Treatment of Aqueous Waste Streams Contaminated With Carbon Dioxide and Crude Oil from an Enhanced Oil Recovery Process

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ABSTRACT

Treatment of Aqueous Waste Streams Contaminated With Carbon Dioxide and Crude Oil from an Enhanced Oil Recovery Process

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In the process of Enhanced Oil Recovery (EOR), carbon dioxide and water are used excessively (8000 ft^3 of CO_2 and 10 bbl of water per 1 bbl oil extracted) to increase the oil production and as a result, a large stream of waste water is generated. The main contaminants of this waste are dissolved gases mainly CO_2 and dissolved petroleum hydrocarbons (referred to as TPH). CO_2, which forms about 70% of the greenhouse gases, is the major cause of global warming and its atmospheric concentration (currently 385 ppm) has been rapidly increasing since the past decades.

In a series of batch experiments, the application of several electron donors such as simple volatile fatty acids and mono and disaccharides were investigated to remove CO_2 and TPH from a synthetic waste stream (containing about 200 mg COD/L of TPH and dissolved CO_2 at the saturation level). About 95% of CO_2 and 76% of TPH were removed using formate (2 g/L) and sucrose (2.5 g/L) respectively at a mesophilic range of temperature (about 35°C). In the second phase of this study, a two-step reactor system was used to treat this waste and the system operation was optimized using the method of evolutionary operation (EVOP) factorial design. For the first reactor with CO_2 reduction and CH_4

production as the target parameters, values of pH, temperature and organic loading rate of 2.5, 38°C and 6240 mg COD/L were obtained. The corresponding values for the second reactor were 6.5, 39.5°C and 394 mg COD/L for the TPH removal.

The energy balance for the system resulted in the calculated net energy ratio (NER) of 3.7 which showed a sustainable biogas production. The kinetic study of the system showed that degradation of formate and sucrose in both reactors is affected by the presence of petroleum hydrocarbons probably due to their inhibitory effects. Also, it was shown that the original differential equations for the substrate concentration and microbial growth can better predict the kinetic behavior of the system than the simplified models.

As the overall conclusion of this study, this method is less complex compared to other competitive methods and it can be easily applied. Moreover, besides its low energy requirements, it can generate CH_4 from CO_2 as a clean source of energy.

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List of Symbols and Abbreviations

AA	Acetic Acid
AGWP	Absolute Global Warming Potential
Alk	Alkalinity
APHA	American Public Health Association
API	American Petroleum Institute
ASTM	American Society for Testing and Materials
BA	Butyric Acid
BZ	Benzene
C	Concentration
COD	Chemical Oxygen Demand
EOR	Enhanced Oil Recovery
EP	Enzymatic Product Complex
ER	Energy Recovery
EVOP	EVolutionary Operation
GC	Gas Chromatography
GHG	Greenhouse Gas
GWP	Global Warming Potential
HPLC	High-Performance Liquid Chromatography
HRC	Hydrogen Release Compound
J	Joule
k	Reaction Rate Constant
K	Equilibrium Constant

k_d	Biomass Death Rate
K	Equilibrium Constant
K_s	Half-Saturation Constant
KHP	Potassium Hydrogen Phthalate
LA	Lactic Acid
LCA	Life Cycle Analysis
MA	Methanogenic Activity
MF_i	Mass Fraction of compound i
MW_i	Molecular Weight of compound i
N	Number of interactions
NER	Net Energy Ratio
NEB	Net Energy Balance
OLR	Operating Loading Rate
ORWARE	ORganic WAste REsearch
P	Pressure, Product
Pa	Pascal
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PFL	Pyruvate Formate Lyase
ppm	Parts Per Million
R	Run of the Reactors
S	Substrate
S_i	Solubility
T	Temperature

TCE	Tetrachloroethane
Tol	Toluene
TPH	Total Petroleum Hydrocarbons
TSS	Total Suspended Solids
UV	Ultraviolet (usually ultraviolet spectrophotometry)
VSS	Volatile Suspended Solids
Xyl	Xylene
X_i	Mole fraction
Y	Yield
ΔG	Changes in Gibbs Free Energy
μ_m	Maximum growth rate of biomass
σ	Standard Deviation
ν, V	Reaction Rates

Chapter 1: Introduction

1.1 Problem Statement

1.1.1 Enhanced Oil Recovery Process and the Waste Stream

The process of Enhanced Oil Recovery (EOR) was first used at large scale in 1972 (Melzer 2004). In this process, CO_2 is injected (CO_2 flood) to increase the oil extraction efficiency. The main factor affecting the efficiency of the process is the miscibility of CO_2 in the oil phase (Orr and Taber 1984, Blunt et al. 1993, Orr et al. 1995). The dissolved CO_2 reduces the viscosity of the oil and also causes swelling of the oil phase (Kovscek and Cakici 2005). Then large volumes of water under pressure are injected into the crude oil zone to sweep the oil along with water. Carbon dioxide and water can alternately be injected as the flood streams to increase the oil production.

During this process, a huge stream of wastewater is generated which is contaminated mainly with dissolved carbon dioxide and dissolved petroleum hydrocarbons. Carbon dioxide is one of the most important gases in the atmosphere, affecting the radiative heat balance of the earth as well as the calcium carbonate ($CaCO_3$) equilibrium of the oceans.

The global problems associated with carbon dioxide emissions can be summarized as:

- Significant regional climate variations
- Continental heartlands will dry out more in summer
- Declining soil moisture in many regions
- Increasing evaporation and average global precipitation, which in turn can increase sea levels and cause intense rainstorms. This will increase flooding in coastal areas and river estuaries.

- Storms and hurricanes will become more frequent and stronger as oceans heat up causing more water to evaporate.

In addition to environmental problems associated with dissolved gases such as CO_2, the wastewater can be very corrosive due to such gases. Moreover, the presence of inorganic salts can create leak problems in the pipelines and other facilities.

The presence of petroleum hydrocarbons in the wastewater and/or the potential of contamination of ground and surface waters with dissolved hydrocarbons are other environmental concerns. Some of the TPH compounds can affect our central nervous system. Typical adverse effects are headaches and dizziness at high levels in the air. TPH compounds can cause effects on the nerve system, blood, immune system, lungs, skin, and eyes (ATSDR 1999).

Moreover, the presence of dissolved petroleum hydrocarbons creates serious operational problems such as fouling in the downstream treatment systems (i.e. reverse osmosis) and emission of light hydrocarbon vapors. Also, these operational problems can significantly increase the costs of maintenance and operation.

1.2 Objectives of the Research

Application of chemical, biological or combined treatment processes for aqueous waste streams containing dissolved gases such as carbon dioxide has always been associated with operational or technical complications such as complexity of the process, application of complex chemicals such as

patented solvents, practical limitations of the methods etc. It is then necessary to investigate and develop new approaches that can be applied with the minimum of the above mentioned problems.

The main objective of this research was to develop a new biological method based on an anaerobic process to remove carbon dioxide and dissolved petroleum hydrocarbons which were the main contaminants of the aqueous waste stream resulting from crude oil extraction processes. The experiments were conducted in two phases. In phase I the batch experiments were performed to evaluate the applicability of this method and application of some test materials for the removal of these contaminants. In phase II which was based on the results of the Phase 1, a two-step reactor system was applied for the treatment of the waste stream. Also in this phase, the system was optimized and its environmental sustainability was assessed. Kinetic analysis of the system was also done in this phase.

1.2.1 Batch Experiments

In this phase of experiments, several batch experiments were conducted to assess the treatability of this waste stream and investigate the effect of addition of some potential electron donors on the treatment efficiency and the removal of the main contaminants from the waste stream. These materials are the intermediate or final products of an anaerobic biomass treatment process. The major activities/goals in this phase can be summarized as follows:

- Simulate the conditions of a typical EOR process to generate a synthetic waste stream similar to a real EOR process with carbon dioxide and dissolved petroleum hydrocarbons as the target contaminants to remove.

- Evaluate the quality of the resulting wastewater and determine if its constituents and their concentrations, such as inorganic species, as well as its conditions/parameters such as pH, alkalinity, concentration of dissolved gases, concentration of dissolved petroleum hydrocarbons, etc., are suitable for using an anaerobic treatment.

- Evaluate the operating conditions and parameters such as pH, alkalinity etc., and their variations, on the activity of the biomass in terms of removal efficiency, methane production, COD removal, etc.

- Evaluate the methanogenic activity for each batch based on methane production and concentration of the biomass to determine the ability of acclimated culture to degrade the fatty acids.

- Determine the critical acidity conditions under which the methanogenic bacteria can function since under acidic conditions, carbon dioxide is mostly in the liquid phase and more hydrogen ion is available for methanogenic reduction of carbon dioxide to methane.

- Select the best test materials for removal of the CO_2 and TPH from the waste stream and use them in the second phase of this work.

1.2.2 Two-Step Reactor System

In this phase and based on the results of the phase 1, a two-step reactor system (system) was used to treat the wastewater in two steps. The major activities/goals in this phase can be summarized as follows:

- Evaluate the operation of the system for CO_2 and TPH removal from the waste stream in two consecutive steps using the materials selected in Phase I: The first reactor was designed for methanogenic reduction of CO_2 and the second reactor was used for TPH removal.

- Evaluate the effectiveness of the materials selected in the batch tests for the removal of target contaminants: CO_2 and TPH in the first and second reactors, respectively.

- Primary optimization of the system operation for the removal of the target contaminants. For this purpose, the single-parameter optimization was first applied for the main operating parameters of temperature, pH, and organic loading rate.

- Evaluate the application of multivariable method of EVOP (EVolutionary OPeration) factorial design for the system optimization based on the primary results of the single-parameter optimization and find the optimum point for the system operation regarding the target parameters.

- Investigate the environmental sustainability of the system operation for the conditions of the optimization step. Application of the method of net energy balance and determine the energy recovery (ER) indices for biogas production.

- Investigate the kinetics of the anaerobic degradations of formate, sucrose and TPH and evaluate the effect of TPH on the kinetic parameters of formate and sucrose.

1.2.3 Organization of the Thesis

This thesis is comprised of 8 chapters as follows:

- Chapter 1 describes the origin of the problem and the objectives of this study
- Chapter 2 provides information about characteristics of the waste stream, its adverse environmental effects and existing treatment methods. It also includes the theory of the method used in this work.
- Chapter 3 introduces the materials used and describes the experimental methods/setups employed as well as the analytical/computational methods used.
- Chapter 4 presents and discusses the results obtained in the two phases of this work; phase (1) the batch experiments and phase (2) the continuous operation.
- Chapter 5 presents the results of optimization methods for the system. In this step of the study, the system was optimized using the single-step optimization method followed by the method of evolutionary optimization (EVOP) factorial design, and the results are discussed.
- In Chapter 6, the environmental sustainability of the system was investigated using the method of net energy balance and the net energy ratio (NER) was estimated.
- Chapter 7 specifies the kinetic study of the system. In this chapter, the kinetic parameters were investigated and based on comparison with the other studies, the results are discussed.
- Chapter 8 contains the overall conclusions drawn from the work and contribution made to knowledge.
- In Chapter 9, for continuation of this work, the directions for future work have been suggested.
- References are listed at the end of the thesis followed by appendix section.

Chapter 2: Literature Review

2.1 The Process of Enhanced Oil Recovery

EOR is preferred for oil with densities ranging from 29 to 48 API (882–788 kg/m^3) and reservoir depths from 760 to 3700 m below ground surface (Taber et al. 1997). The magnitude of CO_2 required in an EOR field is great, and several sources can be used to provide the necessary amounts (Aycaguer et al. 2001). The majority of CO_2 used commercially is for EOR purposes and originates from natural CO_2 reservoirs for the most part. For example, three natural CO_2 reservoirs, Sheep Mountain, Bravo Dome, and Mc Elmo Dome, provide CO_2 for the EOR fields in New Mexico and West Texas through a set of pipelines (USDOE, 1999). Other potential sources for CO_2 include byproducts from ammonia plants, other chemical plants, and oil field acid gas separation plants. The supply available from ammonia plants is 98% pure but very limited. Power plant stacks also contain CO_2 but at low concentration, and therefore, separation and compression of the CO_2 are required (Stalkup 1984).

2.2 The Nature of Contamination

The waste stream contains contaminants in two phases: 1) gas phase and 2) liquid phase. Gas and liquid phases contain environmentally hazardous gases such as carbon dioxide which is one of the major greenhouse gases. In a closed system, the concentration of dissolved gases can be functions of chemical and physical parameters such as temperature, pH, alkalinity, dissolved species etc. The amount of CO_2 present in the gas phase depends on parameters such as temperature and acidic strength of the solution which can shift the equilibrium of the system as indicated in the following reactions (at 25°C):

$$CO_2(aq) + H_2O \Leftrightarrow H^+ + HCO_3^- \qquad K_1 = \frac{[H^+][HCO_3^-]}{[CO_2(aq)]} = 4.47 \times 10^{-7} M \qquad (2\text{-}1)$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \qquad K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} = 4.68 \times 10^{-11} M \qquad (2\text{-}2)$$

Another gas is dissolved oxygen (DO) which like CO_2 creates severe operational problems such as corrosion that significantly increases operational and maintenance costs. In addition to dissolved gases, there exist dissolved petroleum hydrocarbons in the liquid phase. There are several hundred individual hydrocarbon chemicals defined in crude oil.

Crude oils originate from the decomposition and transformation of aquatic, mainly marine, animals and plants that became buried under successive layers of mud and silt some 15-500 million years ago; they are essentially very complex mixtures of many thousands of different hydrocarbons. Depending on the source, the oils contain various proportions of straight and branched-chain paraffins, cycloparaffins, and naphthenic, aromatic, and polynuclear aromatic hydrocarbons (IPCS, 2006).

Paraffinic crude oils are composed of aliphatic hydrocarbons (paraffins), paraffin wax (longer chain aliphatics), and high grade oils. Naphtha is the lightest of the paraffin fraction, followed by kerosene fractions (American Petroleum Institute, 2003). Asphaltic crude oils contain larger concentrations of cycloaliphatics and high viscosity lubricating oils. Petroleum solvents are the product of crude oil distillation and are generally classified by boiling point ranges. Lubricants, greases, and waxes are high boiling point fractions of crude oils. The heaviest, solid fractions of crude oils are the residuals or bitumen.

The aqueous solubilities of the main classes of hydrocarbons present in crude oil differ and increase in the order n-alkanes < isoalkanes < cycloalkanes < aromatics (McAuliffe, 1966). In general, solubility of petroleum hydrocarbons decreases as the number of carbon increases. For example, pentane (C5) has solubility of 360 ppm at 20°C and the corresponding values for hexane (C6) and decane (C9) are 13 ppm and 0.009 ppm respectively. BTX (benzene, toluene and xylene), is the most important petroleum hydrocarbon mixture and the solubilities of its constituents are as follows (Irwin 1997):

Benzene solubility = 1780 ppm at 20°C

Toluene solubility = 515 ppm at 20°C

Xylene solubility = 175 – 1986 ppm

However, the solubility of each component of crude oil is different from its solubility as an individual component and can be related to its mass/mole fraction in crude oil as follows:

$$\text{Effective Solubility} = (X_i)\,(S_i) \qquad (2\text{-}3)$$

$$X_i = (MF_i)(MW_t)/(MW_i) \qquad (2\text{-}4)$$

X_i = mole fraction of compound

S_i = solubility of compound (ppm)

MF_i = mass fraction of compound

MW_t = average molecular weight of crude oil (g/gmol)

MW_i = molecular weight of compound (g/gmol)

The solubilities of the crude oil components may extend up to one or two percent individually, however, total solubility of all components will be dictated by component composition and loading rates of oil to water (API Report, 2003). Since light crude oil has more fractions of light hydrocarbons, it has more solubility in water than heavy crude oil.

2.3 Carbon Dioxide: The Dominant Greenhouse Gas

For 650,000 years prior to the Industrial Revolution, atmospheric CO_2 concentrations remained between 180 to 300 parts per million by volume (ppmv) (Petit et al. 1999, Augustin et al. 2004, Siegenthaler et al. 2005). Increased fossil fuel burning associated with industrialization and increased use of carbon dioxide, have raised atmospheric CO_2 concentrations at rates of 0.25% per year in the 1960s to 0.75% per year in the last five years (Kleypas et al. 2006). The current atmospheric concentration of CO_2 is about 380 ppm and is expected to continue to rise by about 1% per year over the next few decades (Houghton, 2001). The rate of current and projected CO_2 increase is about 100 times faster than which has occurred over the past 650,000 years and the rising atmospheric CO_2 levels are irreversible on human time scales (Royal Society 2005).

2.4 Contamination of Water with Crude Oil

On average, more than seven barrels of water in the United States and three barrels of water worldwide are used for each barrel of oil (Lee et al. 2002). The annual cost of disposing of this water is estimated to be 5-10 billion dollars in the US and around 40 billion dollars worldwide. Figure 2.1 shows a schematic of the EOR process.

Once the oil is mobilized by CO_2, it must be either pushed or pulled to the production well. For improved oil recovery, CO_2 injection frequently is alternately with water injection in a water-alternating-gas (WAG) process. In practice, a "slug" of injected CO_2 is repeatedly alternating with water drive over intervals ranging from several weeks to months (Amarnath 1999).

There are also sources of excess water production (also called production water) in crude oil extraction process which are contaminated with dissolved petroleum hydrocarbons (Figure 2.2). Many different

materials and methods can be used to cope with excess water production problems which can be categorized as chemical or mechanical approaches (such as using well packers or gels and resins). Each of these methods may work very well for certain types of problems but are usually ineffective for other types (Lee et al. 2002).

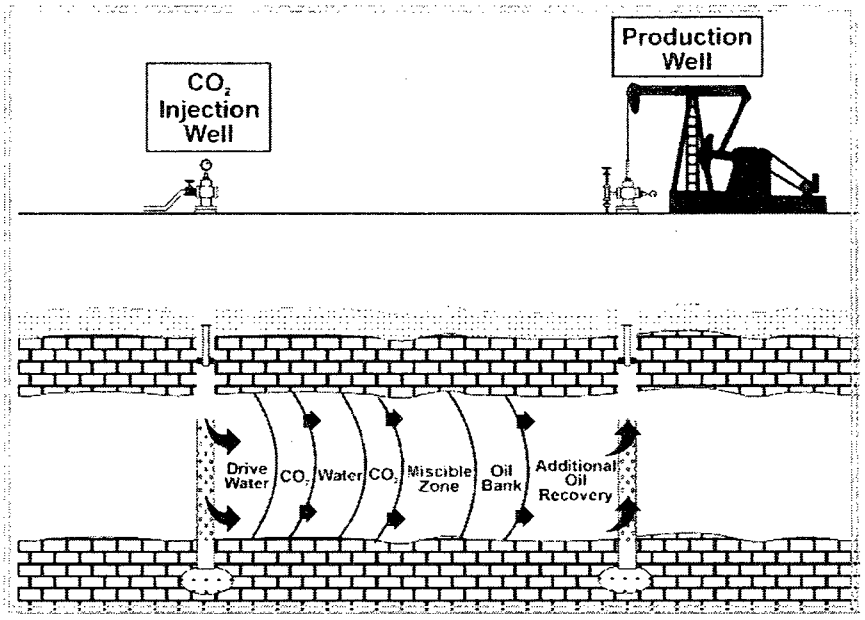


Figure 2.1 Schematic of an enhanced oil recovery process. (From Amarnath 1999).

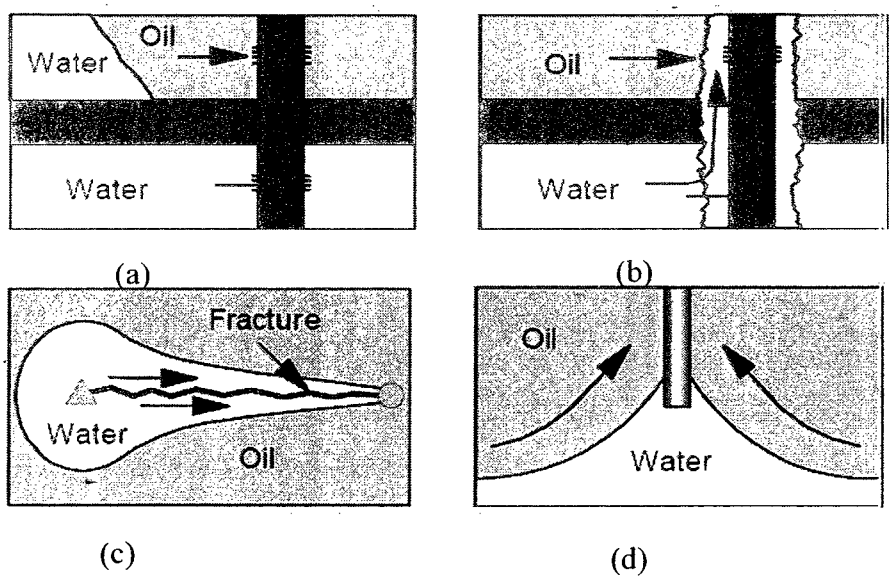


Figure 2.2 Sources of excess wastewater production in the crude oil extraction process: (a) open water one, (b) flow behind pipe and casing leaks (c) channeling from injectors (d) coning and cusping (Lee et al. 2002).

Despite prevention methods, a stream of contaminated wastewater which has to be treated is generated as crude oil is extracted from each well. The presence of dissolved gases and hydrocarbons and also chemical conditions such as low pH values make it very difficult and sometimes impossible to directly apply the conventional treatment methods to this wastewater. Klusman (2003) has reported the chemical characteristics of a waste stream resulted from an EOR process for an oil with API gravity of 34°. The process conditions and concentrations of the main species of the waste stream are shown in Tables 2.1 and 2.2, respectively.

Table 2.1 Operating conditions of an enhanced oil recovery process. (From Klusman 2003).

Parameter	**Value**
Crude Oil Density (°API)	34
Gas/Oil ratio	300
Gas injection (MPa)	34.5
Water injection (MPa)	30.5
Hydrostatic Pressure (MPa)	20.7
CO_2 Flux (g/m.day)	0.33-3.8
Temperature (°C)	70

Table 2.2 Average concentrations of the main ions in the waste stream of an EOR process. (From Klusman, 2003).

Ion/Parameter	Concentration (g/L)
Cl^-	21.5 - 28.1
SO_4^{2-}	0.4 - 0.45
HCO_3^-	0.3- 2.8
Na^+	12.3 – 15.6
Ca^{2+}	1.0 – 1.7
Mg^{2+}	0.1 – 0.3
K^+	0.3 – 0.5
pH	5.5 – 6.7 (dimensionless)

Generally, the pressure and temperature for injection applications range from 7.5 to 30 MPa and 50 to 100°C respectively. Carbon dioxide mixed with the aqueous phase reacts according to the balanced reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad (2\text{-}5)$$

The two steps of this reaction have been described in Equations (2-1) and (2-2). The dissolution ratio of CO_2 increases with pressure and decreases with temperature and the effect of pressure are dominant. In other words, the dissolution ratio of carbon dioxide at the bottom of an injection well is higher than the dissolution ratio at the surface despite the temperature increase due to the geothermal gradient (Doerler et al. 2001).

In general, because of the different physical and chemical characteristics of multiple underground layers, collecting data about the quality of the wastewater generated during the crude oil extraction is difficult.

However it is generally known that it contains dissolved species such as scale-forming ions, organic contamination due to dissolved petroleum hydrocarbons and dissolved gases such as carbon dioxide and oxygen.

2.5 Removal of CO_2

There have been many attempts to reduce carbon dioxide emissions using CO_2 sequestration through its injection in the underground waters e.g. saline waters, aquifers or deep oceans (Herzog, 2003, Reeve 2000, Hitchon 98). As an example, CO_2 has a particular application in western Canada where large fossil fuel users are located close to suitable underground reservoirs (Reeve, 2000). Figure 2.3 shows a schematic for underground storage and disposal of CO_2. In this method, carbon dioxide is transferred from the source to the geological reservoirs for storage or reuse.

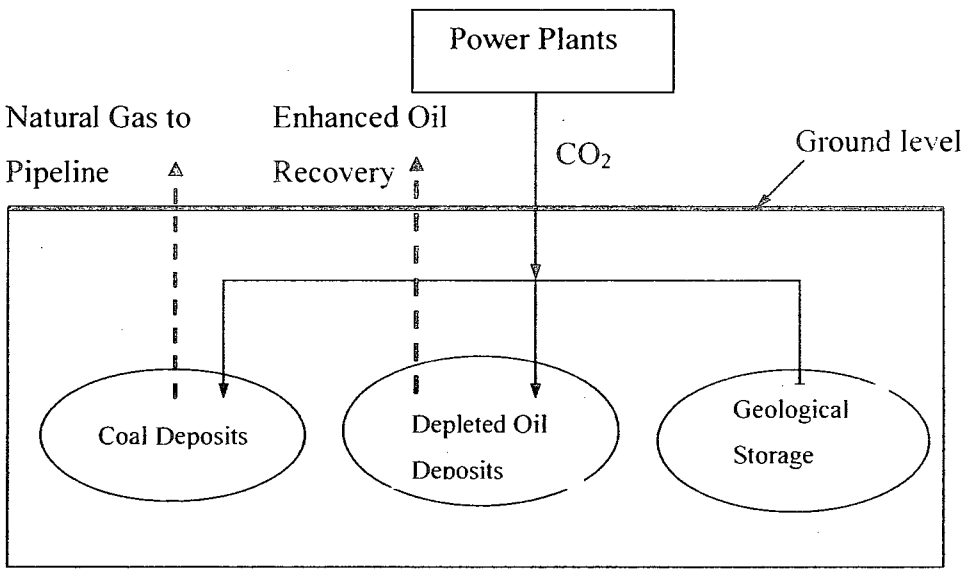


Figure 2.3 Schematic of processes for carbon dioxide capture and storage. [From: (http://www2.nrcan.gc.ca/es/oerd/english/View.asp?x=649&oid=18)]

Injection of CO_2 into local geologic formations or sea floors may be a reasonable component of the carbon management strategy (Herzog et al. 1991, Bachu et al. 1994). Riemer and Ormerod (1995) suggested that deep ocean injection is not immediately applicable due to a lack of information about the physiological effects of dissolved CO_2 on marine life.

However, in all of these processes, CO_2 is transferred from one place to another and there is always the risk of CO_2 release to the atmosphere again. The efforts to reduce CO_2 emissions in power plants can be classified in three categories:

1. Improvements in fuel utilization to reduce CO_2 emissions and increase efficiency.
2. Using biofuels in power generation systems with traditional power cycles or developing new technologies.
3. Using CO_2 separation methods as pre- or post-treatment techniques.

Carbon dioxide can be converted to methane using chemical or biological methods. Some experiments in microchemical catalytic reactors at 250°C have reached 90% CO_2 conversion (Van der Wiel 1999). Biological conversion of CO_2 into sparingly soluble carbonate minerals such as calcite ($CaCO_3$) and siderite ($FeCO_3$) has been studied using Fe(III)-reducing bacteria in conjunction with metal containing fly ash and lime (Roh et al. 2000). Usually it is fly ash that contains the metals.

Examples of biological methods to reduce carbon dioxide emissions in gas phase such as in power plants have been photosynthetic systems with cyanobacteria or microalgae (Maeda et al. 1995, Otaguchi et al. 1997) and bio-electro methods (Kuroda and Watanabe 1995). Lombardi (2003) has compared combined power cycles including application of chemical methods, e.g. chemical absorption, synthesis gas treatment or the use of gas liquefaction units.

As an example of chemical methods, CO_2 removal has been investigated in the iron and steel industry by Gielden (2003). In that study, a special absorbent like Selexol (dimethylether of polyethylene glycol) was used to capture CO_2 which was then compressed and transferred for storage such as to aquifers or oceans.

In addition to complications and the cost associated with most of these methods (especially chemical processes or combined chemical and biological systems) such as the use of complicated processes and instruments, requirements for specific materials such as absorbents or special microbial strains, they are applicable for only the gas phase. Therefore it is necessary to develop new methods that are applicable for carbon dioxide removal both in liquid and gas phase and besides being simple and feasible, they should be applicable onsite with minimal complexity.

2.6 Existing Treatment Methods for the Production Water

Typically, water treatment technologies are limited to treating specific constituents in water, e.g., dissolved solids, organics, conductive ions, etc. Depending on the final use of the water and the desired constituent concentrations, treatment processes are often coupled together to achieve desired water quality.

2.6.1 Reverse Osmosis (RO) Process

The process of natural osmosis occurs when solutions with two different concentrations are separated by a semi-permeable membrane (one that has a high permeability for water but a low permeability for dissolved solids). Osmotic pressure drives water through the membrane; the water dilutes the more concentrated solution. In reverse osmosis process, a hydraulic pressure is applied to the concentrated

solution to counteract the osmotic pressure. Therefore, the pure water is passed through the membrane while the contaminants that are too large to pass through the tiny pores in the membrane are retained on the other side of membrane. The operating pressure for this process is in the range of 850-7000 kPa for a wastewater flux of 320-490 $L/m^2.d$ (Tchobanoglous et al. 2002). This process can be used to concentrate dissolved contaminants [inorganics and relatively high-molecular-weight (greater than 120 g/gmol) organics] in an aqueous waste stream (McArdle et al. 1988). Figure 2.4 shows a schematic of this process.

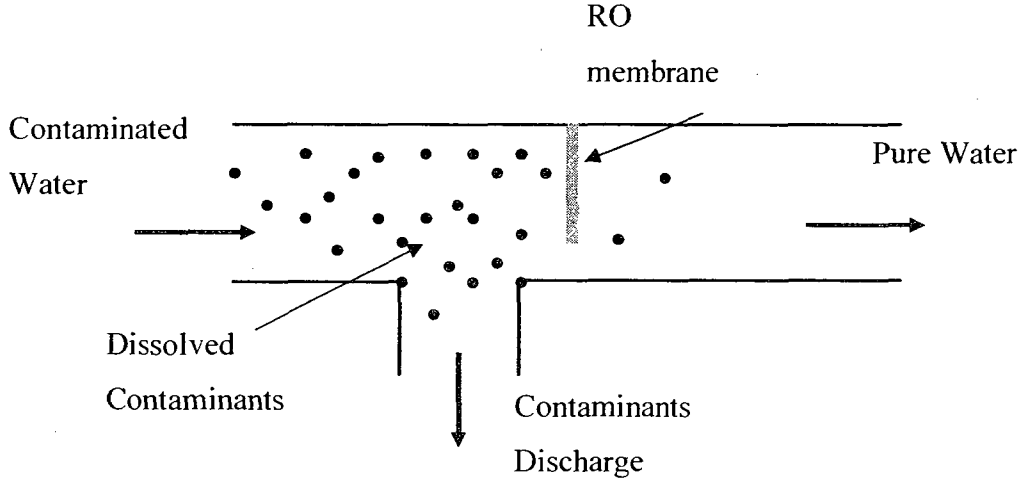


Figure 2.4 Schematic of a reverse osmosis process

Reverse osmosis has rarely been used in the hydrocarbon-production field because it is expensive and the flow rates are limited to a few liters/day. Also, the membranes can be fouled or damaged by organic constituents in raw produced water. Often, produced water must be pretreated before it can be treated with reverse osmosis. Lee et al. (2002) described several pretreatment methods that are being tested at Sandia National Laboratories and the Petroleum Recovery Research Center at New Mexico Tech. These include chemical treatment, filtration, biological treatment, polymeric absorbents, and macroporous polymer extraction.

2.6.2 Ion Exchange Process

Ion exchange is the process of removing dissolved solids ions from an aqueous solution and replacing those ions with other similarly charged dissolved ions. This exchange of ions is performed using cylindrical columns filled with spherical beads of polystyrene or acrylic material about ½ mm in diameter. Production water contains scale-forming ions such as magnesium and calcium which can be removed through an ion exchange process (Amarnath, 1999). Figure 2.5 shows a schematic of an ion exchange column for softening purposes.

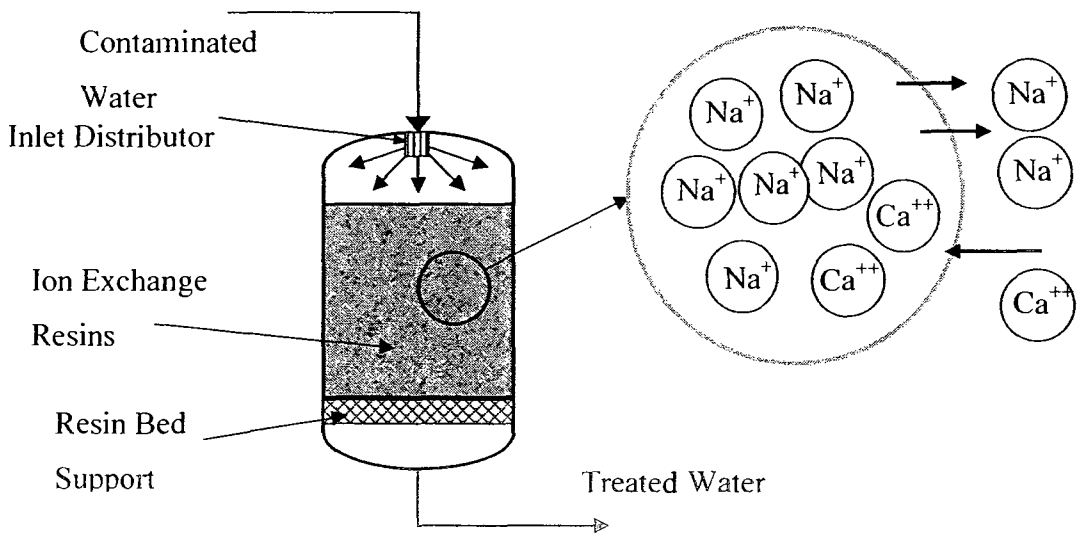


Figure 2.5 Schematic of an ion exchange unit (softening process)

2.6.3 Distillation

As shown in Figure 2.6, the distillation process is capable of removing 99.5% of the impurities concentrated in raw water (Derickson et al. 1992).

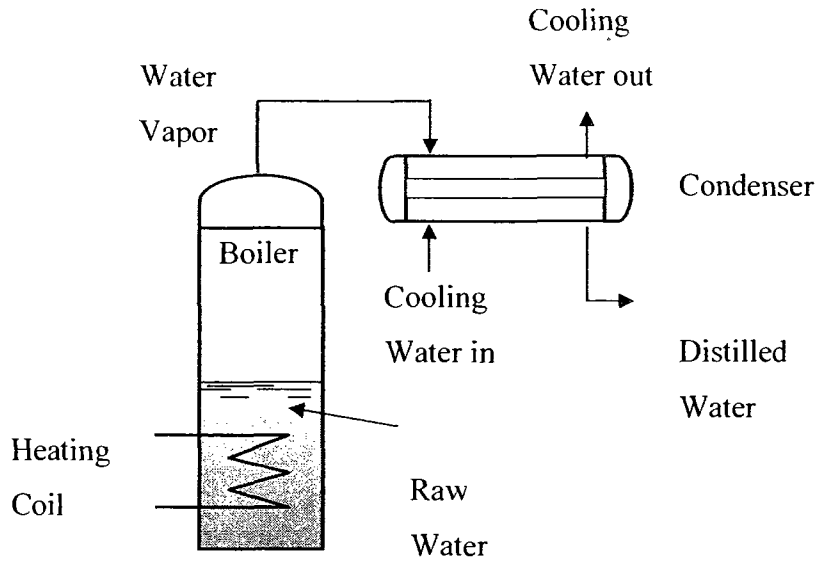


Figure 2.6 Schematic of a simple distillation unit

It relies on evaporation to purify water. Contaminated water is heated to form steam which is cooled and condensed afterwards to form purified water. During this process, inorganic compounds and large nonvolatile organic molecules don't evaporate with the water and are left behind.

2.7 Anaerobic Treatment

The history of anaerobic treatment started in 1776, where Alessandro Volta performed some experiments on combustible gas that were reported to him by a friend, Father Carlo Campi. On a little boat in Lake Maggiore he started to poke and stir the bottom of an area covered with reeds. Upon doing this, Volta noticed a lot of air emerging and decided to collect some in a large glass container. Upon analysis of the gas he noted that it burned a beautiful blue flame. Nearly half a century later, it was shown that the methane formation in these habitats was by a microbial process (Ferry, 1993).

A typical anaerobic process consists of four main steps as shown in Figure 2.7. It is a complex physico-chemical and biological process involving different stages and factors. These steps can be briefly described as follows.

2.7.1 Hydrolysis

The waste materials of plant and animal origin consist mainly of carbohydrates, lipids, proteins and inorganic materials. In the step of hydrolysis, large molecular complex substances are solubilized into simpler ones with the help of extracellular enzymes released by the bacteria.

