Co-conversion of gaseous carbon dioxide and a high strength aqueous organic contaminant (phenol) to methane via a UASB reactor

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ABSTRACT

Co-conversion of gaseous carbon dioxide and a high strength aqueous organic contaminant (phenol) to methane via a UASB reactor

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More economical and efficient means are required to decrease or to recover greenhouse gases (GHG) exhausted into the environment, and to decrease the amount of wastewater that is degrading the water bodies worldwide. Therefore the objective of this research is to develop a system that will treat both carbon dioxide and a high strength wastewater; for this purpose an Up-flow Anaerobic Sludge Blanket (UASB) reactor was used.

Previous studies showed that carbon dioxide could be converted to methane with organic acids. In this study, the addition of carbon dioxide will act as the co-substrate needed in order to treat higher concentrations of the toxic pollutant phenol in wastewater without any recirculation of the effluent. This is based on previous studies that showed that higher concentrations (1260 mg/L phenol) can be treated if a co-substrate is added.

An anaerobic biomass used for this experiment was taken from two reactors treating sucrose and acetic acid. Then, it was acclimated by increasing stepwise the concentration of phenol, while reducing the concentration of sucrose and/or acetic acid. Methanogenic activity tests were performed to evaluate the toxicity limits of phenol toward the biomass. Two sets of two reactors were used. The first set contained the biomass acclimated with sucrose; the second set contained the biomass acclimated with acetic acid. Control reactors had phenol added as the carbon source; whereas in the other reactors, carbon dioxide was added as the co-substrate. The parameters monitored were chemical oxygen demand, volatile suspended

solids, methane content of the biogas and production, phenol concentrations, alkalinity, and dissolved carbon dioxide.

It was shown that both CO_2 and phenol could be degraded in an UASB reactor with reduction values that ranged from 86-88 % and 86-94% respectively. Total COD reduction ranged from 92-97 % and methane content in the biogas ranged from 64-70 %. The biomass showed a steady organic content throughout the experiment after a sudden decrease during the acclimation period. Overall, the reactors with the best performances were R4 (acetic acid-fed biomass with CO_2) and R2 (sucrose-fed biomass with CO_2) although this last one showed some performance similarities with R3 (acetic acid-fed biomass without CO_2) and the reactor which was more prone to loading shock was R1.

Carbon dioxide, a waste gas, poses a great advantage in the anaerobic process since it was proven to be a good co-substrate in the biodegradation of a high strength wastewater, with a higher methane recovery. Adding carbon dioxide to UASB reactors in order to boost degradation instead of releasing it to the environment is a good remediation proposal.

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Table of Contents

List of Figures

List of Tables

List of symbols and abbreviations

APHA	American Public Health Association
CAS	Chemical Abstract Service
CEPA	Canadian Environmental Protection Act
COD	Chemical oxygen demand
EC_{50}	Effective concentration 50
EPA	Environmental Protection Agency
GHG	Greenhouse gas
HRT	Hydraulic retention time
k	Henry's law constant
k_1	Dissociation coefficient
k_{oc}	Organic carbon/water partition coefficient
k_{ow}	Octanol/water partition coefficient
k_w	Water dissociation coefficient
LC_{50}	Lethal concentration 50
OLR	Organic loading rate
pH	Concentration of hydrogen ions $[H^+]$ or acidity
pKa	Ionization constant
pOH	Concentration of hydroxyl $[OH^-]$ ions, or the basicity
PSL	Priority substance list
SMA	Specific methanogenic activity
TA	Total alkalinity
TSS	Total suspended solids
TVSS	Total volatile suspended solids
UASB	Upflow anaerobic sludge blanket
UV/VIS	Ultraviolet-visible spectrophotometer

Chapter 1 . INTRODUCTION

1.1. STATEMENT OF THE PROBLEM

Svante August Arrhenius, a Swedish chemist, mentioned the concept of "Global Warming" on the 19th Century (Lerner et al, 2003). His intention was to explain how greenhouse gases accounted for the earth's climate at that time, and how lower concentrations of this gases might have had a relation with the climate during the ice ages. Arrhenius calculated that human emissions of these gases could affect the earth's climate in the future. Without the greenhouse effect the earth would be inhabitable due to much lower temperatures. However, the concentrations of these greenhouse gases, carbon dioxide in particular, have increased very rapidly due to the increase of burnt fossil fuels for the production of energy used in industrial processing, heating, transportation, etc.

According to Environment Canada in its report for the period 1990-2004, "Total emissions of all GHG in 2004 (758 Mt) were 27% above the 1990 level of 599 Mt and approximately 73% of total GHG emissions in 2004 resulted from the combustion of fossil fuels. CO_2 contributed the largest share of 2004 emissions, at 78% (about 593 Mt), while CH_4 accounted for 15% (110 Mt)" (Environment Canada, 2006).

In addition to gas emissions, the environment is being constantly targeted by liquid contaminants or wastewaters from the industry, households, municipalities, runoff and agriculture, etc. These wastewaters have to comply with the local/general regulations before being returned to the environment, and in order to comply, they have to go

through different processes depending on their nature. Although the tendency in developed countries is to minimize the wastewater or recycle the waste within the production processes, there are still many companies which produce liquid waste streams.

Depending on the sources, these wastewaters could contain carbohydrates, proteins and fat components if it comes from a food processing plant, hydrocarbons from petrochemical processes, heavy metals and arsenic from mining, inorganics such as phosphorous and nitrogen which cause eutrophication, fecal coliforms from households and pesticides, plastics, pharmaceutical, such as hormones which endanger reproduction of species, from complex organics industries. Organic chemical industries produce high strength wastewaters which are not easily degradable. Usually these wastewaters have to undergo dilution before its treatment and later disposal. Phenols are among these wastewaters which require dilution and these contain a variety of phenolic compounds such as o-, m-, p-cresols, dimethylphenol, etc.

1.2 SCOPE OF THE WORK

In this experiment, anaerobic bacteria treating wastewaters from a cheese company was acclimated to two different substrates. These substrates were chosen based on the results from Alimahmoodi (2004) in which he determined that the best substrate for the degradation of dissolved CO_2 was acetic acid. The other selected substrate was based on different experiments treating phenols in which sucrose was determined to be the best substrate for shorter start-ups and for better degradation of phenol (Fang et al., 1998).

For acclimation purposes, the two types of anaerobic bacteria were fed on the respective substrate and later on phenol. In the continuous experiment, each type of bacteria had a control experiment to compare results.

1.3. OBJECTIVES OF THE PROJECT

The objectives of this study were to:

1. Develop a system for wastewater treatment (UASB reactor) with the bioconversion of the carbon dioxide.
2. Evaluate the performance of the system treating a high strength wastewater using carbon dioxide.
3. Evaluate the effects of the composition of wastewater and co-substrate on the performance of the reactor.

1.3. THESIS ORGANIZATION

CHAPTER 1 introduces the problem, the scope of the work and the objectives of the study.

CHAPTER 2 presents all the background information on the two main contaminants, problems related to them, how they have been managed in the past, and information on the process proposed for the degradation.

CHAPTER 3 lists all the materials used to build the proposed reactors, analytical methods used, chemical materials and procedures used in the process.

CHAPTER 4 presents all the results from the experiments from the acclimation period to the continuous experiments.

CHAPTER 5 presents the conclusions of the study, the contributions made and some recommendations for future work.

REFERENCES list all the references used in this project including those from internet.

APPENDICES present the standard curves used for the determination of concentration of methane, phenol, COD and a glossary with important terms.

Chapter 2 . LITERATURE REVIEW

2.1. CARBON DIOXIDE

2.1.1. Greenhouse effect

Solar radiation passes through the atmosphere into the earth, with some of this radiation being absorbed by the earth's surface and the rest being reflected by the earth and atmosphere as well. The greenhouse effect is produced when water vapour, carbon dioxide and other gases trap the terrestrial radiation leaving the surface of the earth in order to warm up the earth's surface and lower atmosphere (IPCC 2001, U.S. EPA Inventory 2006). "Any change in the atmospheric concentration of these gases can cause an unbalance in the energy transfer among the atmosphere, space, land, oceans [increasing the earth's temperature]" (U.S. EPA Inventory 2006).

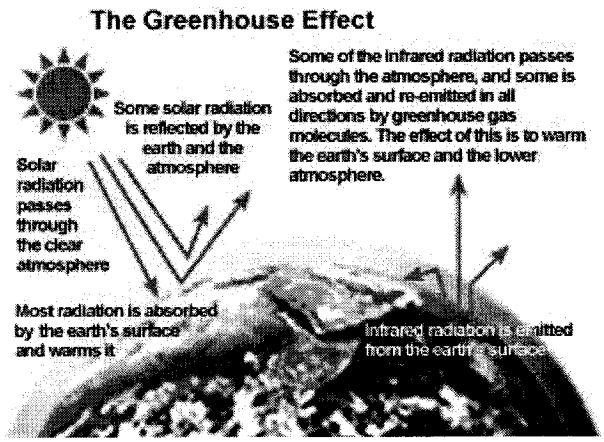


Figure 2.1: The greenhouse effect
http://yosemite.epa.gov/oar/globalwarming.nsf/content/index.html

Carbon dioxide (CO_2), methane (CH_4), nitrogen dioxide (NO_2) and ozone (O_3) are naturally occurring greenhouse gases which are continuously being emitted and removed by natural processes on earth. The problem begins when the emissions of these gases are increased due to anthropogenic activities such as burning fossil fuels for energy production. "Carbon dioxide emissions, a by-product of energy production and use, account for the largest share of greenhouse gases, which are associated with global warming" (World Bank's World Development Indicator Report 2006).

2.1.2. Global Warming due to carbon dioxide emissions

Anthropogenic activities

Human activities have contributed to the 0.6 degree Celsius increase of the earth's climate over the last century. Not surprisingly, this increase is expected to continue in a range of 1.4 to 5.8 degrees Celsius between the years 1990 and 2100, as stated by the World Bank's World Development Indicator Report (2004).

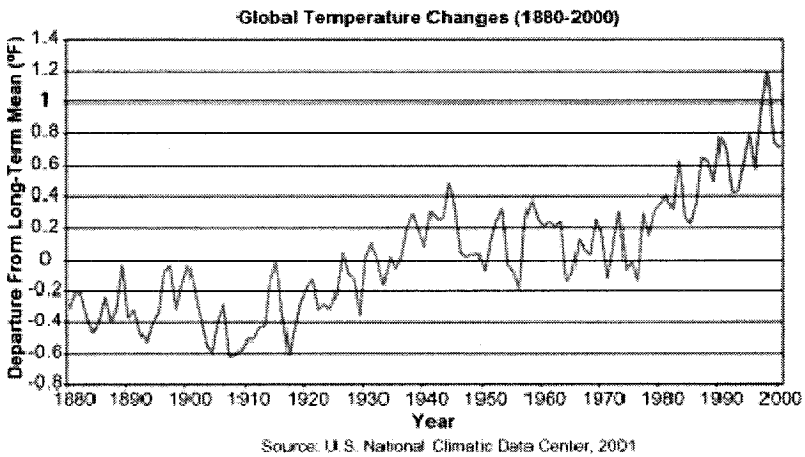


Figure 2.2: Global temperature changes (1880-2000)
Taken from U.S. National Climatic Data Center, 2001

"Out of 6 to 7 billion tons of carbon dioxide released each year by human activities, some 2 billion tons are absorbed by oceans and another 1.5 to 2.5 billion by plants, with the rest being released into the atmosphere" (World Bank, 2004). Carbon dioxide per capita emissions are considerably higher in high-income countries such as North America and Australia, as shown on Figure 2.3.

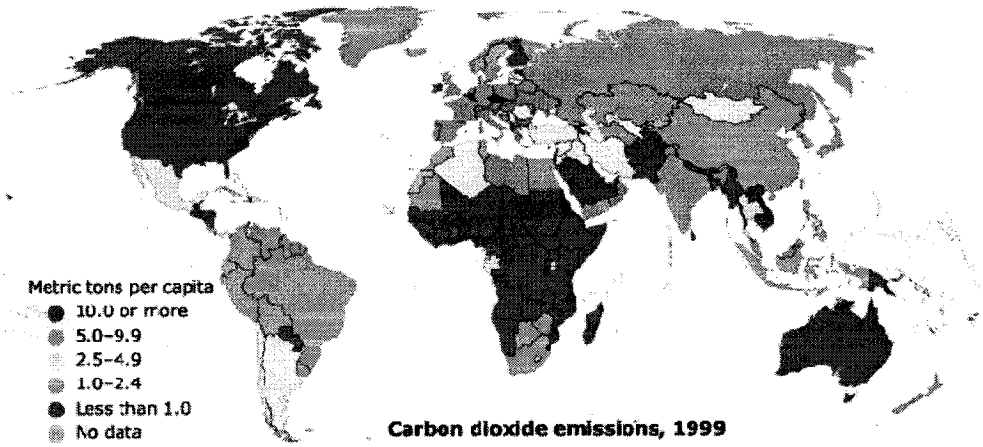


Figure 2.3: Carbon dioxide emissions in 1999.
http://www.developmentgoals.org/Environment.htm#carbon

The Carbon Dioxide Information Analysis Center (CDIAC) calculates annual anthropogenic emissions of carbon dioxide, based on global average fuel chemistry and used, and these estimated are... "Probably within 10% of actual emissions, country estimates may have larger error bounds" (World Bank's World Development Indicator of 2006).

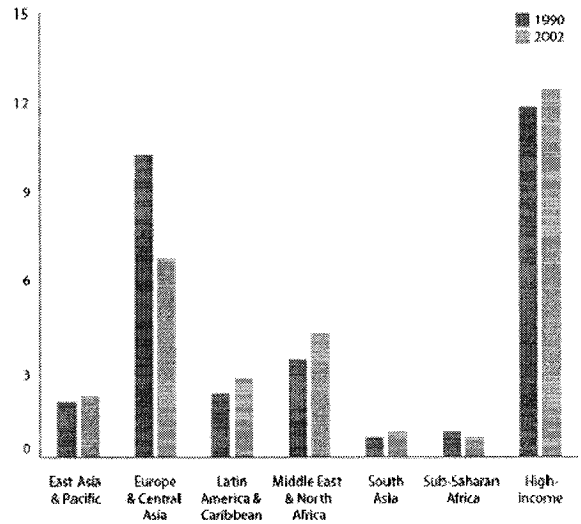


Figure 2.4: World distribution of CO_2 emission (1990-2002)
Taken from World Bank's World Development Indicator Report April 2006.

As shown in Figure 2.5, the five major contributors of carbon dioxide emissions in 1990 and 2002 were the United States, China, the Russian Federation, India and Japan, with the United States being the major contributor in both years.

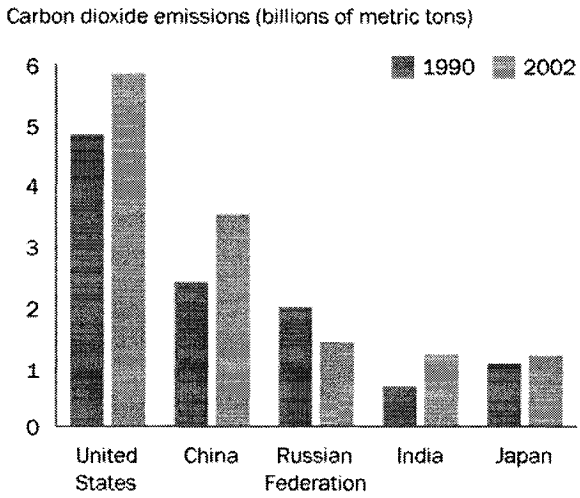


Figure 2.5: Five major contributors of CO_2 emissions until 2002.
Taken from World Bank's World Development Indicator Report April 2006.

2.1.3. Reduction of greenhouse gas emissions

Different approaches have been taken into consideration for the mitigation or reduction of the carbon dioxide emitted to the environment. The use of biofuels, the use of other energy sources (solar, hydro and wind energy), are some of these approaches. Lately, the sequestration of carbon dioxide into underground waters, aquifers and others, is growing among the approaches used to reduced the CO_2 in the atmosphere but the problems is that CO_2 is only being moved from one place to another, no real conversion or elimination takes place, and there is no real knowledge of the impact of this carbon dioxide on groundwater.

In November 2006 at the Workshop on Carbon Capture and Storage organized by The Global Environmental and Climate Change Centre (McGill University), different techniques were discussed in order to sequestrate the carbon dioxide for a longer period

of time and in more stable conditions. Among the techniques was sequestering carbon dioxide in concrete to produce stable products such as calcium carbonate ($CaCO_3$) which will increase concrete's strength and decrease its permeability (Monkman, 2006). In addition, a new carbon-based sorbent for carbon dioxide capture was presented; basically these modified carbons were able to trap carbon dioxide from emissions, and later heat or vacuum was applied to sequestrate the carbon dioxide in tanks (Belanger, 2006).

In this workshop, a new way of capturing and bioconverting carbon dioxide to methane by anaerobic means was also presented, (Mulligan et al., 2006). In this work, methanogenic bacteria were adapted to convert CO_2 into methane using an upflow anaerobic sludge blanket reactor (UASB). The reactors were fed with substrates containing acetic acid, and mixtures of VFA (volatile fatty acids, acetic, propionic, and butyric acid). In this study, all the systems showed high removal rates being acetic acid the best substrate. The efficiency of the reactors was measured by the amount of COD and CO_2 removed from the system. Values above 70% and 75% were achieved respectively for dissolved CO_2 and COD removal.

2.2. PHENOL

2.2.1. Physical and chemical information

Phenol has a white to pink color with an acrid smell and sharp burning taste (CEPA 2000). Its chemical formula is C_6H_6O, with a molecular weight of 94.11 g/mol. Phenol is

identified with a CAS number 108-95-2, and some common names with which can be also identified are carbolic acid; phenylic acid; hydroxybenzene; monohydroxybenzene; phenyl hydroxide, benzene phenol, monophenol, phenic acid, phenol alcohol, phenyl hydroxide, etc. (Environment Canada, 1998; Fisher Scientific's Material Safety Data Sheet).

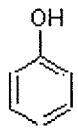


Figure 2.6: Chemical structure of phenol.
Taken from http://omlc.ogi.edu/spectra/PhotochemCAD/html/phenol.html

Table 2-1: Physical properties of phenol

Property	**Value**
Melting Point (°C)	41[1]
Boiling Point (°C)	182[1]
Vapor Pressure (Pa)	47[2]
Henry's law constant ($Pa*m^3/mol$)	0.059[3]
pK_a	9.99[2]
Log K_{oc}	1.15-3.49[4]
Log K_{ow}	1.46[5]
Solubility in water (mg/mL)	8,360[6]

Modified from CEPA, 2000.

1 Verschueren, 1983
2 Dean, 1985
3 Abd-el-Bary et al., 1986
4 DMER and AEL, 1996
5 Fujita et al., 1964
6 Blackman et al., 1955

According to Environment Canada (CEPA 2000), phenol is "toxic to aquatic life, microorganisms and invertebrates at very low concentrations".

2.2.2. Statistics on Phenol Use

According to the Canadian Environmental Protection Act (2000), phenol is no longer produced in Canada since the closure of the last two plants in 1992. Canada imported phenol, in its pure form and as resins and/or polymers, in amounts greater than 76,000 tonnes for the year 1995 and 95,000 tonnes for the year 1996; about 98% of these imports were in its pure form (Environment Canada, 1997b).

Approximately 85% of the phenolic consumption is used for the production of resins which are used to produce panels, insulations, oriented strand board, and lubricants, paints, brakes, etc. It is also used as raw material for the production of other organic substances such as bisphenol A, chlorophenols, aniline and others (SRI International 1993; Environment Canada, 1997b). In addition, phenol is also present in medical products such as ointments, ear and nose drops, cold sore lotions, mouthwashes and in general disinfectants, anaesthetic, and antiseptics (Gosselin et al., 1984; Reynolds, 1989; Gennaro, 1990; CEPA, 2000; Agency for toxic substances and Disease Registry, 2006). An example is the Chloraseptic® spray which has 1.4 % phenol (www.chloraseptic.com/products).

Table 2-2: Phenolic resins produced by various provinces in Canada in 1994*

Province	Amount (tonnes)
Ontario	108,000
Quebec	105,000
British Columbia	35,000
Alberta	18,000

* Data obtained from Camford Information Services (1994), SRI International (1994), and CEPA (2000).

Table 2-3 : Examples of phenolic wastewaters, adapted from Veeresh et al. (2005).

Type of wastewater	Significant phenolic compounds	Phenolic concentration (mg/L)	Phenolic COD (mg/L)	Reference
Coal gasification (10% dilution)	Phenol, o-,m-,p-, cresols (monohydric), resorcinol, hydriquinone (dihydric), dimethyl phenol	207	1102.0	Suidan et al. (1983b)
Petroleum refinery	Phenol, resorcinol	6.42- 88.03	15.3- 210	Tyagi et al. (1993)
Herbicide manufacturing			210	Tyagi et al. (1993)
Aircraft maintenance		200- 400		
Fiberboard factory		150		
Fiberglass manufacturing			40- 400	

2.2.3. Sources of phenol in the environment

Natural Sources

Trace amounts of phenol can be found naturally in water or/and soil as the product of natural decomposition of plants and animal waste; in addition, higher amounts can be found due to forest fires (Dobbins et al., 1987; IPCS, 1994a; CEPA, 2000).

Industrial sources

Phenol is an intermediate product in the production of other chemicals. As shown in Table 2-4, 414.7 tonnes were released to the environment in 1996.

Table 2-4: Release of phenol/total phenol in tonnes by the Canadian industry sector in 1996

Sector	Air	Water	Other [1]	Total
Pulp, paper and wood	205.6	44.3	6.5	256.4
Mineral (non-metallic)	62.7	--	1.3	64.0
Chemical	22.9	--	15.4	38.3
Steel and Metal	23.3	9.2	2.0	34.5
Petroleum refining	1.8	5.0	6.5	13.3
Other [2]	5.5	--	2.7	8.2
Total	321.8	58.5	34.4	414.7

Modified from CEPA, 2000 (Environment Canada, 1997b)

1 Includes discharges to MWTPs, landfills, deep well injection, and land for land farming

2 Includes textiles, transportation equipment, industrial machinery and equipment, and instruments and related products.

2.2.4. Environmental Fate of phenol

Air

According to Eisenreich et al. (1981), phenol exists in the atmosphere mainly in the vapour phase (CEPA, 2000). Due to its short half-life in the air (2.28-22.8 h), it is not expected to be transported long distances. It is removed by photooxidation, photolysis, and wet and dry deposition (Atkinson et al., 1987, 1992; Bunce, 1996; Van Dusen, 1996, CEPA, 2000).

Soil

Soil microorganisms (aerobic and anaerobic) are capable of using phenol for growth which is faster under aerobic conditions (Scott et al., 1982; Howard, 1989). The half life ranges from 2.7 to 552 h depending on the type of soil (Alexander and Aleem, 1961; Federle, 1988; Loehr and Mathews, 1992; DMER and AEL, 1996). Removal processes may include biodegradation, adsorption/desorption, volatilization and oxidation (CEPA, 2000).

Water

In water, phenol reacts as a weak acid but it is not expected to dissociate in the pH found in the environment due to its low pK_a. Some removal processes may include biodegradation, photooxidation, photolysis and volatilization (CEPA, 2000). The suggested half life in water is 55 hours (Mackay et al., 1995; DMER and AEL, 1996; CEPA, 2000), and 12 to 168 hours for groundwater (Howard et al., 1991).

Sediments

It is unlikely that phenol adsorbs on to suspended or bottom sediments due to its low K_{oc} and K_{ow} (U.S. EPA, 1990; DMER and AEL, 1996). A suggested half life of 550 hours has been suggested by Shiu et al. (1994) and DMER and AEL (1996).

2.2.5. Phenol's toxicity

Humans

According to the CEPA (2000), phenol is "highly toxic to humans after oral and dermal exposure". It is rapidly absorbed by the skin, the gastrointestinal tract and the lungs of humans and animals as well. It is also recognized as a potential carcinogen.

Aquatic life

Hill and Robinson (1975) reported that concentrations of 5 to 25 mg/L of phenol could be toxic and/or lethal to fish (Razo-Flores et al., 2003). Moreover, the aquatic life can be affected by phenolic concentrations of 1 mg/L (Chang et al., 1995; Tay et al., 2001; Veeresh et al., 2005). In Quebec, 0.3 mg/L has been set as the limit concentration for the prevention of contamination in waters and aquatic species based on U.S.EPA, 1980; U.S.EPA, 1998a. Beyond this concentration, the organoleptic or aesthetic properties of the drinking water could be altered.

Table 2-5: Toxicity limits for aquatic species

Fish species	Toxicity value
Rainbow trout (*Oncorchynchus mykiss*)	5.02 mg/L (96-hour LC_{50}) [1]
Goldfish (*Carassius auratus*)	85 mg/L (2.5-hour LC_{50}) [1]
Invertebrate specie	
Caddisfly and Mayfly	2 mg/L (48-hour LC_{50}) [2]
Flower fly (*Eristalis sp.*)	2000 mg/L (48-hour LC_{50}) [2]
Fresh algae	
Scenedesmus quadricauda	7.5 mg/L (24-hour $EC_{90,\ assimilation}$) [3]
Scenedesmus subspicatus	1211 mg/L (24-hour $EC_{90,\ assimilation}$) [3]
Vascular plants and macrophytes	
Lemma perpusilla	3 mg/L (12-14 days, abnormal growth) [4]
Lemna minor	1500 mg/L (48-hour $EC_{50,\ chlorosis}$) [4]
Larval stage	
Amphibians	0.04-11.23 mg/L (5-9 days, LC_{50}^{S}) [5]
Fish	0.07-2.67 mg/L (6.5-58 days, LC_{50}^{S}) [6]

Adapted from CEPA, 2000.

1 McLeay, 1976; Kishino and Kobayashi, 1995
2 Kamshilov and Flerov, 1978
3 Bringman and Kühn, 1980, Tisler and Zagorc-Koncan, 1995
4 Rowe et al., 1982
5 Birge et al., 1980
6 Birge et al., 1979; DeGraeve et al., 1980; Millemann et al., 1984

2.3. ANAEROBIC DIGESTION

Anaerobic digestion is the breakdown of organic matter without the presence of oxygen. This type of process has the ability of producing biogas, which is made mostly of methane (CH_4) and carbon dioxide (CO_2), with small amounts of other gases such as hydrogen sulphide (H_2S) which accounts for the rotten egg smell. This process occurs naturally in the environment and has been acknowledged for the production of combustible gas since the 17^{th} Century (Brewer, 1988).

Anaerobic treatment became popular after the up-flow anaerobic sludge blanket (UASB) reactor was developed by Lettinga in 1980 (Singh et al., 1998). It is suitable for the treatment of low to high concentrations of organic chemicals, textiles, petrochemical, food, pulp and paper industry wastewater, chlorinated organics and inorganics (Mulligan, 2002).

This type of degradation offers numerous advantages such as:

- Reduced production of sludge compared to aerobic treatment; (Mulligan, 2002).
- Production of biogas, which can be used as a fuel;
- No energy requirements for aeration due to the mixing produced by the upflow bubbles;
- Low nutrient requirements;

- Anaerobic sludge can be preserved without feeding for months (Lettinga et al., 1980).

Whereas the disadvantages can include:

- Complicated and long start-up due to the slow growth of the bacteria;
- Susceptibility to fluctuations in effluent, temperature, compositions of the substrate and nutrient concentration (Mulligan, 2002).

