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THE FATE OF CHLORINATED PHENOLS

IN UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS

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submitted under the supervision of
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ABSTRACT

Chlorinated compounds are known for their recalcitrance and toxicity in the environment. Five trichlorophenols (2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6-trichlorophenol) and one dichlorophenol (3,5-dichlorophenol) were continuously treated in upflow anaerobic sludge blanket reactors for eight months. Their anaerobic biodegradation pathways and toxicity loading limits were determined. It was found that ortho positioned chlorine was readily removed from tri- and dichlorophenols. With acclimation, meta position chlorine was also removed from 3,4-, 3,5- dichlorophenols. Although there was no evidence of 3- and 4-monochlorophenol being degraded, the mass balance of the chlorophenols indicated that more than 50% of the 3-monochlorophenol formed from its parent compounds had disappeared, and the disappearance of 4-monochlorophenol was 20%. Acclimation and adequate alternate carbon sources reduced inhibition by chlorophenols. Inhibition was caused by : 2,4,5-trichlorophenol at a specific loading rate of 0.3 mg/g volatile suspended solids (hydraulic retention time of 3 days) and 0.9 mg/g volatile suspended solids (hydraulic retention time of 1 day); 2,3,4-trichlorophenol at a specific loading rate of 2.5 mg/g volatile suspended solids (hydraulic retention time of 1 day); 2,3,5-trichlorophenol at a specific loading rate of 1.25 mg/g volatile suspended solids (hydraulic retention time of 1 day); 3,5-dichlorophenol at a specific loading rate of 3.0 mg/g volatile suspend solids (hydraulic retention time of 1 day). No severe

inhibition occurred with 2,4,6-and 2,3,6-trichlorophenol at a specific loading rate of 2.5 mg/g volatile suspended solids.

Sorption played an important role in chlorophenol removal in upflow anaerobic sludge blanket systems. The sorption isotherms of all the mono-, di- tri-chlorophenols as well as pentachlorophenol were determined. Biosorption of chlorophenols by anaerobic granular biomass fitted the Freundlich model. Biosorption of chlorophenols increased with increasing halogenation of the chlorophenols. The conditions used in batch sorption tests were similar to those in upflow anaerobic sludge blanket systems. The biosorption constants determined were successfully used to estimate the amount of chlorophenols sorbed by biomass in upflow anaerobic sludge blanket systems. Biosorption accounted for as much as 6% of the total chlorophenol removal.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY

EQUATION CONSTANT AND VARIABLES

C = equilibrium concentration of adsorbate in solution, μg/L

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

K = Freundlich constant, L/g VSS

K_d = distribution constant

n = exponent constant in Freundlich equation

q = chlorophenol concentration in solid phase, μg/g VSS

ABBREVIATIONS

EPA = Environmental Protection Agency

AOX = adsorbable organic halide

BOD = biological oxygen demand

COD = chemical oxygen demand

DCP = dichlorophenol

HRT = hydraulic retention time [T]

MCP = monochlorophenol

PCP = pentachlorophenol

SLR = specific loading rate

SRT = solids retention time [T]

TCP = trichlorophenol

TeCP = tetrachlorophenol

TOX = total organic halide

TSS = total suspended solids [M/L^3]

UASB = upflow anaerobic sludge blanket reactor

VLR = volumetric loading rate

VSS = volatile suspends solids [M/L^3]

CHAPTER 1

INTRODUCTION

Very little is known of the fate of many hazardous organic pollutants discharged into biological wastewater treatment processes. Volatilization, sorption and biodegradation are all mechanisms leading to the removal of these compounds from the effluent waste streams. Chlorophenols are one class of hazardous wastes produced during chemical processing from wood preservation operations and pulp and paper production that must be treated in wastewater treatment plants. Recent literature reports have shown that anaerobic bacteria may be better suited to reductively dehalogenate highly-chlorinated phenolic compounds while aerobic biological systems have a tendency to be more suitable for biodegrading the less-halogenated phenolic compounds. Most of these studies have been long term, closed system experiments conducted with low biomass concentrations and relatively low concentrations of the target substance used as the only carbon source. The sole purpose of these experiments has been to demonstrate the ability of biological systems to degrade chlorinated phenolic compounds. The role of biosorption in removal of chlorinated aromatic compounds from the aqueous phase, in biodegradation, and in the ultimate fate of these compounds has not been addressed in these studies.

In the wastewater treatment industry, biosorption (sorption onto microbial biomass) of hydrophobic, non-volatile halogenated

aromatics has been shown to play a key role in the final fate of many of these materials. In some integrated biological/chemical treatment processes, sorption of chlorinated aromatic compounds by biologically inactive materials such as activated carbon is used as a method of pretreatment (Bouwer and McCarty, 1982; Ng et al., 1988). In conventional biological treatment systems where sufficient contact time is available for biodegradation to occur, biosorption results in the removal of toxic compounds from the aqueous waste stream and in the accumulation of hazardous pollutants in the microbial sludge. Ultimate disposal of the sludge may pose new environmental problems since the pollutants may be reversibly bound to the microbial biomass.

Anaerobic and aerobic bacteria can biodegrade chlorophenols. Many studies show that anaerobic batch cultures with monochlorophenol (MCP) and dichlorophenols (DCP) as sole their organic carbon sources can mineralize those compounds after long acclimation periods. Recently, a number of researchers have begun to look at the application of advanced anaerobic treatment systems for biodegradation of highly halogenated aromatics (Woods, 1985; Krumme et al., 1988; Ferguson et al., 1990; Fahmy et al., 1990). One such system is the upflow anaerobic sludge blanket (UASB) reactor which relies on the development of anaerobic granular sludge with high metabolic activity and good settleability to maintain a high biomass inventory in the reactor while operating at a short hydraulic retention time (HRT) (Kennedy et al., 1989).

The main objective of this research was to characterize the

biological and nonbiological mechanisms responsible for the removal of chlorophenols during anaerobic wastewater treatment. The specific objectives were:

1. To determine anaerobic biodegradation pathways and toxicity for chlorophenols as a function of the degree of chlorination and the chlorine position,

2. To evaluate the effect of chlorophenol concentration on chlorophenol biodegradation,

3. To evaluate the effect of HRT on chlorophenol removal,

4. To verify the effect of sludge acclimation in chlorophenol biodegradation,

5. To examine the role of sorption of chlorophenols in chlorophenol removal.

CHAPTER 2

LITERATURE REVIEW

2.1 Basic Anaerobic Digestion

Anaerobic processes by definition occur in the absence of oxygen. Two basic groups of bacteria are involved in the breakdown of complex organic compounds to methane and carbon dioxide. First, complex organic compounds are hydrolysed by chemoheterotrophic non-methanogens to free sugars, alcohols, volatile acids, hydrogen and carbon dioxide. Subsequently, the alcohols and volatile acids longer than two carbons are oxidized to acetate and hydrogen. In the last step, acetate and hydrogen are converted to methane by the methanogenic bacteria (Speece, 1983).

Methanogens are the most sensitive group to toxicity of all microorganisms in the overall consortium for anaerobic conversion of organic compounds to methane. In most anaerobic industry wastewater processes, the step of conversion of volatile acids to methane is the rate controlling step as indicated by accumulation of volatile acids.

2.2 Chlorinated Compounds in Pulp Paper Wastewater

Large varieties of chemical compounds are formed in the pulp bleaching process. The purpose of the pulping process is to remove lignin. Lignin, one of the major constituents of wood, is an aromatic polymer containing hydroxyl and phenolic functional groups. The use of chlorine as a pulp bleaching agent results in

depolymerization of the lignin as well as the introduction of chlorine to the aromatic ring. This process results in various acidic lignin components which contain chlorine substituents and which dissolve in the spent chlorination liquor (Kringstad, et al. 1984). More than 200 chlorinated compounds have been identified in effluent from pulp mills, ranging from simple organochlorine compounds (e.g., chlorinated phenolic compounds and hydrocarbons), to a class of chlorolignin with an average molecular weight of approximately 1000 g/mole (M) and higher (Carlberg et al., 1986; Kringstad et al., 1984; Salkinoja-Salonen et al., 1981). Approximately 30% of the organically bound chlorine is associated with compounds with molecular weights less than 1000 (Kringstad and Lindstrom, 1984). It has been estimated that about 5 kg of organically bound chlorine is formed per ton of softwood and 3 kg of these compounds are formed per ton of hardwood kraft pulp during conventional chlorine bleaching. It was reported that the wastewater from the chlorine bleaching of cellulose can contain up to 800 mg chlorinated compounds per L (Vogel and Winter, 1988; Winter et al., 1989).

Chlorinated phenolic compounds have been identified in pulp paper wastewaters by several groups of researchers, but results vary. Salkinoja-Salonen et al. (1981) examined both the chlorination and caustic extraction stage effluents from three mills. Concentrations of chlorinated phenolic compounds in pulp bleaching effluent differed significantly between mills. For example, concentrations of 2,4-DCP in caustic extraction effluent

ranged from non-detectable to 380 μg/L. In the chlorination effluent, 2,4-DCP concentrations ranged from 260 to 950 μg/L. Lindberg and Lund (1980) measured 2,4-DCP, 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP) up to 120 μg/L.

2.3 Mutagenicity of the Organochlorine Compounds

Organochlorine compounds are known for their recalcitrance in the environment. The environmental effects of the industry have been reviewed by Mckean (1980). Properties of chlorophenols, including toxicity, mutagenicity, solubility, vapour pressure, dissociation constant, and octanol-water partition coefficient, have been well reviewed by Suntio et al. (1989). It was found that chlorophenols and related compounds emitted in pulp mill effluents accumulate in sediments of rivers receiving bleaching effluent (Salkinoja-Salonen et al., 1979, 1984) and many of the organochlorine compounds also accumulate in the food chain (Renberg et al., 1980). Their environmental release has been regulated in Europe, Scandinavia and North America. Such regulations can be addressed either by reduction of byproduct generation or by enhanced treatment of the process wastewater. Modifications of the common bleaching sequences have been proposed to reduce the dosage of molecular chlorine. Yet, currently, there is no report of any full-scale implementation of a treatment system in the pulp and paper industry with the primary objective of chlorinated compounds separation and/or destruction.

Although the production of chlorinated aromatic compounds can be decreased through process control, there is a need for understanding the fate of these compounds in the environment and for the development of wastewater treatment processes to remove chlorophenols from pulp mill effluents.

2.4 Volatilization

Volatilization can be an important removal mechanism for compounds with high vapour pressures and low solubilities. 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 two film theory has been used to describe mass transfer between the liquid and gas phases. The Henry's law constant and vapour pressure are useful indicators of a compound's potential for volatilization. Smith et al. (1980) reported that highly volatile compounds typically have Henry's law constants greater than 4.6 x 10^{-3} (m^3 atm)/mole. McCarty (1980) found compounds with constants greater than 10^{-3} (m^3 atm/mole were successfully removed by air stripping (at 3000 m^3 air/m^3 water).

Phenolic and acidic compounds generally have lower vapour pressures and smaller Henry's law constants than neutral compounds (Table 2.1). The vapour pressure of chlorinated compounds is expected to decrease with increasing chlorine numbers. Volatilization from water is not expected to be an important

process, except under conditions of intense air-water contact. For instance, during aeration processes, volatilization contributed very little PCP removal during aerobic and anaerobic treatment processes (Moos et al., 1984; Guthrie et al. 1984). The tendency for volatilization under anaerobic conditions is even smaller compared to aerobic conditions, because the volume of gas produced in anaerobic treatment is less than the gas volume available for mass transfer in an activated sludge system. Woods (1985) found volatilization of chlorophenols only led to removal of less than 1% in a continuous UASB system.

Table 2.1

Vapour pressure and Henry's law constant

Compound	Papour pressure	Henry's law constant
	Pa	(Pa m^3)/mole[1]
2-MCP	316	1.425
3-MCP	41.4	na
4-MCP	20	0.06285
2,4-DCP	18	0.4044
2,6-DCP	12.9	na
2,4,5-TCP	2.48	0.1887
2,4,6-TCP	1.48	0.2424
2,3,4,6-TeCP	0.28	0.2337
PCP	0.231	0.2615

[1] 1 Pa = 1.013×10^{-5} atm
na: not available

2.5 Biosorption of Organic Compounds

2.5.1 Mechanism of Biosorption

The term biosorption is used to describe the process of accumulation of pollutants by microorganisms. The subject of biosorption of organic pollutants has been reviewed by several

researchers. It appears that firm, general conclusions cannot be reached for the mechanism of biosorption. Kenaga (1972) characterized the accumulation process as one involving a temporary equilibrium by adsorption followed by redistribution of the pollutants by absorption partitioning, storage and elimination. Baughman and Paris (1981) concluded that it was not clear whether sorbed compounds reside in or on the cell membranes. Lal and Saxena (1982) also concluded in their review of the subject that the mechanism of biosorption was not clearly understood. Sagiura et al. (1975) proposed a two step process for the biosorption of lindane and other isomers of hexachlorocyclohexane by five types of unidentified bacteria. The first step was rapid surface adsorption followed by passive diffusive penetration. In general, both adsorption and absorption might be involved in biosorption.

Sorption equilibrium can be described by a distribution coefficient, relating the concentration of the sorbate in the solid and liquid phases:

$$q = K_d * C$$

q = concentration of the sorbate in the solid phase, mg/g VSS

C = concentration of the sorbate in the liquid phase, mg/L

K_d = distribution coefficient, L/g

The actual mechanisms of biosorption are poorly understood but it is known that sorption is controlled by the characteristics of the sorbate, sorbent and solvent.

In the case of sorption when bacterial cells act as the sorbent, sorption would be expected to be affected by the mass, surface

area, species, chemical composition, lipid content and age of the bacterial cells. Attempts have been made to relate capacities of biosorbents to the hydrophobic lipid contents of cells. Grimes et al. (1975) investigated the sorption of five organic compounds onto thirteen species of bacteria and reported that the highest sorption of all five organics was consistently achieved by a species of bacteria known to be extremely high in lipid content.

Attempts have been made to relate biosorption to some chemical parameters of the sorbate, for example solubility. It has been demonstrated that there is an inverse relation between solubility of a compound in water and its adsorption onto biomass (Baughman et al., 1981; Bell and Tsezos, 1987; Paris et al., 1977; Steen and Karickhoff, 1981). This relationship is valid for a range of organic pollutants, including chlordane, dieldrin, lindane, PCBs, and PCP.

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

$$K_{ow} = C_O/C_W$$

where:

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

C_O = concentration of the solute in octanol

C_W = concentration of the solute in acidified water

It has been demonstrated that there is a trend: the higher the octanol/water coefficient, the greater the biosorption of the compound. This trend is valid for a wide range of compounds and

various biosorbents (Baughman and Paris, 1981; Bell and Tsezos, 1987; Lal and Saxena, 1982; Paris and Lewis, 1977; Steen and Karickhoff, 1981; Tsezos and Seto, 1986). The octanol/water coefficients of most chlorophenols are listed in Table 2.2.

Tsezos and Bell (1989) examined the sorption and desorption of lindane, diazinon, PCP and 2-chlorobiphenyl by living and dead cells of the fungus R. arrhizus and activated sludge. They found that at an equilibrium liquid phase concentration of 100 μg/L, the solid phase concentration of the pollutants in live activated sludge fit the following equation:

$$\log q = 1.51 \log K_{ow} - 3.73$$

where:

q = the solid phase concentration, μg/g activated sludge.

The sorption of organic compounds by organic sorbents has been related to organic carbon content of the sorbents. Schwarzenbach et al. (1981) developed the following correlation between K_{ow} and the distribution of nonpolar organic compounds (chloromethyl-, ethyl- and butyl-benzene) between water and the organic carbon content of soils:

$$\log K_{oc} = 0.72 \log K_{ow} - 2.51$$

where:

K_{oc} = organic carbon/water distribution coefficient for soil.

However, Bishop et al. (1990) found that most partition coefficients have no significant correlation with the Koc in the selected soil.

Table 2.2

Octanol/Water Partition Coefficients and

Acidity Constants of the Chlorophenols

Compound	log K_{ow}[1]	PKa[1]
phenol	1.46; 1.48	9.96
2-MCP	2.15; 2.19	8.11
3-MCP	2.47	8.80
4-MCP	2.39; 2.44	9.20
2,5-DCP	3.20	na
2,3-DCP	3.5, 3.21	7.7
2,4-DCP	3.23	7.89
2,6-DCP	3.19	6.79
3,4-DCP	3.41	8.59
3,5-DCP	3.68	8.19
2,3,4-TCP	na	
2,3,6-TCP	3.57	na
2,4,5-TCP	4.19	6.94
2,4,6-TCP	3.38; 3.79	5.99
3,4,5-TCP	4.41	7.73
2,3,4,6-TeCP	4.42	5.4
2,3,4,5-TeCP	4.87	6.35
PCP	5.01; 5.24	4.74

[1]Data from Leo et al., 1971; Schellenberg et al., 1984; Callahan et al., 1979, Xie et al., 1984

A key concern regarding the mechanisms of organic pollutant removal from water by biomass is whether the biomass is metabolically degrading the compounds or whether the process is purely sorption. In general, this concern has been examined by observing the difference in uptake by live and dead biomass. In almost all cases reported, the uptake by dead biomass was equal to or greater than that of live biomass. These results suggest that the adsorption process is due to physical adsorption, and not

active uptake or biodegradation (Baughman et al., 1981; Johnson et al., 1973; MacRae, 1985; Paris et al., 1977; Sagiura et al., 1976; Chacko et al., 1967).

2.5.2 Kinetics of Biosorption

The rates of biosorption and desorption have been intensively studied. Most studies have found that biosorption by microorganisms is a relatively rapid process. The time to reach equilibrium has been reported to be from a few seconds to several hours. Weber et al. (1987) reported that equilibrium was achieved in less than 15 minutes for biosorption of lindane by live activated sludge. Herbes (1977) found that equilibrium was achieved within one minute for sorption and desorption of anthracene by autoclaved yeast cells. Some studies show that uptake rate is faster by algae and bacteria than by fungi. Paris et al. (1977) observed that the rates of biosorption and desorption of toxaphene on bacteria, fungi, and algae were the same. Equilibrium in thirty minutes or less is commonly reported for bacteria biosorption (Baughman and Paris, 1981; Bell and Tsezos, 1987; Chacko and Lochwood, 1967; Grimes and Morrison, 1975; Johnson and Kennedy, 1973; Paris and Lewis, 1976; Paris and Lewis, 1977).

2.5.3 Characteristics of the Sorbate

Characteristics of the sorbate such as the presence of functional groups and their position, branching, polarity, hydrophobicity, dipole moment, molecular weight, size, and aqueous solubility affect sorption (Belfort, 1979). For a homologous series, the capacity for sorption increases with molecular weight

of a sorbate. Sorption decreases with solute polarity, increased solubility and branching (Belfort, 1979).

The effect of functional groups on the octanol/water partition coefficients of substituted benzene is shown in Table 2.3. Halogenation increases the compound's tendency to be removed from the liquid phase, as indicated by an increase in the K_{ow}. The octanol/water partition coefficient of PCP (Table 2.2) is more than three orders of magnitude greater than the partition coefficient for phenol (Table 2.2).

Table 2.3

Effect of Function Group Identity on Octanol/water Partition Coefficients of Substituted Benzene

Compound	Functional Group	log K_{ow}[1]
Benzene	-	2.13; 1.56; 2.15
Iodobenzene	-I	3.25
Bromobenzene	-Br	2.99
Cholorobenzene	-Cl	2.84
Toluene	$-CH_3$	2.69; 2.73; 2.11;
Flouorobenzene	-F	2.27
Anisole	$-OCH_3$	2.11; 2.04
Nitrobenzene	$-NO_2$	1.85; 1.88
Benzoic acid	-COOH	1.87
Benzaldehyde	-CHO	1.48
Phenol	-OH	1.46; 1.48
Benzyl alcohol	$-CH_2OH$	1.1

[1] Log of the octanol/water partition coefficient for the neutral species; individual octanol/water partition coefficients were determined by different researchers; values from a review by Leo et al., 1971.

2.5.4 Temperature and pH

The effect of temperature and pH on biosorption of organic compounds onto microbial biomass has not been studied in detail. Most studies have been conducted at constant temperature. Moreover, many studies have not precisely reported the experimental "pH" under which sorption occurred.

Bell and Tsezos (1987) studied the effect of varying temperature on the biosorption of lindane onto Rhizopus arrhizus, and onto activated sludge. The results showed that decreasing temperature enhanced biosorption. The explanation given was that the higher temperature increased the molecule's free energy, therefore increasing the molecule's activity and decreasing its sorption capacity.

Several studies have evaluated pH effects on sorption capacity, but no clear trends were identified. Grimes and Morrison (1979) reported that a pH of 7.0 was optimum for sorption of chlordane and lindane onto bacteria. Hiatt et al. (1960) found that sorption of PCP principally occurs on acidic soils with little or no sorption onto neutral soils. Geller (1979) concluded that pH does not affect sorption of lindane by a single species of bacteria. Amy et al. (1987), in their study of biosorption of organic halides in a kraft mill aerated lagoon, found that more effective sorption occurred at low pH values (pH 3.0 to 5.0), while an elevation in pH was found to increase desorption. Bishop et al. (1990) found that most K_{ow} have no significant correlation with pH in the selected soil.

2.5.5 Competitive Biosorption

The effects of competition by two or more sorbing organic chemicals on microbial biosorption have not been studied intensively. It might be expected that competition between two or more chemicals would result in reduced uptake of each chemical. Tsezos and Seto (1986) reported that individual uptakes of trichloroethane and tetrachloroethane by dead biomass were less when the two compounds were sorbed from a common solution than when each compound was sorbed from a single solute system. On the other hand, Schellenberg et al. (1984) concluded that there was no difference in the individual uptakes of chlorophenols by sediment whether the compounds were sorbed from single solute solutions or from combined solutions. Selvakumar and Hsieh (1988) reported that the phenol uptake capacity by activated sludge in single solute system was 34% lower than in bisolute systems; similarly, the uptake capacity of chlorophenol in single system was 9% lower than in bisolute systems.

2.5.6 Desorption

The possible desorption of organic pollutants from microbial biomass is of importance. Desorption studies (Johnson et al., 1973) have shown that the amount of sorbate that can be desorbed is a function of the organic compound sorbed and the type of microbial biomass utilized as a sorbent. Sorption/desorption was found to be a completely reversible process for lindane, diazinon and 2-chlorobiphenyl sorbed onto either a single species, R. arrhizus, or activated sludge. Sorption/desorption of malathion was 100%

reversible at 5°C, but at 20°C no desorption occurred (Bell and Tsezos, 1987). On the other hand, Isaacson et al. (1984) investigated desorption of phenol, 2-MCP and 2,4-DCP from lake sediment and found that the desorption was not reversible. The time required for the latter three compounds to reach desorption equilibrium was up to three times or more than that required for sorption. Additionally, a significant fraction of those sorbates was irreversibly held by the sediment fraction. The fraction irreversibly sorbed was lower for phenol than for the chlorophenols. Depending on the characteristics of the sediment, 13 to 17 percent of the phenol was irreversibly sorbed, 60 to 90 percent of 2-MCP and 35 to 50 percent of the 2,4-DCP were irreversibly sorbed. Lederman and Rhee (1982) found desorption rates to be lower than sorption rates and also they found incomplete reversibility of sorption. Bishop et al. (1990) also found irreversible sorption in their study of phenol. Their results show that significant desorption occurred when the sorbent was very low in organic matter and significant hysteresis occurred when the sorbent contained more sorbing sites due to higher organic content or fineness of the sediment grain.

2.5.7 Sorption of Chlorophenols by anaerobic sludge

Sorption isotherms of nine chlorophenols were determined by batch sorption tests (Woods, 1985). The biomass was taken from a UASB reactor and pH was 7.1 but test temperature was not reported. The determined sorption constants are shown in Table 2.4.

Table 2.4

Sorption Constants for Chlorophenols[1]

Compound	K	n
PCP	0.85	1.1
2,4,6-TCP	0.16	1.1
2,3,4,5-TeCP	0.98	0.94
3,4,5-TCP	1.16	0.9
2,3-DCP	0.27	1.2
2,4-DCP	0.34	1.2
3,5-DCP	0.37	0.96
3,4-DCP	0.34	1.3

[1]Data after Woods (1985), at pH 7.1

2.6 Biological Treatment of Chlorophenols

2.6.1 Anaerobic Treatment

Anaerobic microbial communities have been shown to degrade a variety of halogenated hydrocarbons, including several chlorinated aromatic compounds. A reaction involved in the anaerobic degradation is replacement of the aromatic chlorine(s) with hydrogen reductive dechlorination (Suflita et al., 1982, Shelton and Tiedje, 1984). This useful reaction makes anaerobic biodegradation advantageous. Aerobic metabolism of highly chlorinated aromatic chemicals is often restricted, since two adjacent ring positions must be free for hydroxylation; Cl is then removed after the ring being opened (Tiedje et al., 1987). In many cases less chlorinated compounds are more biodegradable and less toxic (Tiedje et al., 1987). Recent literature cited that anaerobic bacteria may be better suited to reductively dehalogenate highly-

chlorinated phenolic compounds while aerobic biological systems have a tendency to be more suitable for biodegrading the less-halogenated phenolic compounds (Sahm et al., 1986; Reineke and Knackmuss, 1988; Neison, 1990). The attempts to isolate anaerobic bacteria with the ability to degrade chlorinated phenols have not been very successful, except strain DCB1 which was able to dechlorinate meta position chlorine (Mohn, 1991).

An anaerobic bioprocess is usually more economical than an aerobic process. If a pollutant is bound to soil or sediment, the most cost-effective treatment may be to degrade it in place. If this can be stimulated by making the site anaerobic, e.g., by flooding, the cost would be minimal (Tiedje et al., 1987; Mikesell, 1988). If a waste processing unit is to be constructed, anaerobic systems are often less expensive than aerobic bioprocesses because the cost for pumping oxygen into the system is avoided. Operation of anaerobic systems may be easier and more reliable, since less sludge accumulates, and the system is often more stable to influent and environmental perturbations. Since 1981 more than six full scale anaerobic units (UASB) have been installed for treatment of pulp and paper mill effluent. It was reported that 60 to 75% chemical oxygen demand (COD) removal efficiency is achieved, but the removal of total organic halide (TOX) was not monitored (Habets, 1987).

Hakulinen et al. (1982) and Salkinoja-Salonen et al. (1984) reported that chlorophenols (including PCP) were degraded in an anaerobic fluidized-bed reactor during treatment of paper and pulp

mill effluent. Chlorophenol removal was more rapid in the anaerobic reactor than in either an aerated lagoon or an activated sludge plant.

2.6.2 Inhibition of Anaerobic Bacteria by chlorophenols

Inhibition by chlorophenols in batch and continuous reactors has been observed by many researchers. Two types of inhibition in the anaerobic degradation of chlorinated compounds were often reported. In one case the high loading rate of chlorinated compounds inhibits only the activity of the anaerobes degrading the chlorinated compounds or the activity of methanogens. In another case the high loading rate of chlorinated compounds inhibits the activity not only of those anaerobes degrading the chlorinated compounds, but also of methanogens and acidogens. The second type of inhibition causes total failure of reactors. In general, inhibition increases with chlorine content of compounds and decreases with sludge acclimation of the compounds (Guthrie et al., 1984, Horowitz et al., 1982, Hrudey et al., 1987). Guthrie et al. (1984) reported that methanogenesis was inhibited by PCP at a concentration of 200 μg/L in fresh sludge and 600 μg/L in acclimated sludge. Wang (1991) reported the inhibition concentrations to acetate methanogenic bacteria for MCPs was at 100 to 400 mg/L. Hrudey et al. (1987) did batch and semi-continuous tests on anaerobic sewage sludge to investigate the inhibition by MCPs. Concentrations greater than 30 mg/L of 3-MCP or 4-MCP inhibited ultimate phenol degradation, whereas concentration of 2-MCP less than 97 mg/L was not inhibitory to phenol degradation. Duff and Kennedy (1985) found 50% inhibition

of methanogens at a PCP concentration of 5 mg/L. Acclimated sludge could sustain 12-15 mg/L of PCP without methanogen inhibition.

2.6.3 Acclimation

An acclimation period can stimulate dechlorination. Boyd and Shelton (1984) examined reductive dechlorination of mono-, di-, tri- and PCP in anaerobic sewage sludge. All three MCPs were dechlorinated in nondiluted, fresh sludge. With sludge acclimated to the chlorophenols, the lag time preceding dechlorination decreased, rates of dechlorination increased, distinct patterns of acclimation were observed and the substrate range was extended to include compounds that were not degraded in fresh sludge. For example, when 4-MCP was incubated with digested sludge for 8 weeks, no degradation occurred; however after an additional 8 weeks, 4-MCP was almost completely degraded (Boyd and Shelton, 1984). Another example is that 3,4-DCP and 3,5-DCP were persistent in fresh sludge for 6 weeks, but they were degraded within 14 days in sludge previously acclimated to 3-MCP (Boyd and Shelton, 1984). Acclimation periods for anaerobic reductive dehalogenation vary from several days to 6 months or more. It was suggested that the best explanation for acclimation periods was induction (Linkfield et al. 1989). Woods (1985) investigated dechlorination of chlorophenols in UASB reactors and reported that after 44 days acclimation, 3,5-DCP average removal efficiency was increased to 61%, while within 44 days no removal of 3,5-DCP occurred.

Sludge acclimation may change dechlorination patterns. 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 the degradation of 2-MCP, degraded 2- and 4-MCP at equal rates whereas 3-MCP was not degraded. Sludge acclimated to 3-MCP also degraded 4-MCP but not 2-MCP (Mikesell, 1988).

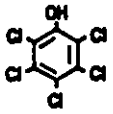
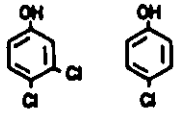
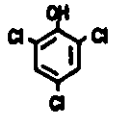
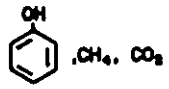
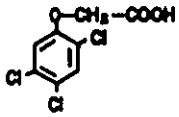
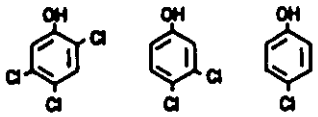
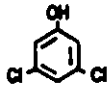
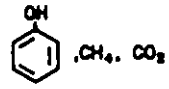
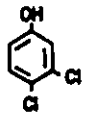
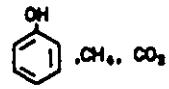
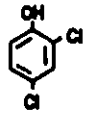
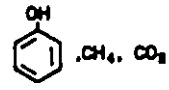
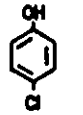
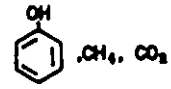
2.6.4 Anaerobic Biodegradation Pathways of Chlorophenols

Anaerobic biodegradation pathways of chlorophenols have been investigated by many researchers and most work was done by batch test. PCP is the most widely studied chlorophenol congener. Ide et al. (1972) suggested reductive dechlorination as a mechanism of anaerobic degradation of PCP. The following degradation products were detected in paddy soil: 2,3,4,5-, 2,3,5,6-, and 2,3,4,6-TeCP, 2,4,5- and 2,3,5-TCP, 3,4-and 3,5-DCP, and 3-MCP (Ide et al.,1972). In general it appeared that the ortho and para positions of PCP were dechlorinated more easily than those in the meta position.

The fate of PCP and other chlorophenols in anaerobic sewage sludge has also been studied by Boyd et al. (1983) and Mikesell and Boyd (1985) . The results are summarized in Table 2.5. In fresh (unacclimated) sludge from primary anaerobic digesters, the ortho positions of PCP were rapidly dechlorinated giving 3,4,5-TCP, which was subsequently converted to 3,5-DCP (Mikesell and Boyd, 1985). MCP was not observed. 2,4,6-TCP followed this pattern of ortho dechlorination yielding 4-MCP as the final product, and mineralization of 4-MCP was also reported. Extensive PCP biodegradation in anaerobic sludge was also observed by Guthrie et al. (1984).

Table 2.4

Summary of anaerobic biodegradation pathways of chlorinated compounds.[1]

Compound	source of activity	Products observed
	fresh sludge	
	fresh sludge	
	fresh sludge	
	fresh sludge	
	sludge enriched on 3-chlorophenol*	
	sludge enriched on 3-chlorophenol*	
	sludge enriched on 2-chlorophenol*	
	sludge enriched on 2-chlorophenol*	

[1] Data after Boyd and Shelton, 1984; Boyd et al., 1983; Mikesell, et al. 1985.

Reductive dechlorination reactions have also been observed for the mono- and DCPs with fresh and acclimated digester sludge. The initial MCP dechlorination was observed for 2-MCP (Boyd et al., 1983). Complete conversion of 30 ppm 2-MCP to phenol required approximately 3 weeks in fresh 10% anaerobic sludge. All three MCPs inhibited methane production. In fresh sludge, the relative rates of disappearance were: 2-MCP > 3-MCP > 4-MCP (Boyd et al., 1983). For DCPs in unacclimated sludge, reductive dechlorination of the chlorine group to phenolic OH was observed for each DCP isomer with such a Cl substituent. 3,4-DCP and 3,5-DCP were persistent during the 6 week incubation (Boyd and Shlton, 1984).

2.6.5 Anaerobic Biodegradation of Chlorophenols in Continuous Flow Reactors

Anaerobic upflow bioreactor systems have been used in continuous treatment of chlorinated phenols. Unfortunately, only a few studies have been done. Hakulinen and Salkinoja-Salonen (1982) developed a two-stage reactor system consisting of an anaerobic upflow bioreactor and an aerobic trickling filter for the treatment of chlorophenolic waste from paper pulp bleaching (see Section 2.6.8). Woods (1985) explored the fate of chlorophenols in an anaerobic upflow reactor which received readily utilizable carbon sources in addition to chlorophenols; Krumme and Boyd (1988) examined the reductive dechlorination of chlorophenols in anaerobic upflow bioreactors using chlorophenols as the sole carbon and energy source.

The degradation studies of chlorophenols and chloromethoxybenzene were performed in a 6.6 L anaerobic upflow sludge blanket reactor with recycle which fed with synthetic waste water (Woods, 1985). The body of the reactor was constructed of Kimax beaded process pipe with Teflon flanged fittings to minimize adsorption of chlorophenols to the reactor vessel. The reactor was housed in a constant temperature room held at 35°C. The influent flow rate was 13.3 L/day and the recycle ratio was 5.3:1. The total biomass in the reactor varied between 5 and 20 g of volatile suspended solids (VSS) throughout the study. The microorganisms (original source of inoculum from municipal sludge digester), were fed diluted caustic extraction effluent and sulfite evaporator condensate from the pulp and paper industry with additional carbon sources, inorganic nutrients and vitamins for approximately 2 years. The synthetic waste water contained three primary carbon sources: acetate, methanol and glucose. Neutralization, inorganic nutrients and vitamins were also provided. The overall COD of the synthetic waste water was 4.3 g/L, resulting in an organic loading rate to the reactor of 8.7 g COD /L day and the specific organic loading rates varied between 3 and 11 g COD/ (g VSS day).

Chlorophenols: 2,3-, 2,4-, 2,6-, 3,5- and 3,4-DCP, 2,4,6-, 3,4,5-TCP, 2,3,4,5-TeCP and PCP and chloromethoxybenzene were added by group to the reactor influent. The total experiment period lasted eight months but the test period of the chlorinated compounds varied from 14 to 21 days. The reactor performance was monitored by measuring the biogas production and analyzing acetate,

methanol, glucose, and chlorinated benzene derivatives in the reactor influent and effluent.

Biodegradation pathways for chlorophenols were determined (shown in Fig. 2.1). Bacteria readily removed ortho chlorines from 2,6-, 2,3- and 2,4-DCP, 2,4,6-TCP, 2,3,4,5-TeCP, and PCP, with acclimation, meta chlorines were also removed from 3,5-, 3,4-DCP, and 3,4,5-TCP. However, throughout 7 months of continuous treatment of synthetic waste water, there was no evidence for dehalogenation of MCPs or removal of chlorines from the para position to the hydroxyl group. The removal efficiency for each chlorophenol increased with increasing chlorination. During 63 days of experiment, total removal of 95% was achieved for PCP, 96% for 2,4,6-TCP and 61% for 3,5-DCP.

