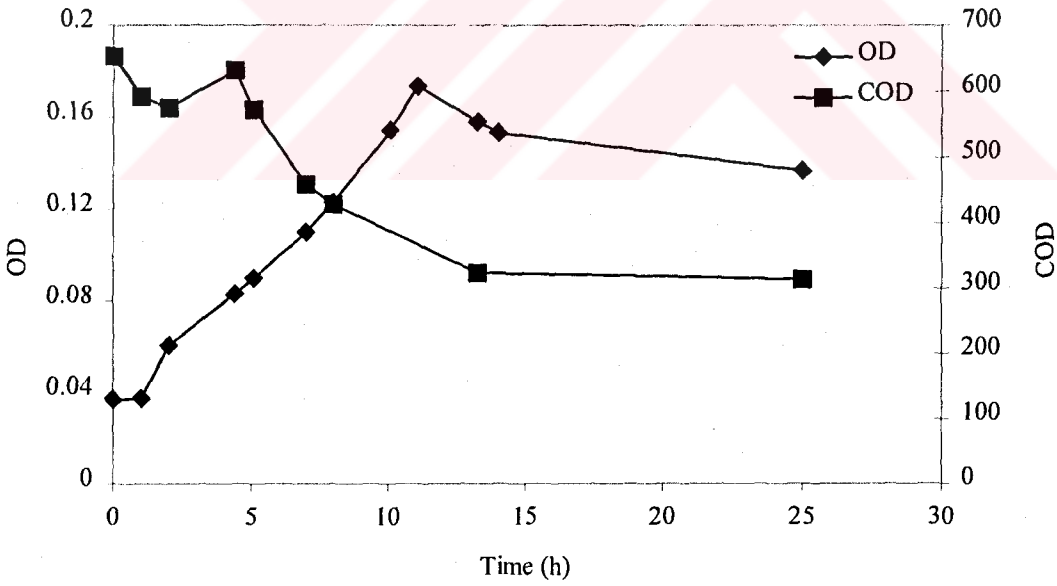

Figure B.16.2. Time course variations of OD and COD concentrations for base-line reactor inoculated with 2,4-DCP acclimated culture

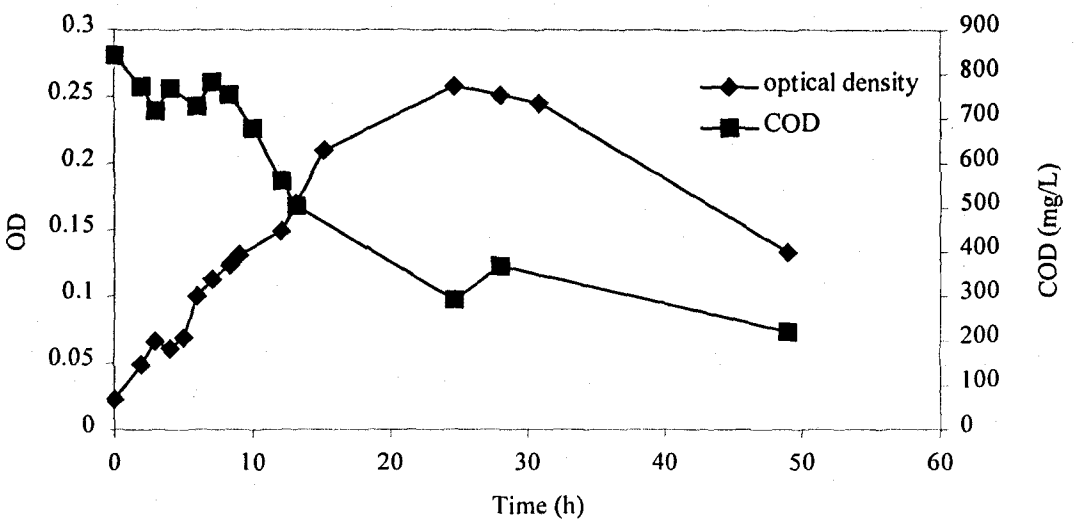

Figure B.17. Time course variations of OD and COD concentrations in the presence of 130 mg/L 4-CP for reactors inoculated with 4-CP acclimated culture

