

**MODELING OF SULFIDE INHIBITION AND CHLORINATED
ALIPHATIC BIOTRANSFORMATION OF ANAEROBIC
TREATMENT OF LANDFILL LEACHATE**

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SIZINTI SUYUNUN ANAEROBİK ARITIMINDA

**SÜLFÜR TOKSİSİTESİNİN MODELLENMESİ VE KLORLU ALİFATİKLERİN
BİYOTRANSFORMASYONU**

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CONTENTS

ABBREVIATIONS	v
LIST OF TABLES	vi
LIST OF FIGURES	vii
NOTATION LIST	viii
ÖZET	ix
SUMMARY	xi
1. INTRODUCTION	1
1.1 General	1
1.2 Objectives	6
2. BACKGROUND	9
2.1 Leachate Characteristics	9
2.2 Anaerobic Biological Treatment of Sanitary Landfill Leachate	14
2.3 Sulfide Toxicity, Metabolism and Modeling	17
2.3.1 Sulfide Toxicity	17
2.3.2 Sulfide Metabolism and Competition Between MPB and SRB	22
2.3.3 Modeling of Sulfide Toxicity	31
2.4 Kinetics of Organic Matter Removal	33
2.5 Toxicity and Biotransformation of Chlorinated Aliphatic Compounds	35
3. MATERIALS AND METHODS	41
4. RESULTS AND DISCUSSION	48
4.1 Overall Treatment Performance	48
4.2 Sulfide Metabolism and Toxicity	54
4.3 Modeling of Sulfide Toxicity	61
4.4 Determination of Kinetic Constants for Organic Matter Removal	64
4.5 Toxicity Effects and Biotransformation of Chlorinated Compounds	69
5. CONCLUSIONS	78

REFERENCES	82
APPENDIX	90
C.V.	92



ABBREVIATIONS

SRB: Sulfate reducing bacteria

MPB: Methane producing bacteria

aSRB: Acetate degrading sulfate reducing bacteria

aMPB: Acetate degrading methane producing bacteria

DS: Dissolved sulfide

CT: Tetrachloromethane

PCA: Tetrachloroethane

PCE: Tetrachloroethylene

TCE: Trichloroethylene

OLR: Organic loading rate

LRR: Leachate receiving reactor

MRR: Methanol receiving reactor

TFS: Total fixed solids

VSS: Volatile Suspended Solids

COD: Chemical Oxygen Demand

VOC: Volatile Organic Carbon

LIST OF TABLES

	<u>Page no</u>
Table 2.1 Characteristics Of Leachates	12
Table 2.2 50 % Inhibition Values Obtained in The Literature	18
Table 2.3 Factors Influencing MPB/SRB Competition	22
Table 2.4 Bacterial Yield of SRB For Acetate, Propionate And Butyrate	23
Table 2.5 Summary of SRB and MPB Competition Depending on COD/SO ₄ Ratio	26
Table 2.6 Optimum, Minimum and Maximum pH Values For aSRB and aMPB	30
Table 3.1 Characteristics of the Leachate Used in the Study	44
Table 3.2 Experimental Feeding and Monitoring Details	44
Table 3.3 Feeding Details during the Experimental Period	45
Table 3.4 Physical and Chemical Data	45
Table 3.5 Operation Parameters for Gas Chromatography Running	47
Table 4.1. System Treatment Performance for 8 Months	49
Table 4.2. Sulfide Metabolism, Gas Production and COD Removal During the Experimental Period	55
Table 4.3 Summary of Inhibition Constants From the Literature	63
Table 4.4 Experimental Data for Determination of Rate Constants	64
Table 4.5 Zero, Half and First Order Reaction Approximations	65
Table 4.6 Reaction Rate Constants	65
Table 4.7 Experimental Data for Determination of 2 nd Order Rate Constants	66
Table 4.8 Comparison of a and b with the Reported Values in the Literature	66
Table 4.9 Experimental Data For Determination of Y and K _d	68
Table 4.10 Summary of Yield Coefficient For The Anaerobic Leachate Treatment	69
Table 4.11 Dissolved Sulfide Production in Chlorinated Compound Receiving and Control Reactors	71
Table 4.12 Biotransformation Rates Observed at Different Chlorinated Compound Concentrations	74
Table 4.13 Biotransformation Rates of PCA And PCE when Spiked Individually and in Mixture	75
Table 4.14 TCE Formation in Reactors	76
Table 4.15 Summary of Chlorinated Aliphatic Spiking to Reactors Fed with Leachate and Methanol	77

LIST OF FIGURES

	<u>Page no</u>
Figure 2.1 Substrate Utilization Pathways With Propionate	23
Figure 2.2 Relationship Between Bicarbonate Alkalinity, pH and Carbon Dioxide Percentage In Anaerobic Treatment	33
Figure 3.1 Batch Reactor Used For Leachate And Methanol Treatment	43
Figure 3.2 Burette Used For The Liquid Displacement Method	43
Figure 3.3 Gas Chromatograph Used For The VOC Analyses	46
Figure 3.4 Experimental Setup of Routine Analyses	46
Figure 4.1 Change in COD Removal and Gas Production Levels Depending on Organic Loading Rate	51
Figure 4.2 Initial COD and Gas Yield Values	51
Figure 4.3 Alkalinity and pH Change with Increasing Initial COD Concentrations	53
Figure 4.4 COD Removal, MPB And SRB Activities (%) and Gas Yield Change with Increasing COD Applied	59
Figure 4.5 Dissolved Sulfide (DS), Gas Production as Percent of Theoretical Value, H ₂ S, and pH Profile For Initial COD Applied	59
Figure 4.6 Dissolved Sulfide Concentration, H ₂ S % and pH Profile During The Experimental Period	60
Figure 4.7 Gas Production, SRB and MPB COD Utilizations (%) for COD Initial Concentrations Applied During The Experimental Period	60
Figure 4.8 Determination of Sulfide Inhibition Pattern	62
Figure 4.9 Determination of Sulfide Inhibition Constant (K _i)	62
Figure 4.10 Evaluation of Zero and Half Order Reaction Rate Constants From Batch Reactors	65
Figure 4.11 Evaluation of a and b Constants for the Second Order Reaction Approximations	67
Figure 4.12 Determination of Yield (Y) and Decay Constant (K _d)	68
Figure 4.13 Determination of μ_{Max} and K _s Using Non-Linear Regression	70
Figure 4.14 Gas Production and COD Removal in Tetrachloromethane Receiving Reactors	72
Figure 4.15 Gas Production and COD Removal in Tetrachloroethane Receiving Reactors	72
Figure 4.16 Gas Production and COD Removal in Tetrachloroethylene Receiving Reactors	73
Figure 4.17 Gas Production And COD Removal in Mixture Receiving Reactors	73

NOTATION LIST

μ	: Specific growth rate, time^{-1}
$\mu_{\max}$	: Maximum growth rate, time^{-1}
S	: Growth limiting substrate concentration, ML^{-3}
S_0	: Initial substrate concentration, ML^{-3}
K_s	: Half-saturation constant, ML^{-3}
K_i	: Inhibition constant, ML^{-3}
Y	: Cell yield, MM^{-1}
k_d	: Specific decay rate, time^{-1}
X	: Biomass concentration, ML^{-3}
r	: Reaction rate, $\text{ML}^{-3}\text{time}^{-1}$
k	: Reaction rate constant, k_0 , $\text{ML}^{-3}\text{time}^{-1}$, $k_{1/2}$, $\text{ML}^{-1/2} \text{L}^{-3/2}\text{time}^{-1}$, k_1, time^{-1} , k_2 , time^{-1}
n	: Reaction order

SIZINTI SUYUNUN ANAEROBİK ARITIMINDA SÜLFÜR TOKSİSİTESİNİN MODELLENMESİ VE KLORLU ALİFATİKLERİN BİYOTRANSFORMASYONU

ÖZET

Genç düzenli çöp depolama sahasından alınan ve yüksek miktarda çözünmüş KOİ (33,000 mg/L) ve sülfat içeren (1300 mg/L) sızıntı suyu kesikli reaktörlerde 35°C'de arıtılmıştır.

Sekiz ay boyunca haftalık periyotlarla, sızıntı suyu artan KOİ konsantrasyonlarında (1300-8400 mg/L) uygulanmıştır. KOİ giderimi %60-89 arasında değişmiştir ve gaz dönüşümü, 35°C'de 119-436 mL gaz/g KOİ giderilen olarak elde edilmiştir. Biyokitlenin organik içeriği, toplam katıların %25'i civarında gerçekleşmiştir.

Alkalinite, bir besleme boyunca 600 mg/L seviyesine kadar üretilmiştir. pH, yükselen alkalinite ile artmış ve asit ilavesi ile kontrol edilmiştir.

Sülfat çözünmüş sülfüre dönüşmüş ve ilk ay yüksek konsantrasyonlarda seyretmiştir (100-450 mg S⁼/L) ve kalan periyotta ise daha düşük seviyelerde gerçekleşmiştir (60-130 mgS⁼/L). H₂S pH'ya bağlı olarak bazik pH'da düşük konsantrasyonlarda (0.7-9 mg S⁼/L) ve nötr pH'da yüksek konsantrasyonlarda (23-32 mg S⁼/L) oluşmuştur. pH'nın nötr değerlere çekilmesi sülfür toksisitesini arttırmıştır (en fazla toksik etkiye sahip olan H₂S'in konsantrasyonu arttığı için), KOİ giderimi %53-64 seviyesine inmiştir ve gaz dönüşümü, 184 mL/g KOİ giderilen olarak elde edilmiştir.

Aktif bakteriyel gruplar belirlenmeye çalışılmıştır. Propiyonik asit fermente eden asetojen bakteri, çözünmüş sülfür ve H₂S seviyelerinden inhibe olmuştur, propiyonik asit oksidasyonu, sülfür çeşitlerine daha dayanıklı olan sülfat bakterileri tarafından gerçekleştirilmiştir. Sülfür ve alkalinite üretimindeki düşüş, diğer bir hassas grup olan asetik asit gideren sülfat bakterilerinin etkilendiğini ve asetik asit kullanan metan bakterileri tarafından faaliyetlerinin devralındığını göstermiştir. Sülfür toksisitesine en dayanıklı gruplar olan hidrojen kullanan metan ve sülfat bakterilerinin, deneysel çalışma boyunca aktif oldukları gözlenmiştir.

Sülfür toksisitesi modellenmiş ve "uncompetitive" inhibisyon modeline uygunluğu gözlenmiştir. İki farklı KOİ konsantrasyon aralığı için farklı inhibisyon sabitleri elde edilmiştir (KOİ:1300-1700 mg/L aralığı için K_i:196 mg S⁼/L ve KOİ: 2200-5500 mg/L aralığı için K_i: 1684 mg S⁼/L).

Reaksiyon hız sabitleri olarak, k₀:4.8 g⁻¹, ve k_{1/2}:2.158 g⁻¹ elde edilmiştir. Büyüme dönüşüm katsayısı (Y) ve içsel solunum hızı (k_d) 0.24 and 0.015 g⁻¹, olarak bulunmuştur. K_s and μ_{max} ise 2500 mg/L ve 0.018 g⁻¹, değerlerinde elde edilmiştir.

Klorlu üç bileşik, CT, PCE ve PCA, arıtım yapan reaktörlere tek ve karışım olarak injekte edilmiştir. Uygulanan konsantrasyonlar, CT için 0.145-1.45 mg/L, PCA için 0.2-2.0 mg/L, ve PCE için 0.197-2.36 mg/L aralığında gerçekleşmiştir. CT, uygulandığı reaktörlerde yüksek derecede toksisiteye yolaçmış, gaz üretimi durmuş, fakat KOİ giderimi etkilenmemiştir. PCA ve PCE, gaz üretimini ve KOİ giderimini önemli derecede etkilememiş ve indirgeyici klor bağlarının kırılma reaksiyonları ile TCE'ye dönüşümü gözlenmiştir.



MODELING OF SULFIDE INHIBITION AND CHLORINATED ALIPHATIC BIOTRANSFORMATION OF ANAEROBIC TREATMENT OF LANDFILL LEACHATE

SUMMARY

Landfill leachate taken from a young municipal landfill site containing high concentration of soluble COD (33,000 mg/L) and sulfate (1300 mg/L) was treated in batch reactors at 35°C.

Eight months of leachate feeding was applied at increasing COD concentrations (1300-8400 mg/L) in weekly periods. COD removal averaged between 60-89% and gas yield between 119-436 mL gas/g COD removed at 35°C were obtained. Organic content of the biomass stayed constant at 25% of total fixed solids.

Alkalinity was produced at high levels up to 600 mg/L per feeding set. pH tended to rise with the alkalinity produced and acid addition was necessary for pH control.

Dissolved sulfide was produced from sulfate at high levels in the first month (100-450 mg S⁼/L) and at relatively lower levels for the rest of the experimental period (60-130 mg S⁼/L). Un-ionized sulfide (H₂S) concentration changed depending on the pH, with low levels at alkaline pH (0.7-9 mg S⁼/L) and high levels around neutral pH (23-32 mg S⁼/L). pH adjustment to neutral values increased the degree of sulfide inhibition (due to H₂S which is the most toxic sulfide specie) where COD removal dropped to 53-64% and gas yield to 184 mL/g COD removed.

An approach to estimate active bacterial groups was made. Propionic acid fermenter acetogens were strongly inhibited by both dissolved sulfide and H₂S levels encountered in the reactors, thus, propionic acid was oxidized by sulfidogens having a much higher tolerance level to sulfide toxicity. Drop in sulfide and alkalinity production throughout the experimental period indicated that another highly sensitive group, aceticlastic sulfidogens were also strongly inhibited and aceticlastic methanogens outcompeted them. As the most tolerant species to sulfide, hydrogenotrophic methanogens and sulfidogens were believed to be highly active throughout the experimental period.

Sulfide toxicity was modeled and data best suited uncompetitive inhibition model with two different K_i values at different COD ranges applied (K_i:196 mg S⁼/L for COD:1300-1700 mg/L and K_i:1684 mg S⁼/L for COD:2200-5500 mg/L).

Reaction rate constants obtained were $k_0:4.8 \text{ d}^{-1}$, $k_{1/2}:2.158 \text{ d}^{-1}$, where biomass yield coefficient (Y) and decay constant (k_d) were found as 0.24 and 0.015 d^{-1} , respectively. K_s and $\mu_{\max}$ were calculated as 2500 mg/L and 0.018 d^{-1} , respectively.

Three chlorinated compounds CT, PCE and PCA were spiked to the reactors (two parallel sets, one fed with leachate and the other with methanol) individually and in mixture at concentrations 0.145-1.45 mg/L for CT, 0.2-2.0 mg/L for PCA, and 0.197-2.36 mg/L for PCE. CT caused severe inhibition on reactors, leachate fed reactor did not recover in gas production where COD removal was not affected. PCA and PCE did not significantly affected gas production and COD removal. PCE and PCA were observed to be biotransformed both to TCE via reductive dehalogenation and dehydrohalogenation reactions.



CHAPTER I INTRODUCTION

1.1 General

Sanitary landfilling is the most preferred and feasible waste stabilization method. Landfills are grand-size anaerobic reactors open at surface undergoing physical and biochemical processes in the waste body. Anaerobic processes proceed in deeper parts where oxygen can not penetrate or is depleted. Each anaerobic stage towards the stabilization of the waste takes different time periods depending on waste degradability. In the acidification stage, complex materials are hydrolized to simpler molecules. Landfills undergoing this stage are considered as young landfills. At the end of acidification stage, acetogenesis and methanogenesis take place where methane gas is produced as the final product and the landfill is of old type with mostly stabilized waste material.

Landfill leachates originating from young landfills contain very high concentrations of soluble organic matter consisting mainly of free fatty acids. In addition to organic matter, several inorganic compounds such as ammonia, sulfate, heavy metals, and also chlorinated organics (especially aliphatics) are present in the leachate. High organic content makes leachate very amenable to anaerobic biological treatment.

Leachate characteristics vary depending on many factors such as waste composition, pH, redox potential and landfill age. Environmental factors (rainfall, climate, seasonal variability) are also effective on the quality and quantity of leachate.

Anaerobic biological treatment of leachate from young landfills brings about many aspects as a very complex treatment process. Inorganic compounds such as ammonia, sulfate and heavy metals are the most important factors affecting system performance.

Ammonia is toxic to bacterial activity at alkaline pH (Speece, 1983) where unionized ammonia rises in concentration and is likely to cause inhibition effects in the leachate treatment due to its neutral charge where alkalinity is produced at high amounts from acetic acid degradation pushing pH to higher values necessitating pH control with acid addition.

Sulfate is used by sulfidogen bacteria as an electron acceptor and reduced to S^{2-} producing HS^- and H_2S depending on pH and temperature. As with ammonia, unionized sulfide form (H_2S) is the most toxic sulfide specie to bacterial activity and rises with decreasing pH. Its high toxicity has been proven due to its ability to penetrate through the cell wall with its neutral charge.

Heavy metals in the leachate are also very undesirable compounds due to their precipitation with sulfide resulting in inorganic solids development which finally causing very low active biomass in the treatment system and also other operational problems (Chang, 1989, Keenan et al., 1993).

Anaerobic leachate treatment in upflow anaerobic sludge blanket reactors have been the most preferred configuration due to better solids retaining and higher capacity to tolerate high and variable organic loading and toxicity (Berrueta and Castrillon, 1992, Ozturk et al., 1999). On-site UASBR also gave a good and stable treatment performance even at low temperatures (Kettunen and Rintala, 1998).

Both ammonia and sulfide toxicity were best modeled using uncompetitive inhibition model where effluent COD rises with increasing toxicant concentration up to a certain concentration at which abrupt failure of the system occurs (Speece, 1986, 1996, Maillacheruvu and Parkin, 1996).

Anaerobic treatment of leachate is proceeded by a very complex bacterial enrichment in which sulfate-reducer sulfidogens are as active as methane-producing methanogens and propionic acid oxidizer acetogens. Sulfidogens compete for the same substrates with methanogens and acetogens. Sulfate-reducers can utilize acetic

acid, propionic acid and hydrogen as substrates resulting in dissolved sulfide and H_2S gas production canalizing some percentage of available COD to sulfidogenic metabolism, lowering methanogenic share of COD utilization and thus methane production. This bacterial competition proceeds depending on several factors such as $\text{COD}/\text{SO}_4^{=}$ ratio, organic loading rate, pH and sensitivity of bacterial groups to sulfide toxicity.

Six major COD utilization pathways from propionic acid have been identified including acetogenic, sulfidogenic and methanogenic activities (Maillacheruvu and Parkin, 1996). These pathways are incomplete propionate oxidation to acetate by sulfidogens, propionate fermentation to acetate and hydrogen by acetogens, acetate utilization by sulfidogens, acetate utilization by methanogens, and hydrogen oxidation by sulfidogens and methanogens.

The competition between bacterial groups depends mostly on the availability of organic matter and sulfate and inhibition level of each bacterial group. Methanogenesis will not shut down even under high sulfate conditions due to tolerance level to dissolved sulfide and H_2S of different classes of methanogens. Each group has a different sensitivity to dissolved sulfide and un-ionized sulfide enabling the prediction of the dominant groups considering all the parameters effective on the system.

Propionic acid fermenter acetogens and aceticlastic sulfidogens are the most sensitive groups to both dissolved sulfide and H_2S with lowest K_i values of 53 mg $\text{S}^{=}/\text{L}$ and 35 mg $\text{S}^{=}/\text{L}$ for dissolved sulfide, respectively (Maillacheruvu and Parkin, 1996). Therefore aceticlastic methanogens can successfully compete for acetate due to their lower inhibition level. The least affected groups are hydrogenotroph methanogens and sulfidogens which will compete for the available hydrogen providing more favorable conditions for acetate production and preventing hydrogen accumulation in the system.

Other important system parameters to define bacterial groups' activity are COD/SO₄⁼ ratio and organic loading rate (Choi and Rim, 1991, Maillacheruvu and Parkin, 1993, Lens et al., 1998). COD/SO₄⁼ ratio of 4-5 is considered as the turnover point for more advantageous conditions for methanogens. For lower ratios down to 1.5, sulfidogens can successfully outcompete methanogens under which no methanogenic activity will proceed. Sulfidogens will consume COD at much higher levels than methanogens.

Under high sulfate conditions, sulfate reduction will proceed mainly via hydrogen utilization and secondly by propionate oxidation both by sulfidogens and acetogens.

Under sulfate-limited conditions, sulfidogens will prefer propionic acid and hydrogen to acetate where acetate degrading *Desulfobacter sp.* will not succeed in competition for the available sulfate due its nature as a poor competitor among other sulfidogens.

Hydrogen-using sulfidogens can outcompete hydrogenotroph methanogens at low hydrogen levels having a higher affinity for hydrogen than methanogens (Yamaguchi et al., 1997).

In the long term competition between methanogens and sulfidogens, methanogens are more advantageous with higher organic loading rates at a constant COD/SO₄ ratio where sulfidogenic bacteria are adversely affected (Maillacheruvu and Parkin, 1993).

Sulfidogenic bacteria are a very diverse group able to shift to different pathways depending on environmental conditions. Acclimation and higher levels of lactate/SO₄ ratios were observed to affect net metabolic pathways of incomplete-oxidizing sulfidogens which grow faster than complete-oxidizing sulfidogens (McCartney and Oleszkiewicz, 1993). Sulfidogens are part of anaerobic environments even in the absence of sulfate with their ability for syntrophic growth in association with hydrogenotrophic methanogens (Ueki et al., 1998).

A general conclusion many studies come to is that under acetate and hydrogen-limited conditions in a sulfate-rich environment, sulfidogens can outcompete methanogens due to their higher affinity for these substrates. At higher acetate and hydrogen levels, $\mu_{\max}$ does not determine the outcompeters, but other system conditions are effective. Under long term high sulfide conditions, 4 groups of bacteria will predominate; aceticlastic methanogens, incomplete propionate-oxidizing sulfidogens, hydrogenotrophic methanogens and sulfidogens.

Halogenated aliphatic compounds are the major contaminants in groundwater and components of hazardous wastes and landfill leachates. Most of them originate from industrial, commercial and agricultural sources and consist of brominated and chlorinated alkanes and alkenes with one to three carbon (Vogel et al., 1987). Dry-cleaning operations and semiconductor manufacturing commonly use chlorinated ethenes and ethanes. Brominated and chlorinated methanes are produced as a result of chlorination of drinking water.

Despite their toxicity effects to bacterial activities in biological treatment systems, their biotransformation abilities under reducing conditions of anaerobic environments have been showed.

Methanogenic bacteria were proved to develop acclimation to chlorinated compounds in time and resume to their normal gas production level even at higher toxicant concentrations (Yang and Speece, 1986).

Several biotransformation reactions were observed where the most common is reductive dehalogenation where a halogen atom is replaced with an hydrogen atom from an electron donor. An external carbon source is required for this reaction and the result is a more reduced molecule. Another common reaction is called dehydrohalogenation where a halogen from a carbon atom is removed with a removal of an hydrogen atom from an adjacent carbon atom to form a double bond. This reaction does not require an external electron supply and does not change the

oxidation state of the reacting molecule. Halogenated alkanes can undergo this reaction to produce alkenes.