Nonbiological mechanisms (biosorption and volatilization) leading to chlorophenols removal in a continuous flow reactor were evaluated. Sorption isotherms of chlorophenols determined by batch sorption test (see Section 2.5.7) were applied in mass balance calculation in continuous flow reactor. Based on the mass balance, biosorption of chlorophneols by sludge contributed 5 to 16% of chlorophenols removal throughout the experiment, while volatilization only contributed 1% of chlorophenols removal.

Krumme and Boyd (1988) studied the reductive dechlorination of chlorophenols in anaerobic upflow bioreactors with chlorinated phenols as the sole carbon source. The bioreactor, with a volume of 1 L, was inoculated with the biomass which was acclimated to

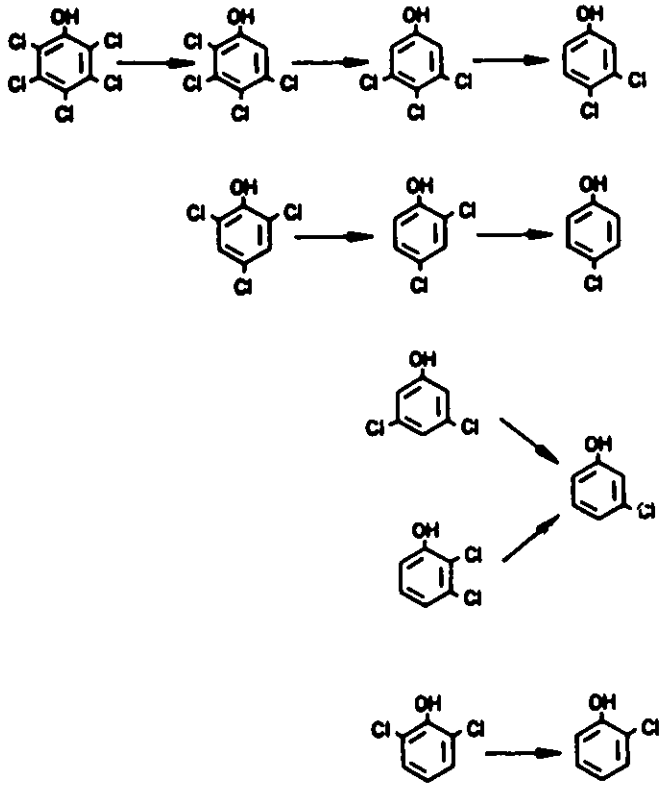


Figure 2.1 Anaerobic Biodegradation Pathways of Chlorophenols

(After Woods, 1985)

chlorophenols for approximately 2 years. The concentration of biomass in the reactor was not reported. Three MCPs, 3,4,5-TCP, 2,4,6-TCP and PCP were tested in three separate bioreactors which were operated over 190 to 400 days. HRTs of 2 and 4 days were used. The temperature was not mentioned. The performance of the reactor was monitored by measuring biogas production, biogas composition and concentration of chlorophenols in the effluent of the reactor. The ability to degrade all three MCPs and 3,4,5-TCP was demonstrated. Approximately 40% of added MCPs was mineralized to CH_4 and CO_2 and a substrate conversion (removal) efficiency of greater than 90% was achieved with substrate loading rates of up to 20 mg/L.day. A 72% removal of 3,4,5-TCP was reported by measuring its concentration in the effluent, but the mineralization of 3,4,5-TCP was not reported. The removal efficiencies of 2,4,6-TCP and PCP were poor (only 23 and 35% respectively). The removal efficiency of chlorophenols was contributed by biodegradation and sludge biosorption, but the role of biosorption of chlorophenols by biomass was not investigated in this study. The chlorophenol biodegradation pathways were not determined. Although utilizing these unique dechlorinating activities for large scale treatment may eventually be useful in remediating chlorophenol contaminated water such as groundwater, supplying other readily utilizable carbon sources to the waste treatment system are much more popular in waste treatment cases.

2.6.6 Alternate Carbon Sources and Nutrients

Nutrient requirements for methanogens have been reviewed by Speece (1983). Rittmann and McCarty (1980a; 1980b) have examined the effect of substrate concentration on the removal of organic compounds by biofilms. But the effect of nutrients on reductive dehalogenation have not been examined extensively. Fathepure et al. (1987) demonstrated that adding nutrients can stimulate dehalogenation of 4-chlororesorcinol. In sewage sludge, yeast extract, rumen fluid, glucose, sludge supernatant and resorcinol, stimulated dehalogenation of 4-chlororesorcinol.

2.6.7 Aerobic Treatment of Chlorophenols

Aerobic treatment of chlorinated compounds has been studied intensively in pure cultures. Pure aerobic bacterial cultures able to metabolize chlorinated phenols have been isolated by a number of research groups. Among these are several strains of a Flavobacterium sp. and a pseudomonad (Watanabe, 1977), and a coryneform bacterium strain KC-3 (Chu and Kirsch, 1973). It was reported that strain KC-3 was able to utilize PCP as its sole source of carbon and energy, releasing 75% of the PCP carbon as CO_2 (Chu and Kirsch, 1973). Strain KC-3 was able to grow on 2,3,4,6-TeCP and 2,4,6-TCP. The Pseudomonas species was isolated from rice paddy soil (Watanabe, 1973; Suzuki, 1977). The mechanisms involved in aerobic degradation of aromatic compounds are methylation and hydroxylation. Conventional aerated lagoons and activated sludge systems have been employed in treatment of pulp and paper mill wastewater in the United States and Scandinavia. The biological oxygen demand (BOD), COD and suspended solids in the

wastewater are efficiently removed; and chlorinated phenolic compounds of the effluent can be reduced by approximately 50% (Bryant et al., 1988,; Gergov et al.,1988). However, chlorinated organic compounds are poorly degraded by these methods, and the major removal mechanism of the chlorinated 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 (Bryant and Amy, 1989; Leuenberger et al., 1985).

The biomass concentration and its production in the system are important factors affecting the removal of organic chlorinated compounds. Haggblom and Salkinoja-Salonen (1990) investigated the enumeration of aerobic PCP degraders in three bleaching effluent treatment plants. The results showed that the PCP degrading microbes only comprised 0.2% of the total heterotrophic bacterial count in an activated sludge plant, and 0.1% in aerated lagoon. The proportion of PCP degraders in the heterotrophic population was 3% in an aerobic trickling filter. The higher proportion of PCP degraders may be because the solid support in the trickling filter prevented those microbes from washing out from the treatment plant.

2.6.8 Sequential Anaerobic and Aerobic

Salkinoja-Salonen et al. (1979) first developed sequential anaerobic and aerobic biological treatment of bleaching wastewater in Finland in the late 70's. The so-called Enso-Fenox process was operated at three mills at a pilot demonstration scale. The anaerobic first stage was not methanogenic, although it was

stage was not methanogenic, although it was effective in removal of chlorinated phenols. But it was not reported whether the effective removal of chlorinated phenols was caused by anaerobic degradation or by sludge biosorption. Little BOD was reduced in the first stage, apparently because the wastewaters were toxic to biomass activity. Treatment of pulp bleaching effluent by the Enso-Fenox process was further studied by Haggblom and Salkinoja-Salonen (1990). Removal of COD was 30 to 65% during the 10 week experimental period, and BOD_7 removal was 60 to 80%. COD and BOD were mainly removed in the aerobic trickling filter phase. Removal of chlorophenols by anaerobic and aerobic treatment was 70 to 80%. Polychlorinated phenols were also removed, mainly in the anaerobic reactor, while dichlorinated phenols were removed in the aerobic reactor. It was reported that anaerobic pretreatment was necessary for the aerobic tricking filter to function properly. No reduction of COD or chlorophenols was observed in the aerobic trickling filter reactor during an operation failure of the anaerobic reactor.

2.6.9 Physical/Chemical Method

Physical/chemical methods (e.g., ultrafiltration, precipitation, oxidation with ozone, ion exchange and active carbon adsorption) have been applied for treatment of pulp mill wastewater. Results showed that ultrafiltration removed 80 to 90% of the colour of wastewater and 50 to 60% BOD_5 but very little chlorinated phenolic compounds. Ion exchange removed the colour efficiently and chlorophenolic compounds. A full scale ion exchange unit is in

operation in Finland (Hakulinen and Salkinoja-Salonen,1982). Ozone, which has a high oxidation potential and can readily oxidize organic compounds has also been tested. Sierka and Bryant (1987) found that removal efficiency of chlorinated compounds was higher by ozone oxidation than by biological treatment, and the wastewater treatment efficiency of the biological treatment was enhanced by ozone pretreatment. Activated carbon used in pulp mill effluent treatment is not promising because the macro-pores are easily blocked by high molecular weight species and its hydraulic loading would be reduced. Although some physical/chemical methods are capable of chlorinated compound removal, high capital and operating costs combined with technical problems associated with physical/chemical methods might limit their application.

2.7 Fate of Organic Pollutants in Wastewater Treatment Plants

Many hazardous organic pollutants have been identified in the influent, effluent, and sludge of publicly owned wastewater treatment plants (Clevenger et al., 1983). In Canada, the Environmental Protection Service identified 57 toxic organic compounds in sludge from 13 sewage treatment plants (Bridle, 1982). In the United States the Environmental Protection Agency (EPA) found numerous hazardous organic pollutants in sludge from 40 treatment facilities (Burns and Roe Industrial Services Corp., 1982). Clevenger et al. (1983) found PCB's, lindane, and other toxic organic in the sludge of 74 municipal treatment plants in Missouri. Maximum concentrations found in the plant influent ranged

from less than one μg/L to several thousand μg/L. Concentration of pollutants associated with sludge have been found in the range of a thousand μg/g of dry solids to less than one μg/g of dry solids. It is apparent from these studies that many hazardous organic compounds were removed from wastewater in biological treatment systems via sorption by sludge. Many compounds appear to remain associated with the sludge through the sludge digestion process (Bridle, 1982).

The United States EPA conducted a pilot plant study to determined the fate of 22 toxic organic compounds discharged into an activated sludge pilot plant (Petrasek et al., 1983). Many compounds were found to accumulate in the primary and secondary sludges. In general, higher concentrations were found in the primary sludge than in the secondary sludge. The fraction of the compounds removed by the primary sludge was reasonably well correlated with the octanol/water partition coefficient of the compounds, however the fraction removed by the secondary sludge was not well correlated. Weber et al. (1987) reported that 14% of PCP was removed by biosorption in the primary sludge, 5% in the activated sludge, and 81% was found in the final effluent. Blackburn et al. (1984), in a lab scale activated sludge process study, reported that approximately 7% of the influent PCP was sorbed on the waste sludge. Amy et al. (1987) reported that one-third to one-half of both TOX and low molecular weight (less than 1000) TOX were removed in a kraft mill aerated lagoon. These studies confirm that sorption of organic pollutants by

microorganisms and other solids is an important removal mechanism in biological wastewater treatment plants.

Volatilization, biosorption and biodegradation are major mechanisms leading to the removal of organic compounds from the effluent waste streams. This study was in an effort to determine the role of biosorption and biodegradation in anaerobic treatment of chlorophenol containing wastewater in a UASB reactor. Six UASB reactors were employed to treat six chlorophenols individually over eight months. The objectives of this study are presented in Chapter 1. In the following chapters, the experimental program and experimental results will be presented.

CHAPTER 3

EXPERIMENTAL PROGRAM AND PROCEDURE

3.1 Introduction

The experimental methods used to examine the fate of chlorinated benzene derivatives during anaerobic wastewater treatment are described in this chapter. The majority of the work was performed using laboratory scale reactors (UASB) continuously fed a synthetic wastewater containing chlorophenols, nutrients, and an alternate carbon source. The reactors were operated in order to determine pathways of anaerobic biodegradation for this class of compounds, and to assess the effect of sludge acclimation and HRT on their removal. The loading concentrations of chlorophenols were increased in steps (from 1.25 to 50 mg/L) in order to determine the biodegradation pathways as well as the limits of the UASB reactor for treatment of the chlorophenols. Batch sorption experiments were performed to determine the biosorption isotherms for most TCPs, DCPs, MCPs, and PCP as well as to determine desorption isotherms of some of the chlorophenols. The biosorption isotherms of chlorinated phenols determined in the batch test were used to complete a chlorophenol mass balance in the UASB systems.

3.2 Chemicals

Organic chemicals used in sorption and continuous flow reactor experiments were purchased from Aldrich Chemicals and were of +99% purity. Water solutions were made from distilled/deionized water

prepared in the laboratory. All chlorinated compounds were made up in 0.01 M NaOH adjusted to pH 7.5. PCP was made up with the sodium salt. Organic solvents used for extraction or analysis were HPLC grade.

3.3 Biomass

Anaerobic granular biomass used in the sorption experiments was taken from a UASB reactor operated at 35°C and used only for the treatment of sucrose and acetic acid based wastewater. This combination of carbon sources ensures the development of a complete acidogenic and methanogenic microbial consortia in the granules. The anaerobic granules were up to 3 mm in diameter and were not acclimated to chlorinated phenols. This would minimize the possibility of chlorophenol biodegradation.

The sludge bed region of an average UASB reactor usually makes up 50-60 % of the reactor empty bed volume, as was the case in this study. Granular biomass removed from the reactor sludge bed contained 50 g total suspended solids/L (TSS) composed of 40 g VSS/L or about 2 times the reactor biomass concentration. In order to simulate UASB conditions, the anaerobic granular biomass was diluted 1 to 1 with dilution media (reduced 0.01 M NaOH) and then adjusted to pH 7.5. Under anaerobic conditions 40 mL of diluted granular sludge was transferred into 60 serum vials and sealed with a butyl rubber stopper. Biomass concentrations in the serum bottles were 19-20 g VSS/L. The whole procedure was carried out at 35°C.

3.4 Batch Sorption Test

Sorption experiments were conducted by contacting the anaerobic sludge (sorbent) in the serum bottles with various chlorinated phenolic compounds (sorbate) at 5 different sorbate concentrations between 5-60 mg/L. Background samples containing biomass but no chlorinated phenols and controls containing dilution media but no biomass were also run. All serum bottles were agitated on an orbital shaker at 80 rpm for 24 hours at 35°C. One mL samples were removed from the serum bottles at 0.25, 2 and 20-24 hours for high pressure liquid chromatographic (HPLC) analysis. Control samples were taken and handled in an identical manner to regular samples. The sample schedule discussed above, was used to evaluate extraneous sorption equilibrium and the possibility of chlorophenol biodegradability. The concentration of chlorophenols in the control bottles determined by HPLC analysis was assumed to be the initial concentration in serum bottles containing biomass. Uptake by the biomass was determined by a mass balance.

3.5 Batch Desorption Test

Desorption experiments were conducted by contacting the biomass with the sorption solution in a manner identical to the sorption test (including sampling) presented above. After 24 hours serum vial mixed liquor was centrifuged at 14,800 g for 20 minutes. The supernatant was removed and the pellet was centrifuged again, then anaerobically transferred into a new serum vial with the same volume of dilution media as removed and then sealed. The serum

bottle was placed back on the shaking platform, maintained at 35°C and samples were taken after 24 hours. Mass balances were made to determine desorption by the biomass.

3.6 Experimental Program for Continuous Flow Reactor Studies

Continuous flow studies were conducted using six UASB reactors containing anaerobic granular sludge with recycle to enhance mixing. Each UASB was fed with the same synthetic wastewater but a different TCP congener. Operating volume of the reactor was 6.1 L. A diagram of the reactor is presented in Figure 3.1. The lower half of the reactor was occupied by anaerobic granular sludge. On the top of the reactor there was a screen made of plastic packing material to prevent the sludge from being washed out through the effluent port. This packing material was made of polyethylene plastic and had a diameter and length of 1.5 cm. The reactors were located in a temperature controlled room maintained at 35 ± 1°C.

A variable speed Masterflex pump (Cole-Palmer model 7555-10,1-600 rpm) provided recycle at recycle to feed ratio of 5:1. Synthetic wastewater feed was pumped to the reactors at the desired rate by a Harvard peristaltic pump (model 5006-033). Feed was maintained at 4°C until used. Chlorophenol solutions were infused into the recycle line by the Harvard Apparatus syringe infusion pumps.

Sampling ports were located along the side of the reactor and in

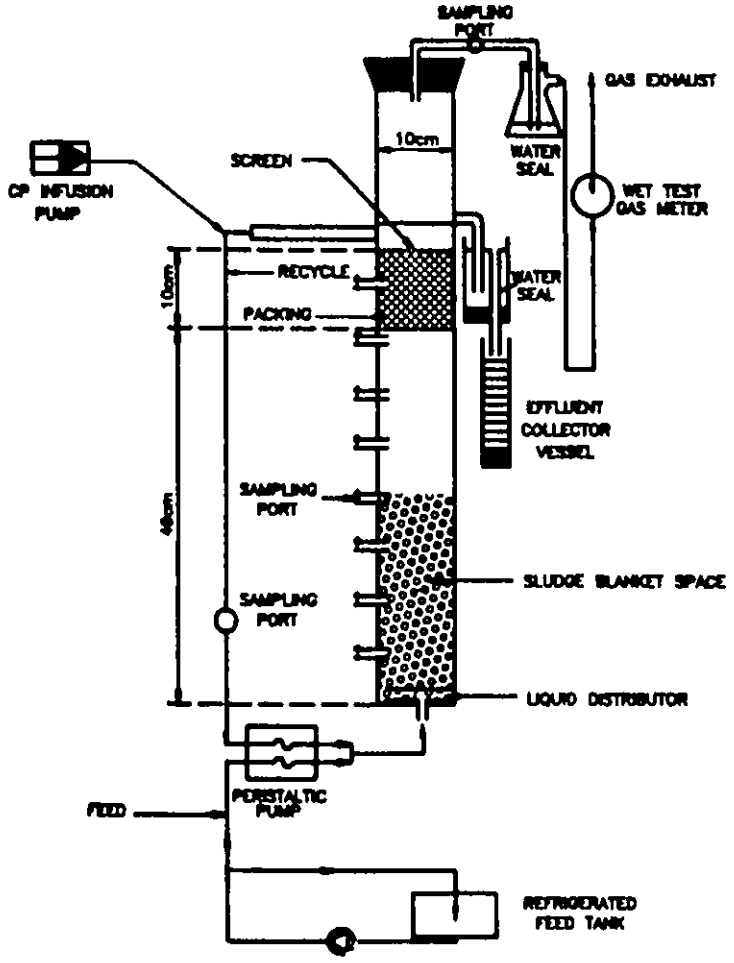


Figure 3.1 Diagram of a UASB Reactor

the recycle line. The recycle line port was a glass tee fitted with a screw cap and butyl rubber septum.

Reactors were inoculated with 3 L of 40 g VSS/L anaerobic granular sludge that had never been exposed to chlorophenols. Reactor biomass was maintained at 20 to 25 g VSS/L during the course of the experiment by removing the extra biomass volume. The experiment period was around 280 days and two HRTs were used: 1 day and 3 day HRT. The recycle feed ratio was maintained at 5:1. In each stage, the chlorophenol loading was increased gradually by increasing the concentration, but the other components (Appendix A) in the synthetic wastewater were not changed. The synthetic wastewater had equal amounts of sucrose and acetic acid as the carbon sources and had a COD of 5000 mg/L.

Gas production and composition, effluent volume, volatile acid and chlorophenol concentrations in the reactor effluent were measured daily. The volume of gas exiting the reactor was measured by a wet tip gas meter and the biogas was emitted into a steel gas collecting pipe. Effluent was collected in a food grade plastic bucket. Soluble COD was analyzed twice a week. pH and alkalinity analysis were conducted once every two weeks. The mass of chlorinated organic compounds associated with the reactor solids was determined by performing extraction of the reactor solids. Throughout the experimental period, mass balances were performed for COD as well as chlorinated phenols.

3.7 Analytical Methods

3.7.1 Analysis of Chlorinated Phenols

Sorption samples and samples (1 mL) for each reactor which were taken daily from the effluent port of the reactor were prepared for HPLC analysis. Samples were centrifuged in a model 235A Fisher Micro-centrifuge for 5 minutes. The supernatant was passed through a Millipore 0.22 µm Millex-GV_{13} filter and stored at 4°C in glass vials with teflon lined caps prior to HPLC analysis. The Miller-GV13 filter was selected over a number of other filters since it minimized the amount of chlorinated phenol retained by the filter.

Chlorophenol concentration in samples was measured with a Hewlett Packard 1090 HPLC equipped with a diode array detector and HP-300 work station for integration of peak areas. Detection wavelengths were 280 and 324 nm which correspond to absorbance maximum for these compounds. 25 µL of sample was injected on to a Shandon Hypersil ODS C18 (2.1mm id x 10cm x 5µm) column maintained at 40°C. Flow rate of the mobile phase was 0.3 mL/min and a gradient was used (Table 3.1).

Table 3.1 HPLC Conditions for Chlorophenol Analysis

Time	Solvent A	Solvent B
	HPLC grade methanol	0.05 M sodium acetate
	pH 4.7	pH 4.7
min	%	%
0	40	60
15	60	40
20	80	20
21	40	60

3.7.2 Analysis Chlorophenols Associated with Biomass

Chlorophenols sorbed to granular biomass in actively dechlorinating UASB reactors were determined by gas chromatographic - mass selective detector (GC-MSD). Ten mL granular biomass samples withdrawn from reactors were centrifuged at 14,800 g for 10 minutes. The supernatant was decanted, the granules washed and suspended in tap water, then recentrifuged. The pellet was then redissolved in 5 mL of dilution media. Depending on the sample one mL of either 2,6-dibromophenol or 2,4,6-TCP was added as an internal standard. Samples were made alkaline with one mL of 1 M NaOH, reacted with 1 mL of acetic anhydride for 3 hours and then extracted into 5 mL hexane.

Acetylated chlorophenols were quantified and verified using a J&W Scientific DB-5 capillary column (30m x 0.25 mm i.d., film thickness 0.25 microns) on a Hewlett-Packard 5890-GC/5971A-MSD equipped with a 7673A autosampler and HP-300 workstation. Splitless injection with a 30 second purge activation was used for all injections. Ultra high purity helium was used as the carrier gas and separation was improved using a temperature program. An initial column temperature of 45°C was held for one minute then increased at 15°C/min to 100°C then 2°C/min to a final temperature of 210°C. Injection port and transfer line temperatures were 250 and 280°C respectively. Chlorophenol recovery with this method was determined to be greater than 95%.

3.7.3 pH and Alkalinity

A Radiometer/Copenhagen pH meter, model 26 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 $\pm$0.05 pH units.

Alkalinity, measured as $CaCO_3$, was determined using a Radiometer Copenhagen pH meter equipped with an automatic titrator. The endpoint of titration was at pH 3.75 and 0.1 N HCl was used as the titrant.

3.7.4 Biogas Analysis

Reactor biogas production was monitored with a wet tip gas meter (Wet Tip Gas Company) which was calibrated prior to use.

The composition of the biogas was performed by GC using a Hewlett Packard model 5710A GC equipped with a thermal conductivity detector and a Hewlett Packard integrator model 3386A. The column was a Porapack T 50/80 mesh which was maintained at 70°C. Helium at a flow rate of 40 mL/min was the carrier gas. The detector temperature was held at 150°C and the temperature of the injection port was set at 100°C. A sample volume of 0.5 mL was injected into the column. Percent biogas fractions were corrected to standard temperature and pressure.

3.7.5 Volatile Fatty Acids

Acetic, butyric and propionic acid were measured by the method of Ackman (1972) using a Hewlett Packard GC model 5840A equipped with an flame ionization detector, an automatic sampler model 7671A and integrator model 3380A. A 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. Helium was passed over formic acid prior to entering the GC. Samples were centrifuged for 5 min in a model 235A Fisher Micro-centrifuge, and then 0.5 mL of sample was diluted with 0.5 mL of internal standard. The internal standard was isobutyric acid.

3.7.6 Chemical Oxygen Demand and Solids

COD was sampled twice a week. A method modified by Knechtel (1978) was used. The minor modification changed the sample heating time at 150°C to 3 hours instead of 2 hours.

TSS and VSS were measured according to Standard Methods (APHA, 1985).

3.7.7 Flow Rate

Positive displacement pumps were used to control the influent and recycle flow rates for the reactors treating the synthetic wastewater. Influent flowrates were monitored daily by measuring effluent volume.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Introduction

Five TCP congeners (2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6-TCP) and a DCP (3,5-DCP) were individually treated in 6.1 L lab scale UASB reactors over an eight month period. Their biodegradation pathways and anaerobic microbial toxicity were determined. In order to evaluate the effect of an alternate readily degradable carbon source on chlorophenol degradation, a synthetic wastewater composed of sucrose and acetic acid was fed to the reactor concurrently with a chlorophenol compound.

It was important that an alternate carbon source was supplied in this experiment. Most previous studies of chlorinated phenol biodegradation involved the chlorophenols as a sole carbon source, but in treatment of wastewater other carbon sources are quite often present. The reactors were operated at a 3 day HRT (from 1 to 5 months), followed by a 1 day HRT for another several months. The concentrations of chlorophenol introduced to the reactor were gradually increased in the first stage, in the second stage the same concentrations were applied but because of the shorter HRT, the volumetric and specific organic loading were tripled. The combination of 1 and 3 day HRT and different chlorophenol concentrations produced information on the effects of HRT and toxic

loading rates on chlorophenols. Biosorption isotherms for most TCPs, DCPs and MCPs were determined by batch sorption tests. Sorption isotherms determined by batch test were used to complete the chlorophenol mass balance in UASB reactors.

4.1 Sorption Isotherms

To better understand the fate of chlorophenols as well as chlorophenol intermediates in UASB reactor, it is important to understand the sorption characteristics of the chlorophenol compounds on or within anaerobic granular biomass. Biosorption isotherms for the uptake of 15 MCPs, DCPs, TCPs, and PCP by anaerobic granular sludge were determined. All batch sorption experiments were set up and run under anaerobic conditions. Physical and chemical conditions were established to simulate conditions that would exist in UASB reactors while minimizing the possibility of biodegradation of the sorbate.

In each experiment a 24 hour contact time was chosen as the time to reach equilibrium conditions. Other researchers have reported that biosorption is a rapid process which will take less than one day to reach equilibrium (Weber et al., 1987; Herbes, 1977; Bell and Tsezos, 1987). However, since the experiment was conducted with live biomass (although non-acclimated) the possibility of biodegradation did exist, therefore samples were also taken after 2 hrs. Figure 4.1 with 2,3,4-TCP shows typical results for 0.25, 2 and 20 hr contact times. It can be seen that the observed uptake by anaerobic biomass at 24 hours was very similar to the 2 hour

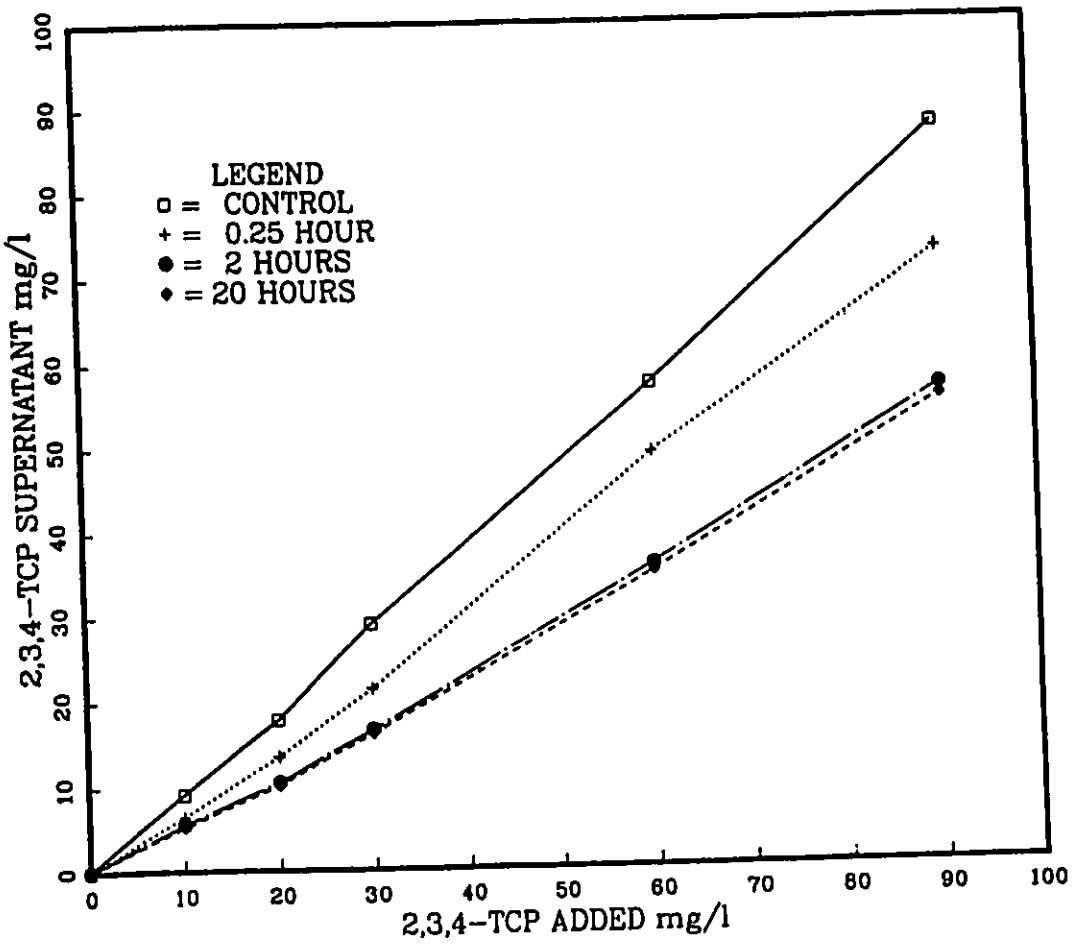


Figure 4.1 2,3,4-TCP Sorption Capacities at Different Sampling Time

contact time. Essentially the same result was seen with each chlorophenol tested. Comparison of results at 0.25 hour with those for 2 and 24 hours indicated that while biosorption began almost instantly the lowest contact time was insufficient for biosorption equilibration. At no time during the course of the sorption test were any reductively dechlorinated intermediates resulting from biodegradation detected.

The similarity of results between 2 and 24 hours indicates that rapid biosorption of these compounds occurs and equilibrium is reached in less than 2 hours. This has important ramifications in terms of UASB reactor operation. Typical HRTs for UASB reactors range from 8 to 36 hours while solid retention times (SRTs) may be between 50 and 200 days. The rapid biosorption of chlorinated phenols to anaerobic biomass combined with reactor operating conditions indicates that sufficient time is available for biosorption equilibration to occur. Hence, for UASB reactor studies it is important to know the biosorption capacity of the original absorbants that may result from biodegradation.

Biosorption equilibrium can be described by the Freundlich equation. Equilibrium concentrations of individual chlorophenols in the liquid and solid (granular biomass) phases were plotted on log-log scales to test their fit to the Freundlich equation (Eq 4.1).

$$q = K*C_{eq}^{1/n} \qquad (4.1)$$

where:

q = equilibrium concentration of adsorbate on live anaerobic granular biomass, $\mu g/g$

C_{eq} = equilibrium concentration of adsorbate in solution, $\mu g/L$

K = Freundlich constant, L/g VSS

1/n = exponent

linearlized least square analysis was run on the equilibrium data to determine the Freundlich constants. The constants, K and 1/n were determined by using the true dependent variable, C_{eq}, and taking the chlorophenol associated with biomass as the independent variables. An example illustrating determination of the constants is presented in Appendix C.

Figures 4.2, 4.3 and 4.4 show live anaerobic granular biomass biosorption data and concomitant Freundlich equation isotherms. Best fit parameters for the Freundlich sorption model as well as K_{ow} and Pka for each individual chlorophenol used in this study are summarized in Table 4.1. Most of the isotherms fit the Freundlich equation very well, with correlation coefficients greater than 0.9. For visual presentation of all the data (Figs 4.2 to 4.4), average Freundlich equation parameters were calculated for selected groups of chlorophenols where possible. The grouped Freundlich parameters and isotherms plots for TCPs and DCPs are shown and explained in Figures 4.5 and 4.6 respectively.

