

ANAEROBIC TREATMENT OF BREWERY WASTEWATER USING A UASB  
REACTOR SEEDED WITH ACTIVATED SLUDGE

by

CAROL CRONIN

B.Sc., McMaster University, 1991

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

Department of Bio-Resource Engineering

We accept this thesis as conforming  
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April 1996

© Carol Cronin, 1996

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Bio-Resource Engineering

The University of British Columbia  
Vancouver, Canada

Date April 17/1996

## ABSTRACT

The start-up of two upflow anaerobic sludge blanket (UASB) reactors seeded with aerobic activated sludge and treating brewery wastewater is presented in this thesis. Both reactors were identical in design and were continuously operated at 19 - 23 ° C. Reactor A was seeded with 1.98 g VSS/l of reactor, while Reactor B was seeded with 5.93 g VSS/l of reactor. The reactors were first operated at a hydraulic retention time of 5 days. The hydraulic retention time was then reduced in a step-wise fashion to 3 days, 1.5 days, 18 hours then 12 hours in order to increase the organic loading rate. This method of increasing the organic loading rate by decreasing the hydraulic retention time allows for higher liquid upflow velocities which act as a selection pressure to retain better settling sludge in the reactor while washing out the poorer quality sludge. The waste strength varied from 600 to 5600 mg/l, and the average organic loading rate ranged from 0.39 g COD/l/d to 3.62 g COD/l/d. The average sludge loading rate was different for both reactors since each was seeded with a different amount of sludge. The sludge loading rate ranged from 0.20 to 3.67 g COD/g VSS/d for Reactor A and from 0.07 to 0.58 g COD/g VSS/d for Reactor B. The maximum methane production rate achieved was 0.98 l CH<sub>4</sub>/l/d for Reactor A and 1.33 l CH<sub>4</sub>/l/d for Reactor B, with COD removal rates of 69% and 89%, respectively, at the maximum organic loading rate of 4.27 g COD/l/d and hydraulic retention time of 12 hours.

# TABLE OF CONTENTS

<b>ABSTRACT.....</b>	<b>ii</b>
<b>TABLE OF CONTENTS.....</b>	<b>iii</b>
<b>LIST OF TABLES.....</b>	<b>v</b>
<b>LIST OF FIGURES.....</b>	<b>v</b>
<b>LIST OF ABBREVIATIONS.....</b>	<b>vi</b>
<b>ACKNOWLEDGEMENTS.....</b>	<b>vii</b>
 <b>I. INTRODUCTION.....</b>	 <b>1</b>
A. Problem Statement.....	1
B. Objectives.....	2
 <b>II. LITERATURE REVIEW.....</b>	 <b>3</b>
A. Anaerobic Digestion.....	3
B. Anaerobic Treatment.....	4
C. UASB Concept.....	5
D. Start-up of UASB Reactors.....	8
1) Seed Sludge.....	9
2) Operational Parameters.....	10
3) Wastewater Characteristics.....	12
4) Environmental Conditions.....	13
 <b>III. MATERIAL AND METHODS.....</b>	 <b>14</b>
A. UASB Reactor Set-up.....	14
B. Feed Substrate.....	16
C. Seed Sludge.....	17
D. Experimental Procedure.....	18
E. Analysis.....	18
 <b>IV. RESULTS AND DISCUSSIONS.....</b>	 <b>19</b>
A. 5 Day Hydraulic Retention Time.....	19
1) Effluent COD and BOD.....	20
2) Volatile Solids and Volatile Suspended Solids.....	21
3) Biogas Methane Composition.....	22

4) Volumetric Methane Production and Methane Yield.....	23
5) Volatile Fatty Acids, P/A Ratio, and pH.....	24
B. 3 Day Hydraulic Retention Time.....	25
1) Effluent COD and BOD.....	26
2) Volatile Solids and Volatile Suspended Solids.....	27
3) Biogas Methane Composition.....	29
4) Volumetric Methane Production and Methane Yield.....	29
5) Volatile Fatty Acids, P/A Ratio and pH.....	30
C. 1.5 Day Hydraulic Retention Time.....	31
1) Effluent COD and BOD.....	32
2) Volatile Solids and Volatile Suspended Solids.....	33
3) Biogas Methane Composition.....	33
4) Volumetric Methane Production and Methane Yield.....	34
5) Volatile Fatty Acids, P/A Ratio and pH.....	34
D. 18 Hour Hydraulic Retention Time.....	35
1) Effluent COD and BOD.....	36
2) Volatile Solids and Volatile Suspended Solids.....	37
3) Biogas Methane Composition.....	37
4) Volumetric Methane Production and Methane Yield.....	37
5) Volatile Fatty Acids, P/A Ratio and pH.....	38
E. 12 Hour Hydraulic Retention Time.....	38
1) Effluent COD and BOD.....	39
2) Volatile Solids and Volatile Suspended Solids.....	40
3) Biogas Methane Composition.....	40
4) Volumetric Methane Production and Methane Yield.....	41
5) Volatile Fatty Acids, P/A Ratio and pH.....	41
F. Summary of Results.....	42
1) Hydraulic Loading Rate and Reactor Performance.....	42
2) COD Loading Rate and Reactor Performance.....	56
3) Sludge Loading Rate and Reactor Performance.....	58
 V. CONCLUSIONS.....	 69
VI. RECOMMENDATIONS.....	72
VII. REFERENCES.....	74
V111. APPENDIX.....	78

## LIST OF TABLES

TABLE 3.1:	Brewery Wastewater Characteristics.....	16
TABLE 3.2:	Seed Sludge Characteristics.....	17
TABLE 4.1:	Summary of 5 Day HRT Results.....	20
TABLE 4.2:	Summary of 3 Day HRT Results.....	26
TABLE 4.3:	Summary of 1.5 Day HRT Results.....	32
TABLE 4.4:	Summary of 18 Hour HRT Results.....	36
TABLE 4.5:	Summary of 12 Hour HRT Results.....	39

## LIST OF FIGURES

FIGURE 1:	Configuration of UASB Reactor.....	15
FIGURE F.1	Liquid Upflow Velocity vs HRT.....	44
FIGURE F.2	COD Loading Rate vs HRT.....	44
FIGURE F.3	Sludge Loading Rate vs HRT.....	45
FIGURE F.4	Effluent COD vs HRT.....	47
FIGURE F.5	COD Reduction vs HRT.....	47
FIGURE F.6	Volatile Suspended Solids vs HRT.....	49
FIGURE F.7	Volatile Suspended Solids Reduction vs HRT.....	49
FIGURE F.8	Biogas Methane Composition vs HRT.....	51
FIGURE F.9	Volumetric Methane Production vs HRT.....	54
FIGURE F.10	Methane Yield vs HRT.....	55
FIGURE F.11	COD Loading Rate vs Time.....	57
FIGURE F.12	COD Reduction vs COD Loading Rate.....	57
FIGURE F.13	Sludge Loading Rate vs Time.....	60
FIGURE F.14	COD Reduction vs Sludge Loading Rate.....	61
FIGURE F.15	Volatile Solids reduction vs Sludge Loading Rate.....	63
FIGURE F.16	Biogas Methane Composition vs Sludge Loading Rate.....	64
FIGURE F.17	Volumetric Methane Production vs Sludge Loading Rate.....	67
FIGURE F.18	Methane Yield vs Sludge Loading Rate.....	68

## LIST OF ABBREVIATIONS

BOD <sub>5</sub>	Five-day biochemical oxygen demand according to <i>Standard methods for the examination for waste and wastewater</i>
COD	Chemical oxygen demand
HRT	Hydraulic retention time
TS	Total Solids
TSS	Total Suspended Solids
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acid
VS	Volatile Solids
VSS	Volatile Suspended Solids

## **ACKNOWLEDGEMENT**

I would like to express my sincere gratitude to my thesis committee members - Dr. K.V. Lo, Dr. A. Lau and Dr. S. Duff- for their guidance and support. I would also like to thank Dr. P.H. Liao who provided much technical and laboratory advice. Finally, I would like to thank my family and friends for their patience and support.



# I. INTRODUCTION

## A. PROBLEM STATEMENT

Brewery plants produce a large quantity of wastewaters which contain high concentrations of organic pollutants, low concentrations of nutrients, and have a large variation in these parameters (Huang *et al.*, 1987). In particular, brewery effluents are generally characterized by high TCOD (TBOD<sub>5</sub>) and TSS concentrations and wide variations in flow and strength (Le Clair, 1984). This results because the effluent stream is made up of the combined discharges of the brewing and packaging sections, whose production rates vary independently of one another. The packaging process produces a high flow, high pH, weak waste composed primarily of spilled beer and caustic bottle cleaning solutions, while the brewing process produces a low flow, neutral pH, high strength alcohol-carbohydrate-protein waste. The temperature of the effluent ranges from about 19° C in the winter to about 24° C in the summer months. In general, pH of the effluent is a function of production activities and may range from pH 7 to 12. However, within a few hours (7 to 16 hours) hydrolysis and anaerobic activity usually reduces the pH to about 4 to 8 since the effluent has a poor buffering capacity. This pH reduction is a function of the carbohydrate concentration which reflects the brewing production rate. Therefore, brewery wastewater tends to be very difficult to treat due to wide variations in strength (in terms of COD), pH and flow.

Aerobic treatment of brewery effluent has had proven success on the industrial scale in the past, as demonstrated by the deep shaft treatment system at Molsons Brewery in Barrie, Ontario (Le Clair, 1984). However, power requirements and sludge handling and disposal significantly raised the projected cost of operation of the treatment system. Anaerobic

treatment, however, produces less sludge, requires little energy to operate, and produces methane which may be burned as an additional energy source. High rate anaerobic treatment systems are able to treat a variety of industrial wastewaters and are proving to be an attractive alternative to aerobic treatment systems.

## **B. OBJECTIVES**

A high rate anaerobic treatment system, the upflow anaerobic sludge blanket (UASB) reactor, was chosen to treat the brewery effluent. Two identical reactors were operated on a continuous feed basis at room temperature (19 to 24 °C). Each reactor was seeded with 2 litres of activated sludge (15.8 g/l VSS) that was acclimatized to brewery effluent for three weeks while Reactor B received an additional 4 litres of unacclimatized activated sludge (15.8 g/l VSS). UASB reactors were chosen for this experiment because they are simple in design and have been successful in treating brewery effluent in lab scale experiments as well as on an industrial scale. The start-up of an upflow anaerobic sludge blanket (UASB) reactor is crucial to successful operation of the system and several guidelines have been given to ensure a proper start-up. These guidelines will be discussed in detail in a later section.

The **objectives** of this experiment are :

- 1) To investigate the effectiveness of activated sludge as a seed sludge for the start-up of the UASB reactor.
- 2) To determine the impact that the amount of seed sludge has on the start-up and reactor performance.
- 3) To determine the impact that the specific sludge loading rate has on reactor start-up and performance.
- 4) To determine the impact the acclimation period has on the start-up of a UASB reactor when using activated sludge as seed sludge.

## II. LITERATURE REVIEW

### A. ANAEROBIC DIGESTION

During anaerobic digestion, organic matter is completely converted to carbon dioxide and methane. This involves four stages : hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Obayashi, 1985). The anaerobic digestion process involves the complex metabolic interactions of three groups of bacteria (Obayashi, 1985) :

- 1) fermentative bacteria hydrolyze the substrate polymers to single soluble compounds which are fermented to volatile acids, carbon dioxide, and hydrogen.
- 2) obligate H<sub>2</sub> producing acetogenic bacteria subsequently oxidize the propionate, butyrate, and longer-chain fatty acids to acetate, carbon dioxide and hydrogen.
- 3) methanogens utilize the acetate and hydrogen to produce methane.

Approximately 70% of the total methane derives from acetic acid while the remaining 30% comes from the conversion of hydrogen and carbon dioxide. The stability of the anaerobic microbial ecosystem is very dependent on methanogenic activity, this activity being characterized by slow growth rates of microorganisms and great sensitivity to inhibition processes and environmental conditions such as pH (Stronach *et al.*, 1986). The ideal pH range for the growth of methanogens is between 6.8 and 7.2, but may vary among species (Stronach *et al.*, 1986). It is essential that growth conditions are suitable for methanogenic bacteria to prevent the breakdown of the treatment process. The rate limiting step in methane fermentation involves the degradation of volatile fatty acids (VFA) such as acetic acid, propionic acid and butyric acid and others during methanogenesis since these acids begin to accumulate in

digesters stressed by high organic loading rates, and/or short retention times and/or inhibitors (Marchaim *et al.*, 1993). Marchaim *et al.*, (1993) found that instability and processes leading to digestion failure can be seen more clearly by means of the propionic : acetic acid ratio rather than a drop in pH when the digester is well buffered and the ratio also indicated bacterial stress in overloaded digesters that are not buffered. In particular, propionic : acetic acid ratios greater than 1: 1.4 and acetic acid levels greater than 800 mg/l indicate impending reactor failure.

## **B. ANAEROBIC TREATMENT**

In recent years, anaerobic treatment of industrial wastes has become increasingly popular due to energy costs and environmental concerns (Lettinga *et al.*, 1980). The anaerobic treatment of wastewater has several advantages over aerobic treatment and few serious drawbacks. Lettinga *et al.*, (1980) have listed some of the advantages and disadvantages of anaerobic wastewater treatment :

### **Advantages**

1. low production of excess sludge
2. stable sludge produced which can easily be dewatered
3. low nutrient requirements
4. no energy required for aeration
5. methane is produced, which can be used as an energy source
6. very high loading rates can be applied under favourable conditions
7. active anaerobic sludge can be preserved unfed for many months

### **Disadvantages**

1. anaerobic metabolism is a sensitive process which can be inhibited by environmental conditions such as the presence of specific compounds ( eg.,  $\text{CN}^-$  )
2. relatively long periods of time are required to start up the process due to the slow growth rate of anaerobic bacteria
3. anaerobic treatment is essentially a pre-treatment method and usually requires post-treatment to meet effluent standards
4. little practical experience has been gained with the application of the process to the direct treatment of wastewaters

In recent years, there has been much research into the development of high rate anaerobic treatment processes for the treatment of a variety of wastewaters. Hulshoff Pol *et al.*, (1982) states that the basic conditions for a high rate anaerobic wastewater treatment are :

- 1) a high sludge retention and a high specific activity of the sludge
- 2) good contact between the sludge and the incoming wastewater

The UASB reactor system is a high rate anaerobic treatment system that has been reported to have achieved high treatment efficiencies at high loading rates (Hulshoff Pol *et al.*, 1982).

### **C. UASB CONCEPT**

The UASB concept was first conceived by Lettinga and his co-workers in the Netherlands in 1971. The first full scale application was realized in 1977, over five years after the first laboratory studies had been made. To date, over 100 full-scale UASB plants have been commissioned in the Netherlands and other Western European countries, Canada, USA, and the Far East (Hickey *et al.*, 1991). In this process, the waste to be treated is introduced at the bottom

of the reactor. The wastewater flows upward through a sludge blanket composed of biologically formed granules or particles and treatment occurs as the wastewater comes in contact with the granules. Lettinga *et al.*, (1980) states that the main principle underlying the UASB concept is that anaerobic sludge inherently has superior flocculation and settling characteristics, provided that the physical and chemical conditions for sludge flocculation are (and remain) favourable. Once this condition is met, the desired retention of the sludge depends mainly on an effective separation of the gas from the sludge and the liquid. This is accomplished in the UASB reactor by the gas-liquid-solid separator device (GSS device) in the upper part of the reactor (see Figure 1) and by keeping mechanical mixing and/or sludge recirculation at a minimum (Lettinga *et al.*, 1980). For a satisfactory operation of the GSS-device it is required to : a) achieve an effective separation of entrapped or attached gases from the sludge; and b) enable the sludge separated from the solution in the settler compartment to return to the digester compartment. The liquid-solids-gas separator consists of a cone, with its larger base either facing up or down, sealed to the reactor inner wall near the top of the reactor. The device achieves high sludge retention by creating a quiescent zone near the top of the reactor which helps rising sludge flocs form larger aggregates and sink back down. The sludge flocs rise either by turbulence (due mainly to gas production) or flotation. The separator used in this experiment is one with the cone's base facing upward. A smaller cone with its tip pointing toward the tip of the larger cone is fitted below the larger cone's tip (Refer to Figure 1). This configuration blocks the uprising liquid from directly hitting the tip of the larger cone and thus minimizes sludge loss. Generated biogas becomes collected in the smaller cone, and when it fills the smaller cone, it escapes from the perimeter of the cone upward and collects in the space between the reactor liquid surface and the base of the larger cone which is sealed to the reactor wall. The biogas collecting in the

cone headspace is then routed to a gas volume measuring device. As well, as flocs of bacteria rise, through bouyancy provided by rising gas bubbles or lack of settleability of the bacteria, and travel through the cone, the liquid velocity tapers off due to the enlargement of the diameter of the cone. Consequently, the rising bacteria particles slow down and tend to gather near the liquid surface and form larger flocs which will then sink down the reactor.

High concentrations of biomass are retained in UASB systems, which allows the process to achieve high removal efficiencies at high volumetric COD loading rates. Weiland *et al.*, (1991) reported that compared to other high rate anaerobic systems, UASB systems lead to the highest biomass accumulation with contents of up to 50 g VSS/l and should achieve removal capacities between 50 and 100 kg COD/m<sup>3</sup> /d at specific sludge loadings of 1-2 g COD/g VSS. In addition, the UASB system is the only anaerobic system that can remove significant amounts of nitrogen (Wentzel *et al.*, 1994). Weiland *et al.*, (1991) have compared the UASB system with other high rate anaerobic treatment systems in terms of its advantages and disadvantages :

#### **Advantages**

1. high removal capacity
2. short retention times
3. high COD removal efficiency
4. low energy demand
5. no need of support media
6. simple reactor construction
7. long experience in practice

### **Disadvantages**

1. granulation process difficult to control
2. granulation depends on wastewater properties
3. start-up eventually needs granulated sludge
4. sensitive to organic shock loads
5. restricted to nearly solids-free wastewater
6.  $\text{Ca}^{++}$  and  $\text{NH}_4^+$  inhibit granule formation
7. re-start can result in granule floating
8. high area demand

### **D. START - UP OF UASB REACTORS**

Good results with the UASB reactor can be primarily attributed to the formation of a highly settleable and active sludge in the reactor. During the start-up of a UASB reactor, the biomass aggregates to form stable, compact, granules or pellets which may be up to 5 mm in diameter (Hulshoff Pol *et al.*, 1982). The development of a completely granulated sludge can be a lengthy process, but is considered vital to the success of the operation of the UASB reactor. This is due to the fact that granules settle well against the upflow of waste to be treated; high biomass retention is possible at hydraulic loading rates which would cause a poorly flocculated sludge to wash out almost immediately (Hulshoff Pol *et al.*, 1982). These hydraulic effects are exacerbated by the degree of turbulence in the bed resulting from high biogas production rates which tends to assist washout of any but the most rapidly settling sludges (Hulshoff Pol *et al.*, 1982). Goodwin *et al.*, (1992) found that although granules can eventually be formed from a



diffuse seed sludge, reactors seeded with granulated sludge achieved high performance levels within a few days, while reactors seeded with diffuse sludge required start-up periods in excess of 60 days. Granulation can be seen to occur in three distinct phases; each phase characterized by different sludge concentration profiles in the reactor and differences in the nature of the sludge itself (Hickey *et al.*, 1991) :

**Phase one** : the sludge bed expands as gas production increases and sludge concentration in the sludge blanket increases, at the same time, granules begin to form in the sludge bed and gradually grow.

**Phase two** : sludge concentration in the blanket continues to increase and washout of the inoculated sludge begins; consequently, the total amount of the sludge in the reactor drops to a minimum.

**Phase three** : the growth of granules exceeds the sludge wash-out rate and the total biomass in the reactor again increases.

Sludge granulation is governed mainly by bacterial growth, and many factors influence the formation of granular sludge in UASB reactors. Such factors include the characteristics of the seed sludge, operational parameters, wastewater characteristics, and environmental factors (Lettinga *et al.*, 1980). These factors will be discussed in detail in the following sections.

### **1. Seed Sludge**

Ideally, UASB reactors should be started up with pre-granulated seed sludge from another UASB reactor. However, this type of sludge is not always available to use as seed sludge, and so alternative seed sludge which can eventually develop into granular sludge under the right conditions are used. Several factors, such as the amount and type of sludge to use, need to be considered. To avoid excessive wash-out of seed sludge, the amount of sludge must be small enough to maintain the sludge bed within the reactor upon increasing the loading rate, but also be large enough to prevent a delay of start-up (Lettinga *et al.*, 1985). Lettinga *et al.*, (1980)

recommend to use a digested sewage sludge of fairly poor methanogenic activity (approximately 0.05 kg COD/kgVSS/d) and an amount of seed sludge of at least 10 kg VSS/m<sup>3</sup>. These requirements can be met by using thick types of sludge with a dry solids content of about 60g DS/l. In the case when a relatively thin seed sludge is used (< 40 kg TS/m<sup>3</sup>) then only 6 kg VSS/m<sup>3</sup> is recommended (Lettinga *et al.*, 1985). Wu *et al.*, (1987) have found that activated sludge is a good alternative to digested sewage sludge because a considerable amount of methanogenic bacteria is found in the activated sludge and it is easy to obtain large amounts of the sludge from activated sludge plants. Another advantage is that activated sludge usually contains little sand and soil and is mainly composed of biomass, thus there is no problem with either dead space or a scum layer coming from the seed material. Wu *et al.*, (1987) have recommended that when using activated sludge as seed material, the amount of seed sludge be kept at approximately 15 g VSS/l and to incubate the seed sludge intermittently or semicontinuously for more than half a month in advance of continuous feed. This incubation period consists of feeding the seed sludge with the feed substrate under anaerobic conditions before seeding the reactor with the seed sludge. Hickey *et al.*, (1991) reported that when non-granular sludge was used as inoculum (digested sewage sludge and activated sludge) at ambient temperatures (19 - 23 °C) the granulation period took 12 months for brewery wastewater and ultimately achieved a COD load of 3- 8 kg COD/ m<sup>3</sup> / d with over 90 % COD removal.

## **2. Operational Parameters**

It is essential to accomplish a sufficient and continuous removal of the lighter sludge fractions from the reactor and retain the heavier sludge as well as promote bacterial growth in or on the heavier sludge (Lettinga *et al.*, 1980). Lettinga *et al.*, (1980) have recommended several

guidelines for the start-up of UASB reactors :

1. washed out or dispersed sludge should not be returned
2. apply dilution or effluent recycle at COD influent greater than 5000 mg/l
3. increase the organic loading rate stepwise, always after at least 80 % reduction in the biodegradable COD has been achieved
4. maintain the acetate concentration below 1000 mg/l
5. start with 12- 15 kg VSS /m<sup>3</sup> of thick seed sludge ( > 60 kg TSS/m<sup>3</sup> )

In particular, the substrate concentration and liquid upflow velocity have considerable influence on granulation and acts as a selection process for the biomass cultivated in the system. Higher hydraulic loading rates are beneficial to the development of granular sludge. At higher hydraulic loading rates, light and bulking sludge tends to migrate upwards, whereas heavy sludge tends to collect at the bottom of the reactor. Thus, the heavy sludge receives the substrate first (which is introduced at the bottom of the reactor) and granulation takes place more quickly than if no selection pressure is imposed. Campos *et al.*, (1992) have suggested that the start-up should be carried out with a medium concentration of substrate (around 1000 mg/l COD) to allow a high hydraulic loading rate and thus a higher upflow liquid velocity. The volumetric loading rate should be increased by decreasing the retention time instead of increasing the COD concentration. This allows washout of the poor sludge and improves the mixing characteristics of the system. They found that the best upflow liquid velocity was in the range of 0.72 to 0.96 m/d.

It is vital to the granulation process to control the sludge loading rate. Underloading leads to the development of voluminous sludge, while overloading is detrimental because of the

gas production that will occur in the liquid-solids-gas separator which will hamper the settling of the sludge in the separator (Hulshoff Pol *et al.*, 1983). Wu *et al.*, (1985) found that granules began to appear at a sludge loading rate of 0.3 kg COD/kg VSS/day for brewery wastewater. Wu *et al.*, (1985) also suggested that after start-up the sludge loading rate be raised to over 0.6 kg COD/kgVSS/day in order to speed up granulation.

### **3. Wastewater Characteristics**

Granulation is very much dependent on the type of wastewater to be treated; granulation may not develop with some types of wastewater. Lettinga *et al.*, (1985) have suggested characteristics of the wastewater to take into consideration :

1. The strength of the waste is important; faster granulation occurs with lower strength waste. The waste needs to be strong enough to maintain conditions for bacterial growth (COD > 1000 mg/l).
2. Dispersed matter slows or may even inhibit granulation.
3. Granulation occurs faster on mainly soluble carbohydrate substrates as compared to mainly volatile fatty acid substrates.
4. High ion concentrations will lead to chemical precipitation, resulting in the formation of a granular sludge with a high ash content.

Due to its high carbohydrate content, granulation proceeds rather easily on brewery wastewater. At operating temperatures of 35 °C , granulation can occur in as little as 2 months, while at sub-optimal temperatures (19 - 24 °C), granulation usually takes as long as 12 months of operation (Hickey *et al.*, 1991). Lettinga *et al.*, (1985) have reported that high suspended solids inhibit granulation and recommend that the suspended solids level not exceed 500 mg/l.

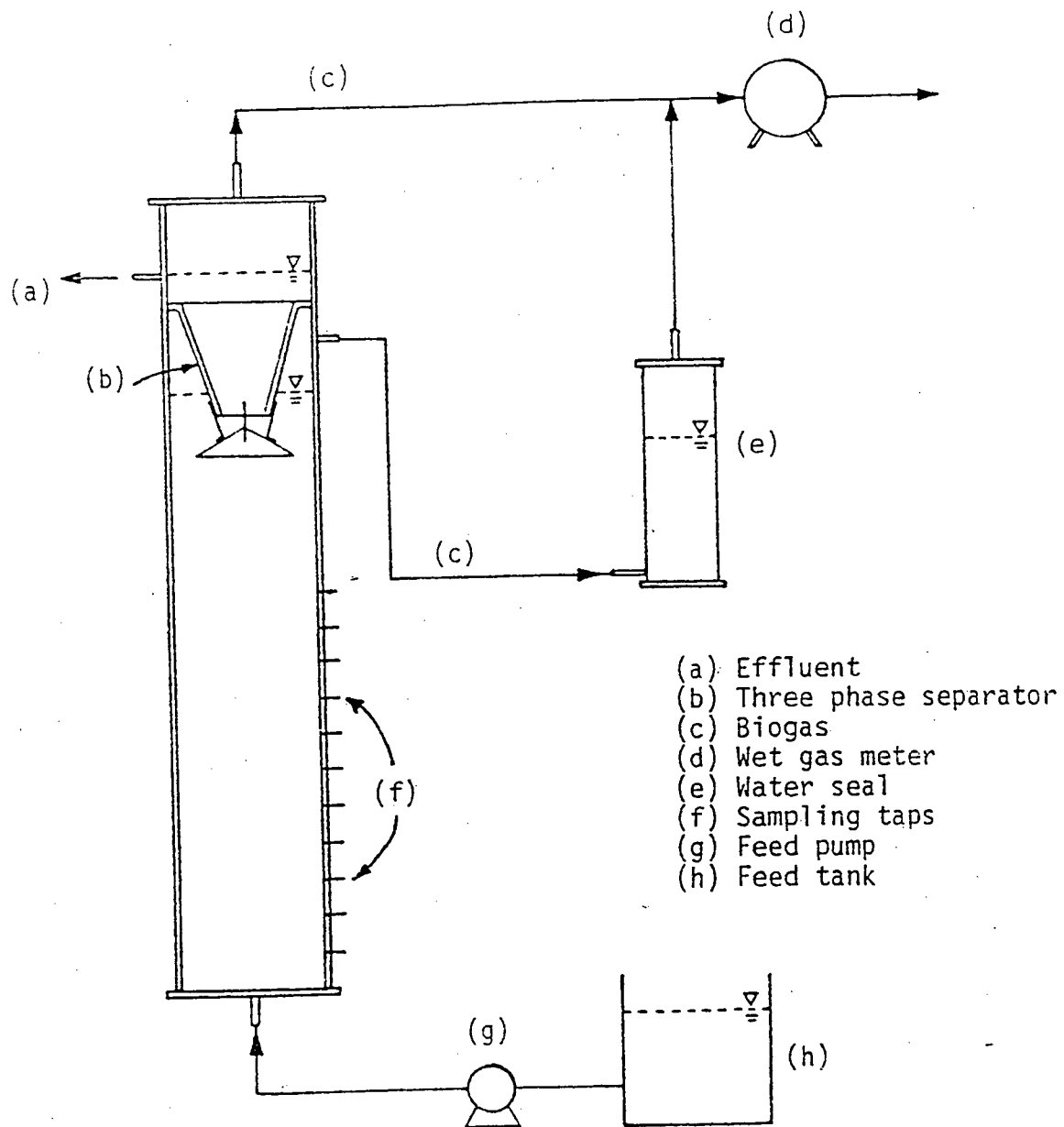
#### **4. Environmental Conditions**

Environmental conditions must be favourable for bacterial growth in order for granulation to take place. Such conditions include : pH between 6.8 to 7.5, all essential nutrients (N, P, K) should be present in sufficient amounts and in available form, and toxic compounds should be absent at inhibitory concentrations or sufficient time should be allowed for bacterial acclimatization. With regard to brewery wastewater, pH levels need to be monitored and carefully controlled. In addition to wide variations in pH (4 to 12), brewery wastewaters do not have any buffering capacity and therefore depend completely on buffer from an external source to control the minimum pH in the reactor (Moosbrugger *et al.*, 1993). In adjusting the pH with a strong base such as NaOH, the pH may increase to such high levels that some of the trace elements may precipitate and become unavailable to the micro-organisms giving rise to partial or complete failure of the process (Moosbrugger *et al.*, 1993).

### **III. MATERIALS AND METHODS**

#### **A. UASB REACTOR SET-UP**

Both reactors were identical and of the UASB reactor design. The UASB reactor design configuration is depicted in Figure 1. The reactor was a cylinder made of acrylic plastic with an inner diameter of 11.5 cm and a total length of 168 cm. The corresponding surface-to-volume ratio was 32.1. The working volume was 16.0 litres. Eleven sampling ports were installed along the length of each reactor starting from near the bottom of the reactor. The spacing between two neighboring sampling ports was such that it widened towards the top of the reactor. The conical three-phase separator is a unique design feature of the UASB reactor. The separator separates and collects the biogas produced from the wastewater. The generated biogas is routed to a water column (which regulates the liquid level around the separator) and passes through a wet gasmeter. The separator is constructed so that its conical base faces upward. As the cross section area increases, the velocity of the fluid flow drops and causes the sludge to be retained in the reactor. The effluent overflows from the top of the reactor and is collected in a bucket. A peristaltic pump was used to continuously pump the influent (brewery effluent) from the feed tank to the reactors, introduced at the bottom of the reactor. The pumping rate was controlled by a speed controller, and a single pump drive with two pump heads was used to ensure both reactors received the same amount of influent (and therefore had identical hydraulic retention times). Both reactors and the influent feed tank were kept at room temperature (19 - 24 °C).



**Figure 1: Configuration of UASB Reactor**

## B. FEED SUBSTRATE

The feed substrate consisted of brewery wastewater obtained from a local brewery and adjusted from a COD concentration ranging from 600 to 5600 mg/l to the desired COD concentration (approximately 1000 to 2000 mg/l). The unadjusted wastewater characteristics are summarized in Table 3.1. The wastewater characteristics vary a great deal in terms of pH, COD and solids concentration. The suspended solids concentration was lowered to less than 500 mg/l by settling the waste before feeding to the reactors. The concentration of nitrogen to support biological growth is estimated to be 20:1 in terms of BOD:TKN, the minimum requirement of 300 mg/l of nitrogen is met without any nitrogen supplement needed. Due to the lack of buffering capacity of the waste, a phosphate buffer was used to keep the pH of the influent at around pH 7. The amount of buffer used was approximately 15 ml buffer/ l influent. The brewery waste was kept in a walk-in cooler at approximately 4 °C and brought to room temperature before it was fed to the reactors. The pH was adjusted by adding either NaOH or HCl to bring the pH to about 7 and then the buffer solution was added since the pH tended to drop to about pH 4 within a few hours.

Table 3.1 Brewery Wastewater Characteristics\*

<b>5 day BOD</b>	750- 3000
<b>COD</b>	600-5600
<b>Total Solids</b>	900-6000
<b>Volatile Solids</b>	400-3000
<b>Total Suspended Solids</b>	40-1000
<b>Volatile Suspended Solids</b>	40-600
<b>Total Kheldahl Nitrogen</b>	300
<b>pH</b>	4-12

\* all values are in mg/l except pH



### C. SEED SLUDGE

Activated sludge that was available in the laboratory was incubated at room temperature (19 to 24°C ) in an airtight glass container for three weeks before being seeded into the reactors. The characteristics of the seed sludge are described in Table 3.2. During the three week acclimation period, the seed sludge was fed daily approximately 250 ml of brewery effluent, (a sludge loading rate of 0.01 g COD/g VSS/d) that had been adjusted to pH 7 with either NaOH or HCl. The purpose of acclimatizing the seed sludge was to cultivate anaerobic bacteria that could use brewery wastewater as a food source. Before seeding it was observed that methane was being produced by the bacteria in the sludge, and so it was concluded that the seed sludge contained a population of methanogens. Each reactor was seeded with 2 litres of this sludge at 15.8 g/l VSS; or 31.6 g (1.98 g VSS/l). In addition, Reactor B was seeded with an additional 4 litres of unacclimatized sludge at 15.8 g/l VSS (i.e 63.2 g) for a total of 94.8 g of seed sludge (5.93 g VSS/l). The purpose of using different amounts of seed sludge was to determine the impact that the amount of seed sludge has on reactor start-up and treatment efficiency. Reactor B was seeded with the same amount of acclimatized seed sludge as Reactor A as well as an additional amount of unacclimatized seed sludge in order to determine the impact that the acclimatization of the seed sludge has on reactor start-up.

Table 3.2 : Seed Sludge Characteristics\*

<b>COD</b>	27251-27998
<b>Total Solids</b>	26860-30540
<b>Volatile Solids</b>	20500-22350
<b>Total Suspended Solids</b>	20250-22280
<b>Volatile Suspended Solids</b>	16000-18220
<b>Total Kheldahl Nitrogen</b>	2430-2835
<b>Ortho-phosphate</b>	1215-1458

\* all values are in mg/l

#### **D. EXPERIMENTAL PROCEDURE**

The reactors were continuously fed with brewery waste adjusted to a COD concentration of about 1000 -2000 mg/l (periodic fluctuations from 600- 5600 mg/l occurred). The sludge loading rate was gradually increased by reducing the hydraulic retention time in a step-wise fashion. This method of increasing the COD loading rate by reducing the retention time acts as a selection pressure in which poorly settling sludge is washed out of the reactor due to an increased liquid velocity and an increased gas production rate so that the heavier sludge is retained in the reactor. The reactors were first operated with a hydraulic retention time of 5 days, from day 1 to 28 of operation. From day 29 to 101, the HRT was 3 days. The HRT was decreased to 1.5 days from day 102 to 132, then 18 hours from day 133 to 162. The final HRT was 12 hours, from day 163 to 189, the last day of reactor operation.

#### **E. ANALYSIS**

Identical analyses were done on both the feed substrate and the effluent. The total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS), and BOD<sub>5</sub> were determined according to the Standard methods (A.P.H.A., 1975). COD was determined by the colorimetric method (Knechtel, 1978). Biogas composition and volatile fatty acids (VFA) were analyzed on a Hewlett Packard 5890A gas chromatograph. The volume of the biogas was measured with a wet gasmeter; the measured volume was corrected to the standard temperature and pressure (STP) which are 0° C and one atmosphere. Total Kjeldahl nitrogen (TKN) was determined using a block digester and a Technicon Auto Analyzer II (Schumann *et al.*, 1983).

## **IV. RESULTS AND DISCUSSIONS**

### **A. Five Day Hydraulic Retention Time**

Both reactors were first operated at a hydraulic retention time (HRT) of 5 days, from day 1 to day 28. The average COD loading rate at this time was 0.48 g COD/l/d and the liquid upflow velocity was 0.48 m/d. The relatively low COD loading rate and low liquid velocity was intended to minimize sludge loss and thus establish an active and healthy bacteria population in the sludge bed region as quickly as possible. Sludge can be washed out of the reactor due to liquid velocity and buoyancy effects caused by gas bubbles produced in the reactor sludge blanket region. Table 4.1 summarizes the results of the 5 day HRT results (the values given are averages).

**Table 3.1 : Summary of the 5 day HRT Results**

<b>Influent Characteristics:</b>			
<b>COD (mg/l)</b>		2415 ± 573	
<b>BOD (mg/l)</b>		1619 ± 183	
<b>VS (mg/l)</b>		1113 ± 433	
<b>VSS (mg/l)</b>		260 ± 173	
		<b><u>Reactor A</u></b>	<b><u>Reactor B</u></b>
<b>COD loading rate (g COD /l /d)</b>		0.48 ± 0.11	0.48 ± 0.11
<b>Sludge loading rate (gCOD/gVSS/d)</b>		0.24 ± 0.06	0.08 ± 0.02
<b><u>Effluent Characteristics:</u></b>			
<b>COD (mg/l)</b>		1451 ± 564	1997 ± 798
<b>BOD (mg/l)</b>		618 ± 337	890 ± 82
<b>VS (mg/l)</b>		1237 ± 361	1456 ± 575
<b>VSS (mg/l)</b>		110 ± 19	199 ± 111
<b>pH</b>		7.7 ± 0.4	7.6 ± 0.5
<b>VFA (mg/l)</b>	<b>acetic</b>	566 ± 545	995 ± 743
	<b>propionic</b>	276 ± 260	157 ± 205
	<b>butyric</b>	0 ± 0	0 ± 0
	<b>iso-butyric</b>	9 ± 15	0 ± 0
<b>COD reduction (%)</b>		42 ± 15	20 ± 6
<b>BOD reduction (%)</b>		64 ± 16	45 ± 4
<b>VS reduction (%)</b>		- 28 ± 49	- 44 ± 63
<b>VSS reduction (%)</b>		39 ± 29	- 21 ± 82
<b>Biogas methane composition (% , v/v)</b>		36 ± 10	28 ± 12
<b>Methane production (l CH<sub>4</sub> /l/d)</b>		0.06 ± 0.03	0.04 ± 0.03
<b>Methane yield (l CH<sub>4</sub> /g COD dest./d)</b>		0.28 ± 0.03	0.15 ± 0.26

### 1) Effluent COD and BOD

As bacteria utilize nutrients in the feed wastewater for maintenance energy and cell material synthesis, the organic strength of the feed decreases. However, if the bacteria in the sludge bed are washed out of the reactor and end up in the effluent stream, the organic strength of the effluent may not be lowered even though there may be some COD (and BOD) removal in the reactor itself. The lack of good settling characteristics of the sludge is often a problem during start-up which leads to excessive sludge loss.

The COD and BOD results indicate that Reactor A was performing better than Reactor B in terms of COD and BOD removal. Reactor B experienced increases in effluent COD frequently during the first twenty days of operation while Reactor A only experienced decreases in effluent COD. This is due to the fact that Reactor B had a considerable amount of sludge washout during this time while Reactor A experienced very little sludge loss. The excessive sludge washout in Reactor B can be explained by the fact that the sludge bed region in Reactor B occupied a larger volume of the reactor than in Reactor A (38% of reactor volume in Reactor B compared to 13% in Reactor A) and thus was not able to settle as well back into the reactor region. In addition, most of the seed sludge in Reactor B had not been acclimatized to the brewery effluent and so sludge washout due to flotation caused by cell lysis may also have been a factor. The COD results in Figures A.1 and A.2 indicate that both reactors eventually achieved a consistent lowering of effluent COD and an increase in COD reduction. For Reactor A, this trend was observed from day 6 and not until day 18 for Reactor B. By the end of the 5 day HRT operation period (day 28), Reactor A was performing better than Reactor B with COD removal rates of 49% and 32%, respectively. The BOD results in Figures A.3 and A.4 support these COD results.

## **2) Volatile Solids and Volatile Suspended Solids**

The volatile suspended solids levels in the influent and effluent were quite low, often below 100 mg/l. In contrast, the volatile solids levels were considerably higher, with levels usually higher than 1000 mg/l. Therefore, most of the solids in the influent (brewery effluent) are dissolved, with the suspended solids component comprising only a small fraction. The low suspended solids levels in brewery effluent makes this wastewater suitable to

treatment by UASB reactors since suspended solids concentrations above 500 mg/l inhibit granulation (Lettinga *et al.*, 1985).

Information about the solids levels in the effluent is useful in that it gives an indication about the amount of sludge being washed out of the reactors, since volatile suspended solids (VSS) levels are usually taken to mean the amount of biomass in the sample. Figures A.5 and A.6 indicate that the VS levels in both reactors were increasing until day 20 then declined until day 27 when they experienced a sharp increase. An increase in effluent VS is expected at the beginning of the start-up as the sludge is not yet adapted to the reactor environment and has poor settling properties. The sharp increase in VS levels on day 27 was due to an accidental temporary increase in the flow rate. This problem was corrected quickly and the flow rate was then adjusted to ensure a 5 day HRT. The increase in flow rate and subsequently, the rising liquid velocity on day 27 of operation, seemed to be responsible for this incidence of sludge washout since gas production at this time was very low and bouyancy effects due to gas bubbles would be minimal. The effluent VS levels in Reactor B were considerably higher than Reactor A. It was observed during this period that Reactor B had experienced considerable sludge loss compared to Reactor A.

Figures A.7 and A.8 indicate that Reactor A experienced only a decline in VSS levels in the first 28 days of operation, and confirm that Reactor A had not experienced the sludge loss that occurred in Reactor B.

### **3) Biogas Methane Composition**

The biogas methane composition is an important indicator of the stability of the anaerobic reactor. As seen in Figure A.9, the methane percentage of the produced biogas

increased steadily for both reactors as the seed sludge adapted to the feed substrate and the reactor environment, and as the COD reductions for both reactors increased.

The biogas methane composition results confirm the COD and VSS results that Reactor A had a higher treatment efficiency than Reactor B. Reactor B was seeded with more seed sludge than Reactor A, however, most of this sludge was not acclimatized to the brewery effluent prior to seeding into the reactor. The lower biogas methane composition for Reactor B was due to the fact that for the first few weeks of reactor operation, the seed sludge in Reactor B had not yet acclimatized to the brewery effluent and to the anaerobic reactor environment, and so had a smaller population of active anaerobic bacteria than the sludge in Reactor A. The lack of acclimation period for the seed sludge in Reactor B resulted in very low COD reductions, and consequently, low methane purity of the produced biogas. After three weeks of operation, the seed sludge in Reactor B appears to have acclimatized to the influent and reactor environment, resulting in increased COD reductions and a methane content in the produced biogas equal to that in Reactor A.

#### **4) Volumetric Methane Production and Methane Yield**

Figure A.10 depicts the volumetric methane production for both reactors in terms of  $1 \text{ CH}_4 / 1 \text{ reactor / day}$ . The volumetric methane production for both reactors fluctuated considerably, but tended to increase over the 28 day period. The volumetric methane production reflects the COD loading rate; as the COD loading rate increases, the methane production rate increases, since there is more influent COD per litre of reactor per day to convert to methane. The volumetric methane production rate of both reactors was very low due to the low COD loading rates. Reactor B was producing less methane than Reactor A at this HRT. This is

again due to the lack of an acclimatization period for most of the seed sludge in Reactor B, and consequently, the poor methanogenic activity of the seed sludge.

The methane yield for the two reactors is expressed in  $l\ CH_4/g\ COD/d$  where the COD has been destroyed. This per-g COD-destroyed methane yield is often preferred to the per-g COD added since it reflects the extent to which sludge is being washed out of the reactors. A negative methane yield can result if the effluent COD has increased instead of decreased, which often results when a considerable amount of sludge has been washed out of the reactor.

Figure A.11 depicts the methane yield per g COD destroyed for both reactors. The methane yield for Reactor A increased slightly from day 6 to day 28. The positive methane yields for Reactor A confirm previous results that Reactor A had not experienced effluent COD increases or sludge loss. Reactor B experienced a few negative methane yields, indicating increases in effluent COD as a result of sludge loss, near the beginning of the operation. After three weeks of operation, in which the sludge quality had improved considerably, Reactor B experienced only positive methane yields, and the methane yield for Reactor B increased to equal the methane yield of Reactor A.

### **5) Volatile Fatty Acids, P/A Ratio and pH**

The VFA concentrations in the form of acetic, propionic, butyric, and iso-butyric acids were measured and are shown for both reactors in Figures A.12 and A.13. An increase in VFA concentration occurs when the reactor is under stress and cannot convert the fatty acids as soon as they are made. In particular, an acetic acid concentration of higher than 800 mg/l may be an indication that the treatment process in the reactor is under some stress. However, high acetic acid levels can be expected when the organic loading rate increases due to an increase in COD



concentration or decrease in the HRT. The VFA concentrations will decrease as the microbial population in the sludge blanket adjusts to the increase in organic loading rate. The presence of iso-forms, such as iso-butyric acid also indicate reactor stress.

For the first 10 days of operation, Reactor A had acetic acid levels higher than 800 mg/l which gradually decreased to 67 mg/l on day 28 as the sludge in the reactor had adjusted to the operating conditions. Similarly, Reactor B had acetic acid levels over 800 mg/l until day 18 which gradually decreased to 39 mg/l on day 28.

A propionic-acetic acid ratio of more than 1.4 is another indicator of reactor stress. Figure A.14 indicates that for both reactors, the P/A ratio was always under 1.4 for the first 28 days of operation.

The pH of the effluent for both reactors was around 7 as seen in Figure A.15. The elevated acetic acid levels did not result in a drop of the effluent pH due to the buffer which was added to the influent to prevent drops in pH prior to feeding to the reactors.