Anaerobic treatment can achieve good performance under mesophilic (30-40 °C) and thermopilic (50-75 °C) temperatures (Brewer, 1988); in addition, recent studies confirmed that this type of degradation could be carried under low temperature conditions, such as 3-9 °C (Nozhevnikova et al, 2000). This type of process depends on other parameters, as important as the temperature, including the pH/alkalinity, food requirements, retention times and loading rates. According to Mulligan (2002), the ideal pH for this type of process is 7; moreover, a broader pH range of 6.6 to 7.6 has been mentioned by Brewer (1988). The required retention times will relate to the volume of substrate to be treated and the rate of the degradation.

Anaerobic treatment (also called methane fermentation) is performed by various groups of microorganisms that undergo a series of metabolic reactions divided in three main stages. The first stage is hydrolysis and acidogenesis, in which the microorganisms convert polymeric material, e.g., lipids, proteins, and carbohydrates, into monomers such as glucose and amino acids. Then these monomers are converted into higher volatile fatty

acids (such as propionic, formic, acetic, *n*-valeric, *n*-caprionic and *n*-butyric acids), and hydrogen (Heukelekian, 1958; Brewer, 1988; FAO, 1999).

Decomposition of saturated fatty acids according to Neave and Buswell (1927) is shown as follows:

$$C_nH_{2n}O_2 + \frac{(n-2)}{2} H_2O \rightarrow \frac{(n+2)}{2} CO_2 + \frac{(3n-2)}{4} CH_4 \qquad (2.1)$$

Where n is the number of carbon atoms in the molecule.

Lipases are required to convert lipids (esters) to long-chain fatty acids, which are then degraded by β-oxidation to produce acetyl CoA. Proteases hydrolyzed the proteins into amino acids, which are then degraded to volatile fatty acids such as acetic, propionic and butyric acids. Cellulases, amylases and pectinases are in charge of hydrolyzing cellulose, starch and pectin (FAO, 1999).

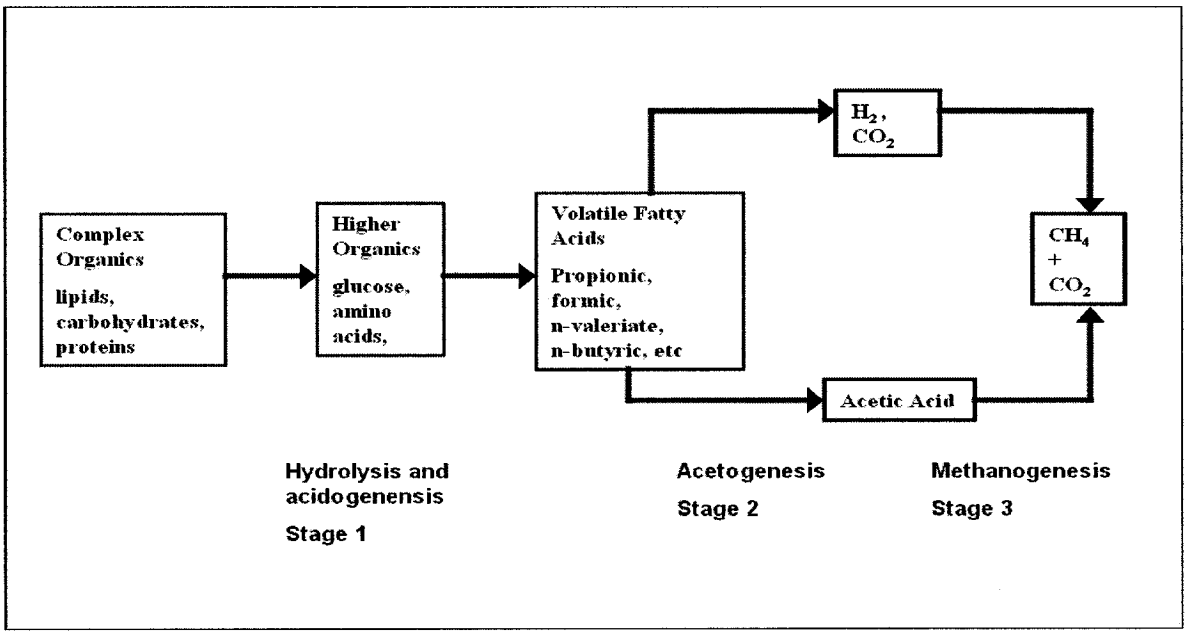


Figure 2.7: Schematic diagram of the anaerobic process.
Adapted from Chapter 4: Methane production (FAO, 1999)
http://www.fao.org/docrep/w7241e/w7241e0s.gif

The second reaction is acetogenesis, in which the bacteria convert the volatile fatty acids into hydrogen, and acetic acid. Although these two products are produced during acidogenesis, acetogenesis accounts for the major production of hydrogen and acetic acid during these degradation processes (FAO, 1999). The third stage is methanogenesis, in which the methanogenic bacteria produce methane and carbon dioxide from acetic acid, or methane and water from carbon dioxide and hydrogen (FAO, 1999; Mulligan, 2002).

Examples of carbon dioxide reduction are shown as follows by Heukelekian (1958):

$$2CH_3CH_2CH_2CH_2OH + 2H_2O + CO_2 \leftrightarrow 2CH_3CH_2CH_2COOH + CH_4 + 2H_2O \quad (2.2)$$

Butyl alcohol Butyric acid

$$2CH_3CH_2CH_2COOH + 4H_2O + CO_2 \leftrightarrow 4CH_3COOH + CH_4 + 2H_2O \quad (2.3)$$

Butyric acid Acetic acid

The general equation for methane fermentation is as follows:

$$4H_2A + CO_2 = 4A + CH_4 + 2H_2O, \quad (2.4)$$

Where H_2A represents any hydrogen donor for the reduction of carbon dioxide.

2.3.1. Methanogenic Bacteria

Anaerobic degradation is performed by groups or consortia of microorganisms working at different stages at the same time. These bacteria are called methanogenic bacteria, and can be divided into two groups:

- H_2/CO_2-consumers, called hydrogenophilic. H_2/CO_2-consuming methanogens reduce CO_2 as an electron acceptor via the formyl, methenyl, and methyl levels through association with coenzymes, to finally produce CH_4, (FAO, 1999) and
- Acetate-consumers, called acetoclastic. This acetate consuming bacteria such as *Methanosarcina spp.* and *Methanothrix spp.* (currently named, *Methanosaeta*) are the most important anaerobic organisms found in UASB reactors (Sing et al, 1998; FAO, 1999). *Methanosarcina spp.* are known to have high growth rates, good performance in the treatment of low strength wastewaters but are also

known to be easily washed out in continuous flows; whereas *Methanosaeta* are good for promoting granulation in the system (Singh et al, 1998).

Table 2-6: Methane species and their substrates according to Baker (1956).

Methane Bacteria	Substrate
Methanobacterium formicicum	Formate, CO, H_2
Methanobacterium omelianskii	Primary and secondary alcohols, H_2
Methanobacterium propionicum	Propionate
Methanobacterium sohngenii	Acetate and butyrate
Methanobacterium suboxydans	Butyrate, valerate, and caproate
Methanococcus mazei	Acetate, butyrate
Methanococcus vanielli	Formate, H_2
Methanosarcina methanica	Acetate, butyrate
Methanosarcina barkerii	Methanol, acetate, HCO

Anaerobic degradation can be achieved with sulphate, carbon dioxide, nitrate and some organics instead of oxygen. Oxygen is lethal for this type of bacteria and thus, has to be removed from the wastewater to be treated. However, some studies suggest that certain acetogenic bacteria can cope with low oxygen concentrations under in situ conditions (Karnholz et al.., 2002).

2.4 *UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTOR*

The upflow anaerobic sludge blanket (UASB) reactor has been widely used during the last decades to successfully degrade various types of wastewaters (Riera et al., 1985; Fang et al., 1994; Chou et al., 2004). These wastewaters range from waste from the food processing industry to wastes from petrochemical industry including pharmaceutical, pulp and paper, slaughterhouses and domestic wastes (Sing et al., 1997; Chou et al., 2004).

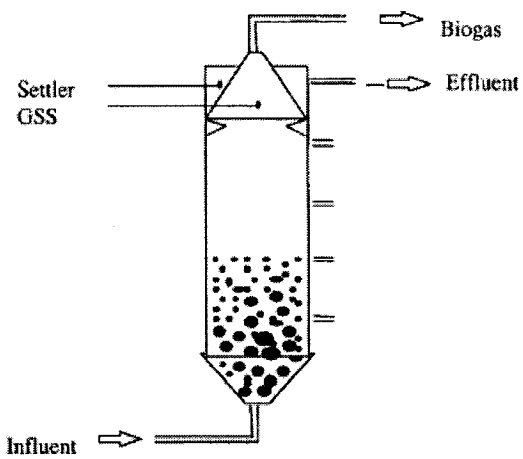


Figure 2.8: General UASB reactor design (Karim and Gupta, 2003).

As mentioned by Sing et al. (1998), the UASB reactor consists mainly of sections or stages:

- The first stage consists of a bed made of sludge (bacteria) where all the organics present in the influent wastewater is converted anaerobically into methane and carbon dioxide.

- These gases then flow upward through the second stage, where all the mixing takes place due to the upflow bubbles.
- The last part of the system is the gas-solid separator, which enables the gas to move upward towards the gas output, and retains the solids in the reactor, preventing washout.

It is very important to ensure the appropriate physical and chemical conditions to the system during the start up of it. These conditions include pH, temperature and nutrients and minerals for proper bacterial growth.

2.4.1. UASB REQUIREMENTS: Nutrients and trace metals

The microorganisms involved in the process as for any living being need food to grow and live. According to Brewer (1988), the most important nutrients required by methanogens are nitrogen and phosphorus. Also other minerals and trace metals are necessary but in doses which will allow them to degrade the organic contaminant without causing toxic shocks. Example of these nutrients may be calcium, nitrates, and ammonium salts (Singh et al., 1999). The concentration of each nutrient may vary and reported values can be obtained from previous studies.

- Nitrogen: The concentration should be between the required values for the proper growth of the sludge. Singh et al. (1999) suggest that these values should be higher than 0.3 g/L but smaller than 1 g/L.

- Along with nitrogen, phosphorous is essential for the proper growth of the bacteria. According to Sing (1999), the amount of phosphorous should account for 20% of the amount of nitrogen. In this experiment, phosphorus is usually added in the form of KH_2PO_4 or K_2HPO_4.
- Calcium ions help enhance the flocculation of the sludge by improving the mechanical strength of the flocs. (Lettinga et al., 1980). Concentrations between 100-200 mg/L have been shown to improve the granulation (Mahoney et al., 1987; Yu et al., 2001).
- The magnesium concentration should be maintained around 35 mg/L to promote growth. Magnesium in the form of $MgCl_2$*6 H_2O will be used. (Singh et al., 1999).
- The *Methanosarcina* species is known to use sodium for cell growth, amino acid transportation, pH regulation and methanogenesis. (Sing et al., 1999).
- Potassium is required for proper cell growth, and sufficient amounts could be achieved by adding the phosphate buffers KH_2PO_4 or K_2HPO_4; in addition, it could be supplemented by adding KCl. (Singh et al., 1999).
- In addition to these minerals, yeast extract is added as a source of vitamin B, which promotes growth in cells, and peptone which supplies nitrogenous compounds needed as well for growth (Singh et al., 1999).

In addition to the nutrients, there are some elements needed for the synthesis of various anaerobic microorganisms (Jarrell et al., 1987; Sing et al., 1999). These elements include iron, zinc, molybdenum, manganese, nickel, cobalt, boron and copper. Also, methanogens and acetogens need nickel, cobalt and boron for stability. The forms of

$MgSO_4*H_2O$, $CaCl_2$, $CuCl_2$, $ZnCl_2$, NaCl, $NiCl_2*6H_2O$, $CoCl_2*2H_2O$ and $FeCl_3*$ $6H_2O$ were used in this experiment.

2.4.2. Phenolic Treatment

The study of aerobic degradation of phenolic compounds has been replaced by the anaerobic biodegradation in the last years (Fang et al., 2000). Anaerobic processes have gained acceptance in the degradation of high strength wastewaters in the last years contrary to what Lettinga believed in 1980 when he developed the UASB reactor (Fang et al., 2000; Veeresh et al., 2005). Anaerobic processes can be considered as good alternatives since the costs are relatively lower than aerobic processes. They can treat small volumes of wastewaters, and produce lower amounts of sludge (Samson, 1991; Charest et al., 1999).

The biodegradation of phenolic compounds from petrochemical industries in lab-scale UASB reactors has been reported (Vogel and Winter, 1988; Parker and Farquhar, 1989; Fang et al., 1996; Fang and Zhou, 2000; Razo-Flores at al., 2003; Veeresh et al., 2004; Fang et al., 2004). A petroleum refinery plant is involved in numerous processes with effluents of high phenolic concentrations (Charest et al., 1999).

Phenols are prime materials used in a variety of chemical processes, which include production of adhesives, resins, antioxidants, herbicides, photo-developing chemicals. It

is also found in processes such as coal conversion, coke ovens, oil refinery, fibreglass manufacturing and petrochemical industry (Fang et al., 2000; Veeresh et al., 2005). The concentration of phenols in these waters can vary from 1.0 to 1.7 x 10^4 mg/L; contributing from 40 to 80 % of the total COD of these wastewaters (Veeresh et al., 2005).

REPORTED START-UPS OF PHENOLIC DEGRADATIONS

Previous studies have shown that degradation of high strength wastewaters can be enhanced by adding an easily degradable co-substrate such as carbohydrates or volatile fatty acids (VFAs) (Fang et al., 2000). According to Tay et al. (2001), a concentration of 1000 mg/L of glucose provided the fastest sludge acclimation and sludge removal. They also reported that the systems supplemented with glucose were less sensitive to thermal shocks.

During the start up, the bacteria were fed with easily degradable substrates in order to enhance the initial activity. Once the performance has reached 80% of COD removal, and a stable gas production rate is reached, the system is ready to be loaded with increasing concentrations of phenol.

Veeresh et al. (2005) have reported 6 weeks to 10 months for the duration of start-up, whereas Fang et al. (1996) reported 45 days of acclimation for a sucrose-fed inoculum (83% of COD removal and 420 mg/L of phenol concentration). In addition, Tay et al.

(2001) reported that acclimation with glucose as co-substrate showed shorter start-up and granulation period. Also Kobayashi (1989) found that adding peptone as a co-substrate could enhance the biodegradation of phenol. Some delays during the start-up could be related to the rate of transformation of phenols into biogas or to the adaptability of the biomass to the chemical (Veeresh et al., 2005).

According to Veeresh et al. (2005), UASB reactors treating phenols as the sole substrate without re-circulation can have drawbacks, due to the toxicity of the chemical, such as a decrease in the degradation rate at higher loading rates, sensitivity to temperature and loading shock without the ability to recuperate quickly. Adding a co-substrate, dilution or re-circulation can solve this problem. Some common co-substrates may include sucrose, glucose, VFAs, methanol, etc. (Veeresh et al., 2005).

REPORTED BIODEGRADATION OF PHENOL

Fang et al. (2000) have reported degradation of up to 97% of phenol at an HRT of 12 hours for phenol concentrations of 1260 mg/L (which corresponds to 3000 mg/L of COD) and a loading rate of 6 g COD/(L-d) and up to 97-99% of benzoate for loading rates up to 30.6 g COD/(L-d). To ensure good performance of the reactor, detectable levels of phenol, benzoate, VFAs and sucrose should be less than 1 mg/L (Fang et al., 2000).

Phenolic biodegradation pathway

Kobayashi et al. (1989) proposed a biodegradation pathway for phenol under anaerobic conditions. The first step in this pathway occurs when phenol is converted into benzoate or benzoic acid (Kobayashi et al., 1989; Charest et al., 1999; Veeresh et al., 2005). After, benzoate is de-aromatized to form cyclohexane carboxylic acid; it is then cleaved into heptanoate. Heptanoate is degraded (fragmented) to form

a) By β- oxidation: acetate, valerate and propionate (Keith et al., 1978; Fang et al., 1996; Veeresh et al., 2005); or
b) Directly propionate and butyrate, which can be transformed into acetate (Fina et al., 1978; Fang et al., 1996).

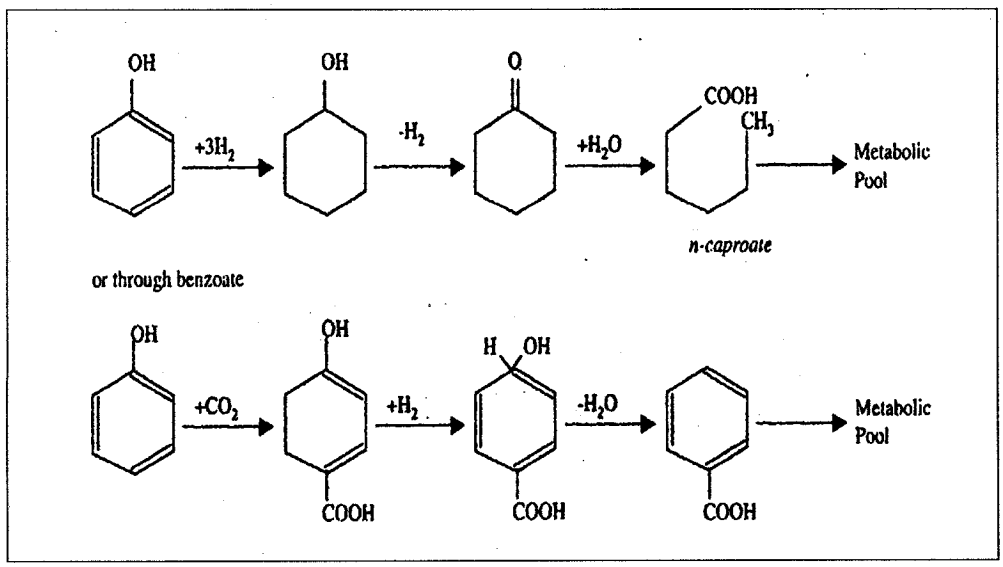


Figure 2.9: Phenolic biodegradation pathway (Jothimani et al., 2003).

Fang et al. (1996) reported that phenol conversion into benzoate was shown to be the rate-limiting step in the anaerobic degradation of phenol. According to Fang et al. (1996), the benzoate degradation into acetate occurs inside the cell of the bacteria. They

stated this after no propionate or butyrate was detected in the effluent. The phenol-degrading granules lacked a layered microstructure. They stated that probably due to the high reaction rate of the conversion of phenol into benzoate, the phenol may be diffusing into the granules instead of being converted on the surface.

Phenol is also known as a disinfectant and it is inhibitory to the activity of some bacteria to a concentration of about 800 mg/L, approximately 2000 mg/L COD (Kirk-Othmer, 1978; Fang et al., 1996). Since phenol poses harm and recirculation is not intended, an appropriate organic loading rate (OLR) has to be found in order to reduce the danger to the activity of the methanogenic bacteria.

Having a co-substrate has been proven to help in the degradation of phenol and it is intended in this experiment to use CO_2 (which is a GHG and a major contributor in Global Warming) as the co-substrate needed for boosting the phenol degradation process. The use of carbon dioxide poses many advantages such as the use of a waste gas instead or releasing it to the environment with higher methane recovery and less toxic shocks in the system due to the co-substrate.

Chapter 3 . MATERIALS AND METHODS

3.1. EQUIPMENT USED

The equipment used for the experiment consisted of four acrylic UASB reactors adapted with ports, two peristaltic pumps for feed and water bath, thermometer to check that temperature was around 35 °C, heater to maintain the hot water bath, pH probe and meter, manometer and syringe to measure gas production rate and content. In addition, analytical equipment was used such as a gas chromatograph (GC), high pressure liquid chromatograph (HPLC), UV/VIS spectrophotometer, and COD block heater to achieve a temperature of 150° C for the digestion of the COD samples were used. Other equipment consisted of a Blue Lindberg Oven, an Isotemp Muffle furnace, a vacuum pump, a titration burette, and a fume hood to host the setup.

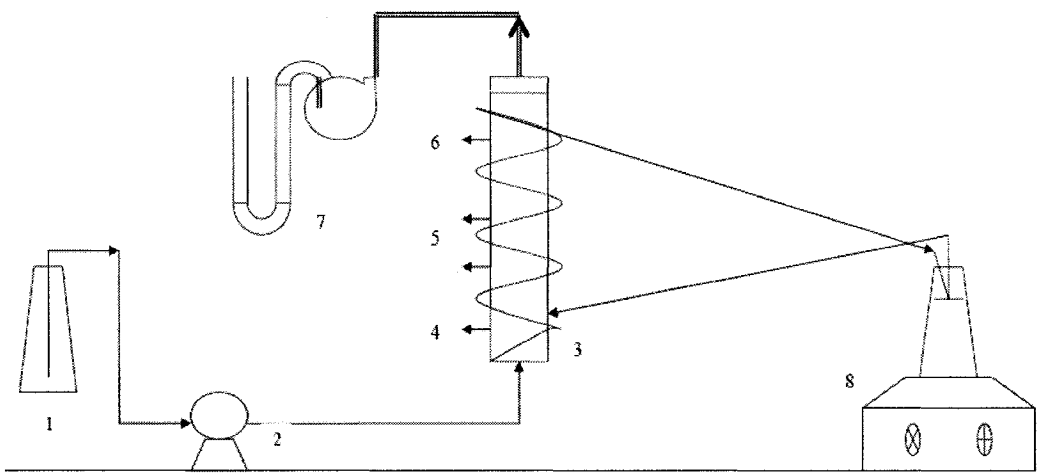
3.1.1. UASB Reactors

A set of four acrylic custom-made UASB reactors of 45 cm in height and 8.2 cm in diameter were used for this experiment. The inside volume used of each reactor was 1.2 L. The reactors had three sampling ports along its height: the lowest one for biomass sampling, one in the middle for the thermometer and one on the top for effluent sampling.

In addition, the reactors had one opening at the bottom of the reactor as the wastewater inlet, and a gas sampling port at the top of the rubber stopper which served to collect the

gas for its qualitative and quantitative measurements. To avoid the escape of biomass and any channelling due to the flow, the bottom of the reactors was covered with a mesh made from fibreglass with openings of 1.0 cm, and glass beads 4.0 – 5.0 mm.

Also, each reactor was provisioned with a gas-solid separator, made from a funnel, which was attached to the reactor. These separators retained the solids that were transported by the output gas flow to be returned to the reactor system. According to Lettinga (1980), the top of the sludge blanket should remain well below the solid-gas separator to avoid excessive washout. Only under steady state conditions this may be accepted due to the fact that sludge washout equals the sludge growth.



1. 1L flasks with caps (4)
2. Peristaltic pump (with 4 lines)
3. UASB reactor (4)
4. Biomass sampling port
5. Effluent sampling ports
6. Temperature sampling port with thermometer
7. Manometer with bubbling tube for gas production monitoring
8. Heater with hot water coil to maintain 35° C

Figure 3.1: Schematic representation of the experimental setup

3.1.2. Peristaltic Pumps

Two peristaltic pumps were used. One pump Masterflex model 7553-70 was used to re-circulate the hot water needed to reach the mesophilic temperature, and the other, Masterflex L/S model 7519-15 with 6 parallel lines, was used to inject the substrate in continuous mode. The pumps were purchased from Cole Parmer.

3.1.3. Water Bath and Thermometers

Since the biomass worked under mesophilic conditions, a hot-water bath had to be used to achieve a constant temperature of 35° ±2 C. Hot water was re-circulated inside a tube that surrounded the reactors, and the water was heated using a Fisher stirring hot plate (from Fisher Scientific Ltd.); Fisherbrand thermometers with a range of -10 to 110 ° C bought from Fisher Scientific were installed on a sample port to keep track of the temperature changes.

3.1.4. pH Probe and Meter

The pH was controlled by a pH probe (purchased from Fisher Scientific Inc.) and Chemocadet pH controller from Cole-Parmer. Solutions of 1 N KOH and 0.1 N HCl were prepared in order to adjust the pH whenever necessary.

3.1.5. Manometer

A manometer was used to measure the gas production rate of each reactor. The manometer was made with two syringes of 100 ml joined together with tubes. A direct line from the reactor was allotted with a three way valve. The manometer also had one Dudley tube filled with an acid solution of 5N HNO_3 (nitric acid) to retain any moisture from the biogas.

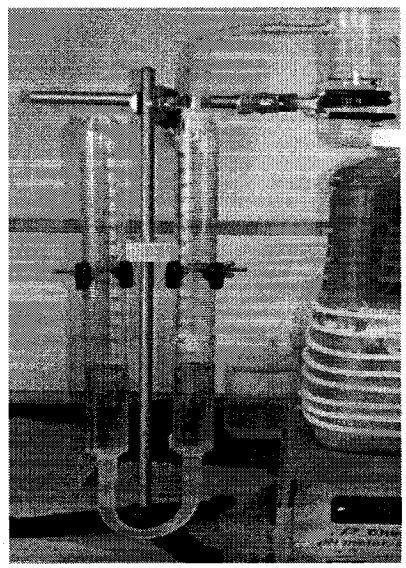


Figure 3.2: Manometer used in the experiments

3.1.6. Syringe

A plastic syringe with volume of 10 mL and a valve was used to transfer the gas to be measured in the gas chromatograph (GC).

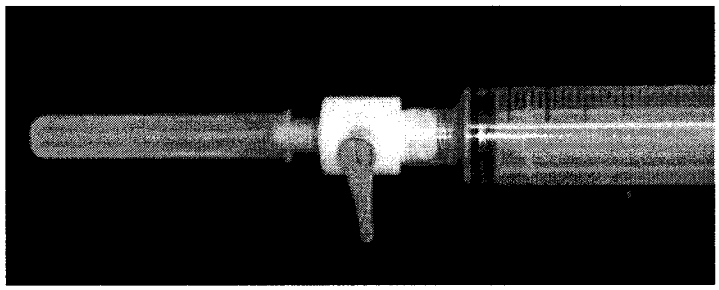


Figure 3.3: Syringe with valve for gas sampling.

3.2 Materials

3.2.1. Biomass

The reactors were inoculated with acclimated anaerobic biomass collected from the treatment plant of a cheese factory in Granby, Quebec, Canada. Agropur Coop is the largest dairy cooperative in Canada and processes about 1.9 billion litres of milk annually with sales exceeding $1.9 billion. Agropur processes fluid milk, butter, cheese, fine cheeses, and yogurt in their 18 plants. Characterization of the initial biomass was performed, and samples were taken to determine the organic content; TSS was 106.3 g/L, TVSS was 76.5 g/L and the organic ratio (TVSS/TSS) was 0.719 (71.9%).

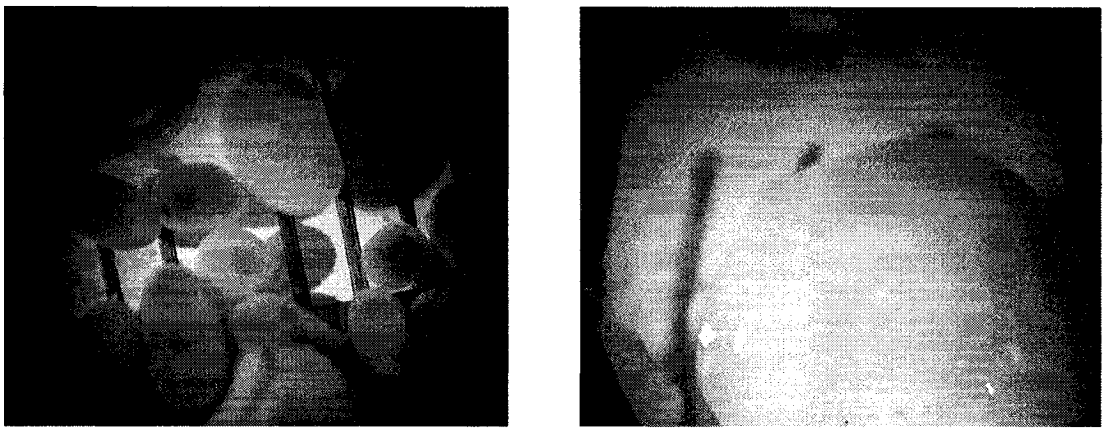


Figure 3.4 a and b: Pictures of the mixed-culture bacteria (size of the bacteria range from 1 – 3 mm)
Taken with a microscope and magnification of 6x and 5.0 mega pixels digital camera.