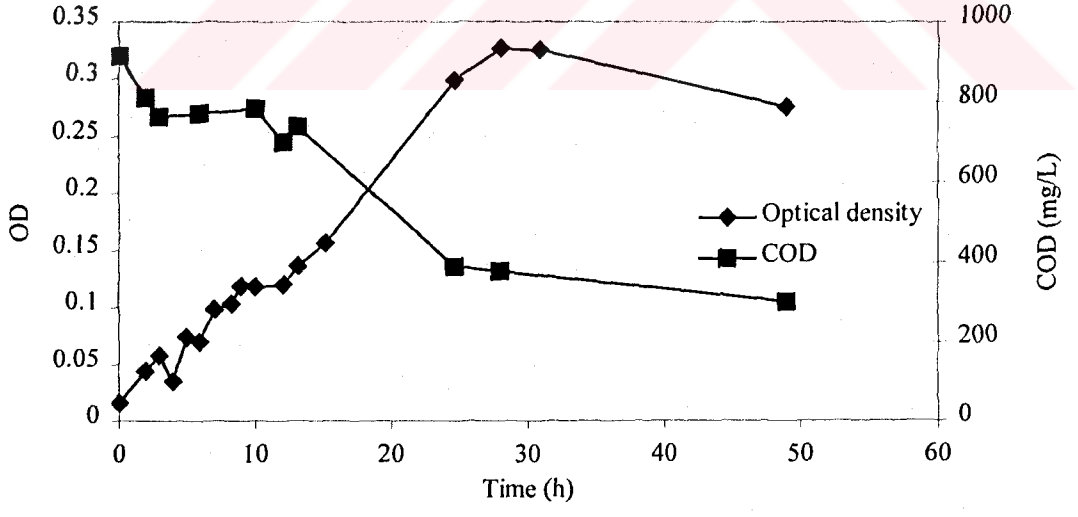

Figure B.18. Time course variations of OD and COD concentrations in the presence of 200 mg/L 4-CP for reactors inoculated with 4-CP acclimated culture

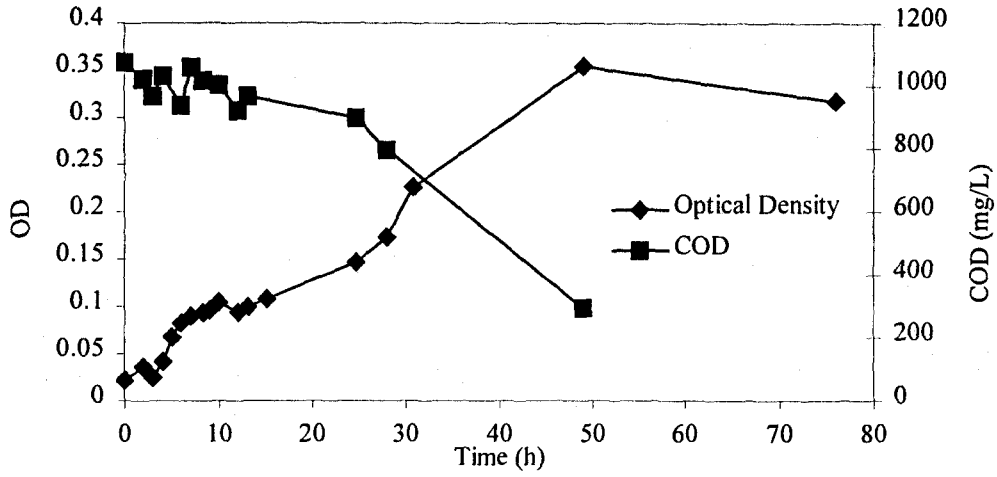

Figure B.19. Time course variations of OD and COD concentrations in the presence of 300 mg/L 4-CP for reactors inoculated with 4-CP acclimated culture