Dechlorination was observed in many studies from highly chlorinated aliphatic compounds to less chlorinated ones which are mostly less toxic and more amenable to further treatment. Lower dechlorination rates were also observed as the chlorine number of the molecule decreased (Mohn and Tiedje, 1992, Vogel et al., 1987).

As three of the most common contaminants, tetrachloroethylene (PCE) and tetrachloromethane (CT) were observed to be transformed to less chlorinated compounds. PCE was degraded to trichloroethylene (TCE), dichloroethylene (DCE) and vinyl chloride (VC) (Vogel and McCarty, 1985) and to ethane with further transformation (DeBruin et al., 1992). CT was transformed to CO₂ via hydrolysis (Bouwer and McCarty, 1983).

Mostly methanogenic bacteria were held responsible for the reductive dechlorination but several reports showed the acetogenic bacteria involvement in the reaction as well (DiStefano et al., 1991).

1.2 Objectives

Many studies concentrated on the engineering of suitable systems for the treatment of leachate which today has to be stabilized as the problematic product of sanitary landfilling posing grave operational diversity with its changing characteristics depending on many factors. Anaerobic treatment seems most promising treatment alternative for the leachate containing high amounts of volatile fatty acids readily degradable anaerobically. As other biological systems, anaerobic treatment of leachate is affected by many factors especially toxicity (sulfide, ammonia, chlorinated organic compounds, heavy metals and hazardous materials). pH plays an important role in determining toxicity levels (sulfide and ammonia) and precipitation of metals. The presence of sulfate promotes sulfidogenic bacteria whose activity lowers gas production, results in the production of H₂S and lowers methanogenic activities. Also, another significant component of leachate is chlorinated compound family

which easily can be found in the waste body originating from domestic and industrial use. These compounds can evaporate, dissolve in leachate or are adsorbed on the solid waste.

In this study, anaerobic treatment of leachate from Istanbul sanitary landfill site was studied with in simple batch reactors receiving leachate at increasing organic loading rates with its different bacterial response characteristics to inborn sulfide toxicity and possible chlorinated compounds' effects due to its highly complex structure. Batch reactors are the simplest tools to determine any toxicity effect, kinetic behavior and short-term response of the system. Gas production as well as other important parameters were monitored and many different system conditions could be implemented to observe corresponding response pattern at lowest cost in paralel reactors fed with methanol. Substantial and guiding data were obtained for further more complex treatment of complex wastewaters.

In this study, different bacterial responses to sulfide toxicity (and to chlorinated aliphatic compounds toxicity with further biotransformation characteristics) depending on pH with sulfide, alkalinity and gas production monitored daily as well as overall COD removal characteristics were observed with increasing organic loading rate and changing pH. Competition between two major bacterial groups, sulfidogens and methanogens with changing system conditions was observed. These two groups of bacteria are also known for their reductive dehalogenation capabilities, that's why three chlorinated aliphatic compounds were spiked at increasing concentrations individually and in mixture to the reactors fed with methanol (where methanogenic activity is promoted) and with leachate (where sulfidogenic, methanogenic, acetogenic and fermenting bacteria activities are obtained) to observe any biotransformation and toxicity characteristics on system performance.

Sulfide toxicity was observed depending on pH at different levels for different groups of bacteria. Shift in bacterial species was observed during the experimental period. As contribution to the literature where specific substrates were used artificially, organic matter removal and sulfide toxicity were modeled and kinetic

constants were obtained for leachate. Much lower levels of inhibition on system performance were observed with leachate where Monod kinetics were used to determine biokinetic constants. Uncompetitive inhibition suited best to the data obtained also supported by previous inhibition studies with sulfide and inhibition constant was determined for this specific substrate feeding. Biotransformation rates and products of chlorinated compounds were determined where no significant overall performance effect due to chlorinated aliphatic toxicity was observed. As addition to reductive dehalogenation, dehydrohalogenation reaction also was observed which was not reported previously from tetrachloroethane.



CHAPTER II BACKGROUND

2.1 Leachate Characteristics

Formation of leachate in landfills follows slow biochemical reactions undergoing inside the waste body. Environmental factors such as rainfall, climate, seasonal variability, type of waste received to the depony all affect the leachate quality and quantity. Leachate characteristics also vary depending on waste composition, pH, redox potential and landfill age.

Leachate production is related to:

- meteoclimatic conditions (annual rainfalls distribution, average temperature and humidity, direction and strength of predominant winds, evapotranspiration phenomena),
- planimetric characteristics of the site and neighbouring areas (surface runoff from/to the landfill),
- MSW intrinsic humidity and liquids resulting from biodegradation processes,
- infiltration and moisture storage characteristics of soil and solid waste layers,
- landfilling operation (recirculation ratio of the leachate onto the MSW mass, compaction level of the waste).

Hazardous and toxic materials in the waste body can easily pass into the leachate rendering it less degradable. pH and redox potential influence all the chemical processes inside the landfill. A landfill's waste body can be considered as a partly open anaerobic reactor undergoing all the anaerobic stages in deeper parts where oxygen can not penetrate or is depleted. All three anaerobic stages proceed in time, even at the same time at different parts of the landfill; acidification (hydrolysis), acetogenesis and methanogenesis. Waste nature affects each process' duration. Biodegradation phenomenon start with chemical and enzymatic hydrolysis of the complex organic macromolecules (proteins, lipids, carbohydrates) occurring at the

liquid-solid interface of the wet MSW masses. In the acidogenic stage, occurring at low pH environment, the hydrolyzed organic matter is degraded into short-chain (C1-C3) volatile acids, which are further degraded into methane and carbon dioxide at neutral pH conditions in the methanogenic stage.

Readily degradable organic matter lowers time necessary for acidification but for some resistant-to-degradation materials, it takes longer for their stabilization (cellulose, e.g.). Leachate produced will vary on quality and quantity depending on the stage the waste body is undergoing. Leachate characteristics can be used as an indicator of the degree of stabilization within a landfill.

Landfills are classified based on their age (operation time) as young and as old types. In young landfills, stabilization of the organic waste is in the acidification stage which may take from 1 to 9 years in time scale (Harmsen, 1983). Leachate originating is rich in organic load consisting mainly of free fatty acids making it readily degradable for biological treatment. In old landfills, stabilization of the organic matter is almost complete and organic loading is much lower consisting mainly of high molecular weight compounds which are more resistant to bacterial degradation.

In general, knowledge of the qualitative and quantitative characteristics of the leachate produced at municipal solid waste landfilling sites allows for the following returns in terms of design and management operations (McBean et al., 1993, Liberti et al., 1997):

- proper design of the landfill based on scientific data,
- long term planning of the leachate treatment operations,
- accurate economic evaluation and timing of the leachate management operations,
- preventive management of rebound effects after specific actions on the landfilling operations,
- minimization of the environmental impacts on air, groundwater, soil and biota.

In the characterization of leachates mainly two types are considered: leachates from old and young landfills. The chemical and physical structure of these leachates differ

significantly. Harmsen (1983) identified organic compounds in two different types of leachate, one collected from a waste tip in the acidification stage. The organic load was high and consisted mainly of free volatile acids (over 95% of TOC). Volatile amines and ethanol were also present. Alcohols, hydrocarbons, esters, terpenes and phthalates were identified with a low concentration of high molecular weight compounds.

The second type of leachate was collected from a waste tip in the methane fermentation stage. The organic load was relatively low. An important part of the organics was identified as high molecular weight compounds (32 % of organic carbon belonged to compounds with a molecular weight over 1000 belonging to the humic compounds group).

Heavy metal content of the two types of leachate was remarkably different, due to lower pH in the acidification stage where metals are more soluble also by complexation with free volatile acids. In the methane fermentation stage, pH is much higher, no acids are present resulting in a significant fall in soluble metal concentrations (except for Lead which is able to form very stable complexes with the humic acids).

Först et al., (1989) determined volatile organic pollutants in leachate from different landfills (six hazardous waste landfills and five domestic refuse dumps). Chlorinated hydrocarbons were found in concentrations up to 20 000 µg/L in the samples from the hazardous waste landfills and 14 µg/L in samples from the domestic refuse dumps. Methylene chloride, trichloroethylene and tetrachloroethylene were identified as the main components. Benzene and alkylated benzenes were determined at concentrations between 5 and 13 000 µg/L in the samples from the hazardous waste landfills and between 1 and 500 µg/L from domestic refuse dumps.

Mendez et al. (1989) characterized two types of leachate from two landfill sites in the same area receiving similar type of waste (Table 2.1). The leachate from the older tip had much lower organic load with a 40 % refractory content to anaerobic treatment.

Analysis results from another long term study are also given in Table 2.1 (Krug and Ham, 1997). They observed long-term leachate quality from 13 municipal solid waste landfills. In general, leachate constituents rose in concentration during landfill operation, reached peak values approximately at closure, followed by a gradual decrease after closure. Transition from acidogenic phase to methanogenic-dominated decomposition led to most significant BOD, COD, hardness, iron and cadmium decreases and pH rising.

Moreover in another leachate composition (Chang, 1989) a wide difference was observed between the young landfill leachates. Usage of gel permeation technique enabled him to characterize molecular weight distribution of the leachate. Molecules having molecular weight over 2000 constituted 11% of COD in the raw leachate.

Table 2.1 Characteristics of leachates (all except pH in g/L)

	Old Landfill Leachate (Mendez et al.)	Young Landfill Leachate (Mendez et al.)	Young Landfill Leachate (Chang)	Acid Anaerobic Phase (Krug&Ham)	Methano- genic Phase (Krug&Ham)
pH	7.6	7.30	7.0	4.5-7.5	7.5-9
COD _t	0.83	4.1	58.4	6-60	0.5-4.5
COD _s	0.65	3.5	(BOD ₅ :40)	(BOD ₅ :4-40)	(BOD ₅ :0.02-
SS	0.2	1.2	43.8		0.6)
VSS	0.12	0.6	20.7		
N-NH ₄ ⁺	0.28	0.8	1.72		
P-PO ₄ ³⁻	0.12	0.0	0.063		
Protein	0.00	0.5			
Alkalinity	1.9	3.16	8.53		

Leachate samples contain a significant amount of colloidal matter for which fractionation procedure reveals size distribution and metal content. In samples collected from four Danish landfills colloids were found but dissolved fraction with a size smaller than 0.001 µm constituted more than 78% of the total solids (Jensen and Christensen, 1997). The majority of the colloids were small and found in the fraction between 0.001 and 0.40 µm (characterized as dissolved passing through 0.45 µm

filter paper). Up to 10% of the total solids were found as suspended matter larger than 1.2 μm . For Zinc and Nickel, up to 95% and 60% (respectively) of total content were associated with the colloidal fractions.

Similar results were obtained in another leachate characterization (Lavagnolo and Palmas, 1997) study with volatile solids, where the ratio between dissolved and non settleable solids was smaller than 1. This phenomena also affects determination of BOD₅ and COD, values decrease when considering raw leachate, sedimented sample and filtered leachate. The result is that organic substance is concentrated in volatile solids, particularly in dissolved solids, where the highest fraction of volatile solids belongs to the dissolved quotient and is characterized by a ratio between dissolved and total solids higher than 80%.

Leachates originating from simulated landfill column reactors operating under sulfate-reducing and methanogenic conditions treating a refractory organic matter, cellulose and lignocellulosic material, differed in composition (Kim et al., 1997). Degradation of cellulose was faster under sulfate reducing conditions, therefore, leachate was high in organic content.

Leachate monitoring is also a useful tool to follow the anaerobic stages in a landfill, especially of volatile fatty acids potentially posing delaying effect on methanogenesis, such as acetic acid (James et al., 1998). Possible remedial strategies can be designed to reduce concentrations of VFA (such as bicarbonate addition to promote methanogenesis under acidogenic conditions).

Gel permeation chromatography technique was used to better characterize landfill leachate samples in depth and to evaluate different treatment systems' efficiency on different level of molecular weight substances (Milot et al., 1987). They obtained good results on the efficiency of aerobic treatment of low molecular weight and physical-chemical treatment of compounds with molecular weight of 5000.

2.2 Anaerobic Biological Leachate Treatment

Two alternatives are usually practiced for proper management of MSW landfill leachate: the first is based on leachate recirculation onto the waste body by over- or sub-irrigation (Scudato, 1993), the second is based on the treatment of the wastewaters in- or off- the landfilling site. In the case of over-irrigation of leachate by sprinkling, evaporation phenomena allows volume reduction of liquids, but, resulting in increase of pollutants concentrations which further precipitate onto the MSW body (Vizler et al., 1993). This is most certain for heavy metals precipitation which necessitates removal of metals by lime treatment prior to irrigation. Odor problem also rises with young landfills, where sub-irrigation is a solution in terms of smell control but shows inefficiency due to short circuiting and channeling. Storing leachate in the rainy season where high volumes of leachate are produced is another practice to keep seasonal leachate production under control (Higuchi et al., 1997).

Treatment alternatives off-the landfill site for leachate are transfer (to a municipal treatment plant, to the same landfill), biological methods (aerobic, anaerobic), and physico-chemical methods (precipitation, oxidation, adsorption, stripping, reverse osmosis).

Leachates from young landfills are highly amenable to biological treatment, especially to anaerobic treatment (high oxygen consumption and sludge production do not make aerobic treatment feasible) due to high volatile fatty acid content. High metal content necessitates pretreatment to protect the anaerobic biomass against mineralization. Post-treatment of effluents from anaerobic digestion is also needed for further COD removal to acceptable levels. pH control is one of the most important operational challenge regulating toxicity levels frequently encountered in anaerobic digesters treating leachate (ammonia or sulfide toxicity, e.g.).

Combined treatment processes are often used to obtain acceptable effluent quality: leachate treatment with coupling biological nitrification-denitrification and oxidation by ozone (Primi et al, 1997), anaerobic pre-treatment followed by reverse osmosis (Bauchot et al., 1997), biological nitrification-denitrification + chemical oxidation by ozone, final biological treatment with rotary disk filter and ion

exchanger (Kettern et al., 1997), biological treatment with a membrane bioreactor followed by a concentration treatment via alternatively used thermal process and reverse osmosis and a UV/ozone rays process (Bressi and Favali, 1997), biological pre-treatment followed by reverse osmosis and evaporation and drying of the concentrate (Hippen et al., 1997), anaerobic lagoons followed by aerobic lagoons (Lavagnolo and Muntoni, 1997), reverse osmosis systems (tubular and spiral membrane) and a double stage under vacuum evaporator with ammonia stripping from the permeate and condensate (Beone et al., 1997).

A co-treatment of leachate with municipal wastewater could be practiced successfully at leachate/wastewater volume ratios between 1-2% (Viviani and Torregrossa, 1997) without any drop in efficiency.

As the most practiced treatment application for leachate, in anaerobic treatment case, Mendez et al. (1989) treated two types of leachate, in a semi-continuous attached growth system, one from old and the other from a young landfill located in the same area. Leachate treatment from old landfill located was ineffective due to the high refractory content of the organic load. Other leachate was treated successfully to levels similar to the leachate from old landfill.

Upflow Anaerobic Sludge Blanket Reactor is a commonly preferred configuration to treat a variety of wastewaters. It can successfully treat high organic load wastewaters due to its great biomass retaining capability. It can also tolerate higher toxicant levels.

Kettunen and Rintala (1998) treated successfully municipal landfill leachate in a pilot-scale UASB reactor, on-site and at low temperatures (13-23°C). Despite changes in leachate quality, 65-75 % COD and up to 95 % BOD₇ removals were achieved with organic loading rates of 2-4 kg COD/m³.d at 18-23°C. At 13-18°C, 50-55 % COD and 72 % BOD₇ removals were obtained at organic loading rates of 1.4-2 kg COD/m³.d. Methane yield averaged 320 ml CH₄/g COD removed. Specific methanogenic activity at 11°C was one third of that at 22-24°C. A problem encountered was the inorganic material accumulation (Calcium and Iron accounted

for 24% and 7% of total solids of the sludge, respectively, at the end of the study). Excessive solids washout was not observed despite low process temperatures.

Ozturk et al (1998) investigated anaerobic treatability of young municipal leachate from Istanbul Sanitary Landfill in a laboratory scale UASBR. A total COD removal of 94 % at an organic loading rate: 3.7 kg COD /m³.d was obtained. For higher organic loading rates as high as 11 kg COD/m³.d, a COD removal of 92-94 % with a 0.39 m³ CH₄ per kg of COD removed was achieved. A significant degree of ammonia inhibition was experienced in some parts of the study due to high alkalinity and pH values. pH control was considered as the most important operational challenge.

In another study with UASBR (Berrueta and Castrillon, 1992), they obtained a maximum of 88 % of COD removal and CH₄ production close to theoretical values at OLR:6.8 kg COD/m³.d at an HRT of 2.4 days. Influent COD ranged from 3553 mg/L to 16394 mg/L. COD removals between 46-88% still resulted in high effluent concentrations in the range of 1331-3005 mg/L which necessitated further treatment such as aerobic and physico-chemical treatment.

Another young landfill leachate was treated in anaerobic sequencing batch reactors (ASBR) and anaerobic hybrid bed filter (AHBF) at mesophilic conditions (Timur and Ozturk, 1997). ASBR achieved 73.9% TOC removal at maximum organic loading rate of 2.8 kg TOC/m³.d at an HRT:1.5 days. A HBF reached 81.4% TOC removal at 1.2 kg TOC/m³.d and an HRT:2.4 days. ASBR performed better than AHBF above OLR:1.5 kg TOC/m³.d. AHBF remained also limited in volumetric removal rate.

Chang (1989) treated efficiently landfill leachate using an upflow anaerobic reactor combined with a sludge bed and a filter where he obtained a removal of 92 % of soluble COD at an OLR<13 kg COD/m³.d. Removal dropped to 70 % at an OLR:21.77 kg COD/m³.d. Accumulation of iron was observed. Influent iron concentration ranged from 160 mg/L to 515 mg/L, whereas it reached 7100 mg/L after 185 days of operation. Sulfate loading affected the system, resulting in less energy recovery (from 90% to 52%). Biogas yield was 0.31 L at STP per gram of

COD removed. Molecules having molecular weight over and below 2000 both underwent a 74% reduction during anaerobic digestion, where larger molecules could be broken down to smaller molecules during anaerobic digestion.

Inorganic solids development resulted in a shut-down of a pilot-scale anaerobic reactor after 9 months of operation on-site (Keenan et al., 1993). COD removals exceeded 85 % at an organic loading rate of 10 kg COD/m³.d . After 9 months of operation, 80 % of the total solids became of inorganic nature due to metal precipitates making prior treatment of metals necessary. Inorganic precipitates were suggested to have caused mass-transfer limitations preventing contact between bacteria and substrate or end-product accumulation inside the granules.

2.3 Sulfide Toxicity, Metabolism And Modeling

2.3.1 Sulfide Toxicity

Anaerobic bacteria can easily produce H₂S from sulfate in the wastewater using it as an alternative electron acceptor to CO₂, thus, resulting in lower CH₄ production. Methane producing (MPB) and SO₄-reducing bacteria (SRB) will compete for the same substrates (acetate and H₂) where one's dominance will depend on the environmental conditions such as pH, toxicity, organic loading rate, trace metal bioavailability, chelators, and vitamins (Speece, 1997). H₂S production and iron precipitation of sulfide may cause undesirable operational problems with industrial wastewaters having low COD/SO₄ ratios.

Factors affecting sulfur concentration in the off-gas and in the aqueous phase are rather dependent on the temperature and pH which directly affects sulfide ionization, thus partitioning between HS⁻ and H₂S. Sulfur sinks in anaerobic digestion are SO₄²⁻ in influent and effluent, H₂S in off-gas, total sulfide in effluent, microbial synthesis of sulfur, and internal precipitation of sulfur.

H₂S poses as a severe toxicant and problem both in the aqueous phase and in the off-gas. pH and temperature affects its balance between H₂S and dissolved sulfide (DS) where H₂S is far more toxicant than DS due to its neutral charge enabling it to affect the bacterial cell contents.

H₂S toxicity levels reported in the literature vary for each study owing to different organic and sulfate loading rates, pH and predominant bacteria groups. Several studies investigated controlling factors on the predominance of the competing groups of bacteria and their findings will be presented in the next section.

Inhibition levels obtained in studies on the sulfide toxicity on the anaerobic bacteria are summarized in Table 2.2. Inhibition type is given as H₂S-originated. aSRB and aMPB stand for acetate-utilizing SRB and MPB. All values differ depending on the system configuration, the type of substrate used, organic loading rate and COD/SO₄ ratio applied.

Table 2.2 Inhibition levels obtained in the literature (Speece, 1997)

Inhibition Level	Reference
• aSRB inhibited before aMPB • No inhibition of SRB at H₂S:178 mg/L • No inhibition at H₂S:200 mg/L (prop+acet.) • 50% inhibition of aMPB at H₂S:1200 mg/L • 50% inhibition of aMPB at H₂S:250 mg/L • 50% inhibition of MPB at H₂S: 5%of gas • SRB inhibited at 83 mg/L • Propionate accumulation at H₂S:100 mg/L • No inhibition on propionate use up to TS:100 mg/L and then severe inhibition • 25-90% inhibition of aMPB at H₂S:150 mg/L • Propionate use less inhibited than acetate and butyrate use at H₂S:20-300 mg/L • Process failure of acetate and propionate degradation at H₂S:60-70 mg/L • Inhibition of acetate and propionate use at H₂S:60-75 mg/L • Inhibition of lactate and glucose use at H₂S:100-150 mg/L • 50% Inhibition of methanogenesis at H₂S:50-250 mg/L	Maillacheruvu et al., 1993, McCartney and Oleszkiewicz 1991 Uberoi and Bhattacharya, 1995 Maillacheruvu et al., 1993 Isa et al., 1986 Koster et al., 1986 Karhadkar, 1987 McCartney and Oleszkiewicz, 1993 McCartney and Oleszkiewicz, 1991 Rinzema and Lettinga, 1988 Lettinga et al., 1984 McCartney and Oleszkiewicz, 1991 Parkin et al., 1990 Maillacheruvu et al., 1993 Maillacheruvu et al., 1993 Visser et al., 1996

Visser et al. (1996) reported sulfide inhibition caused by un-ionized sulfide (H_2S) with a 50% inhibition level where 350-550 mg/L H_2S caused complete inhibition of *Desulfovibrio* species at pH:6.2-6.7. Inhibition of aSRB was reported to have caused a process failure above 50 mg/L H_2S .

Two different studies reported similar inhibition levels (50%) of acetoclastic methanogens at two different concentrations of H_2S (1200 mg/L in Isa et al. (1986) and 250 mg/L in Koster et al. (1986)) where Isa et al. (1986) kept organic and sulfate loading rates very high compared to Koster et al. (1986)., so, the system tolerated much higher H_2S levels. Kharadkar et al. (1987) obtained 50% inhibition levels at total sulfide concentrations of 100-224 mg/L. Parkin et al. (1990) observed process failure with acetate and propionate fed chemostats at H_2S concentrations of 60-70 mg/L.