## **B. Three Day Hydraulic Retention Time**

The HRT was reduced from 5 days to 3 days for both reactors on day 29; this HRT was maintained until day 101. The average COD loading rate at this time was 0.75 g COD/l/d and the liquid upflow velocity was 0.8 m/d. This liquid upflow velocity is within the recommended range of 0.72 - 0.96 m/d ( Campos *et al.*, 1992). As mentioned in the literature review, increasing the COD loading rate by decreasing the hydraulic retention time acts as a selection pressure in which the lighter sludge is washed out of the reactor and the heavier, better settling sludge is retained in the reactor. Periodic increases or decreases in the organic loading rate can result however, since the COD concentration is quite variable and a change in the influent COD

concentration will result in a change in the organic loading rate if the hydraulic retention time is kept constant. The results for the 3 day HRT are summarized in Table 4.2 (all values are based on averages).

**Table 4.2 Summary of the 3 day HRT Results**

<b><u>Influent Characteristics:</u></b>		
<b>COD (mg/l)</b>		2248 ± 965
<b>BOD (mg/l)</b>		1718 ± 624
<b>VS (mg/l)</b>		1319 ± 771
<b>VSS (mg/l)</b>		168 ± 114
		<b><u>Reactor A</u></b>
		<b><u>Reactor B</u></b>
<b>COD loading rate (g COD /l /d)</b>		0.75 ± 0.32
<b>Sludge loading rate (gCOD/gVSS/d)</b>		0.38 ± 0.16
<b><u>Effluent Characteristics:</u></b>		
<b>COD (mg/l)</b>		648 ± 367
<b>BOD (mg/l)</b>		430 ± 362
<b>VS (mg/l)</b>		297 ± 309
<b>VSS (mg/l)</b>		921 ± 345
<b>pH</b>		63 ± 47
<b>VFA (mg/l) acetic</b>		7.4 ± 0.5
<b>propionic</b>		7.5 ± 0.5
<b>butyric</b>		374 ± 339
<b>iso-butyric</b>		515 ± 570
<b>COD reduction (%)</b>		222 ± 161
<b>BOD reduction (%)</b>		0 ± 0
<b>VS reduction (%)</b>		0 ± 0
<b>VSS reduction (%)</b>		0 ± 0
<b>Biogas methane composition (% v/v)</b>		71 ± 15
<b>Methane production (l CH<sub>4</sub>/l/d)</b>		80 ± 21
<b>Methane yield (l CH<sub>4</sub>/g COD dest./d)</b>		19 ± 43
		46 ± 48
		29 ± 73
		48 ± 8
		56 ± 8
		0.19 ± 0.12
		0.21 ± 0.14
		0.31 ± 0.03
		0.33 ± 0.04

### 1) Effluent COD and BOD

Figures B.1 and B.2 depict the effluent COD and COD reduction, respectively, for both reactors. The COD removal rates increased drastically over this HRT operating period, as the

sludge in both reactors increased in methanogenic activity and had adjusted to the new operating conditions (HRT and organic loading rate). As can be seen by Figure B.1, there were fluctuations in the influent COD. The COD concentration of the brewery effluent varied considerably which made adjusting the influent COD to the desired concentration of 1000 mg/l difficult. Despite these fluctuations, both reactors consistently performed well in terms of COD removal. The BOD results for both reactors confirm the COD results and are depicted in Figures B.3 and B.4.

## **2) Volatile Solids and Volatile Suspended Solids**

The transition from a 5 day to a 3 day HRT and the corresponding increase in liquid velocity and gas production resulted in immediate increases in effluent VS and a corresponding negative reduction in VS in both reactors, as seen in Figures B.5 and B.6. Some of the poorer quality, lighter sludge was washed out of the reactors, but this sludge washout only appears to have occurred for the first few days of the new operating regime (until day 33 of operation). After this time, the VS levels for both reactors tended to decrease, with a sharp increase around day 61 for both reactors. Before samples were taken on day 61, it was discovered that air was accidentally pumped in overnight, resulting in excessive sludge washout. Although the VS levels tended to decrease for both reactors over this time period, the reductions fluctuate considerably. These fluctuations reflect the large fluctuations in influent VS concentrations.

Figures B.7 and B.8 depict the effluent VSS and VSS reductions for both reactors. These results are similar to the VS results for both reactors. For the first few days operating at the new HRT (until day 33), there was some sludge loss in Reactor B as seen in the large increase in

effluent VSS. There was no increase in effluent VSS for Reactor A, indicating that there was little, if any, sludge loss for this reactor. This can be explained by the fact that Reactor A was seeded with much less seed sludge than Reactor B, and so the sludge bed did not expand upward into the settling zone and wash out of the reactor, as occurred with Reactor B. By day 102, when the HRT was reduced to 1.5 days, distinct zones in the sludge bed had appeared in Reactor B, with the bottom of the reactor appearing darker and more concentrated, and becoming less concentrated further up the reactor, until nearly clear near the three phase separator at the top of the reactor. In contrast, the sludge bed appeared uniform in Reactor A, with the sludge bed occupying a distinct region in the bottom of the reactor, and a clear zone immediately above this region. It appears that shortening the HRT, and therefore increasing the liquid upflow velocity, was effective in acting as a selection pressure in removing the poorer quality sludge and causing the better settling sludge to settle at the bottom of the reactor for Reactor B. However, this was not the case for Reactor A, as there had been little, if any, sludge washout in this reactor thus far, and consequently even the light, poorer quality sludge was retained in Reactor A.

At this time, the concentration of the sludge in each reactor was measured to calculate a new sludge loading rate. It was necessary to re-calculate the sludge loading rate for each reactor since the concentration and amount of the sludge in each reactor had changed considerably since the beginning of the operation. The new sludge loading rate for Reactor A was based on 1.9 litres of sludge at a concentration of 8.3 g/l VSS, or a total of 15.77 g VSS (0.99 g VSS/l reactor). The sludge loading rate for Reactor B was based on 5.2 litres of sludge at an average concentration of 19.2 g/l VSS, or a total of 99.84 g VSS (6.24 g VSS/l reactor). Due to the small amount of seed sludge used, it was not possible to sample the seed sludge before this time since sampling results in some sludge loss.

### **3) Biogas Methane Composition**

The biogas methane composition for both reactors is depicted in Figure B.9. The methane composition fluctuated considerably for both reactors, reflecting the fluctuations in the COD removal rate. Overall, there was an increase for both reactors over the 3 day HRT operating period, as there was in the COD reduction rate for both reactors.

There was a sharp decline in methane composition for both reactors on day 61 until day 69. The VS results indicate that there was a sharp increase in effluent VS for both reactors around this time. Prior to sampling on day 61, it was discovered that air was accidentally pumped into both reactors overnight when the tubes pumping to the reactors had slipped above the liquid level in the influent tank. The COD results indicate that COD removal was not affected in either reactor, so it is assumed that the methane composition dropped so drastically because the sample taken contained an unusually high concentration of air and not because the reactors were unstable. Even though reactor stability does not seem to have been compromised by this intake of air, it resulted in some sludge loss in both reactors.

### **4) Volumetric Methane Production and Methane Yield**

The volumetric methane composition for both reactors is shown in Figure B.10. Due to difficulties with the gasmeters for both reactors, no gas production readings were available from day 54 to day 69, and no data for volumetric methane production or methane yield is available for this time period.

The volumetric methane production for both reactors increased considerably at the 3 day HRT compared to the 5 day HRT as the organic loading rate had increased, and more COD was

converted to methane. As well, the methanogenic activity of the sludge improved with time as the sludge continued to adapt to the influent and the reactor environment. The large increase in methane production from day 97 to 100 can be explained by the large increase in the COD loading rate due to a sudden increase in influent COD concentration.

Figure B.11 depicts the methane yield-per g COD destroyed for both reactors. Both reactors experienced only positive methane yields, indicating that there was no increase in effluent COD during this time period. The methane yield increased for both reactors due to the increase in COD reduction rates and volumetric methane production rates for both reactors.

### **5) Volatile Fatty Acids, P/A Ratio and pH**

Figures B.12 and B.13 depict the VFA concentrations for Reactor A and Reactor B, respectively. Both reactors experienced periodic increases in the acetic acid levels above 800 mg/l during the 3 day HRT operating period. As the COD and gas results at the time of these acetic acid increases indicate that the reactors were operating well, it is reasonable to conclude that these increases in acetic acid concentrations were a response to the change in organic loading rates and not the result of an instability in the reactor itself.

Another indicator of reactor stress is a propionic to acetic acid ratio of greater than 1.4. The P/A ratios of both reactors is shown in Figure B.14. The P/A ratio in Reactor A was mostly above 1.4 until day 50. The P/A ratio in Reactor B only rises above 1.4 once in this time period, on day 40. This ratio seems to be a more reliable indicator of reactor stress when looking at the COD and BOD reductions at these time periods which show a reduction in the COD removal rate at these particular times.

Figure B.15 shows the effluent pH of both reactors. The pH of both reactors was close to

pH 7. Buffer was added to the feed influent, so high acetic acid levels did not result in a drop in the effluent pH. The effluent pH readings was not a reliable indicator of reactor stress due to the addition of this buffer to the influent.

### **C. 1.5 Day Hydraulic Retention Time**

The HRT was reduced from 3 days to 1.5 days on day 102 until day 132. The COD loading rate was increased to an average of 1.68 g COD/l/d and the surface upflow velocity increased to 1.6 m/d. The superior performance of Reactor B was evident at this HRT. Reactor A could not handle the increase in the organic loading rate and this resulted in decreases in the COD reductions for Reactor A. Table 4.3 gives a summary of the 1.5 day HRT results (all values are based on averages).

**Table 4.3 : Summary of the 1.5 day HRT Results**

Influent Characteristics:			
COD (mg/l)		2513 ± 1362	
BOD (mg/l)		1889 ± 566	
VS (mg/l)		1225 ± 745	
VSS (mg/l)		94 ± 61	
		<u>Reactor A</u>	<u>Reactor B</u>
COD loading rate (g COD /l /d)		1.68 ± 0.49	1.68 ± 0.49
Sludge loading rate (gCOD/gVSS/d)		1.70 ± 0.50	0.27 ± 0.08
<u>Effluent Characteristics:</u>			
COD (mg/l)		1733 ± 1065	559 ± 424
BOD (mg/l)		499 ± 296	221 ± 78
VS (mg/l)		975 ± 522	825 ± 489
VSS (mg/l)		41 ± 33	80 ± 129
pH		6.4 ± 0.5	7.1 ± 0.3
VFA (mg/l)	acetic	312 ± 152	176 ± 138
	propionic	176 ± 138	64 ± 19
	butyric	10 ± 16	3 ± 10
	iso-butyric	10 ± 17	0 ± 0
COD reduction (%)		33 ± 22	79 ± 12
BOD reduction (%)		72 ± 17	86 ± 8
VS reduction (%)		12 ± 45	17 ± 66
VSS reduction (%)		46 ± 48	17 ± 130
Biogas methane composition (% , v/v)		58 ± 6	66 ± 3
Methane production (l CH <sub>4</sub> /l/d)		0.23 ± 0.14	0.51 ± 0.24
Methane yield (l CH <sub>4</sub> /g COD dest./d)		0.26 ± 0.22	0.32 ± 0.02

### 1) Effluent COD and BOD

The effluent COD and COD reductions for both reactors are depicted in Figures C.1 and C.2, respectively. The superior treatment efficiency of Reactor B over Reactor A in terms of COD removal became apparent during this operating period. Reactor B adjusted more quickly to the new operating regime and corresponding increase in the COD loading rate than Reactor A. The COD removal rate for Reactor B was often more than twice that of Reactor A during this time period. The advantage of the additional seed sludge was obvious at this point. One reason



for this advantage was that Reactor A with less sludge and therefore a higher specific sludge loading rate than Reactor B, was becoming overloaded at this increased COD loading rate, and could only remove a small portion of the influent COD. Reactor B, with more seed sludge and therefore more biomass, was able to remove a larger portion of the influent COD. The BOD results for both reactors shown in Figures C.3 and C.4 support these COD results.

## **2) Volatile Solids and Volatile Suspended Solids**

Figures C.5 and C.6 depict the VS results for both reactors. The VS levels for both reactors fluctuated considerably. Although the effluent VS for both reactors tended to decrease over this time period, there were periodic increases, which appeared to be a result of increases in the influent VS. The effluent VS decreased for Reactor B as the sludge had become more stable and had improved settling characteristics and did not wash out despite the increase in gas production and liquid upflow velocity.

The VSS results in Figures C.7 and C.8 depict a trend of decreasing VSS over this time period, which supports the VS results.

## **3) Biogas Methane Composition**

The biogas methane composition is depicted in Figure C.9. The gas composition fluctuated for both reactors. The biogas methane composition actually decreased slightly for both reactors over this time period, as both reactors experienced periods of low COD reductions due to the sharp increases in sludge loading rates that occurred at this HRT.

#### **4) Volumetric Methane Production and Methane Yield**

In terms of volumetric methane production, Reactor B was superior to Reactor A, as shown in Figure C.10. Difficulties with the gas meters for both reactors made gas readings between day 112 and day 124 impossible.

As mentioned earlier, the methane production is closely related to the COD loading rate, and as the COD loading rate increases, gas production increases as the COD is converted to methane, provided that the digestion process is not under any stress. The fluctuations in volumetric methane production during this time period reflected the fluctuations in influent COD concentrations and therefore, COD loading rate. Despite fluctuations in volumetric methane production, Reactor B consistently produced more methane than Reactor A. This can be explained by the fact that Reactor B had more sludge than Reactor A, and was therefore able to convert more of the influent COD into methane. The higher COD removal rates of Reactor B over Reactor A confirm these results.

The methane yield of both reactor was fairly consistent over this time period, as seen in Figure C.11.

#### **5) Volatile Fatty Acids, P/A Ratio and pH**

The VFA concentrations for Reactor A is depicted in Figure C.12. The acetic acid concentration under 800 mg/l suggests that the reactor was not under stress, and was operating well. However, the appearance of iso-forms such as iso-butyric acid indicate that Reactor A may have experienced some stress. The COD loading rate was very high at this time and it appears that Reactor A was being overloaded and the treatment process was starting to break down, as there were reductions in the COD removal rate. However, Reactor A seemed to recover from

this stress when the organic rate was lowered, as seen by the disappearance of the iso-forms and increase in COD removal rates.

The VFA concentrations of Reactor B were considerably lower than Reactor A, as seen in Figure C.13. These low concentrations of VFA, together with the absence of iso-butyric acid suggests that the digestion process was not under any stress for Reactor B. It appears from these VFA results that Reactor B was able to adjust to the increased COD loading rate better than Reactor A.

The P/A ratio was less than 1.4 for both reactors as shown in Figure C.14.

Figure C.15 depicts the effluent pH of both reactors. The effluent pH of Reactor A dropped below pH 6, apparently due to the elevated VFA levels, however, as the acetic acid concentration lowered, the pH again increased to above pH 6.

#### **D. 18 Hour Hydraulic Retention Time**

From day 133 to day 162, the reactors were operating at a HRT of 18 hours. The liquid upflow velocity increased to 3.2 m/d, but the average COD loading rate actually decreased to 1.53 g COD/l/d. This decrease in COD loading rate was a result of the large drop in influent COD concentration to below 1000 mg/l from day 155 to 162. These frequent and large fluctuations in the COD of the brewery effluent makes it difficult to control the operating conditions of the reactors such as COD loading rate. The results for the 18 hour HRT period are summarized in Table 4.4 (all values are based on averages).

**Table 4.4 : Summary of the 18 hour HRT Results**

Influent Characteristics:			
COD (mg/l)		1147 ± 374	
BOD (mg/l)		1876 ± 567	
VS (mg/l)		638 ± 194	
VSS (mg/l)		161 ± 91	
		<u>Reactor A</u>	<u>Reactor B</u>
COD loading rate (g COD /l /d)		1.53 ± 0.50	1.53 ± 0.50
Sludge loading rate (gCOD/gVSS/d)		1.55 ± 0.51	0.25 ± 0.08
<u>Effluent Characteristics:</u>			
COD (mg/l)		550 ± 211	141 ± 179
BOD (mg/l)		63 ± 59	41 ± 51
VS (mg/l)		556 ± 297	417 ± 212
VSS (mg/l)		74 ± 30	58 ± 62
pH		6.1 ± 0.6	6.8 ± 0.2
VFA (mg/l)	acetic	110 ± 34	50 ± 8
	propionic	50 ± 14	44 ± 6
	butyric	3 ± 9	0 ± 0
	iso-butyric	0 ± 0	0 ± 0
COD reduction (%)		52 ± 13	89 ± 10
BOD reduction (%)		97 ± 3	98 ± 2
VS reduction (%)		8 ± 61	30 ± 40
VSS reduction (%)		47 ± 30	57 ± 55
Biogas methane composition (% v/v)		58 ± 4	67 ± 2
Methane production (l CH <sub>4</sub> /l/d)		0.24 ± 0.11	0.43 ± 0.12
Methane yield (l CH <sub>4</sub> /g COD dest./d)		0.30 ± 0.03	0.32 ± 0.03

### 1) Effluent COD and BOD

Figures D.1 and D.2 depict the COD results for both reactors. The influent COD dropped below 1000 mg/l on day 155 until day 162. A COD concentration above 1000 mg/l is recommended as the minimum influent COD, concentrations below this level lead to underloading and low gas production. Despite these fluctuations in influent COD, Reactor B was able to achieve COD removal rates above 80% for most of this time period, while Reactor A had much lower COD removal rates, as well as large fluctuations in COD reductions. These

reductions in the COD removal rate for Reactor A indicate that the reactor was not able to operate well at the higher organic loading rates and could not break down much of the influent COD. The BOD results shown in Figures D.3 and D.4 confirm these COD results.

## **2) Volatile Solids and Volatile Suspended Solids**

The VS results for both reactors are shown in Figures D.5 and D.6. The influent VS and the effluent VS levels for both reactors fluctuated considerably. There was a trend of decreasing VS levels for both reactors, with periodic increases, which reflected increases in influent VS.

The VSS results are depicted in Figures D.7 and D.8. These results also show a lowering in the effluent VSS levels. The effluent VS and VSS of Reactor B was lower than Reactor A at this HRT since Reactor B had denser and better settling sludge than Reactor A and was able to settle well despite the increase in liquid upflow velocity and gas production. The sludge in Reactor A was less dense, resulting in some sludge washout at this higher liquid upflow velocity.

## **3) Biogas Methane Composition**

The biogas methane composition for both reactors is depicted in Figure D.9. The biogas methane composition for both reactors increased slightly over the 18 hour HRT operating period. The biogas methane content was fairly consistent, indicating reactor stability. This stability was reflected in the relatively consistent COD reductions for Reactor B. However, Reactor A had fluctuations in COD reductions, but not in methane content.

## **4) Volumetric Methane Production**

The volumetric methane production for both reactors is shown in Figure D.10. As mentioned earlier, the amount of methane produced depends largely on the COD loading rate; a

higher COD loading rate leads to a higher methane production. The COD loading rate was inadvertently lowered from day 155 to day 162, resulting in a reduction of volumetric methane production over this time period. Although the biogas methane composition was not significantly higher for Reactor B than Reactor A, the volume of methane produced was significantly higher. Reactor B, with more biomass in its sludge bed, produced more methane since it was able to convert more of the influent COD to methane than Reactor A. This was confirmed by the much lower effluent COD concentrations of Reactor B compared to Reactor A.

Figure D.11 depicts the methane yield per COD destroyed for both reactors. There was an overall increase in methane yield for both reactors over this time period, as the methanogenic activity of the sludge continued to increase with time.

#### **5) Volatile Fatty Acids, P/A Ratio, and pH**

The VFA concentrations for both reactors are shown in Figures D.12 and D.13. The low VFA concentrations for both reactors indicate that the digestion process was not under stress, and reflect the relative stability of both reactors.

As can be seen in Figure D.14, the P/A ratio for both reactors remained well below 1.4 from day 133 to 162. These results agree with the VFA concentration results in that the treatment process was not under stress.

The effluent pH of both reactors is shown in Figure D.15.

### **E. 12 Hour Hydraulic Retention Time**

The reactors were operated at a final HRT of 12 hours, from day 162 to 189. The COD loading rate increased to 2.91 g COD/l/d and the liquid upflow velocity increased to 5.4 m/d.

The results for the 12 hour HRT time period are summarized in Table E.1 (all values are based on averages).

**Table 4.5 : Summary of the 12 hour HRT Results**

Influent Characteristics:			
COD (mg/l)		1455 ± 477	
BOD (mg/l)		1411 ± 608	
VS (mg/l)		789 ± 379	
VSS (mg/l)		174 ± 89	
		<u>Reactor A</u>	<u>Reactor B</u>
COD loading rate (g COD /l /d)		2.91 ± 0.95	2.91 ± 0.95
Sludge loading rate (gCOD/gVSS/d)		2.95 ± 0.96	0.47 ± 0.15
<u>Effluent Characteristics:</u>			
COD (mg/l)		774 ± 246	305 ± 261
BOD (mg/l)		171 ± 100	30 ± 10
VS (mg/l)		610 ± 226	562 ± 223
VSS (mg/l)		70 ± 20	53 ± 25
pH		6.3 ± 0.6	6.9 ± 0.5
VFA (mg/l)	acetic	1634 ± 736	204 ± 313
	propionic	45 ± 6	244 ± 379
	butyric	15 ± 18	0 ± 0
	iso-butyric	16 ± 19	0 ± 0
COD reduction (%)		45 ± 12	80 ± 12
BOD reduction (%)		87 ± 8	96 ± 1
VS reduction (%)		9 ± 44	17 ± 40
VSS reduction (%)		50 ± 25	67 ± 17
Biogas methane composition (% , v/v)		49 ± 15	59 ± 10
Methane production (l CH <sub>4</sub> /l/d)		0.45 ± 0.24	0.79 ± 0.26
Methane yield (l CH <sub>4</sub> /g COD dest./d)		0.32 ± 0.03	0.34 ± 0.02

### 1) Effluent COD and BOD

Figures E.1 and E.2 depict the COD results for both reactors. The treatment efficiency of Reactor B was significantly higher than Reactor A in terms of COD removal. It appeared that Reactor A could not adjust to the increase in COD loading rate and reduced HRT, as seen by its very low COD removal rates. Reactor B adjusted to the increase in COD loading rate and

reduced HRT without a decline in treatment efficiency. Figures E.3 and E.4 show the BOD results for both reactors.

## **2) Volatile Solids and Volatile Suspended Solids**

The VS results for both reactors are depicted in Figures E.5 and E.6. The VS reductions fluctuated for both reactors. As can be seen in Figure E.5, the influent VS levels fluctuated considerably, from 225 mg/l to 1550 mg/l, which may explain the fluctuations in effluent VS. Overall, the effluent VS levels tended to decrease for both reactors, with periodic increases for both reactors. Reactor B had larger reductions in effluent VS than Reactor A, since it had a denser, better settling sludge.

The VSS results shown in Figures E.7 and E.8 tend to support the VS results. The VSS levels in both reactors decreased, although these reductions vary. These VSS results confirm the VS results that there was little sludge washout in either reactor. Reactor B had higher VSS reductions at this HRT than Reactor A, which reflects the better settling properties of its sludge in the sludge bed.

## **3) Biogas Methane Composition**

The biogas methane composition for both reactors is depicted in Figure E.9. The methane composition remained fairly stable until day 178, when it decreased to 38% for Reactor A and 44% for Reactor B. The COD reductions for both reactors decreased significantly at this time, in response to a large increase in the organic loading rate. The methane composition remained low for Reactor A, but Reactor B recovered quickly and the methane content of its biogas rose to 57% on day 183. The biogas methane results indicate that Reactor B was more



stable and able to recover from upsets such as an increase in organic loading rate faster than Reactor A.

#### **4) Volumetric Methane Production and Methane Yield**

The volumetric methane production for both reactors is shown in Figure E.10. The methane production declined for both reactors over this 12 hour HRT operating period. This decline in methane production was a result in the overall reduction in the organic loading rate from the beginning to the end of this HRT operating period. Reactor B, in addition to having a higher methane content in its biogas, produced significantly more methane than Reactor A. At the higher organic loading rates, both reactors experienced higher volumetric methane production rates. Both reactors reached a maximum methane production rate on day 176, at 0.98 l CH<sub>4</sub>/l/d for Reactor A, and 1.33 l CH<sub>4</sub>/l/d for Reactor B, when the COD loading rate was at a maximum of 4.27 g COD/l/d.

The methane yield per COD destroyed is depicted in Figure E.11. The methane yield for both reactors did not fluctuate to any great extent, and was at its highest at this HRT, due to the high methane production rates and COD reductions.

#### **5) Volatile Fatty Acids, P/A Ratio and pH**

The VFA concentrations for Reactor A are shown in Figure E.12. The acetic acid concentration quickly rose from 69 mg/l on day 163 to 2857 mg/l on day 187. These extremely high acetic acid levels were in response to the decrease in HRT and corresponding increase in organic loading rate. However, the acetic acid levels remained high, indicating that the reactor may have been under stress. The appearance of iso-butyric acid on days 179 and 189 was another indication that the reactor may have experienced stress.

The VFA concentrations for Reactor B are shown in Figure E.13. In contrast to Reactor A, the acetic acid levels remained quite low, and ranged from 42 mg/l to 46 mg/l until day 181, but increased to 1007 mg/l on day 189. The acetic acid concentrations indicate that the reactor was able to adjust to the increases in organic loading rate at this HRT.

The P/A Ratio of both reactors is depicted in Figure E.14. The P/A ratio for Reactor A remained well under 1.4. The P/A ratio of Reactor B rose above 1.4 on day 184.

The effluent pH is depicted in Figure E.15.

## **F. Summary of Results**

In this section, the impact of the hydraulic retention time on reactor performance is examined in order to give a clearer picture of the reactors' performance over the entire operating time span. The impact of the COD loading rate and the sludge loading rate on reactor performance is also discussed in this section.

### **1) Hydraulic Loading Rate and Reactor Performance**

In this experiment, the hydraulic retention time was decreased in a stepwise fashion in order to increase the organic loading rate. As mentioned earlier, this method of increasing the organic loading rate by decreasing the hydraulic retention time instead of increasing the COD concentration allows for higher liquid upflow velocities. The higher liquid upflow velocities act as a selection pressure in which the lighter, poorer settling sludge is gradually washed out of the reactor, while the heavier, better settling is retained in the reactor. However, if the liquid upflow velocity is too high, it might lead to excessive washout, especially if the sludge in the reactor has poor settling characteristics. Campos *et al.*, (1992) recommend that during the start-up, the liquid

upflow velocity be kept between 0.72 to 0.96 m/d. The liquid upflow velocity versus the hydraulic retention time is depicted in Figure F.1. From the 1.5 day HRT to the 12 hour HRT, the liquid upflow velocity is well above the range recommended by Campos *et al.*, (1992). The high liquid upflow velocities at the shorter HRTs resulted in some sludge washout in Reactor A since its sludge had poor settling properties. The sludge in Reactor B did not wash out since it had denser sludge which was able to settle well despite the high liquid upflow velocities and increase in gas production.

Due to fluctuations in the influent COD concentration, it was difficult to keep a constant organic loading rate at each hydraulic retention time. The average organic loading rate for each HRT is shown in Figure F.2. The organic loading rate increased as the HRT shortened, except from the 1.5 day HRT to the 18 hour HRT, when the average organic loading rate actually decreased slightly. This was due to a drop in the influent COD concentration at this time.

Since each reactor was seeded with different amounts of seed sludge, the sludge loading rate was different for each reactor, even though the organic loading rate was the same. Reactor B was seeded with 5.93 g VSS/l, while Reactor A was seeded with 1.98 g VSS/l, so that the sludge loading rate of Reactor A was about three times that of Reactor B. As mentioned earlier, the sludge loading rate was re-calculated after the 3 day HRT ( on day 102) due to changes in the amount and concentration of sludge in each reactor. At this point, the sludge in Reactor B had increased to 6.24 g VSS/l, and the sludge in Reactor A had decreased to 0.99 g VSS/l. As a result, the sludge loading rate for Reactor A for the 1.5 day, 18 hour and 12 hour HRTs was approximately six times that of Reactor B. The sludge loading rate versus HRT is depicted in Figure F.3.

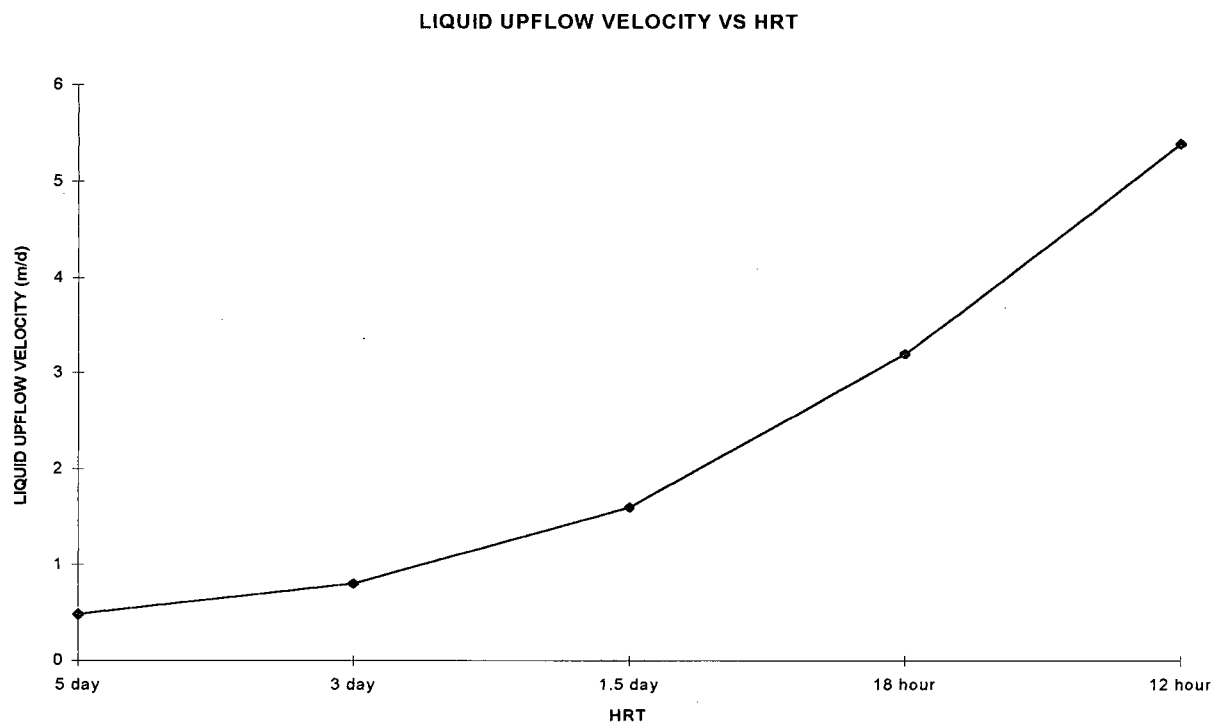


Figure F.1 Liquid Upflow Velocity versus Hydraulic Retention Time

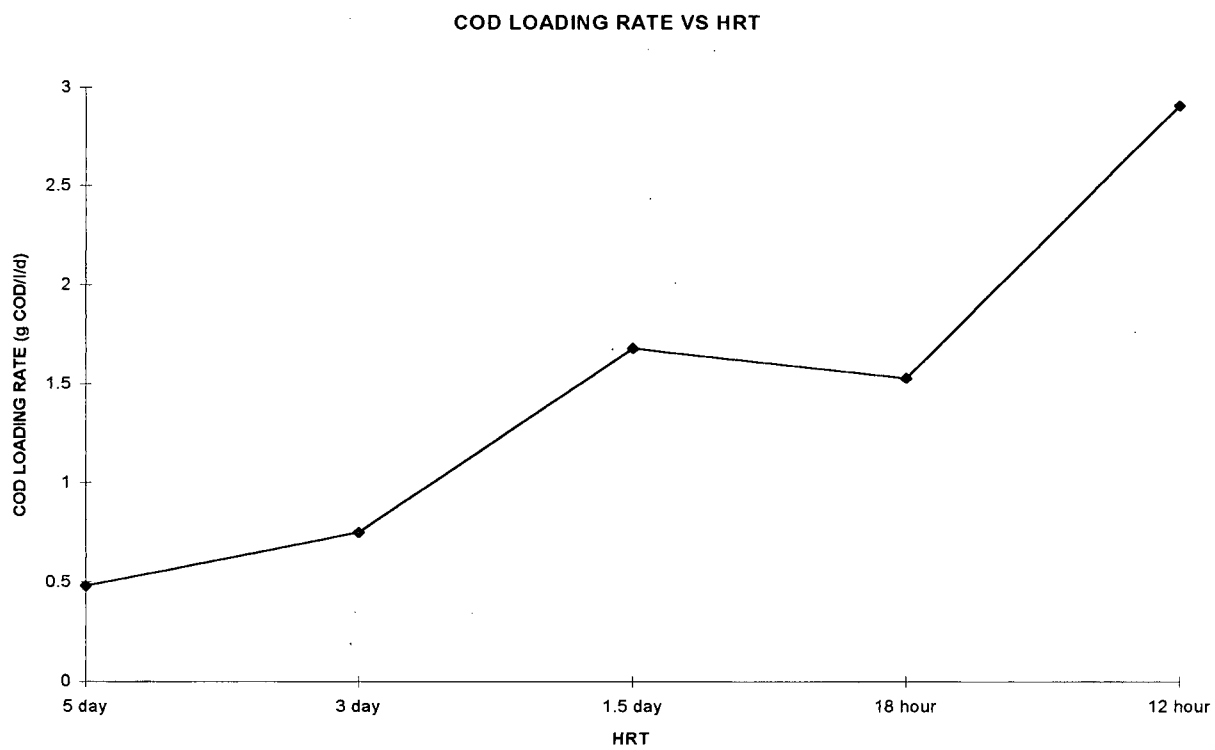


Figure F.2 COD Loading Rate versus Hydraulic Retention Time

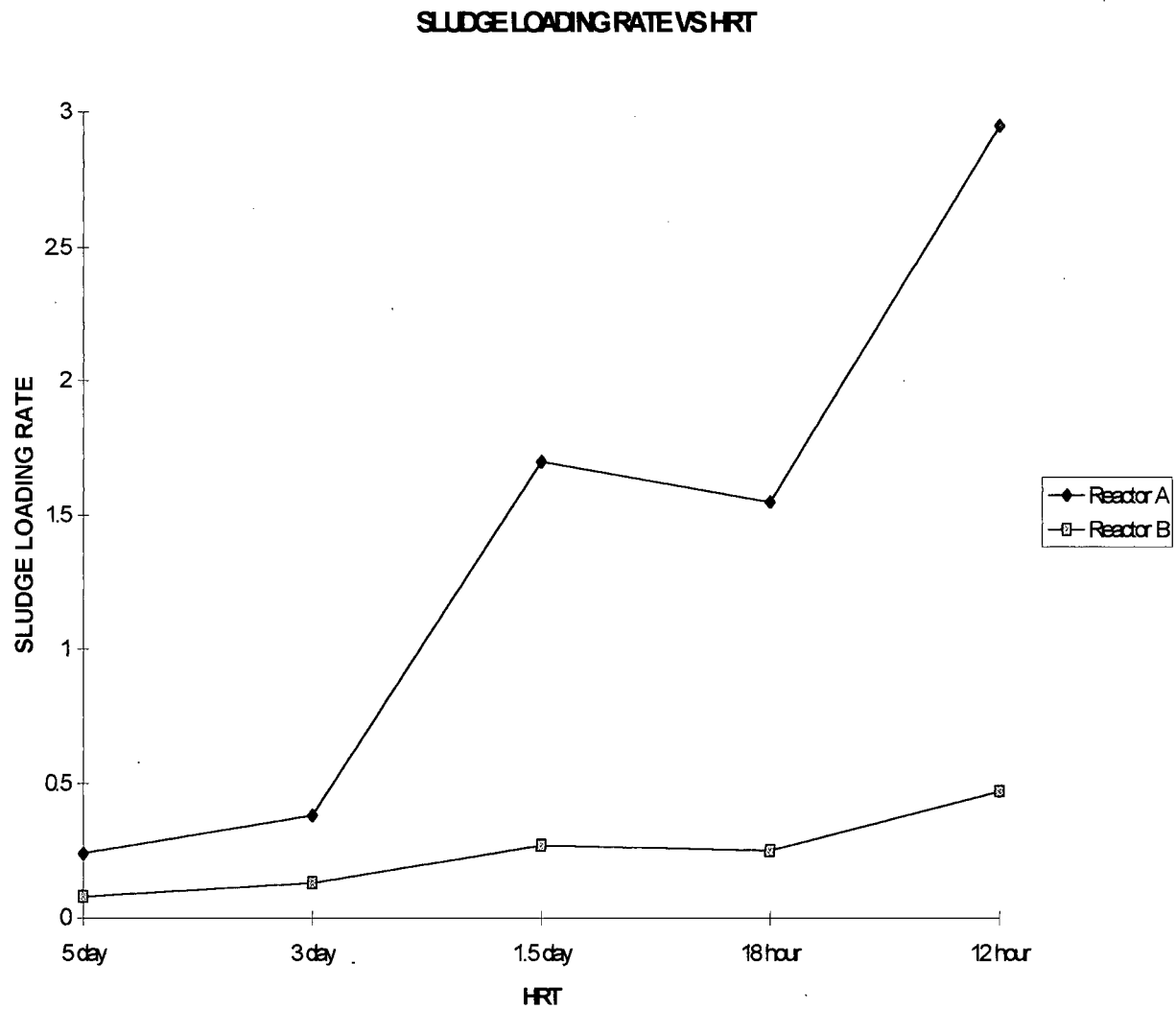


Figure F.3 Sludge Loading Rate versus Hydraulic Retention Time

Figures F.4 and F.5 depict the effluent COD and COD reduction versus HRT, respectively. The COD reduction rate for Reactor A increased from the 5 day HRT to the 3 day HRT as the sludge adapted to the reactor environment. From the 3 day HRT to the 1.5 day HRT, there was a large reduction in the COD removal rate due to the large increase in the sludge loading rate for Reactor A. The sludge loading rate for Reactor A was too high and the reactor could not break down a large portion of the influent COD. When the average sludge loading rate decreased at the 18 hour HRT, the COD reduction rate for Reactor A increased. At the 12 hour HRT, the COD reduction decreased, since the sludge loading rate at this time was extremely high, averaging 2.95 g COD/g VSS/d. The COD reduction rate was highest for Reactor A at the 3 day HRT, when the sludge loading rate was 0.38 g COD/g VSS/d, which is within the range of sludge loading rates of 0.3-0.5 g COD/g VSS/d recommended during start-up by Wu *et al.*, (1987).

There was a large increase in the COD reduction rate for Reactor B from the 5 day HRT to the 3 day HRT since three-quarters of the seed sludge in Reactor B was unacclimatized. This unacclimatized seed sludge consisted of mostly aerobic bacteria, and therefore needed time to adapt to the brewery effluent and the anaerobic conditions of the reactor environment before it contained a large enough anaerobic bacteria population to break down the influent COD. The COD reduction continued to increase for Reactor B at the shorter HRTs. Reactor B achieved high COD reductions (above 80%) even at the 18 and 12 hour HRTs, when the average sludge loading rate was between 0.25 g COD/g VSS/d and 0.47 g COD/g VSS/d.

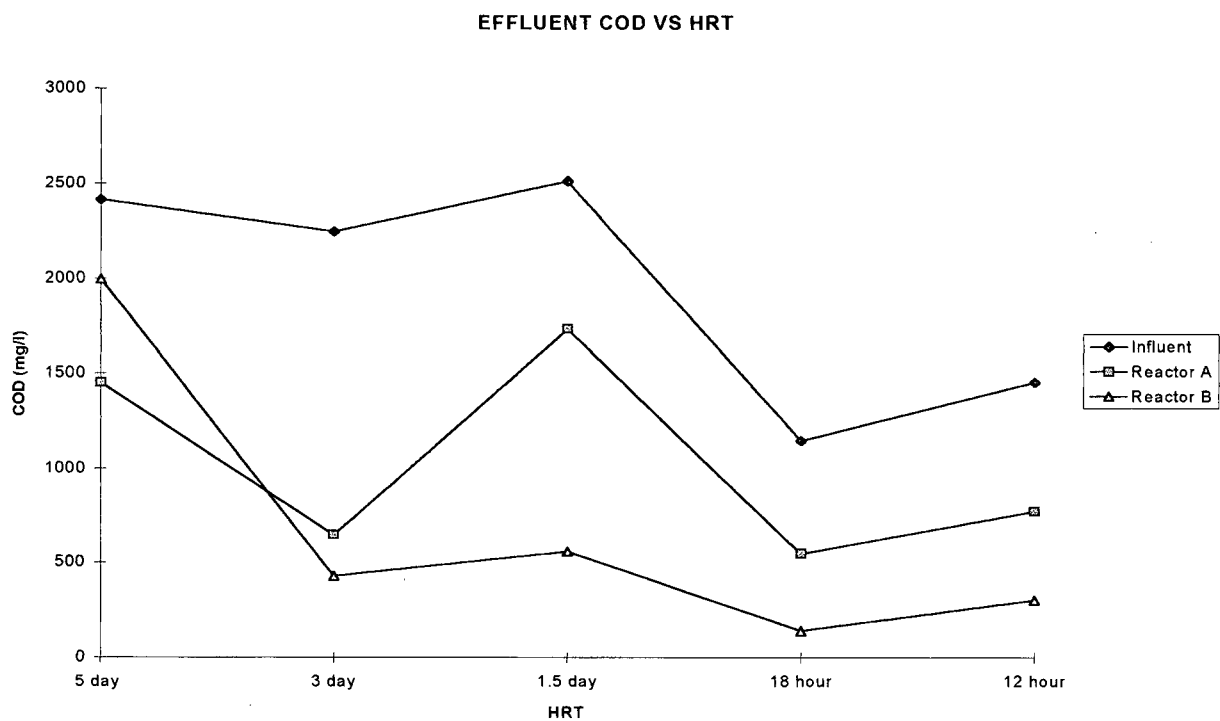


Figure F.4 Effluent COD versus Hydraulic Retention Time

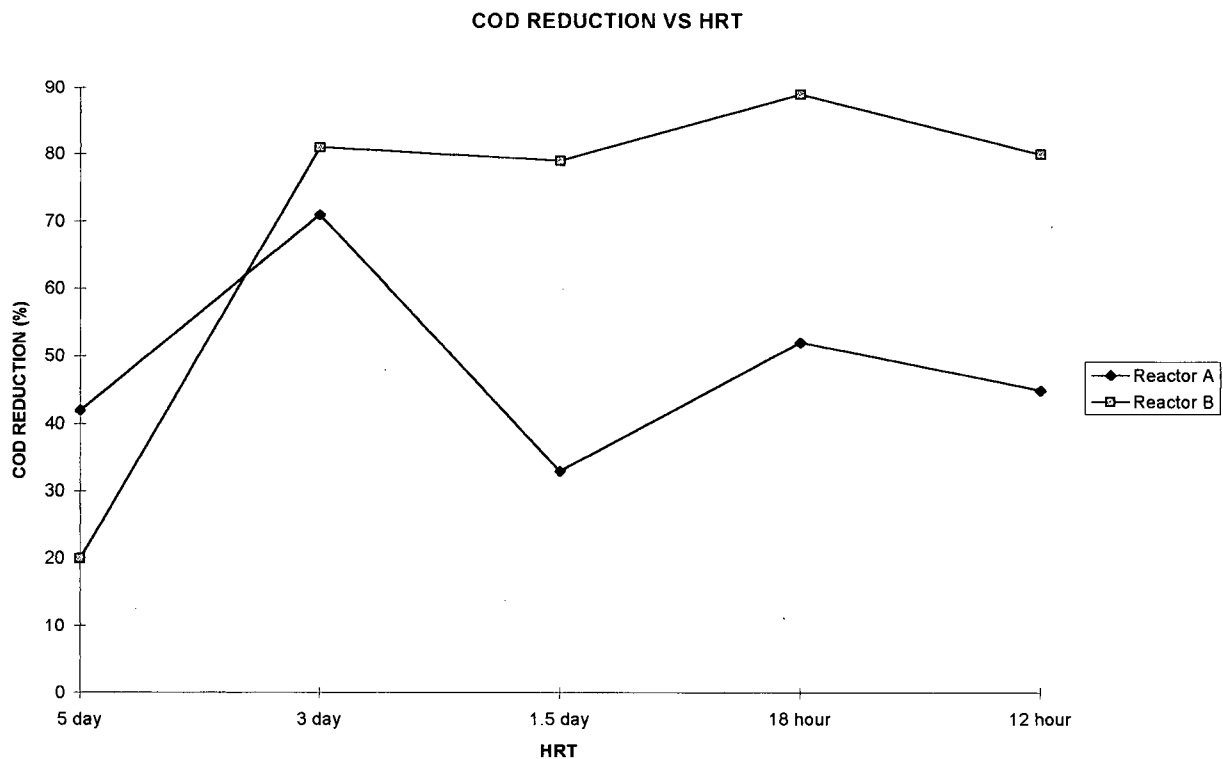


Figure F.5 COD Reduction versus Hydraulic Retention Time

The Volatile Suspended Solids results versus HRT are shown in Figures F.6 and F.7. The VSS results indicate that Reactor A experienced larger reductions in effluent VSS at the longer HRTs. Reactor A was seeded with very little seed sludge (1.98 g VSS/l) which was able to settle well at the lower liquid velocities. At the 5 day and 3 day HRT, Reactor A experienced very little sludge washout compared to Reactor B. However, as the liquid upflow velocity increased at the shorter HRTs (18 and 12 hours), the sludge in Reactor A, which had poor settling properties, was washed out of the reactor.

