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BIODEGRADATION OF 2,4-DICHLOROPHENOL IN SEQUENCING BATCH ANAEROBIC REACTORS

by

Yisu Zeng

M.A.Sc Thesis

Submitted to the School of Graduate Studies and Research

under the Supervision of Dr. L. Fernandes

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ABSTRACT

A synthetic wastewater containing 20 mg/L 2,4-dichlorophenol (2,4-DCP) and medium-strength carbonaceous co-substrate (7 g COD/L) was used to investigate the feasibility of treating chlorophenols in laboratory-scale sequencing batch upflow anaerobic sludge blanket reactors, designated as SBAR. The specific organic loading rate (SOLR) and specific 2,4-DCP loading rate (SDCPLR) tested were 0.35, 0.53, 0.70 g COD/g VSS/d and 1.0, 1.5 and 20 mg 2,4-DCP/g VSS/d, respectively. For each SOLR and SDCPLR operating condition, three different fill/react ratios, 0.33, 0.66 and 1.00 were examined in order to assess the performance of SBAR operating strategies. One continuous upflow anaerobic sludge blanket (UASB) reactor used as the control was operated at three SOLR and SDCPLR corresponding to those applied to SBARs.

Experimental results show that laboratory-scale SBARs were able to treat 2,4-DCP contaminated wastewater in addition to medium-strength carbonaceous co-substrate. The over all removal efficiencies of SBARs were almost 100% for 2,4-DCP and ranged from 72.5% to 94.2% for carbonaceous co-substrate. Under studied conditions, the performance of the continuous flow reactor was quite similar to that of sequencing batch reactors. Removal efficiencies for the continuous flow reactor were almost 100% for 2,4-DCP and ranged from 84.2% to 93.3% for COD. The major biodegradation product of 2,4-DCP in both sequencing batch and continuous UASB reactors was 4-monochlorophenol.

This experimental work also confirmed earlier observations that SOLR significantly influenced the overall COD removal efficiency of the SBAR, and SOLR based on fill time $(SOLR)_f$ is an important design parameter for SBAR systems.

Experimental data from this study were also compared with the simulated results obtained using a previously developed dynamic SBAR model. It was verified that this model can predict the SBAR performance reasonably well.

TABLE OF CONTENTS

LIST OF FIGURES

Figures

LIST OF TABLES

Tables

GLOSSARY

c = HS fraction in total COD

COD = chemical oxygen demand

DCP = dichlorophenol

HS = unionized volatile fatty acids concentration, g/L

k = maximum specific substrate uptake rate constant, h^{-1}

K_a = ionization constant

K_i = product inhibition constant, g/L

K_s = half velocity constant, g/L

K'_i = apparent product inhibition constant, g/L

K'_s = apparent half velocity constant, g/L

MCP = monochlorophenol

PCP = pentachlorophenol

Q = influent flow rate in SBAR fill stage, L/h

R = rate of substrate uptake, g/L/h

R1C1 = the first SBAR cycle of reactor #1

R1C2 = the second SBAR cycle of reactor #1

R1C3 = the third SBAR cycle of reactor #1

R2C1 = the first SBAR cycle of reactor #2

R2C2 = the second SBAR cycle of reactor #2

R2C3 = the third SBAR cycle of reactor #2

R3C1 = the first SBAR cycle of reactor #3

R3C2 = the second SBAR cycle of reactor #3

R3C3 = the third SBAR cycle of reactor #3

R4C1 = the first cycle of control reactor #4

R4C2 = the second cycle of control reactor #4

R4C3 = the third cycle of control reactor #4

SBAR = sequencing batch anaerobic reactor

SBR = sequencing batch reactor

SDCPLR= specific 2,4-dichlorophenol loading rate, mg DCP/g VSS/d

$(SDCPLR)_f$ = specific 2,4-dichlorophenol loading rate based on fill time, mg DCP_{in}/g VSS/d

SOLR = specific organic loading rate, g COD/g VSS/d

$(SOLR)_f$= specific organic loading rate based on fill time, g COD_{in}/g VSS/d

S_{VFA} = volatile fatty acids concentration, g/L

t = total reaction time, h

t_f = fill time, h

t_r = react time, h

TCP = trichlorophenol

TeCP = tetrachlorophenol

v = culture volume, L

V_T = culture volume at the end of fill stage, L

VSS = volatile suspended solids

X = biomass concentration, g/L

CHPTER ONE

INTRODUCTION

1.1 Introduction

Chlorophenols, in particular 2,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol have been placed on the US Environmental Protection Agency list of priority pollutants (LaGrega et al., 1994). There are many pollution sources of chlorophenols in the environment. Chlorophenols are widely used as biocides for wood preservation; they are formed as by-products when chlorine is used for bleaching of pulp and for disinfecting drinking water; they are also formed during combustion of organic matter and as biological breakdown products of chlorophenoxyacetic acid herbicides. The highly toxic nature and widespread use of chlorophenols have brought an increased concern over the removal of these compounds from waste streams (Paasivirta, 1988).

The biodegradation of chlorophenols has been studied in both aerobic and anaerobic systems (Murray and Richardson, 1993; Randle et al., 1991). Under anaerobic conditions, chlorine can be removed from the aromatic ring by reductive dechlorination resulting in partially or fully dehalogenated products, that are then more susceptible to either aerobic or anaerobic attack (Tiedje et al., 1987). Reductive dechlorination of chlorophenols has been demonstrated in anaerobic sludges. Upflow anaerobic sludge blanket (UASB) reactors have been used as a continual treatment system in order to enhance the biodegradation of chlorophenols. It was reported by Woods and co-workers (1989) that chlorophenols can be effectively biodegraded in continuous flow UASB reactors and the anaerobic dechlorination of chlorophenol had very strong position preference. However, different chlorophenol biodegradation

pathways have been reported using continuous UASB reactors (Hendriksen et al., 1991; Lu, 1992; Woods, 1989; Krumme and Boyd, 1988).

Although there are several reports of chlorophenol treatment in continuous flow reactors, no information regarding chlorophenol treatment in sequencing batch reactor (SBR) system has been reported yet. Compared to continuous flow systems, the major advantages of SBR systems include the ability to handle periodical flows and the ability to periodically change environmental conditions within biological reactors. Hence, the latter type of treatment system may be useful in selecting or enriching specific microbial populations, that could be more suitable for anaerobic dechlorination and may eventually be practical for treating chlorophenol contaminated industrial wastewaters.

Limited research has been done on the application of SBR technology to anaerobic treatment. Previous laboratory studies by Kennedy et al. (1991) have shown that SBARs were able to treat medium-strength soluble carbonaceous substrates, and the removal efficiencies of these substrates were significantly affected by the operating strategy of the reactor at high organic loadings. On the basis of this experimental work, a mathematical model was developed to describe soluble carbonaceous substrate degradation in SBARs (Fernandes et al., 1993). By examining the dynamic response and testing the validity of this model, it was found that the model can predict SBAR performances fairly well. However, this model was based on limited experimental data that were available, and more experimental data is required for this model verification.

1.2 Objectives

The general objective of this thesis is to demonstrate the treatablity of 2,4-dichlorophenol contaminated wastewater in sequencing batch upflow anaerobic sludge blanket reactors, and it is designated throughout the rest of the thesis as the SBAR treatment system.

The specific objectives of this thesis are:

1) To assess the feasibility and flexibility of sequencing batch anaerobic reactors to treat 2,4-dichlorophenol with medium strength easily biodegradable co-substrate.

2) To examine the fate of 2,4-dichlorophenol in sequencing batch and continuous UASB reactors.

3) To compare the performance of sequencing batch and continuous UASB treatment systems.

4) To test the validity of the dynamic model describing soluble carbonaceous substrate degradation in SBARs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

It was first recognized in the 1960s that some of the chemicals that had been used or disposed of in the environment were not disappearing and were becoming a hazard to humans and other animals. This environmental concern stimulated the development of a body of knowledge on persistence and biodegradation of a variety of organic chemicals. Chlorophenols are one group of these chemicals that have been intensively studied. Chlorinated phenols constitute a series of 19 compounds consisting of mono-, di-, tri-, and tetrachloroisomers and one pentachlorophenol (Fig. 2.1). At room temperature they all are crystalline solids with the exception of orthochlorophenol, which is a liquid. These compounds are toxic and/or mutagenic, difficult to degrade, have a propensity to bioaccumulate and pose a serious human health hazard (Ahlborg and Thunberg, 1980). Extensive studies have shown that pentachlorophenol and other lower chlorinated phenols are quite ubiquitous in the environment. So it is necessary to investigate the environmental impact of chlorophenols, to elucidate the mechanisms of chlorophenol removal, and to develop appropriate processes for chlorophenol treatment.

2.2 Chlorophenols in the environment

2.2.1 Chlorophenols: occurrence and sources

The introduction of modern analytical techniques has revealed the prevalent occurrence of chlorophenols in the environment. Chlorophenols have been detected in

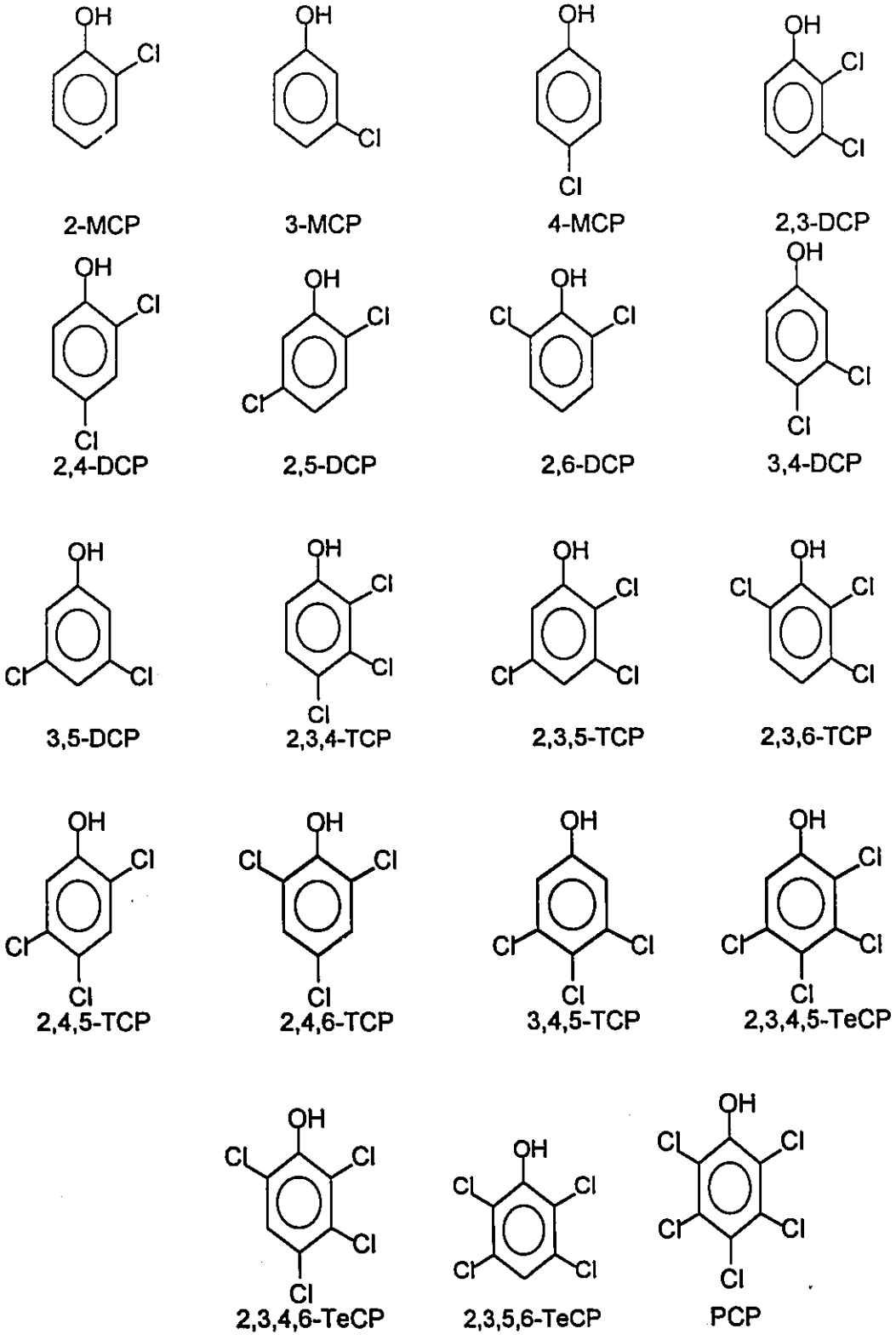


Fig. 2.1 Chemical structure of chlorophenols

waters (surface water, ground water, wastewater and rainfall), soils and in aquatic life in many parts of the world (Ahlborg and Thunberg, 1980). Chlorophenols have also been found in the fly ash and flue gases from different types of waste combustion incinerators (Paasivirta, 1988). In addition, several surveys have shown the occurrence of pentachlorophenol in human urine of exposed workers and nonoccupationally exposed persons. Furthermore, pentachlorophenol and other lower chlorinated phenols have been identified as metabolites of other organochlorine compounds in some aquatic creatures and other mammals. Fig. 2.2 is a summary of the main sources of human exposure to chlorophenols. It can be seen that chlorinated phenols are widespread in the environment, and there is an increased concern over the pollution source of chlorophenols.

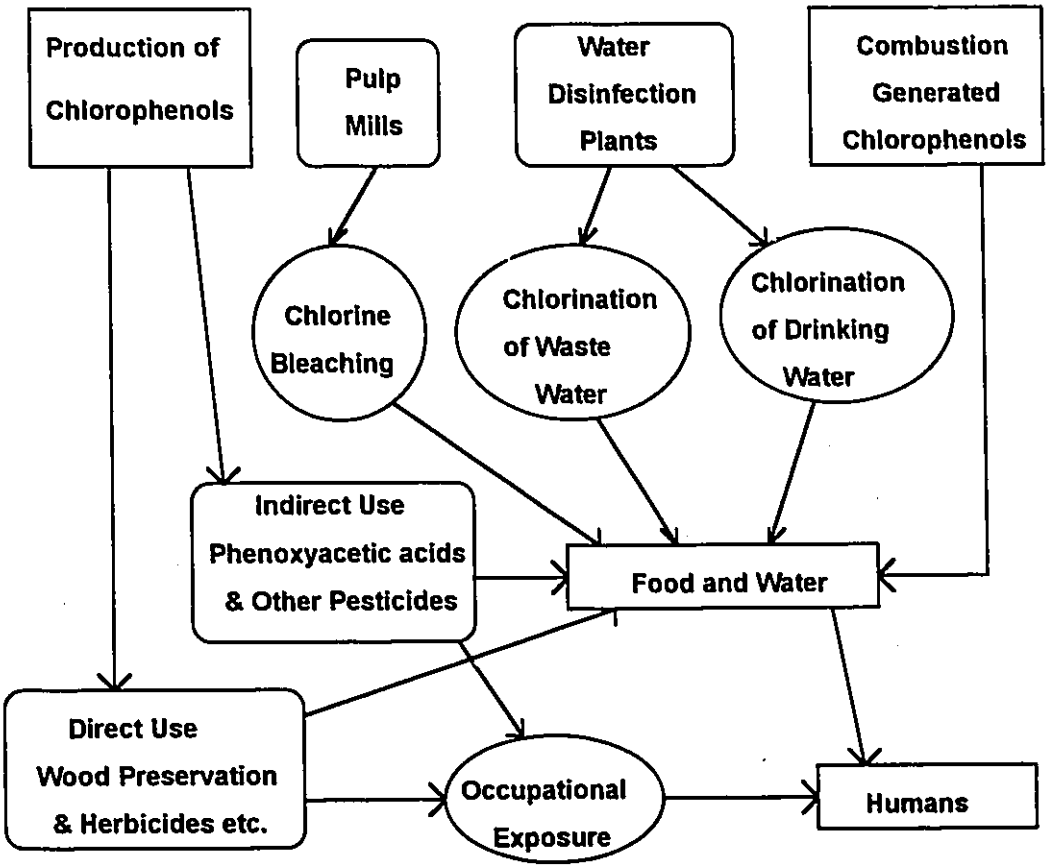


Fig. 2.2 Potential sources of human exposure to Chlorophenols

One extensive pollution source of chlorophenols in the environment is their production wastes. Since the early 1930s, pentachlorophenol and lower chlorinated phenols, tetra- and trichlorophenols, have been extensively used as fungicides, herbicides, insecticides, and as the precursors in the synthesis of other pesticides (Ahlborg and Thunberg, 1980). Due to the large amount of chlorophenols manufactured, dumping of production waste is one of the most serious environmental problems in the industrial area. More than 40 sites in the state of Missouri, USA, were heavily contaminated with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) containing chlorophenol wastes (Yanders, 1986). Large amounts of persistent toxic compounds were buried around Niagara City, USA (Vianna, 1983), in the surrounding of the Dow Chemical Company, Midland, Michigan, USA (Nestrik et al., 1986), near Amsterdam, the Netherlands (Heida, 1983), and in hundreds of other sites in the USA and Europe. The phenolic components of these dumps are a threat to aquatic life in polluted waters. The metabolites of phenols, such as methyl ethers, pose a long term risk to the environment because they are more persistent and fat-soluble aromatic chloroethers. (Paasivirta et al., 1988).

Other significant sources of organochlorine compounds entering the aquatic ecosystem are chloro-disinfecting of water and chloro-bleaching of pulp. It has been estimated that chlorine used by water disinfecting plants contributed to 8% of total amount of chlorophenols discharged into Finnish aquatic ecosystems (Ahlborg and Thunberg, 1980). In Canada, the pulp and paper industry is responsible for 50% of all the waste dumped into the nation's waters (Sinclair, 1990). In the U.S., pulp and paper mills are the nation's third largest polluter (Springer, 1986). Many pulp and paper mills use chlorine as a bleaching agent to produce high quality white pulp. As a result, a wide range of organochlorinated compounds are formed in the bleaching process. The wastewaters from the chlorine stage have been reported to contain up to 800 mg/L of chlorinated compounds (Vogel and Winter, 1988). Over 200 different chlorinated

compounds have been identified in effluents from bleaching pulp mills. The lower molecular weight organochlorinated compounds such as chlorophenols are responsible for almost all the toxicity of bleaching effluent due to their mutagenicity and possible carcinogenicity (Kringstad and Lindstorm, 1984). It was estimated by Lindberg and Lund (1980) that the concentration of 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol was up to 120 μg/L in mixed bleaching effluent. In general, these low molecular weight organochlorine compounds are only partially removed before the effluent is discharged to waterbodies. They will accumulate either in the sediment or in the body of aquatic creatures and eventually gain access into the food chain (Carberg et al., 1986; Salkinoja-Salonen et al., 1984).

The third important pollution source is combustion-generated chlorophenols. It was noticed that all organic matter will produce chlorophenols and other organochlorine compounds during combustion if chloride is present (Chiu et al., 1983; Eklund and Stromberg, 1983). Several reports have shown that chlorophenols were detected in the fly ash and flue gases of municipal waste incinerators and other types of incinerators (Paasivirta et al., 1985; Ahling and Bjorseth, 1978). This confirmed that chlorophenols were discharged to the atmosphere from various types of combustion. Paasivirta and coworkers (1985) observed that snow near heavy traffic routes contained exceptionally high levels of chlorophenols, especially 2,4,5-trichlorophenol. This suggested that automobile exhaust gas had very similar pattern of isomers as the emissions from municipal incinerators. These combustion-generated chlorophenols are transported into the atmosphere and in the sewage, and finally discharged into watercourses.

2.2.2 Toxicity of chlorophenols

2.2.2.1 Acute toxicity

The toxic nature of chlorophenols has been well established. The acute toxicity of chlorophenols was reviewed extensively by Ahlborg and Thunberg (1980). The toxic effects of monochlorophenols on humans include general pain, tremors, weakness, shaking, and finally collapse. Pentachlorophenol produces an intoxication, characterized by an initial period of central depression followed by an increase in respiratory rate and pulse rate, with increasing blood pressure and body temperature death is followed as a result of heart failure. The minimum lethal dose of pentachlorophenol for humans was estimated to be 29 mg/kg of body weight.

2.2.2.2 Chronic toxicity

The studies of chronic toxicity of chlorophenols on humans are rather limited. Brandt et al. (1977) reported a case study of a woman continuously exposed to pentachlorophenol vapors for several years in a wooden house that had been extensively treated with chlorophenol preservatives. The patient had suffered from liver damage and increased activity of liver enzymes. The liver enzymes decreased in activity almost every time the patient was away from her house for several days. Another recent case study revealed the medical consequences of drinking water polluted with phenol and chlorophenolic compounds, as a result of an industrial accident in Russia (Bulatova and Tereulova, 1992). When 25,000 people were examined after their exposure to the polluted water supply, irritation of health state was observed. The health state aggravation was displayed by contact reaction of the skin, oral mucous membranes, respiratory organs and primary reaction to general toxic

effect. Clinically they were revealed by prolonged recurrence of chronic diseases of organs in combination with allergic dermatoses.

2.2.2.3 Ecotoxicity

The harmful ecological effects of chlorophenols have been reported globally. Paasivirta et al. (1988) discovered chlorophenols in fish and mussels in Finland's lake biota. Pulp mills were thought to be the source of these compounds. However analyses of all chlorinated organic compounds in water, sediment and biota samples suggested that pulp bleaching was not the only major pollution source of chlorophenols. Use of wood preservatives and their dispersion in the air also appeared to be very important (Paasivirta et al., 1985). A similar conclusion was drawn by Grimvall et al., (1991) from studies on the long term fate of chlorophenols and absorbable organic halogens (AOX) in two large recipients, the Baltic Sea and Lake Vaettern in Sweden, of bleach-plant effluents. These researchers concluded that non-point sources (as a result of precipitation) can contribute considerably to the background levels of toxic compounds, which were normally regarded as indicators of bleach-plant effluents.

Furthermore, the possibility of ground water contamination by chlorophenols has also been investigated. Kitunen and Salkinoja-Salonen (1990) examined soils at an abandoned sawmill area in Finland, and discovered that the dry soil contained 50-1000 mg of polychlorinated phenols (CPs) per kg of soil. CPs were found to be mobile and leaching deep into the soil where they remained stable. Another study was conducted by Juergens and Roth (1989) at the site of a closed herbicide plant in West Germany. It was reported that the ground water below contaminated soil was polluted by chlorophenols. The high mobility of chlorophenols has the potential to contaminate potable ground water supplies.

In conclusion, the highly toxic nature coupled with mobility and wide spread use

of chlorophenols constitutes a threat to the environment and also to the top of food chain, humans. Therefore, removal of these compounds from waste streams is of great concern to the research community.

2.3 Fate of chlorophenols in biological wastewater treatment processes

2.3.1 Background

Various treatment processes have been studied on chlorophenol removal from waste streams. Among them, the biological treatment process is popular due to its practical and economical features. Volatilization (air stripping), biosorption and biodegradation are principal mechanisms for chlorophenol removal in biological wastewater treatment processes. Although considerable research has been conducted on the aerobic biodegradation of chlorophenols, relatively little is known about its biodegradability and fate in anaerobic system. Better understanding the fate of chlorophenols in an anaerobic environment would aid in developing efficient biological treatment systems. In the following sections, three major mechanisms of chlorophenol removal in biological reactors -- volatilization, biosorption and biodegradation will be discussed.

2.3.2 Volatilization

The release of volatile organic compounds (VOCs) from wastewater surfaces to the atmosphere is termed volatilization. VOCs are released because they partition between the gas and water phases until equilibrium concentrations are reached. Generally, the concentration of VOCs in the atmosphere is extremely low. Their transfer usually occurs from wastewater to the atmosphere. VOCs have high vapor pressures

and low solubility. Other factors influencing the rate and extent of volatilization are temperature, pressure, chemical reactions, ionic strength, mixing, the interfacial area available for transport and the concentration of the compound in solution (Weber, 1972; Smith et al., 1980).

The saturation concentration of a VOC in wastewater is a function of its partial pressure in the atmosphere in contact with the wastewater. This relationship is given by Henry's law, as follows:

$$\frac{C_g}{C_s} = H_c \qquad (2.1)$$

Where C_g = concentration of VOC in gas phase, μg/m³

C_s = saturation concentration of VOC in liquid, μg/m³

H_c = Henry's law constant, dimensionless

Henry's law constant is a useful indicator of a compound's potential for volatilization. Smith et al. (1980) reported that highly volatile compounds typically have Henry's law constants greater than 454 $m^3Pa/mole$. McCarty (1980) found compounds with H_c constants greater than 98.7 $m^3Pa/mole$ could be successfully removed by air stripping. The Henry's law constants for chlorinated phenols are generally low, ranging from 0.26 $m^3Pa/mole$ to 1.43 $m^3Pa/mole$. Another important indicator of a compound's potential for volatilization is the vapor pressure. Volatilization increases with vapor pressure. The vapor pressure of chlorophenols is quite low and it decreases with increasing chlorine numbers. Therefore, there is little tendency for chlorophenol volatilization under anaerobic conditions. Woods (1985) found that volatilization contributed to less than 1% chlorophenol removal in a continuous UASB system.

2.3.3 Biosorption

Biosorption is another potential removal mechanism of chlorophenols in a biological reactor. The uptake or accumulation of chemicals by microbial biomass is termed biosorption. The accumulation of toxic organic compounds by sorption onto wastewater solids not only affects the efficiency of the treatment system, but also impacts the management of wastewater solids.