Hilton and Oleszkiewicz (1988) showed that SRB were more severely inhibited than MPB at higher pH.

Methanogenesis will not shut down under high sulfate conditions due to the tolerance level to dissolved sulfide (DS) and H_2S of different classes of MPB, acetogens, and SRB. In Parkin's study (1995), the most sensitive groups to DS and H_2S toxicity were propionate fermenter acetogens (K_i :53 mg/L) and acetoclastic SRB (K_i :35 mg/L for DS and 8 mg/L for H_2S). Therefore, acetoclastic methanogens can successfully compete for acetate due to their lower inhibition level. The least affected groups are hydrogenotrophic MPB (K_i :1430 mg/L) and SRB (K_i :422 mg/L) with similar specific substrate utilization rates and maximum growth rates. Different tolerance levels to sulfide inhibition will determine the dominant groups in the substrate utilization metabolism.

H_2S can be highly toxic due to its ability to penetrate through the cell wall and combining with the iron cytochromes and other essential iron-containing compounds in the cell (McCartney and Oleszkiewicz, 1993, Maillacheruvu and Parkin, 1996). Once inside the cytoplasm, H_2S may denature native proteins. Parkin et al. (1980) controversially observed that H_2S inhibition was reversible, stating that Coenzyme A and M sulfide linkages may be interfered.

Sulfide toxicity was correlated to the free hydrogen sulfide concentration in the pH range of 6.4-7.2 (Koster et al., 1986), where 50% inhibition was caused by 250 mg S^{2-} /L. 90 mg S^{2-} /L resulted in the same inhibition level at pH:7.8-8.0.

In acetate and propionate-fed systems, responses to sulfide toxicity were different (Maillacheruvu et al., 1993). With acetate as substrate, sulfate removal was affected with the inhibition and no significant recovery of SRB to sulfide toxicity did occur but COD removal approached 100% due to methanogenic activity. Complete recovery of SRB was reported in propionate-fed system. Anaerobic filters were able to tolerate higher toxicity levels than suspended growth reactors. A propionate-fed filter could tolerate more than 150 mg H_2S /L and 1000 mg S^{2-} /L of dissolved sulfide. For acetate-fed filter, these levels were 125 mg S^{2-} /L of H_2S and 400 mg/L of DS.

In the treatment of high sulfate wastewaters, anaerobic filters seem advantageous due to better retaining capacity of MPB in attached growth systems (Koster et al., 1986, Isa et al., 1986, Nielson., 1987). Isa et al. (1986) suggested that anaerobic processes could be affected by feedback inhibition of H_2S on SRB, iron availability for SRB, inocula and substrate concentration effects on SRB and the capacity of SRB to colonize and adhere in the carrier matrix of the reactor.

The predominance of aceticlastic MPB was related to the better biomass retention of MPB in the upflow reactor and inferior attachment capacity of aceticlastic SRB promoted their washout from the reactor at higher upflow velocities (O'Flaherty et al., 1998).

Parkin et al. (1991) treated propionate and sulfate in their upflow anaerobic filter with OLR:3-5 g COD/L.d and at COD/S ratios of 20 to 8. They experienced performance decline at unionized sulfide and dissolved sulfide concentrations of 110 and 350 mg S^{2-} /L, respectively, which were significantly higher than the levels encountered in completely mixed-reactors.

They also observed that most of the biological activity; sulfate reduction, propionate removal and methanogenesis occurred in the same location in the filter.

As the most sensitive reactor configuration, H_2S gas inhibited SRB at concentrations greater than 100 mg S^{2-}/L in serum bottles where a lesser degree of inhibition was obtained in chemostats (Uberoi and Bhattacharya, 1995).

In Choi and Rim's study (1991) more inhibition levels were observed with synthetic substrates than industrial wastewaters. Inhibitory concentrations for sulfate reducers and methane producers were 160-200 mg S^{2-}/L and 120-140 mg S^{2-}/L , respectively, with synthetic substrate.. Inhibition increased proportionally with the organic loading rate. Methanogenesis was not affected by 240 mg S^{2-}/L with sea-food waste treatment.

McCartney and Oleszkiewicz (1991) reported that under strictly sulfidogenic conditions ($\text{COD}/\text{SO}_4^{2-}:0.67$), lactate degradation proceeded unaffected up to total sulfide and H_2S concentrations of 1060 mg/L and 320 mg/L, respectively. Acetate utilization was inhibited proportionally up to 80 mg/L H_2S concentration and was independent of total sulfide concentration.

Increasing organic loading rate resulted in higher H_2S stripping due to increased levels of methanogenesis (Speece, 1997) which will be discussed in more detail in the next section as providing more advantageous conditions to MPB on the competition with SRB.

Another important finding was that at high pH values with high sulfide conditions, even though aMPB's growth was strongly inhibited, their specific methanogenic activity was relatively little inhibited, implying that under stress, bacteria spends energy derived only for cell maintenance, not for growth (Visser et al., 1996).

To prevent sulfide inhibition from affecting methanogenesis, Lens et al. (1998) implemented a staged anaerobic reactor to see the capability of sulfidogens to use sulfate and acetate. They treated a mix of volatile fatty acids (acetic acid, propionic acid and butyric acid) at a COD/SO_4 ratio of 0.5 under strictly sulfidogenic conditions in a USSB (upflow staged sludge bed) reactor, in order to establish acidogenesis and sulfate reduction in acidification stage where more H_2S stripping was expected due to acidic pH. They obtained 35% of sulfate reduction in the

reactor. Acetate using SRB developed very slowly and acetate degradation was the rate-limiting step. The reactor configuration was not found successful to treat acetate and sulfate.

2.3.2 Sulfide Metabolism and Competition Between Sulfate Reducing Bacteria And Methane Producing Bacteria

Sulfate reducing bacteria (sulfidogens) compete with methane producing bacteria (methanogens) for acetate and H_2 . Additionally, sulfidogens also compete with propionate fermenter acetogens for propionate (Parkin et al., 1990, Visser et al., 1993, Uberoi and Bhattacharya, 1995), for butyrate (Mizuno et al., 1994) and benzoate (Li et al., 1996,).

Both SRB and MPB have an absolute Fe^{2+} requirement and in the presence of sulfide are able to successfully compete for Fe^{2+} (Speece, 1997). Factors influencing MPB/SRB competition are given in Table 2.3.

Table 2.3 Factors influencing MPB/SRB competition (Speece, 1997)

- | |
|--|
| <ul style="list-style-type: none">• Substrate concentration in feed• Sulfate concentration in feed• Maximum specific utilization rate (k_{max})• Half velocity constant (K_s)• Thermodynamics/free energy of the reaction• Nutrient availability• Adhesion properties• Proximity of cells• Temperature• Substrate type• Long term shifts |
|--|

Bacterial yield values reported in the literature are given in Table 2.4.

Maillacheruvu and Parkin (1996) examined COD utilization pathways from propionate degradation and concluded in 7 major pathways as shown in Figure 2.1.

Table 2.4 Bacterial yield of SRB for acetate, propionate and butyrate (Speece, 1997)

Electron acceptor	Electron donor	Yield
SO ₄	H ₂	1.9 g cells/mole H ₂
SO ₄	Acetate	4.8 g cells/mole acetate
SO ₄	Propionate	0.086 mg cells/mg propionate

In their comprehensive work, they also determined inhibition constants for each group of bacteria involved in these pathways to better establish competition conditions.

The reactions for SRB utilizing acetate and H₂ are as follows:

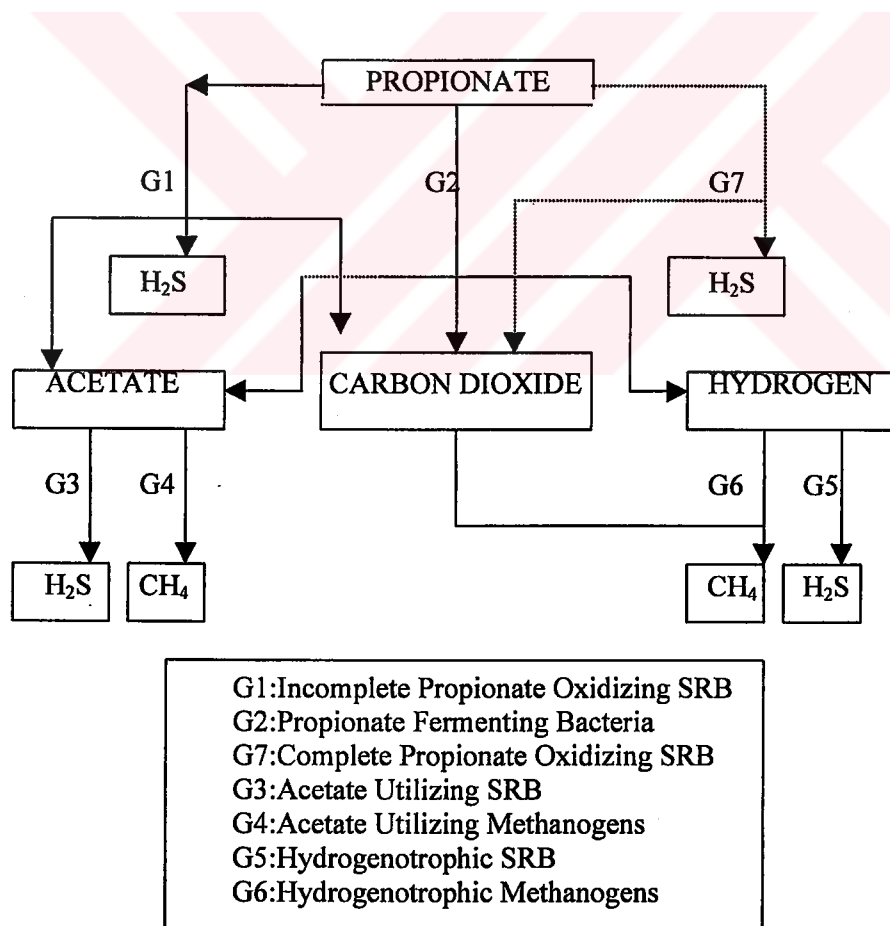
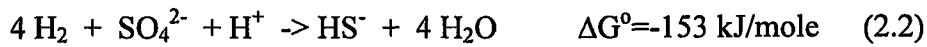
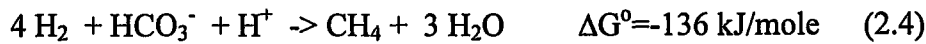
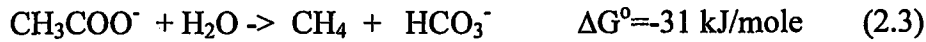


Figure 2.1 Substrate Utilization Pathways with Propionate (Maillacheruvu and Parkin, 1996)



The reactions for MPB utilizing acetate and H_2 are as follows:



Propionate degradation by syntrophic acetogens:



$$\Delta G^0 = 76 \text{ kJ/mole} \quad (2.5)$$

Incomplete propionate oxidation by SRB :



$$\Delta G^0 = -37.8 \text{ kJ/mole} \quad (2.6)$$

Thermodynamically, SRB seems more advantageous. In real systems, it is hard to predict which bacteria will dominate due to system's various parameters. SRB compete not only with MPB but also with hydrogen producing syntrophic acetogens. The predominant species will be the ones surviving under sulfide inhibition conditions depending on their tolerance level (lower K_i values show higher sensitivity to inhibition and higher values are for lower sensitivity) to stressed conditions and also to other parameters such as organic and sulfate loading rate, and pH.

Conversion stoichiometry is 0.395 L CH_4 and H_2S per gram of COD degraded at 35°C . There is a proportional relationship between H_2S in the gas and feed COD concentration (Speece, 1997).

According to Maillacheruvu and Parkin's study (1996), the most sensitive species to sulfide inhibition are propionate fermenter acetogens and acetate utilizing SRB. The least affected groups are hydrogenotrophic MPB and SRB having also similar specific substrate utilization rates and maximum growth rates. Under high sulfate conditions sulfate reduction takes place mainly via hydrogen utilization keeping hydrogen partial pressure low enough to ensure proper system running where propionate oxidation also is realized by SRB which are less inhibited than acetogens.

As a summary of their research, under long term high sulfide loading conditions, four groups of bacteria predominated; aceticlastic MPB, propionate oxidizing SRB, hydrogenotrophic MPB and SRB.

Two other important factors to predict the system performance and dominance of species are organic loading rate and $\text{COD}/\text{SO}_4^{=}$ ratio. Choi and Rim (1991) worked with different $\text{COD}/\text{SO}_4^{=}$ ratios from 1 to 13.3 and concluded that SRB and MPB were very competitive between $\text{COD}/\text{SO}_4^{=}$ ratios of 1.7 and 2.7 and CH_4 production (no production at $\text{COD}/\text{SO}_4^{=}:1.0$) took place increasingly above $\text{COD}/\text{SO}_4^{=}>2.7$. Below this ratio SRB predominated and also survived at $\text{COD}/\text{SO}_4^{=}:0.4$. Inhibitory concentrations were found as 2000 mg SO_4/L (160-200 mg $\text{S}^{=}/\text{L}$) for SRB and 1200 mg $\text{SO}_4^{=}/\text{L}$ (120-140 mg $\text{S}^{=}/\text{L}$) for MPB. MPB and SRB competition occurred at different levels depending on the availability of carbon source. When substrate was limited, SRB competed more with MPB for the same substrates, but under non-limiting substrate conditions, SRB preferred other substrates to acetate and CH_4 yield rose much faster with increasing $\text{COD}/\text{SO}_4^{=}$ ratios. A summary of SRB and MPB competition is given in Table 2.5.

In Harada's research (1994) low influent COD concentrations were fed with different sulfate concentrations. SRB outcompeted increasingly with the increasing sulfate concentrations. They fed low levels of starch and glucose with three COD/SO_4 levels (0.8, 3.3, and 16). At the lowest ratio they obtained 75% of COD utilization by SRB. No decrease in methanogenic activity were observed in acetate-fed vials with the addition of sulfate, with only exception of long term dominating behavior of aSRB, having higher affinity for acetate and higher substrate removal rate. No propionate accumulation was observed in the presence of sulfate where

Table 2.5 Summary of SRB and MPB competition depending on COD/SO₄⁼ ratio (Choi and Rim, 1991)

% COD degraded	COD/SO ₄
60-70 to CH ₄ 22 to H ₂ S 6-14 to cells	>2.7
29-47 to CH ₄ 46-59 to H ₂ S 6-1 to cells	1.7
No CH ₄	1.0

SRB were observed to contribute strongly to propionate degradation. SRB shifted to using hydrogen (their most favorite substrate) when sufficient proton-reducing bacteria (syntrophs) for propionate degradation were active in the reactor and also predominated over MPB on the use of hydrogen.

McCartney and Oleszkiewicz (1991) reported that under strictly sulfidogenic conditions (COD/SO₄:0.67), lactate degradation proceeded unaffected up to total sulfide and H₂S concentrations of 1060 mg/L and 320 mg/L, respectively. Acetate utilization was inhibited proportionally up to 80 mg/L H₂S concentration and was independent of total sulfide concentration. SRB did not outcompete MPB for acetate showing higher sensitivity to both total sulfide and unionized sulfide levels. Propionate accumulated above 112 mg/L H₂S concentration. SRB was believed to take an active role in the propionate degradation.

In a later study, McCartney and Oleszkiewicz (1993) treated lactate and observed different degradation pathways depending on the degree of acclimation and COD/SO₄ ratios. At a turnover ratio of 3.7 for COD/SO₄, lactate was converted to propionate and acetate via a non-sulfate reducing pathway, whereas at a ratio of 1.6, only acetate was produced from propionate coupled to sulfate reduction. Acclimation and higher lactate:SO₄ ratios affected the net metabolic pathways of propionate oxidation by SRB. Incomplete-oxidation of propionate to acetate by a sulfidogen specie growing faster than complete-oxidizing SRB was observed with acclimated sludge. Aceticlastic methanogenesis was the rate-limiting step in lactate degradation. Different sensitivities to inhibition and rate-limiting steps were observed at changing COD:SO₄ ratios. Sulfate reduction with lactate degradation was much less sensitive

to sulfide than aMPB (possibly role of hydrogen utilizing SRB). Sulfate reduction with propionate degradation was slightly more sensitive to sulfide than aMPB.

Role of sulfidogens in propionate degradation was also emphasized in another study (Ueki, et al., 1986). Lactate was converted to propionate and acetate with and without sulfate, but addition of sulfate speeded up propionate degradation.

Mizuno et al. (1994) observed methane bacteria's predominance over COD/S ratio of 6 with the treatment of butyrate where over 80% of the total electron flow was used by methanogens. At COD/S:1.5, SRB utilized over 50% of electron flow. Butyrate degradation pathways changed also with COD/S ratio. In the absence of sulfate, butyrate was degraded to acetate and hydrogen in the presence of methanogenesis. Under sulfate-rich conditions, butyrate was degraded to acetate and sulfide where acetate was further utilized by both SRB and MPB.

Oonge and Parkin (1991) reported system failure at $\text{COD}/\text{SO}_4 < 3.3-5$ for acetate and propionate fed systems.

Rintal and Lepisto (1992) reported more than 80% sulfate reduction with their high feed COD/ SO_4 ratio reactor, but only 20-50% reduction with a low ratio.

Yamaguchi et al. (1997) observed that hydrogenotrophic SRB outcompeted hydrogenotrophic MPB below a hydrogen level of 0.3-0.9 mg COD/L in the degradation of palmitate, a long chain fatty acid, their activity ratio was obtained as (SRB/MPB) 123, where aceticlastic MPB outcompeted aceticlastic SRB under sulfate rich conditions. SRB was proved to be an efficient hydrogen scavenger having a higher affinity for hydrogen than MPB which enabled them to keep hydrogen level at much lower levels than MPB. Palmitate was degraded to acetate by a symbiosis between acetogens and methanogens in the absence of sulfate, whereas sulfidogens replaced methanogens in the presence of sulfate.

Explanation brought in the same study to the predominance of aceticlastic MPB to aceticlastic SRB (which have higher affinity for acetate especially below the critical

acetate level (40 mg COD/L)) was that sodium concentration was not enough for halophilic SRB needing higher sodium levels for optimum growth.

Another finding of this research was that SRB could grow in sulfate free mediums proving that SRB can use other pathways such as fermentation of organic substances in the absence of sulfate (e.g. alcohols or aldehydes).

O'Flaherty et al. (1998) observed their full scale upflow reactor treat citric acid production wastewater (COD/SO₄:3) for 5 years and concluded that MPB dominated on acetate degradation and SRB competed for butyrate and ethanol. Hydrogen was utilized by SRB which are able to keep hydrogen concentration below the threshold level for MPB. Propionate was degraded by SRB via incomplete oxidation to acetate and then MPB converted acetate to methane. An active population of butyrate and ethanol degrading syntrophic bacteria competed well with SRB. The predominance of aceticlastic MPB was related to the better biomass retention of MPB in the upflow reactor and inferior attachment capacity of aceticlastic SRB promoted their washout from the reactor at higher upflow velocities.

Propionate and butyrate degradation was again observed to be completely or incompletely mediated by SRB with another citric acid production wastewater with full and lab-scale fixed bed digester (Colleran et al., 1994). After a long term operation on a high sulfate influent with COD/SO₄⁼:4, very low methanogenic activity against propionate and butyrate was observed. Influent sulfate, COD concentrations and OLR were 4.3 g/L, 10-15 g/L, and 857 kg COD/m³.d, respectively. Their sludge contained 4% VSS and obtained a COD removal rate of 0.65 kg/kg VSS.d. COD removal was between 75-81% where methanogenesis was successfully obtained.

A general conclusion many studies came to was that under acetate and hydrogen-limiting conditions in a sulfate rich environment, SRB could outcompete methanogens. At higher concentrations of acetate and hydrogen, $\mu_{\max}$ did not determine the outcompeters, but other system conditions were effective.

Sulfate reducing bacteria not only competes for substrate but also for sulfate as electron acceptor. Different types will also compete, such as acetate degrading *Desulfobacter postagei* is the poorest competitor compared to propionate degrading *Desulfobulbus propionicus* and the hydrogen oxidizing *Desulfovibrio baculatus* (Visser et al., 1996).

Uberoi and Bhattacharya (1995) observed propionate degradation by SRB in the presence of acetogens and methanogens. Under sulfate-limiting conditions they found that acetate was the least favored substrate compared to hydrogen and propionate by SRB. Under sulfate-rich conditions, lower substrate loading increased acetate utilization by SRB. Under sulfate-limited conditions, most of the sulfate was used by either propionate oxidizing or hydrogen utilizing SRB. Two possible mechanisms predominated: the first mechanism where propionate was degraded by both SRB and syntrophic acetogens and acetate and hydrogen were oxidized by MPB. The second metabolism was the degradation of propionate by acetogens, acetate by SRB and hydrogen use by both SRB and MPB.

As another study on the competition characteristics depending on pH, Visser et al. (1996) conducted a study in batch reactors to observe the competition between acetotrophic MPB and SRB at different pH levels and observed that below a pH of 6.9 aMPB predominated and above pH:7.7 aSRB outcompeted MPB due to their higher maximal specific growth rate and lower inhibition degree from sulfide. The reported optimal, minimum and maximum pH values for aSRB and aMPB are summarized in Table 2.6.

Table 2.6 Optimum, minimum and maximum pH values for aSRB and aMPB (Visser et al., 1996)

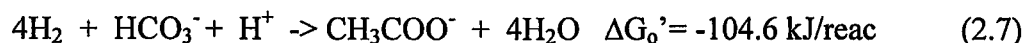
	Optimum	pH	
		Minimum	Maximum
<i>Methanothrix soehngenii</i> (acetotrophic)	7.1-7.8	6.8	8.3
<i>Methanosarcina sp.</i> (acetotrophic)	6.5-7.5	5.5	8.0
aSRB	7.3-7.6	6.0	9.0

As observed from Table 2.6, aSRB can survive at higher pH levels compared to aMPB. At pH values between 6.9 and 7.7, aMPB and aSRB are very competitive with similar growth rates and the predominance of any bacteria will depend on the sulfate concentration. They report from their previous work that no aSRB was observed at COD/SO₄ ratios exceeding 20. They also emphasized on the sludge type to determine affinities and sulfide toxicity. For granular sludge aSRB and aMPB showed similar affinities for acetate, whereas in suspended sludge aSRB had a lower affinity. With granular sludge, total sulfide caused the inhibition, whereas, free H₂S was the toxicant specie. At high pH levels growth was more severely affected than the activity.

The conclusion they obtained is that for a complex wastewater, acetate will be degraded by aMPB and propionate and hydrogen will be utilized by SRB. Inhibition level of SRB above pH:7 will be determined by total sulfide level.

Sulfidogenic bacteria's growth and ability to ferment organic matter in the absence of sulfate was observed by Yamaguchi (1997). Similarly, Ueki et al. (1998) in their study on the intrinsic ability of SRB, have shown that a strain of SRB can survive as the degrader of a wide range of organic compounds in methanogenic environments in the absence of sulfate. For some other strains, syntrophic growth in association with hydrogenotrophic methanogens had already been demonstrated.