3.2.2. Substrates

Two different substrates were used in this experiment. The first substrate was based on acetic acid or sucrose, depending on the reactor, for the acclimation of the bacteria. After the acclimation of the bacteria, a substrate containing a mixture of acetic acid/sucrose and phenol was used.

A feed with phenol as the carbon source was used for the acclimation of the biomass. Adding specific nutrient and mineral solutions (Table 3-1) made the substrate appropriate for the biomass growth. The solution was prepared according to Alimahmoodi (2004). In addition, 10 ml of trace mineral solution (Table 3-2) was added to allow bacterial growth.

All the chemicals were purchased at Fisher Scientific Ltd. Also, peptone and yeast extract were added in different quantities (Table 3-3).

Table 3-1: Nutrients used per litre of solution.

Compound	Amount(g/L)
NaCl	0.6
KH_2PO_4	0.44
$MgCl_2$	0.2
$CaCl_2$	0.2
NH_4Cl	1.2
Trace mineral solution (Table 3-2)	10 ml

Table 3-2: Trace mineral solution.

Minerals	Amount(g/L)
$MnSO_4.H_2O$	0.1
$CoCl_2$	0.0131
$CaCl_2$	0.076
$CuCl_2$	0.02
$ZnCl_2$	0.1
NaCl	1.0
$NiCl_2.6H_2O$	0.12
$FeCl_3.6H_2O$	1.34

Table 3-3: Co-substrates in the synthetic wastewater.

Solution	COD(g/L)	
	Up to 0.8 g/ L	**0.8- 1.2 g/L**
Sucrose (g/L)	0.4- 1.0	----------
Acetic acid (ml/L)	0.4- 1.0	----------
Yeast extract (g/L)	0.8	1.2
Peptone (g/L)	0.4	0.8

3.2.3. Gas

A nitrogen tank of an ultra high purity 5.0 was used throughout the whole experiment to purge the wastewater and provide a non-oxygen environment for the bacteria. Also, a carbon dioxide tank with purity of >99.99% was also used in order to saturate the wastewater containing the phenol. These tanks were purchased from Praxair.

3.3. EXPERIMENTAL SETUP

In these experiments, the UASB reactors were used under two different conditions or processes. The first process was a batch-wise experiment and the latter was a continuous-fed experiment. In addition, the reactors were tested under different co-substrates (sucrose and acetic acid).

3.3.1. Incubation Period

During the incubation, the biomass was acclimated to the mesophilic temperature of 35° ± 2°C for 4 weeks. Two reactors of 4 L were used, the first reactor, with 2 L of biomass, for sucrose-acclimation, and the second reactor with 2 L of biomass for acetic acid-acclimation. Temperature was the only parameter under observation at this stage

3.3.2. Acclimation Period

During the acclimation process both reactors were fed with the corresponding wastewater, sucrose and acetic acid. The initial concentration of each substrate was 1.0 g/L (approximately of g COD/L) and later it was increased step-wise until a concentration of 10.0 g COD/L was achieved.

The acclimation period was approached as a batch-wise experiment. In this type of process the gas production had fluctuations. First, there was low production since the bacteria had to adapt to the newly received substrate. The reactor showed an improvement in the gas production when it reached half of the process, and then it declined again when it reached the end of the process. In this period, the substrate was fed in fixed volumes of 2L each time, and the new load was added only after a portion of the organic load was digested in each of the reactors. The degradation of 80% of the initial COD and a retention time of one day was used as the set point for the next loading.

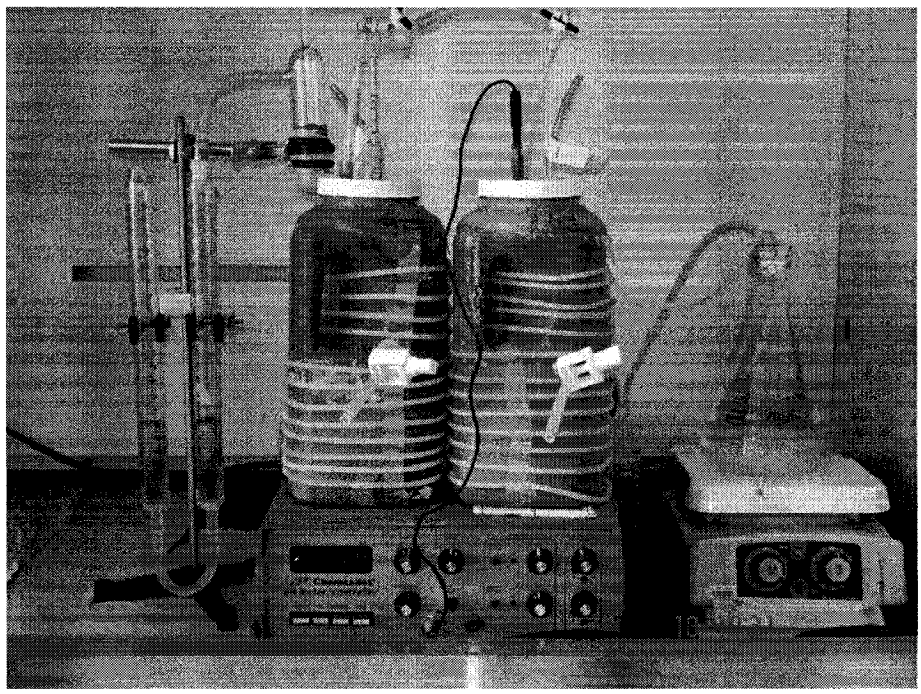


Figure 3.5: Acclimation set-up

TOXICITY TEST

A specific methanogenic activity (SMA) test (Ince et al., 1994) was carried out to determine the highest possible concentration of phenol that could be degraded with the biomass. This helped to determine the toxicity limit of the bacteria. The units to express the SMA were L CH_4/gTVSS-d.

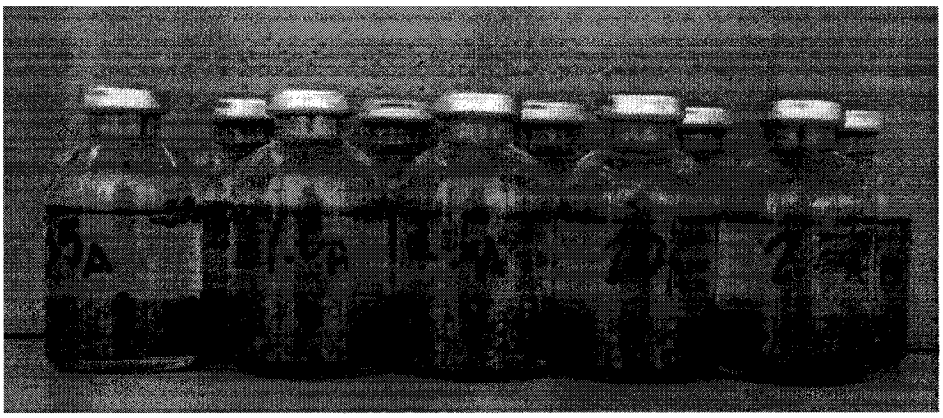


Figure 3.6: Toxicity test to determine the inhibitory concentration of phenol towards the bacteria

BIOMASS ACCLIMATION TO PHENOL

After determining the range of values at which the biomass was not inhibited by the phenol, the system was ready to be loaded with phenol. At the beginning, a concentration of 1000 mg/L of the co-substrate (sucrose or acetic acid) was loaded with a concentration of 300 mg/L of phenol. When the system reached a degradation of approximately 80% COD, the co-substrate concentration was reduced in steps which were inversely proportional to the increase in the concentration of phenol. So far, 1260 mg/L of phenol has been reported as the maximum value of phenol in an UASB reactor (Fang et al., 1996). Parameters such as temperature, pH, COD, DO, biogas rate, methane content, SMA and TVSS were monitored (Table 3-4).

Table 3-4: Frequency of testing during biomass acclimation to phenol

Parameter	Frequency
Temperature	Constant measurement
pH	Constant measurement
Dissolved oxygen (DO)	Before every new batch
Methane content of the biogas	Every day
COD	Every day
TVSS	At the end of the acclimation

3.3.3. Continuous Experiment

Two sets of 2 reactors were used during this stage. In each set one reactor was the control (in this reactor the wastewater will be composed solely of phenol), and the other reactor was used with the substrate containing phenol supplemented with CO_2. For this last reactor, carbon dioxide gas was added to the wastewater, until a saturation point was reached, approximately pH 4.5 - 5.2, and then, the wastewater was pumped into the system. The final pH for the continuous experiment was 6.5 - 7.3.

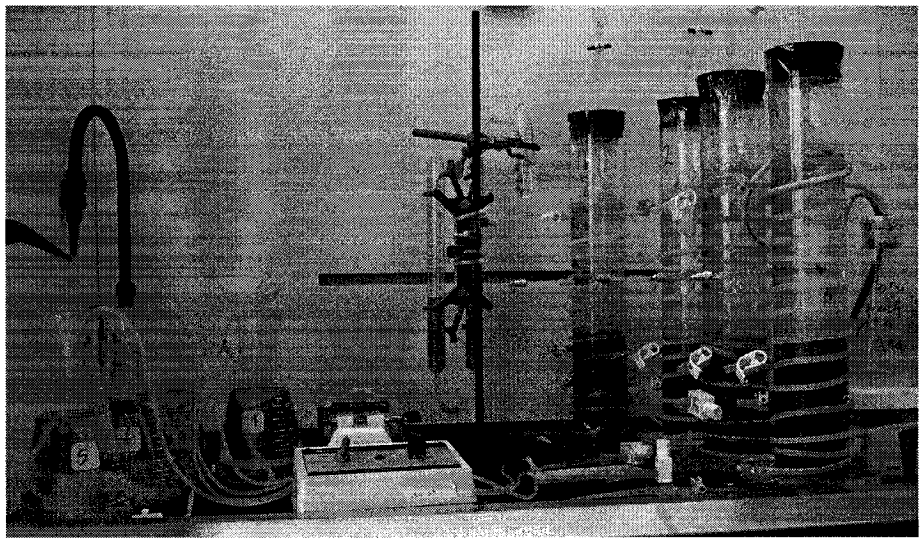


Figure 3.7: Continuous test setup

A constant volume was always kept inside the reactor. The system was checked for degradation values and the concentration of phenol was adjusted according to the performance of the reactor. The initial concentration corresponded to the last concentration used in the acclimation period. Parameters such as temperature, pH, chemical oxygen demand (COD), dissolved oxygen (DO), biogas rate, total alkalinity (TA), methane content, and total volatile suspended solids (TVSS) and total suspended solids in the effluent (TSS) were monitored (Table 3-5).

Table 3-5: Frequency of testing during the continuous test

Parameter	Frequency
Temperature	Constant measurement
pH	Every two hours
TA	Every two hours
Biogas rate	Every half hour
Methane content of the biogas	Every day
COD	Every day
Phenol concentration	Every day
TSS, TVSS (biomass)	Every week
TSS (effluent)	Every week

Table 3-6: Summary of the experimental schedule

Incubation Period	Incubation of the biomass at 35 ° C. t1 = 4 weeks
Acclimation Period	
I. Acetic acid / sucrose	HRT= 1 day COD_i= 1.0 g COD/l, COD_f=10.0 g COD/l t2 = 9 weeks
II. Toxicity test	Concentrations of 0, 0.5, 1.0, 1.5, 2.0 g phenol/L for each co-substrate.
III. Phenol acclimation	Increasing concentrations of phenol while reducing concentrations of the co-substrate. HRT= 1 day COD_i= 0.3 g COD/l of phenol and 1.0 g COD of acetic acid or sucrose t3 = 4 weeks
Continuous system	Loading of the system continuously with wastewater saturated with CO_2. t5 = 5 weeks

COD_i = Initial COD

COD_f = Final COD

3.4. ANALYTICAL METHODS

3.4.1. Alkalinity Test

Alkalinity is the capacity to neutralize acids. The alkalinity test was used to determine the amount of carbonate, bicarbonate and hydroxide content in natural waters and wastewaters. Alkalinity tests in order to determine the $CaCO_3$ concentration were performed by titration with sulfuric acid (0.02 N) following the method 2320 B of the standard methods (APHA, 1998). The total alkalinity of 1 ppm $CaCO_3$ is equivalent to 1 ml of sulfuric acid. Alkalinity (A) depends on the pH end point.

Definition of alkalinity (Nazaroff and Alvarez-Cohen, 2001; Alimahmoodi 2004),

$$A= [OH^-] + [HCO_3^-] + 2[CO_3^{2-}] - [H^+] \quad (3.1)$$

Definition of pH defined by Sorensen (1909)

$$[H^+] = 10^{-pH} \quad (3.2)$$

Procedure

To test the alkalinity in reactors R2 and R4, 5 mL of effluent were filtered through a 45 µm syringe filter. The pH was measured and TA was determined by titration of 0.1 N H_2SO_4. Testing was done in duplicate.

3.4.2. Dissolved Carbon Dioxide Concentration Determination

This value is based on the total alkalinity, pH values and the equilibrium relationships of the carbonate species (Alimahmoodi, 2004).

Equations for the dissociation of carbon dioxide in water

$$CO_2\ (aq) + H_2O \rightarrow H^+ + HCO_3^- \qquad K_1 = 4.47 \times 10^{-7}\ M \tag{3.3}$$

$$HCO_3^- \rightarrow H^+ + CO_3^{2-} \qquad K_2 = 4.68 \times 10^{-11}\ M \tag{3.4}$$

Combining these together,

$$K_1K_2 = 2.1 \times 10^{-17}\ M = \frac{[H^+]^2\,[CO_3^{2-}]}{[CO_2\ (aq)]} \tag{3.5}$$

Equation for the dissociation of water

$$Kw = [H^+]\,[OH^-] \tag{3.6}$$

The dissolved carbon dioxide can be found by using equation 3.1 and equations 3.3 and 3.4 after determining the value of $[H^+]$ from the alkalinity test and $[OH^-]$ from the dissociation of water. Since there are two unknown concentrations ($[CO_3^{2-}]$ and $[CO_2\ (aq)]$), a system of two equations is needed (eqs. 3.1 and 3.5).

3.4.3. Specific Methanogenic Activity Test (SMA)

The SMA test was used as a parameter in determining the optimum conditions in the anaerobic reactor. This test was performed by injecting substrate and sludge into a 40 ml flask with a rubber septum at 35 °C with anaerobic conditions (Yang et al., 1996). A ratio of 1:1 (V/V) was maintained between the substrate and the sludge. The methane was

collected with a syringe and measured daily. The SMA was calculated by determining the CH_4 production rate over a range of TVSS values. This test was also performed at the end of the continuous test to determine the performance of the reactors.

3.4.4. Biomass Characterization

The characterization of the biomass was based on its organic content. For this, a mix sample of biomass, approximately 2-3 mL, was taken from the reactors (lowermost sampling port of the reactors), and filtered by vacuum filtration on to gooch crucibles with filter papers. Later, these crucibles were ignited as per Method 2540 of the standard methods (APHA, 1998) for the determination of TSS and TVSS. The samples were done in duplicate.

3.4.5. COD Test

According to the standard methods (APHA 1998), the chemical oxygen demand (COD) is the amount of an oxidant that reacts with a sample. The reduced amount of the oxidant is expressed in terms of its oxygen equivalence. In this research, the closed reflux, colorimetric method (5250 D) from the APHA was used to test the biomass, the inlet substrate, and the effluent using a UV Spectrophotometer Perkin Lambda 40 UV/ VIS at a 600 nm wavelength with a cell adapter for the COD vials.

When a sample is digested, Cr^{6+} is oxidized into Cr^{3+}. These Cr species are both green-colored and they absorb in the visible region of the spectrum. The density of the green color is then measured against a standard curve made with different concentrations of potassium phthalate (KHP) solutions ranging from 0- 500 mg/L.

Procedure

COD testing vials (purchased from Fisher Scientific), containing potassium dichromate, sulfuric acid, silver sulphate, mercury sulphate, were used to measure the COD values. A sample from the effluent of each reactor was diluted and a volume of 2.5 ml was added to each testing vial containing 7.5 ml of the dichromate mixture. The samples were digested for two hours in a heating block at 150°C, and cooled. After this, the samples were ready for the measurements in the spectrophotometer. Each reactor's samples were taken in duplicate and the average value was reported.

Some theoretical COD values used during the experiment are according to Razo-Flores et al. (2003) and Vereesh et al. (2005), 1.0 g of phenol is equivalent to 2.38 g COD, and 1.0 g CH_4 is equivalent to 4.0 g COD according to Alimahamoodi (2004) and Fang et al. (1996).

3.4.6. Solids

The total solids of a sample are the remaining solids after evaporation and drying at a specific temperature. The total solids accounts for the total suspended solids and total

dissolved (settled) solids of a sample. In this experiment, the effluent was tested to determine the total suspended solids. The tests were all performed in duplicate based on method 2540 of the standard methods (APHA, 1998). In the case where TVSS was measured, the gooch crucible and the filter paper were ignited at 550° ± 2 C for 2 hours to remove any organic particles.

TOTAL SUSPENDED SOLIDS

In order to determine the amount of suspended solids in the sample, approximately 2 ml of the effluent was filtered with Whatman Gf/C filter paper (purchased at Fisher Scientific) and dried until a constant weight at a temperature of 105 ± 2°C overnight. The crucible was allowed to cool down for 10 minutes in a desiccator. The increase in weight of the gooch crucible represents the total suspended solids.

TOTAL VOLATILE SUSPENDED SOLIDS

The remaining solids from the previous test were then ignited until reaching a constant weight in a muffle furnace at 550 ± 2°C for 2 hours. After cooling down in the desiccator, weight loss was recorded. This weight loss accounts for the total volatile suspended solids (TVSS). The remaining material represents the fixed solids or inorganic matter.

3.4.7. Gas Analysis

Gas produced through anaerobic processes contains large amounts of CH_4, CO_2, and trace amounts of H_2, H_2S, O_2, and N_2, and it is saturated with water vapor (American Public

Heath Association, 1998). The biogas volume and composition were determined in order to assess the percentage of methane being produced (to determine the reactor's efficiency).

The volume of the methane produced was measured by the water displacement method over a period of time (L/d). This was achieved using a manometer, and a stop watch. Dudley tubes containing a solution of 5 N H_2SO_4 were used in order to remove water vapor.

Based on the Method 2720 C of standard methods (APHA, 1998), the methane percentage was analyzed by injecting 3 ml of the gas (using a syringe) to a gas chromatograph. The system was equipped with a thermal conductivity detector (TCD). The carrier gas was helium applied at a flow rate of 5 ml/ min. The chromatograph was a Varian model CP 3800. The column was a Carboxen 1010 Plot from Supelco. The injector temperature was 225° C. The column oven temperature was increased from 50 to 100 ° C at a rate of 5° C/min and the gas retention time was 15 min. The biogas was analyzed by comparing the concentration of the gases to reference curves of the standard gases. Samples of CH_4, CO_2, and N_2 of known purity were used.

3.4.8. Dissolved Oxygen

The amount of dissolved oxygen in the substrate was measured using a DO meter Aquacheck, (model 51600) after every purge. A probe was inserted in the 1 L flask containing the water after purging, and the value was recorded (less than 5 mg/L). It was

determined that 15 min was enough to reduce the DO of the water to the desired value of < 5 mg/L.

3.4.9. Phenolic concentration analysis

Phenolic concentrations and its derivative, benzoic acid, were measured based on the US EPA Method 610 using a High Pressure Liquid Chromatograph (HPLC) Beckman Coulter supplied by Fisher Scientific. The column used was a Supelcosil LC-PAH of 5μm, 15 cm by 4.6 mm. The mobile phase used was composed of 60:40, acetonitrile and water respectively at a flow rate of 1 mL/min and at a temperature of 25°C. The pressure used was 918 psi (6.3 MPa). The lamp used for the detection was UV at a wavelength of 254 nm and a sample size of 10 μL. The phenol retention time was 2.0 minutes and the retention time for benzoic acid was 1.2 minutes.

Procedure

To measure the phenolic concentration, 1 ml of effluent was filtered through a 45μm syringe filter and added to a sampling vial. This vial was placed in the HPLC for measurement. The area of the peak obtained was compared to a standard curve made with known values of phenol.

Chapter 4 . RESULTS AND DISCUSSION

4.1 Toxicity Tests

Toxicity tests were performed to evaluate the inhibitory concentration of phenol. A set of 10 flasks of 30 ml with rubber septa were used. The amount of biomass used per flask was determined based on the recommendations of Kleerebezem and Macarie (2003) who indicated that the maximum concentration of biomass is 25g TVSS/working volume and the initial TVSS values determined from the previous test corresponded to 10 ml of biomass. The rest of the flasks were filled with 16 ml of simulated wastewater containing 0, 0.5, 1.0, 1.5, 2.0 g/L of phenol (in duplicate). The flask was sealed and biogas measurements were taken for 6 days. Since this test is based on methane production over TVSS of the biomass, at the end of the experiment the biomass was dried.

The results are shown in Table 4-1. For the acetic acid-fed biomass, the methane production was reduced once the concentration passed 0.5 g/L, meaning that after this concentration the biomass could have been inhibited by phenol. In the other case, the sucrose-fed biomass showed an overall lower activity, and the inhibition was present after 1.0 g/L. The acetic acid-fed biomass performed better than the sucrose one.

Table 4-1: SMA for two types of biomass

Acetic Acid		Sucrose	
Phenol concentration (g/L)	SMA (L CH_4/gTVSS-d)	Phenol Concentration (g/L)	SMA (L CH_4/gTVSS-d)
0.0	0.0031	0.0	0.0031
0.5	0.1850	0.5	0.0039
1.0	0.0128	1.0	0.0051
1.5	0.0089	1.5	0.0025
2.0	0.0064	2.0	0.0022

4.2 Biomass Acclimation to Phenol

After determining the inhibitory concentration of phenol, the system was ready to be loaded with the first batch of synthetic wastewater containing phenol. Since the amount of biogas recovered from this test was very low, this test was not taken into consideration when determining the initial loading concentration of phenol. The initial concentration of phenol was decided to be 300 mg/L with the addition of 800 mg/L of the sucrose/ acetic acid based on Fang et al. (1996; 2004) and Chou et al. (2005) who stated that the initial loading should be below 500 mg of phenol/L. The idea during this step was to acclimate the biomass in the reactor by increasing step-wise the concentration of phenol and reducing the concentration of the co-substrate until it was completely removed. Since phenol is considered a high strength contaminant, in this part of the experiment a reduction of 80% of the initial COD and retention time of 24 hours was the set point for the next load. The problem encountered during this period was that when the concentration of 800 mg/L was reached, the HRT wasn't able to be decreased to 24 h probably because for this concentration more co-substrate was needed after all this is the

inhibitory concentration for anaerobic bacteria, and this was taken as the starting point for the continuous experiment with the addition of CO_2 as the co-substrate.

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COD trends

For the acclimation period, acetic acid showed an overall better COD removal compared to sucrose. Figures 4-1 to 4-8 show the results. Samples were analysed for COD in duplicate and average values were obtained with an error of 8 %. Figures 4-1 and 4-2 represent the total COD degradation for acetic acid-fed biomass for four different concentrations of phenol and the respective degradation for sucrose-fed biomass. For all four concentrations of phenol (300, 400, 600 and 800 mg/L), the acetic acid-fed biomass showed lower phenol values in the effluent. Figures 4-3 and 4-4 represent the reduction percentage for acetic acid and sucrose-fed biomass respectively, showing better removal percentages for the acetic acid-fed biomass (values over 90 % reduction compared to approximately 80 % reduction for the sucrose-fed biomass).

Once a reduction of 80% of the initial COD was achieved, the retention time was lowered from approximately 4 days to 1 day. Figures 4-5 and 4-6 represent the total COD degradation for acetic acid-fed biomass at retention time of 24 hours and the respective degradation for sucrose-fed biomass. At a retention time of 24 hours, the reactors followed the same trend showing that acetic acid-fed biomass presented lower amounts of phenol in the effluent. Figures 4-7 and 4-8 represent the reduction percentage for acetic acid and sucrose-fed biomass respectively. As shown in Figure 4.7, when the retention time was reduced to 1 day, the reactor treating acetic acid removed 80% of the total COD

(at concentrations of 300, 400 and 600 mg phenol/L) whereas the reactor treating sucrose achieved approximately 60% (at concentrations of 300 and 600 mg phenol/L) and 70% (at a concentration of 400 mg phenol/L).