2.3.3.1 Process description of biosorption

Biosorption of chlorinated phenolic compounds is an important step in the dechlorination process. In the literature, there is a lack of information on the actual biosorption mechanism of toxic organic compounds by wastewater solids. Most published reports on sorption of toxic organic compounds on biomass only correlate the experimental data using empirical equations. The biosorption could be affected by the biomass, surface area, microbial species, chemical composition, lipid content and age of the bacterial cells. High biosorption capacities usually correspond to biosorbents having high lipid contents. The type of membrane lipid present is also an important factor (Kennedy et al., 1992).

Generally, the Freundlich equation has been successfully used to construct biosorption isotherms for various hazardous organic pollutants.

$$q = KC_{eq}^{1/n} \qquad (2.2)$$

Where: q = equilibrium concentration of adsorbate on biomass, μg/g VSS

C_{eq} = equilibrium concentration of adsorbate in solution, μg/l

K = Freundlich constant,

1/n = empirical constant

When the exponent is equal to 1.0, the Freundlich constant reduces to a distribution coefficient K_d, describing a linear relationship between adsorbate concentration in the liquid and solid phase.

$$q = K_d C_{eq} \tag{2.3}$$

Where

K_d = distribution coefficient, l/g VSS

The octanol/water coefficient also has been demonstrated to be an indicator of chemical's biosorption potential. The octanol/water partition coefficient, K_{ow}, is defined as follows:

$$K_{ow} = C_o / C_w \tag{2.4}$$

Where

K_{ow} = octanol/water partition coefficient, dimensionless

C_o = concentration of the solute in octanol, µg/l

C_w = concentration of the solute in acidified water, µg/l

The higher the octanol/water coefficient the greater the biosorption of the compound. This trend is valid for a wide range of organic compounds and various biosorbents (Baughman and Paris, 1981; Bell and Tsezos, 1987).

Regarding the mechanisms of organic pollutant removal from wastewater by biomass, it is a key concern whether the biomass is metabolically degrading the compounds or whether the process is pure sorption. This concern was addressed by examining the difference in uptake of organic chemicals by live and dead biomass.

Tsezos and Bell (1989) reported that the uptake by dead biomass was equal or greater than that of live biomass. Higher sorption by dead biomass was suggested to be influenced by such factors as the absence of metabolic protection against cellular transport of pollutants, increased permeability of dead cell membrane, and the change in the surface adsorptive properties of the cells following death. These results suggest that the adsorption process is due to physical sorption, and not active uptake or biodegradation (Baughman, et al., 1981; Tsezos and Bell, 1989).

Biosorption by microorganisms is a relatively rapid process. Time to reach equilibrium was reported to be from a few seconds to several hours (Weber et al., 1972; Bell and Tsezos, 1987).

2.3.3.2 Sorption of chlorophenols by anaerobic sludge

In order to better understand the fate of chlorophenols and their degradation products in upflow anaerobic sludge blanket reactors, Kennedy et al. (1992) studied the sorption of chlorophenols by anaerobic sludge granules. The sorption isotherms were determined and the obtained data fitted the Freundlich equation quite well. These results confirmed that chlorophenols had linear sorption isotherms, which could be defined by simple distribution coefficients. However, these distribution coefficients were only weakly correlated to octanol-water partition coefficients. No obvious relationship was found between sorption and numbers or positions of chlorine substituents. All sorption equilibrium was reached within two hours.

Using granular and dispersed sludge from five different industrial sources Kennedy and Pham (1995) studied PCP biosorption isotherms. The biosorption capacities between dispersed and granular sludge showed no significant difference. PCP sorption equilibrium by anaerobic biomass was reached within three hours.

To evaluate the role of biosorption in continuous UASB reactors, Woods et al.

(1989) conducted mass balance of 2,4,6-TCP and it's two biodegradation products. The results showed that under steady state conditions, the sum of the mean molar concentrations of 2,4,6-TCP, 2,4-DCP and p-MCP in the treated effluent accounted for 99.4% of the influent 2,4,6-TCP concentration. These authors concluded that the removal of 2,4,6-TCP by sorption was not significant in their study. Similar conclusion was drawn by Lu (1992). By investigating the fate of several chlorophenol isomers in continuous UASB reactors, she found that biosorption on granular sludge only contributed up to 6% of total chlorophenols removal.

2.3.4 Biodegradation of chlorinated phenolic compounds

2.3.4.1 Advantages and disadvantages of aerobic and anaerobic dechlorination processes

The biodegradation of chlorophenols has been studied in both aerobic and anaerobic systems, especially in pure aerobic cultures. Chlorophenols may be aerobically degraded by two distinct mechanisms. Mono- and dichlorophenols are usually degraded by hydroxylation to chlorocatechols, followed by dechlorination only after ring cleavage. In contrast, polychlorophenols are completely dechlorinated before cleavage of the aromatic ring (Murray and Richardson, 1993). In addition, several species of bacteria and fungi have been shown to O-methylate chlorinated phenols under aerobic conditions (Haggblom et al., 1988). Methoxylated compounds are usually more persistent and toxic to the ecosystem than their precursors, because methylation increases lipophilicity and the potential to bioaccumulate. Apparently, the aerobic degradation of polychlorophenols presents potential problems that must be taken into consideration in the design of wastewater treatment processes (Murray and Richardson, 1993). The dechlorination ability of a full scale conventional aerobic

biological treatment process was assessed by a number of researchers (Bryant et al., 1987; Leuenberger et al., 1985). Although certain removal rates of chlorinated compounds were achieved, the degradation of chlorinated organic compounds in aerobic environment was in general very low. The major removal mechanism of the chlorinated organic compounds seems to be sorption onto biomass and subsequent settling to the benthic layer, where the sorbed chlorinated compounds undergo anaerobic degradation via reductive dehalogenation.

Compared to an aerobic method, the advantages of anaerobic processes stem directly from the slow growth rate of methanogenic bacteria (Tchobanoglous and Burton, 1991). A slow microbial growth rate requires less nutrients and energy consumption, and has a low operation cost. The sludge production of an anaerobic system is also lower than that of an aerobic system. Besides, the methanogenic bacteria can convert most of the organic waste to methane gas, which is combustible and therefore a useful end product. In addition, anaerobic methods for chlorophenol treatment are also environmentally safe since the potential for stripping toxic chemicals into the ambient air is quite low. Furthermore, it has been proven that an anaerobic treatment system is more efficient for treating wastewater containing chlorophenols than an aerobic treatment system (Randle et al., 1991; Salkinoja-Salonen et al., 1984).

Although there are many advantages, the anaerobic dechlorination process also possesses some disadvantages. One of the major disadvantages is the high sensitivity of anaerobic microorganisms. Optimum environmental control is very important in anaerobic processes. Another major disadvantage is the inhibitory effects of chlorinated compounds on anaerobic organisms. But, upon substrate acclimation, the inhibitory effects can be substantially reduced.

2.3.4.2 Anaerobic dechlorination process microbiology

Three types of anaerobic consortia, acidogenic bacteria, acetogenic bacteria and methanogenic bacteria, are involved in ordinary anaerobic biodegradation of organic substrates. Acidogenic bacteria break down large organic polymers into short chain fatty acids such as acetic, propionic and butyric acids, as well as carbon dioxide and hydrogen. Acetogenic bacteria are responsible for the conversion of propionic and butyric acids to acetic acid, carbon dioxide and hydrogen. Methanogenic bacteria are able to utilize simple substrates such as formate, acetate, carbon dioxide, hydrogen and methyl alcohol to produce methane and carbon dioxide.

It is not clear whether ordinary bacterial strains in anaerobic communities or unusual microbes are responsible for anaerobic dechlorination. Conflicting information has been produced in this area. It was reported that any anaerobic digester sludge, i.e., an ordinary anaerobic microbial community, can dehalogenate chlorophenols (Ferguson and Dalentoft, 1991). In addition, the supplementary carbon sources such as acetate, hydrogen and methanol were found to increase the rate of dehalogenation. This might imply those methanogens carry out dehalogenation as a cometabolic process (Hendriksen et al., 1992). However, studies conducted by Mohn and Kennedy (1992a) and Hakulinen et al. (1985) indicated that the dehalogenating organisms were not necessarily ordinary members of anaerobic methanogenic communities. In fact, Hakulinen et al. (1985) found that in pure cultures, *Pseudomonas-like* organisms and yeast are responsible for chlorophenol degradation. On the other hand, Mohn and Kennedy (1992a) found that in mixed culture, *P.aeruginosa* and *K.oxytoca* were able to degrade 2,4,6-trichlorophenol. The microbe *Desulfomonile tiedjei DCB-1* was shown to reductively dehalogenate chlorophenols (Mohn and Kennedy 1992b) .

Microorganisms under anaerobic conditions are very sensitive to environmental changes in the reactor. In order to optimize the process for efficient treatment, a strict

control of the minimum conditions is required. The basic requirements to establish and maintain a healthy anaerobic dechlorination process are as follows:

Anaerobic conditions - Oxygen is toxic to obligate anaerobes, thus a complete absence of dissolved oxygen is required for optimum conditions.

Temperature control - It has been proven that reductive dechlorination occurred only in the temperature range from 15 to 40°C and chlorophenol degradation rate reached the peak at 35°C (Kohring et al. 1989). In order to maintain a stable wastewater treatment process, most research and industrial scale anaerobic digesters are temperature controlled between 30 to 38°C (Tchobanoglous and Burton, 1991).

pH control - The pH is a key factor in the growth of organisms. Generally, the optimum pH for anaerobic bacterial growth lies between 6.5 and 7.5. Bicarbonate ion is the common buffer used in anaerobic systems for providing alkalinity. The preferred concentration of alkalinity is in the range 1000 - 5000 mg/L expressed as $CaCO_3$. The volatile fatty acids in the system should be less than 500 mg/L (Tchobanoglous and Burton, 1991).

Good mixing - Good mixing is required to ensure an effective contact of the reactive components in any microbial or chemical system. Mixing can be achieved by mechanical mixing, supernatant recirculation and self generated mixing resulting from gas production.

Carbon and energy source - Dechlorinating activity can be maintained with chlorophenols as the sole carbon and energy source (Krumme and Boyd, 1988). However, the addition of easily biodegradable carbonaceous substrate stimulates the dechlorination of chloroaromatic compounds (Hendriksen et al., 1992). Hydrogen, methanol, acetate and glucose can be used as additional carbon sources.

Nutrient requirement - In order to optimize process treatment efficiencies, an adequate supply of nutrients must be provided. A COD:N:P ratio of 100:1:0.5 should be maintained to ensure a slight excess of nitrogen and phosphorous.

2.3.4.3 Inhibition and acclimation of anaerobic bacteria

The inhibitory effects of chlorophenols on anaerobic bacteria have been reported in both batch and continuous flow reactors. Two types of inhibition have been detected. In the first type of inhibition, the high loading rate of chlorinated compounds inhibited only the activity of the dechlorinating organisms and methanogenic bacteria. Less methane production and less AOX removal were usually observed in this type of inhibition (Wang et al., 1991; Hrudey et al., 1987). In the second type of inhibition, the high loading rate of chlorinated compounds inhibited activity not only of dechlorinating microorganisms but also of acidogenic, acetogenic and methanogenic bacteria. This type of inhibition caused total failure of the reactors (Guthrie et al., 1984).

Upon acclimation to certain toxic chemicals, the inhibition effects on anaerobic bacteria may be reduced. The microorganisms can develop a capacity to actually degrade compounds that showed initial toxicity. Most previous studies have focused on the microbial activity following acclimation. The acclimation process itself is poorly understood. Based on limited information, the characteristics of the acclimation period of anaerobic microbes can be summarized as follows:

1. The acclimation time periods varied for different substrates, but they were reproducible over time and among sampling sites. Also, they were characteristic of the chemicals tested. The concentrations of the chemical may or may not result in any significant differences in the acclimation periods (Linkfield et al., 1989).

2. Acclimation periods appeared to be a function of chemical structure. With the same average initial concentration, the lag periods for the ortho, meta, and para isomers of bromobenzoate were 20, 23, and 39 days, respectively (Linkfield et al., 1989). It was found that ortho-chlorophenol was apparently degraded without a lag period, while meta- and para-chlorophenols each required 28 days

before significant degradation occurred (Boyd and Shelton, 1984).

3. A microbial population acclimated to a particular substrate is often simultaneously acclimated to other substrates with very similar molecular structure. An acclimation period can stimulate subsequent dechlorination processes. With anaerobic sludge acclimated to chlorophenols, the lag time preceding dechlorination decreased and rates of dechlorination increased (Boyd and Shelton, 1984).

4. Sludge acclimation may change its substrate specificity. The same sludge acclimated to either 2-, 3-, or 4-MCP gave patterns of degradation distinctly different from those of fresh sludge. For example, sludge acclimated to 2-monochlorophenol degraded 2- and 4-monochlorophenol at equal rates, and 3-monochlorophenol was not degraded; also sludge acclimated to 3-monochlorophenol degraded 4-monochlorophenol but not 2-monochlorophenol. However, sludge acclimated to 4-monochlorophenol could degrade all three monochlorophenol isomers (Boyd and Shelton, 1984).

5. Possible explanations for the mechanisms effecting the acclimation period have been proposed: (1) genetic change, (2) induction, (3) exhaustion of a preferred substrate or (4) growth of an active population from a very low initial density. It was suggested that induction best explains the observed patterns of the acclimation process (Linkfield et al., 1989).

Acclimation periods for anaerobic reductive dechlorination vary from several days to 6 months or more. Because of its lengthy nature, the acclimation period cannot be ignored in evaluating anaerobic biodegradation potential of these chemicals.

2.3.4.4 Biodegradation pathway of chlorophenols in anaerobic environment

Microbial degradation of chlorophenols in anaerobic environments has not been

totally understood yet. Recognition of their biodegradation mechanisms is of cardinal importance for understanding the fate of these chemicals in the environment, and for developing methods for biological treatment of wastes containing chlorinated compounds.

Chlorinated compounds are highly oxidized and in a reducing environment may serve as electron acceptors for either biological or nonbiological reactions. In anaerobic environments, direct elimination of chlorine substituents from the aromatic ring usually occurs by displacement with either hydroxyl groups or hydrogen atoms (reductive dechlorination). Many researchers have investigated the pathways of this process. Interestingly, various patterns of chlorophenol degradation have been reported in the literature.

Fig. 2.3 summarizes the degradation pathways of chlorophenols in unacclimated sludge. Using conventional enrichment culture techniques, Boyd and Shelton (1984) studied the anaerobic biodegradation of mono- and dichlorophenol isomers using unacclimated sludge. Each of the monochlorophenol isomers was degraded and the relative rates of disappearance were ortho > meta > para. For dichlorophenols, reductive dechlorination of the ortho position chlorine was observed, and the monochlorophenol compounds released were subsequently degraded to methane and carbon dioxide. The dechlorinations of tri-, tetra- and pentachlorophenol were studied by Boyd and Shelton (1983) and Mikesell and Boyd (1986), and they found that chlorine in the ortho position could be easily degraded. Dechlorination may also occur in the meta position, but seldom in the para position. As Ferguson and Dalentoft (1991) concluded in their study, the dechlorination of a group of congeners proceeds with the most highly chlorinated congeners and with the very strong position

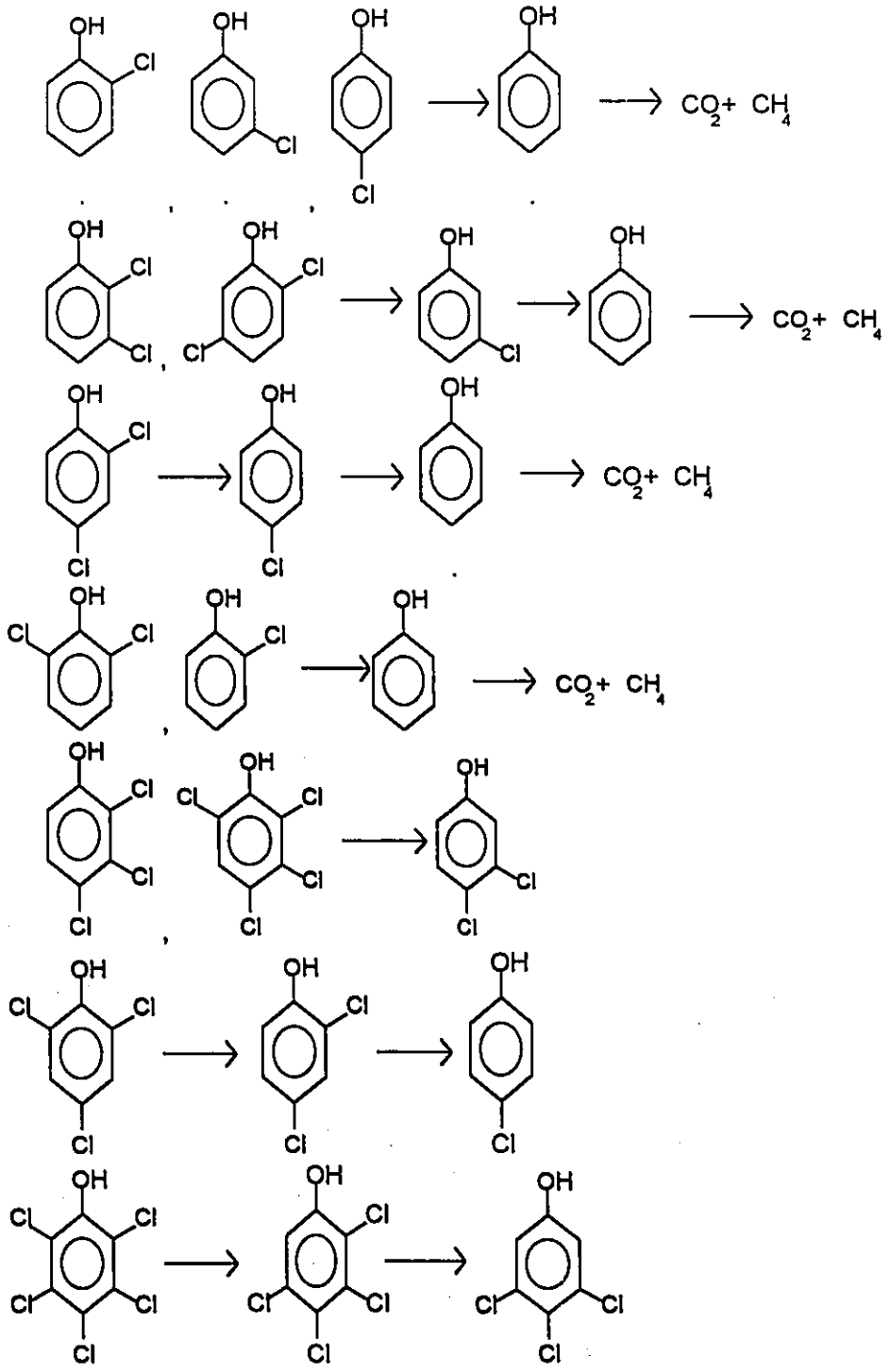


Fig. 2.3 Anaerobic biodegradation pathways of chlorophenols in unacclimated sludge (Source: Boyd and Shelton, 1983; and Mikesell and Boyd, 1986; Ferguson and Dalentoft, 1991)

preference for chlorine removed. The complete dechlorination of pentachlorophenol has not been observed in unacclimated sludge. Although complete dechlorination of PCP to phenol is the ideal situation, it should be noted that the partial removal of chlorine atoms yields lightly chlorinated phenols, that are generally less toxic, less likely to bioaccumulate and more amenable to possible subsequent aerobic degradation.

It was found that PCP could be degraded with sludges acclimated to each individual MCP (Mikesell and Boyd, 1986). The resulting biodegradation products are shown in Fig. 2.4. PCP degradation occurred most rapidly, in about three days, when using 2-MCP acclimated sludge. PCP degradation in the 3- and 4-MCP-acclimated sludge was considerably slower, requiring 12 and 9 days, respectively. When the 2-, 3- and 4-MCP-acclimated sludges were mixed in equal volumes, PCP was completely dechlorinated to methane and carbon dioxide. The pathway of this reaction is shown in Fig. 2.5.

As an alternative to conventional enrichment culture, the upflow anaerobic sludge blanket reactors also have been used to study biodegradation pathways of chlorophenols. Different chlorophenol biodegradation pathways were demonstrated using an unacclimated sludge. Woods and co-workers (1989) examined the fate of nine chlorophenol isomers in the continuous UASB reactors. They reported that ortho chlorine could be easily removed and with acclimation, meta chlorine was also removed. There was no evidence for dehalogenation of para chlorine. A summary of the degradation pathways for the studied chlorophenols is shown in Fig. 2.6. Later in 1991, Hendriksen et al. studied PCP dechlorination in two UASB reactors. One reactor received glucose (0.9 g/L) as an additional carbon source while the other one served as the control. As shown in Fig. 2.7, the major pathway for PCP dechlorination in both reactors was via 2,3,5,6-TeCP as an intermediate product. This result is in disagreement with the results obtained by Woods et al. (1989), in which an initial ortho cleavage leading to 2,3,4,5-TeCP was found.

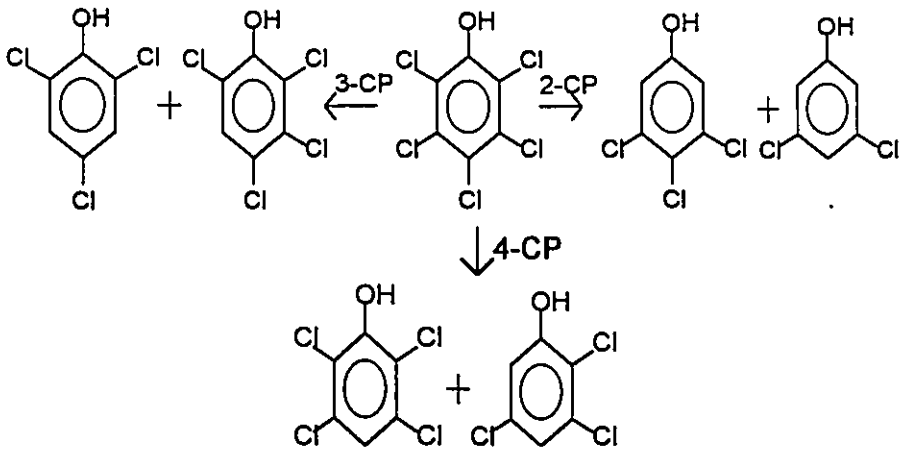


Fig. 2.4 Products resulting from the dechlorination of PCP using sludges acclimated to 2-,3- or 4-MCP.(Mikesell and Boyd, 1986)

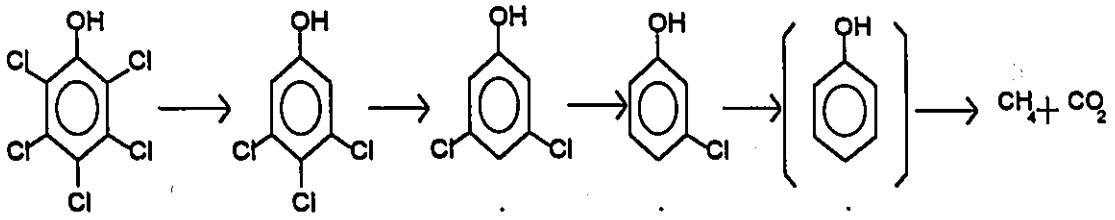


Fig. 2.5. PCP biodegradation pathway in mixed monochlorophenol acclimated sludge (Mikesell and Boyd, 1986)

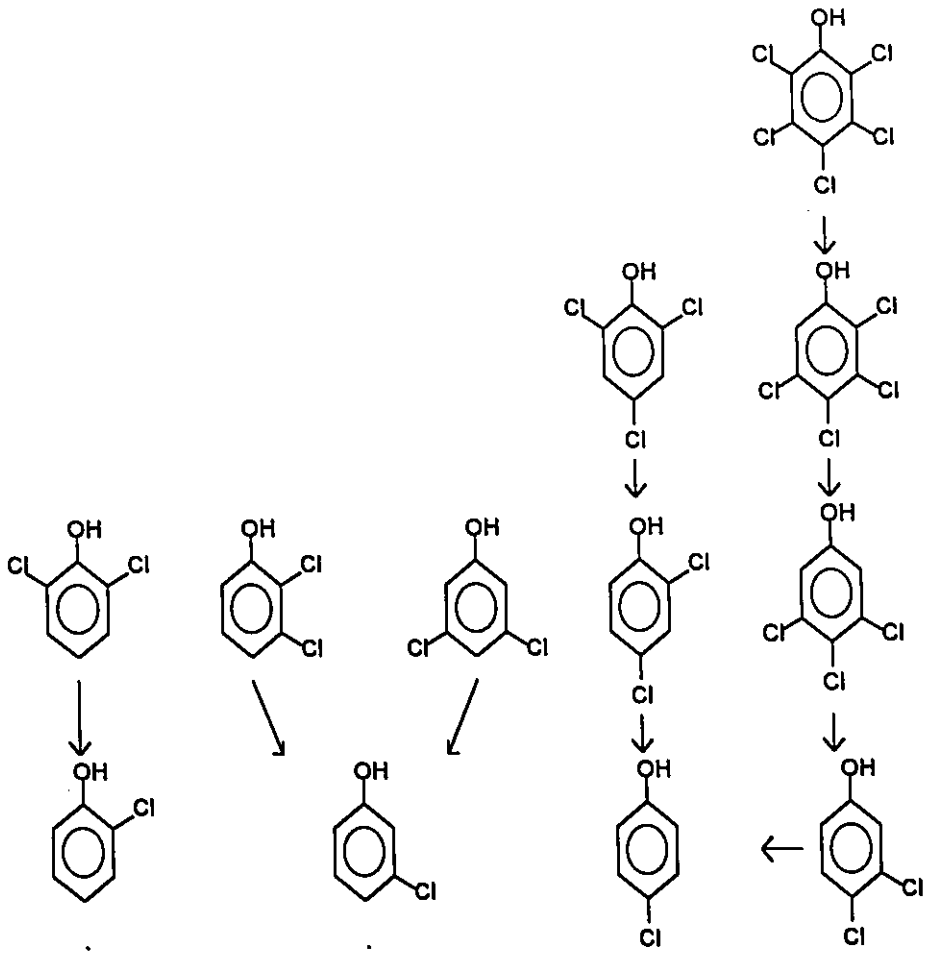


Fig. 2.6 Summary of observed biodegradation pathways in UASB reactors (Source: Woods et al, 1989)

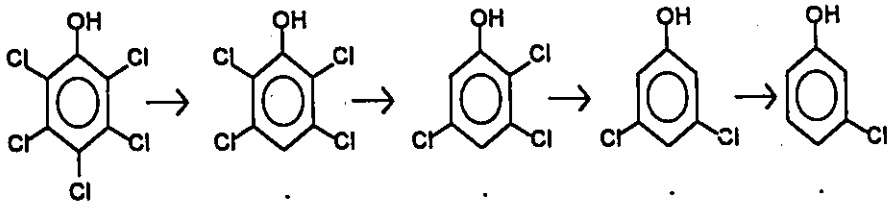


Fig. 2.7 Major PCP dechlorination pathway in UASB reactors (Source: Hendriksen et al., 1991).