4.2 Linear Biosorption

As can be seen from Table 4.1 a number of the compounds tested had 1/n values close to 1. This indicates linear sorption isotherms

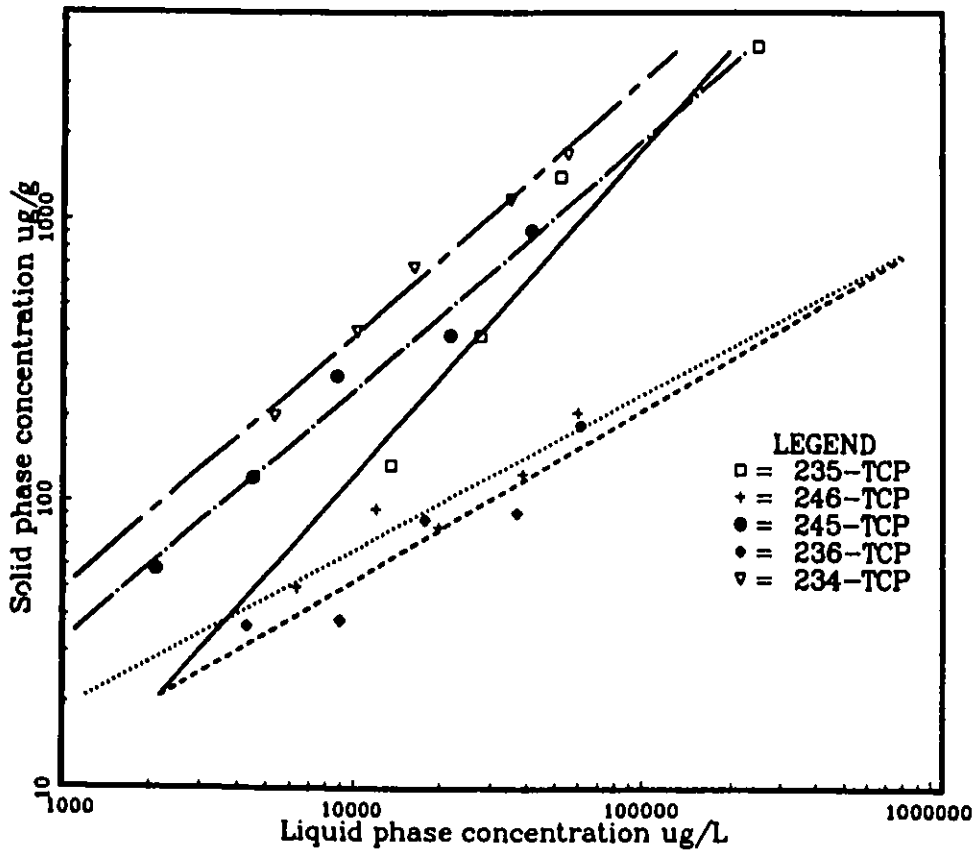


Figure 4.2 Trichlorophenols Sorption Isotherms

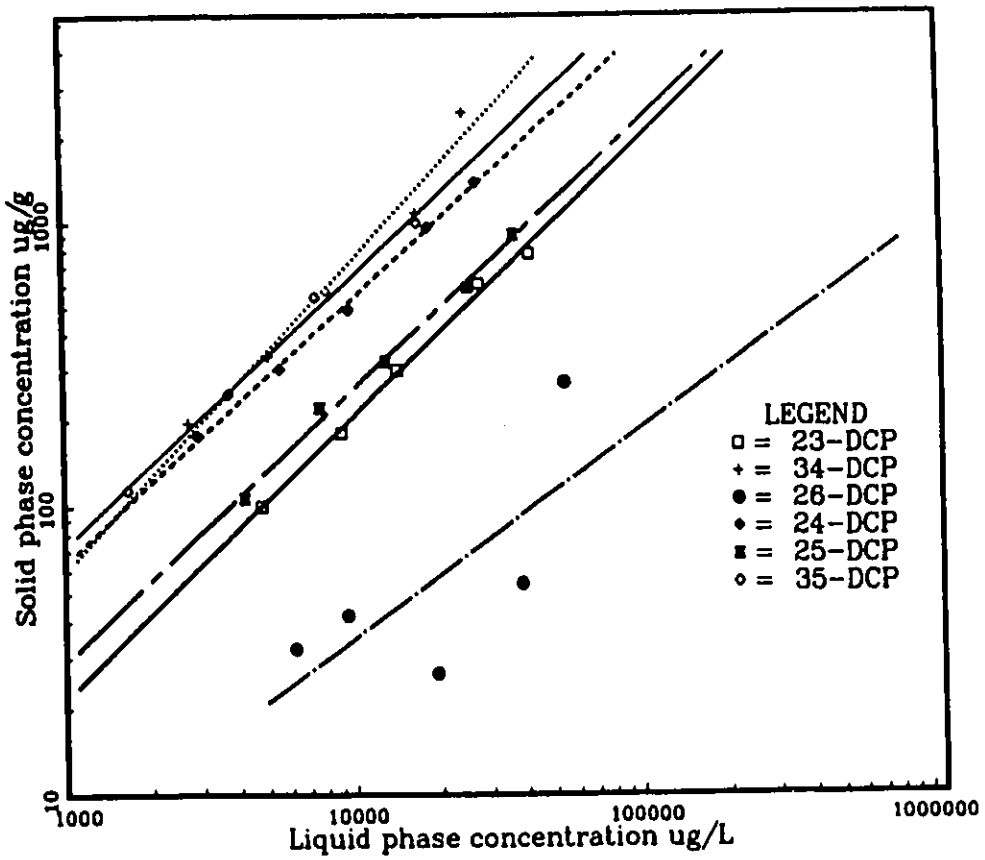


Figure 4.3 Dichlorophenols Sorption Isotherms

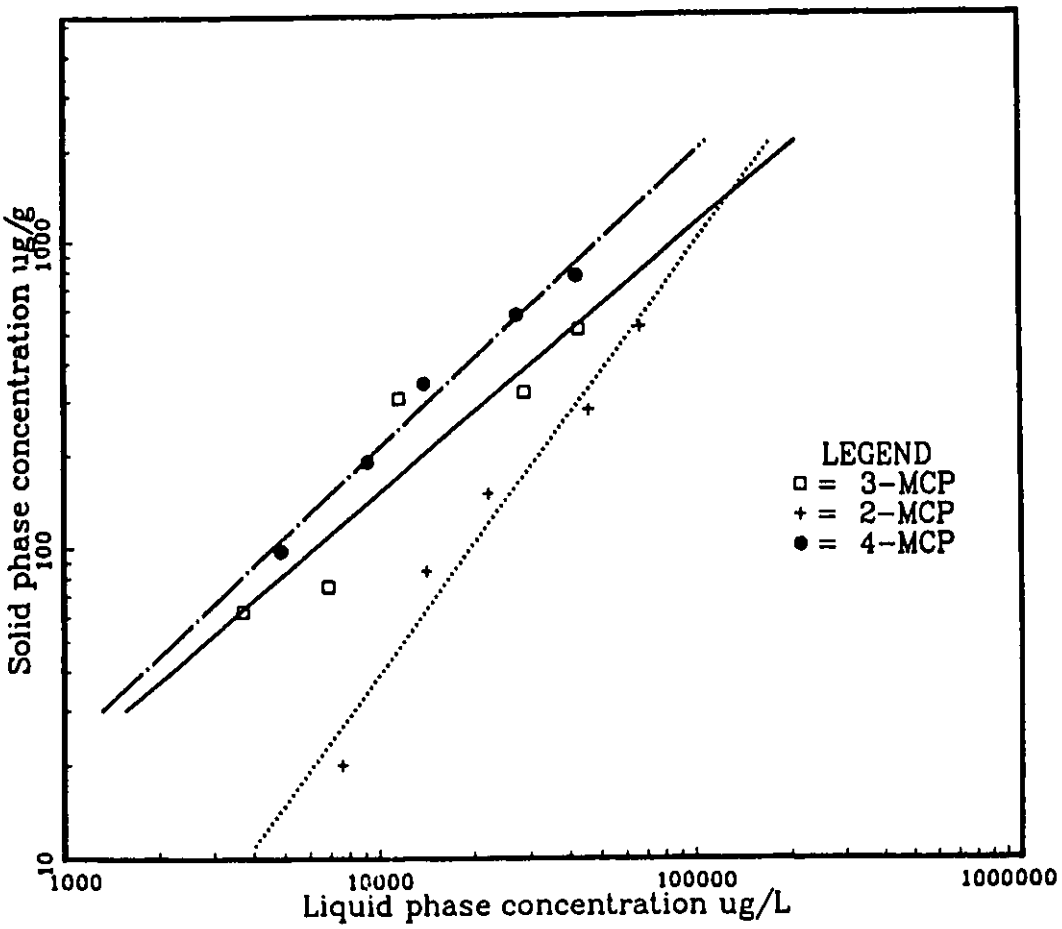


Figure 4.4 Monochlorophenols Sorption Isotherms

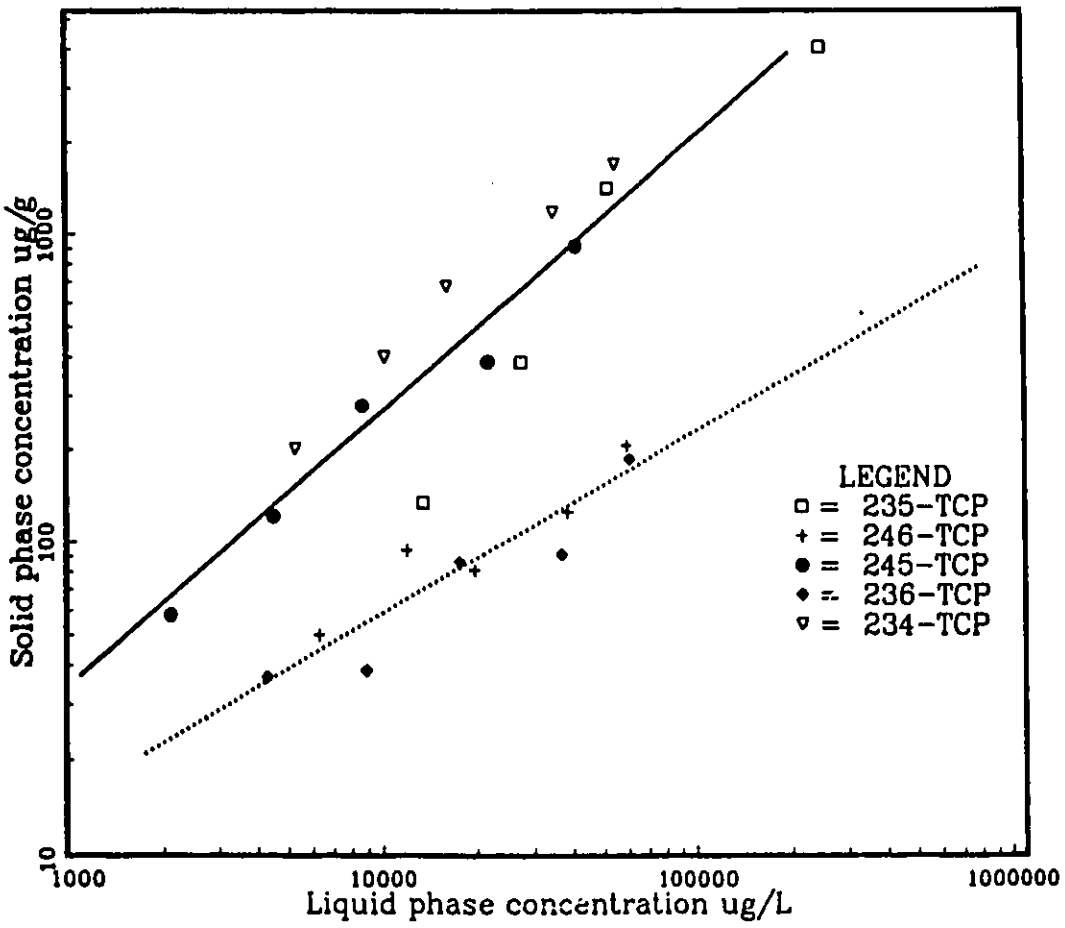


Figure 4.5 Grouped Trichlorophenols Sorption Isotherms

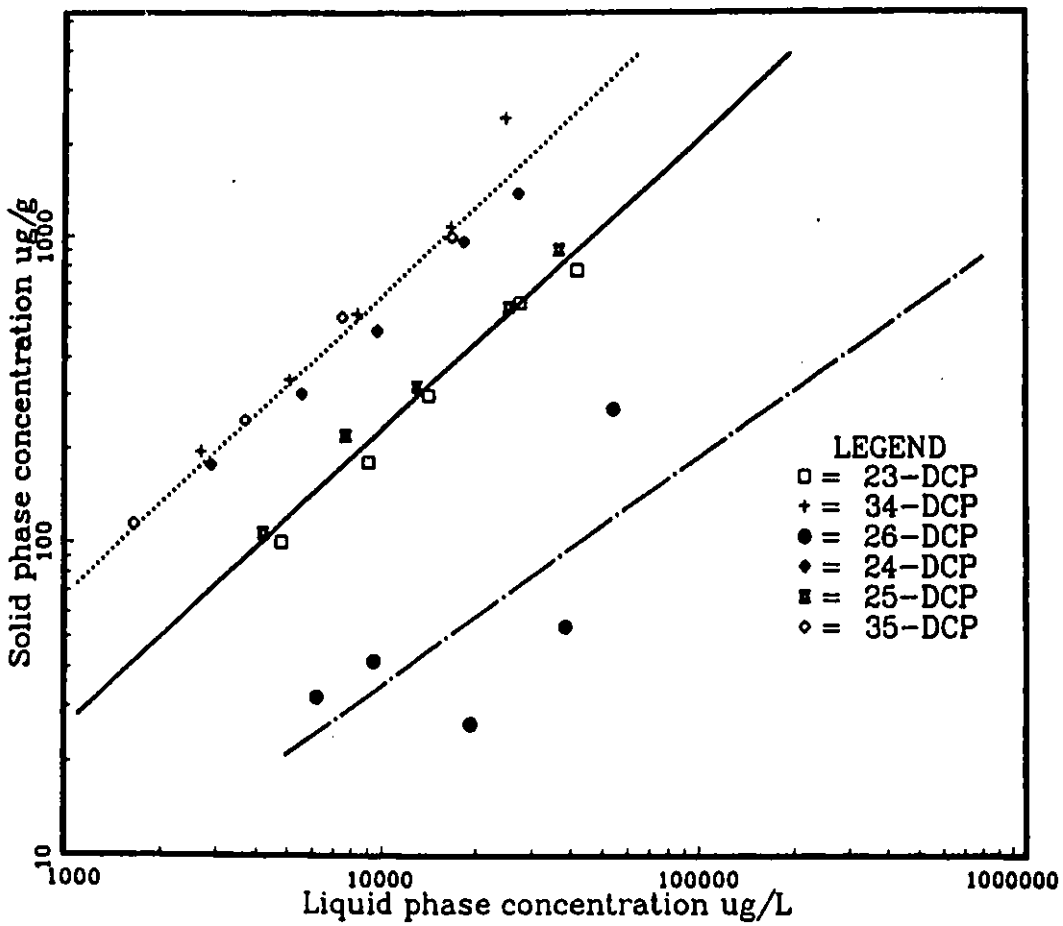


Figure 4.6 Grouped Dichlorophenols Sorption Isotherms

Table 4.1 Parameters for Biosorption by Anaerobic Granular biomass

compound	K	1/n	r^2	log K_{ow}[1]	Pka
phenol	nd		1.46		na
2-MCP	0.000107	1.390	0.96	2.15	8.49
3 MCP	0.051370	0.866	0.86	2.47	8.85
4-MCP	0.030570	0.952	0.98	2.39	9.18
2,3 DCP	0.025363	0.977	0.99	3.50	7.70
2,4-DCP	0.107103	0.925	0.99	3.23	7.89
2,5-DCP	0.042238	0.946	0.99	3.20	6.35
2,6-DCP	0.043471	0.727	0.52	3.19	6.80
3,4-DCP	0.031754	1.091	0.98	3.41	8.59
3,5-DCP	0.103920	0.948	0.99	3.68	8.19
2,3,4-TCP	0.098175	0.899	0.99	na	
2,3,5-TCP	0.002929	1.156	0.84	na	
2,3,6-TCP	0.208641	0.600	0.90	3.57	7.13
2,4,6-TCP	0.420860	0.551	0.89	3.38	6.0,7.42
2,4,5-TCP	0.073232	0.882	0.97	4.19	7.43
3,4,5-TCP	nd		4.87		6.35
PCP	2.466039	0.680	0.91	5.01	4.81

nd: not done

[1] Octanol/water partition coefficient (data from Leo et al., 1971; Schellenberg et al., 1984; Callahan et al., 1979; Xie et al. 1984).

and suggests a constant-partitioning sorption mechanism. This type of sorption isotherm can occur when the absorbent can readily penetrate into the sorbent (in this case a microbial granule). The availability of sorption sites remains constant at all concentrations up to saturation, typical of the partitioning of a solute between two immiscible solvents. Linear sorption isotherms commonly occur when relatively pure, porous sorbents are used and

the sorption is being carried out over a relatively small concentration range. It could be argued that some of these conditions were present in the experiments. The sorbent concentration range used was narrow, (1 order of magnitude) simulating conditions that might be found in a UASB reactor. Anaerobic granules are known to be very porous (Wiegant and de Man, 1986; MacLeod et. al., 1990) allowing penetration and sorption sites in the inner portion of the granule. It appears that the granules which are a consortium of anaerobic bacteria behave mainly as a homogeneous, pure sorbent.

When the exponent (1/n) is equal to 1.0, the Freundlich equation reduces to a distribution coefficient, describing a linear relationship between chlorophenol concentrations in the liquid and solid phases (4.2). For cases where 1/n was approximately equal to one, the distribution coefficient, K_d, was determined by linear regression analysis.

$$q = K_d * C_{eq} \qquad (4.2)$$

where

K_d = distribution coefficient, L/g VSS

Table 4.2 provides the distribution sorption constants, K_d for the chlorophenols on anaerobic granular sludge in case where 1/n was approximately equal to 1. K_d values have been used as indicators of sorption of many chemicals by soils and organic material. Higher values of K_d indicate higher sorption capacity. Several studies (Bailey and White, 1964; Lambert, 1967) have reported high correlation between organic content of the sorbent and organic

chemical sorption, and such studies have determined the distribution sorption constant for a given chemical per unit of organic matter, K_{om}. In this study it is assumed that the organic fraction of the granular biomass (i.e. VSS) is responsible for sorbing the chlorophenols and so the K_d values take into account the percent VSS in the granule (in this study granules were 80% VSS).

Table 4.2

Sorption Distribution Constants of Chlorophenols

Compound	K_d L/g VSS	r^2
4-MCP	0.018	0.98
2,3-DCP	0.019	0.98
2,4-DCP	0.049	0.99
2,5-DCP	0.024	0.99
3,4-DCP	0.096	0.94
3,5-DCP	0.057	0.99
2,3,4-TCP	0.029	0.99
2,4,5-TCP	0.221	0.97

For cases where 1/n was equal to 1, a separate plot of log K_{ow} vs. log K_d (Fig.4.7) was made to see if a correlation could be established between K_d and the octanol/water partition coefficient. The following relationship was developed:

$$\log K_d = 0.092 \log K_{ow} - 1.786 \qquad r^2 = 0.3 \qquad (4.3)$$

The fit to the data was very poor, indicating that for those chlorophenols which exhibited linear biosorption isotherms, a

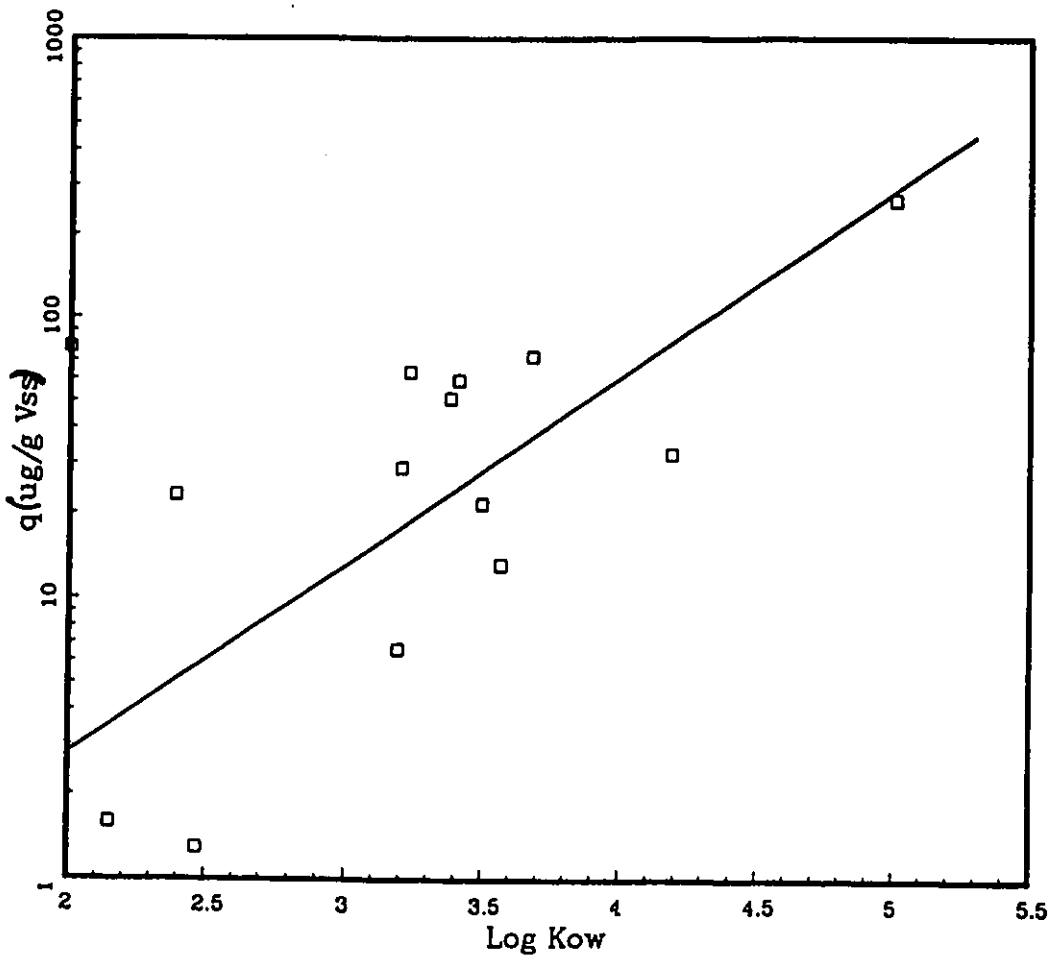


Figure 4.7 Correlation of Kow and Kd

poor correlation between K_d and K_{ow} can be stated that there was an increase in K_d with increasing K_{ow}. These observations suggest that chlorophenol sorption by anaerobic sludge granules is more complex than partitioning of chlorophenols between two solvents, such as water and octanol.

4.3 Sorption Capacities of Chlorophenols

Table 4.3 gives the equilibrium sorption capacities of anaerobic granular biomass at 2 different solution concentrations as calculated by the Freundlich equation. For the most part TCPs, DCPS, and MCPs sorption capacities were in the same range. For a liquid equilibrium concentration of 100 μg/L, biosorption capacities were between 2.5 and 9.2 μg/g VSS. The exception was 2-MCP which had a very low biosorption capacity. PCP the most halogenated of the chlorophenols had the highest biosorption capacity.

A number of studies have correlated the uptake of sorbate on biological sludge (Matter-Muller et al., 1980; Tsezos and Bell, 1989), Lipids (Neeley et. al., 1974) and aquifer material (Schwarzenbach and Westfall, 1981) to the octanol/water partition coefficient. In this study, (equilibrium liquid phase concentration of 1000 μg/L) the correlation between K_{ow} and the solid phase concentration, q in μg/g Vss, Figure 4.8 was best described by equation 4.4:

$$\log q = 0.65 \log K_{ow} - 0.81 \qquad (4.4)$$

The correlation coefficient was rather low (0.6) indicating that K_{ow} only partly describes sorption of chlorophenols to granular biomass. Tsezos and Bell (1989) found good correlation for the three compounds they tested (correlation coefficient of 0.99). Perhaps the good correlation was the result of using fewer compounds. For this study all that can be concluded is, that in general, the uptake capacity of live anaerobic biomass tends to increase with increasing octanol/water partition coefficient.

Table 4.3 Equilibrium Sorption Capacity of Live Anaerobic Granular Sludge

	Biosorption Capacity at Stated aqueous phase Concentration, µg/g VSS	
compound	100 µg/L	1000 µg/L
2-MCP	0.002	0.05
3-MCP	4.1	30.3
4-MCP	2.9	25.9
2,3-DCP	2.5	23.5
2,4-DCP	8.9	75.4
2,5-DCP	3.9	34.5
2,6-DCP	2.9	15.5
3,4-DCP	3.5	43.4
3,5-DCP	9.2	81.6
2,3,4-TCP	7.8	61.7
2,3,5-TCP	4.6	36.3
2,3,6-TCP	6.2	24.6
2,4,5-TCP	5.8	44.1
2,4,6-TCP	7.8	27.9
PCP	42.4	202.7

Some other studies have attempted to relate sorption to the type or position of the functional groups on the sorbate (Boyd, 1982).

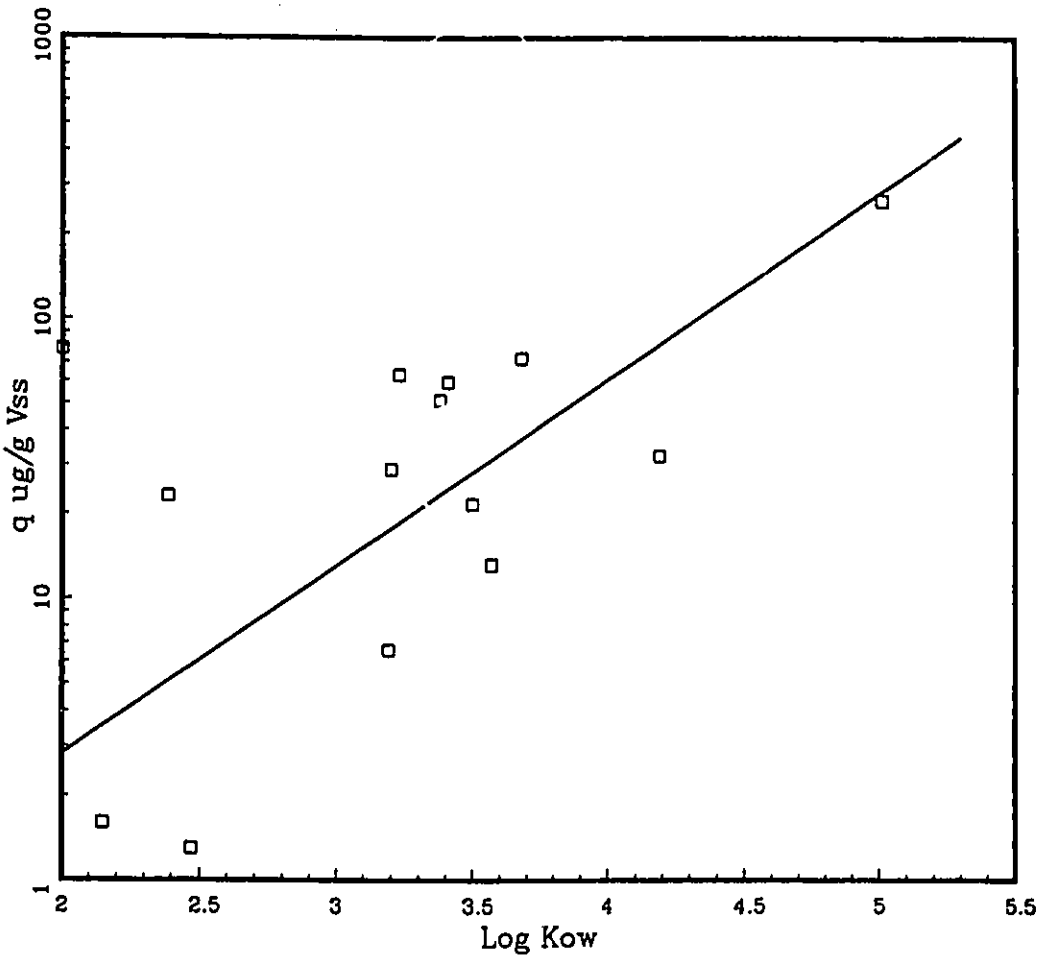


Figure 4.8 Correlation of Kow to q

In this study it was found that biosorption of chlorophenols having a chlorine in the para position or containing 2 meta chlorine(s) tended to be higher than chlorophenols containing chlorine in only ortho or a single meta position.

The characteristics of sorbent and solvent as well as sorbate affect sorption. Being the same sorbent and solvent used, the difference of sorption capacity was only caused by the characteristics of sorbate. Belfort (1979) found that for a homologous series, the capacity for adsorption increases with molecular weight, solubility and branching. The results in this sorption study shows that there is not a strong trend that sorption capacity increases with chlorination or increasing molecular weight. But, it was found that the sorption capacity is strongly dependent on chlorine position. In general, chlorophenols with para position chlorine have larger sorption capacity. For example, the sorption capacity among three MCPs decreased in following order: 4-MCP > 3-MCP > 2-MCP.

4.4 PCP Sorption to Biomass

Bell and Tsezos (1987) and Tsezos and Bell (1989) reported on the sorption of PCP to living and dead biomass. Two types of biomass were reported on, a mixed culture of aerobic activated and a pure culture of Rhizopus arrhizus. They found that biosorption was nonlinear, could be described by the Freundlich Equation and that biosorption can account for part of the removal of PCP from the aqueous phase. They also reported that it was impossible to

make any generalizations regarding the relative magnitude of biosorptive uptake between live and dead cells. Baughman and Paris (1981) stated that in general biosorption occurs to the same extent or slightly better on dead rather than live cells.

Figure 4.9 is a Freundlich sorption isotherm plot of the results of this study using live anaerobic granular biomass and extrapolated results from Bell and Tsezos (1987) and Tsezos and Bell (1989) with live and dead aerobic activated sludge and Rhizopus arrhizus fitted to the Freundlich model. Freundlich parameters and conditions for PCP sorption isotherms for these plots are summarized in Table 4.4. The Freundlich constant (K) for the live anaerobic granular sludge was an order of magnitude lower than for the live aerobic cultures and 4 fold lower than the lowest constant reported for dead aerobic sludge. This indicates that the biosorption capacity of live anaerobic sludge was much less than that of live or dead aerobic material. For an equilibrium liquid phase PCP concentration of 1000 μg/L, biosorption to anaerobic biomass would be about 202 μg/g VSS which is approximately 4.5 fold and 8 fold less than for live and dead activated sludge, respectively.

The differences in biosorption capacity for anaerobic and aerobic biomass may be related to differences in their lipid composition or content. Canton et al. (1977) reported that biosorption of hexachlorocyclohexane by chlamydomonas was almost twice that of Dunaliella on a dry weight basis. When normalized to the lipid content of the cells, biosorption was identical.

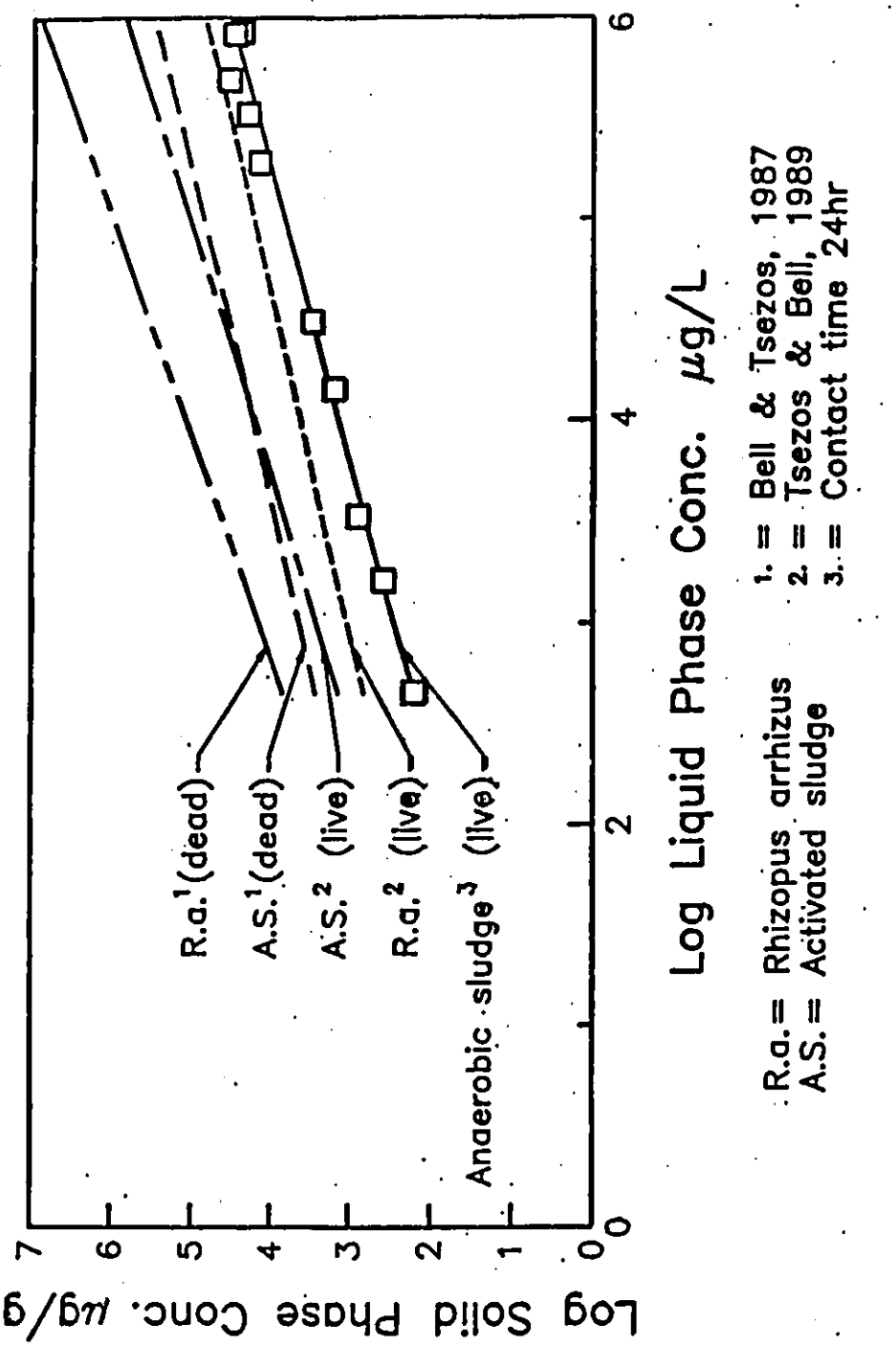


Figure 4.9 PCP Sorption Isotherms and Conditions

Canton et al.'s (1977) study demonstrated the important role of membrane lipids to biosorption. Total lipid content of anaerobes seems to be similar to aerobic microorganisms and so should not be an influencing factor (Sprott et al., 1983). However, there may be a qualitative difference between the lipids of the aerobes from Tsezos and Bell (1989) and those of the anaerobes used in this study. The aerobes were presumably mostly eubacteria, while the anaerobes were largely archebacteria (methanogens). The former have membrane lipids that contain fatty acids esterified to glycerol, while the latter instead contain unique neutral squalene and ether linked polar lipids (Balch et al., 1979).

It seems likely that the different lipids might have different sorptive properties. Another factor influencing the lower sorptive capacity of anaerobic granular biomass relative to aerobic biomass may be the test temperature (35°C vs 20°C, respectively). Higher temperatures have been shown to reduce the sorptive capacity of biomass.

Table 4.4 Freundlich Parameters and Conditions for PCP Sorption Isotherms

	Temp	K	1/n	Reference
Rhizopus arrhizus				
live	20	32.1	0.56	Ref 1
dead	20	28.8	0.90	Ref 2
Activated sludge				
live	20	85.1	0.6	Ref 1
dead	20	10.1	0.8	Ref 2
Anaerobic Granular sludge				
live	35	2.47	0.68	this study

Ref 1: Tsezos and Bell (1989)

Ref 2: Bell and Tsezos (1987)

4.5 Desorption of Chlorophenols

Quantities of sorbed 3-MCP, and 3,4-DCP that desorbed from anaerobic granular biomass were determined. Figure 4.10 shows experimentally determined desorption equilibrium data (24 hrs) for 3,4-DCP against the corresponding biosorption isotherm. Figure 4.11 shows 3-MCP desorption isotherms.

A few desorption points lie above the 3,4-DCP sorption isotherm (Fig. 4.10) suggesting a small degree of hysteresis and concomitantly slight irreversibility. The desorption equilibrium result for 3,4-DCP follows its respective biosorption isotherms suggesting almost complete reversibility of the biosorption phenomenon. For 3-MCP (Fig. 4.11) the majority of points were above the biosorption curve suggesting a degree of irreversibility. As discussed in the introduction, desorption of toxic compounds that accumulate in microbial biomass can lead to problems in the ultimate disposal of the sludge, should biodegradation not occur or be incomplete.

4.6 Chlorophenols Associated with Biomass in UASB Reactors - Sludge Extraction

The Freundlich isotherm model and the parameters developed for it were used to predict the concentration of chlorinated phenols sorbed to anaerobic granular biomass in continuous 6.1 L UASB reactors reductively dehalogenating DCPs and TCPs wastewater. Measured chlorophenols were determined by sludge extraction. The reactors operated at 35°C, pH between 7.4-7.7 and contained 20 g VSS/L reactor of acclimated anaerobic granular biomass.