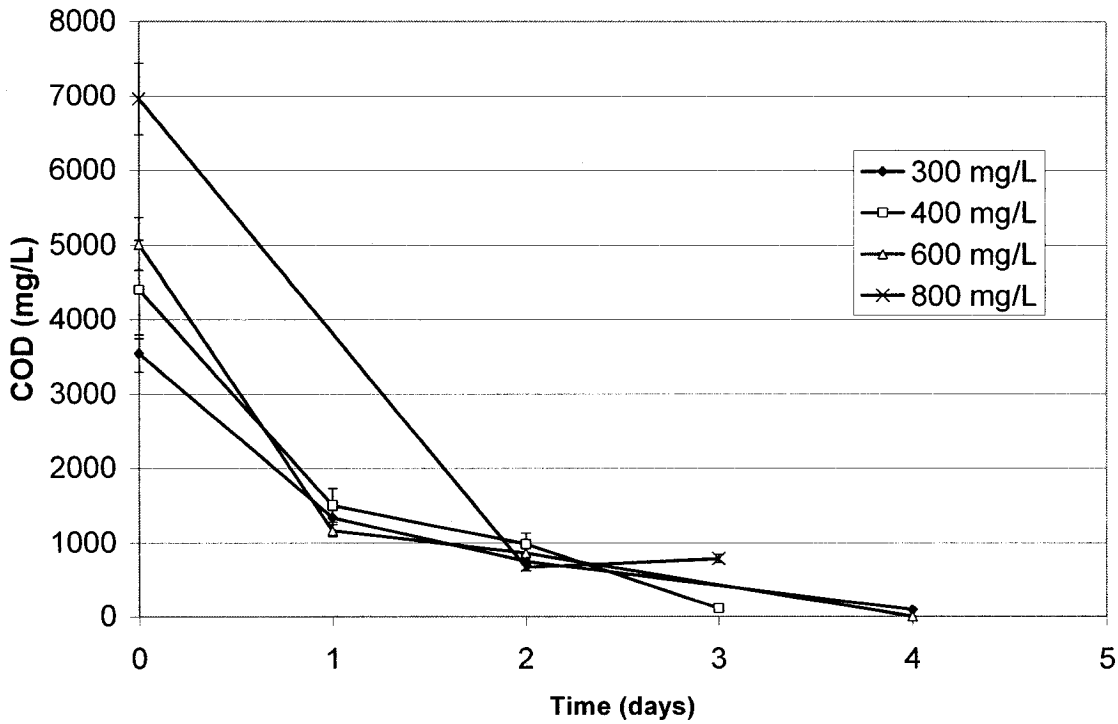

Figure 4.1: Total COD degradation for acetic acid-fed biomass at different concentrations of phenol

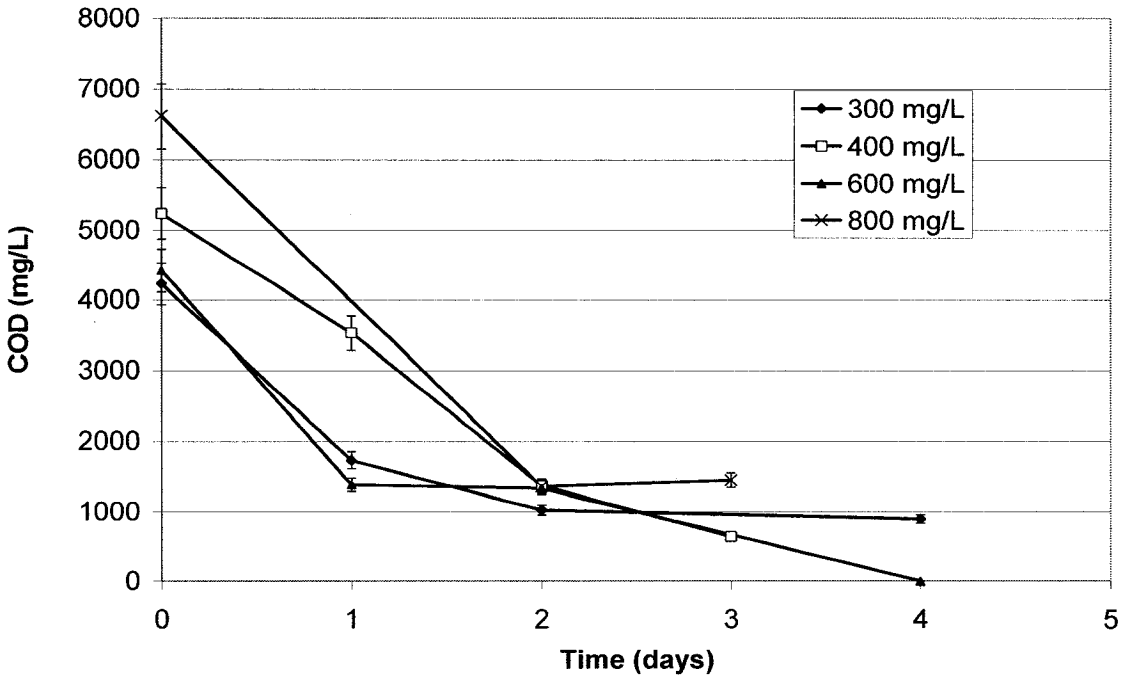

Figure 4.2: Total COD degradation for sucrose-fed biomass at different concentrations of phenol

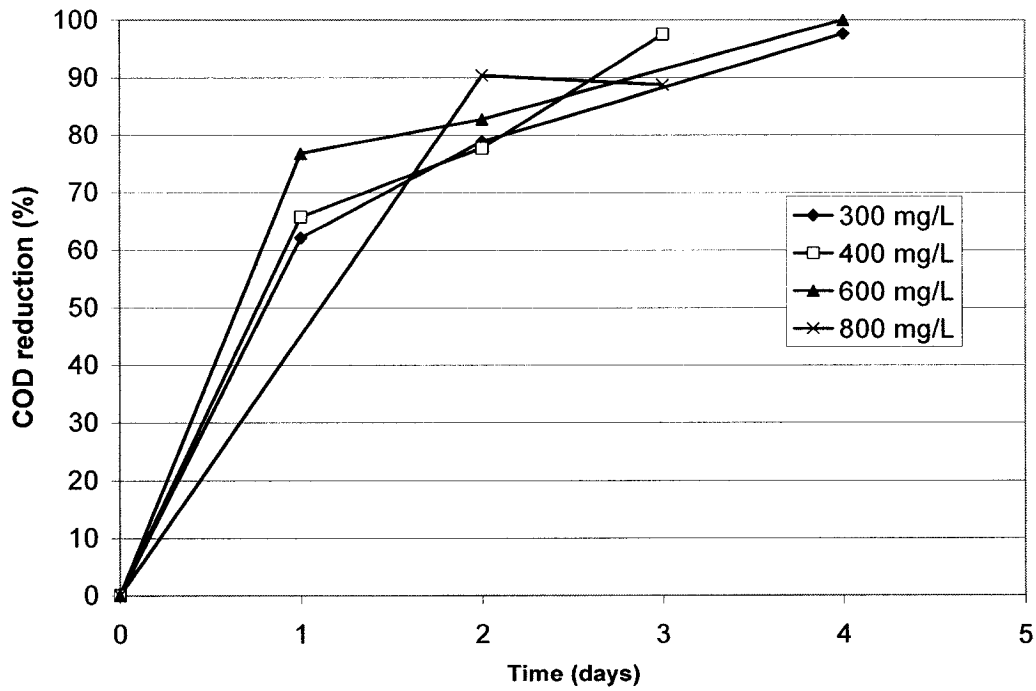

Figure 4.3: Total COD reduction (%) for acetic acid-fed biomass at different concentrations of phenol

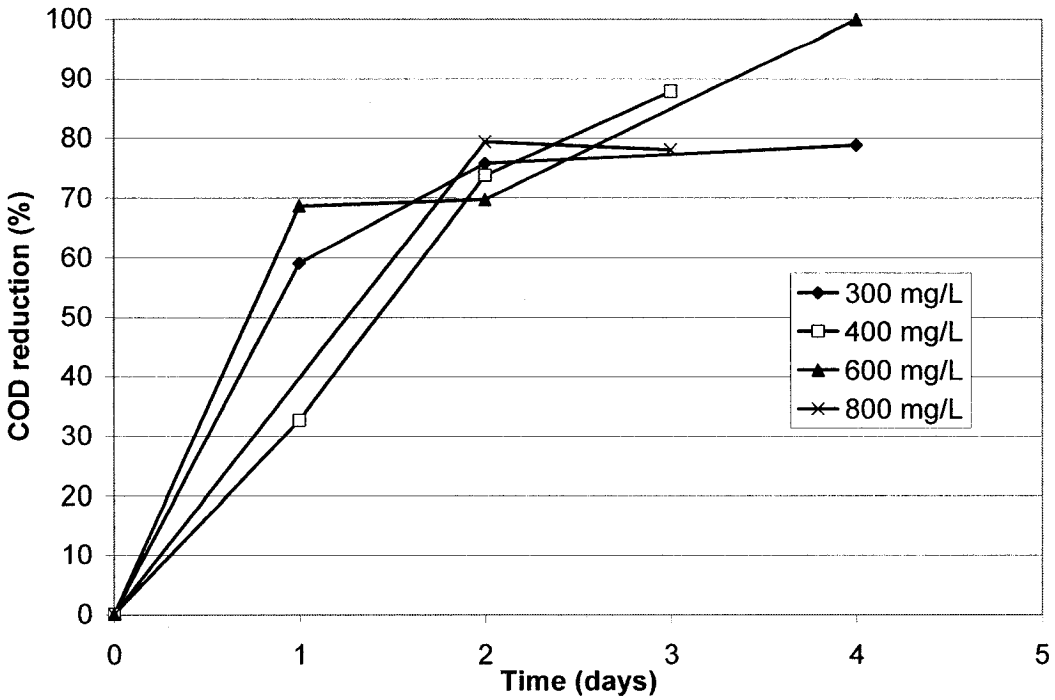

Figure 4.4: Total COD reduction (%) for sucrose-fed biomass at different concentrations of phenol

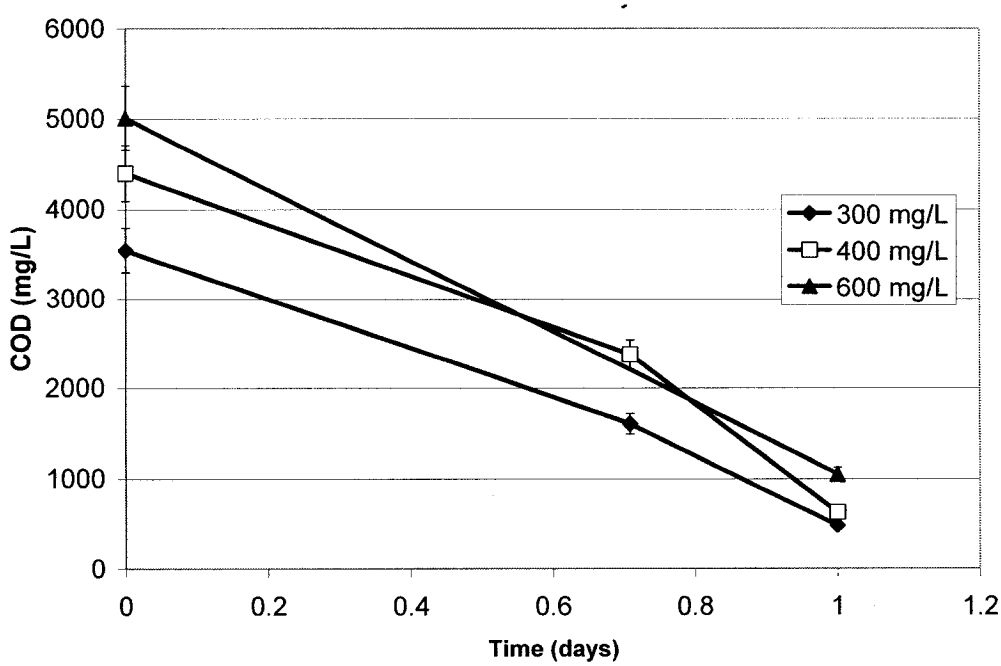

Figure 4.5: Total COD degradation for acetic acid- fed biomass at different concentrations of phenol and HRT= 1 day

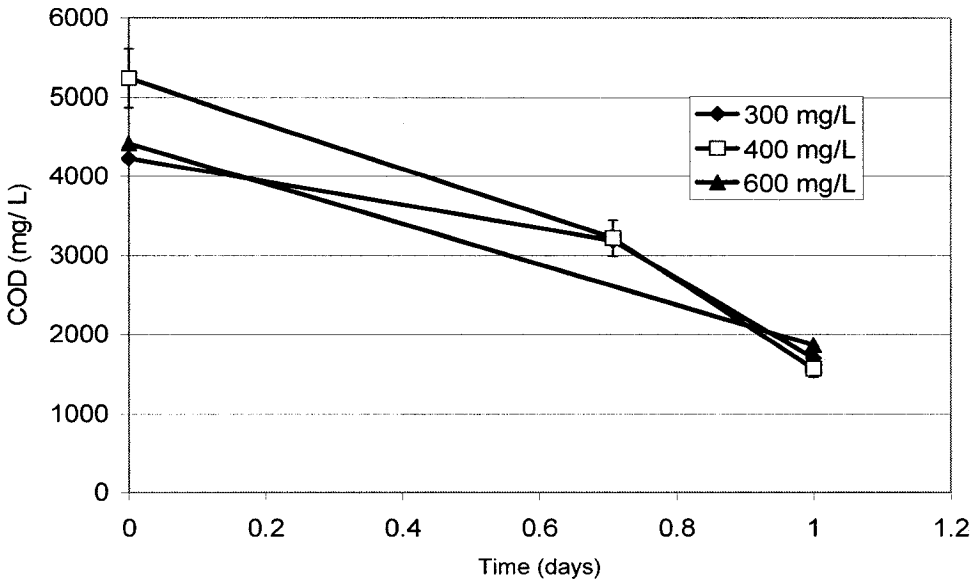

Figure 4.6: Total COD degradation for sucrose-fed at different concentrations of phenol and HRT=1 day

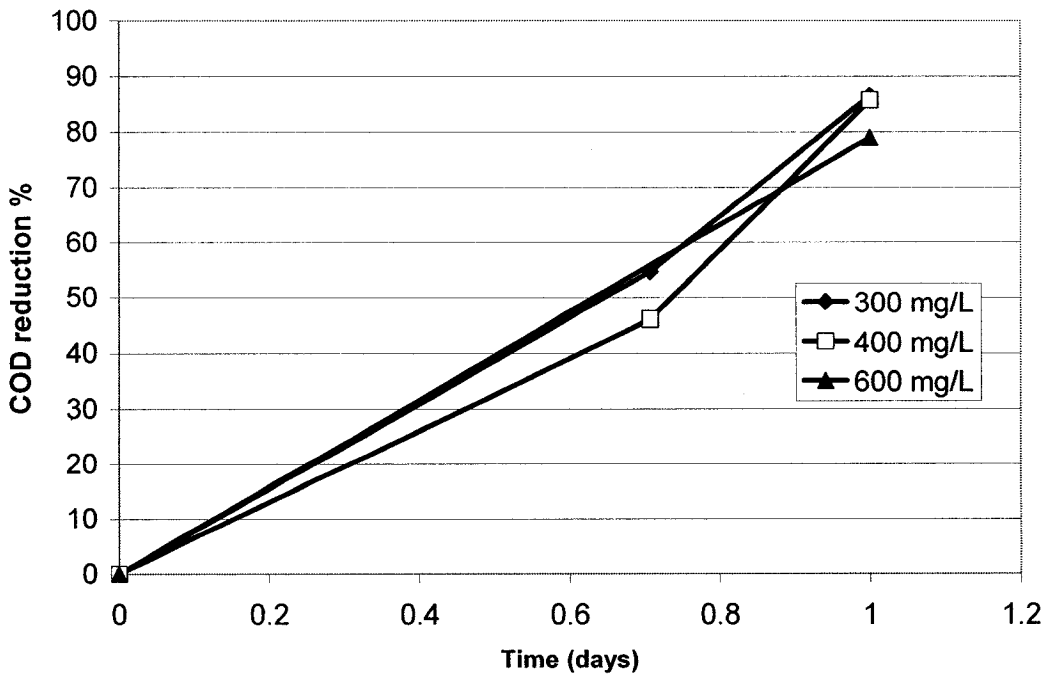

Figure 4.7: COD reduction (%) for acetic acid-fed biomass at different concentrations of phenol and HRT=1 day

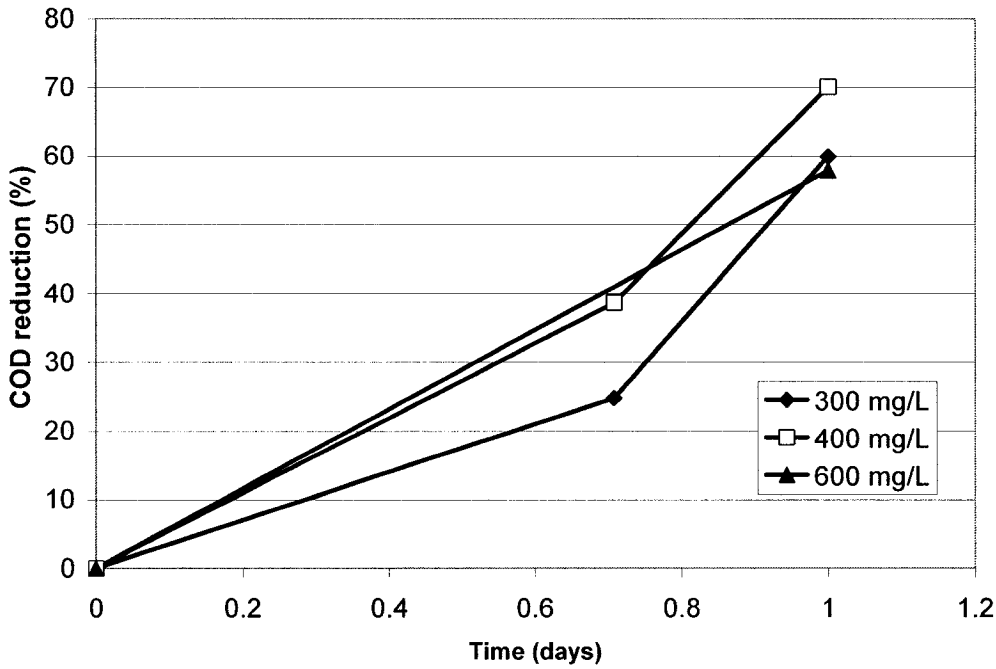

Figure 4.8: COD reduction (%) for sucrose-fed biomass at different concentrations of phenol and HRT= 1 day

Phenolic trends

As mentioned before, phenol loading was increased step-wise once 80% degradation was reached. An initial concentration of 300 mg/L initially took 4 days to reach 80% removal and the same concentration reached 80% in 24 hours in a second batch. Once the 24 hour period was achieved as the retention time, the phenol concentration was increased. The loading schemes (initial phenol concentration and the corresponding retention times to reach 80% degradation) are shown in Table 4-2 and Figure 4.9. The phenolic trends (Figures 4.12 and 4-13) showed some similarities with the COD trends. The reactor treating acetic acid showed more removal of the contaminant in comparison to the reactor acclimated with sucrose. This might be due to the fact that even though the reactor has a mixed culture of bacteria, the reactor treating acetic acid might have developed more

acetotrophic bacteria (*Methanotrix spp.*, *Methanosarccina spp.*) which is more closely related to the bacteria needed in the degradation of phenol (the same bacteria are needed for benzoate degradation - layered *Sytrophus buswellii* with a center core of acetotrophic bacteria). The reactor treating sucrose, on the other hand, might have developed more hydrogenotrophic bacteria (*Methanospirillum hungatei*) which are responsible for the degradation of hydrogen (Fang et al., 1996) or acetic acid degradation that is part of the phenolic pathway degradation (Fang et al., 1996). Sucrose follows its own degradation pathway making the phenol degradation longer for these sucrose-fed bacteria. Phenol samples were done in duplicate and the average values were shown with an error of 2%.

Table 4-2: Phenol loading scheme for the acclimation period

Initial Phenol Concentration (mg /L)	**HRT (hours)**	**COD loading rate (mg/L-day)**
300	4	180
300	1	710
400	3	320
400	1	950
600	4	360
600	1	1400
800	3	630

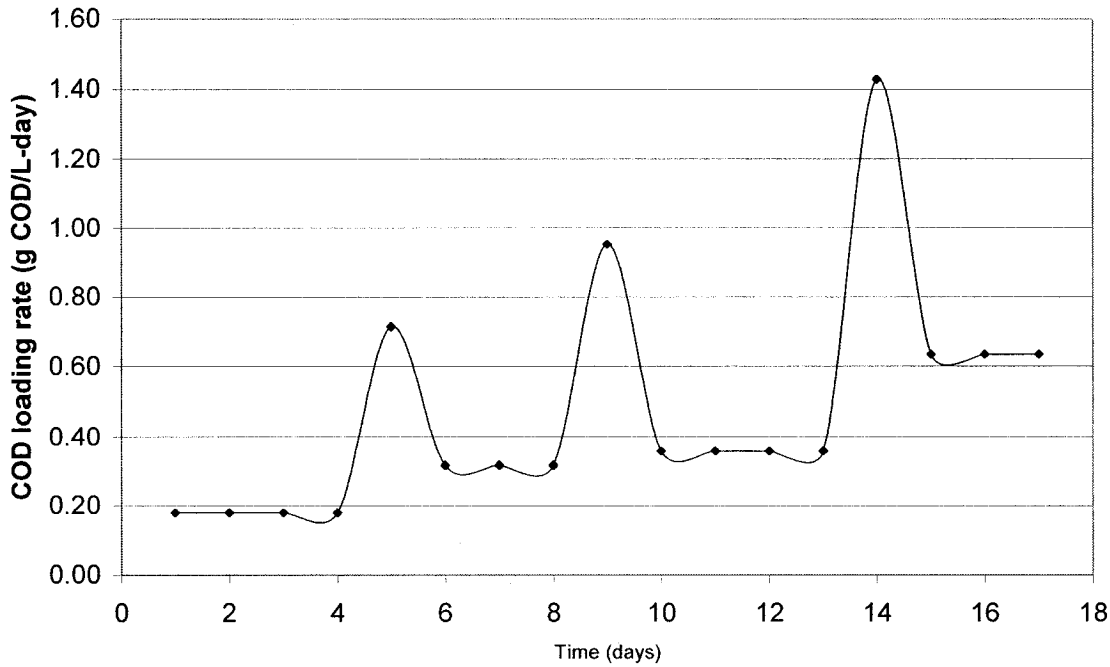

Figure 4.9: COD loading scheme for the acclimation period

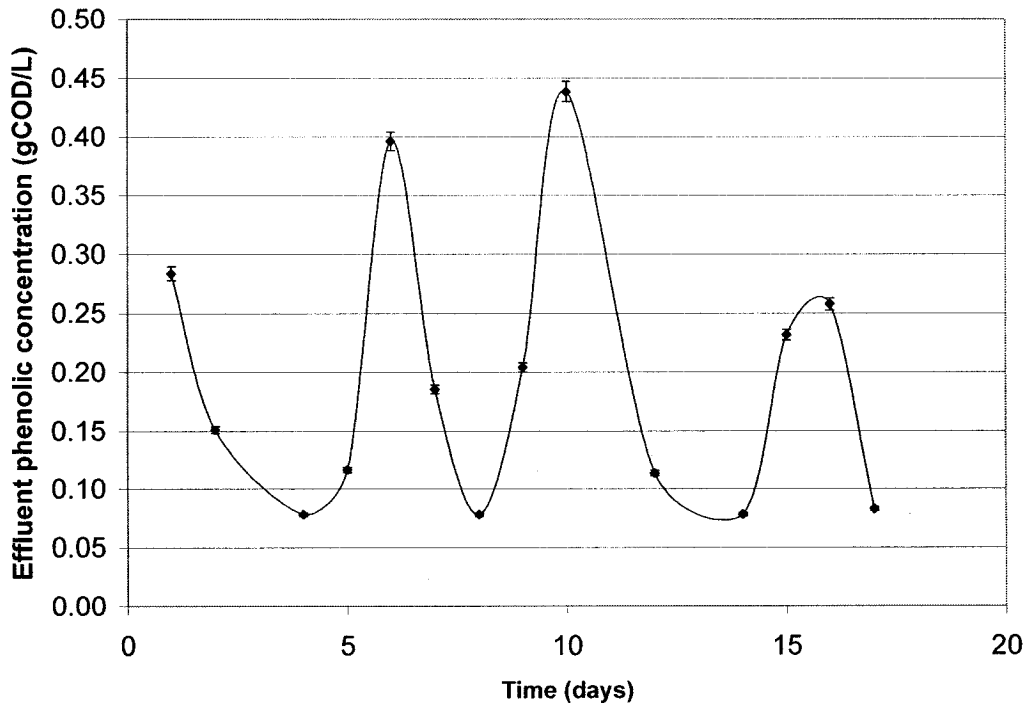

Figure 4.10: Effluent phenolic concentration for acetic acid-fed biomass

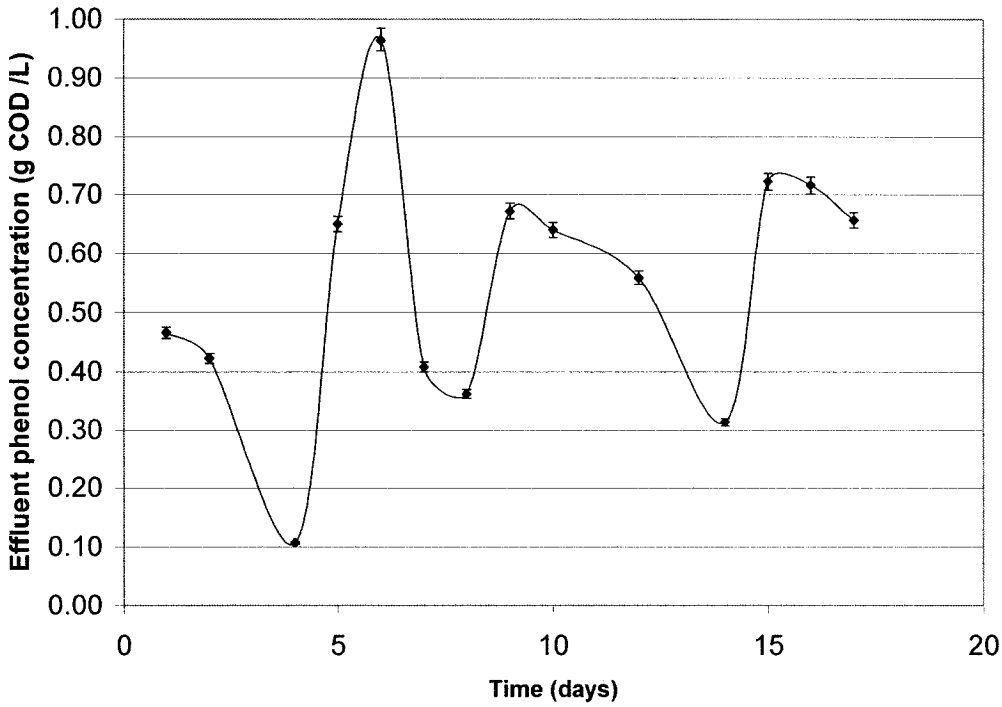

Figure 4.11: Effluent phenolic concentration for sucrose-fed biomass

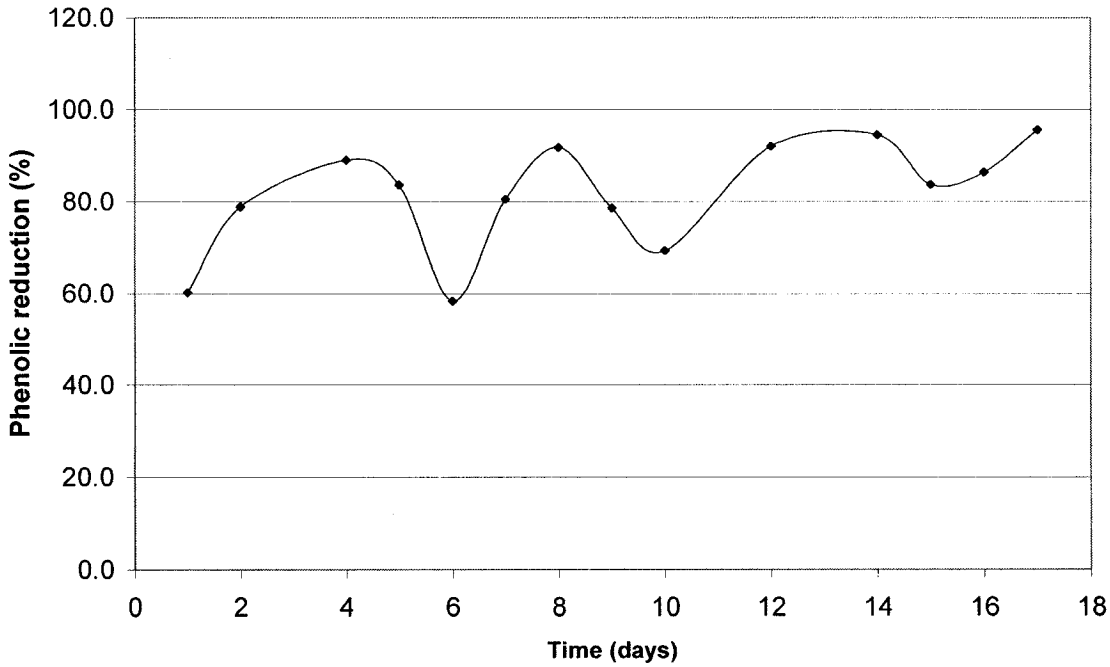

Figure 4.12: Phenolic reduction (%) for acetic acid-fed biomass

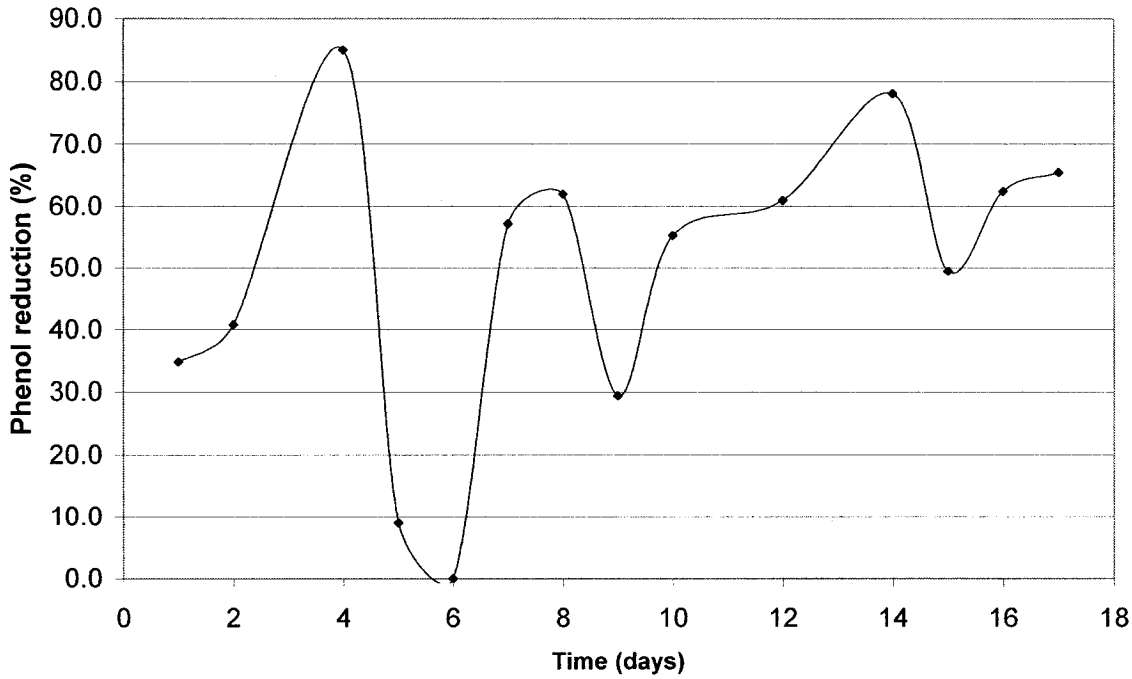

Figure 4.13: Phenolic reduction (%) for sucrose-fed biomass

On average, the methane percentages in the biogas produced by the reactors were above 60 %. A higher methane percentage was achieved around the second day for each batch followed by a decrease in its concentration. The methane content showed a decreased for the sucrose-fed biomass at a concentration of 400 mg/L and this may be due to the fact that sucrose-fed biomass was more susceptible to shocks once a new concentration was loaded but on the second day showed an improvement. In Table 4-3 the average values are shown.