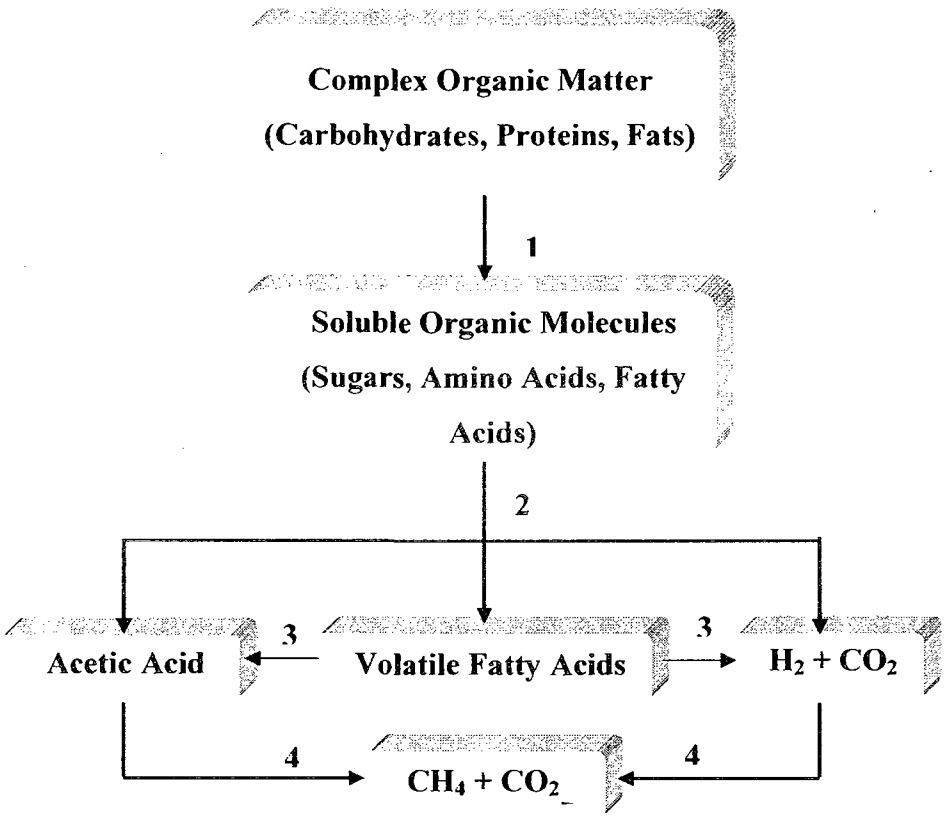


Figure 2.7 Major steps in a typical anaerobic treatment process. (1-Hydrolysis, 2-Fermentation, 3-Acetogenesis, 4- Methanogenesis).

This stage is also known as the polymer breakdown stage. For example, cellulose which is polymerized glucose, is broken down to dimeric, and then to monomeric sugar molecules (glucose) by cellulolytic bacteria.

2.7.2 Fermentation or acidogenesis

In this step, the monomeric molecules such as glucose, which are produced in the previous step, are fermented under anaerobic conditions into various acids with the help of enzymes produced by the acid-forming bacteria. They break down molecules of six atoms of carbon (glucose) into molecules of fewer atoms of carbon (acids). The principal acids produced in this process are acetic, propionic and butyric acids.

2.7.3 Acetogenesis

In this step, the fermentation products are converted into acetate, hydrogen and carbon dioxide by acetogenic bacteria.

2.7.4 Methanogenesis

In methanogenesis, methane (CH_4) is formed from acetate and hydrogen/carbon dioxide by a special group of anaerobic bacteria called methanogens. Simple organic molecules including short-chain fatty acids, along with carbon dioxide and hydrogen, are converted to biogas. Therefore, it is possible to simulate this step and provide conditions to convert CO_2 to CH_4 using methanogenic bacteria.

2.7.4.1 Methanogenic bacteria

Methanogenic archaea are obligate anaerobes. In fact, they are the strictest anaerobes discovered (Harley et al. 1909, Holt et al. 1994). Methanogens can be found in a variety of waters from freshwaters to

hypersaline waters. There are many types of methanogens, but a few known to be extremely halophilic are methylotrophs belonging to the *Methanosarcinaceae*. Sowers and Gunsalus (1988) reported a type of salt-adapted *Methanosarcina* (Ferry, 1993). Methanogens have different morphological structures. Figure 2.8 shows some micrographs of these microorganisms.

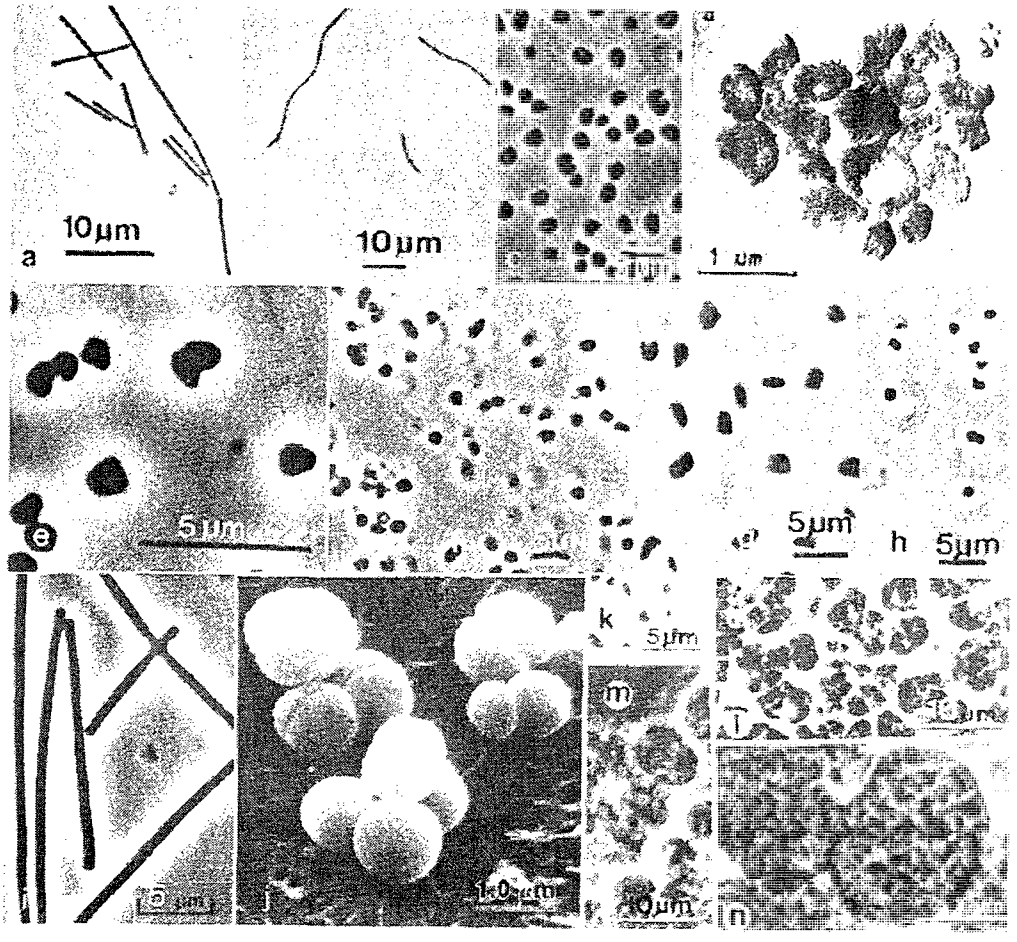


Figure 2.8 Morphological structures of methanogenic cells. (From Ferry 1993).

Rod-shaped methanogens are illustrated by *Methanobacterium spp.* or *Methanopyrus kandleri* (a). Some methanogens have such a distinctive shape that they can be tentatively identified by light microscopy even in mixed cultures. These are *Methanospirillum* (long thin spirals, Figure 2.8b), *Methanosaeta*

("*Methanothrix*") (Patel 1992) (also long but thicker filaments, Figure 2.8i), and *Methanosarcina* (clusters of round cells, Figure 2.8, l-n). Examples of the round-shaped or coccoid *methanogens* are *Methanogenium* (Figure 2.8 c), *Methanococcus* (Figure 2.8 h), *Methanocorpusculum* (Figure 2.8 d), *Methanococcoides* (Figure 2.8e), *Methanolobus* (Figure 2.8f), *Methanohalophilus* (Liu et al. 1990), and *Methanoculleus* (Blotevogel et al. 1991).

2.7.4.2 Environmental Growth Factors

Some environmental factors can affect the function and activity of methanogens and therefore the performance of the treatment system. So it is important to know how these factors affect the efficiency of the treatment system. These factors are described as follows.

Temperature

Methanogens are tolerate a wide variety of temperatures. They are generally divided into two groups: mesophilic methanogens with an optimum temperature of about 35°C and themophilic methanogens with an optimum temperature of about 65°C. There are also special strains of methanogens such as marine methanogens which can function at temperatures of about 2°C and geothermal methanogens living at temperatures above 100°C (Ferry 1993).

pH

The optimum pH for most methanogens is near neutrality (Jones et al. 1987). Some methanogens like those in peat bogs can produce methane at pH values of 4.0 or less. There are also some alkaliphilic methanogens that can grow at pH values of 8 and 9 (Blotevogel et al. 1985). In order to stabilize the pH in a typical anaerobic process, a certain amount of alkalinity is needed to maintain a buffering capacity

in the solution. In order to achieve a pH around 7 in a reactor at a CO_2 concentration of 30% in the biogas, roughly, 40 equivalents of bicarbonate per cubic meter of wastewater must be present in the reactor (Kleerebezem and Macarie 2003).

Oxygen

Methanogens are known to be strict anaerobes. They are unable to grow or produce methane in aerobic media, but they can tolerate certain levels of dissolved oxygen. In a study by Kiener and Leisinger (1983), it was found that there is a wide range of oxygen tolerance for methanogens from 3 to 24 hours, before dying because of peroxides and toxic byproducts. On the other hand, some adaptations to oxygen peroxides have been reported (Kiener et al. 1988).

Toxicity

Mineral ions, heavy metals and detergents are some of the toxic materials that inhibit the normal growth of bacteria in a digester. Small quantities of mineral ions (e.g. sodium, potassium, calcium, magnesium and sulfur) stimulate the growth of bacteria, while very high concentrations of these ions will have a toxic effect. For example, NH_4 concentrations of 50 to 200 mg/l stimulate the growth of microbes, whereas concentrations above 1500 mg/l produce toxicity. Similarly, heavy metals such as copper, nickel, chromium, zinc, etc. in small quantities are essential for the growth of bacteria but higher concentrations have toxic effects.

Likewise, detergents including soap, antibiotics, organic solvents, etc. inhibit the activities of methane producing bacteria and addition of these substances in the digester should be avoided. Although there is

a long list of substances that are toxic and have adverse effects on bacterial growth, the inhibiting levels of some of the major ones are given in Table 2.3.

Table 2.3 Toxic levels of various inhibitors for methanogens.
[From: The Biogas Technology in China, BRTC, China (1989)]
(http://www.fao.org/sd/EGdirect/EGre0022.htm)

Inhibitor	**Inhibiting Concentration**
Sulphate (SO_4^{2-})	5000 ppm
Sodium Chloride or Common salt (NaCl)	40000 ppm
Nitrate (Calculated as N)	0.05 mg/ml
Copper (Cu^{2+})	100 mg/l
Chromium (Cr^{3+})	200 mg/l
Nickel (Ni^{2+})	200 - 500 mg/l
Sodium (Na^{+})	3500 - 5500 mg/l
Potassium (K^{+})	2500 - 4500 mg/l
Calcium (Ca^{2+})	2500 - 4500 mg/l
Magnesium (Mg^{2+})	1000 - 1500 mg/l
Manganese (Mn^{2+})	Above 1500 mg/l

2.7.4.3 Methanogenic pathways and reactions

As shown in Figure 2.9, the catabolic pathways of methanogens can be divided into three groups: CO_2-reducing, methylotrophic, and acetoclastic pathways. However, there are similarities among these pathways in terms of some intermediate reactions and their products. Most methanogens can grow using H_2 as a source of electrons via hydrogenase. H_2 is a major fermentation product in many species of

anaerobic bacteria, fungi and protozoa. In many methanogenic environments, this H_2 is utilized rapidly even when it is present at very low concentrations (Wolin 1976).

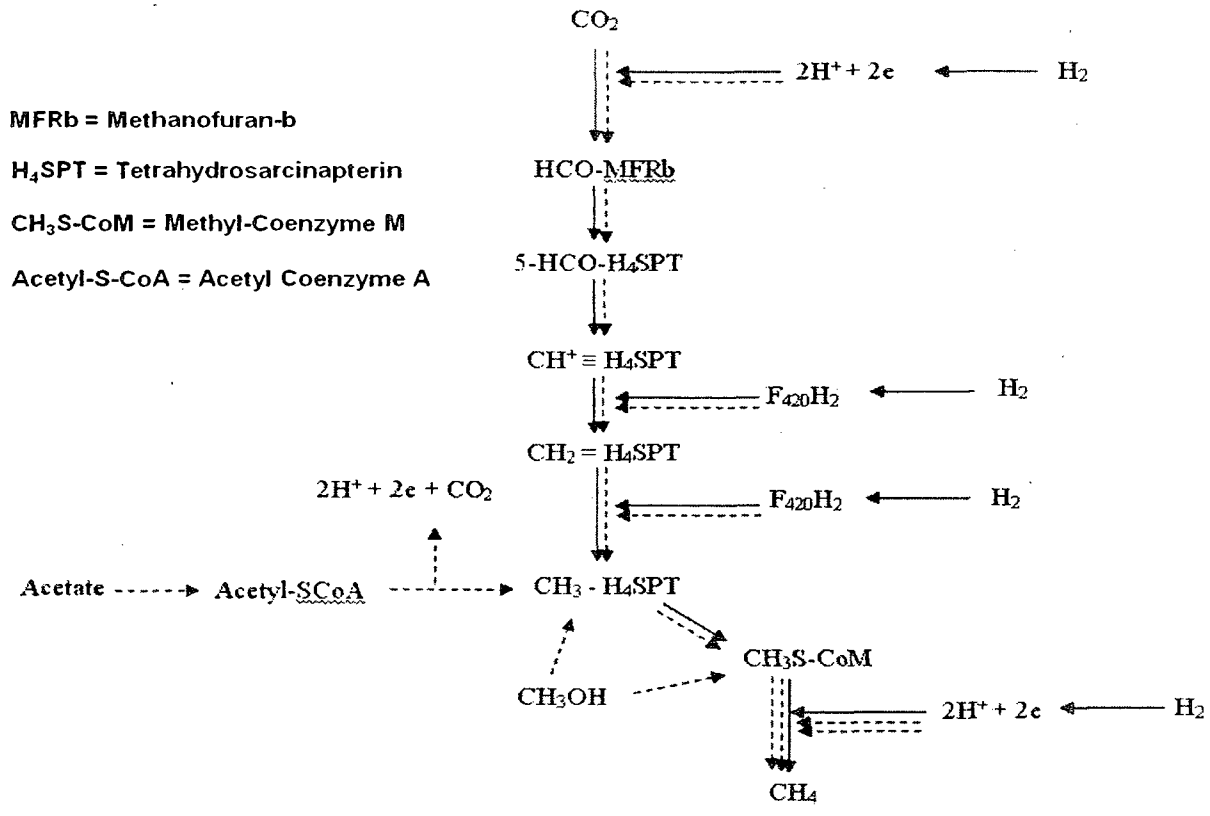


Figure 2.9 Methanogenic pathways from H_2 and CO_2, acetate and methanol including the intermediate products and coenzymes. (Reproduced from Ferry 1993).

Many H_2-using methanogens also can use formate as an electron donor for the reduction of CO_2 to CH_4. Like H_2, formate may be an important substrate for methanogenesis even though its concentration in methanogenic environments is low, because it is rapidly produced and consumed (Boone et al. 1989, Hungate et al.1970, Thiele and Zeikus 1988). A limited number of methanogens can also utilize secondary alcohols for CO_2 reduction to methane, and an even smaller number can use some primary alcohols (Bleincher et al. 1989, Maestrojuan et al. 1990, Widdel 1986, Widdel et al. 1988, Zellner and Winter 1987a).

Methylotrophic pathways catabolize compounds that contain methyl groups, such as methanol (Schnellen, 1947), trimethylamine (Hippe et al. 1979), and dimethyl sulfate (Kiene et al. 1986, Mathrani et al. 1988, Oremland et al. 1989). Typically the methyl group is transferred to a methyl carrier (ultimately to coenzyme M) and reduced to methane. Electrons for methyl reduction may be obtained by oxidizing a fraction of methyl groups to CO_2 or by using H_2 as an electron donor. The most widespread catabolic reactions carried out by methanogens are shown in Table 2.4. As shown in this table, the hydrogentrophic reduction of CO_2 to CH_4 is the most favorable reaction of methanogens (with the maximum energy released).

Table 2.4 Energy-yielding reactions of methanogens. (Reproduced from Ferry 1993).

Reaction	$\Delta G°$(kJ/mol CH_4)
$4\ H_2 + CO_2 \longrightarrow CH_4 + 2\ H_2O$	-130.4
$CH_3OH + H_2 \longrightarrow CH_4 + H_2O$	-112.5
$4\ CH_3OH \longrightarrow 3\ CH_4 + CO_2 + 2\ H_2O$	-106
$4\ CH_3NH_2 + 2H_2O \longrightarrow 3\ CH_4 + CO_2 + 4\ NH_3$	-76.7
$4\ (CH_3)_3N + 6H_2O \longrightarrow 9\ CH_4 + 3\ CO_2 + 4\ NH_3$	-75.8
$2\ (CH_3)_2NH + 2H_2O \longrightarrow 3\ CH_4 + CO_2 + 2\ NH_3$	-74.8
$(CH_3)SH + H_2 \longrightarrow CH_4 + H_2S$	-69.3
$2(CH_3)_2S + 2H_2O \longrightarrow 3\ CH_4 + CO_2 + 2\ H_2S$	-52.1
$4(CH_3)SH + 2H_2O \longrightarrow 3\ CH_4 + CO_2 + 4\ H_2S$	-51.0
$CH3COO^- + H^+ \longrightarrow CH_4 + CO_2$	-36.0
$2\ Butyrate^- + HCO_3^- + H_2O \longrightarrow 4\ Acetate^- + CH_4 + H^+$	-39.4
$4\ Propionate^- + 3H_2O \longrightarrow 4\ Acetate^- + HCO_3^- + H^+ + 3\ CH_4$	-34.0

Bioconversion of CO_2 in the waste stream from EOR process to methane has been the main objective of this work which was investigated using direct hydrogen gas and other alternative materials. On the other

hand, biodegradation of dissolved petroleum hydrocarbons can occur under anaerobic conditions. This novel approach is still under development and could eventually include other steps to meet some of the criteria such as hydrocarbon removal, methane collection etc. In this method, the input oilfield stream enters an anaerobic system where most of the organic material is removed along with the majority of the dissolved gases such as oxygen and carbon dioxide. Off-gas methane is collected.

Depending on the treatment objectives, the brine can undergo further aerobic polishing for removal of the remaining organic material prior to entering the additional treatment systems such as reverse osmosis or ion exchange processes.

This process is under consideration because it has many benefits which are mainly as follows:

1- It is a simple and easily applicable method which can be applied either as a pretreatment or as a main treatment process.
2- It needs low energy requirements compared to aerobic treatment and no need to use special solvents as in chemical processes.
3- In this method, co-treatment of CO_2 and dissolved hydrocarbons is possible.
4- Application of this method involves production of methane as a clean source of energy.

As mentioned by Lee et al. (2002), for a detailed study, this process may require additional laboratory and bench scale investigation.

Chapter 3: Materials and Methods

3.1 Materials

The materials used in this work can be divided into the inoculum, basic elements and nutrients, mineral solution, test materials, crude oil and gases. The composition of the solutions were chosen based the recommended media for anaerobic treatment (Atlas 1997) and it was considered to be a suitable growth medium for a mixed-culture biomass with more focus on methanogenic bacteria.

3.1.1 Inoculum

The granulated biomass used in the system was collected from the upflow anaerobic sludge blanket (UASB) reactor in Agropur at a cheese factory in Notre Dame du Bon Conseil, Quebec. Before using the biomass, it was first kept in the incubator at 35°C for a period of two weeks for temperature acclimation. Figure 3.1 shows a microscopic image of this biomass taken by the laboratory microscope (WILD Heerbrugg, model Wild M5A).

3.1.2 Basic Elements and Nutrients

Basic components and nutrients necessary for cell growth considered in this work, with their concentrations are shown in Table 3.1.

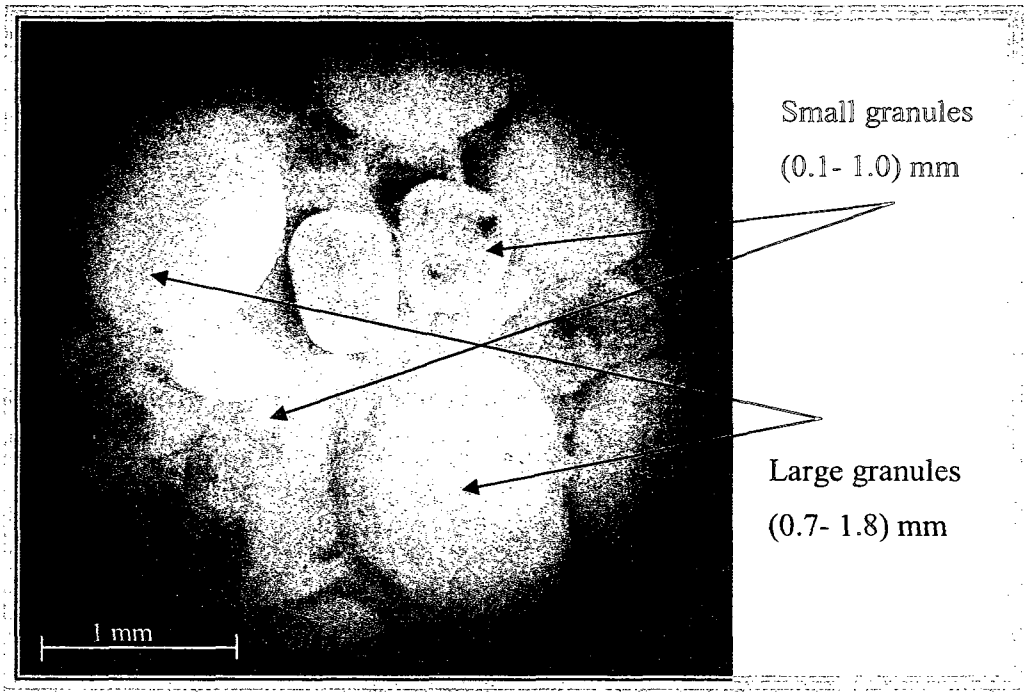


Figure 3.1 Microscopic image (25X magnification) of the mixed culture cheese whey biomass used in this work.

Table 3.1 Composition of the basic elements and nutrients

Component	Purity (%)	Concentration (g/L)
NaCl	99.7	1.0
KCl	99.0	0.3
KH_2PO_4	100.0	0.2
$MgCl_2.6H_2O$	99.8	0.5
$CaCl_2.2H_2O$	98.6	0.2
NH_4Cl	99.5	1.0

3.1.3 Trace Mineral Solution

A stock solution of trace inorganic elements was made and each time, 10 ml of this solution was added to 1L of the solution in Table 3.1. Table 3.2 shows these compounds and their concentration. All

chemicals were supplied by Fisher Scientific Ltd. All chemicals were of either biological grade or certified ACS (American Chemical Society).

Table 3.2 Composition of the trace mineral solution

Minerals	Purity (%)	Concentration (g/L)
$MgSO_4.7H_2O$	99.9	2.0
$ZnSO_4.7H_2O$	99-108.7	0.18
$MnCl_2$	98-101	0.1
$CoCl_2$	97.0	0.05
$CaCl_2.2H_2O$	98.6	0.1
$CuSO_4$	99.0	0.01
$ZnCl_2$	97-100.5	0.1
NaCl	99.7	1.0
$NiCl_2.6H_2O$	99.88	0.02
$FeSO_4.7H_2O$	100.0	1.34
$KAl(SO_4)_2.12H_2O$	100.0	0.02
H_3BO_3	99.8	0.01

3.1.4 Test Materials

In this study, a number of materials were selected to be used in the anaerobic systems as alternatives to hydrogen gas. The selection was based on either their ability to generate hydrogen in a reductive environment or their utilization by methanogenic bacteria as alternative electron donors for CO_2 reduction. Biological hydrogen production has been known over a century and a review of different approaches and critical limiting factors is given by Hallenbeck and Benemann (2002). Application of various materials for anaerobic hydrogen production has been the subject of much research and a review was done by Nandi and Sengupta (1998).

The test materials with their individual concentrations are shown in Table 3.3. These materials are the typical intermediate or final products of acidogenesis or hydrolysis steps in an anaerobic process and their application in this work was investigated.

Table 3.3 Test materials used in the batch experiments

Component	Molecular Formula	Purity (%)	Concentration (g/L)
Formic acid	CH_2O_2	88-99	2.0
Butyric acid	$C_4H_8O_2$	100	2.0
Lactic acid	$C_3H_6O_3$	>98	6.0
*HRC	*NA	NA	2.0
Galactose	$C_6H_{12}O_6$	100	2.0
Glucose	$C_6H_{12}O_6$	>99	2.0
Sucrose	$C_{12}H_{22}O_{11}$	100	1.2

* HRC (Hydrogen Release Compound). NA = Not available

3.1.4.1 Formic, Acetic, Butyric and Lactic Acids

These organic acids were selected as representative of low to intermediate fatty acids which in a typical anaerobic system are generated by acidogenic bacteria from sugars, amino acids and fatty acids. Formic acid is the simplest fatty acid (HCOOH). Many hydrogentrophic methanogens are capable of using formic acid as a substrate (Ferry 1993) and its metabolism has been studied in the past (Schauer et al. 1982). Formate can serve as an electron donor instead of hydrogen for the reduction of carbon dioxide. Also, most nonphotosynthetic anaerobic bacteria can produce hydrogen from formic acid (Nandi and Sengupta 1998).

Application of acetic acid and some other light fatty acids in methanogenic reduction of CO_2 have been investigated in a series of batch and continuous experiments (Alimahmoodi and Mulligan 2008). It was

shown that under certain conditions in an anaerobic system with acetic acid/sodium acetate at mesophilic range of temperature (about 35°C), CO_2 removal and CH_4 production can be improved.

Butyric acid ($C_4H_8O_2$) is an intermediate fatty acid in an anaerobic system and lactic acid ($C_3H_6O_2$) is an intermediate product of fermentation of sugars such as glucose and can release hydrogen upon fermentation. Both butyric and lactic acid have been used as electron donors in many studies. As an example, the effect of butyric and lactic acids as electron donors for reductive dechlorination of tetrachloroethane (TCE) has been investigated (Fennell et al. 1997).

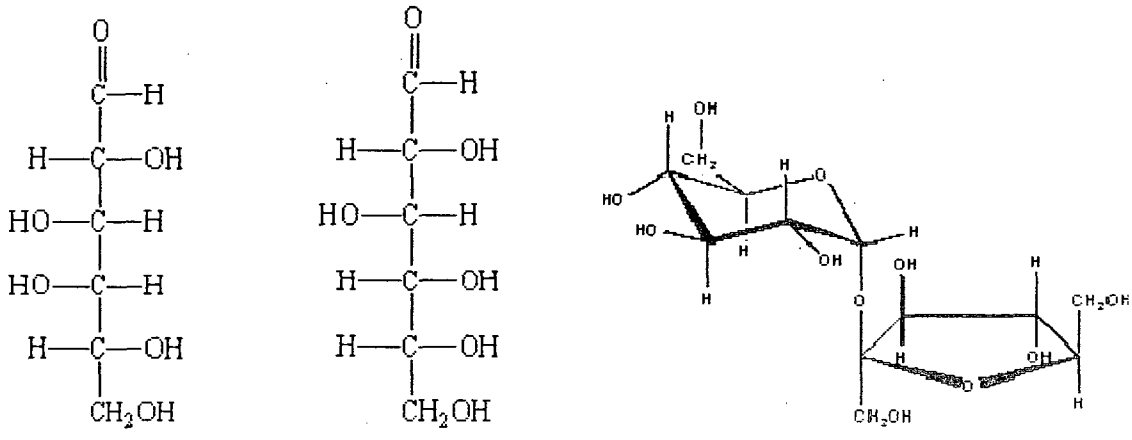
It has been reported that under anaerobic conditions, hydrogen atoms in lactic acid can be converted to hydrogen (Smith et al. 2003). Another reason for using lactic acid in this study was the use of a commercial substance called hydrogen release compound (HRC) that allegedly produces hydrogen under anaerobic conditions after its conversion to lactic acid. This is discussed in the next section.

3.1.4.2 Hydrogen Release Compound (HRC)

HRC is a proprietary, food grade, polylactate ester (glycerol tripolylactate and glycerol) that is produced by Regenesis Ltd. for anaerobic bioremediation of chlorinated aliphatic hydrocarbons (CAHs). It is a highly viscous and flowable liquid that is formulated for slow release of lactic acid upon hydration which is a multi-step process. Anaerobic microbes degrade lactic acid and release hydrogen which is used in reductive dehalogenation of CAHs (Koenigsberg and Norris 1999). The use of this material for bioremediation of contaminated sites has been investigated in several works and about 410 applications of this material have been reported (Environmental Security Technology, 2002). Samples of this material were obtained from Regenesis Bioremediation Products (San Clemente CA, USA) to investigate its application in this work.

3.1.4.3 Galactose, Glucose and Sucrose

The effect of adding monosaccharides and disaccharides was investigated using D-galactose and D-glucose as monosaccharides and sucrose as a disaccharide. Applications of saccharides as electron donors to anaerobic nitrogen transformations and denitrification (Wang et al. 2007) and on biological sulfate reduction (Liamleam and Annachhatre 2007) have been investigated. Ogino et al. (2005) have studied hydrogen production from glucose using several strict and facultative anaerobes. Chang and Lin (2004) have studied the conversion of sucrose to hydrogen in an upflow anaerobic sludge blanket (UASB) reactor.



(a) D-Galactose (b) D-Glucose (c) Sucrose

Figure 3.2 Structural formula for sugars used.

In another study by Woodward et al. (2002), use of several sugars including monosaccharides such as galactose and glucose and disaccharides such as sucrose for anaerobic hydrogen production have been investigated. Recently, H_2 production from glucose by mixed anaerobic cultures at various temperatures in the mesophilic range has been studied (Yang et al. 2006) and Chena et al. (2006) have investigated the kinetics of biological H_2 production by anaerobic fermentation of sucrose. Therefore, based on the ability of these materials to release hydrogen under anaerobic conditions, they were used in this work to investigate their effect on the treatment process.

3.1.5 Crude Oil

Crude oil used in the experiments was supplied from the PetroCanada Refinery in Montreal. It is categorized under CAS number 8002-05-9. Some of the properties of this crude oil are listed in Table 3.4 with the typical percentages of the different cuts for this category of crude oil. This is a light crude oil which compared to the heavy crude oil, contains a higher percentage of light cuts and therefore has more soluble components in water.

3.1.6 Gases

Carbon Dioxide

A carbon dioxide gas tank with industrial purity of 99% purchased from Praxair Inc. was used as a source of CO_2 gas. The tank pressure was reduced from 0-8000 kPa to 0-400 kPa with a gas regulator to use low gas flow rates.

Table 3.4 General properties of the crude oil used in this work

Property	Description/Value
Physical State	Viscous liquid
Color	Dark brown
Boiling Point	Varies with crude sources
Density	0.7-1.1 (Water = 1)
Reid Vapor Pressure	19 kPa
Flash Point	below -20 °C
Aromatic	9-50 (%vol)
Naphthenes	18-54 (%vol)
Paraffins	37-80 (%vol)

Nitrogen

Nitrogen was used to strip out the free chlorine from the tap water used for the solutions. An industrial grade nitrogen gas tank purchased from Praxair Inc. was used as the source of N_2. Nitrogen pressure was reduced from 0-12000 kPa to 0-400 kPa with a gas regulator.

Hydrogen

Hydrogen gas was used in a batch test as a control for direct application of hydrogen gas. As is shown in equation (3-1), the anaerobic reduction of CO_2 with hydrogen is highly favorable and can be done by most methanogens.

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \qquad \Delta G° = -130.4 \text{ kJ/mole } CH_4 \qquad (3\text{-}1)$$

An industrial grade hydrogen gas tank purchased from Praxair Inc. was used. Hydrogen pressure was reduced from 0-5000 kPa to 0-400 kPa with a gas regulator.

Helium

Helium was used in the gas chromatograph (GC) as a carrier gas. It was purchased from Praxair Inc. with the purity of grade 5 and pressure of 15000 kPa. The operating pressure was 600 kPa and the pressure was controlled by a regulator.

Argon

Argon was used in the gas chromatograph (GC) as a carrier gas for determination of hydrogen in gas samples. It was purchased from Praxair Inc. with the purity of grade 5 and pressure of 14000 kPa. The operating pressure was 600 kPa and the pressure was controlled by a regulator for argon.

Air

An air cylinder was connected to the GC to maintain a minimum flow through the GC column as the make-up gas. It was purchased from Praxair Inc. with the purity of 5 (very dry) and pressure of 17000 kPa which was reduced to 300 kPa by a regulator for air.

3.2 Analytical Methods

3.2.1 Chemical Oxygen Demand (COD) Test

Values of COD were measured according to a colorimetric method [Standard Method (1998) – Method 5220B] at 600 nm using a Perkin Elmer Lambda 40 UV/VIS spectrometer. In this method, a hot mixture of chromic and sulfuric acid is used to oxidize most types of organic matter. A sample is refluxed in a strongly acid solution containing an excess amount of potassium dichromate ($K_2Cr_2O_7$) through which Cr^{6+} is reduced to Cr^{3+}. The intensity of the green color of the chromium ion is measured against a known value in the standard calibration curve.

3.2.1.1 Solution Preparation

A standard potassium hydrogen phthalate (KHP) was prepared according to the following procedure:

1- Lightly crush 450 g of KHP ($HOOCC_6H_4COOK$).

2- Dry crushed KHP in the oven (Linderberg/Blue Gravimetric Oven) at 120°C to a constant weight.

3- Dissolve 425 mg of KHP in distilled water (made in the laboratory) and dilute the solution to 1000 ml. This solution has a theoretical COD of 500 mg O_2/L.

3.2.1.2 Test Procedure

In the standard COD test procedure, twist-cap vials purchased from Bioscience Inc. were used. This test is approved by the EPA as a micro-COD test (EPA Method 410.4). The total volume of the COD reagent is 5 ml. The procedure to prepare the standard curve is as follows:

1- Preheat a COD block heater (do not use oven) to 150°C.

2- Remove the cap from a COD twist-cap vial.

3- Carefully add 2.5 ml of sample down the side of the vial such that it forms a layer on top of the reagents.

4- Replace the twist cap.

5- Thoroughly mix the contents of the sealed vial by shaking.

6- Process standards and blanks exactly as the samples.

7- Place the twist-cap vial in a COD heater block capable of maintaining 150°± 2°C for 2 hours.

8- Remove the vial from the heater block and allow it to cool.

9- Allow any suspended precipitate to settle and wipe the outside of the twist cap clean.

10- Set the wavelength of the spectrophotometer to 600 nm, and, using a procedural blank, zero the absorbance reading.

11- Read the absorbance of each standard and sample on the spectrophotometer.

12- Prepare a graphic calibration curve by plotting the absorbance of the standards versus their known concentrations. Compare sample absorbance to the graphic calibration curve to determine COD concentration.

3.2.1.3 COD Standard Curve

The COD standard curve was prepared using standard solutions of the concentrated KHP solution (original concentration of 500 mg/L). Five concentrations of the KHP solution and two blanks as shown in Table 3.5 were prepared. COD values of these vials were measured according to the test procedure (Sec. 3.2.1) and the absorbance values were determined using ultraviolet spectroscopy. The data obtained showed a linear relationship between absorbance values and COD of the standard solutions in the range of 0-500 mg COD/L (Figure A.1 shows the reference curve of absorbance vs. COD values). The reactor samples were filtered through syringe filters (pore size of 0.45 μm purchased from Fisher Scientific Ltd.) and their concentration was measured using the value of absorbance read by the UV spectrometer and using the reference curve obtained.

Table 3.5 Standard sample concentrations for COD standard curve.

COD Value (mg/L)	Amount of KHP solution (ml)	Amount of Distilled Water (ml)
500	2.5	0
400	2.0	0.5
300	1.5	1.0
200	1.0	1.5
100	0.5	2.0
0 (Blank)	0	2.5

3.2.2 Alkalinity

Alkalinity (alk) refers to the capacity of water to neutralize acids. This parameter is determined by the abundance of four ions: carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), hydroxyl (OH^-), and hydrogen (H^+). For many engineering purposes, the alkalinity is defined as follows:

$$\text{Alk} = [OH^-] + [HCO_3^-] + 2[CO_3^{2-}] - [H^+] \qquad (3\text{-}2)$$

where the concentrations of ionic species are measured in mole per liter and alkalinity is expressed in equivalent per liter (Nazaroff and Alvarez-Cohen 2001).

The alkalinity was measured by titration according to the method No.2320 B (Standard Method, 1998). The indicator was Bromcresol green solution, which has a color change at pH 4.5. To prepare the solution, 100 mg dry Bromcresol green was dissolved in 100 ml distilled water. The standard used was sulfuric acid (0.02 N). Each ml of this acid is equivalent to a total alkalinity of 1 ppm $CaCO_3$. The end point for the titration test was determined based on a color change of the solution from blue to pale green (greenish yellow).

3.2.3 Dissolved Carbon Dioxide

The dissolved carbon dioxide concentration in the reactor liquid was measured based on the alkalinity (Alk) and pH values and using the equilibrium relationship among carbonate species. When carbon

dioxide is dissolved in water, it is converted to its dissolved form $CO_2(aq)$ or carbonic acid (a weak acid) and its conjugate base, bicarbonate (an even weaker acid). $CO_2(aq)$ then dissociates in water according to the following and the equilibrium reactions (at 25°C):

$$CO_2(aq) + H_2O \Leftrightarrow H^+ + HCO_3^- \qquad K_1 = \frac{[H^+][HCO_3^-]}{[CO_2(aq)]} = 4.47\times10^{-7} M \qquad (3\text{-}3)$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \qquad K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} = 4.68\times10^{-11} M \qquad (3\text{-}4)$$

By combining two equations,

$$K_1K_2 = \frac{[H^+]^2[CO_3^{2-}]}{[CO_2(aq)]} = 2.1\times10^{-17} \qquad (3\text{-}5)$$

By definition of pH, $[H^+]$ can be calculated as:

$$[H^+] = 10^{-pH} \qquad (3\text{-}6)$$

By measuring the alkalinity and calculating the concentration of $[OH^-]$ from the following equation for water dissociation,

$$K_w = [H^+][OH^-] \qquad (3\text{-}7)$$

the concentrations of species CO_3^{2-} and HCO_3^- were calculated from equations 3-2, 3-4 and 3-6. Then expression 3-5 was used to determine the concentration of dissolved CO_2. All calculations were programmed into Excel with pH, alkalinity and temperature as the input data. Table 3.6 shows a sample of output results for the computer program in Excel with the values of input parameters.