Reactor B experienced considerable sludge washout at the beginning of the operation, especially at the 5 day HRT. One reason for this sludge washout was that Reactor B was seeded with a large volume of seed sludge, (38% of the reactor volume) which tended to expand upward into the separator area, and wash out of the reactor. A second, and more important factor, was the lack of an acclimation period for most of the seed sludge. In Reactor B, 1.98g VSS/l of the seed sludge was acclimatized and 3.95 g VSS/l was unacclimatized. Since the seed sludge used was activated sludge, which consists mostly of aerobic bacteria, the acclimation period is important to cultivate a population of anaerobic bacteria prior to seeding into the reactor. Anaerobic bacteria is denser, and therefore has better settling properties than aerobic bacteria (Lettinga *et al.*, 1980). When the aerobic bacteria in the unacclimatized seed sludge was seeded into the anaerobic reactor environment, cell lysis and subsequent sludge washout occurred. There was also sludge washout due to the poor settling properties of the activated sludge. Toward the end of the 3 day HRT, the settling properties of the sludge had improved considerably, and there was a significant increase in VSS reductions for Reactor B. There was a drop in the VSS reductions at the 1.5 day HRT, due to the large increase in the liquid upflow velocity and the



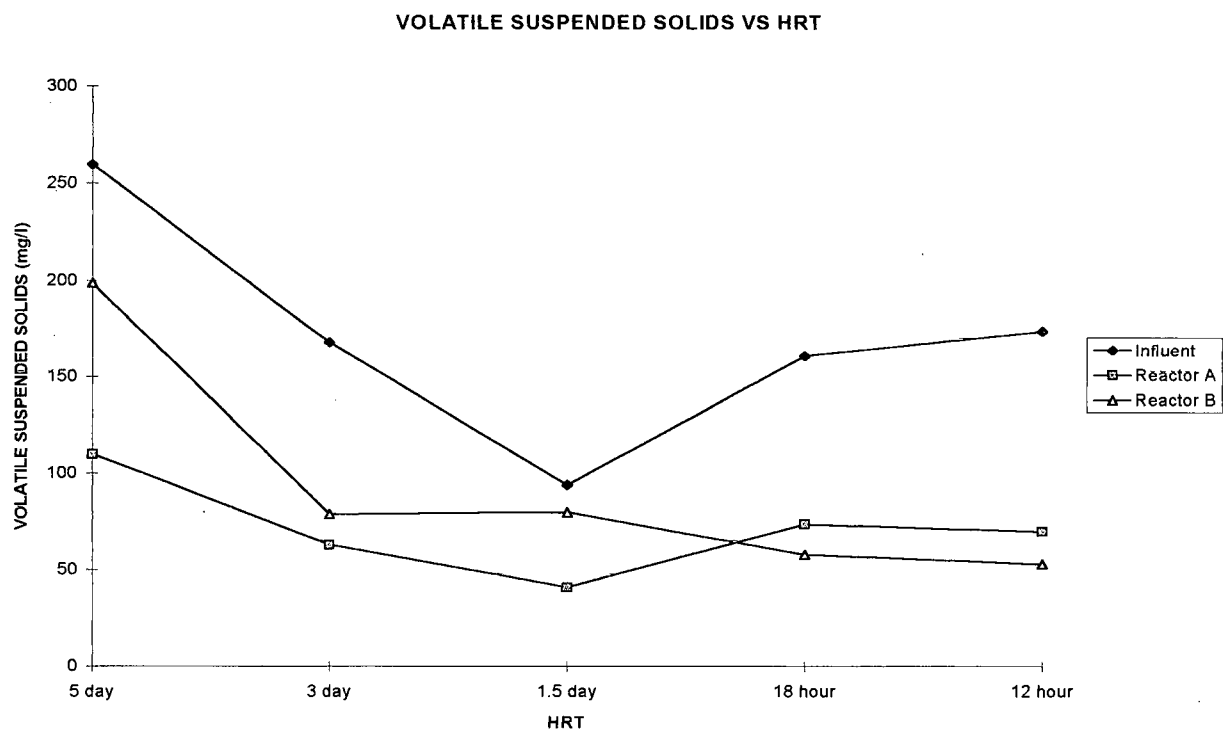


Figure F.6 Volatile Suspended Solids versus Hydraulic Retention Time

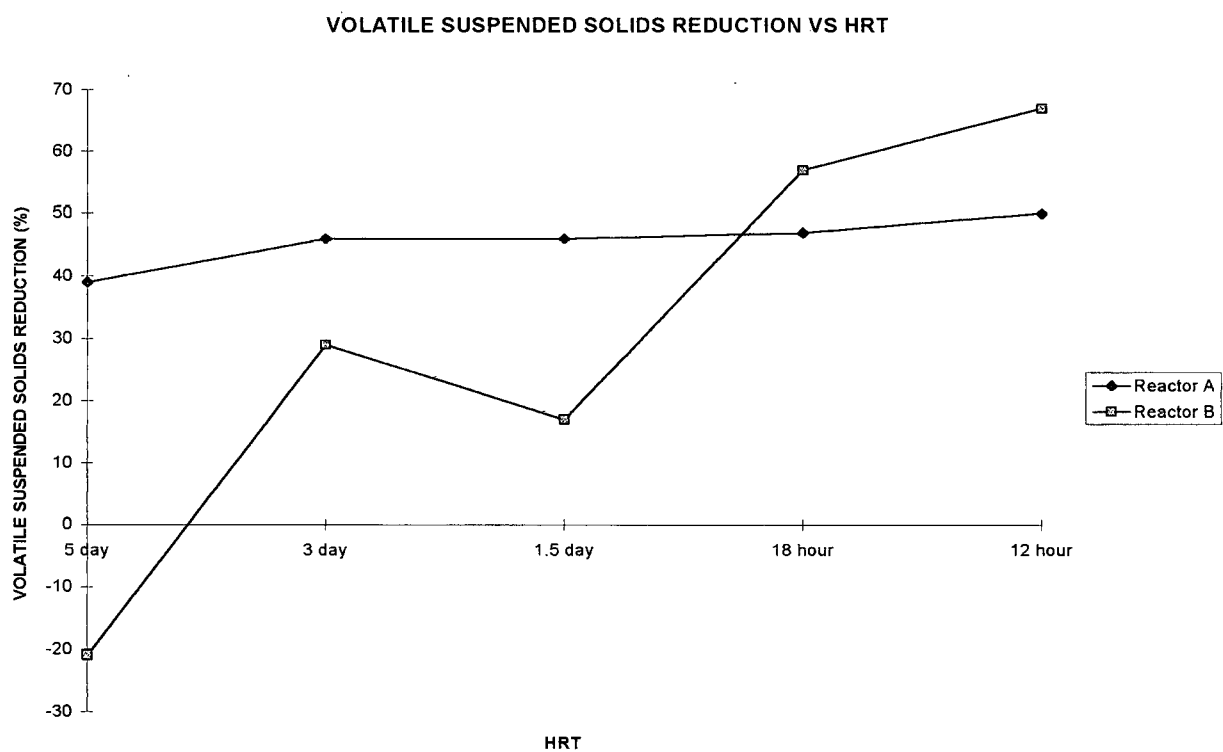


Figure F.7 Volatile Suspended Solids Reduction versus Hydraulic Retention Time

organic loading rate which resulted in increased gas production. However, as the sludge continued to improve, the VSS reductions increased at the shorter HRTs of 18 and 12 hours.

The Biogas Methane Composition versus HRT is shown in Figure F.8. The methane content of the biogas was low for both reactors at the 5 day HRT, at 36% methane for Reactor A, and 28% for Reactor B. The methanogenic activity of the sludge was quite low at the beginning of the operation, as the sludge was still adapting to the anaerobic reactor environment, and the sludge did not yet contain a very large population of active methanogenic bacteria. It is interesting to note that even though Reactor B had the same amount of acclimatized sludge as Reactor A, 1.98 g VSS/l, the methane content of its biogas was significantly lower than Reactor A. This was due to the additional 3.95 g VSS/l of unacclimatized activated sludge used as seed sludge in Reactor B, which had very low methanogenic activity compared to the acclimatized sludge, and resulted in low COD reductions for Reactor B. The methane content of the biogas for both reactors increased as the HRT was reduced. The methane content increased from 48 % at the 3 day HRT to 58% at the 1.5 and 18 hour HRT for Reactor A. Reactor B experienced similar increases of 56% at the 3 day HRT, and 66% and 67% at the 1.5 day and 18 hour HRTs, respectively. Sax (1985) reported that shorter HRTs result in a higher methane purity of produced biogas. However, at the shortest HRT of 12 hours, the average methane content of the biogas for both reactors decreased. This was due to a large decrease in the biogas methane content of both reactors from day 178 to 189, resulting from a large decrease in the COD reductions for both reactors. Around this time, the organic loading rate increased drastically, due to a large increase in the influent COD concentration and the reactors had not yet adjusted to the increase, so the COD reductions were lowered. The methane content of the biogas for Reactor B was starting to increase on day 183, and would likely have continued to increase further had the

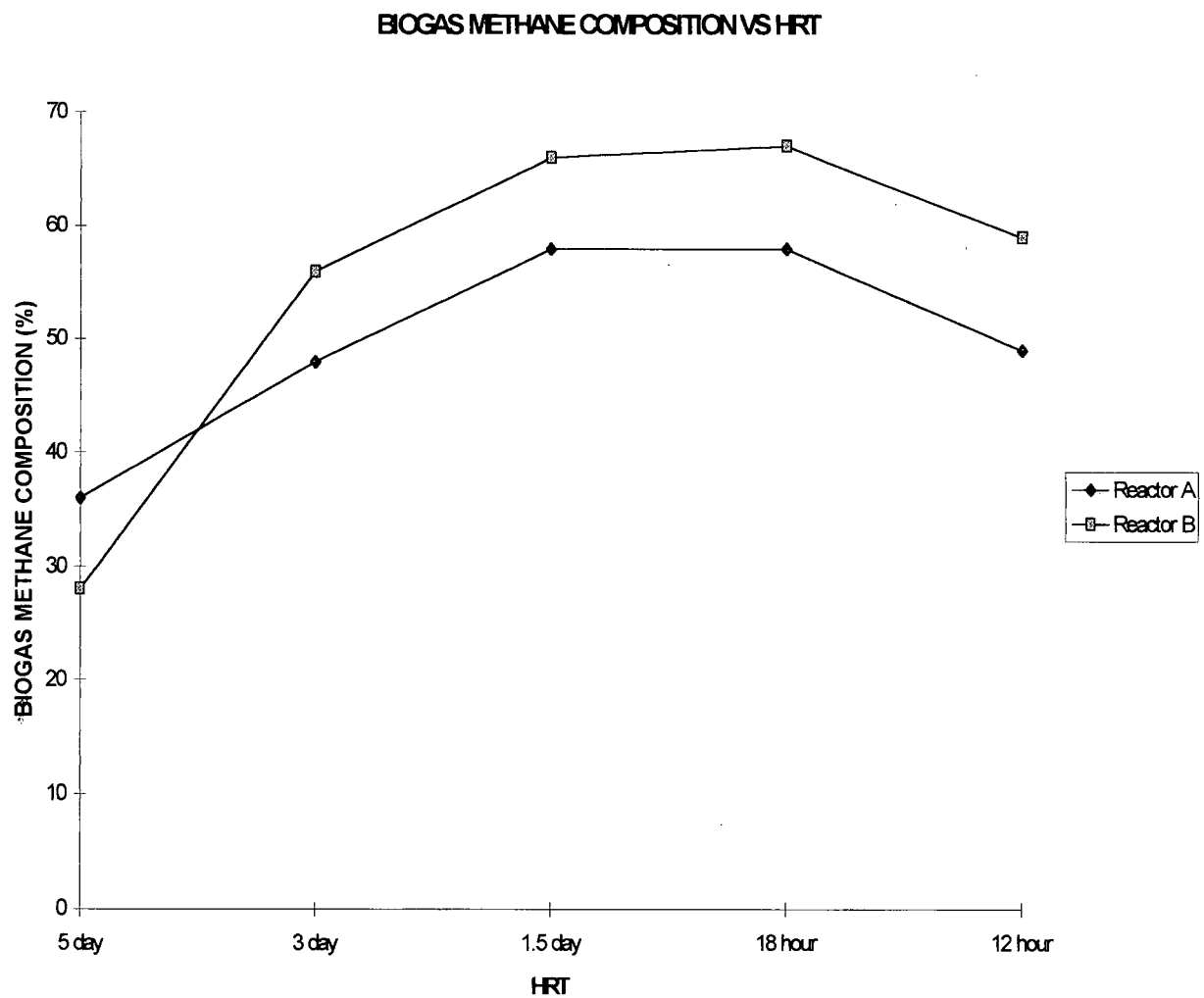


Figure F.8 Biogas Methane Composition versus Hydraulic Retention Time

operation been extended at this HRT. The biogas content of Reactor A showed no indication of increasing, and did not appear to be able to handle a sludge loading rate higher than the average 2.95 g COD/g VSS/d imposed at this HRT.

The Volumetric Methane Production versus HRT is shown in Figure F.9. The amount of methane produced per litre of reactor per day was very low at the 5 day HRT for both reactors. As mentioned earlier, the volumetric methane production increases as the organic loading rate increases. At higher organic loading rates, there is more influent COD per liter of reactor per day to convert to methane, provided that the reactor is able to break down this influent COD. In addition, the volumetric methane production also tends to increase with time as the quality of the sludge improves with continued conditioning or adaptation. This phenomenon was observed by Lettinga *et al.*, (1988). The volumetric methane production rate was lower for Reactor B than Reactor A at the 5 day HRT even though it had the same amount of acclimatized sludge (1.98 g VSS/l). The reason for this difference is the additional 3.95 g VSS/l of unacclimatized seed sludge in Reactor B. In effect, the acclimatized sludge in Reactor B had a lower sludge loading rate than the acclimatized sludge in Reactor A, resulting in a lower methane production rate.

As the organic loading increased at the 3 day HRT, both reactors experienced increases in methane production. The average methane production rate was 0.19 l CH<sub>4</sub>/l/d for Reactor A, and 0.21 l CH<sub>4</sub>/l/d for Reactor B. At the 1.5 day HRT, there was a significant difference in the methane production rates between the reactors, with an average methane production of 0.23 l CH<sub>4</sub>/l/d for Reactor A, and 0.51 l CH<sub>4</sub>/l/d for Reactor B. Reactor B had at this point, approximately six times the biomass in its sludge bed than Reactor A. Therefore, Reactor B was able to convert more of the influent COD to methane, which is confirmed by the higher COD

reductions of Reactor B at this time. The methane production drops for both reactors at the 18 hour HRT, as the average organic loading rate was lowered from the 1.5 day HRT, due to a large decrease in the influent COD concentration. At the 12 hour HRT, when the organic loading rate was its highest, both reactors experienced their highest average methane production rates of 0.45 l CH<sub>4</sub>/l/d for Reactor A, and 0.79 l CH<sub>4</sub>/l/d for Reactor B.

The Methane Yield versus HRT for both reactors is shown in Figure F.10. At the 5 day HRT, the methane yield is significantly lower for Reactor B than Reactor A, at 0.15 l CH<sub>4</sub>/g COD destroyed/d, and 0.28 l CH<sub>4</sub>/g COD/d, respectively. At the 5 day HRT, Reactor B, on several occasions, experienced negative methane yields, due to increases in the effluent COD. The increases in COD were a result of the washed out sludge contributing to the effluent COD.

The methane yield increased considerably to 0.33 l CH<sub>4</sub>/g COD/d for Reactor B at the 3 day HRT, as the sludge quality improved and resulted in increases in methane production and COD reductions. The methane yield also increased less dramatically for Reactor A, to 0.31 l CH<sub>4</sub>/g COD/d at the 3 day HRT. There was a decrease in the average methane yield for Reactor A at the 1.5 day HRT, due to several occasions of increases in effluent COD resulting in negative methane yields. The average methane yield was relatively consistent for both reactors at the 18 hour and 12 hour HRTs, with values of 0.32 l CH<sub>4</sub>/g COD/d and 0.34 l CH<sub>4</sub>/g COD/d for Reactor B, and 0.30 l CH<sub>4</sub>/g COD/d and 0.32 l CH<sub>4</sub>/g COD/d for Reactor A.

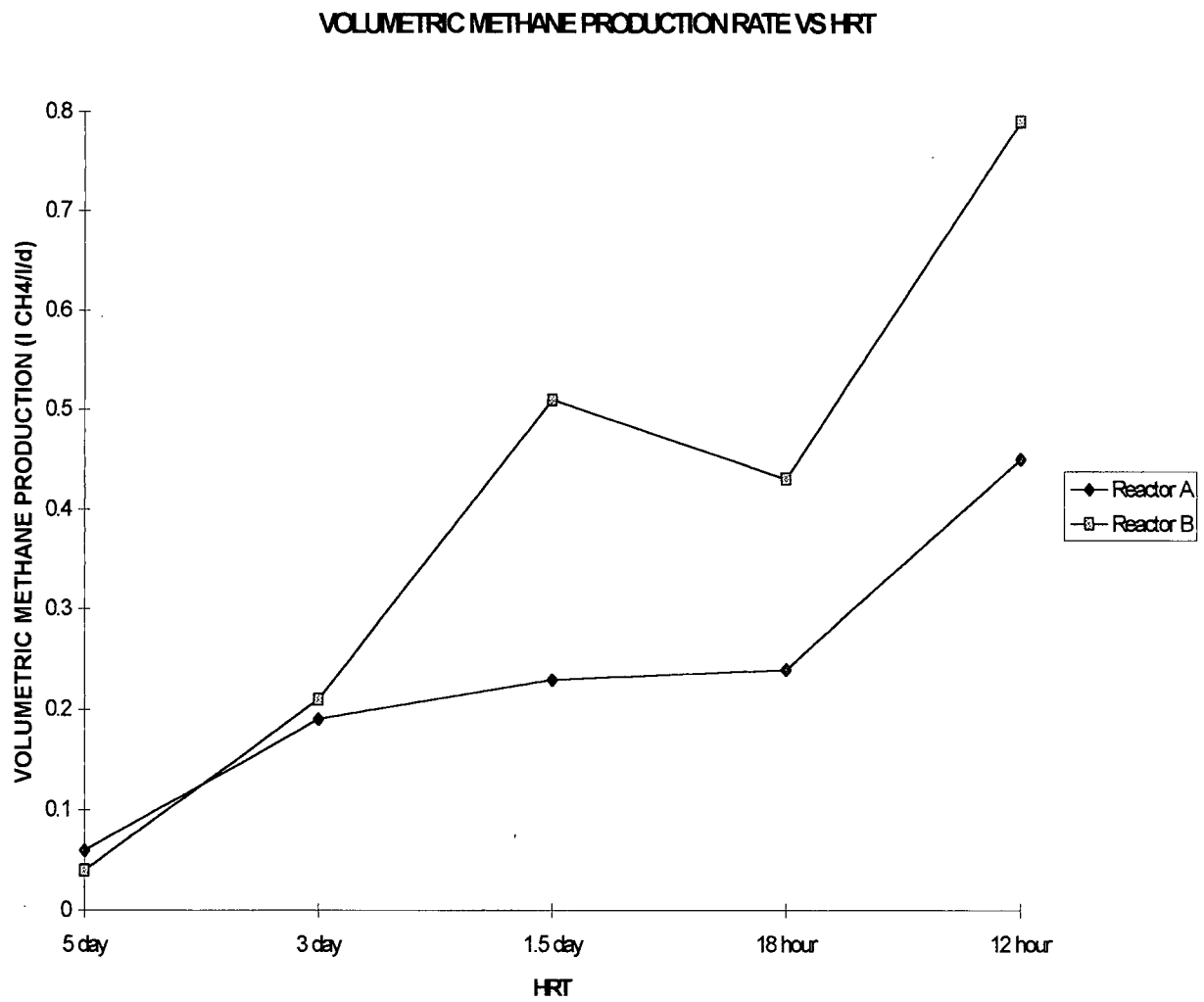


Figure F.9 Volumetric Methane Production versus Hydraulic Retention Time

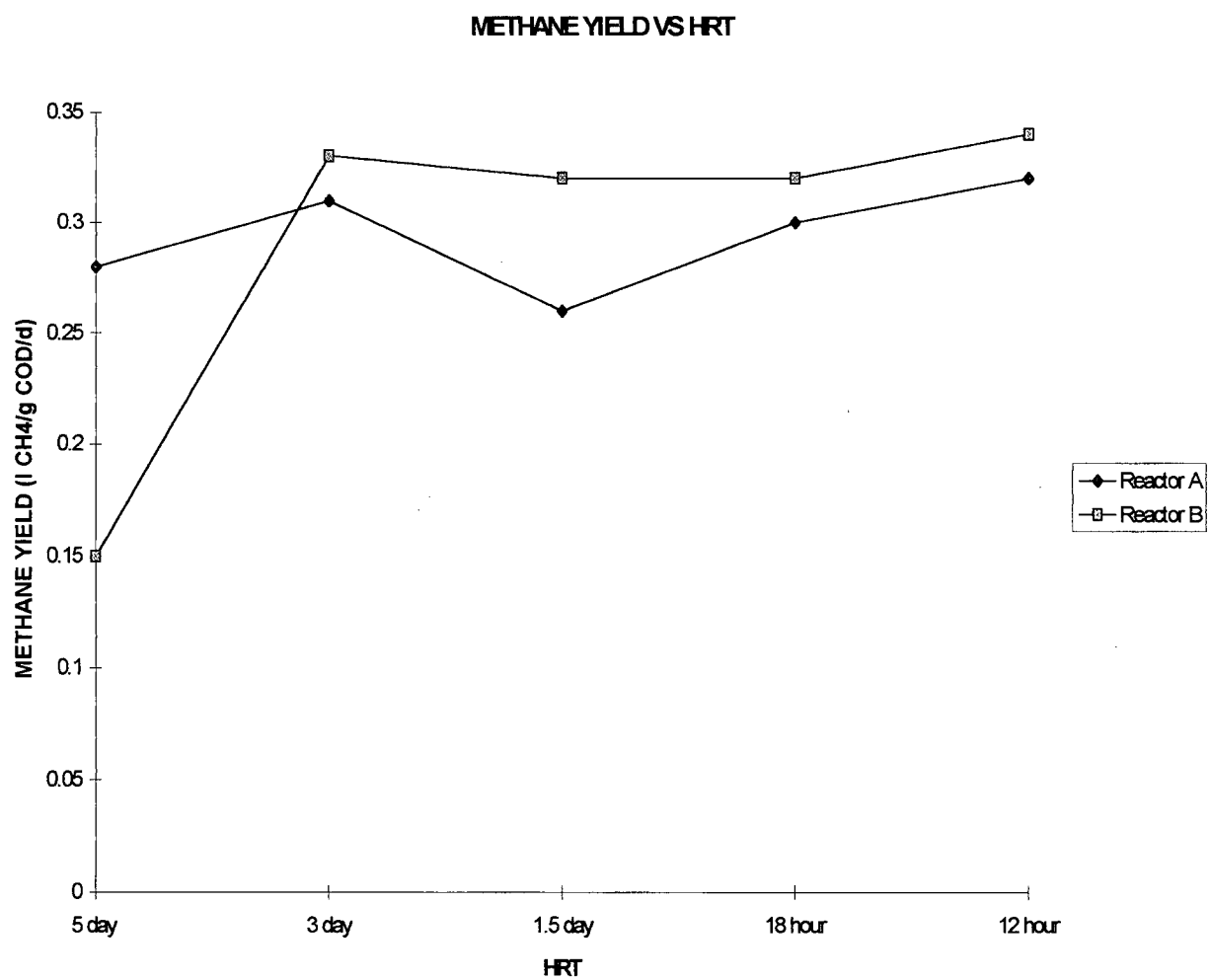


Figure F.10 Methane Yield versus Hydraulic Retention Time

## 2) COD Loading Rate and Reactor Performance

During the course of operation of both reactors, the organic loading rate was increased in a stepwise fashion by decreasing the hydraulic retention time. Due to fluctuations in influent COD concentrations, the organic loading rate also fluctuated and sometimes even decreased when the HRT was lowered. The COD loading rate from day 1 to 189 for both reactors is shown in Figure F.11. Figure F.12 depicts the COD reduction versus the COD loading rate for both reactors. The difference in COD reductions was greatest at the higher organic loading rates, with Reactor A having COD reductions mostly under 50%, at the 18 hour and 12 hour HRTs. This is because Reactor A was becoming overloaded at these higher organic loading rates. The maximum average COD reduction of 89% for Reactor A was at a COD loading rate of 0.59 g COD/l/d on day 80 (3 day HRT). At the maximum COD loading rate of 4.27 g COD/l/d, on day 176, the COD reduction for Reactor B was 89%. The performance of both reactors is comparable to other reactors treating brewery waste operating at similar temperatures. For example, Hickey *et al.*, (1991) reported that brewery wastewater treated at an operating temperature of 19 - 23 °C inoculated with digested sewage sludge and activated sludge took 12 months to achieve granulation and ultimately achieved a COD load of 3-8 kg COD/m<sup>3</sup>/d with over 90% COD removal. Fang *et al.*, (1990) operated a pilot plant for the treatment of brewery effluent and indicated that the process operated at 26 °C could reduce over 89% of the COD and 92% of the BOD with a HRT of 13.3 hours and a COD loading rate of 4.9 kg COD/m<sup>3</sup>/d, with an average influent of 2692 mg/l COD and 1407 mg/l BOD. UASB reactors operating at ambient temperatures seeded with granular sludge had more favourable results. For example, de Vegt *et al.*, (1992) reported that a full scale UASB plant seeded with granular sludge operating



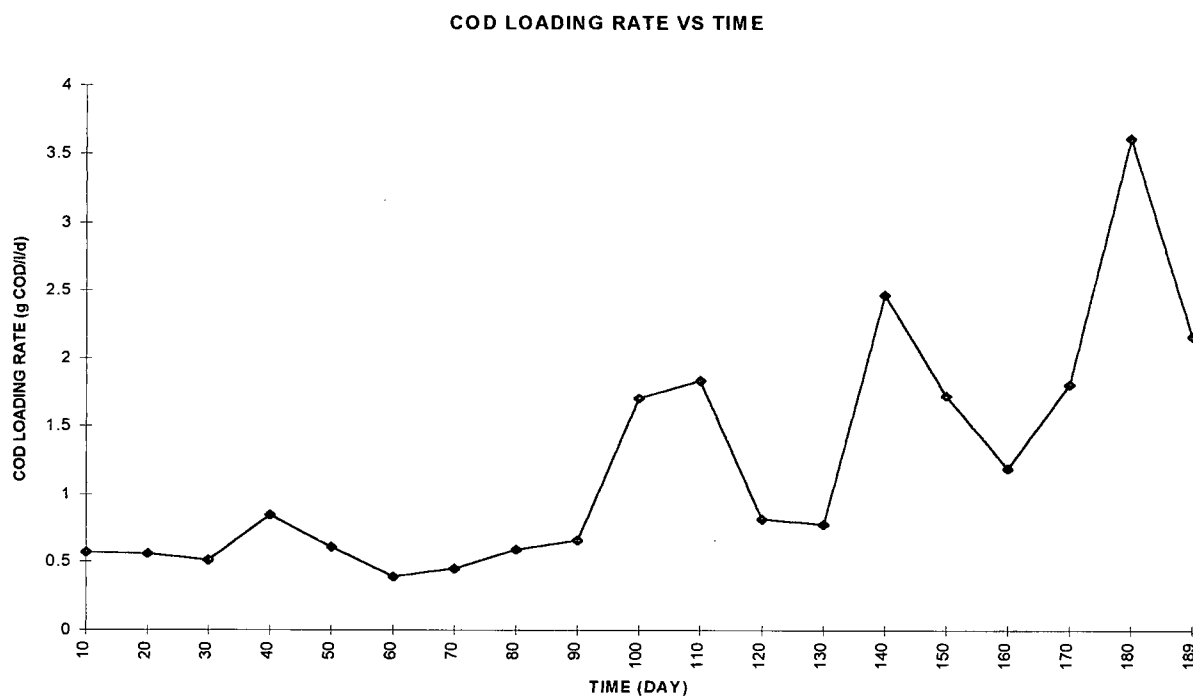


Figure F.11 COD Loading Rate versus Time

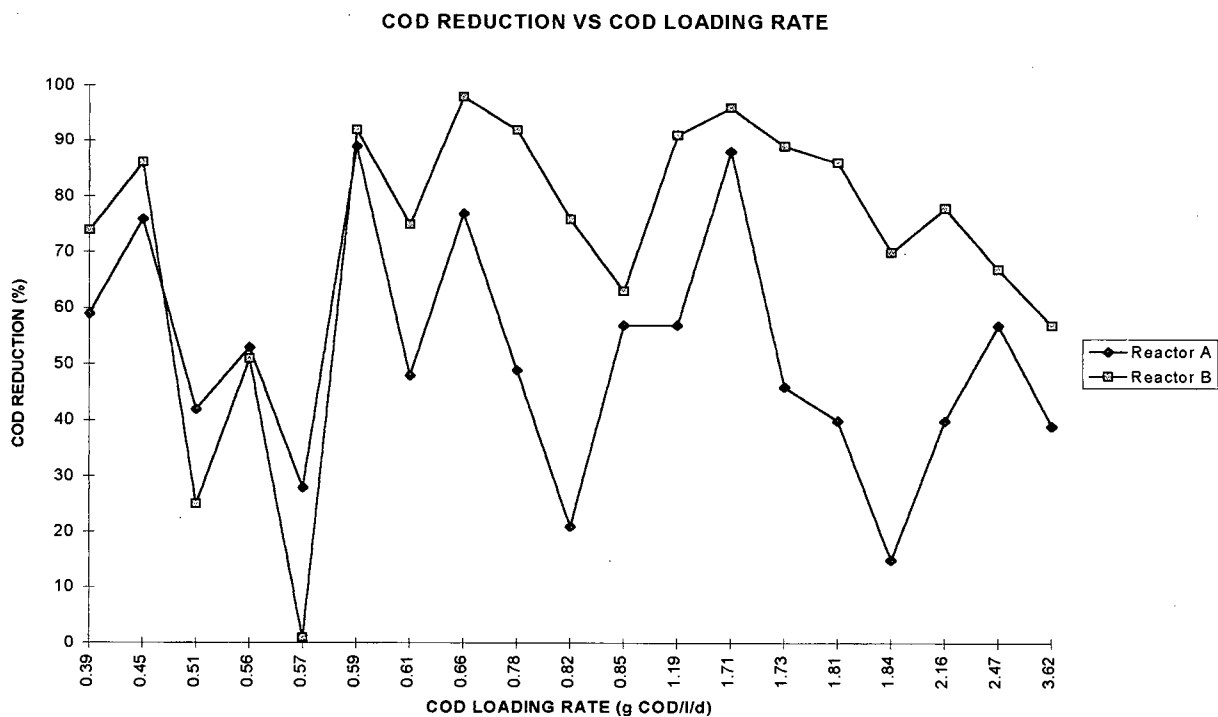


Figure F.12 COD Reduction versus COD Loading Rate

at temperatures of 15-27° C treating brewery waste at an influent COD concentration of 2400 mg/l ( BOD of 1650 mg/l) after 6 years of operation had a total COD removal of 75% and a BOD removal of 88% at a HRT of 4.3 hours and organic loading rate of 11.7 kg COD/m<sup>3</sup>/d.

### **3) Sludge Loading Rate and Reactor Performance**

The sludge loading rate is perhaps a more important parameter in this experiment since each reactor was seeded with different amounts of seed sludge. Therefore, even though the COD loading rate was the same for both reactors, the sludge loading rate was much different. The sludge loading rate is defined as the amount of organic matter (measured as COD) added per amount of reactor sludge biomass (measured as VSS) per day. The sludge loading rates from day 1 to 189 for Reactor A and Reactor B are shown in Figure F.13. The sludge loading rates were much higher for Reactor A than Reactor B since it was seeded with much less seed sludge. The sludge loading rate from day 1 to 102 (5 day and 3 day HRT) was based on 1.98 g VSS/l of sludge for Reactor A, and 5.93 g VSS/l for Reactor B. On day 102, when the HRT was lowered to 1.5 days, the sludge in both reactors was measured. The sludge in Reactor A decreased to 0.99 g VSS/l, while the sludge in Reactor B increased to 6.24 g VSS/l. Since there was a relatively small amount of seed sludge in Reactor A, the sludge was not measured before this time in order to prevent sludge loss. It is interesting to note that the sludge in Reactor A appears to have deteriorated, and actually decreased in concentration from 15.8 g/l to 8.3 g/l VSS. The sludge in Reactor B had become thicker and denser, increasing from 15.8 g/l to 19.2g/l VSS. As well, there was the appearance of some granules in Reactor B, but none in Reactor A. This increase in VSS in Reactor B was actually very low compared to increases in other reactors operating under similar conditions. Fang *et al.*, (1990) operated a pilot plant treating brewery

wastewater at 26 °C and reported that the average VSS in the sludge bed increased from 11.6 g/l to 24.5 g/l over a 4 month period. Wu *et al.*, (198 ) reported that a modest amount of seed sludge such as 15-16 g VSS/l is advisable to obtain a high COD volumetric loading rate as early as possible.

The average sludge loading rates for Reactor A ranged from 0.20 g COD/g VSS/d to 3.67 g COD/g VSS/d and from 0.07 g COD/g VSS/d to 0.58 g COD/g VSS/d for Reactor B. Hickey *et al.*, (1991) reported that higher sludge loading rates tended to increase the process of granulation and sludge loading rates of 0.3 to 0.5 kg COD/kg VSS/d should be applied as soon as possible to accelerate granule formation. Wu *et al.*, (1987) using activated sludge as seed sludge, to treat citrate at 35 °C reported that once the sludge loading rate was increased to 0.6 kg COD/kg VSS/d or higher, granulation was completed within approximately 20 days.

The COD reduction versus the sludge loading rate is depicted in Figure F.14. Reactor A achieved its highest COD reduction rates at sludge loading rates of 0.30 to 0.87 g COD/g VSS/d. (between day 80 to 100, at 3 day HRT). Reactor B was able to achieve high COD removal rates at the sludge loading rates imposed during the course of operation. The highest sludge loading rate for Reactor B was 0.68 g COD/g VSS/d on day 176, and the COD reduction was 89%. Sax (1985) reported treating brewery effluent at 35 °C using an industrial scale UASB reactor which was seeded with granular sludge (with an influent COD of 2900 mg/l and an effluent COD of 500 mg/l) achieved a COD removal of 80% with a COD loading of rate of 8.6 kg COD/m<sup>3</sup> /d and a sludge loading rate of 0.7 kg COD/kg VSS/d at an operating HRT of 5.9 hours. Sax (1985) also reported that the digester effluent COD concentrations remains consistent despite the fluctuations in the COD of the incoming wastewater. The capability of this system to perform in

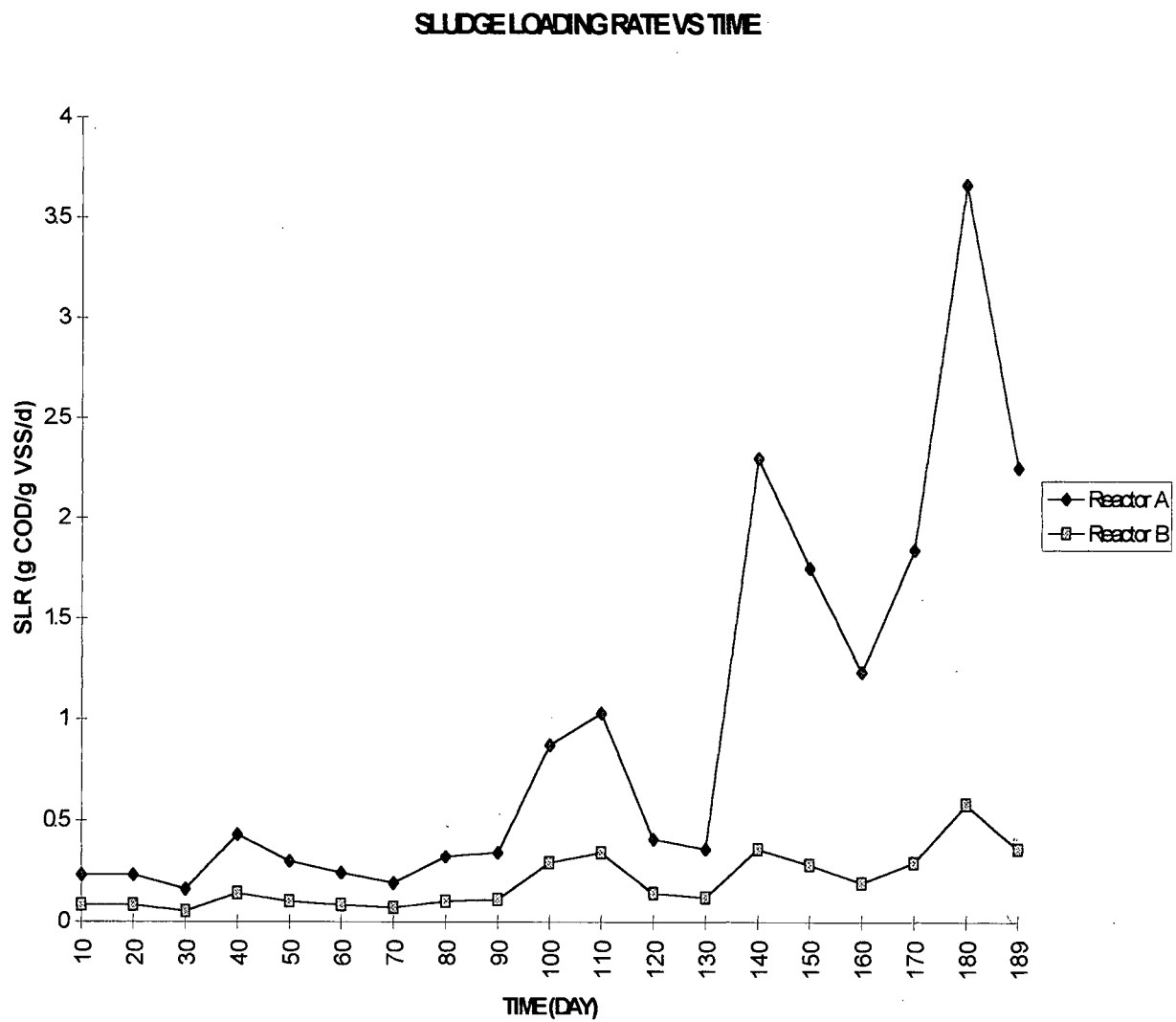


Figure F.13 Sludge Loading Rate vs Time

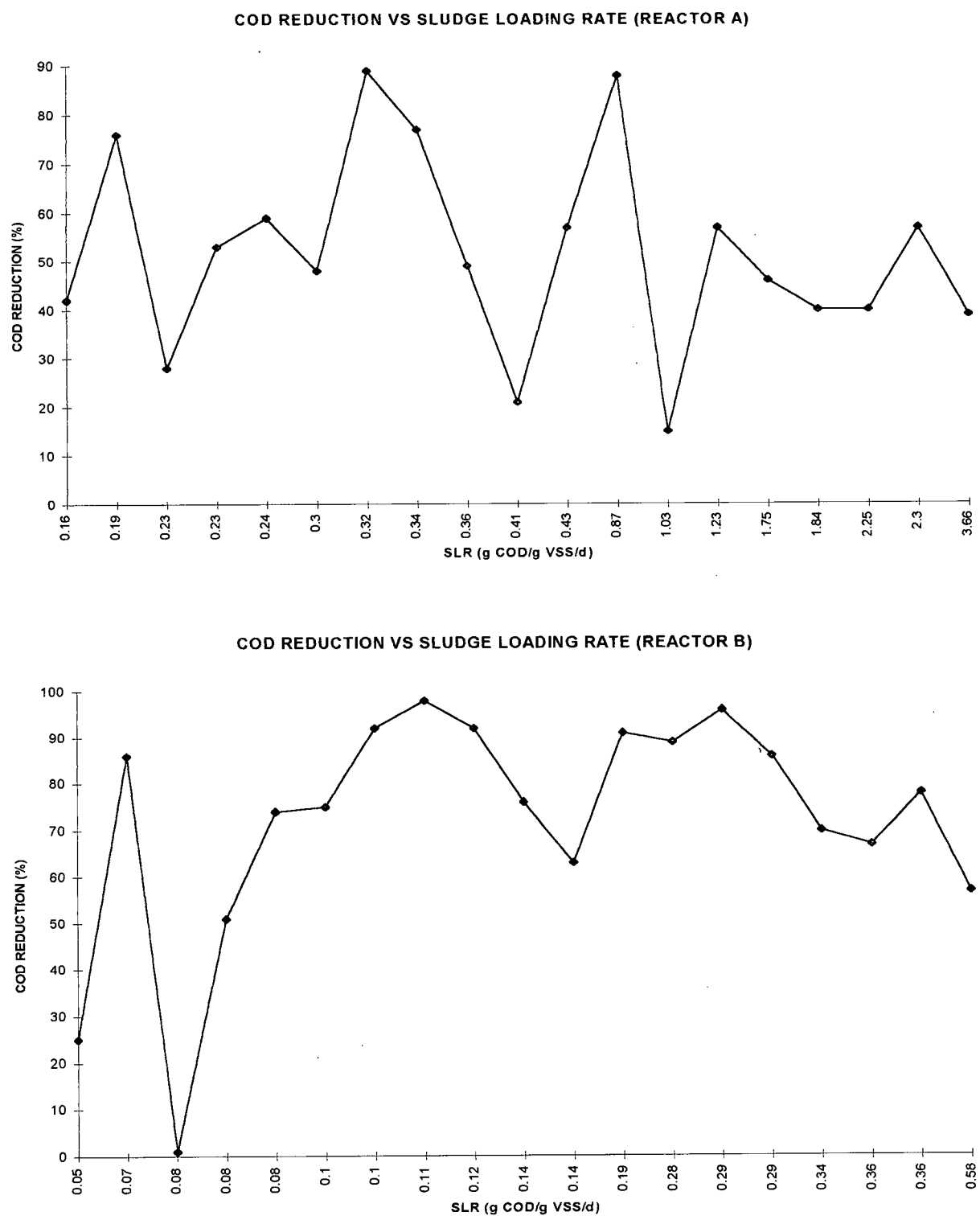


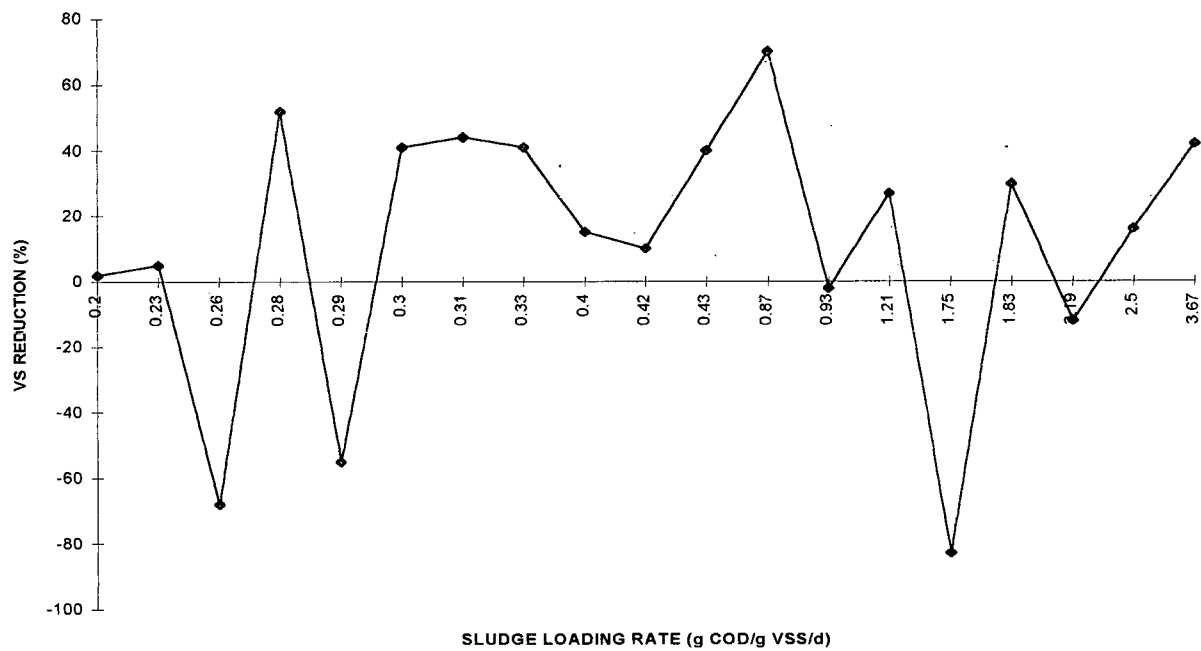
Figure F.14 COD Reduction vs Sludge Loading Rate

a very stable manner despite receiving highly fluctuating wastewater loadings attests to the anaerobic treatability of brewery effluent.

The VS reduction versus the sludge loading rate is shown in Figure F.15. The VS reductions for both reactors fluctuated considerably. The effluent VS concentration appeared to be a function of the influent VS concentration, and the HRT. Most of the decreases in VS reduction for Reactor B occurred at the lower sludge loading rates, which also corresponded to the beginning of the experiment when the sludge was still adapting to the reactor environment. At the higher sludge loading rates, Reactor A experienced more sludge washout than Reactor B since its sludge was less dense and tended to wash out with the higher liquid upflow velocities at the shorter HRTs of 18 hours and 12 hours.

The biogas methane composition versus the sludge loading rate is shown in Figure F.16. Reactor A had an average maximum biogas methane content of 63% on days 110 and 170, when the sludge loading rates were 0.87 g COD/g VSS/d and 1.83 g COD/g VSS/d, respectively. Reactor B had an average maximum biogas methane content of 68% on days 80 and 170, when the sludge loading rates were 0.10 g COD/g VSS/d and 0.29 g COD/g VSS/d, respectively. Sax (1985) reported that at a HRT of 6.5 hours and a sludge loading rate of 0.38 kg COD/kg VSS/d, the methane purity was 84% when treating brewery wastewater at 35°C. de Vegt *et al.*, (1992) reported a methane purity of 85% while treating brewery wastewater at 15 -27 °C and a HRT of 4.3 hours with an organic loading rate of 11.7 kg COD/m<sup>3</sup>/d. However, the UASB reactor was seeded with granular sludge. The low methane content of the biogas in Reactors A and B compared to other UASB reactors may be a result of the lower methanogenic activity of this sludge compared to other reactors.