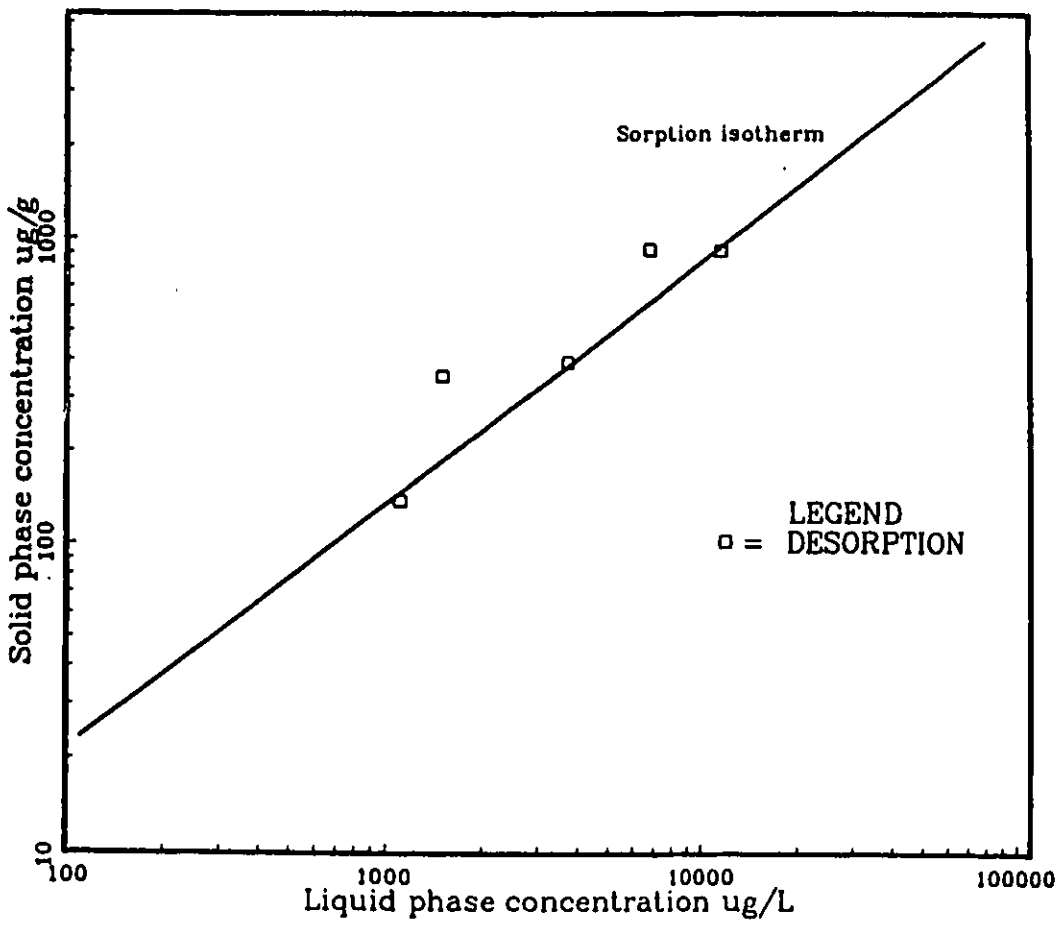


Figure 4.10 3,4-DCP Desorption Isotherms

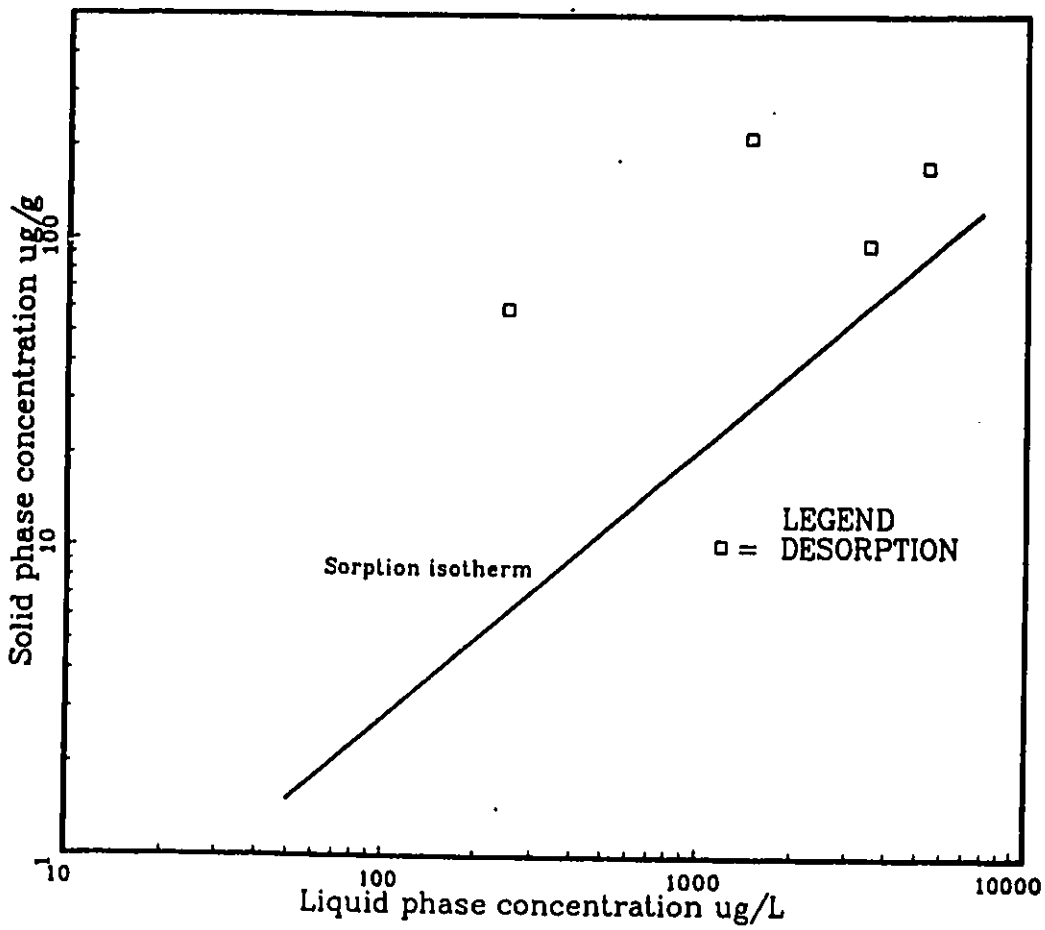


Figure 4.11 3-MCP Desorption Isotherms

Table 4.5 Measured and Predicted Concentration of Chlorophenols in Granular Biomass from UASB Reactors

Reactor feed	Days of operation	Compound	Concentration in granular biomass		Difference
			Measured*	Predicted	
			µg/g	µg/g	µg/g
3,5-DCP	229	3 MCP	23.0	27.4	.4
		3,5-DCP	0.3	0**	-0.3
2,3,4-TCP	229	3,4-DCP	13.5	13.6	0.1
		2,3,4-TCP	49.5	35.4	-14.1
2,3,5-TCP	229	3 MCP	111.7	90.7	-21.0
		3,5-DCP	2.4	0	-2.4
		2,3,5-TCP	0	**	0
2,3,6-TCP	229	3 MCP	101.5	41.8	-59.7
		2,5-DCP	7.8	16.1	8.3
		2,3,6-TCP	1.7	8.5	6.8
2,4,5-TCP	229	3,4-DCP	59.5	76.8	17.3
		2,4,5-TCP	3.5	0**	-3.5
2,4,6-TCP	229	4-MCP	289.0	251.9	-37.1
		2,4-DCP	19.4	48.5	28.1
		2,4,6-TCP	6.4	21.3	14.9

* Average of duplicate samples

** Concentrations less than detection limit

Since the biomass was reductively dechlorinating DCPs and TCPs present in the wastewater, the reactor liquor was a multi-component mixture of MCPs, DCPs, and TCPs. Except for the acclimated granular biomass and the multi-component nature of the reactor mixed liquor, UASB reactor conditions and the conditions of the batch sorption

tests were similar. Liquid and granular sludge samples from UASB reactors were analyzed and the concentrations of tri-, di-, or MCP in the liquor and granular biomass phases were determined. Freundlich parameters determined from batch sorption tests and reactor liquor chlorophenol analyses were coupled in the Freundlich model to predict the concentrations of individual chlorophenols sorbed to the granular biomass. Table 4.5 gives removed and predicted results for the original chlorophenol substrate as well as all chlorophenol intermediates produced, in each reactor.

UASB reactor conditions were similar to the conditions of the batch sorption tests. Chlorophenols associated with biomass based on the Freundlich model prediction and determined by direct analysis of the granular biomass were compared (Table 4.5). In general, there was relatively good agreement between predicted granular biomass chlorophenol concentrations and measured values. Results for most compounds were within the same order of magnitude which is reasonable for biological systems.

Differences might be explained by differences by acclimated vs. unacclimated granular biomass. There may be differences in the type of microbial flora in acclimated vs unacclimated granules, which may change surface properties and enhance sorption. Additionally, actively dechlorinating biomass may not allow equilibrium between the liquid and solid phase to occur. The fact that the original parameters were determined in batch tests with individual compounds while the reactor liquor is a more complex mixture in a continuous state of flux might also affect results.

4.7 Dechlorination of Chlorophenols in UASB Reactors

Five TCPs (2,3,4-, 2,3,6-, 2,3,5-, 2,4,5-, 2,4,6-DCP) and one DCP (3,5-DCP) were respectively treated in UASB reactors for approximately eight months. The HRTs of 3 and 1 day were used. A readily degradable co-substrate (sucrose plus acetate) was fed to the reactors at the volumetric loading rates (VLR) of 1.6 and 5 g COD/L.d for 3 and 1 day HRT, respectively. The corresponding specific loading rates (SLR) were 0.08 and 0.25 g COD/g VSS.d respectively. The biomass in the reactors R234, R236, R246, and R245, which were used to treat 2,3,4-, 2,3,6-, 2,4,6-, 2,4,5-TCP respectively, had never been previously exposed to chlorinated compounds. All reactors were operated in parallel, i.e., changes in operating conditions, were made in identical ways, so as to make toxic loading rates comparable for different reactors. Reactors R235 and R35 which were used to treat 2,3,5-TCP and 3,5-DCP were also run in parallel.

Several chlorophenol loading rates were applied and increased stepwise. Most chlorophenol loading rates were maintained for a minimum operating time of 14 days in order to allow biomass to acclimate to a higher loading rate change. It was observed that at low loading rates of 1.25 and 2.5 mg/L, neither parent chlorophenols nor intermediates were detected in most reactors, i.e, a 100% removal of chlorophenols was achieved. Chlorinated compounds tend to sorb to biomass, so it was assumed that 100% removal might be largely due to biosorption but the biodegradation of chlorophenols might occur.

Reactor performance was monitored by measuring biogas volume and its methane component, acetic acid concentration in the influent and effluent daily; COD in the influent and effluent three times a week; pH and alkalinity once every two weeks. It was found that throughout the experiment, alkalinity and pH in all reactors were approximately 3500 mg/L as CaCO3 and 7.5 respectively.

In the following sections, general reactor performance, chlorinated phenol anaerobic biodegradation pathways and reactor anaerobic granular sludge toxic loading limits will be described individually.

4.7.1 General R246 Reactor Performance

Reactor 246 (R246) treated 2,4,6-TCP in the presence of a readily degradable co-substrate. In the course of the experiment, R246 was operated for 245 days, 175 days at 3 day HRT followed by 70 days at 1 day HRT. At 3 and 1 day HRT, the co-substrate VLR were 1.6 and 5 g COD/L.d. The corresponding SLR were 0.08 and 0.25 g COD/g VSS.d, respectively. The inoculum sludge in R246 had never been previously exposed to chlorinated compounds. After 4 days of operation with the sucrose/acetate wastewater, 2,4,6-TCP was continuously applied to R246 reactor and the 2,4,6-TCP loading rate was increased stepwise. During the 3 day HRT stage, the applied 2,4,6-TCP concentration range was from 1.25 mg/L (SLR of 0.02 mg/g VSS.d) to 25 mg/L (SLR of 0.42 mg/g VSS.d), and six 2,4,6-TCP loading concentrations (1.25, 2.5, 6, 12, 18, 25 mg/L) were applied. The specific COD loading rate (0.08 g/g VSS.d) was in a low range and was not able to support adequate biomass growth;

however, under these conditions 100% removal of 2,4,6-TCP was achieved at most loading rates and no methanogensis or acidogensis inhibition occurred which is discussed in Section 4.7.4.

At 1 day HRT, the 2,4,6-TCP concentration ranged from 4 mg/L (SLR of 0.2 mg/g VSS.d) to 50 mg/L (SLR of 2.5 mg/g VSS.d), again six 2,4,6-TCP loading concentrations (4, 6, 12, 18, 25, 50 mg/L) were applied. The specific co-substrate COD loading rate (0.25 mg/g VSS.d) was in an adequate range to promote growth of granular biomass. Removal efficiency of 2,4,6-TCP was enhanced in this stage but inhibition of 2,4,6-TCP removal occurred at a SLR of 2.5 mg/g VSS.d. At this loading rate, the concentration of 2,4,6-TCP and its metabolites 2,4-DCP and 4-MCP increased (Fig. 4.12), their removal efficiency decreased, however the concentration of acetic acid (Fig. 4.13) and COD removal efficiency (Fig. 4.14) in the reactor were unchanged indicating the activities of acidogens and methanogens were not inhibited by this 2,4,6-TCP concentration.

During the course of the experiment, COD removal efficiency was in the range of 96.0 to 99.8%; acetic acid concentration was in range of 0.0 to 80 mg/L, and average biogas composition was 65% methane. pH and alkalinity were stable at 7.5 and 3500 mg/L as $CaCO_3$ respectively. Between days of 235 and 240, chlorophenol syringe pump was stuck three times and the 2,4,6-TCP loading was reduced, but the steady state was finally reached at this loading rate (Fig. 4.12).

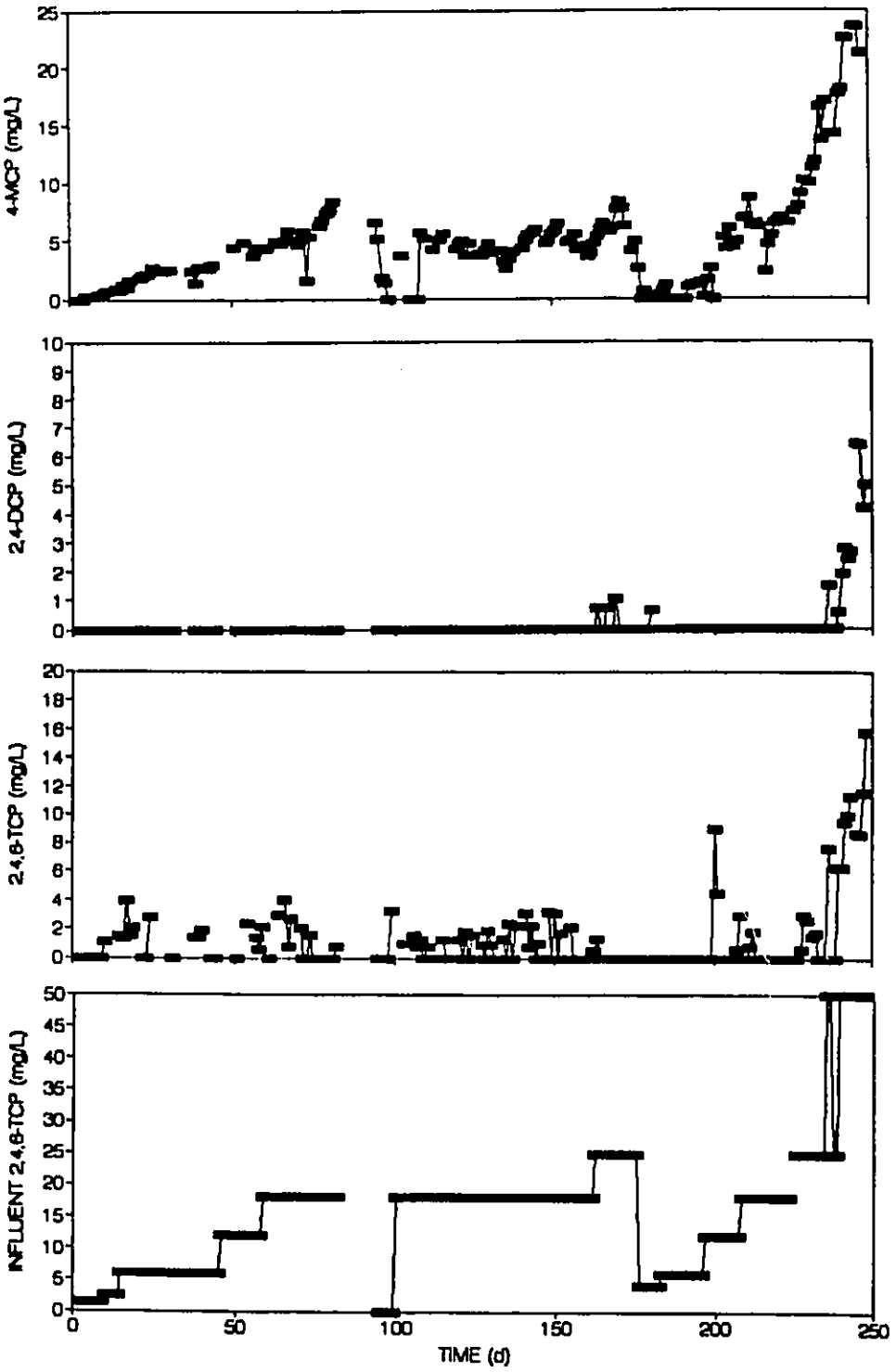


Figure 4.12 Soluble Concentration of 2,4,6-TCP and its Metabolite in R246

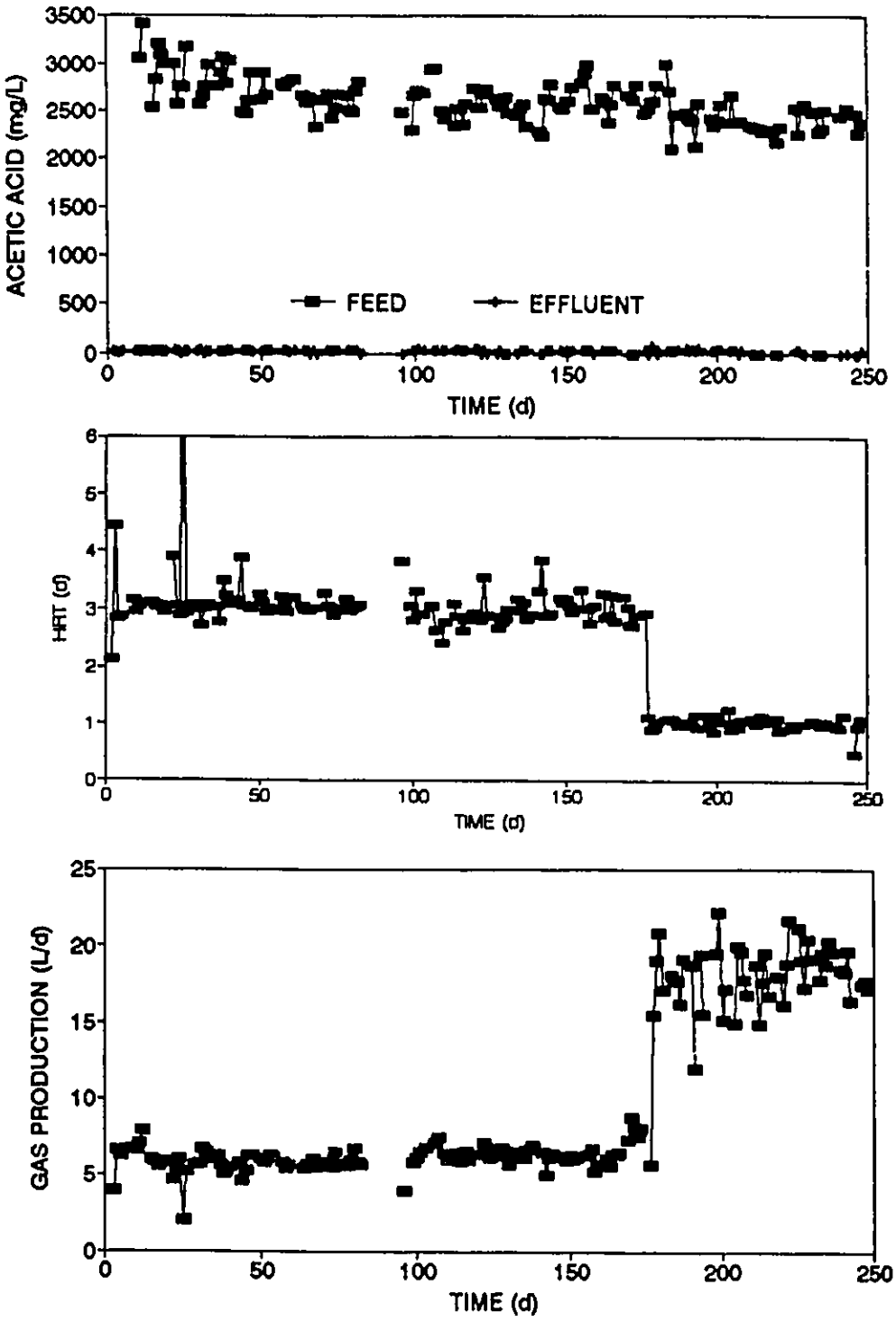


Figure 4.13 General R246 Reactor Performance.

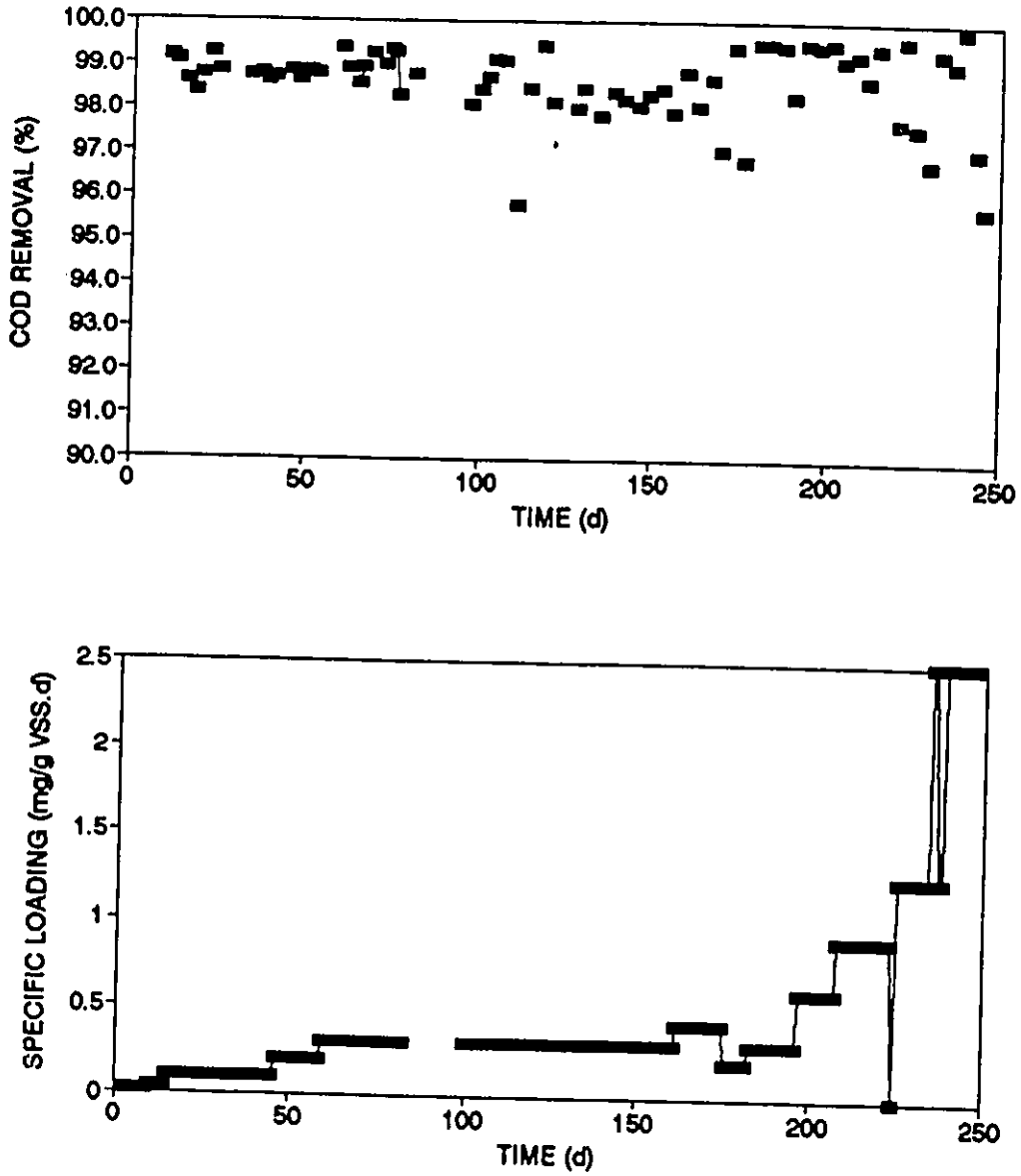


Figure 4.14 Treatment Efficiency of the R246 Reactor under Toxic Loading

4.7.2 Biodegradation Pathway for 2,4,6-TCP

Biodegradation of 2,4,6-TCP and its biodegradation pathway were determined by HPLC analysis. At the low 2,4,6-TCP loading rate (1.25 mg/L) used at the beginning of 3 d HRT, 2,4,6-TCP and its metabolites were not detected in the effluent liquid (Fig. 4.12). Chlorophenols tends to sorb to solids, so it was assumed that 100% removal might be largely due to anaerobic sludge biosorption. It also indicates that the biosorption isotherms determined in batch tests are not valid for these low concentrations.

Within 2 days of 2,4,6-TCP addition at 2.5 mg/L (SLR 0.042 mg/g VSS.d), 0.3 mg/L of 4-MCP (a reductive dehalogenation product of 2,4,6-TCP) was detected in the reactor effluent. The fact that the reactor sludge had never been exposed to chlorinated compounds before suggests that a significant population of bacteria were present in R246 with the inherent ability to degrade 2,4,6-TCP.

At higher 2,4,6-TCP loading concentrations (25 mg/L at 3 day HRT and 50 mg/L at 1 day HRT), 2,4-DCP was also detected in the reactor effluent. The detection of 4-MCP at low loading rates followed by 2,4-DCP at high loads indicates that 2,4,6-TCP degradation begins with removal of an ortho-Cl producing 2,4-DCP, followed by removal of a second ortho-Cl to produce 4-MCP. At no time during the experiment were 2,6-DCP or 2-MCP detected in the effluent, in addition 2,6-DCP and 2-MCP were not detected when sludge samples were extracted and analyzed by GC-MS. The fact that 4-MCP was the main product in the reactor would suggest that para dechlorination of 4-MCP activity was poor. The fact that there were no significant

amounts of 2,4,6-TCP or 2,4-DCP accumulating in the reactor at most loading rates suggests that the rate of reductive dechlorination of ortho-Cl is high. The pathway of 2,4,6-TCP anaerobic biodegradation (Fig. 4.15a) observed in this study is identical to that observed by Woods (See Section 2.6.5, Fig. 2.1).

4.7.3 Fate of 2,4,6-TCP in R246

The removal efficiency and average concentrations of 2,4,6-TCP and its biodegradation products during steady state in R246 reactor are shown in Tables 4.6a and 4.6b. There was a minimum operating time of 14 days at each loading rate in order to allow biomass to acclimate to a higher loading rate changes. It was assumed that steady state was reached in the last four days at each loading rate. Table 4.6a indicates that at 3 day HRT 2,4,6-TCP disappeared completely (100% removal) when the loading concentrations were 1.25 and 2.5 mg/L. This was due to a combination of biodegradation and biosorption. When the loadings were increased to 6 and 12 mg/L, 2,4,6-TCP removal efficiencies were 86.3 and 75.7% respectively. When the loadings were shifted to 18 and 25 mg/L, 100% removal of 2,4,6-TCP was achieved at steady state. 2,4-DCP (a 2,4,6-TCP degradation intermediate) tended not to accumulate in the reactor and 100% removal of 2,4-DCP was achieved at most loading rates. There was no analytical evidence to indicate 4-MCP was further degraded in the reactor although it was removed in part by sludge sorption.

At 1 day HRT, a 100% removal of 2,4,6-TCP and 2,4-DCP was achieved at most loading concentrations from 4 to 50 mg/L (VLR of

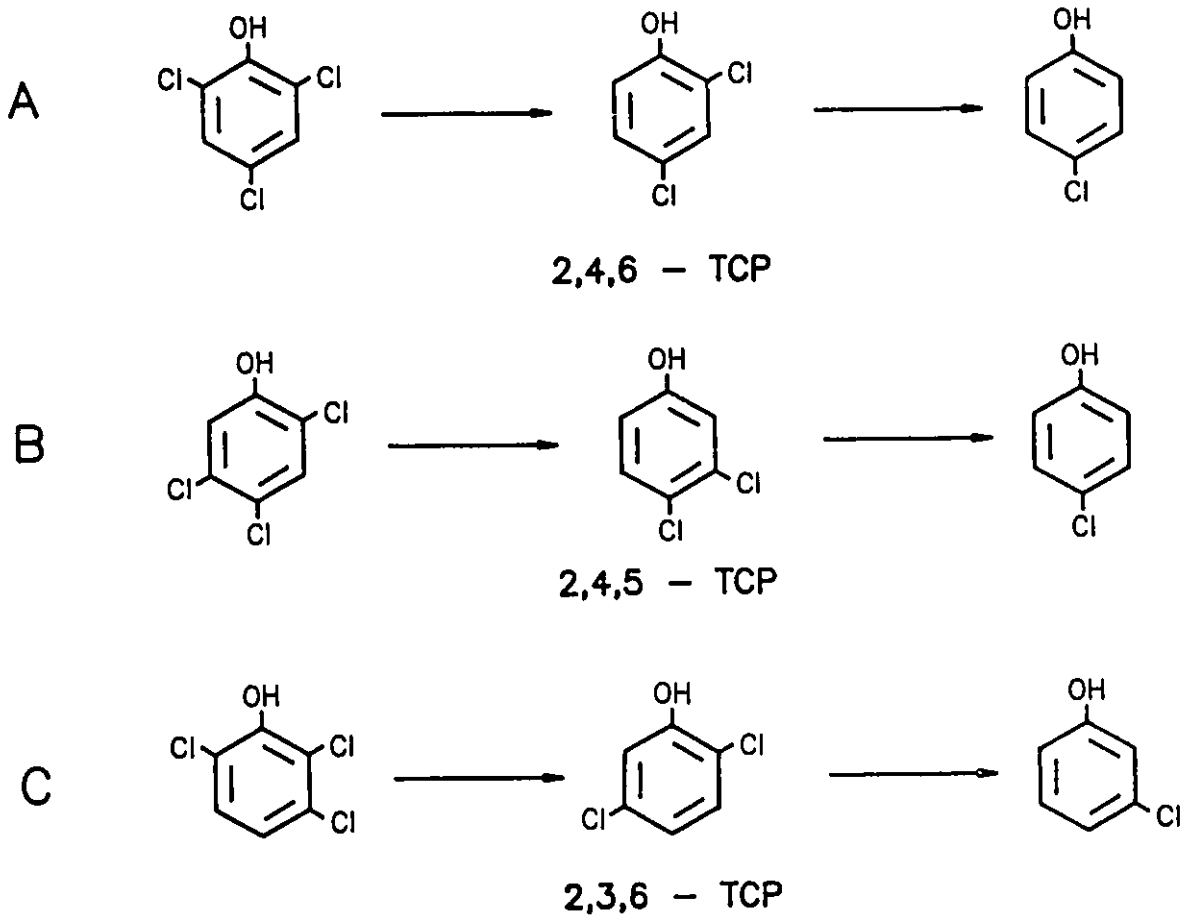


Figure 4.15a Chlorophenol Biodegradation Pathways

(a) 2,4,6-TCP, (b) 2,4,5-TCP, (c) 2,3,6-TCP

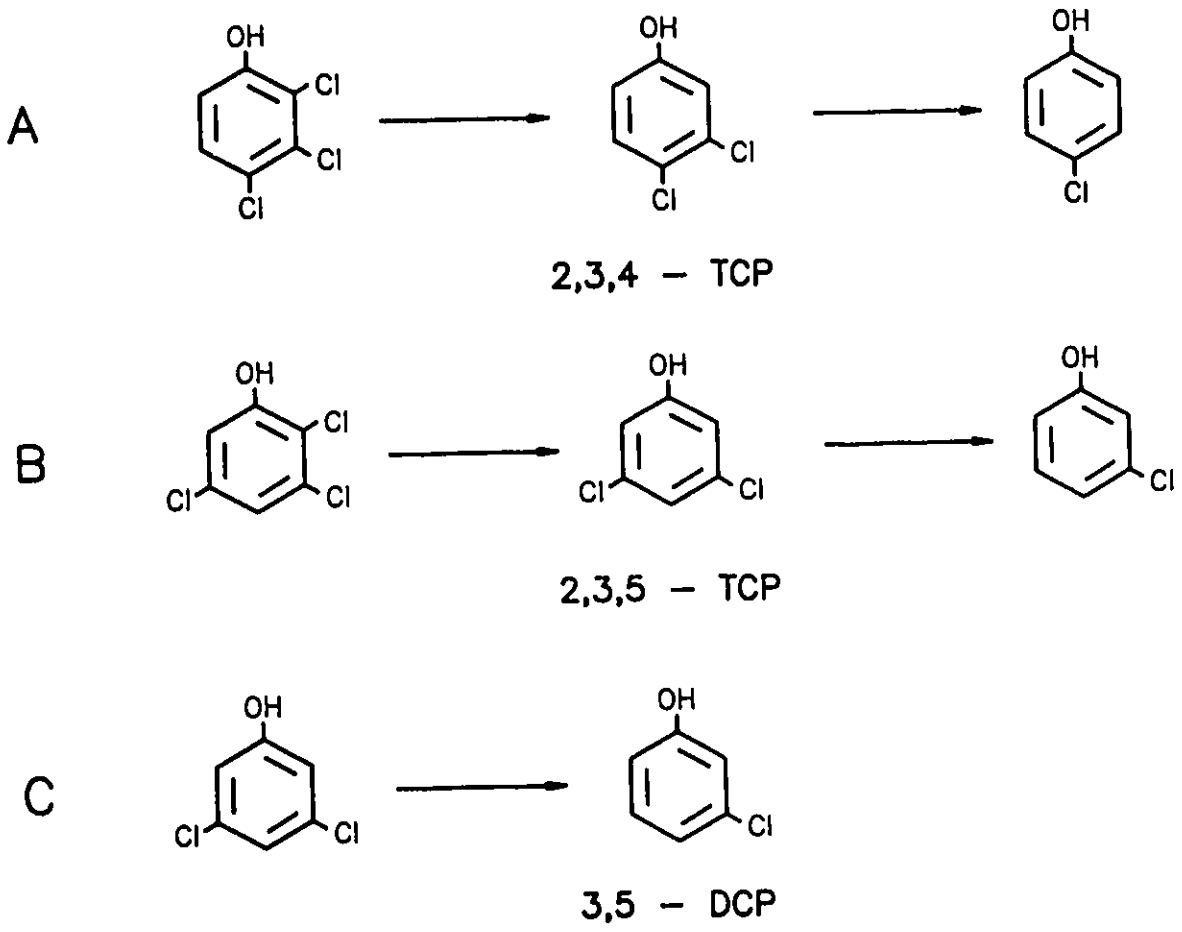


Figure 4.15b Chlorophenol Biodegradation Pathways

(a) 2,3,4-TCP,(b) 2,3,5-TCP,(c) 3,5-DCP

Table 4.6a Steady State 2,4,6-TCP and its Metabolites Removal Efficiency, 3 day HRT

3 day HRT

TIME PERIOD								
FROM DAY:		3	10	15	46	59	100	162
TO DAY:		9	14	45	58	82	161	175
2,4,6-TCP								
INFLUENT CONC. (mg/L)		1.25	2.5	6	12	18	18	25
STEADY STATE REACTOR	Ave	0	0	0.82	1.46	0	0	0
CONC. (mg/L)	STD	0	0	0.843	0.736	0	0	0
SORBED BY BIOMASS (mg/L)				0.3	0.5	0.0	0.0	0.0
TOTAL REMOVAL (%) *		100.0	100.0	86.3	87.8	100.0	100.0	100.0
REMOVAL BY SORPTION (%) *				5.7	3.9	0.0	0.0	0.0
2,4-DCP								
STEADY STATE REACTOR	Ave	0	0	0	0	0	0	0
CONC. (mg/L)	STD	0	0	0	0	0	0	0
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL REMOVAL (%)		100.0	100.0	100.0	100.0	100.0	100.0	100.0
REMOVAL BY SORPTION (%)		0.0	0.0	0.0	0.0	0.0	0.0	0.0
4-MCP								
STEADY STATE REACTOR	Ave	0.2	0.5	2.4	4.23	7.47	4.45	5.98
CONC. (mg/L)	STD	0.155	0.098	0.587	0.449	0.576	0.687	1.521
SORBED BY BIOMASS (mg/L)		0.1	0.2	1.0	1.7	3.0	1.8	2.4
TOTAL REMOVAL (%)		75.3	69.1	23.3	35.0	35.8	61.8	63.0
REMOVAL BY SORPTION (%)		11.7	14.0	23.3	26.6	25.6	15.6	14.9

Table 4.6b Steady State 2,4,6-TCP and its Metabolites Removal Efficiency, 1 day HRT

1 day HRT

TIME PERIOD							
FROM DAY:		176	183	197	209	225	236
TO DAY:		182	196	208	224	235	248
2,4,6-TCP							
INFLUENT CONC. (mg/L)		4	6	12	18	25	50
STEADY STATE REACTOR	Ave	0	0	0.15	0	0.93	11.8
CONC. (mg/L)	STD	0	0	0.261	0	1.106	2.572
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.1	0.0	0.4	1.5
TOTAL REMOVAL (%)		100.0	100.0	98.8	100.0	96.3	76.4
REMOVAL BY SORPTION (%)		0.0	0.0	1.1	0.0	1.5	2.9
2,4-DCP							
STEADY STATE REACTOR	Ave	0.1	0	0	0	0	4.55
CONC. (mg/L)	STD	0.23	0	0	0	0	1.34
SORBED BY BIOMASS (mg/L)		0.2	0.0	0.0	0.0	0.0	5.2
TOTAL REMOVAL (%)		97.0	100.0	100.0	100.0	100.0	85.0
REMOVAL BY SORPTION (%)		4.6	0.0	0.0	0.0	0.0	17.1
4-MCP							
STEADY STATE REACTOR	Ave	1.33	0.8	4.95	6.32	10	22
CONC. (mg/L)	STD	1.815	0.469	0.654	0.535	2.828	0.978
SORBED BY BIOMASS (mg/L)		0.6	0.4	2.0	2.5	3.9	8.3
TOTAL REMOVAL (%)		44.3	79.4	34.7	45.7	34.8	
REMOVAL BY SORPTION (%)		24.1	9.1	26.6	21.8	25.6	51.7

4 to 50 mg/L.d, SLR of 0.2 to 2.5 mg/g VSS.d). Removal of 4-MCP was largely attributed to sludge sorption. However, when the loading concentration was increased to 50 mg/L, 2,4,6-TCP removal efficiency decreased to 76.4% coupled with accumulation of 2,4-DCP and 4-MCP; 2,4-DCP steady state concentration increased to 4.6 mg/L, with removal efficiency of 85%. Biosorption was attributed to account for 17.1%; 4-MCP steady state concentration increased to 22 mg/L and was removed mainly by sorption (Table 4.6b). The concentration of acetic acid in the reactor was not elevated. These results indicate that the reactor had approached the 2,4,6-TCP loading limit but the activities of acidogens and methanogens were not inhibited.