Table 4-3: Methane content at different concentrations of phenol

Initial phenol concentration	Methane content using acetic acid	Methane content using sucrose
300 mg/L	76.7 ± 3.5 %	69.6 ± 1.7 %
400 mg/L	69.3 ± 10.8 %	49.1 ± 13.6 %
600 mg/L	71.4 ± 1.2 %	75.3 ± 0.8 %

Biomass organic content

The biomass was dried to determine if the organic content had changed during this period. The TVSS/TSS ratio for the bacteria of both reactors decreased from 0.72 to 0.60 and this may be due to the change in substrate. These bacteria might have been acclimated to rich calcium products, and now there was a change in the substrate. This change in substrate might have affected the granulation, strength and density of the biomass since calcium has been reported as a metallic ion necessary for proper metabolism and aggregation (Mahoney et al., 1987; Kosaric and Blaszczyk, 1990; Grotenhius et al., 1991; Schmidt and Ahring, 1996; Yu et al., 2001). In addition, it has been stated that calcium might be the constituent of extracellular proteins used as linking materials and its removal could lead to either disintegration or weakening of the structure of the biogranules (Grotenhius et al., 1991; Morgan et al., 1991; Yu et al., 2001). In general, both types of bacteria had a similar TVSS/TSS ratio of approximately 0.6. Results are shown in Table 4-4.

Table 4-4: Biomass organic content of two types of biomass

Parameter	Sucrose-fed biomass	Acetic acid-fed biomass
TSS_{av} (g/L)	78.5	74.2
$TVSS_{av}$ (g/L)	50.3	49.5
TVSS/TSS ratio	0.64	0.66

4.3 Continuous Tests

Based on the TVSS of the biomass from the previous test, the volume of biomass per reactor was determined as 500 mL. In the continuous experiment, the reactor was fed continuously and showed a more consistent gas production rate. The continuous experiment was carried out using a peristaltic pump, and the flow was controlled through a speed controller attached to the pump. The inlet flow was very hard to control due to the low flow rate needed. On average the retention time was 4 hours ± 15 minutes but for calculation purposes, 4 hours was used unless otherwise specified.

The biomass was fed with a solution based on phenol as the only carbon source plus minerals, yeast and peptone (Atlas, 1997). Two sets of two reactors were used to determine which co-substrate was better. The first and third reactors, named R1 and R3

respectively, were kept as a control and the second and fourth reactors, R2 and R4 respectively, were run with CO_2 as a co- substrate. The stability and the performance of both systems were determined by assessing the total COD, soluble COD, into and out of the reactors, the degradation of phenol and the dissolved CO_2 in the last two systems (R2 and R4). Because the addition of CO_2 lowered the pH of the wastewater, 0.1 N HCl was added to the control reactors (R1 and R3) to adjust the pH to the same as the other reactors (R2 and R4).

The continuous loading scheme presented in Table 4-5 consisted in increasing the initial load once 80 % of degradation was achieved (day 1- 19). Once the initial load reached 5.4 g COD/ L-day (comparable to 1260 mg phenol/L mentioned by Fang et al. (1996) on day 20, the system was allowed to reach a stable state before taking final values. For better performance of the reactors and better spread out of results, the HRT was increased to 6 hours with the same loading rate. The final degradation values for all reactors are shown in Table 4-6.

Table 4-5: Phenol loading scheme for continuous tests

Day	HRT (hours)	Initial Phenol Concentration (mg /L)	COD loading rate (g/L-day)
1-3	4	50.0	0.7
4-5	4	100.0	1.4
6-11	4	150.0	2.1
12	4	245.0	3.5
13-14	4	256.0	3.6
15-16	4	285.0	4.1
17-19	4	380.5	5.4
20-26	6	570.0	5.4

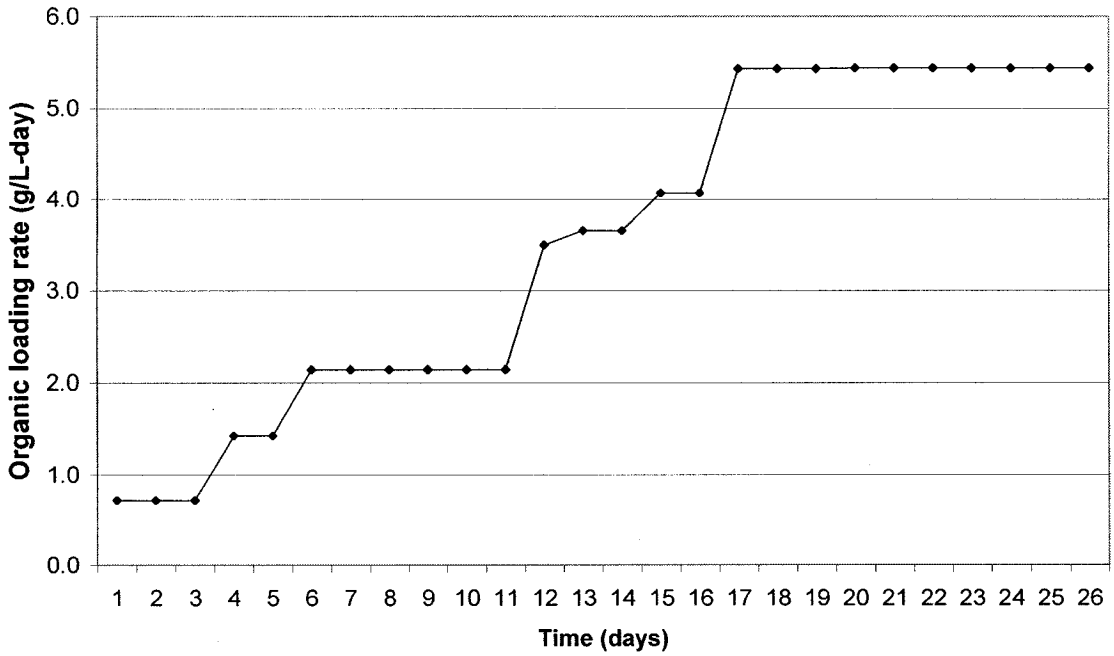

Figure 4.14: Phenol loading scheme for continuous tests

The initial concentration for this continuous step should have been 800 mg/L (the final input from the acclimation period) but this was not used because this concentration was not easily degraded, and a toxic shock loading must be avoided. The organic loading rate from the last batch was calculated and the continuous fed was started with 50 mg phenol/L (approximately 714 mg COD/L-day) and the HRT used for this step was 4 hours. The reactors reacted well to the initial load, and the concentration increased immediately to 100 mg phenol/L as shown in Table 4-5. When the load reached 150 mg/L, the reactors produced biogas with a decreased methane percentage and the phenol and COD removal percentages were reduced as well. This inefficiency (which affected sucrose-fed biomass the most) to degrade the phenol may be due to the fact that the bacteria preferred to decompose first the easily degradable substrate rather than phenol. Throughout the rest of the experiment, the ratio of wastewater to biomass was kept at 1:1 (v/v), and HRT was maintained at 4 hours. Since the reactors were fed in 1:2 ratio (v/v) of substrate to biomass. On day 11, it was decided to reduce the substrate to 0.5 litres per 0.5 litres of biomass in order to keep the 1:1 ratio in each system. No more problems were encountered due to overloading. The final values for all parameters are shown on Table 4-6.

Table 4-6: Final degradation values for the continuous test from day 20-26

	R_1	R_2	R_3	R_4
Initial COD (mg/L)	3589.6	3732.0	3589.6	3732.0
Final COD (mg/L)	267.4	215.9	265.7	90.0
COD reduction (%)	92.6	94.2	92.6	97.6
Initial Phenol Conc. (g/L)	0.6	0.6	0.6	0.6
Final Phenol Conc. (g/L)	0.18	0.15	0.15	0.08
Phenol reduction (%)	86.5	89.0	89.1	94.5
CH_4 production (mL/day)	218.7	229.2	242.46	249.3
CH_4 %	67.6	64.9	70.7	67.0
CH_4/ g COD reduced	0.1	0.1	0.1	0.1
Final Dissolved CO_2 (M)		0.07		0.07
CO_2 reduction (%)		88.5		86.6

COD trends

Dissolved carbon dioxide increased the total COD concentration of reactors R2 and R4 in an average of 8% over that of R1 and R2 and with this consideration in mind, the total COD reduction percentage was used to determine which reactor performed better than the

others. During the continuous test, the reactors followed a similar trend to the acclimation period. On average, reactors R2 and R4 showed a slightly better performance for reducing COD. The removal of COD was compromised when the reactors could only degrade around 50% of the initial COD without any increase in the subsequent days (days 8-10). The initial COD was kept constant but the volume of the feed was reduced to 0.5 L (after day 10) and an improvement of the reduction was noticed on day 11 (Figures 4.14 to 4.18).

The removal of COD during the continuous experiment is shown in Figures 4.15-4.18. The COD samples were done in duplicate and the average values were shown with an error of 8 %. Figure 4.15 shows the total COD concentration in the effluent for R1, on day 11 is shown how the COD concentration was greatly reduced after adjusting the substrate. Figure 4.16 shows the total COD concentration in the effluent for R2 and also shows how on day 11 the final COD value was reduced. Figure 4.17 presents the total COD concentration in the effluent of R3 and it also presents the reduction on day 11 but it is believed that the load shock affected this reactor less because the total COD values reported were lower than those of the reactor containing the sucrose-fed bacteria. Figure 4.18 presents the total COD concentration in the effluent for R4. This was the least affected reactor because this reactor showed the lowest COD values for the initial loading rate of 2.1 g COD/L-day.

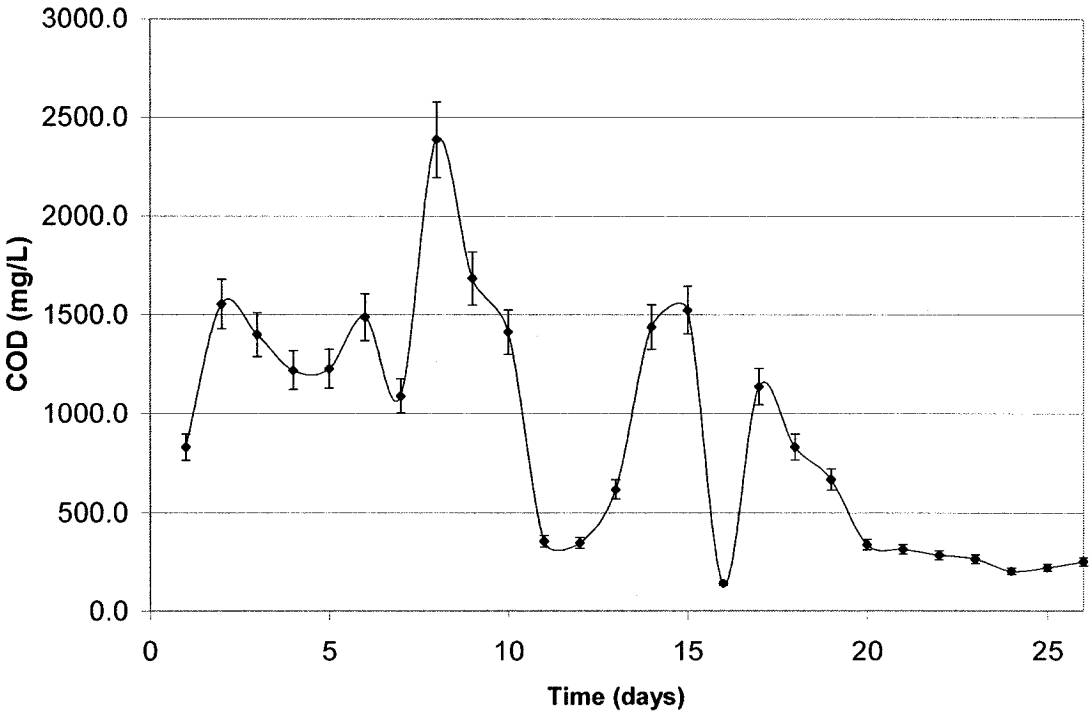

Figure 4.15: Total COD in effluent for reactor 1.

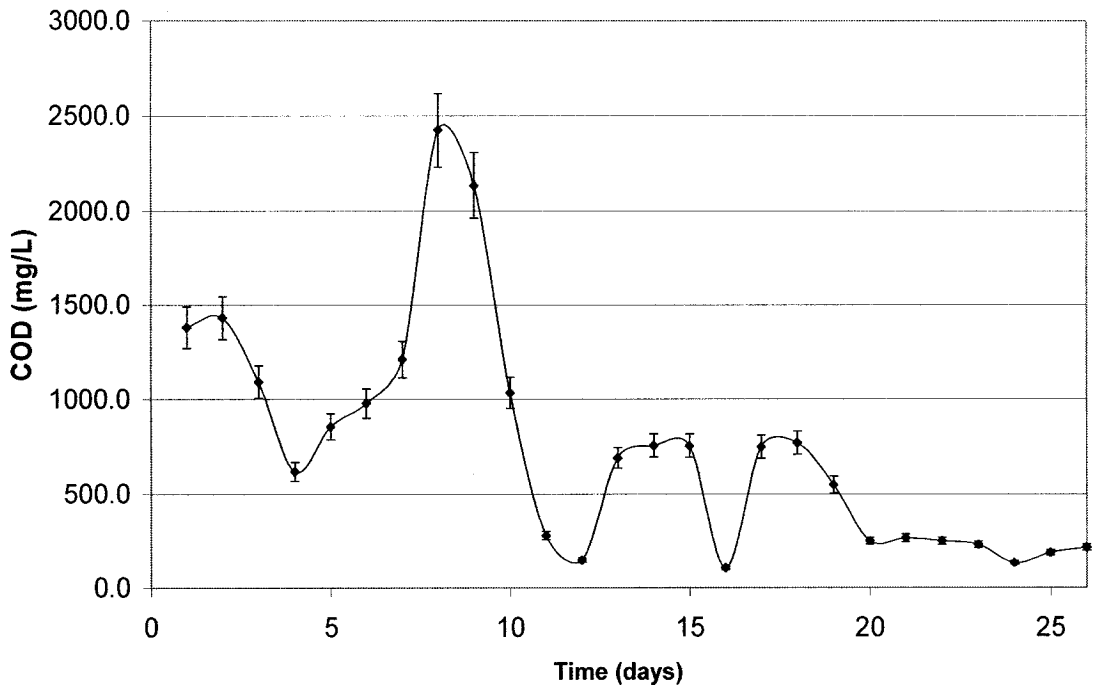

Figure 4.16: Total COD in effluent for reactor 2.

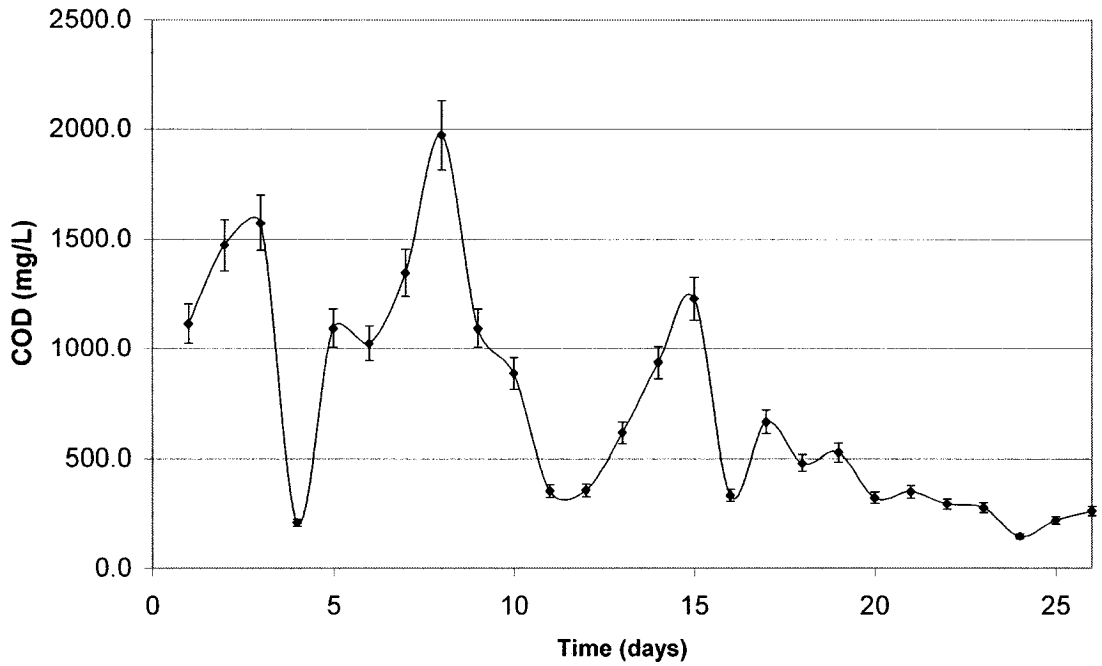

Figure 4.17: Total COD in effluent for reactor 3

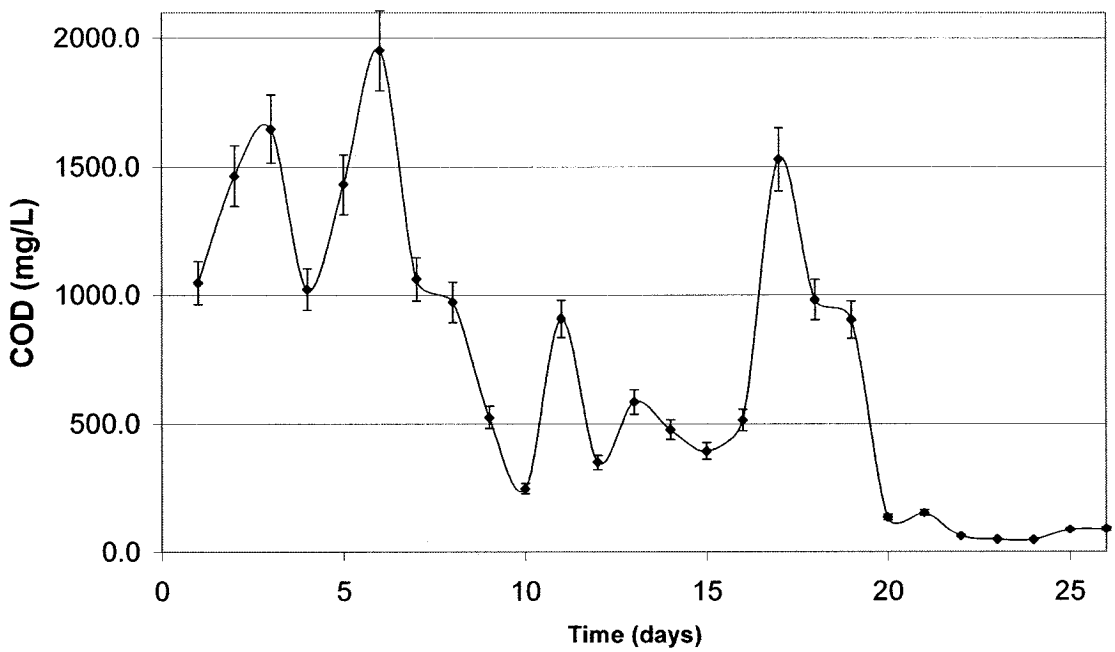

Figure 4.18: Total COD in effluent for reactor 4.