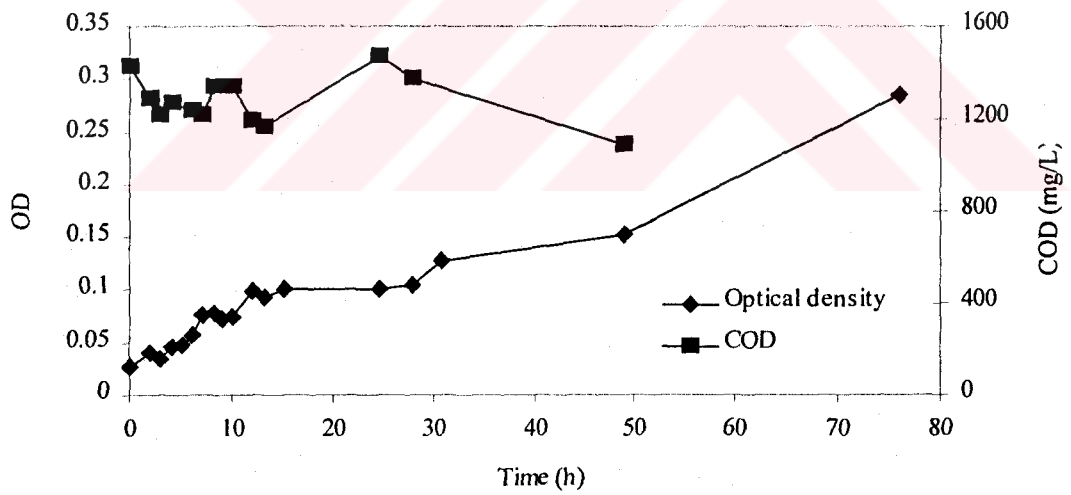

Figure B.20. Time course variations of OD and COD concentrations in the presence of 390 mg/L 4-CP for reactors inoculated with 4-CP acclimated culture

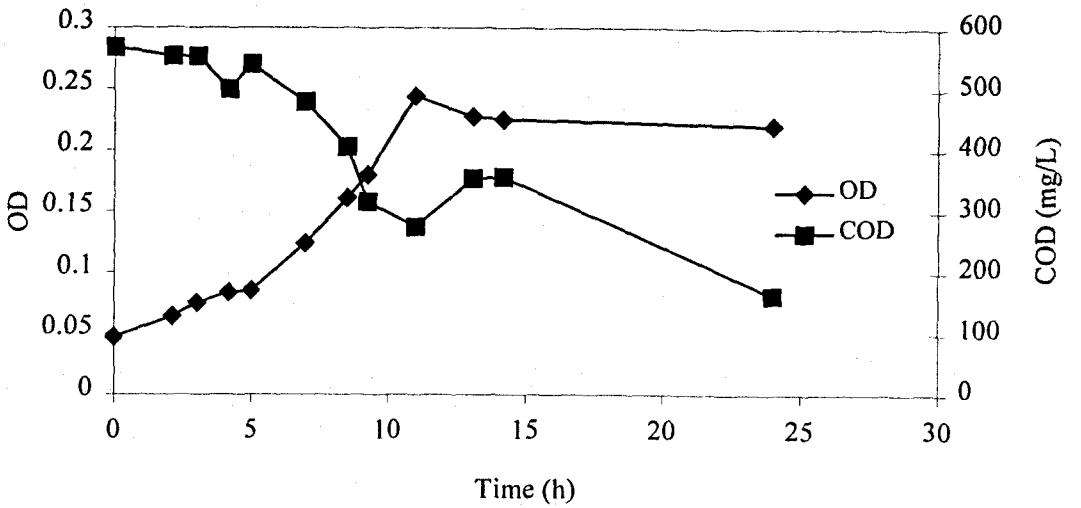

Figure B.21. Time course variations of OD and COD concentrations in the presence of 76.6 mg/L 2,4-DCP for reactors inoculated with 2,4-DCP acclimated culture