A mass balance of chlorophenols in R246 was done to verify the observed 2,4,6-TCP biodegradation pathway and to determine the role of sludge sorption in chlorophenols removal. The observed 2,4,6-TCP biodegradation pathway was as follows: 2,4,6-TCP degradation began with reductive dehalogenation of an ortho chlorine to produce 2,4-DCP, then 2,4-DCP was further reduced at the ortho chlorine to produce 4-MCP. Detectable levels of phenol were not observed and no effort was made to monitor aromatic ring cleavage products throughout the experiment.

Ideally, if there was no aromatic ring cleavage, (no transformation of MCP to methane gas), the total mass of TCP and its metabolites in the reactor should be identical to the total mass added minus the amount sorbed by granular biomass. Cumulative values for the above during the 3 day HRT and for the whole

experimental period are shown in Table 4.7. The sorption isotherms were employed to estimate chlorophenols sorbed by biomass, and the measured liquid phase concentration on the last day of each experimental period was used as the liquid phase concentration. Molar concentrations were used in the mass balance calculation. The conversion factors for mole concentration and pure concentration of chlorophenols are shown in Appendix D.

Figure 4.16 shows a mass balance of 2,4,6-TCP and its metabolites. In the whole experimental period, total 2,4,6-TCP added was 50855 μM, total chlorophenols (tri- plus di- plus MCP) washed out was 38947 μM, the total amount of chlorophenols sorbed by biomass was 1342 μM, therefore the mass deficit in R246 was 10566 μM (20.8%). The final product detected in the reactor was 4-MCP. The mass of 4-MCP in the reactor was fairly close to the amount produced from its parent compound indicating most 4-MCP was washed out and 4-MCP was sparsely degraded in R246.

4.7.4 Toxicity of 2,4,6-TCP

No severe acidogens and methanogens inhibition occurred in R246 reactor throughout the experiment. However, at the highest loading rate of 50 mg/L, i.e., the SLR of 2.5 mg/g VSS.d, there was accumulation of 2,4,6-TCP, 2,4-DCP and 4-MCP in the reactor. As

Table 4.7 Mass Balance of CPs in UASB Reactors

3 day HRT

FEED	AMOUNT ADDED uM	MASS OUT			SORPTION CPs 1		SUM. SOR&OUT uM	MASS DEFICIT	
		TCP uM	DCP uM	MCP uM	uM	%		uM	%
2,3,4-TCP	10757.2	479.9	3255.2	28.3	0.0	0.00	3763.4	6993.8	65.0
2,3,6-TCP	16525.6	844.5	915.5	7650.1	15.2	0.09	9425.3	7100.3	43.0
2,3,5-TCP	10635.3	4846.8	1923.5	69.7	847.4	7.97	7687.4	2947.9	27.7
2,4,6-TCP	16764.9	1318.6	52.1	8118.1	132.0	0.79	9620.8	7144.1	42.6
2,4,5-TCP	6498.4	1853.0	2815.8	0.0	365.4	5.62	5034.2	1464.2	22.5
3,5-DCP	10357.1		3236.6	1336.2	48.6	0.47	4621.4	5735.7	55.4

3 day & 1 day HRT

FEED	MASS ADDED uM	MASS OUT			SORPTION CPs		SUM. SOR&OUT uM	MASS DEFICIT	
		TCP uM	DCP uM	MCP uM	uM	%		uM	%
2,3,4-TCP	47448	1385.3	4340.8	28.2	94.1	0.20	5848.4	41599.6	87.7
2,3,6-TCP	57021	3420.3	2840.4	17896	254.5	0.45	24411.2	32609.8	57.2
2,3,5-TCP	43670.2	7604.4	2989.1	839.9	757.5	1.73	12190.9	31479.3	72.1
2,4,6-TCP	50855.1	5778.5	1575.4	31593.5	1341.8	2.64	40289.2	10565.9	20.8
2,4,5-TCP	24907.5	4548.8	5436.7	117.6	282.8	1.14	10385.9	14521.6	58.3
3,5-DCP	79862.9		15967.5	25359.6	4898.7	6.13	46225.8	33637.1	42.1

1 The amount of CPs sorbed by biomass was estimated by Freundlich isotherms and effluent concentrations of CPs on the last day of each running period.

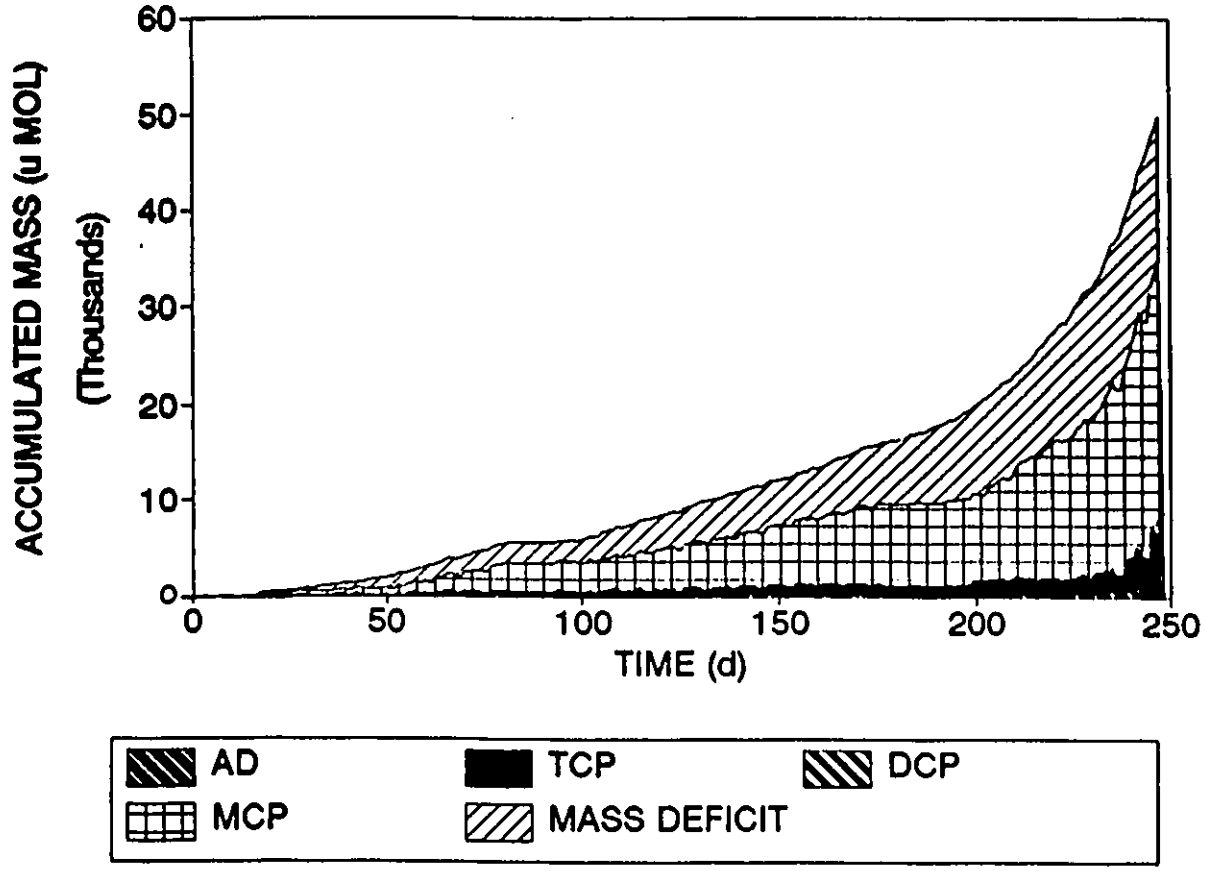


Figure 4.16 Mass Balance of 2,4,6-TCP

mentioned above this indicates that 2,4,6-TCP was beginning to inhibit biomass dechlorination in the reactor. However, the rate of degradation of co-substrate was not affected (Figure 4.12), indicating that the activities of acidogenic and methanogenic bacteria were not inhibited by this 2,4,6-TCP loading rate.

4.8 Dechlorination of 2,4,5-TCP in a UASB Reactor

4.8.1 General Reactor Performance

The general performance of reactor R245 used to treat 2,4,5-TCP was fairly similar to R246. In the course of the experiment, R245 was operated for 223 days, 122 days at 3 day HRT followed by 101 days at 1 day HRT. During the 3 day HRT, the 2,4,5-TCP was applied continuously at concentrations between 1.25 mg/L (0.02 mg/g VSS.d) to 18 mg/L (SLR of 0.3 mg/g VSS.d). Five 2,4,5-TCP loading concentrations (1.25, 2.5, 6.0, 12.0, 18.0 mg/L) were applied. Complete removal of 2,4,5-TCP was achieved at a low loading rates (1.25, 2.5 and 6.0 mg/L). Severe inhibition occurred at a loading rate of 18 mg/L (SLR of 0.3 mg/g VSS.d). Under these condition 2,4,5-TCP and its metabolite 3,4-DCP increased to 16 and 4.5 mg/L, respectively (Fig. 4.17). The acetic acid concentration increased to 300 mg/L and biogas production decreased to 4 L/d (Fig. 4.18). In order to recover the activities of bacteria in R245, R245 was not fed for 15 days but the recycle pump was kept on. After this time period, R245 was supplied with synthetic feed and 2,4,5-TCP at concentration of 12 mg/L at 3 day HRT until the HRT was shifted to 1 day.

At 1 day HRT, the 2,4,5-TCP concentration treated ranged from 4 mg/L (SLR of 0.2 mg/g VSS.d) to 18 mg/L (SLR of 0.9 mg/g VSS.d) and four concentrations (4.0, 6.0, 12.0, 18.0 mg/L) were applied. The removal efficiencies of 2,4,5-TCP were enhanced in this stage but inhibition still occurred at SLR of 0.9 mg/g VSS. Similar to the situation at 3 day HRT, the removal efficiencies of 2,4,5-TCP and 3,4-DCP decreased, biogas production decreased and acetic acid concentration in the reactor elevated to 280 mg/L (Fig. 4.18), indicating the activities of bacteria responsible for degradation of both chlorinated compounds and COD were inhibited and the reactor had reached the treatability limit.

Biogas volume, acetic acid concentration in influent and effluent, and COD removal are shown in Figures 4.18 and 4.19 respectively. Biogas component was approximately 65% for non-inhibition period.

4.8.2 Biodegradation Pathway for 2,4,5-TCP

Biodegradation of 2,4,5-TCP and its biodegradation pathway were determined by HPLC analysis. At the low 2,4,5-TCP loading rate (1.25 mg/L), 2,4,5-TCP and its metabolites were not detected in the reactor effluent (Fig. 4.17). However, within 3 days of 2,4,5-TCP addition at 6.0 mg/L (SLR of 0.1 mg/g VSS.d), i.e., with acclimation period of 12 days, 0.64 mg/L of 3,4-DCP (a reductive dehalogenation product of 2,4,5-TCP) was detected in the reactor effluent. The fact that the reactor sludge had never been exposed to chlorinated compounds indicated that a significant population of bacteria was present in the reactor with the inherent ability to

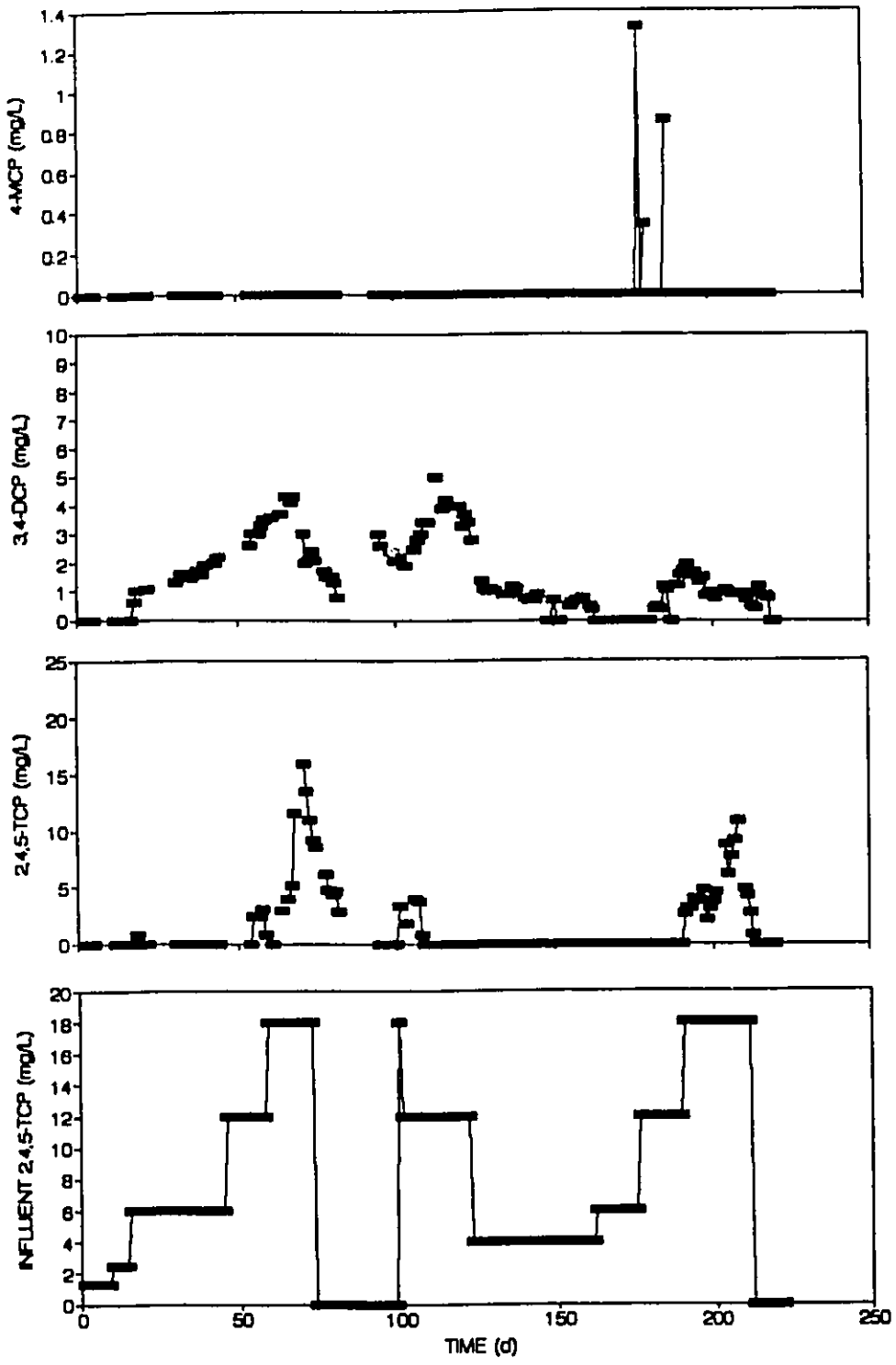


Figure 4.17 Soluble Concentration of 2,4,5-TCP and its Metabolite in R245

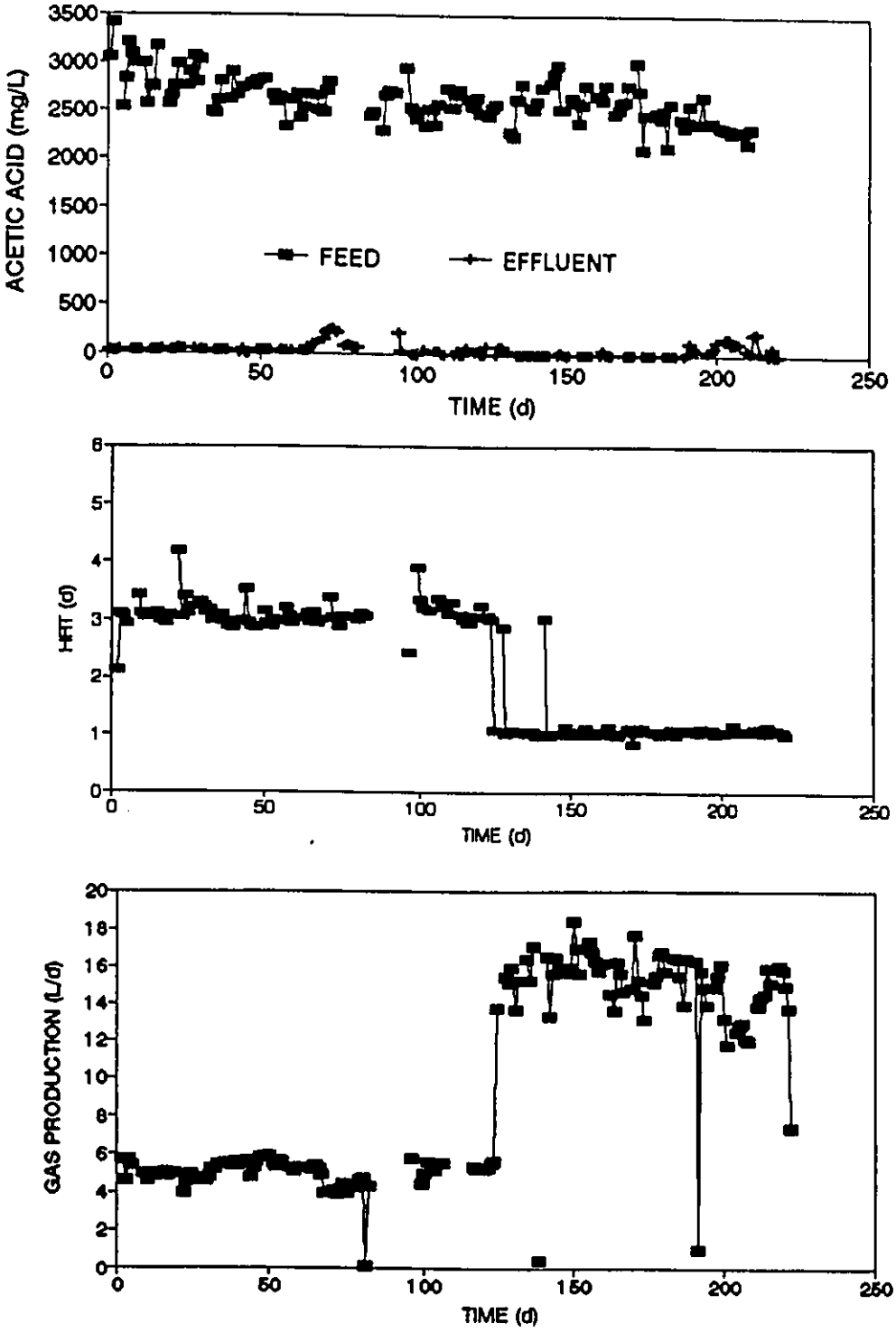


Figure 4.18 General R245 Reactor Performance.

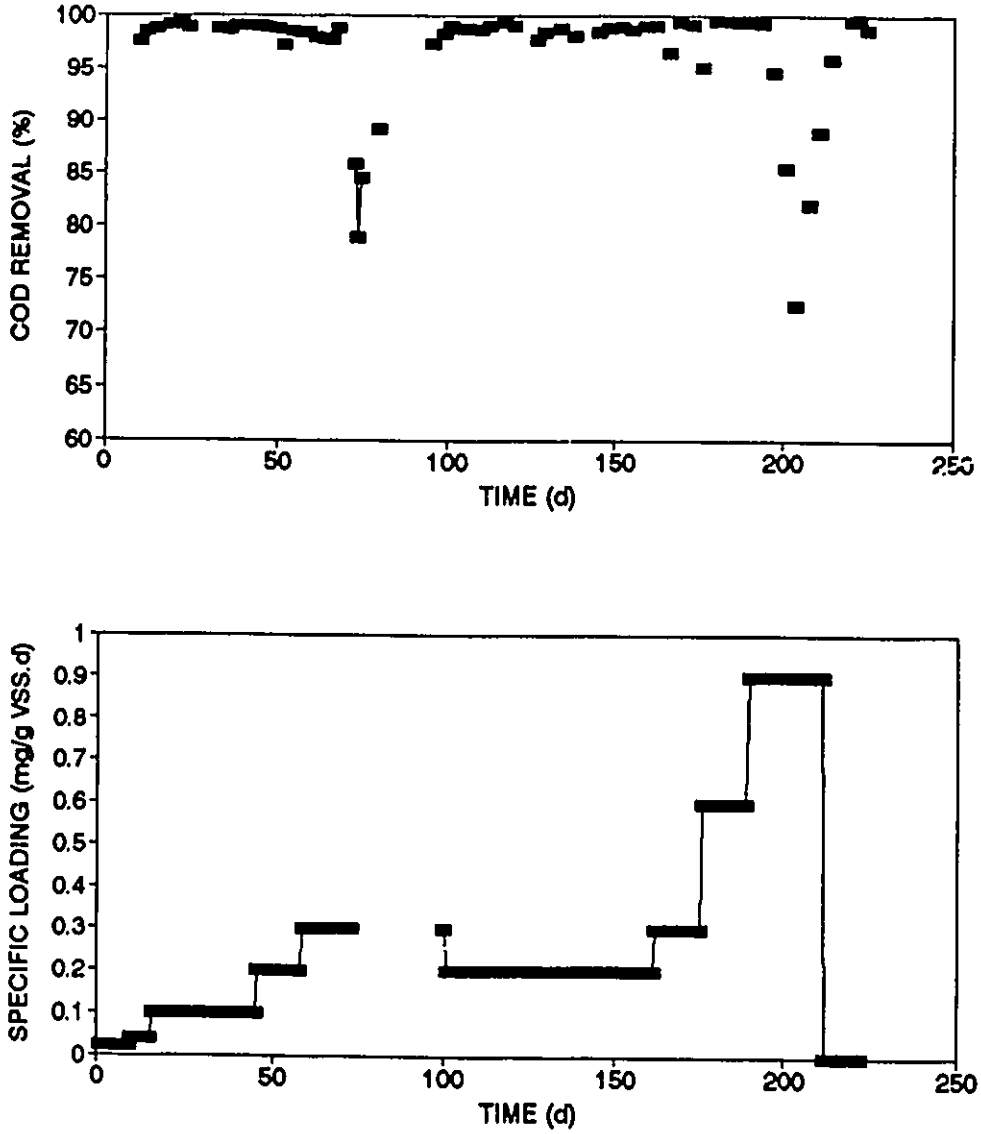


Figure 4.19 Treatment Efficiency of the R245 Reactor under Toxic Loading

degrade 2,4,5-TCP.

Following a long acclimation period (180 days), higher 2,4,5-TCP concentration (12 mg/L) and higher loading (1 day HRT), 4-MCP was also detected in the reactor effluent. The detection of 3,4-DCP at low loads followed by 4-MCP at high load rates indicates that 2,4,5-TCP degradation begins with removal of an ortho-Cl producing 3,4-DCP, followed by removal of a meta-Cl to produce 4-MCP. At no time during the experiment (3 or 1 day HRT) was 3-MCP detected in the reactor or effluent. Additionally, 3-MCP was not detected when sludge samples from R245 were extracted and analyzed by GC-MS. The fact that 3,4-MCP was the main intermediate accumulated in the reactor would suggest that 3,4-DCP dechlorination was poor. Since no significant amount of 2,4,5-TCP accumulated in the reactor (except in inhibition period), it suggested that the rate of reductive dechlorination of ortho-Cl was high. The observed 2,4,5-TCP biodegradation pathway is shown in Fig. 4.15a.

As with 2,4,6-TCP, phenol was not detected in the reactor. On day 120 of the experiment, one mg of C-14 labelled radioactive 2,4,5-TCP was added to R245 in order to monitor the mineralization of the compound. However the amounts of radioactive CH_4 and CO_2 observed was small, indicating that only partial 2,4,5-TCP removal was occurring and that it was not being completely mineralized. Dechlorination of the meta chlorine of 3,4-DCP would seem to be the rate limiting step in the dechlorination process in R245.

4.8.3 The Fate of 2,4,5-TCP in R245

The removal efficiency and average concentrations of 2,4,5-TCP

and its biodegradation products during steady state conditions in R245 reactor are shown in Tables 4.8a and 4.8b. Table 4.8a indicates that at 3 day HRT, 2,4,5-TCP disappeared completely when the loading concentration was 1.25, 2.5, and 6 mg/L. This was due to a combination of biodegradation and sludge biosorption. When the loadings were increased to 12 mg/L, TCP removal efficiency was 79%. When the loading was 18 mg/L, the removal efficiency decreased to 28%. 3,4-DCP tended not to be degraded and based on sorption data the removal of 3,4-DCP was largely attributed to sludge biosorption.

At 1 day HRT, 100% removal of 2,4,5-TCP was achieved at most loading conditions. However, when the loading concentration was increased to 18 mg/L, the steady state concentration of 2,4,5-TCP in the reactor increased to 7.5 mg/L, its corresponding removal efficiency was 59% of which 20% was attributed to sludge sorption. The concentrations of 3,4-DCP and 4-MCP at steady state were not elevated at this loading rate. The removal efficiency of 3,4-DCP was enhanced at 1 day HRT, a 100% removal was achieved at a loading concentration of 6 mg/L of 2,4,5-TCP. There was no analytical evidence to indicate that 4-MCP was degraded. The fact that 4-MCP was detected at only one concentration (12 mg/L, 1 day HRT) coupled with the poor mineralization with C-14 labelled 2,4,5-TCP suggests that 3,4-DCP may be a difficult intermediate to be dechlorinated.

Similar to the mass balance for R246 (Section 4.7.3), a mass balance of chlorophenols in R245 was also done to verify the

Table 4.8a Steady State 2,4,5-TCP and its Metabolites Removal Efficiency, 3 day HRT

3 day HRT

TIME PREIOD									
FROM DAY:		3	10	16	46	59	74	95	100
DAY:		9	15	45	58	73	82	99	122
2,4,5-TCP									
INFLUENT CONC. (mg/L)		1.25	2.5	6	12	18	0	0	12
STEADY STATE REACTOR	Ave	0	0	0	2.53	13	5.75	0	0
CONC. (mg/L)	STD	0	0	0	0.32	1.91	2.18	0	0
SORBED BY BIOMASS (mg/L)				0.0	1.5	6.2	3.0	0.0	0.0
TOTAL REMOVAL (%)		100.0	100.0	100.0	78.9	27.8			
REMOVAL BY SORPTION (%)				0.0	12.2	27.8			0.0
3,4-DCP									
STEADY STATE REACTOR	Ave	0	0	1.03	3.1	2.85	1.61	2.2	3.67
CONC. (mg/L)	STD	0	0	0.124	0.141	0.923	0.485	0.864	0.286
SORBED BY BIOMASS (mg/L)		0.0	0.0	1.2	4.1	3.7	2.0	2.8	4.9
TOTAL REMOVAL (%)		100.0	100.0	79.2	53.0				62.9
REMOVAL BY SORPTION (%)		0.0	0.0	24.9	53.0				49.8
4-MCP									
STEADY STATE REACTOR	Ave	0	0	0	0	0	0	0	0
CONC. (mg/L)	STD	0	0	0	0	0	0	0	0
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL REMOVAL (%)									
REMOVAL BY SORPTION (%)									

Table 4.8b Steady State 2,4,5-TCP and Its Metabolites Removal Efficiency, 1 day HRT

1 day HRT

TIME PREIOD						
FROM DAY:		123	162	176	190	212
DAY:		161	175	189	211	223
2,4,5-TCP						
INFLUENT CONC. (mg/L)		4	6	12	18	0
STEADY STATE REACTOR	Ave	0	0	0	7.45	0.43
CONC. (mg/L)	STD	0	0	0	1.75	
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	3.8	0.3
TOTAL REMOVAL (%)		100.0	100.0	100.0	58.6	
REMOVAL BY SORPTION (%)		0.0	0.0	0.0	21.2	
3,4-DCP						
STEADY STATE REACTOR	Ave	0.675	0	0.875	0.95	0.58
CONC. (mg/L)	STD	0.108	0	0.508	0.033	0.404
SORBED BY BIOMASS (mg/L)		0.8	0.0	1.0	1.1	0.7
TOTAL REMOVAL (%)		79.5	100.0	91.1	82.9	
REMOVAL BY SORPTION (%)		23.5	0.0	10.4	20.3	
4-MCP						
STEADY STATE REACTOR	Ave	0	0	0.2	0	0
CONC. (mg/L)	STD	0	0	0.36	0	0
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.1	0.0	0.0
TOTAL REMOVAL (%)						
REMOVAL BY SORPTION (%)						

observed 2,4,5-TCP biodegradation pathway and to determine the role of the sludge biosorption in chlorophenols removal. The sum of 2,4,5-TCP added, 2,4,5-TCP and 3,4-DCP and 4-MCP in the effluent for the 3 day HRT and the whole experimental period are shown in Table 4.7 and Figure 4.20. For the whole experimental period, total 2,4,5-TCP added was 24908 μM, the total chlorophenols (2,4,5-TCP, 3,4-DCP and 4-MCP) washed out from R245 were 10386 μM, the total amount of chlorophenols sorbed by biomass was 283 μM. The mass deficit in R245 for the whole experimental period was 14522 μM, i.e., 58.3%. At 3 day HRT, the percentage of mass deficit was 22.5%. However based on a single radioactive test on day 120 of the experiment, it is impossible to conclude that the unclosed mass balance was attributed to mineralization of 2,4,5-TCP.

4.8.4 Toxicity of 2,4,5-TCP

Severe inhibition occurred in R245 reactor at loading concentration of 18 mg/L of 2,4,5-TCP at both 3 day HRT and 1 day HRT (i.e. the SLR of 0.3 and 0.9 mg/g VSS.d at 3 day and 1 day HRT respectively). When these loadings were applied, the concentration of 2,4,5-TCP in reactor effluent increased to 13.0 and 7.5 mg/L for 3 day HRT and 1 day HRT, respectively (Fig. 4.17). The acetic acid concentration in the reactor increased to 400 mg/L (at normal conditions the acetic acid concentration was about 40 mg/L in the reactor, Fig. 4.18). The COD removal efficiency also decreased to 73% (Fig. 4.19) and biogas production decreased. This indicates that 2,4,5-TCP at 18 mg/L inhibited the activities of acidogens and methanogens in addition to inhibiting dechlorination of 2,4,5-TCP.

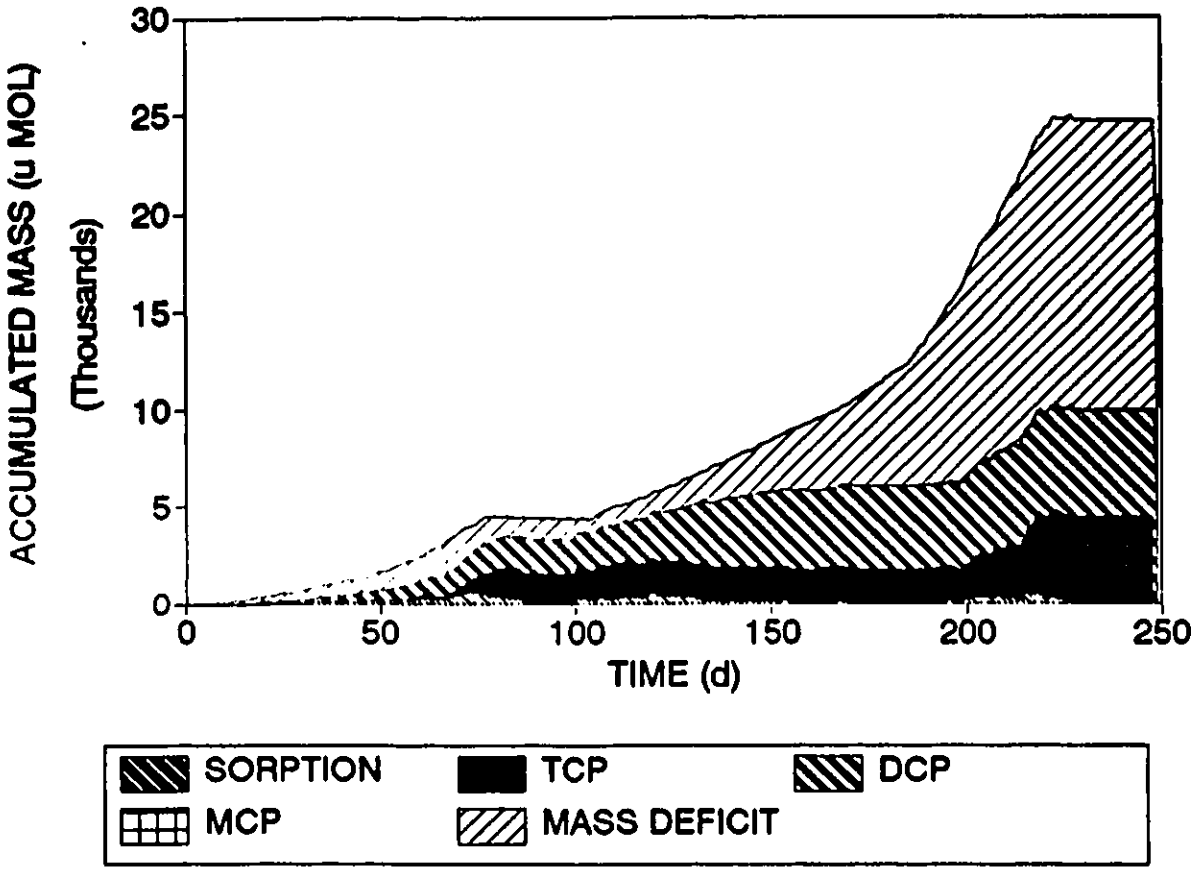


Figure 4.20 Mass Balance of 2,4,5-TCP

4.9 Dechlorination of 2,3,6-TCP in a UASB Reactor

4.9.1 General Reactor Performance

The general performance of R236 reactor used to treat 2,3,6-TCP was in general similar to R246. R236 was operated for 258 days, 175 days at 3 day HRT, followed by 83 days at 1 day HRT. Following 4 days of treatment of sucrose/acetic synthetic wastewater, R236 was dosed continuously with 2,3,6-TCP at stepwise increase in loading rate. During the 3 day HRT, six 2,3,6-TCP loading rates (1.25, 2.5, 6.0, 12.0, 18.0, 25.0 mg/L) were used corresponding to SLR between 0.02 and 0.42 mg/g VSS.d. The specific COD loading rate of was 0.08 g/g VSS, however, a 100% removal of 2,3,6-TCP was achieved at most loading rates and no methanogenic or acidogenic inhibition was observed.