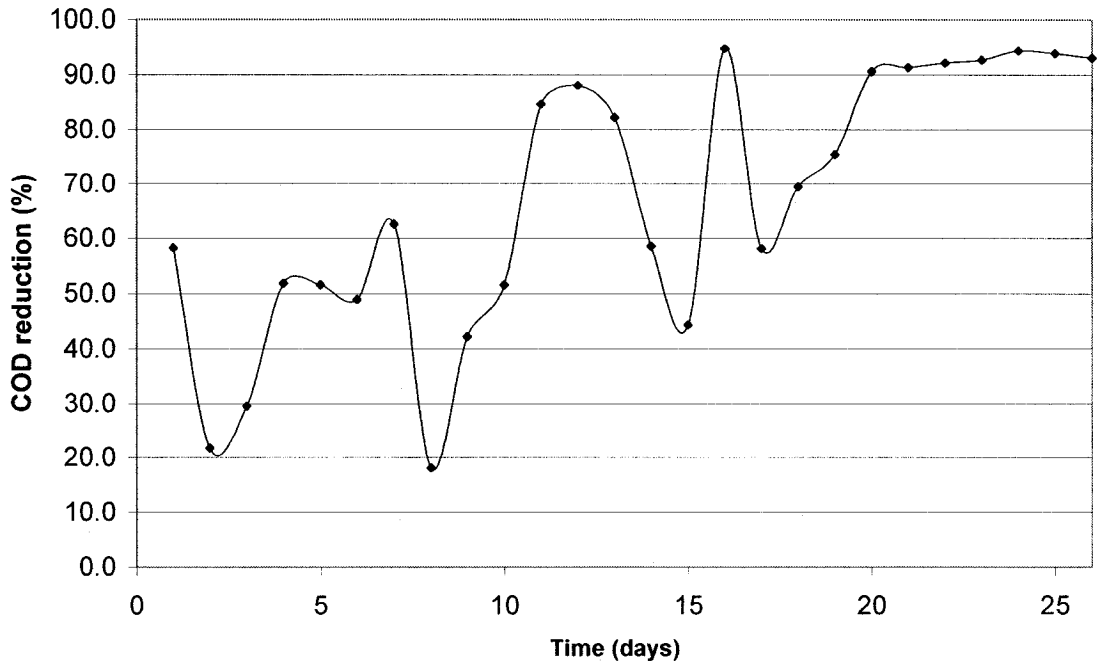

Figure 4.19: Total COD reduction (%) in reactor 1

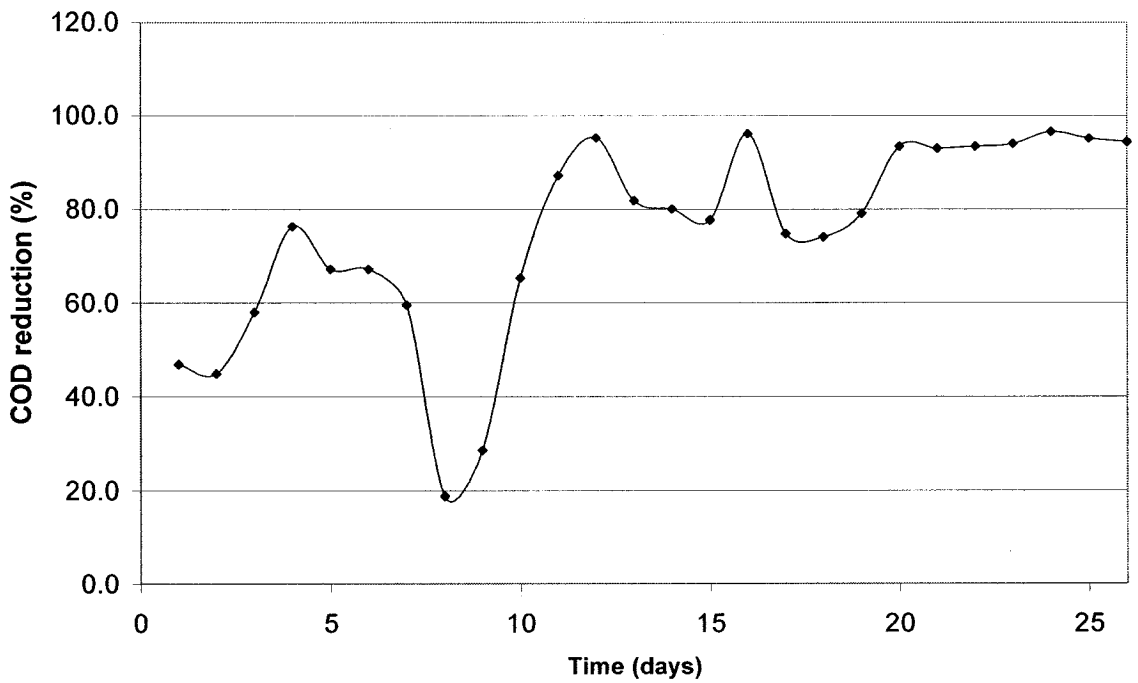

Figure 4.20: Total COD reduction (%) in reactor 2

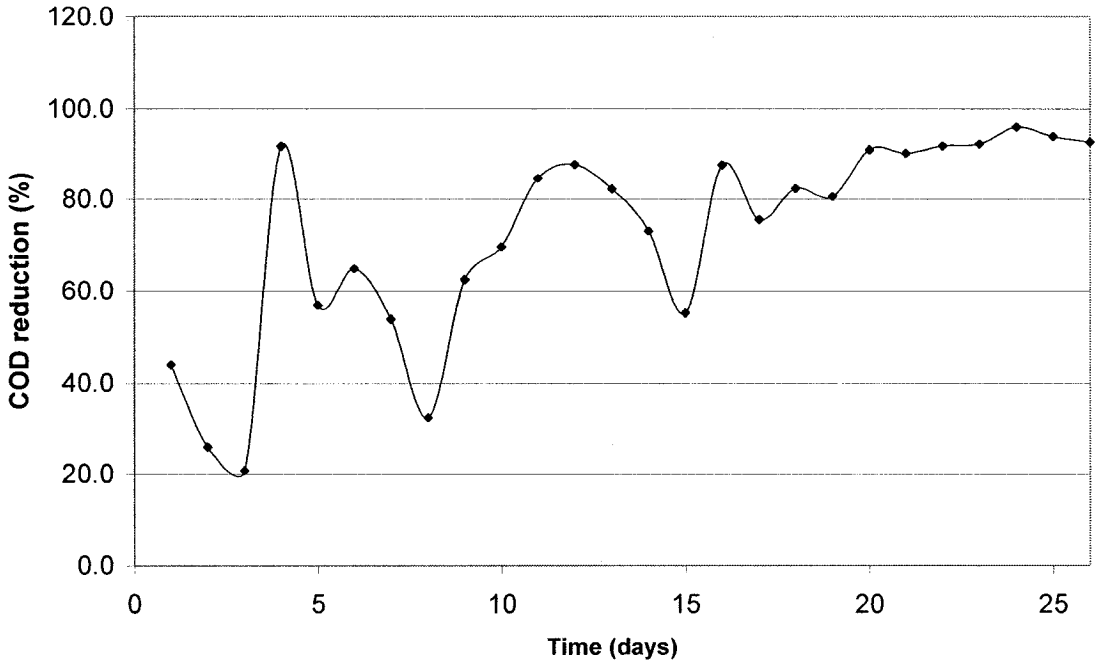

Figure 4.21: Total COD reduction (%) in reactor 3

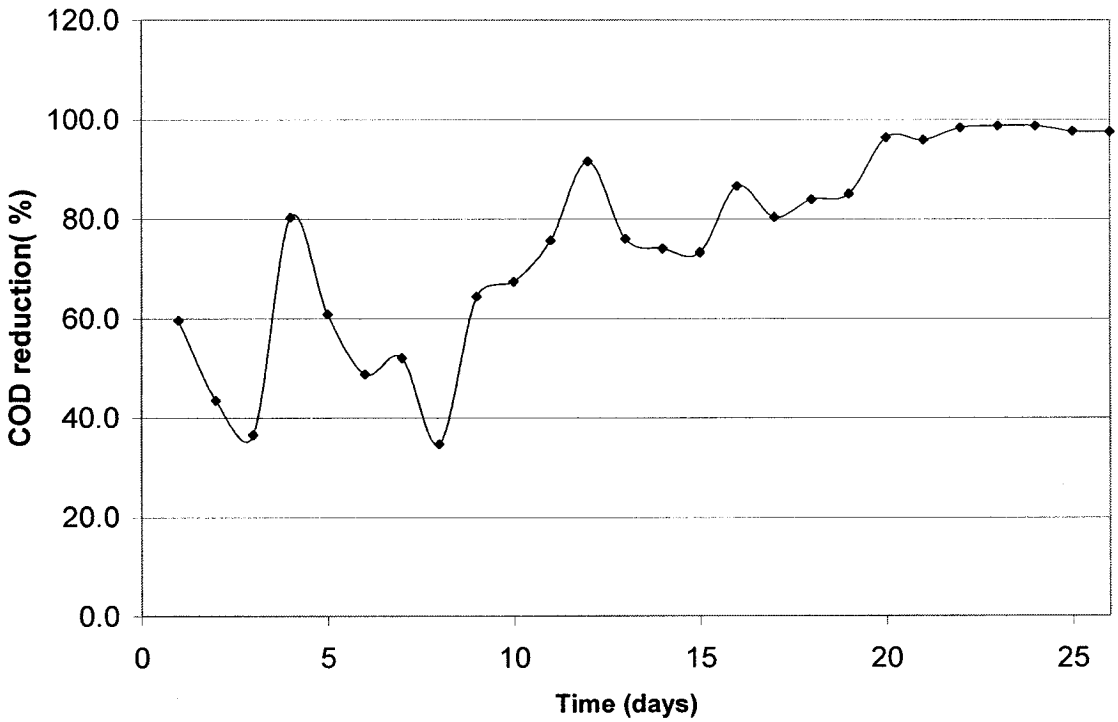

Figure 4.22: Total COD reduction (%) in reactor 4

Phenolic trends

On average both systems (sucrose and acetic acid) could treat phenol with a slightly better performance of R4, followed by R3. Although the sucrose-fed bacteria (R1 and R2) were more likely to suffer more shocks when the load was increased than the acetic acid-fed bacteria (R3 and R4), it was noticed that it adapted quickly after the shock (comparison of days 11-12 based on methane production and COD degradation of R1 and R2). Phenol samples analyses were done in duplicate and average values were shown (Figures 4.23- 4.30) with an error of 2 %.

Figure 4.23 shows the final phenolic concentration in the effluent of R1. In this graph is also shown how the phenol concentration was reduced after adjusting the volume of the substrate. The system degradation decreased from day 6 to 10 with an improvement on day 11. Figure 4.24 shows the final phenolic concentration in the effluent of R2. This graph also presents the improvement after day 10. Figure 4.25 indicates the final phenolic concentration in the effluent of R3 also presenting the same trend after day 10. Figure 4.26 presents the final phenolic concentration in the effluent of R4 following the same pattern as the other 3 reactors. Figures 4.27 – 4.30 show the corresponding reduction percentages for R1 through R4.

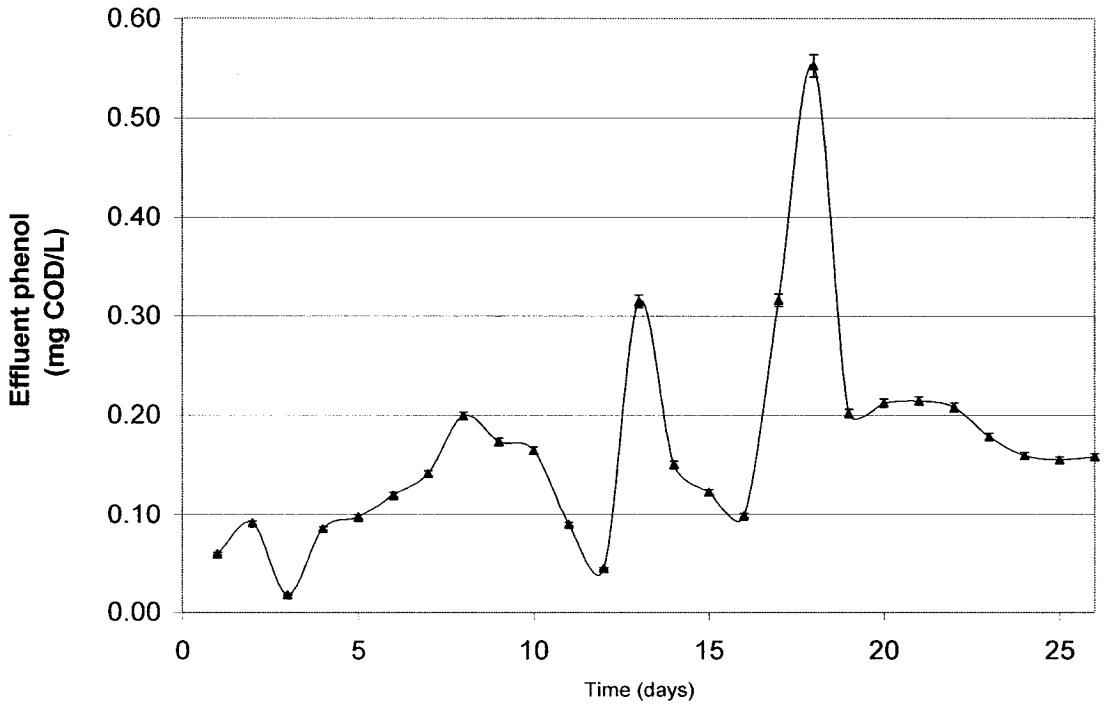

Figure 4.23: Effluent phenol concentration in reactor 1.

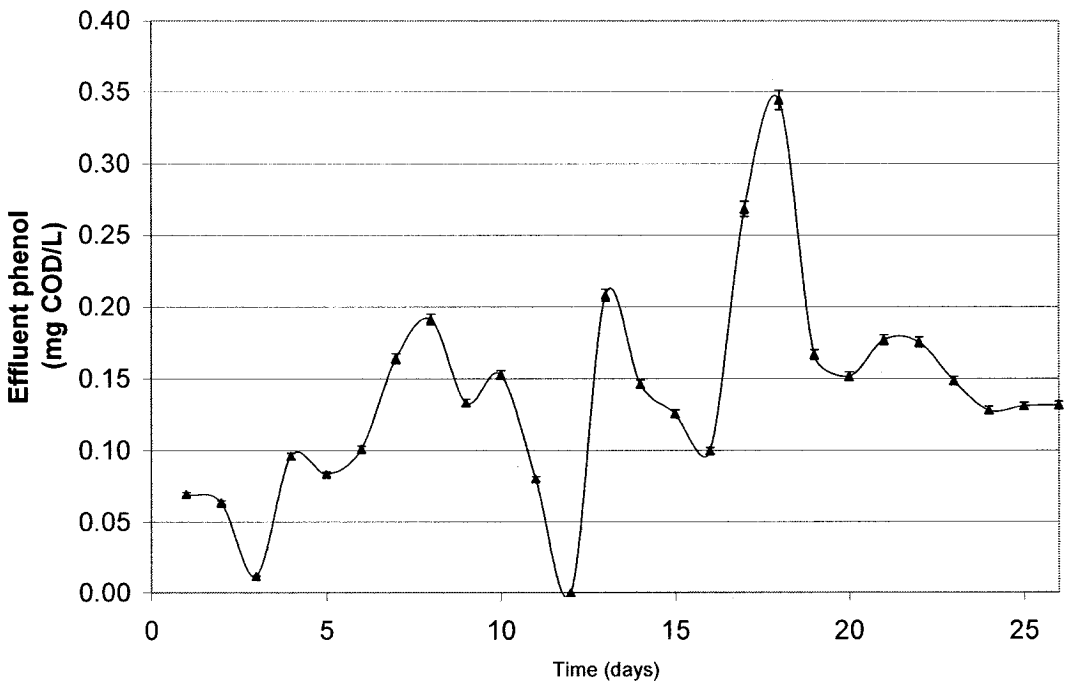

Figure 4.24: Effluent phenol concentration in reactor 2

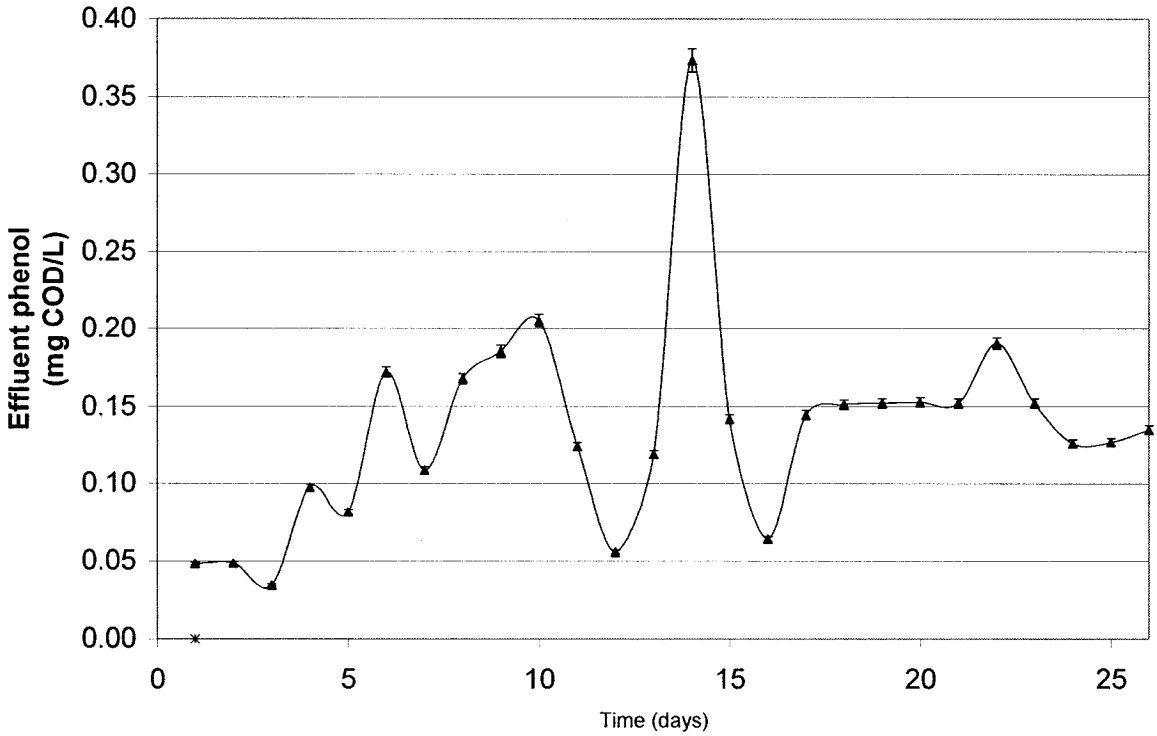

Figure 4.25: Effluent phenol concentration in reactor 3.

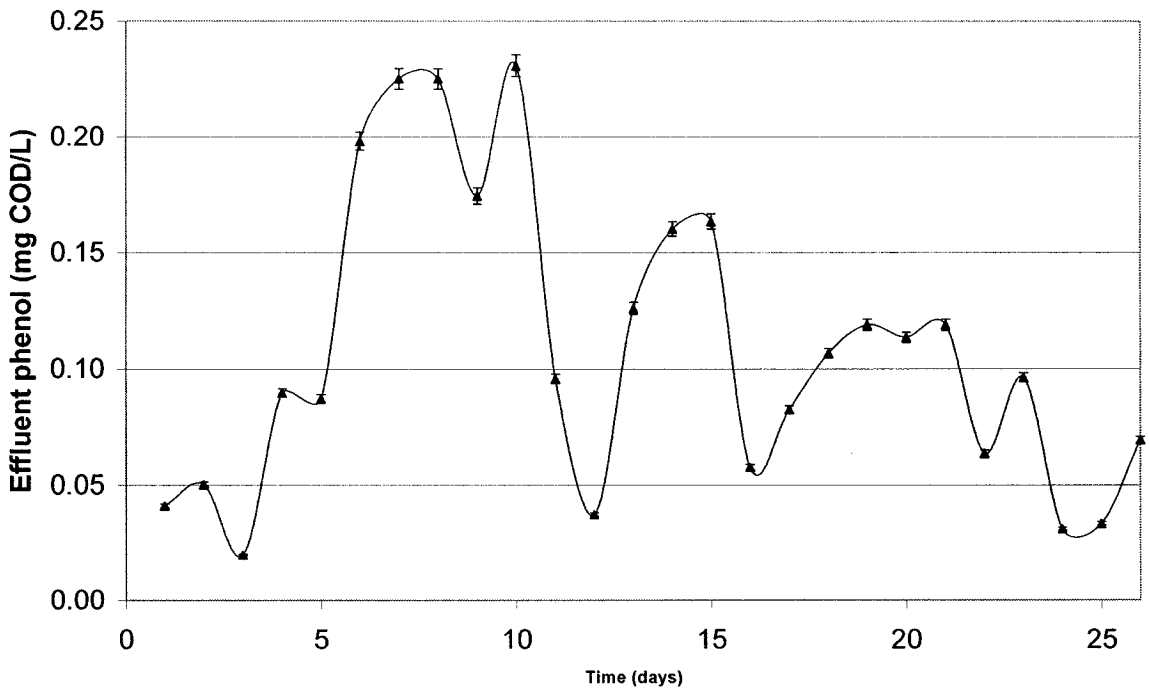

Figure 4.26: Effluent phenol concentration in reactor 4

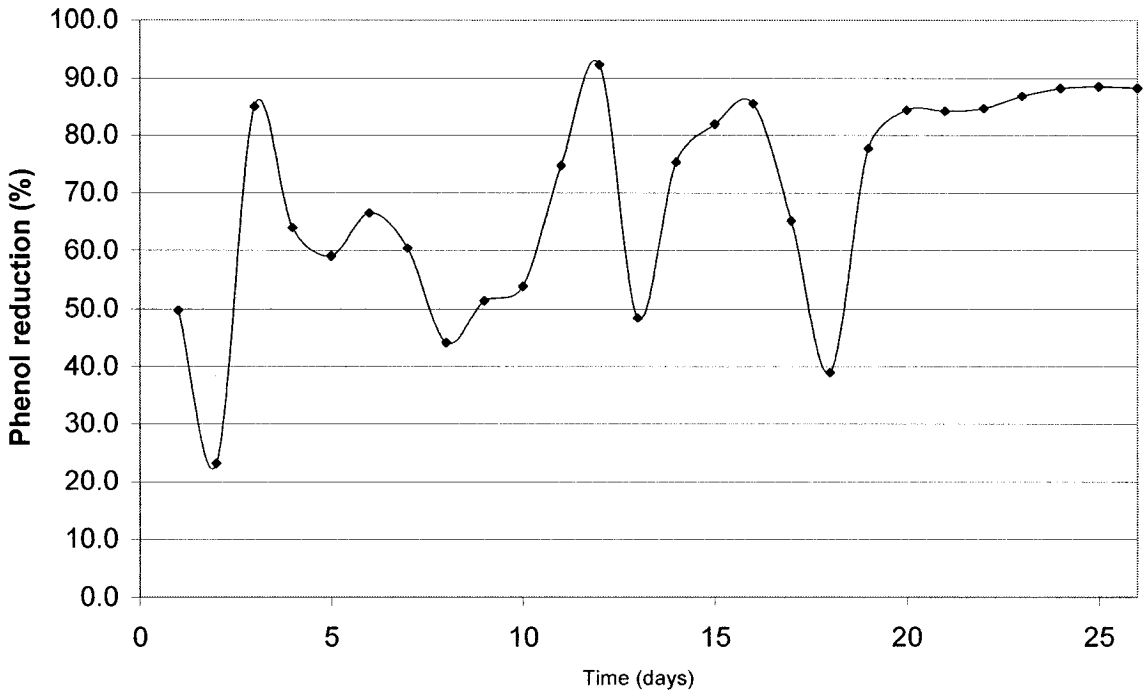

Figure 4.27: Phenol reduction (%) in reactor 1.

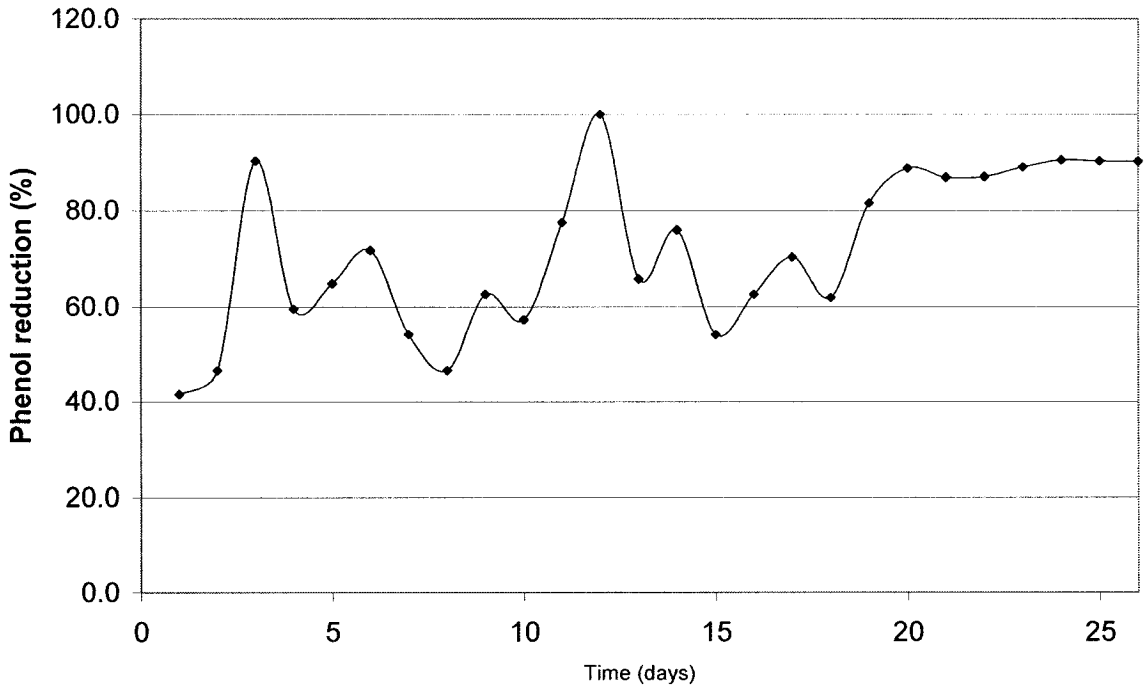

Figure 4.28: Phenol reduction (%) in reactor 2.

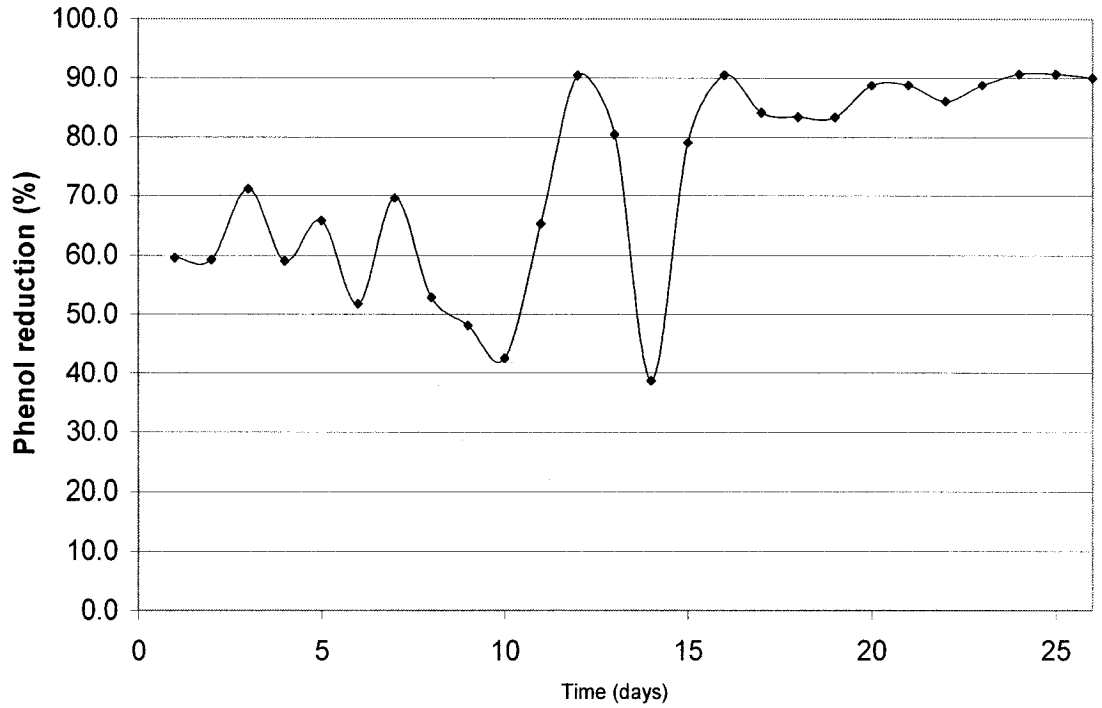

Figure 4.29: Phenol reduction (%) in reactor 3.

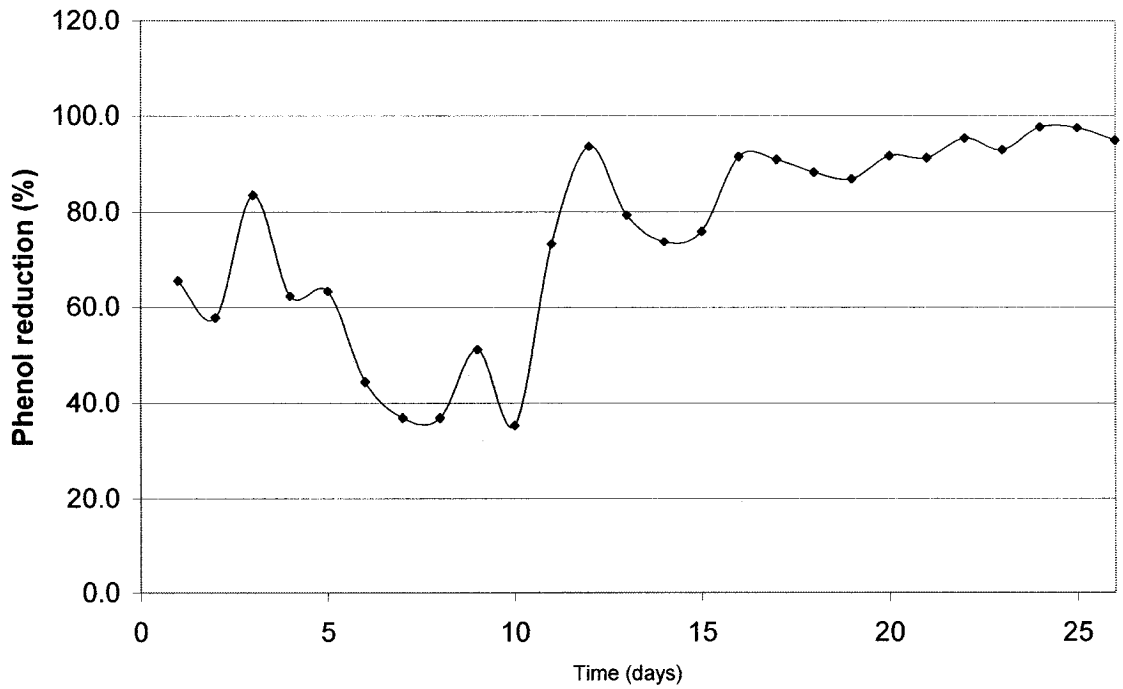

Figure 4.30: Phenol reduction (%) in reactor 4.

Gas production trends

On average, R1 and R3 have slightly better methane contents (66% and 70% compared to 64% and 65% of R2 and R4) but due to the higher amount of gas produced by R2 and R4, the latter had higher yields of methane. The methane content varied according to Figure 4.31. During the experiment, the problem that persisted was the escape of gas due to the inside pressure of the reactors (with the eventual breakage of the cylinders and leak of the gas). This occurred in all of the reactors, and the cylinder had to be changed for all reactors. The results for the methane production are shown in Figures 4.33 to 4.36. Figure 4.33 shows the methane yield for R1 and a leakage on day 3. Figure 4.34 show the methane yield for R2. Figure 4.35 shows the methane yield for R3 and leakage on days 1, 12 and 15. Figure 4.36 shows the methane yield for R4 with leakage on days 8 and 10.