Table 3.6 Sample calculation of dissolved carbon dioxide concentration.

Input Data	
Parameter	**Value**
$T\,(^{\circ}C)$	35
pH	7
$Alk\ (\frac{g}{L}CaCO_3)$	1
Output (Calculated) Values	
Parameter	**Value**
$T\ (K)$	298.15
$*\ pK_1$	6.351
$*\ pK_2$	10.329
K_1	4.46 E-07
K_2	4.69 E-11
$[HCO_3^-]$(M)	9.99 E-3
$[CO_3^{2-}]$(M)	4.68 E-6
$[CO_2](\frac{g}{L})$	9.86 E-2
$[CO_2](\frac{L}{L(H_2O)})$	5.48 E-2

* pK = -Log K. Correlations for values of pK at different temperatures are from Lide (2003).

3.2.4 Total Petroleum Hydrocarbon (TPH) Concentration

The concentration of dissolved petroleum hydrocarbons was determined using High Pressure Liquid Chromatography (HPLC). In order to select the proper wavelength, an Ultraviolet (UV) spectrometry was performed to scan a sample of the waste stream.

3.2.4.1 UV Scan Method

A sample of contaminated water was scanned according to Standard Method APHA 5910, using A UV spectrometer (Lambda 40) with UV-Visible light and a wavelength range of 190-400 nm and the resulting spectrum is shown in Figure A.2.

3.2.4.2 HPLC Analysis

Based on the scan results, the UV detection wavelength for HPLC analysis was selected as 210 nm where the maximum absorbance is about 1 (0.999 according to Standard Method 5910). Samples of treated wastewater were filtered with 0.45 μm syringe filters (purchased from Fisher Scientific Ltd.) and were tested by HPLC analyzer (Beckman Coulter Inc.) under the conditions shown in Table 3.7. HPLC spectra were analyzed using the software (32 Karat Software licensed by Beckman Coulter Inc.) installed on the HPLC. TPH concentrations were compared based on the total area under the peaks and the results were compared with that of untreated wastewater and a control containing none of the test materials.

Table 3.7 Operating conditions for measuring TPH with the HPLC

Parameter	Setting
Column	SUPELCOSIL(58318) 4.5× 15 cm
Solvent type	Acetonitrile/water (60%/40%)
Solvent flow-rate (ml/min)	1.0
Run time (min)	5-10
Column temperature (°C)	35
Injection volume (μL)	10
UV detection wavelength (nm)	210

Figures 3.3 and 3-4 show the typical chromatograms for untreated and treated samples of the synthetic wastewater. The total area under the peaks was determined by the instrument and was considered as the concentration of total dissolved petroleum hydrocarbons (TPH). The peaks for the aromatic compounds such as benzene, toluene and mixed xylene were identified and shown. The concentration of each compound was determined using a reference curve made based on known concentrations.

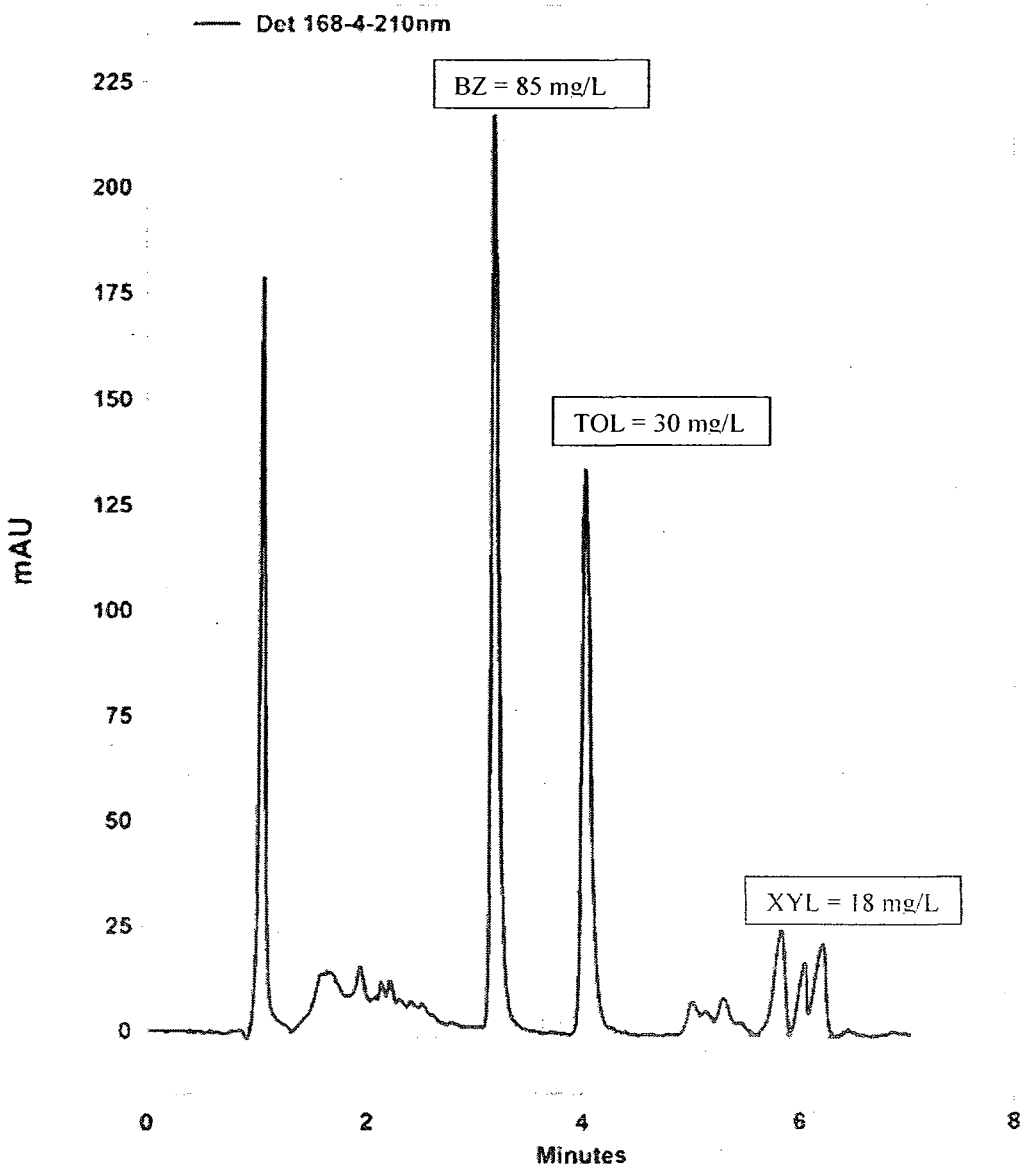


Figure 3.3 Chromatograms of HPLC analysis of TPH for a sample of untreated wastewater. (BZ = Benzene, TOL = toluene, XYL = xylene, UV detector, 210 nm).

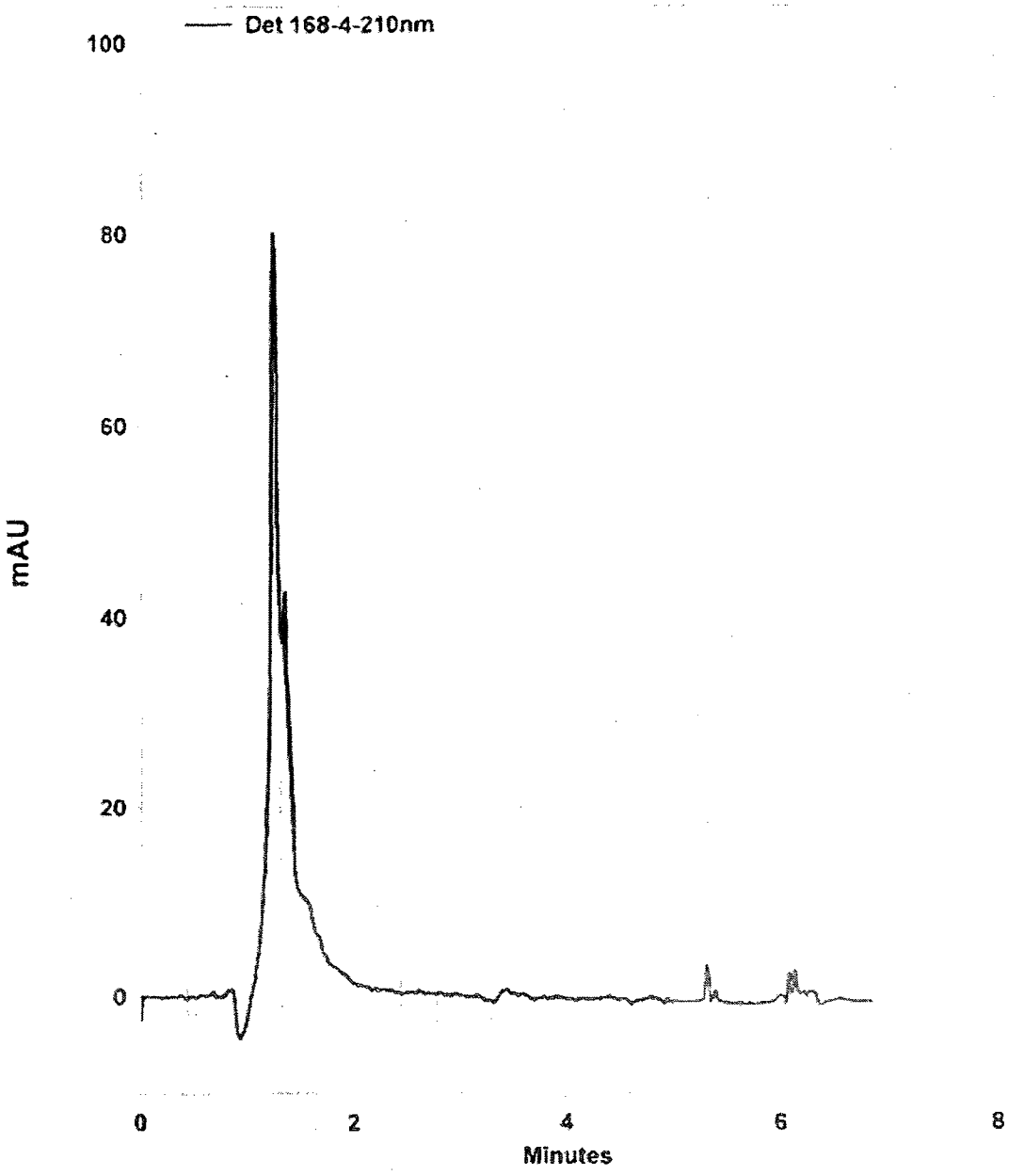


Figure 3.4 Spectrum of HPLC analysis of TPH for a sample of treated wastewater. (UV detector at wavelength of 210 nm).

3.2.5 Purity (Methane Content) of the Biogas

Gas samples were analyzed using the gas chromatograph (GC). The samples were injected into a Varian type 1041 on-column injector, fitted with a *Valco instruments Co. Inc.* (VICI) pressurized valve delivery system as a 0.2 ml sample plug. The operating conditions and column specification are shown in Table 3.8.

Table 3.8 Test parameters and conditions for gas chromatography.

Parameter	Setting or type
Column (30mm×0.53mm)	CARBOXEN 1010 PLOT (Capillary Column) from SUPELCO
Carrier gas	Helium/Argon
Detector	TCD
Sample Delivery	VICI Pressurized Valve System
Injector	1041 On-column
Injector temperature (°C)	225
Column oven temperature (°C)	50-100 (5°C/min)
Injection flow (ml/min)	5
Gas retention time (min)	15

The resulting GC spectrum consisted of two peaks (Figure A-3): The first peak was that of CH_4 with an average retention time of 7.5 minutes and the second peak was that of CO_2 with an average retention time of 13 minutes. To measure the methane content of the biogas using the GC analyzer, a reference curve was made. For this purpose, samples containing known compositions of pure carbon dioxide and methane were injected to the GC spectrometer and the peak ratio of CH_4/CO_2 for the resulted spectrum was measured and plotted against the methane content. This reference curve is shown in Figure A.4.

3.2.6 Measuring the Volume of the Biogas

The total volume of the biogas for each batch was measured by collecting the biogas in a Tedlar plastic bag purchased from Fisher Scientific Ltd. and using a water displacement method. The schematic of the setup used for this purpose is shown in Figure 3.5. The water was acidified using 1N sulfuric acid to prevent any dissolution of biogas in water. The volume of water collected was measured using a graduated cylinder and it was reported as the biogas volume. The volume of methane was calculated by multiplying the biogas volume and the biogas purity.

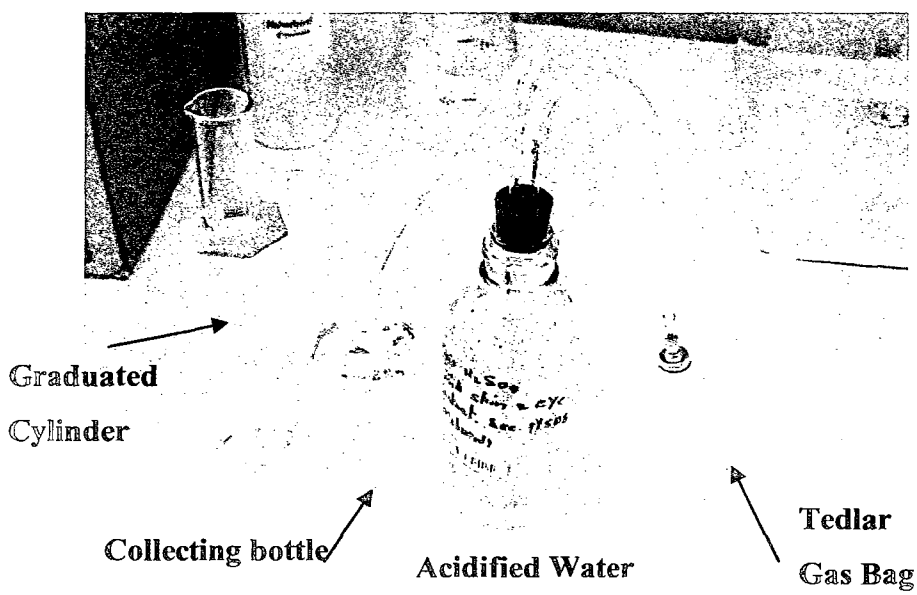


Figure 3.5 Setup used to measure the volume of biogas

For each gram of chemical oxygen demand (COD) converted, a theoretical value of 0.35 L methane is expected at standard temperature (0°C) and pressure (101.3 kPa). This value was calculated for the actual test conditions. Because all gas samples were collected under atmospheric pressure and temperatures around 35°C, only a correction for temperature was needed for the theoretical value. Assuming methane as an ideal gas, this correction can be done according the following T-V equation:

$$\frac{V_s}{T_s} = \frac{V_a}{T_a} \qquad (3\text{-}8)$$

where V and T denote volume and temperature and indices "s" and "a" refer to "standard "and "actual" conditions respectively. So, for 35°C, the actual volume for each gram of COD can be calculated as:

$$V_a = V_s(\frac{T_a}{T_s}) = 0.35(\frac{273+35}{273+0}) = 0.395L \qquad (3\text{-}9)$$

3.2.7 Volatile Suspended Solids (VSS) and Total Suspended Solids (TSS)

TSS is a measure of the total suspended solids in water, both organic and inorganic, and VSS is the organic portion of the TSS that is lost after ignition. Both are expressed in mass per volume as g/L or mg/L in this work. These tests were done according to the Standard Method (Clesceri et al. 1998), to measure the concentration of the sludge in the reactor and effluent. The test procedure was as follows:

1- Pre-dry a gooch crucible with Whatman GF/C filter paper in a Fisher Scientific Isotemp muffle furnace at 550°C± 2°C for 1 hour and allow it to cool down in a desiccator (Sanpla Dry Keeper, automatic dehumidifying desiccator) for 30 min. Then weigh it immediately and record it as weight "A".

2- Take a sample from the biomass and transfer it to the gooch crucible and filter it by vacuum filtration.

3- Put the gooch crucible in the oven (Linderberg/Blue Gravimetric Oven) and let it dry at 105°C ± 2°C for 1 hour and allow it to cool down in the desiccator for 10 min. Then weigh it and record it as weight "B".

The TSS can be determined using the following relationship:

$$TSS = \frac{A-B}{1(mL)} \times 1000 \ (\frac{g}{L}) \qquad (3\text{-}7)$$

4- Put the gooch crucible in the muffle furnace at 550°C ± 2°C for 2 hours, and then allow it to cool down in a desiccator for 30 min. Weigh the crucible and record it as weight "C".

5- Calculate the VSS from the following expression:

$$VSS = \frac{B-C}{1(mL)} \times 1000 \quad (\frac{g}{L}) \qquad (3\text{-}8)$$

The residual material in the gooch crucible, represents the ash content of the biomass, and can be calculated per mass of dry solids as follows:

$$Ash = 1 - \frac{VSS}{TSS} \quad (\frac{g}{g}) \qquad (3\text{-}9)$$

Three samples were taken each time to measure TSS and VSS concentrations.

3.3 Experimental Approach

3.3.1 Experimental Setup for the Synthetic Waste Stream

To simulate the conditions of CO_2 and water flooding in the enhanced oil recovery process, an experimental setup was used as shown in Figure 3.6. Tap water and the crude oil were mixed in 4:1 proportions using a magnetic stirrer and CO_2 was injected from the CO_2 cylinder during the mixing process to produce a mixture of dissolved petroleum hydrocarbons in water saturated with carbon dioxide (In a real process, the amount of water is much more than CO_2 and the ratio above was considered to be the worst case). The CO_2 exit tube was submerged in a bottle containing a 5 N KOH solution to absorb any CO_2 released from the system.

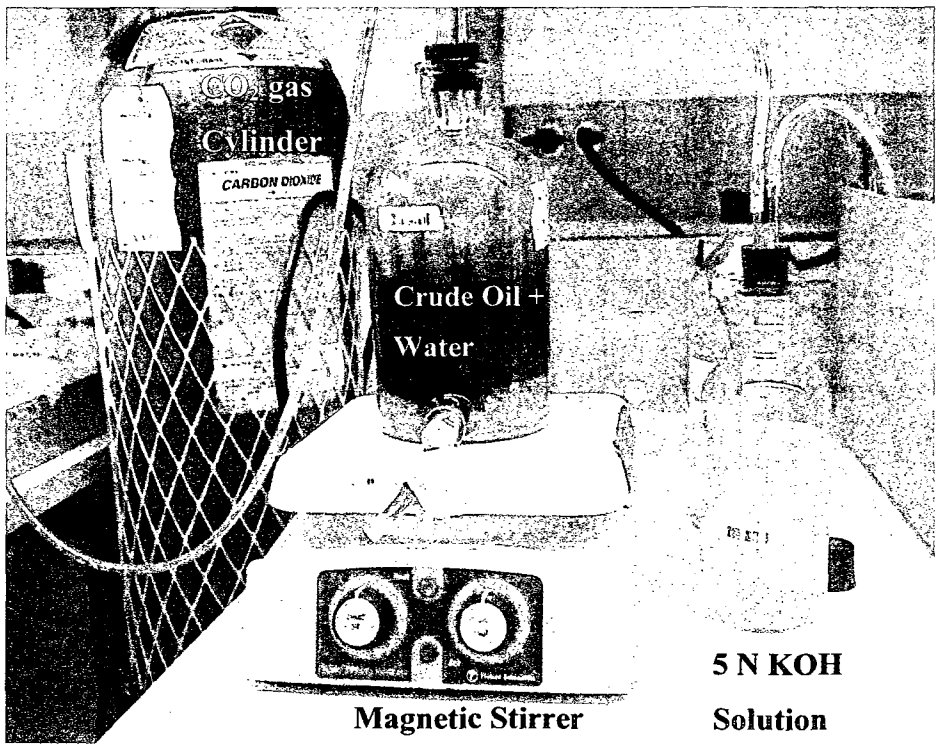


Figure 3.6 Experimental setup used to generate the synthetic waste water

The basic materials and nutrients along with the trace element solutions were added to this mixture to prepare a stock solution which was the base solution for all experiments.

3.3.2 Batch Experiments

3.3.2.1 Batch Reactors

Experiments were performed in 9 batches of glass containers. The graduated Wheaton bottles each of 1 L total volume purchased from Fisher Scientific Ltd. were used in this work. Each bottle was equipped with a flexible cap with a rubber septum as shown in Figure 3.7. This cap and septum enabled the bottle to be sealed and preserve the produced biogas. Also, it was possible to take gas and liquid samples by using syringes without opening the cap.

Figure 3.7 Wheaton graduated glass container with rubber septum cap used for the batch tests

3.3.2.2 Operation and Sampling

For each batch, the corresponding test material was added to the waste stream generated in section 3.3.1 with the concentration shown in Table 3.3. The working volume of each batch and initial biomass concentration were 500 ml and 25.0 gVSS/L, respectively. The headspace of each batch was purged with nitrogen gas for 5 minutes. Then the batches were stored in the incubator at 35°C.

The bottles and all other vessels and accessories were kept clean and isolated throughout the experiments. A 10 ml sterile plastic syringe purchased from Pharmaprix and a 10 ml gas-tight glass syringe purchased from Fisher Scientific Ltd. were used to take liquid and gas samples respectively.

Every day, liquid and gas samples were taken from each batch. GC and HPLC analyses were performed for gas and liquid samples respectively to determine the methane content of the biogas and TPH concentration in the liquid phase. Also, for the liquid samples, pH and alkalinity were measured to calculate the dissolved carbon dioxide and COD was determined using the method described in sections 3.2.1 and 3.2.3.

3.3.3 Two-Step Reactor System

Based on the results of the first part of this work, the materials were selected to be used in the second part. A two-step reactor system was used in this step. The main apparatus was the New Brunswick Scientific Bioflow 2.0 L Fermenter. The device has a number of measuring and control devices to calibrate, adjust and monitor conditions such as pH, dissolved oxygen, agitation, temperature, nutrient feed and foam. A schematic of this system is shown in Figure 3.8. Figure 3.9 shows a photograph of this system with microscopic pictures of the anaerobic bacteria for each step.

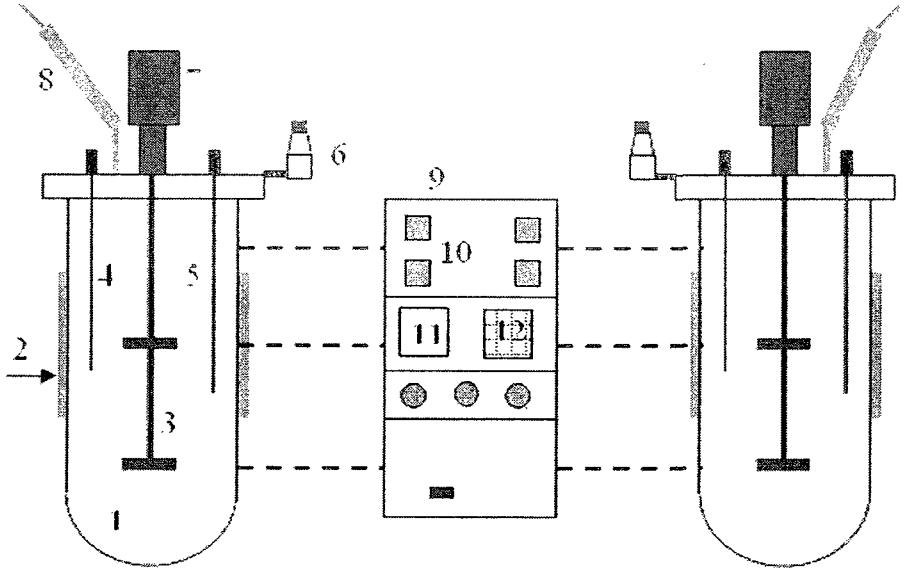


Figure 3.8 Schematic of the two-step reactor system: 1-Reactor, 2-Heating jacket, 3-Agitator, 4- pH probe, 5- DO probe, 6- pH solution, 7- Motor, 8- Condenser, 9-Control unit 10- Pumps, 11- Display, 12- Local panel

The motor was located directly on top of the reactor vessel. A sampling port was used for all samples taken during the reactor's operation. There were two addition pumps to control pH by adding acid/base solutions. Also, other pumps were available for addition of antifoam and nutrients. There were also pumps working with switches. The display panel was located above the power and agitation switches. The heating jacket located below the reactor vessel was used to keep the temperature constant. The dissolved oxygen probe located atop the reactor vessel was used to measure the dissolved oxygen in the vessel at all times. The condenser was used to condense the water vapor and return it to the reactor vessel for a constant-volume operation.

The initial temperature of the reactors was set at 35°C. In order to control the pH, a concentrated alkaline solution (2N NaOH) and an acid solution (85% phosphoric acid) were used. The reactors were loaded initially with 50% (volume) of the sludge. The mixer was used intermittently at the minimum speed of 50 rpm because in an anaerobic system, the gas production and movement through the medium also helps the agitation.

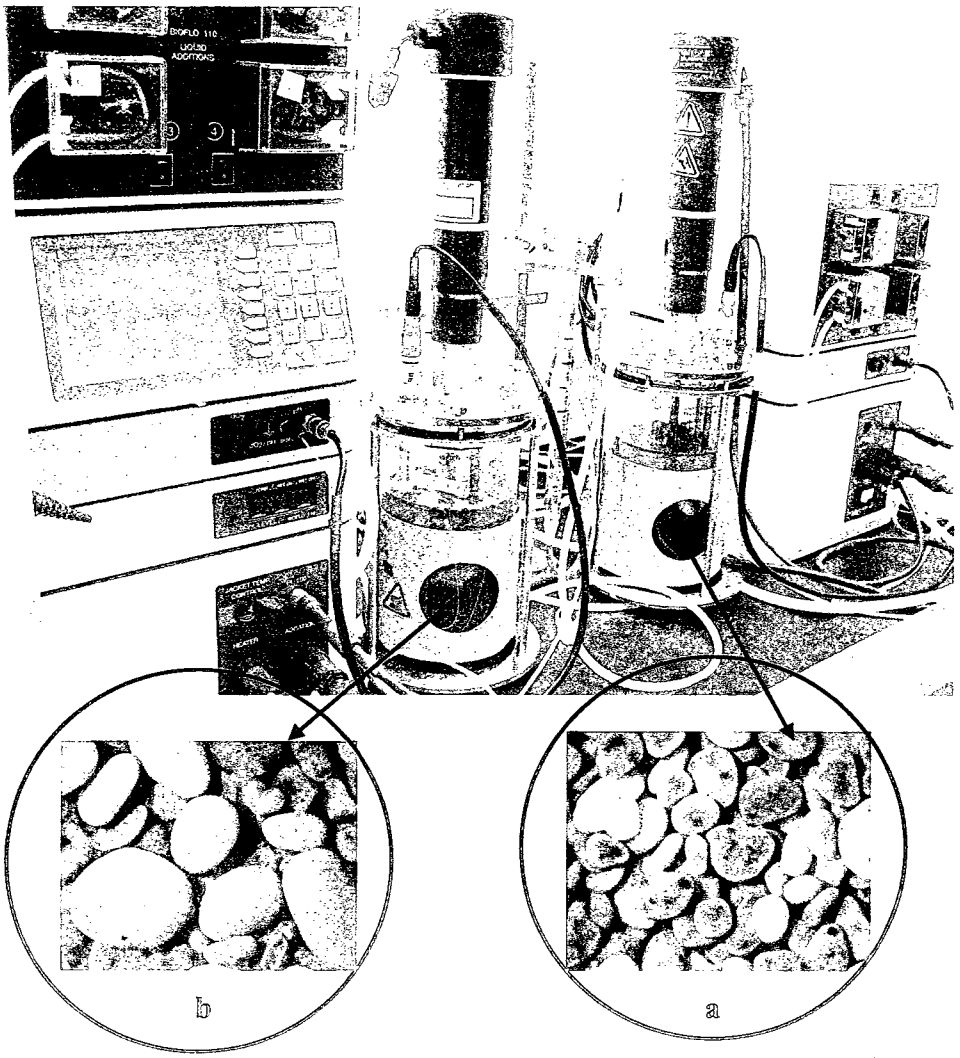


Figure 3.9 Photograph of the two-step reactor system with microscopic pictures of microcolonies of the bacteria in each step: (a) reactor 1 for methanogenic removal of CO_2 and (b) reactor 2 for removal of TPH.

Chapter 4: Results and Discussion

In this chapter the results from the batch experiments and the continuous operations are provided and discussed. Based on the sampling schedules for both steps, the measurements and calculations were done according to the standard methods and procedures described in Chapter 3. In the batch experiments, the results of the first part are discussed and the best materials were selected to be used in the second part. Then, in the second part or continuous operation, the application of these materials for CO_2 and TPH removal was investigated.

4.1 Batch Experiments

4.1.1 Carbon Dioxide Reduction

The concentrations of dissolved carbon dioxide for the batch tests with different materials and those of the blank and the batch with direct hydrogen gas were calculated based on daily sampling and measurements and the results are shown in Figures 4.1 to 4.4. All the results showed decreasing trends for dissolved CO_2 and more reduction was observed for the batches containing fatty acids compared to those with the complex materials such as sugars. The best results were observed for formic acid which can be due to its utilization by hydrogentrophic methanogens as an alternative for hydrogen.

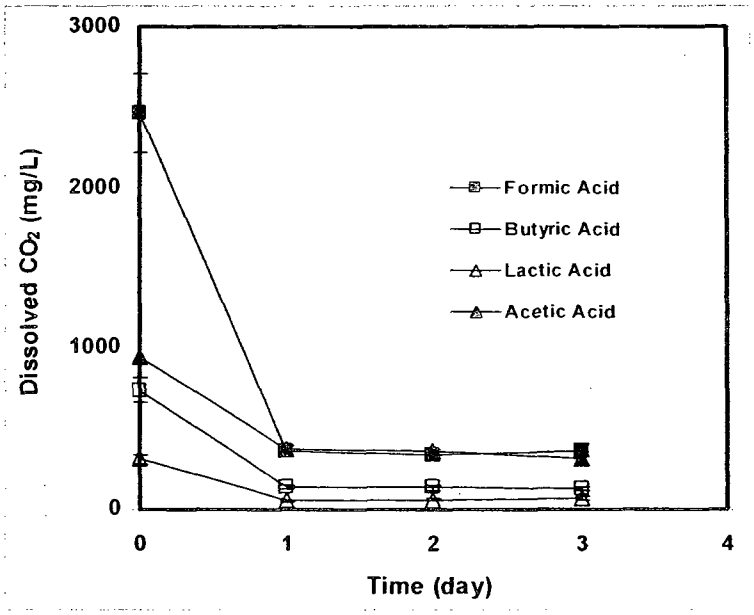


Figure 4.1 Trends of dissolved carbon dioxide for the batch tests with light- to medium-molecular weight fatty acids.

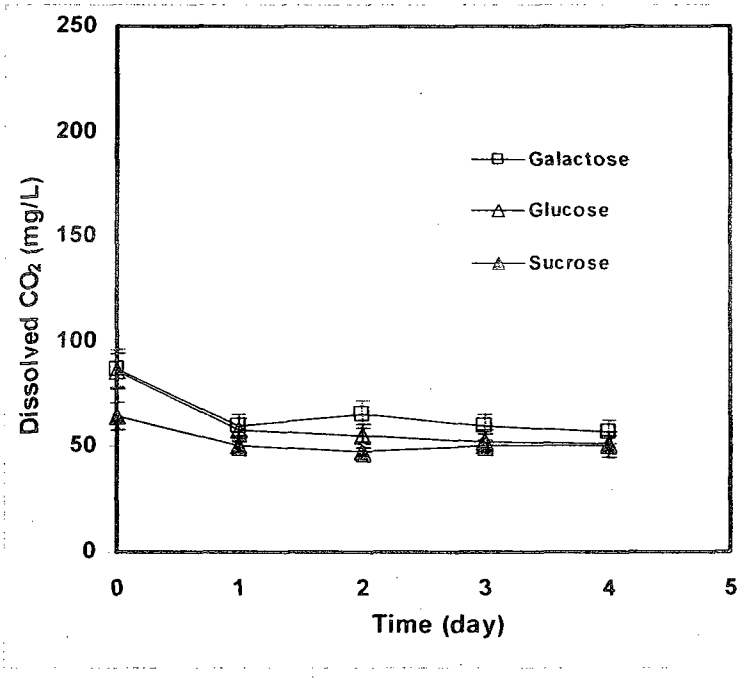


Figure 4.2 Trends of dissolved carbon dioxide for the batch tests with mono- and disaccharides.

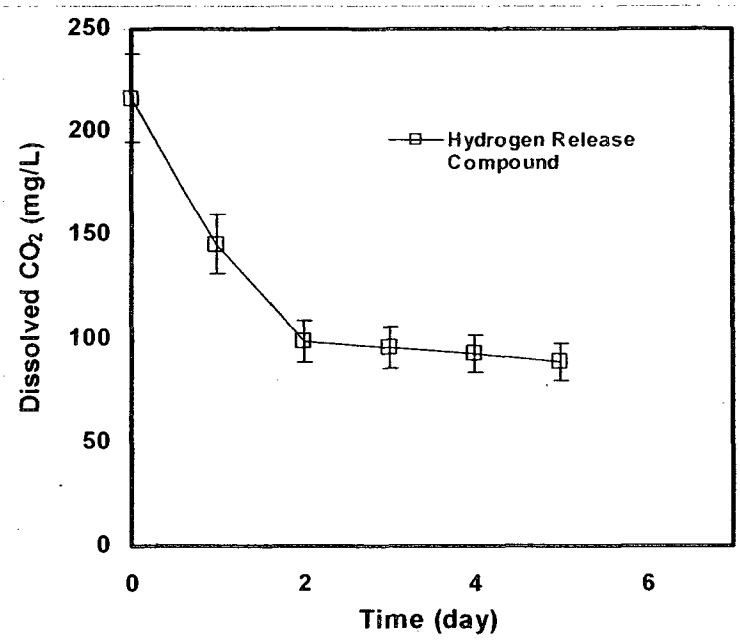


Figure 4.3 Trends of dissolved carbon dioxide for the batch experiments with HRC.

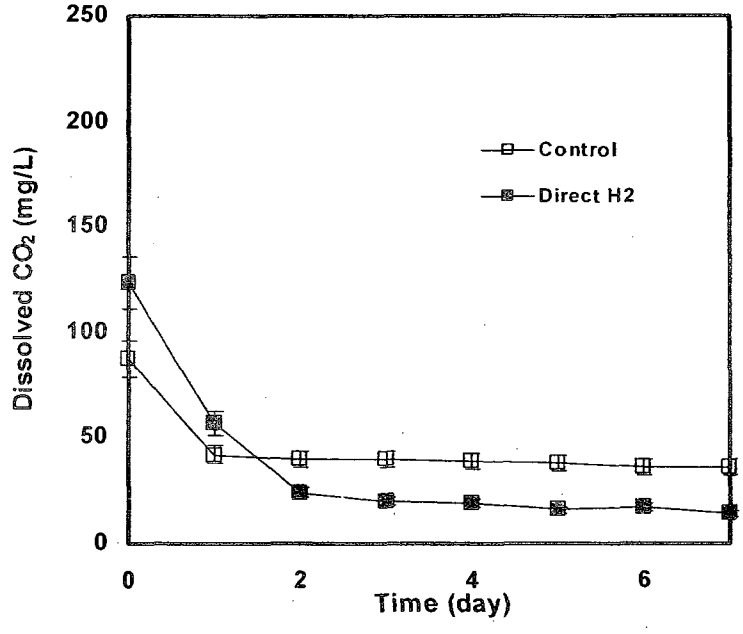


Figure 4.4 Trends of dissolved carbon dioxide for the batch experiments with pure hydrogen and the control.

The initial and final (in the effluent after the retention time) values of dissolved CO_2 were determined and the results in terms of reduction percentage are shown in Table 4.1.

Table 4.1 Percent dissolved CO_2 reduction for the batch tests with the light crude oil

Material	Carbon Dioxide Concentration (g/L)		$(CO_2)_r$ (%)
	$(CO_2)_i$	$(CO_2)_f$	
Formic Acid	2.43	0.37	85.1
Acetic Acid	0.94	0.32	66.1
Butyric Acid	0.74	0.13	82.2
Lactic Acid	0.31	0.08	75.3
HRC	0.22	0.09	58.9
Galactose	0.09	0.06	34.8
Glucose	0.09	0.05	39.7
Sucrose	0.06	0.05	20.0
Blank	0.09	0.04	58.6
Direct H_2	0.12	0.01	88.6

Indices: i = initial, f = final, r = removed

The results showed the light and medium fatty acids such as formic and butyric acids can be used as alternative sources of electron donors for CO_2 reduction. The best results for CO_2 reduction to methane were observed for the batch containing formic acid. As mentioned before, many H_2-using methanogens also can use formate as an electron donor for the reduction of CO_2 to CH_4. From the methanogenic pathways (Figure 2.9), it can also be seen that the production of CH_4 from H_2 and CO_2 involves the formation of the intermediate products such as formyl methanofuran (HCO-MFRb) and 5-

formyl-tetrahydrosarcinapterin (5-HCO-H_4SPT) (Ferry, 1993). Also, the influent acidic condition makes the hydrogen ion and dissolved CO_2 readily available for CO_2 reduction to methane which is highly favorable with the overall energy release of -131 kJ/mole (Table 2.4).