VOLATILE SOLIDS REDUCTION VS SLUDGE LOADING RATE (REACTOR A)



VOLATILE SOLIDS REDUCTION VS SLUDGE LOADING RATE (REACTOR B)

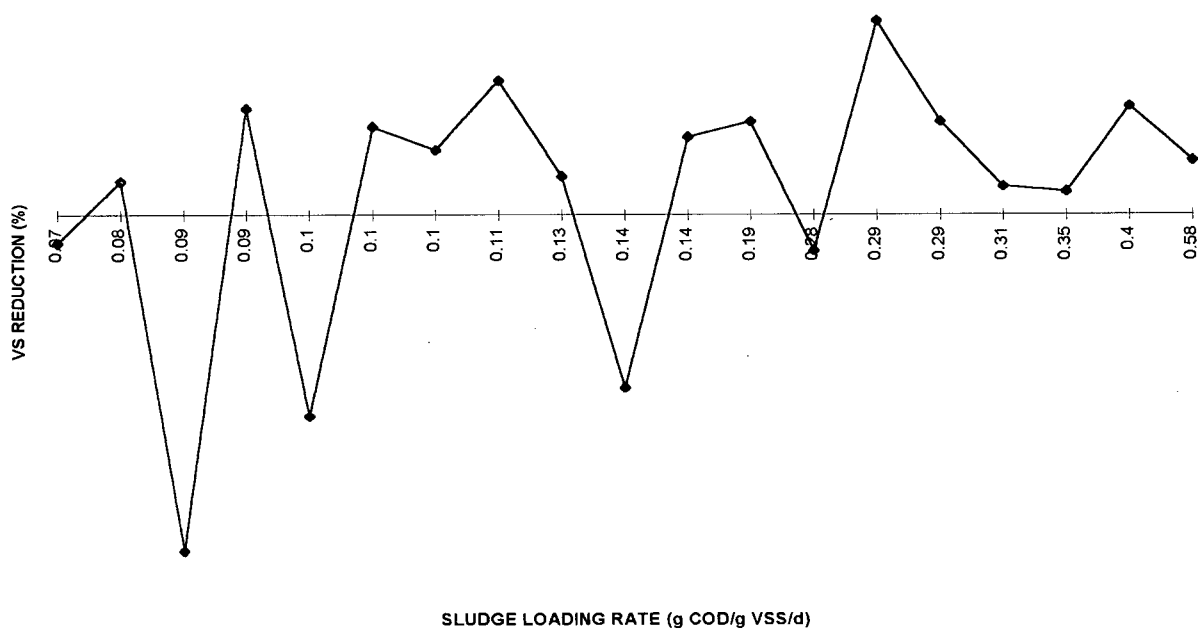
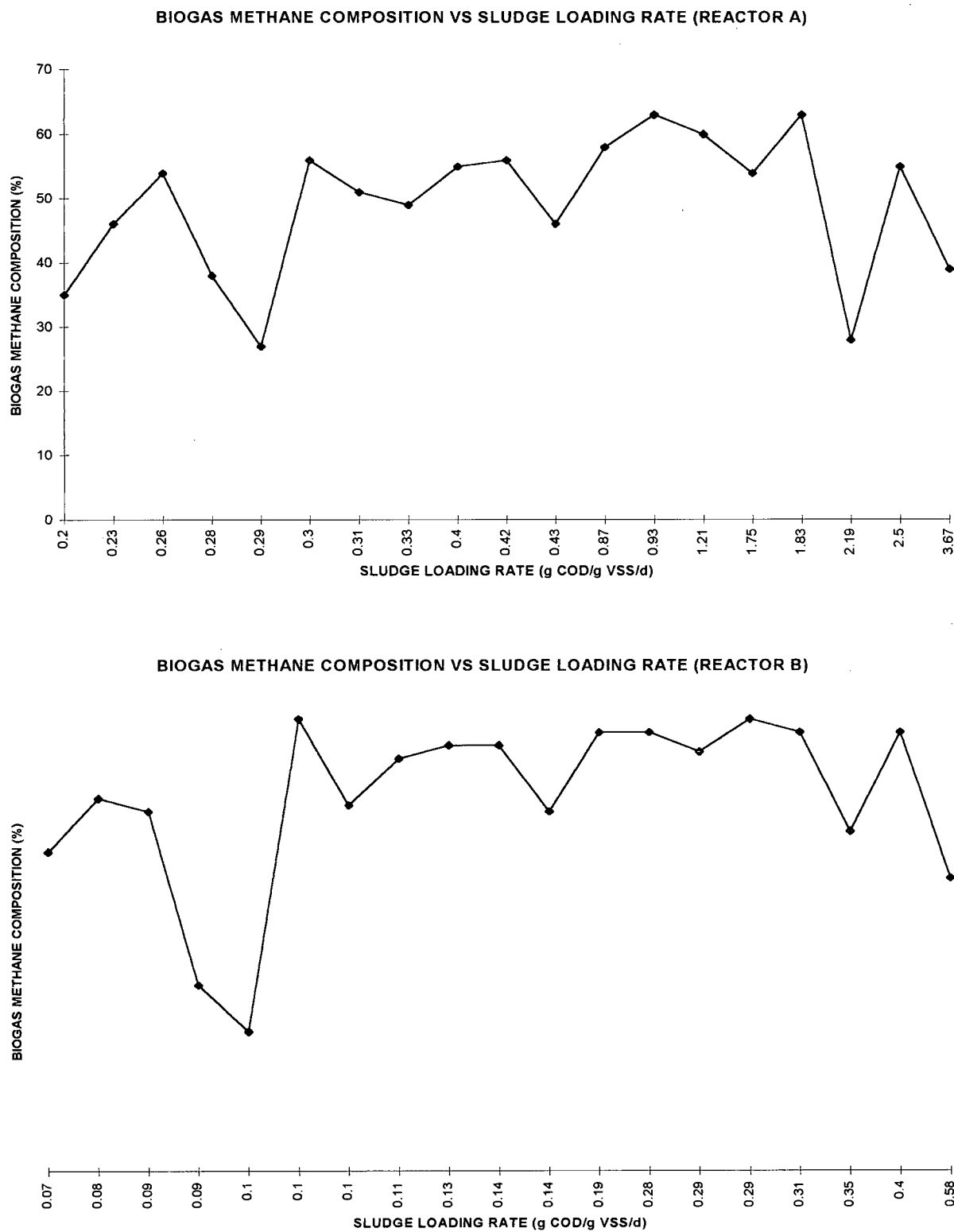


Figure F.15 Volatile Solids Reduction vs Sludge Loading Rate





#### Figure F.16 Biogas Methane Composition vs Sludge Loading Rate

The volumetric methane production rate is shown in Figure F.17. Reactor A had its highest methane production rate of  $0.98 \text{ l CH}_4/\text{l/d}$  on day 176, at a sludge loading rate of  $4.32 \text{ g COD/g VSS/d}$ . Reactor B had its highest methane production rate of  $1.33 \text{ l CH}_4/\text{l/d}$  on day 176, when the sludge loading rate was  $0.68 \text{ g COD/g VSS/d}$ . The corresponding organic loading rate was  $4.27 \text{ g COD/l/d}$ . There were no references to the volumetric methane production of UASB reactors treating brewery wastewater in the literature, so comparisons to other wastewaters have been made instead. Lo *et al.*, (1994) reported a volumetric methane production of  $0.71 \text{ l CH}_4/\text{l/d}$  at an organic loading rate of  $3.51 \text{ g COD/l/d}$  with a UASB hybrid reactor treating swine wastewater at  $35^\circ \text{C}$ . In a UASB hybrid reactor treating molasses wastewater at  $35^\circ \text{C}$ , methane productions of  $0.36 \text{ l CH}_4/\text{l/d}$  at an organic loading rate of  $2.3 \text{ g COD/l/d}$ , and  $0.91 \text{ l CH}_4/\text{l/d}$  at an organic loading rate of  $5.8 \text{ g COD/l/d}$  was reported by Lo *et al.*, (1991). The higher volumetric methane productions in this experiment may be a result of higher sludge loading rates than those used in these references, since only the COD loading rates were reported.

Figure F.18 depicts the methane yield per COD destroyed. Reactor A had a negative methane yield of  $-0.08 \text{ l CH}_4/\text{g COD destroyed}$  on day 110, when the sludge loading rate was  $0.93 \text{ g COD/g VSS/d}$ . This reflects the increase in effluent COD which occurred on this day. Otherwise, the methane yields were similar for both reactors, ranging from  $0.25$  to  $0.39 \text{ l CH}_4/\text{g COD destroyed}$  for Reactor A, and  $0.26$  to  $0.41 \text{ l CH}_4/\text{g COD destroyed}$  for Reactor B. These values are close to the stoichiometric quantity of  $0.35 \text{ m}^3 \text{ CH}_4/\text{kg COD destroyed}$ , and comparable to values obtained in the literature. Sax (1985) reported that at sludge loading rates of  $0.38 \text{ kg COD/kg VSS/d}$ , the methane yield was  $0.37 \text{ m}^3/\text{kg COD}$  treating brewery wastewater at  $35^\circ \text{C}$  on an industrial scale. Fang *et al.*, (1990) operated a pilot plant treating brewery

effluent at 26 °C and consistently achieved a methane yield of 0.45 m<sup>3</sup> of methane for each kg of COD removed and had a biogas methane content of 70% or higher.

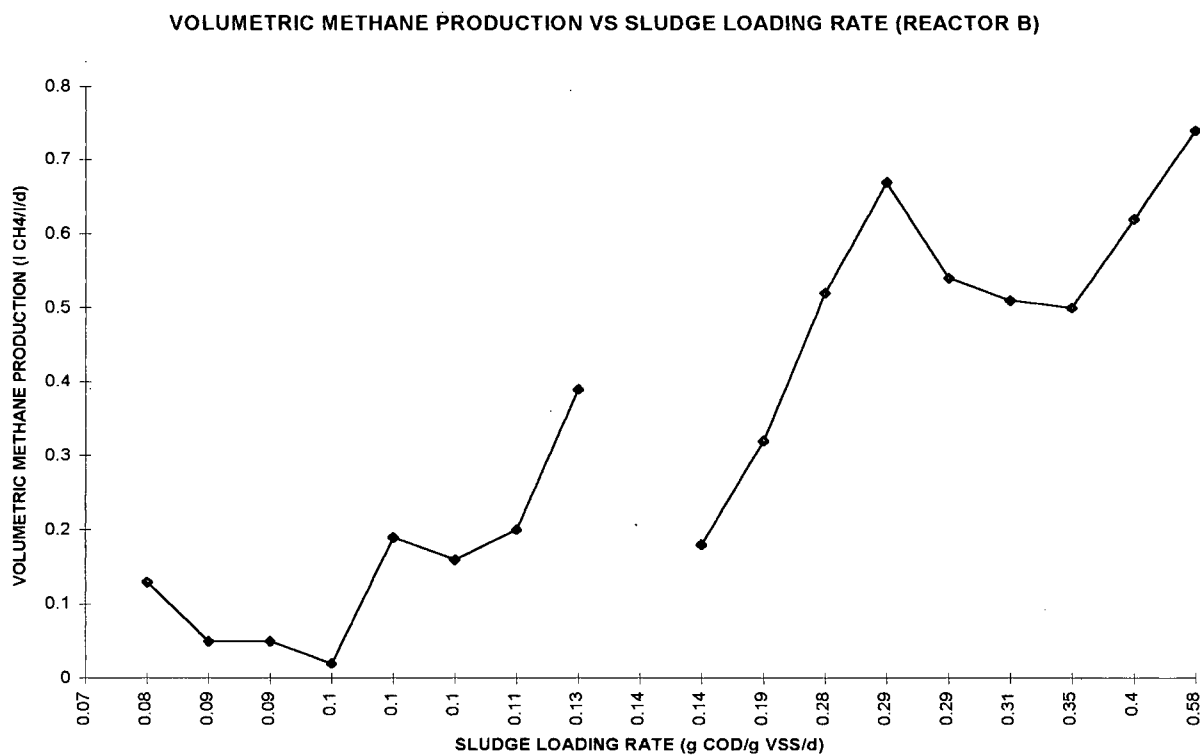
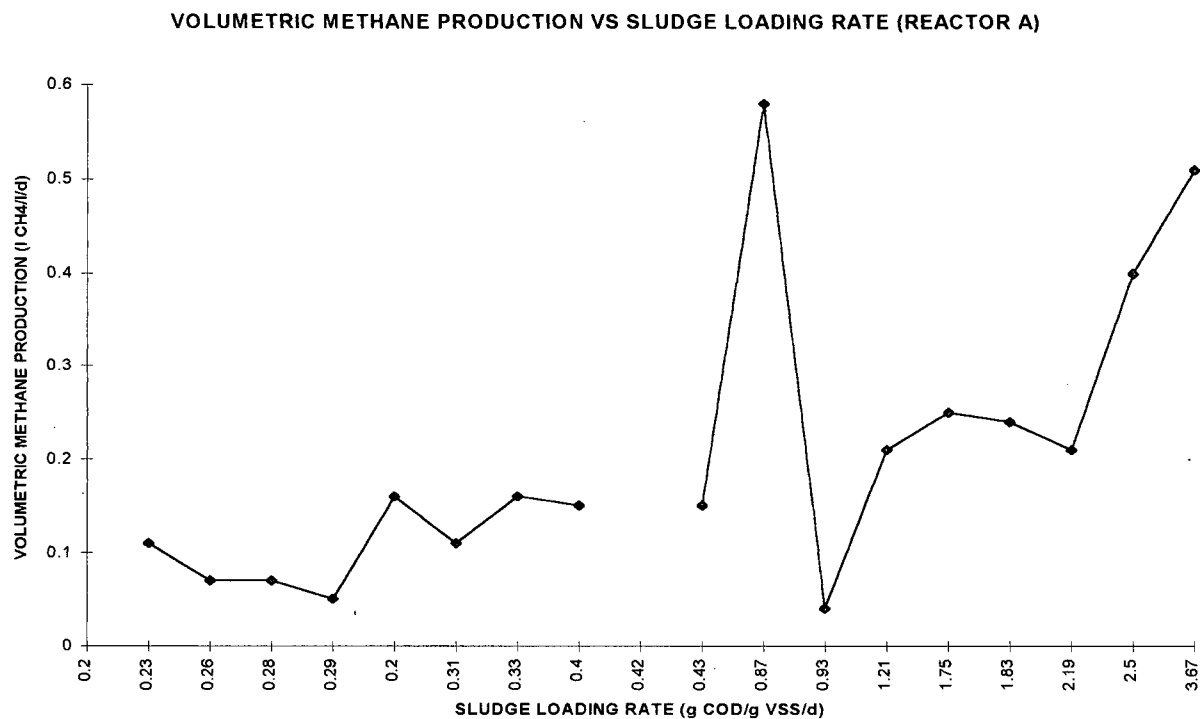


Figure F.17 Volumetric Methane Production vs Sludge Loading Rate

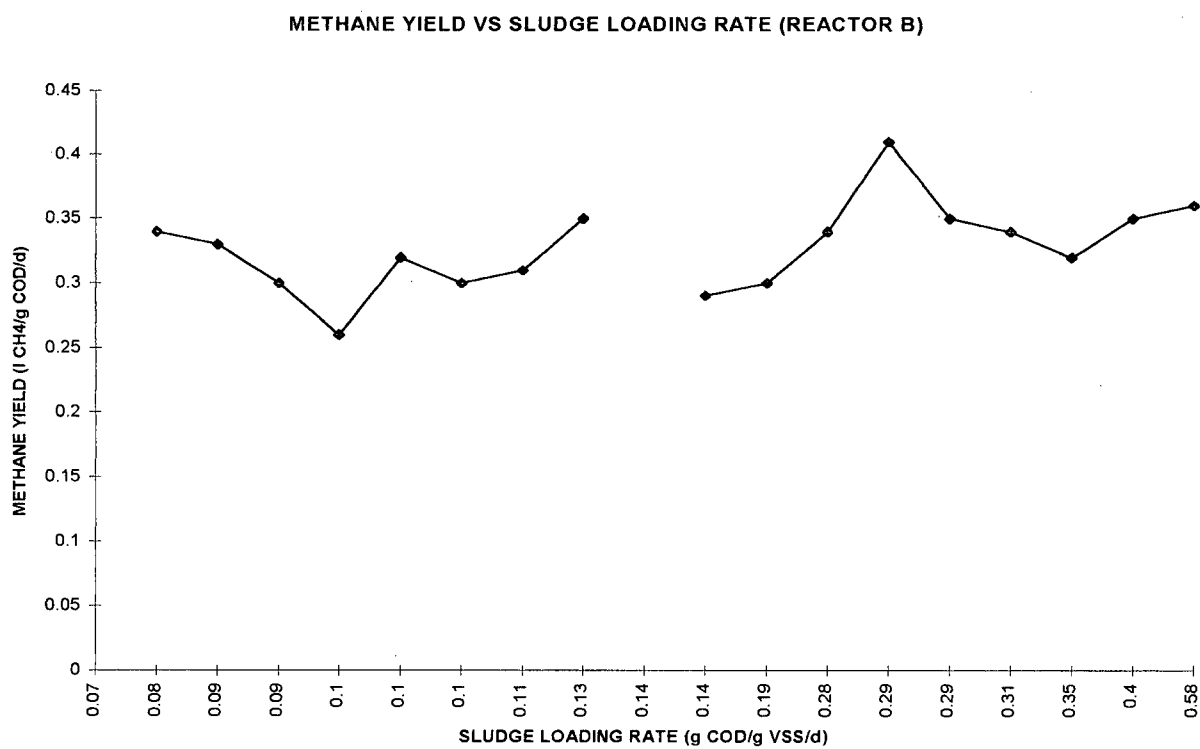
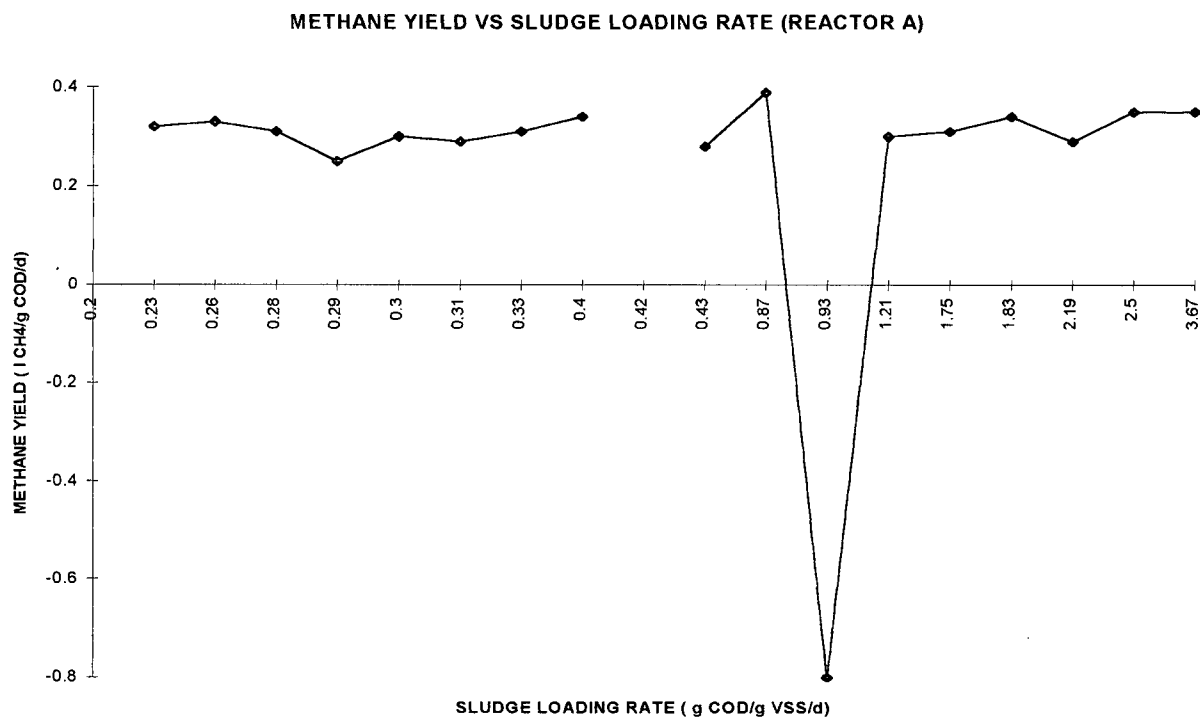


Figure F.18 Methane Yield vs Sludge Loading Rate

## V. CONCLUSIONS

1) Activated sludge is suitable as a seed sludge for the start-up of UASB reactors, as methanogenic bacteria can be cultivated under anaerobic conditions with this type of sludge.

2) The acclimation period is vital to the start-up of UASB reactors seeded with activated sludge. An acclimation period of at least three weeks is important since activated sludge is unstable and has a low density and the acclimation period allows the sludge to stabilize and become more dense (Wu *et al.*, 1987). Activated sludge consists mostly of aerobic bacteria and acclimation to the wastewater to be treated under anaerobic conditions is necessary to cultivate an active anaerobic bacteria population. The lack of an acclimation period delays start-up and leads to excessive sludge washout, as the aerobic bacteria lyse under anaerobic conditions. Reactor B, which was seeded with 3.95 g VSS/l unacclimatized seed sludge (and 1.98 g VSS/l of acclimatized seed sludge) had increases in effluent COD, low methane production, and excessive sludge washout in the first 20 days of reactor operation. After this time, the seed sludge appeared to have acclimatized to the influent and the anaerobic reactor environment, and the performance of Reactor B improved considerably.

3) The amount of seed sludge is important in the start-up process and reactor performance. Reactor A was seeded with 1.98 g VSS/l and Reactor B was seeded with 5.93 g VSS/l. Wu *et al.*, (1987) recommend to use approximately 15 g VSS/l of seed sludge when using activated sludge as seed. Reactor B, with more seed sludge was able to achieve higher COD removals, and higher methane production at the higher organic loading rates than Reactor A. The performance

of Reactor B was comparable to other reactors operating at similar temperatures and organic loading rates. Thus, it is possible to start up a UASB reactor and achieve a satisfactory treatment efficiency with less than the 15 g VSS/l of acclimatized activated sludge recommended by Wu *et al.*, (1987).

4) The sludge loading rate is important in the start-up of UASB reactors. The maximum average sludge loading rate of 3.67 g COD/g VSS/d for Reactor A was too high. At this high sludge loading rate, Reactor A was overloaded and had a low COD reduction of 39%. The quality of the seed sludge and consequently, the performance of the reactors, improved as the sludge loading rate increased with time. Lettinga *et al.*, (1988) reported that the efficiency of a UASB reactor improved considerably as the organic load increases and postulated that this was an effect of the conditioning or adaptation of the sludge with time.

5) Increasing the organic loading rate by decreasing the hydraulic retention time was effective in acting as a selection pressure in washing out the lighter sludge and retaining the denser, better settling sludge in Reactor B. Reactor B experienced sludge washout at the beginning of the experiment, however, Reactor A had virtually no sludge washout. The VSS concentration in Reactor B increased from 15.8 g/l VSS to 19.2 g/l VSS, while the VSS concentration in Reactor A decreased from 15.8 g/l VSS to 8.3 g/l VSS. As a result, at the 18 hour and 12 hour HRTs, Reactor A experienced more sludge washout than Reactor B.

6) The initial HRT of 5 days that the reactors were operated at was too long, and resulted in sludge loading rates less than the recommended range for start-up in Reactor B. However, at the shorter HRTs, the upflow liquid velocity was well above the recommended range of 0.72 to 0.96

m/d by Campos *et al.*, (1992).

7) The use of a mixture of acclimatized sludge and unacclimatized sludge confounded the reliability of the results by changing two variables at once (i.e. amount of seed sludge and acclimation of seed sludge). Therefore it was not possible to determine if the differences in the reactors' performance in the first few weeks of operation was due to the amount of seed sludge added or the acclimation period. The poor performance of Reactor B in the first few weeks of operation could have been due to a lack of an acclimation period of the seed sludge or due to underloading as a result of the amount of seed sludge used.

8) The effluent pH was not a reliable indicator of reactor stress due to the buffer that was added to the influent prior to feeding into the reactors. As a result, the effluent pH did not drop when the acetic acid levels increased.

## VI. RECOMMENDATIONS

The following are recommendations to take into consideration for anyone attempting to do follow up work or repeat this experiment. These recommendations represent suggestions which would improve the results obtained in this experiment or represent areas which did not receive enough attention in the present experiment:

- 1) The initial hydraulic retention times should be shortened to achieve a minimum sludge loading rate of approximately 0.3 g COD/g VSS/d.
- 2) To ensure equal hydraulic retention times, the same pumping rate should be achieved by using double pump heads on the same pump drive.
- 3) The influent VSS should be kept below 500 mg/l. This can be accomplished by settling prior to feeding into the reactors.
- 4) An improved reactor design would optimize the operating conditions. The cross sectional area should be increased to allow the liquid upflow velocity to be within the recommended range of 0.72 to 0.96 m/d during start-up. With an increased cross-sectional area, the number of feed inlets should be increased to more than one to allow the feed to be evenly distributed throughout the sludge bed.
- 5) A thick activated sludge should be used to allow approximately 15 g/l VSS to be seeded into the reactors. The sludge used in this experiment was too thin to allow enough sludge to be added for a proper start-up. If a thick sludge is not available, the acclimation period should be extended to allow the sludge to become more dense.
- 6) Instead of a phosphate buffer,  $\text{CaCO}_3$  should be used so that the effluent doesn't have elevated phosphate levels, which are a concern in effluent quality.
- 7) The influent COD should be carefully monitored and kept between 1000-1500 mg/l during the start-up period. The UASB reactor system has shown to be able to handle



fluctuations in influent COD so that after start-up, the influent COD need not be adjusted.

8) Periodic cleaning of the influent tube is recommended to eliminate clogging and ensure that the pumping rates are accurate.

9) The impact of the amount of seed sludge and the acclimation period on reactor start-up and performance should be determined separately. The impact of the amount of seed sludge should be done by varying the amount of seed sludge only. Similarly, the impact of the acclimation period should be studied using two reactors seeded with the same amount of seed sludge.

## VIII. REFERENCES

- American Public Health Association (1975). *Standard Methods for the examination for waste and wastewater*, 14th edition, Washington, D.C.
- Barford, J.P. (1988). Start-up dynamics and control of anaerobic digesters. *Handbook on anaerobic fermentations*. Edited by L.E. Erickson and D. Yee-Chak Fung; Marcel Dekker Inc., Chapter 23.
- Campos, C.M.M. and Anderson, G.K. (1992). The effect of liquid upflow velocity and substrate concentration on the start-up and steady-state periods of lab-scale UASB reactors. *Water Science and Technology*, 25, 41-50.
- Christensen, D.R., Gerick, J.A. and Eblen, J.E. (1984). Design and operation of an upflow anaerobic sludge blanket reactor. *Journal of the Water Pollution Control Federation*, 56, 9: 1059-1062.
- Dangcong, P., Xingweng, Z., Quiting, J., LiangKui, X. and De, Z. (1994). Effects of the seed sludge on the performance of UASB reactors for the treatment of toxic wastewater. *Journal of Chemical Technology and Biotechnology*, 60, 171-176.
- Fang, H.H.P., Guohua, L., Jintu, Z., Bute, C. and Guowei, G. (1990). Treatment of brewery effluent by UASB process. *Journal of Environmental Engineering*, 16, 3 : 454-460.
- Goodwin, J.A.S., Wase, D.A.J. and Forster, C.F. (1992). Pre-granulated seeds for UASB reactors: how necessary are they. *Bioresource Technology*, 41, 71-79.
- Heertjes, P.M. and van der Meer, R.R. (1979). Comparison of different methods for anaerobic treatment. *Proceedings of the 34th Industrial Waste Conference*, Purdue University, 790-803.
- Hickey, R.F., Wu, W.M., Veiga, M.C. and Jones, R. (1991). Start-up, operation, monitoring and control of high-rate anaerobic treatment systems. *Water Science and Technology*, 24, 207-225.
- Huang, C.W. and Hung, Y.I. (1987). Brewery wastewater treatment by contact oxidation process. *Proceedings of the 41st Industrial Waste Conference*, Purdue University, 90-98.
- Hulshoff Pol, L.W., deZeeuw, W.J., Velzeboer, C.T.M. and Lettinga, G. (1983). Granulation in UASB reactors. *Water Science and Technology*, 15, 291-304.

- Jian, C. and Shi-yi, L., (1993). Study of mechanism of sludge granulation in UASB reactors. *Water Science and Technology*, 28, 171-178.
- Khan, N. and Mehfotra, I. (1990). Performance of laboratory-scale upflow anaerobic sludge blanket (UASB) reactors. *Water Science and Technology*, 22, 285-286.
- Knetchel J.R. (1978). A more economical method for the determination of chemical oxygen demand. *Water Pollution Control*, 116, 25-27.
- Kosaric, N., Blaszczyk, R. and Orphan, L. (1990). Factors influencing the formation and maintenance of granules in anaerobic sludge blanket reactors. *Water Science and Technology*, 23, 275-282.
- LeClair, B.P. (1984). Performance monitoring program - Molson's brewery deep shaft treatment system. *Proceedings of the 9th Industrial Waste Conference*, Purdue University, 257-268.
- Lettinga, G. van Velsen, A.F.M., Hobma, S.W., de Zeeuw, W. and Klapwijk, A. (1980). Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, 22, 699-734.
- Lettinga, G., Hulshoff Pol, L.W., Koster, I. W., Weigant, W.M., de Zeeuw, W.J., and Rinzema, A., Grin, P.C., Roersma, R.E. and Hobma, S.W. (1984). High rate anaerobic wastewater treatment using the UASB reactor under a wide range of temperature conditions. *Biotechnology and Genetic Engineering Reviews*, 2, 253-284.
- Lettinga, G., de Zeeuw, W., Hulshoff Pol, L., Weigant, W., and Rinzema, A. (1985). Anaerobic wastewater treatment based on biomass retention with emphasis on the UASB process. *Proceedings of the 4th International Symposium on Anaerobic Digestion*, Guangzhon, China, 279-295.
- Lettinga, G. and Hulshoff Pol, L.W. (1986). Advanced reactor design, operation, and economy. *Water Science and Technology*, 18, 99-108.
- Lettinga, G. and Hulshoff Pol, L.W. (1988). UASB process design for the various types of wastewaters. *Design of Anaerobic Processes for the treatment of Industrial and Municipal Wastes*, Edited by J.F. Malina and F.G. Pohland.
- Lettinga, G. and Hulshoff Pol, L.W. (1991). UASB process design for various types of wastewaters. *Water Science and Technology*, 24, 87-107.
- Lo, K.V. and Liao, P.H. (1985). High-rate anaerobic digestion of screened dairy manure. *Journal of Agricultural Engineering Research*, 32, 349-358.

- Lo, K.V. and Liao, P.H. (1990). Anaerobic treatment of baker's yeast wastewater: I. Start-up and Sodium Molybdate addition. *Biomass*, 21, 3, 207-218.
- Lo, K.V., Chiu, C. and Liao, P.H. (1991). Anaerobic treatment of molasses wastewater: Start-up and steady-state performance. *Canadian Agricultural Engineering Journal*, 33, 295-301.
- Lo, K.V., Liao, P.H. and Gao, Y.C. (1994). Anaerobic treatment of swine wastewater using hybrid UASB reactors. *Bioresource Technology*, 47, 153-157.
- Marchaim, U. and Krause, C. (1993). Propionic to acetic acid ratios in overloaded anaerobic digestion. *Bioresource Technology*, 43, 195-203.
- Moosbrugger, R.E., Ekama, G.A. and Marais, G.R. (1993). Lauter tun (brewery) waste in UASB systems- feasibility, alkalinity requirements, and pH control. *Water, S.A.*, 19, 1: 41-51.
- Morvai, L., Mihaltz, P. and Czako, L. (1992). The kinetic basis of a new start-up method to ensure the rapid granulation of anaerobic sludge. *Water Science and Technology*, 25, 113-122.
- Obayashi, A. (1985). *Management of Industrial Pollutants by Anaerobic Processes*. Edited by J.W. Patterson. Chapters 1 and 23.
- Paula, D.R. and Foresti, E. (1992). Kinetic studies on a UASB reactor subjected to increasing COD concentration. *Water Science and Technology*, 25, 103-111.
- Samson, R. and Guiot, S. (1985). Mixing characteristics and performance of the anaerobic upflow sludge blanket filter (UBF) reactor. *Journal of Chemical Technology and Biotechnology*, 65-74.
- Sam-Soon, P., Loewenthal, R.E., Wentzel, M.C. and Marais, G.R. (1990). Effect of nitrogen limitation on pelletisation in upflow anaerobic sludge bed (UASB) systems. *Water S.A.*, 16, 3: 165-170.
- Sax, R.I. (1985). Anaerobic pretreatment of brewery wastewater on the industrial scale. *Proceedings of the 40th Industrial Wastewater Conference*, Purdue University, 59-68.
- Schumann, G.E., Stanley, M.A. and Knudsen, D. (1983). Automated total nitrogen analysis of soil and plant samples. *Proceedings of Soil Sci. Soc. Amer.*, 480-481.
- Souza, M.E. (1986). Criteria for the utilization, design, and operation of UASB reactors. *Water Science and Technology*, 18, 55-69.
- Stonach, S.M., Rudd, T., and Lester, J.N. (1986). *Anaerobic Digestion Processes in Industrial Wastewater Treatment*. Public Health Engineering Laboratory, Imperial College, London, Chapter 4.

- de Vegt, A., Vereijken, T. and Dekkers, F. (1992). Full-scale anaerobic treatment of low strength brewery wastewater at sub-optimal temperatures. *Proceedings of the 47th Industrial Waste Conference*, Purdue University, 409-417.
- Weiland, P. and Rozzi, A. (1991). The start-up, operation and monitoring of high-rate anaerobic treatment systems: discussor's report. *Water Science and Technology*, 24, 257-277.
- Wentzel, M.C., Moosbrugger, R.E., Sam-Soon, P.A., Ekama, G.A. and Marais, G.R. (1994). Tentative guidelines for waste selection, process design, operation and control of upflow anaerobic sludge bed reactors. *Water Science and Technology*, 30, 31-42.
- Wu, W.M., Hu, J.C. and Gu, X.S. (1985). Properties of granular sludge in upflow anaerobic sludge blanket (UASB) reactor and its formation. *Proceedings of the 4th International Symposium on Anaerobic Digestion*, Guangzhon, China, 339-351.
- Wu, W., Hu, J., Gu, X., Zhao, H. and Gu, G. (1987). Cultivation of anaerobic granular sludge in UASB reactors with aerobic activated sludge as seed. *Water Research*, 21, 7: 789-799.
- Wu, M., Voice, T.C., and Hickey, R.F. (1992). Hydraulic characteristics of an upflow anaerobic sludge blanket (UASB) reactor with granular sludge. *Proceedings of the 47th Industrial Waste Conference*, Purdue University, 425-435.
- Yan, I.Q., Lo, K.V. and Liao, P.H. (1985). Anaerobic digestion of cheese whey using an upflow anaerobic sludge bed reactor. *Biological Wastes*, 27, 289-305.
- Yang, G. and Anderson, G.K. (1993). Effects of wastewater composition on stability of UASB reactors. *Journal of Environmental Engineering*, 119, 5: 958-977.
- de Zeeuw, W. and Lettinga, G. (1983). Acclimation of digested sewage sludge during start-up of an upflow anaerobic sludge blanket (UASB) reactor. *Proceedings of the 35th Industrial Waste Conference*, Purdue University, 39-47.
- de Zeeuw, W.J. (1985). Acclimation of anaerobic sludge for UASB reactor start-up. *Netherlands Journal of Agricultural Sciences*, 33, 81-84.

## VII. APPENDIX

### LIST OF FIGURES

Figure A.1: Effluent COD versus Time (5 day HRT) .....	80
Figure A.2: COD Reduction versus Time (5 day HRT).....	80
Figure A.3: Effluent BOD versus Time (5 day HRT).....	81
Figure A.4: BOD Reduction versus Time (5 day HRT).....	81
Figure A.5: Effluent Volatile Solids versus Time (5 day HRT).....	82
Figure A.6: Volatile Solids Reduction versus Time (5 day HRT).....	82
Figure A.7: Effluent Volatile Suspended Solids versus Time (5 day HRT).....	83
Figure A.8: Volatile Suspended Solids Reduction versus Time (5 day HRT).....	83
Figure A.9: Biogas Methane Composition versus Time (5 day HRT).....	84
Figure A.10: Volumetric Methane Production versus Time (5 day HRT).....	85
Figure A.11: Methane Yield versus Time (5 day HRT).....	85
Figure A.12: Volatile Fatty Acids versus Time ( Reactor A : 5 day HRT).....	86
Figure A.13: Volatile Fatty Acids versus Time (Reactor B : 5 day HRT).....	86
Figure A.14: Propionic-acetic Acid Ratio versus Time (5 day HRT).....	87
Figure A.15: Effluent pH versus Time (5 day HRT).....	87
Figure B.1: Effluent COD versus Time (3 day HRT).....	88
Figure B.2: COD Reduction versus Time (3 day HRT).....	88
Figure B.3: Effluent BOD versus Time (3 day HRT).....	89
Figure B.4: BOD Reduction versus Time (3 day HRT).....	89
Figure B.5: Effluent Volatile Solids versus Time (3 day HRT).....	90
Figure B.6: Volatile Solids Reduction versus Time (3 day HRT).....	90
Figure B.7: Effluent Volatile Suspended Solids versus Time (3 day HRT).....	91
Figure B.8 Volatile Suspended Solids versus Time (3 day HRT).....	91
Figure B.9: Biogas Methane Composition versus Time (3 day HRT).....	92
Figure B.10: Volumetric Methane Composition versus Time (3 day HRT).....	93
Figure B.11: Methane Yield versus Time (3 day HRT).....	93
Figure B.12: Volatile Fatty Acids versus Time (Reactor A : 3 day HRT).....	94
Figure B.13 : Volatile Fatty Acids versus Time (Reactor B : 3 day HRT).....	94
Figure B.14: Propionic-acetic Acid ratio versus Time (3 day HRT).....	95
Figure B.15: Effluent pH versus Time (3 day HRT).....	95
Figure C.1: Effluent COD versus Time (1.5 day HRT).....	96
Figure C.2: COD Reduction versus Time (1.5 day HRT).....	96
Figure C.3: Effluent BOD versus Time (1.5 day HRT).....	97
Figure C.4: BOD Reduction versus Time (1.5 day HRT).....	97
Figure C.5: Effluent Volatile Solids versus Time (1.5 day HRT).....	98
Figure C.6: Volatile Solids Reduction versus Time (1.5 day HRT).....	98
Figure C.7: Effluent Volatile Suspended Solids versus Time (1.5 day HRT).....	99
Figure C.8: Volatile Suspended Solids Reduction versus Time (1.5 day HRT).....	99
Figure C.9: Biogas Methane Composition versus Time (1.5 day HRT).....	100
Figure C.10: Volumetric Methane Composition versus Time (1.5 day HRT).....	101

Figure C.11: Methane Yield versus Time (1.5 day HRT).....	101
Figure C.12: Volatile Fatty Acids versus Time (Reactor A : 1.5 day HRT).....	102
Figure C.13: Volatile Fatty Acids versus Time (Reactor B : 1.5 day HRT).....	102
Figure C.14: Propionic-acetic Acid Ratio versus Time (1.5 day HRT).....	103
Figure C.15: Effluent pH versus Time (1.5 day HRT).....	103
Figure D.1: Effluent COD versus Time (18 hour HRT).....	104
Figure D.2: COD Reduction versus Time (18 hour HRT).....	104
Figure D.3: Effluent BOD versus Time (18 hour HRT).....	105
Figure D.4: BOD Reduction versus Time (18 hour HRT).....	105
Figure D.5: Effluent Volatile Solids versus Time (18 hour HRT).....	106
Figure D.6: Volatile Solids Reduction versus Time (18 hour HRT).....	106
Figure D.7: Effluent Volatile Suspended Solids versus Time (18 hour HRT).....	107
Figure D.8: Volatile Suspended Solids Reduction versus Time (18 hour HRT).....	107
Figure D.9: Biogas Methane Composition versus Time (18 hour HRT).....	108
Figure D.10: Volumetric Methane Composition versus Time (18 hour HRT).....	109
Figure D.11: Methane Yield versus Time (18 hour HRT).....	109
Figure D.12: Volatile Fatty Acids versus Time (Reactor A : 18 hour HRT).....	110
Figure D.13: Volatile Fatty Acid versus Time (Reactor B : 18 hour HRT).....	110
Figure D.14: Propionic-acetic Acid Ratio versus Time (18 hour HRT).....	111
Figure D.15: Effluent pH versus Time (18 hour HRT).....	111
Figure E.1: Effluent COD versus Time (12 hour HRT).....	112
Figure E.2: COD Reduction versus Time (12 hour HRT).....	112
Figure E.3: Effluent BOD versus Time (12 hour HRT).....	113
Figure E.4: BOD Reduction versus Time (12 hour HRT).....	113
Figure E.5: Effluent Volatile Solids versus Time (12 hour HRT).....	114
Figure E.6: Volatile Solids Reduction versus Time (12 hour HRT).....	114
Figure E.7: Effluent Volatile Suspended Solids versus Time (12 hour HRT).....	115
Figure E.8: Volatile Suspended Solids Reduction versus Time (12 hour HRT).....	115
Figure E.9: Biogas Methane Composition versus Time (12 hour HRT).....	116
Figure E.10: Volumetric Methane Production versus Time (12 hour HRT).....	117
Figure E.11: Methane Yield versus Time (12 hour HRT).....	117
Figure E.12: Volatile Fatty Acids versus Time (Reactor A : 12 hour HRT).....	118
Figure E.13: Volatile Fatty Acids versus Time (Reactor B : 12 hour HRT).....	118
Figure E.14: Propionic-acetic Acid Ratio versus Time (12 hour HRT).....	119
Figure E.15: Effluent pH versus Time (12 hour HRT).....	119

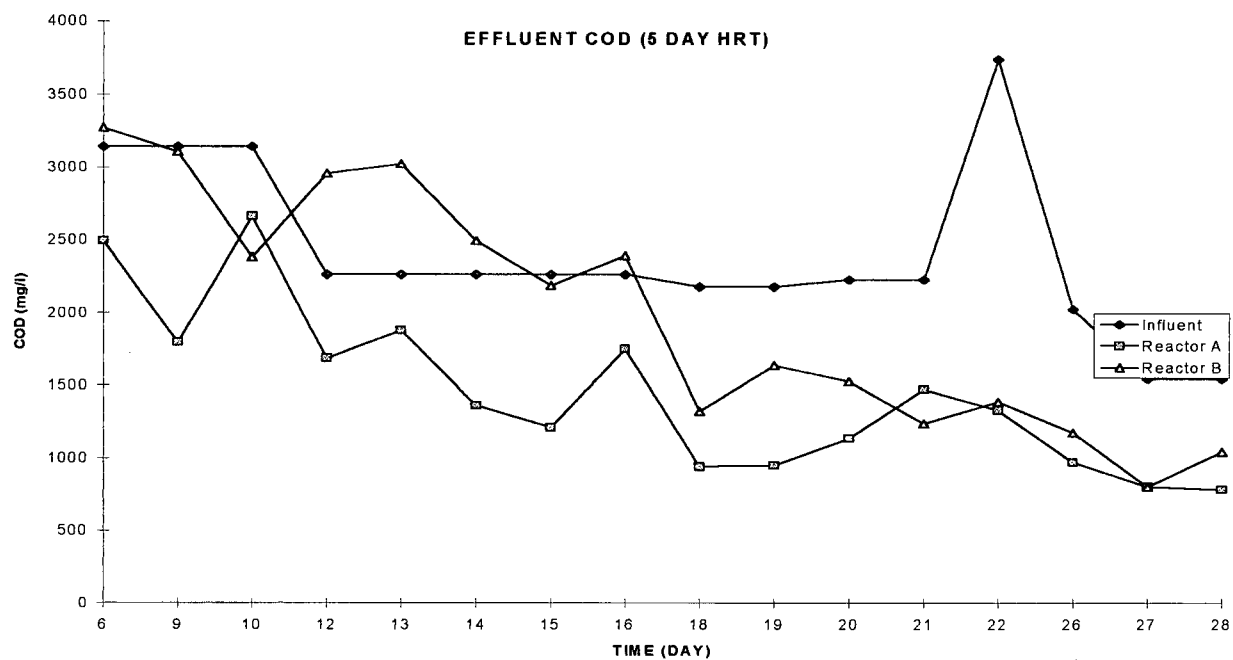


Figure A.1 Effluent COD vs Time (5 day HRT)

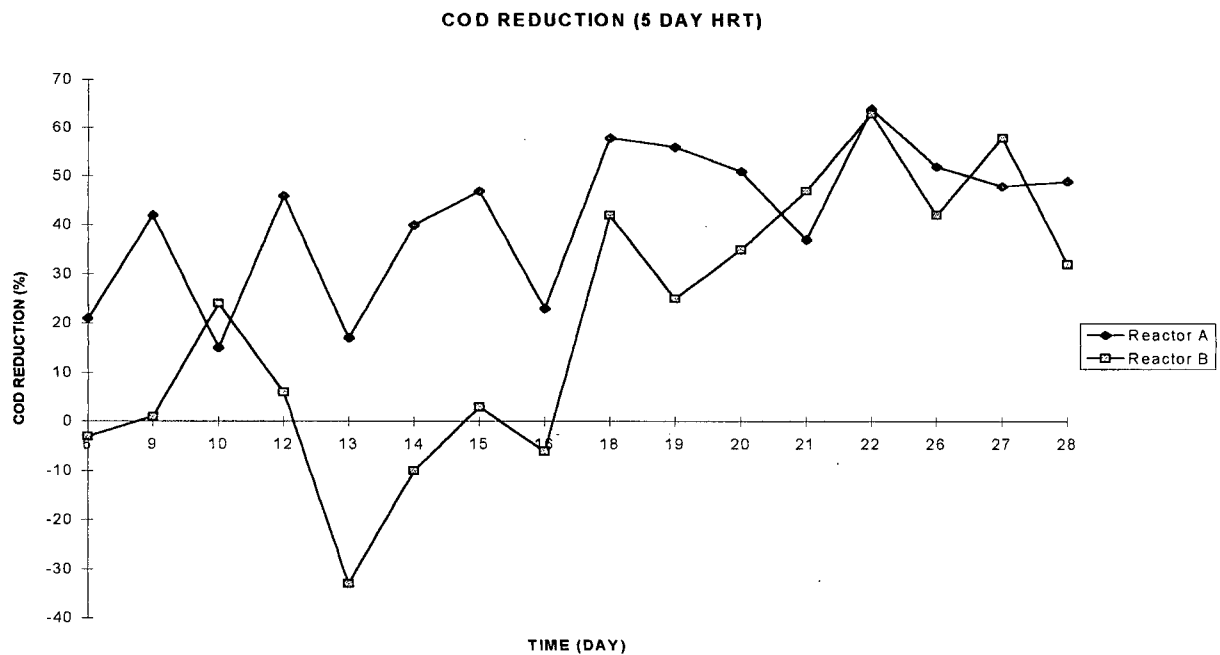


Figure A.2 COD Reduction vs Time (5 day HRT).