Mohn and Kennedy (1992b) isolated one pure culture of chlorophenol-degrading bacteria. This microbe, *Desulfomonile tiedjei DCB-1* was shown to reductively dehalogenate PCP and other chlorophenols. The pathway of PCP dehalogenation by *D. tiedjei* is shown in Fig. 2.8.

The anaerobic dissimilatory pathway for phenol has not been completely established yet. An anaerobically grown, nitrate-reducing *Pseudomonas sp* was found to carboxylate phenol to 4-hydroxybenzoate as the first step in the anaerobic degradation of phenol (Tschech and Fuchs, 1987). In contrast, an obligates syntrophic consortium of anaerobic bacteria was found to decarboxylate 4-hydroxybenzoate to phenol, followed by carboxylation of the phenol to benzoate (Knoll and Winter, 1989). Genthner et al. (1989) proved that carboxylation occurred para to the phenolic hydroxyl group. This strongly suggested that 4-hydroxybenzoate was the intermediate compound between phenol and benzoate. In an extensive study, Zhang and Wiegel (1990) demonstrated the complete sequential degradation of 2,4-DCP to carbon dioxide and methane by a microbial community isolated from freshwater sediment. By separating the various activities into individual enrichment cultures, the type of microbes and the specific sequential steps involved were elucidated. Fig. 2.9 shows the proposed pathway of 2,4-DCP mineralization. This anaerobic pathway may be the one by which most chlorinated phenolic compounds are degraded and mineralized.

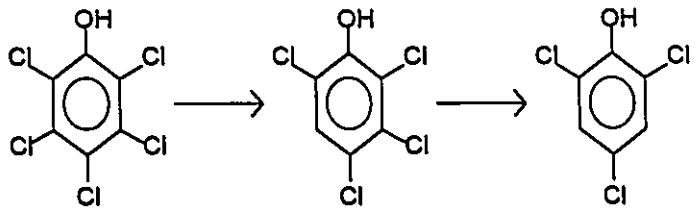


Fig. 2.8 Pathway of PCP dehalogenation by *D. tiedjei* (Source: Mohn and Kennedy, 1992)

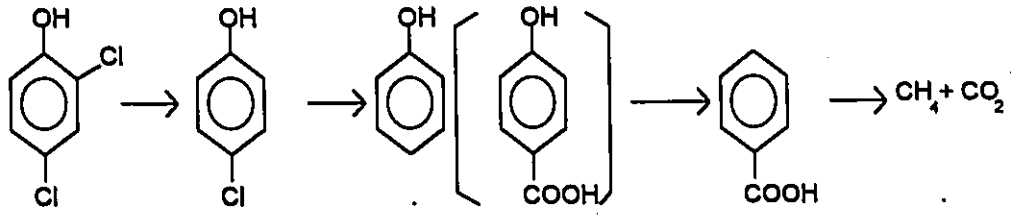


Fig. 2.9 Proposed pathway for the anaerobic degradation of 2,4-dichlorophenol (Source: Zhang and Wiegel, 1990).

2.3.5 Anaerobic dechlorination of chlorophenol in UASB reactors

2.3.5.1 Upflow anaerobic sludge blanket reactors

Among various anaerobic biological reactors, the UASB system has gained acceptance by many researchers. An upflow anaerobic sludge blanket reactor is shown schematically in Fig. 2.10. In this system, anaerobic bacteria form dense granules that settle and remain in a bed at the bottom of the reactor. The influent is distributed over the bottom of the reactor to maintain even flow distribution in the sludge bed. Mixing of the bed to bring the granules into contact with the soluble organics is achieved by hydraulic flow distribution and turbulence resulting from biogas generation. A recycle flow is normally used to maintain a constant hydraulic loading on the reactor and to dilute influent waste as needed. Compared with other high-rate anaerobic systems, the UASB reactor has the advantages of shorter start-up time, lower washout rate, the ability for the high biomass concentration in the reactor to withstand organic or toxic shock loads with minimal adverse effects on process performance, and no requirement for mechanical mixing in the reactor (Lee et al., 1989).

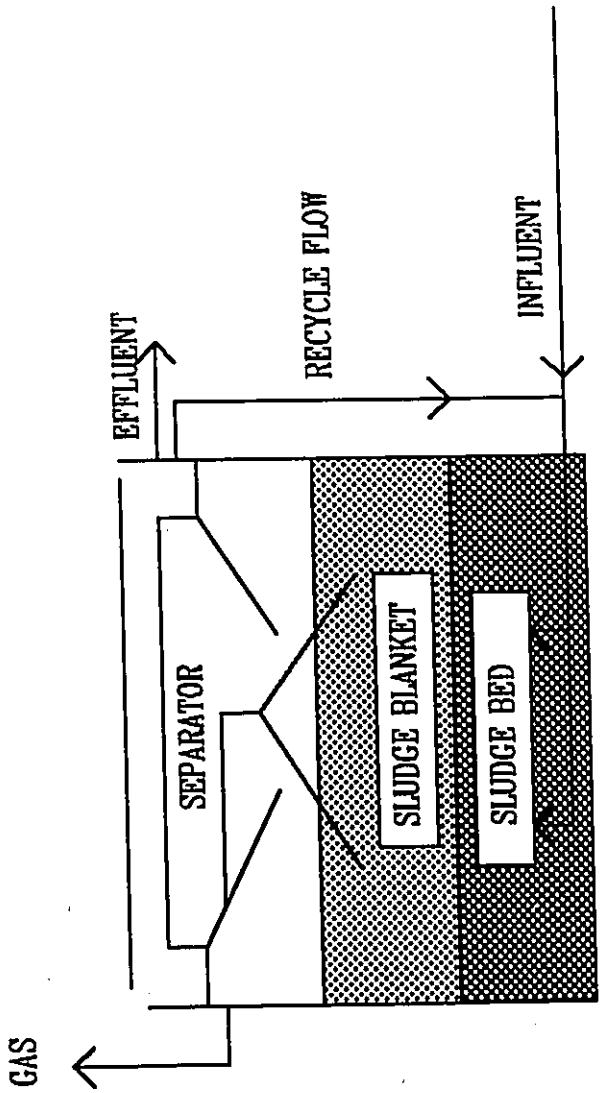


Fig. 2.10 Schematic view of an UASB-reactor

2.3.5.2 Feasibility of UASB reactor for treatment of wastewater containing chlorophenols

Laboratory scale continuous UASB reactors have been used for removal of chlorophenols by many investigators. The treatment efficiencies and dechlorination pathways of chlorophenols in UASB reactors are summarized in Table 2.1.

The following conclusions can be made from this table:

1. It is possible to effectively remove a highly recalcitrant compound such as chlorophenols in a continuous UASB reactor with conventional granular sludge. The chlorophenol removal efficiencies ranged from 23% to 99% in previous studies.

2. Reports on the extent of chlorophenol biodegradation were varied. The major dechlorination end products were monochlorophenols and dichlorophenols. Complete mineralization was also observed.

3. The addition of a readily utilizable carbon source stimulated the dechlorination of chloroaromatic compounds. Under the same operating conditions, the removal of PCP was 99% in the glucose-amended reactor, whereas the removal varied between 32 and 77% in the control reactor which did not receive glucose substrate.

Table 2.1 Summary of previous studies on chlorophenol treatment in continuous UASB reactors

Influent Carbon & energy source	Micro-organisms	Study length	Hydraulic retention time	Removal efficiency	Effluent Major end products	Source
PCP, TeCP, TCP, DCP & Glucose	Municipal sludge	8 months	0.5 day	61-96%	MCP, DCP	Woods et al., 1989
PCP and Glucose	Granular sludge	10 months	2 days	99%	TeCP, TCP, DCP	Hendriksen et al., 1991
PCP	Granular sludge	10 months	2 days	32-77%	PCP, TeCP, TCP, DCP	Hendriksen et al. 1991
MCP	Sewage sludge acclimated for 2 yr.	n/a*	2-10 days	90-100%	CH_4, & CO_2	Krumme & Boyd, 1988
TCP, PCP	Sewage sludge acclimated for 2 yr.	7-13 months	2-5 days	23.2-71.5%	n/a*	Krumme & Boyd, 1988
TCP, DCP & Sugar	Granular sludge	8 months	1-3 days	23-100%	MCP, DCP	J. Lu, 1991

* non applicable

2.4 Process description of sequencing batch reactor (SBR) for biological wastewater treatment

The sequencing batch reactor is a time-oriented, mixed-culture, suspended-growth system. The reactor in a SBR system is filled during a discrete period of time and then it is operated in a batch mode. After desired treatment, the mixed liquor is allowed to settle and the clarified supernatant is drawn from the reactor. A typical SBR cycle is shown in Fig. 2.11.

Operating of SBRs under aerobic and anoxic conditions was used for treatment of synthetic, domestic, industrial and hazardous wastewaters as well as landfill leachate. 70%-90% organic waste removal was obtained in bench-scale SBR studies (Irvine and Ketchum, 1989; Herzbrun et al., 1985). The major advantages associated with SBRs are: operational flexibility, potential to select a specific microbial population and plug flow kinetics. Many studies have focused on the understanding of both microbiological aspects and operating conditions of the SBR. It has been proven that the operating cycle (fill and react ratio), mixing and aeration strategies affect the organisms settling characteristics and waste removal efficiencies (Irvine and Ketchum, 1989).

Moreover, sequencing batch reactors have been proven useful as a technology for the degradation of toxic or hard-to-degrade wastes. Herzbrun et al. (1985) demonstrated the applications of SBR for phenol degradation in cold weather. The effects of periodic operation and extended idle periods on the treatment of a multisubstrate landfill leachate in SBRs were studied by Weber (1986). Ying (1986) studied the treatment of a landfill leachate in bench-scale SBRs with the addition of powdered activated carbon. These researchers found that (1) SBRs were effective for the biodegradation of recalcitrant chemicals, and (2) the addition of various supplements to SBRs stimulated toxic chemical degradation, although the associated

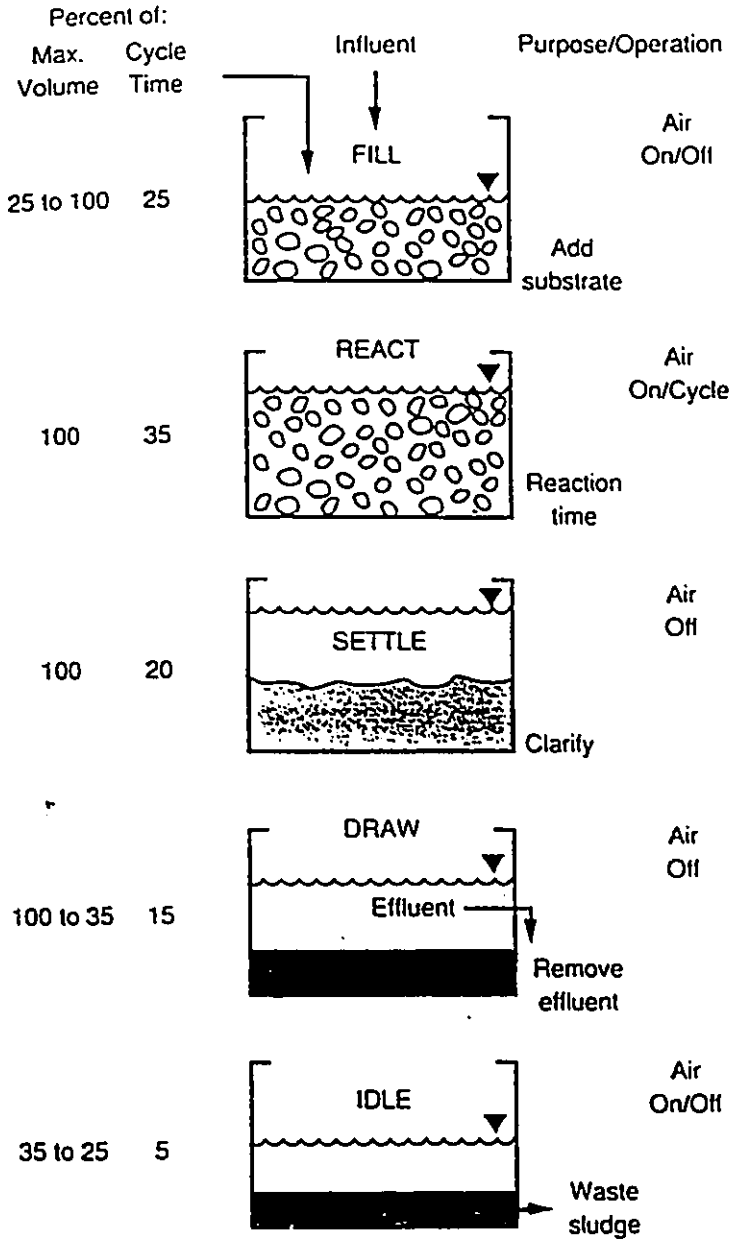


Fig. 2.11 Typical operating sequence for the sequencing batch reactor (Source: Tchobanoglous and Burton, 1991).

mechanisms were not identified.

Reports on the application of SBR technology to anaerobic treatment are scarce. Kennedy et al. (1991) conducted laboratory studies on soluble carbonaceous substrate degradation in sequencing batch anaerobic reactors (SBARs). They reported: (1) Lab-scale SBARs were able to treat medium-strength soluble organic substrates at organic loadings of up to 9 g COD/L/d, with soluble COD removal efficiencies above 80%. (2) Removal efficiencies were found to be significantly affected by the operating strategy of a reactor at high organic loadings. (3) The specific organic loading during the fill period appeared to be a critical design parameter for SBAR process. Based on this experimental work, a dynamic model for soluble carbonaceous substrate degradation in SBARs was developed by Fernandes et al. (1993). Modified Haldane-type and non-competitive inhibition functions were used in the model development. It was found that both inhibition functions can predict SBAR performance fairly well.

2.5 Summary

The above review yields several important points. First, the common occurrence and toxic nature of chlorophenols have brought an increased concern over the elimination of these compounds from waste streams. Among the various removal processes, the anaerobic bio-dechlorination process is the most appropriate one.

Second, it is possible to effectively remove chlorophenols in a continuous UASB reactor with conventional granular sludge. The addition of an easily biodegradable carbon source stimulates the dechlorination of chlorophenols.

Third, sequencing batch reactors were proven to be capable in removing soluble carbonaceous substrate anaerobically. Also, SBRs were found to be efficient in biodegrading recalcitrant chemicals aerobically.

This study is designed to assess the feasibility and flexibility of sequencing

batch anaerobic reactors (SBARs) to treat chlorophenol contaminated wastewater. In addition, the fate of 2,4-dichlorophenol (2,4-DCP) in SBARs is examined.

2,4-DCP was chosen as the recalcitrant chemical in this study for the following reasons: First, it is listed in the US Environment Protection Agency's (US EPA) priority pollutants list (LaGrega et al., 1994). There is a clear need for investigating the anaerobic fate of this compound to evaluate its risk to humans and to the environment. Second, 2,4-DCP possesses two chlorines that occupy two distinct positions (ortho and para positions) on the aromatic ring. As reviewed earlier, contrasting information has been reported on the removal preference of these chlorines by anaerobic microbes. Therefore, investigating its anaerobic dechlorination pathway could be very informative. Third, 2,4-DCP can be readily analyzed by High Performance Liquid Chromatography (HPLC).

The application of SBARs for treating chlorophenol is of interest because of following important advantages. First, the SBAR process has the ability to handle periodic flows. This type of treatment system may eventually be practical in remediating chlorophenol contaminated industrial waste. Second, the SBAR process is able to periodically change environmental conditions in the biological reactor in a controlled manner. Hence, it may be useful in selecting or enriching specific microbial populations that are suitable for anaerobic dechlorination. Third, as supernatant withdrawal occurs in nearly ideal quiescent conditions, the SBAR process may better ensure biomass retention. At last, although there are some studies done on chlorophenol treatment in continuous UASB reactors, no information on sequencing batch UASB reactors has been reported yet. This study should provide some useful information regarding dechlorination of organic compounds in SBAR.

CHAPTER THREE

EXPERIMENTAL MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Chlorophenol compounds and other required chemicals were of high grade (99% purity) and purchased from *Aldrich Chemicals*. All chlorophenol solutions were made up in 0.01 M NaOH solution and adjusted to pH 7.5. Other chemical solutions were made with distilled/deionized water prepared in the laboratory. Organic solvents used for analysis were HPLC grade.

3.1.2 Synthetic Wastewater

For these experiments, a synthetic wastewater was prepared consisting of soluble macro and micro nutrients, sucrose/acetate substrate and chlorophenols, respectively. The composition of the synthetic wastewater is shown in Table 3.1. During the initial acclimation period, the average COD concentration of the wastewater was 5 g/L, and the concentration of chlorophenols (including 2-MCP, 4-MCP and 2,4-DCP) ranged from 0 to 20 mg/L. To study the operation of the sequencing batch anaerobic reactor, the average COD and 2,4-DCP concentrations of the wastewater were 7 g/L and 20 mg/L, respectively.

This concentration of 2,4-DCP was selected for SBAR study because it is more representative of chlorophenol concentrations present in industrial wastewater. Furthermore, the detection limit for chlorophenol by HPLC (Hewlett Packard 1090

HPLC) is 0.5 mg/L. Thus, an initial concentration of 20 mg/L 2,4-DCP should minimize analytical measurement error.

A concentrated stock solution of the wastewater was prepared weekly and was stored in a cold room at 4°C. The diluted wastewater feed (1:10) was made up daily and maintained at 10 - 15 °C until use.

Table 3.1 Composition of Synthetic Wastewater

Constituent	*Per liter	**Per liter
Acetic acid	2.5 ml	3.5 ml
Sucrose	2.5 g	3.5 g
Ammonia bicarbonate	1.0 g	1.4 g
Sodium bicarbonate	2.5 g	3.5 g
Potassium bicarbonate	3.1 g	4.3 g
Ammonia sulfate	0.25 g	0.35 g
Di-potassium hydrogen phosphate	0.13 g	0.18 g
Potassium di-hydrogen phosphate	0.10 g	0.14 g
Yeast extract	0.05 g	0.07 g
+Chlorophenols	0 - 20 mg	20 mg
Chemical oxygen demand (COD)	5 g	7 g

* This dosage was used during SBAR acclimation period
** This dosage was used to study SBAR performance
+ 2-MCP, 4-MCP and 2,4-DCP were used in this study

3.1.3 Biomass

The anaerobic granular sludge used as seeding material in these experiments was obtained from anaerobic digesters located at *Quesnel River Pulp*, Quesnel, British Columbia. The anaerobic sludge was of a non hazardous type, with granules' size up to 3 mm in diameter. The initial concentration of volatile suspended solids (VSS) was 40 g/L. During pseudo-steady state experimental runs, the mean cell residence time of the sludge was 50 days and it was controlled by withdrawing the sludge periodically.

3.1.4 Laboratory-scale Reactors

Figure 3.1 shows the basic configuration of the laboratory-scale upflow anaerobic sludge blanket reactor used in this study. The reactor consisted of a cylindrical glass column with an operating liquid volume of 5.25 L. During operation, wastewater entered horizontally at the bottom under a bed of active granular sludge through a T-inlet. The reactors were operated with a sludge bed volume of 1.75 L, representing 33% of the liquid operating volume.

The UASB reactors were operated in either sequencing batch or continuous operation mode. In *sequencing batch* operation mode, two recycling lines were employed for each reactor. During the *fill period*, the recycle flow was withdrawn from a port three to five centimeters above the effluent port and pumped into the reactor with the influent waste stream. For the purpose of mixing during the *react period*, the recycle flow was withdrawn three to five centimeters below the top of water level, and was subsequently pumped back through the influent port. In *continuous operation* mode, only the upper recycling line was used for supernatant recirculation. The recirculation-to-feed ratio was 5:1 and provided better mixing and contact between the influent wastewater and the biomass granules. The produced biogas exited through a port at the top of the reactor and was measured with a wet tip gas meter. A system of solenoid valves and a controller were used to allow the biogas in or out of the reactors depending on the cycle period.

The temperature of the reactors was maintained at 35 ± 3°C with the aid of a hot water bath during the experimental period.

3.2 Sludge Acclimation Process

For most chlorinated organic substrates, the acclimation period is confirmed by

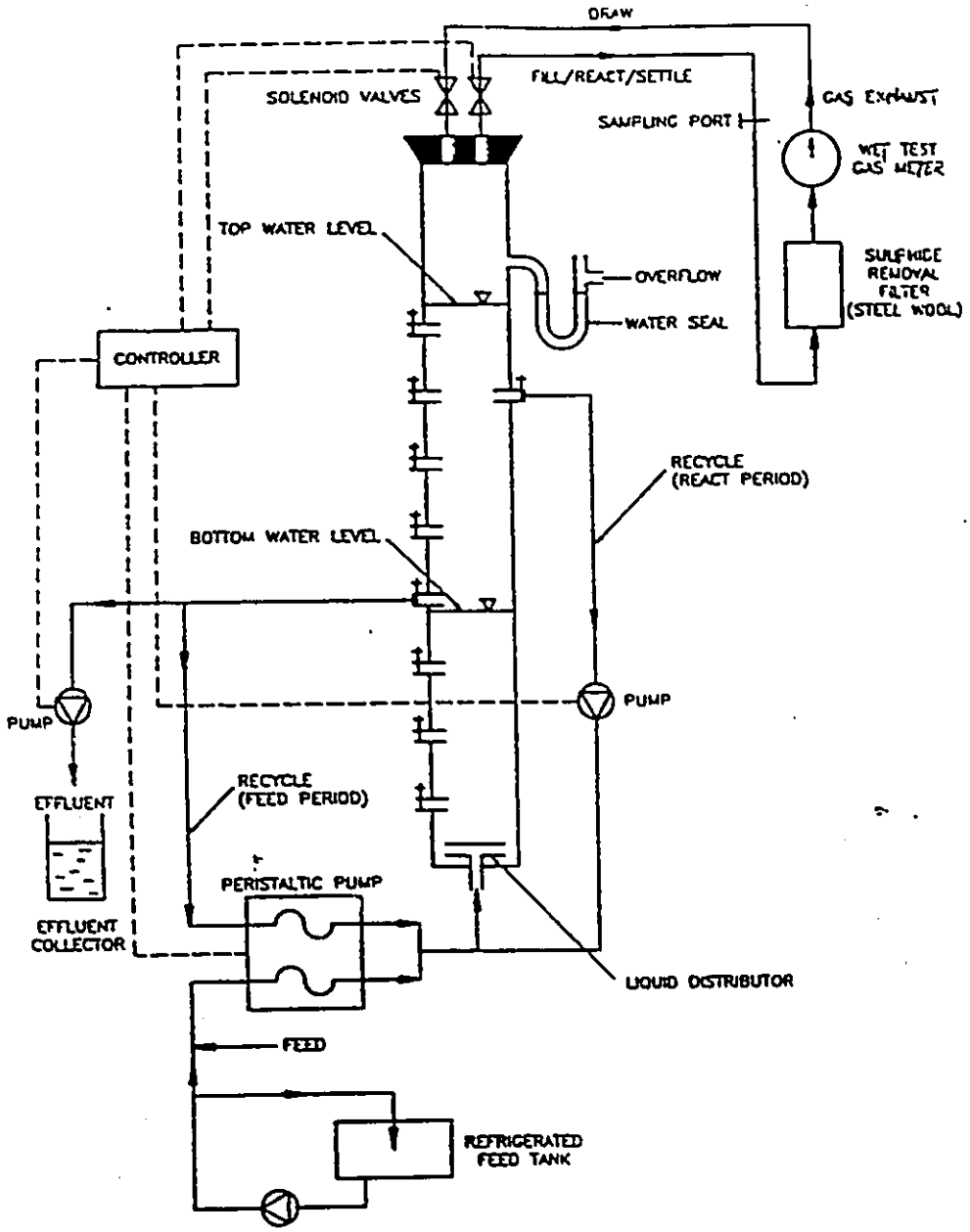


Figure. 3.1 Configuration of Laboratory-Scale Sequencing Batch UASB Reactor

(Source: Kennedy, et al., 1991)

the occurrence of a de-chlorinated product, and it should be present in quantities greater than 3% of the parent chlorinated substrate (Linkfield, 1989). In this study, the acclimation process was conducted in two stages. In the first stage of the sludge acclimation process, two continuous UASB reactors were used for 2-monochlorophenol and 4-monochlorophenol acclimation, respectively. The second stage of sludge acclimation was conducted in four continuous UASB reactors for 2,4-dichlorophenol acclimation. The motivation for the initial MCP-acclimation prior to the 2,4-DCP acclimation is discussed in Chapter Four.