At 1 day HRT, the 2,3,6-TCP concentrations of 4.0, 6.0, 12.0, 18.0, 25.0, 50.0 mg/L were applied. The removal efficiency of 2,3,6-TCP was enhanced in this stage. At a loading concentration of 50 mg/L (SLR of 2.5 mg/g VSS.d) the removal efficiency of 2,3,6-TCP was 95% (Table 4.9b). Reactor acetic acid remained low and biogas production was unaffected indicating no inhibition of methanogenic and acidogenic activities occurred. Among the four TCPs (2,3,4-; 2,3,6-; 2,4,5-; 2,4,6-TCP) which were run in parallel, 2,3,6-TCP was the least toxic to anaerobic bacteria.

Biogas volume and methane component, and acetic acid concentration in influent and effluent were measured daily. In general, the biogas production was approximately 3 L per 5 g of synthetic feed (Fig. 4.21) and methane component in the biogas was

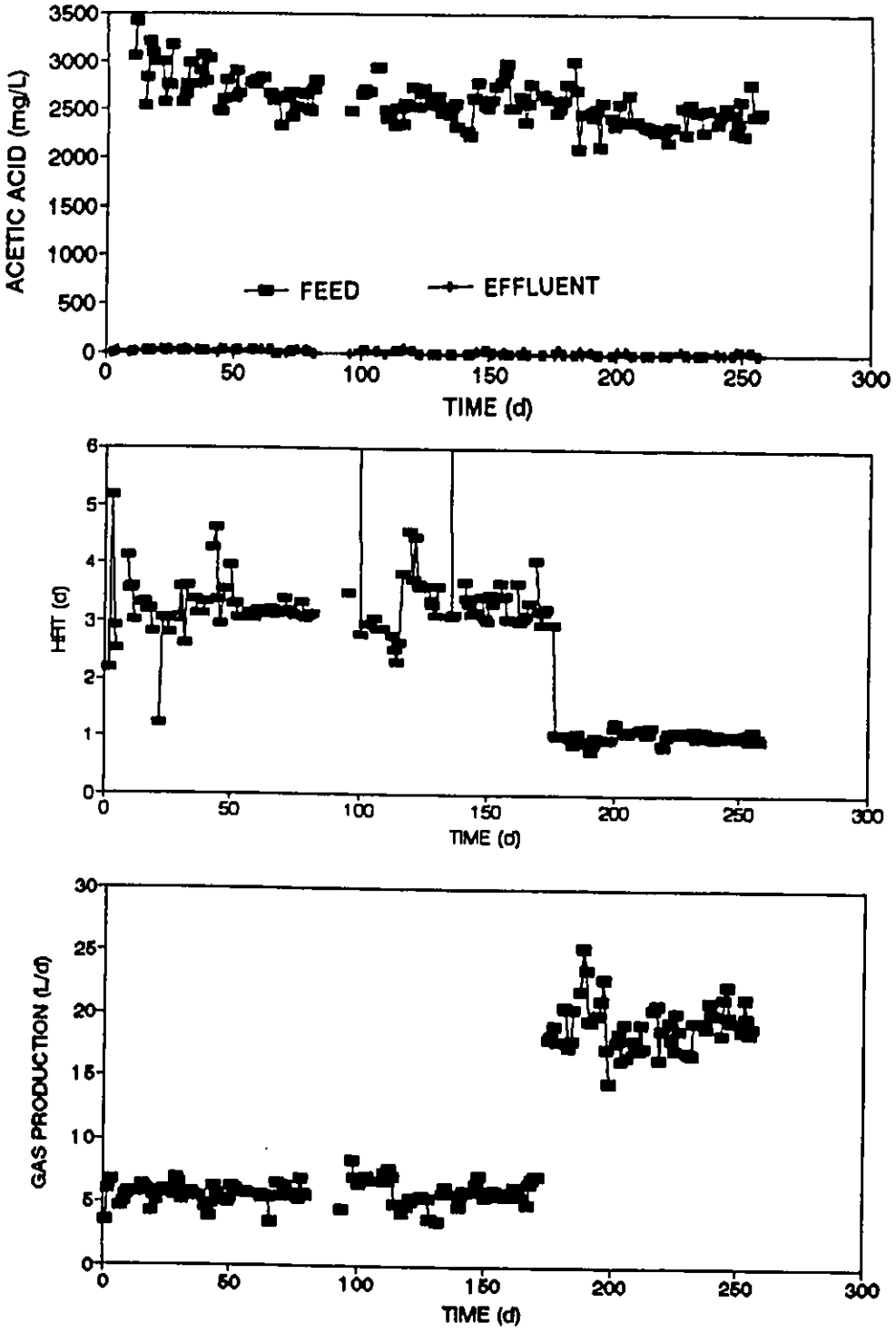


Figure 4.21 General R236 Reactor Performance.

approximately 65%. The COD removal efficiency was in a range of 96.0 to 99.8% (Fig. 4.22). In the course of the experiment, the pH was in the range of 7.4 to 7.6 and alkalinity was in range of 3200 to 3600 mg/L as $CaCO_3$. High COD removal efficiency coupled with the low concentration of acetic acid in the R234 indicated that acidogenic and methanogenic anaerobes were not inhibited by 2,3,4-TCP and its metabolites.

During 3 day HRT, the feed pump failed twice. Consequently, 2,3,6-TCP concentrations in the reactor were elevated (Fig. 4.23). The chlorophenol syringe pump was stuck three times at the loading concentration of 50 mg/L, 1 day HRT. But the reactor finally reached the steady state at this loading rate.

4.9.2 Biodegradation Pathway for 2,3,6-TCP

Biodegradation of 2,3,6-TCP and its biodegradation pathway were determined by HPLC analysis. At a 2,3,6-TCP loading rate of 1.25 mg/L, 2,3,6-TCP and its metabolites were not detected in the reactor or liquid effluent (Fig. 4.23). However, within 2 days of 2,3,6-TCP addition at a concentration of 6.0 mg/L (SLR 0.11 mg/g VSS.d), 0.4 mg/L of 2,5-DCP (a reductive dehalogenation product of 2,3,6-TCP) was detected in the reactor effluent. The fact that the reactor sludge had never before been exposed to chlorinated compounds indicated that a significant population of bacteria was present in the reactor with the inherent ability to remove the ortho chlorine from 2,3,6-TCP. one day after 2,5-DCP was detected, 0.5 mg/L of 3-MCP (a metabolite of 2,5-DCP) was detected in the reactor.

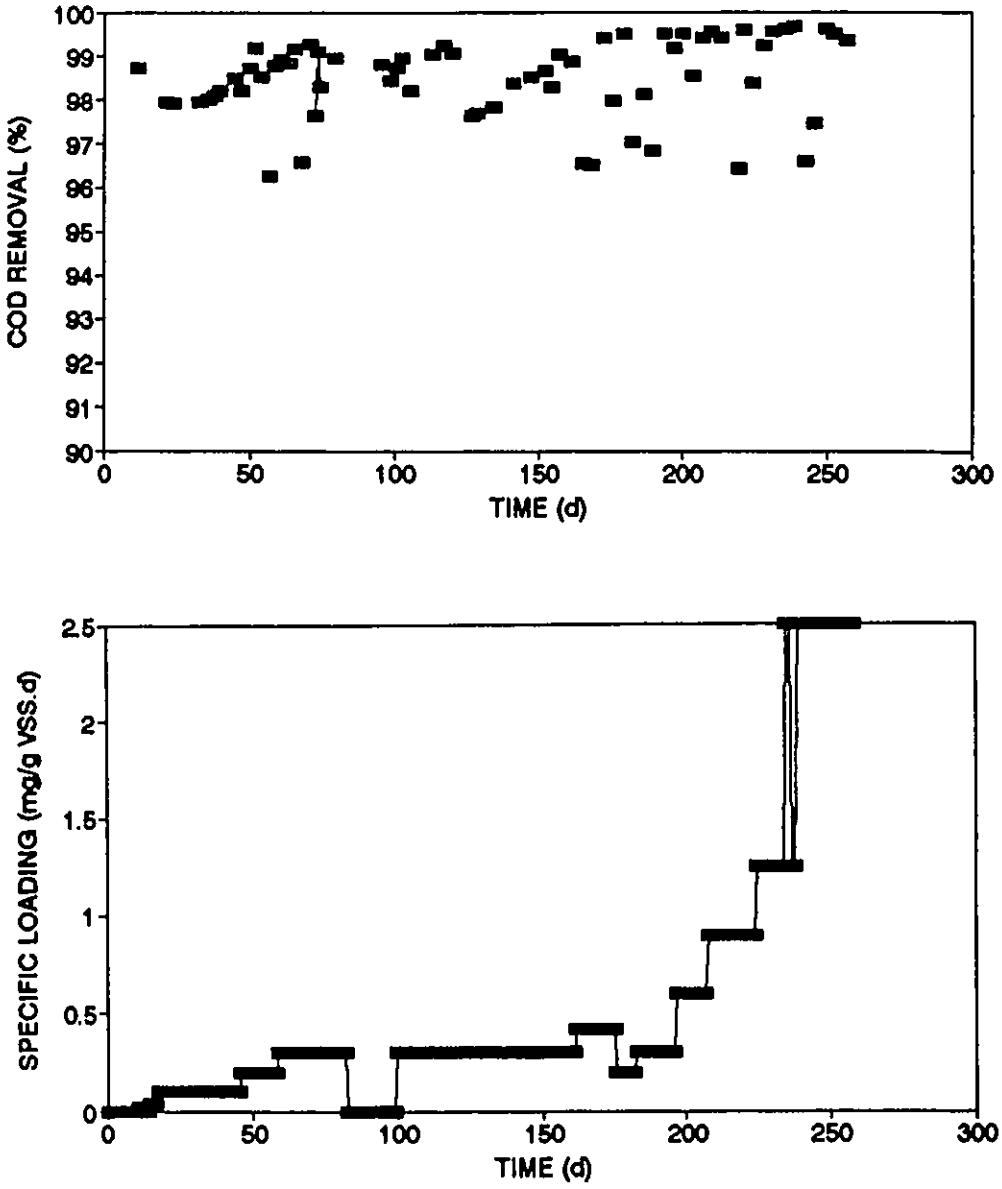


Figure 4.22 Treatment Efficiency of the R236 Reactor under Toxic Loading

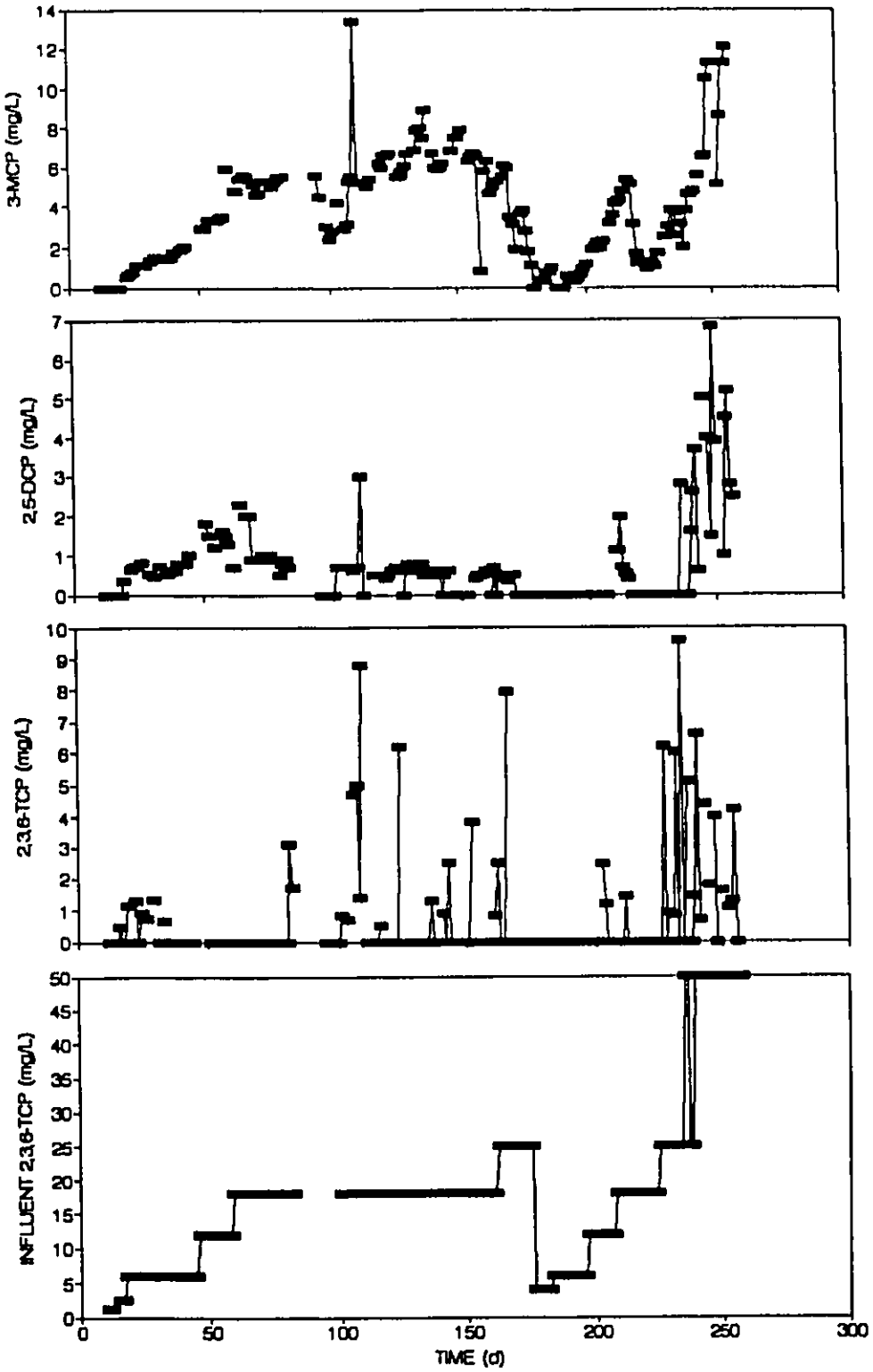


Figure 4.23 Soluble Concentration of 2,3,6-TCP and its Metabolite in R236

Detection of 2,5-DCP and 3-MCP in reactor R236 indicates that 2,3,6-TCP degradation begins with removal of an ortho-Cl producing 2,5-DCP, followed by removal of a second ortho-Cl to produce 3-MCP. At no time during the experiment was 2-MCP detected in the effluent but 2,3-DCP was occasionally detected at high loading rates of 2,3,6-TCP. The fact that 3-MCP was the main intermediate accumulated in the reactor would suggest that meta-Cl dechlorination was poor, additionally since no significant amounts of 2,3,6-TCP or 2,5-DCP accumulated in the reactor (at most loading rates) suggests that the rate of reductive dechlorination of both ortho-Cls was high for both 2,3,6-TCP and 2,5-DCP (Fig. 4.23). At no time was phenol detected in the R236. The pathway of 2,3,6-TCP anaerobic biodegradation is summarized in Figure 4.15a.

4.9.3 The Fate of 2,3,6-TCP in R236

The removal efficiencies and average concentrations of 2,3,6-TCP and its biodegradation products during steady state in reactor R236 are shown in Table 4.9a and 4.9b. Removal efficiencies and steady state concentrations were calculated in the same way used for R246 (see Section 4.4.3). Table 4.9a indicates that at 3 day HRT, 2,3,6-TCP was removed to below detective limits when the influent concentration was 1.25 mg/L. When the loading concentrations were increased to 6, 12, 18 and 25 mg/L, the removal efficiency of 2,3,6-TCP was above 98%. 2,5-DCP tended to not accumulate in the reactor and 2,5-DCP removal efficiency was greater than 84% at all loading rates. There was no analytical evidence that 3-MCP was further degraded in the reactor. However, mass balance calculation

indicated that approximately 72% of the 2,3,6-TCP was biodegraded.

At 1 day HRT, a 100% removal of 2,3,6-TCP and 2,5-DCP was achieved at most loading concentrations. The removal of 59 to 90% of 3-MCP was achieved while sorption to biomass accounted for 4 to 17% removal. However, when the loading concentration was increased to 50 mg/L, 2,3,6-TCP removal efficiency decreased slightly to 94.8% with concomitant accumulation of 2,5-DCP and 3-MCP (Fig. 4.23). 2,5-DCP steady state concentration increased to 2.9 mg/L from 0, calculated removal efficiency of 93%. 3-MCP steady state concentration increased to 6.9 mg/L removal efficiency of 74% (Table 4.9b). However, removal efficiency of chlorophenols in R236 remained high at a loading concentration 50 mg/L indicating that 2,3,6-TCP loading capacity of the reactor had not been exceeded.

A mass balance of chlorophenols in R236 was also done. The accumulation of 2,3,6-TCP added, 2,3,6-TCP and 2,5-DCP and 3-MCP in the effluent for 3 day HRT and the complete experimental period are shown in Table 4.7 and Figure 4.24. Chlorophenols sorbed to biomass were estimated by the individual chlorophenol sorption isotherms, biomass concentration data and measured liquid phase concentrations of the compounds. Throughout the experiment, total 2,3,6-TCP added was 57021 μM, total chlorophenols (2,3,6-TCP, 2,5-DCP and 3-MCP) washed out from R236 was 24156 μM, the amount of chlorophenols sorbed to biomass was 255 μM, therefore the mass deficit in R236 in the whole experimental period was 31479 μM (55%). At 3 day HRT, the

Table 4.9a Steady State 2,3,6-TCP and its Metabolites Removal Efficiency, 3 day HRT

3 day HRT

TIMR PREIOD							
FROM DAY:		11	15	18	46	59	100	162
TO DAY :		14	17	45	58	82	161	175
2,3,6-TCP								
INFLUENT CONC. (mg/L)		1.25	2.5	6	12	18	18	25
STEADY STATE REACTOR	Ave	0	0.1	0	0	0.32	0.2	0
CONC. (mg/L)	STD	0	0.192	0	0	0.854	0.346	0
SORBED BY BIOMASS (mg/L)			0.1	0.0	0.0	0.1	0.1	0.0
TOTAL REMOVAL (%)		100.0	96.0	100.0	100.0	98.2	98.9	100.0
REMOVAL BY SORPTION (%)			2.6	0.0	0.0	0.7	0.6	0.0
2,5-DCP								
STEADY STATE REACTOR	Ave	0	0	0.8	1.4	1.1	0.4	0.23
CONC. (mg/L)	STD	0	0	0.141	0.158	0.52	0.234	0.233
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.5	0.8	0.6	0.2	0.1
TOTAL REMOVAL (%)		100.0	100.0	83.8	85.8	92.4	97.3	98.9
REMOVAL BY SORPTION (%)		0.0	0.0	9.5	8.1	4.4	1.7	0.7
3-MCP								
STEADY STATE REACTOR	Ave	0	0	1.77	3.37	5.3	6.36	3.18
CONC. (mg/L)	STD	0	0	0.19	0.08	0.34	0.4	0.69
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.7	1.2	1.7	2.0	1.1
TOTAL REMOVAL (%)		100.0	100.0	38.6	44.1	46.9	41.9	80.0
REMOVAL BY SORPTION (%)		0.0	0.0	23.2	19.3	17.3	18.5	7.0

Table 4.9b Steady state 2,3,6-TCP and its Metabolites Removal Efficiency, 1 day HRT

1 day HRT

TIMR PREIOD							
FROM DAY:		176	184	198	208	225	235
TO DAY :		183	197	207	224	234	258
2,3,6-TCP							
INFLUENT CONC. (mg/L)		4	6	12	18	25	50
STEADY STATE REACTOR	Ave	0	0	0	0	3.3	2.62
CONC. (mg/L)	STD	0	0	0	0	3.869	1.83
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.0	0.5	0.5
TOTAL REMOVAL (%)		100.0	100.0	100.0	100.0	86.8	94.8
REMOVAL BY SORPTION (%)		0.0	0.0	0.0	0.0	2.2	0.9
2,5-DCP							
STEADY STATE REACTOR	Ave	0	0	0	0	0	2.9
CONC. (mg/L)	STD	0	0	0	0	0	1.8
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.0	0.0	1.6
TOTAL REMOVAL (%)		100.0	100.0	100.0	100.0	100.0	92.5
REMOVAL BY SORPTION (%)		0.0	0.0	0.0	0.0	0.0	4.1
3-MCP							
STEADY STATE REACTOR	Ave	1.06	0.4	2.11	1.22	2.87	6.86
CONC. (mg/L)	STD	0.96	0.29	0.13	0.24	0.49	3.18
SORBED BY BIOMASS (mg/L)		0.4	0.2	0.8	0.5	1.0	2.2
TOTAL REMOVAL (%)		59.0	89.7	72.8	89.5	79.0	74.4
REMOVAL BY SORPTION (%)		16.6	4.7	10.0	4.2	7.4	8.0

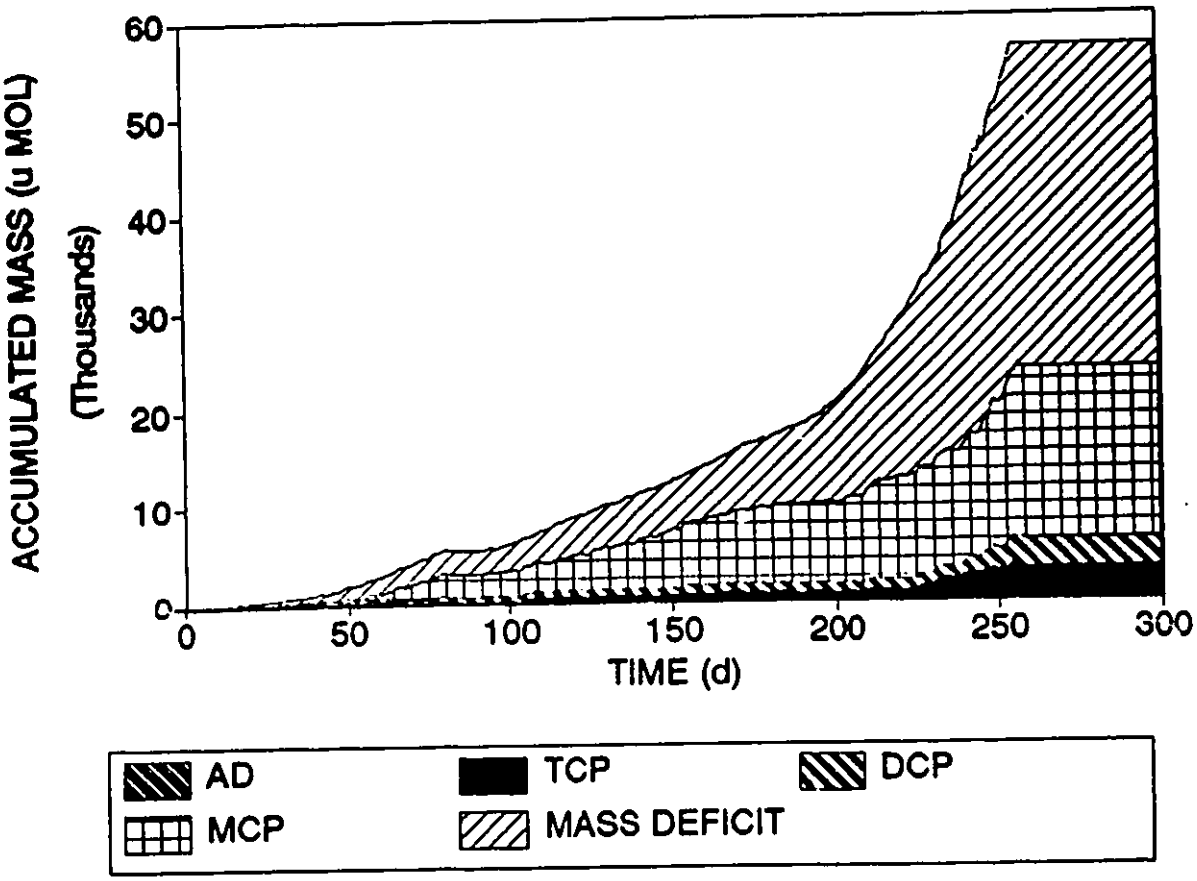


Figure 4.24 Mass Balance of 2,3,6-TCP

percentage of mass deficit was 43%. The mass balance deficit indicates that 2,3,6-TCP was mineralized however, there was no direct analysis evidence to prove that 3-MCP was being mineralized in R236, because phenol was not detected. The radioactive test was not done in R236 reactor.

4.9.4. Toxicity of 2,3,6-TCP

No inhibition of acidogens and methanogens occurred in R236 reactor throughout the experiment. Even at the highest loading rate (50 mg/L at 1 day HRT, i.e. SLR of 2.5 mg/g VSS.d), removal efficiencies of 2,3,6-TCP, 2,5-DCP and 3-MCP were high (95, 93 and 74% respectively). As mentioned in Section 4.9.3, these results indicate that the reactor had not approached the 2,3,6-TCP loading limit. Compared with the COD removal, volatile acid and chlorophenol degradation of other reactors, 2,3,6-TCP was less toxic to anaerobes than 2,4,6-TCP, 2,4,5-TCP and 2,3,5-TCP.

4.10 Dechlorination of 2,3,4-TCP in a UASB Reactor

4.10.1 General Reactor Performance

Reactor R234 treated 2,3,4-TCP in the presence of a readily degradable co-substrate. R234 was operated for 250 days, 189 days at 3 day HRT followed by 61 days at 1 day HRT. Because of shortage of chlorophenol syringe infusion pumps, 2,3,4-TCP was not applied to reactor R234 until 65 days after the reactor was fed with synthetic wastewater. Several 2,3,4-TCP loading rates were applied, most loading rates were maintained for a minimum of two weeks. During the 3 day HRT, six 2,3,4-TCP loading concentrations were

applied to R234 reactor, which were 1.25, 2.5, 6.0, 12.0, 18.0, and 25.0 mg/L (the SLR of 0.02 to 0.42 mg/g VSS.d). Although the COD SLR was 0.08 g/g VSS.d, a 100% removal of 2,3,4-TCP was achieved at most loading rates and no inhibition of methanogens and acidogens occurred.

During the 1 day HRT, the 2,3,4-TCP concentrations ranged from 4 mg/L (SLR of 0.2 mg/g VSS.d) to 50 mg/L (SLR of 2.5 mg/g VSS.d). Removal efficiencies of 2,3,4-TCP were enhanced in this stage. Inhibition of acidogens and methanogens occurred at SLR of 2.5 mg/g VSS.d. At this loading rate, the concentration of 2,3,4-TCP and its metabolite, 3,4-DCP increased to 9 and 3 mg/L in the reactor respectively (Fig. 4.25). The acetic acid concentration increased to 500 mg/L and COD removal efficiency decreased to 81.3% (Fig. 4.26 and 4.27, respectively). This indicated that the activities of acidogens and methanogens were inhibited. It was noticed that a layer of dark substance gradually formed outside granular sludge particles in 1 day HRT period, and the dark substance came off from the granular sludge particle when inhibition of acidogens and methanogens occurred.

Reactor performance was monitored by measuring biogas volume and methane component, acetic acid concentrations in influent and effluent, and COD removal, as shown in Figures 4.26 and 4.27 respectively. In normal conditions, the ratio of volume of biogas produced to volume of synthetic feed was approximately 3:1. Methane component in the biogas was approximately 65%; COD removal

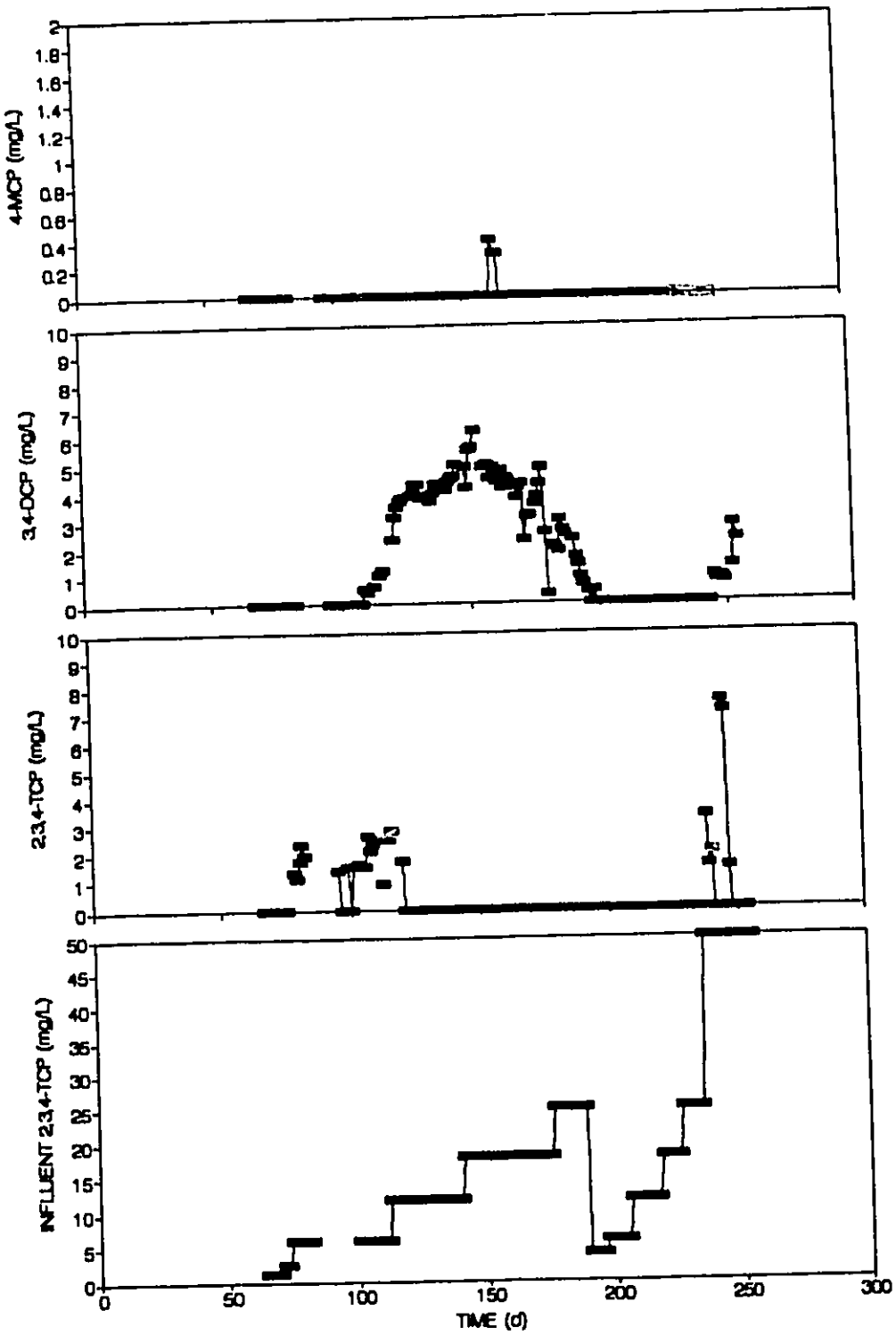


Figure 4.25 Soluble Concentration of 2,3,4-TCP and its Metabolite in R234

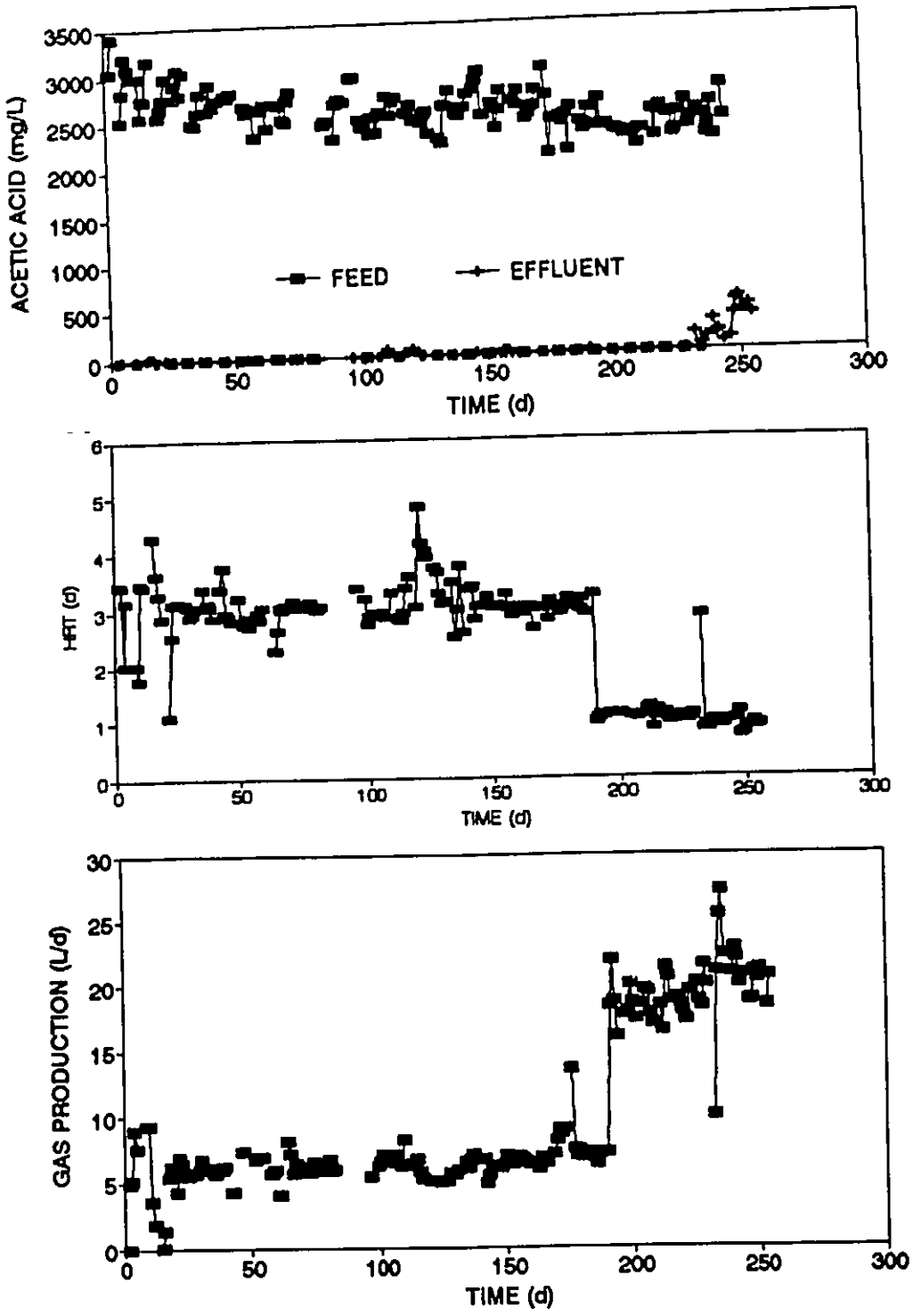


Figure 4.26 General R234 Reactor Performance.