Mizuno et al. (1998) used chemostats fed with sucrose to investigate the effects of sulfate-reduction on the acidogenic phase of anaerobic digestion. 90% sulfate removal efficiencies and no inhibition on sucrose degradation were obtained at H₂S concentration of 99 mg S⁼/L. During the syntrophic oxidation of volatile fatty acids and neutral products, SRB predominated as an hydrogen scavenger in the interspecies hydrogen transfer resulting in decreased methanogenesis. SRB could not only outcompete MPB but also competed successfully with acetogenic bacteria for butyrate. Addition of sulfate also changed anaerobic degradation of sucrose where hydrogen was predominantly consumed by SRB with no hydrogen removal under low sulfate conditions. The reaction for homoacetogenic bacteria was given as follows :



Sulfate removal efficiency dropped with influent sulfate concentration over 1200 mg/L.

Reis et al. (1988) examined anaerobic acidogenic phase response to high sulfate concentrations at different pH values (5.8-6.6) in two different reactor configurations. Sulfate removals obtained for upflow fixed film fixed bed reactor (UFBR) and continuous stirred tank reactor (CSTR) were 31-100% and 33-71%, respectively, depending on pH and HRT. They obtained lowest sulfate utilization at lower pH and HRT levels.

Lens et al. (1998) conducted a similar study treating a mix of VFA at COD/SO₄=0.5 and found that acetate removal capacity was the limiting factor of sulfidogenic VFA removal where acetate using SRB had a low growth rate and did not increase over the duration of the study. Maximum 80% of COD removal was obtained at an OLR:30 g COD/L.d where 80 % of COD was used by sulfate reducing bacteria.

As the relationship among alkalinity, pH and carbon dioxide Figure 2.2 is used to calculate carbon dioxide percentages occurring at known levels of alkalinity and pH.

2.3.3 Modeling of Sulfide Toxicity

Speece et al., (1986) evaluated models used to describe toxic response of anaerobic treatment. Toxicity effects were modeled in the literature according to competitive and uncompetitive inhibition models. Ammonium and sulfide toxicity best suited uncompetitive inhibition model.

In literature, uncompetitive inhibition pattern was proved to best suit toxicity of inorganic substances (Speece, 1997). Uncompetitive toxicity causes the effluent COD concentration to rise with toxicant concentration up to a certain concentration at which abrupt failure of the system occurs .

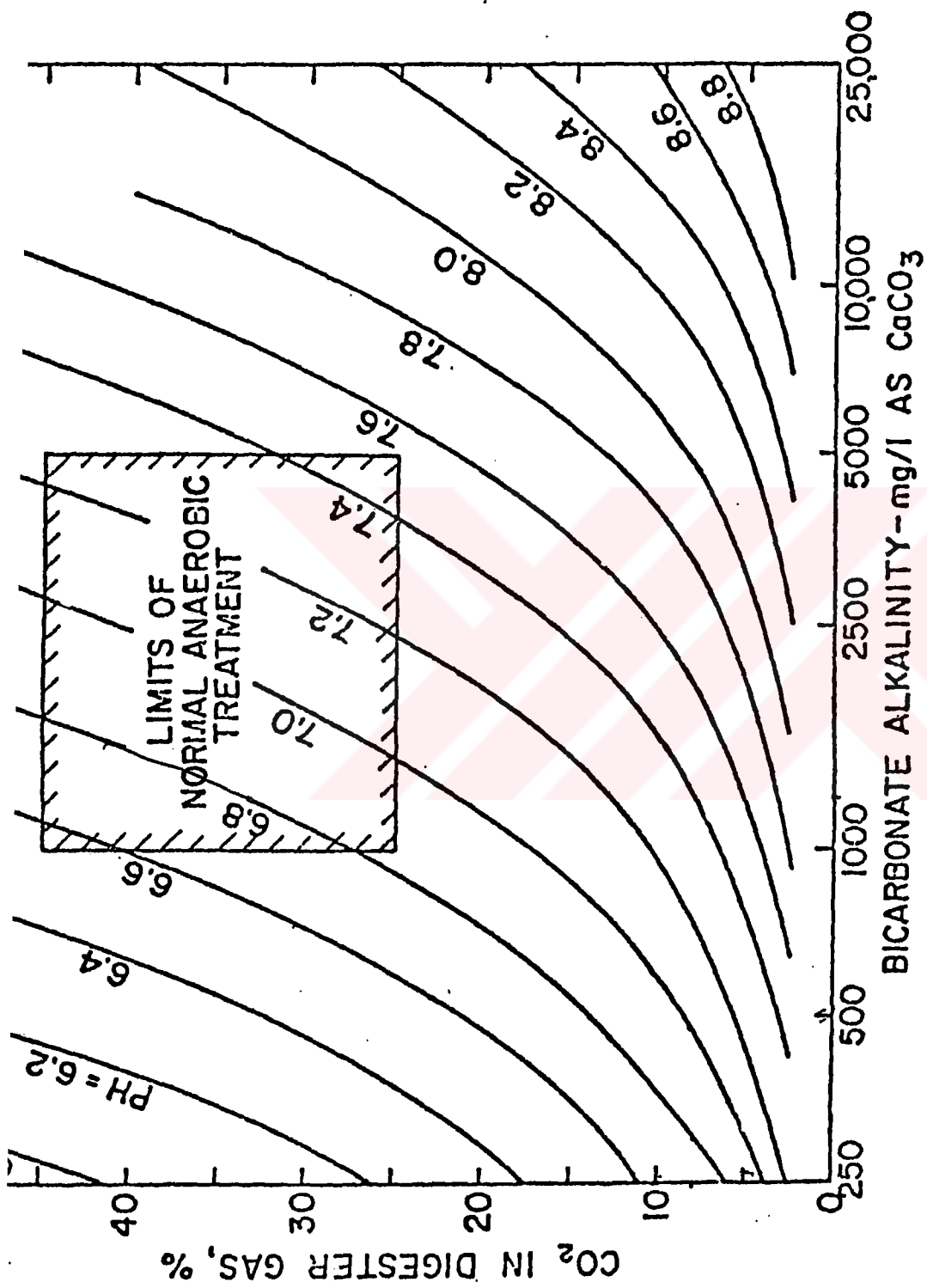


Figure 2.2 Relationship between bicarbonate alkalinity, pH and carbon dioxide percentage in anaerobic treatment (Speece, 1997)

The most comprehensive study on the kinetics of growth, substrate utilization and sulfide toxicity in anaerobic systems fed propionate and acetate is conducted by Maillacheruvu and Parkin (1996). In their study, they determined major groups active in these systems and kinetic and inhibition constants for each group. They observed that sulfide inhibition best suited uncompetitive inhibition model in agreement with previous studies indicating that inorganic compounds usually causes uncompetitive inhibition response.

Uncompetitive inhibition is modeled as follows where change in k is observed:

$$-dS / dt = k.S.X / (K_s + S [1 + I/K_i]) \quad (2.8)$$

Noncompetitive inhibition is a special case of mixed inhibition where change in K_s and k are observed and also would be expected to describe sulfide toxicity:

$$-dS / dt = k.S.X / (K_s + S)(1 + I/K_i) \quad (2.9)$$

Inhibition pattern is determined with plotting S_0/q at different substrate concentrations versus toxicant concentration. Lines are constructed for each S_0 and if the lines intercept above x-axis, then the inhibition pattern suits un-competitive inhibition model. S_0 , is the initial COD concentration and q is the unit substrate removal rate.

Inhibition constant (K_i) is determined with plotting $1/q$ versus toxicant concentration. The slope of the line is equal to $1/k.K_i$.

2.4 Kinetics of Organic Matter Removal

Reaction orders are important to express different behavior under changing conditions for organisms, substrates, or environmental conditions. Different rates of reactions may occur and can be defined with the expression given below:

$$r = - \frac{dS}{dt} = k S^n \quad (2.10)$$

Where we can take the ln of both sides:

$$\ln r = \ln (dS/dt) = \ln k + n \ln S \quad (2.11)$$

where

r = reaction rate, $g L^{-1} day^{-1}$

S = substrate concentration, $g L^{-1}$

n = reaction order

k = reaction rate constant

If we plot $\ln r$ versus $\ln S$, we will obtain a linear plot with a slope of reaction order, n and intercept of reaction rate constant, k .

Experimentally, the effect of a limiting substrate or nutrient was adequately defined by Monod with the following expression (Metcalf and Eddy, 1991):

$$\mu = \mu_{\max} \frac{S}{(K_s + S)} = \frac{dX}{dt} \frac{1}{X} \quad (2.12)$$

where

μ = Specific growth rate, $time^{-1}$

$\mu_{\max}$ = Maximum specific growth rate, $time^{-1}$

S = Concentration of growth-limiting substrate in solution, mass/unit volume

K_s = Half-velocity constant, mass/unit volume

X = Biomass concentration, mass/unit volume

dX/dt = Rate of bacterial growth, mass/unit volume. $time^{-1}$

Specific substrate utilization in microbial cultures is the substrate utilized per unit time per unit mass of biomass and expressed as:

$$\frac{dS}{dt} \cdot \frac{1}{X} = q \quad (2.13)$$

where

dS/dt = Substrate utilization rate, mass/unit volume. time⁻¹

q = Specific substrate utilization rate, time⁻¹

Growth yield Y is defined as:

$$Y = \frac{dX / dt}{dS / dt} \quad (2.14)$$

Where

Y = Growth yield coefficient, mass /mass

The relationship between specific substrate utilization rate, specific growth rate and growth yield is given below:

$$\frac{dS / dt}{X} = q = \frac{(dX / dt) / X}{dX / dS} = \frac{\mu}{Y} \quad (2.15)$$

If we introduce endogenous decay term :

$$\mu = Y q - k_d \quad (2.16)$$

Which is equal to :

$$q = 1/Y \cdot \mu + k_d / Y \quad (2.17)$$

If we plot specific substrate utilization rate versus specific growth rate, we will obtain a line with a slope $1/Y$ and intercept k_d .

2.5 Toxicity and Biotransformation of Chlorinated Aliphatic Compounds

Halogenated aliphatic compounds are the major contaminants in groundwater and components of hazardous wastes and landfill leachates (Vogel et al., 1987). Most of them originate from industrial, commercial and agricultural sources and consist of brominated and chlorinated alkanes and alkenes with one to three carbon atom. Dry-cleaning operations and semiconductor manufacturing commonly use chlorinated

ethanes and ethenes. Brominated and chlorinated methanes are produced as a result of chlorination of drinking water .

Toxicity effects of chlorinated compounds commonly found in sewage and industrial wastewaters are most severe on methanogenesis which is considered the rate-limiting step in anaerobic digestion. Methanogens are highly sensitive to toxicity and decrease in their activity results in hydrogen and acetic acid accumulation, further stopping propionic acid oxidation and system can undergo complete failure with a significant drop in pH.

Investigations on chlorinated aliphatics started in late 60s (Thiel, 1969). He investigated the toxicity effects of chloromethanes. Inhibition to methanogenesis occurred and the extracellular hydrogen levels increased in the system. Concentrations which caused 50 % reduction on methane production were 0.96, 2.2 and 200 mg/L for chloroform, carbon tetrachloride and methylene chloride, respectively.

Another study on the toxicity effect on bacterial groups showed that hydrogenotrophic methanogens were the most sensitive group and toxicity was least effective on acetogens (Rhee and Speece, 1992).

Despite their high sensitivity, methanogens proved to develop acclimation to chlorinated aliphatic compounds with time and gas production resumed to its normal level (Yang and Speece, 1986).

Despite their toxicity effects on biological treatment systems, discovery of their biological treatment attracted much attention and started investigations on their biotransformation characteristics.

Chlorinated aliphatic and aromatic compounds are observed to undergo biotransformation under anaerobic conditions more specifically dechlorination process under reducing conditions of anaerobic digestion (Mohn and Tiedje, 1992) mostly resulting in less chlorinated, thus less harmful products more amenable to

further treatment . In many studies, methanogenic bacteria were held responsible for the dehalogenation process.

Bouwer and McCarty (1983) showed that carbon tetrachloride, chloroform and dichloroethane were biodegraded to CO₂ using Carbon-14 labeling under methanogenic conditions with acetate as the primary substrate. Carbon tetrachloride was believed to be transformed to CO₂ by hydrolysis (biooxidation).

Vogel and McCarty (1985) reported the transformation of tetrachloroethylene (PCE) to trichloroethylene (TCE), dichloroethylene (DCE) and vinyl chloride (VC) in anaerobic column studies. Labeled CO₂ was detected but mineralization to CO₂ could not be determined from any specific halogenated compound.

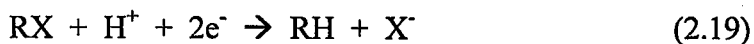
Reductive dehalogenation requires the presence of an electron donor (substrate) and nutrient necessary for bacterial growth (Vogel et al., 1987). Transformation of halogenated compounds can be divided into two general classes: reactions that require external electron transfer (oxidations and reductions) and those do not (substitutions and dehydrohalogenations). External electron is provided from an electron donor to the halogenated compound.

Halogenated alkanes can undergo elimination reactions that produce alkenes. Elimination of HX is called dehydrohalogenation where a halogen from one carbon atom is removed with a removal of an hydrogen atom from an adjacent carbon atom. The reaction is presented as follows:



This reaction does not require external electron supply and does not change the oxidation state of the reacting molecule. Polychlorinated alkanes undergo dehydrohalogenation under extreme basic conditions and at pH 7.

Reductive dehalogenation is another process with a removal of a halogen atom replaced by an hydrogen atom. The reaction is presented as follows:



Methanogens have the ability to reduce halogenated compounds using nickel-containing enzymes and cofactors such as F₄₂₀ specific to methanogens (Balch et al., 1979). Less chlorinated compounds are observed to have a slower dehalogenation rate than more chlorinated ones under anaerobic conditions.

Methanogens were not the only group of bacteria which were studied for their reducing capabilities. Several studies have reported that acetogens were also able to transform halogenated compounds. Egli et al. (1988) showed direct tetrachloromethane (CT) degradation to CO₂ with pure cultures of sulfidogens and methanogens. Experiments showed that CO₂ was formed directly from CT transformation and used in the carboxyl group of acetate by acetyl CoA pathway. Ninety percent of the labeled CT was recaptured as CO₂ and acetate. Following this pathway, bacteria were believed to use CT as a carbon source for autotrophic growth. This pathway is used in the autotrophic growth of acetogens, methanogens and sulfate-reducers (Wood et al., 1986). Several acetogens were shown to obtain carbon from CO₂ and energy from hydrogen through this pathway (Ljundahl, 1986, Fuchs, 1986).

Reductive dechlorination of CT led to the intermediate trichloromethane with a further transformation to dichloromethane and unidentified products (Galli and McCarty, 1989) using a pure culture isolated from a methanogenic culture.

The effect of substrate and mineral elements on the anaerobic bioremediation of CT was investigated with feeding different substrates, such as glucose, acetate, humic acid in the presence of nitrate as the electron acceptor (Doong and Wu, 1995). Removal of CT was observed to rise from 45% to 80% for glucose and from 18% to 49% for acetate-fed systems when substrates and mineral ions were added, thus, they enhanced biotransformation efficiency. Glucose was a better substrate than the others (Doong et al., 1996) where CT removal was modeled with pseudo-first-order kinetics.

Complete biotransformation of PCE and TCE to ethylene was observed both under methanogenic conditions (Freedman and Gossett, 1989) in the presence of an electron donor and in the absence of methanogenesis (DiStefano et al., 1991) where 32 % of the substrate (methanol) was consumed for dechlorination and 69 % for acetate production, emphasizing the role of acetogens in the process. Dechlorination stopped when methanogenesis was suppressed with BES or absence of substrate where acetogenic activity proceeded (Skeen et al., 1995). A methanol-enriched sludge removed PCE and metabolites at lower rates (approximately one order of magnitude).

Another study emphasizing the role of methanogens on reductive dechlorination of PCE showed that when methanogenesis was selectively inhibited by BES, dechlorination was also inhibited (Fathepure and Boyd, 1988)

DeBruin et al. (1992) observed complete dechlorination of PCE to ethene which was further converted to ethane.

PCE is one of the most common groundwater contaminants and remediation of contaminated sites using high-rate anaerobic filters was shown to be efficient on the removal of low and high concentrations of PCE (2.8 mg/L and 80 mg/L, respectively) using only 100 mg/L and 200 mg/L ethanol as electron donor (Komatsu et al., 1997).

Another high-rate bioremediation system, a laboratory-scale continuously fed anaerobic attached film expanded bed (AAFEB) obtained removals of PCE/TCE over 98% with initial concentrations of 8.2-26 mg/L (Chu and Jewell, 1994). Sucrose was used as the electron donor. Intermediates TCE and DCE remained at low concentrations indicating no rate-limiting. Most of the PCE and TCE were converted to vinyl chloride.

Different electron donors were observed to promote dechlorination of PCE such as glucose, formate, acetate, and hydrogen (Aguirre et al., 1997). With hydrogen as substrate, dechlorination process could not be supported as efficiently as other

substrates, possibly due to nutritional deficiencies. Also, PCE and TCE dechlorination ability decreased over time.



CHAPTER III MATERIALS AND METHODS

Anaerobic leachate treatment, sulfide inhibition, toxicity and biotransformation of chlorinated aliphatic compounds were studied in 250 mL batch reactors. Reactors were Schott bottles bearing a glass tube threaded on one side to bear butyl rubber septa and plastic cap, connected via heat treatment to the reactors to enable headspace and other sampling (Figure 3.1).

An approximate sludge volume of 40-50 mL was completed to 150 mL with nutrient and substrate solutions at the beginning of each set due to insufficient phosphorous levels. Anaerobic seed sludge was provided from Pakmaya UASB reactors in İzmit where molasses were fed as the substrate. Three weeks of feeding with leachate (half of the reactors) and methanol was applied to allow adaptation to the new substrates.

Leachate and methanol were fed to two sets of reactors (5 fed with leachate and other 5 with methanol). Landfill leachate was provided from Istanbul Municipal Landfill site on March, 1998. The characteristics of the leachate were presented in Table 3.1. Details of the feeding and monitoring are summarized in Table 3.2 and 3.3.

Fourteen sets of leachate feeding were monitored in batch reactors during 8 months. The sets consisted of 7 to 15 day feeding periods. The treatment performance of the system was monitored with daily measurements of COD, pH, total gas production, dissolved sulfide, alkalinity and bacterial growth with TFS/VSS measurements at the beginning of each set.

Three COD ranges were applied to the reactors. For the first 8 sets, 1300-2200 mg/L, for the following 3 sets, 3300-5500 mg/L COD and for the last three sets

6000-8400 mg/L COD initial values were used. Initial and final COD's and COD removals are given as the average values from 5 reactors.

The study was conducted in 10 reactors of which 5 reactors received leachate at the beginning of each feeding and other 5 reactors received methanol (2 controls did not receive chlorinated compound). The reactors were kept at 35°C and all sampling and feeding was conducted with syringe through sampling device. Analyses and experimental setup were summarized in Figure 3.4.

Monitored parameters were pH, COD, Alkalinity, dissolved sulfide, total gas production, total fixed solids (TFS) and volatile suspended solids (VSS). pH was measured on a Jenway 3040 Ion Analyzer. For COD measurements, closed reflux method in 20 mL Schott tubes were used (5220.C, APHA). Alkalinity and dissolved sulfide were titrimetrically determined according to 2320.B and 4500.E (APHA). TFS and VSS were determined according Standard Methods (APHA). Gas production was measured via liquid displacement method (Figure 3.2).

Chlorinated aliphatic compounds were tetrachloromethane (CT), tetrachloroethane (PCA), and tetrachloroethylene (PCE). Chemical and physical data of these compounds are given in Table 3.3.

Stock solutions of these compounds were prepared in Ecological Chemistry and Waste Analysis Department of Braunschweig Technical University, Germany.

Spiked concentrations of chlorinated compounds ranged from 0.145 mg/L to 1.45 mg/L for tetrachloromethane, 0.2 mg/L to 2 mg/L for tetrachloroethane, and 0.197 mg/L to 2.36 mg/L for tetrachloroethylene.

Chlorinated aliphatics were spiked 10 times and their disappearance and biotransformation rates were monitored weekly in the first 5 sets and every 2-3 days at the rest of the spikings.



Figure 3.1 Batch Reactor used for leachate and methanol treatment

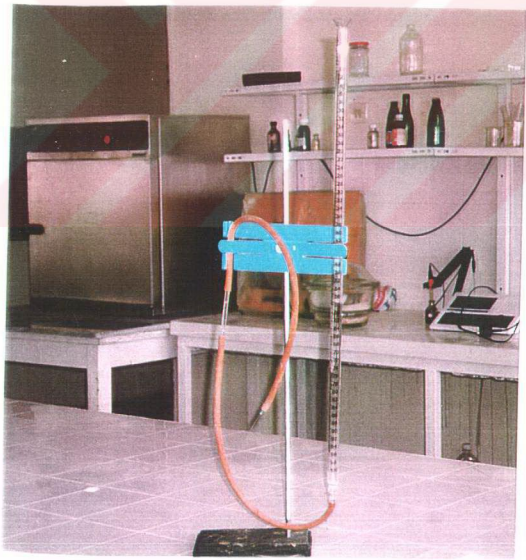


Figure 3.2 Burette used for the liquid displacement method

Table 3.1 Characteristics of the leachate used in the study taken in March, 1998

Parameter	Values
Alkalinity, mg / l	11480
pH	6,8
COD, mg / l	36350
COD (dissolved), mg / l	32950
SO ₄ ²⁻ , mg / l	1100
SS, mg / l	390
TKN, mg / l	4490
NH ₃ -N, mg / l	1210
Zinc, mg / l	0,4
Iron, mg / l	60
Chromium, mg / l	<0,5
Nikel, mg / l	0,8
Lead, mg / l	<1,0
Copper, mg / l	<0,5
Cadmium, mg / l	<0,2
Silver, mg / l	<0,5
Manganese, mg / l	1,3
Propionic a., mg / l	3350
Isobutyric a., mg / l	850
Butyric a., mg / l	3450
Isovaleric a., mg / l	800
Valeric a., mg / l	1950
Caproic a., mg / l	3150
Heptanoic a., mg / l	800

Table 3.2 Experimental feeding and monitoring details

Reactors	Feeding	COD applied (mg/L)	Monitored Parameters
LRR (leachate receiving reactors)	Leachate	1500-8400	COD, DS, pH, Gas Prod.* TFS/VSS, Alkalinity** Chlorinated aliphatics***
MRR (methanol receiving reactors)	Methanol	1500-3000	Gas Prod.* Chlorinated aliphatics ***

*Daily, **Weekly, ***Every other day

Table 3.3 Feeding details during the experimental period (COD and chlorinated aliphatics in mg/L)

Parameter	Set 1 31.3.98	Set 2 14.04.98	Set 3 20.04.98	Set 4 27.04.98	Set 5 4.5.98	Set 6 11.5.98	Set 7 18.5.98
COD applied to LRR	1500	2300	1660	1500	1300	1650	2200
Reten. T. (d)	13	6	5	7	7	7	7
COD applied to MRR	1580	3000	5833	-	3000	3000	2330
CT			0.145	0.29		0.43	0.43
PCA			0.2	0.4		0.6	0.6
PCE			0.197	0.394		0.59	0.59
	Set 8 25.5.98	Set 9 20.7.98	Set 10 3.8.98	Set 11 10.8.98	Set 12 9.11.98	Set 13 24.11.98	Set 14 1.12.98
COD applied to LRR	2200	5500	3300	5500	6000	7700	8400
Reten. T. (d)	3	14	7	11	15	7	7
COD applied to MRR	2700	3200	5000	2500	6700	8920	2200
CT	0.58	0.58	0.58		1.16	1.45	1.45
PCA	0.8	0.8	0.8		1.63	2.0	2.0
PCE	0.78	0.78	0.78		2.36	2.36	2.36

Table 3.4 Physical and chemical data (Handbook of Chemistry and Physics, 1982)

Compound	Formula	Molecular Weight	Boiling Point, °C	Melting Point, °C	Henry's Constant* (Gossett, 1987)
CT	CCl_4	153.82	76.5	-23	1.823
PCA	$\text{C}_2\text{H}_2\text{Cl}_4$	167.85	146.2	-36	-
PCE	C_2Cl_4	165.83	121	-19	1.116
TCE	C_2HCl_3	131.2	87	-73	0.591

*dimensionless: $\text{conc(gas)/conc(liq)}$ at 34.6°C

Headspace samples taken with a 1 mL gas tight syringe (Hamilton) were analyzed in a HP-5890 Series IIA Gas Chromatograph equipped with an electron capture detector (ECD) and HP-624 capillary column using split/splitless mode (Figure 3.3). Operation parameters were summarized in Table 3.5.