3.2.1 Sludge Acclimation to Monochlorophenols

Two reactors as shown in Fig. 3.1 were inoculated with 2.5 L of anaerobic granular sludge for the purpose of acclimation to 2- and 4-monochlorophenol. The initial granular sludge bed had an average organic solid content of 40 g VSS/L. The sludge VSS/TSS ratio was 0.80. The reactors were started in a continuous operating mode with a substrate concentration of 5 g COD/L (Table 3.1). The starting hydraulic retention time (HRT) was 20 days. It was then decreased gradually to 1 day HRT over a four week period and maintained at this level for the rest of the experiments. From the fifth week chlorophenol feed was started in addition to the easily biodegradable carbonaceous substrate. The first of two reactors received 2 mg/L of 2-monochlorophenol while the other received 2 mg/L of 4-monochlorophenol. The chlorophenol concentrations were raised gradually up to 20 mg/L within a five week period, and maintained at that level for an additional three weeks. The chlorophenol concentrations in the treated effluent were measured every other day.

3.2.2 Sludge Acclimation to 2,4-dichlorophenol

After an eight week acclimation period to monochlorophenol, the sludges were removed from both reactors, mixed and used to inoculate four identical UASB reactors, as illustrated in Fig. 3.1. It was assumed that this mixed sludge might easily adapt to 2,4-DCP. Therefore, a starting concentration of 10 mg/L 2,4-DCP was used for the purpose of acclimating the sludge to this substance. However, after four days of continuous feeding with 10 mg/L 2,4-DCP, high volatile fatty acids (VFA) concentrations (2700 - 3100 mg/L) were detected in the reactor's supernatant, which could have eventually led to process failure. Consequently, chlorophenol feeding was discontinued for the following thirty days until supernatant VFA concentrations dropped back to within a normal range (i.e. below 500 mg/L). Following this, the mixed sludge was acclimated to 2,4-dichlorophenol over a period of ten weeks. During this period, the influent 2,4-DCP concentration increased from 2 mg/L to 20 mg/L stepwise at an incremental rate of 2 mg/L per week. The reactor's supernatant VFAs were monitored and maintained below 500 mg/L throughout the acclimation period. Reactor performances were routinely assessed by daily determinations of biogas production rate, biogas composition, VFA concentration, soluble COD concentration, mono- and di-chlorophenol concentrations, sludge bed height, and reactor temperature.

3.3 Sequencing Batch UASB Reactor

3.3.1 Reactor Start-up

Four UASB reactors, as described in section 3.1.4, were inoculated with 1.75 L granular sludge that was acclimated to 2,4-dichlorophenol over a period of ten weeks. In order to obtain pseudo-steady state conditions, all reactors were started in a

continuous operating mode and supplied with 20 mg/L of 2,4-DCP as well as an organic co-substrate with a concentration of 5 g COD/L. The latter was increased stepwise during the following four weeks to a level of 7 g COD/L .

3.3.2 SBAR Operation

Three identical UASB reactors were set up to study the sequencing batch operation mode. Reactors were operated at three different cycle lengths: 12, 18 and 24 hours. Each reactor received an identical volume and type of feed. This resulted in three different specific organic loading rates: 0.35, 0.525 and 0.7 g COD/g VSS/d. The operating parameters used for this experimental work are presented in Table 3.2.

A typical cycle for the SBAR consisted of the following four stages: FILL, REACT, SETTLE and DRAW. A description of these stages appears below.

FILL — During the *FILL* stage, the influent wastewater was added to the biomass remaining in the reactor from the previous cycle. In the reactor, complete mixing was achieved by supernatant recycling. The liquid volume increased from 1.75 L to 5.25 L. The fill periods investigated in these experiments ranged from 2.5 hours to 11 hours.

REACT — Reactions that initiated during the FILL stage were completed during the *REACT* stage. Reactor liquid level remained at the maximum level. Complete mixing was achieved as a result of supernatant recirculation and the turbulence caused by the rising biogas bubbles that were produced in the reactor. The react periods used in this experimental work ranged from 5 to 16.5 hours.

SETTLE — At this stage, the waste/sludge mixture was allowed to settle for 75 minutes to insure that the sludge blanket remained below the effluent

port of the reactor and did not rise due to biogas formation before *DRAW* was completed.

DRAW — Treated clarified wastewater was withdrawn from the reactor during the *DRAW* stage. The draw period used in this experimental work was 45 minutes.

The effect of fill-to-react ratio was also studied. At each organic loading rate, three fill-to-react ratios were investigated; these being 0.33, 0.66 and 1. These operating conditions are summarized in Table 3.2.

Each of these experimental runs was operated for 4 to 5 weeks to obtain pseudo-steady state conditions. At the end of each SBAR experimental run, intensive hourly sampling was conducted throughout the entire cycle to obtain complete profiles of the substrate's biodegradation. Each of the intensive sampling schedules was repeated three times, with a time interval of 3 days.

3.3.3 Control Reactor

One continuous upflow sludge blanket reactor, identical in configuration to the SBARs, was used as the control. The control reactor treated the same wastewater and was maintained at the same temperature (35 ± 3 °C). It was also operated with a 1.75 L sludge bed seeded with the same granular sludge used in the SBARs. Operating details are shown in Table 3.2.

The continuous-flow reactor was operated at three different organic loading rates, corresponding to those applied for the SBAR system (Table 3.2). After each of the required loading rates were established, samples were taken during a 4- to 5-week period to evaluate steady-state conditions of the UASB system. The only difference between the SBARs and the control reactor was their mode of operation. Wastewater

Table 3.2 Operating Parameters Tested in the Experimental Program

Reactor	Run	Cycle length	Flow rate	Specific organic loading rate	Specific 2,4-DCP loading rate	Operation sequence (hr).			
#	#	(hr)	(L/d)	g COD /g VSS/d	mg DCP /g VSS/d	FILL	REACT	SETTLE	DRAW
1	R1C1	24	3.5	0.35	1.0	5.5	16.5	1.25	0.75
1	R1C2	24	3.5	0.35	1.0	8.8	13.2	1.25	0.75
1	R1C3	24	3.5	0.35	1.0	11	11	1.25	0.75
2	R2C1	18	5.3	0.53	1.5	4	12	1.25	0.75
2	R2C2	18	5.3	0.53	1.5	6.4	9.6	1.25	0.75
2	R2C3	18	5.3	0.53	1.5	8	8	1.25	0.75
3	R3C1	12	7.0	0.70	2.0	2.5	7.5	1.25	0.75
3	R3C2	12	7.0	0.70	2.0	4	6	1.25	0.75
3	R3C3	12	7.0	0.70	2.0	5	5	1.25	0.75
4	R4C1	*n/a	3.5	0.35	1.0	Continuous Mode			
4	R4C2	*n/a	5.3	0.53	1.5	Continuous Mode			
4	R4C3	*n/a	7.0	0.70	2.0	Continuous Mode			

* non applicable

samples were taken on a regular basis and were analyzed for COD, VFA and chlorophenol concentrations.

3.4 Analytical methods

3.4.1 Wastewater Analyses

3.4.1.1 Measurement of Flow Rates

Peristatic pumps (Harvard Apparatus) and Masterflex positive displacement pumps were used to control the influent and recycle flow rates for the reactors. Also influent flowrates were monitored daily by measuring the discharged effluent volume. A fluctuation in the flow rate of approximately 5% was observed during the experiments.

3.4.1.2 Wastewater Sampling

A wastewater sample of approximately 10 ml was taken either from the effluent port or from the port connected to the reactor recycling line. These samples were centrifuged in a micro-centrifuge (Fisher 235A) for 5 minutes. After centrifugation, 5 ml of supernatant was prepared for COD, VFA and pH analyses, and the rest of sample was passed through a Millipore 0.22 μ m Milex-GV13 filter and stored at 4°C in glass vials with Teflon line caps prior to HPLC analysis.

3.4.1.3 Analysis of Chlorophenols

The concentrations of 2- and 4-monochlorophenol and 2,4-dichlorophenol in the

wastewater samples were measured with a Hewlett Packard 1090 HPLC equipped with a diode array detector and *HP-300* work station for the integration of peak areas. The detection wavelength was 280 nm corresponding to the maximum absorbency for these compounds. A sample size of 25 μL was injected onto a Shandon Hypersil ODS C18 column (2.1mm id x 10cm x 5μm) maintained at 40°C. The flow rate of the mobile phase was 0.3 ml/min and the composition of the solvents used is presented in Table 3.3.

Table 3.3 HPLC Conditions for Chlorophenol Analysis

Time	Solvent A HPLC Grade Methanol pH 4.7	Solvent B 0.05 M Sodium Bicarbonate pH 4.7
min	%	%
0	40	60
15	60	40
20	80	20
21	40	60

3.4.1.4 Analysis of Volatile Fatty Acids

The concentration of acetic, butyric and propionic acids in the samples was measured using the method by Ackman (1972) using a Hewlett Packard 5840A Gas Chromatograph (GC) equipped with a flame ionization detector, a 7671A automatic sampler and 3380A integrator. The chromosorb 101 glass column and injection port were maintained at 180°C and 200°C, respectively. The carrier gas was helium at a flow rate of 15 ml/min, and it was passed over formic acid prior to entering the GC. Liquid samples were prepared by centrifuging for five minutes in a 235A Fisher Micro-centrifuge. Following this, 0.5 ml of sample was diluted with 0.5 ml of internal standard (isobutyric acid) and injected into the GC.

3.4.1.5 Analyses of Chemical Oxygen Demand

A method modified by Knechtel (1978) was used to analyze wastewater chemical oxygen demand (COD). Instead of two hours, one and a half hour of sample heating time was used for COD digestion.

3.4.1.6 Measurement of pH

A 26 Radiometer/Copenhagen pH meter, with a glass combination electrode, was used for all pH measurements. The meter was standardized prior to pH measurements and it had a sensitivity of 0.05 pH units.

3.4.2 Biogas Production Rate and Composition

The biogas production rate in the reactors was measured by a wet tip gas meter (Wet Tip Gas Company) which was calibrated prior to use.

During the acclimation period, approximately 0.5 ml of biogas sample was taken from the gas sample port of each reactor, daily, for determination of biogas composition. The analyses were performed with a Hewlett Packard 5710A Gas Chromatograph (GC) equipped with a thermal conductivity detector and a Hewlett Packard Integrator. A Porapack T 50/80 mesh column was used and helium gas at a flow rate of 40 ml/min was used as the carrier gas. Temperatures of the detector, the injection port and the column were maintained at 150 °C, 100 °C and 70 °C, respectively.

3.4.3 Biomass Analyses

In order to analyze reactor biomass accurately, approximately 5 ml sludge samples were taken from five different sludge sampling ports of the reactor. The mixture of sludge samples was used as representative of the sludge in the reactor.

TSS and VSS concentrations of the sludge were measured according to Standard Methods (APHA, 1985).

CHAPTER FOUR

PERFORMANCE OF SEQUENCING BATCH ANAEROBIC REACTORS

This chapter describes performance of sequencing batch anaerobic reactors (SBARs). There are four sections in this chapter. The first section describes the acclimation process that was studied in this experimental work. The second section discusses the basic process microbiology. The SBAR process operation is outlined in section three. Section four evaluates the performance of SBAR treatment systems.

4.1 Acclimation of granular sludge to chlorophenols

Generally, the observed acclimation period of microorganisms to a substrate could be due to either a "true" acclimation, i.e., a period of no biodegradation followed by an initiation and acceleration of degradation, or an "apparent" acclimation, in which biodegradation would proceed from the time of the substrate addition, but at rates so low as to be almost non detectable. In the present study, an initial monochlorophenol-acclimation stage prior to a 2,4-dichlorophenol acclimation was conducted based upon the following: First, a previous study by Boyd and Shelton (1984) suggested that a microbial population acclimated to a particular substrate can be easily acclimated to another substrate with a very similar molecular structure. Also, the lag periods for anaerobic biodegradation of monochlorophenols were usually shorter than that of higher chlorinated phenols. Second, complete dechlorination of pentachlorophenol was reported using a mixture of 2-, 3- and 4-MCP-acclimated sludge (Boyd and Shelton, 1984). Hence, it was felt that an initial 2- and 4-MCP-acclimation stage may shorten subsequent sludge acclimation period for 2,4-DCP, and also complete dechlorination of 2,4-DCP could be achieved using a mixture of 2- and 4-MCP-acclimated sludge.

Consequently, the acclimation process was conducted in two stages. In the first stage, two continuous flow UASB reactors were used for 2-monochlorophenol and 4-monochlorophenol acclimation over a 60-day period, respectively. Next, the mixture of the 2- and 4-MCP-acclimated sludge was used for 2,4-dichlorophenol acclimation for another 60-day period. The influent and effluent chlorophenol concentrations were measured daily, and detailed results will be described in the following sections. In order to maintain a healthy operating anaerobic system during the acclimation period, the concentration of volatile fatty acids in the reactor was monitored and maintained under 500 mg/L. If VFA concentration increased above 500 mg/L, the supply of influent substrate would be controlled until the reactor returned to acceptable VFA operating values.

4.1.1 Sludge acclimation to 2-monochlorophenol

Fig. 4.1 shows UASB reactor performance in terms of influent and effluent 2-MCP, as well as phenol concentration curves during the 60 day acclimation period. The reactor was fed with 2-MCP as well as an easily biodegradable carbonaceous substrate (5 g COD /L). The starting concentration of 2-MCP was 2 mg/L and it was raised to 20 mg/L within 35 days. During the first four weeks of the acclimation period, the effluent chlorophenol concentration was 10% - 40% lower than that of the influent waste streams. This indicated that sorption played a significant role in the removal of chlorophenol in the first stage. However, during the second stage (week five and six), phenol was detected as a 2-MCP biodegradation product, and the concentration of phenol in the reactor effluent represented 15% of the influent 2-MCP. From week seven to week nine, the effluent 2-MCP concentration was less than 10% of the influent 2-MCP. These findings suggest that phenol was further biodegraded to methane and carbon dioxide, and almost complete mineralization of 2-MCP was achieved. The

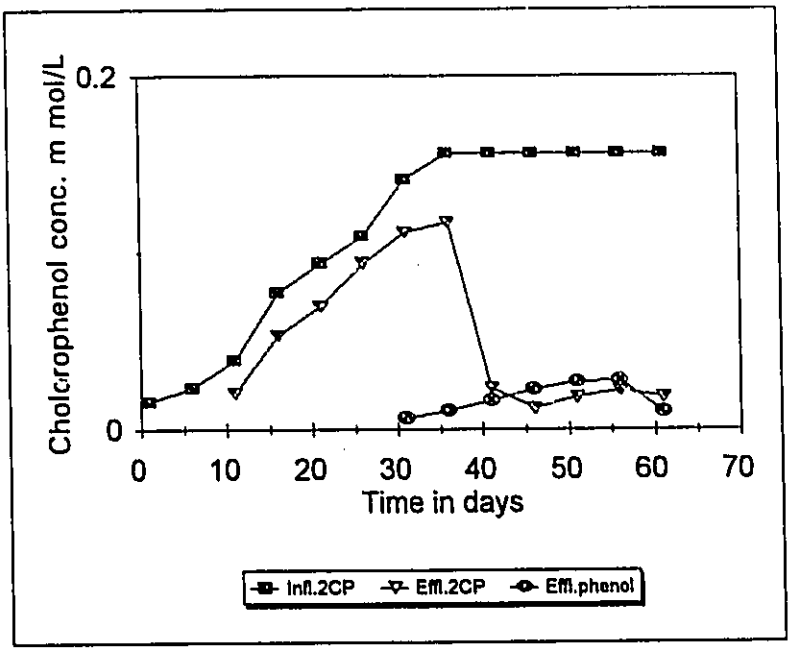


Fig 4.1. 2-monochlorophenol acclimation curve

anaerobic biodegradation pathway of 2-MCP during the acclimation period was via phenol to methane and carbon dioxide.

4.1.2 Sludge acclimation to 4-monochlorophenol

For 4-MCP acclimation, the continuous flow UASB reactor was fed with 4-MCP in addition to soluble carbonaceous substrate (COD 5 g/L). The initial concentration of 4-MCP was 2 mg/L and it was raised to 20 mg/L within 35 days, and maintained at this level thereafter. During the 60 day acclimation period, no 4-MCP biodegradation product was detected. At the end of this period, the measured effluent 4-MCP concentration was over 90% of that in the influent waste stream as shown in Fig. 4.2. Boyd and Shelton (1984) reported, however, that the average acclimation period for 4-monochlorophenol was 28 days when using municipal sludge while 2-monochlorophenol was apparently degraded without a lag time. Under the present study conditions the acclimation period for 2-MCP was 28 days, which was much longer than previously reported results. Therefore, it was predicted that the required acclimation period for 4-MCP would be a lengthy one. Since the aim of this study was to evaluate 2,4-dichlorophenol biodegradation in a UASB reactor operating in a sequencing batch mode, it was decided to discontinue the 4-MCP acclimation process after a period of sixty days.

4.1.3 Sludge acclimation to 2,4-dichlorophenol

In the subsequent stage, sludge that was acclimatized to 2-MCP and exposed to 4-MCP was mixed. It was then used for 2,4-DCP acclimation in four continuous flow UASB reactors. The 2,4-DCP acclimation period began with an average influent COD concentration of 5 g/L and 2,4-DCP concentration of 10 mg/L for all reactors. After four

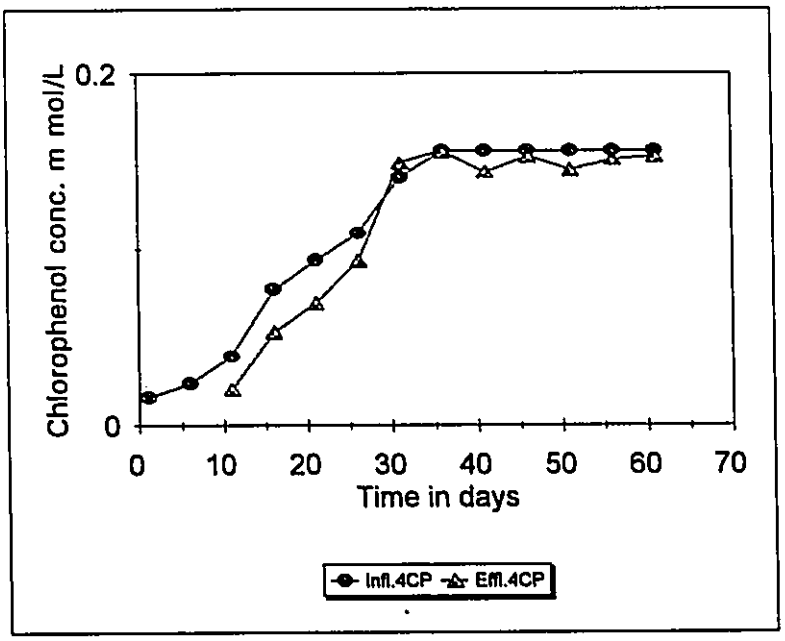


Fig 4.2. 4-monochlorophenol acclimation curve

days of continuous feeding, the supernatant total VFA concentration was over 3000 mg/L in all four reactors. Also, the gas production rates of these reactors were reduced by 30% to 35%. These conditions suggested that the initial 10 mg/L of 2,4-DCP loading inhibited the activity of all three groups of bacteria, including acidogenic, acetogenic and methanogenic bacteria. These UASB reactors were in the process of failing. 2,4-DCP feeding was therefore discontinued for the subsequent thirty days until the supernatant VFA concentration dropped back to below 500 mg/L.

The concentration of 2,4-DCP in the influent feed was then re-started from 2 mg/L and gradually increased to 20 mg/L within 55 days. The biodegradation product 4-MCP was detected during the ninth week. No further biodegradation products were detected during the 60 day acclimation period. A typical 2,4-DCP acclimation curve is shown in Fig. 4.3.

4.1.4 Discussion

(1) In the present study, the initial acclimation of granular sludge to 2- and 4-MCP did not appear to shorten the length of the subsequent 2,4-dichlorophenol acclimation period. This finding contradicts the earlier reports by Boyd and Shelton (1984), in which a microbial population that was acclimated to a particular substrate is often simultaneously acclimated to other substrates with very similar molecular structures. As well, complete dechlorination of 2,4-DCP was not observed under studied conditions.

(2) In this experiment, high continuous loading of 2,4-DCP (10 mg/L/d) exhibited a strong inhibition effect on carbon oxidizing bacteria, while low loading of 2,4-DCP (2 mg/L/d) did not have any negative impact on the microbial populations. Concomitantly, it can be deduced that a higher loading of 2,4-DCP requires gradual and a longer

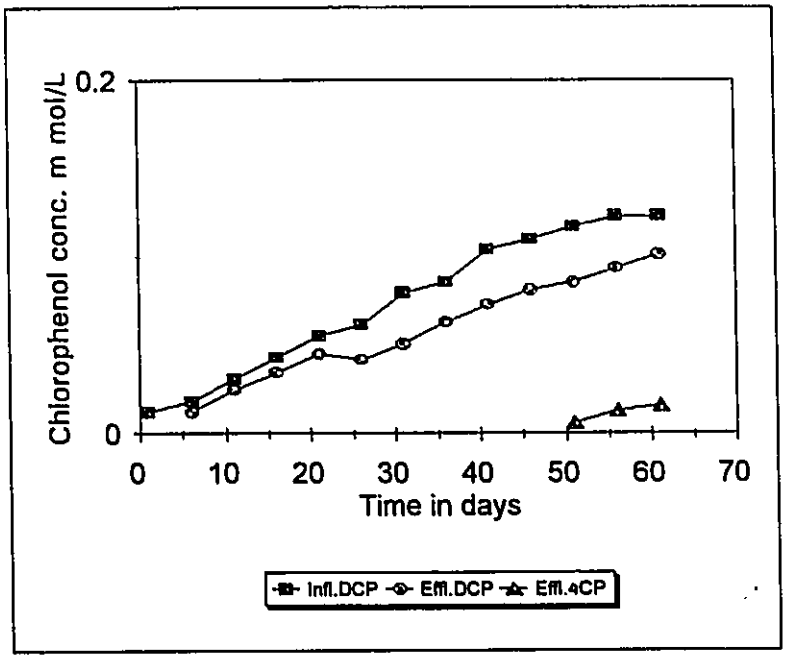


Fig 4.3. 2,4-dichlorophenol acclimation curve

acclimation period. This finding suggests that the length of the acclimation period of anaerobic sludge to 2,4-DCP is a function of this chemical's concentration.

4.2 Process microbiology

Although anaerobic process microbiology has been well established, the specific microbes responsible for reductive dechlorination activities have not yet been identified. Identification of these microbes is beyond the scope of this study. This section will focus on the microbiological aspects of ordinary anaerobic bacteria. The relationship between the various groups of bacteria in the studied sequencing batch and continuous flow reactors is shown in Fig. 4.4.