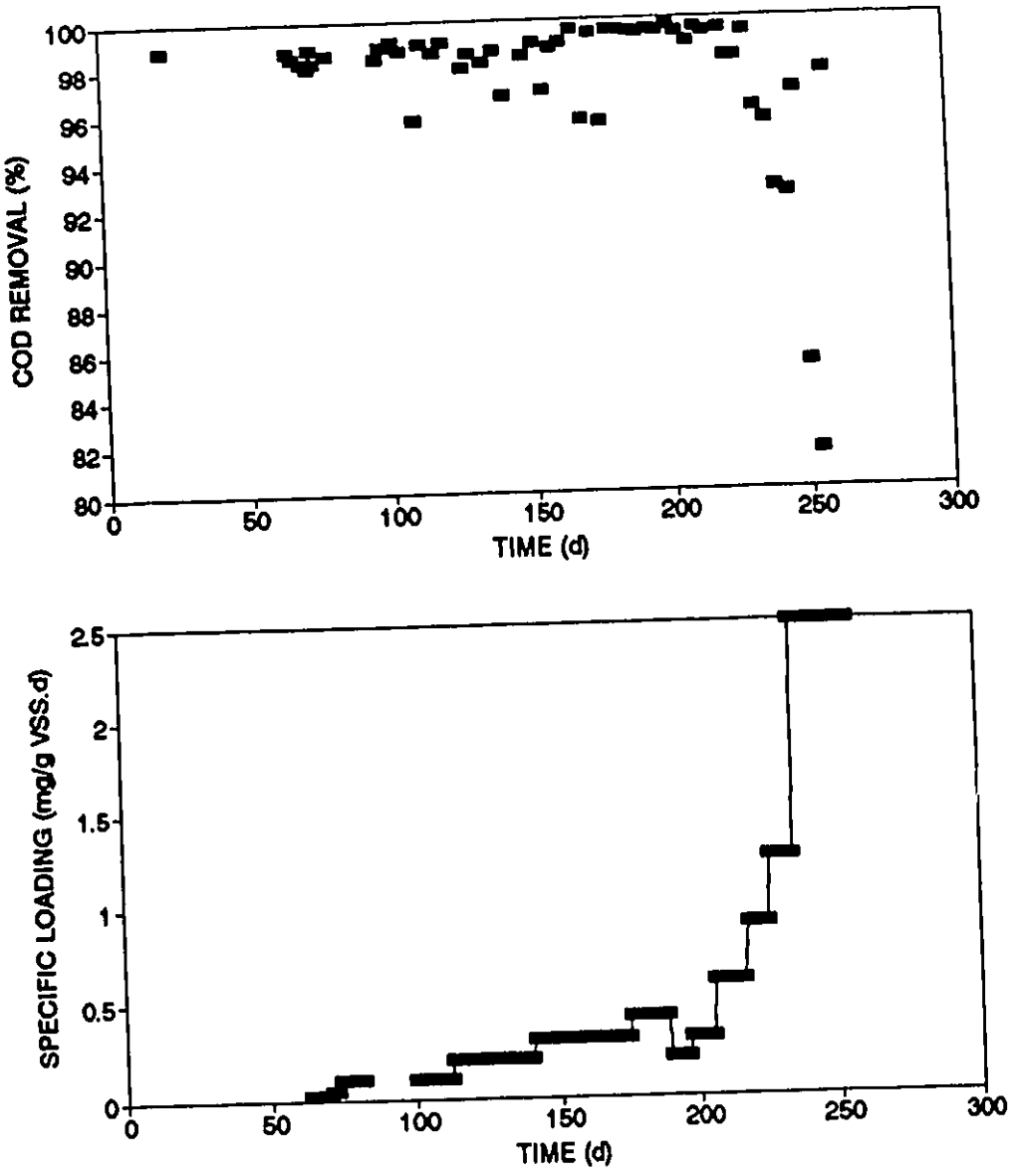


Figure 4.27 Treatment Efficiency of the R234 Reactor under Toxic Loading

efficiency was between 92 and 99.7%; the acetic acid concentration was below 80 mg/L.

4.10.2 Biodegradation Pathway for 2,3,4-TCP

Biodegradation of 2,3,4-TCP and its biodegradation pathway were determined by HPLC analysis. At 3 day HRT the low 2,3,4-TCP loading rates (1.25 and 2.5 mg/L) were applied at the beginning of the experiment, and 2,3,4-TCP and its metabolites were not detected in the reactor (Fig. 4.25). However, within 12 days of 2,3,4-TCP addition at 6 mg/L (SLR of 0.1 mg/g VSS.d), 0.5 mg/L of 3,4-DCP (a reductive dehalogenation product of 2,3,4-TCP) was detected in the reactor effluent. The fact that the reactor sludge had never been exposed to chlorinated compounds but within 35 days of acclimation a metabolite was detected in the R234 indicated that a certain population of bacteria was present in the reactor with the inherent ability to degrade 2,3,4-TCP.

At higher 2,3,4-TCP concentrations and loading rates (25 mg/L at 1 day HRT), a small amount of 4-MCP was detected in the reactor effluent. The fact that detection of 3,4-DCP at low loads followed by 4-MCP at high loads indicated that 2,3,4-TCP degradation began with removal of an ortho-Cl producing 3,4-DCP, followed by removal of a meta-Cl to produce 4-MCP. At no time during the experiment was 3-MCP detected in the effluent. Since 3,4-DCP was the main intermediate accumulated in the reactor this would suggest that 3,4-DCP dechlorination in R234 was poor. The fact that there was no significant amount of 2,3,4-TCP accumulating in the reactor at most loading rates suggested that the rate of reductive dechlorination

of ortho-Cl was high. The anaerobic biodegradation pathway of 2,3,4-TCP is summarized in Fig. 4.15b.

4.10.3 The Fate of 2,3,4-TCP in R234

The removal efficiency and average concentrations of 2,3,4-TCP and its biodegradation products during steady state in reactor R234 are shown in Tables 4.10a and 4.10b. Similar to other reactors, the compounds steady state concentrations were estimated by averaging their last four days reactor concentrations for each loading rate. Table 4.10a indicates that at 3 day HRT 2,3,4-TCP disappeared completely when the loading concentration was 1.25 and 2.5 mg/L, this was due to a combination of biodegradation and sludge biosorption. When the loadings were increased to 6 mg/L, 2,3,4-TCP removal efficiency was 65%.

Complete removal of 2,3,4-TCP was achieved when the loading concentrations were at 12.0, 18.0, and 25.0 mg/L. Accumulation of 2,3,4-TCP at concentration of 6 mg/L while not at 18, 25 mg/L indicates that sludge became acclimated during the 6 mg/L loading period. 3,4-DCP tended to accumulate in the reactor with the increasing loading of 2,3,4-TCP. At a 2,3,4-TCP loading rate of 12 mg/L only 58% of 3,4-DCP was removed, largely by sorption. There was no analytical evidence of 4-MCP being further degraded in the reactor, but it was removed partly by sludge sorption.

At 1 day HRT, a 100% removal of 2,3,4-TCP and 3,4-DCP was achieved at most loading concentrations from 4 to 50 mg/L (VLR of 4 to 50 mg/L.d, SLR of 0.2 to 2.5 mg/g VSS.d). Removal of 4-MCP was

Table 4.10a Steady State 2,3,4-TCP and Its Metabolites
Removal Efficiency, 3 day HRT

		3 day HRT					
TIME PERIOD							
FROM DAY:		64	71	74	100	113	142
TO DAY:		70	73	82	112	141	175
2,3,4-TCP							
INFLUENT CONC. (mg/L)		1.2	2.5	6	6	12	18
STEADY STATE REACTOR	Ave	0	0	1.66	2.08	0	0
CONC. (mg/L)	STD	0	0	0.427	0.614	0	0
SORBED BY BIOMASS (mg/L)				1.5	1.9	0.0	0.0
TOTAL REMOVAL (%)		100.0	100.0	72.3	65.3	100.0	100.0
REMOVAL BY SORPTION (%)				25.7	31.5	0.0	0.0
3,4-DCP							
STEADY STATE REACTOR	Ave	0	0	0	0.3	4.13	4.4
CONC. (mg/L)	STD	0	0	0	0.252	0.108	0.778
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.3	5.6	6.0
TOTAL REMOVAL (%)		100.0	100.0	100.0	82.1	58.2	70.3
REMOVAL BY SORPTION (%)		0.0	0.0	0.0	19.1	56.6	40.4
4-MCP							
STEADY STATE REACTOR	Ave	0	0	0	0	0	0
CONC. (mg/L)	STD	0	0	0	0	0	0
SORBED BY BIOMASS (mg/l)		0.0	0.0	0.0	0.0	0.0	0.0
TOTAL REMOVAL (%)							
REMOVAL BY SORPTION (%)							

Table 4.10b Steady State 2,3,4-TCP and its Metabolites
Removal Efficiency, 1 day HRT

1 day HRT

TIME PERIOD							
FROM DAY:		190	197	206	219	226	235
TO DAY:		196	205	218	225	234	255
2,3,4-TCP							
INFLUENT CONC. (mg/L)		4	6	12	18	25	50
STEADY STATE REACTOR	Ave	0	0	0	0	0	0
CONC. (mg/L)	STD	0	0	0	0	0	0
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.0	0.0	0.0
TOTAL REMOVAL (%)		100.0	100.0	100.0	100.0	100.0	100.0
REMOVAL BY SORPTION (%)		0.0	0.0	0.0	0.0	0.0	0.0
3,4-DCP							
STEADY STATE REACTOR	Ave	0.84	0	0	0	0	1.93
CONC. (mg/L)	STD	0.572	0	0	0	0	0.649
SORBED BY BIOMASS (mg/L)		1.0	0.0	0.0	0.0	0.0	2.4
TOTAL REMOVAL (%)		74.5	100.0	100.0	100.0	100.0	95.3
REMOVAL BY SORPTION (%)		29.9	0.0	0.0	0.0	0.0	5.9
4-MCP							
STEADY STATE REACTOR	Ave	0	0	0	0	0	0
CONC. (mg/L)	STD	0	0	0	0	0	0
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.0	0.0	0.0
TOTAL REMOVAL (%)							
REMOVAL BY SORPTION (%)							

largely attributed to sludge sorption. When the influent concentration was increased to 25 mg/L, complete removal of 2,3,4-TCP was achieved and 3,4-DCP removal efficiency was 95%. When the loading rate increased to 50 mg/L, the acetic acid concentration increased to 500 mg/L. These results indicated that the SLR of 2.5 mg/g VSS.d inhibited the activities of acidogens and methanogens.

As mentioned in Section 4.10.1, an interesting phenomenon was observed that black material formed outside the granule sludge particles during 1 day HRT period and the black material stripped off from sludge particles when inhibition occurred. DiGeronimo et al. (1979) also reported that dark polymeric substances were formed in treatment of haloaromatics by conventional industrial sewage treatment systems. Therefore, it is doubtful that the total disappearance of 2,3,4-TCP and 3,4-DCP in R234 at most higher loading rates was due to the degradation, sorption or formation of the dark substance.

A mass balance of chlorophenols was also done for R234. The sum of 2,3,4-TCP added, 2,3,4-TCP and 3,4-DCP and 4-MCP in the effluent during 3 day HRT for the whole experimental period are shown in Table 4.7 and Figure 4.28. The amount sorbed by biomass was estimated by the sorption isotherms and measured liquid phase concentrations of the compounds on the last day of the experimental period. During 3 day HRT period, the mass deficit was 6994 μM (65%). No phenol was detected in R234 but the mass deficit in R234 was extremely high. Possibly, the large amount of chlorophenol that disappeared was transformed to the dark substance.

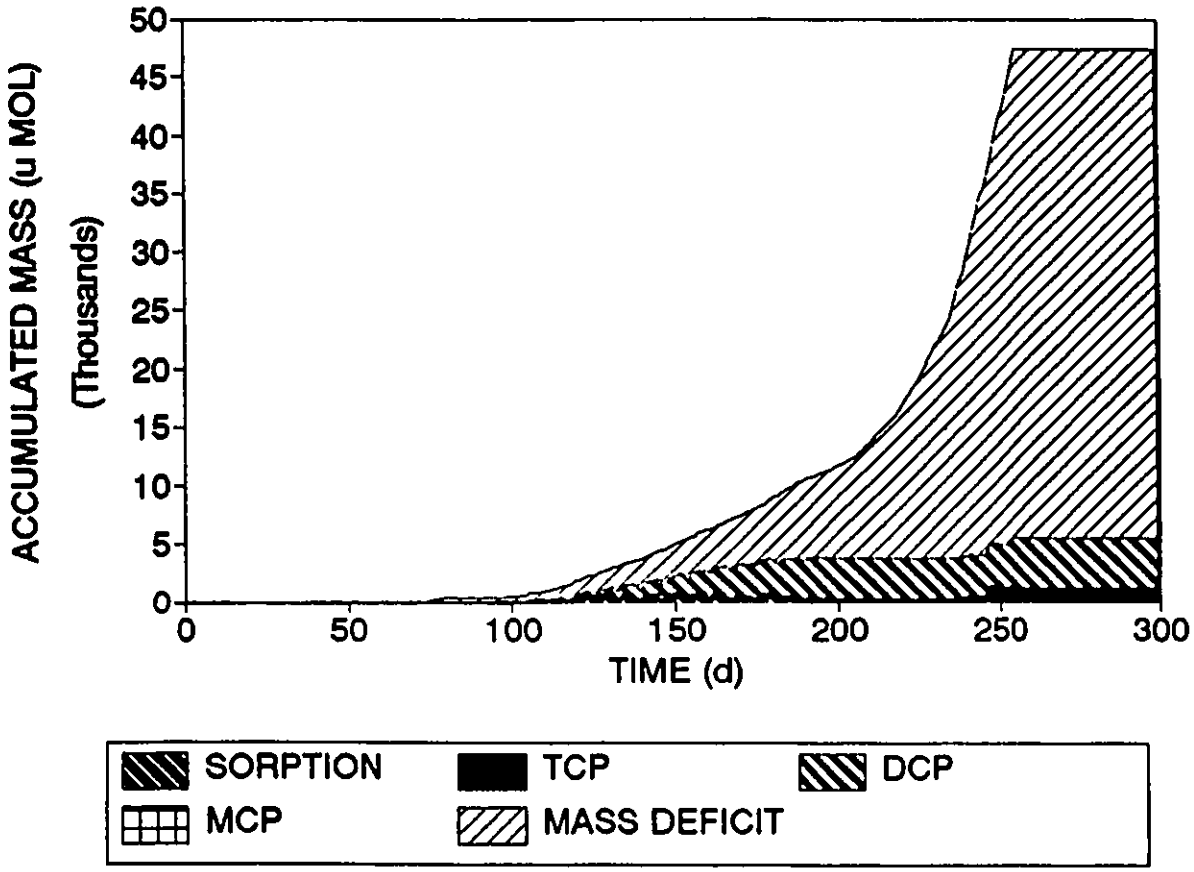


Figure 4.28 Mass Balance of 2,3,4-TCP

4.10.4. Toxicity of 2,3,4-TCP

Inhibition occurred in R234 reactor at the highest loading concentration of 50 mg/L, i.e., the specific loading of 2.5 mg/g VSS.d. Although the concentrations of 2,3,4-TCP and 3,4-DCP in the reactor were not extremely elevated, the rate of degradation of co-substrate decreased (Figure 4.26), indicating that the activities of acidogenic and methanogenic bacteria were inhibited by this 2,3,4-TCP loading rate.

4.11 Dechlorination of 2,3,5-TCP in R235 UASB Reactor

4.11.1 General Reactor Performance

Reactor R235 treated 2,3,5-TCP in the presence of readily degradable co-substrate. In the course of experiment R235 was operated for 213 days, 72 days at 3 day HRT followed by 141 days at 1 day HRT, the corresponding co-substrate VLR of 1.6 and 5 g COD/L.d respectively. The reactor R235, which was used to treat 2,3,5-TCP for five months, had been shut off for six months before this study was done. After 4 days of supply with sucrose/acetic synthetic wastewater, R235 was continuously loaded with 2,3,5-TCP at concentration of 25 mg/L at 3 day HRT (VLR of 8.3 mg/L.d or SLR of 0.42 mg/g VSS.d). Although the specific COD loading rate (0.08 g/g VSS.d) was in a considerable low range not able to support adequate biomass growth, 92% of removal of 2,3,5-TCP was achieved at this loading rate. Only one loading rate (25 mg/L) was applied.

During 1 day HRT, four 2,3,5-TCP loading rates, which were increased stepwise, were applied. These four loading rates were 6.0, 12.0, 18.0, 25.0 mg/L and the reactor reached the treatability limit at loading rate at 25 mg/L. The specific COD loading rate (0.25 mg/g VSS.d) was in a more adequate range to cause growth of biomass. Removal efficiency of 2,3,5-TCP was enhanced in this stage but inhibition of acidogens and methanogens occurred at the highest loading rate of 25 mg/L. At this loading rate, the concentrations of 2,3,5-TCP and its metabolite 3,5-DCP increased to 8.9 and 2.1 mg/L respectively (Fig. 4.29), the concentration of acetic acid increased to 1000 mg/L (Fig. 4.30) and COD removal efficiency decreased to 75% (Fig. 4.31), which indicated severe inhibition occurred to the biomass in the reactor.

During non-inhibition period, the methane component was around 65%, biogas production was 2.5-3.0 L per L of synthetic feed, acetic acid concentration in the reactor was below 60 mg/L , and COD removal efficiency was between 92 and 99.8%. pH and alkalinity were approximately 7.5 and 3500 mg/L as $CaCO_3$ respectively.

4.11.2 Biodegradation Pathway for 2,3,5-TCP

Biodegradation of 2,3,5-TCP and its biodegradation pathway were determined by HPLC analysis. Within 3 days loading of 2,3,5-TCP at loading concentration of 25 mg/L (SLR of 0.42 mg/g VSS.d), 0.5 mg/L of 3-MCP (a reductive dehalogenation product of 2,3,5-TCP) was detected in the reactor effluent (Fig. 4.29), 0.9 mg/L of 3,5-DCP

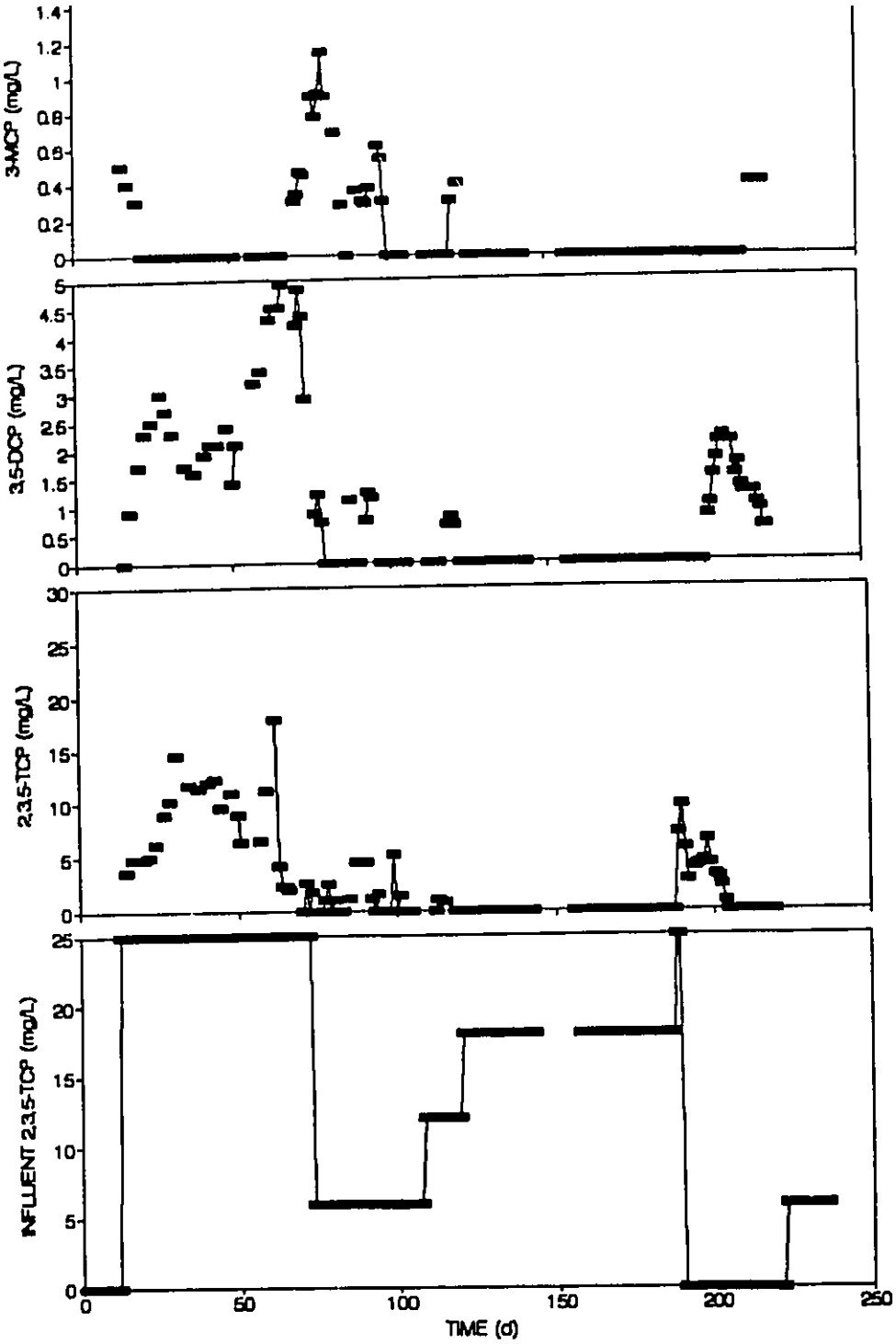


Figure 4.29 Soluble Concentration of 2,3,5-TCP and its Metabolite in R235

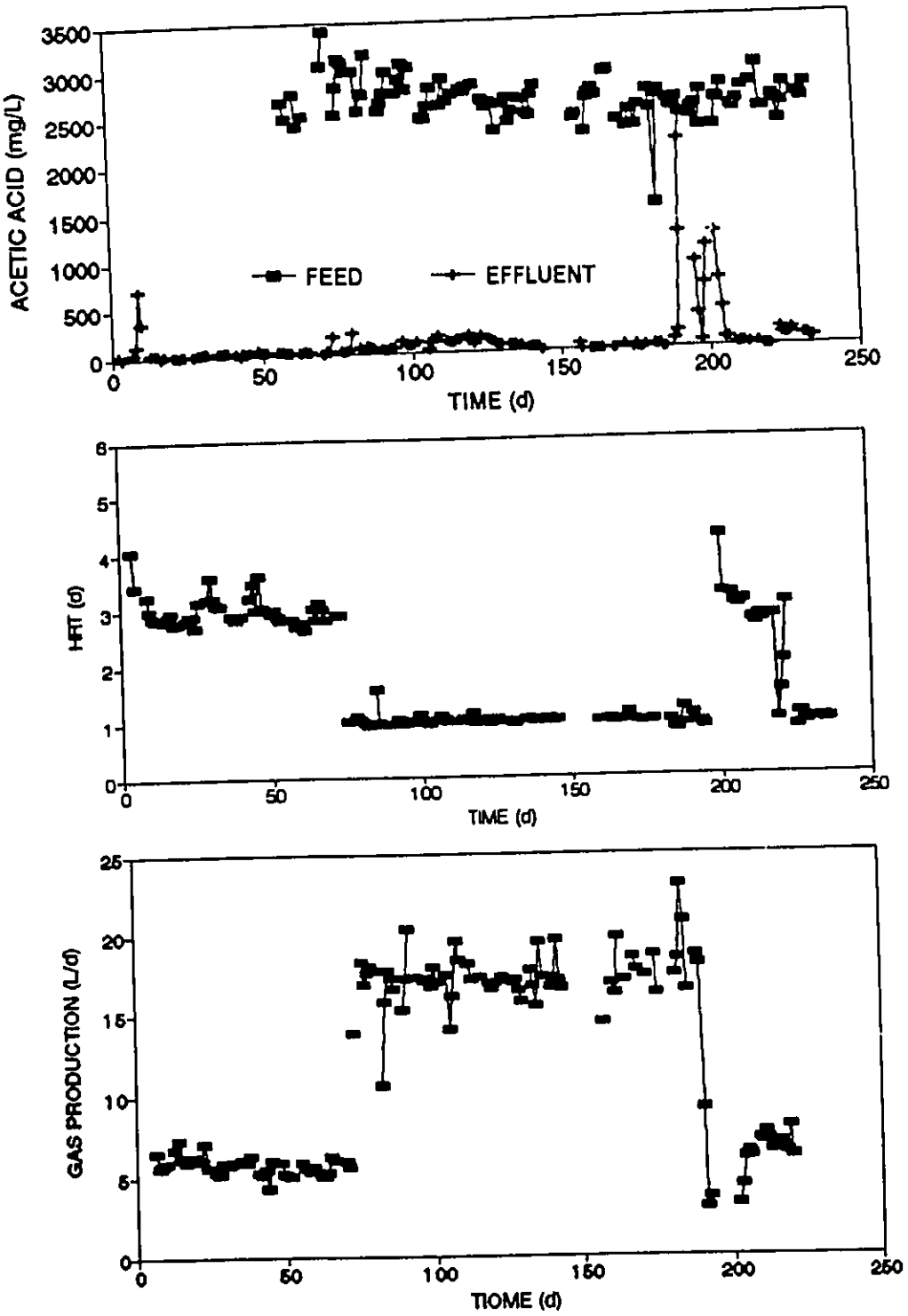
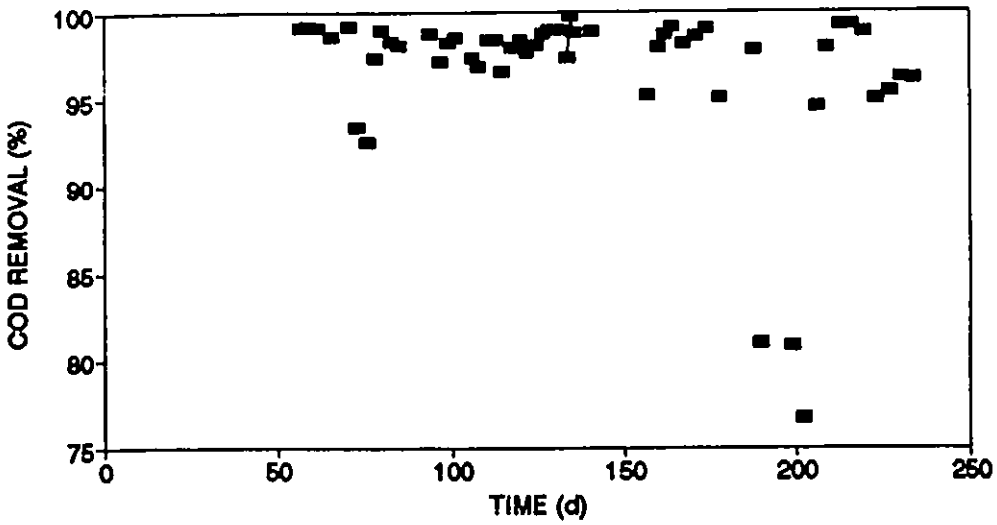


Figure 4.30 General R235 Reactor Performance.



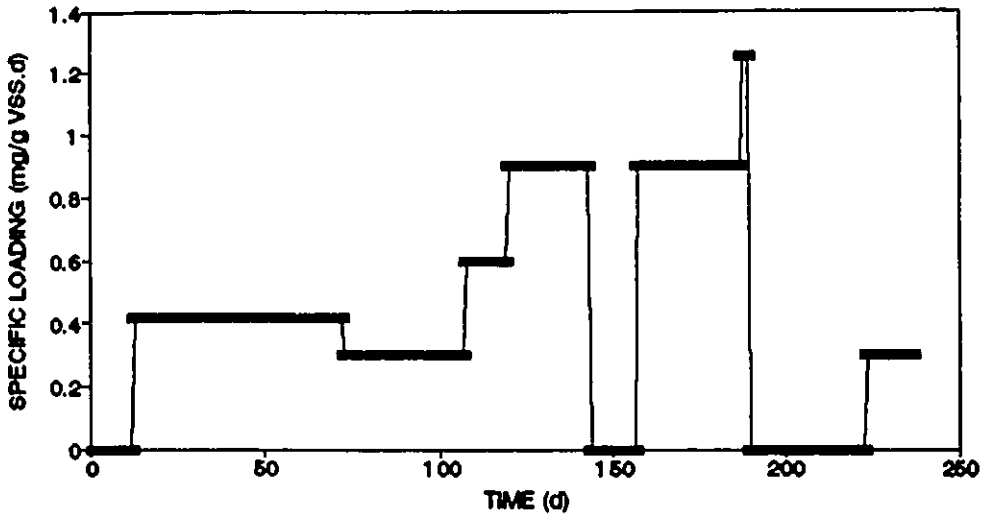


Figure 4.31 Treatment Efficiency of the R235 Reactor under Toxic Loading

was detected in the reactor another day after. The fact that the reactor had been shut off for six months before this loading was applied, indicated that there was a significant amount of anaerobes able to degrade 2,3,5-TCP in the reactor sludge and those anaerobes activities were recovered very quickly.

Throughout the course of experiment, only two metabolites 3,5-DCP and 3-MCP were detected in the reactor, which was also proved by doing sludge sample extraction. These results indicated that 2,3,5-TCP degradation began with removal of an ortho-Cl producing 3,5-DCP, followed by removal of a meta-Cl from 3,5-DCP to produce 3-MCP (shown in Fig. 4.15b). The fact that 3,5-DCP was the main intermediate accumulated in the reactor would suggest that 3,5-DCP dechlorination was poor.

4.11.3 The Fate of 2,3,5-TCP in R235

The removal efficiency and average concentrations of 2,3,5-TCP and its biodegradation products during steady state in reactor R235 are shown in Tables 4.11. Similar to other reactors, steady state concentration was estimated by averaging last four days reactor concentrations at each loading rate. Table 4.11 indicates that at 3 day HRT and the loading concentration of 25 mg/L, 2,3,5-TCP removal efficiency was 92%, 3,5-DCP removal efficiency was 75%, and 3-MCP removal efficiency was 98%. Sludge biosorption attributed to 4.0, 33, 1.5% removal, respectively.

At 1 day HRT, the removal efficiencies of 2,3,5-TCP and 3,5-DCP were enhanced. Complete removal of 2,3,5-TCP and 3,5-DCP was

Table 4.11 Steady State 2,3,5-TCP and Its Metabolites Removal Efficiency
3 day & 1 day HRT

		3 day HRT	1 day HRT					
TIME PERIOD (DAY)			73	110	120	160	188	190
FROM		12	109	119	143	187	189	213
TO		72						
2,3,5-TCP								
INFLUENT CONC. (mg/L)		25	6	12	18	18	25	0
STEADY STATE REACTOR	Ave	1.9	0.47	0.45	0	0	8.45	0
CONC. (mg/L)	STD	1.379	0.66	0.445	0	0	1.25	0
SORBED BY BIOMASS (mg/L)		1.0	0.3	0.3	0.0	0.0	3.7	
TOTAL REMOVAL (%)		92.4	92.2	96.3	100.0	100.0	66.2	
REMOVAL BY SORPTION (%)		3.9	4.6	2.2	0.0	0.0	14.8	
3,5-DCP								
STEADY STATE REACTOR	Ave	4.54	0	0.36	0	0	0	1.26
CONC. (mg/L)	STD	0.229	0	0.368	0	0	0	0.551
SORBED BY BIOMASS (mg/L)		6.1	0.0	0.6	0.0	0.0	0.0	1.8
TOTAL REMOVAL (%)		75.1	100.0	90.2	100.0	100.0	100.0	
REMOVAL BY SORPTION (%)		33.4	0.0	5.9	0.0	0.0	0.0	
3-MCP								
STEADY STATE REACTOR	Ave	0.15	0	0	0	0	0	0.15
CONC. (mg/L)	STD	0.18	0	0	0	0	0	0.19
SORBED BY BIOMASS (mg/L)		0.1	0.0	0.0	0.0	0.0	0.0	0.1
TOTAL REMOVAL (%)		97.5	100.0	100.0	100.0	100.0	100.0	
REMOVAL BY SORPTION (%)		1.5	0.0	0.0	0.0	0.0	0.0	

achieved at the loading concentration of 18 mg/L (VLR of 18 mg/L.d or SLR of 0.9 mg/g VSS.d). However, when the loading concentration was increased to 25 mg/L, 2,3,5-TCP concentration in the reactor increased to 8.45 mg/L and its removal efficiency was decreased to 66% (Table 4.11), but no 3,5-DCP was detected. The acetic acid concentration in the reactor increased to 1050 mg/L, and consequently biomass production decreased slightly (Fig.4.30). These results indicate that the reactor had approached the 2,3,5-TCP loading limit.

A mass balance of chlorophenols in R235 was also done. The sum of 2,3,5-TCP added, 2,3,5-TCP and 3,5-DCP and 3-MCP in the effluent during 3 day HRT and whole experimental period were shown in Table 4.7 and Figure 4.32. The amount sorbed by biomass was estimated by the sorption isotherms and measured liquid phase concentrations of the compounds on the last day of the experimental period. During 3 day HRT period, the mass deficit was 2948 μM (28%). During the whole experimental period, the mass deficit in R235 in whole experimental period was 31479 μM, i.e., 72%. Phenol was not detected in the R235 reactor and 2,3,5-TCP radioactive test was not done in R235.

4.11.4 Toxicity of 2,3,5-TCP

There was severe inhibition of acidogens and methanogens in R235 reactor at the highest loading rate of 25 mg/L, i.e., the SLR of 1.25 mg/g VSS.d. There was an accumulation of 2,3,5-TCP (Fig. 4.29) and co-substrate (Fig. 4.30) in the reactor indicating that the

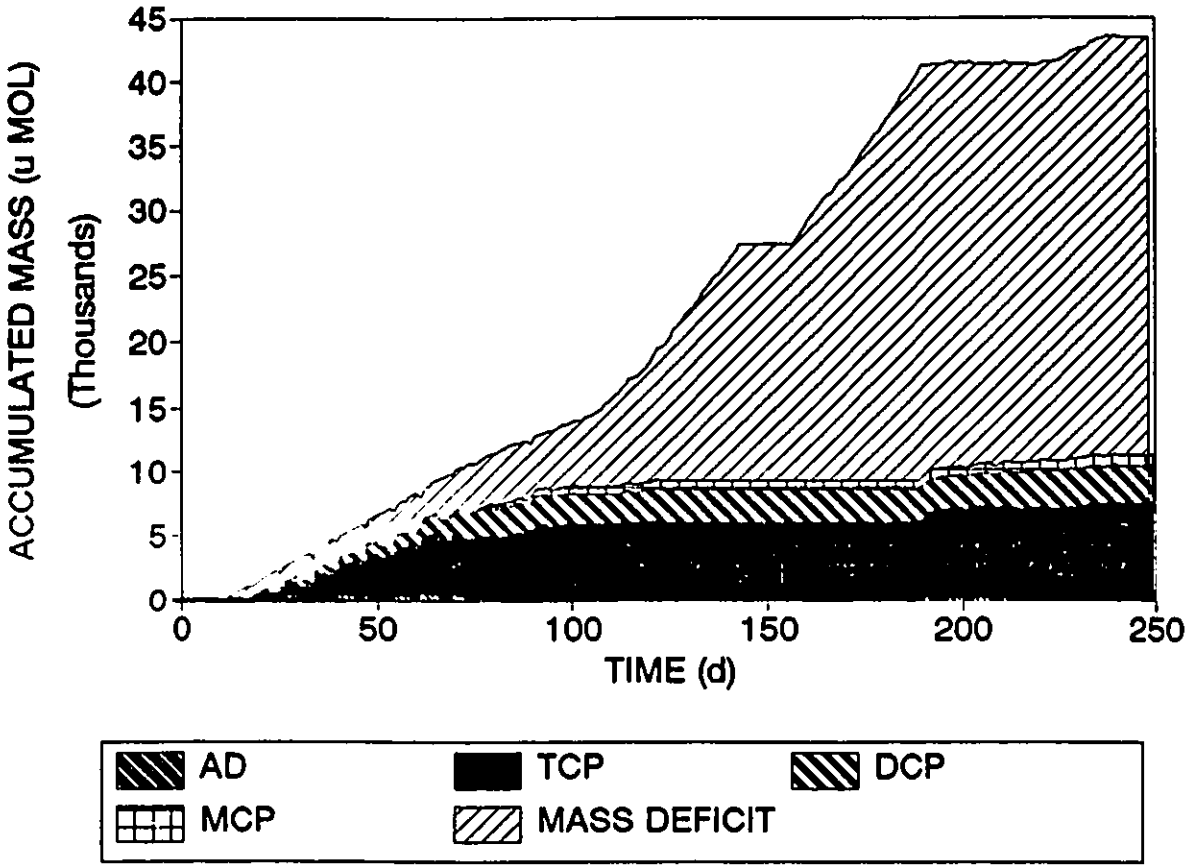


Figure 4.32 Mass balance of 2,3,5-TCP

activities of acidogenic and methanogenic bacteria were severely inhibited by this 2,3,5-TCP loading rate. As mentioned above this indicated that reactor approached the 2,3,5-TCP degradation limit.