The result of the batch with acetic acid (about 66% of CO_2 removal with CH_4 purity of 67%) was not as observed in another experiment with CO_2 as the only contaminant to remove (85% of CO_2 removal with CH_4 purity of 78% - Alimahmoodi and Mulligan, 2008). This is despite the similarities between the methanogenic pathways from acetate and H_2 and CO_2. The reason could be the presence of dissolved oil hydrocarbons in this work. It has been investigated (Warren et al. 2003) that crude oil compounds have an inhibitory effect on the degradation of acetate. They also reported mild effects on methane production from formate and hydrogen.

As shown in Tables 2.4 and 2.5, the anaerobic degradation of medium molecular weight organic acids such as butyric and lactic acid yields acetate. To complete the degradation process, methanogenic bacteria will degrade the acetate and produce CH_4. This requires existence of a mixed-culture of acetogens and methanogens. Therefore the lack of enough methanogenic bacteria in the batches with butyric and lactic acids could be the reason for the lower CO_2 reduction to CH_4 in these batches. For more investigation on the methanogenic reactions, the results of CH_4 production, measured methanogenic activity and microbial observations are discussed in the following sections.

4.1.2 Methane Production

The result for methane concentration in the headspace for each batch is shown in Figures 4.5 through 4.8. The methane content of the headspace increased for each batch. The total volume of methane production was also determined from the amount of gas volume collected.

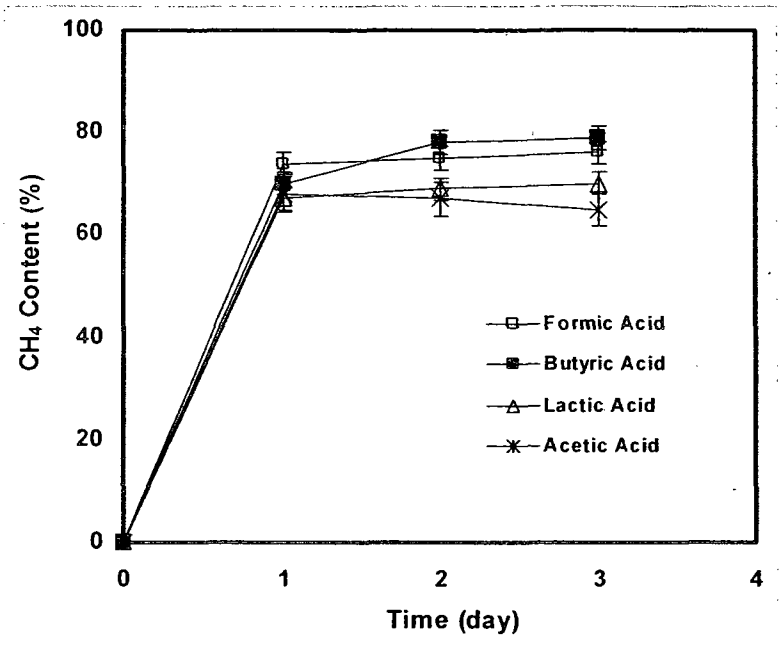


Figure 4.5 Trends of methane concentration in the headspace for the batch tests with light-to medium-molecular weight fatty acids.

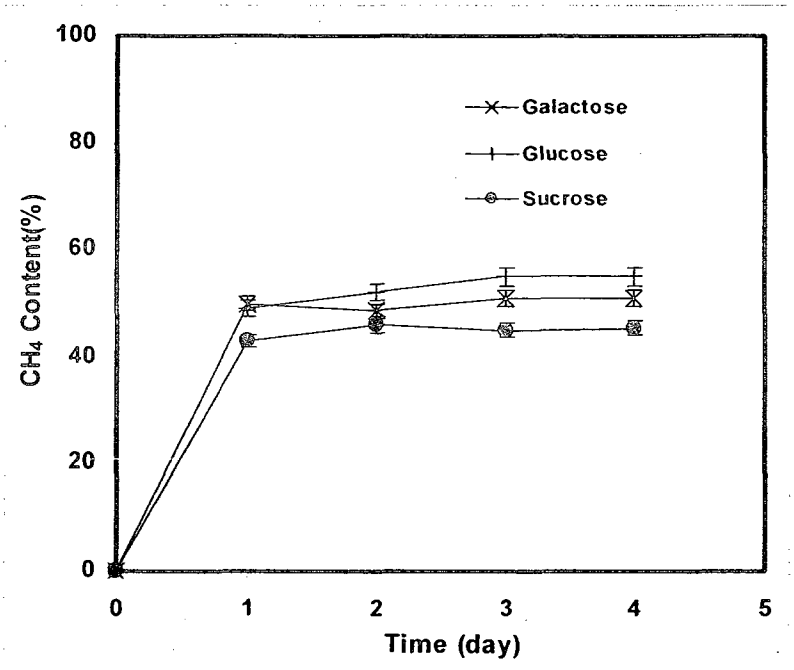


Figure 4.6 Trends of methane concentration in the headspace for the batch tests with mono- and disaccharides.

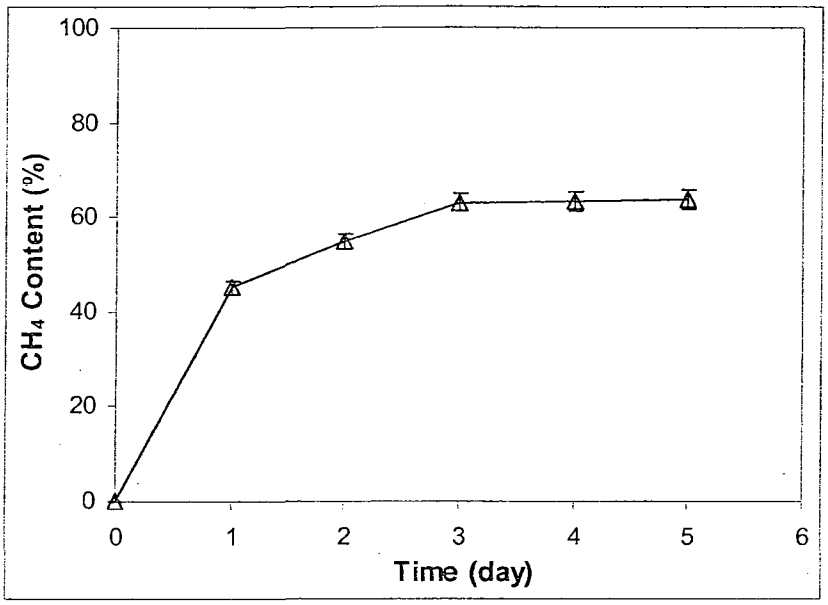


Figure 4.7 Trends of methane concentration in the headspace for the batch tests with HRC.

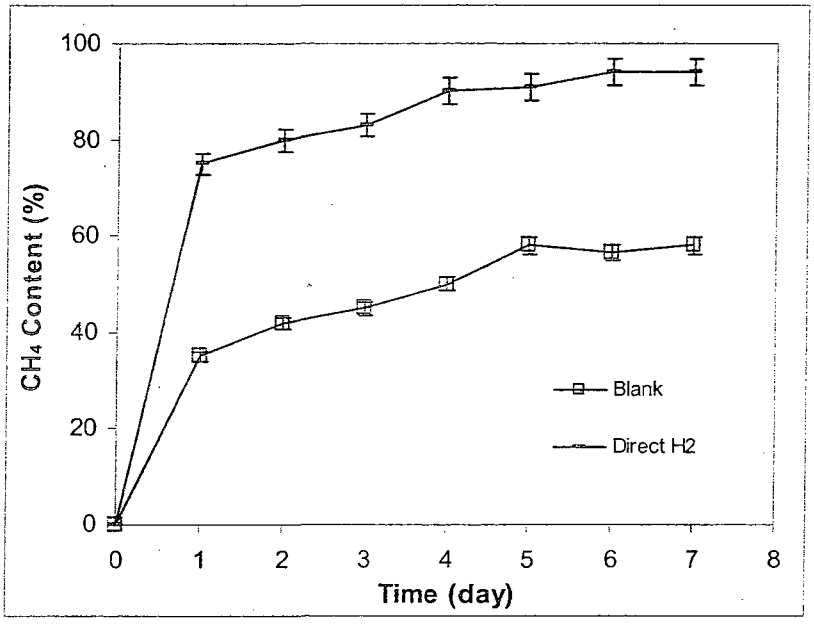


Figure 4.8 Trends of methane concentration in the headspace for the batch experiment with pure hydrogen and the blank.

The volumes of CH_4 production per COD removal were calculated and compared to the theoretical values and the results are shown in Table 4.2. The highest value was calculated for the batch experiment

with formic acid. This could be related to its simple organic structure and also the higher affinity of the bacteria for this material compared to the others for methane production in methanogenic reactions

Table 4.2 Volume of methane for the batch tests with light crude oil

Material	Methane Volume (ml)		CH_4 L/gCOD$_{rem.}$
	Measured	Theoretical	
Formic Acid	45.6	49.4	0.42
Acetic Acid	58.5	73.9	0.31
Butyric Acid	158.0	177.3	0.35
Lactic Acid	90.5	98.2	0.36
HRC	76.4	88.6	0.34
Galactose	114.8	132.1	0.34
Glucose	99.0	113.9	0.34
Sucrose	77.0	86.9	0.35
Blank	19	22.6	0.34

4.1.3 Methanogenic Activity

This parameter was calculated for each batch based on the initial rate of methane production and the amount of biomass. As shown in Figure 4.9, it can be estimated from the maximum rate of methane production and the available biomass in the system. It is a useful parameter to assess the activity of an anaerobic culture for the production of methane. The result of the MA calculation is shown in Table 4.3. The results show the highest value for the batch with formic acid. The maximum activity for the pure or enriched methanogenic culture is about 10 g COD removed/g VSS.d (Harper and Pohland 1986), while the observed activity in both industrial and laboratory digesters ranges from 0.1 to 1.0 gCOD removed/gVSS.d (Dolfing and Bloemen 1985; Field et al. 1988; Guiot 1991; Soto et al. 1993). This can be due to the fact that in activity tests for a mixed culture, only a fraction of the inoculated microorganisms will be able to produce methane. If the ratio between the actual activity and the

maximum pure culture activity can be assumed as the fraction of acetoclastic bacteria in sludge, this fraction will range between 1 and 10% (Soto et al. 1993).

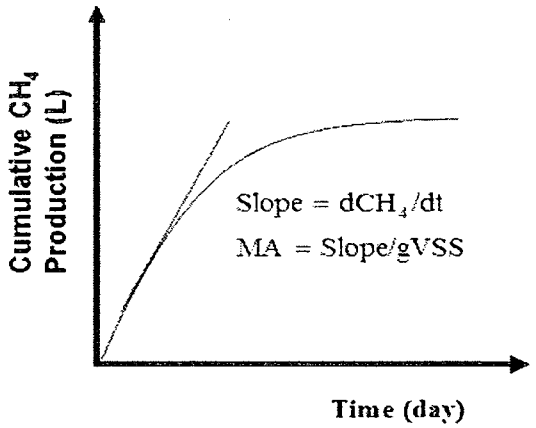


Figure 4.9 Determining methanogenic activity (MA) from the cumulative methane production. (VSS = Biomass concentration as volatile suspended solids).

Table 4.3 Values of methanogenic activity for the batch tests

Material	MA (L CH_4/gVSS.d)
Formic Acid	0.92
Acetic Acid	0.42
Butyric Acid	0.74
Lactic Acid	0.35
HRC	0.29
Galactose	0.30
Glucose	0.37
Sucrose	0.28
Blank	0.16

James et al. (1991) used a laborious method with a special respirometer to measure MA at 35°C. They used different solutions of acetic acid, mixed VFAs and sodium acetate as substrates with seed sludge

from UASB reactors treating low to medium-strength wastewaters. Their values were mostly in the range of 0.2 - 0.4 $\frac{L(CH_4)}{gVSS.d}$.

Soto et al. (1993) used a simpler method using a 126 ml vial as described in Figure 4.10 with a VFA mixture (acetic, 2.0 g/L; propionic, and n-butyric acid, 0.5 g/L each) and also individual VFA, neutralized with NaOH. Seed sludge was from an industrial processing of mussel (Lema et al. 1987), whose most important carbon source was glycogen. They obtained values of 1.047 and 1.25 $\frac{gCH4-COD/d}{gVSS}$ for maximum methanogenic activity with a VFA mixture and acetic acid, respectively, at 37°C.

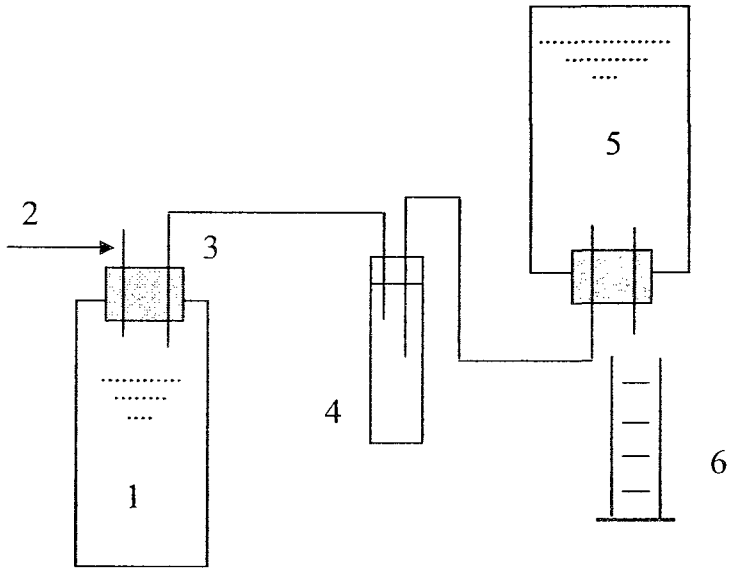


Figure 4.10 Digester of 126 mL connected to the alkaline solution displacement system, used for determination of specific methanogenic activity: (1) Culture medium (2) Sample point (3) Biogas circuit (4) Security vessel (5) Mariotte flask (6) Calibrated cylinder.

4.1.4 Results of Total Petroleum Hydrocarbon (TPH) Concentration

Results of HPLC analysis for TPH concentration are shown in Figure 4.11. For each batch, the percent reduction in TPH was calculated and the percent remaining was reported. The results showed that in the batch experiments with complex materials such as sugars and HRC more TPH reduction was observed compared to the other batches. This showed that these complex materials were more effective for the removal of dissolved petroleum hydrocarbons compared to CO_2 removal.

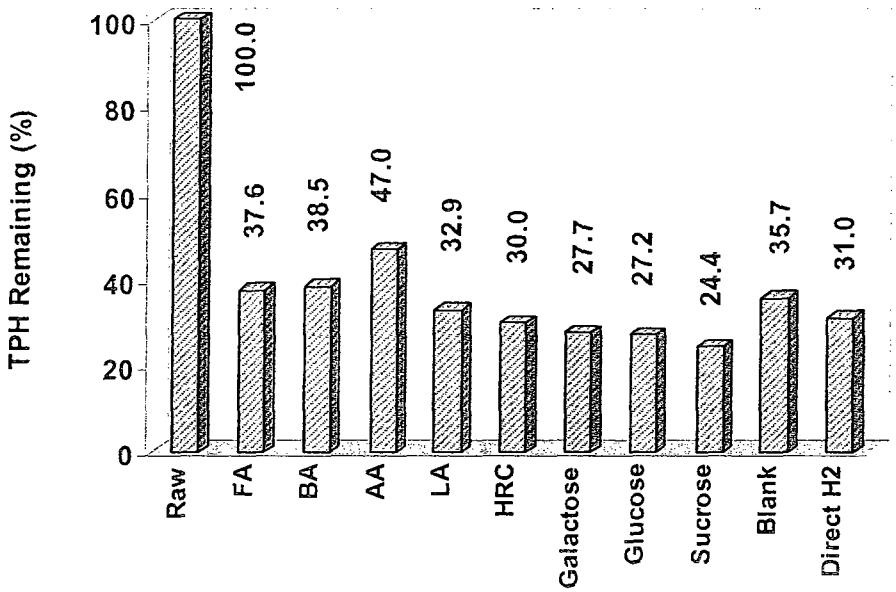


Figure 4.11 Relative concentrations of TPH for the batches with light crude oil. (Symbols: FA = Formic acid, BA = Butyric acid, LA = Lactic acid, HRC = hydrogen release compound, Raw = Raw wastewater).

This can be due to their molecular complexity which requires longer degradation times and existence of a mixed culture biomass both of which will help the degradation of dissolved hydrocarbons under reductive conditions. Higher removal efficiency was obtained for the batches containing sugars with the best result for the sucrose. Boopathy (2003) showed the usefulness of a mixed microbial system containing various groups of anaerobic bacteria for degradation of TPH. About 81% of TPH was removed in his experiments.

Under anaerobic conditions, sucrose is mobilized from several other pathways to enter glycolysis via its conversion to glucose and fructose (Dey 1997). As shown in Figure 4.12, the end product of this pathway is pyruvate which in turn is converted to either lactate or ethanol.

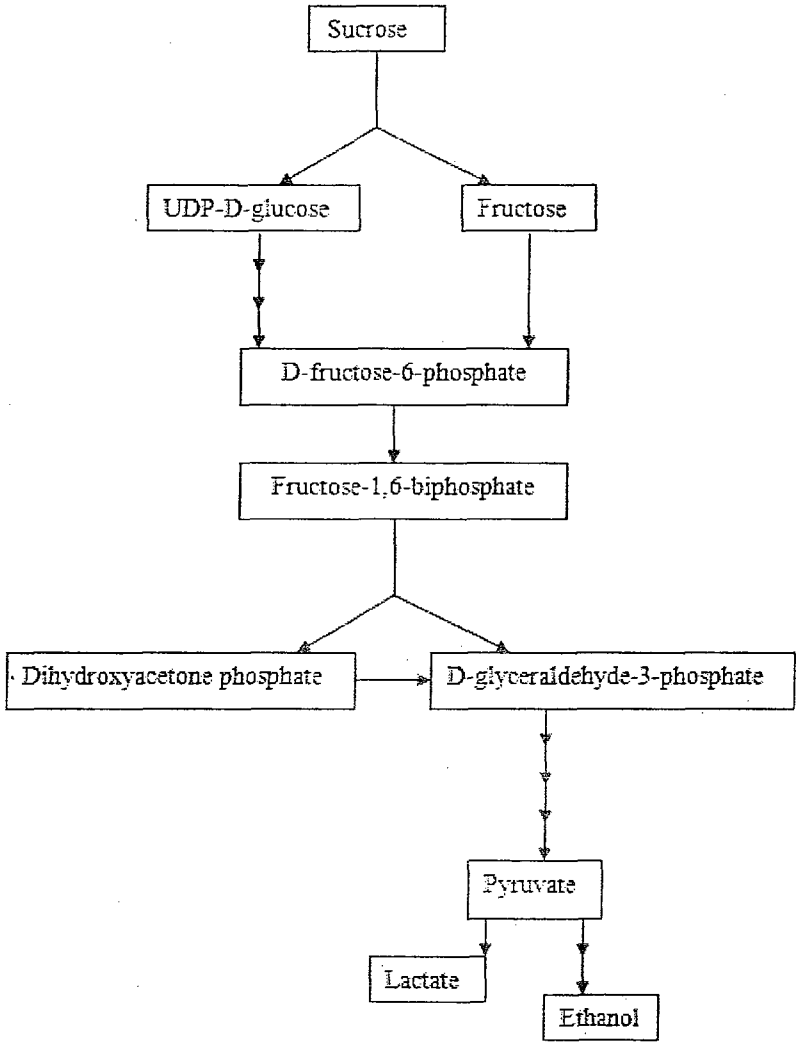


Figure 4.12 Metabolic pathways of anaerobic degradation of sucrose. (Reproduced from Dey 1997).

Pyruvate plays an important role in biochemical processes. It was shown (Yuan and Chang 2007, Baba and Katayama 2007, Dudkova and Demnerova 2007) that the addition of electron donors such as pyruvate, lactate and acetate improved the anaerobic degradation of polycyclic aromatic

hydrocarbons (PAHs) and polychlorinated biphenols (PCBs). Also, Widdel and Rabus, (2001) discussed the formation of pyruvate formate lyase (PFL) in the mechanism of the anaerobic degradation of aromatic hydrocarbons. For instance, one of the enzymes of the PFL group can catalyse the addition of fumarate to the methyl group of the aromatic ring, therefore making toluene accessible for ring cleavage (Heider et al. 1999, Boll et al. 2002).

4.1.5 COD Reduction

The values of COD were measured for each batch on a daily basis until the difference between each measured value was less than 10%. The trends for COD reduction are shown in Figure 4.13. The results shows faster COD reductions for simpler organic materials since these materials are easier to break down by the bacteria compared to the complex materials.

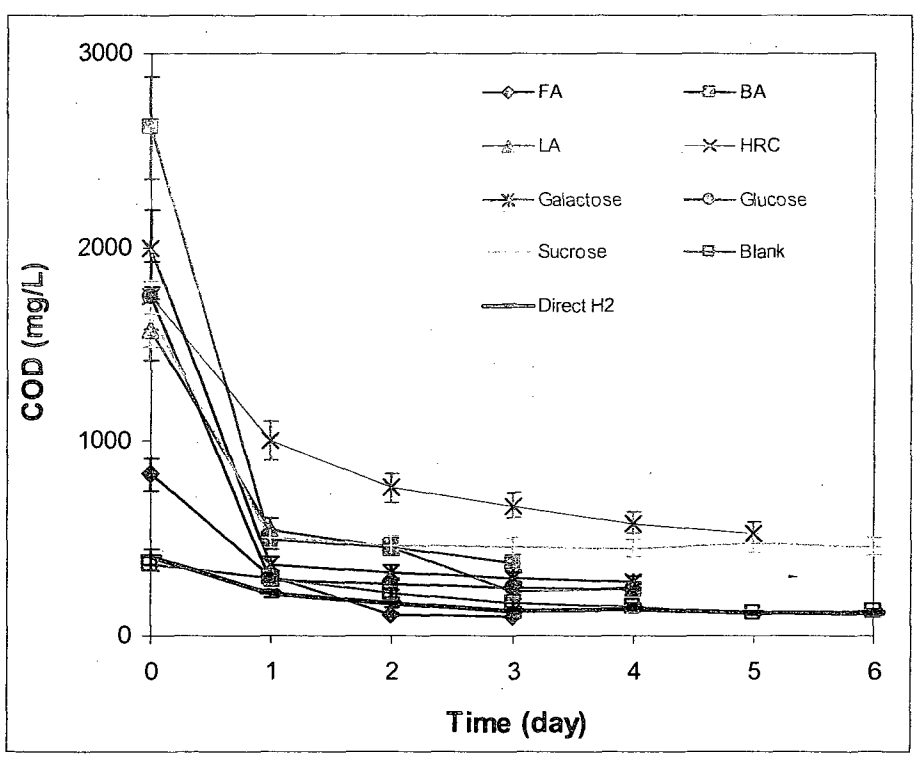


Figure 4.13 Trends of COD reduction for the batch tests with light crude oil

4.1.6 pH and Alkalinity Trends

The pH and alkalinity of the batches were monitored during the experiments and the trends for each parameter are shown in Figures 4.14 and 4.15. The pH values of the initial solutions were different depending on the material used and the overall trends were increasing with less increase for complex molecules due to possibly CO_2 generation associated with their degradation. This can also be the reason for the lower CO_2 reduction observed for these materials (Table 4.1).

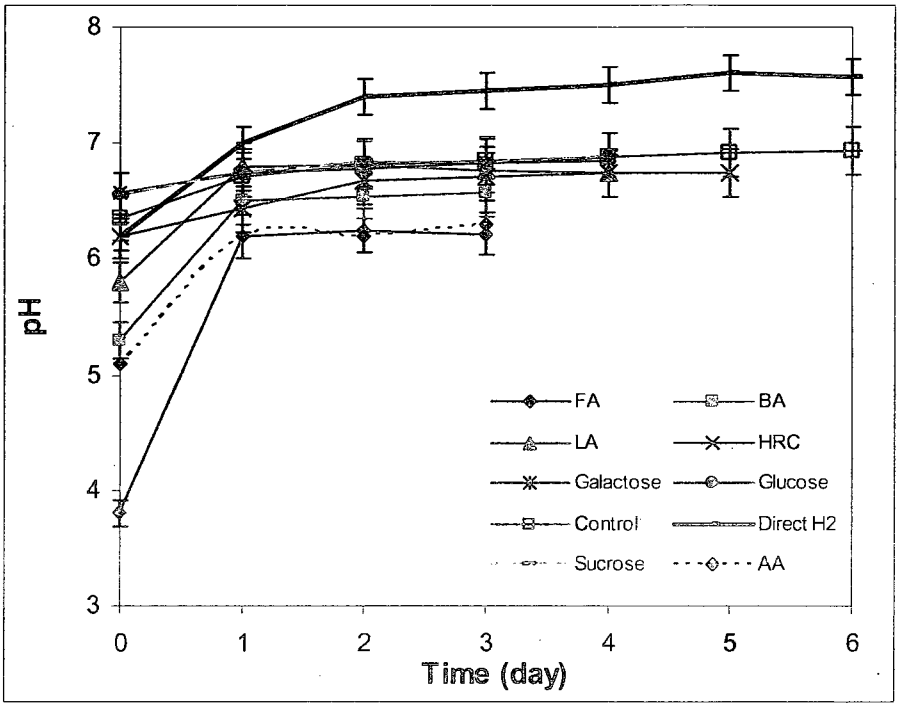


Figure 4.14 Trends of pH variation for the batch tests with the light crude oil

The increasing trends of pH show the degradation of and consumption of organic acid by the bacteria. These acids are consumed by most methanogens and it was observed that even at pH values as low as 4.0 for formic acid, the methanogens are viable. This can also be related to the simple structure of this organic acid.

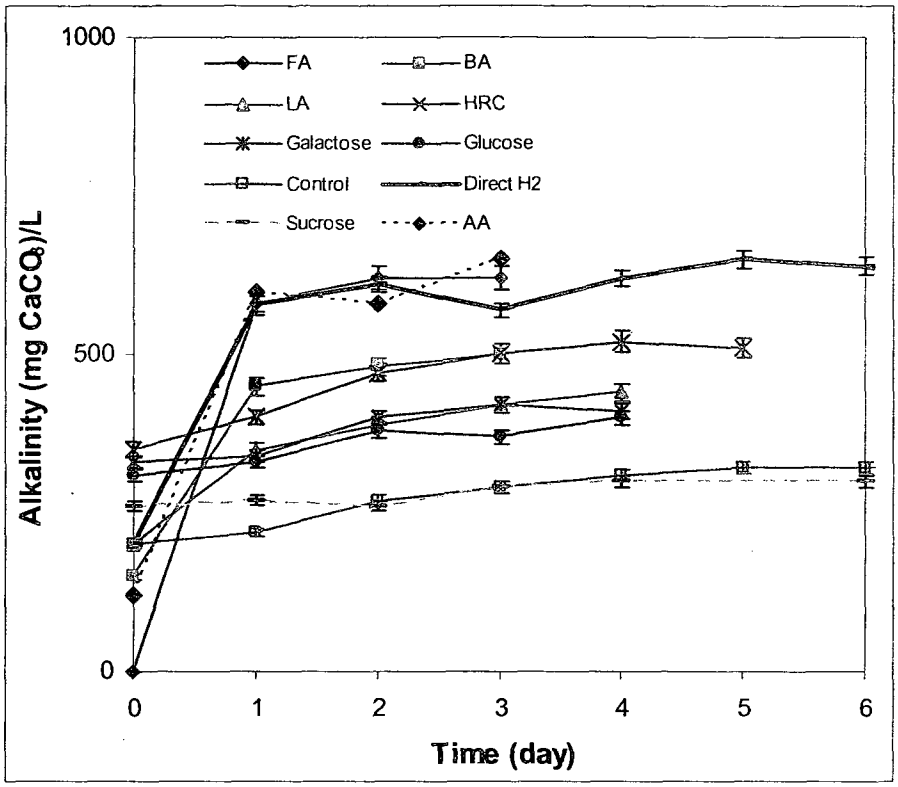


Figure 4.15 Alkalinity trends for the batch tests with the light crude oil

4.1.7 Summary and Conclusions of Phase I

A new biological approach was introduced and tested in this research work for the treatment of a waste stream from an enhanced oil recovery process with multiple contaminants in aqueous phase. Carbon dioxide, the dominant greenhouse gas, and dissolved petroleum hydrocarbons are the major contaminants in this waste stream.

In the first phase of this work a set of preliminary tests was performed to evaluate the applicability of this waste stream using the process of anaerobic treatment. A series of materials including the intermediate and mid-products of an anaerobic process have been used in this phase to perform a preliminary investigation of the efficiency of these materials to remove the contaminants from the waste stream.

The initial results from several batches showed that under anaerobic conditions and a mesophilic temperature of 35°C, addition of certain intermediate and final products of an anaerobic process such as low molecular weight fatty acids and mono and disaccharides can accelerate the removal process with different effects.

It was shown that using low molecular weight carboxylic acids such as formic and butyric acids are more beneficial for CO_2 removal. Among the fatty acids used, formic acid can be used even at low pH values (an observed minimum pH of 4.0) as an alternative to hydrogen for methanogenic reduction of CO_2. The intermediate products of the methanogenic pathway of CO_2 reduction by H_2 suggest that formate can be used as an alternative for hydrogen. Methanogenic activity measurements showed that the bacteria have more tendency to degrade formic acid than the other fatty acids used and the highest value for formic acid and highest CO_2 reduction in this batch showed that more population of methanogens exist in this batch compared to the other batches. Despite similarities between pathways of CH_4 production from H_2 and CO_2 and acetate, the result of CO_2 reduction using acetate was not as expected probably because of the inhibitory effect of the petroleum hydrocarbons.

On the other hand, using complex materials such as mono- and disaccharides can improve removal of TPH from the liquid phase compared to removal of dissolved CO_2. This effect can be related to the pathway of sucrose metabolism which involves the formation of pyruvate and lactate that have been shown to play an important role in anaerobic degradation of the petroleum hydrocarbons.

For most of the experiments, pH as an important operating condition whose control is vital for methanogenic bacteria was increasing. This increase was more for the batches with fatty acids because

of their consumption in methanogenic reactions. However, pH values remained mostly within the suitable range for most methanogens (6.5-7.6).

4.2 Continuous Operation (Two-Step Reactor System)

4.2.1 General Remarks

It was observed that low molecular weight fatty acids can contribute to reduction of carbon dioxide. Also, the processing time regarding the ultimate CO_2 and COD removal, CH_4 content, etc. is less compared to that of complex materials used in the first part. Formic acid as a C1 molecule showed the best results for CO_2 removal and the short reaction time compared to sucrose, suggesting that it is possible to do the treatment in two steps: In the first step using formic acid, CO_2 can be removed (with the possibility of partial TPH removal) and in the second step using sucrose, TPH can be removed. In the two-step reactor system, the effluent from the first step was used for the second step. The operating conditions of the reactors such as organic loading, pH, and temperature were optimized and the sustainability of the method was investigated.

4.2.2 Pre-run of the Two Step Reactor System

Before using this system for the main experiments and for acclimation of the anaerobic bacteria, the reactors were run at 35°C with the solutions containing the selected materials from the first phase. The first reactor was fed with solutions containing formic acid while the second one was fed with sucrose. The necessary materials and elements for microbial growth were added according to sections 3.1.2 and 3.1.3. During this period, the parameters such as COD reduction, biogas rate, methane volume and methanogenic activity, etc. were measured and calculated.

4.2.3 System Operation and Primary Optimization

The two step reactor system was run with the simulated waste stream. This stream was fed into the first step with the addition of formic acid and after a certain retention time and based on the test results including CO_2 reduction, the solution was pumped to the second reactor with the addition of sucrose for TPH removal.

For the optimization of the system, two methods were applied. At first, the system was run by changing the operating parameters such as retention time, pH and temperature (one at a time) to find the primary optimum point of operation for the target parameters of CO_2 reduction and CH_4 production for the first reactor and TPH removal for the second reactor. These conditions were selected to be the base conditions for the method of EVOP factorial design which was then applied to optimize the target parameters in each reactor.

4.2.3.1 Effect of Retention Time

The waste stream was processed in the reactor system and the retention time was determined for both reactors. After 3 weeks, a retention time of 4 hours was obtained in the first reactor leading to 95% CO_2 conversion. For the second reactor, the retention time of 7 days was obtained with a TPH removal of about 76%. Figure 4.16 shows the results of CO_2 and TPH removal for several runs at this point. The results were consistent with the types of reactions expected in each reactor: In the first reactor, methanogenic reduction of CO_2 to CH_4 under the operating and microbial conditions of this reactor, is very favorable (Eq. 3-1) and can take place faster than removal of TPH comprising of a variety of low to high- molecular weight hydrocarbons. However, effluent analysis from the first reactor showed a partial TPH removal which could be due to partial degradation of some of the light hydrocarbons in this

reactor. In the second reactor, more retention time is required to degrade the more-complex hydrocarbons by a mixed culture of biomass. After 7 days no significant improvement in the TPH removal was observed.

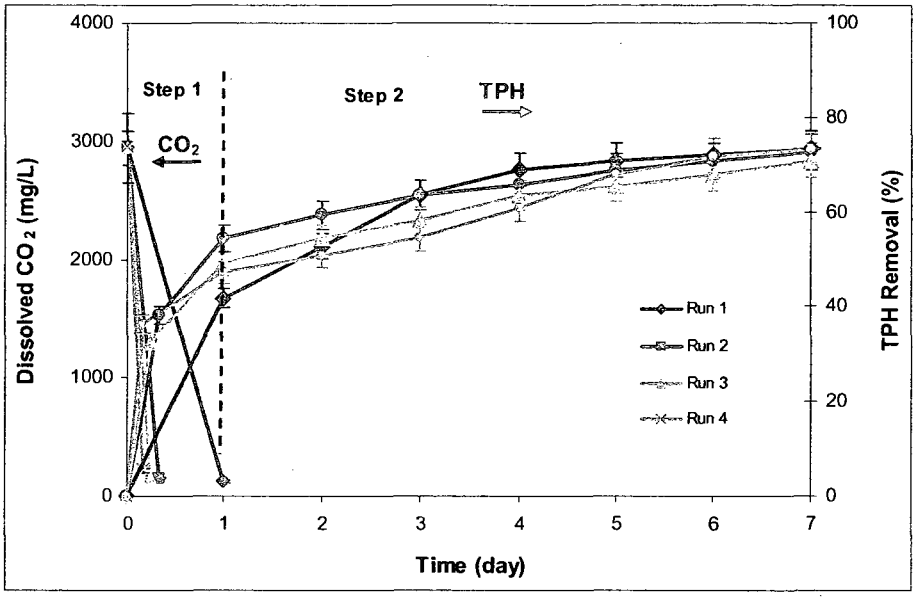


Figure 4.16 Results of CO_2 and TPH removal for several runs of the system.

4.2.3.2 Effect of pH

First reactor

For the first reactor, the effect of low pH values was evaluated to achieve the minimum pH for which the methanogenic reactions could take place. The advantage of a low pH value in this step is that under a low pH, more hydrogen ions and dissolved CO_2 are available for methanogenic reduction of CO_2. After several runs, the minimum pH of 3.5 was achieved for a retention time of 4 hours. Figure 4.17 shows the results for CO_2 reduction and CH_4 production for this reactor.

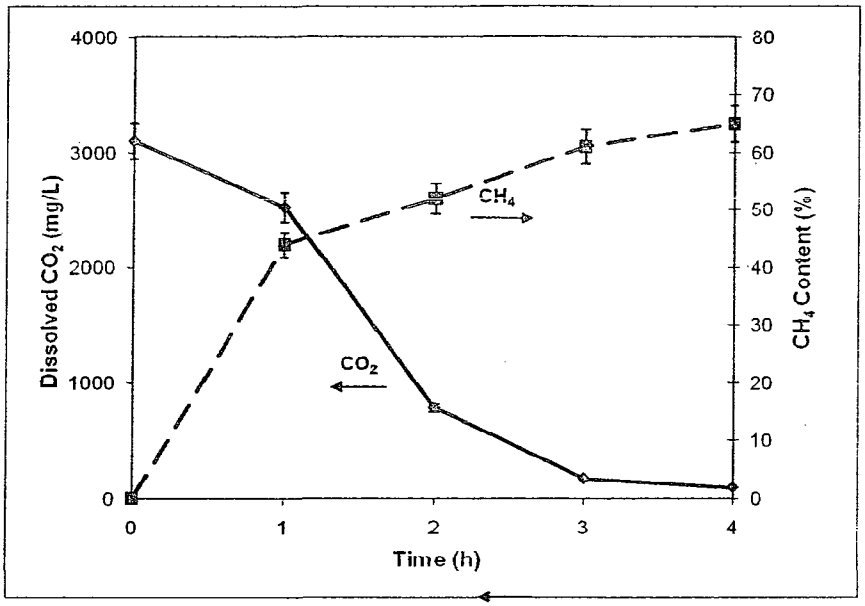


Figure 4.17 Result of CO_2 reduction and CH_4 content of the biogas in reactor 1 (pH 3.5, T = 35°C and OLR = 7800 mg COD/L.d, retention time = 4h).

Although the conditions of reactor 1 were set for methanogenic reduction of the CO_2, some TPH was removed in this reactor which can be due to the methanogenic degradation of light hydrocarbons under the conditions of reactor 1. Figure 4.18 shows the variation of TPH.