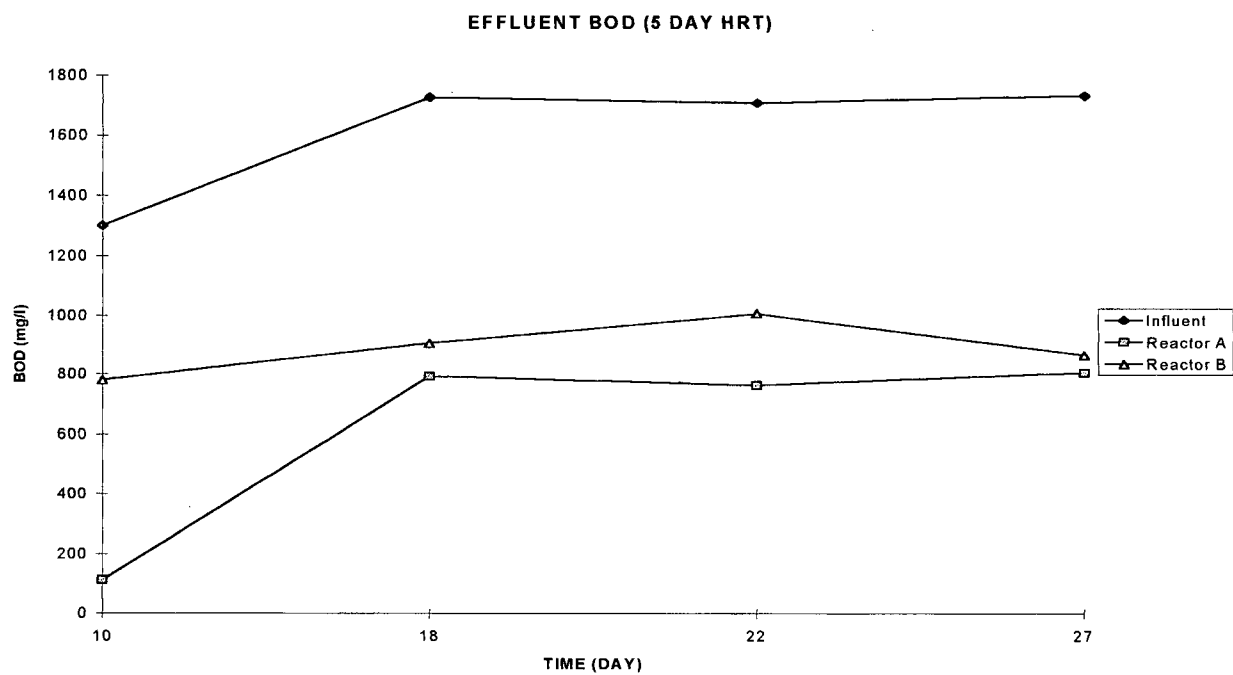


Figure A.3 Effluent BOD vs Time (5 day HRT)

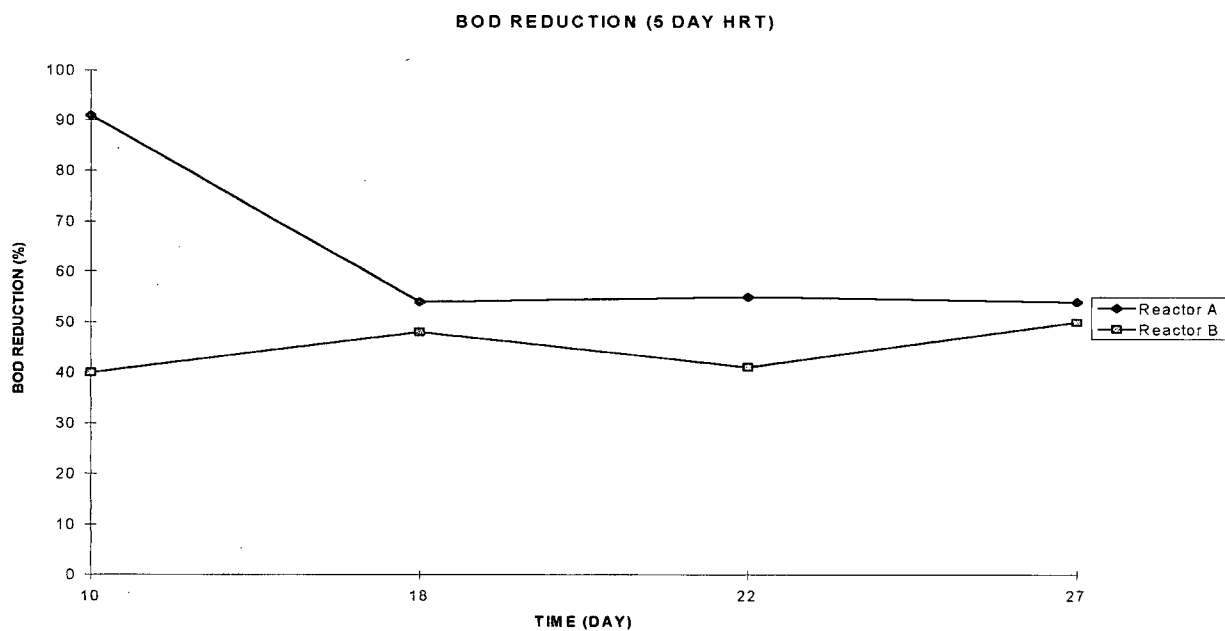


Figure A.4 BOD Reduction vs Time (5 day HRT)

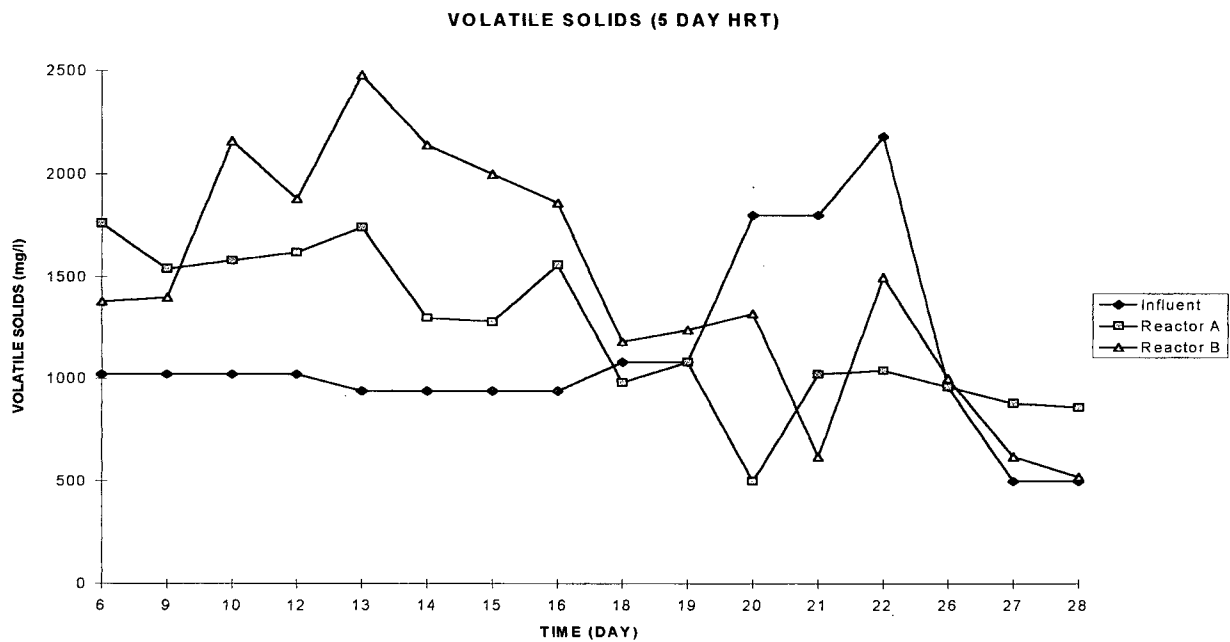


Figure A.5 Volatile Solids vs Time (5 day HRT)

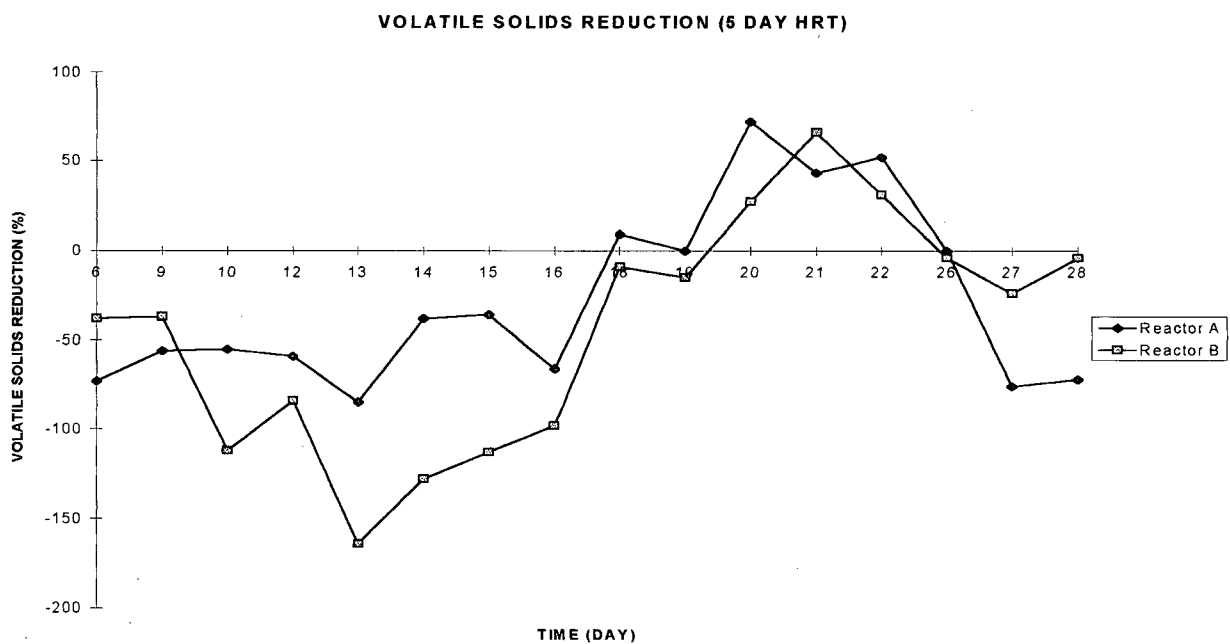


Figure A.6 Volatile Solids Reduction vs Time (5 day HRT)

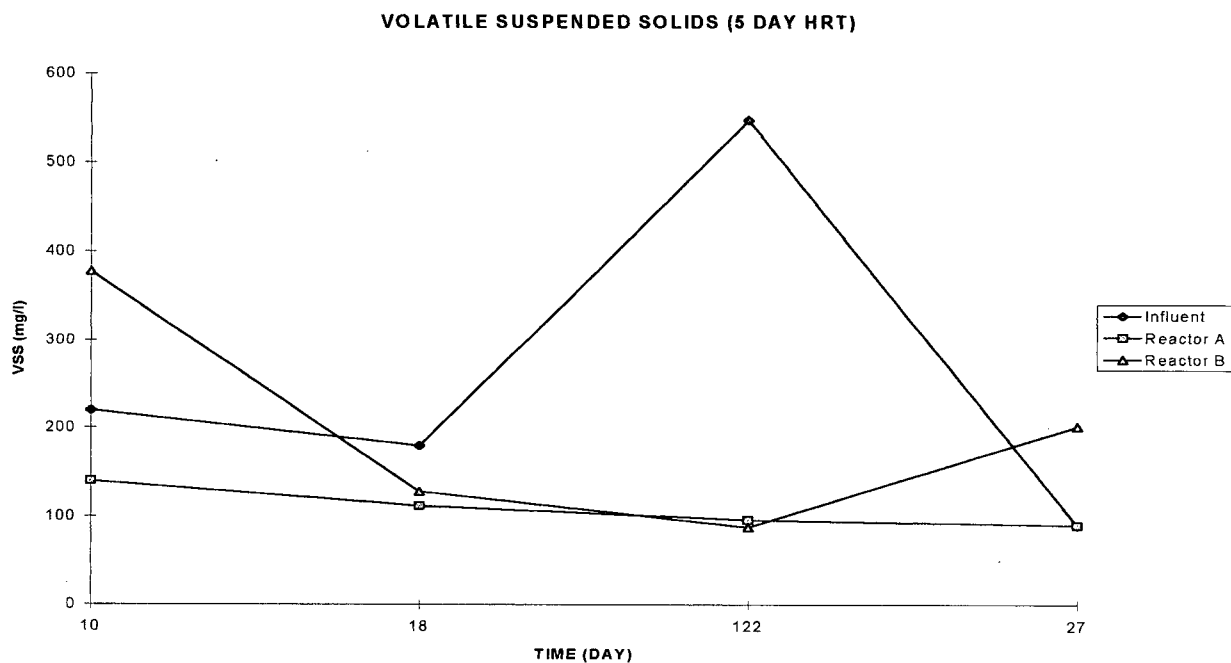


Figure A.7 Volatile Suspended Solids vs Time (5 day HRT)

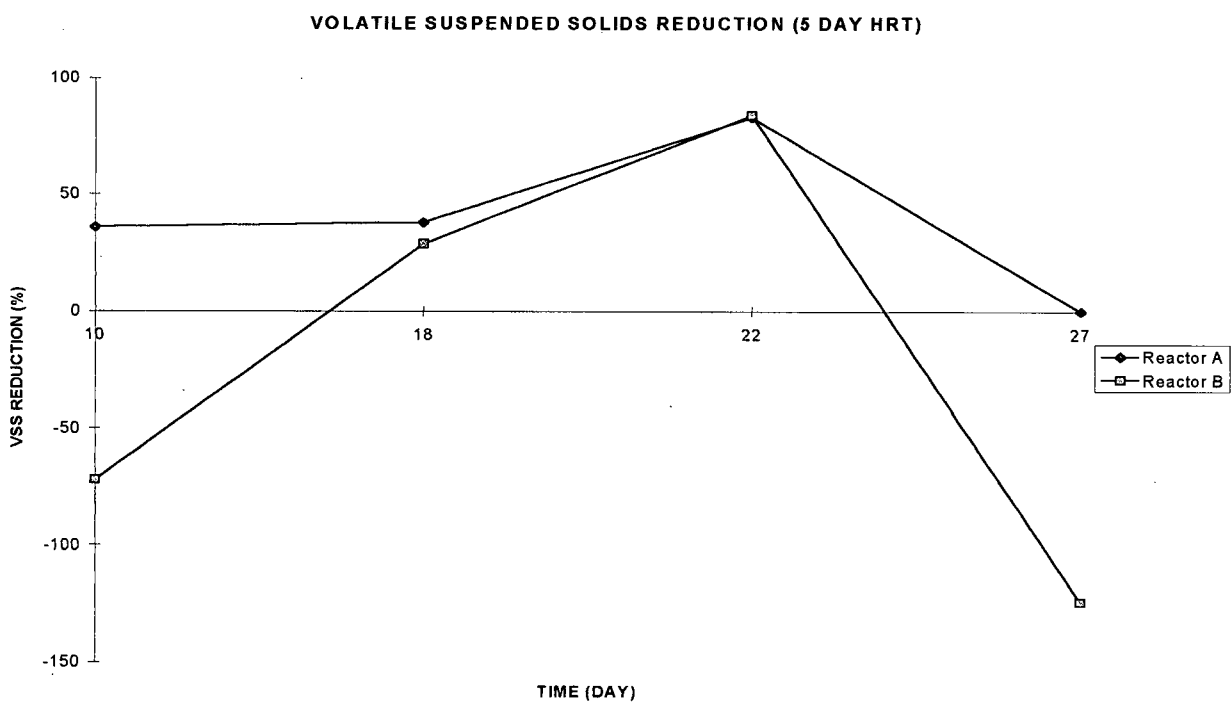


Figure A.8 Volatile Suspended Solids Reduction vs Time (5 day HRT)

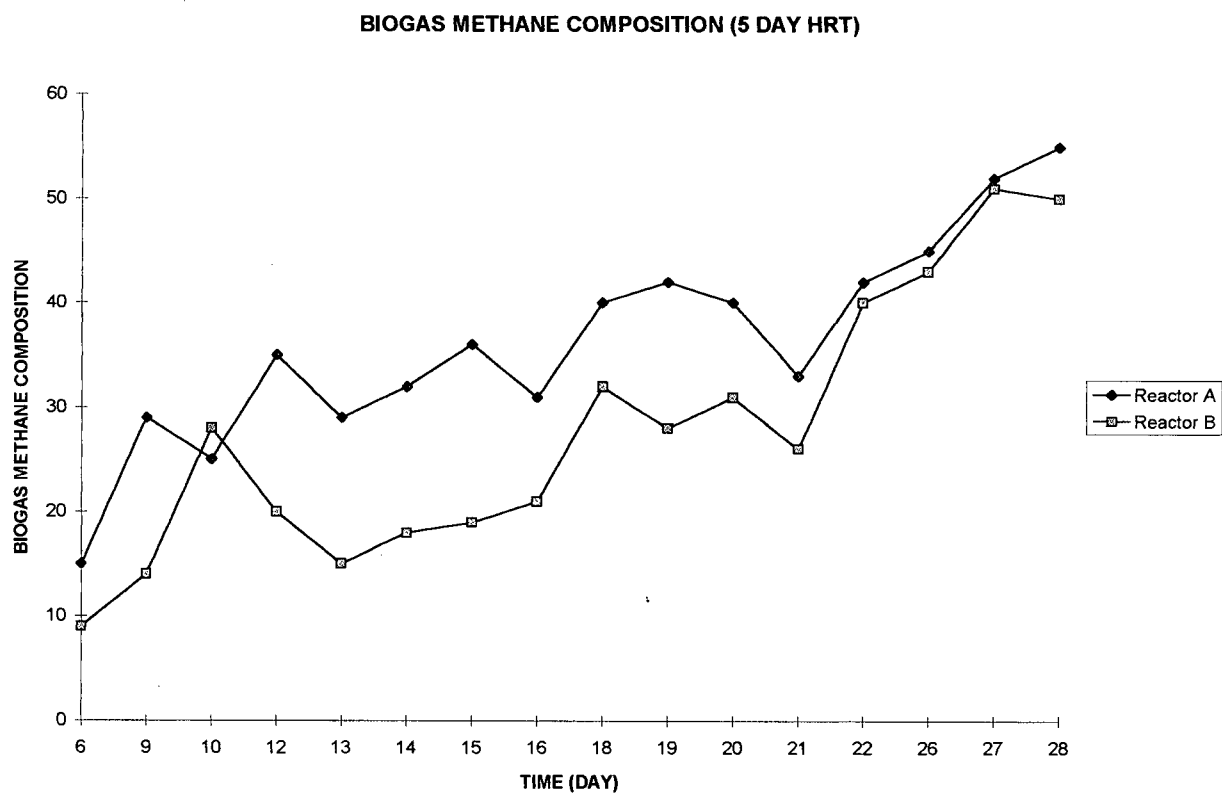


Figure A.9 Biogas Methane Composition vs Time (5 day HRT)

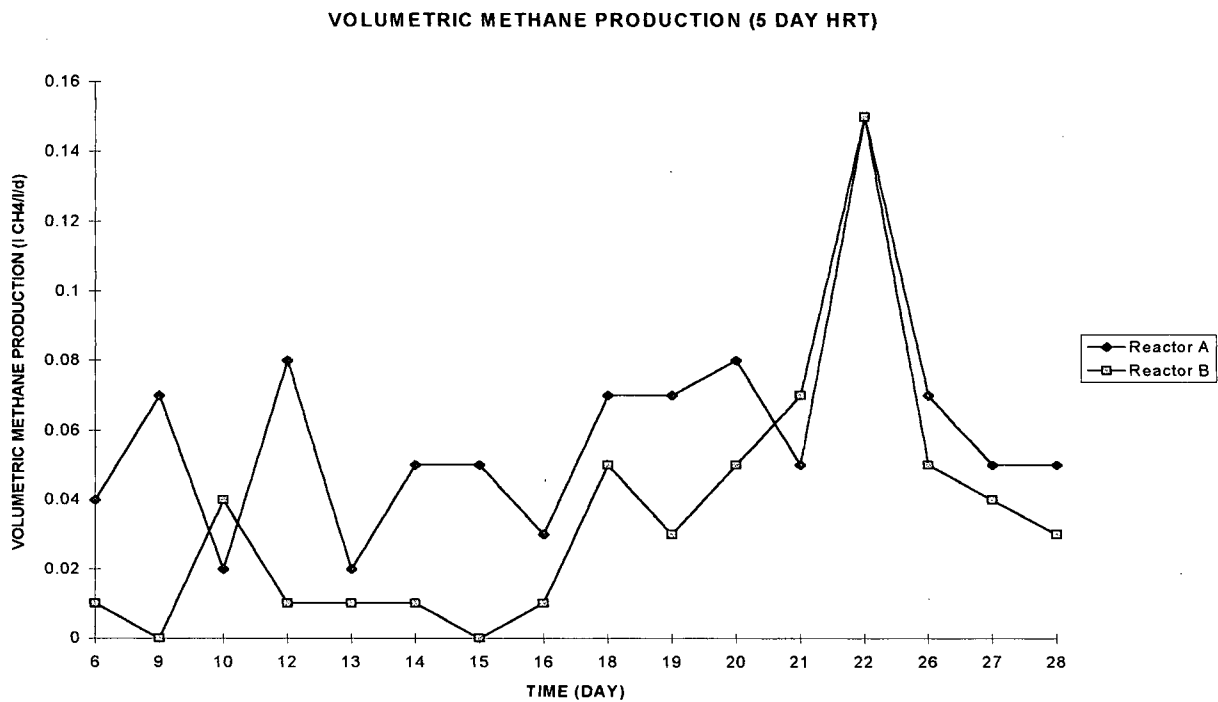


Figure A.10 Volumetric Methane Production vs Time (5 day HRT)

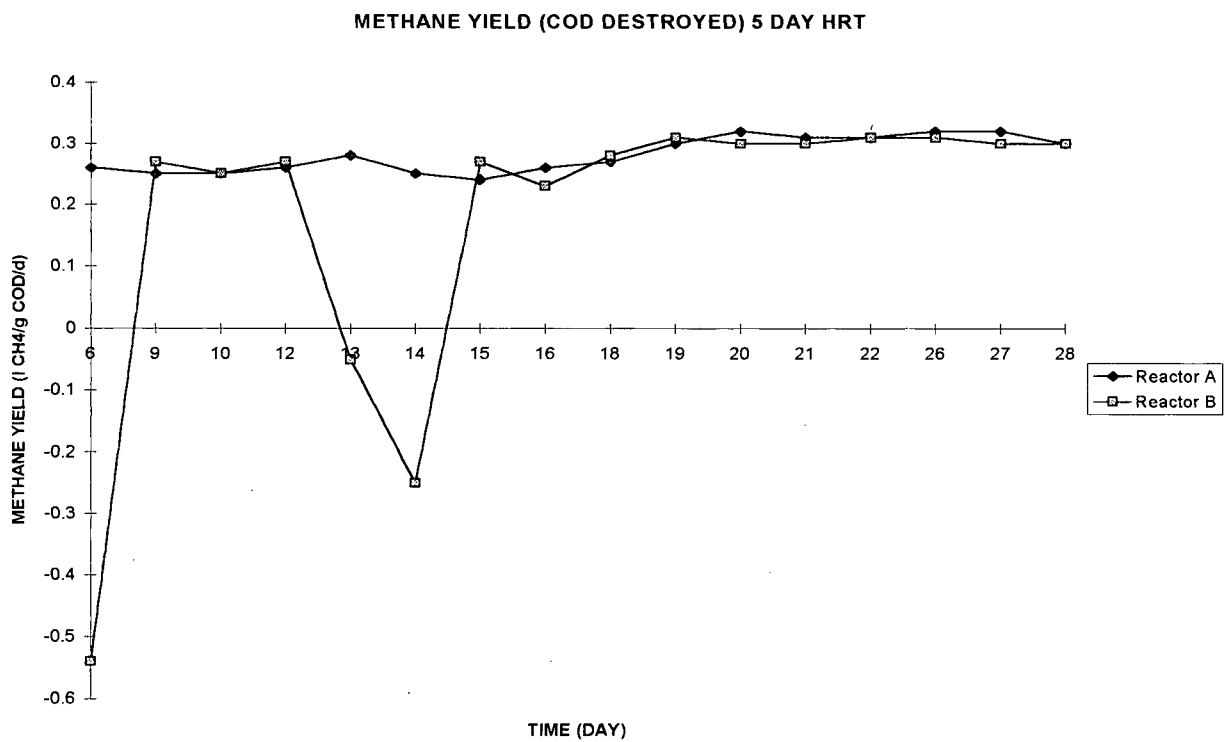


Figure A.11 Methane Yield vs Time (5 day HRT)

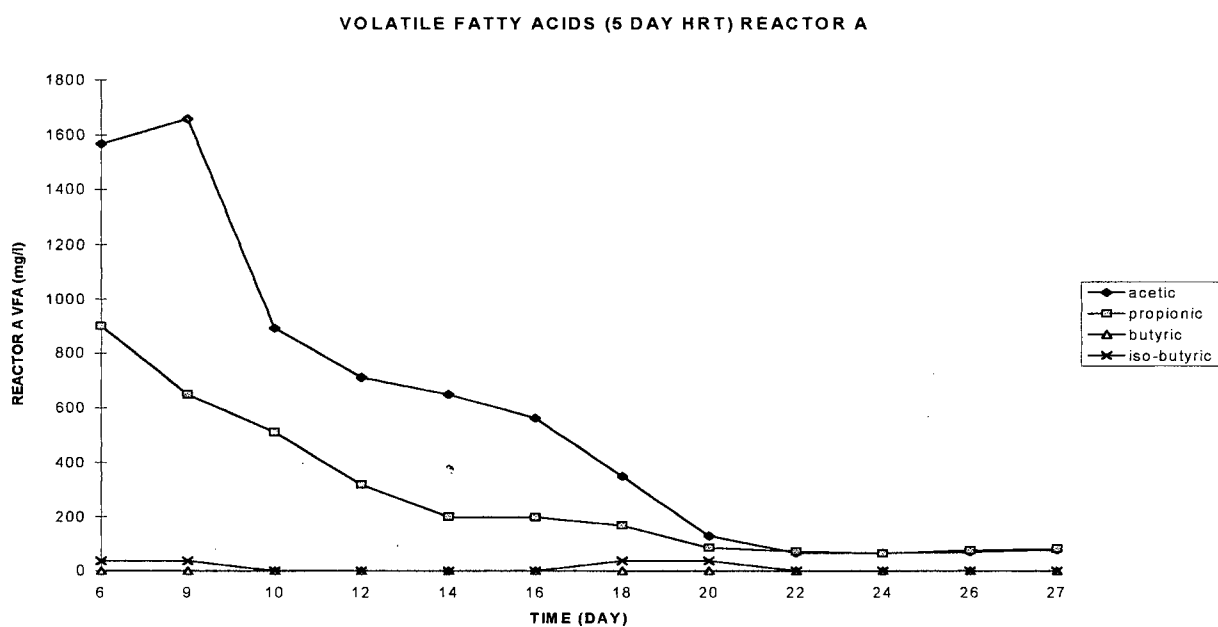


Figure A.12 VFA concentration vs Time (Reactor A : 5 day HRT)

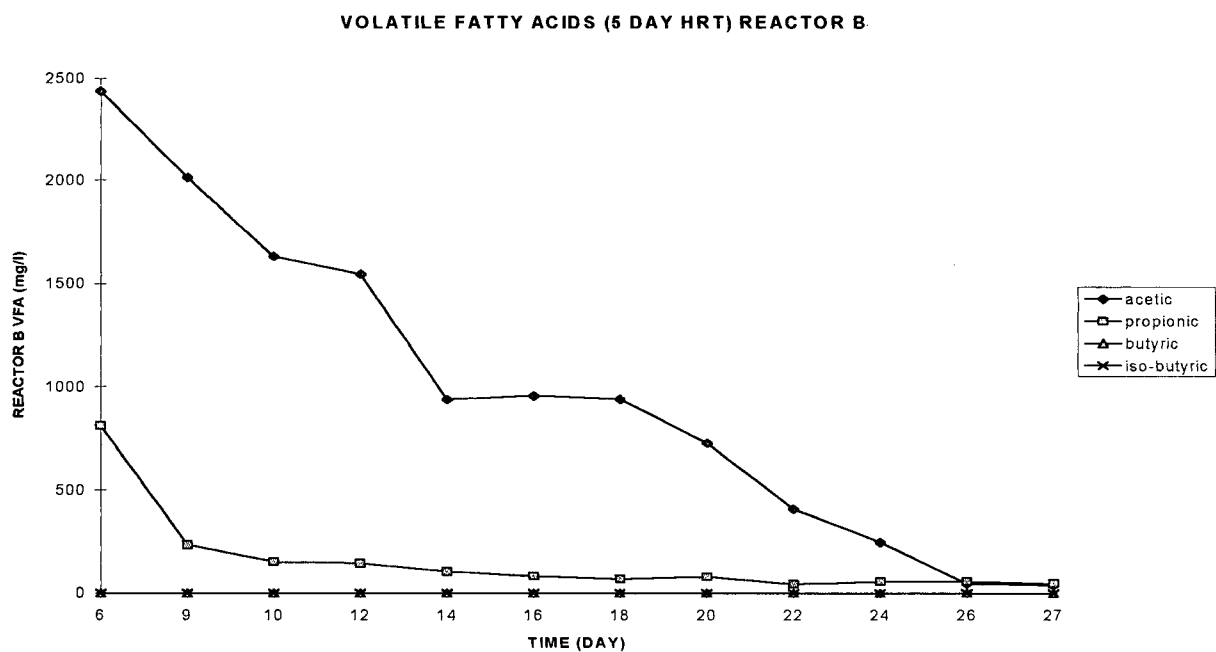


Figure A.13 VFA concentration vs Time (Reactor B : 5 day HRT)

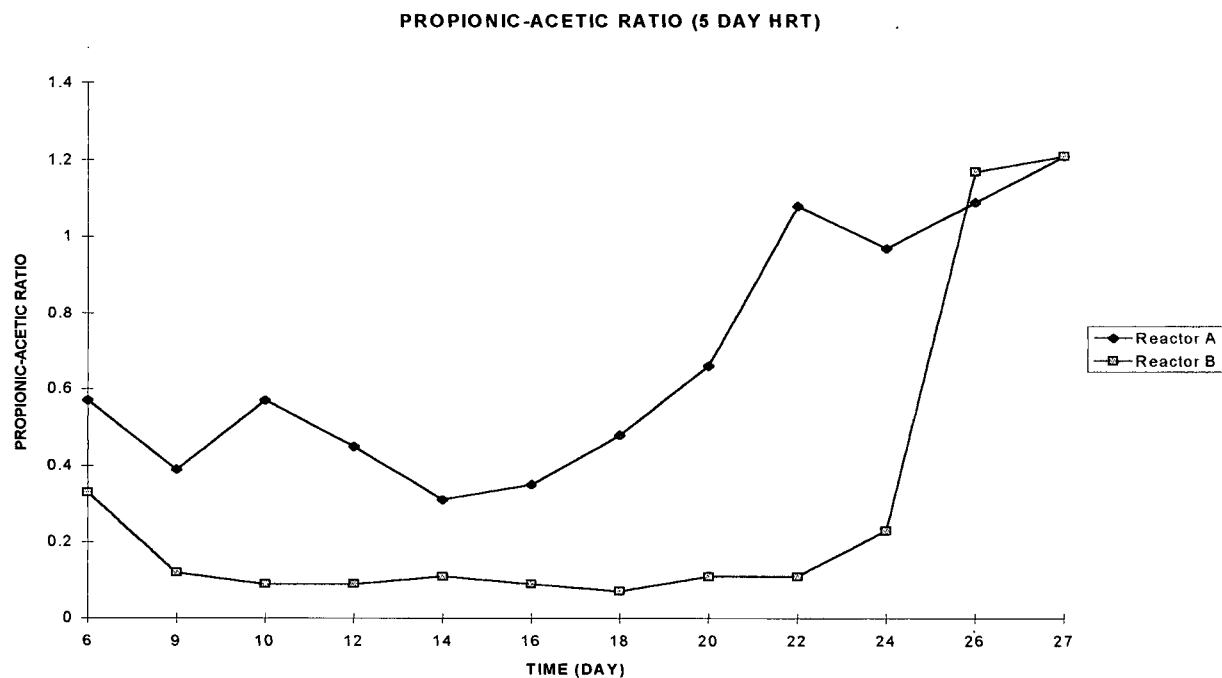


Figure A.14 Propionic-acetic acid Ratio vs Time (5 day HRT)

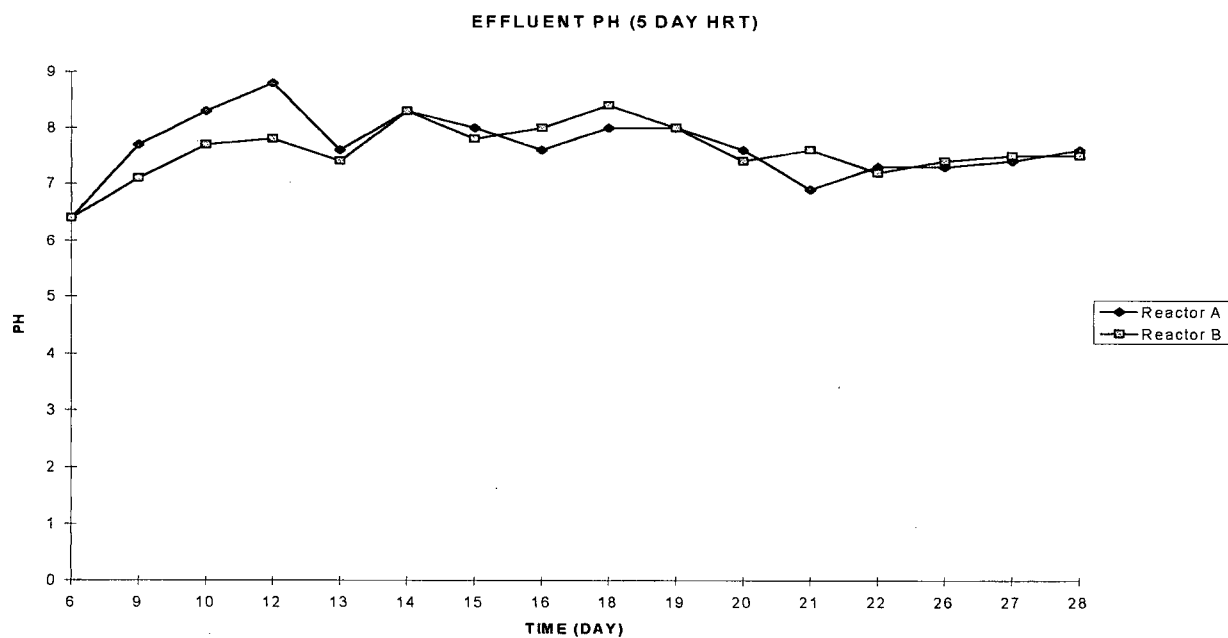


Figure A.15 Effluent pH vs Time (5 day HRT)

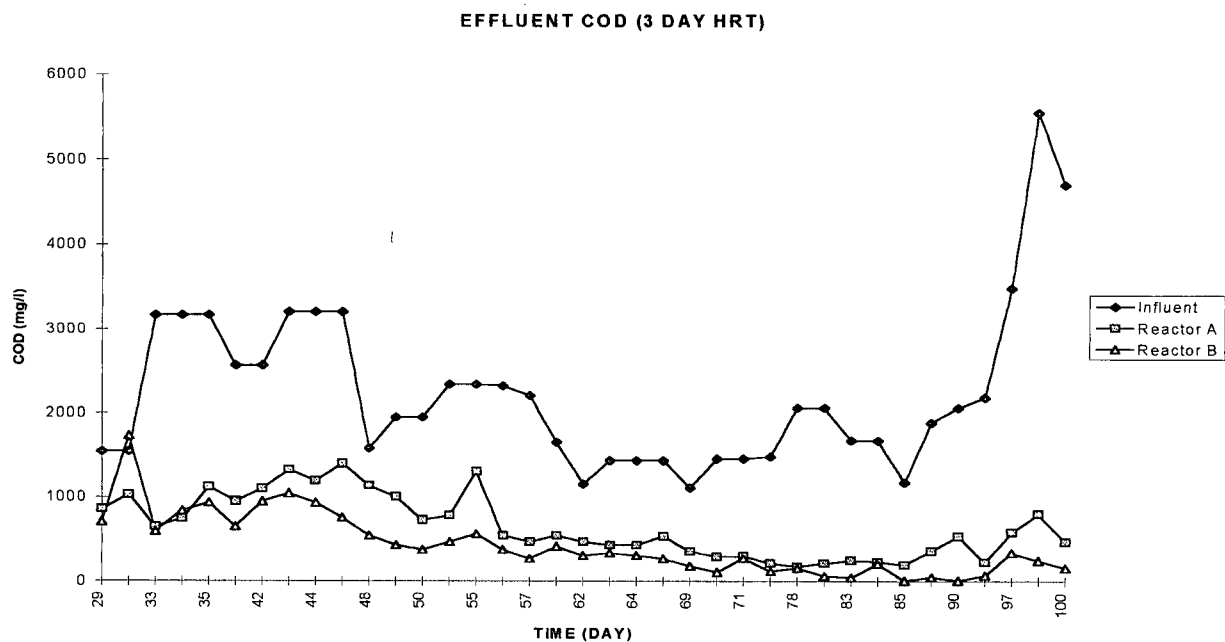


Figure B.1 Effluent COD vs Time (3 day HRT)

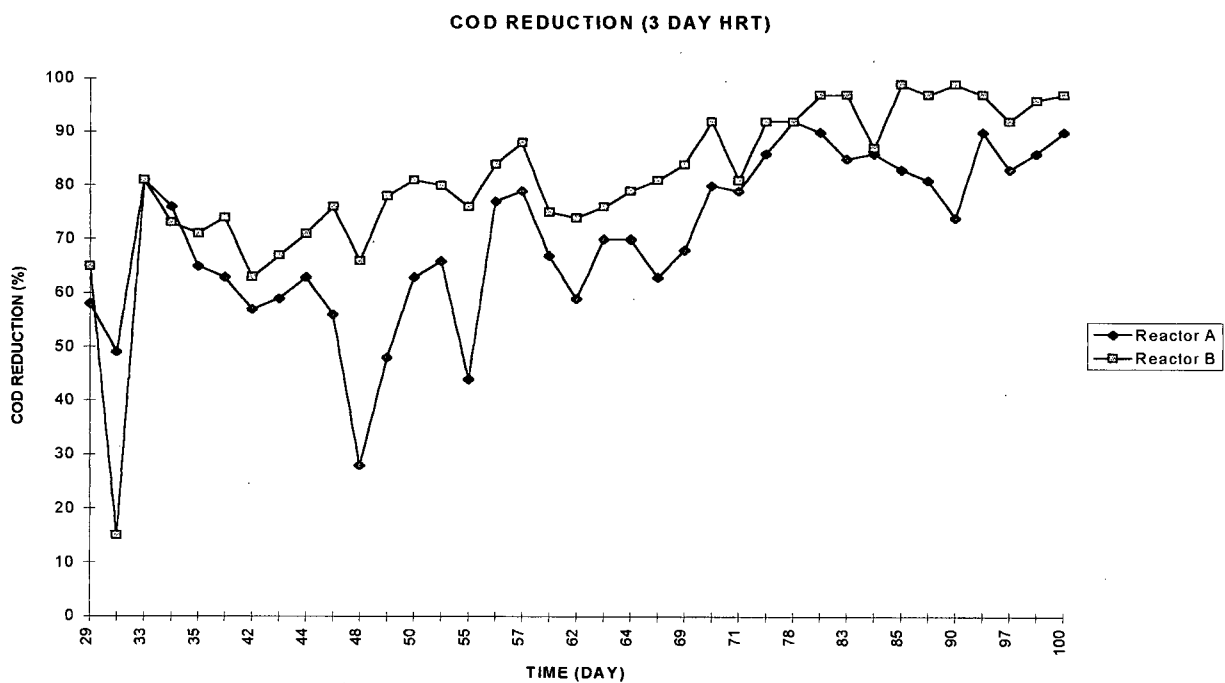


Figure B.2 BOD Reduction vs Time (3 day HRT)



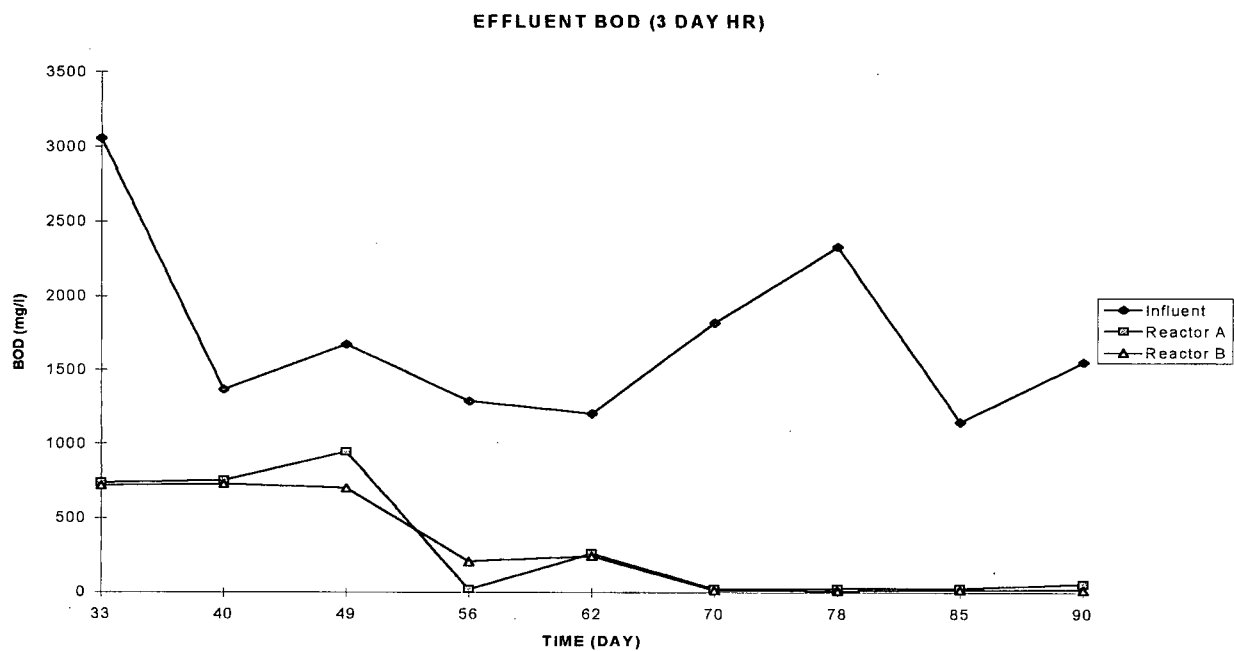


Figure B.3 Effluent BOD vs Time (3 day HRT)