Figure 3.3 Gas Chromatograph used for the VOC analyses

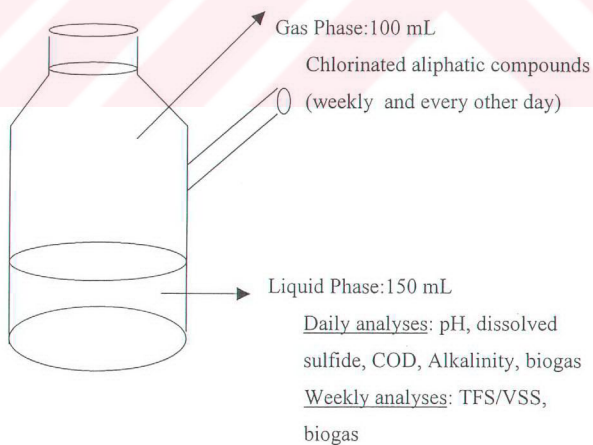


Figure 3.4 Experimental setup of routine analyses

Table 3.5 Operation parameters for Gas Chromatography running

Operation Parameters	
Injection Temperature, °C	300
Detector Temperature, °C	300
Temperature Program	40 to 140 (10°C/min) to 240 (20°C/min)
Column flow, mL/min	1.5
Make-up Gas Flow, mL/min	40
Injection Mode	split/splitless

Acclimation of the reactors to the chlorinated compounds were started at low concentrations spiked weekly. Analyses were done in about one hour equilibration time from the injection and after one week. Compounds disappearance was observed at the end of each week. For the last 5 sets, headspace analyses of chlorinated compounds were conducted every other day to better observe biotransformation rates. The chlorinated compounds could be detected in solvent samples down to 333 ng/L and 0.3 ng/L in headspace samples.

CHAPTER IV RESULTS AND DISCUSSION

4.1 Overall Treatment Performance

COD removal efficiencies differed in each set depending on the other parameters than were given here such as pH, dissolved sulfide (DS) and H_2S balance which will be discussed in the next section. The results obtained are summarized in Table 4.1. Despite the stressed conditions of methanogenesis, COD removal stayed mostly over 70 % and close to the range of 80 to 90 %. This supports the evidence of a good competition between sulfate reducing bacteria (SRB) and methane producing bacteria (MPB). As stated earlier (Maillacheruvu and Parkin, 1996, Speece, 1997), competition for the same substrates between SRB and MPB depends on the inhibition level of both bacteria. Detailed data showing this competition will be presented in the next section.

Each feeding set's time period is given as hydraulic retention time where periods longer than 7 days are mostly encountered at first feeding sets after intervals (9th and 12th sets). In these sets, no new feeding was started before COD dropped to approximately 20 % of the initial value.

pH rising in the 2. set and drop to values close to 7.0 caused stress on both the methanogenetic bacteria and sulfate-reducing bacteria where COD removal dropped to around 60 % in the 5th set. Degradation of acetic acid produced alkalinity pushing pH to higher values at the end of each feeding set. The system seemed less stressed at pH values close to 7.8-8.0 level. This is in agreement with Oleszkiewicz et al. (1989) who observed that at pH 8.0, H_2S toxicity to MPB could be avoided whereas SRB were inhibited by products.

Table 4.1. System Treatment Performance for 8 months (with leachate)

Parameter	Set 1 31.3.98	Set 2 14.04.98	Set 3 20.04.98	Set 4 27.04.98	Set 5 4.5.98	Set 6 11.5.98	Set 7 18.5.98	Set 8 25.5.98	Set 9 20.7.98	Set 10 3.8.98	Set 11 10.8.98	Set 12 9.11.98	Set 13 24.11.98	Set 14 1.12.98
COD (mg/L)														
initial	1500	2300	1660	1500	1300	1650	2200	2200	5500	3300	5500	6000	7700	8400
final	348	239	438	452	525	224	278	(52)190	595	530	614	928	1755	1472
% COD Rem..	77	89	74	70	60	86	87	81	89	84	89	85	77	82
pH														
initial	7.3-7.8			7.1-7.4	7.2-7.5	8.2-8.4	7.7-8.0	7.0-7.1	8.1-8.3	7.0-7.2	7.4-7.5	9.3-9.7	7.8-8.0	7.9-8.0
final	8.4-9.3	9.2-9.4	7.9-8.5	7.3-7.5	7.6-7.7	7.0-7.4	7.0-7.1	7.2-7.3	8.0-8.3	7.5-7.9	7.9-8.1	7.9-8.2	7.8-8.2	7.9-8.0
Alkalin.. (g/l)														
initial	1.75-2.5	3.2-4.5	2.5-3	2.25-2.5			1.5-1.6	1.5-1.6	2.5-3.2	2.8-3	3.9-4.4	3.4-4.4	4.7-5.2	5.2-6
final	4-6	1.7-2.5	3	3-4.3				1.6-2.0	2.8-3	3.1-3.5	4.6-4.9	4-4.5	4.9-5.4	5.7-6.4
CO ₂ %	5-17		<1	15-27			3-5	25	3-5	33-45	25	<<	10	8
	2-2.5	<2	5	25-27				23	16	6-17	8	8	8	10
Gas Yield*	329	119	306	436	184			231	166	393	290	373	319	364
VSS/TFS (%)														
SRB	25-26.6				21-27	25-26.5	23-26		25-29	26-29				
MPB	25-27				24-26	24-25	25-26		24-26	27-29				

*mL/g COD removed

Most leachate treating systems are under the threat of increasing inorganic content of biomass due to sulfide precipitation of heavy metals present (Chang, 1989, Keenan et al., 1993, Kettunen and Rintala, 1998). In this study, VSS/TFS ratio remained around 25 %, during 10 sets, no considerable change in inorganic content was observed. The leachate's high iron content's contribution to the inorganic part of the biomass through precipitation with sulfide was believed to be cancelled off by the volatile solid content of the leachate itself.

Gas yield was measured in the range of 119-436 mL/g COD removed staying mostly close to the theoretical value (400 mL/g COD removed, in Speece, 1997). Gas yield gives good evidence of the inhibition level of methanogens. Increasing COD applied caused mixed response of gas yield which dropped to low levels indicating inhibition caused both by pH and toxicity with the role of other bacteria on the COD removal consisting mainly of volatile fatty acid degradation.

Carbon dioxide percentage in the gas produced was calculated from alkalinity and pH values for each set. CO₂ reached 45% as maximum level when pH dropped to neutral values indicating CO₂ release from the bicarbonate form.

In Figure 4.1, the effect of increasing organic loading rate on the COD removal and gas production was shown. COD removal mostly stayed over 80 % and did not seem to be affected by changes in organic loading rate (0.1-1.2 g/L.d), whereas gas production differed from the theoretical value in a wide range. This mixed response can be interpreted as the stressed conditions due to parameters affecting methanogenesis, such as pH and H₂S/DS level. No toxicity effect on COD removal also indicates that bacteria involved in the degradation of volatile fatty acids are more tolerant to toxicity present than methanogens.

Any inhibition pattern in the first six sets is believed to be due to pH and H₂S/DS since chlorinated aliphatic compounds have been applied starting from the 7th set. The gas yield and production values in Table 4.1, Figure 4.1 and Figure 4.2, are taken from the uninhibited reactors from chlorinated compound applied.

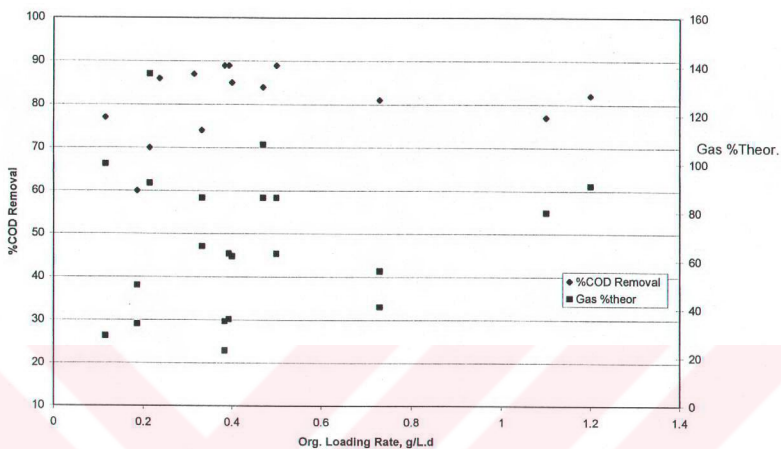


Figure 4.1 Change in COD removal and gas production levels depending on organic loading rate

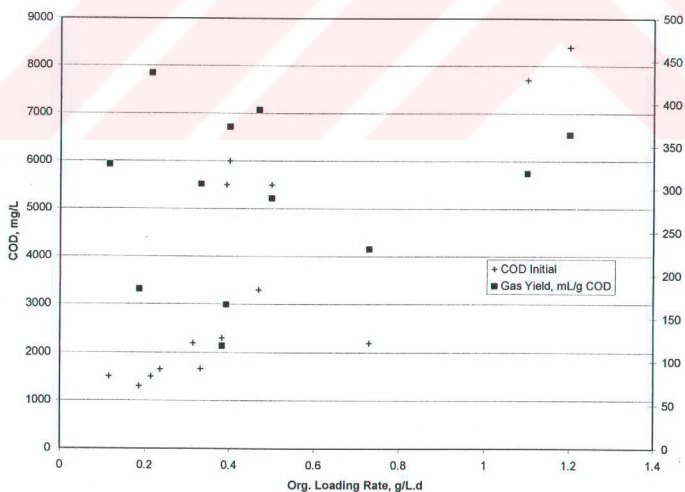


Figure 4.2 Initial COD and gas yield values

In Figure 4.2, gas yield variation versus organic loading rate and initial COD concentrations showed a similar pattern similar to the previous figure results. COD was increased from 1300 mg/L to 8400 mg/L. Gas yield values mostly stayed over 220 ml/g COD removed, showing no dependence on the organic loading rate. Lower yield values indicate stressed methane production where other parameters need to be considered to clarify system conditions.

pH values varied in a wide range throughout the experiments. Alkalinity production, as a result of acetic acid degradation both by MPB and SRB raised pH during each feeding set. Acid addition was applied in some sets to the reactors for pH control.

The relationship between pH, COD degradation and alkalinity production can be better seen in Figure 4.3 where alkalinity increases with rising pH in a close dependence on each other. Increasing initial COD applied also caused higher levels of alkalinity due to the leachate characteristics. Alkalinity production and/or pH rising is also a good indicator of activity for aceticlastic methanogens and sulfidogens where rising concentrations during sets give evidence on their increasing activities, especially in the first sets where alkalinity production was at its highest. Starting from set 5, alkalinity production tended to decline and was not produced at significant levels indicating lower acetate degradation as the rate-limiting step of the system.

More evidence of the rate-limiting characteristic of the aceticlastic bacteria's activity, higher pH levels around days 45 and 62 were reached at the end of intervals given between feedings for some weeks. These high pH values indicate further acetic acid degradation after feeding set was ended, thus, incomplete acetic acid degradation and accumulation took place at the end of time period taken for the last sets, thus, acetic acid degradation seemed to be the rate-limiting step possibly due to higher sensitivity level of aceticlastic bacteria groups.



Figure 4.3 Alkalinity and pH change with increasing initial COD concentrations in the time scale

4.2 Sulfide Metabolism and Toxicity

Dissolved sulfide (DS) was measured daily with pH and H₂S percentage and concentration were calculated based on these data. The concentration equilibrium between DS and H₂S is given as below:

$$[\text{HS}^-][\text{H}^+] / [\text{H}_2\text{S}] = K_1 \quad (4.1)$$

K₁ value at 35°C is given as 14.9×10⁻⁸ (Speece, 97).

The fraction for un-ionized sulfide (H₂S) is calculated as follows:

$$(\text{H}_2\text{S fraction}) = 1 / (1 + (K_1/10^{-\text{pH}})) \quad (4.2)$$

COD used by sulfidogens was calculated by 32 g of S²⁻ reducing 64 g of COD and given in Table 4.2 during the experimental period. Maximum values of DS and H₂S from 5 reactors are summarized in Table 4.2.

Sulfidogens compete with methanogens for acetic acid and hydrogen, also with propionate fermenter acetogens for propionic acid. They both share COD utilized at different percentages depending on various parameters, such as inhibition level of each group of bacteria. Maillacheruvu and Parkin (1996) determined relative sensitivities of SRB and MPB and concluded that propionate fermenter acetogens and aceticlastic SRB were the most sensitive groups to both DS and H₂S. H₂S is proved to be the most toxic specie due to its neutral charge (McCartney and Oleszkiewicz, 1993).

In anaerobic treatment, several parameters provide evidence on dominant or active groups of bacteria, such as, alkalinity production and/or pH rise as the evidence of aceticlastic bacteria activity, gas yield or production as an indicator of overall methanogenic activity where sulfide production gives evidence of overall sulfidogenic activity.

Table 4-2. Sulfide metabolism, gas production and COD removal during the experimental period

Parameter	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11	Set 12	Set 13	Set 14
pH														
initial	31.3.98	14.04.98	20.04.98	27.04.98	4.5.98	11.5.98	18.5.98	25.5.98	20.7.98	3.8.98	10.8.98	9.11.98	24.11.98	1.
final	7.3-7.8 8.4-9.3	9.2-9.4	7.9-8.5	7.1-7.4 7.3-7.5	7.2-7.5 7.6-7.7	8.2-8.4 7.0-7.4	7.7-8.0 7.0-7.1	7.0-7.1 7.2-7.3 (52)7.6- 7.8	8.1-8.3 8.0-8.3	7.0-7.2 7.5-7.9	7.4-7.5 7.5-7.7	9.3-9.7 7.9-8.2	7.8-8.0 7.8-8.2	7.9-8.0 7.9-8.0
DS (max)	290-450	250-400	200-220		100-300	60-108	80-130	72-120	70-150	100-120	80-130	n.d.	n.d.	n.d.
H₂S														
%	0.003-25	4-2.7	7.8-2.1		12-30	3-40	6-40	25-40	3-6	8-40	12-18			
(mg/l)	1-39	11-16	17-5		17-62	3-16	3-21	7-28	0.5-7	3-7	7-24			
COD (%)	78-87	71-86	72-76	60-76	53-64	84-89	85-89	78-83	88-91	78-87	87-90	83-86	78-83	80-86
Removal														
% MPB	60%	41%	68%		45%			63%	63%	80%	63%	60%	84%	92%
mg COD	143	110	14		53			170	462	417	460	450	750	960
%SRB	32%	35%	32%		50%	15%	10%	9%	4%	7%	4%			
mg COD	85	94	66		57	32	36	24	30	29	29			
DS (mg/l)														
Initial	50-160	150-330	200-220		100-300	40-80	30-60	40-70	10-20	90-110	80-120			
Final	290-450	130-400	180-220		77-91	50-90	30-60	30-50	60-110	80-120	50-130			
Biogas ^														
LRR*	29-100	27-40	66-86	92-137	34-50			0-56	0-63	0-108	0-86	8-73	0-84	0-94
**								56	63	86-108	63-86	71-100	86-100	87-92
MRR*	80-100	68-100		85-100	69-100			0-100	2-100	10-100	0-100	44-100	42-100	0-68

^ % of theoretical value, * results from all reactors, ** results from only uninhibited reactors by the chlorinated compound applied

LRR: leachate receiving reactors, MRR: methanol receiving reactors

In this study, sulfide production took place at high concentrations in the first sets (220-450 mg S⁼/L) where high pH levels kept H₂S concentration at low levels (around 1 mg/L) and COD removal seemed not to be effected until pH was adjusted to 7.1-7.4 in the 4th set. In the following sets, H₂S concentration rose significantly with close to neutral pH values increasing toxicity effect. In the 7th and 8th sets, pH dropped to 7.0-7.1 and did not rise significantly, implying acetic acid accumulation (alkalinity concentrations belonging to these sets stayed constant at 1.5-1.6 g/L, thus, only hydrogenotrophic activity contributed to the gas production). At the beginning of 9th set after 2 month interval, both alkalinity and pH values were found at much higher values indicating a late/delayed acetoclastic bacteria activity, where the same pattern was observed at the 12th set.

Evidence on the competition between SRB and MPB is presented as percent COD utilization by these bacteria groups. MPB and SRB competed at similar activities at COD concentrations applied between 1300 and 1660 mg/L until the 6th set where percent COD utilization by SRB dropped significantly to below 15 percent and dissolved sulfide to 30-130 mg S⁼/L level. This phenomenon can be explained as SRB activity using sulfate as electron donor staying constant and independent from increasing initial COD applied (or organic loading rate) which promoted methanogenic activity. This phenomenon is believed to be due to more sulfidogenic involvement in the degradation of volatile fatty acids through fermentation and no acetoclastic activity due to toxic levels of dissolved sulfide. COD removed by bacterial groups in Table 4.2 shows that COD utilization by SRB decreased in time from 85-94 mg COD equivalent per reactor to 29-32 mg COD equivalent per reactor staying constant at this value while SRB share of COD utilization through sulfate reduction diminished from the set 5 to the end of set 11. In the last three sets, MPB consumed much higher percentages of COD (61, 80 and 91 %). Adverse effect of increasing loading rate on SRB at a constant COD/SO₄ ratio was also reported in Maillacheruvu and Parkin's study (1993). The leachate characteristics may have pushed sulfidogens to ferment higher volatile fatty acids forming most of the leachate's organic content.

Methanogens' outcompeting of sulfate reducing bacteria in the long term or at higher loading rates at a constant COD/SO₄ (also higher than 4-5) ratio was proven in

previous studies (Harada et al, 1994, Yamaguchi et al., 1997). Long term competition rendered acetoclastic methanogens dominant to acetoclastic sulfidogens (aSRB) and consequently sulfidogenic activity shifted to competition for hydrogen and propionate (Uberoi and Bhattacharya, 1995, O'Flaherty et al., 1998). In another study (McCartney and Oleszkiewicz, 1993), acclimation ability of methanogens and shift in SRB's substrate degradation pathways seemed to have affected MPB's dominance over SRB. In this study COD/SO₄ ratio was 30 which promoted favorable conditions for methanogenic activity and where SRB was expected to use hydrogen and propionate and other volatile fatty acids.

Increasing initial COD applied (organic loading rate) promoted methanogenic activity and methanogens seemed to reach much higher COD utilization percentages (Figure 4.4). Gas yield values also improved as well as COD removal percentages.

In Figure 4.5, dissolved sulfide and H₂S (%) were plotted against COD applied with pH and gas production (as percent of theoretical value). Gas production approached the theoretical maximum value depending on the pH of the reactors. At high (above 9.0) and neutral pH levels, gas production stayed at very low amounts (27-50%), due to very alkaline pH out of optimum range for methanogens and high un-ionized sulfide (H₂S) concentration around neutral pH. Gas production stayed very close to theoretical values in the last five sets (Table 4.2), sometimes higher than theoretical values due to increase in H₂S and CO₂ content because of lower pH levels.

Dissolved sulfide was produced at high levels (100-450 mg/L) in the first 5 sets. Lower COD applied or alkaline pH seemed to promote sulfidogenic activity in agreement with previous studies (Harada et al, 1994, Visser et al., 1996, Yamaguchi et al., 1997). In following sets, pH adjustment to neutral values caused shifting on the sulfide species and resulted in much higher H₂S concentration (12-40% of DS) and sulfidogenic activity dropped to lower levels (DS=10-130 mg S⁼/L). Sulfidogenic bacteria seemed more inhibited by their own product where pH played a crucial role. Dissolved sulfide concentration, H₂S fraction, pH and gas ratio to theoretical value were plotted on the time basis in Figure 4.6. Dissolved sulfide production dropped in time and un-ionized sulfide went up and down as a function of pH.

From the start to the end, activity profile of the bacteria changed significantly. As observed in previous studies (McCartney and Oleszkiewicz, 1993, Harad et al., 1994, Yamaguchi et al., 1997) long term behavior should be monitored in pilot and real scale digesters on the terms of months to better establish system's performance and bacterial characteristics. In this Figure, competition between SRB and MPB for organic matter could be observed in the first five sets (first two months) despite favorite conditions for methanogenic growth and activity ($\text{COD}/\text{SO}_4:30 \gg 4-5$) (McCartney and Oleszkiewicz, 1993). Following the first three months, sulfidogenic COD utilization stayed constant at a 30 mg COD consumption per reactor despite increasing organic loading rate, whereas, methanogenic share rose significantly.

In the literature on SRB and MPB activities, it was demonstrated that under favorable conditions ($\text{COD}/\text{SO}_4 > 4-5$ and pH between 6.9-7.7 (Visser et al., 1996)), acetoclastic MPB outcompeted easily acetate-oxidizing SRB under long term high sulfide loading conditions (Maillacheruvu and Parkin, 1996, Yamaguchi et al., 1997), especially under sulfate-limited conditions and in the presence of other more favorite substrates for sulfidogens such as propionic acid and hydrogen (Harada et al., 1994, Ueroi and Bhattacharya, 1995, O'Flaherty et al., 1998). Higher sensitivity of propionic acid fermenter acetogens to both dissolved and un-ionized sulfide facilitate incomplete propionate oxidation by sulfidogens (most preferred pathway by SRB (McCartney and Oleszkiewicz, 1993)).