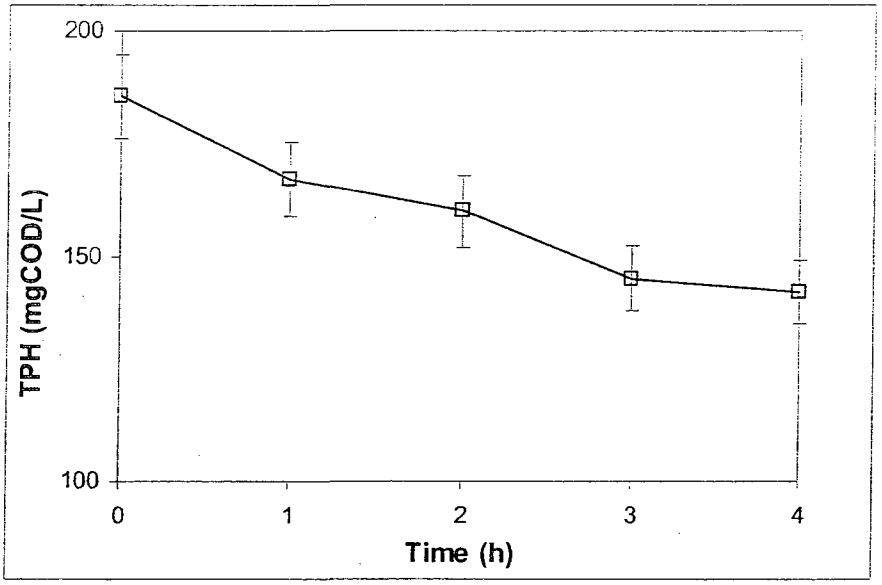


Figure 4.18 Degradation trend of TPH in reactor 1. (pH 3.5, T = 35°C and OLR = 7800 mg COD/L.d, retention time = 4h).

Second reactor

For the second reactor, the pH was controlled at different values around 7 for several runs. It was observed that for a retention time of 7 days, the TPH removal improved as the pH increased from 6 to 7, but slightly decreased for a pH of 8. With another run of the reactor at pH 7.5, better results were observed (about 80% of TPH removal). Figure 4.19 shows these results. Also, the TPH concentration was calculated as the COD equivalent (mg/L) and the results are shown in Figure 4.20. The pH obtained for this reactor is within the optimal range for most methanogens (6.5-7.6) and probably higher pH values will reduce the activity of these bacteria to complete the degradation processes in conjunction with sucrose and TPH degradation. Other studies have shown similar pH ranges: Hunkeler et al. (1998) obtained 65% removal of TPH at pH of 7.5. Boopathy (2003) obtained about 43% TPH removal for the contaminated sediments under methanogenic conditions at pH of 6.5. Cuenca et al. (2006) obtained about 83% TPH removal in a fluidized bed reactor within a pH range of 6.7-7.3 which is the optimal pH range for anaerobic microorganisms (Maier et al. 2000).

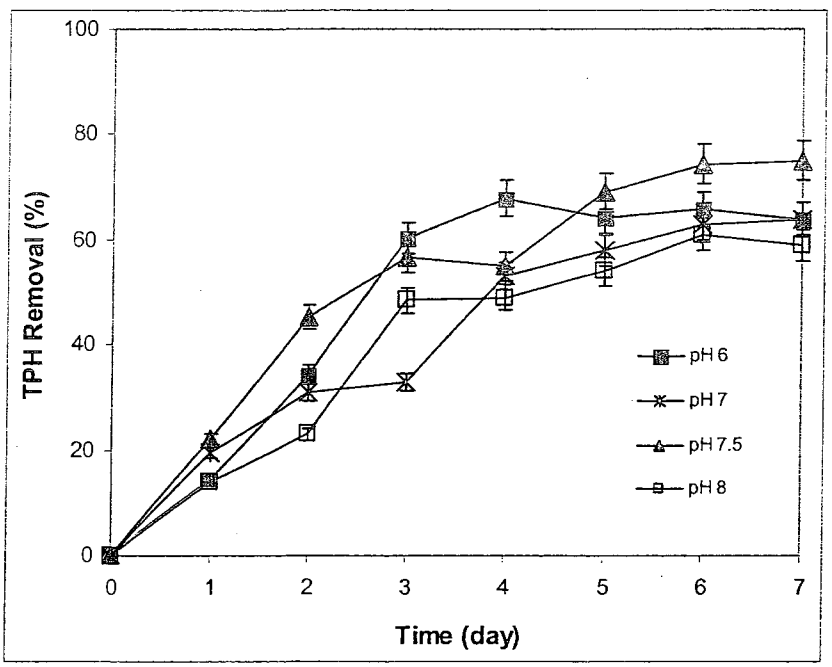


Figure 4.19 Results of TPH removal (percentage) for reactor 2. (T = 35°C and OLR = 450 mg COD/L.d, retention time = 7d).

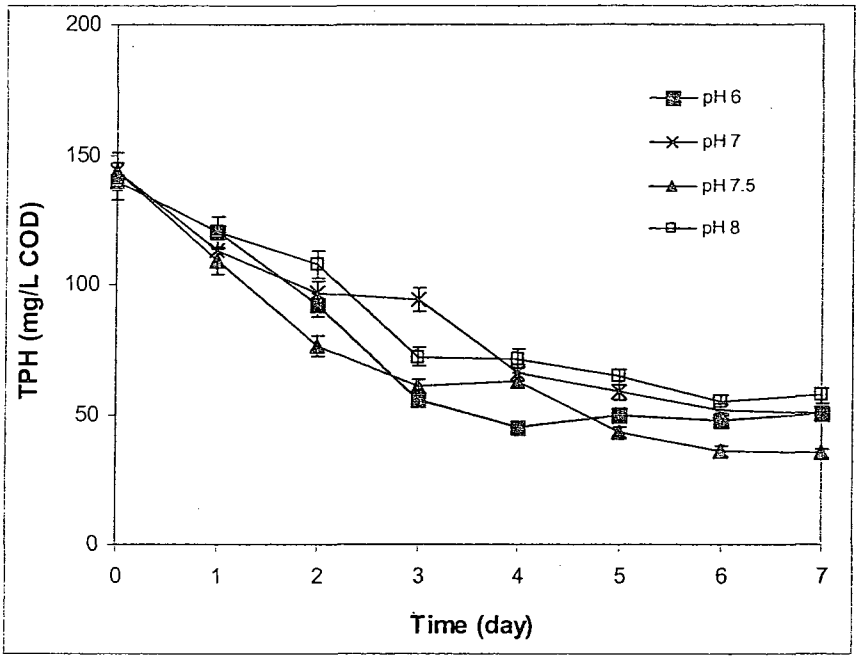


Figure 4.20 Trends of TPH removal (COD units) for reactor 2. (T = 35°C and OLR = 450 mg COD/L.d, retention time = 7d).

4.2.3.3 Effect of Temperature

In this section and regarding the retention times obtained in the previous section, the effect of temperature on the CH_4 production and CO_2 removal in the first reactor and TPH removal in the second reactor was investigated. The temperature was varied in the mesophilic range of temperature from 33°C to 39°C. The temperature was controlled using the control loop on each reactor including an on-off controller connected to a heating jacket and a flow controller of the cooling water loop.

In general, temperature can affect the rate of biomass growth and substrate utilization in biological reactions. The influence of temperature on the coefficients such as endogenous decay rate, the maximum rate constant or the first order constant can be described by the Arrhenius equation (Celenza, 1999):

$$\ln k = \ln A - \frac{E_a}{RT} \qquad (4\text{-}1)$$

k = rate constant

A = constant

Ea = activation energy (J/mol)

R = gas constant (8.3 J/K)

T = temperature (K)

Equation 4-1 can be solved for two temperatures as follows:

$$k_2 / k_1 = \Phi(T_2 - T_1) \qquad (4\text{-}2)$$

The value of Φ depends on the biological system. The effects of temperature on other parameters such as yield and half-velocity constant is not well documented and could depend on the system configuration (Celenza, 1999). Generally speaking, an increase in the temperature results in an increase of the rate constant. However, within the mesophilic range of temperature, it is estimated that there is an approximate doubling of the rate of biochemical activity with every 10°C rise between 0°C and 30°/35°C (Gounot 1991, Standing and Killham 2007). Gibb et al. (2001) have shown that a low temperature will affect microbial growth and propagation, and under normal circumstances, rates of degradation decrease accordingly. This is primarily due to decreased rates of enzymatic activity. The optimum temperature is typically in the range of 30 to 40°C. At temperature above this range, enzymatic activities are inhibited as proteins denature (Leahy and Colwell, 1990).

First reactor

Figure 4-21 shows the effect of temperature change on the CH_4 content of the biogas and Table 4.4 shows the CO_2 conversion and CH_4 production for each run of reactor 1. It seems that despite a slight

decrease in methane content of the biogas, increasing the temperature in the mesophilic range of temperature didn't have a significant effect on the target parameters of CO_2 conversion and CH_4 production in this reactor. Therefore, the base temperature was considered to be 35°C.

According to Bouallagui et al. (2004), the production of biogas has two optima, one in the mesophilic range (about 35°C) and the other in the thermophilic range (about 55°C). Results of several studies in the mesophilic range of temperature (Ahn and Forester 2000, Gallert and Winter 1997, Zabranska et al. 2000) have shown that the methane content of the biogas is mainly affected by the types of substrate, rather than the temperature conditions. Moreover, the degradation of formate is rapid and is less affected by the temperature variation in this reactor.

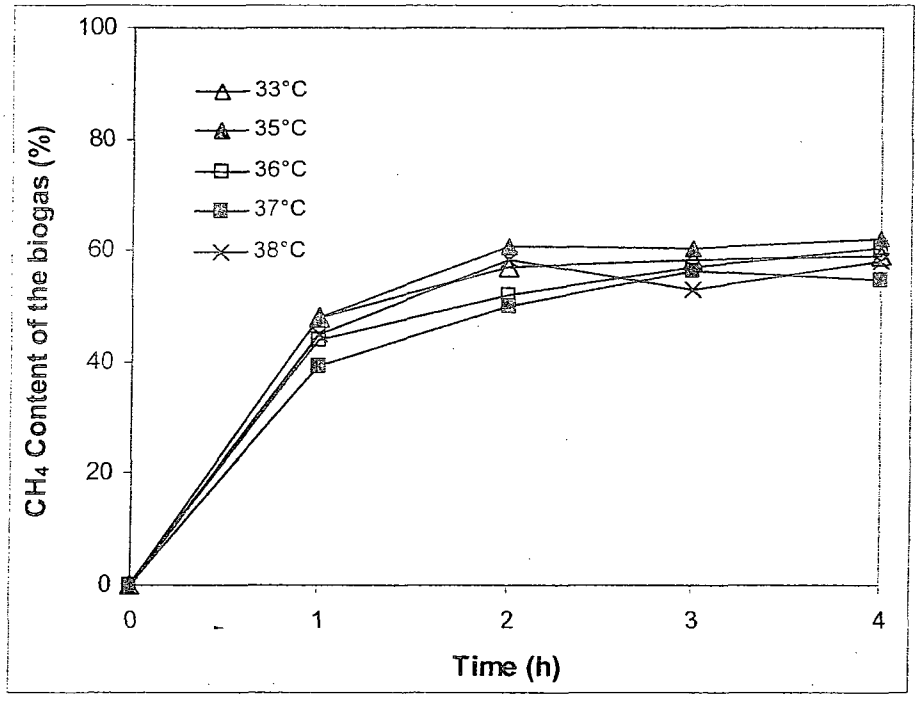


Figure 4.21 Results of CH_4 content of the biogas for reactor 1. (pH 4, OLR = 7800 mg COD/L.d, retention time = 4h).

Table 4.4 Performance at different temperatures in the first reactor

Temperature (°C)	CO_2 Removed (%)	Average CH_4 Content (%)	CH_4 Volume (mL)	CH_4 /VSS (ml/g)
33	95.0	55.7	168.6	10.1
35	97.0	56.3	174.2	10.5
36	96.2	55.0	170.3	9.7
37	94.0	51.3	158.7	9.1
38	94.8	54.0	167.2	9.7

Second reactor

For the second reactor as shown in Figure 4.22, it seemed that increasing the temperature had a positive effect on TPH removal. However no improvement was observed for temperatures of 37°C and higher and the best results were obtained for the reactor temperature of 36.5°C.

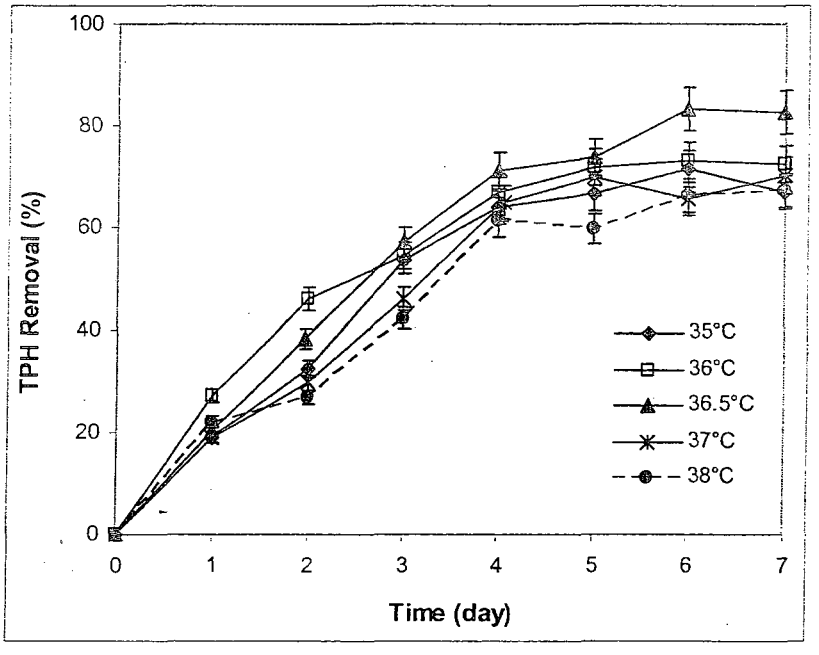


Figure 4.22 Effect of temperature on the TPH removal for reactor 2. (pH 7.5, OLR = 450 mg COD/L.d, retention time = 7d).

Biodegradation of hydrocarbons can occur over a wide range of temperatures and the highest degradation rates generally occur in the range of 30–40 °C (Okoh 2006). Unlike the first reactor, it seems that for the second reactor, degradation of the hydrocarbons is more affected by the temperature. This could be primarily due to the change in the solubility of the hydrocarbons as investigated by Foght et al. (1996). Also, more of the light hydrocarbons are removed due to their higher solubility and degradability.

4.3 Microbial Results

In order to check the quality and quantity of the anaerobic bacteria in the reactors, the concentration and morphological structure of the biomass for both reactors were measured and monitored. Biomass samples from both reactors were taken during the experiments and for the analytical methods and microscopic observations. The average particle size of random samples was recorded and the values of total suspended solids (TSS), volatile suspended solids (VSS) and the ratio of VSS/TSS were measured throughout the experiments.

4.3.1 Volatile and Suspended Solids of the Sludge (VSS and TSS)

T

he values of VSS, TSS and the ratio of VSS/TSS are important parameters in a biological treatment system indicating changes in the bacterial conditions. The variations in these parameters are related to structural changes of the biomass particles, which in turn are influenced by multiple factors, including the composition of influent. The VSS, TSS and VSS/TSS ratios for the sludge in the reactor were measured and monitored in each stage of the work. Each time, three samples were taken and tested according to the method illustrated in section 3.2.7 and the average value of the three samples was

reported. The average value had a maximum of 3% difference from lowest and highest values, so it was considered to be the maximum error for all measurements.

4.3.1.1 Reactor 1

The results for this reactor are shown in Figures 4.23 and 4.24. As shown, the concentration of the biomass in this reactor decreased for the first weeks of operations and this decrease was not significant after the week 11^{th}. The initial reduction could be related to the change of the substrate and the removal of some of the floating biomass particles due to poor settling characteristics. The original culture (a mixed culture biomass) was previously fed with cheese whey and in this work, it was acclimated to the new substrate and operating conditions.

Microscopic observations showed morphological changes to the biomass colonies and it seems that in the course of the reactor operation, a new culture of biomass was being developed. The new colonies were more uniform in size and color. This can due to the fact that the conditions of this reactor were set for the methanogenic reactions and the methanogenic bacteria were the most abundant culture in this reactor. This can also explain the decrease in VSS after the week 11^{th}. Microscopic observations will be further discussed in the next section. The ratio of VSS/TSS in Figure 4.23 changed from 0.66 (0.75 the original value) to 0.62. This is in accordance with the changes in biomass colonies and loss of some of their volatile fraction as the newer colonies were smaller in size and had thinner outer layers.

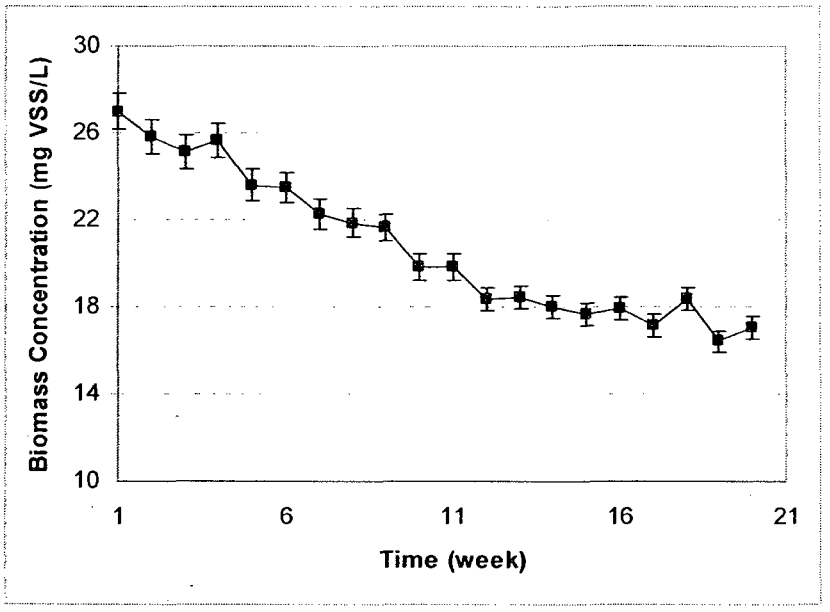


Figure 4.23 Variation of concentration of the anaerobic biomass for reactor 1.

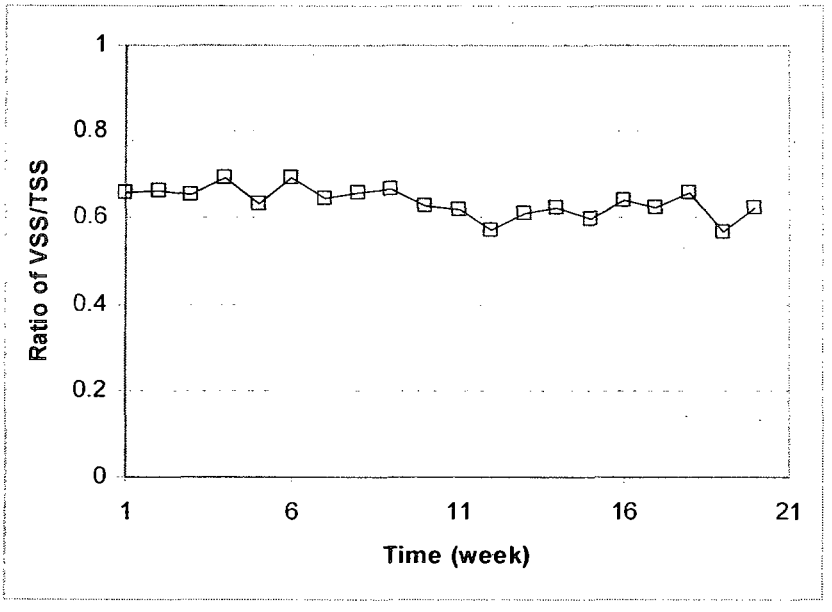


Figure 4.24 Variation of the ratio of VSS/TSS for the anaerobic biomass in reactor 1.

4.3.1.2 Reactor 2

For this reactor, the variations in concentration of reactor biomass and the ratio of VSS/TSS are shown in Figures 4.25 and 4.26. Unlike reactor 1, less reduction in the concentration of reactor biomass was observed. This could be due to the fact that in this reactor, the bacteria were acclimated to a complex substrate including sucrose and the multi-step nature of the anaerobic processes requires a mixed culture of biomass. Also, fewer morphological changes were observed for this culture as will be discussed in the next section. Also as shown in Figure 4.24, the ratio of VSS/TSS changed from the initial value of 0.75 to the final value of 0.78.

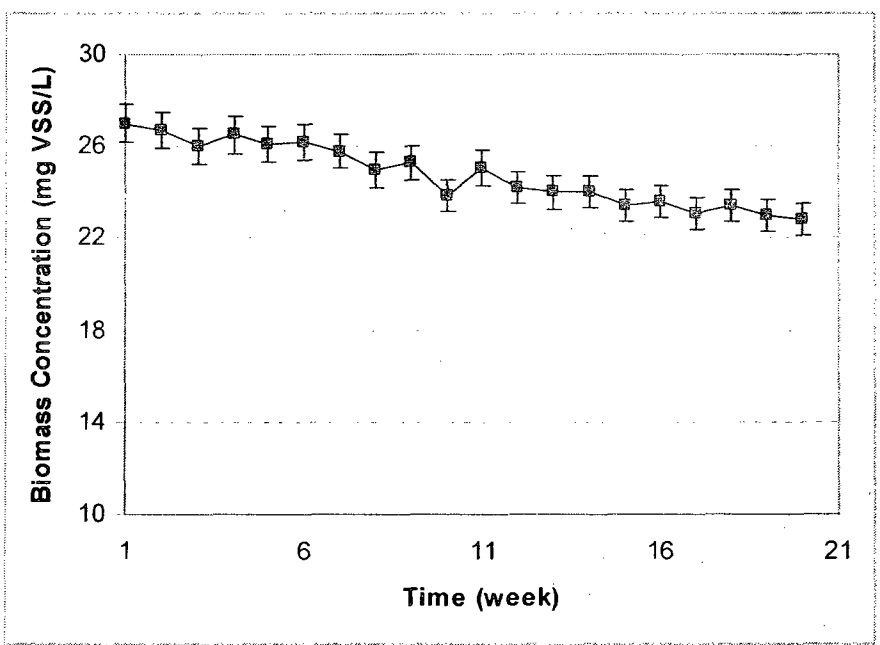


Figure 4.25 Variation of concentration of the anaerobic biomass for reactor 2.

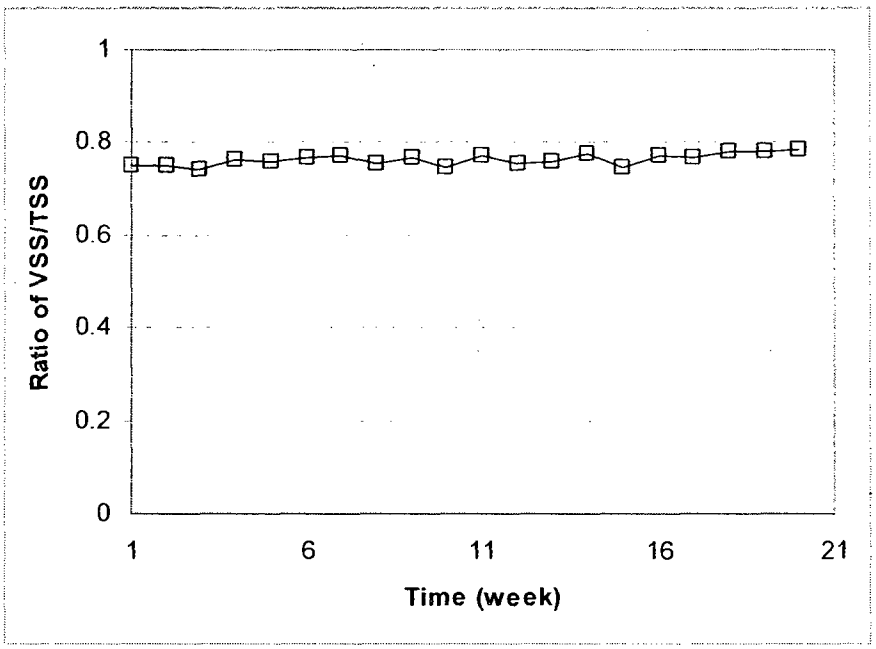


Figure 4.26 Variation of the ratio of VSS/TSS for the anaerobic biomass in reactor 2.

The higher VSS/TSS ratio in this reactor compared to the first reactor shows that the amount of insoluble organic or inorganic particulates contributing to TSS is less compared to organic constituents. Also, as will be discussed in the next section, the bacterial colonies in the second reactor are different in size and morphological structure than those in the first reactor due to the different reactions and conditions in both reactors. In a study by Dolfing et al. (1985) on granular methanogenic sludge grown on wastewater of a liquid sugar factory, values of 10 to 20% (80%-90% VSS/TSS) were reported for the ash content of the biomass. Fang (2003) reported the formation of granules of 1.6 mm in diameter, 1.038 g/ml in density and 11% in ash content for H_2-producing acidogenic sludge in a stirred reactor treating sucrose-rich wastewater at 26°C and pH 5.5 with 6 hours of hydraulic retention. Gonzalez et al. (2001) reported an ash content of 15%, for the treatment of a brewery wastewater which did not vary significantly during one year of reactor operation. On the other hand, Grotenhuis et al. (1991) reported a minimum value of 45.4% for ash content of granular sludge grown on propionate in an UASB reactor. These examples show that the organic constituents of microbial cultures are affected by different factors

such as substrate and environmental conditions and in general, microbial cultures treating more complex substrates, such as those containing proteinaceous materials, have coarser bacterial granules with higher VSS/TSS ratios.

4.3.2 Microscopic Observations

In order to determine the structural changes of the original biomass with the progress of this work and to monitor its morphological changes, microscopic photographs were taken from samples of biomass from both reactors. Microscopic observations along with the other biomass characteristics determined by microbial and activity tests can help to investigate the gradual changes in the microbial population and interpretation of the results. A photograph of the original biomass was already shown in Figure 3.1. Another photograph (Figure 4.27) showed that the original biomass also contained some filamentous type bacteria which could be an indication that this culture was a mixture of different bacterial cultures.

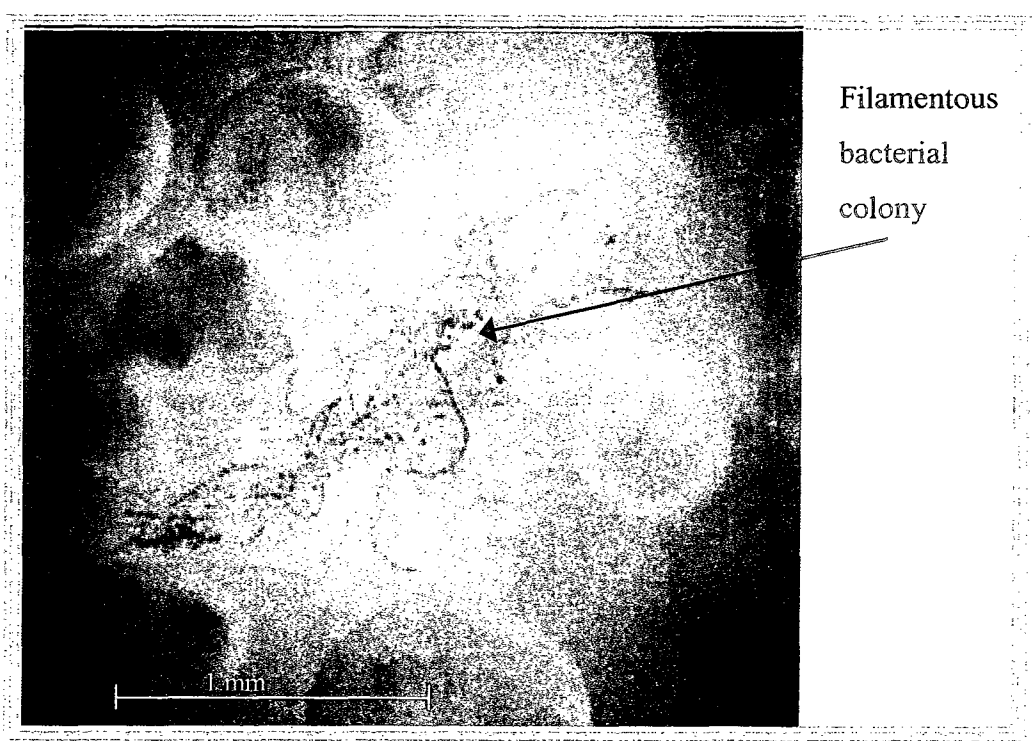


Figure 4.27 A microscopic picture of the original biomass showing a filamentous type of bacteria (WILD Heerbrugg, model Wild M5A - 50X magnification).

4.3.2.1 Microscopic Observations in Reactor 1

Figure 4.28 shows a microscopic picture of the biomass in reactor 1 and Figure 4.29 shows the general image of a sample of bacteria in this reactor. With the progress of experiments, changes in appearance (shape, color and size) of the original biomass particles in this reactor were observed showing their possible structural changes. The bacterial colonies in this reactor were characterized by granules with a particle size range of 0.1-1.0 mm and light to dark gray colors. As can be seen from these pictures, the original layer of light brown seemed to become thinner and in some particles it disappeared and the number of smaller dark gray particles increased. This could be another indication that methanogenesis was the dominant reaction and the population of methanogenic bacteria was increasing in this reactor. As an example of methanogenic colonies, a photo of methanogenic granular sludge studied by Yamada et al. (2007) is shown in Figure 4.30 in comparison to Figure 4.29.

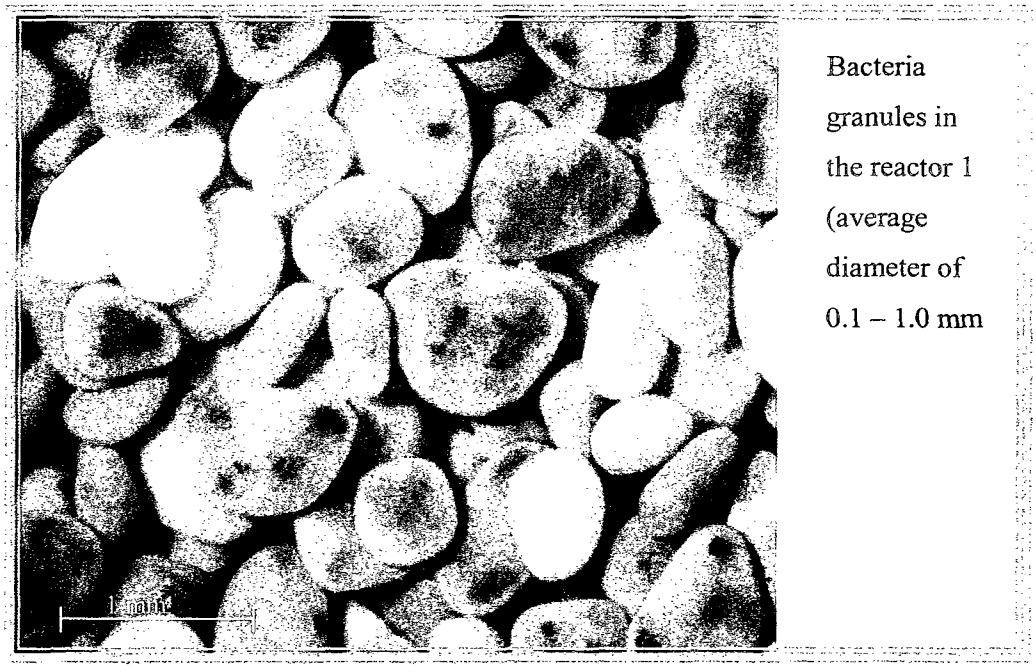


Figure 4.28 Microscopic image of the biomass in reactor 1. (WILD Heerbrugg, model Wild M5A - 25X magnification).

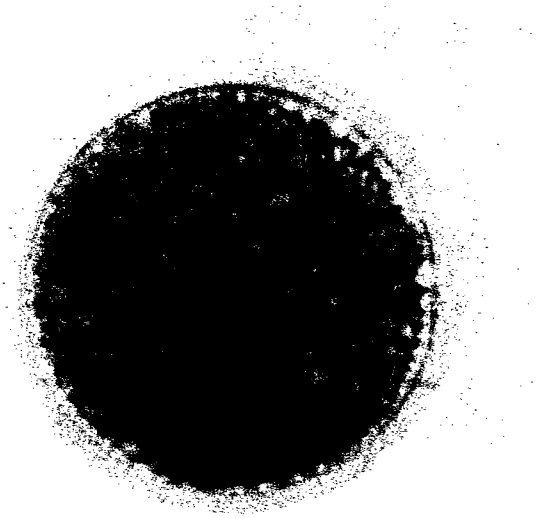


Figure 4.29 Photo of a sample of the bacteria in reactor 1 (scale 1:1).

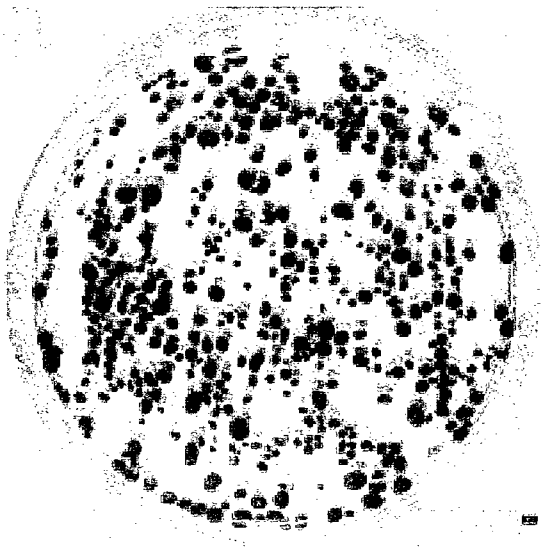


Figure 4.30 Photo of a sample of the methanogenic bacteria. (From Yamada et al. 2007- scale 1:1).

Also, in another study (Alimahmoodi and Mulligan 2008), biomass particles of this type were observed which were very different from the original culture. In that work, the biomass was acclimated to an acetic acid/sodium medium and as shown in Figure 4.31, they contained whitish spots on their surfaces. This was probably due to the effect of inorganic compounds, such as sodium salts as discussed by Peinemann et al. (1988) and/or inorganic precipitates such as $CaCO_3$ or $Ca_3(PO_4)_2$. Since bicarbonate is produced during anaerobic conversion of acetate, calcium precipitates can form within the sludge, even

at reactor calcium concentrations as low as 200 mg/liter (Guiot et al. 1992, Uemura et al. 1995). Zehnder et al. (1991) reported that for granules grown on propionate as a sole carbon and energy source, larger granules were obtained by using high substrate concentrations.

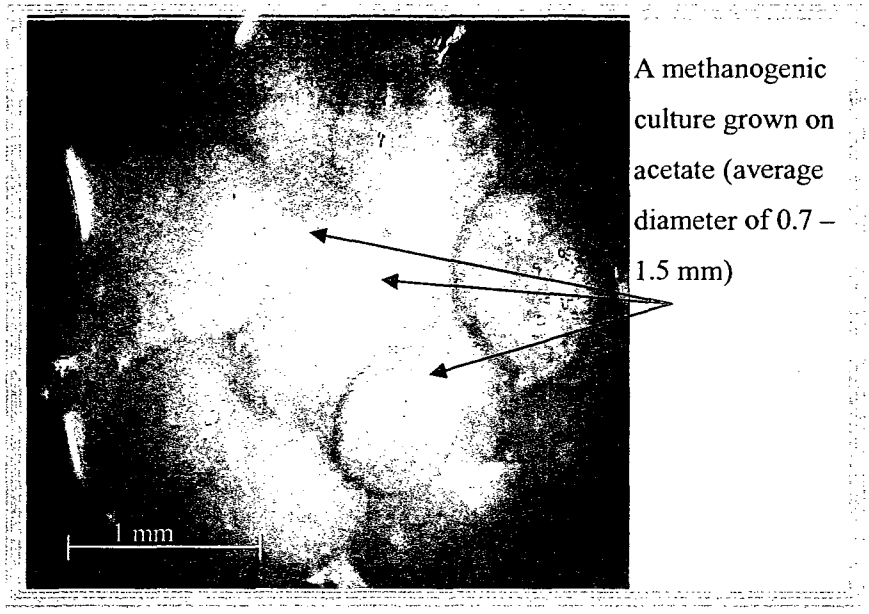


Figure 4.31 Photo of a sample of the methanogenic biomass. (Alimahmoodi and Mulligan, 2008- WILD Heerbrugg, model Wild M5A - 25X magnification)

4.3.2.2 Microscopic Observations in Reactor 2

The bacterial colonies observed in reactor 2 were very different from reactor 1. Figure 4.32 shows a microscopic picture and Figure 4.33 shows a general image of the bacteria in this reactor. In this reactor, the size of biomass particles were in the range of 0.8- 2.3 mm (with some particles of 3.0 mm diameter) and the color of most particles was light brown. Also, some biomass particles similar to those in reactor 1 were observed.