4.12 Dechlorination of 3,5-DCP in a UASB Reactor

4.12.1 General Reactor Performance

General reactor performance of reactor R35, which was used to treat 3,5-DCP, was quite similar to reactor R235. In the course of experiment R35 was operated for 276 days, 91 days at 3 day HRT followed by 185 days at 1 day HRT. Similar to R235, the initial sludge in R35 had been used to treat 3,5-DCP for five months then it was shut down for six months before this study was started. Adding synthetic wastewater for four days at a rate of two L per day, 18 mg/L (VLR of 6 mg/L.d or SLR of 0.3 mg/g VSS.d) of 3,5-DCP was continuously applied to R35 reactor. The biodegradation of 3,5-DCP in R35 began within three days. Although the SLR (0.08 g/g VSS.d) was in a considerably low range not adequate to support biomass growth, complete removal of 3,5-TCP was achieved at this loading rate and no inhibition of occurred.

At 1 day HRT, five 3,5-DCP loading concentrations of 12.0, 18.0, 25.0, 30.0, and 60.0 mg/L were applied to R35 reactor in order. Inhibition occurred at the highest loading rate of 60.0 mg/L (SLR of 3.0 mg/g VSS.d). The specific COD loading rate (0.25 mg/g VSS.d) was in a more adequate range to prompt growth of biomass. The removal efficiency of 3,5-DCP was above 90% at most loading rates except at SLR of 3.0 mg/g VSS. At this loading rate, the

concentration of 3,5-DCP and its metabolites 3-MCP in the reactor increased (Fig. 4.33), the concentration of acetic acid elevated to 1000 mg/L (Fig. 4.34) and COD removal efficiency decreased to 78% (Fig. 4.35), which indicated severe inhibition occurred to the biomass and the reactor reached its treatability limit.

Reactor performance was monitored by measuring biogas volume and methane component, acetic acid concentration in influent and effluent, COD removal, shown in Figures 4.34 to 4.35. During non-inhibition period, methane component was around 65%, COD removal efficiency was between 92 and 99.7%. pH and alkalinity were quite stable at around 7.5 and 3500 mg/L as $CaCO_3$ respectively.

4.12.2 Biodegradation Pathway for 3,5-DCP

Biodegradation of 3,5-DCP and its biodegradation pathway were determined by HPLC analysis. Although the reactor had been shut off for six months, within 3 days of loading 3,5-TCP at SLR of 0.3 mg/g VSS.d, 0.6 mg/L of 3-MCP (a reductive dehalogenation product of 3,5-DCP) was detected in the reactor effluent (Fig. 4.33). This indicated that the activities of anaerobes able to degrade 3,5-DCP was not lost with storage and recovered very quickly. Throughout the course of the experiment, only 3-MCP was detected in the reactor, which indicated that 3,5-DCP degradation began with removal of a meta-Cl to produce 3-MCP. There was no analytical evidence that 3-MCP was degraded. The pathway of 3,5-DCP anaerobic biodegradation is summarized in Figure 4.15b.

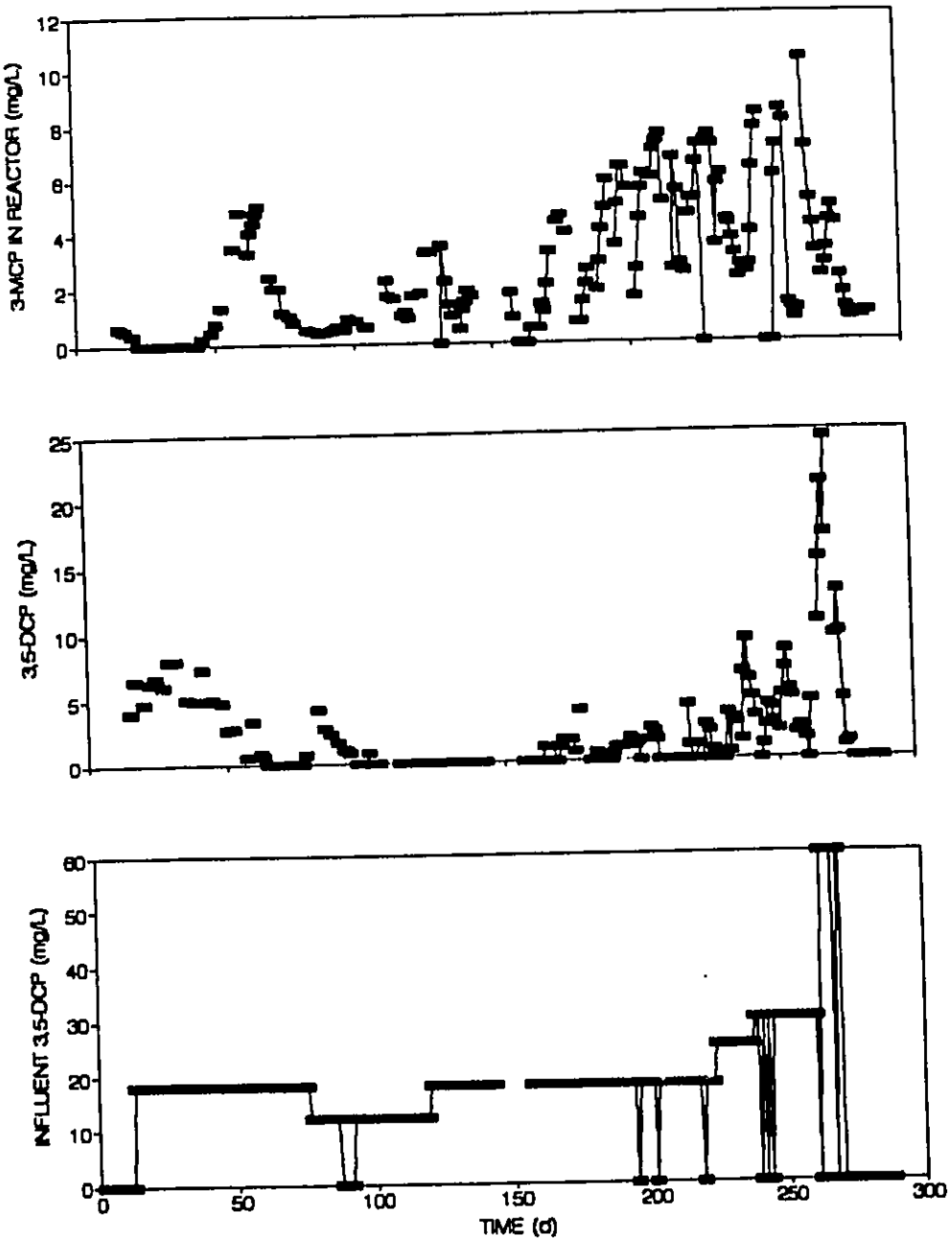


Figure 4.33 Soluble Concentrations of 3,5-DCP and its Metabolite in R35

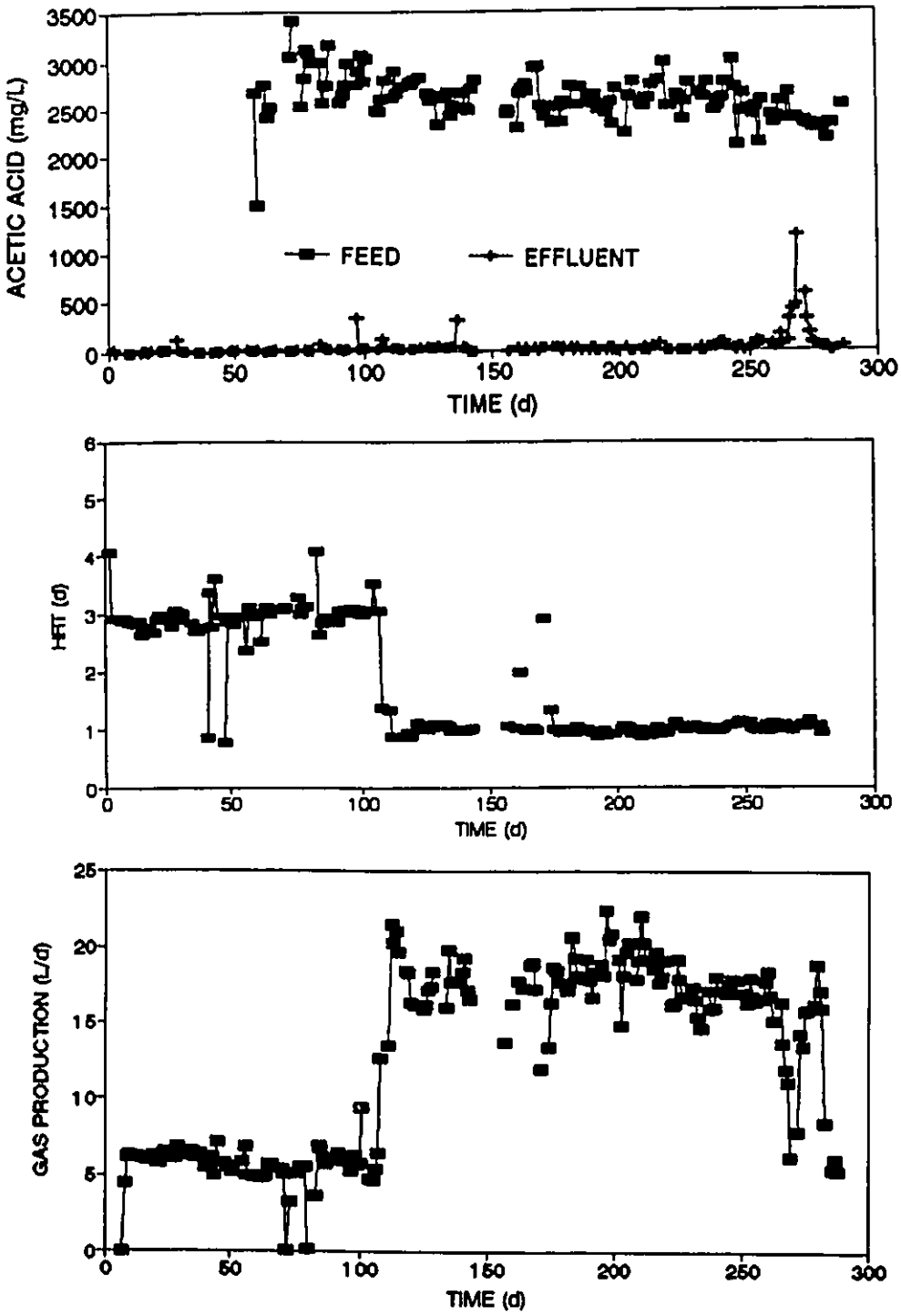


Figure 4.34 General R35 Reactor Performance.

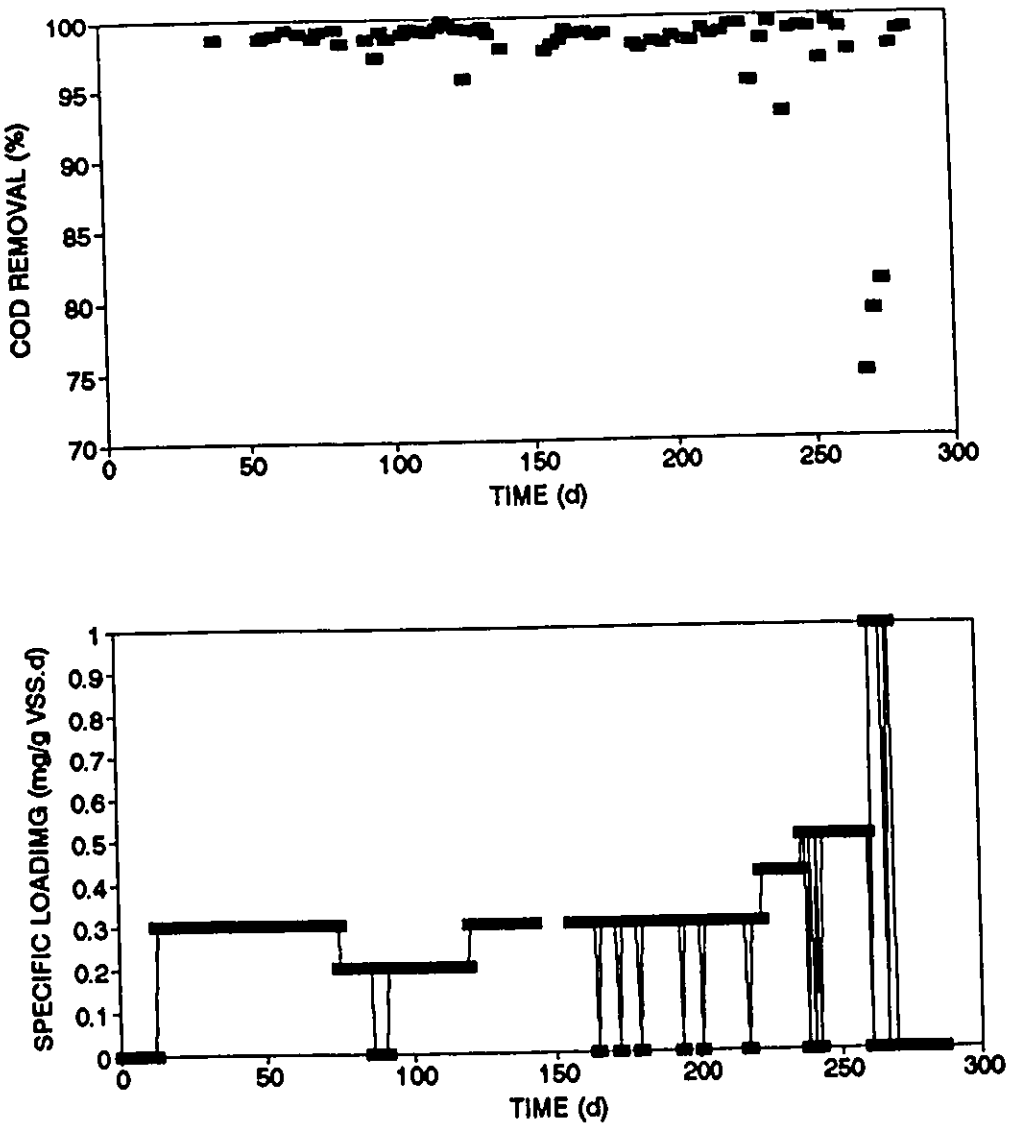


Figure 4.35 Treatment Efficiency of the R35 Reactor under Toxic Loading

4.12.3 The Fate of 3,5-DCP in R35

The removal efficiency and average concentrations of 3,5-DCP and its biodegradation products during steady state in R235 reactor are shown in Tables 4.12. The methods to estimate the steady state concentration and removal efficiency of chlorophenols were the same as the methods used in other reactors. Table 4.12 indicates that at a 3 day HRT, 100% removal of 3,5-DCP was achieved at the loading concentrations of 18 and 12 mg/L (6 mg/L.d and 4 mg/L.d), and the removal efficiency of 3-MCP was above 87%.

At 1 day HRT, more than 90% of removal of 3,5-DCP and more than 64% removal of 3-MCP was achieved at most loading rates. Sludge biosorption attributed 2.8 to 13.7% removal of 3,5-DCP, and 5.4 to 14.2% removal of 3-MCP. However, when the loading concentration increased to 60 mg/L, 3,5-DCP removal efficiency decreased to 67%, 3-MCP removal efficiency was 64%. Consequently, the acetic acid concentration in the reactor increased to 1000 mg/L and biogas production decreased by one-third, which indicated that the reactor had approached the loading limit.

A mass balance of chlorophenols in R35 was also done. The sum of 3,5-DCP added, 3,5-DCP and 3-MCP in the effluent during 3 day HRT and the whole experimental period were shown in Table 4.7 and Figure 4.36. During 3 day HRT period, the mass deficit was 5736 μM (55%). During the whole experimental period, mass deficit in R35 was 33637 μM (42%). Phenol was not detected in the R35 reactor and 3,5-DCP radioactive test was not done in R35.

Table 4.12 Steady State 3,5-DCP and its Metabolites Removal Efficiency, 3 & 1 day HRT

		3 day HRT		1 day HRT					
TIME PERIOD									
FROM DAY:		13	77	92	121	156	223	237	262
TO DAY:		76	86	120	143	222	236	261	269
3,5-TCP									
INFLUENT CONC. (mg/L)		18	12	12	18	18	25	30	60
STEADY STATE REACTOR	Ave	0	0	0	0	0.33	2.47	1.95	19.4
CONC. (mg/L)	STD	0	0	0	0	0.471	1.16	0.326	3.608
SORBED BY BIOMASS (mg/L)		0.0	0.0	0.0	0.0	0.5	3.4	2.7	24.1
TOTAL REMOVAL (%)		100.0	100.0	100.0	100.0	98.2	90.1	93.5	67.7
REMOVAL BY SORPTION (%)		0.0	0.0	0.0	0.0	2.8	13.7	9.1	40.2
3-MCP									
STEADY STATE REACTOR	Ave	1.71	1.22	1.22	1.83	3.3	5.64	4.8	4.93
CONC. (mg/L)	STD	0.429	0.275	0.275	1.025	0.927	1.346	3.508	1.416
SORBED BY BIOMASS (mg/L)		0.8	0.6	0.6	0.8	1.3	2.2	1.9	1.9
TOTAL REMOVAL (%)		88.5	87.7	87.7	87.7	76.7	64.2	77.0	63.7
REMOVAL BY SORPTION (%)		5.1	5.6	5.6	5.4	9.5	13.7	9.0	14.2

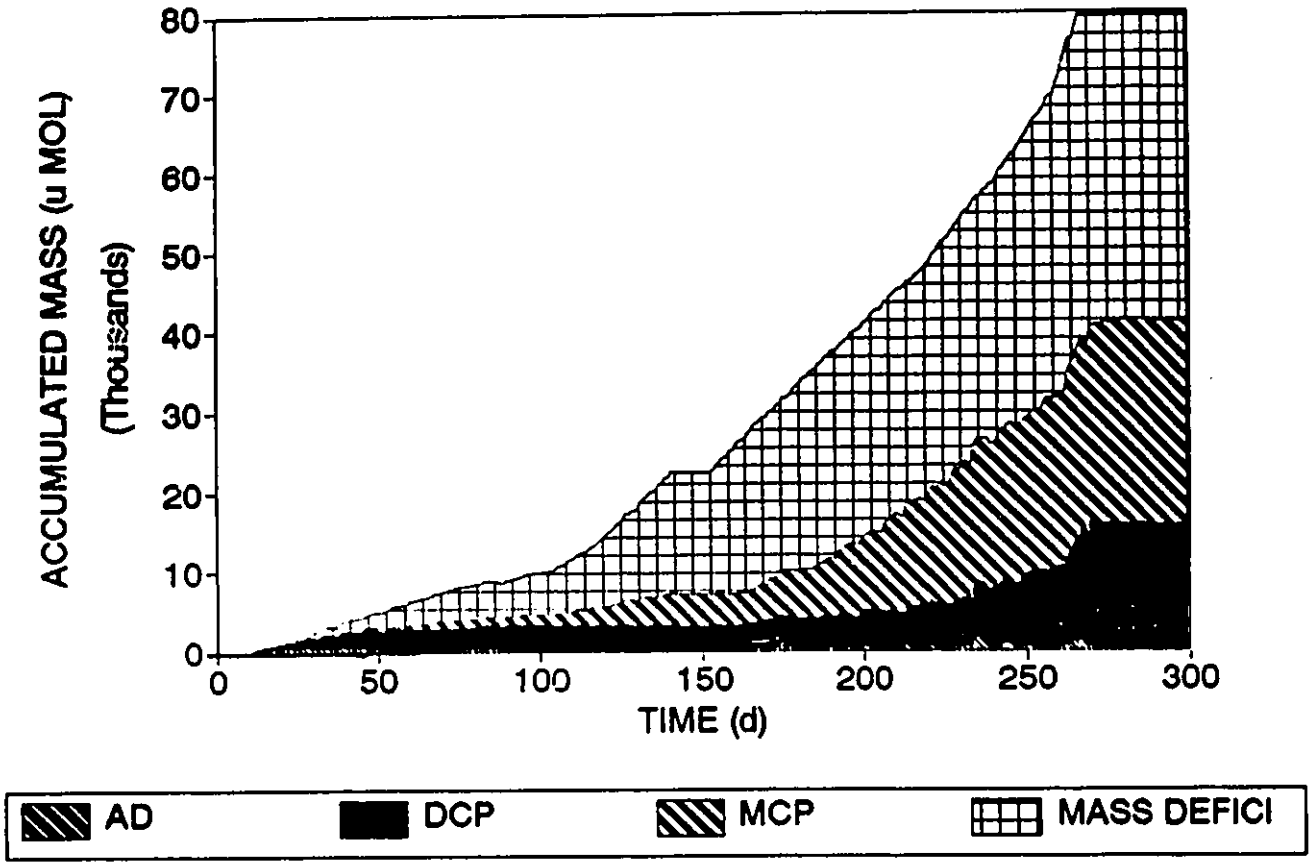


Figure 4.36 Mass Balance of 3,5-DCP

4.12.4. Toxicity of 3,5-DCP

Inhibition occurred in R35 reactor at the 3,5-DCP highest loading rate of 60 mg/L.d, i.e., the specific loading of 3.0 mg/g VSS.d. Accumulation of 3,5-DCP and 3-MCP in the reactor and accumulations of co-substrate and volatile fatty acid (Figure 4.33, 4.34), indicated that the activities of acidogenic and methanogenic bacteria were inhibited at this 3,5-DCP loading rate.

4.13 Biomass Yield in the Reactors

Biomass growth in an anaerobic system is a slow process because a major portion of readily biodegradable COD is converted into methane and carbon dioxide. Consequently, anaerobic biomass yield is much smaller than in the activated sludge process. Biomass yield can be estimated by two methods. One method is to monitor net biomass volume increase in the reactor and to measure the amount of biomass washed out in effluent; another method is using an empirical model to estimate biomass yield. The initial biomass volume in the reactors was 3 L. In order to keep a constant volume of biomass, the increased volume of biomass was wasted at the end of the 3 day HRT period (See Appendix D), but the amount of the biomass washed out from the reactor daily via the effluent was not recorded. Here, an empirical model and COD mass balance were employed to estimate the biomass yield in each reactor:

1 g COD = 0.39 L methane gas(at 35°C)

1 g biomass = 1.42 g COD

The amount of COD converted to biomass in the whole experimental period was calculated by the total amount of COD added minus the amount of COD washed out in effluent minus the amount of equivalent COD converted into biogas, shown in Table 4.13. The estimated biomass yields for each reactor were in a range of 0.02 to 0.08 g VSS/ g COD removal. The amount of biomass wasted during the experiment for each reactor is shown in Appendix E. It should be pointed out that during the period of inhibition of acidogens and methanogens, biomass production would be expected to decrease; the loss of biomass through the effluent increased. Furthermore, unexpected operation failures of the reactors also caused biomass losses.

Table 4.13

Biomass Yield in Each Reactor

Reactor	COD (mg)		Methane	Biomass	Yield
	In	Out	Volume (L)	Produced (mg)	(mg VSS/g COD)
R246	3648829	50585	1346.8	102094.4	0.03
R245	3668342	121354	1261.7	219716.8	0.06
R236	3685800	54537	1361.2	99361.2	0.03
R234	3494695	79556	1292.4	71379.2	0.02
R235	6696021	197342	2232.7	545084.1	0.08
R35	5913309	127219	2030.7	408031.9	0.07

4.14 Transient Effect

The stepwise increases in influent chlorophenol concentration led to transient changes in the chlorophenol concentrations in the

reactor. Unlike the results reported by Woods (1985), the transient effect was only exhibited in R246 reactor in the early stage of the experiment. When 2,4,6-TCP was added at a concentration of 6 mg/L at 3 day HRT, during the first two days its concentration increased to a level approximately twice the subsequent mean steady state concentration. Similarly, when the chlorophenol concentration was increased again after 35 days, the 2,4,6-TCP concentration increased to a concentration 2.5 times that of the subsequent steady state (Fig. 4.14). After acclimating for a longer period of time, the transient effect was not clear.

4.15 Toxicity of Chlorophenols

Inhibition of chlorophenol to anaerobes was observed in many reactors during the experiment. As cited in Chapter 2, inhibition not only inhibited the activity of bacteria corresponding to chlorophenol degradation but also inhibited the activity of acidogens and methanogens. Table 4.14 gives the observed anaerobes behaviours at the highest chlorophenol loading rate in each reactor. In general, chlorophenols with para position chlorine were more toxic than those without para position chlorine; chlorophenols having both para and meta chlorine would be more toxic than those that only had a para chlorine. More halogenated chlorophenols were more toxic than less halogenated ones. Severe inhibition occurred to R245 at SLR of 0.3 mg 2,4,5-TCP/g VSS.d at 3-day HRT and at the SLR was 0.9 mg/g VSS.d at 1-day HRT. While R236 and R246 functioned very well at those loading rate. At SLR of 2.5 mg/g VSS.d at 1-day

Table 4.14

Minimum Concentrations of Chlorophenols with Toxic Effects

3 day HRT

REACTOR	FEED ADDED	LOADING[1] CON.(mg/L)	SLR mg/g VSS	DAYs[2]	INHIBI.	REMARK
R234	2,3,4-TCP	25	0.42	115	NO	
R236	2,3,6-TCP	25	0.42	150	NO	
R235	2,3,5-TCP	25	0.42	2	NO	
R246	2,4,6-TCP	25	0.42	160	NO	
R245	2,4,5-TCP	18	0.30	70	YES	SEVERE[3]
R35	3,5-DCP	18	0.30	2	NO	

1 day HRT

REACTOR	FEED ADDED	LOADING[1] CON.(mg/L)	SLR mg/g VSS	DAYs[2]	INHIBI.	REMARK
R234	2,3,4-TCP	50	2.50	175	YES	MILD[4]
R236	2,3,6-TCP	50	2.50	230	NO	
R235	2,3,5-TCP	25	1.25	190	YES	SEVERE
R246	2,4,6-TCP	50	2.50	235	NO	
R245	2,4,5-TCP	18	0.90	210	YES	SEVERE
R35	3,5-DCP	60	3.00	260	YES	SEVERE

[1] The listed loading concentrations of chlorophenols was the minimum concentration with toxic effect to anaerobes.

[2] The reactor had been operated for the specified days when the loading concentration was applied.

[3] Severe inhibition indicated that not only the chlorophenols removal rate decreased but also the acetic acid concentration in the reactor increased over 500 mg/L and COD removal efficiency decreased to less than 80% and biogas production reduced by 10%.

[4] Mild inhibition indicated that the concentration of chlorophenols increased but the biogas production was not effected.

HRT, 2,3,4-TCP exhibited severe inhibition, 2,4,6-TCP exhibited minor inhibition while 2,3,6-TCP did not. 3,5-TCP exhibited inhibition at SLR of 3.0 mg/g VSS.d while 2,3,5-TCP exhibited severe inhibition at SLR of 1.25 mg/g VSS.d.

CHAPTER 5

CONCLUSIONS

The following conclusions can be made for anaerobic granular biosorption:

Biosorption can be described using the Freundlich model. Biosorption of chlorophenols to live anaerobic granular biomass was found to be strongly dependent on chlorine position. A number of the chlorophenols tested exhibited linear sorption isotherms that can be described by a simple distribution coefficient, K_d. While there was a trend for increased biosorption with increasing K_{ow}. Good empirical correlation describing q or K_d in terms of K_{ow} was not found.

Desorption of 3,4-DCP from anaerobic sludge was found to be almost completely reversible while 3-MCP exhibited a greater degree of irreversibility. PCP biosorption capacity of anaerobic granular biomass was about 16 and 10 fold less than reported capacities of aerobic activated sludge biomass and Rhizopus arrhizus respectively. This result may be due to differences in the test conditions and the unique lipids present in anaerobic bacteria.

Application of the sorption results and the Freundlich model to actively dechlorinating UASB systems provided a reasonable method of predicting chlorophenol accumulation in anaerobic granular biomass. It should be realized that the sorption tests reported on in this study are specific to conditions found in anaerobic sludge bed reactors.

The following conclusions can be made for anaerobic biodegradation of chlorophenols in UASB reactors:

There was a significant population of anaerobes in anaerobic sludge with the inherent ability to degrade chlorophenols. Without acclimation, anaerobic granular sludge was readily able to degrade most tested trichlorophenols at low trichlorophenol loading rates within 10 days. The observed typical trichlorophenol biodegradation pathways is: an ortho chlorine was removed from trichlorophenols. Another ortho (on 2,4-,and 2,5-DCP) was easily removed, and with sludge acclimation the meta chlorine (on 3,5-, and 3,4-DCP) was removed. 3-, or 4-MCP was the final product detected in the reactors.

At 3 day HRT, although the specific COD loading rate (0.08g/g VSS.d) was in a low range and was not able to support adequate biomass growth, over 80% removal of ortho-chlorine (s) from TCPs and DCPs, over 70% removal of meta-chlorine from 3,5-DCP and over 50% removal of meta-chlorine from 3,4-DCP were achieved. At 1 day HRT, chlorophenols' removal efficiencies were enhanced, which was attributed to the combination of sludge acclimation and an adequate amount of carbon for growth being supplied.

The treatability limits for six compounds varied from 0.3 mg/g VSS to 3.0 mg/g VSS. Inhibition of acidogens and methanogens occurred when a specific loading limit of a chlorophenol was applied to a reactor. Inhibition of chlorophenols had strong correlation to their molecular structures and the extent of halogenation. The more halogenated the chlorophenol, the more toxic

it was. The trichlorophenols with para or meta chlorine were much more toxic than those chlorophenols which do not have chlorine in those positions. 2,4,5-TCP was the most toxic compound among the tested trichlorophenols. Sludge acclimation and supply of adequate readily biodegradable carbon source and nutrients reduced the inhibition and increased the toxic specific loading limit.

Mass balance calculations showed that during the whole experimental period, the mass deficit of 3-MCP and 4-MCP were over 40% and 20% respectively, which might indicate that mineralization of 3-MCP or 4-MCP did occur in the reactors, but the mineralization could not be detected with the current HPLC technique.

Mass balance showed that sludge biosorption of chlorophenols contributed as high as 6% of total chlorophenol removal. However, during the inhibition period or for those chlorophenols having poor biodegradability, biosorption was the major mechanism leading to chlorophenol removal.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE RESEARCH

The following areas of future research are recommended:

1. Investigation of biosorption of additional organic pollutants and additional types of biomass.
2. Study of the effects of wastewater characteristics and treatment plant operating variables on the biosorption process.
3. Investigation of the fate of sorbed pollutants during sludge digestion and other sludge processing operations.
4. Examination of the behaviour of sorbed pollutants after land disposal of wastewater.
5. Development of models to predict the fate of hazardous organic pollutants in biological wastewater treatment plants.
6. Development of models to predict the behaviour of sorbed pollutants in landfilled sludges.
7. Evaluate the effect of carbon sources on biodegradation of chlorinated compounds in continuous flow reactor systems.

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APPENDIX A

COMPOSITION OF SYNTHETIC SUCROSE/ACETATE WASTE*

Feed Volume	150L
Sucrose	1500 g
Acetic acid (glacial)	1500 g
NH_4HCO_3	600 g
$NaHCO_3$	1500 g
$KHCO_3$	1860 g
$(NH_4)_2SO_4$	15 g
K_2HPO_4	78 g
KH_2PO_4	60 g
Yeast extract	30 g

TO MAKE STOCK FEED:

Put about 1/2 the required water into feed container. Measure all chemicals except acetic acid into dry pail. Add chemicals to water and stir. Measure acetic acid into graduated cylinder and add the acetic acid to feed in small amount, stirring between additions. Bring volume up to final level using tap water.

TO MAKE WORKING FEED:

Dilute stock feed 1:3 (1L stock + 3L tap water).

* Source: Kennedy, PhD thesis (1985), pp. 114

APPENDIX B

CONVERSION FACTOR OF CHLOROPHENOL

PURE CONCENTRATION TO MOLE CONCENTRATION

Compound	Molecular weight g/mol	Pure concen. mg/L	Mole concen. uM/L
Phenol	94	1	10.6
MCP	128	1	7.81
DCP	163	1	6.13
TCP	198	1	5.05
TeCP	232	1	4.31
PCP	266	1	3.76

APPENDIX C

AN EXAMPLE OF DETERMINATION OF CHLOROPHENOL BIOSORPTION ISOTHERM CONSTANTS

File: CA:\DATA\ADSORB\AD21.WK1
Date: 01/18/91
Samples: Live biomass.
Compounds: 4-MCP

Vial	AD	4-MCP (mg/L) 0h	2h	20h	average	sd
A	5	6.8	6.8	6.7	6.77	0.06
B	10	12.8	12.8	12.8	12.80	
C	20	20.5	20.5	20.5	20.50	0.00
D	40	38.7	38.5	38.5	38.57	0.09
E	60	57.3	57.4	56.8	57.17	0.26
F	5	5.4	5.0	4.9	5.10	0.22
G	10	9.2	9.2	9.2	9.20	
H	20	14.9	14.2	14.0	14.37	0.39
I	40	31.4	28.0	27.7	29.02	1.68
J	60	45.4	42.6	42.6	43.53	1.32
Control		none.				

Calculation

AD	Liquid phase ug/L	Solid phase ug/g VSS	Log (L)	Log (S)
5	4900	98.2	3.69	1.99
10	9200	189.5	3.96	2.28
20	14000	342.1	4.15	2.53
40	27700	571.9	4.44	2.76
60	42600	766.7	4.63	2.88

Regression Output:

Constant		-1.5
Std Err of Y Est		0.1
R Squared		0.984394
No. of Observations		5
Degrees of Freedom		3
X Coefficient(s)	0.959153	
Std Err of Coef.	0.069724	

Where log K = -1.5, K = 0.031623
1/n = 0.959152

APPENDIX D

An Example of Calculation of Chlorophenols Removal Efficiency

Formula:

Trichlorophenol total removal efficiency:

r_T = TCP removed/TCP added * 100%

= $(C_{T,in} - C_{T,out})/C_{T,in}$ * 100%

Dichlorophenol total removal efficiency:

r_D = DCP removed/DCP formed * 100%

= $((C_{T,in} - C_{T,out} - S_T)*5.05 - C_{D,out}*6.13)/((C_{T,in} - C_{T,out} - S_T)*5.05)*100\%$

Monochlorophenol total removal efficiency:

r_M = MCP removed/MCP formed x100%

= $((C_{T,in} - C_{T,out} - S_T)*5.05 - (C_{D,out} + S_D)*6.13 - C_{M,out}*7.81)/((C_{T,in} - C_{T,out} - S_T)*5.05 - (C_{D,out} + S_D)*6.13)*100\%$

The amount of chlorophenol sorbed by sludge in one litre liquid:

$S_i = K*(C*1000)^{1/n}(ug/g)/1000(mg/ug)$ * 20 (g/L)

The removal percentage by sorption:

$r_s = S_i/C_i$ * 100%

Example:

In Table 4.3a column 4, 2,4,6-TCP influent conecentration was 12 mg/L, the effluent concentrations of 2,4,6-TCP was 1.46 mg/L, 2,4-DCP was 0 mg/L, and 4-MCP was 4.23 mg/L.

$r_{2,4,6\text{-TCP}}$ = (12-1.46)/12*100% = 10.54/12*100 = 87.8 %

$S_{2,4,6\text{-TCP}}$ = 0.42086*(1.46*1000)$^{0.551}$/1000*20

= 0.47 (mg/L)

$r_{s,2,4,6\text{-TCP}}$ = 0.47/12*100% = 3.9 %

$r_{2,4\text{-DCP}}$ = 100 %

$S_{2,4\text{-DCP}}$ = 0.0 (mg/L)

$r_{4\text{-MCP}}$ = ((12-1.46-0.47)*5.05-(0.0+0.0)*6.13-4.23*7.81)/((12

-1.46-0.47)*5.05-(0.0+0.0)*6.13)x100 %

= 35.8 %

$S_{4\text{-MCP}}$ = 0.03057*(4.23x1000)$^{0.952}$/1000*20

= 1.7 (mg/L)

$r_{s,4\text{-MCP}}$ = 1.7*7.81/((12-1.46-0.47)*5.05)*100 = 26.6 %

APPENDIX E

BIOMASS WASTED IN EACH REACTOR DURING EXPERIMENT

Reactor	Date	on Days	Sludge Wasted g VSS
R245	Mar/08/91	154	41.2
R235	Mar/08/91	200	17.6
R35	Mar/08/91	200	59.6
R236	Jun/03/91	246	22.0
R234	Jun/03/91	246	50.0