Other evidence on shifting of bacterial species was the alkalinity production from acetic acid degradation of both SRB and MPB. Higher sulfide and alkalinity production (and more survivable conditions for acetoclastic SRB at pH above 9) in the first sets gave over to much lower production levels in the last sets where a pH range around 7.8-8.0 provided acetoclastic methanogens more favorable conditions.

Gas production close to theoretical values indicated that hydrogenotrophic methanogens, the least sensitive group to sulfide toxicity, were also fully active (Figure 4.7).

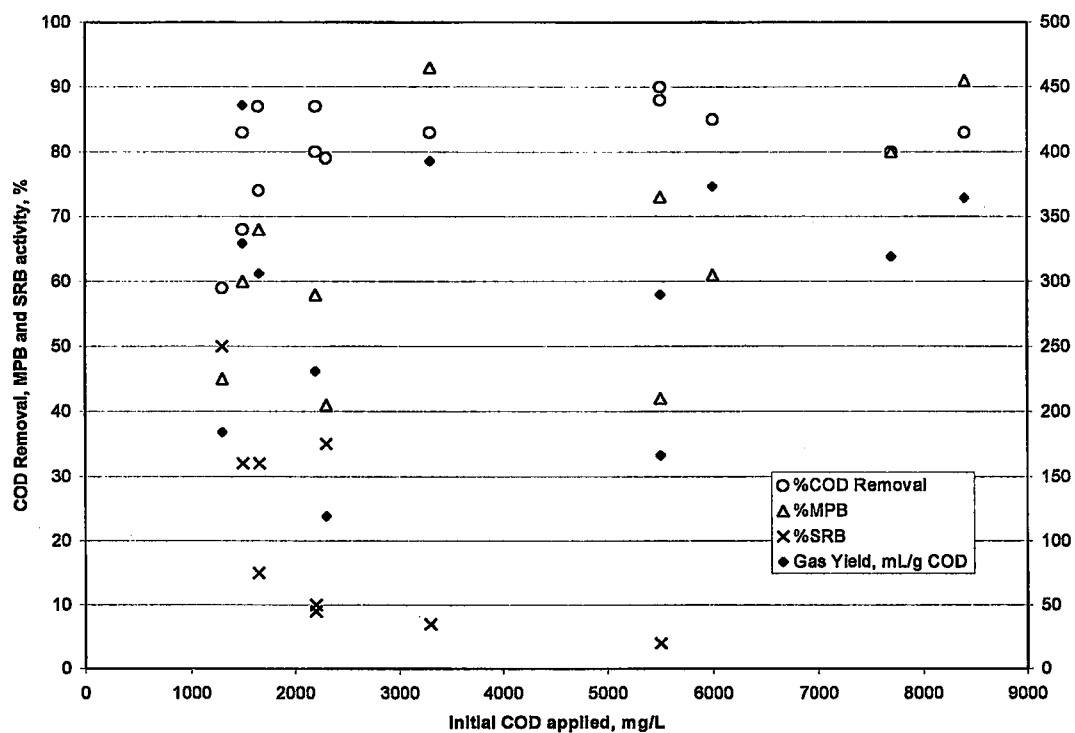


Figure 4.4 COD removal, MPB and SRB activities (%) and gas yield change with increasing COD applied

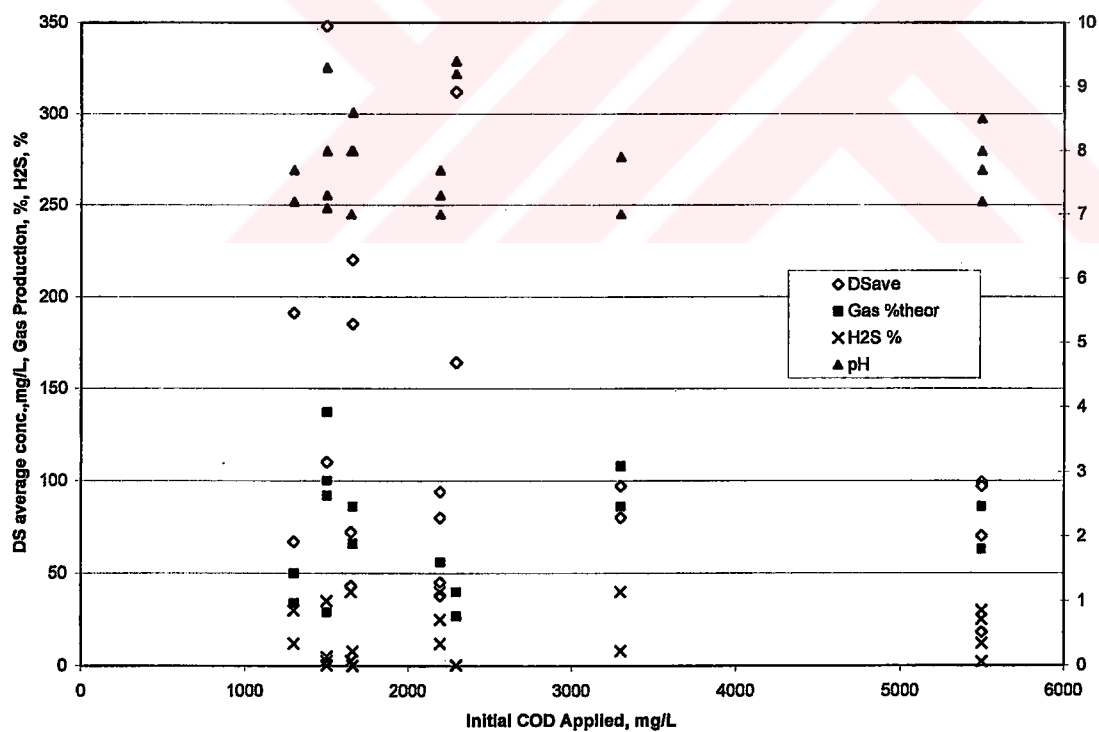


Figure 4.5 Dissolved sulfide (DS), gas production as percent of theoretical value, H₂S, and pH profile for initial COD applied

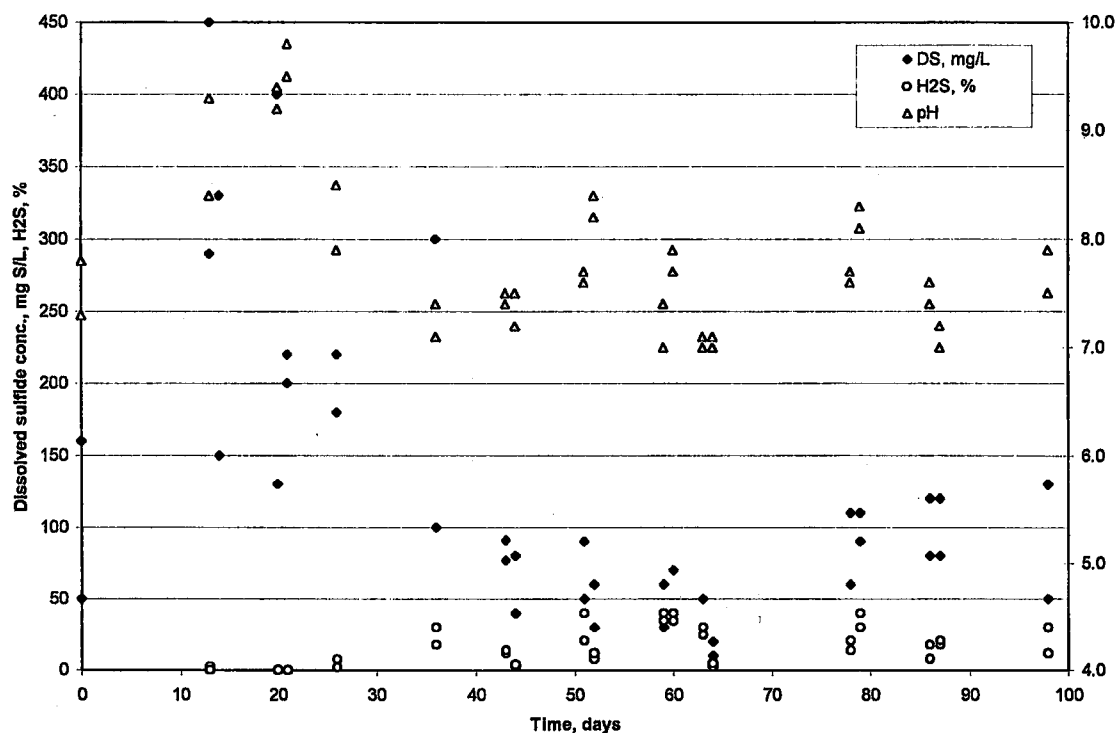


Figure 4.6 Dissolved sulfide concentration, H_2S % and pH profile during the experimental period

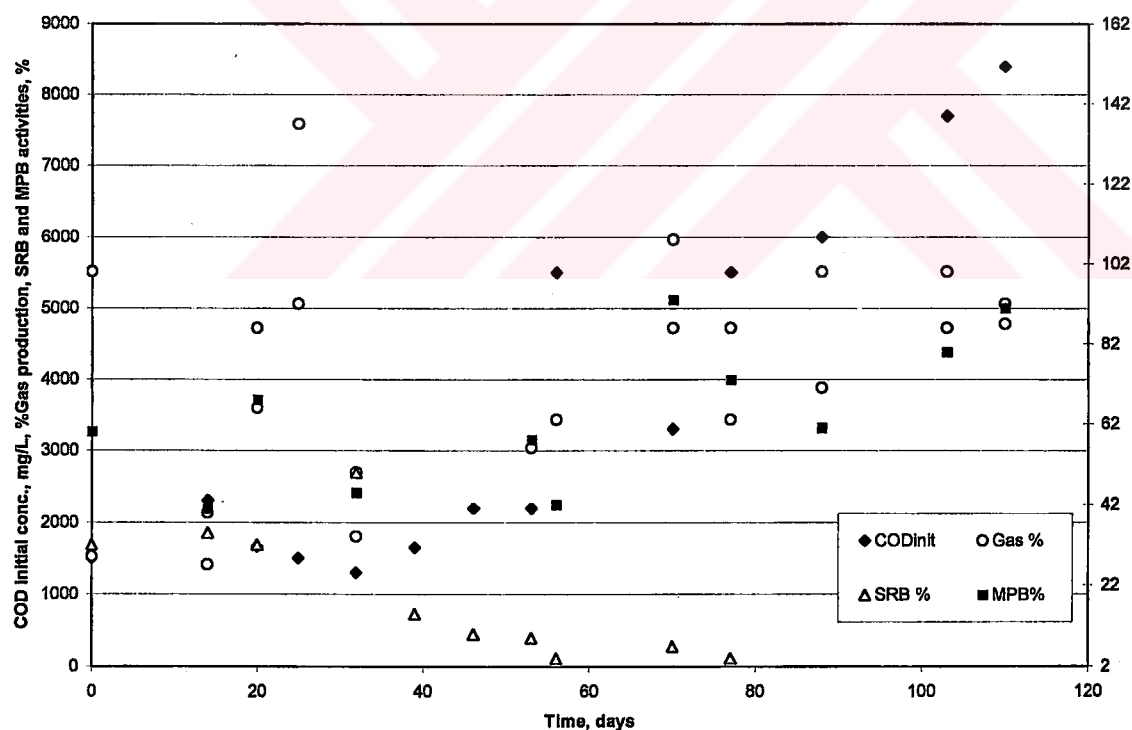


Figure 4.7 Gas production (as percentage of the theoretical value), SRB and MPB COD utilizations (%) for COD initial concentrations applied during the experimental period

Propionic acid fermenting acetogens are highly sensitive to sulfide toxicity. Dissolved sulfide levels of 100-400 mg/L in the first sets and 60-130 mg/L in the following sets were much higher than toxicity threshold ($K_i=53 \text{ mg S}^-/\text{L}$, Maillacheruvu and Parkin, 1996) of this bacteria group. Consequently, propionic acid seemed to be degraded by sulfidogens having more favorable conditions to outcompete acetogens ($K_i=681 \text{ mg S}^-/\text{L}$, Maillacheruvu and Parkin, 1996) throughout the experimental study.

4.3 Modeling of Sulfide Toxicity

In the literature, it has been observed that inorganic compounds cause uncompetitive inhibition which results in higher effluent COD with increasing toxicant concentration to a certain concentration at which abrupt failure of the system occurs (Bhattacharya and Parkin, 1986, Speece, 1997). Maillacheruvu and Parkin (1996) showed in their comprehensive study that the best model to describe sulfide toxicity was uncompetitive inhibition model.

To determine the inhibition pattern, S_0/q (where S_0 , is the initial COD concentration and q , rate of reaction as mg COD/mg VSS.d) at different substrate concentrations was plotted versus dissolved sulfide concentration in Figure 4.8. Lines which were constructed for COD ranges intercepted above x-axis showing uncompetitive inhibition pattern (Maillacheruvu and Parkin, 1996).

Inhibition constant (K_i) was determined from plotting of $1/q$ versus toxicant concentration (dissolved sulfide) in Figure 4.9. The slope of the line is equal to $1/k.K_i$ (where k is specific substrate utilization rate, mg COD/mg VSS.d). K_i value obtained was $275 \text{ mg S}^-/\text{L}$ which is lower than some K_i values found in Maillacheruvu and Parkin's study (1996) for different groups of bacteria (Table 4.3).

Dissolved sulfide was chosen as the inhibitor specie because it was directly measured, was produced at much closer levels to K_i values reported in the literature and gave good correlation for the inhibition pattern. H_2S concentration was calculated from dissolved sulfide and pH data and was produced at relatively low levels compared to dissolved sulfide due to pH levels. No inhibition pattern was

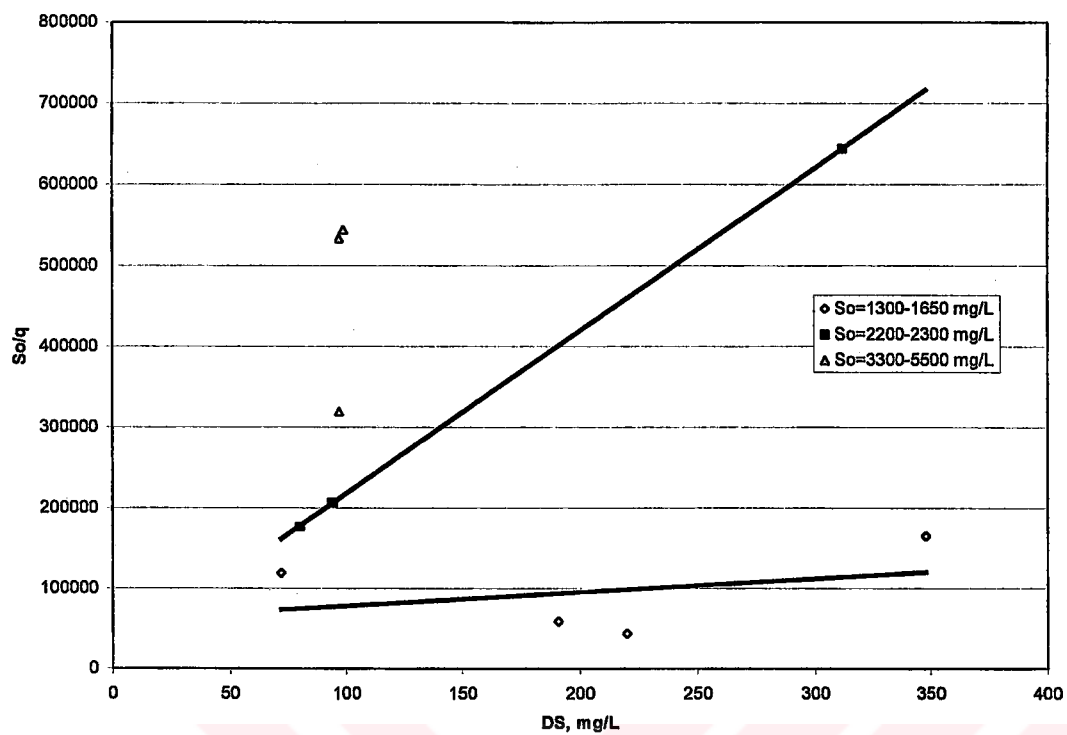


Figure 4.8 Determination of sulfide inhibition pattern

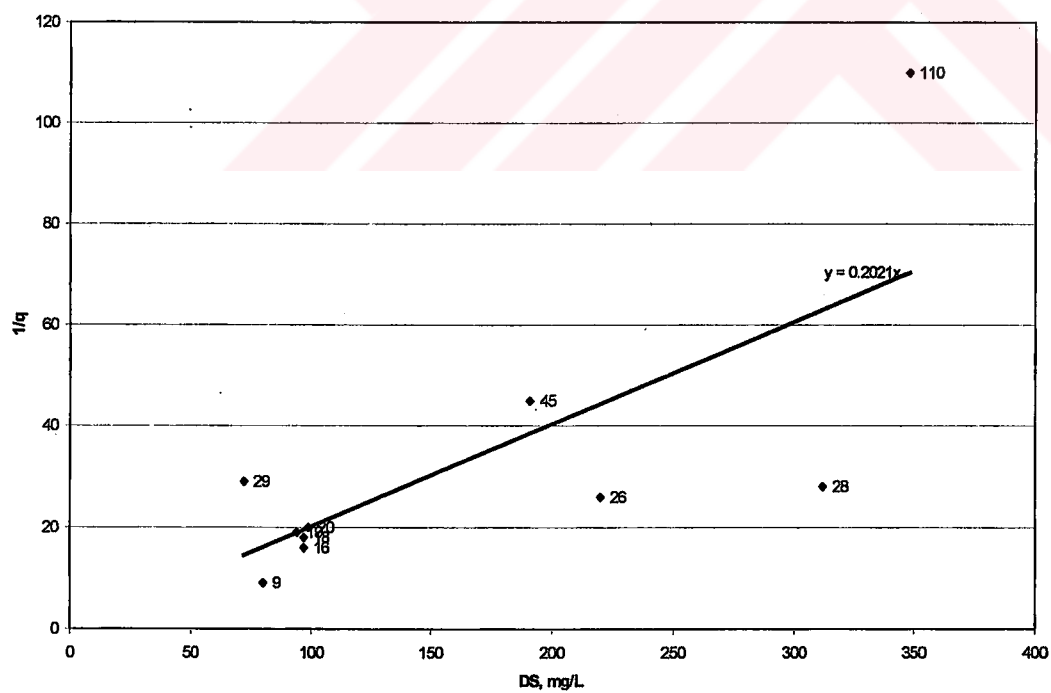


Figure 4.9 Determination sulfide inhibition constant (K_i)

obtained with H_2S on COD removal which was believed to consist mainly of the fermentation of volatile fatty acids present at high levels in the leachate. A relatively low K_i value compared to the reported ones in the literature for methanogens and sulfidogens degrading propionic acid and hydrogen is significant when considering the complex biomass activities treating leachate. Sulfate reducing bacteria are believed to be mainly involved with propionic acid and hydrogen utilization through oxidation-reduction reactions.

Dissolved sulfide level was observed above K_i value only in the first two sets where high pH values (7.8-9.3) kept H_2S levels below 15 mg/L with no inhibition effect. In the following sets, H_2S affected gas production/methanogenesis at close to neutral pH values with no effect on COD removal.

For the higher COD ranges applied a higher level of methanogenic activity was observed resulting in the predominance over sulfidogens using sulfate as electron donor which stayed at a constant activity independent of organic loading rate, yielding less dissolved sulfide production. The shift in bacterial groups' activity was interpreted as more sulfidogenic involvement in the degradation of volatile fatty acids using no sulfate with less involvement in the acetate degradation which is highly sensitive to dissolved sulfide levels occurred throughout the experimental period which is clearly supported by lower sulfide production, high COD removal rates and lower acetate degradation in the second half of the experimental period.

Table 4.3 Summary of inhibition constants from the literature

Bacteria Group	K_i (mg S^{2-} /L) (based on DS)	Reactor Configuration	Reference
Propionate-oxidizing SRB	681	Batch	Maillacheruvu and Parkin (1996)
Propionate fermenters	53	//	
Acetate-oxidizing SRB	35	//	
Aceticlastic MPB	222	//	
Hydrogenotrophic SRB	422	//	
Hydrogenotrophic MPB	1430	//	
Mixed (leachate)	275	//	This study

4.4 Determination Of Kinetic Constants for Organic Matter Removal

Organic matter removal was observed with daily COD measurements. Hydraulic retention time values of batch feeding sets varied from 4 to 15 days. A total of 14 sets was completed and data obtained from were used to determine kinetic constants for organic matter removal and bacterial growth kinetics. Three COD ranges were applied as 1300-2200 mg/L, 3300-5500 mg/L, and 6000-8400 mg/L. Related data are given in Table 4.4.

Zero, half and first order reaction approximations of the generalized reaction rate equation given in Eq. 2.10 are summarized in Table 4.4.

Experimental data were plotted as $\ln$ of the observed reaction rate versus $\ln$ of the substrate concentration obtained at the end of the batch feeding set. Two reaction rates were observed in Figure 4.10, for the lowest COD range applied (1300-1500 mg/L) reaction rate was observed as zero order and for the higher ranges, half-order reaction rate was observed. Calculated reaction rate constants are given in Table 4.5.