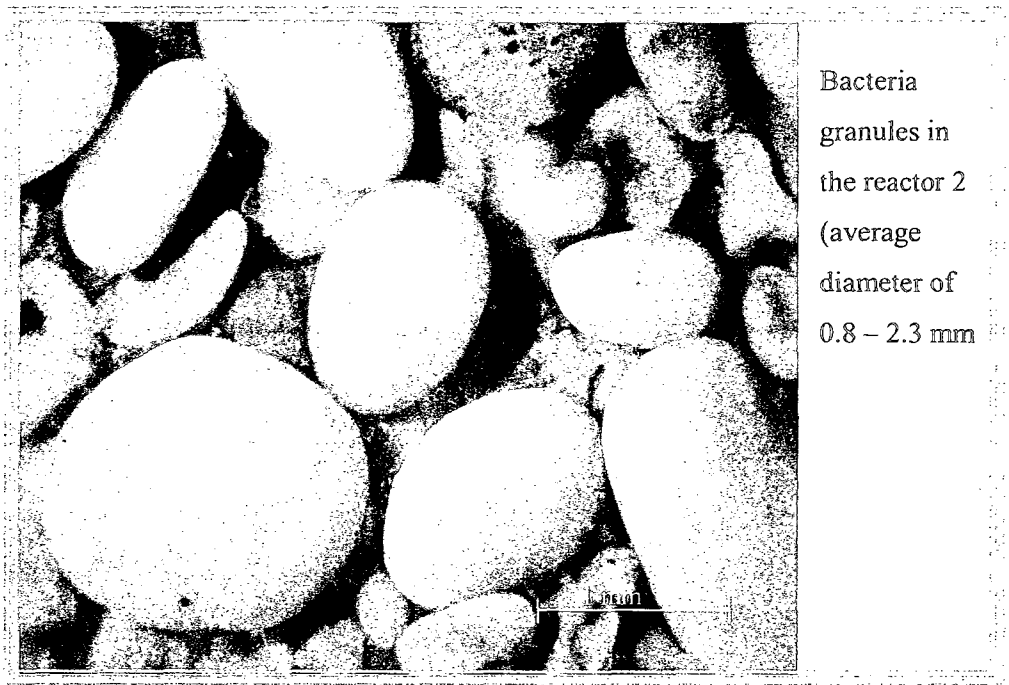


Figure 4.32 Microscopic image of the biomass in reactor 2. (WILD Heerbrugg, model Wild A - 25X magnification).

More microscopic images showed new bacterial colonies [Figures 4.34 (a) and (b)] having red colors that sometimes had different shapes from the original biomass.

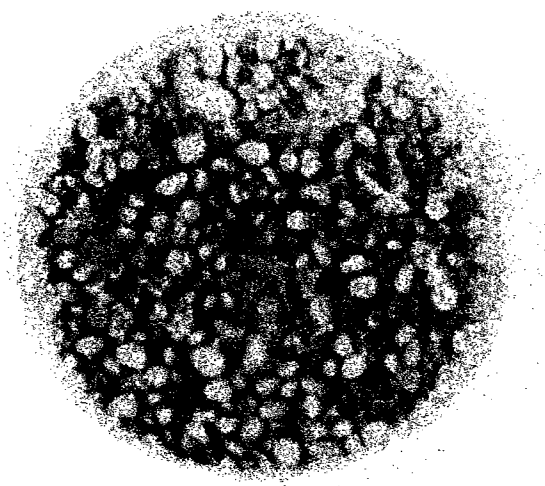


Figure 4.33 Photo of a sample of the biomass in reactor 2 (scale 1:1).

Figure 4.34 Microscopic images of the biomass in reactor 2 showing different colonies of bacteria. (WILD Heerbrugg, model Wild M5A - 25X magnification).

These figures could be another indication of the occurrence of complex anaerobic reactions in this reactor and existence of a mixed culture biomass in accordance with the degradation of sucrose and petroleum hydrocarbons. Bakalova et al. (2007) reported the creation of colonies with red color as shown in Figure 4.35 while growing a culture on soluble hydrocarbons that might be of a similar type observed in this work.

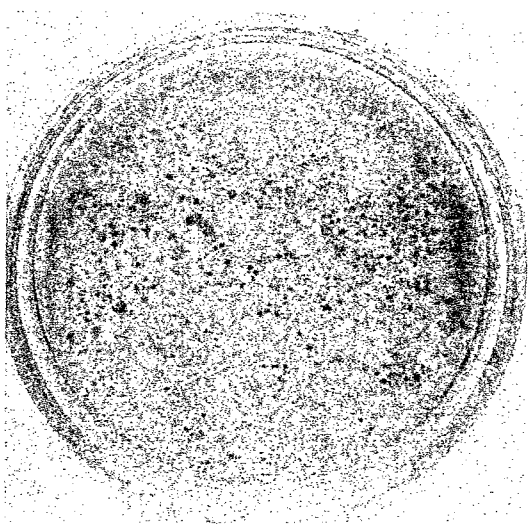


Figure 4.35 Red colonies grown on soluble hydrocarbons (Bakalova et al. 2007-scale 1:1).

In a study by Belyaev et al. (1983), microscopic observation of methanogenic enrichment cultures inoculated with injection water, groundwater, or oil-bearing sandstone revealed a heterogeneous population of microorganisms that included irregular spherical shapes, spores, and a variety of rod types. A methanogenic enrichment culture from an oil-bearing sandstone inoculums contained both *Methanobacterium* spp. and *Methanosarcina spp.* as indicated by the presence of long filamentous rods and irregular spherical packets. The maximum colony diameter observed was 3 to 6 mm, 2 to 3 mm and less than 1 mm for the three types of strains in that study. The rod type colony observed in Figure 4.34b might be of the same group of colonies.

4.3.2.3 Discussion

In an anaerobic process, many factors can affect the quality and quantity of biomass particles such as:

1) the type of wastewater, the biodegradability of the organic matter, the presence of finely dispersed non-biodegradable organic and inorganic matter, the ionic-composition (concentration of uni- and divalent cations) and the presence of inhibitory compounds.
2) the availability of essential nutrients,
3) the pH, which is different depending on the substrate and
4) the temperature, since the specific activity of methanogenic sludge is highly temperature dependent.

In this study, it seems that the dominant factor in the formation of different bacterial cultures was the composition of the influents to each reactor. More specifically, the morphological structure of the particles was affected by the composition and concentration of the substrates and metabolites, such as

sucrose, formate, petroleum hydrocarbons, etc. As was seen for reactor 1, the particles had a smaller size range compared to reactor 2 and their appearance and color were also different.

A schematic of microbial structure shown in Figure 4.36 has been proposed by Fang et al. (1995) for granules treating soluble hydrocarbons. As can be seen in this figure, a multi-layer structure has been proposed for an anaerobic biomass particle. It is shown that acidogens which are responsible for breaking down macromolecules in a complex substrate are concentrated in the outer layer of the granule and they can be part of the constituents of the light brown layer for the biomass degrading the complex substrate in this study (reactor 2). The methanogens, on the other hand, are mostly concentrated in the core layer having a darker color. Since for reactor 1 the methanogenic reactions were predominant, more population of methanogens were expected compared to reactor 2. The fact that smaller particles with dark gray color were observed in reactor 1, suggests that the population of acidogens might have been decreasing in this reactor resulting in a change in the microstructure of the biomass particles.

Also for reactor 2, observation of new microbial colonies (as shown in Figure 4.33 a and b) suggests the creation of new colonies, possibly responsible for biodegradation of petroleum hydrocarbons in this reactor.

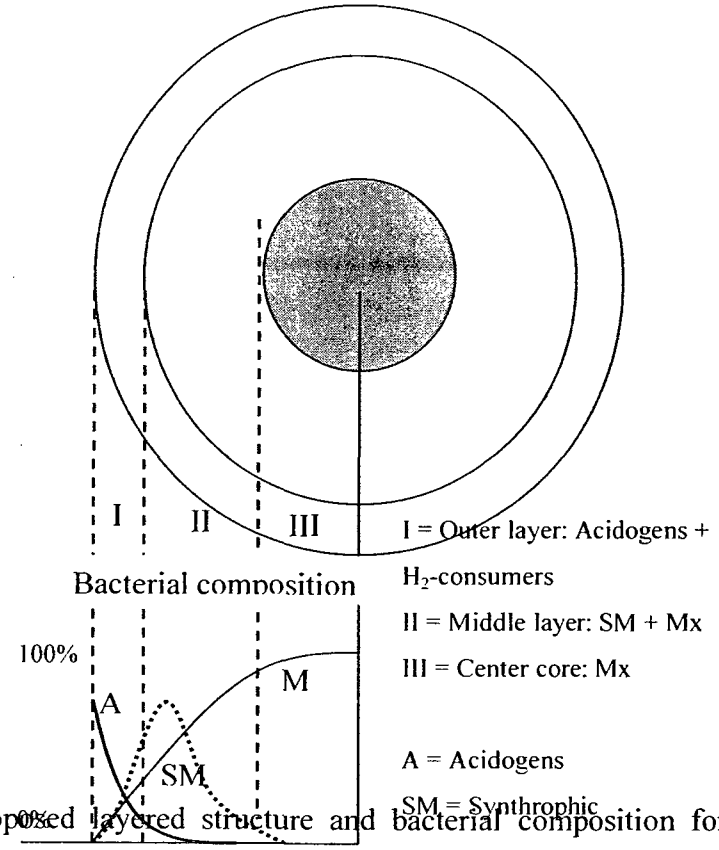


Figure 4.36 Proposed layered structure and bacterial composition for the granules treating soluble carbohydrates. (Reproduced from Fang et al. 1995).

Chapter 5: Multivariable Optimization: Method of Evolutionary Operation (EVOP) Factorial Design

5.1 Introduction

The EVOP method is a very efficient and robust technique to optimize a multivariable process. This technique uses a combination of the factorial method for designing experiments and the EVOP methodology for analyzing the experimental results with a certain level of confidence and leads to a conclusion based on the best combination of the process parameters. It has many advantages over existing and traditional techniques such as those considering only one parameter at a time.

For biological approaches, this technique has been used mostly for enzymatic experiments. Examples of this kind are production of gallic acid using filamentous fungi from tannin-rich mixed substrates (Mukherjee and Banerjee 2004) and amylase and protease production from *Aspergillus awamori* (Negi and Banerjee 2006).

This technique has been used in this research as an engineering approach to optimize the system for the three operating conditions of pH, temperature and organic loading rate. The base conditions were those obtained in the previous section with the method of single-variable optimization. The target parameters were CO_2 reduction and CH_4 production for the first reactor and TPH reduction for the second reactor. As a result, 8 (2^3) runs representing 8 combinations of the operating parameters were investigated for each reactor.

5.2 Description of the Method

The EVOP methodology can be considered to be a multivariable sequential search technique, in which the effects of two or three factors are studied together and the responses are analyzed statistically to reach a decision. The search is made sequentially and the design of the next phase of experiments requires the results of the earlier phase of experiments (Tunga et al. 1999).

The variables under investigation are set to a particular value (level) during the experiment. These variables may be quantitative or qualitative. The levels of these variables should be set with the specific purpose of understanding their impact on the response variables. These variables are also called parameters, inputs, controlled variables, independent variables or X variables (Lynch 2003).

The results or the response variables from the experimental run are the output that is of special concern to the experimenter. An understanding of the relationship between the response variables and the inputs is important to optimize the response variable by setting the input variables to their optimal levels. The term response variable is also called response, output, uncontrollable variable, dependent variable, effect, Y variable, result and outcome (Lynch 2003).

In a system having n variables, the total number of new experiments to be conducted is 2^n in addition to 2 control (search level) experiments. The parameters or variables (n) for the above experiments are considered in two states: both the higher level (+) and lower level (-) compared to the parameters in the base conditions which is normally assumed to be the initial optimum level. The new experiments are divided into two blocks (Block I and Block II) and each of these has one set of control experiments (base conditions).

For each block, new experiments will be done based on different combinations of lower and higher level parameters. Each new experiment in Block I must have an odd number of lower level (-) parameter(s), such as, 1, 3, 5, 7, n, if n is an odd number, or (n-1), when n is an even number. Block II, should have an even number of lower level (-) parameter(s), such as, 2, 4, 6, 8,. . . . or n/2, if n is an even number, or (n-1)/2, when n is an odd number. For Block II, one more experiment will have n higher level (+) parameters. The above mentioned arrangements distinguish the overall responses of Block I from those of Block II. When Block I exhibits lower level (-) responses, Block II exhibits higher level (+) responses compared to those of control experiments. Table 5.1 shows how the experiments are distributed between Block I and Block II for n=3.

Table 5.1 Distribution of experiments between block 1 and block 2 for n = 3

Parameter	**B1**	**R1**	**R2**	**R3**	**R4**	**B2**	**R5**	**R6**	**R7**	**R8**
Temperature (°C)	0	-	-	+	+	0	+	-	+	-
pH	0	-	+	-	+	0	+	-	-	+
Organic Loading Rate (g COD/L.d)	0	-	+	+	-	0	+	+	-	-
Response Parameter	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	a_9	a_{10}

The response parameters can be obtained in duplicate by repeating all the experiments in two cycles (cycles I and II). Therefore, the standard deviation and error limits can be minimized. The results of cycles I and II are recorded separately to determine their differences and average values. The effects of each parameter and also their interactions are evaluated based on the average results of two cycles. The standard deviation and error limits based on a 95% confidence level are estimated from the differences according to the Table 5.2 (Tunga et al. 1999). For an n-variable system, the effect of each parameter is called a zero order interaction and the total number of such effects can be estimated as N_1. When the

interaction of two parameters are considered, their effects may be called a first order interaction and the total number of such

Table 5.2 Calculation of standard deviation and error limits

Parameter	Expression
Standard Deviation (σ)	$(\sigma_1+\sigma_2)/2$
σ_1	$R_1 \times f$
σ_2	$R_2 \times f$
R_1	Largest difference – Smallest difference in Block I
R_2	Largest difference – Smallest difference in Block II
f	Statistical Constant (0.3 for number of cycles 2 and number of experiments per cycle up to 32)
Error limits	For average = $\pm$ 1.414 σ For effects = $\pm$ 1.004 σ For change in mean effects = $\pm$ 0.891 σ

effects is expressed as $N_2 = n(n - 1)/2!$. For an n-variable system there will be up to (n-2)th order interaction and the number of such interactions is 1. There will also be one 'change in mean effect'. Therefore, the total number of all kinds of effects in an n-variable system can be expressed as follows:

Total number of interactions = $N = N_1 + N_2 + \ldots\ldots + N_n$ + change in mean effect (5-1)

To describe this method for a three-parameter system (the present work), let's consider the parameters as P_1, P_2 and P_3. In this case according to Eq. 4-3, the number of zero order interactions is 3. These are the effects of P_1, P_2 and P_3 as individual parameters. The number of first order interactions where two parameters are involved is 3 or P_1P_2, P_1P_3 and P_2P_3. The number of second order interactions is 1 or $P_1P_2P_3$.

The single and multiple-effects of parameters for the three-variable system in Table 5.1 is presented in Table 5.3. The magnitudes of effects, error limits and change in mean effect were examined as per the decision making procedure to arrive at the optimum.

Table 5.3 Calculation worksheet for effects of the three-variable system in Table 4.5

Effect of	Calculation of effects
T*	$(-a_2 -a_3 +a_4+ a_5+ a_7 -a_8+ a_9 -a_{10})/4$
P*	$(-a_2 +a_3 -a_4+ a_5+ a_7 -a_8- a_9 +a_{10})/4$
Load*	$(-a_2 +a_3 +a_4 -a_5 +a_7 +a_8 -a_9 -a_{10})/4$
TP	$(+a_2 -a_3 -a_4 +a_5 +a_7 +a_8 -a_9 -a_{10})/4$
TLoad	$(+a_2 -a_3 +a_4 -a_5 +a_7 -a_8 -a_9 +a_{10})/4$
PLoad	$(+a_2 +a_3 -a_4 -a_5 +a_7 -a_8 +a_9 -a_{10})/4$
TPLoad	$(-a_2 -a_3 -a_4 -a_5 +a_7 +a_8 +a_9 +a_{10})/4$
Change in mean	$(a_2 +a_3 +a_4 +a_5 +a_7 +a_8 +a_9 +a_{10} -4a_1 -4a_6)/10$

*T=Temperature, P = pH and Load = Organic Loading Rate

5.3 Analysis of the Results

The effect of changes in individual parameters and multiple input variables are assessed compared to the control (base) conditions to check if whether any change in the control experimental conditions will help to improve the objective variables (i.e. response) and if so, which is the desired direction of change. The magnitudes of the effects are compared with that of the error limits for this purpose. If all or any of the effects are larger than the error limits, the change in the experimental conditions may lead to better

results. In order to make a decision on the desired direction of change of a variable, the following procedure is to be considered (Tunga et al. 1999).

1- If the effect of the parameter under consideration is positive and larger than the error limit and the change in mean effect is small, then, a) increasing the value of the variable(s) will help to maximize the response or objective function, b) reducing the value of the variable(s) may help to minimize the response.

2- If the effect is negative and larger than the error limit and the change in mean effect is small, then reducing variable(s) will help maximize the response function and vice versa.

3- If the effect is smaller than the error limit while the change in mean effect is large, then, a) the maximum has been reached if the change in the mean effect is negative, b) the minimum has been reached if the change in the mean effect is positive.

4- If the effects are negative or positive and smaller than the error limit and the change in the mean effect is also small, then it will be advisable to select a new search region and start a new phase of experiments.

5.3.1 Application of the Method for the Two-Step Reactor System

As indicated in Table 5.3, the input parameters were pH, temperature and organic loading rate and the target parameters were CO_2 removal and CH_4 generation for the first reactor and TPH removal for the second reactor. The control or search level experimental conditions were selected based on the results of the single-parameter optimization (indicated as B in the results). Then the new experimental

conditions were selected based on the lower and higher levels of the input parameters and are indicated as R1 to R8. The reactors were run under the operating conditions at least twice for each case and the target parameters were measured and recorded for each reactor. This was called cycle I (from R1 to R8). Then the same experiments were repeated for the same cases of R1 to R8 and the results were recorded as cycle II. Tables 5.4 to 5.7 show the results for both reactors.

Table 5.4 Results of application of the EVOP method for the two-step reactor system (Reactor 1)

Parameter \ Run	B	R1	R2	R3	R4	B	R5	R6	R7	R8
Temperature(°C)	35	32	38	38	32	35	32	38	38	32
pH	3.5	2.5	4.5	2.5	4.5	3.5	2.5	4.5	2.5	4.5
Organic Load (mgCOD/L.d)	7800	6240	6240	10400	10400	7800	10400	10400	6240	6240
CH_4 (mL-cycle I)	160	155	180	100	126	160	83	184	240	225
CH_4 (mL-cycle II)	140	170	192	91	133	140	94	136	210	190
Difference	20	-15	-12	9	-7	20	-11	48	30	35
Average	150	162.5	186	95.5	129.5	150	88.5	160	225	207.5
CO_2 Removal (%-cycle I)	90	97	96.5	87.2	67.3	95	61.2	72	96	62
CO_2 Removal (%-cycle 2)	95	97	90	83	70	93	74	67	98	97
Difference	-5	0	6.5	4.2	-2.7	2	-12.8	5	-2	-35
Average	92.5	97	93.25	85.1	68.65	94	67.6	69.5	97	79.5

Table 5.5 Error analysis of the results for the two-step reactor system (Reactor 1)

Effects	CH_4 Production	CO_2 Removal
T	19.63	8.03
P	27.88	-8.95
Load	-76.88	-18.98
TP	-15.13	-0.73
TLoad	-0.88	1.15
PLoad	24.88	1.68
TPLoad	26.88	-7.60
Change in the mean effect	5.45	-8.84
Standard Deviations	12.45	4.40
Error limits		
For Average (±)	17.60	6.21
For effects (±)	12.50	4.41
For change in the mean effect (±)	11.09	3.92

*T=Temperature, P = pH and Load = Organic Loading Rate

Table 5.6 Results of the application of the EVOP method for the two-step reactor system (Reactor 2)

Parameter \ Run	B	R1	R2	R3	R4	B	R5	R6	R7	R8
Temperature(°C)	36.5	33.5	39.5	39.5	33.5	36.5	33.5	39.5	39.5	33.5
pH	7.5	6.5	8.5	6.5	8.5	7.5	6.5	8.5	6.5	8.5
Organic Load (mgCOD/L.d)	450	394	394	525	525	450	525	525	394	394
TPH Removal (%-cycle I)	75	55	60	63	62	75	54	58	72	62
TPH Removal (%-cycle II)	77	70	67	70	66	77	65	67	83	75
Difference	-2	-15	-7	-7	-4	-2	-11	-9	-11	-13
Average	76	62.5	63.5	66.5	64	76	59.5	62.5	77.5	68.5
CH_4 (mL-cycle I)	560	360	460	620	450	560	650	520	550	454
CH_4 (mL-cycle II)	600	420	500	650	480	600	670	470	580	500
Difference	-40	-60	-40	-30	-30	-40	-20	50	-30	-46
Average	580	390	480	635	465	580	660	495	565	477

Table 5.7 Error analysis of the results for the two-step reactor system (Reactor 2)

Effects	TPH Removal	CH_4 Production
T	3.88	45.75
P	-1.88	-83.25
Load	-4.88	85.75
TP	-7.13	-29.25
TLoad	-1.13	-43.25
PLoad	2.13	-84.25
TPLoad	2.88	56.75
Change in the mean effect	-8.35	-47.30
Standard Deviations	2.25	18.00
Error limits		
For Average (±)	3.18	25.45
For effects (±)	2.26	18.07
For change in the mean effect (±)	2.00	16.04

*T=Temperature, P = pH and Load = Organic Loading Rate

Based on these results, the following conclusions can be made for the operation of the two reactor system. These conclusions are based on the effects on the target (output) parameters:

5.3.1.1 Conclusions for Reactor 1

- The individual and interactive effects are more significant for CH_4 production compared to CO_2 reduction. This could be due to the presence of light components of dissolved hydrocarbons whose partial degradation occurs in this step and is affected by the operating conditions (mostly pH and organic load as described below).

- The importance of the effect of individual parameters on CH_4 production is in the following order:

Organic loading > pH > Temperature

This behavior is due to the short retention time (about 4 h) in this reactor and sensitivity of methanogens to high loading rates and pH.

- The pH and temperature have direct effects on the reactor operation for CH_4 production while it is inversely affected by the organic loading. The methanogenic reactions are mostly affected by pH as methanogens are more sensitive to pH and higher organic loading is equivalent to lower retention time in the reactor and therefore less degradation time available for the biomass.

- The interactive effects of pH-organic load and temperature-pH-organic load are more important for CH_4 production in this reactor. This is due to relative importance of pH and organic load compared to the temperature for this reactor. As an example of application of interactive parameters, it was observed that it is possible to operate this reactor at even lower pH values (2.5) compared to the base conditions (3.5) with decreasing the organic loading rate.

- The overall results of CO_2 removal and CH_4 production obtained in Cycle II (90% -98% and 91ml -210 ml respectively) were better in comparison to those in Cycle I (61.2% -97% and 83 ml -240 ml respectively) which could be due to improved acclimation of the reactor biomass to the operating conditions.

As the overall conclusion for this reactor, the optimum operating conditions for this reactor was found to be the conditions of R7 (T= 38°C, pH 2.5 and organic loading rate = 6240 mg COD/L.d) for which CO_2 removal of 98% and CH_4 production of 240 ml) were obtained. These conditions will be assessed further for sustainability in the next chapter.

5.3.1.2 Conclusions for Reactor 2

For this reactor, the primary optimum conditions of 36.5°C, 7.5 and 450 g COD/L.d for the temperature, pH and organic loading rate were selected as the base conditions (Table 6) and the same order of higher and lower-level conditions as in the first reactor were investigated for the target parameter of TPH removal. Although not a main target parameter, CH_4 production was monitored in this reactor. Results of Table 5.6 were evaluated based on a statistical analysis according to the importance of the effects regarding their magnitude and sign as shown in Table 5.7.

In this reactor and for the TPH removal, the change in mean effect is greater than the error limits and the effects are within or around the error margins. The order of effects of the individual variables on the TPH removal can be expressed as follows:

Organic loading > Temperature > pH

Organic loading rate was shown to have an indirect effect on the TPH removal and the effect of temperature was less significant. As a general rule, rate of enzymatic reactions in the cell approximately doubles for a temperature rise of 10°C (Nester et al., 2001). However, there is an upper limit for the temperature that microorganisms can function. Many bacteria that degrade petroleum hydrocarbons are mesophiles which have an optimum temperature ranging from 25°C to 45°C (Nester et al., 2001). The effect of pH was within the error limits showing no considerable effect on the TPH removal. From the Table 5.6 it can be seen that the TPH removal is maximum (83%) for the conditions of the run 7.

For CH_4 production, the change in mean effect is larger compared to the error limits whilst the effects are out of the error margins. The importance of effect of the individual parameters on CH_4 production is in the following order:

$$\text{Organic loading} \cong \text{pH} > \text{Temperature}$$

Increasing organic loading between R3 and R7 showed to have direct effects on the CH_4 production. The maximum CH_4 production (650 mL) was obtained in R3 with the same pH but higher organic loading compared to R7, suggesting an increase in the organic loading due to carry over from the first reactor. Regarding the pH, for lower-level cases such as R3 and R7, the CH_4 production generation was higher than the higher-level cases (pH 8.5). This could be due to the fact that pH of 6.5 is around the neutrality and still in the proper range for most methanogens (6.5-7.6).

The overall results of TPH removal obtained in Cycle II (65% -80%) were better compared to those in Cycle I (55% -72%). Also CH_4 production was 420 ml -670 ml for Cycle II compared to 360 ml-650 ml for Cycle I. Again as observed for reactor 1, the improved results could be due to improved acclimation of the reactor biomass to the substrate and operating conditions.

As the overall decision for the second reactor, the best operating conditions were found to be the conditions of R7 (T= 39.5°C, pH 6.5 and organic loading rate = 394 mg COD/L.d) for which the TPH removal was maximum (83%). For these conditions, CH_4 production was considerably high (580 ml). Although this is less than 650 ml obtained for the conditions of R3, the TPH removal as the main target parameter for this run was 66.5% which was less than 80% for R7.

Chapter 6: Sustainability Analysis

6.1 Anaerobic Treatment and Sustainability

During the last few decades technologies based on the anaerobic treatment of wastewaters and organic wastes have been applied successfully to a wide variety of problems (Lema and Omil, 2001). Anaerobic digestion and treatment processes, when properly applied, are successful sustainable processes. They are yet to be improved for aspects such as effect of trace elements, environmental factors, etc. (Lettinga 2005).

It has been a challenge to prove the feasibility of anaerobic treatment, despite the obvious advantages in energy consumption, sludge production, and required land area. Issues such as required effluent polishing, odors, sensitivity to toxic compounds, have made the potential users reluctant to choose this process rather than the conventional aerobic systems. It has been shown that intensive research has overcome most of these drawbacks (Lexmond et al. 2001).

Anaerobic processes have been extensively used for the digestion of primary and secondary sludge in wastewater treatment plants based on conventional aerobic systems such as the activated sludge and trickling filter systems. More attention has been paid to anaerobic technology for improving the sustainability of sewage treatment after the energy crisis in the 1970s. Now, there is more opportunity for designing new treatment systems based on information obtained on biological and physical-chemical processes related to the recovery or removal of nitrogen, phosphorus and sulfur compounds. The design of domestic sewage treatment plants with the anaerobic reactor as a core unit coupled to the pre- and

post-treatment systems in order to promote the recovery of resources and the polishing of effluent quality can improve the sustainability of treatment systems (Eugenio et al. 2006).

A combination of anaerobic pre-treatment followed by photosynthetic post-treatment is suggested by Gijzen (2002) for the effective recovery of energy and nutrients from sewage. This approach is based on the nutrient assimilating capacity of photosynthetic plants. It is claimed to be energy efficient, cost effective and applicable under a wide variety of rural and urban conditions. It is concluded that a natural systems approach towards waste management could generate affordable eco-technologies for effective treatment and resource recovery.

In addition to net energy production, anaerobic treatment produces biosolids that are good soil conditioners. It also requires less reactor volume and destroys troublesome hazardous chemicals. According to McCarty (2001), anaerobic treatment has met the 1995 NRC criteria for sustainable development.

In Alberta, a study has been done on the improvement of the feasibility and utility of bioreactor projects in the agricultural and food processing industry, and to help reduce the capital investment needed to initiate these projects. According to this study, the produced biogas can be utilized onsite or be integrated with the upstream oil and gas (UOG) industry. Some of the results of this study for establishing a feasible biogas plant are as follows (Jamin et al. 2005):

1- Most of the capital costs of a biogas project are for the anaerobic digesters and feed preparation equipment. The incremental costs of any biogas processing and utilization equipment normally amount to less than 16 percent of the total capital cost.

2- The best biogas production opportunity areas are near meat processing plants and the maximum economic benefit occurs when meat waste has a disposal cost. Biogas plants are most viable when all excess energy can be utilized by the owner and they are located close to adequate sources meat waste and manure. Maximum environmental and economic benefit is achieved when the biogas plant is considered a waste handling facility that disposes of manure and meat waste while producing clean water, liquid fertilizer, solid soil amendment and finally biogas.

3- Storage, mixing and reactor tanks represent the bulk of initial capital costs. Choosing shop fabricated tanks instead of more expensive field constructed tanks is an effective choice for minimizing total plant cost and improving economic acceptability for potential proponents up to medium sized applications (i.e. bioreactor volume up to 3400 m^3).

Nowadays, more and more advanced technologies make benefit of new techniques based on anaerobic treatment to generate sustainable fuels such as biogas. For such technologies, certain criteria should be met to consider them as being feasible, and more importantly, environmentally sustainable. There are different methods of evaluating sustainability such as life cycle analysis (LCA) or net energy balance (NEB) analysis and simulation models such as ORWARE (Organic Waste Research) can be used depending on the application.

6.2 Sustainability of Biofuels

6.2.1 Global Warming Effect of the Greenhouse Gases

For each biogas generating plant, direct greenhouse gas (GHG) offset credits can be calculated. Direct GHG offset credits are a potential source of revenue and for a small-scale bioreactor might range from 2000 to 5000 tonnes of carbon dioxide equivalent (CO_2E) per year (Jamin et al. 2005). CO_2E represents the amount of global warming of greenhouse gases in terms of the amount of carbon dioxide (CO_2) that would have the same global warming potential (GWP). It is measured over a specified timescale (generally, 100 years). GWP is a measure of the contribution of a greenhouse gas to global warming and is developed by the Intergovernmental Panel on Climate Change (IPCC- Jamin et al. 2005).

For a given gas, GWP is calculated over a specific time interval compared to carbon dioxide (GWP = 1) using equation 6-1. In this equation, RF is radiative forcing and for each gas, it is positive if the gas has global mean surface warming effect and is negative if it has global mean surface cooling effect. GWP is estimated based on the time-integrated global mean RF of a pulse emission of 1 kg of the gas relative to that of 1 kg of the reference gas CO_2.

$$GWP \equiv \frac{\int_0^{TH} RF(t)dt}{\int_0^{TH} RF_r(t)dt} = \frac{\int_0^{TH} a[C(t)]dt}{\int_0^{TH} a_r[C_r(t)]dt} \qquad (6\text{-}1)$$

where TH is the time horizon and C(t) and $C_r(t)$ are the time-dependent abundance of the gas under question and the reference gas respectively. The terms in the numerator and denominator are called the

absolute global warming potential (AGWP). Table 6.1 shows values of GWP for some greenhouse gases (Forester et al. 2007).

Table 6.1 Values of GWP for some greenhouse gases. (IPCC/TEAP* 2005).

Gas	Lifetime (years)	GWP time horizon		
		20 years	100 years	500 years
Methane (CH_4)	12	72	25	7.6
Nitrous oxide (N_2O)	114	310	298	153
Hydrofluorocarbon (HFC-23)	270	12000	14800	12200
Hydrofluorocarbon (HFC-134a)	14	3830	1430	435
Sulfur hexafluoride (SF_6)	3200	16300	22800	32600

* Technology and Economic Assessment Panel

6.2.2 Net Energy Balance (NEB) and Net Energy Ratio (NER) Concept

In order to produce biofuels, an initial energy is required. The concept of net energy balance is to evaluate the net energy requirement to produce a fuel. The value of NEB can be calculated as shown in Equations (6-2) (Dewulf 2006). NER as indicated in Equation (6-3) can be measured to assess the sustainability of biofuels. The higher value of NER means the biofuel is more sustainable.

$$NEB = A - B \qquad (6\text{-}2)$$

A= Energy content of a fuel

B = Energy content of the petroleum and other fossil energy sources used over the fuel's entire production cycle

$$NER = A/C \qquad (6\text{-}3)$$

C = Energy input to the process for fuel production

NEB is negative and NER is less than 1 for all petroleum fuels because it takes energy to convert one form of fuel to a more useful form. Calculation of NEB for biofuels is more complicated and more factors such as energy used for energy extraction, energy transportation, feedstock production and feedstock conversion should be considered. Also, the energy content of other auxiliary materials that are made from energy resources such as fertilizers, pesticides and other petrochemicals need to be included.

NER has been calculated for some biofuels such as ethanol (International Energy Agency 2004). Most recent values of NER for corn and sugar cane ethanol have been reported to be 1.35-1.67 (Dewulf 2006) and 8.3-10.2 (Macedo et al. 2004), respectively.

Börjesson (2004) has studied the energy efficiency of producing transportation fuels from energy crops; ethanol from wheat, and biogas from wheat or lea crops. The results are presented as energy balances (transportation fuel output divided by total energy input) and net energy output per hectare and year (transportation fuel output minus total energy input). The calculations are based on the total primary energy input, including both direct and indirect energy inputs. Reported values were 1.3 to 2.4 for the net energy balance of the transportation fuel chains for the net energy output from about 20 up to about 60 GJ per hectare.

According to the European Biomass Industry Association (EUBIA) which is committed to reduce GHG emissions by 8% from 2008 to 2012, biogas can contribute substantially to the sustainable energy recovery from agriculture and the organic fraction of wastes. They have reported values of energy inputs and outputs for these wastes as shown in Table 6.2.

Table 6.2 Energy inputs and outputs for biogas production from different agricultural wastes. (reproduced from European Biomass Industry Association website (http://www.eubia.org/108.0.html)

Raw material	Estimated dry matter content (%)	Energy input			Energy output	
		Handling of raw material (GJ/dry t)	Transportation of raw material (MJ/dry t, km)	Transportation of digested residues (MJ/dry t, km)	Estimated biogas yield (GJ/dry t)	
Lea crops	23	1.7	4.8	20	11	(9.5-17)
Sugar beets tops+ leaves	19	0.6	5.8	20	11	(8.5-14)
Manure-pig	8	n/a	14	14	7	(5.6-8.5)
Manure-cow	8	n/a	14	14	6.2	(5.6-8.5)
Slaughter waste	17	n/a	11	20	9.4	-
Grease separator sludge	4	n/a	80	20	22	(20-27)
Municipal organic waste	30	0.6	15	20	14	(8.8-19)

6.2.3 Calculation of NER for the Two-Step Reactor System

This parameter was calculated for the two-step reactor system with input and output streams as shown in Figure 6.1. For the energy input, the energy yield reactions and values of Gibbs free energy for formate and sucrose were considered which are given in Table 6.3. For the dissolved crude oil, the methanogenic degradation data were used where available and for the remaining hydrocarbons, the energy input was calculated based on the COD equivalent concentration and theoretical methane yield. Since the original temperature of the waste is 70°C, it was assumed no energy is required for the heating the waste to the mesophilic range of temperature (about 35°C). For the energy output, an energy content of methane (39.2 kJ/L at standard conditions of 101.3 kPa and 20°C) in the biogas was calculated and the energy content of methane dissolved in the effluent (treated stream) is considered as a loss. Then NER was calculated based on the energy inputs and outputs.

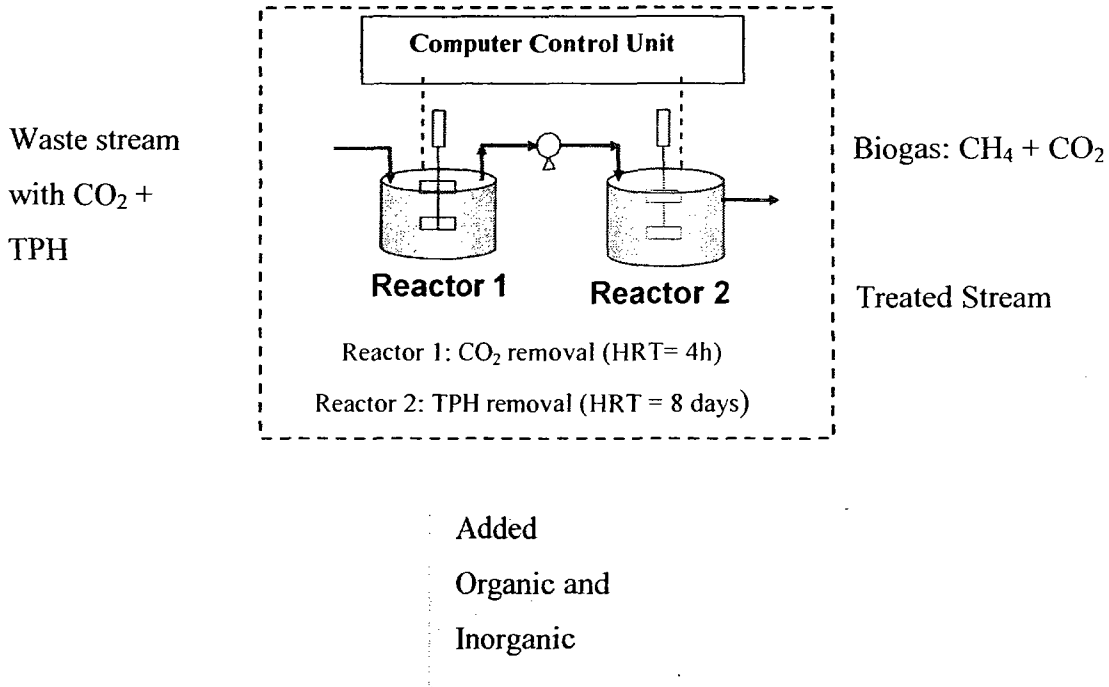


Figure 6.1 Inputs and outputs of the two-step reactor system

Table 6.3 Energy yield reactions of anaerobic degradation of formate, sucrose and BTX.