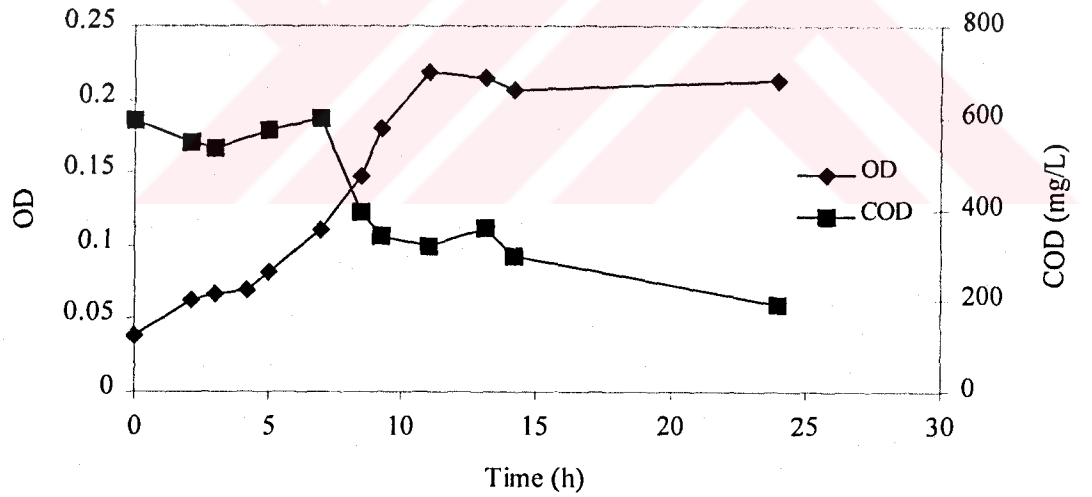

Figure B.22. Time course variations of OD and COD concentrations in the presence of 101.2 mg/L 2,4-DCP for reactors inoculated with 2,4-DCP acclimated culture

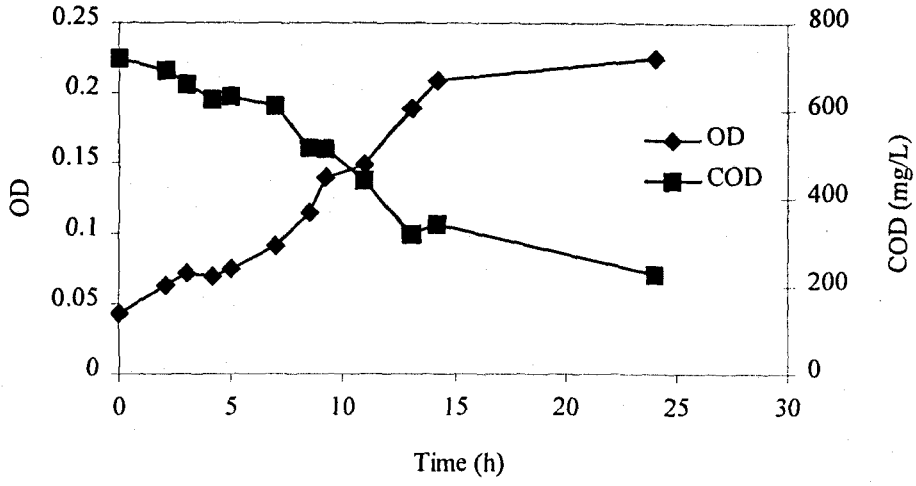

Figure B.23. Time course variations of OD and COD concentrations in the presence of 148.7 mg/L 2,4-DCP for reactors inoculated with 2,4-DCP acclimated culture