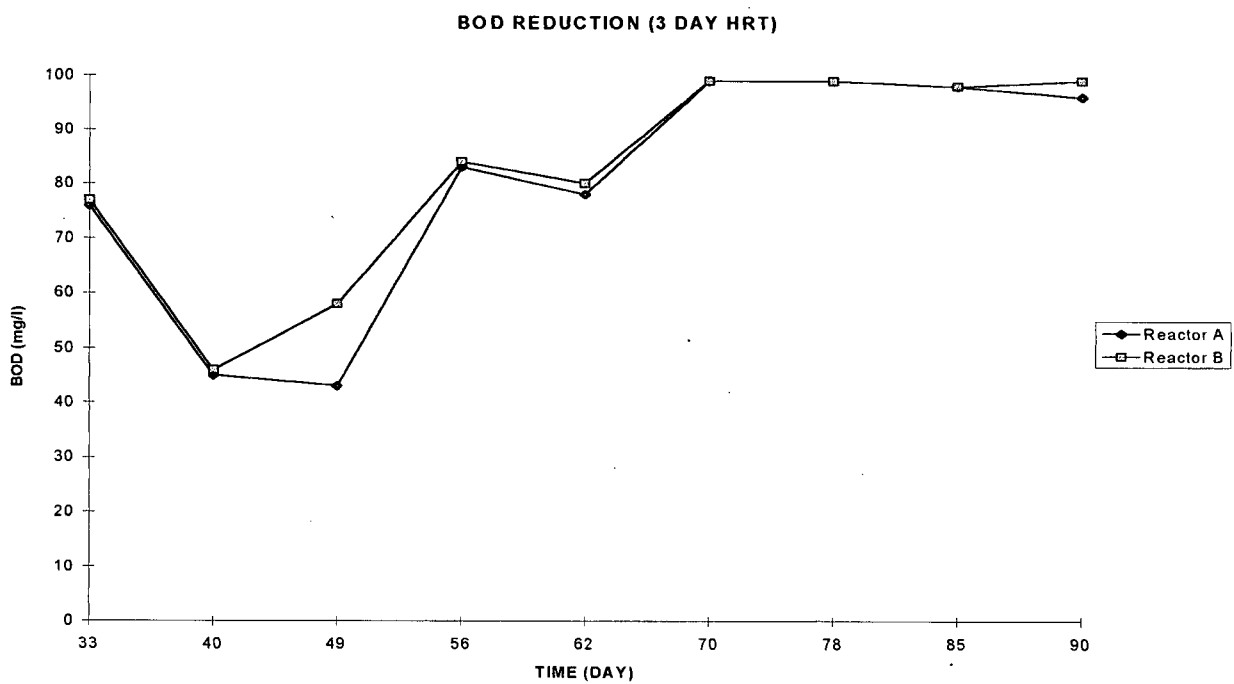


Figure B.4 BOD Reduction vs Time (3 day HRT)

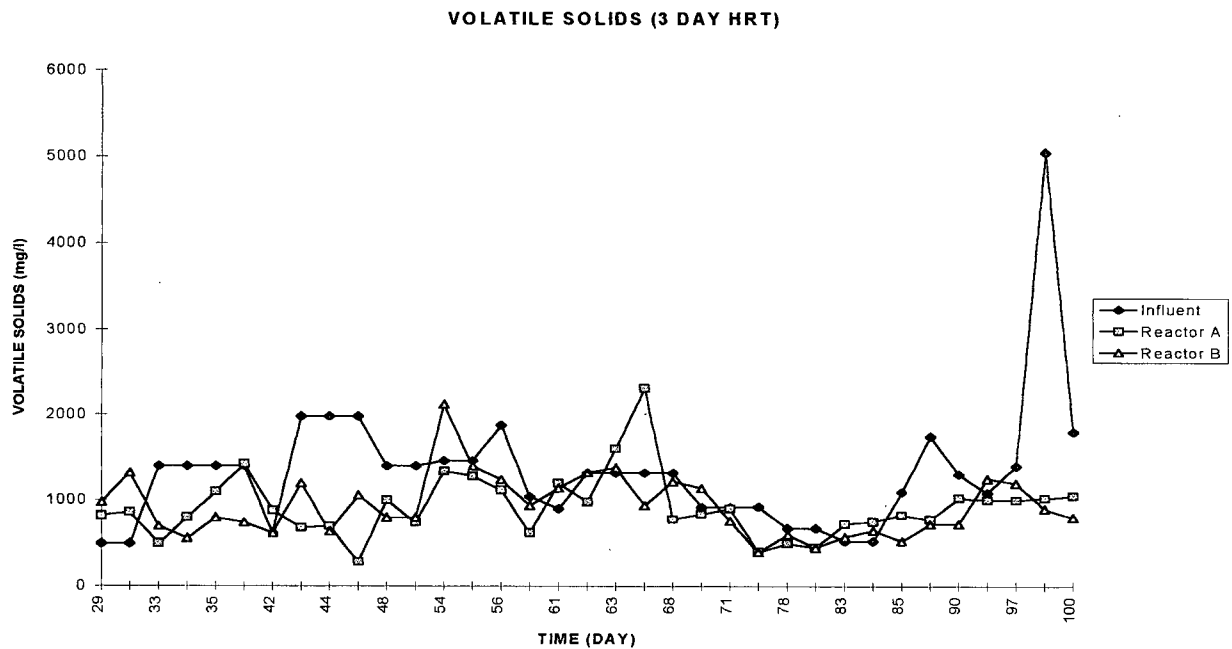


Figure B.5 Volatile Solids vs time (3 day HRT)

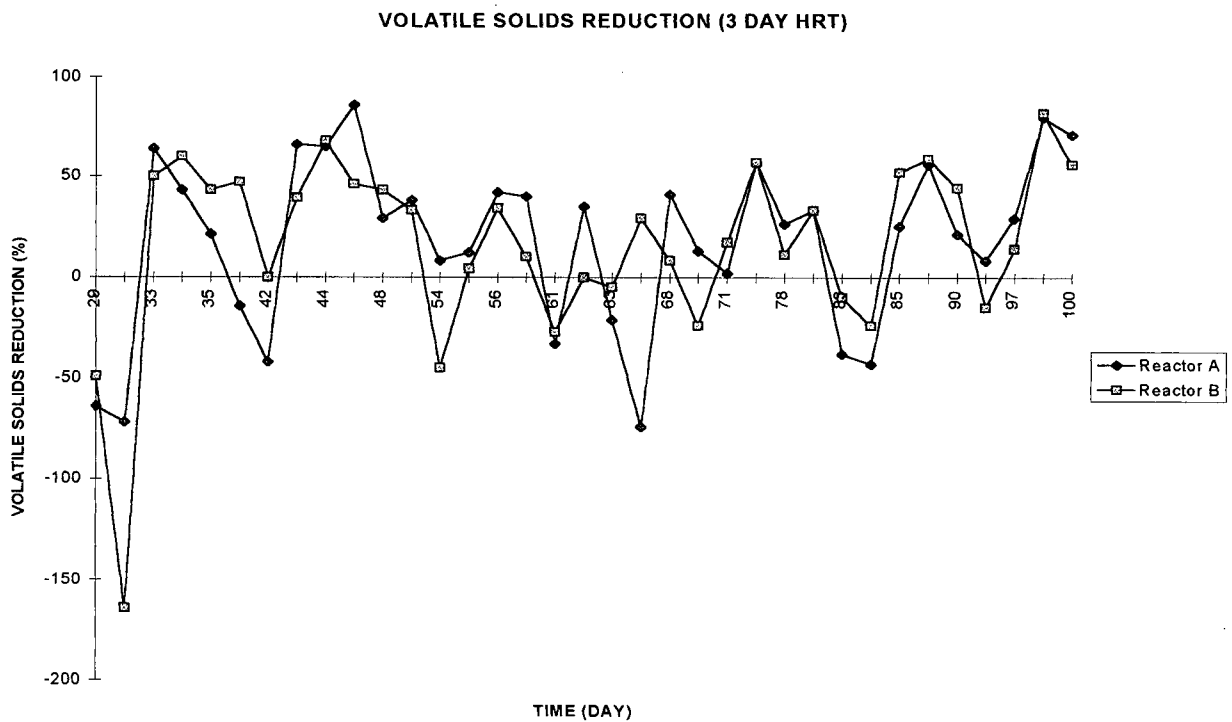


Figure B.6 Volatile Solids Reduction vs time (3 day HRT)

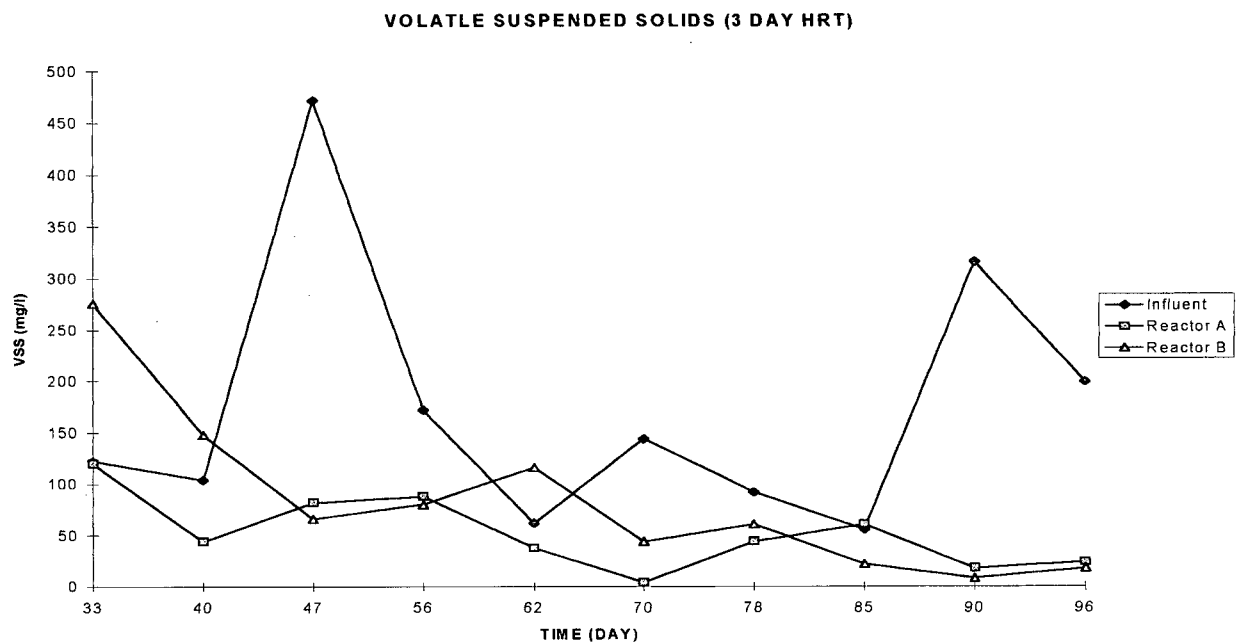


Figure B.7 Volatile Suspended Solids vs Time (3 day HRT)



Figure B.8 Volatile Suspended Solids Reduction vs Time (3 day HRT)

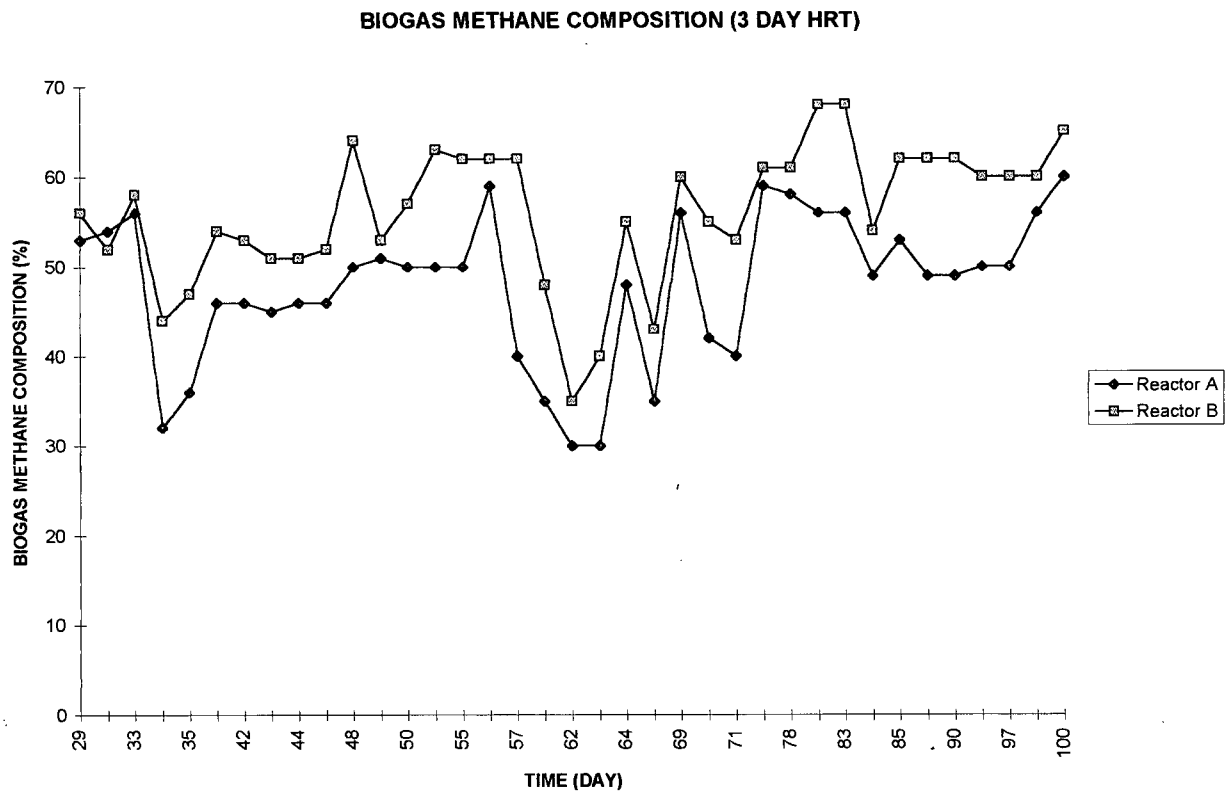


Figure B.9 Biogas Methane Composition vs Time (3 day HRT)

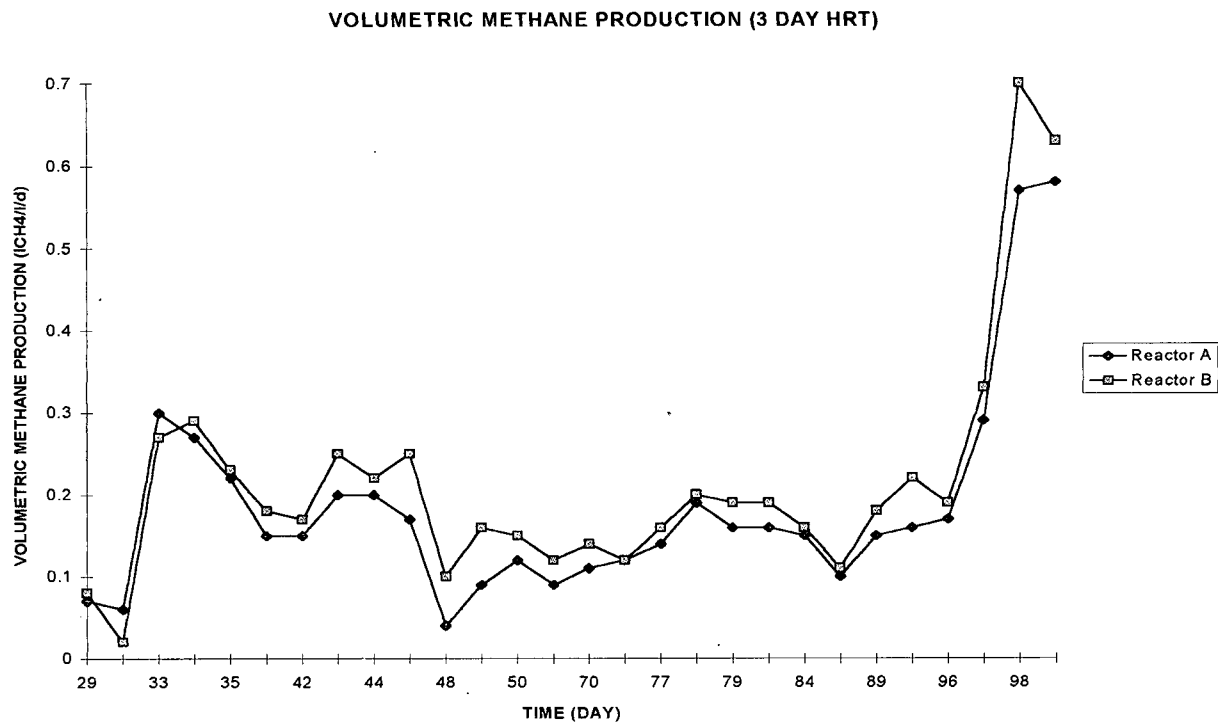


Figure B.10 Volumetric Methane Production vs Time (3 day HRT)

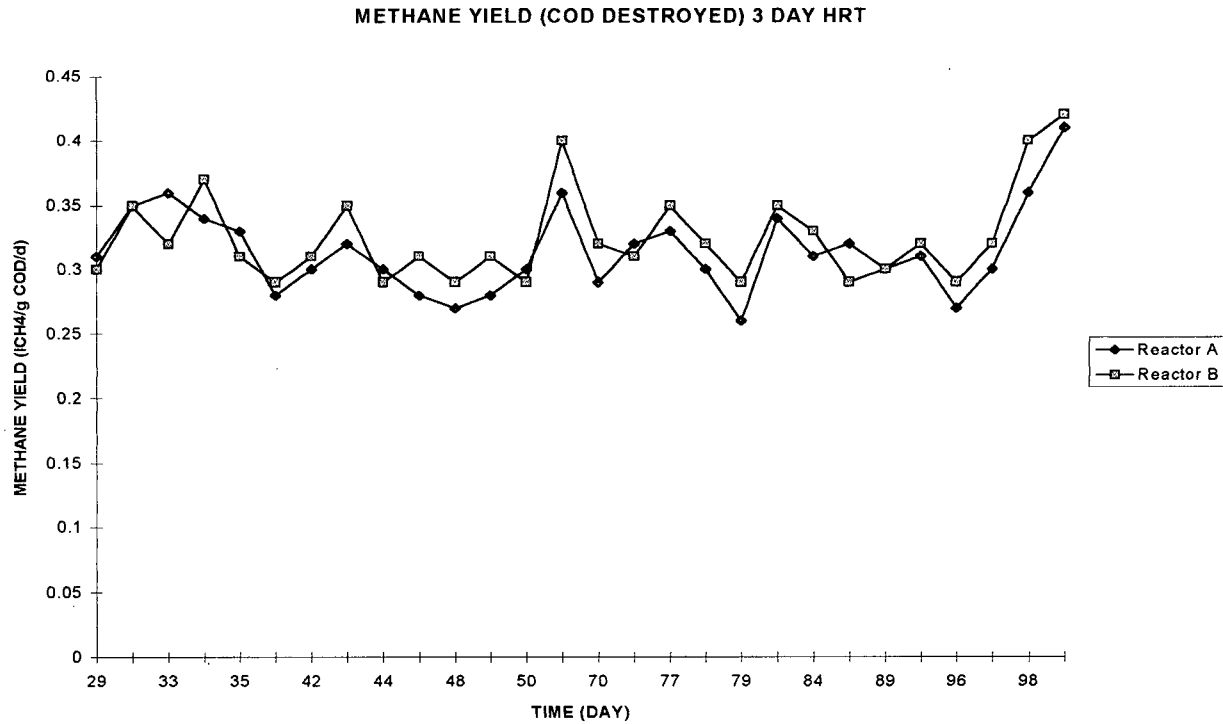


Figure B.11 Methane Yield vs Time (3 day HRT)

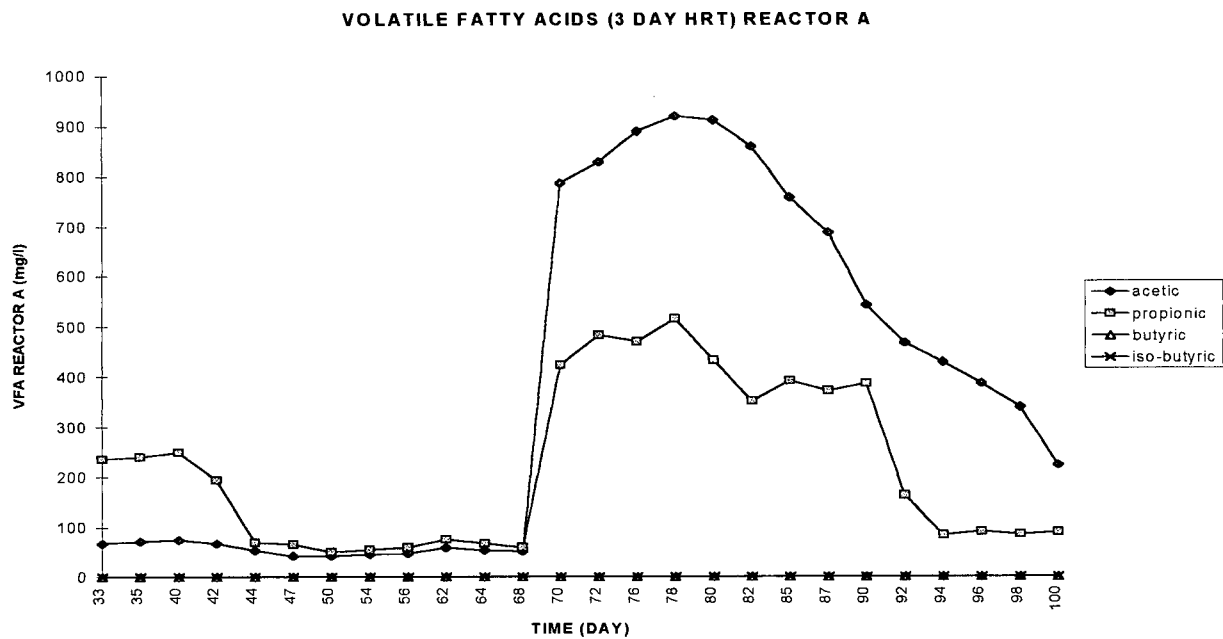


Figure B.12 VFA concentration vs Time ( Reactor A : 3 day HRT)

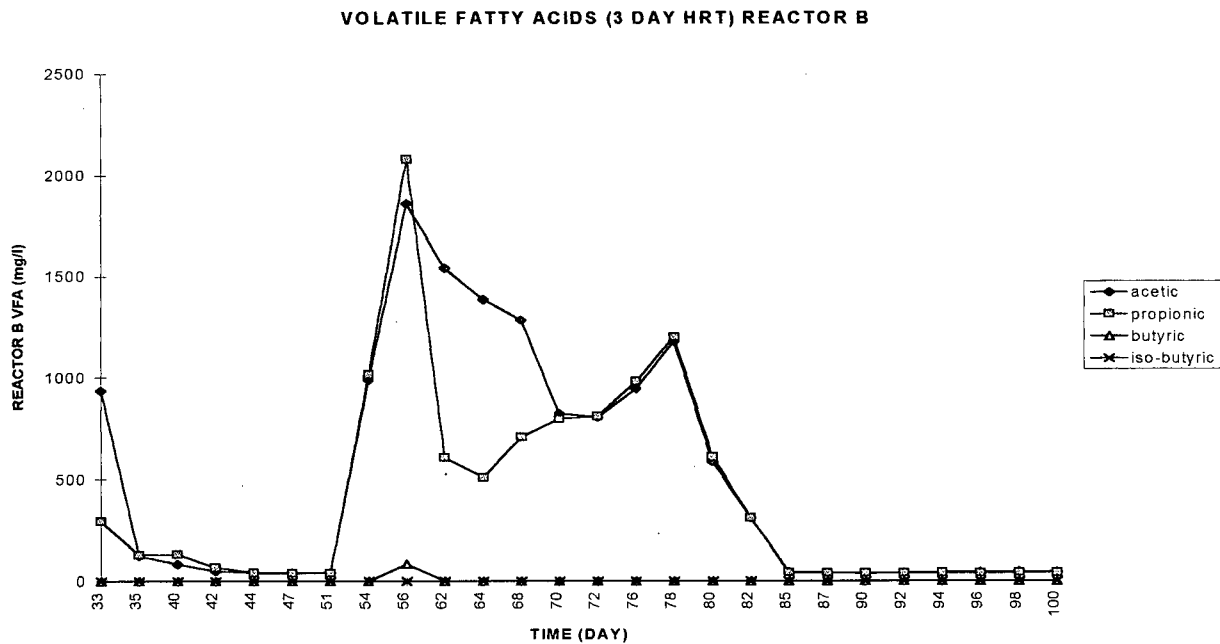


Figure B.13 VFA concentration vs Time (Reactor B : 3 day HRT)

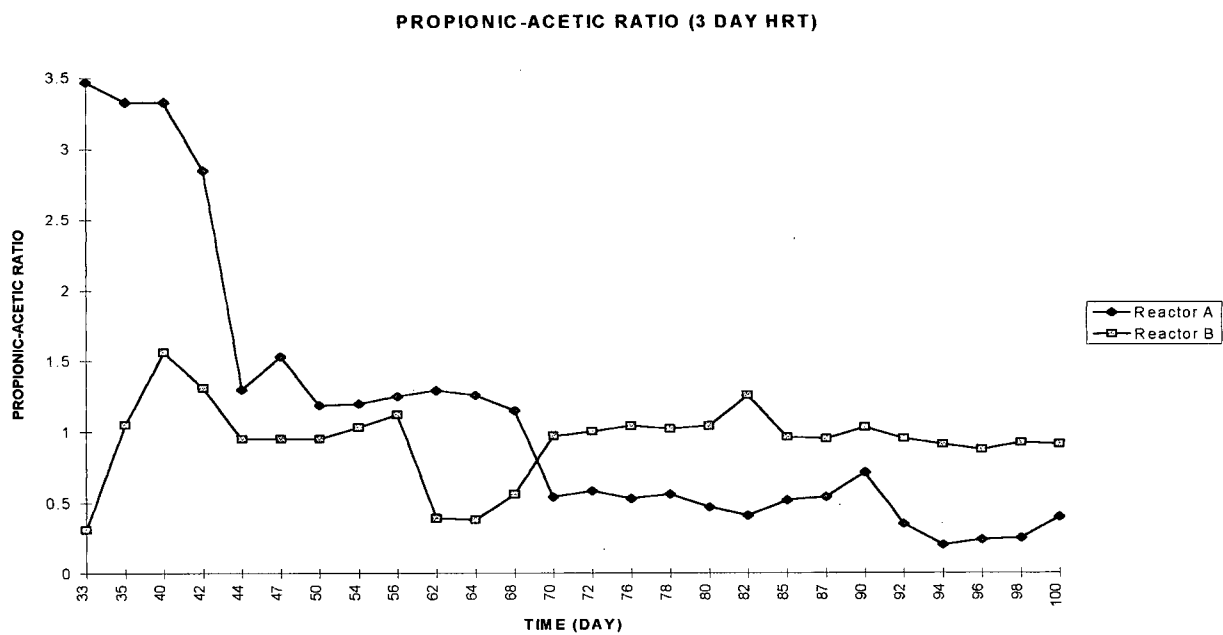


Figure B.14 Propionic-acetic acid Ratio vs Time (3 day HRT)

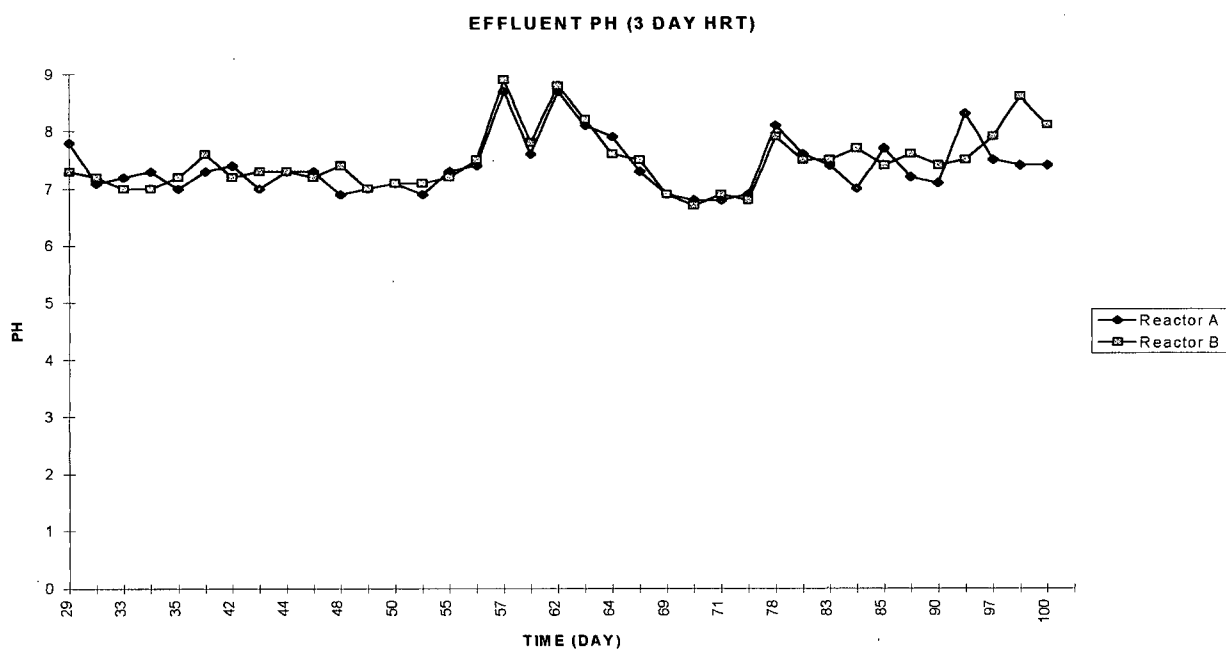


Figure B.15 Effluent pH vs Time (3 day HRT)

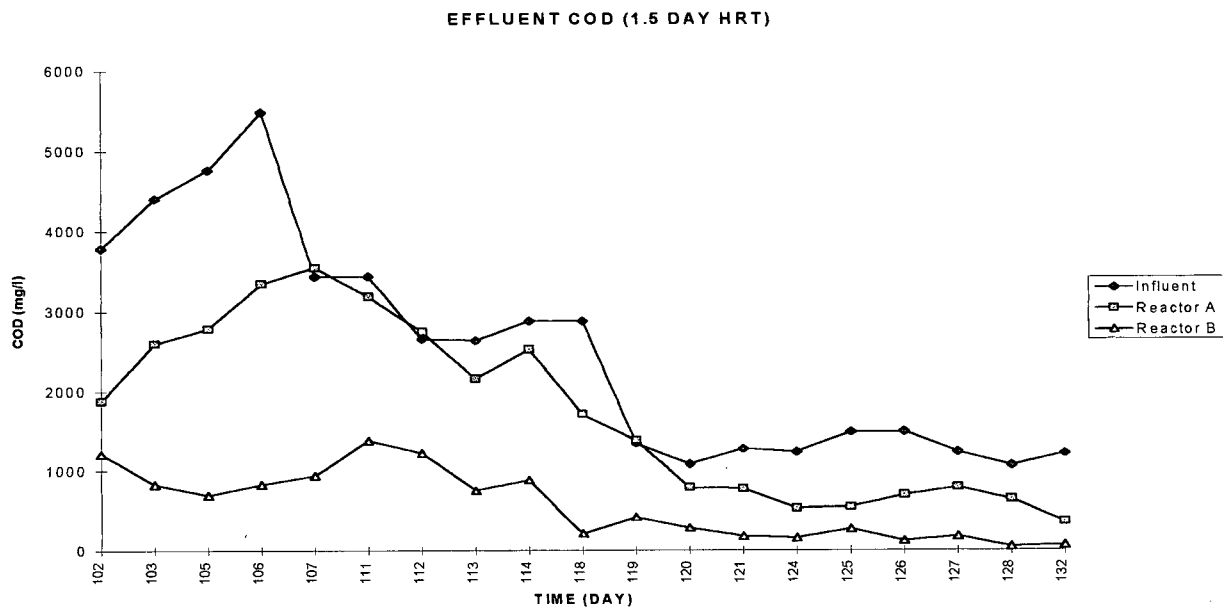


Figure C.1 Effluent COD vs Time (1.5 day HRT)

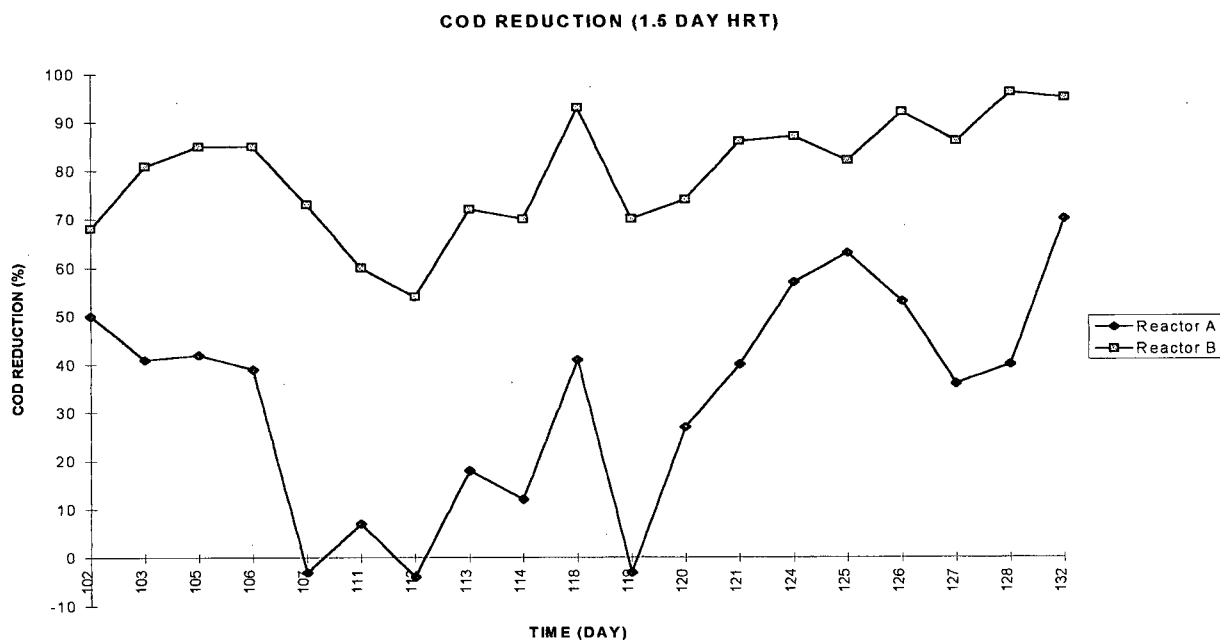


Figure C.2 COD Reduction vs Time (1.5 day HRT)



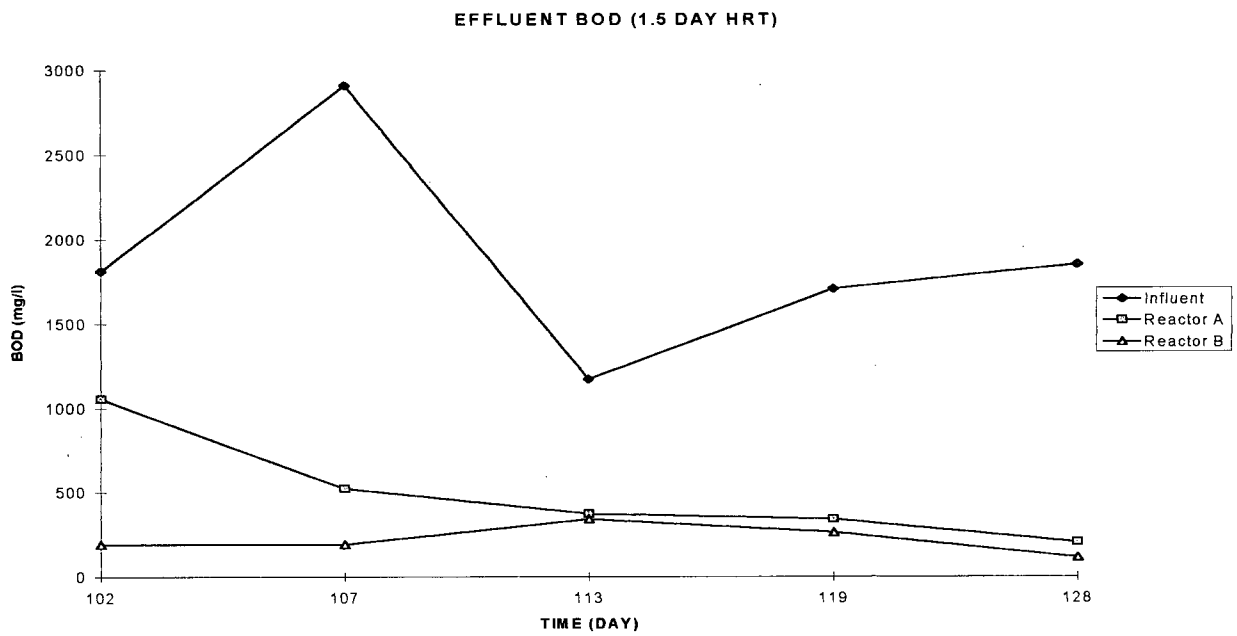


Figure C.3 Effluent BOD vs Time (1.5 day HRT)

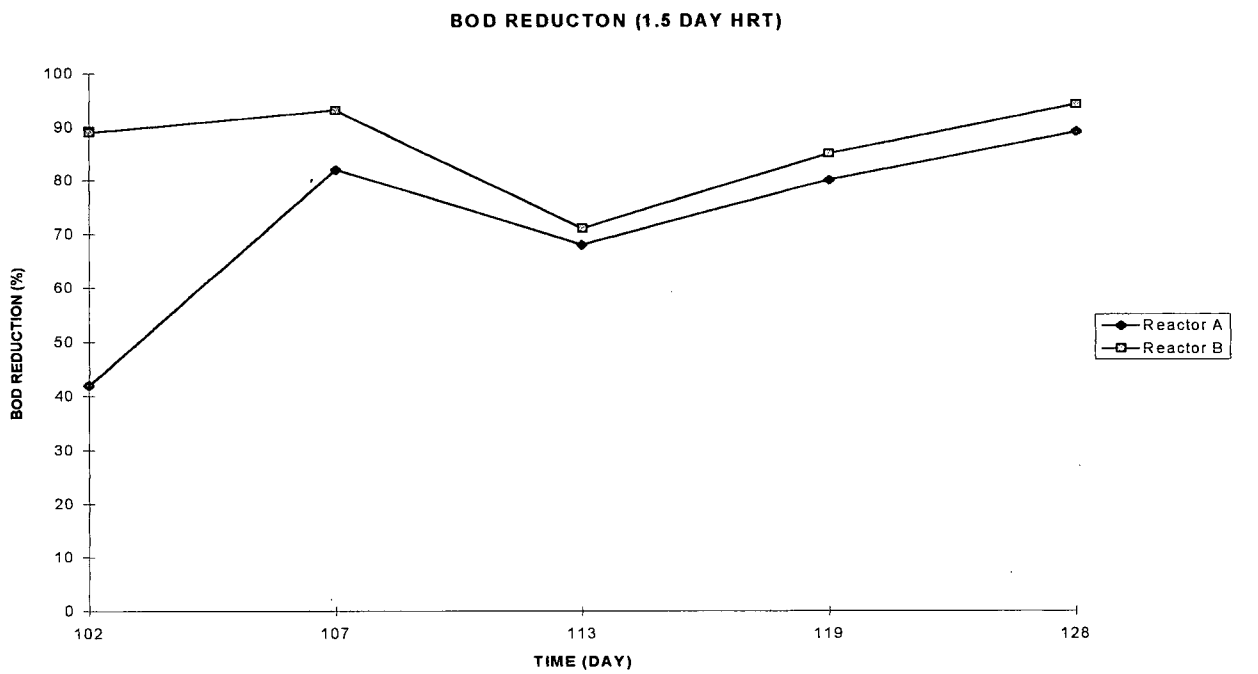


Figure C.4 BOD Reduction vs Time (1.5 day HRT)

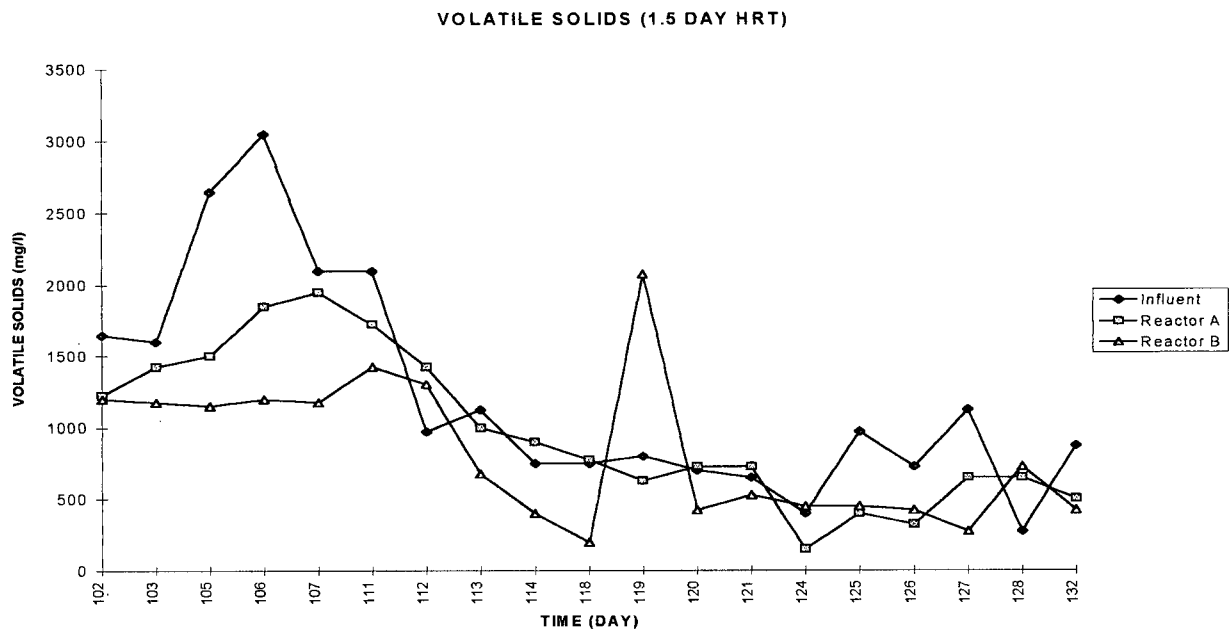


Figure C.5 Volatile Solids vs Time (1.5 day HRT)

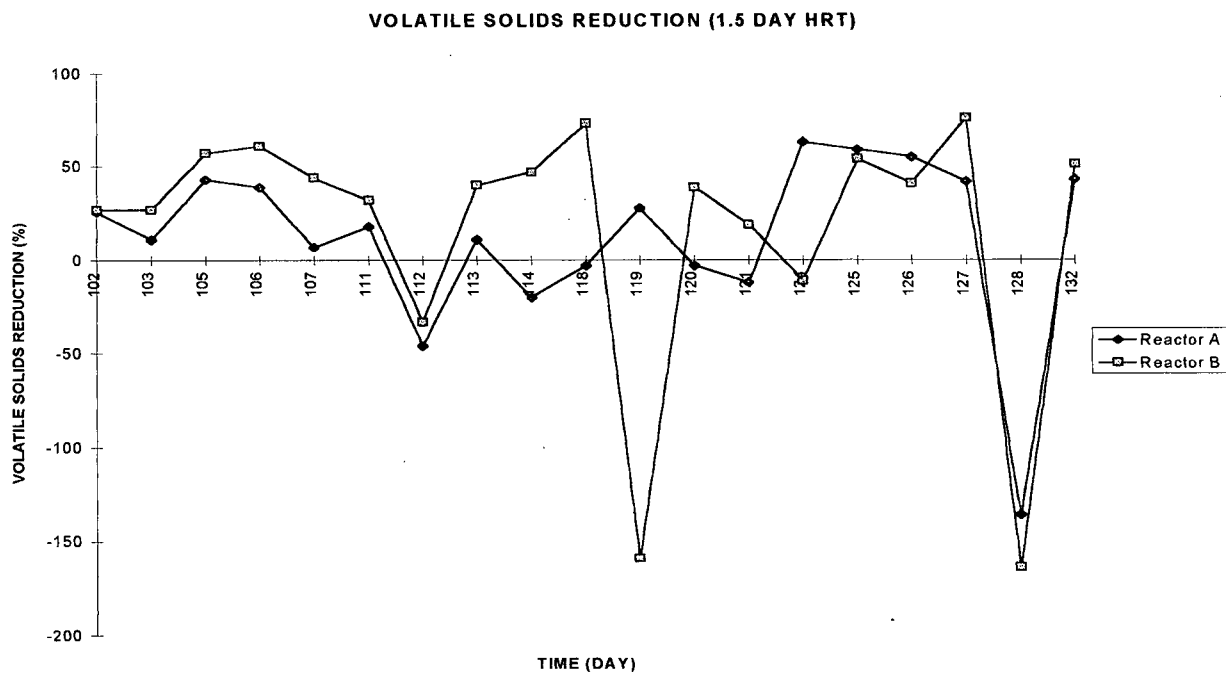


Figure C.6 Volatile Solids Reduction vs Time (1.5 day HRT)

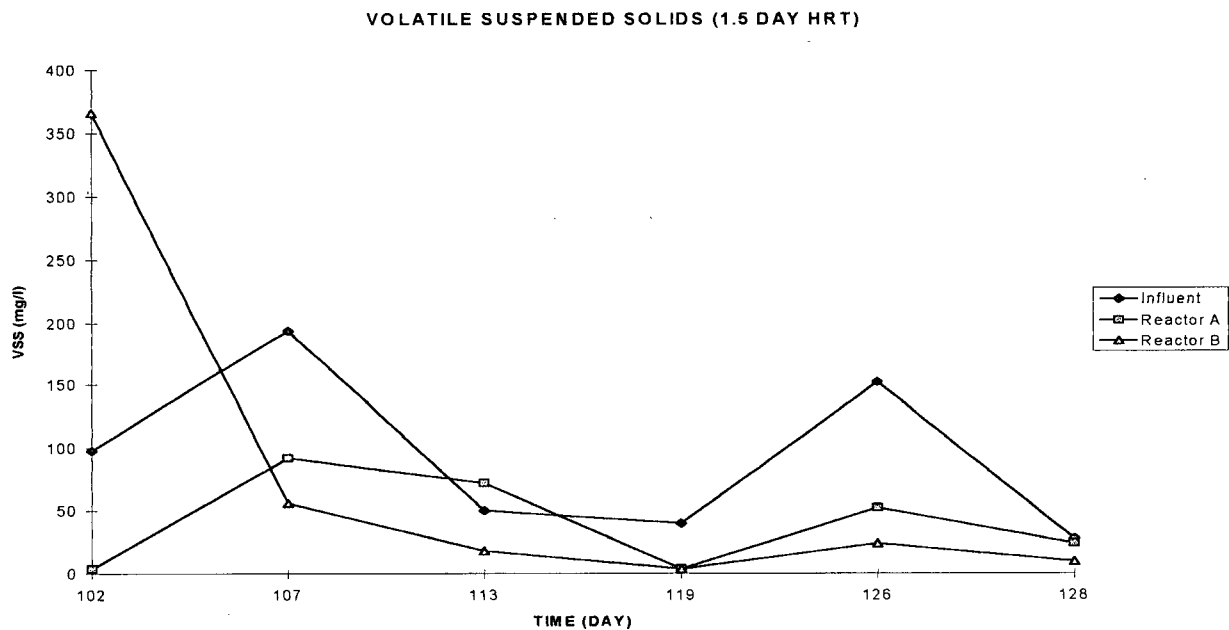


Figure C.7 Volatile Suspended Solids vs Time (1.5 day HRT)