Material	Reaction	ΔG° (KJ/mole)	Source
Formate	$4HCOO^- + H^+ + H_2O \longrightarrow CH_4 + 3HCO_3^-$	-130.1*	Ferry (1993)
Sucrose	Sucrose + $H_2O \longrightarrow 6CO_2 + 6CH_4$	-790	Valdez-Vazquez et al. 2005
Benzene	$C_6H_6 + 6.75H_2O \longrightarrow 2.25HCO_3^- + 3.75CH_4 + 2.25H^+$	-116	Ulrich and Edwards (2003)
Toluene	$C_7H_8 + 7.5H_2O \longrightarrow 2.5HCO_3^- + 4.5CH_4 + 2.5H^+$	-131	Edwards and Grbic-Galic (1994)
Xylene	$C_8H_{10} + 8.25H_2O \longrightarrow 2.75HCO_3^- + 5.25CH_4 + 2.75H^+$	-169	Edwards and Grbic-Galic (1994)

*Negative values of the Gibbs free energy indicate a feasible thermodynamic process (exergonic) (in comparison to positive values that indicate impossible processes (endergonic)).

The values of NER were calculated using Equation 6.3 for all runs of the system (R1 to R8) in the optimization step and the results are shown in Table 6.4. It can be seen that for each run of the reactors and for a certain value of the energy input, the energy output of the system changed based on the selected operating conditions. This shows how interactive effects of the operating conditions can

influence the net energy produced in the system. These effects were previously discussed in the optimization step and the results here show the consistency between the overall results from the optimization step and the sustainability assessment. The results in Table 6.4 showed that the operation of the system was more sustainable for the conditions of R7, for which the maximum value of NER=3.7 was calculated.

Table 6.4 Calculated NER values for the biogas in all runs of the two-step reactor system under different operating conditions.

Run	Reactor	Operating Conditions			Total Energy input (kJ)	Total Energy output (kJ)	NER
		T (°C)	pH	OLR (mgCOD/L.d)			
R1	1	32.0	2.5	6240	6.1	23.1	2.5
	2	33.5	6.5	394			
R2	1	38.0	4.5	6240	6.1	27.1	2.9
	2	39.5	8.5	394			
R3	1	38.0	2.5	10400	8.8	31.0	2.6
	2	39.5	6.5	525			
R4	1	32.0	4.5	10400	8.8	24.0	2.0
	2	33.5	8.5	525			
R5	1	32.0	2.5	10400	8.8	29.9	2.5
	2	33.5	6.5	525			
R6	1	38.0	4.5	10400	8.8	28.1	2.3
	2	39.5	8.5	525			
R7	1	38.0	2.5	6240	6.1	34.5	3.7
	2	39.5	6.5	394			
R8	1	32.0	4.5	6240	6.1	30.2	3.3
	2	33.5	8.5	394			

The value of NER for the biogas production in an anaerobic process can be affected by the raw material used and the energy input. Other factors such as transportation can also be influential as indicated in Table 6.2. Table 6.5 shows typical values of NER estimated for the biogas production in several anaerobic processes.

Table 6.5 NER values for biogas production in anaerobic processes

Raw material	NER	Reference
Biomass crop	1.5-3.1	Demuynck et al. (1984)
Mixed crop and livestock	1-2	Bender (2001)
Organic waste (municipal)	5-12	RIS International (2002)
Biomass feedstock	4.1	Banks (2005)
Brewery wastewater	5.4	Getz et al. (2007)
Algal biomass	5.5-7.5	Chisti (2008)

The estimated NER value of 3.7 in this study can be compared to the anaerobic processes treating wastewaters containing soluble organic materials. However, the processes using renewable sources of energy such as biomass or those treating industrial and/or municipal wastewaters generally have higher NER values. The main reason for this difference is the use of finished products such as sucrose and formic acid in this study whose production involves consumption of energy. Nevertheless, the use of such materials was minimized to obtain the highest possible NER.

Chapter 7: Kinetic Study of the Systems

7.1 Introduction

In this chapter, the kinetics of the system for both reactors are discussed. The experimental data for anaerobic degradation of materials for both reactors are compared with the data obtained from the kinetics models. The best kinetics parameters were estimated based on the best fit between the experimental data for substrate utilization and biomass production and those predicted from the numerical analysis. The results were compared with the available results from the literature and a discussion has been made.

7.2 Kinetics of Anaerobic Degradation

Biodegradation reactions are empirically driven and their kinetics could follow one of the following general forms as shown in Table 7.1.

Table 7.1 General rate expressions for biodegradation reactions

Reaction	Reaction kinetics	Order
A ⟶ P	$\frac{dC_A}{dt} = -k_0$	Zero
	$\frac{dC_A}{dt} = -k_1 C_A$	First
A + B ⟶ P	$\frac{dC_A}{dt} = -k_2 C_A C_B$	Second

where:

k_0, k_1, k_2 = rate constants mol/l.sec, 1/sec, 1/mol.sec, respectively

C_A, C_B = reacting species

t = time

These rate expressions are applicable for the reaction of the compounds with a surface such as a metal catalyst, a soil surface or an enzyme. It happens that in a biodegradation reaction, there are few molecules of reactant, C_A, compared to many of the surface (Case 1) or C_A is so large that covers all surface sites so that the surface is saturated with A (Case 2). In case 1, the reaction rate dC_A/dt is proportional to the concentration of C_A (first order reaction) whereas in case 2, the rate is constant (zero order reaction). Therefore, it is possible to combine the two cases in a general rate expression as follows:

$$\frac{dC_A}{dt} = \frac{-k_0 C_A}{k' + C_A} \qquad (k' = \frac{k_0}{k_1}) \qquad (7\text{-}1)$$

This equation is the general biological form of the equation for growth on a substrate which can be modified to yield the kinetic models such as Michaelis-Menten or Monod type kinetics.

In an enzymatic reaction with the following general equation:

$$S \longrightarrow P \qquad (7\text{-}2)$$

With S and P as the representatives for substrate and product, several steps can be involved:

1) binding the substrate (S) to the enzyme (E) to form the enzyme-substrate complex (ES),
2) conversion of substrate to the product (P) and formation of a new enzyme-product complex (EP);
3) dissociation of the new enzyme-product complex (EP) to the enzyme (E) and the free product (P).

The whole process can be expressed as follows:

$$S + E \rightleftharpoons ES \longrightarrow EP \longrightarrow E + P \qquad (7\text{-}3)$$

In a treatment system using bacteria, the substrate consumption is related to the biomass growth and the general differential equations expressing the biomass (X) production and substrate (S) consumption are as follows:

$$\frac{dX}{dt} = \frac{\mu_m SX}{K_s + S} - k_d X \qquad (7\text{-}4)$$

$$\frac{dS}{dt} = \frac{-1}{Y}\frac{dX}{dt} \qquad (7\text{-}5)$$

where the parameters are defined as:

μ_m = Maximum growth rate of biomass (1/d)

K_s= Half-saturation constant (g/L or mM)

k_d = Biomass death rate (1/d)

Y= Yield (g biomass/ g COD)

In Michaelis-Menten model, the relationship between the rate of reaction (v) and the concentration of (S) in the reaction follows the following equation:

$$-\frac{dS}{dt} = v = \frac{V_{max}.S}{K_m + S} \qquad (7\text{-}6)$$

where: V_{max} = maximal reaction rate (gCOD/m^3.d)

S = substrate concentration (gCOD/m^3)

t = time (d)

Figure 7.1 shows a typical curve resulting from this equation. The parameter K_m is the value of S when $V = V_{max}/2$ and may approximate how strong is the binding of E to S and therefore, shows the substrate affinity of the enzyme.

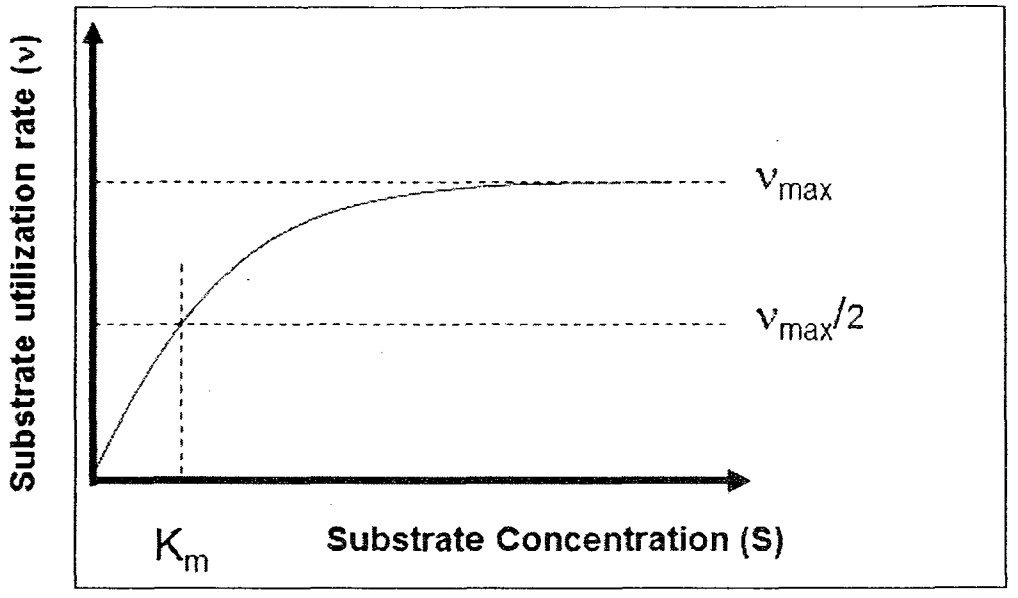


Figure 7.1 Relationship between substrate utilization rate and substrate concentration in Michelis-Menten model.

Michelis-Menten equations often can be used to estimate kinetic parameters of biological processes. Michaelis-Menten kinetics is formulated on the basis of constant catalyzing material (enzymes), and is applicable to a situation in which the growth of microbial cells participating in the degradation is not significant. In other words, the value of V_{max} should not change during substrate consumption (De Zeeuw, 1984).

Bacterial growth kinetics is slightly more complex and follows the classical kinetics proposed by Monod (1950), who studied the fermentation of grape sugars to alcohol. In this case, the rate of substrate utilization is proportional to the concentration of the microorganisms present, X, and is a function of the substrate concentration. The Monod bacterial growth kinetics is traditionally written as:

$$\mu = \mu_{max} \frac{S}{K_s + S} \qquad (7\text{-}7)$$

where

S = Substrate concentration

μ = Specific growth rate of microorganisms (1/d)=Relative increase of mass per time unit

μ_{max} = Maximum specific growth rate (1/d)

K_S = Monod constant (at which the rate of growth is half the maximum rate) (g COD/L)

In this case, K_S is the value of S when $\mu = \mu_{max}/2$. Monod model yields an S-shaped (sigmoidal) substrate depletion curve in batch experiments (Robinson and Tiedje, 1983). Since $\frac{dX}{dt} = \mu X$ and $Y = \frac{dX}{dS}$, where Y= growth yield factor (gVSS/gCOD), it can be derived that:

$$-\frac{dS}{dt} = \frac{\mu_{max} S}{K_s + S} \cdot \frac{X}{Y} \qquad (7\text{-}8)$$

The original set of equations (Equations 7-4 and 7-5) can be solved using numerical techniques. Sometimes simplified models have been used to determine the kinetic parameters. For example, Ahring and Westermenn (1987) used the following integrated solution to determine the kinetic constants for butyrate and acetate hydrogen utilization:

$$\frac{\ln S_0 / S_t}{t} = \left(\frac{-1}{K_m}\right)\left(\frac{S_0 - S_t}{t}\right) + \frac{V_{max}}{K_m} \qquad (7\text{-}9)$$

Where,

V_{max} = Maximum initial velocity or reaction rate that can be reached (the units of K_m/t i.e.1/d)

K_m = Michaelis constant or half-saturation constant (with the same units as "S" i.e. g/L)

S_0 = Substrate concentrations at time = 0 and time = t, respectively (in g/L)

t = time (d)

Degradation of volatile fatty acids (VFAs) in anaerobic media has been investigated in the past (Lawrence & McCarty 1969, Heyes and Hall 1983, Min and Zinder 1989, Fukazaki et al. 1990, Aguilar et al. 1995). Sometimes, a specific substrate utilization rate (**v**) is used as in the following expression (Lin et al. 1986):

$$v = \frac{D(S_0 - S)}{X} \qquad (7\text{-}10)$$

where:

D = Dilution rate (1/d) = $\frac{1}{HRT}$ for a completely mixed reactor

X = Microbial concentration (mg/L)

In that study, values for v_{max}, K_S, Y_g and k_d were determined using the Lineweaver-Burk equation which is a linear form of the Michaelis-Menten model. They plotted data of v vs. D and $\frac{S}{v}$ vs. effluent VFA concentration as suggested by the following equations:

$$\frac{S}{v} = \frac{K_S}{v_{max}} + \frac{1}{v_{max}} S \qquad (7\text{-}11)$$

$$v = \frac{1}{Y_g} D + \frac{K_d}{Y_g} \qquad (7\text{-}12)$$

7.3 Kinetic Parameters of the System and Optimization

In the present study, the original equations (7-4 and 7-5) were solved numerically using a Runge-Kutta 4th order using the initial values of substrate and biomass concentration determined by the routine tests.

The integration was done for the degradation reactions over the retention time and at the operating conditions of the reactors. The kinetic parameters were estimated by fitting the experimental data and the numerical results. To optimize the results, the method of Levenberg-Marquardt (Press et al, 1992) was used to find the best fit. This method uses a non-linear least square regression which minimizes the sum of the square errors for a set of empirical and calculated data as follows:

$$E(\alpha) = \sum_{i=1}^{n} [(y_i - y_i(x_i, \alpha)]^2 \qquad (7\text{-}13)$$

Where;

n = number of data points

E = calculated sum of error squares

y_i, y_i (x_i, α) = empirical and calculated data.

The working volume of each reactors was 1L. For each run of the reactors, the data was obtained for the steady-state conditions. It was assumed that the steady-state condition for the measured parameters (such as effluent quality, biogas rate and methane content) was established. The steady state condition was assumed if the values for two consecutive runs were within ± 5% error limit after the target parameters such as CH_4 production, CO_2 removal, etc. were at their maximum value.

For improved accuracy of the calculations, small time increments were used and the two equations were solved simultaneously. No simplifications were made to solve the equations. Figure 7.2 shows an example of an S-X graph obtained.

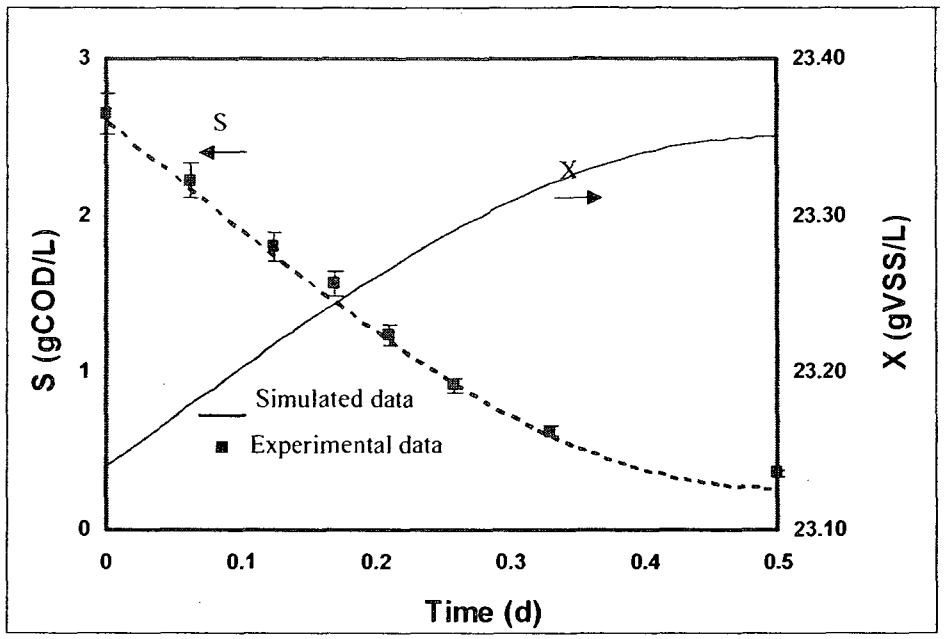


Figure 7.2 Sample of results of the numerical solution for substrate concentration and microbial growth in comparison with the experimental data.

7.3.1 Reactor 1

Since in this reactor the methanogenic reduction of CO_2 was the main process, the kinetic parameters were obtained for anaerobic degradation of the formate based on the experimental data obtained. In many research studies, the kinetic parameters for methanogenic reactions have been estimated for different cultures and experimental conditions such as the substrate used, pH, temperature, etc. Also, since methanogenic strains are sensitive to the environmental conditions and the composition of medium, often different values have been suggested for a specific type of reaction. Also, it has been shown that inhibitory effects on methanogenic reactions from some reaction products or byproducts can affect the kinetic parameters.

7.3.1.1 Kinetic Parameters for Methanogenic Reactions

A review of kinetic parameters for methanogenesis from hydrogen and carbon dioxide and acetoclastic methanogenesis is given by Harper and Poland (1986) as shown in Table 7.2. A large number of studies, especially those about anaerobic degradation of undefined complex substrates, have yielded kinetic parameters, a summary of which has been complied by Henze and Harremoës (1983). Based on an extensive literature review, various researchers proposed a set of kinetic values for the acid-phase and the methane-phase of anaerobic process. Some of these parameters are shown in Table 8.3. With the exception of the hydrolysis step, all other subprocesses of anaerobic treatment have been successfully modeled by Monod kinetics (Switzenbaum, 1990).

Table 7.2 Summary of kinetic data for methanogenesis from H_2 and CO_2 and acetoclastic methanogenesis. (Harper and Poland, 1986).

Culture	T (°C)	k (g COD/ g VSS.d)	K_s (mg COD/L)	μ_{max} (1/d)	Y (g VSS/ g COD)	b (1/d)	Reference
B/Mixed Culture	30	2.6-5.1	-	-	0.02	-	Van den Berg (1977)
B/Mixed Culture	35	2.6-5.1	-	0.08-0.09	0.02	-	Van den Berg (1977)
B/Pure Culture	36	-	320	0.5-0.7	0.03-0.04	-	Smith and Mah(1978)
Methanobacterium sp.	30	26.0	11	0.26	0.01	-	Cappenberg (1975)
Methanobrevibacter arboriphilus	33	-	0.6	1.4	0.04	-	Gujer and Zehnder (1983)
Rumen Bacteria	37	2-8[1]	0.016	-	-	-	Hungate et al.(1970)
Methanospirillum hunguei JF-I[2]	37	1.92[3]	0.093-0.177	0.05	0.017-0.025	-	Robinson and Tiedje (1984)
B/Mixed Culture	35	1.0-1.14	0.17-0.2	0.074-0.081	0.065-0.08	0.033-0.04	Alimahmoodi and Mulligan (2008)

B = Batch; $k = \mu_{max}/Y$; b = Decay rate (similar to k_d in this study)

1 = Expressed as mg COD/g rumen liquid-day

2 = Batch grown on H_2 - CO_2 gas mixture

3 = Assuming a protein content of 60% of dry weight

Other researchers such as Kaspar and Wuhrmann (1978) studied the acetate degradation at 33°C and a retention time of 40 days in a lab-scale digester and obtained values of 13 mg/L to 29 mg/L (COD basis) for the half-saturated constant, which is in the range for K_s given in Table 7.3.

Table 7.3 Representative values of kinetic constants for anaerobic digestion in mesophilic range.

Process	k (g COD/ g VSS.d)	K_s (mgCOD/L)	μ_{max} (1/d)	Y (g VSS/ g COD)	b (1/d)
Acidogenesis[1]	13	200	2.0	0.15	-
Methanogenesis[1]	13	50	0.4	0.03	-
Overall[1]	2	-	0.4	0.18	-
Anaerobic oxidation[2]	6.2-17.1	12-500	0.13-1.2	0.025-0.047	0.01-0.027
Acetoclastic Methanogenesis[2]	2.6-11.6	11-421	0.08-0.7	0.01-0.054	0.004-0.037

1 = Henze and Harremoës, 1983, T = 35°C

2 = Switzenbaum 1990

7.3.1.2 Kinetic Parameters of Formate Degradation in Reactor 1

The data obtained for anaerobic degradation of the formate was based on the results of the last step of the optimization step. The results of the numerical analysis are shown in Figure 7.3 and the kinetics data are tabulated in Table 7.4. The results of curve-fittings and their correlation coefficients are shown in Table 7.5.

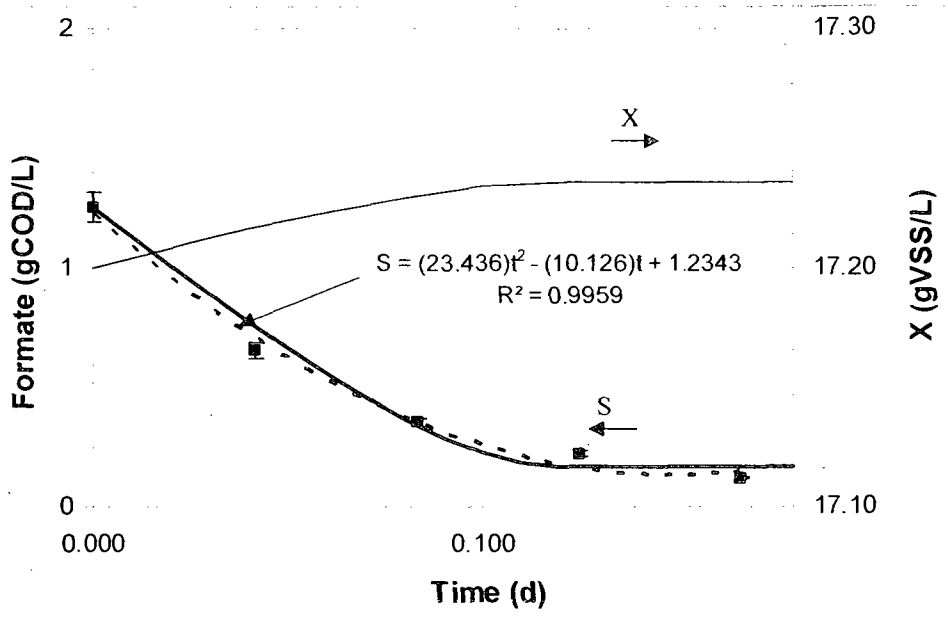


Figure 7.3 Results of formate degradation and bacterial growth in reactor 1. [(T=38°C, pH 2.5, organic loading = 6240 mg COD/L.d, ( ) numerical results, (■) experimental data, (- - -) second-order curve fitting)].

Table 7.4 Values of kinetic parameters for formate degradation in reactor 1.

Parameter	Description	Value
S_0 (g COD/L)	Initial Substrate Conc.	1.250
X_0 (g VSS/L)	Initial Biomass Conc.	17.20
μ_{max} (1/d)	Max. Growth Rate	0.098
K_s (g COD/L)	Half-Saturation Constant	0.070
Y (g VSS/g COD)	Yield	0.033
k_d (1/d)	Biomass Death Rate	0.069
T (°C)	Reactor Temperature	38.000
Δt (d)	Time Increment	0.020

Table 7.5 Comparison of the results for formate degradation in the first reactor.

Time (d)	Formate Concentration (mg COD/L)		
	Exp.	Calc.	Calc. (2^{nd}- order)
0.000	1.25	1.25	1.25
0.042	0.64	0.68	0.65
0.083	0.35	0.32	0.34
0.125	0.20	0.17	0.19
0.167	0.12	0.14	0.17
R^2	-	0.993	0.996

The results from the second-order curve regression showed a good fit with the experimental data suggesting a linear relationship between the formate consumption rate and time. Also the results from the original set of S-X equations showed very good consistency (R^2 = 0.993) with the experimental data. It can be seen from Figure 7.3 that the rate of formate degradation is mostly linear over the degradation time probably because of its simple structure which makes it readily biodegradable. That could be the reason that the second-order model is also a good fit for the experimental data.

Table 7.6 shows some of the results for the kinetics parameters for formate degradation in different anaerobic media. The values of K_m and V_m for Michaelis-Menten model in this study was estimated using the Lineweaver-Burk equation in the differential form as follows:

$$\frac{1}{V} = -\frac{dt}{dS} = \frac{K_m}{V_m}\frac{1}{S} + \frac{1}{V_m} \qquad (7\text{-}13)$$

By plotting the reciprocal of the substrate consumption rate $(\frac{1}{V})$ versus the inverse of substrate concentration $(\frac{1}{S})$ and applying a linear regression, the values of K_m and V_m can be estimated. Figure 7.4 shows these results.

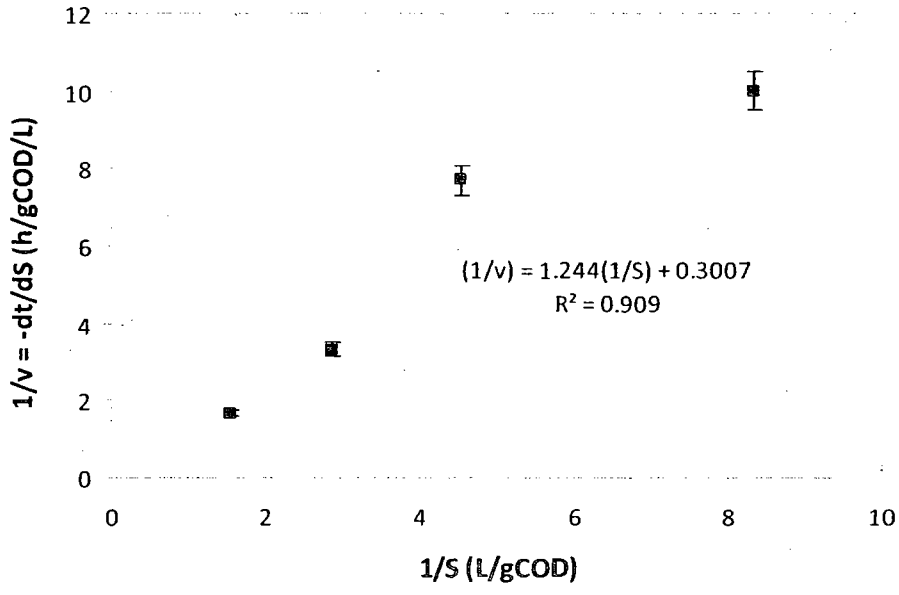


Figure 7.4 Estimation of K_m and V_m for anaerobic formate degradation in reactor 1 using Michaelis-Menten model.

Although the Michaelis-Menten model showed a good fit for the experimental data of formate degradation (R^2 = 0.910), the results obtained from the original set of S-X equations showed a better fit (R^2 = 0.996). The difference could be as a result of factors influencing the degradation of formate. In general, the factors affecting the kinetic parameters in a biological treatment process are the substrate composition, the type of microorganisms and the environmental factors such as temperature, pH, etc. Other factors can also be important. For example, it has been reported that the size of microbial aggregates imposes mass transfer limitations that reduce the half saturation constant (Goodwin et al.

1991). Dolfing (1985) has stated that for the Monod kinetics the effects of mass transfer resistance depend on (1) the bulk substrate concentration, (2) the K_m value of the bacteria for the substrate, (3) the thickness of the biolayer, and (4) the maximum specific activity of the biolayer. K_m values of 0.15-0.30 mM for anaerobic degradation of formate were obtained when the mass transfer resistance was not significant. The corresponding results obtained by Schauer et al (1982) were 0.22- 0.58 mM when the mass transfer increased the K_m values. Therefore, in the kinetic models which are usually based on some assumptions, the values of kinetic parameters can be affected by different physiochemical and/or biological phenomena, while using the original equations seems to be more reliable.

Table 7.6 Values of kinetic parameters for the anaerobic degradation of formate.

Culture	T (°C)	Kinetic Model	K_m	V_m	K_s	μ_m	Y	k_d	Reference (s)
Strains 10-16B, RMB	35	M-M[1]	5-26 μM	0.24 (1/h)	-	-	NA	NA	Lovley et al. (1984)
Methanobacterium formicicum *Methanospirillum hungatei*	35	M-M	0.22- 0.58 mM	0.037 (mole formate/h.g) 0.044 (")	-	-	NA	NA	Schauer et al. (1982)
Formate-/H_2-utilizing Bacteria	35	M[2]	-	NA	0.22 mM	NA	NA	NA	Voolapalli and Stuckey (1999)
Strain FDH_H	24	M-M	7.8-29.5 mM	0.024 - 0.041(min/mM)	-	-	NA	NA	Axley and Grahame (1991)
Formate-fed anaerobic culture	38	Original kinetic equations	16.4 mM*	3.32 (1/h)*	0.64 mM	0.098 (1/d)	0.033 (g/g)	0.069 (1/d)	This work

1-Michelis-Menten Model

2- Monod Model

* Using Michaelis-Menten model

7.3.1.3 Kinetic Parameters for TPH Degradation in Reactor 1

As mentioned in section 4.2.3, some of the TPH was degraded in reactor 1. The trend of TPH concentration in this reactor is shown in Figure 7.5 with the results of the numerical analysis. The kinetic parameters and the experimental and numerical data are shown in Tables 7.7 and 7.8.

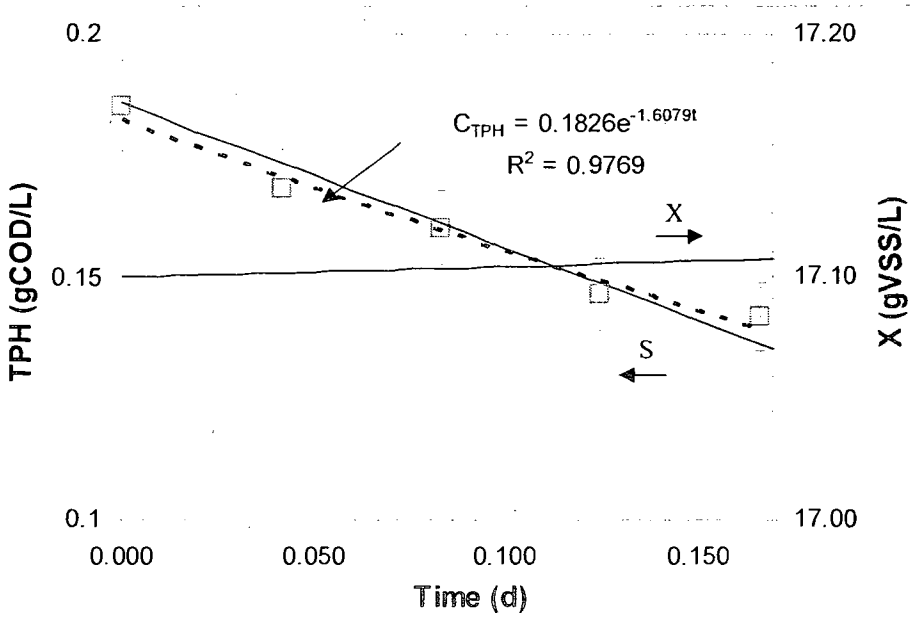


Figure 7.5 Trend of TPH degradation and bacterial growth in reactor 1. [(T=38°C, pH 2.5, organic loading = 6240 mg COD/L.d, ( ) numerical results (▣) experimental data, (- - -) second-order curve fitting)].

It seems that the first order kinetic model is a good fit for TPH degradation in this reactor although not much data is available due to the short retention time in this reactor. More discussion will follow after the results of TPH degradation in reactor 2.

Table 7.7 Values of kinetic parameters for the TPH degradation in reactor 1.

Parameter	Description	Value
S_0 (g COD/L)	Initial Substrate Conc.	0.186
X_0 (g VSS/L)	Initial Biomass Conc.	17.10
μ_{max} (1/d)	Max. Growth Rate	0.0237
K_s (g COD/L)	Half-Saturation Constant	0.0006
Y (g VSS/g COD)	Yield	0.15
k_d (1/d)	Biomass Death Rate	0.0212
T (°C)	Reactor Temperature	39.5
Δt (d)	Time Increment	0.01

Table 7.8 Comparison of the results for the TPH degradation in reactor 1.

Time (d)	TPH Concentration (gCOD/L)		
	Exp.	Calc.	Calc. (2^{nd}- order)
0	0.185	0.186	0.182
1	0.168	0.172	0.170
2	0.160	0.158	0.159
3	0.147	0.145	0.149
4	0.142	0.131	0.139
R^2	-	0.925	0.977

Comparison of the K_s values for Voolapalli and Stuckey (1999) and the present work shows that the bacteria in reactor 1 have shown less affinity for the formate degradation and that could be the result of the presence of TPH in this reactor. Some of the TPH degradation occurred in this reactor (about 23%) as shown in Figure 7.5. Also, the results of Michaelis-Menten showed good consistency (R^2=0.91) and the K_m value is in the range suggested by Lovley et al. (1984) and Axley and Grahame (1991).

7.3.2 Reactor 2

In this reactor, the kinetic parameters were estimated for anaerobic degradation of the sucrose and petroleum hydrocarbons. In reactor 2, unlike reactor 1, the reactions are complex and there is a mixed culture of bacteria undergoing a complex degradation of sucrose and TPH. The kinetic parameters for sucrose and TPH are investigated using the set of differential equations for substrate utilization (S) and microbial growth (X). Then, the numerical solutions were fitted to the experimental data for each substrate and the results were compared to those of other curve-fitting models.

7.3.2.1 Kinetic Parameters for Sucrose Degradation

Previous investigations have concluded that mono- and disaccharides can be used in cometabolic processes to remove persistent materials. Examples of such studies, are the biodegradation of dichloroethenes (DCEs) (Olaniran et al. 2006), reduction of chromium 6 (Cr^{+6}) to chromium 3 (Cr^{+3}) (Rege et al. 1997) and use of sucrose as electron donor in attached-film expanded bed reactors to treat tetrachloroethylene (PCE) (Carter and Jewell, 1993; Chu and Jewell, 1994). Hatzinger et al. (2001) studied the biodegradation of methyl tertiary butyl ester and found out that MTBE was rapidly degraded by sucrose-grown cells. A review of enhanced anaerobic bioremediation of chlorinated solvents has been provided (Parsons Corporation, 2004). Also, Britto (2000) has reviewed the effect of synthetic substrates such as sucrose on perchlorate degradation. As for reactor 1, the final results of the optimization step for sucrose degradation in reactor 2 has been used for estimation of the kinetic parameters. The computed and experimental data are shown in Figure 7.6 and are tabulated in Table 7.9.

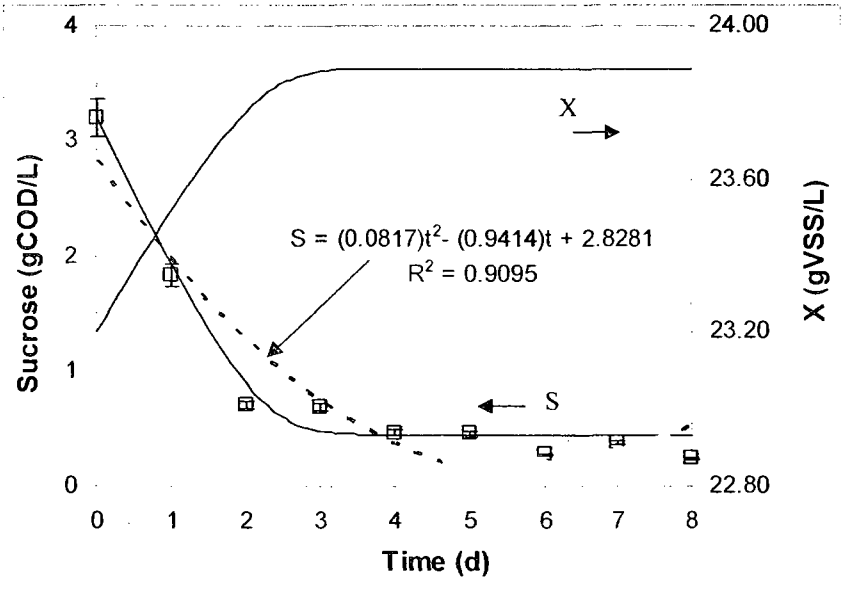


Figure 7.6 Results of sucrose degradation and bacterial growth in reactor 2. [(T=39.5°C, pH 6.5, organic loading=394 mg COD/L.d, ( ) numerical results, (■) experimental data, (- - -) second-order curve fitting)].

Table 7.9 Values of kinetic parameters for the sucrose degradation in reactor 2.

Parameter	Description	Value
S_0 (g COD/L)	Initial Substrate Conc.	3.200
X_0 (g VSS/L)	Initial Biomass Conc.	23.200
μ_{max} (1/d)	Max. Growth Rate	0.96
K_s (g COD/L)	Half-Saturation Constant	0.032
Y (g VSS/g COD)	Yield	0.30
k_d (1/d)	Biomass Death Rate	0.069
T (°C)	Reactor Temperature	39.50
Δt (d)	Time Increment	0.100

Table 7.10 shows the comparison between the computed data for the kinetic equations and the second-order curve fitting. It seems that in this reactor, the results of the quadratic equation for the degradation

of sucrose is less consistent (R^2 = 0.91) with the experimental data compared to the numerical results (R^2 = 0.95) obtained from the original set of S-X equations. Also, the quadratic trend cannot predict the end results. The experimental data shows that most of the degradation for sucrose occurred in the first 2-3 days of the reactor operation and suggests that only for this period there could be a linear relationship between the sucrose degradation rate and time. The numerical results obtained from solving the original differential equations are a better fit instead.

Table 7.10 Comparison of the results for the sucrose degradation in reactor 2.

Time (d)	Formate Concentration (mg COD/L)		
	Exp.	Calc.	Calc. (2^{nd}- order)
0	3.2	3.20	2.83
1	1.83	1.93	1.97
2	0.70	0.90	1.27
3	0.69	0.48	0.74
4	0.46	0.44	0.37
5	0.45	0.44	0.17
6	0.27	0.40	0.12
7	0.38	0.40	0.24
8	0.24	0.40	0.53
R^2	-	0.952	0.91

Michaelis-Menten kinetics has been fitted to the experimental data and the results are shown in Figure 7.7. It seems that this model is not a good fit for the degradation of sucrose in this reactor. A reason could be the quasi-steady state assumption which is associated with such models that may lead to incorrect results (Szigeti and Tanner, 1993). In those cases, the full set of differential equations should be solved. Uhlenhut and van Lessen (2002) have shown that biological treatment processes with

complex reaction mechanisms (such as those with intermediate products) can be described better using appropriate differential equations without the assumptions of Michaelis-Menten model.