Table 4.4 Experimental Data for Determination of Rate Constants

Influent COD (mg/L)	HRT (day)	S (mg/L)	LnS	ln dS/dt
1300	7	525	6.26	4.70
1500	7	452	6.1	5.0
1500	13	279	5.63	4.54
1660	5	438	6.08	5.50
1650	7	224	5.41	5.31
2100	7	278	5.62	5.56
2200	4	415	6.03	6.1
3300	7	530	6.27	5.98
5500	14	595	6.39	5.86
5500	11	614	6.42	6.1
6000	15	928	6.8	5.82
7700	7	755	7.47	6.74
8000	13	983	6.89	6.29

Table 4.5 Zero, half and first order reaction approximations

Order	Equation	Linearized form	Slope	Equation No
Zero	$r = -dS/dt = k_0 S^0 = k_0$	$\ln r = \ln k_0$	0	4.3
Half	$r = -dS/dt = k_{1/2} S^{1/2}$	$\ln r = \ln k_{1/2} + 1/2 \ln S$	0.5	4.4
First	$r = -dS/dt = k_1 S^1$	$\ln r = \ln k_1 + \ln S$	1	4.5

Table 4.6 Reaction rate constants

Rate constant (1/day)		COD Range (mg/L)
k_0	4.8	1300-1500
$k_{1/2}$	2.158	1650-8000

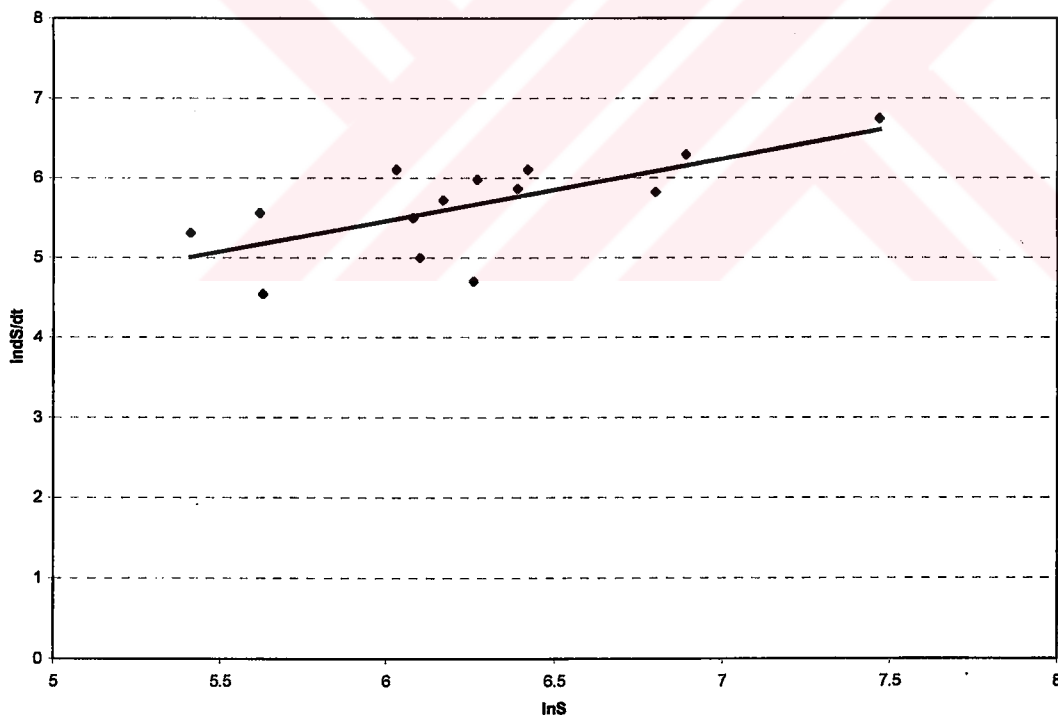


Figure 4.10 Evaluation of zero and half order reaction rate constants from batch reactors

Second order multiple reaction kinetics was also applied to the experimental data of this study. Substrate removal rate is:

$$r = - dS / dt = k_2.X.(S/S_0)^2 \quad (4.6)$$

Linearized form after integration of this equation is:

$$S_0.t / (S_0-S) = t + S_0 / k_2.X \rightarrow S_0.t / (S_0-S) = a + b.t \quad (4.7)$$

The plot of $S_0.t / (S_0-S)$ versus time is given in Figure 4.11. Experimental data are summarized in Table 4.7, a and b of the line are found to be 0.47 and 1.124, respectively ($r:0.99$). Comparison with the reported values in the literature is given in Table 4.8. HRT was kept in the range of 4-15 days. Greater b's are expected for high loaded wastewaters.

Table 4.7 Experimental Data for Determination of 2nd order Rate Constants

HRT (days)	$S_0.T/(S_0-S)$ (d ⁻¹)	MLVSS (mg/L)
4	4,93	
5	6,79	6400
6	7,57	8372
7	8,1	4900
7	8,07	5720
7	8,34	5252
11	12,38	
13	14,82	9808
13	15,97	9740
14	15,7	7140
15	17,74	

Table 4.8 Comparison of a and b with the reported values in the literature

a	b	Reference	Configuration
0.2	1.099	Ozturk et al., 1994	UASBR
0.1765	1.15848	Timur,H., 1997	ASBR
0.47	1.124	This study	BR

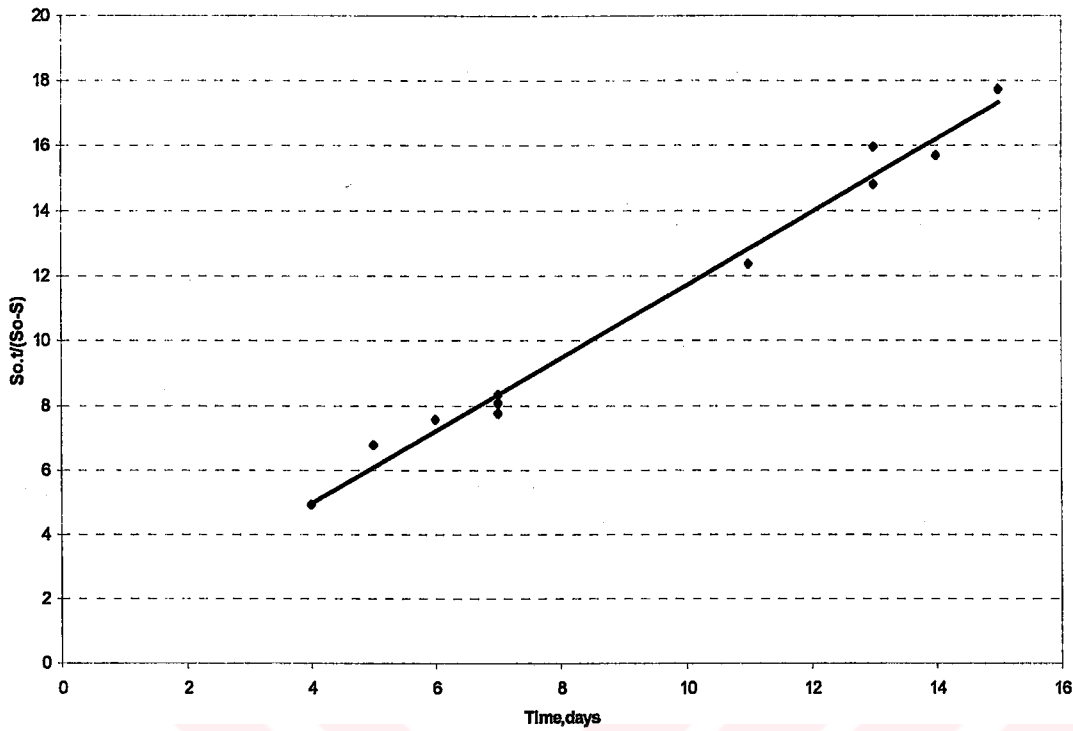


Figure 4.11 Evaluation of a and b constants for the second order reaction approximations

In order to determine bacterial growth-related constants Y and k_d , biomass balance is written as follows:

$$\text{Net Growth} = \text{Total Growth} - \text{Biomass Lost by Endogenous} \quad (4.8)$$

Respiration:

$$(dX/dt)_g = (dX/dt)_t - (dX/dt)_e \quad (4.9)$$

$$(dX/dt)_g = Y (dS/dt) - k_d X \quad (4.10)$$

Dividing of both sides of the equation gives:

$$1/\theta_c = \mu = Y q - k_d \quad (4.11)$$

$$q = \frac{1}{Y \cdot \theta_c} + k_d / Y \quad (4.12)$$

Experimental data for determination of Y and k_d are summarized in Table 4.9 and q versus $1/\Theta_c$ (μ) plot is represented in Figure 4.12. Y and k_d values were obtained as 0.24 and 0.015 d^{-1} , respectively.

Table 4.9 Experimental Data for determination of Y and k_d

COD (mg/L)		HRT (day)	VSS (mg/L)	$\mu(1/\theta_c)$ (1/day)	Specific	
Influent	Effluent				Loading (L_x)	Removal (q)
1300	525	7	4900	0,0048	0,038	0,0226
1500	452	7	6176	0,006	0,034	0,0242
1500	279	13	9808	0,0023	0,0118	0,0096
1650	224	7	5720	0,0043	0,041	0,0356
1660	438	5	6400	0,008	0,05	0,038
2300	478	6	8372	0,0064	0,046	0,036
3300	530	7	7154	0,0043	0,066	0,055
5500	595	14	7140	0,002	0,055	0,049

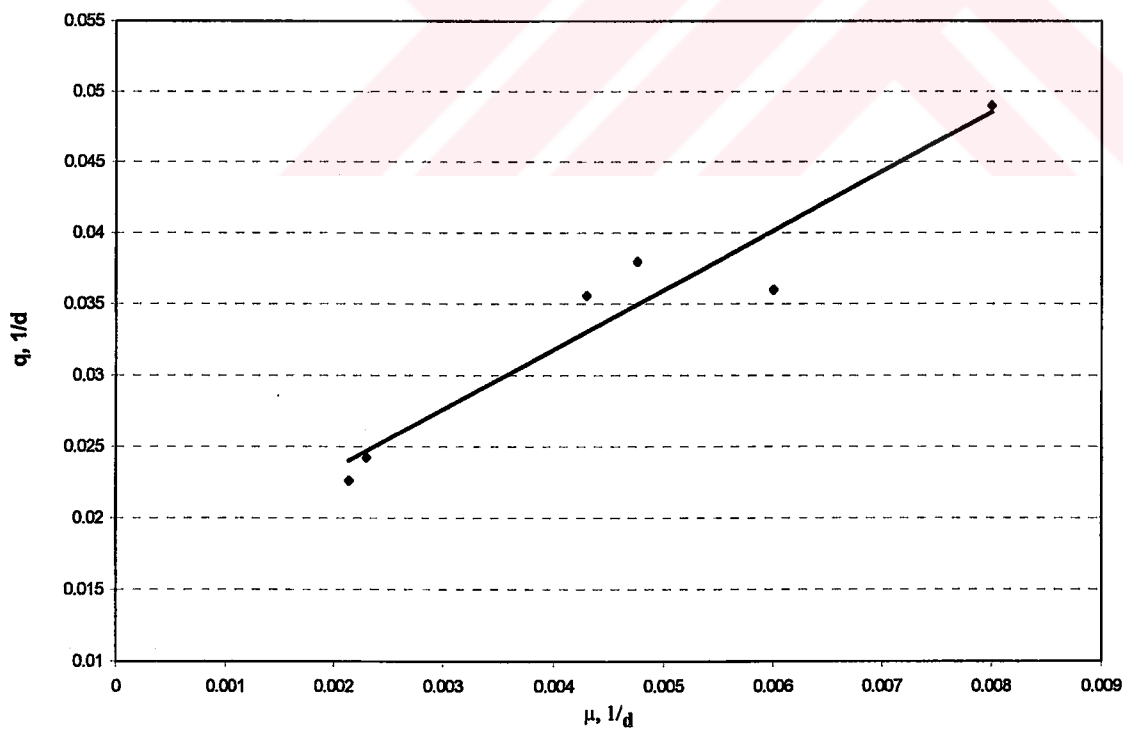


Figure 4.12 Determination of yield (Y) and decay constant (k_d)

Table 4.10 Summary of yield coefficient for the anaerobic leachate treatment

Influent COD (g/L)	Y (gVSS/g removed)	k_d (day ⁻¹)	Configuration	Reference
13	0,1	0,17	ASBR	Pohland & Harper,1985
12.9	0,33			//
5.7*	0.041	0,003- 0,022		Timur,1997
19*	0.45-0.3			Timur,1997
1.3-5.5	0.24	0,015		This study

* TOC

Y and k_d values obtained in this study are close to the values given in the literature, with only a magnitude lower k_d from Pohland and Parker's study (1985). Leachate characteristics may be the most significant factor in determining the kinetic constants. When compared to Timur's work, the constants found in these studies are in close proximity.

μ_{max} and K_s were determined using non-linear regression (Sigma Plot 3.0) (Figure 4.13) as 0.018 d⁻¹ and 2500 mg/L, respectively.

4.5 Toxicity Effects and Biotransformation of Chlorinated Compounds

Four chloro-aliphatic compounds, tetrachloromethane (CT), tetrachloroethane (PCA), and tetrachloroethylene (PCE) were injected to two sets of reactors fed with leachate and methanol at concentrations of 0.145-1.45 mg/L, 0.2-2.0 mg/L, and 0.197-2.36 mg/L, respectively. Methanol fed reactors were set up as paralel experiments to the leachate fed reactors in order to observe any difference in biotransformation characteristics of chlorinated compounds where bacterial species are quite different. Many research in literature have shown dominantly dechlorinating abilities of methanogenic bacteria. The presence of sulfate-reducing bacteria in leachate treating reactors might have had different dechlorinating ability or inhibition level than methanogenic bacteria. The two sets consisted of 5 reactors,

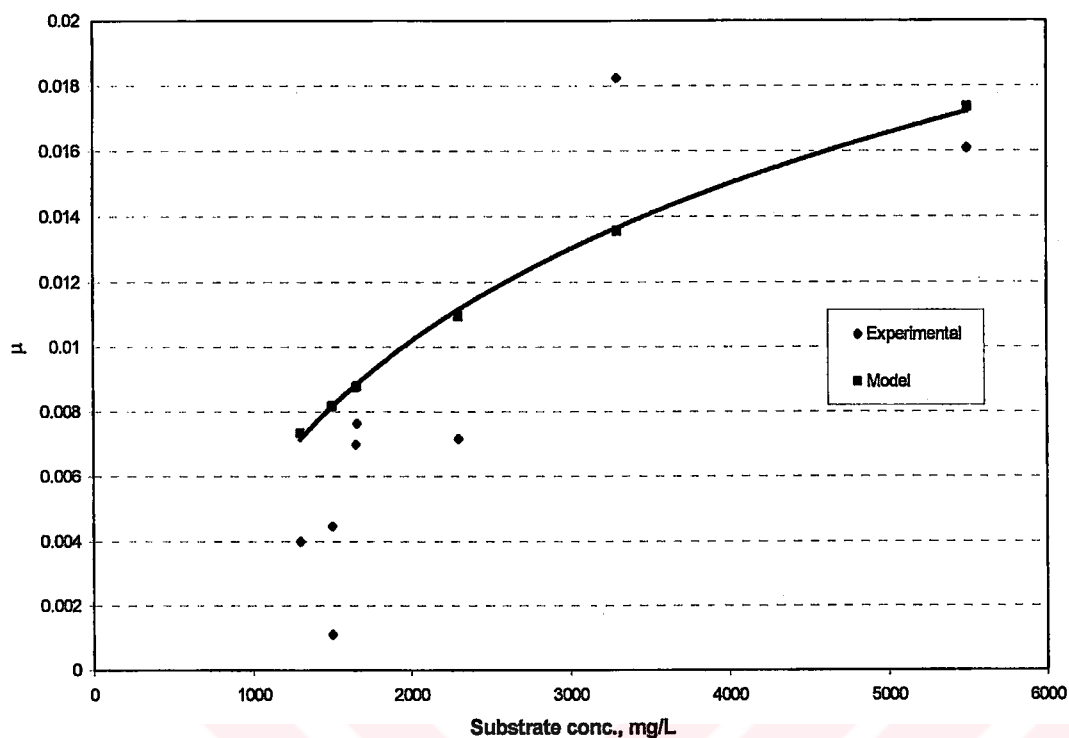


Figure 4.13 Determination of $\mu_{\max}$ and K_s using non-linear regression

one control, three receiving only one chlorinated compound and one receiving a mixture of three compounds.

Toxicity results are presented in Figures 4.14-17 as gas production (as percentage of control) in leachate receiving reactors (LRR) and methanol receiving reactors (MRR) and as COD removal only in leachate receiving reactors (LRR). Sulfide production for each chlorinated compound receiving reactor was compared with control values to see any possible toxicity effect to sulfidogens (Table 4.11).

Tetrachloromethane caused severe inhibition in both leachate and methanol receiving reactors (Figure 4.14). Gas production did not recover in leachate fed reactors during the experimental period, whereas methanol receiving reactors started to produce gas in the last 3 sets, despite increasing CT concentration indicating faster recovery of methanogenic bacteria. COD removal occurred at the same numbers as the control reactor, COD utilization was not affected despite inhibited methanogenesis.

Tetrachloroethane did not show any inhibitory effect on methane production in both reactors (Figure 4.15). Gas production was obtained at very close amounts to the control. COD removal was at the same levels as control.

Reactors receiving tetrachloroethylene showed some degree of inhibition with a faster recovery in methanol receiving reactors (Figure 4.16). COD removal was not affected.

Three chlorinated aliphatics were spiked together to leachate and methanol receiving reactors (Figure 4.17). Toxicity effect was more significant in methanol fed reactor with a late recovery compared to the other one. COD removal was not affected again.

Sulfide production with only a slight decrease in 3-5. sets implies higher tolerance level of sulfate-reducing bacteria to CT toxicity (Table 4.11). In PCA spiked reactor, in three sets lower levels compared to the control were obtained but it recovered in the last two sets. PCE, when fed alone and with other chlorinated compounds, did not affect sulfide production.

Table 4.11 Dissolved sulfide production in chlorinated compound receiving and control reactors

Concentration (mg/L)			Dissolved Sulfide Production (mgS ⁼ /L)				
CT	PCA	PCE	Control Reactor	CT Receiving Reactor	PCA Receiving Reactor	PCE Receiving Reactor	Mixture Receiving Reactor
0.145	0.2	0.197	56-293	127-256	37-176	68-96	77-136
0.29	0.4	0.394	12-96	20-88	36-80	40-133	8-100
0.43	0.6	0.59	28-128	20-64	18-80	40-120	28-80
0.43	0.6	0.59	28-120	52-72	30-60	40-68	32-80
0.58	0.8	0.78	36-131	16-68	18-78	18-150	11-100
0.58	0.8	0.78	78-97	88-97	78-93	40-97	107-116
0.58	0.8	0.78	74-107	20-120	78-97	40-78	116-126

Increasing toxicant concentrations applied resulted in higher biotransformation rates for PCA (Table 4.12). PCE degradation occurred independently from initial

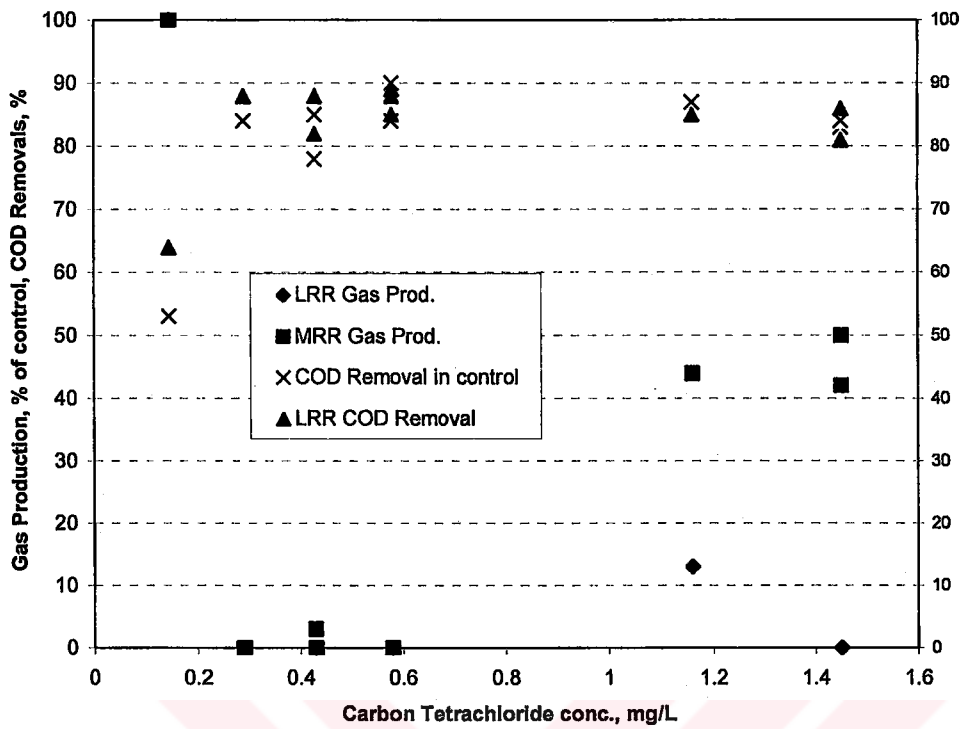


Figure 4.14 Gas production (% of control) and COD removal in tetrachloromethane receiving reactors (LRR:Leachate receiving reactor, MRR:Methanol receiving reactor)

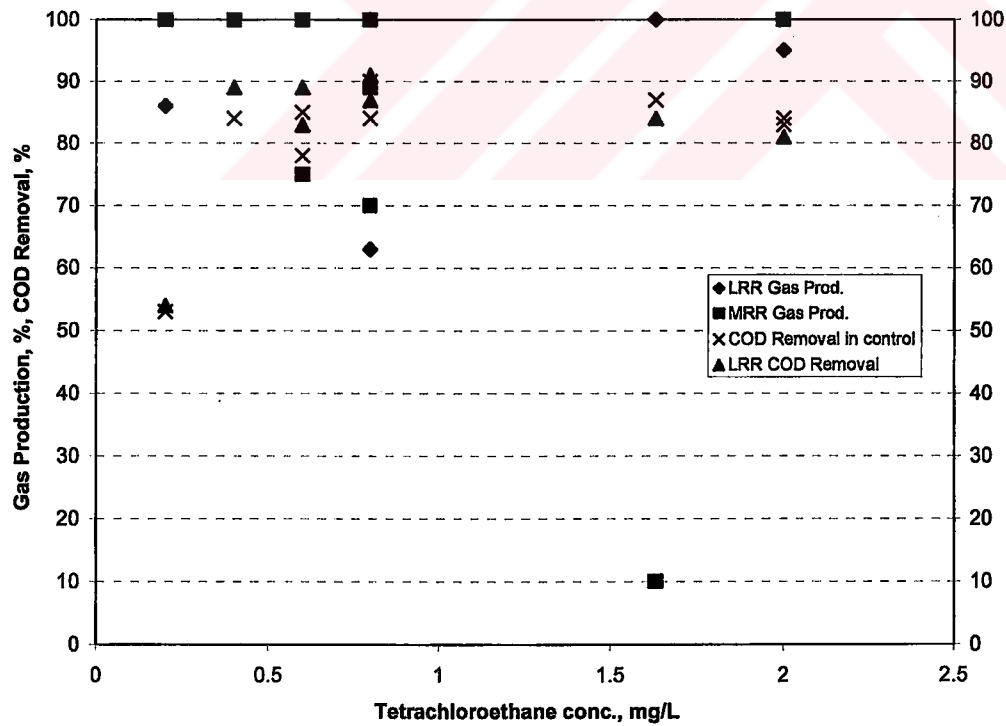


Figure 4.15 Gas production (% of control) and COD removal in tetrachloroethane receiving reactors (LRR:Leachate receiving reactor, MRR:Methanol receiving reactor)