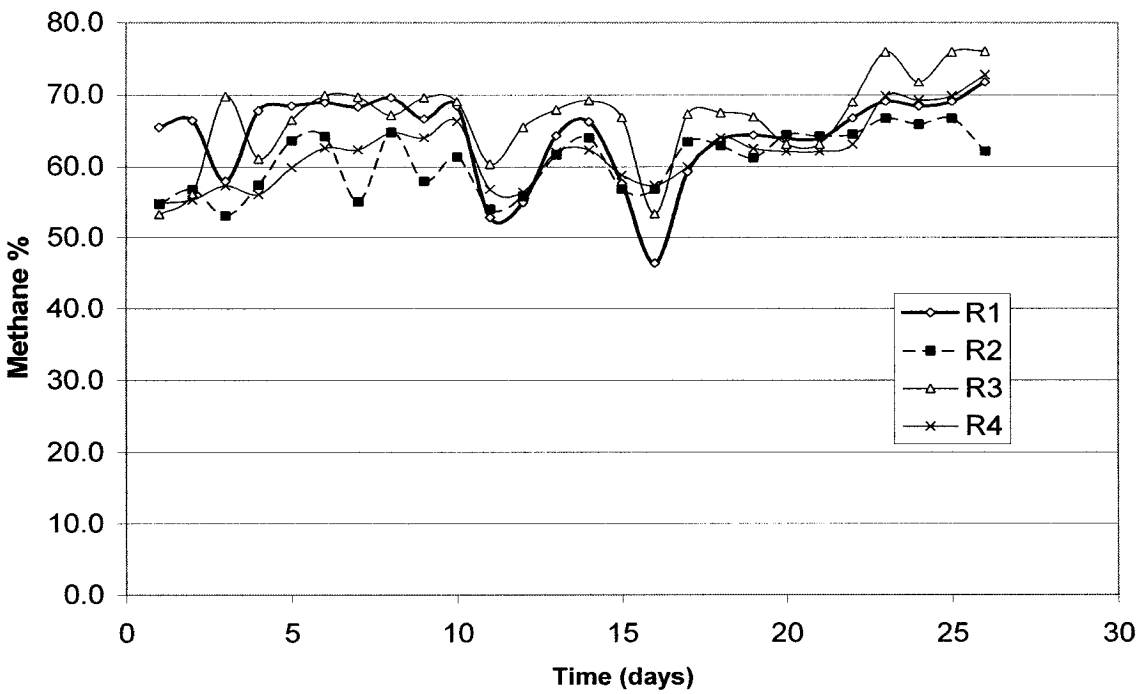

Figure 4.31: Methane percentage in biogas

Alimahmoodi (2004) mentioned that every gram of total COD reduced had a theoretical yield of 0.395 L (corrected value at 35° C) of methane and that at higher loading rates the systems could present deviations. This experiment showed similar tendencies and higher deviations were presented at a higher loading as shown in Figure 4.32.

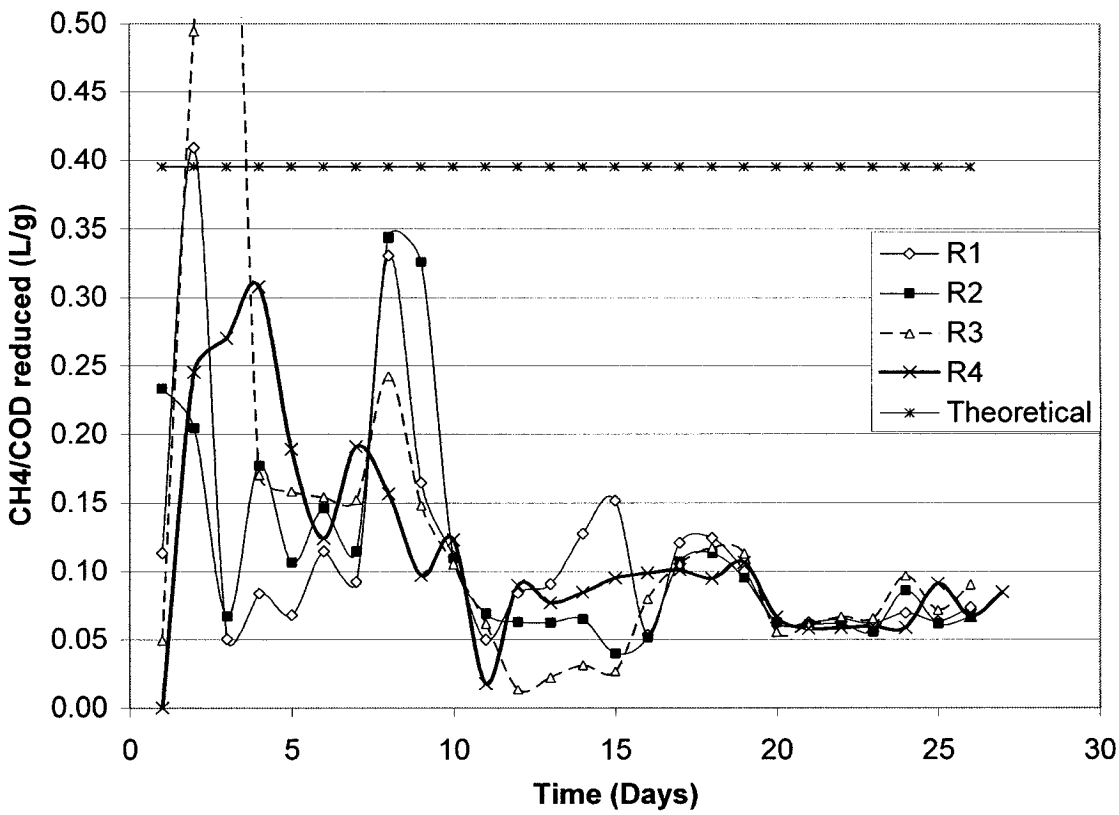

Figure 4.32: Methane yield per g COD reduced

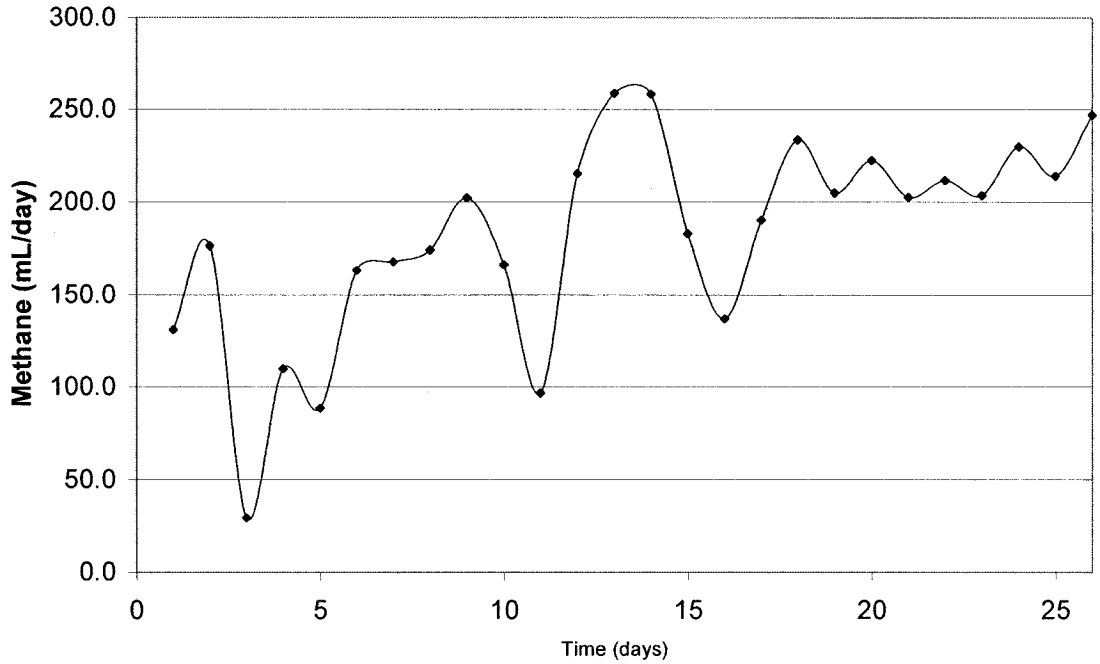

Figure 4.33: Methane production rate for reactor 1.

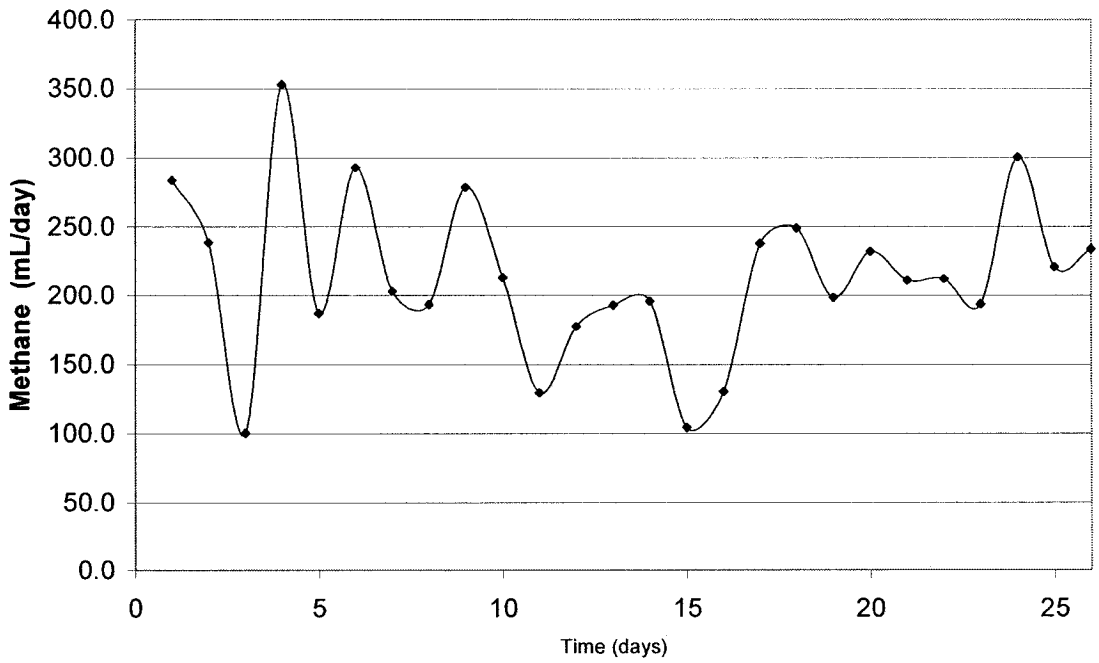

Figure 4.34: Methane production rate for reactor 2.

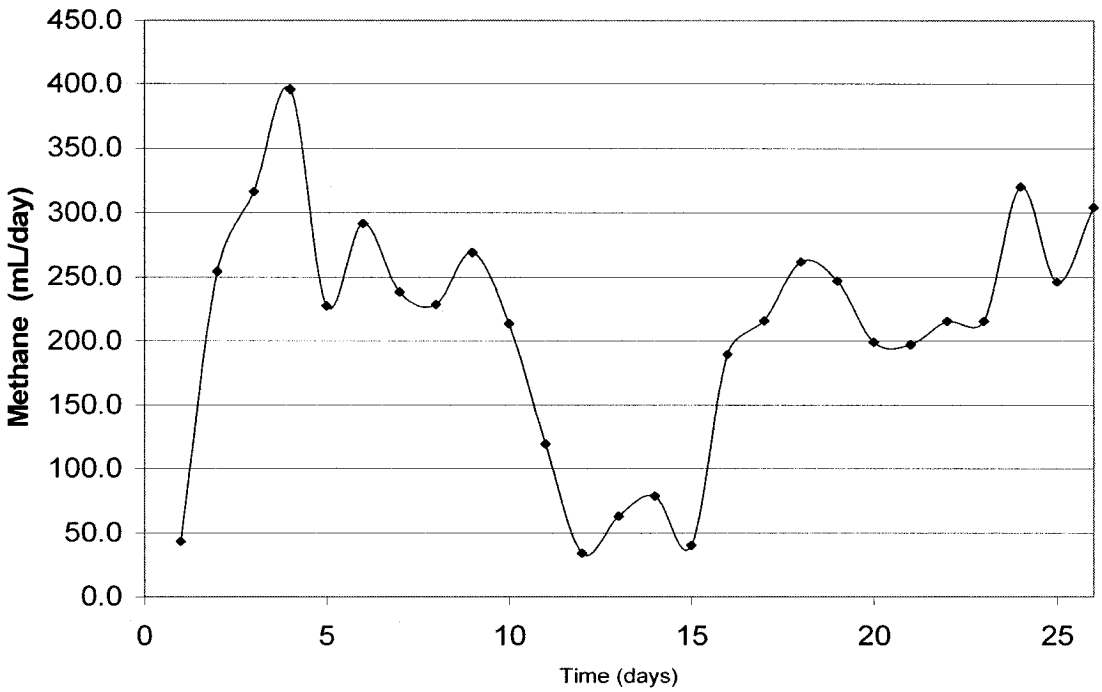

Figure 4.35: Methane production for reactor 3.

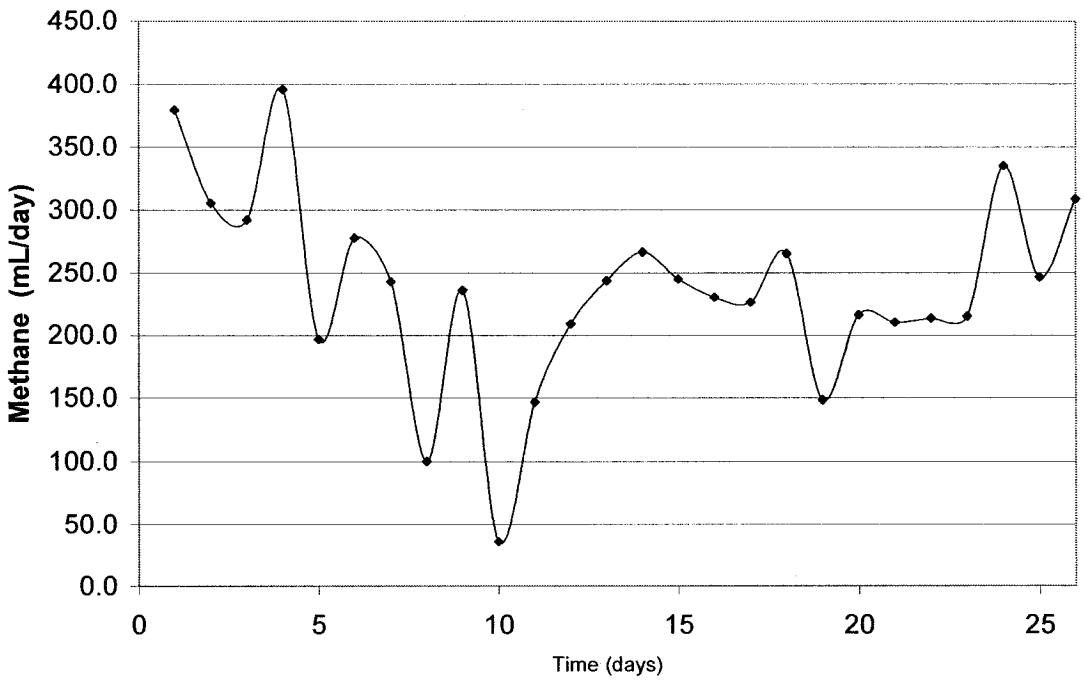

Figure 4.36: Methane production for reactor 4.

Dissolved carbon dioxide trends

Dissolved carbon dioxide was monitored in R2 and R4, and both reactors performed well proving that the system could treat both phenol and reduced carbon dioxide. High values of approximately > 80% reduction were achieved which can be compared to >75% reduction of CO_2 reported by Alimahmoodi et al. (2004). The concentrations of dissolved carbon dioxide (initial and final) and the removal efficiencies of R2 and R4 are shown in Figures 4-37 to 4-40 respectively. Carbon dioxide showed high compatibility with both types of bacteria, with a slightly better compatibility with sucrose-fed biomass which reduced CO_2 by 80% most of the time. Acetic acid-fed biomass showed more fluctuations reducing CO_2 at the beginning but rapidly increased the reduction (%). The dissolved carbon dioxide wastewater proved to be a good co-substrate in the treatment of phenol in an UASB reactor even though the pH values were below the typical pH values used in anaerobic processes. As the system reduced the dissolved carbon dioxide the pH values increased to 6.5 – 7.3. The advantages of using dissolved carbon dioxide wastewater can increase the applicability of the process since no pure substances are needed such as glucose and VFA (Hwang et Cheng, 1991; Kennes et al., 1997; Tay et al., 2001; Veeresh et al., 2006).

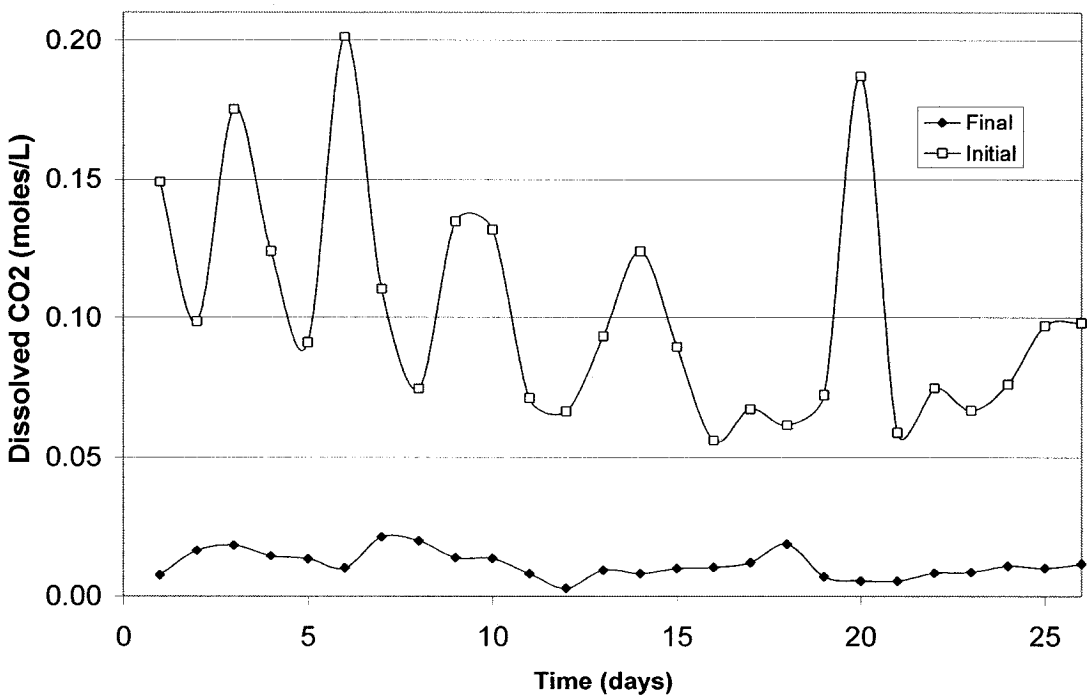

Figure 4.37: Dissolved CO_2 in the effluent for R2

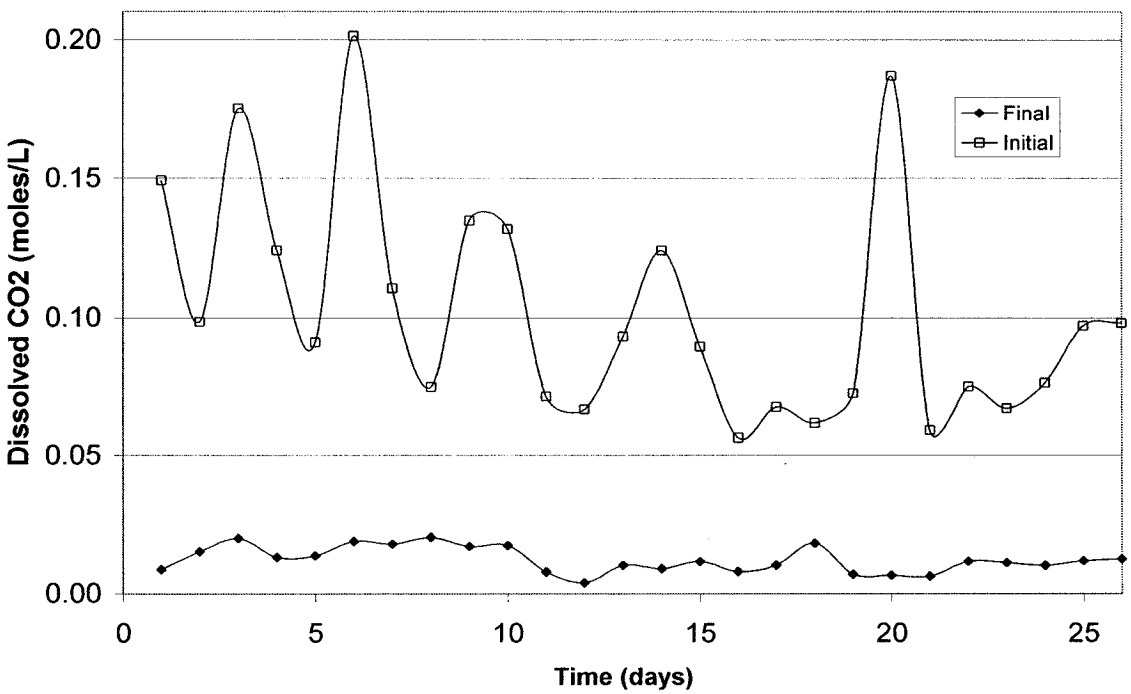

Figure 4.38: Dissolved CO_2 in the effluent for R4

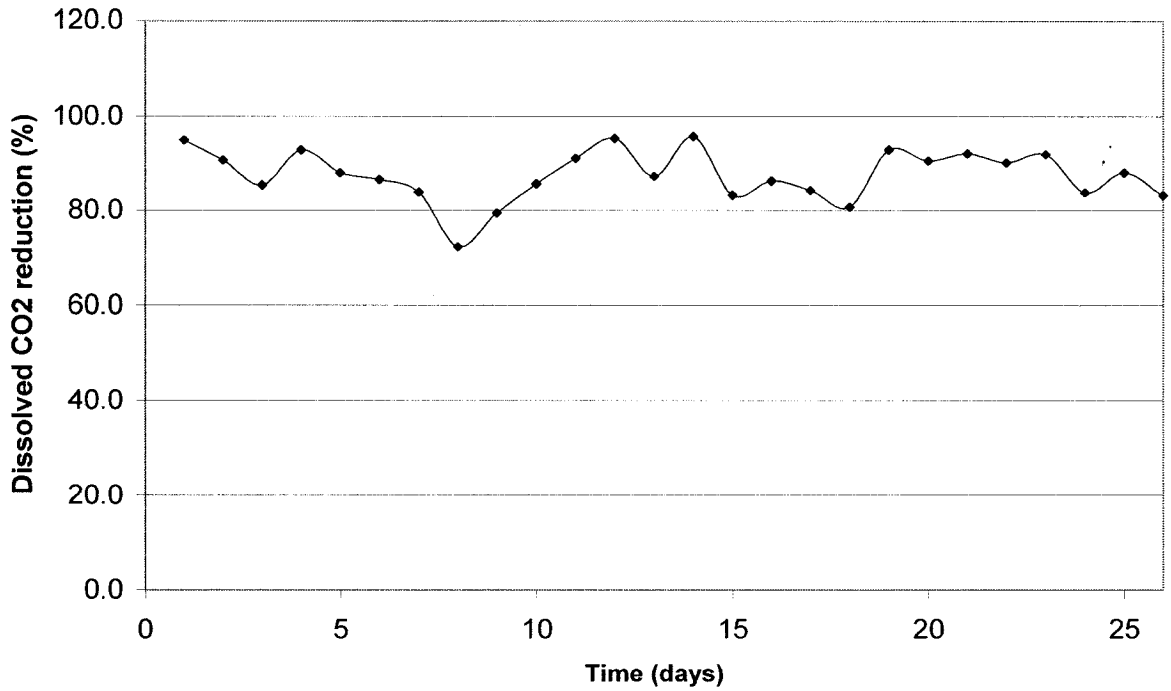

Figure 4.39: CO_2 reduction (%) for R2

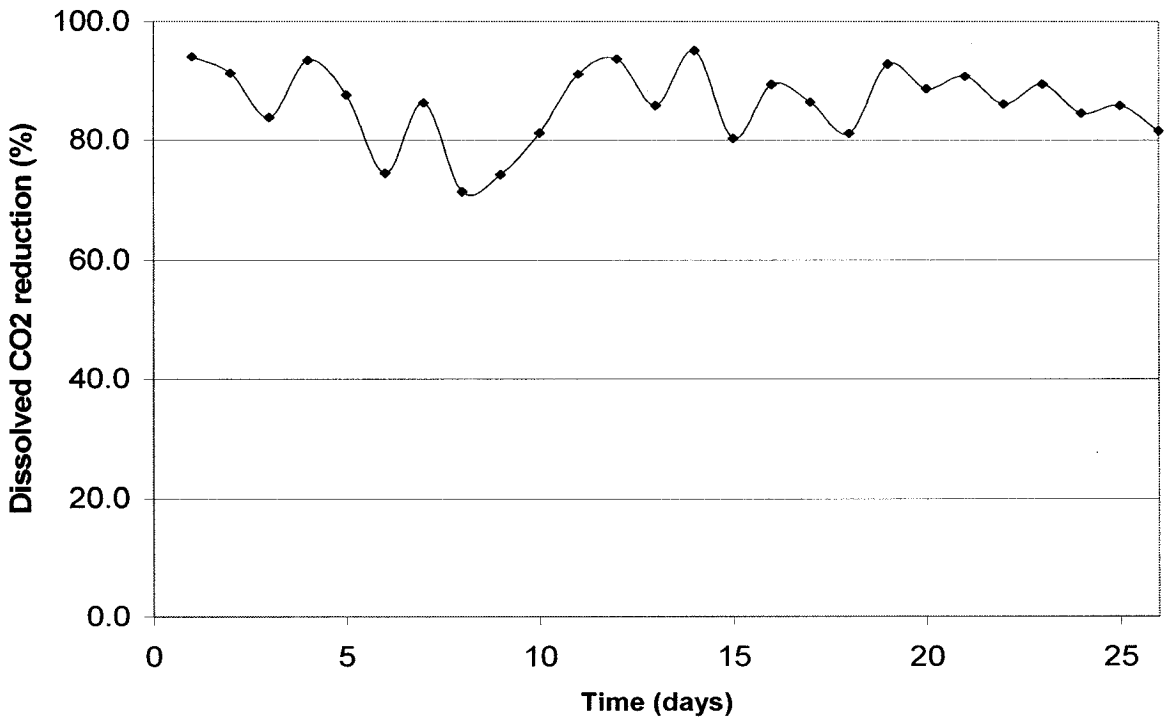

Figure 4.40: CO_2 reduction (%) for R4

Organic content of the biomass

The organic content of the biomass was monitored throughout the experiment by measuring the TVSS and TSS. At the end of the experiment, the biomass on all four reactors measured between 1 to 3 mm, as shown in Figure 4.41. The TVSS and TSS were measured every week, and the results are shown in Table 4-7. All the experiments were done in duplicate. Also the TSS of the effluent was measured every two weeks in order to determine how much biomass was being washed out. The TSS variations in the influents are shown in Table 4-8. Based on the results, more biomass was washed out from R1 and R2 but still these values were very low. VSS and TSS samples were done in duplicate and average values are presented with an error of 5%.