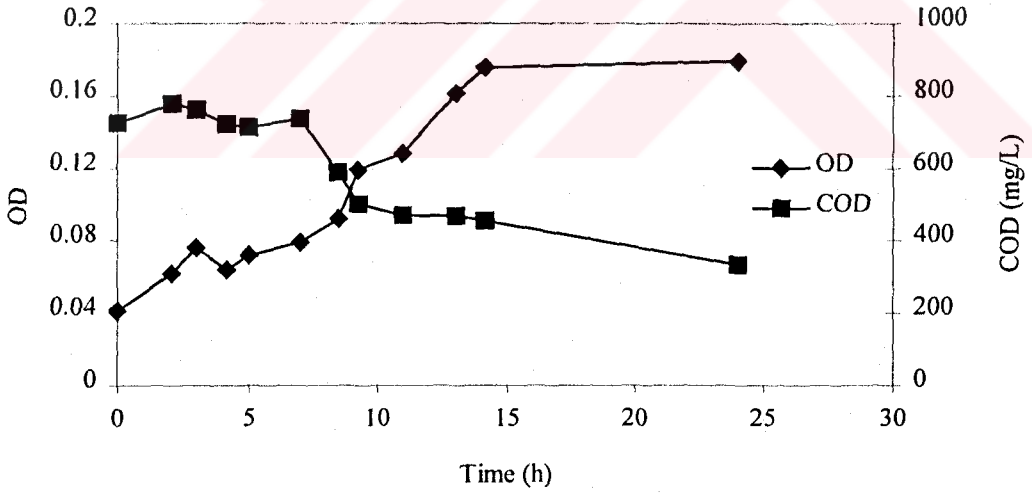

Figure B.24. Time course variations of OD and COD concentrations in the presence of 195 mg/L 2,4-DCP for reactors inoculated with 2,4-DCP acclimated culture

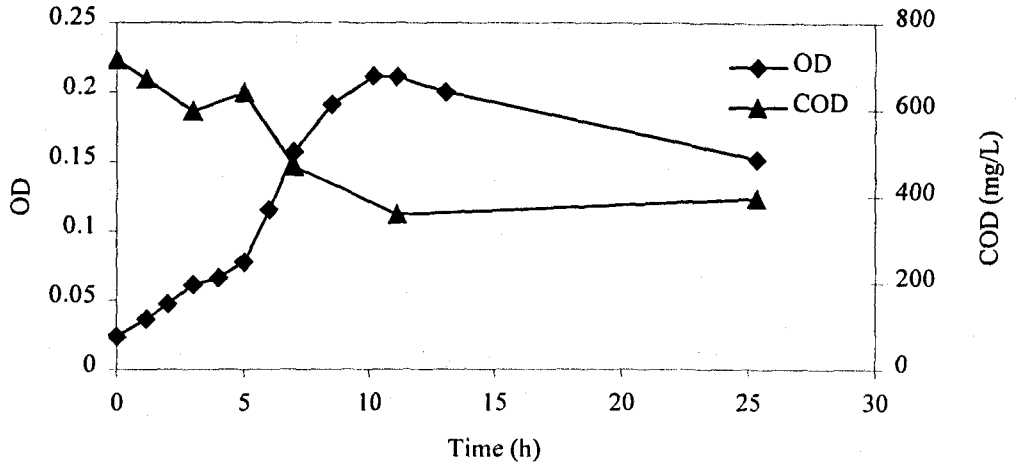

Figure B.25. Time course variations of OD and COD concentrations of batch experiment conducted on effluent of fed-batch reactor treating 130 mg/L 4-CP.