In this experiment, the major components of the wastewater were sucrose, acetate and 2,4-dichlorophenol. The average COD concentration of the synthetic wastewater was 7 g/L, about 50% of which was contributed by acetate. It was detected that the major intermediates formed in the reactor were acetate and propionate. Three groups of anaerobic microorganisms, i.e., acidogenic bacteria, acetogenic bacteria and methanogenic bacteria, participated in the biodegradation of the easily degradable carbonaceous substrates fed to the reactors. Acidogenic bacteria were able to break down sucrose into short chain fatty acids, such as acetic and propionic acids. Propionic acid was converted to acetic acid, carbon dioxide and hydrogen by acetogenic bacteria. Methanogenic bacteria can utilize acetate, carbon dioxide and hydrogen with the formation of methane. These energy-yield reactions are as follows:

Conversion of sucrose into acetic acid

$$C_{12}H_{22}O_{11} + 5\,H_2O \rightarrow 4\,CH_3COOH + 4\,CO_2 + 8\,H_2 \qquad (4.1)$$

Conversion of sucrose into propionic acid

$$C_{12}H_{22}O_{11} + 4\,H_2 \rightarrow 4\,CH_3CH_2COOH + 3\,H_2O \qquad (4.2)$$

Conversion of propionic into acetic acid

$$CH_3CH_2COOH + 2\,H_2O \rightarrow 2\,CH_3COOH + CO_2 + 4H_2 \quad (4.3)$$

Conversion of acetic acid into methane and carbon dioxide

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (4.4)$$

Conversion of hydrogen and carbon dioxide into methane and water

$$4H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O \quad (4.5)$$

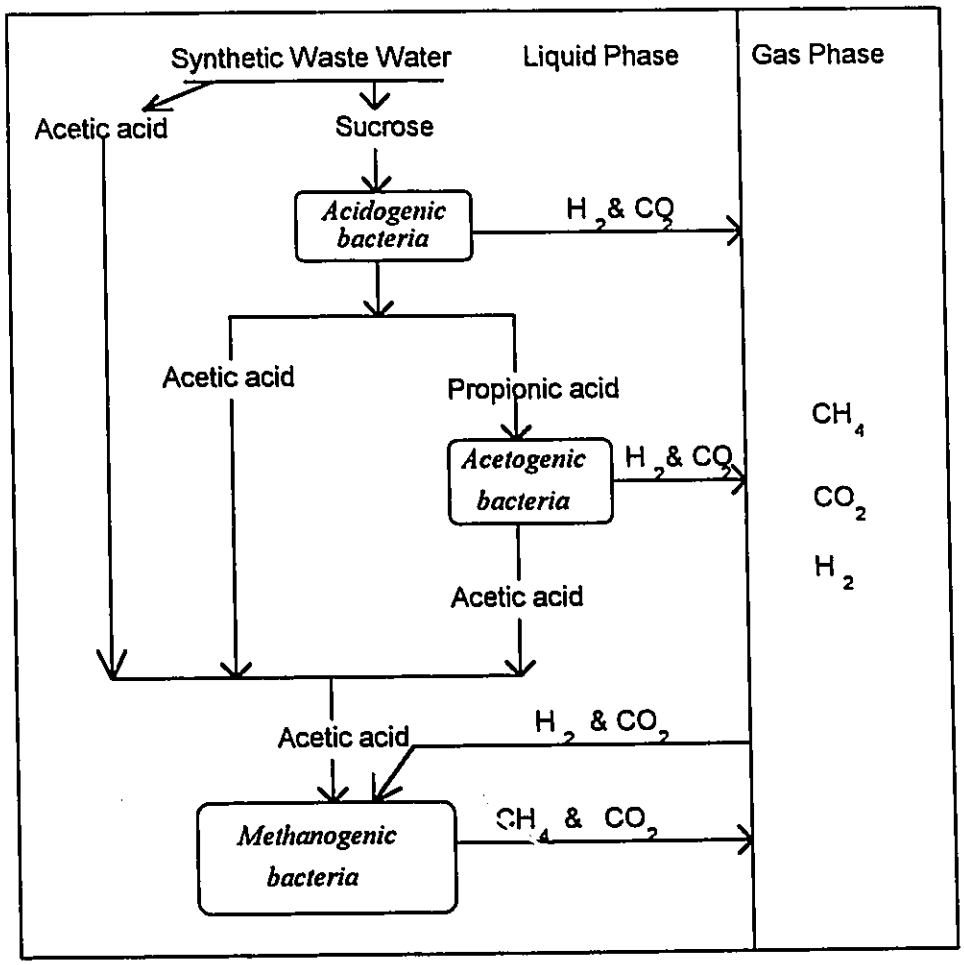


Fig 4.4. Schematic of the relationship between bacteria groups in the studied anaerobic treatment systems

4.3 Operation of the SBAR process

4.3.1. Evaluation of pseudo steady-state of SBARs

In the present study, all four UASB reactors were started in a continuous mode and three of them were switched to sequencing batch operating mode after the influent substrate concentration was increased to desired operational level, i.e., 7g COD and 20 mg 2,4-DCP per liter. These sequencing batch anaerobic reactors were operated at three different cycle lengths (12, 18 and 24 hours) which resulted in three different specific organic loadings (0.35, 0.53 and 0.70 g COD/g VSS/d), one per reactor. The sludge acclimation period necessary to reach pseudo steady-state conditions for these reactors was about six weeks, and it was assessed by monitoring effluent VFA and 2,4-DCP concentrations on a regular basis. The averaged weekly results in terms of substrate removal are reported in Table 4.1.

Table 4.1. Effluent characteristics during the first six weeks of SBAR operation

Reactor	SOLR	Substrate	Substrate removal %					
#	g COD/g VSS/d		Week #1	Week #2	Week #3	Week #4	Week #5	Week #6
R1C1	0.35	VFA	86	91	95	92	94	93
R1C1	0.35	2,4-DCP	42	53	59	79	96	100
R2C1	0.53	VFA	62	66	70	84	91	89
R2C1	0.53	2,4-DCP	26	38	53	81	91	98
R3C1	0.70	VFA	50	58	66	73	70	75
R3C1	0.70	2,4-DCP	21	55	52	76	90	94

It is seen that the effluent qualities in terms of VFA and 2,4-DCP removal of the last two weeks (week #5 and #6) are reasonably close. Concomitantly, it was determined that the length of the sludge acclimation period necessary to reach pseudo steady-state was six weeks.

4.3.2. Biodegradation of carbonaceous substrates at pseudo steady-state conditions

The SBARs were also operated and monitored for another six-week period at different F:R ratios, 0.33, 0.66 and 1, in order to establish the pseudo steady-state at these studied conditions. Furthermore, with the pseudo steady-state established, three intensive experimental runs were conducted by sampling hourly the treated wastewater characteristics and biogas production from all four reactors. The averages obtained from these three sets of intensive sampling data were used to generate representative cycle profiles of the SBAR system. These cycle profiles were obtained for the total VFA, the soluble COD and the 2,4-DCP concentrations as well as the biogas production rate. About 10% - 15% variation in the VFA and COD values and 5% variation in the gas production rate were observed. Fig 4.5 shows typical results of VFA variation of three replicated experimental runs obtained for SBAR cycle R3C1; the cycle length was 12 hours, and other information regarding this cycle is included in Table 3.2. This figure shows that the standard deviations of the three sets of data are small, and high reproducibility among the data was obtained from the three intensive experimental runs. Similar results were obtained for other SBAR cycles. Detailed data are presented in Appendix A. Some of the typical profiles are discussed in the following sections.

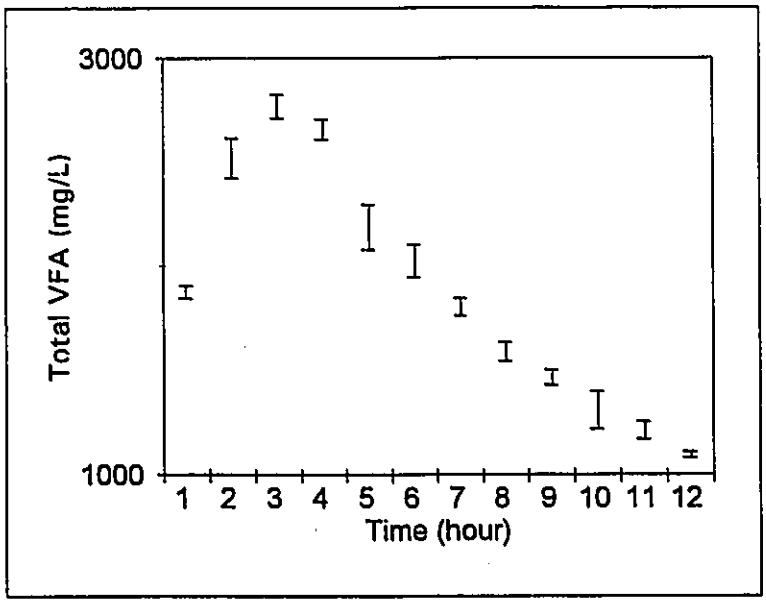


Fig. 4.5. VFA variation for three replicated experimental runs for R3C1 (Cycle length: 12 hours. SOLR: 0.70 g COD/g VSS/d. Fill/react ratio: 0.33)

4.3.2.1 Biodegradation of volatile fatty acids

Generally, the volatile fatty acid concentration in the treated supernatant provides a useful measure of the reactor's performance. Low VFA levels indicate stable operation, while high VFA concentrations are invariably associated with reactor failure (Hill et al., 1987). For the SBAR fed with sucrose and acetic acid, the major intermediates detected in the reactor supernatant were simple volatile fatty acids such as acetic and propionic acids. Acetate has been described as the least toxic of the volatile fatty acids, while propionate has often been implicated in the literature as a major cause of digester failure (Tchobanoglous and Burton, 1991).

VFA biodegradation profiles: Fig. 4.6 shows total VFA concentration profiles of three cycles for reactor #1. The length of these cycles was 24 hours. The average influent total VFA concentration was 3600 ± 250 mg/L, and the resulting specific organic loading rate was 0.35 g COD/g VSS/d. The fill and react ratios of these cycles, R1C1, R1C2 and R1C3, were 0.33, 0.66 and 1, respectively. This figure shows that during the SBAR operating cycle, the total VFA concentration in the supernatant increased sharply during fill period to a maximum value of 2480 mg/L. It then started to decrease during react and was below 300 mg/L at the end of each cycle. The sharp peak observed for the first cycle R1C1, which had the shortest fill time, even out as the fill time was increased. As well, the peak VFA concentration value decreased from 2500 mg/L to 1400 mg/L as the fill time increased from 5.5 hours to 11 hours. Similar behavior was observed for other SBAR cycles. Over 90% of the total VFA removal was achieved in all three operating cycles of this reactor. Apparently, fill and react ratios did not significantly influence overall VFA removal efficiency at this organic loading rate. Fig. 4.6 also indicates that anaerobic granular sludge had the ability to handle periodic VFA shock loadings of up to 2500 mg/l. This is significantly different from previous

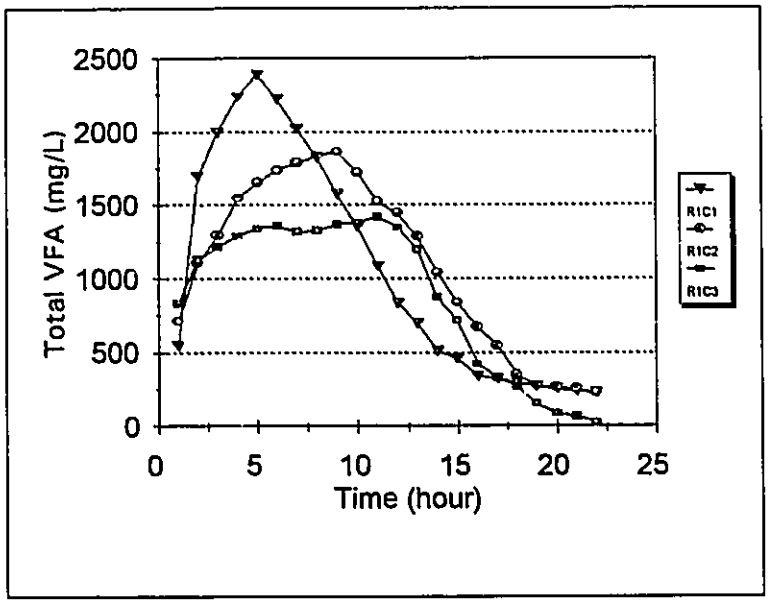


Fig. 4.6. Total VFA concentration profiles for reactor #1
(Cycle length: 24 hours. SOLR: 0.35 g COD/g VSS/d)
(Fill/react ratio: R1C1, 0.33; R1C2, 0.66; R1C3: 1.00)

studies where a VFA concentration below 500 mg/L was recommended for operating stable anaerobic systems.

Fig 4.7 shows the VFA profiles for reactor #3. The specific organic loading rate of R3C1, R3C2 and R3C3 was 0.70 g COD/g VSS/d. The cycle length was 12 hours and the fill/react ratios ranged from 0.33 to 1. This figure shows that the discrepancies of peak values and shapes among these cycle profiles, decreased when the specific organic loading rate increased. Only 50-70% VFA removal was achieved at the end of these cycles. Obviously, the reactor performance was influenced by the specific organic loading rate of the SBAR cycle. The effluent quality deteriorated and the VFA concentration was between 1100 to 1500 mg/L.

VFA Composition: The varying concentration of individual organic acids, such as acetic and propionic acid, in a reactor gives one of the best insights into the dynamic behavior of the SBAR process. These VFAs directly and indirectly affect the physical and biological behavior of the reactor. The accumulation and consumption of VFA are a clear indication of differences in the growth rates of the acid-producers and acid-consumers. In the reactor supernatant, the total concentration of volatile fatty acids was contributed by acetate and propionate. Fig. 4.8 illustrates the ratio of propionate and total VFA concentration in the supernatant for a typical SBAR cycle (R3C3). The total VFA profile for the same experimental run is also shown in this figure. Both fill and react periods of this cycle were five hours and the specific organic loading was 0.70 g COD/g VSS/d. This figure shows that as total VFA concentrations increased from 1850 mg/L to 2580 mg/L during the fill period, the relative propionate and acetate concentrations in the reactor supernatant remained virtually unchanged. However, the relative concentration of propionate increased significantly during the react period. This phenomenon of propionate accumulating in the reactor's supernatant was observed during the react periods of all tested SBAR cycles. These findings are in good agreement with previous studies. Possible explanations for accumulation of propionate

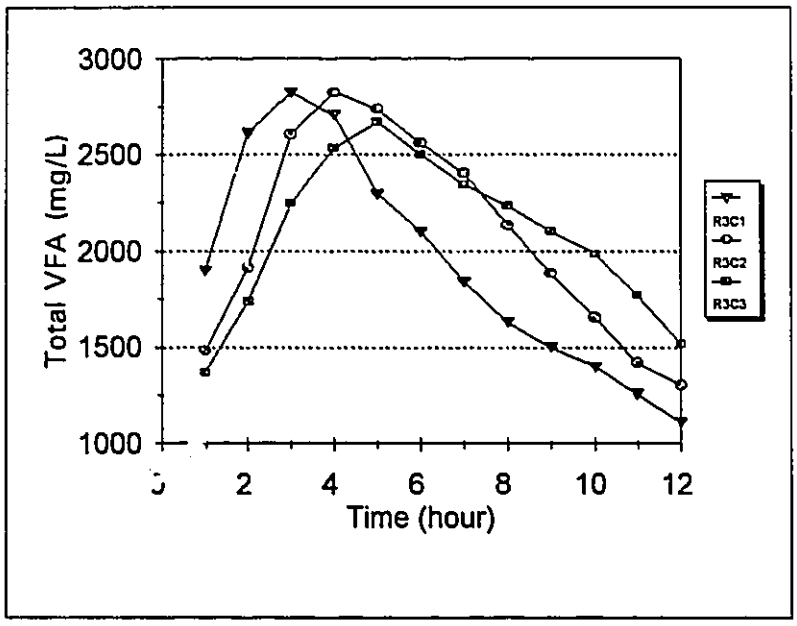


Fig. 4.7. Total VFA concentration profiles for reactor #3
(Cycle length: 12 hours. SOLR: 0.70 g COD/G VSS/d)
(Fill/react ratios: R3C1, 0.33; R3C2, 0.66; R3C3,1.00)

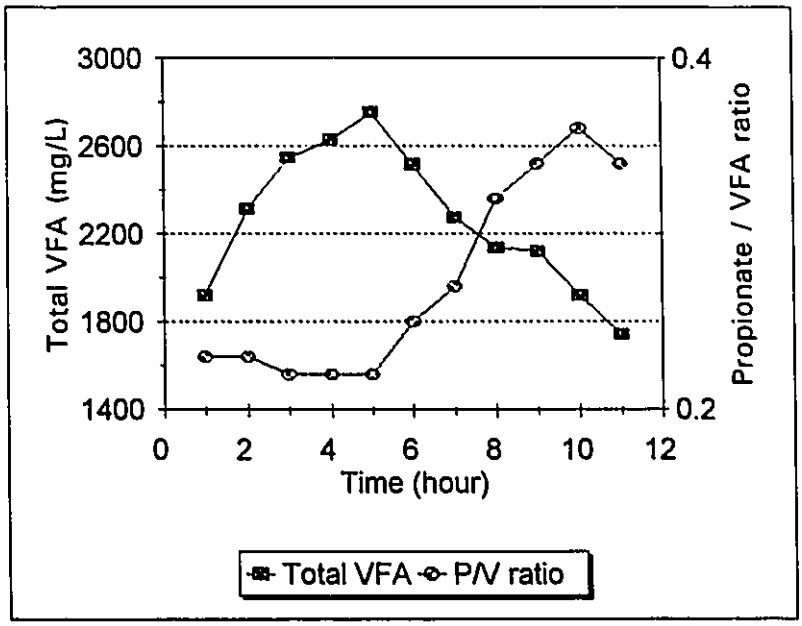


Fig. 4.8 Profiles of total VFA and propionate/VFA ratio
(Cycle length: 12 hours. Fill/react ratio: 1)

in anaerobic reactors include: (1) low natural populations of propionate-degrading bacteria in many methanogenic sludges (Ozlurk, 1991); (2) product inhibition of propionate degradation by acetate accumulation (Mawson et al., 1991); (3) thermodynamic limitation, such as build-up of hydrogen, reduces the energy available for propionate degradation (Hickey and Switzenbaum, 1991).

In the present study, the concentration of acetic acid in the SBARs was up to 3500 mg/L, which could have affected to some extent propionate utilization in this system. The accumulation of propionate during the react period indicated that the propionate degradation rate was lower than the acetate degradation rate which reveals possible inhibition effects on propionate degrading bacteria. However, complete inhibition of propionate degrading bacteria was not observed, indicating that an SBAR system has the capacity to tolerate inhibitory effects.

4.3.2.2 Biodegradation of total soluble COD

Commonly, the strength of carbonaceous substrates in wastewater is measured in terms of COD concentration. In this study, the feed COD was mainly contributed by sucrose and acetate. The average influent COD concentration was 6.71g ± 0.40g COD/L.

COD biodegradation profiles: The COD degradation profiles obtained from the experiments are similar to that of the VFAs. The typical soluble COD profiles for the three cycles of reactor #1 is shown in Fig. 4.9. The cycle length was 24 hours and the specific organic loading rate was 0.35 g COD/g VSS/d. The fill/react periods for these cycles were 5.5/16.5, 8.8/13.2, and 11/11 hours, respectively. Fig. 4.10 shows the soluble COD profiles (R2C1, R2C2, and R2C3) for reactor #2. The cycle length of this reactor was 18 hours and the specific organic loading rate was 0.53 g COD/g VSS/d. The fill/react periods for these cycles were 4/12, 6.4/9.6 and 8/8 hours, respectively.

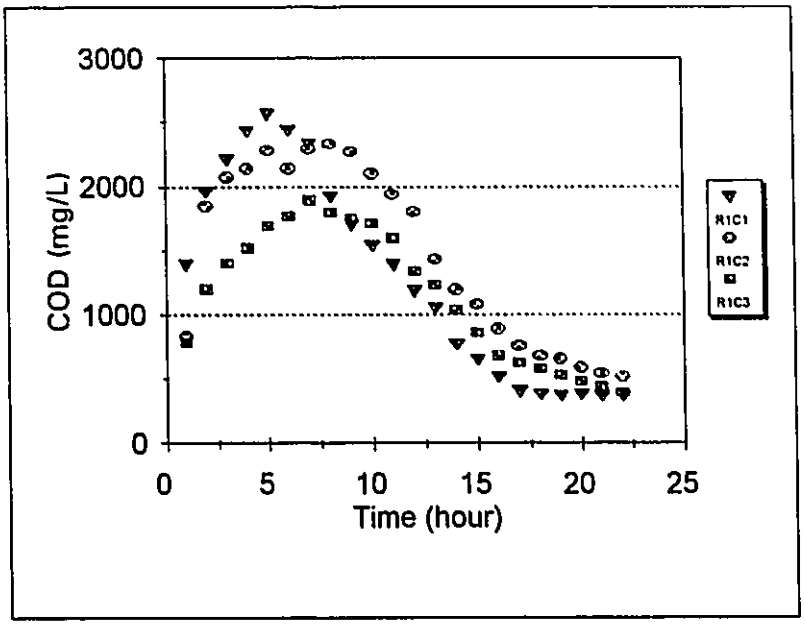


Fig. 4.9 Soluble COD concentration profiles for reactor #1
(Cycle length: 24 hours. SOLR: 0.35 g COD/g VSS/d)
(Fill/react ratios: R1C1, 0.33; R1C2, 0.66; R1C3, 1)

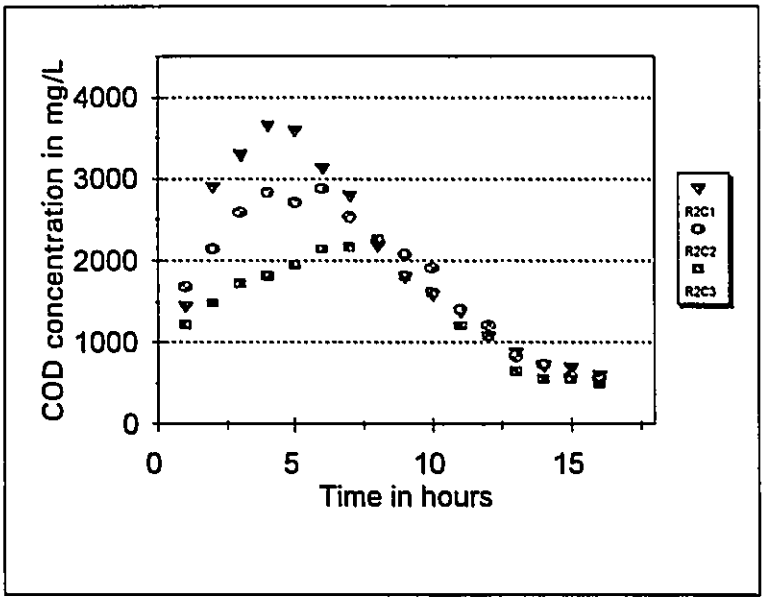


Fig. 4.10. Soluble COD concentration profiles for reactor #2
(Cycle length: 18 hours. SOLR: 0.53 g COD/g VSS/d)
(Fill/react ratios: R2C1, 0.33; R2C2, 0.66; R3C3, 1)

Comparing Fig. 4.9 with Fig. 4.10, it is seen that a significant fraction (over 90%) of influent COD was utilized within the first 18 hours of the cycle. The average treated effluent COD concentrations were 430 mg/L and 560 mg/L for reactor #1 and #2, respectively. Since the quality of the effluent did not change significantly, it can be concluded that an 18 hour cycle is sufficient to effectively treat this particular wastewater.

COD composition: It was observed that the average supernatant VFA/COD concentration ratio of all SBAR cycles was 0.84 ± 0.10 while the incoming feed VFA/COD concentration ratio was 0.5. Little variations of that were observed during the SBAR cycles. Figure 4.11 illustrates the superimposition of the soluble COD profile and the VFA/COD concentration ratio curve for reactor cycle R3C3. It appears that as the feed entered the reactor during the fill period, a large portion of the sucrose was quickly converted to acetate and propionate, which contributed to the increase of the total VFA concentration in the treated supernatant. This suggests that the degradation of the sucrose component to acetic and propionic acids by acidogenic bacteria was a rapid process, while VFA conversion by acetogenic bacteria and methanogenic bacteria was a much slower process.

4.3.2.3 Biogas production

Biogas production profiles: The biogas production profiles illustrate the performances of methanogenic bacteria during SBAR cycles. It was determined that methane accounted for 65% to 85% of the biogas produced during these experiments. It should be noted that in the studied system methanogenic bacteria could only use a limited number of substrates, such as carbon dioxide, hydrogen and acetate, for the formation of methane. Acetate was the most important precursor of methane production. Fig. 4.12 shows typical biogas production profiles. The length of these

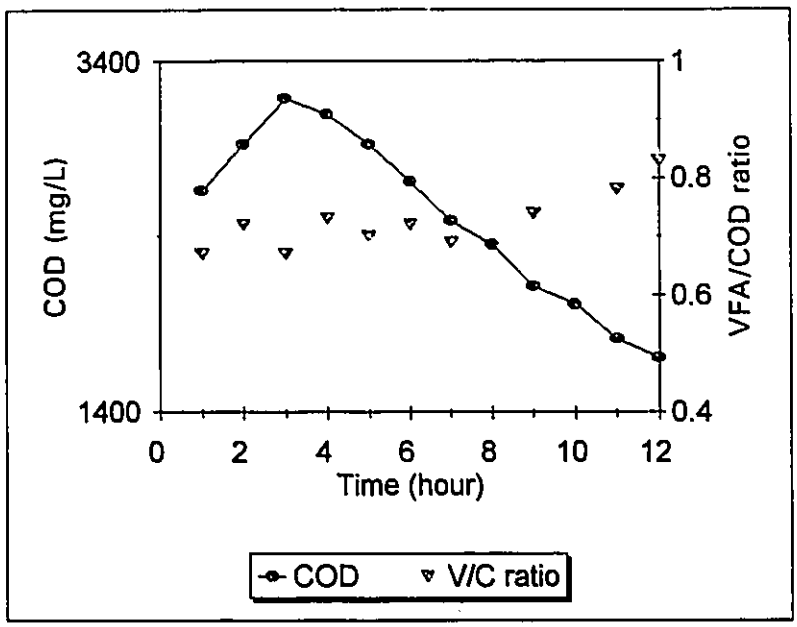


Fig. 4.11. Profiles of COD and VFA/COD ratio
(Cycle length: 12 hours. SOLR: 0.70 g COD/g VSS/d. Fill/react ratio: 1)

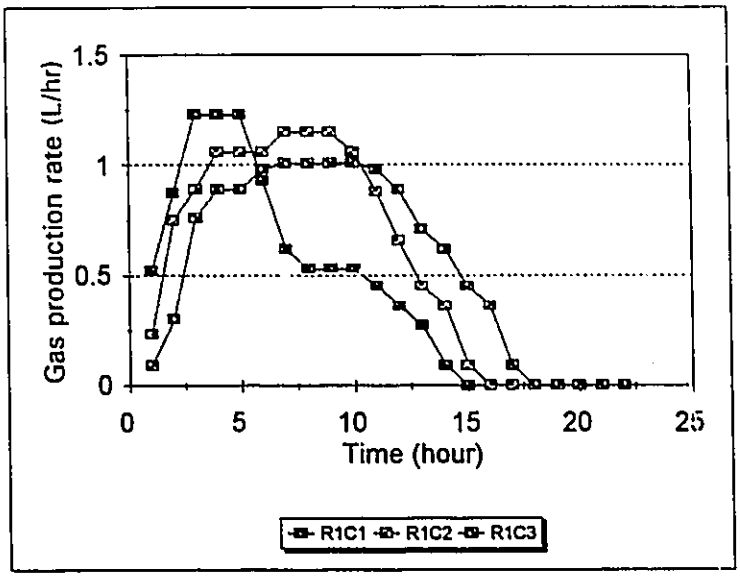


Fig. 4.12. Gas production rate profiles for reactor #1
(Cycle length: 24 hours. SOLR: 0.35 g COD/g VSS/d)
(Fill/react ratios: R1C1, 0.33; R1C2, 0.66; R1C3, 1)

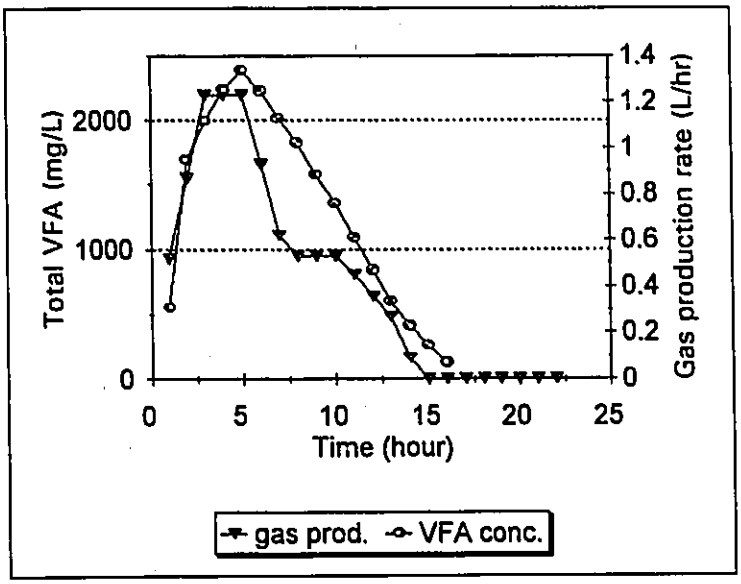


Fig. 4.13. VFA concentration and gas production rate profiles for R1C1
(Cycle length: 24 hours. Fill/react ratio: 0.33)

cycles is 24 hours, and the corresponding specific organic loading rate was 0.35 g COD/g VSS/d, while the fill/react ratios varied from 0.33 to 1.0. These profiles show that during the fill period, the gas production rate increased as waste entered the system and it remained stationary within some intervals of the SBAR cycle. By superimposing VFA and gas production profiles for the same experimental run (Fig. 4.13), it can be seen that the VFA degradation rate was not synchronous with the gas production rate. Similar patterns were observed with the other two SBARs. This phenomenon probably reflects the "true" behavior of different microorganism species adjusting to the available type of substrate under the studied condition.