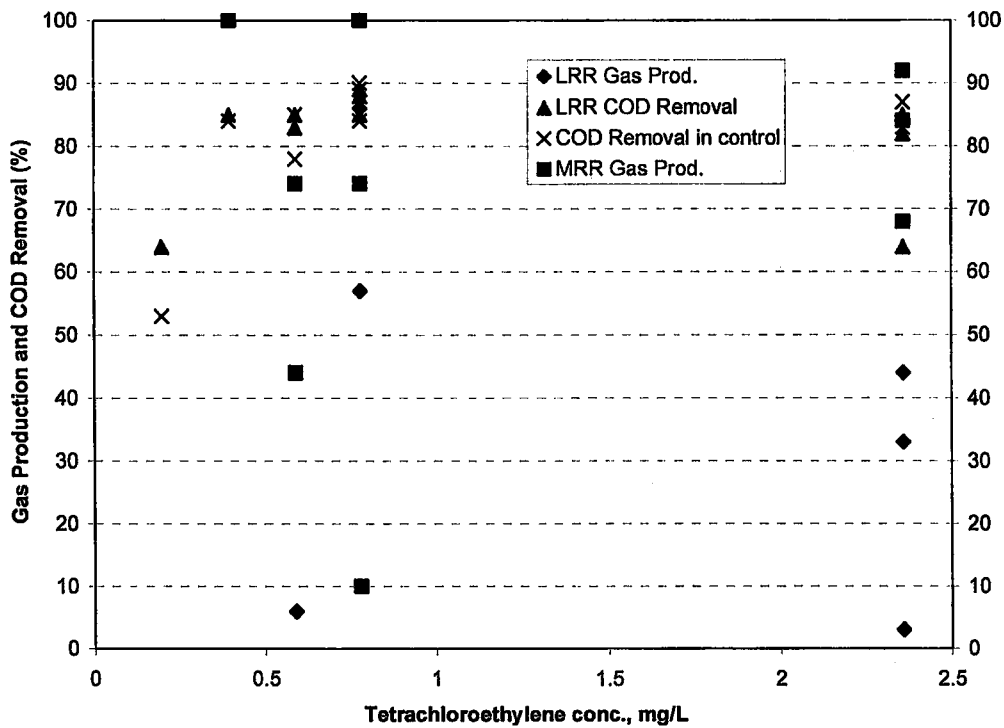


Figure 4.16 Gas production (% of control) and COD removal in tetrachloroethylene receiving reactors (LRR:Leachate receiving reactor, MRR:Methanol receiving reactor)

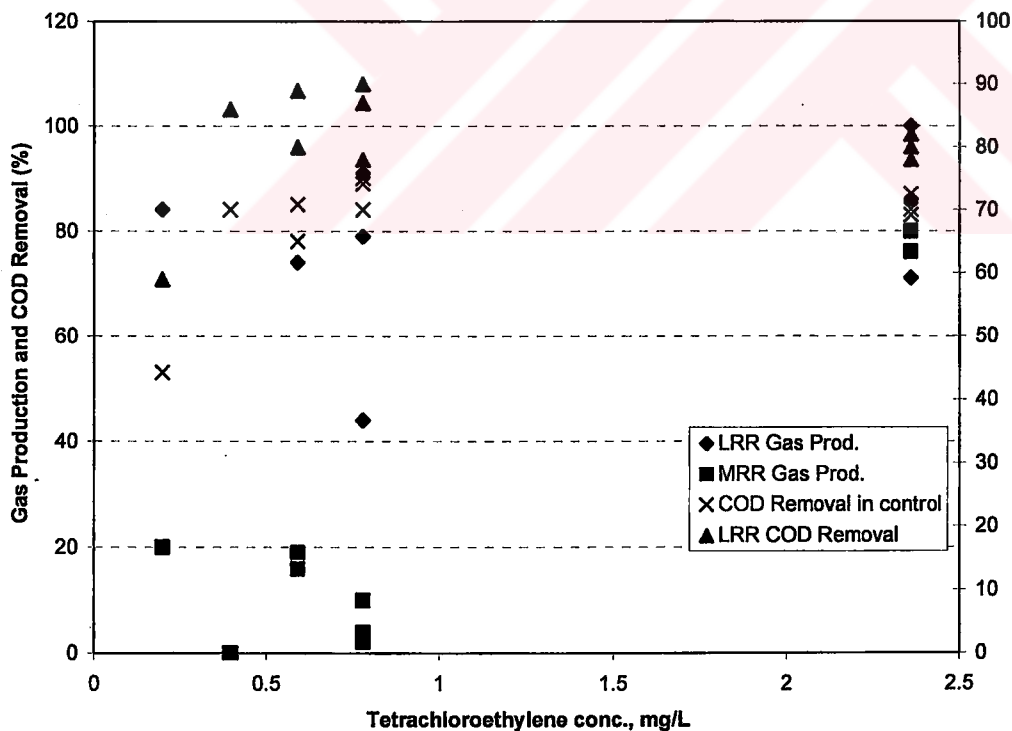


Figure 4.17 Gas production (% of control) and COD removal in mixture receiving reactors (LRR:Leachate receiving reactor, MRR:Methanol receiving reactor)

concentration with higher rates in the first sets. This may be correlated to different bacterial group profile between the sets belonging to the first and second half of the experiments. Tetrachloromethane could not be detected after injection, therefore, no biodegradation was determined.

Individual and mixed biotransformation rates are summarized in Table 4.13. Both PCA and PCE were degraded faster when spiked in mixture.

Carbon tetrachloride could only be detected after the injection and its disappearance rate could not be determined. Chloroform formation was expected but it was not detected. Direct carbontetrachloride biooxidation to CO₂ was reported in the literature using C-14 labeling (Bouwer and McCarty, 1983, Egli et al., 1988) where it was believed that bacteria used CT as a carbon source for autotrophic growth.

Trichloroethylene (TCE) was expected to be produced from the dechlorination of PCE literature in the presence of methanogenesis (Bouwer and McCarty, 1983, Vogel and McCarty, 1985, Freedman and Gossett, 1989) and in the absence of

Table 4.12 Biotransformation Rates Observed at Different Chlorinated Compound Concentrations

	Initial Applied Conc., mg/L			Biotransformation Rates, µg/L.d			
	CT	PCA	PCE	PCA		PCE	
				LRR	MRR	LRR	MRR
Set 6 11.5.98	0.43	0.6	0.59	0.314 0.46	0.08 0.106	1.19 2.8	0.5 1.14
Set 7 18.5.98	0.43	0.6	0.59	1.4 0.5	0.17	2.94 6.3	4.1 5.86
Set 8 25.5.98	0.58	0.8	0.78	0.4 0.47	0.19 0.29	2.02 1.91	2.36 4.36
Set 9 20.7.98	0.58	0.8	0.78	0.31 0.89	.29 0.28	0.94 3.46	1.45 1.5
Set 10 3.8.98	0.58	0.8	0.78			0.129 0.25	0.29 0.33
Set 12 9.11.98	1.16	1.63	2.36	1.235	0.575	0.533 1.054	0.65 0.98
Set 13 24.11.98	1.45	2	2	2.37	1.62	0.76 1.96	2.14 1.1
Set 14 1.12.98	1.45	2.36	2.36	1.1	2.4		

Table 4.13 Biotransformation rates of PCA and PCE when spiked individually and in mixture (rates in $\mu\text{g/L.d}$)

PCA (individual)		PCA (in mixture)		PCE (individual)		PCE (in mixture)	
LRR	MRR	LRR	MRR	LRR	MRR	LRR	MRR
0.31-1.4	0.08-0.29	0.47-2.37	0.106-2.4	0.129-2.94	0.29-4.1	0.25-6.3	0.33-5.86

methanogenesis (DiStefano et al., 1991) where acetogens were believed to reductively dechlorinate.

TCE was found in increasing and then decreasing pattern after the injection of PCE. Each chlorinated compound was observed to disappear to below the detection level at the end of each set. Trichloroethane was not produced as much as expected from PCA degradation, instead, TCE was found in PCA injected reactors. This is also supported by much higher TCE formation in reactors injected with both PCA and PCE, than PCE alone (Table 5.5.4). TCE production from PCA is believed to be produced via dehydrohalogenation (Vogel and McCarty, 1987) which is another biotransformation reaction where no electron donor is required and no change on the oxidation state of the reacting molecule occurs (at basic pH or pH 7).

Accumulation of TCE was observed in methanol fed reactors, explanation can be brought as some level of toxicity, also mixture receiving reactor was in a low gas production level.

A summary of chlorinated aliphatic spiking results is given in Table 4.15. COD removal was not affected by the toxicant concentrations applied, only methanogenesis seemed to be inhibited severely by CT, recovery in methanol receiving reactor was obtained in the last 3 sets. Recovery with PCE was fast. Mixture receiving reactors recovered well. TCE was degraded as fast as it was produced.

Methane formation All chlorinated aliphatics were biodegraded very efficiently in all reactors. COD removal was not affected by the concentrations applied. Completely stopped in CT receiving leachate fed reactor, but it did not start to recover as in methanol fed reactor in the last three sets.

Table 4.14 TCE formation in reactors

	Maximum Concentration Reached ($\mu\text{g/L}$)					
	TCE (individual)		TCE (mixed with PCA)		TCE (in PCA fed reactors)	
	LRR	MRR	LRR	MRR	LRR	MRR
Set 6	0.16	10*	4.3	4	5.9	0.4
Set 7	0.36	15.4*	4	6	3.7	0.3
Set 8	1.9	-	5.3	6.2	0.93	0.15
Set 9	3.3	5.3	3.6	35*	-	-
Set 12	3.4	7	27.6	12.3	-	-
Set 13	6	5	23	14.3	-	-

* accumulation

Reactors receiving chlorinated compounds in mixture showed some degree of inhibition but both did recover well. PCE and PCA did not significantly affect gas production.

Parameter	Set 3 20.04.98	25.4.98	Set 6 11.5.98	Set 7 18.5.98	Set 8 25.5.98	Set 9 20.7.98	Set 10 3.8.98	Set 12 9.11.98	Set 13 24.11.98	Set 14 1.12.98
Initial conc. (mg/l, aq):										
CT	0.145	0.29	0.43	0.43	0.58	0.58	0.58	1.16	1.45	1.45
PCE	0.2	0.4	0.6	0.6	0.8	0.8	0.8	1.63	2.0	2.0
PCE	0.197	0.394	0.59	0.59	0.78	0.78	0.78	2.36	2.36	2.36
% Degradation:										
CT	LRR	MRR	LRR	MRR	LRR	MRR	LRR	MRR	LRR	MRR
PCE	a	a	a	a	a	a	a	a	a	a
PCE b	a	a	a	a	a	a	a	a	a	a
C	83	88	78	89	97	99	99	97	98	99
	95	73	56	54	74	98	99	91	92	99
Duration of the set (day)										
Gas Production		10	7	6	8	14	7	15	4	6
(%control):										
LRR										
CT	100	100	n.m.	n.m.	0	0	0	13	0	0
PCE	100	100	n.m.	n.m.	6	57	86	(44)	(33)	(3)
PCA	100	86	n.m.	n.m.	100	63	100	100	100	95
Mixture	100	84	n.m.	n.m.	74	44	91	71	86	100
MRR										
CT	n.m.	100	0	3	0	0	0	44	42	50
PCE	n.m.	100	100	74	44	74	100	84	92	68
PCA	n.m.	100	100	75	100	100	89	100	100	-
Mixture	n.m.	20	0	19	16	2	100	76	80	-
%COD Removal:										
LRR: Cont.	71	53	84	85	78	89	84	87	83	84
CT	60	64	88	88	82	88	85	85	81	86
PCE	69	64	85	85	83	88	85	85	64	82
PCA	73	54	89	89	83	91	87	84	81	81
Mixture	76	59	86	89	80	90	78	82	78	80

a : not detected, b : only PCE receiving reactor, c : mixture receiving reactor, () experimental error n.m.: not measured

CHAPTER V CONCLUSIONS

- Three COD concentration ranges were applied to the reactors; 1300-1660 mg/L, 2200-5500 mg/L, and 6000-8400 mg/L. Sulfide inhibition did not significantly affect COD removal (with a lowest of 53-64%) when pH was adjusted to neutral values promoting rise in H_2S concentration only in 1 set. In following sets, COD removal proceeded between 78-91% where system seemed adjusted and seemed to tolerate dissolved sulfide levels (below 100 mg S^{2-}/L). COD removal was believed to consist of mainly volatile fatty acid fermentation by sulfidogens with no use of sulfate or CO_2 .
- Sulfidogenic activity was at its highest level in the first four sets (dissolved sulfide production at a 200-400 mg S^{2-}/L level). COD percentage utilized by SRB was between 32-50%. pH was mostly alkaline during this period (7.3-9.8) providing relatively low H_2S concentrations (less than 15 mg/L). Methanogenic activity used 41-68% of COD and gas yield proceeded between 119-436 mL/g $\text{COD}_{\text{removed}}$. Adjustment of pH to neutral level increased H_2S level up to 62 mg/L concentration level and COD utilization percentage by sulfidogens dropped to 4-15%, and dissolved sulfide production to 60-130 mg S^{2-}/L . COD utilized by SRB stayed constant at 24-36 mg COD removed per reactor throughout the rest of the feeding sets, independently from increasing OLR which promoted methanogenic activity with higher COD percentages (from 42% to 93%) (COD applied increased from 2200 mg/L to 8400 mg/L). MPB outcompeted SRB at higher organic loading rates and seemed to acclimate to sulfide levels in the system.
- Most sensitive bacteria groups to sulfide declined in activity. Shift in bacterial species was observed throughout the experimental period due to several parameters such as sulfide, pH and organic loading rate. Acetate-oxidizing SRB

seemed to be outcompeted by aceticlastic MPB (alkalinity and sulfide production dropped). Dissolved and un-ionized sulfide levels were above the inhibition threshold for propionate-fermenter acetogens. Low alkalinity production revealed also inhibited acetate degradation by methanogens resulting in a rate-limiting step of the system. Propionic acid seemed to be degraded by sulfidogens having higher tolerance level to inhibition compared to acetogens. Sulfidogens were believed to be highly involved in the volatile fatty acid fermentation with no use of sulfate evidenced by high COD removal levels.

- Gas production was close to theoretical value (66-137%) in most of the feeding sets with inhibition effects due to very alkaline pH in the first two sets (26-40%), to H₂S rising in the 5. set (34-50%), and to chlorinated compounds applied to the results in the last 7 sets (0-108%). Gas yield proceeded in the range of 119-184 mL gas per gram COD removed. Lower yield values (119-184 mL gas per gram COD removed) were obtained in the sets mentioned above. Gas production close to theoretical values showed that hydrogenotrophic methanogens, the least sensitive group to sulfide toxicity were fully active.
- pH tended to increase to alkaline values (7.8-8.0) with the production of alkalinity from volatile fatty acid degradation and the system seemed to perform better at this pH level.
- Sulfide inhibition best suited uncompetitive inhibition model and K_i : 275 mg S⁼/L was obtained. System tolerated dissolved sulfide occurring throughout the experimental period below this level.
- Kinetic constants for organic matter removal were determined as zero-order rate constant (k_0 :4.8 d⁻¹) for COD range of 1300-1500 mg/L, half-order rate constant ($k_{1/2}$:2.158 d⁻¹) for COD range of 1650-8400 mg/L. a and b were found as 0.47 and 1.124, respectively, in second-order approximations. Yield coefficient (Y) and decay coefficient (k_d) were obtained as 0.24 and 0.015 d⁻¹. Maximum growth rate (μ_{max}) and half-saturation constant (K_s) were determined as 0.018 d⁻¹ and 2500 mg/L, respectively.

- Chlorinated aliphatic compounds, tetrachloromethane, tetrachloroethane and tetrachloroethylene, were applied individually and in mixture to the leachate fed and methanol fed reactors at concentrations 0.145-1.45 mg/L for CT, 0.2-2.0 mg/L for PCA, and 0.197-2.36 mg/L for PCE. Among three chlorinated aliphatics applied individually, CT caused severe inhibition to methane production (no gas production in leachate-receiving reactor) which recovered only in methanol-fed reactor to 42-50% gas production level which is believed to be due to more stressed methanogenesis in leachate receiving reactors going through sulfide inhibition. Sulfate-reducing bacteria seemed more tolerant to chlorinated compound toxicity. Sulfide production was not affected significantly by chlorinated compounds applied. No biotransformation for CT was observed. PCA did not exhibit any toxicity effect on gas production and COD removal. Reactors receiving PCE showed some degree of inhibition with a faster recovery in methanol-receiving reactors. COD removal was not affected.
- Each chlorinated compound was observed to disappear to below the detection limit. PCA and PCE were biotransformed at rates 0.31-2.37 $\mu\text{g/L.d}$ for leachate-receiving reactor (LRR) and 0.08-2.4 $\mu\text{g/L.d}$ for methanol fed reactor (MRR) for PCA, and 0.129-6.3 $\mu\text{g/L.d}$ for LRR and 0.29-5.86 $\mu\text{g/L.d}$ for MRR for PCE. PCA and PCE disappeared faster when spiked together than individually. Chloroform formation from tetrachloromethane was not observed. TCE was transformed from PCE and also was further degraded. PCE and PCA were biotransformed faster when spiked together than individually. For PCA, individual transformation rates were 0.31-1.4 $\mu\text{g/L}$ for LRR and 0.08-0.29 $\mu\text{g/L}$ for MRR and rates for mixture spiking were 0.47-2.37 $\mu\text{g/L}$ for LRR and 0.106-2.4 $\mu\text{g/L}$ for MRR. For PCE, individual transformation rates were 0.129-2.94 $\mu\text{g/L}$ for LRR and 0.29-4.1 $\mu\text{g/L}$ for MRR and rates for mixture spiking were 0.25-6.3 $\mu\text{g/L}$ for LRR and 0.33-5.86 $\mu\text{g/L}$ for MRR.
- TCE was also produced in PCA receiving reactors which is also supported by much higher TCE formation in reactors injected with both PCE and PCA than

PCE alone. TCE production from PCA is believed to occur via dehydrohalogenation where no change on the oxidation state of the reacting molecule occurs.



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APPENDIX A

Tablo A.1 EXPERIMENTAL DATA OBTAINED AS COD, DS, TFS/VSS

Sets/Reactors	COD Initial	COD				DS					TFS/VSS (g/L)				
		Day 13				Day 1	2	3	4	13					
1. Set/ LRR-1	1500	199				156	288	308	188	368	53.2/13.6				
LRR-2		332				88		328	328	288	54.7/14.4				
LRR-3		332				128	288	288	268	288	27.2/6.9				
LRR-4		232				128	148	348	208	348	32.7/8.1				
LRR-5		299				48	228	248	328	448	22.9/6.1				
		Day 6				Day 1	2	3	4						
2. Set/ LRR-1	2300	664				268		188	408						
LRR-2		531				328	308	288	408						
LRR-3		664				148	248	88	248						
LRR-4		398				268	228	148	228						
LRR-5		333				268	248	108	268						
		Day 1				3	4	5	Day 2	4					
3. Set/ LRR-1	1660	930	664	398	465	220		180							
LRR-2		1726	398	465	465	220		220							
LRR-3		664	531	465	398	200		160							
LRR-4		1062	664	465	465	220		180							
LRR-5		930	598	531	398	200									
		Day 1				2	7								
4. Set/ LRR-1	1500	664	598	432											
LRR-2		531	598												
LRR-3		664	598	465											
LRR-4		800	598	398											
LRR-5		664	598	365											
		Day 0.4				2	3.8	7	Day 2.8	3	4	7			
5. Set/ LRR-1	1300		1062	664	564	293	56	117			27/6				
LRR-2		1262	1129	697	465	256	127	130	91		33/7				
LRR-3		1062	1129	664	465	96	68	77	77		14/3.5				
LRR-4		1461		564	598	76	45	37	77		17/4				
LRR-5		1129		730	531	136	80	144	77		15/4				
		D.0.4	1	2	3.8	7.7	1	1.4	2	4.8	7.7				
6. Set/ LRR-1	1650	1381	913	797	318	257		68	40	45	46	25.4/6.4			
LRR-2		1248		540	223	199	80	40	88	45	24	31.1/7.6			
LRR-3		1089	996	575	608	252	40	108	80	133	60	16.6/4.4			
LRR-4		1116	1162	487	318	186	40	20	80	61	44	20.4/5.1			
LRR-5		1142	747	443	440	226	40	32	48	53	40	19.3/5.1			
		Day 1				3	7	0	0.16	1	2	3	4	7	

7. Set/ LRR-1	2200	1770	593	320	28	44	60	52	128	96	48	23.74/5.78
LRR-2		1106	443	265	48	60	64	40	64	20	26	31.72/7.26
LRR-3		1483	708	320	52	40	64	48	120	92	56	14.52/3.7
LRR-4		1704	673	234	60	40	60	18	80	80	44	20.62/5.12
LRR-5		1527	797	252	40	28	60	30	80	60	53	17/4.4
		Day 1	2.16	3.16	52	0.16	1	1.16	2.16	3.16	52	
8. Set/ LRR-1	2200	1355	534	493	195	36	120	74	40	28	20	
LRR-2		1158	402	400	144	70	72		52	28	5	
LRR-3		1355	723	370	212	40	68	40	60	48	15	
LRR-4		1355	542	382	202	44	60	30	60	32	7	
LRR-5		1725	616	431	198	60	80	32	48	52	7	
		D.2	4	8	11	14	0	2	4	8	11	
9. Set/ LRR-1	5500	4158	2984	1147	684	605		78	122	116	107	31.6/8.2
LRR-2			3750	1273	867	680	18	49	61	20	20	36.3/9.1
LRR-3		4360	2984	895	677	655	18	110	150	97	93	19.1/5.6
LRR-4		4561	3427	730	562	480	18	59	61	50	49	23.8/6.4
LRR-5		4990	3387	643	698	554	11	68	100	88	97	22.2/6.4
		Day 1	2	3	4	7	2	3	4	7		
10. Set/ LRR-1	3300	1940	1915	983	864	523	97	88	93	78		35.7/9.16
LRR-2		2322	2255	2079	1663	484	97	97	97	88		35.7/9.36
LRR-3		2696	1827	1360	612	504	97	84	88	40		18.5/5.3
LRR-4		1814	1638	605	576	428	88	93		78		22.5/6.3
LRR-5			1701	1172	864	712	107	116		116		19.3/5.65
		Day 8	10	11	1	7	9	10	11			
11. Set/ LRR-1	5500	1228	640	528	74	97	107	88	97			
LRR-2		1386	672	634	120	20	49	59	59			
LRR-3		1153	720	624	78	40	59	40	49			
LRR-4		1292	552	576	97	78	88	78	88			
LRR-5		1134	816	710	116	116	116	113	126			
		Day 4	8	11	15	-	-	-	-	-	-	
12. Set/ LRR-1	6000	5664	3304	1357	826							
LRR-2		4248	2360	1770	944							
LRR-3		5428	3147	2478	944							
LRR-4		5286	2989	1947	1022							
LRR-5		3776	2989	1947	905							
		Day 2	3	4	7	-	-	-	-	-	-	
13. Set/ LRR-1	7700	4850	5136	3000	1498							
LRR-2		5136	4280	3000	1284							
LRR-3		6391	5992	4494	2782							
LRR-4		5136	4280	3000	1498							
LRR-5		5707	4850	3424	1712							
		Day 1	3	6	7	-	-	-	-	-	-	
14. Set/ LRR-1	8400	6420	4280	1926	1370							
LRR-2		5992	3424	1926	1198							
LRR-3		7276	4565	2354	1540							
LRR-4			2853	1712	1540							
LRR-5		6420	4280	1926	1712							

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