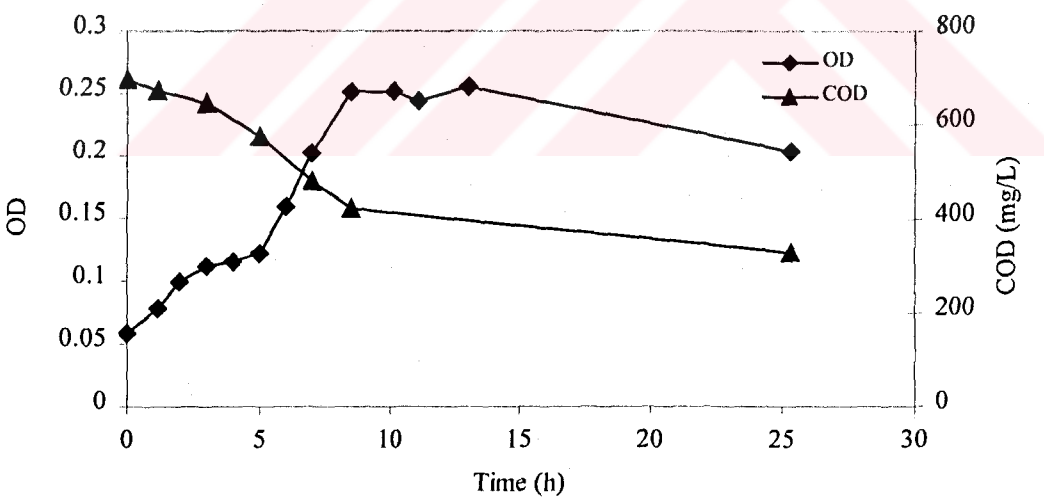

Figure B.26. Time course variations of OD and COD concentrations of batch experiment conducted on effluent of fed-batch reactor treating 75 mg/L 2,4-DCP.

APPENDIX C

RESULTS OF BATCH EXPERIMENTS UNDER ANOXIC CONDITIONS

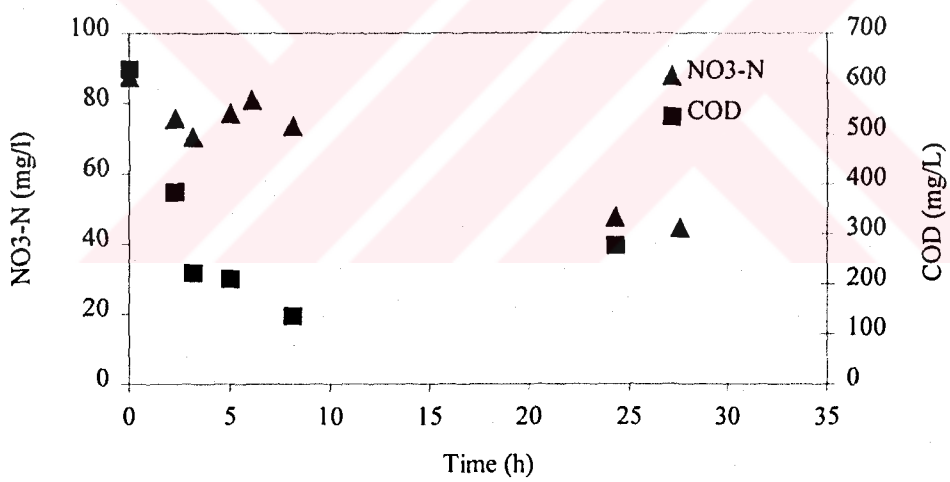

Figure C.1. NO_3-N and COD variations with time for reactor with 20 mg/L 4-CP.