4.3.3 Biodegradation of 2,4-dichlorophenol

The reductive dechlorination is a complex process. The microbiological aspects of this process have not been completely understood yet. However, the experimental data obtained can still provide some useful information.

4.3.3.1 Biodegradation pathway of 2,4-dichlorophenol

Analyses of the reactors' supernatant and treated effluent samples have shown that the major biodegradation product of 2,4-dichlorophenol, in both sequencing batch and continuous flow UASB reactors, was 4-monochlorophenol. This confirms that anaerobic dechlorination activities have a very strong preference for the position occupied by chlorines that are removed. In the study of 2,4-dichlorophenol, ortho chlorine was removed first. The major biodegradation pathway of 2,4-DCP is shown in Fig. 4.14.

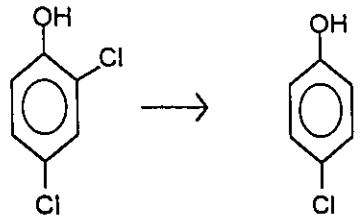


Fig. 4. 14 Major biodegradation pathway of 2,4-DCP in sequencing batch and continuous UASB reactors

As well, trace amounts of benzoate were detected in most reactors' supernatant. This finding is consistent with the work carried out by Zhang and Wiegel (1989). The complete biodegradation pathway proposed by these authors is illustrated in Fig. 4.15. It shows that 2,4-DCP was de-chlorinated to 4-MCP, which was further de-chlorinated to phenol. The later was then carbonxylated to benzoate, and benzoate was degraded via acetate to methane and carbon dioxide. In the SBAR system, the biodegradation of 4-MCP was the rate limiting step of the dechlorination process.

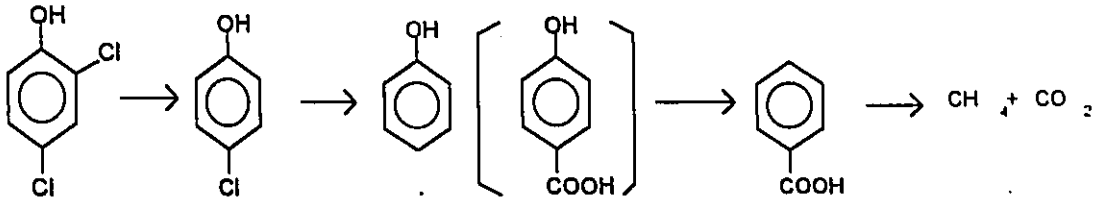


Fig. 4.15 Proposed complete biodegradation pathway of 2,4-DCP (Source: Zhang and Wiegel, 1989).

4.3.3.2 Mass balance of chlorophenol isomers in SBARs

During this experimental study, the influent 2,4-DCP concentrations were measured twice daily and the average values obtained were used for calculating the 2,4-DCP concentration in the reactors. Fig. 4.16 is a schematic mass balance of chlorophenol isomers for a typical SBAR cycle (R1C2). This figure shows that the normalized curve of total chlorophenol concentration (2,4-DCP and its metabolic

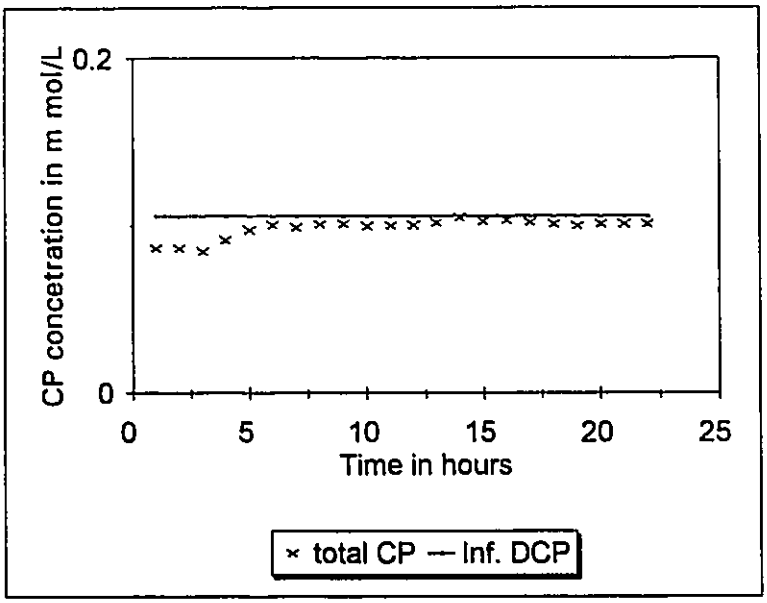


Fig. 4.16. Schematic mass balance of chlorophenol for R1C2 (Cycle length: 24 hours. SDCPLR: 1mg 2,4-DCP/g VSS/d)

product, 4-MCP) in the supernatant closely follows the average influent 2,4-DCP concentration plot during the cycle. These results clearly support that a good mass balance was achieved for 2,4-DCP and its metabolites throughout the SBAR cycle. Moreover, the mass balance obtained for chlorophenols suggests that the major removal mechanism of 2,4-DCP was biodegradation in the SBAR system, since the concentrations of 2,4-DCP and produced 4-MCP remaining in the treated effluents were almost equal to the influent 2,4-DCP concentration. The removals of 2,4-DCP as a result of sorption and volatilization were apparently negligible during this cycle.

At the pseudo-steady state, the average normalized influent and effluent chlorophenols concentration and their mass balance for all nine SBAR cycles studied are shown in Table 4.1. In all experimental runs, the 2,4-DCP concentration in the treated effluent was below detectable limits, while the mass of effluent 4-MCP accounted for 83 to 96% of the influent 2,4-DCP mass.

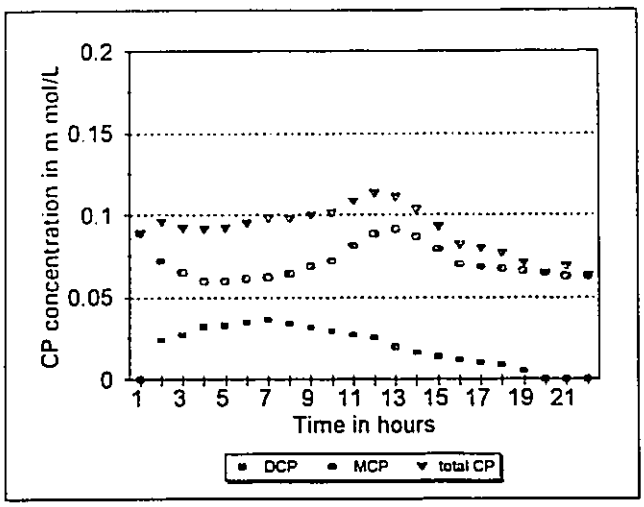
Table 4.1 Mass balance of chlorophenols in SBARs

Cycle #	Specific DCP loading rate mg DCP/g VSS/d	Avg. influent 2,4-DCP m mol / L	Avg. effluent 4-MCP m mol / L	Mass balance %
R1C1	1.0	0.104 ± 0.08	0.100 ± 0.008	96.2
R1C2	1.0	0.106 ± 0.08	0.102 ± 0.007	96.2
R1C3	1.0	0.109 ± 0.08	0.099 ± 0.005	90.8
R2C1	1.5	0.104 ± 0.08	0.091 ± 0.007	86.2
R2C2	1.5	0.106 ± 0.08	0.095 ± 0.009	89.6
R2C3	1.5	0.109 ± 0.08	0.092 ± 0.008	84.4
R3C1	2.0	0.104 ± 0.08	0.088 ± 0.011	83.3
R3C2	2.0	0.106 ± 0.08	0.090 ± 0.009	84.9
R3C3	2.0	0.109 ± 0.08	0.096 ± 0.010	88.1

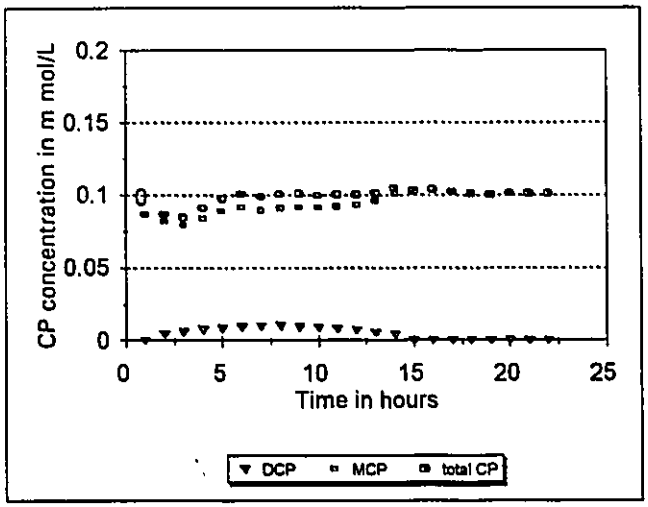
4.3.3.3 Chlorophenol biodegradation profiles

Fig. 4.17a to Fig. 4.17c display chlorophenol biodegradation profiles. These profiles were generated with data obtained from the three extensive experimental runs. About 10% to 20% standard deviation was observed. Fig. 4.17a presents chlorophenol profiles for the first cycle of the reactor #1, R1C1. The cycle length of this reactor was 24 hours and the fill/react ratio was 0.33. This figure shows that at the beginning of the cycle, as much as 0.1 m mol/L of 4-MCP had accumulated in the reactor. It was the major 2,4-DCP biodegradation product from the previous cycle and it represented about 80-90% of the influent 2,4-DCP. As wastewater entered the reactor during the fill period, the supernatant 2,4-DCP concentration increased and the 4-MCP concentration decreased accordingly. Apparently the 2,4-DCP biodegradation rate was much lower than its fill rate at this stage, since there was an increase of 2,4-DCP concentration, from 0 to a maximum of 0.04 m mol/L, in the reactor at the end of the 5.5 hour fill period. The 2,4-DCP concentration decreased from 0.04 m mol/L to 0 and the 4-MCP concentration increased from 0.06 m mol/L to 0.09 m mol/L during the react period, which suggested that the 2,4-DCP biodegradation was the major dechlorination process at that stage. At the end of the cycle, the increase of 4-MCP to a concentration of approximately 0.1 m mol/L in the supernatant confirmed that 2,4-DCP was completely biodegraded to 4-MCP.

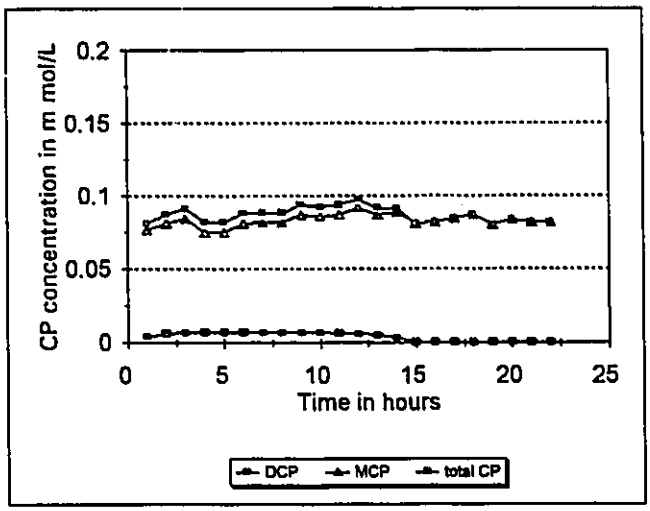
Fig. 4.17b and Fig. 4.17c present the chlorophenol profiles for the second and the third cycles of the same reactor, and the length of these cycles was 24 hours. The fill/react ratios of these cycles were 0.66 and 1, and the resulting $(SDCPLR)_f$ was 2.73 and 2.18 mg DCP_{in}/g VSS/d. These figures show that there was no 2,4-DCP mass build up in the reactor supernatant during cycles' fill period. Obviously, 2,4-dichlorophenol was being dechlorinated at the same rate as it was being supplied to the reactor. However, the concentrations of 4-MCP in the reactor supernatant were



(a)



(b)



(c)

Fig. 4.17. Chlorophenoi degradation profiles, (a) R1C1; (b) R1C2; (c) R1C3

almost unchanged. Therefore, it can be predicted that anaerobic sludge has the ability to handle a much higher dichlorophenol loading and the reactors were actually under loaded during this experimental work.

4.4 SBAR process evaluation

4.4.1 Characteristics of treated effluents from SBAR

The characteristics of treated effluents from SBARs gives one of the best insights of the reactor's performance. Table 4.2 summarizes the effluent characteristics. These results are based on averages of data obtained from experimental runs under pseudo steady state conditions.

Table 4.2 Characteristics of treated effluents in SBARs

Cycle	SOLR (g COD/ g VSS/d)	COD Removal efficiency (%)	Effluent COD/VFA ratio*	Effluent Propionate/ VFA ratio*	DCP Removal efficiency (%)
R1C1	0.35	94.1	0.70 ± 0.09	0.26 ± 0.08	99.9
R1C2	0.35	92.6	0.70 ± 0.09	0.26 ± 0.08	99.9
R1C3	0.35	94.2	0.70 ± 0.09	0.26 ± 0.08	99.9
R2C1	0.53	90.4	0.90 ± 0.12	0.76 ± 0.14	99.9
R2C2	0.53	91.9	0.90 ± 0.12	0.76 ± 0.14	99.9
R2C3	0.53	92.9	0.90 ± 0.12	0.76 ± 0.14	99.9
R3C1	0.70	72.5	0.80 ± 0.12	0.38 ± 0.08	99.9
R3C2	0.70	75.9	0.80 ± 0.12	0.38 ± 0.08	99.9
R3C3	0.70	76.1	0.80 ± 0.12	0.38 ± 0.08	99.9

The following information can be obtained from this table:

1. Overall COD removal efficiency can be significantly influenced by the SOLR of the SBAR cycle. The overall COD removal efficiency versus the specific organic loading rates of SBAR cycles is plotted in Fig. 4.18. This figure shows that COD removal efficiency decreases with increasing specific organic loading rates. Actually, the overall COD removal efficiency decreased from 94% to 75% when the specific organic loading rate increased from 0.35 to 0.7 COD/g VSS/d. This reveals the limited biodegradation abilities and possible inhibition effects of anaerobic bacteria under the higher loading conditions.

2. It is obvious that the reactors were under loaded in terms of 2,4-DCP mass in the influent wastewater. No detectable amounts of 2,4-dichlorophenol were observed in the treated effluents in all reactors operating under the pseudo steady-state conditions. Almost complete removal of 20 mg/L of 2,4-DCP in the wastewater was achieved

3. For reactor #1, high COD removal efficiency (92.6%-94.2%) associated with low ratio of propionate in the treated effluent (0.26 ± 0.05) indicates that all three groups of bacteria, acetogenic bacteria, acidogenic bacteria and methanogenic bacteria, were functioning well, and the inhibition effect was negligible at this specific organic loading (0.35 g COD/g VSS/d). However, for reactor #2, lower COD removal efficiency (90.4%-92.9%) and relatively higher effluent propionate composition (0.76) indicates that there was an accumulation of propionate in the reactor. This suggests an inhibitory effect on propionate degrading bacteria when applying a SOLR of 0.53 g COD/g VSS/d. Low COD removal efficiency (72.5-76.1%) and low propionate ratio (0.38) for reactor #3 suggests that all three substrate components, sucrose, acetate and propionate, were not being utilized efficiently. There is a possible inhibitory effect on all three groups of carbon oxidizing anaerobic bacteria at this specific organic loading.

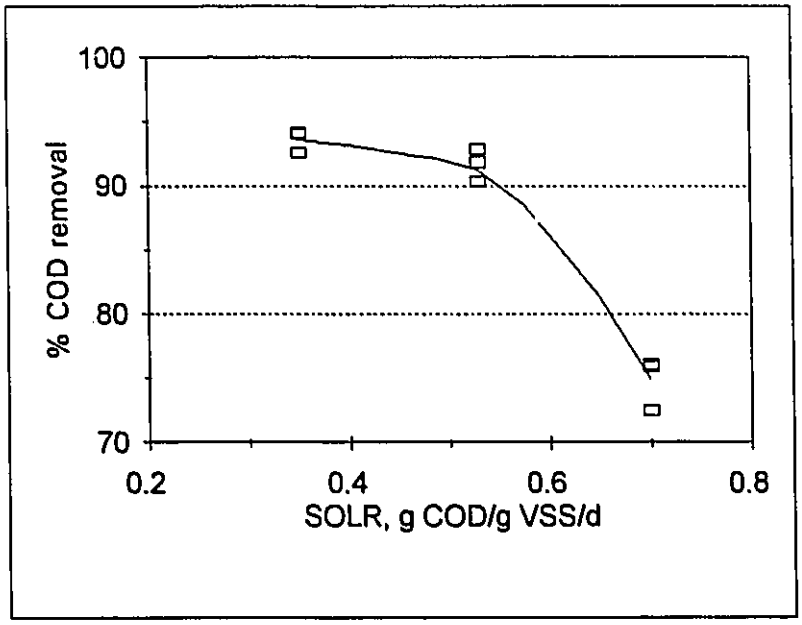


Fig 4.18. COD removal efficiencies for SBARs

4.4.2 The effect of SBAR operating strategy

In the SBAR operation, the biomass in the reactor is exposed to a much higher substrate loading during the fill period than the estimate based on a complete cycle basis. Therefore, the influences of operating strategies, i.e., fill/react ratios, on treatment efficiency of SBARs were investigated through examing the effect of specific organic loading rates (SOLR) which was defined as a function of the fill time and expressed as $(SOLR)_f$. The unit of $(SOLR)_f$ is g COD_{in}/g VSS/d. In the present study, the $(SOLR)_f$ of tested SBAR cycles ranged from 0.76 to 3.4 g COD/g VSS/d. A plot of $(SOLR)_f$ versus percentage soluble COD removal of these SBAR cycles is shown in Fig. 4.19. This figure indicates that a strong relationship exists between overall COD removal efficiency and $(SOLR)_f$ of SBAR treatment system. A significant decrease (from 95 to 75%) in overall COD removal efficiency was observed when a $(SOLR)_f$ greater than 1.5 g COD_{in} /g VSS /d was applied. Results in the same order of magnitude were reported by Kennedy et al. (1991). With similar feed composition but without chlorophenol addition, these researchers recommended a $(SOLR)_f$ of 1 g COD_{in}/g VSS/d for SBAR systems.

The unit COD uptake rate by biomass versus $(SOLR)_f$ is plotted in Fig. 4.20 to further investigate the influence of $(SOLR)_f$ on a reactor's performance. From this figure it can be seen that when $(SOLR)_f$ is less than 1.5 g COD_{in}/g VSS/d the unit COD uptake rate by biomass increases from 350 to 500 mg COD/g VSS/d. However, a further increase in $(SOLR)_f$ does not show any increase in the biomass COD uptake rate. It was almost constant at approximately 500 mg COD/g VSS/d when the $(SOLR)_f$ increased from 2 to 3.5 g COD_{in}/g VSS/d. This finding probably reflects the limited biodegradation ability of anaerobic sludge, and it further supports the fact that the fill-specific loading rate is an important design parameter for sequencing batch anaerobic reactors.

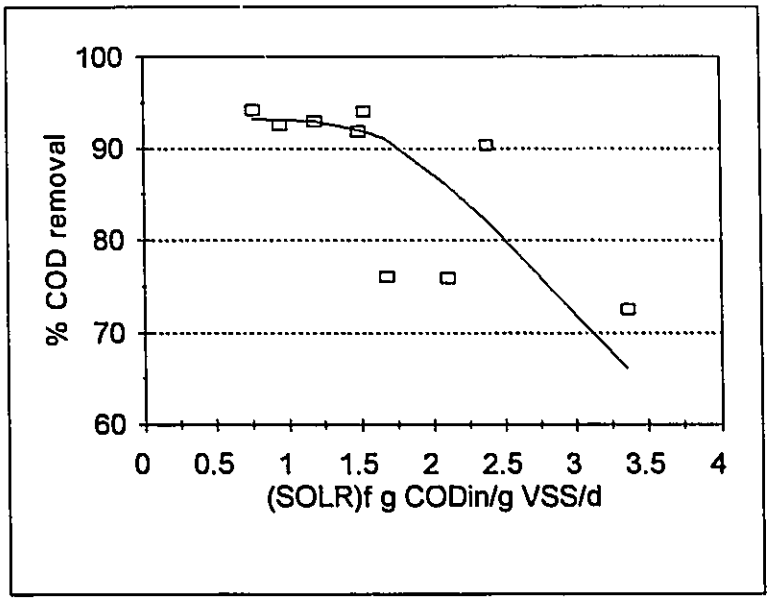


Fig. 4.19. COD removal efficiencies versus SOLR based on fill time of SBARs

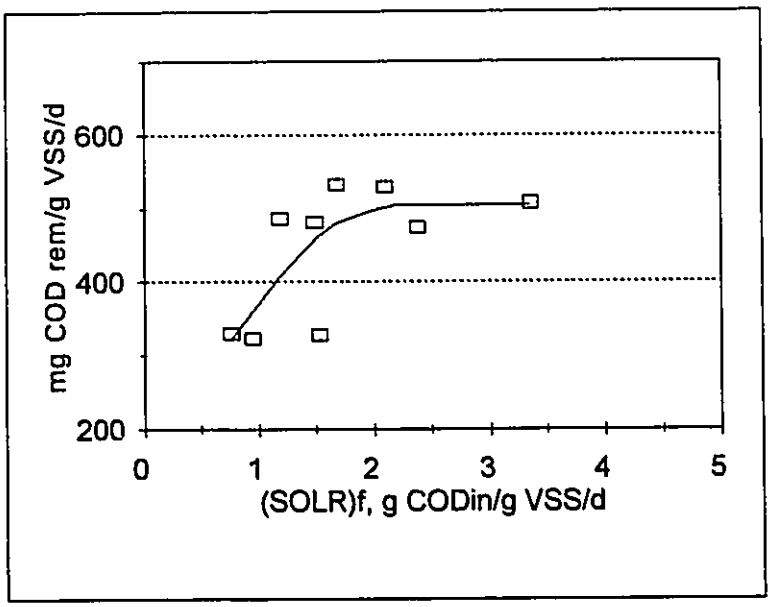


Fig. 4.20. SBAR treatment efficiencies versus SOLR based on fill period

4.4.3 Cumulative biogas production

The cumulative biogas production rates per unit biomass for all SBAR cycles versus SOLR are plotted in Fig. 4.21. This figure shows that as the specific organic loading rate of a SBAR cycle increases, the cumulative biogas production rate increases accordingly. The unit biogas production is highly correlated to the SOLR of the cycles, and the correlation coefficient is 0.92. The yield coefficients of biogas in this experimental work ranged from 0.3 to 0.5 L/g COD removed. Since the methane composition in the produced biogas ranged from 65-85%, the averaged methane yield coefficients for these reactors were 0.23 to 0.38 g L CH_4/g COD removed. These results are similar to those reported by Kennedy, et al., (1991), where the averaged methane yield coefficients ranged from 0.22 to 0.32 g L CH_4/g COD removed.