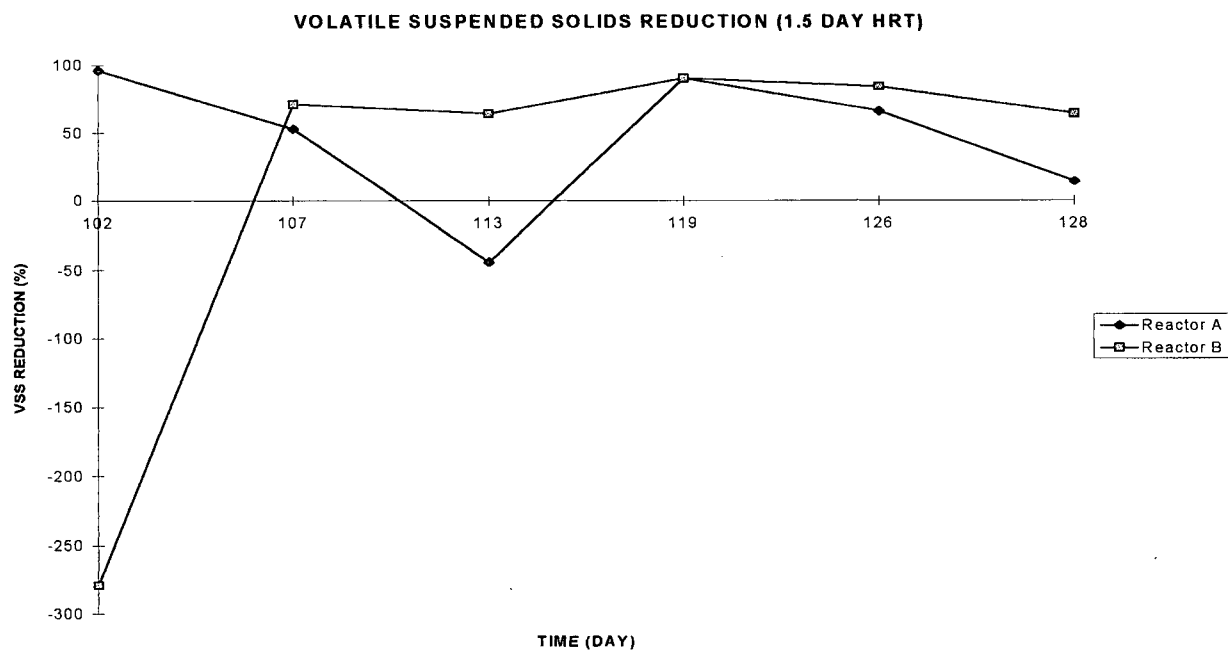


Figure C.8 Volatile Suspended Solids Reduction vs Time (1.5 day HRT)

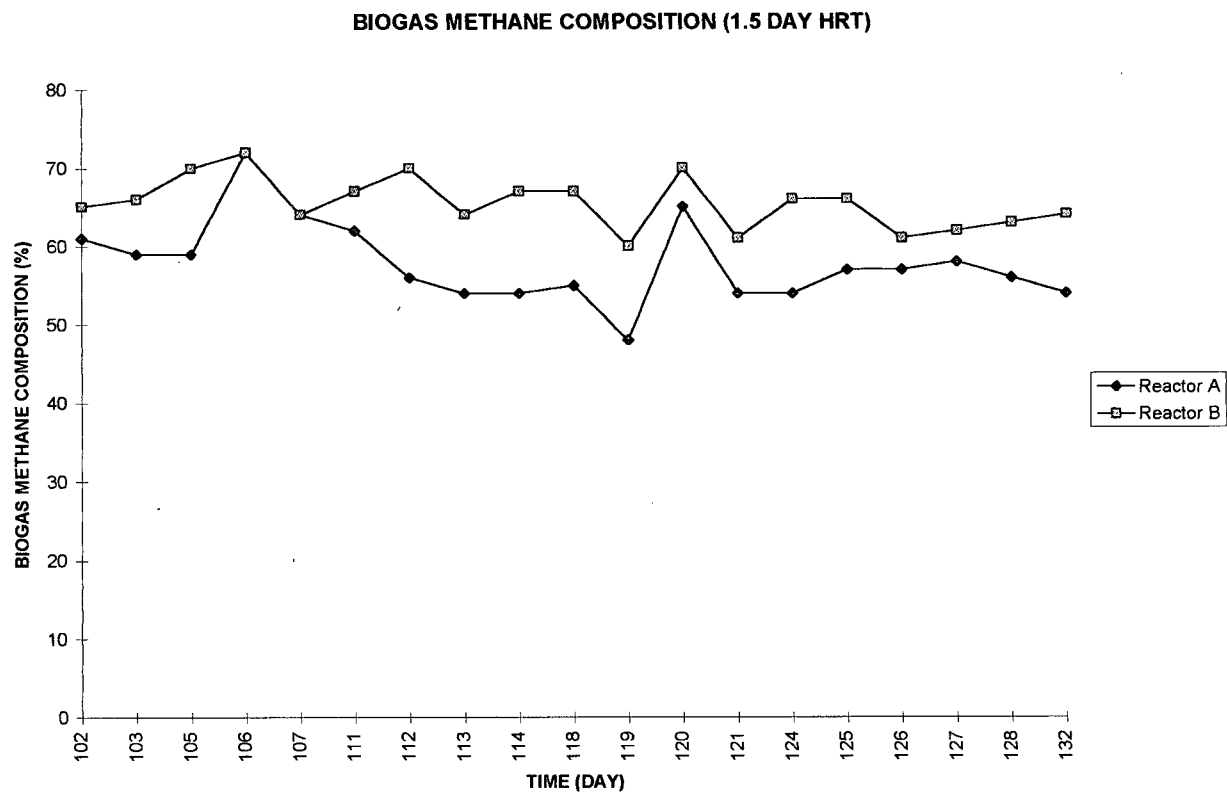


Figure C.9 Biogas Methane Composition vs Time (1.5 day HRT)

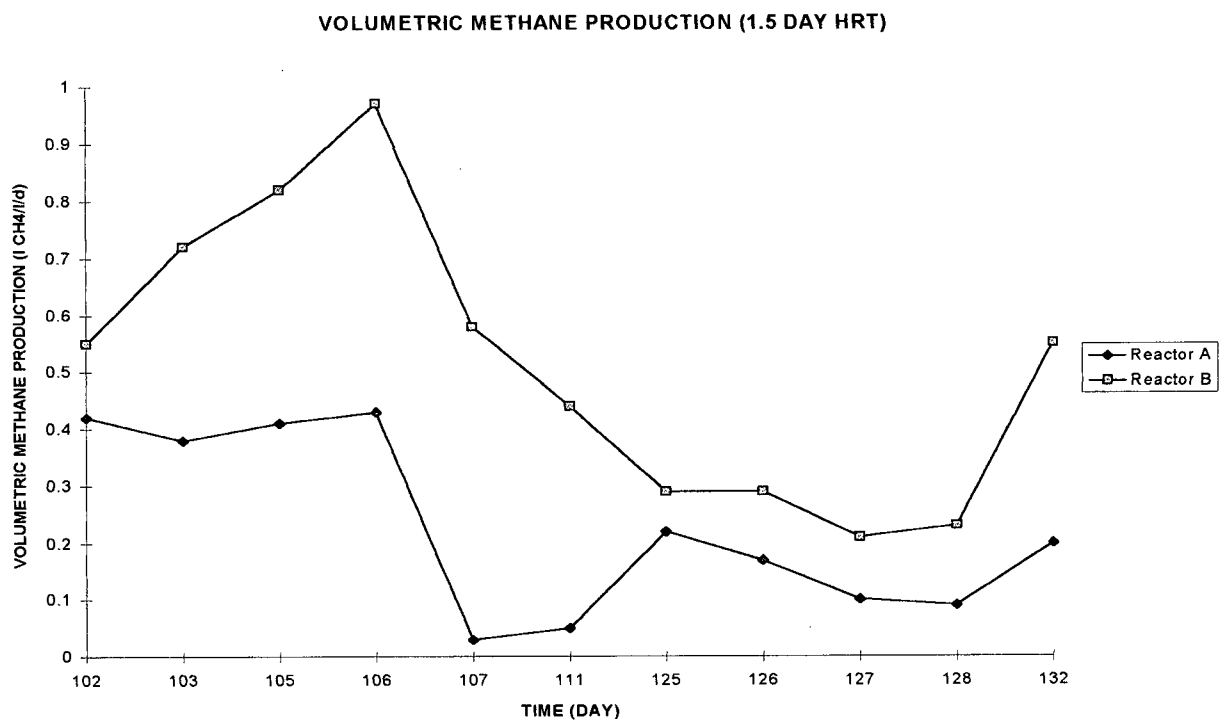


Figure C.10 Volumetric Methane Production vs Time (1.5 day HRT)

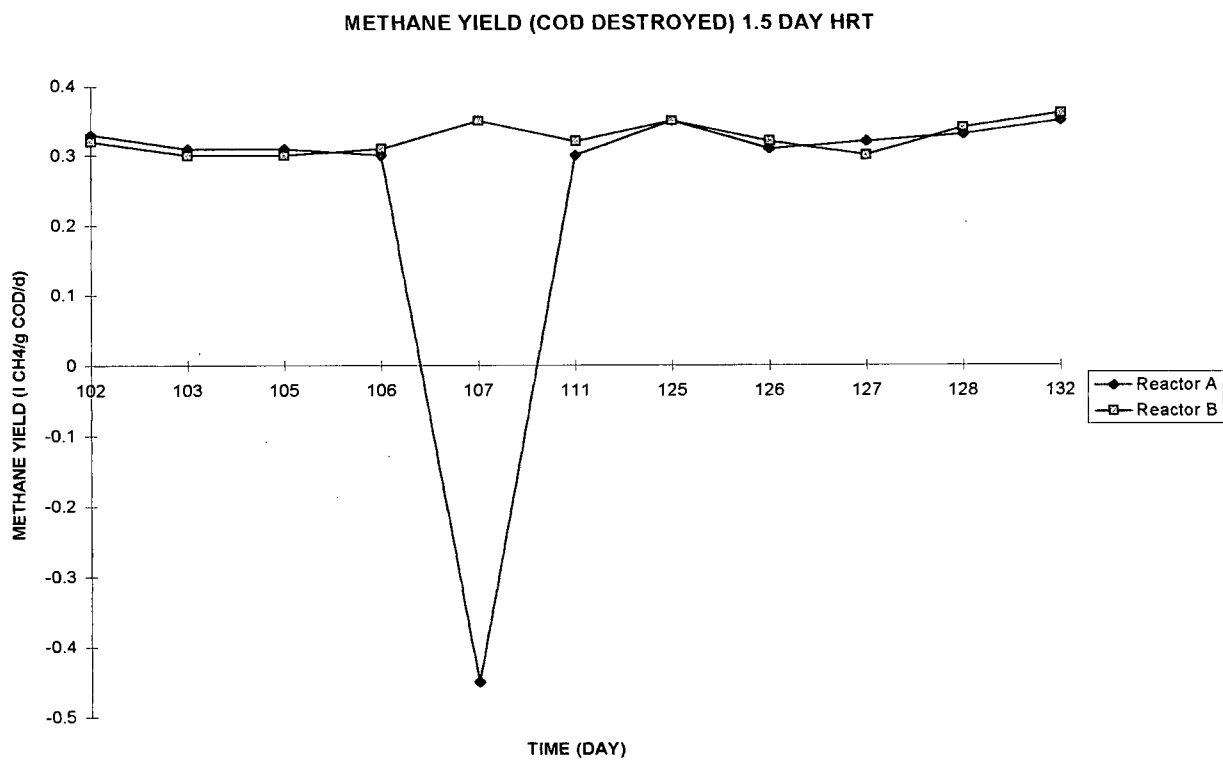


Figure C.11 Methane Yield vs Time (1.5 day HRT)

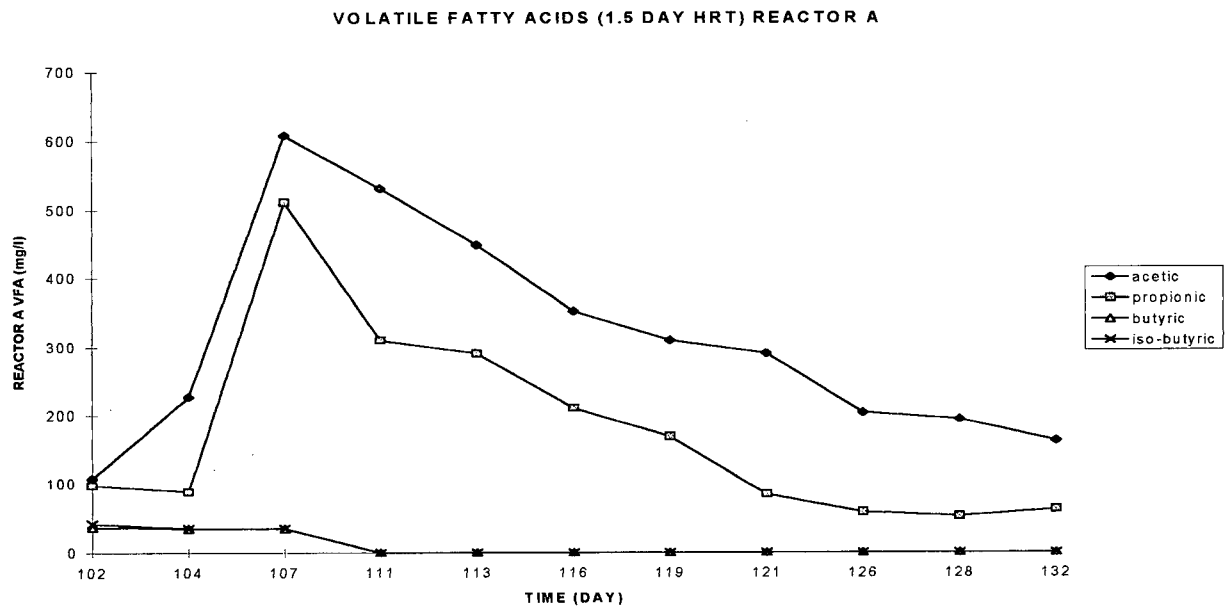


Figure C.12 VFA concentration vs Time (Reactor A : 1.5 day HRT)

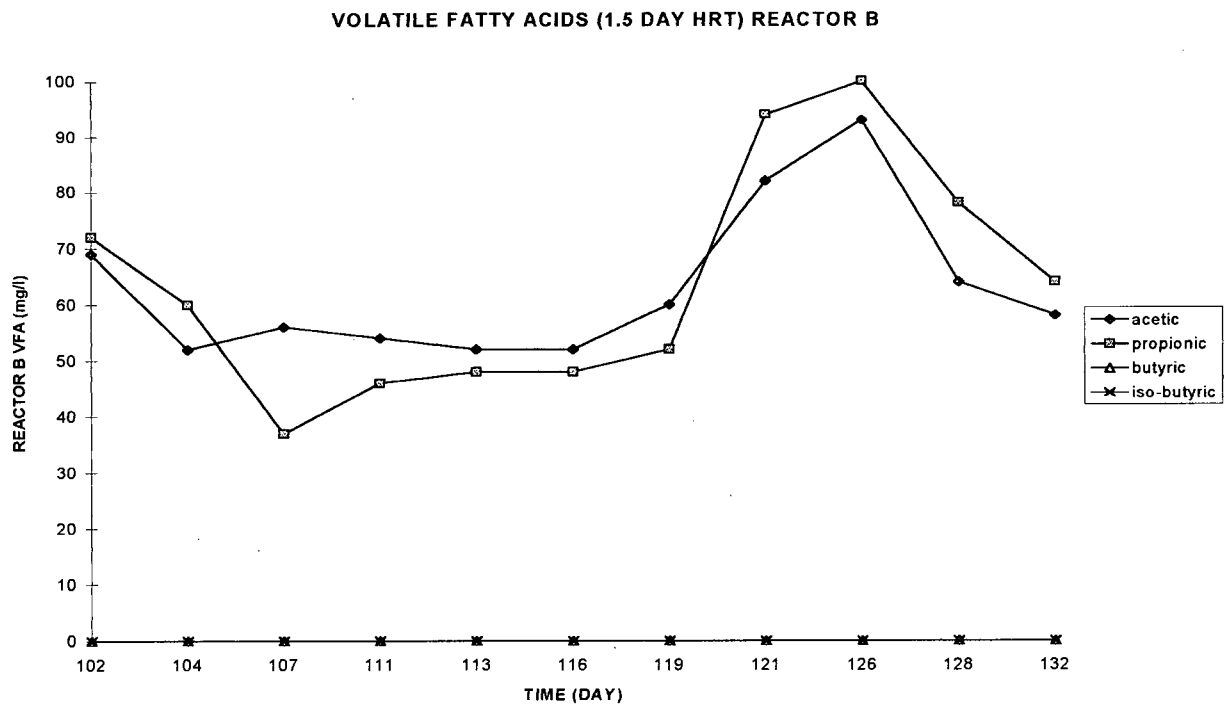


Figure C.13 VFA concentration vs Time (Reactor B : 1.5 day HRT)

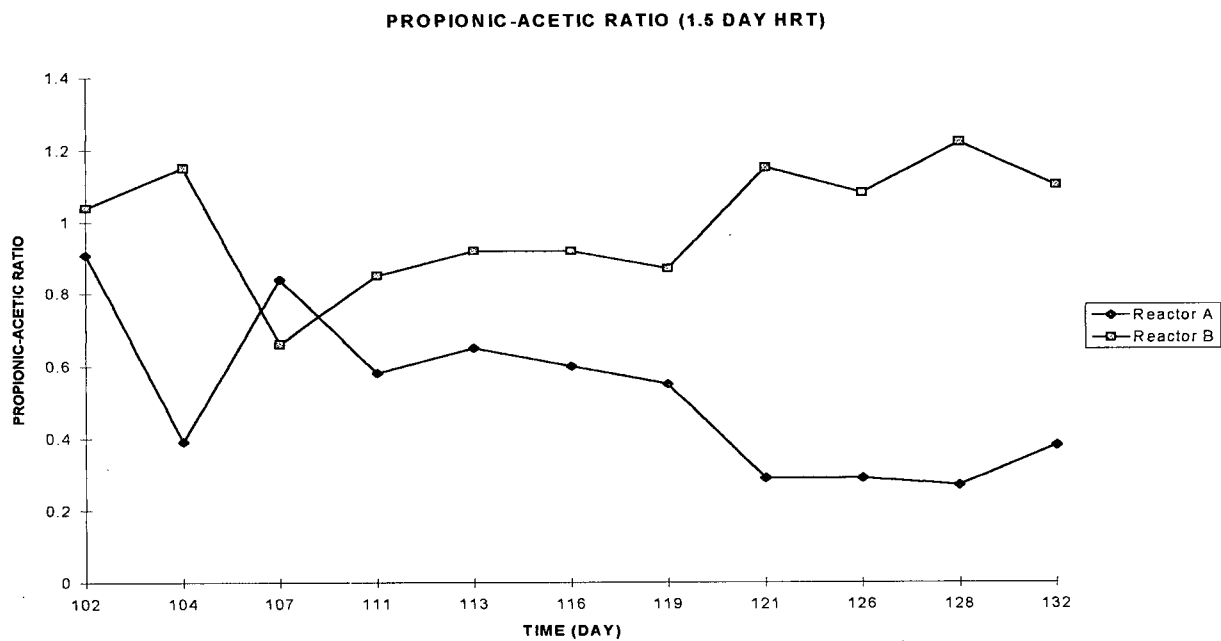


Figure C.14 Propionic-acetic acid Ratio vs Time (1.5 day HRT)

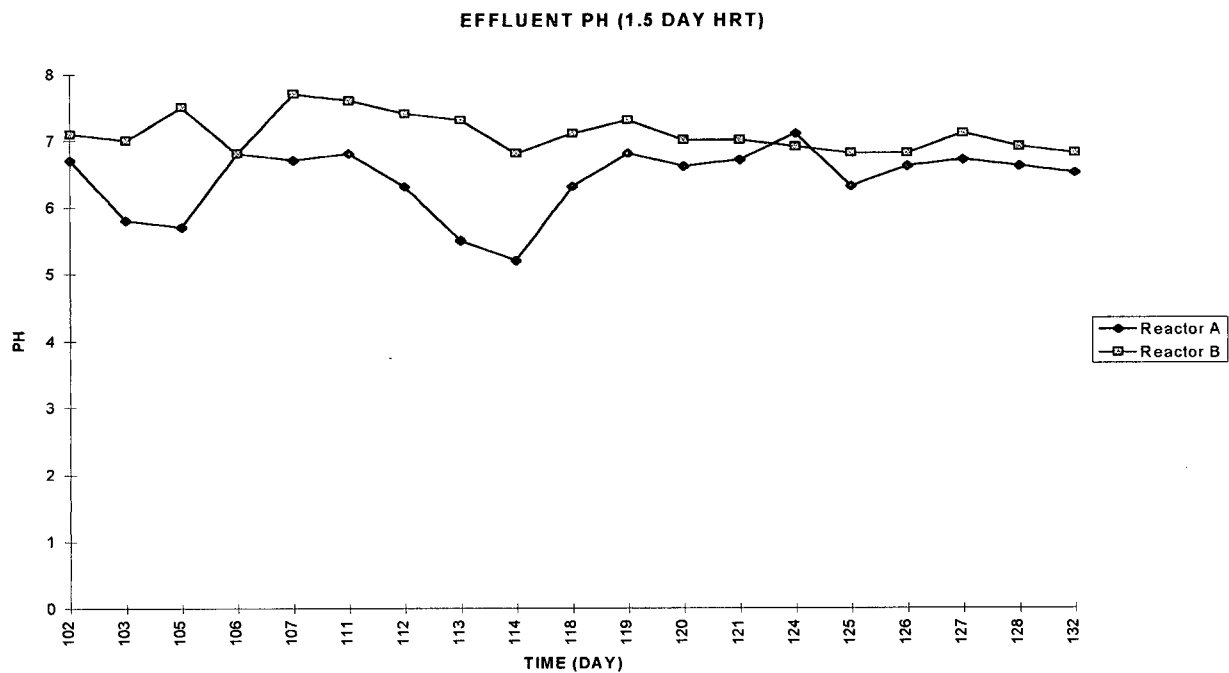


Figure C.15 Effluent pH vs Time (1.5 day HRT)

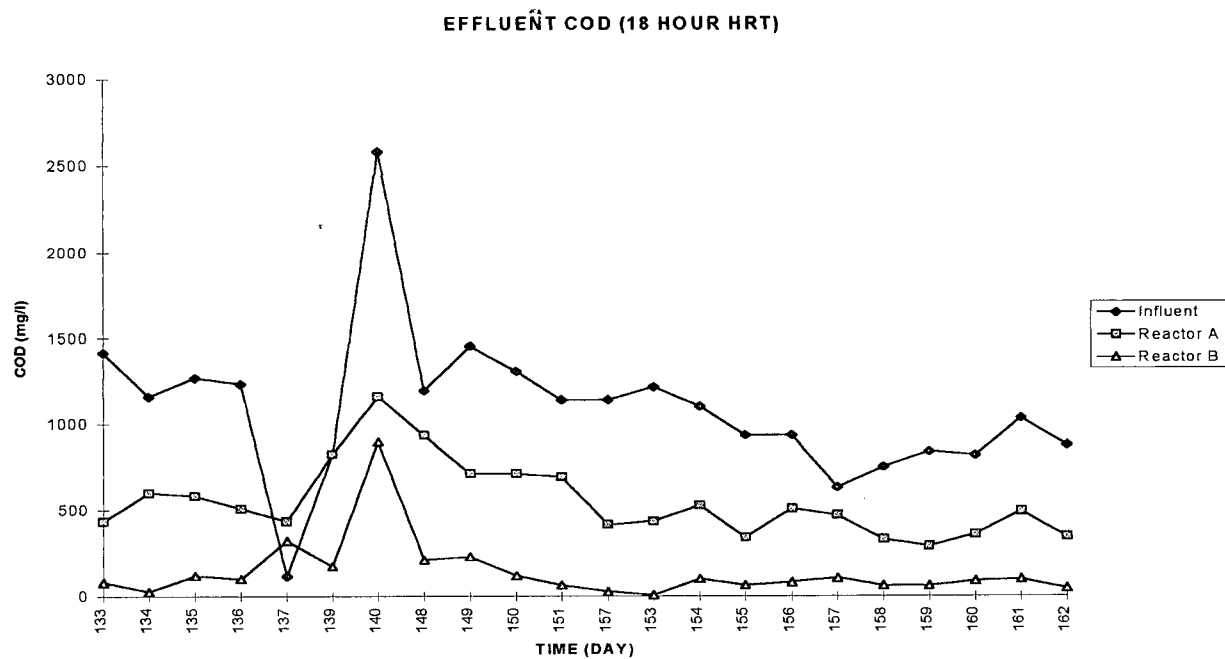


Figure D.1 Effluent COD vs Time (18 hour HRT)

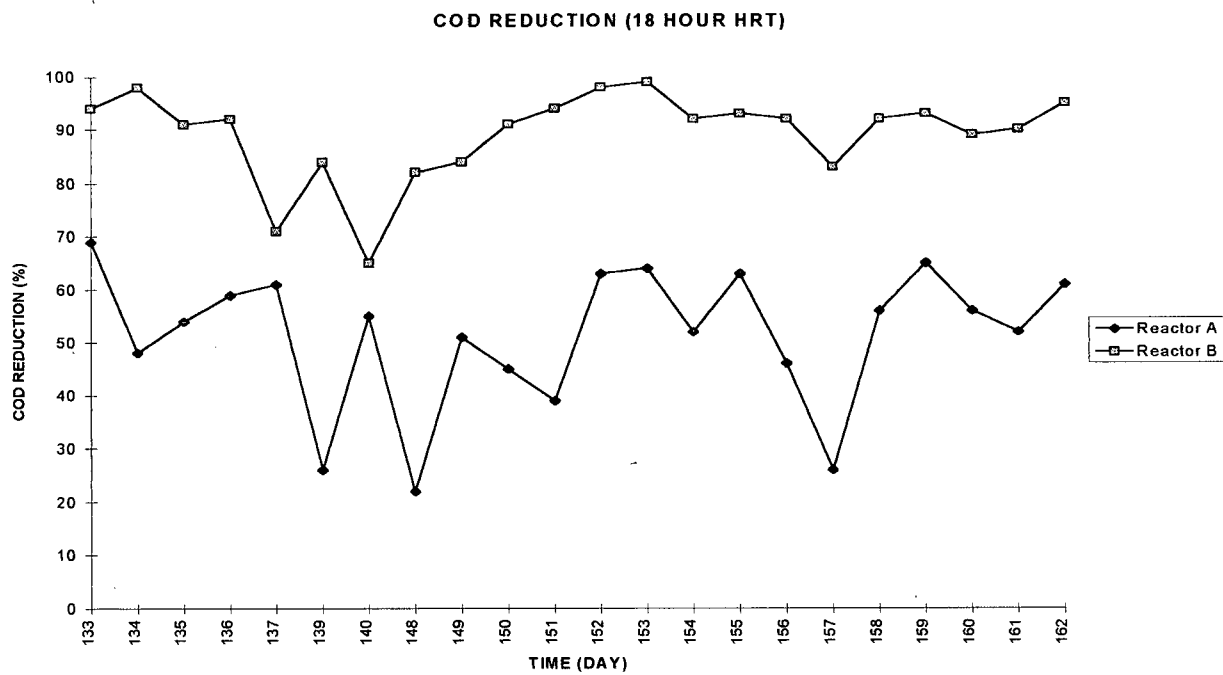


Figure D.2 COD Reduction vs Time (18 hour HRT)



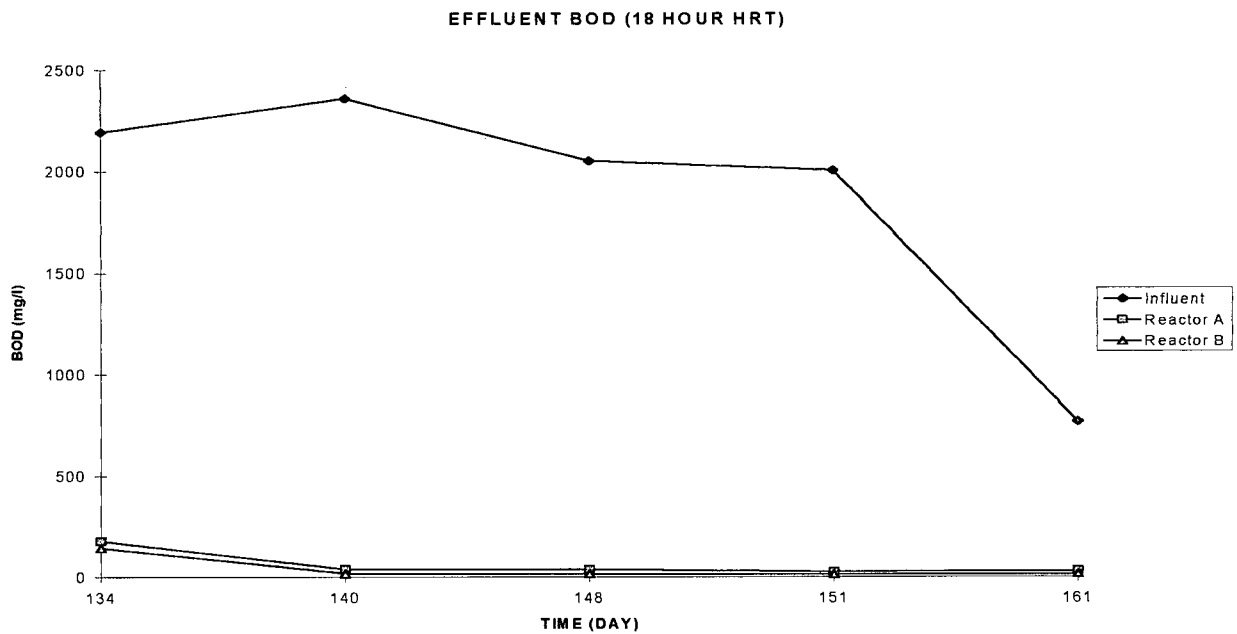


Figure D.3 Effluent BOD vs Time (18 hour HRT)

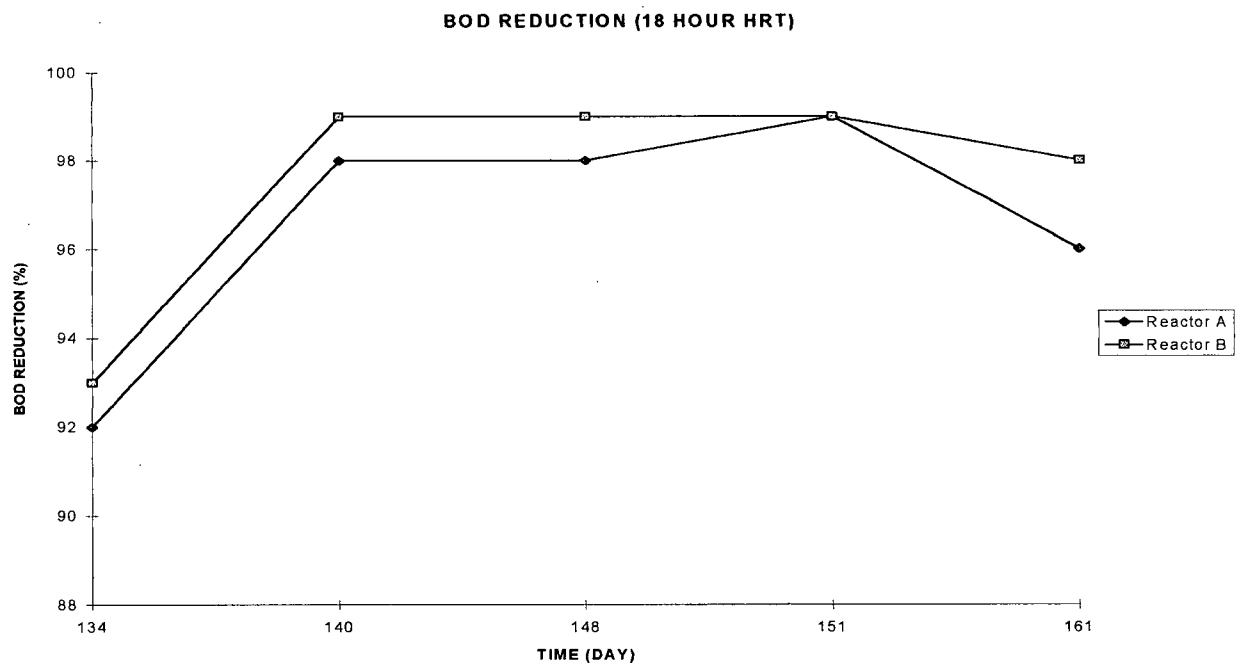


Figure D.4 BOD Reduction vs Time (1.5 day HRT)

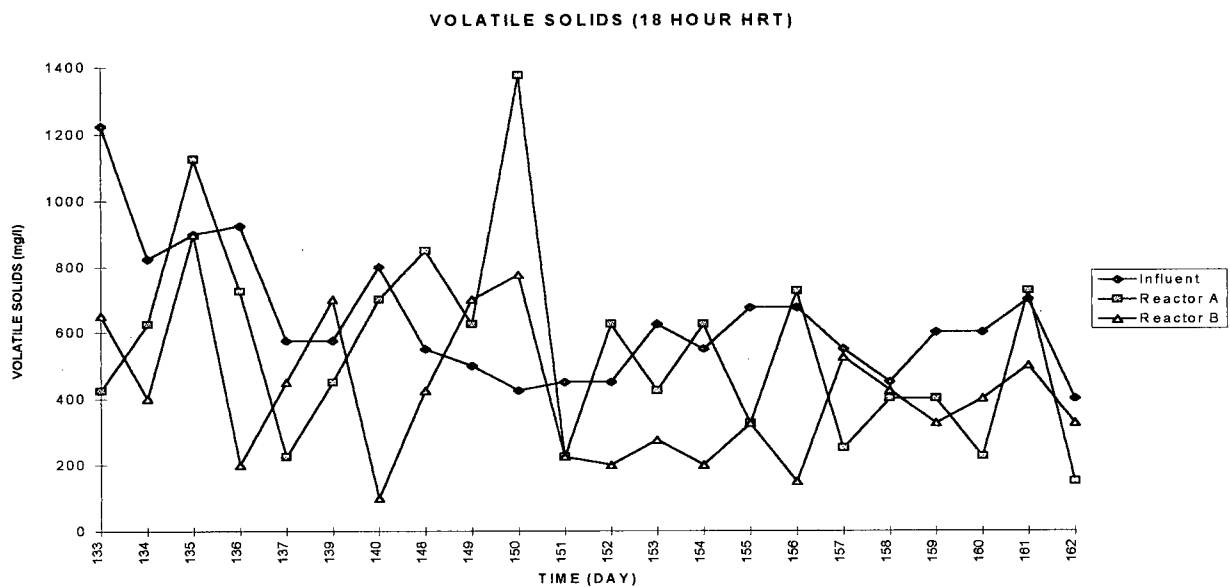


Figure D.5 Volatile Solids vs Time (18 hour HRT)

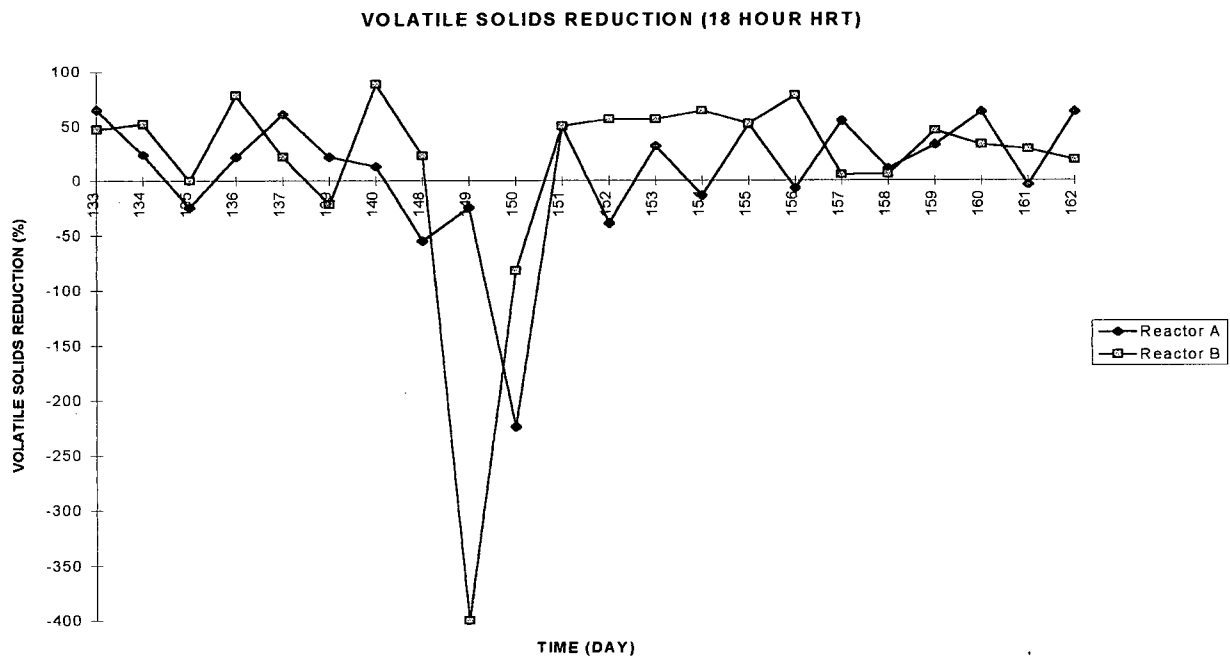


Figure D.6 Volatile Solids Reduction vs Time (18 hour HRT)

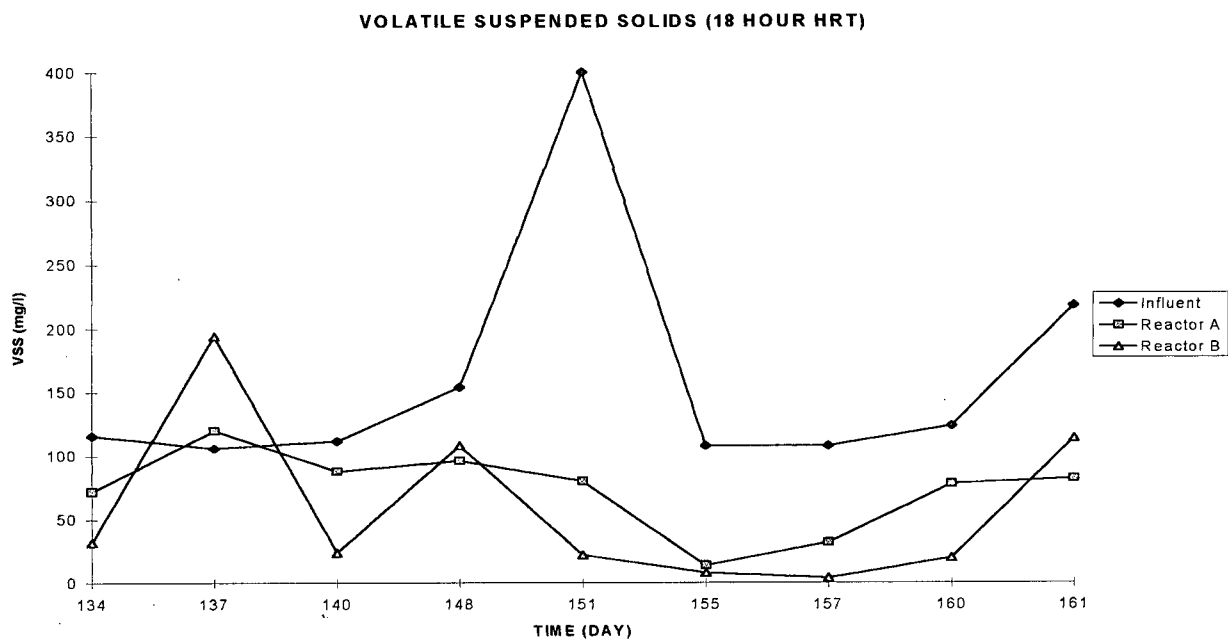


Figure D.7 Volatile Suspended Solids vs Time (18 hour HRT)

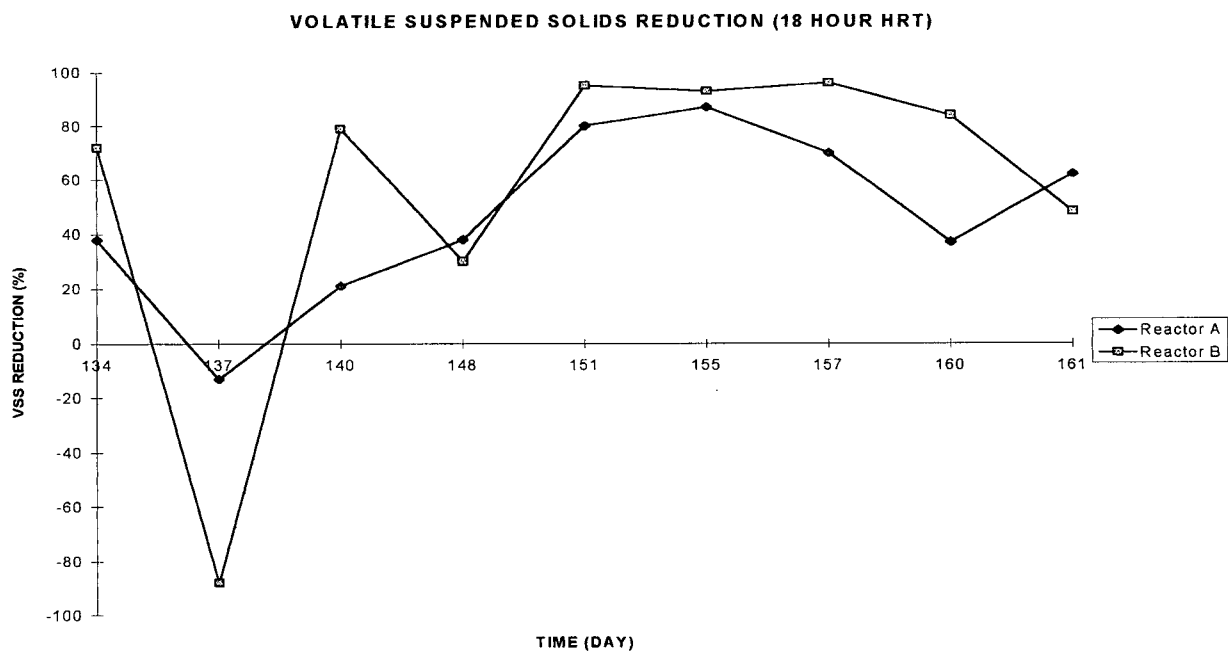


Figure D.8 Volatile Suspended Solids Reduction vs Time (18 hour HRT)

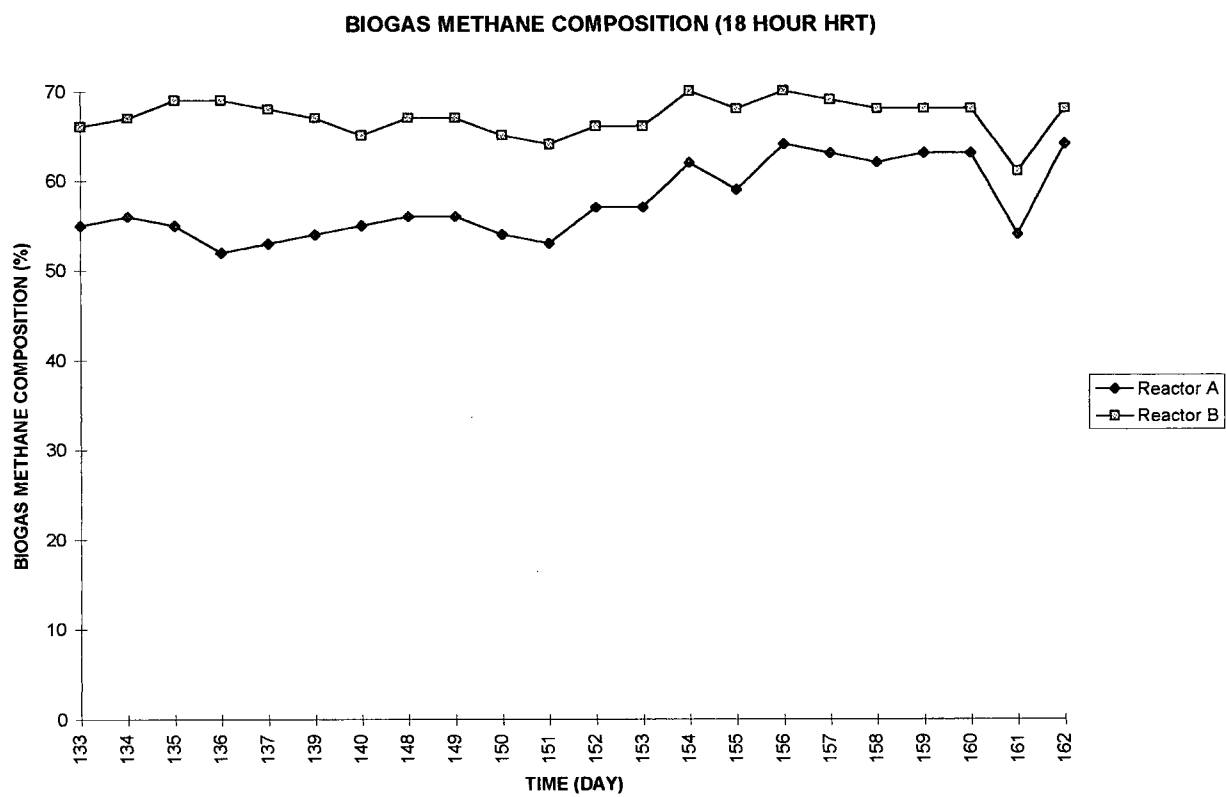


Figure D.9 Biogas Methane Composition vs Time (18 hour HRT)