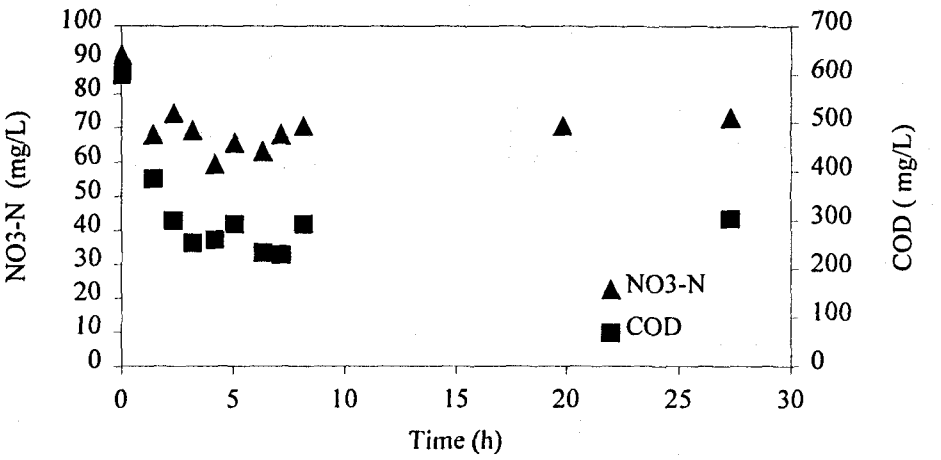

Figure C.2. NO_3-N and COD variations with time for reactor with 33 mg/L 4-CP.

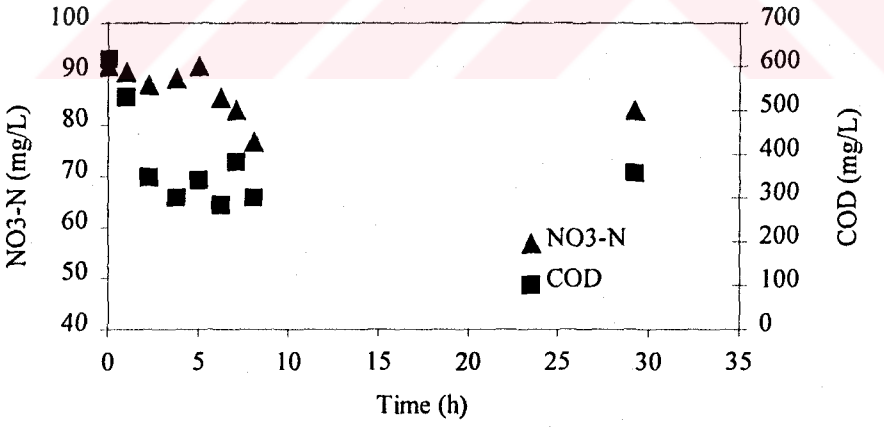

Figure C.3. NO_3-N and COD concentrations with time for reactor with 38 mg/L 4-CP.

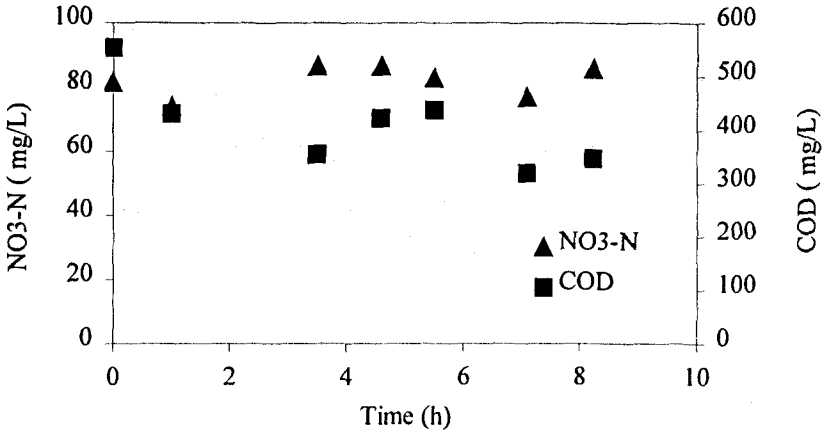

Figure C.4. NO_3-N and COD concentrations with time for reactor receiving 50 mg/L 4- CP.