4.4.4. Comparison of sequencing batch and continuous UASB reactors

The results regarding influent and effluent quality as well as biogas production for SBAR and continuous flow UASB reactors are summarized in Table 4.3. These are averaged results at each specific organic loading under pseudo steady state conditions. This table shows that the performance of the continuous flow reactor was satisfactory under the studied conditions. As indicated in Table 4.3, the COD removals are 327, 458 and 589 mg COD/g VSS/d in the continuous flow UASB reactor. The low ratios of propionate/VFA (0.14 -0.26) and VFA/COD (0.47 - 0.76) in the treated effluent indicates low accumulation of acetic and propionic acid. This suggests that all three groups of anaerobic bacteria were functioning quite well in the continuous flow UASB reactor. A state of dynamic equilibrium was reached and a "syntrophic" (mutually beneficial) relationship was formed among these bacteria. However, under the studied condition, the performance of SBARs was not necessarily superior to that of the

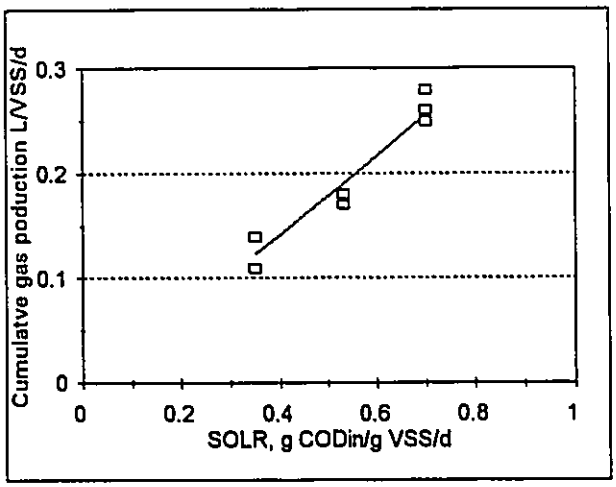


Fig. 4.21. Cumulative gas production per unit biomass versus SOLRs

Regression Output:	
Constant	-0.01001
Std Err of Y Est	0.01729
R Squared	0.926828
No. of Observations	9
Degrees of Freedom	7
X Coefficient(s)	0.379761
Std Err of Coef.	0.04033

Table 4.3 Comparison of sequencing batch and continuous UASB reactors

Operation mode	Continuous UASB reactor			Sequencing batch UASB reactors		
Influent specific organic loading rate (mg COD_{in} /g VSS/d)	350	525	700	350	525	700
Unit biomass COD removal rate (mg COD_{rem}/g VSS/d)	327	458	589	328	481	532
Effluent total VFA and COD concentration ratio	0.47	0.61	0.76	0.70	0.90	0.80
Effluent propionate and VFA concentration ratio	0.14	0.25	0.26	0.26	0.76	0.38
Unit biomass gas production rate (L/g VSS /d)	0.17	0.22	0.29	0.15	0.21	0.29

continuous reactors. The unit COD removal rates of SBARs are close to those obtained from the continuous UASB reactors. However, the high propionate/VFA ratio (0.26 to 0.76) in the treated effluent is an indication of an inhibitory effect on propionate degrading bacteria in the SBAR system. Ozturk (1991) has reported that the biodegradation of propionate is thermodynamically unfavorable in anaerobic digestion. In this study propionate conversion appeared to be more easily inhibited by periodical environment change caused by the SBAR operation mode.

CHAPTER FIVE

SBAR MODEL SIMULATION

A dynamic model considering product inhibition to describe soluble carbonaceous substrate degradation in SBARs was presented by Fernandes et al. (1993). This model was validated by comparing the simulated results with previously reported experimental data by Kennedy and co-workers (1991). The SBAR system and the substrate used in the present study were quite similar to the ones used in previous study. The only difference between the two systems was the addition of 20 mg/L of 2,4-dichlorophenol to the influent feed. Although modelling of chlorophenol biodegradation is not applicable due to the scale of the present study, it is believed that the degradation of carbonaceous substrate can be described by the same model as the one presented by Fernandes et al. (1993). This chapter examines the dynamic response and tests the validity of this model.

5.1 Model description

5.1.1 Background

In this study, a typical SBAR cycle consists of four stages: FILL, REACT, SETTLE, DRAW. Physical descriptions of these stages were presented in chapter three. The variation of culture volume during one SBAR cycle is graphically shown in Fig. 5.1.

In SBARs, the biodegradation of carbonaceous substrate involves three major groups of bacteria: acidogenic bacteria, acetogenic bacteria and methanogenic bacteria. Each of these groups may suffer from various types of inhibition under

unbalanced metabolic conditions resulting from the SBARs' operation. These inhibition factors include pH, hydrogen concentration and intermediate products, mainly volatile fatty acids. In these experiments, the reactors' pH was controlled within an acceptable range (7.8 - 8.2). Therefore, it is assumed that the most important inhibition factor would be the intermediate products.

The following assumptions were made in the model development:

(1) Substrate biodegradation takes place in the fill and react stages only. Substrate utilization during settle, and draw stages is negligible.

(2) The reactor is completely mixed during fill and react stages.

(3) Biomass increase due to growth in one cycle is insignificant compared with the total amount of biomass present in the reactor.

(4) No pH and hydrogen inhibition effect are expected.

(5) Upon acclimation, 2,4-dichlorophenol inhibition effect is negligible in the studied range of 20 mg/L.

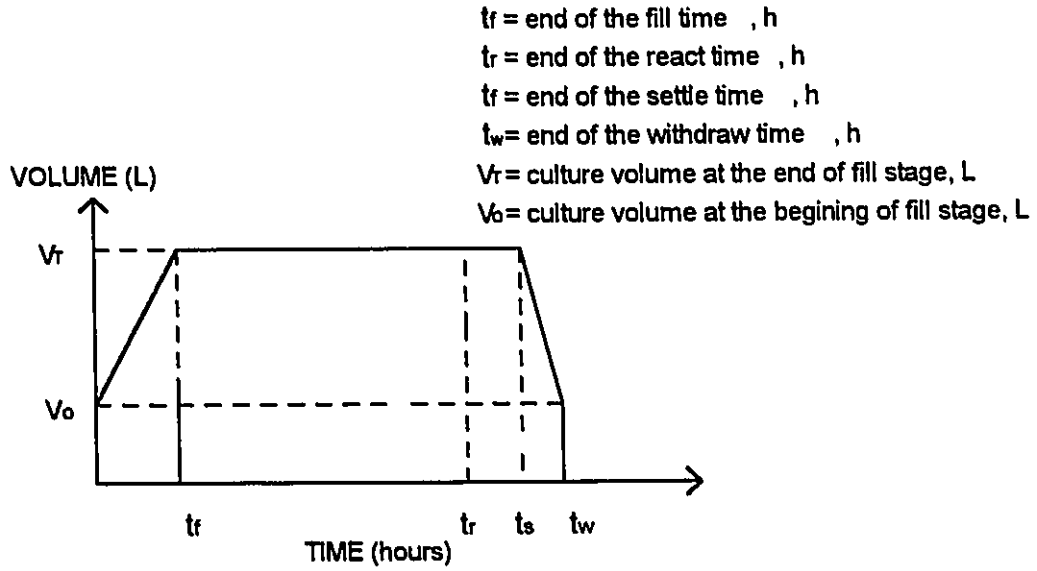


Fig. 5.1 Variation of the culture volume in the reactor operating in SBAR mode

5.1.2 Mathematical description of carbonaceous substrate degradation

Based on mass balances, the variations of soluble COD during fill and react stages in the SBAR can be expressed as follows:

For fill stage:

$$\frac{dS_{COD}}{dt}v = Q(S_{COD,0} - S_{COD}) - Rv \qquad (0 \le t \le t_f) \qquad (5.1a)$$

For react stage:

$$\frac{dS_{COD}}{dt} = -R \qquad (t_f \le t \le t_r) \qquad (5.1\ b)$$

where:

S_{COD} – total COD concentration in the supernatant of the reactor, g/L

$S_{COD,0}$ – total COD concentration in the influent, g/L

Q – influent flow rate during fill stage, L/h

t – time, h

R – rate of COD degradation, g/L.h

v – culture volume, L

Prior to solving equations 5.1a and 5.1b, R must be determined. The Monod equation is often used to describe the rate of substrate degradation in continuous flow reactors. However, it does not account for process inhibition.

Costello et al. (1991) used a non-competitive inhibition function, indicated below as Eq. 5.2, to describe product inhibition for the acidogenic phase of anaerobic digestion in continuous flow reactors with total volatile acids as the inhibitor.

$$R = \frac{kX(HS)}{(K_s + HS)(1 + HS/K_i)} \quad (5.2)$$

Where: k -- maximum specific substrate uptake rate constant, h^{-1}

K_i -- product inhibition constant, g/L

K_s -- half velocity constant, g/L

X -- biomass concentration, g/L

HS -- unionized volatile fatty acids concentration determined at a fixed pH and substrate concentration by the following equation, g/L

$$[HS] = \frac{[H^+][S_{VFA}]}{K_a} \quad (5.3)$$

Where, K_a is the ionization constant. S_{VFA} is the total volatile fatty acid's concentration, g/L.

Eq. 5.2 can be further simplified by expressing HS in terms of COD, based on Eq. 5.3:

$$HS = cS_{COD} \quad (5.4)$$

Where

$$c = \frac{10^{-pH}}{K_a} \times \frac{S_{VFA}}{S_{COD}} \quad (5.5)$$

Substituting Eq. 5.4 into Eq. 5.2, results in:

$$R = \frac{kXS_{COD}}{[(K_s/c)+S_{COD}][1+S_{COD}/(K_i/c)]} \tag{5.6}$$

Where K_s/c and K_i/c are referred to as apparent K_s and K_i and designated as K_s' and K_i', respectively. Hence, Eq. 5.6 is written as:

$$R = \frac{kXS_{COD}}{(K_s' + S_{COD})(1+S_{COD}/K_i')} \tag{5.7}$$

Substituting equation 5.7 into 5.1 and considering culture volume changes that resulted from SBAR operation, a dynamic model describing substrate degradation with a non-competitive inhibition function for SBARs is developed.

For fill stage:

$$\frac{dS_{COD}}{dt}v = Q(S_{COD,0} - S_{COD}) - \frac{kXS_{COD}v}{[K_s'(V_T/v)+S_{COD}]\{1+S_{COD}/[K_i'(V_T/v)]\}}$$

$$(0 \le t \le t_f) \tag{5.8a}$$

For react stage:

$$\frac{dS_{COD}}{dt} = -\frac{kXS_{COD}}{(K_s'+S_{COD})(1+S_{COD}/K_i')}$$

$$(t_f \le t \le t_r) \tag{5.8 b}$$

Where: t -- total reaction time, h

t_f -- fill time, h

t_r -- react time, h

V_T -- culture volume at the end of fill stage, L

5.2 Determination of model parameters

5.2.1 Experimental parameters

In the present study, three sequencing batch UASB reactors were used to study the performance of SBARs. One continuous UASB reactor identical to SBARs was used as the control. The same influent feed with 7 g COD/L and 20 mg 2,4-DCP/L was used for all reactors. Each sequencing batch reactor was operated at a different organic loading rate by changing the fill/react sequence length. The control reactor was operated at three different specific organic loading rates (SOLR), which corresponded to the SOLR applied to the SBARs. The experimental parameters for the various SBAR operating cycles are listed in Table 5.1.

5.2.2. Determination of biological kinetic parameters k and K_s'

The biological kinetic parameters are based on a Monod-type relationship for substrate utilization. There are two kinetic parameters that must be specified to simulate the uninhibited substrate uptake. These are: the maximum specific substrate uptake rate constant k and apparent half velocity constant K_S'. Based on supernatant and effluent composition analyses in chapter four, it was concluded that the product inhibition effect in the continuous flow reactor was negligible. Therefore, the data derived from the control reactor are used to determine the biological kinetic coefficients. The k and K_s' values were determined to be 0.044 h^{-1} and 1.16 g/L respectively. Detailed computations of these values are listed in Appendix B1. It should be noted that these values are lower than the values reported by Fernandes et al., (1993), where the k and K_s' values were found to be 0.104 h^{-1} and 3.9 g/L. This discrepancy may be caused by

Table 5.1 Experimental SBAR parameters

Cycle #	V_T (L)	V_0 (L)	*$S_{COD,0}$ (g/L)	**$S_{COD,I}$ (g/L)	X_0 (g/L)	Operating Strategies Fill/React (h)	Fill stage		React stage	
							pH	S_{VFA}/S_{COD}	pH	S_{VFA}/S_{COD}
R1C1	5.25	1.75	6.23	0.43	73.5	5.5/16.5	8.0	0.83	8.2	0.75
R1C2	5.25	1.75	7.15	0. 52	73.5	8.8/13.2	8.0	0.80	7.8	0.40
R1C3	5.25	1.75	6.80	0.40	73.5	11/11	7.8	0.74	7.9	0.79
R2C1	5.25	1.75	6.23	0.57	73.5	4/12	7.9	0.84	8.1	0.55
R2C2	5.25	1.75	7.15	0.57	73.5	6.4/9.6	7.9	0.80	7.9	0.76
R2C3	5.25	1.75	6.80	0.56	73.5	8/8	8.0	0.83	8.0	0.82
R3C1	5.25	1.75	6.23	1.71	73.5	2.5/7.5	8.1	0.80	8.1	0.84
R3C2	5.25	1.75	7.15	1.71	73.5	4/6	8.1	0.81	7.9	0.87
R3C3	5.25	1.75	6.8	1.71	73.5	5/5	7.8	0.859	8.2	0.87

*$S_{COD,0}$ - Influent COD concentration, g/L.

**$S_{COD,I}$ - Supernatant COD concentration at the beginning of fill stage in the reactor, g/L.

the different type of sludge used in the experiments and the addition of 2,4-dichlorophenol to the influent feed.

5.2.3. Determination of the apparent inhibition parameter K_i'

The value of K_i' was determined by non-linear regression using the rate equation for the fill and react period (Eq. 5.8a & Eq. 5.8b). Nine sets of SBARs experimental data were obtained from the present study. One set of these data, corresponding to R3C3 with fill/react of 5/5 h, was used to determine inhibition constant K_i' with a non-linear data-fitting procedure based on the least squares principle. From the regression analysis, K_i' was found to be 8.39 g/L., which is in the same order of magnitude as K_i' (1.15 g/L) reported by Fernandes et al. (1993). The results are presented in Appendix B2.

5.2.4. Determination of physical-chemical parameter K_a

The ionization constant K_a was determined based on the average temperature (35°C)of the experimental runs. A ±3°C fluctuation in temperature was observed during the experiments. At 35°C, the ionization constant value is 1.73×10^{-5}.

5.3 Implementation of the model

Model verification and validation was carried out with experimental data obtained from eight different SBAR operating strategies. These experimental parameters are shown in Table 5.1. The Runge-Kutta method was employed to solve the proposed dynamic model as expressed by equation 5.8. Model implementation was performed with ***FORTRAN 77*** software on an IBM personal computer.

5.4. Discussion

The proposed dynamic model incorporating the modified non-competitive function was implemented for eight different operation strategies. The results simulated by the model and the measured experimental data are presented in Figs 5.2 to 5.9. It can be seen that a good agreement was obtained between the simulated results and the experimental data. The correlation coefficients of regression analysis ranged from 0.93 to 0.98. The closeness of the correlation coefficients to 1 further supports that the previously developed dynamic SBAR model was able to provide a good comparison with the experimental data examined. The results of the dynamic simulation studies were very good considering the complexity of SBAR experimental conditions.

Differences between the predicted and measured results can be linked to the simplification of the model and possible experimental measurement error. Due to the scope of the present study, the major simplification made in the model simulation was to consider only the biodegradation of the total soluble COD at a constant rate. However, the experimental results show that three major components of COD, such as sucrose, acetate and propionate, were degraded at different rates. Sucrose was converted to acetate and propionate immediately after entering the SBAR system, while acetate was degraded at a much faster rate than propionate. The biodegradation in this model was considered simply as the degradation of total COD in the digesters, and it could lead to some error in the prediction of results. Sensitivity analysis regarding SBAR modelling has shown that an error of 0.1 pH unit measurement could contribute to as much as 13% deviation in the simulated result, and 5% deviation in influent COD measurement may result in a 8.8% deviation in model simulation (Fernandes et al., 1993).

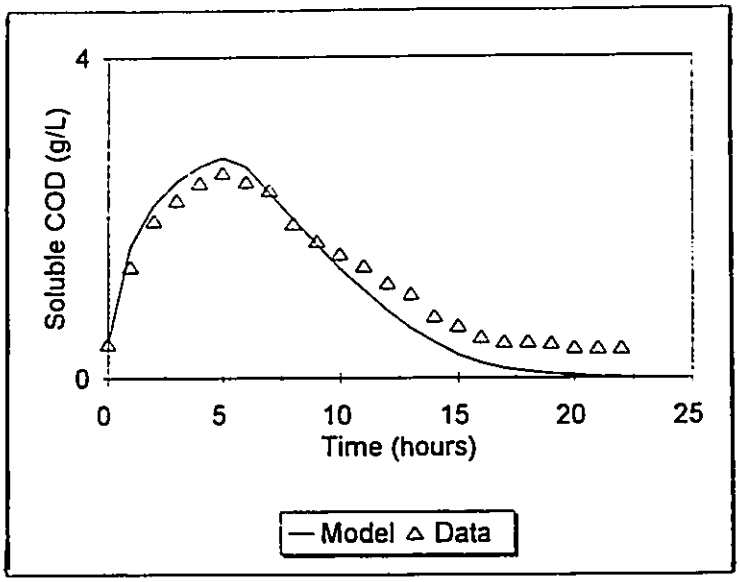


Fig. 5.2 Simulation of SBAR cycle, fill/react 5.5/16.5 h.

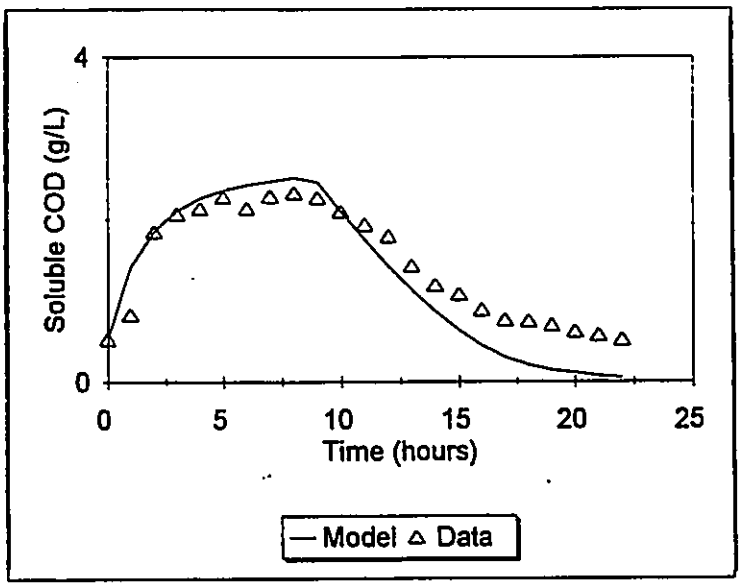


Fig. 5.3 Simulation of SBAR cycle, fill/react 8.8/13.2 h.

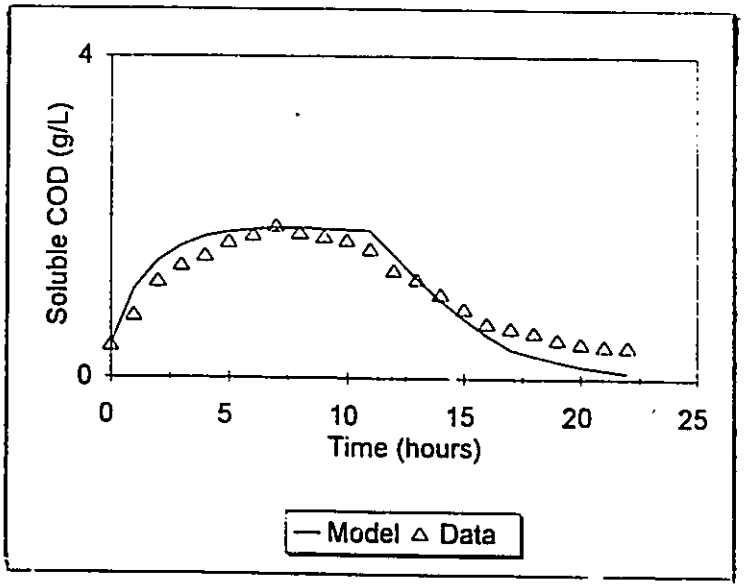


Fig. 5.4 Simulation of SBAR cycle, fill/react 11/11 h

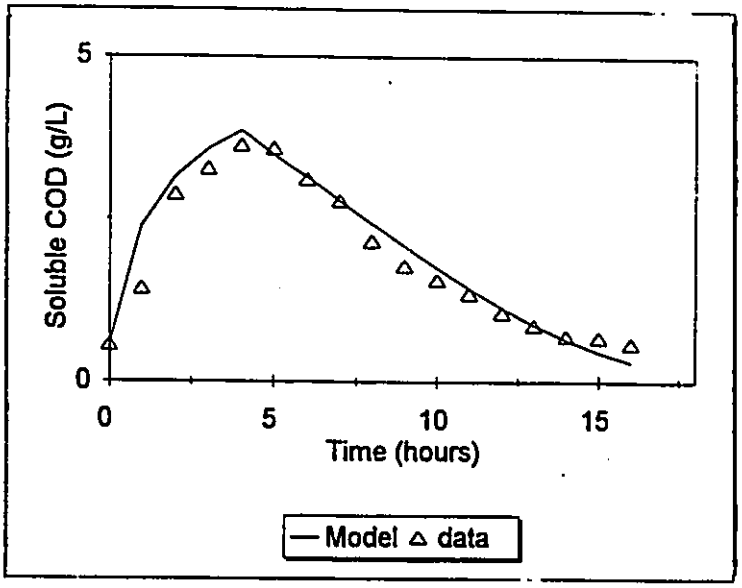


Fig. 5.5 Simulation of SBAR cycle, fill/react 4/12 h

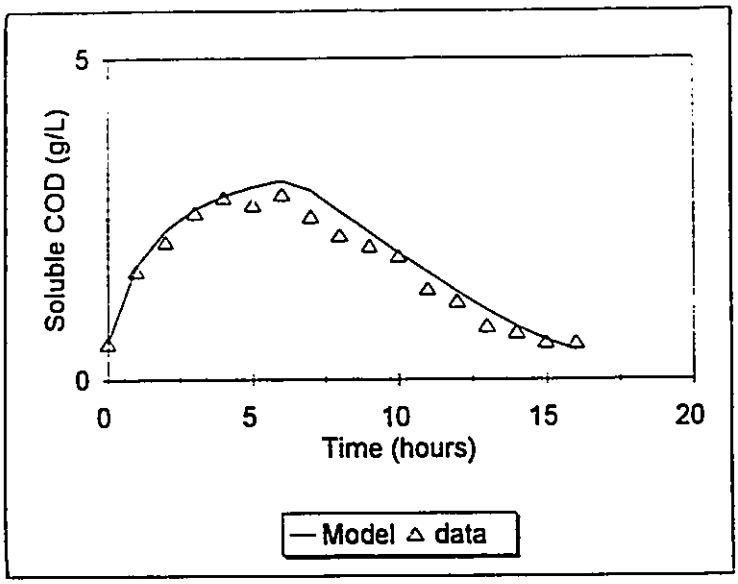


Fig. 5.6 Simutation of SBAR cycle, fill/react 6.4/9.6 h.

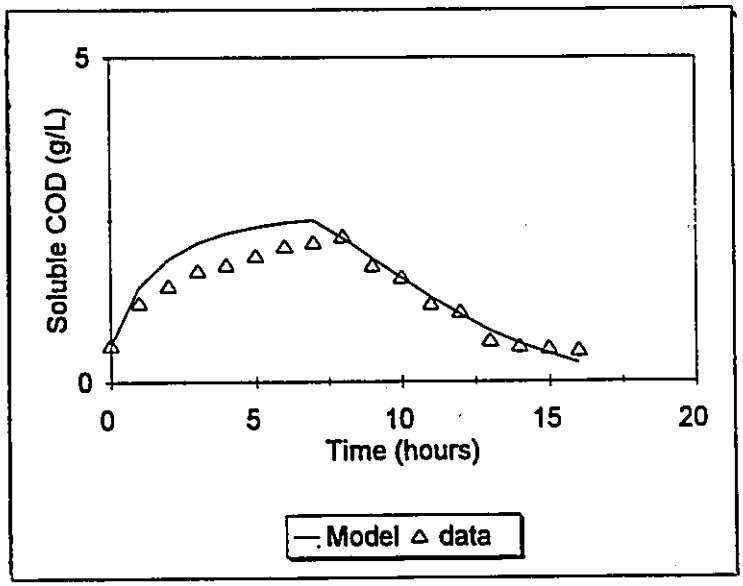


Fig. 5.7 Simutation of SBAR cycle, fill/react 8/8 h.