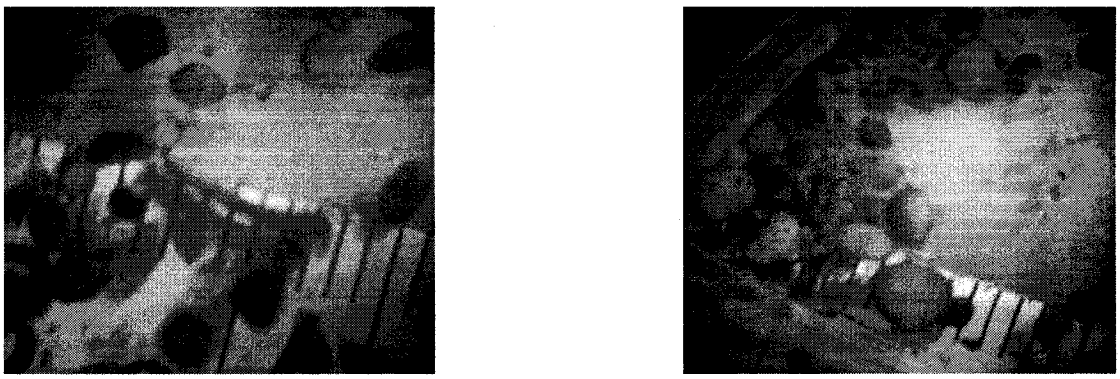

Figure 4.41 a and b: Mixed-culture bacteria (size ranges from 1 – 3 mm)
Taken with a microscope and magnification of 6x and 5.0 mega pixels digital camera.

Table 4-7: Organic Content of biomass during continuous tests

	Week 1			Week 2			Week 3			Week 4		
	TSS (g/L)	TVSS (g/L)	TVSS / TSS	TSS (g/L)	TVSS (g/L)	TVSS / TSS	TSS (g/L)	TVSS (g/L)	TVSS / TSS	TSS (g/L)	TVSS (g/L)	VSS / TSS
R1	74.4	48.4	0.7	73.2	49.3	0.7	62.9	42.2	0.7	70.91	48.46	0.7
R2	70.9	49.7	0.7	68.4	46.6	0.7	67.0	45.2	0.7	70.66	47.08	0.7
R3	80.8	50.4	0.6	66.6	45.5	0.7	67.1	44.0	0.7	71.42	46.88	0.7
R4	68.8	44.2	0.6	69.5	46.0	0.7	59.6	38.4	0.6	72.46	50.15	0.7

Table 4-8: Effluent TSS during continuous tests

	TSS (g/L)	TSS (g/L)	TSS (g/L)	TSS (g/L)
R1	0.06	0.12	0.18	0.23
R2	0.10	0.30	0.20	0.10
R3	0.13	0.02	0.02	0.02
R4	0.07	0.11	0.09	0.09

SMA trend

Based on the methane production of all the reactors and the VSS of both types of bacteria, a final specific methanogenic activity test was performed to determine the efficiency of the reactors. The final volume occupied by the bacteria in each reactor was measured in order to find the total VSS. The SMA values for all reactors are presented in Table 4-9. Overall, the reactors presented a similar trend but R1 showed a slightly lower value. As mentioned before, this may be due to the fact that the reactor treating sucrose was more susceptible to shocks but could recover fast. The reason for the low values could be due to the toxicity that phenol poses to the bacteria and the gas losses during the experiment due to cracking of the reactors and gas accumulation in the reactor's head space. The final values for the SMA of both type of biomass compare to that of the toxicity test for a concentration higher than 0.5 g phenol which corresponds to the final concentration used in the continuous test.

In this test the SMA was low compared to other values reported in literature (Fang et al., 1996; Tay et al., 2001; Veeresh et al., 2006) and this may be due to different types of biomass used such as the partially granulated sludge from digesters already in continuous operation (Veeresh et al., 2006) and sludge from a wastewater treatment plant from Hong Kong (Fang et al., 1996) which may already had high toxicity response values.

Table 4-9: SMA for the continuous test.

	R1	R2	R3	R4
Methane (mL/day)	218.7	229.2	242.5	249.3
Initial volume of biomass (mL)	500	500	500	500
Final volume of biomass (mL)	490 ± 5	490 ± 5	495 ± 10	495 ± 10
Initial VSS of biomass (g)	25.5	25.5	24.8	24.8
Final VSS of biomass (g)	23.7	23.1	23.2	24.8
SMA (L CH_4/ g VSS-day)	0.009	0.01	0.01	0.01

Based on the average values for all trends presented in Table 4-6, R4 (acetic acid-fed biomass plus carbon dioxide) was chosen as the best reactor. A summary of all the values for this reactor is shown in Figures 4.42 to 4.44. Figure 4.42 shows the final COD and phenol degradation values. Figure 4.43 presents the methane production and methane content, which increased along with the loading; and Figure 4.44 shows the final pH and carbon dioxide concentration. The final pH value shows that the bacteria were able to degrade the substrates (including CO_2) and reach a comfort pH zone.

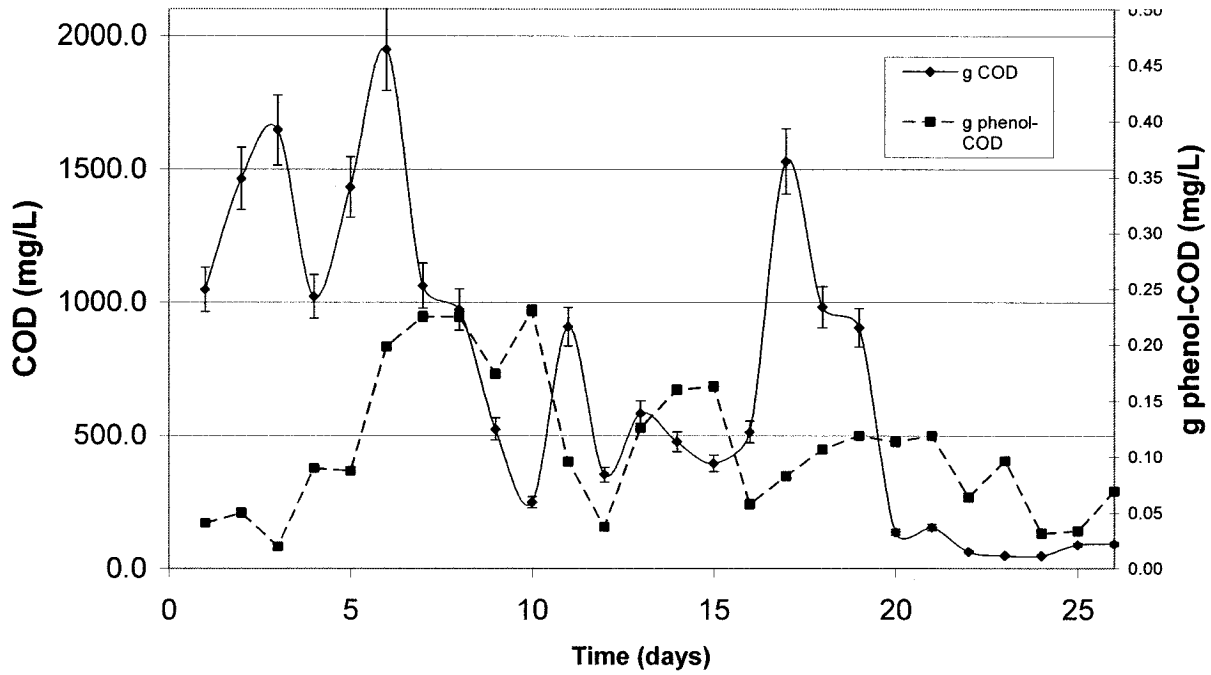

Figure 4.42: COD and phenol final degradation values for R4

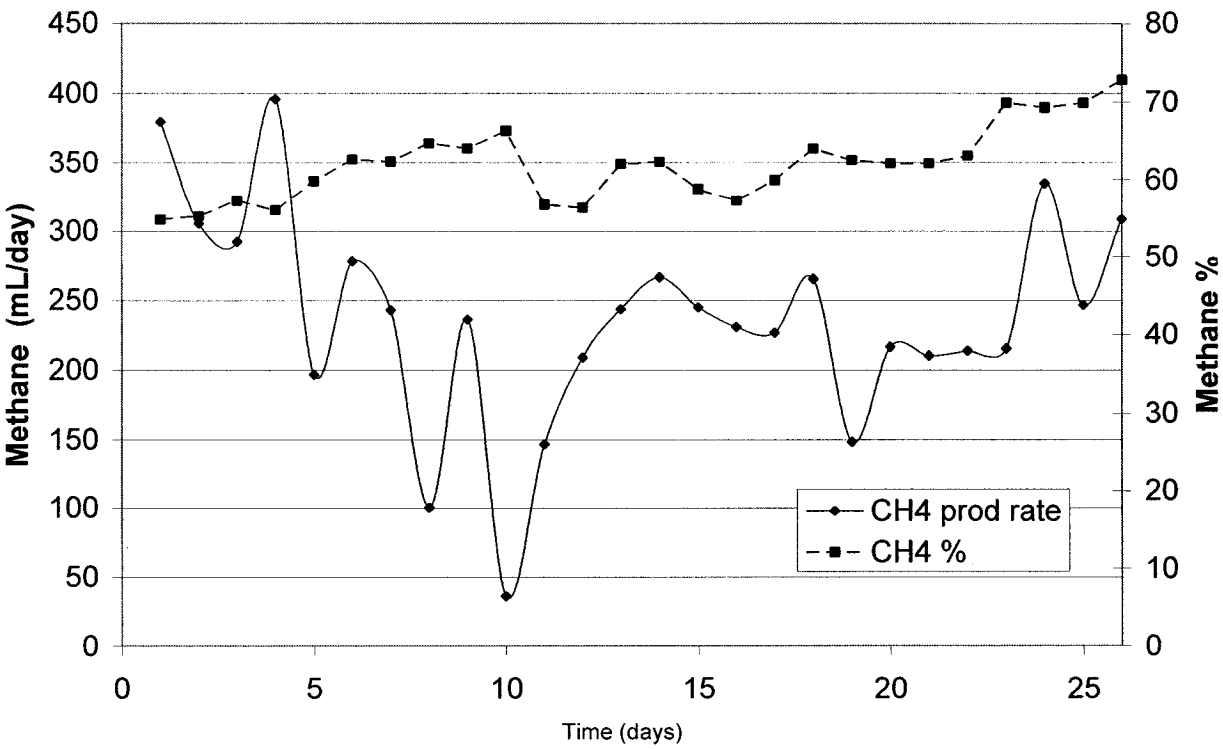

Figure 4.43: Methane production and percentage for R4

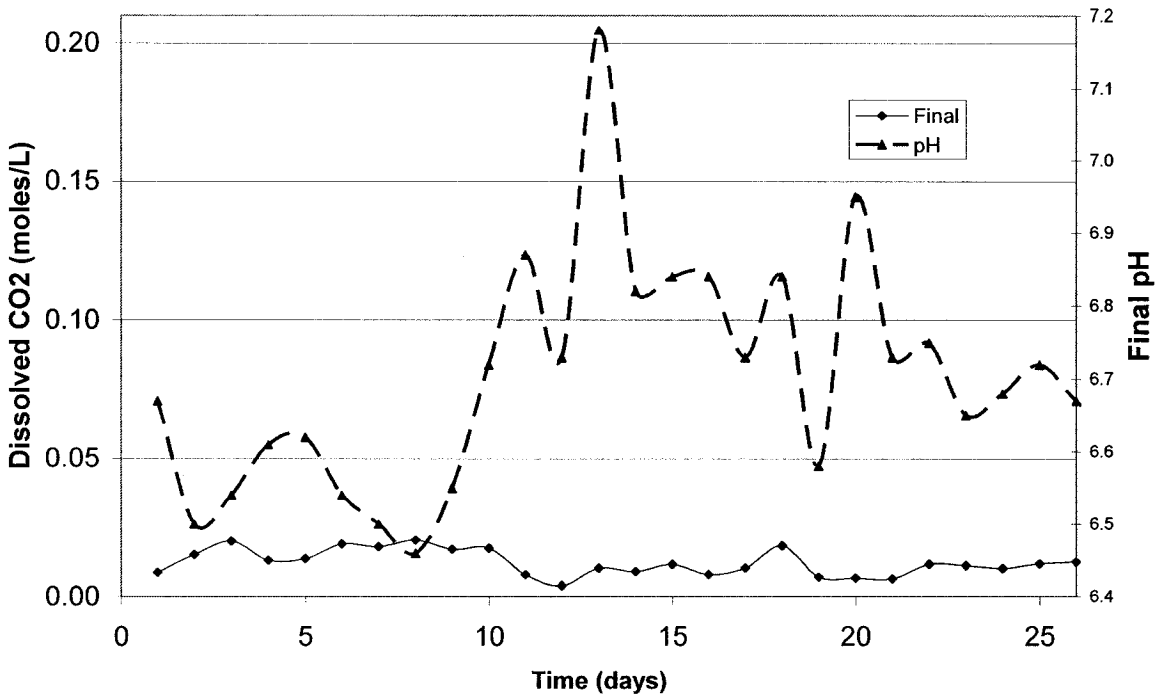

Figure 4.44: Final degradation for carbon dioxide and final pH for R4

Chapter 5 .CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

After finishing these tests, there are some conclusions that can be drawn from the data obtained. It was shown that both CO_2 and phenol could be degraded in an UASB reactor without the need of recirculation of the wastewater. Both systems were able to remove phenol with the only difference that sucrose-fed bacteria were more susceptible to loading shocks (which were rapidly overcome). This may indicate that sucrose-fed bacteria need a longer period for start-up for acclimation to phenol.

Dissolved carbon dioxide removal ranged from 80-90% in most cases; and both reactors showed good performance. This means that CO_2 can be biodegraded in an anaerobic reactor. R1 and R3 showed higher methane contents and this may be due the fact that no dissolved carbon dioxide was added to the wastewater. One design change could be to recirculate the CO_2 and add it to the influent.

The biomass suffered a decrease in TVSS/TSS ratio at the beginning of the acclimation period. This may be due to the fact that these bacteria were acclimated to cheese which contains calcium and this calcium concentration was drastically reduced. After this sudden decrease, the TVSS/TSS ratio was around 0.6-0.7 throughout the experiment for

all the reactors. Although methanogenic bacteria have been known to grow at pH 7 (Mulligan, 2002), in this experiment the bacteria performed under pH 4.5-5.0 with a final pH of 6.5 – 7.3.

Overall, carbon dioxide (a greenhouse gas and by product of combustion which is emitted in the atmosphere) can be captured and used as a co-substrate for the biodegradation of high strength wastewaters under anaerobic conditions which lead to the production of methane which can be used as an energy source in any industrial process.

5.2 RECOMMENDATIONS

For future work, this experiment should be performed with other types of wastewater and its respective medium (depending on the type of waste to be treated) to determine the compatibility of CO_2 towards these compounds.

At the beginning of the continuous experiment, the amount of reactors treating CO_2 was reduced due to problems encountered in keeping the right temperature for all reactors. One recommendation on this aspect will be the use of a jacket to maintain a more stable temperature regardless of the amount of reactors in use.

Another recommendation would be the recirculation of CO_2 contained in the biogas to the influent to reduce the amount of carbon dioxide as a waste product of the system.

If the same type of biomass is used in the future, the addition of more calcium would be better to avoid a drastically reduced inorganic content as in this test.

Increasing the volume of the reactor could help overcome the variations encountered in the influent flow rate by increasing the flow.

For future experiments involving phenol degradation, monitoring of the VFA could help identify the degradation pathways and the intermediate products during the process, and the type of bacteria present in the consortia.

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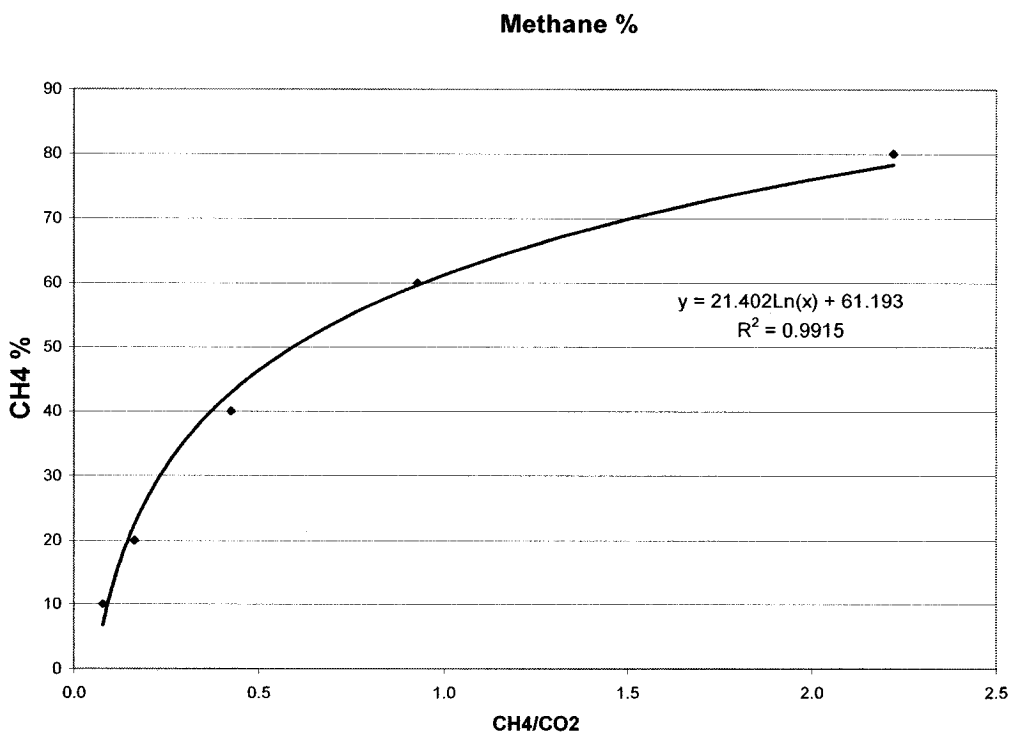
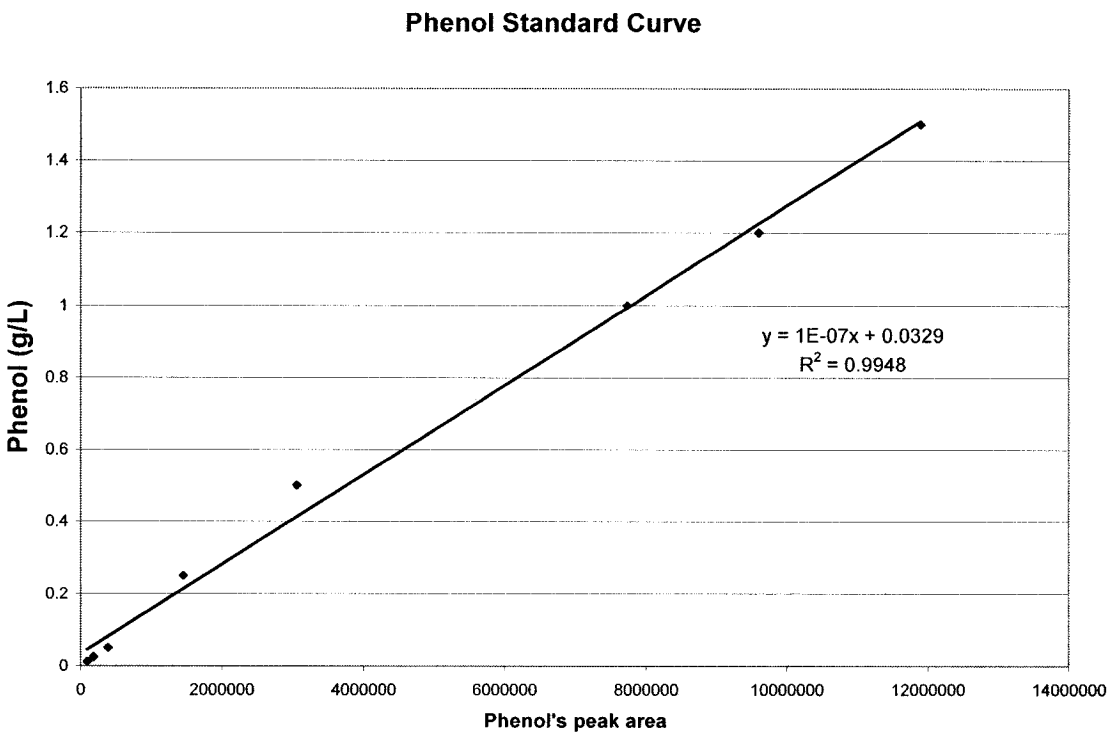
APPENDIX

A. Methane Standard Curve



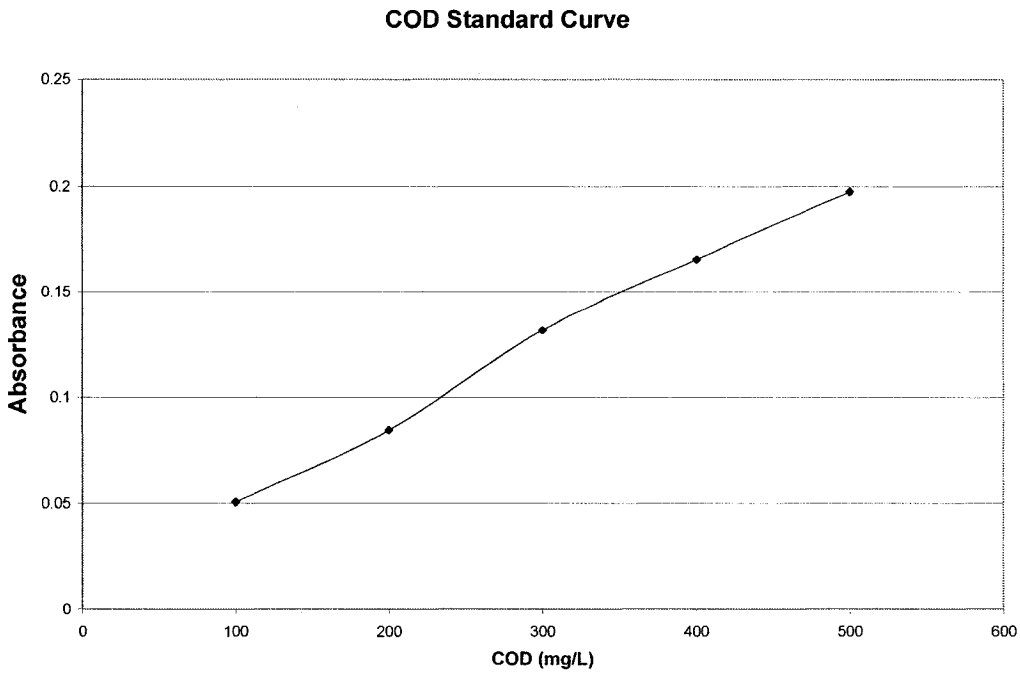
CH_4 (mL)	CO_2 (mL)	CH_4/CO_2	CH_4/CO_2	CH_4/CO_2 av
10	90	0.0851	0.0721	0.078
20	80	0.1615	0.1658	0.163
40	60	0.4192	0.4313	0.425
60	40	0.9201	0.9375	0.928
80	20	2.0964	2.3487	2.222

B. Phenol Standard Curve



Phenol Concentration (g/L)	Area 1 Min*volts	Area 2 Min*volts	Area av Min*volts
0.0125	102295	93493	97894
0.025	182382	192639	187510.5
0.05	398797	383654	393355.7
0.25	1296534	1621421	1458978
0.5	3053397	3069501	3061449
1	7747387	7715017	7731202
1.2	9616908	9580803	9598856
1.5	11889281	11898420	11893851

C. Standard curve for COD



Absorbance	COD concentration (mg/L)
0.05061	100
0.08435	200
0.13174	300
0.16546	400
0.19756	500

D. Glossary

1. **Acetyl CoA:** Important molecule in metabolism, used in many biochemical reactions. Its main use is to convey the carbon atoms within the acetyl group
2. **Adsorption/desorption:** Removal of contaminants from air/water by collecting it on the surface of a solid material.
3. **Acetoclastic bacteria:** Acetate consuming bacteria.
4. **Acetogenesis:** Conversion of volatile fatty acids into hydrogen and acetic acid by bacteria.
5. **Acetogenic bacteria:** Organisms responsible of the conversion of organic matter to acetic acid and other intermediate volatile fatty acids.
6. **Anaerobic digestion:** Degradation of organic matter in the absence of oxygen.
7. **Alkalinity:** The capacity of bases to neutralize acids.
8. **Aromatics:** A type of hydrocarbon, such as benzene or toluene, with a specific type of ring structure.
9. **Beta-oxidation:** Process by which fatty acids, in the form of Acyl-CoA molecules, are broken down in the mitochondria to generate Acetyl-CoA
10. **Biochemical Oxygen Demand (BOD):** A measure of the amount of oxygen consumed in the biological processes that break down organic matter in water. The greater the BOD, the greater the degree of pollution
11. **Biodegradable:** Capable of decomposing under natural conditions.
12. **Carboxylic acid:** are organic acids characterized by the presence of a carboxyl group usually written -COOH or $-CO_2H$.

13. **COD:** Organic content of a wastewater based on the organic oxygen equivalent susceptible to permanganate or dichromate oxidation in an acid solution. A measure of the oxygen required to oxidize all compounds, both organic and inorganic, in water
14. **Dehydrogenation:** Chemical reaction that involves the elimination of hydrogen (H_2).
15. **EC $_{50}$:** Effective concentration 50. Molar concentration of an agonist, which produces 50% of the maximum possible response for that agonist.
16. **Half-Life:** The time required for a contaminant to lose one-half of its original concentration.
17. **Hydrolysis/acidogenesis:** Conversion of polymeric material into monomers.
18. **Hydrogen donor:** Any chemical compound that has a hydrogen atom available for chemical interaction; from simple mineral acids to ordinary acids, carboxylic acids, amines, sulfonic acids.
19. **Heptanoate:** An organic compound composed of a seven-carbon chain terminating in a carboxylic acid.
20. **Kow:** Ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature, usually used to determine the fate of chemicals in the environment.
21. **Kw:** The self-ionization of water (1.011×10^{-14} in pure water at 25°C). Often K_w is more simply expressed in logarithmic terms as pK_w.
22. **LC_{50}:** Lethal concentration 50. Is the concentration of a chemical which kills 50% of a sample population.

23. **Mesophilic:** Growing in the temperature range of 30-40 ° C
24. **Methanogenesis:** Production of methane and carbon dioxide from acetic acid or methane and water from carbon dioxide and hydrogen.
25. **Methanogenic bacteria:** Organisms responsible for the production of methane from intermediate volatile fatty acids
26. **Oxidation:** Chemical addition of oxygen to break down contaminants or organic waste by bacterial and chemical means.
27. **pKa:** specific equilibrium constant for the reaction of an acid with its conjugate base in aqueous solution, often expressed in log terms.
28. **Photolysis:** Light-driven oxidation
29. **Photooxydation:** conversion of a reduced molecule to an oxidized form in the presence of molecular oxygen via a set of chemical reactions that are initiated by photolysis.
30. **Radiation:** Energy in the form of electromagnetic waves which have no mass and are not electrically charged.
31. **Reduction:** Addition of hydrogen, removal of oxygen, or addition of electrons to an element or compound.
32. **Substrate:** Food used by microorganisms to sustain metabolism.
33. **Suspended solids:** Solids which do not settle nor dissolved in the suspension liquid.
34. **Thermal conductivity detector:** Detector for the transfer of heat between two solid materials that are physically touching each other.
35. **Thermophilic:** Growing in the temperature range of 50-75° C

36. **Volatile fatty acids:** Fatty acids with a carbon chain of six carbons or fewer

37. **Volatilization:** The tendency of a material to pass into the gas state at a particular temperature from a solid or liquid state.