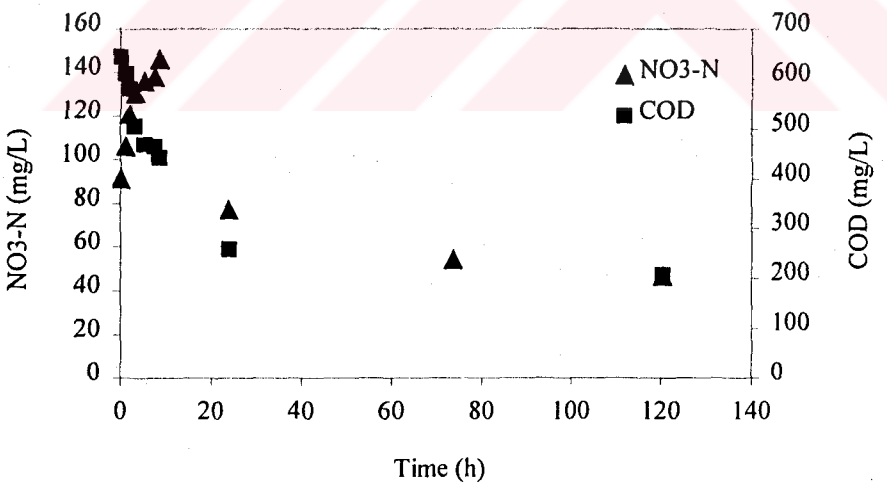

Figure C.5. NO_3-N and COD variations with time for reactor with 10 mg/L 2,4-DCP.

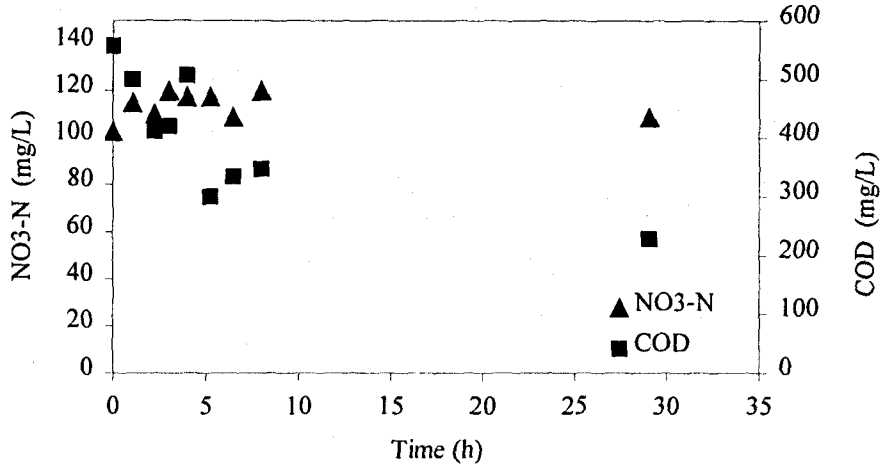

Figure C.6. NO_3-N and COD variations with time for reactor with 18 mg/L 2,4-DCP.

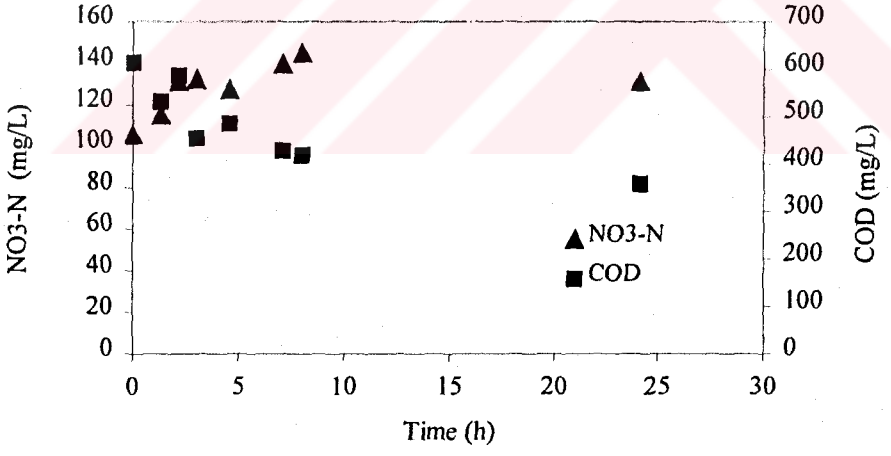

Figure C.7. NO_3-N and COD variations with time for reactor receiving 27 mg/L 2,4-DCP