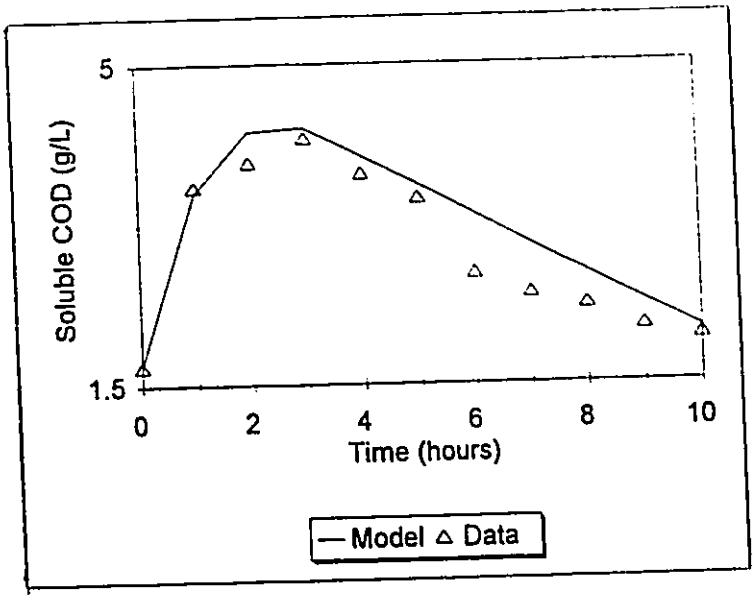


Fig 5.8 Simulation of SBAR cycle, fill/react 2.5/7.5 h.

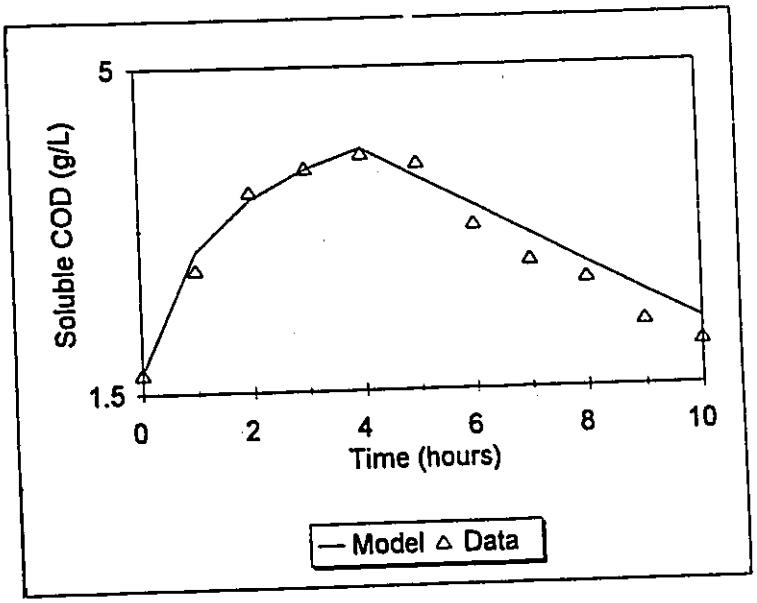


Fig 5.9 Simulation of SBAR cycle, fill/react4/6 h.

CHAPTER SIX

CONCLUSIONS AND RESEARCH RECOMMENDATIONS

6.1 Conclusions

This laboratory study shows that the application of the SBAR process to treat low concentrations of 2,4-dichlorophenol, in the range of 2 to 20 mg/L, is a feasible and practical technology. It could be an attractive alternative for treating chlorophenol contaminated industrial wastewater. According to the experimental results obtained, the following conclusions can be drawn:

(1) Laboratory-scale sequencing batch anaerobic reactors were able to treat 2,4-dichlorophenol contaminated wastewater in conjunction with medium-strength (7g COD/L) carbonaceous co-substrate. Almost 100% 2,4-dichlorophenol removal was achieved and the overall COD removal efficiencies ranged from 72.5% to 94.2% in the SBARs.

(2) Under the studied conditions, the performances of sequencing batch and continuous UASB reactors are quite close in terms of treatment efficiency. For the continuous UASB reactor the overall removal efficiency for 2,4-DCP was about 100% and COD removal efficiencies ranged from 84.2% to 93.3%.

(3) In both sequencing batch and continuous flow UASB reactors the major anaerobic biodegradation product of 2,4-DCP was 4-MCP. In treated effluents, 4-MCP accounted for 83.3% to 96.6% of influent 2,4-DCP. Good mass balance based on influent and effluent 2,4-DCP and 4-MCP indicated that the 2,4-DCP removals due to volatilization and biosorption were negligible.

(4) This experimental work also confirmed earlier observations that the specific organic loading rate (SOLR) significantly influenced the overall COD removal efficiency

of the sequencing batch anaerobic reactors. To achieve over 90% COD removal efficiency, a SOLR less than 0.53 g COD/g VSS/d is recommended for a similar SBAR system. The specific organic loading rate based on fill time $(SOLR)_f$ appeared to be an important design parameter of the SBAR process. The highest contaminant removal efficiency as well as the best performance was achieved at a $(SOLR)_f$ of 1.5 g COD_{in} /g VSS/d.

(5) In terms of the biodegradation of carbonaceous substrate, the simulation results obtained in this study using the dynamic SBAR model by Fernandes et al., (1993) can predict the SBAR performance reasonably well.

6.2 Recommendation for further research

The treatment of chlorophenols in sequencing batch upflow anaerobic sludge blanket reactors demonstrated in this study has shown that continuation of this research work is worthwhile. In order to determine treatment efficiency and to model the biodegradation of chlorophenols in a SBAR system, the higher loading rates of these compounds should be investigated.

The varying concentrations of individual VFAs in the reactor's supernatant gives the best insight into the dynamic behavior of the SBAR process. In order to better simulate SBAR performance, a more detailed model considering the biodegradation of individual VFAs should be developed.

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Appendix A1

Reactors' supernatant chlorophenol concentration during acclimation study

2-chlorophenol acclimation

Time	Infl.2CP	Effl.2CP	Effl.phenol
days	m mol/L	m mol/L	m mol/L
1	0.0156		
6	0.0234		
11	0.0391	0.0203	
16	0.0773	0.0531	
21	0.0938	0.0695	
26	0.109	0.0938	
31	0.141	0.111	0.0064
36	0.156	0.117	0.0106
41	0.156	0.023	0.016
46	0.156	0.0117	0.0227
51	0.156	0.018	0.0266
56	0.156	0.0219	0.0277
61	0.156	0.018	0.0096

4-chlorophenol acclimation

Time	Infl.4CP	Effl.4CP
days	m mol/L	m mol/L
1	0.0156	
6	0.0234	
11	0.0391	0.0203
16	0.0773	0.0531
21	0.0938	0.0695
26	0.109	0.0938
31	0.141	0.149
36	0.156	0.156
41	0.156	0.144
46	0.156	0.153
51	0.156	0.145
56	0.156	0.151
61	0.156	0.153

2,4-dichlorophenol acclimation

Time	Infl.DCP	Effl.DC	Effl.4CP
days	m mol/L	m mol/L	m mol/L
1	0.0123		
6	0.0184	0.0123	
11	0.0307	0.0247	
16	0.0429	0.0345	
21	0.0552	0.045	
26	0.0613	0.0419	
31	0.0797	0.0506	
36	0.0859	0.0632	
41	0.104	0.0728	
46	0.11	0.0811	
51	0.117	0.0853	0.006
56	0.123	0.0934	0.0133
61	0.123	0.101	0.0158

Appendix A2

COD concentration profiles for all cycles of SBARs

Time hr	R1C1 COD mg/l	R1C2 COD mg/l	R1C3 COD mg/l	R2C1 COD mg/l	R2C2 COD mg/l	R2C3 COD mg/l	R3C1 COD mg/l	R3C2 COD mg/l	R3C3 COD mg/l
influent	6230	7015	6795	6230	7015	6795	6230	7015	6795
1	1390	830	780	1435	1680	1220	3660	2830	2690
2	1960	1850	1200	2895	2140	1480	3930	3660	3080
3	2210	2070	1400	3285	2590	1720	4090	3910	3320
4	2430	2140	1520	3640	2830	1810	3780	4050	3590
5	2560	2280	1690	3585	2710	1950	3530	3950	3600
6	2440	2140	1770	3120	2880	2140	2710	3280	3630
7	2330	2290	1890	2790	2530	2160	2490	2880	2860
8	1920	2330	1800	2170	2230	2250	2365	2685	2440
9	1700	2270	1750	1785	2070	1810	2115	2210	2150
10	1540	2100	1710	1569	1910	1615	2014	1975	1975
11	1390	1940	1605	1360	1400	1205	1818	1800	1760
12	1180	1800	1345	1074	1210	1075	1712	1698	1625
13	1050	1195	1230	866	830	645			
14	770	1080	1040	706	730	555			
15	648	890	860	689	584	545			
16	512	760	680	594	566	485			
17	480	740	630						
18	465	742	574						
19	432	697	487						
20	389	624	435						
21	370	573	405						
22	370	523	395						
effluent	370	523	395	689	466	385	1712	1698	1625

Appendix A3

Total VFA concentration profiles for three cycles of reactor #1

Time HR	R1C1 Acetate mg/l	Propionate mg/l	Total VFA mg/l	R1C2 Acetate mg/l	Propionate mg/l	Total VFA mg/l	R1C3 Acetate mg/l	Propionate mg/l	Total VFA mg/l
1	1039	82	1121	629	83	712	695	135	830
2	1436	258	1694	923	184	1107	882	247	1129
3	1688	310	1998	1400	225	1625	1053	263	1316
4	1878	360	2238	1563	273	1836	1114	273	1387
5	1991	395	2386	1577	305	1882	1048	293	1341
6	1815	408	2223	1329	498	1827	1056	308	1364
7	1623	395	2018	1205	535	1740	1086	334	1420
8	1427	396	1823	1010	643	1653	1054	314	1368
9	1194	382	1576	977	627	1604	1057	325	1382
10	986	368	1354	986	553	1539	1045	332	1377
11	748	339	1087	967	482	1449	1060	327	1387
12	532	306	838	829	454	1283	960	317	1277
13	329	270	599	710	297	1007	876	304	1180
14	182	230	412	577	284	861	617	258	875
15	84	176	260	398	254	652	499	219	718
16	77	160	237	370	231	601	283	137	420
17	64	151	215	256	212	468	227	104	331
18	57	139	196	207	210	417	184	98	282
19	49	123	172	189	202	391	156	87	243
20	44	110	154	126	198	324	112	79	191
21	40	98	138	87	192	279	65	54	119
22	36	91	127	41	189	230	28	31	59

Appendix A3

Total VFA concentration profiles for three cycles of reactor #2

Time HR	R2C1 Acetate mg/l	Propionate mg/l	Total VFA mg/l	R2C2 Acetate mg/l	Propionate mg/l	Total VFA mg/l	R2C3 Acetate mg/l	Propionate mg/l	Total VFA mg/l
1	785	109	894	758	98	856	788	178	966
2	1465	255	1720	1065	155	1220	922	233	1155
3	1830	366	2196	1314	313	1627	1095	332	1427
4	2201	413	2614	1570	431	2001	1113	402	1515
5	1923	439	2362	1630	496	2126	1086	436	1522
6	1644	446	2090	1608	528	2136	1025	454	1479
7	1384	473	1857	1608	630	2238	1025	444	1469
8	1164	557	1721	1349	680	2029	1139	518	1657
9	981	507	1488	1104	580	1684	906	522	1428
10	731	423	1154	885	535	1420	846	399	1245
11	573	295	868	627	528	1155	658	337	995
12	425	268	693	538	541	1079	499	321	820
13	358	222	580	427	492	919	417	303	720
14	226	182	408	333	455	788	253	278	531
15	119	138	257	280	365	645	168	227	395
16	89	114	203	112	207	319	85	157	242

Appendix A3

Total VFA concentration profiles for three cycles of reactor #3

	R3C1			R3C2			R3C3		
Time HR	Acetate mg/l	propionate mg/l	Total VFA mg/l	Acetate mg/l	propionate mg/l	Total VFA mg/l	Acetate mg/l	propionate mg/l	Total VFA mg/l
1	1571	331	1902	1236	246	1482	1255	321	1576
2	2165	449	2614	1614	313	1927	1458	461	1919
3	2425	601	3026	2214	404	2618	1812	536	2348
4	2615	614	3229	2336	489	2825	2003	581	2584
5	2170	620	2790	2150	589	2739	2019	613	2632
6	1869	623	2492	1962	599	2561	2023	651	2674
7	1628	584	2212	1824	582	2406	1854	649	2503
8	1522	569	2091	1593	542	2135	1663	621	2284
9	1310	535	1845	1387	499	1886	1422	625	2047
10	1080	520	1600	1173	482	1655	1292	610	1902
11	877	490	1367	954	468	1422	1175	599	1774
12	652	457	1109	867	437	1304	986	534	1520

Appendix A4

Biogas production rate during SBAR cycles

Time hour	R1C1 Gas prod L/hr	R1C2 Gas prod L/hr	R1C3 Gas prod L/hr	R2C1 Gas prod L/hr	R2C2 Gas prod L/hr	R2C3 Gas prod L/hr	R3C1 Gas prod L/hr	R3C2 Gas prod L/hr	R3C3 Gas prod L/hr
1	0.52	0.23	0.09	0.48	0.26	0.12	0.72	0.6	0.54
2	0.87	0.75	0.3	0.78	0.55	0.43	0.96	0.9	0.72
3	1.23	0.89	0.76	0.97	0.76	0.65	1.26	1.14	1.26
4	1.23	1.06	0.89	0.97	0.89	0.87	1.05	1.14	1.32
5	1.23	1.06	0.89	0.92	0.89	0.87	0.9	1.14	1.32
6	0.93	1.06	0.98	0.92	0.89	0.87	0.9	1.08	1.14
7	0.62	1.15	1.01	0.72	0.89	0.87	0.9	1.08	1.08
8	0.53	1.15	1.01	0.72	0.82	0.87	0.9	1.08	1.08
9	0.53	1.15	1.01	0.72	0.82	0.82	0.84	0.84	1.02
10	0.53	1.06	1.01	0.55	0.82	0.82	0.84	0.84	1.02
11	0.45	0.88	0.98	0.55	0.82	0.71	0.84	0.84	0.89
12	0.36	0.66	0.89	0.55	0.71	0.82	0.18	0.38	0.24
13	0.27	0.45	0.71	0.55	0.71	0.71			
14	0.09	0.36	0.62	0.33	0.69	0.69			
15	0	0.09	0.45	0.33	0.55	0.63			
16	0	0	0.36	0.33	0.33	0.55			
17	0	0	0.09						
18	0	0	0						
19	0	0	0						
20	0	0	0						
21	0	0	0						
22	0	0	0						
L/day	9.39	12	12.05	15.5	15.2	15.1	20.6	22.1	23.3

Appendix A5

Chlorophenol concentration profiles for reactor #1

Time hr	R1C1 DCP m mol/L	MCP m mol/L	total CP m mol/L	R1C2 DCP m mol/L	MCP m mol/L	total CP m mol/L	R1C3 DCP m mol/L	MCP m mol/L	total CP m mol/L
1	0	0.0891	0.0891	0	0.0864	0.0864	0.0039	0.0771	0.081
2	0.0239	0.0719	0.0958	0.0046	0.0817	0.0863	0.0059	0.0813	0.0872
3	0.027	0.0648	0.0918	0.0056	0.0789	0.0846	0.0067	0.0847	0.0914
4	0.0319	0.0594	0.0913	0.0073	0.0836	0.0909	0.007	0.075	0.082
5	0.0325	0.0594	0.0919	0.0085	0.0885	0.097	0.007	0.0753	0.0823
6	0.0344	0.0609	0.0953	0.0093	0.0909	0.1002	0.0069	0.0811	0.088
7	0.0362	0.0617	0.0979	0.0095	0.0891	0.0986	0.0067	0.0819	0.0886
8	0.0337	0.0641	0.0978	0.0098	0.0907	0.1005	0.0067	0.0818	0.0885
9	0.0313	0.0688	0.1	0.0093	0.0914	0.1007	0.0067	0.0874	0.0941
10	0.0294	0.0719	0.1013	0.0086	0.091	0.0996	0.0065	0.0858	0.0923
11	0.027	0.0813	0.1082	0.008	0.0919	0.0999	0.0064	0.0876	0.094
12	0.0252	0.0883	0.1134	0.0072	0.093	0.1001	0.0057	0.0921	0.0978
13	0.0196	0.0914	0.111	0.0052	0.0961	0.1013	0.0049	0.087	0.0919
14	0.0166	0.0867	0.1033	0.004	0.1008	0.1048	0.0031	0.0888	0.0918
15	0.0137	0.0789	0.0926	0	0.1023	0.1023	0	0.0809	0.0809
16	0.0117	0.0697	0.0814	0	0.1032	0.1032	0	0.0821	0.0821
17	0.0103	0.0682	0.0795	0	0.1021	0.1021	0	0.0845	0.0845
18	0.0085	0.0671	0.0765	0	0.1008	0.1008	0	0.0872	0.0872
19	0.0049	0.0654	0.0704	0	0.0998	0.0998	0	0.0801	0.0801
20	0	0.0646	0.0646	0	0.1011	0.1011	0	0.0837	0.0837
21	0	0.0625	0.0688	0	0.1008	0.1008	0	0.0821	0.0821
22	0	0.0625	0.0625	0	0.1008	0.1008	0	0.0821	0.0821

Appendix A5
Chlorophenol concentration profiles for reactor #2

	R2C1			R2C2			R2C3		
Time	DCP	MCP	total CP	DCP	MCP	total CP	DCP	MCP	total CP
hr	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L
1	0	0.0922	0.0922	0	0.0875	0.0875	0.0054	0.0768	0.0822
2	0.0209	0.082	0.1029	0.004	0.0697	0.0737	0.0035	0.0832	0.0867
3	0.0258	0.0688	0.0945	0.0117	0.0763	0.0879	0.0039	0.0852	0.0891
4	0.0294	0.057	0.0865	0.0128	0.0728	0.0856	0.0078	0.0814	0.0892
5	0.0337	0.0469	0.0806	0.0136	0.0827	0.0962	0.0064	0.0743	0.0807
6	0.0325	0.0492	0.0817	0.0128	0.0759	0.0887	0.0058	0.0835	0.0893
7	0.038	0.0555	0.0935	0.0196	0.0805	0.1	0.0075	0.0715	0.0789
8	0.038	0.0609	0.099	0.0172	0.0773	0.0945	0.006	0.0745	0.0805
9	0.035	0.0617	0.0967	0.0201	0.067	0.0871	0.0069	0.0763	0.0832
10	0.0337	0.0664	0.1001	0.0172	0.0852	0.1023	0.0082	0.0738	0.082
11	0.0313	0.0688	0.1	0.0169	0.0648	0.0817	0.0066	0.0744	0.0809
12	0.0301	0.0711	0.1012	0.0168	0.075	0.0918	0.0078	0.0856	0.0934
13	0.0239	0.0727	0.0966	0.0137	0.0672	0.0809	0.0055	0.0836	0.0891
14	0.0215	0.0875	0.109	0.008	0.073	0.081	0	0.0855	0.0855
15	0	0.0875	0.0875	0.0067	0.0898	0.0966	0	0.0902	0.0902
16	0	0.082	0.082	0	0.0891	0.0891	0	0.0948	0.0948

Appendix 5
Chlorophenol concentration profiles for reactor #3

Time	R3C1			R3C2			R3C3		
	DCP	MCP	total CP	DCP	MCP	total CP	DCP	MCP	total CP
hr	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L	m mol/L
1	0	0.0766	0.0766	0.0117	0.0734	0.0851	0.0069	0.0857	0.0925
2	0.0294	0.0641	0.0935	0.0129	0.0805	0.0934	0.0087	0.0813	0.09
3	0.0258	0.0641	0.0898	0.016	0.0766	0.0925	0.0094	0.0751	0.0845
4	0.0288	0.0617	0.0906	0.0221	0.0703	0.0924	0.0096	0.0741	0.0837
5	0.0221	0.0859	0.108	0.0178	0.0789	0.0967	0.0102	0.0728	0.083
6	0.0264	0.0758	0.1022	0.0252	0.0805	0.1056	0.0097	0.0777	0.0874
7	0.0221	0.0625	0.0846	0.0196	0.0984	0.1181	0.0095	0.0751	0.0847
8	0.0209	0.0594	0.0802	0.0209	0.0781	0.099	0.0067	0.0849	0.0916
9	0.0245	0.057	0.0816	0.0172	0.0906	0.1078	0.0067	0.0916	0.0983
10	0.0166	0.1008	0.1173	0.0123	0.0883	0.1006	0	0.0968	0.0968
11	0	0.0906	0.0906	0.0086	0.0828	0.0914	0	0.1077	0.1077
12	0	0.0898	0.0898	0	0.0883	0.0883	0	0.0926	0.0926

Appendix B

Determination of biological kinetic parameters k and K_s'

The biological kinetic parameters are based on a Monod-type relationship for substrate utilization. There are two kinetic parameters that must be specified to simulated the uninhibited substrate uptake. These are: the maximum specific substrate uptake rate constant k and apparent half velocity constant K_S'. Based on supernatant and effluent composition analyses in chapter four, it was concluded that the product inhibition effect in continuous reactor is negligible. Therefore, the data derived from control reactor are used to determine biological kinetic coefficients.

By definition, the substrate utilization rate r_{su} can be expressed as follows (Tchobanoglous and Burton, 1991)

$$r_{su} = -\frac{kXS}{K_s'+S} = -\frac{S_0 - S}{\theta} \qquad \text{(B1)}$$

Dividing by X yields

$$\frac{kS}{K_s'+S} = \frac{S_0 - S}{X\theta} \qquad \text{(B2)}$$

The linearized form of Eq. 5.9, obtained by taking its inverse, is

$$\frac{X\theta}{S_0 - S} = \frac{K_s'}{k}\frac{1}{S} + \frac{1}{k} \qquad \text{(B3)}$$

The continuous reactor was operated at three different organic loadings corresponding these of SBARs as control. At each organic loading, three experimental runs were conducted. Therefore, nine sets experimental data were obtained. Following steps are involved to compute K_s and k.

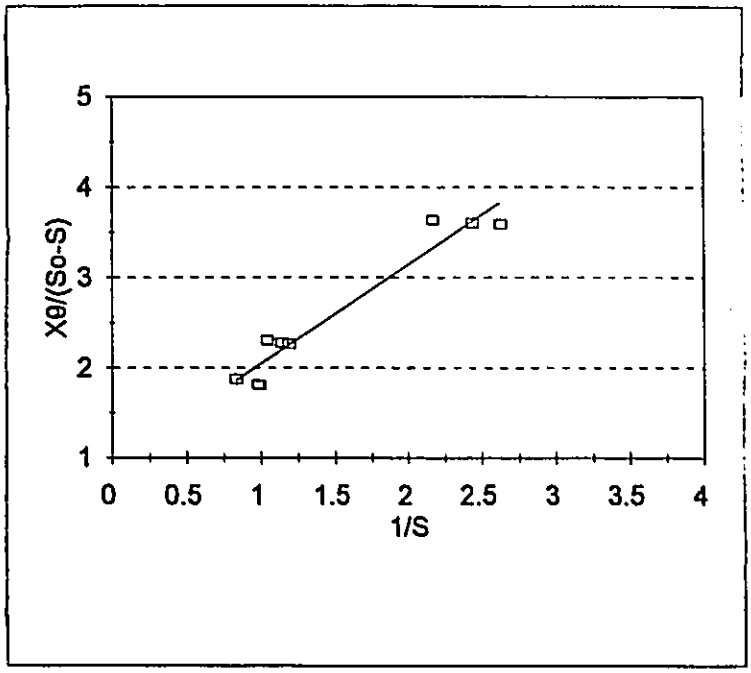
(a) Set up computation table (Table B1)

(b) Plot the term ($X\theta/S_0$-S) versus (1/S) as shown in Fig B1.

i. From Eq. B3, the y intercept equals (1/k)

Table B1. Computation table for determine k & K_s'

Run #	$S_{COD,0}$ (g/L)	S_{COD} (g/L)	$S_{COD,0}$-S_{COD} (g/L)	X (g/L)	θ (day)	$X\theta$ (g.day/L)	$\frac{X\theta}{S_{COD,o}-S_{COD}}$ (day)	$\frac{1}{S}$ (1/g)
C1R1	6.23	0.38	5.85	14	1.5	21	3.59	2.63
C1R2	6.23	0.41	5.82	14	1.5	21	3.61	2.44
C1R3	6.23	0.46	5.77	14	1.5	21	3.64	2.17
C2R1	7.02	0.84	6.18	14	1.0	14	2.27	1.19
C2R2	7.02	0.96	6.06	14	1.0	14	2.31	1.04
C2R3	7.02	0.88	6.14	14	1.0	14	2.28	1.14
C3R1	6.80	1.02	5.78	14	0.75	10.5	1.82	0.98
C3R2	6.80	1.20	5.60	14	0.75	10.5	1.88	0.83
C3R3	6.80	1.01	5.79	14	0.75	10.5	1.81	0.99



(XΘ/So-S) V.S. (1/S)

Regression Output:

Constant	0.948373
Std Err of Y Est	0.208358
R Squared	0.940694
No. of Observations	9
Degrees of Freedom	7
X Coefficient(s)	1.094306
Std Err of Coef.	0.103852

y=0.948373+1.094306x

$$\frac{1}{k} = 0.948373 \text{ day}, \quad k = 1.054 \text{ d}^{-1} = 0.044 \text{ h}^{-1}$$

ii. From Eq. B3, the slope of the curve in Fig. 5.2 equals K_s/k

$$\frac{K_s'}{k} = 1.094 \text{ g/L.d} = 26.26 \text{ g/L.h}$$

$$K_s' = 26.26 \text{ g/L.h} \times 0.044 \text{ h}^{-1} = 1.16 \text{ g/L}$$

The values determined were $k=0.044 \text{ h}^{-1}$ and $K_s'=1.16$ g/L respectively.