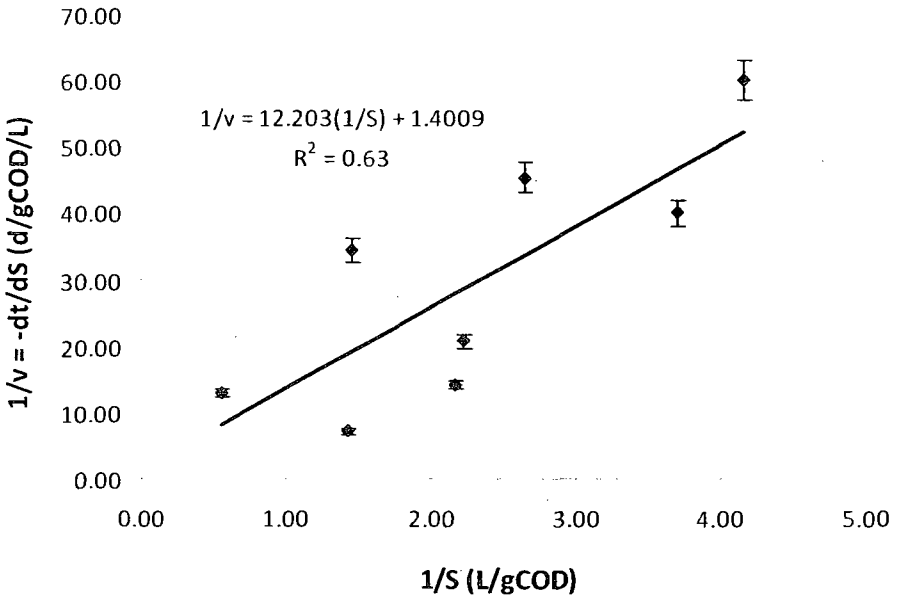


Figure 7.7 Estimation of K_m and V_m for anaerobic sucrose degradation in reactor 2 using Michaelis-Menten model.

The results of kinetic parameters for sucrose in this study and those of other work are shown in Table 7.11. Also, a typical set of kinetic parameters for anaerobic cheese whey treatment has been shown to compare with the results in this study. It seems that the rate of degradation of sucrose has been affected by the presence of petroleum hydrocarbons as the maximum rate (μ_m) obtained is less compared to those of other studies in Table 7.11. However, the anaerobic bacteria have shown a high affinity for the sucrose when comparing K_s values. Also, the biomass yield is still comparable for a complex substrate as it is for the cheese whey. This yield is higher than that of reactor 1. This could be another indication that in reactor 2, the bacteria have a more complex structure and the cell material some of which are also degrading the TPH compounds.

Table 7.11 Values of kinetic parameters for the anaerobic degradation of sucrose.

Process	T (°C)	Kinetic Model	K_m	V_m	K_s	μ_m	Y	k_d	Reference (s)
Anaerobic H_2 production	35	M[1]	-	-	68 mg COD/L	0.172 (h^{-1})	0.1	NA	Chen et al. (2001)
Anaerobic Fermentation	35	MM[2]	1.4 g COD/L	NA	-	-	NA	NA	Chen et al. (2006)
Anaerobic H_2 production	35	mixed anaerobic cultures	13.5 g/L	0.28 (1/h)	-	-	-	-	Yang et al. (2006)
Anaerobic treatment (cheese whey)	36.2	Monod, the Contois equations	-	-	134 mg COD/L	9.9 (1/d)	0.29	NA	Demirel et al. (2005)
Anaerobic treatment	,39.5	Original kinetic equations	8.7 g COD/L*	0.03 (1/h)*	32.0 mg COD/L	0.96 (1/d)	0.30	0.069 (1/d)	This work

1 Monod kinetic model

2 Michelis-Menten kinetic model

* Using Michaelis-Menten model

7.3.2.2 Kinetic Parameters for TPH Degradation in reactor 2

In reactor 2, the data of reduction of TPH obtained in the optimization step has been fitted to the numerical solution and the quadratic equation. The results are shown in Figure 7.8 and the kinetic parameters are tabulated in Table 7.12. Table 7.13 shows the regression results of the two numerical solutions. The results show that the results from the original set of equations is a better fit (R^2 = 0.95) for the TPH degradation compared to the quadratic equation (R^2 = 0.90) that fails to predict the results after the retention time of 5 days. Also, as shown in Figure 7.9, the Michaelis-Menten kinetic model didn't show a good fit to the experimental data (R^2= 0.62). Some fluctuations in the data of TPH concentrations were observed for the end points of TPH analysis with the HPLC analysis which could be due to very low final concentrations.

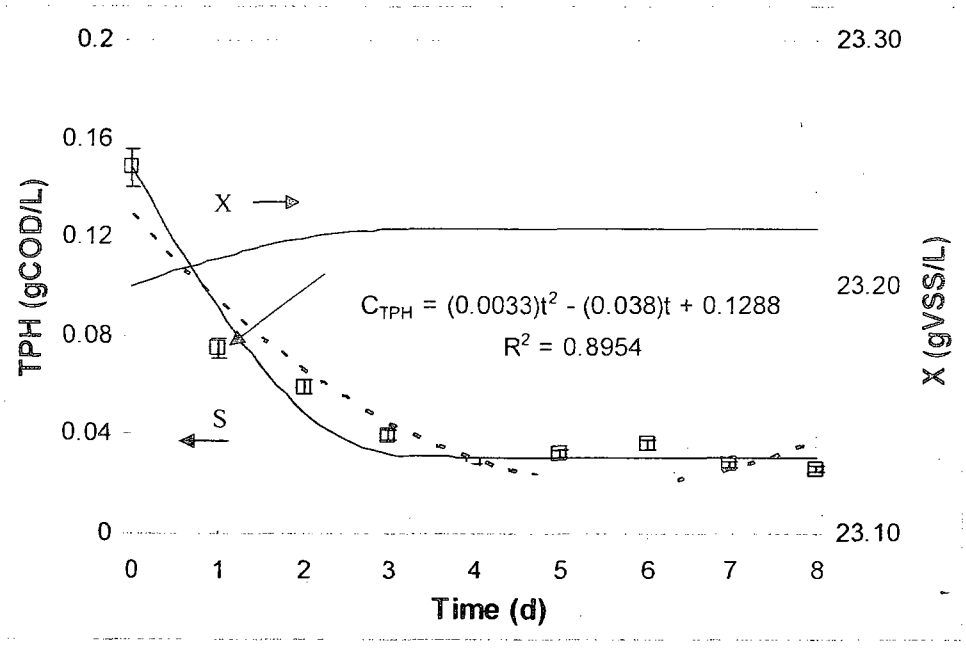


Figure 7.8 Results of TPH degradation and bacterial growth in reactor 2. [(T=39.5°C, pH 6.5, organic loading =394 mg COD/l.d, ( ) numerical results, ( ) experimental data, (- - -) second-order curve fitting)].

Table 7.12 Values of kinetic parameters for the TPH degradation in reactor 2.

Parameter	Description	Value
S_0 (g COD/L)	Initial Substrate Conc.	0.148
X_0 (g VSS/L)	Initial Biomass Conc.	23.20
μ_{max} (1/d)	Max. Growth Rate	0.0508
K_s (g COD/L)	Half-Saturation Constant	0.0004
Y (g VSS/g COD)	Yield	0.20
k_d (1/d)	Biomass Death Rate	0.05015
T (°C)	Reactor Temperature	39.5
Δt (d)	Time Increment	0.20

Table 7.13 Comparison of the results for the sucrose degradation in reactor 2.

Time (d)	Formate Concentration (mg COD/L)		
	Exp.	Calc.	Calc. (2^{nd}- order)
1	0.148	0.148	0.127
2	0.075	0.092	0.095
3	0.059	0.049	0.069
4	0.039	0.033	0.049
5	0.027	0.031	0.035
6	0.032	0.031	0.027
7	0.036	0.031	0.025
8	0.028	0.031	0.029
R^2	-	0.948	0.895

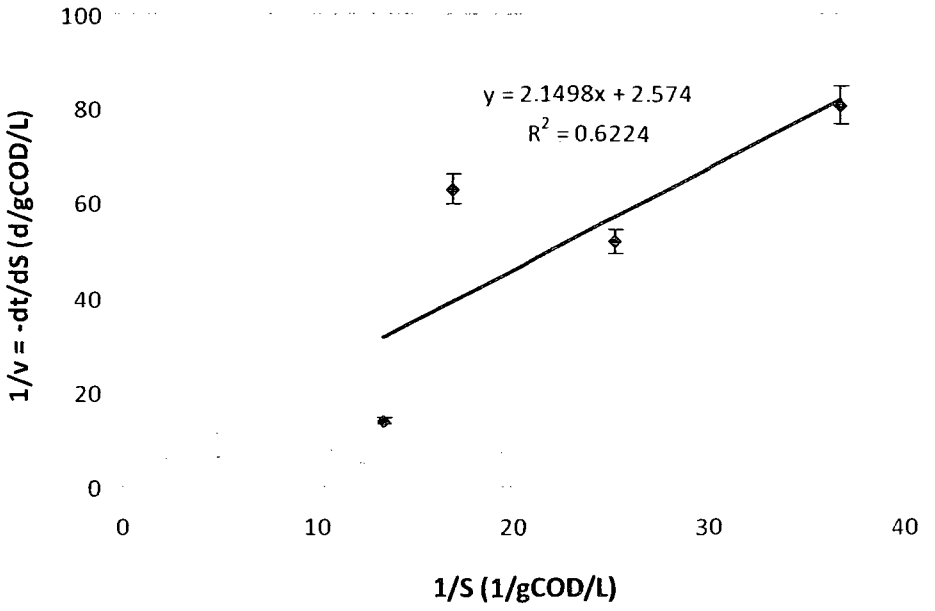


Figure 7.9 Estimation of K_m and V_m for anaerobic TPH degradation in reactor 2 using the Michaelis-Menten model.

A summary of the kinetic parameters obtained for TPH degradation in this study and those of other work regarding individual compounds is shown in Table 7.14. Following this table, a summary of studies on degradation of petroleum hydrocarbons is presented. Most of the studies are done on the kinetics of anaerobic or aerobic degradation of individual petroleum hydrocarbons and/or BTEX compounds. Also, there is some information about the aerobic degradation of TPH. There seems to be a lack of information about the kinetics of TPH degradation under anaerobic conditions. The overall results of this study show that the kinetic parameters for TPH could be quite different from those of the individual components due to the possibly different consortia of bacteria and difference in their affinity towards compound.

Table 7.14 Values of kinetic parameters for the anaerobic degradation of petroleum hydrocarbons in reactor 2.

Hydrocarbon	T (°C)	Kinetic Model	K_m	V_m	K_s	μ_m	Y	k_d	Reference (s)
Toluene	35	M[1]	-	-	30 ±30% µM	0.11± 20% (1/d)	11 g cell dry)/mole	-	Edwards and Grbic-Galic (1994)
O-Xylene					20 ±30% µM	0.07± 20% (1/d)	13 g cell (dry)/mole		
Toluene[2]	NA	M	-	-	0.15 mmol OCtol /l	1 (1/d)	0.02 mol OC cell /mol OCtol	0.1 (1/d)	Schafer et al. (1998)
TPH	39.5	Original kinetic equations	83.7 mg COD/L*	0.39 (1/d)*	0.036 g COD/L	0.002 (1/d)	0.24	0.0011 (1/d)	This work

1- Monod Model

* Using Michaelis-Menten model

2- For sulfate-reducing conditions. OC = organic carbon

Subsurface waters are typically anaerobic and there is increasing evidence of the occurrence of viable anaerobic hydrocarbon degradation processes (Aitken et al. 2004). It is only in the past twenty years that the use of hydrocarbons as substrates by anaerobic microorganisms has been investigated and the identification of metabolites and possible metabolic pathways reported (Boll et al. 2002, Widdel and Rabus 2001). A list of metabolites in anaerobic degradation of crude oil in subsurface reservoirs is given by Aitken et al. (2004).

The anaerobic degradation of petroleum hydrocarbons has been studied in the field and in the lab scale. The ability of anaerobic microbial consortia to degrade BTEX, PAH, and linear and branched alkanes has been studied in the past (Aeckerberg et al. 1998; Anderson and Lovley, 2000; Evans et al. 1991; So and Young, 1999a,b; Zengler et al. 1999; Boopathy, 2003). It has been shown that bacterial communities can convert long-chain alkanes to methane. Anaerobic degradation of hexadecane has been studied (Zengler et al. 1999) over a long period of 800 days. Boopathy (2003) has studied the TPH removal for No.2 diesel fuel (including 65%-85% of the normal, branched, and cyclic alkanes (paraffin), 10–30% Aromatic components and trace amounts of BTEX compound) from several sediments of wetlands. He has shown that 43% of TPH has been removed under methanogenic conditions at 20-22°C in a period of 500 days. However, Anderson and Lovley (2000) showed that hexadecane can be readily converted to methane using adapted microorganisms (*in situ* tests) in a relatively short period of 25 days.

Recently, anaerobic degradation of BTEX compound has been studied using several electron acceptors in periods of 1-15 days (Chakraborty et al, 2005). Also, Cuenca et al. (2006) have studied the anaerobic degradation of a diesel fuel (39.7% paraffins and 50.8 % cycloparaffins) contaminated tap water with 100, 200 and 300 mg/L of diesel fuel and nutrients. They obtained COD removal of 61.9 and 84.1% (with maximum diesel fuel removal of about 90%). In their experiments, the concentration of diesel fuel

in the effluent was less than 50 mg/L which was in the range of Level II groundwater standards of the MUST guidelines of Alberta.

Reinhard et al. (2007) have reported zero-order kinetics for anaerobic degradation of BTEX compounds under sulfate- and nitrate- reducing conditions. It has been shown that for simultaneous presence of BTEX, anaerobic biotransformation is sequential with toluene being the most readily degraded compound followed by *p*- and *o*-xylene (Ball and Reinhard 1996, Haag et al. 2001, Edwards et al., 1992). For ethylbenzene, the degradation rate was reported to be high under nitrate-reducing conditions but lower under sulfate-reducing conditions. Benzene is generally the most persistent compound, although it has been shown to be degraded under methanogenic (Grbic-Galic and Vogel, 1987), sulfate-reducing (Lovley et al. 1995, Edwards and Grbic-Galic, 1992), and iron-reducing conditions (Lovley et al. 1994). Brauner and Widdowson (1997) have proposed a sequential model for aerobic-anaerobic degradation of petroleum hydrocarbons in groundwater in which an aerobic approach has been considered for benzene degradation.

In a recent study by Hu et al. (2007), biodegradation performance of benzene, toluene, ethylbenzene and three xylene isomers (BTEX) under nitrate, sulfate and ferric iron reducing conditions has been investigated. They have shown that toluene, ethylbenzene, *m*-xylene and *o*-xylene could be degraded independently by the mixed cultures coupled to nitrate, sulfate and ferric iron reduction. They have suggested a decreasing order of degradation rate as toluene > ethylbenzene > *m*-xylene > *o*-xylene> benzene > *p*-xylene.

Prommer (2003) has described the anaerobic degradation of toluene under sulfate reducing conditions. He used Michaelis-Menten type kinetics and assumed the yield of 10% for the toluene conversion to biomass cells.

Nardi et al. (2007) has recently studied the anaerobic degradation of BTEX in a bench-scale packed-bed anaerobic reactor. They used a synthetic substrate composed of protein, carbohydrates and BTEX solution in ethanol, as well as a BTEX solution in ethanol as the sole carbon source. They fitted a first-order kinetic model to their experimental data with the apparent first-order coefficient values, ranged from 8.4±1.5 day^{-1} for benzene to 10.7±1.4 day^{-1} for *o*-xylene in the presence of ethanol, protein and carbohydrates, and from 10.0±2.0 day^{-1} for benzene to 13.0±1.7 day^{-1} for *o*-xylene in the presence of ethanol. These rates were said to be 10- to 94-fold higher than those found in reports on microcosm studies. The results of TPH degradation in reactor 1 shows that the first-order degradation can be a good fit for the TPH degradation with an apparent first-order coefficient value of 1.61 day^{-1} which is less than the above values. This could be as a result of different substrate/biomass conditions since in this work, the conditions of reactor 1 were set for methanogenic reduction of CO_2.

As the summary of this review, it can be said that there is less information available on the kinetic parameters when a variety of hydrocarbons is present at the same time in an aqueous waste stream medium. Also, fewer studies have been done on strictly anaerobic media such as in methanogenic conditions. Although some of the heavier hydrocarbons have less solubility compared to the lighter compounds such as BTEX, they could be persistent even at very low concentrations. For the category of the crude oil used in this study, the percent of aromatic compound could vary between 14-19 percent while paraffins and naphthenes could consist of 78-86 percent of the crude oil (API Report 2003).

Chapter 8: Conclusions and Contributions

8.1 Overall Conclusions of the Study

This study was aimed at exploring a new application of anaerobic process for the treatment of a waste stream generated in an enhanced oil recovery (EOR) process which is extensively used to increase oil production. The two major contaminants were defined to be CO_2 and dissolved petroleum hydrocarbons (TPH). The initial physiochemical conditions of this waste (such as pH, temperature, etc.) and its primary analysis of organic and inorganic constituents (including the toxicity limits of inorganic species) showed that a wastewater with the similar characteristics to that of a typical EOR process in the lab is treatable using a biological method. The original temperature of this waste (70°C- Table 2.1) suggested that the treatment can be done in a mesophilic range of temperature. The applicability of this method was examined in a series of batch experiments with a variety of the test materials. Then based on the results of the first phase, this waste was treated in a lab-scale two-step reactor system. Next, this system was optimized for the target parameters of CO_2 and CH_4 production in the first and TPH removal in the second reactor. Moreover, a sustainability assessment was done for the operation of the whole system based on the net energy production. Finally, a kinetic study was done regarding the experimental data and the kinetic models and the available results in the literature. As the summary of the conclusions for this research, the following items can be mentioned:

1- The method can be successfully applied under certain physiochemical conditions in the mesophilic range of temperature (about 35°C).

2- A variety of the electron donors can be used for the removal of CO_2 and TPH. Some of these materials such as light fatty acids have been demonstrated in a precedent work to this study to reduce CO_2 to methane in a simulated methanogenic process. It has been shown that they can be used in this work for a different substrate composition with a maximum of 85% of CO_2 removal using the formic acid. For the TPH removal, conditions were different and complex materials such as mono- and disaccharides showed better TPH removals. A maximum of 75.6% was achieved when sucrose was used as the electron donor.

3- The commercial material of hydrogen release compound (HRC) showed good results for TPH removal (about 70%). Although its formulation was not given, the HPLC analysis showed that it contains lactic acid and glycerol which upon anaerobic degradation can act as electron donors. It has been shown in the literature that HRC has successful applications for site remediation and removal of recalcitrant compounds such as chlorinated aromatics. However, its application for the aqueous solutions such as in this study should be with special precautions since the pH drops suddenly if excessive amount of this material is used. This sudden decrease in pH can cause inhibitory effects especially on the methanogenic reactions since they are pH-sensitive.

4- The results of the experiments with the control batch showed partial removals of the both contaminants. This can be due to the utilization of proteinaceous cell material of the original biomass as the co-substrate in the absence of the additional electron donors. The structural change of the bacterial colony could be a result of this process.

5- The results of activity measurements showed that the original bacteria which were previously used in a different treatment process, showed a good adaptability to the new materials. They

were acclimated to the new organic materials used such as the volatile fatty acids and the complex materials. The maximum yield of 0.42 L CH_4/g VSS and methanogenic activity of 0.92 L CH_4/gVSS.d for the formic acid showed a good acclimation to this material which is a very simple fatty acid and easy to degrade. The corresponding results of 0.35L CH_4/gVSS and 0.28 L CH_4/gVSS.d for yield and methanogenic activity of the sucrose also showed good acclimation of the biomass to this material. This is a good measure of the degree of bacterial acclimation to the new conditions which is also necessary for the selection of the proper electron donor.

6- The batch experiments showed that the removal conditions of CO_2 and TPH are different: the operating conditions, material used, etc. So it was concluded that the whole treatment process can be done in two steps: One step for methanogenic reduction of CO_2 and another step for removal of TPH. The set of two-step reactor system was used for this purpose. Also, pH and alkalinity were found to be the most important operating parameters especially for the bioconversion of CO_2 to CH_4.

7- The operation of the two-step reactor system showed that this system can be efficiently used for the treatment of the waste. The primary operation of this system showed that 95% of CO_2 and 76% of TPH can be removed in the first and second reactor respectively. However, since the operation of the second reactor can be influenced by the first one, the operating conditions should be set so that the treatment objectives be met efficiently. In addition to checking the operating conditions of the system, it is very important to monitor the environmental conditions such as pH, temperature, substrate conditions, etc. since they can directly affect the system performance. Also, it is important to monitor the status of bacteria in the reactors and possible

changes in their morphological structure parallel to the results of microbial tests such as activity measurements, population change, etc.

8- The results of application of evolutionary factorial (EVOP) design approach for optimization of the system showed several advantages over the single-parameter optimization: (1) it allows consideration of more alternatives for the operating conditions affecting the optimized parameters; (2) simultaneous changes in factors affecting the target parameters is possible; (3) it is possible to check interactive effects of the factors on the target parameters and (4) the relative importance of the factors on the optimization can be evaluated. The operation of reactor 1 was found to be more important since it precedes reactor 2 and its operation can affect the performance of reactor 2.

9- The results of the sustainability assessment showed a net energy production in the system and an EOR index of 3.3 for the whole process was calculated. Although the objective of the process was not just the energy production, the operation of the system was shown to be sustainable. It was also observed that the results of the optimization process coincide with that of the sustainability assessment (both occur at the operating conditions of R7). This could be a result of considering methane production as one of the parameters to be optimized. Sustainability assessment is an important aspect of the processes to be considered not only to evaluate their positive outcome, but also sometimes to check if there are no adverse effects on the environment.

10- The kinetic study showed that the kinetic parameters for anaerobic degradation of formate and sucrose can be affected by the presence of petroleum hydrocarbons which contain a variety of compounds even at low concentrations. This effect is more significant for reactor 2 since the

retention time is longer and the complex reactions are happening in this reactor. The bacteria in both reactors showed more affinity for the lighter TPHs (higher initial reaction rates) than for the heavier ones. Although sometimes simple models can be applied, it is has shown that it is better to solve the original governing differential equations to predict the kinetic behavior of the system which also will result in a complete set of kinetic parameters.

8.2 Contribution to Knowledge

This study showed a novel use of anaerobic treatment for a waste that is generated at large scale. It also demonstrated a practical application of a two-step reactor system for this purpose which can be implemented for a real case. As the contributions of this study, the following can be mentioned:

- Development of a method that allows the co-treatment of carbon dioxide and dissolved petroleum hydrocarbons in a waste stream that could be the case for many industries.
- Development of a new application of anaerobic treatment for an industrial waste stream which is generated at very large scale. This method is simple, easily applicable and has many benefits such as low energy requirements and production of methane as a source of energy.
- Development of a new aspect for an on-stream optimization of a process applicable for a two-step reactor system and determination of multiple interactions of the process parameters. This eliminates the limitations of the simple and one-parameter optimization methods and will provide more flexibility for the plant operation in terms of the objective and target of optimization.
- A comprehensive kinetic study of the anaerobic degradation of petroleum hydrocarbons has been done and the kinetic parameters are estimated when all ranges of hydrocarbons are presented in a waste stream.

- Development of a method of evaluating the environmental sustainability based on an energy analysis and net energy balance for a biological treatment system. This approach is applicable for a process in which methane is produced as a clean source of energy.

Chapter 9: Recommendations for Future Work

The results of this study have demonstrated the applicability of anaerobic treatment and effectiveness of using a two-step reactor system in treating an industrial waste stream with the major contaminants of CO_2 and petroleum hydrocarbons.

However this work could be a starting point to improve the existing system or to develop other treatment systems.

- Using a system of upflow anaerobic sludge blanket (UASB) reactors

As an alternative to the reactor system used, a reactor system of two upflow anaerobic sludge blanket (UASB) reactor can be used. The first UASB reactor was developed at Wageningen Agricultural University, Netherlands (Lettinga, 1978). In this reactor, bacteria having self-immobilized granular structures and with good settling properties form a blanket zone inside the reactor. The influent flows up through this zone where the decomposition of organic matter happens under anaerobic conditions. Figure 9.1 shows a schematic of this reactor.

These reactors due to their high biomass concentrations can achieve conversions higher than that possible by conventional anaerobic processes and tolerate fluctuations in influent feed, temperature and pH (Kosaric & Blaszcyk, 1990). Moreover, since no support medium is required for attachment of the biomass, it decreases the capital cost and minimizes the possibility of plugging. The energy requirement also is small because there is no mechanical mixing within the reactor, no recirculation of sludge, and no

high recirculation of effluent (Praveen and Ramachandran 1993). There is no need for a separate settler with sludge return pump as in the anaerobic contact process. Unlike the anaerobic filter and fixed film reactors, there is no loss of reactor volume through filter or carrier material. Also, compared to fluidized bed reactors, there is no need for high rate effluent recirculation and therefore, high pumping energy.

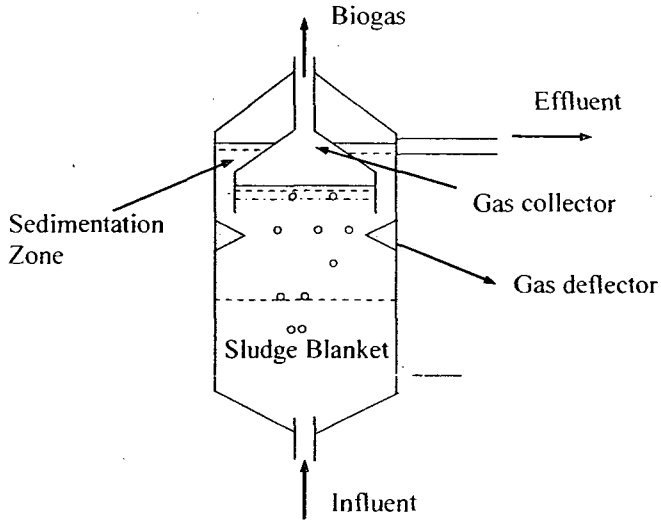


Figure 9.1 A schematic of an upflow anaerobic sludge blanket (UASB) reactor.

- Co-treatment of CO_2 and individual petroleum compounds in waste streams from petroleum processing plants

Some of the waste streams that are produced in refineries or petrochemical plants are contaminated with individual petroleum compounds such as phenol or styrene. It can be investigated that how these waste streams are capable of capturing carbon dioxide as a CO_2 sink and how the resulting waste stream can be treated in the two-step reactor system or other systems using different types of reactors.

- Co-treatment of TPH-contaminated streams with other wastewaters

As a result of this study, the co-treatment of this waste stream with the wastewaters from certain industries such as sugar cane production can be investigated. Also, the reduction of TPH in the control batch in the absence of the electron donors suggested that bacteria in this batch could have used their

proteinaceous extracellular material. Therefore, it seems to be possible to co-treat this waste stream with the wastewaters containing proteins such as cheese whey of diary production.

- Using strains of petroleum degrading bacteria for TPH removal

In order to achieve higher TPH removal in the second reactor, special types of bacteria such as those already cultured and isolated in a lab or naturally occurring bacteria can be used. Examples are anaerobic strains found in gasoline-contaminated groundwater (Kasai et al. 2006), a BTX degrading strain (Chakraborty et al. 2005) and anaerobic bacteria to degrade polycyclic aromatic hydrocarbons (PAHs) found in a marine harbor sediments (Coates et al. 1997). This can reduce the acclimation time and allow higher organic loadings. Also a secondary electron donor such as sucrose may not be required.

- Application of biosurfactants for the removal of higher TPH concentrations

During oil extraction in an EOR process, the concentration of TPH could increase in the waste stream due to turbulent mixing or mechanical action such as in the supply pumps. In this case, a brownish waste could be produced as a result and it may be necessary to use biosurfactants. Batch experiments should be performed to find the required dose of the biosurfactant prior to using the two-step reactor system.

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Appendix

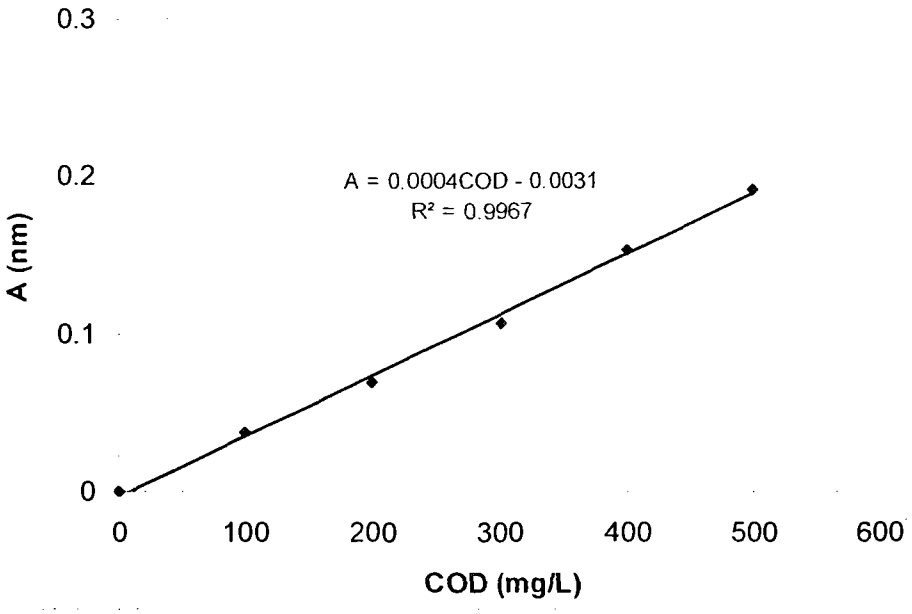

Figure A.1 Standard curve of UV Absorbance vs. COD concentration. (Wavelength = 600 m).

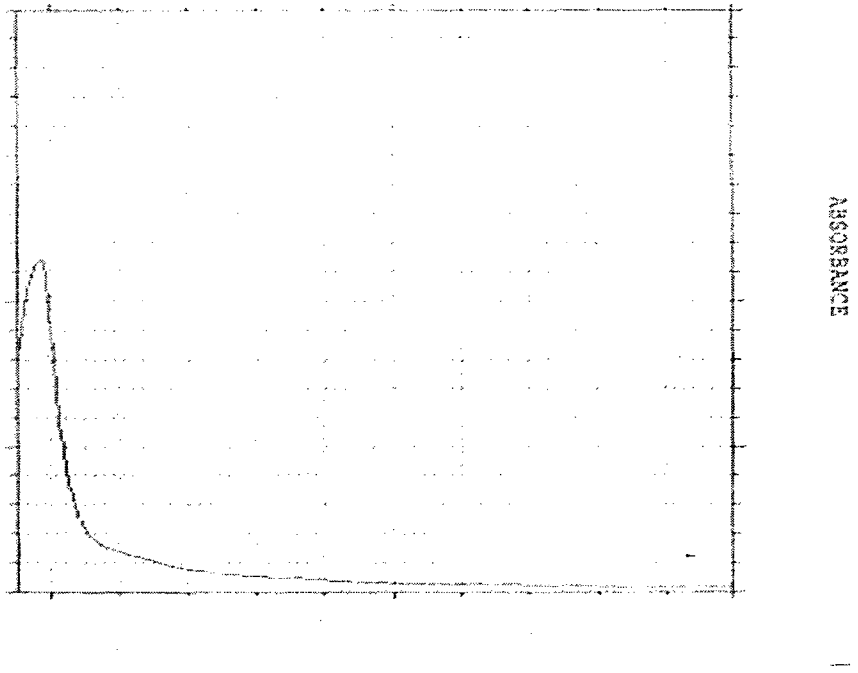

Figure A.2 Scanned UV spectrum of an untreated sample of CO_2 and crude oil-contaminated wastewater. (Wavelength 190-400 nm, UV Spectrometer Lambda 40).

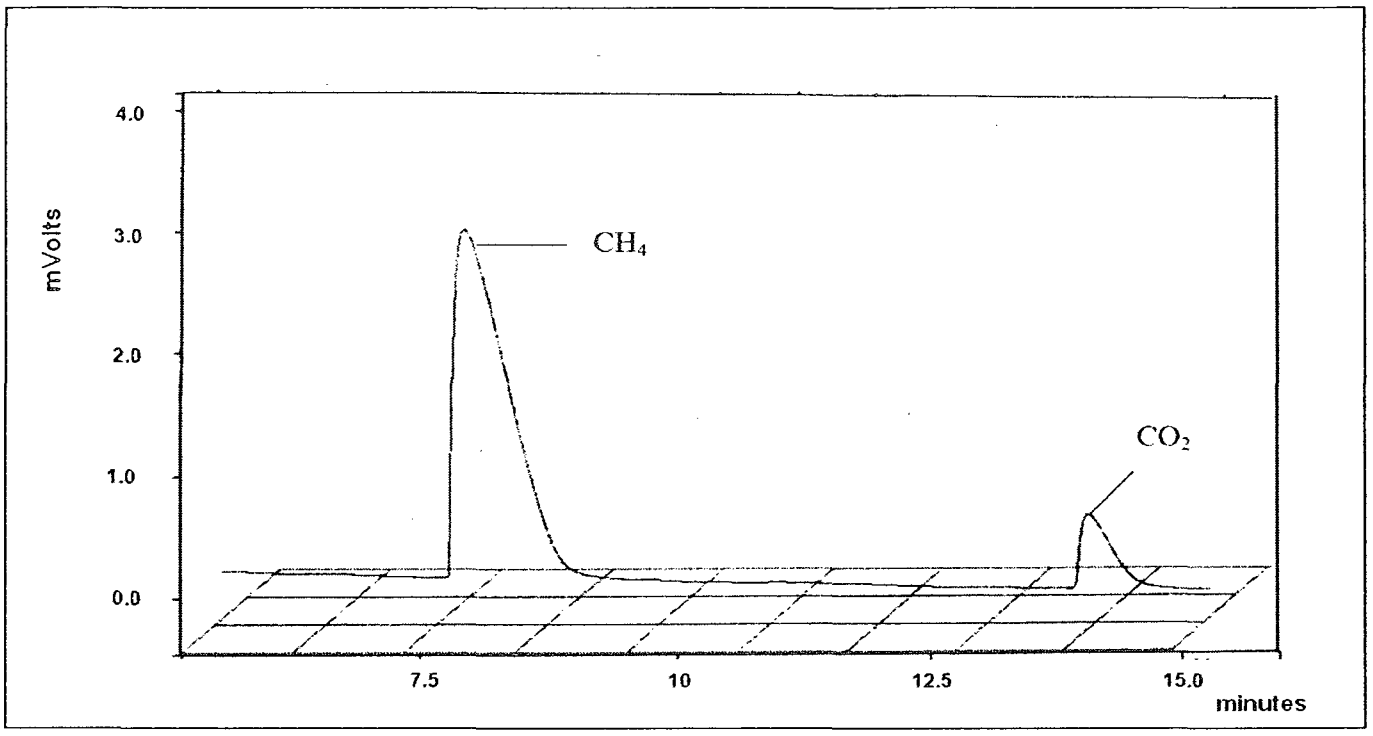

Figure A.3 A sample spectrum of GC for a gas mixture of CH_4/CO_2. [85%/25% (v/v)].

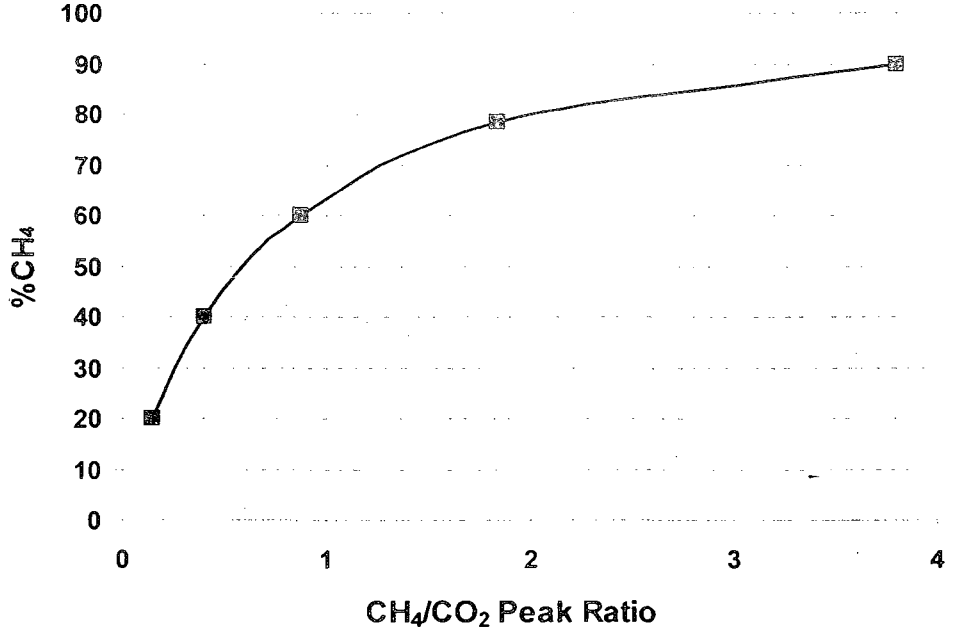

Figure A.4 Reference curve for methane content of the biogas obtained by gas chromatography.