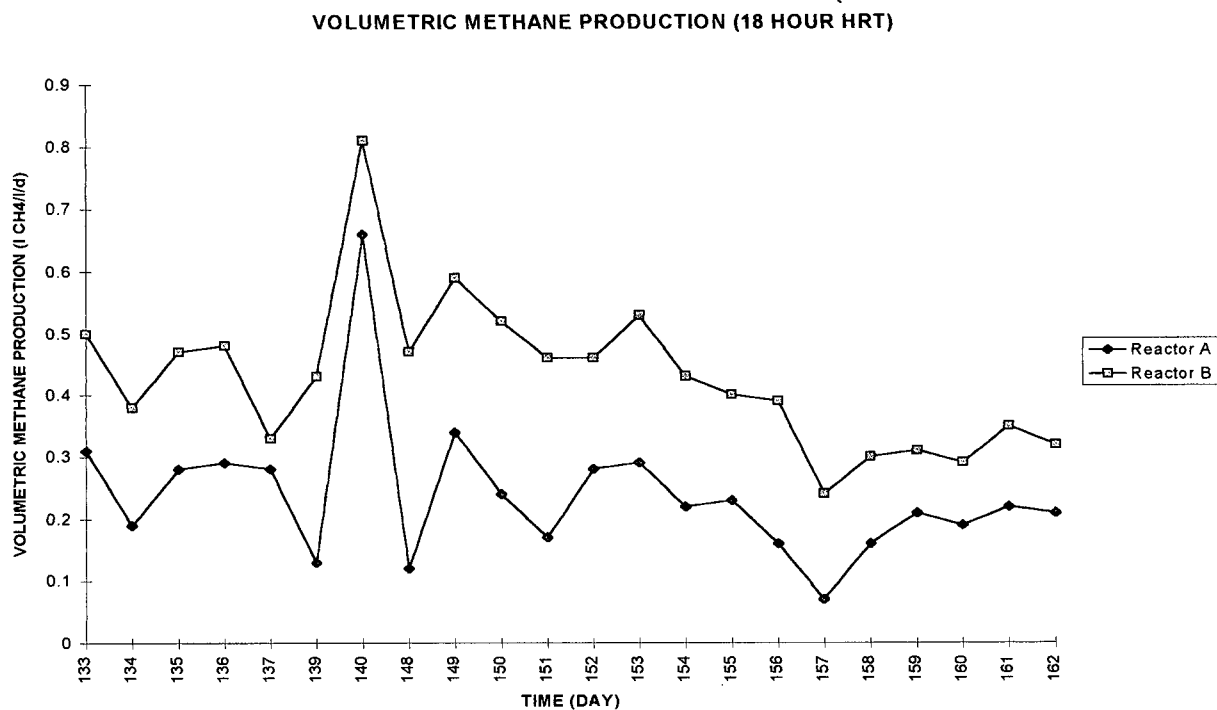


Figure D.10 Volumetric Methane Production vs Time (18 hour HRT)

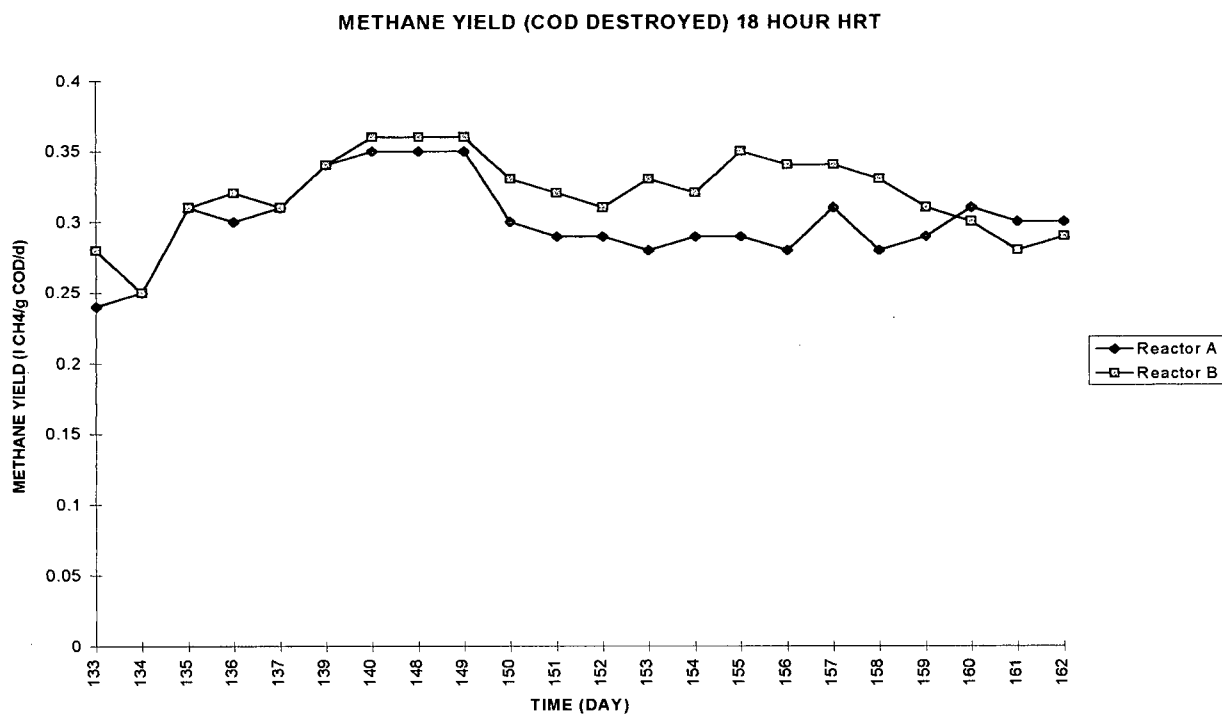


Figure D.11 Methane Yield vs Time (18 hour HRT)

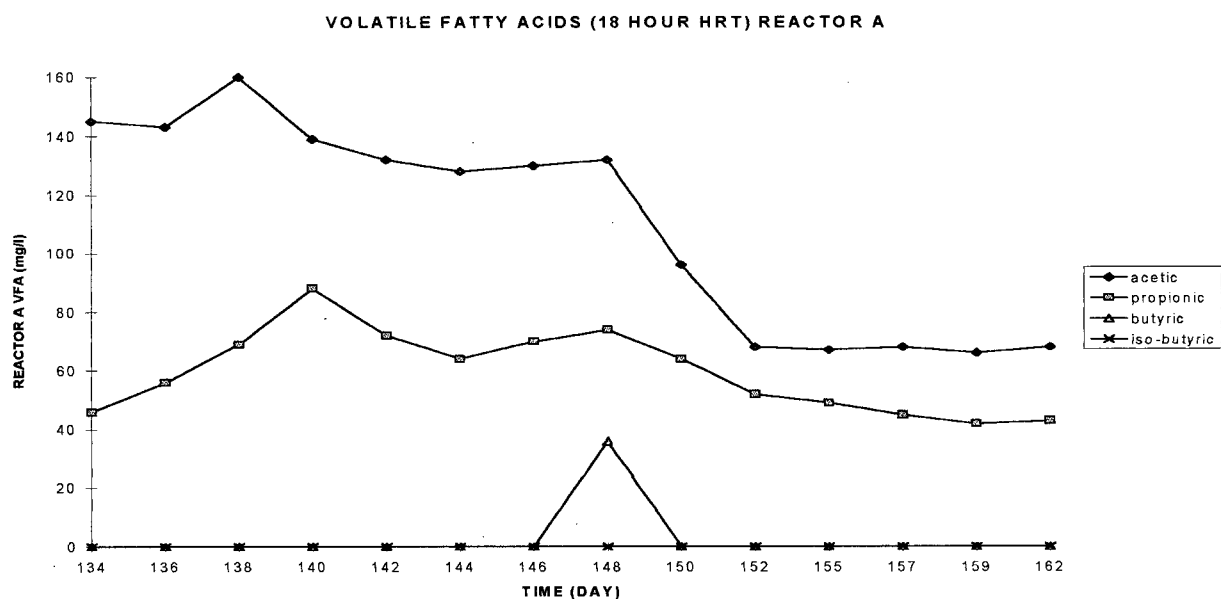


Figure D.12 VFA concentration vs Time (Reactor A : 18 hour HRT)

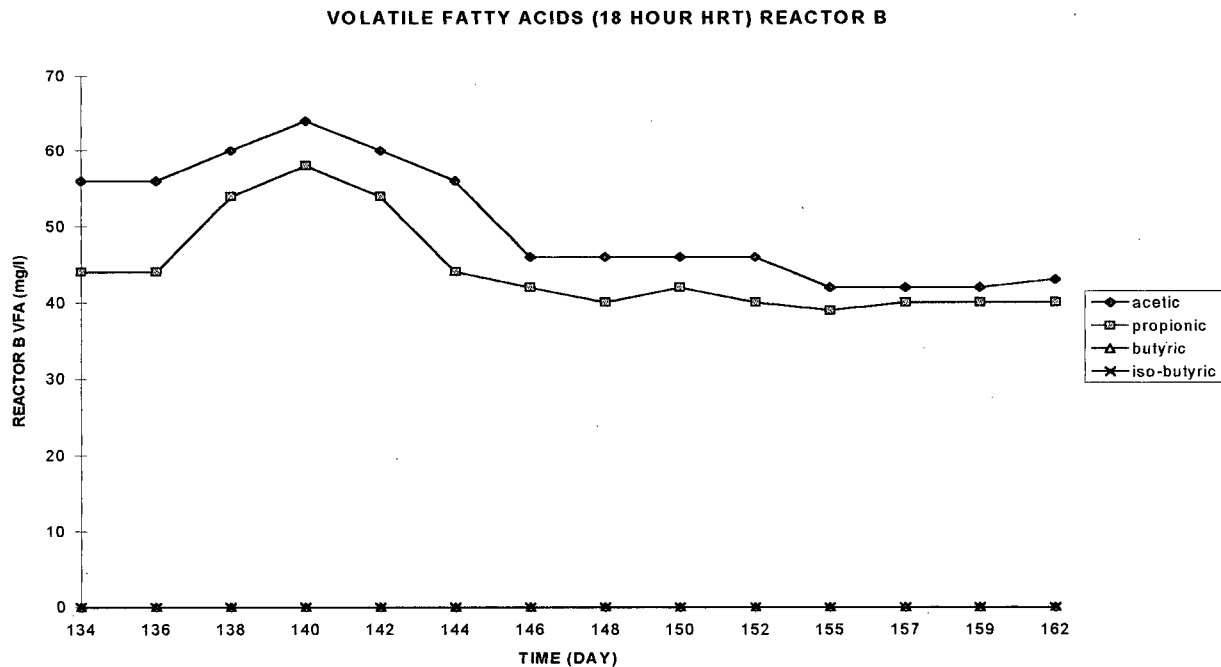


Figure D.13 VFA concentration vs Time (Reactor B : 18 hour HRT)

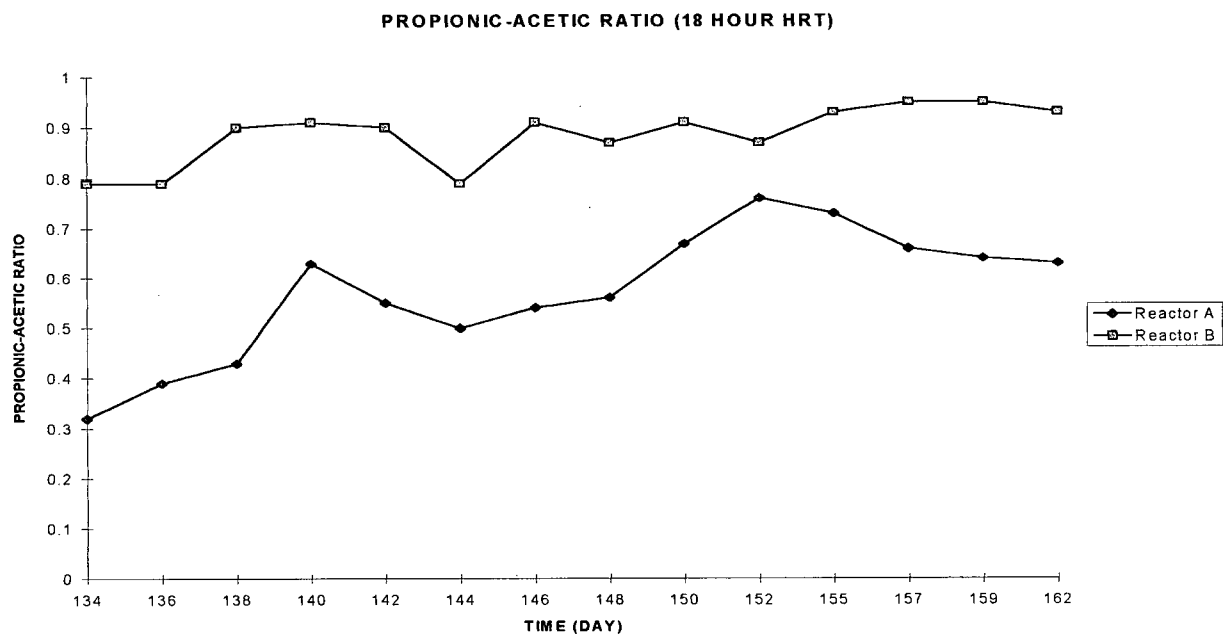


Figure D.14 Propionic-acetic acid Ratio vs Time (18 hour HRT)

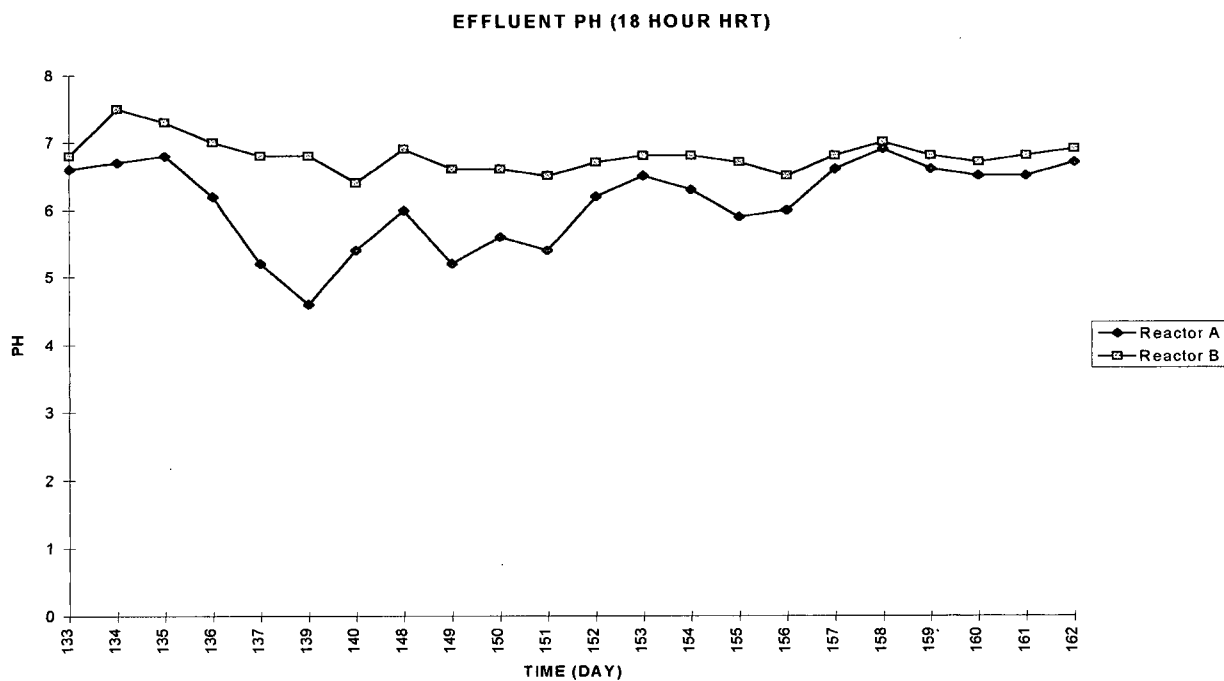


Figure D.15 Effluent pH vs Time (18 hour HRT)

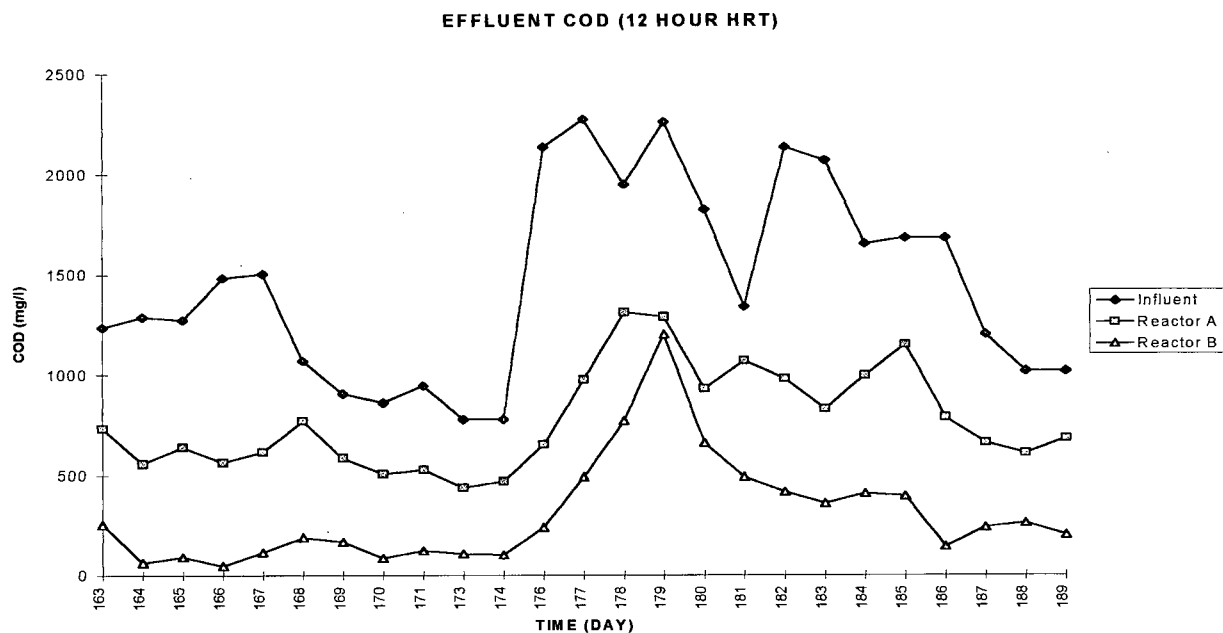


Figure E.1 Effluent COD vs Time (12 hour HRT)

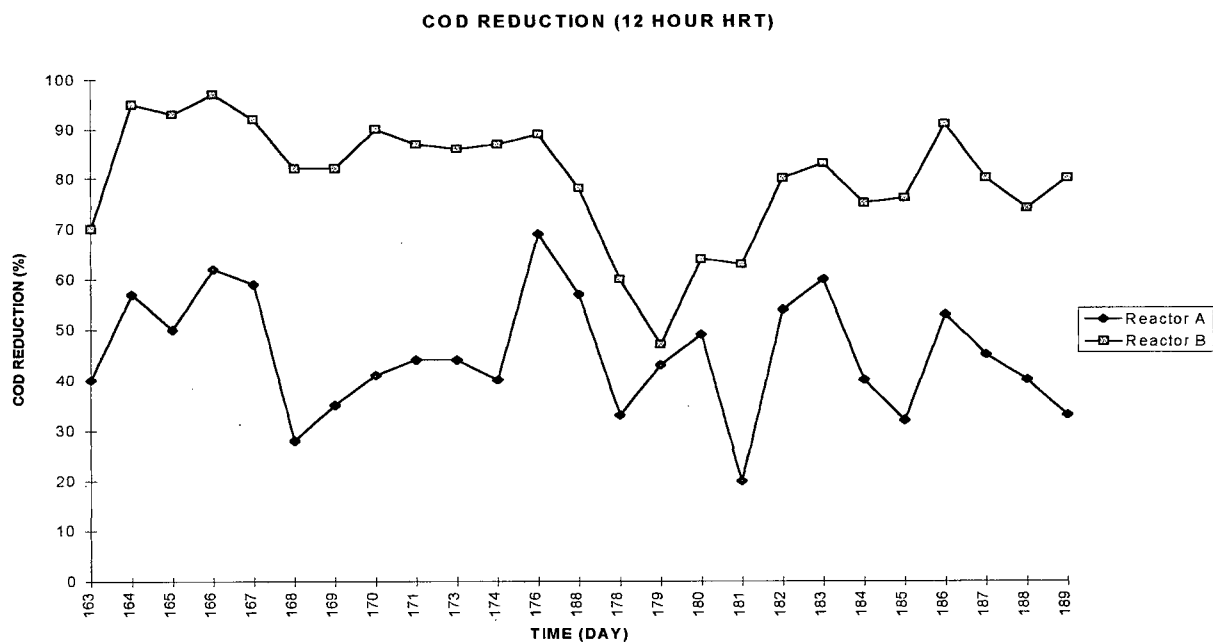


Figure E.2 COD Reduction vs Time (12 hour HRT)



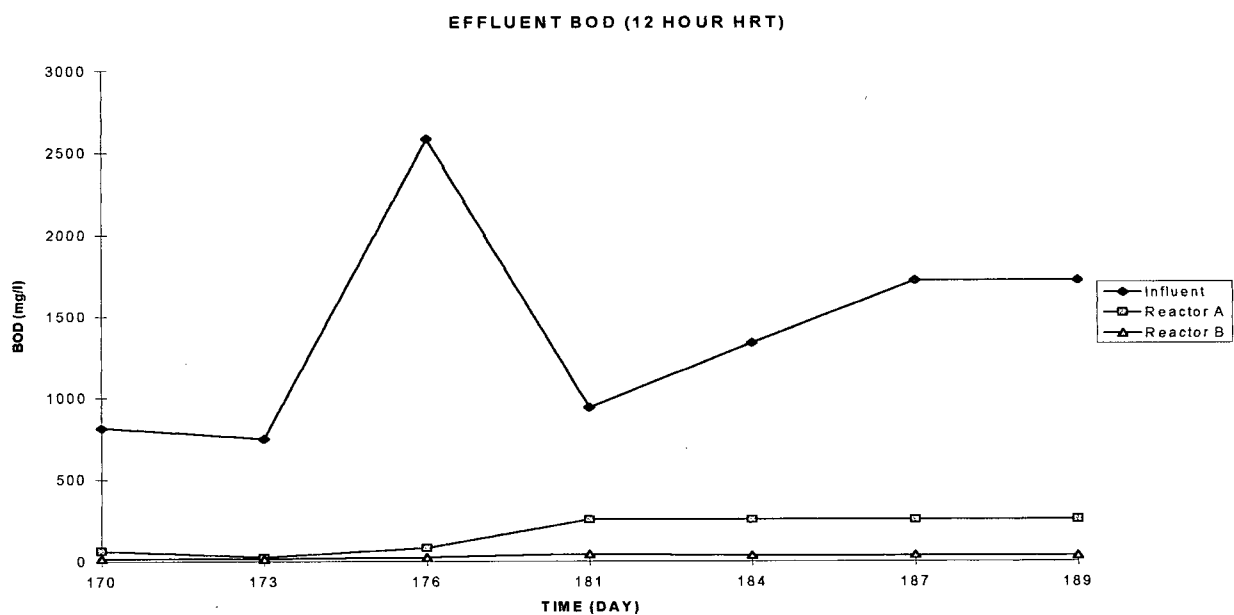


Figure E.3 Effluent BOD vs Time (12 hour HRT)

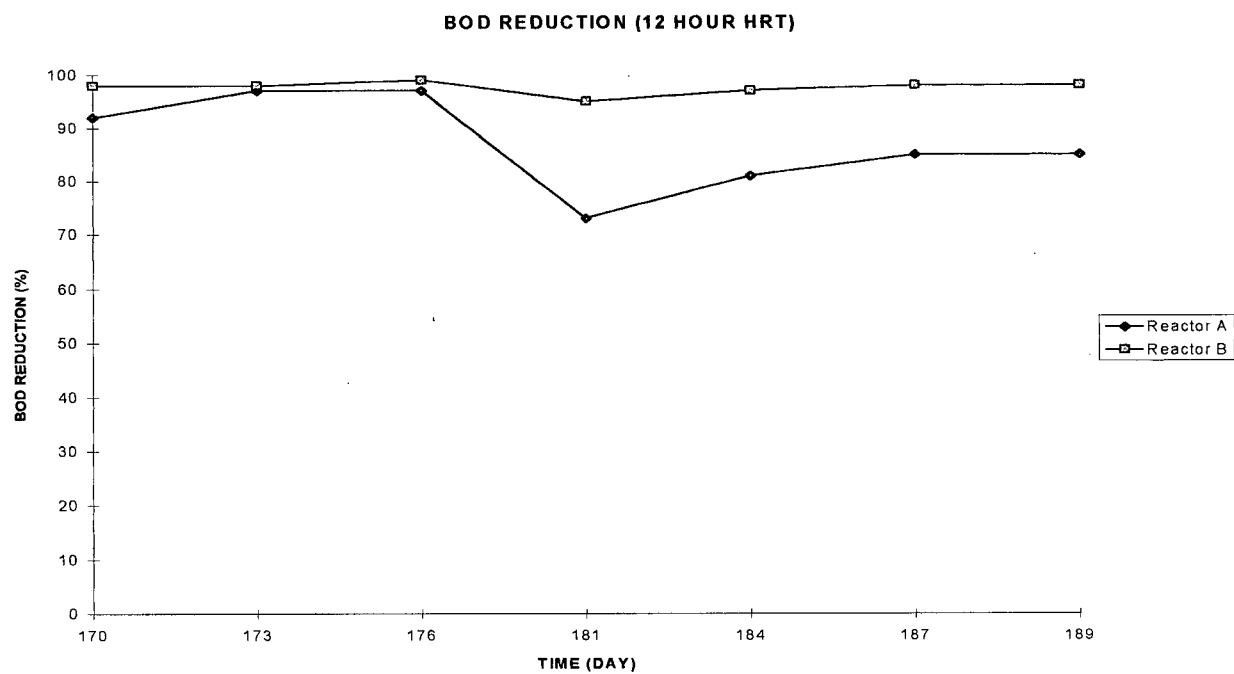


Figure E.4 BOD Reduction vs Time (12 hour HRT)

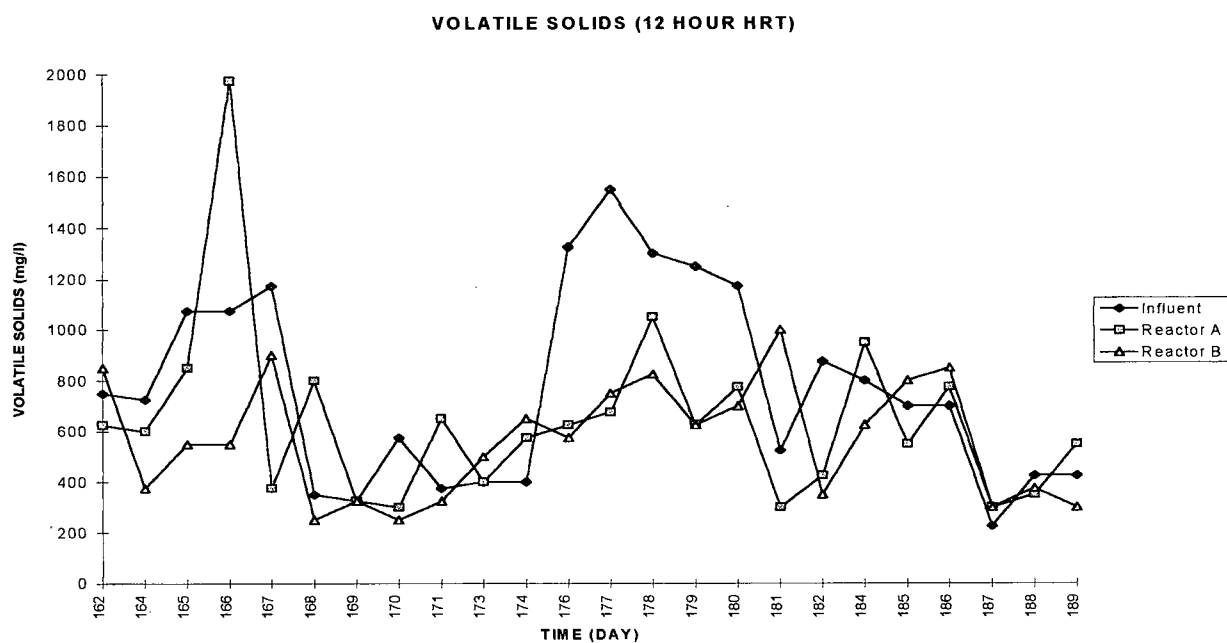


Figure E.5 Volatile Solids vs Time (12 hour HRT)

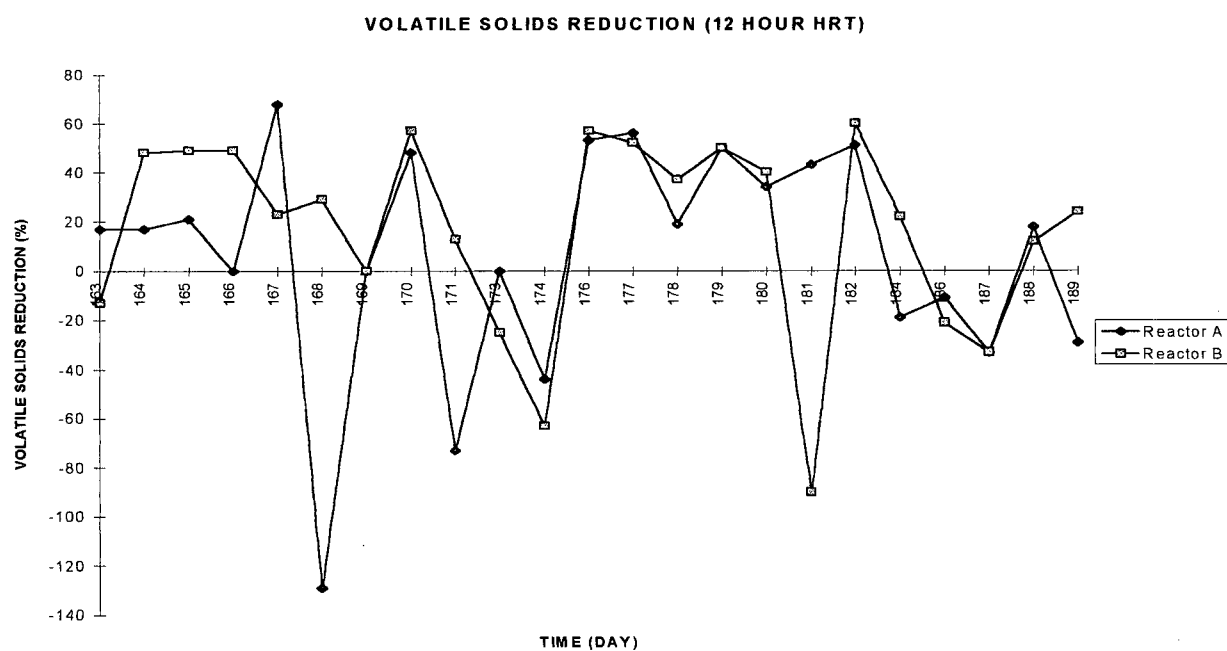


Figure E.6 Volatile Solids Reduction vs Time (12 hour HRT)

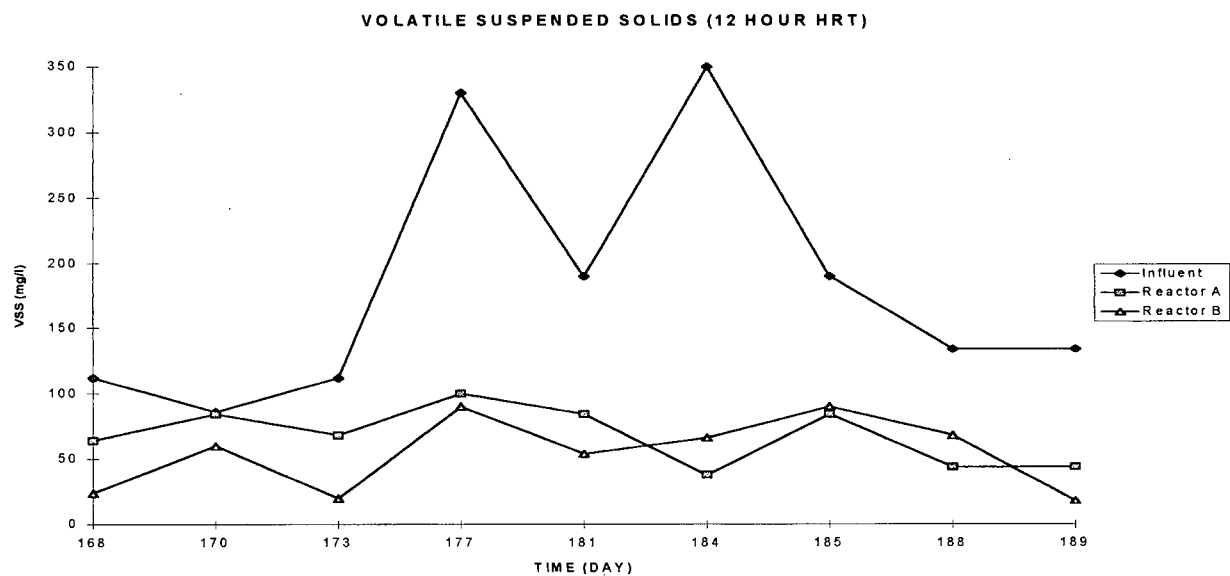


Figure E.7 Volatile Suspended Solids vs Time (12 hour HRT)

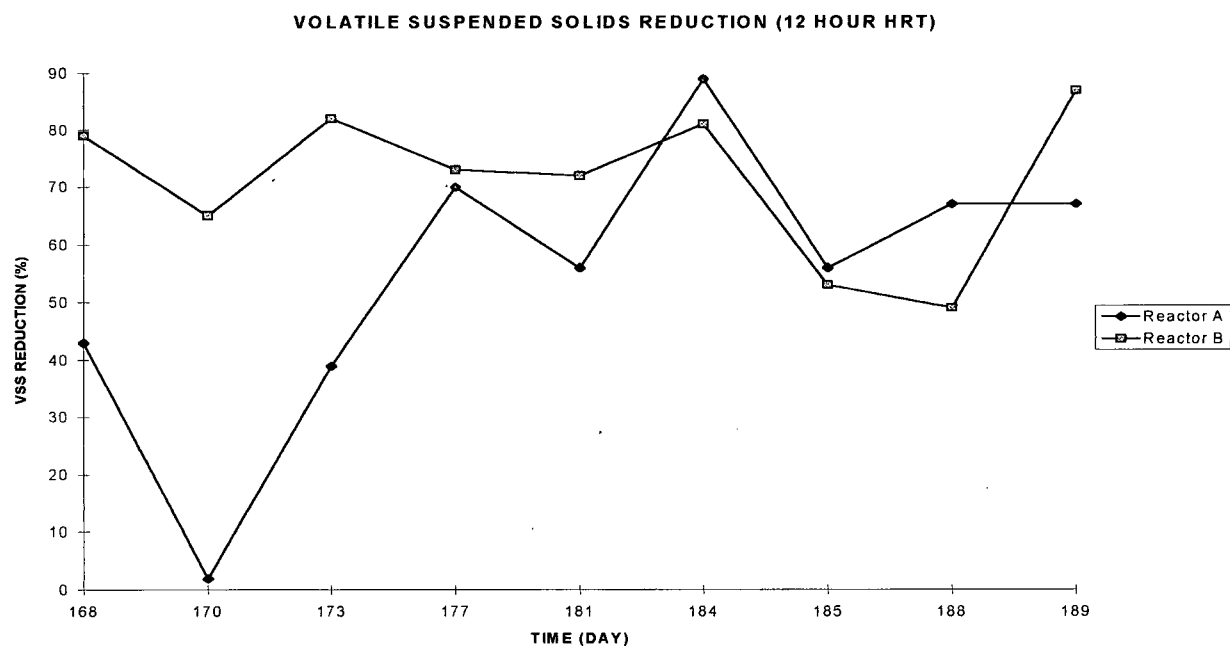


Figure E.8 Volatile Suspended Solids Reduction vs Time (12 hour HRT)

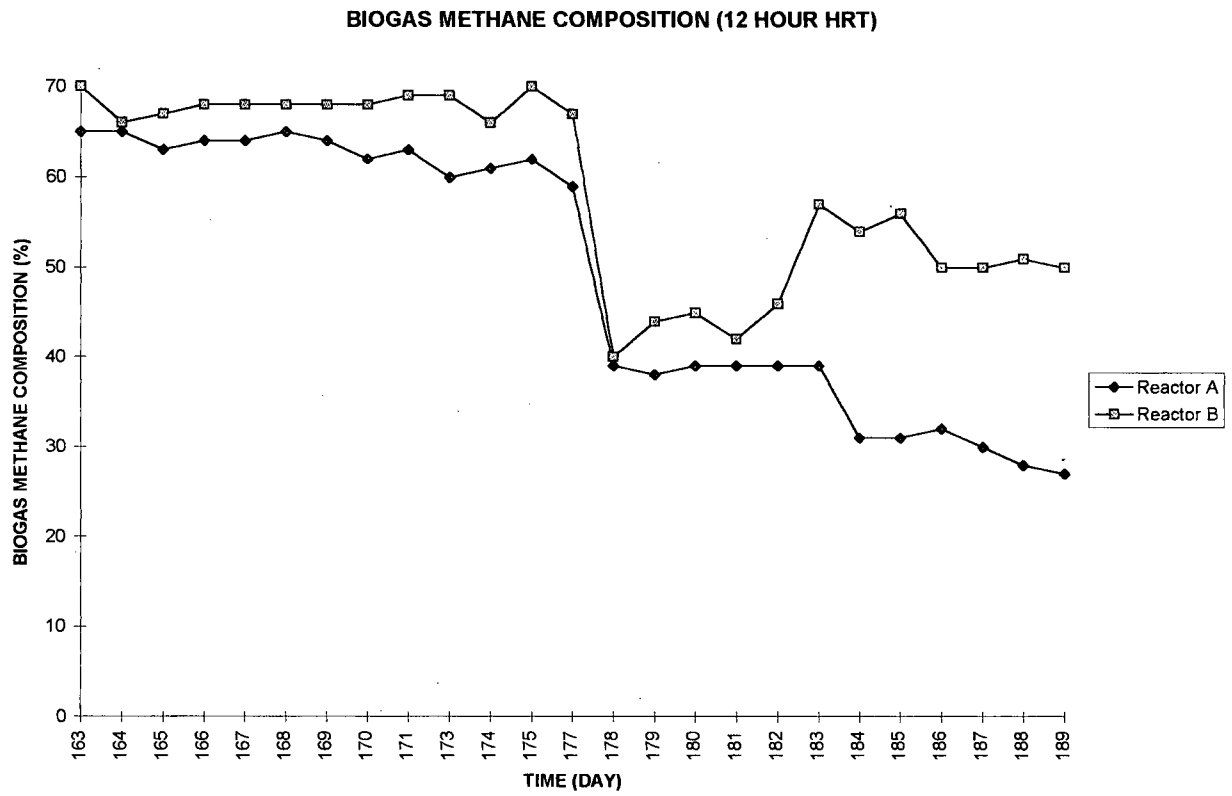


Figure E.9 Biogas Methane Composition vs Time (12 hour HRT)

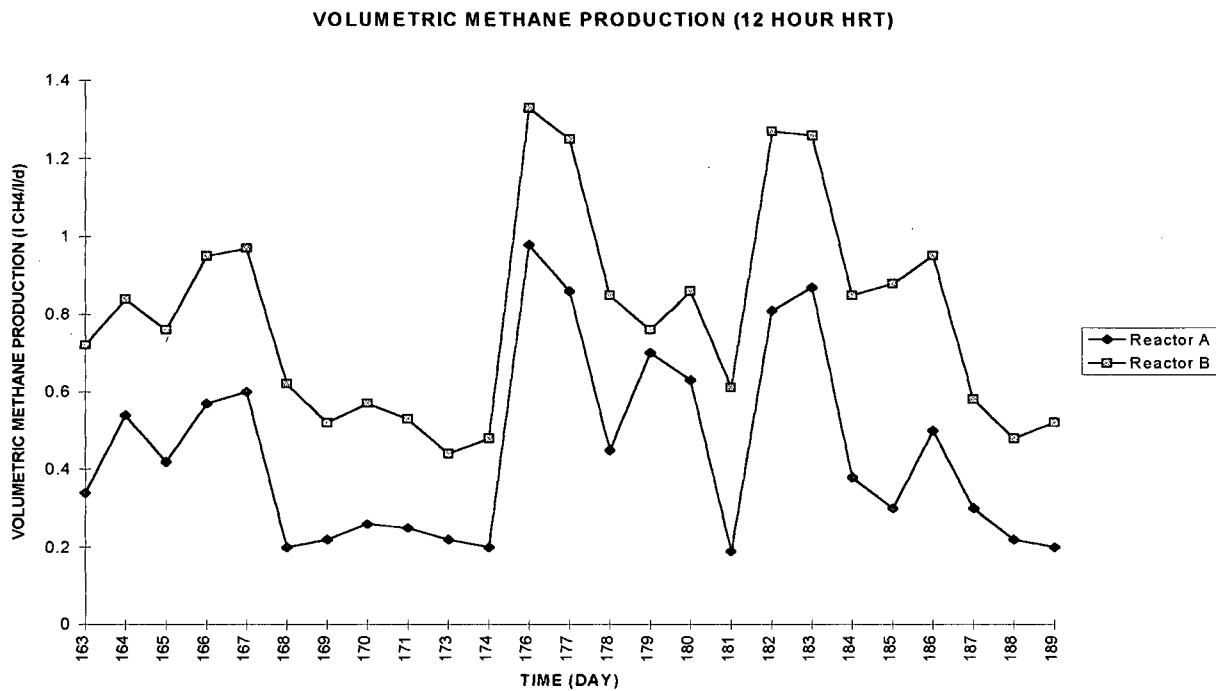


Figure E.10 Volumetric Methane Production vs Time (12 hour HRT)

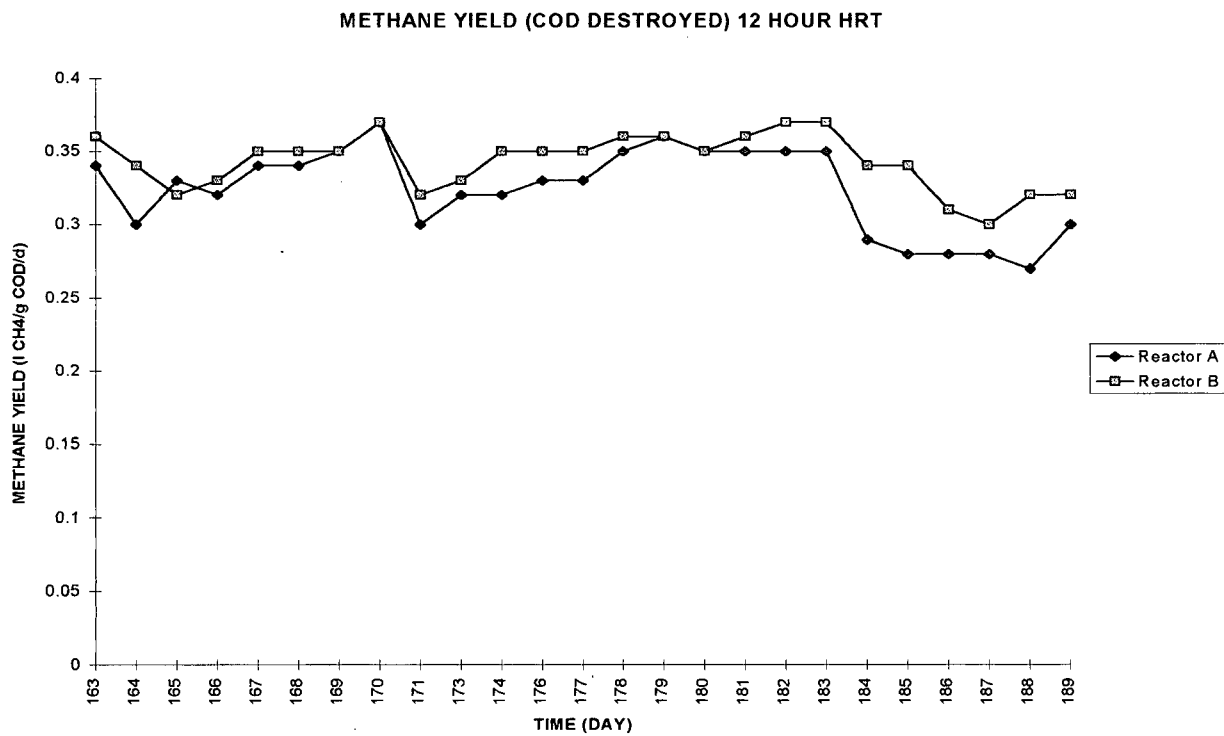


Figure E.11 Methane Yield vs Time (12 hour HRT)

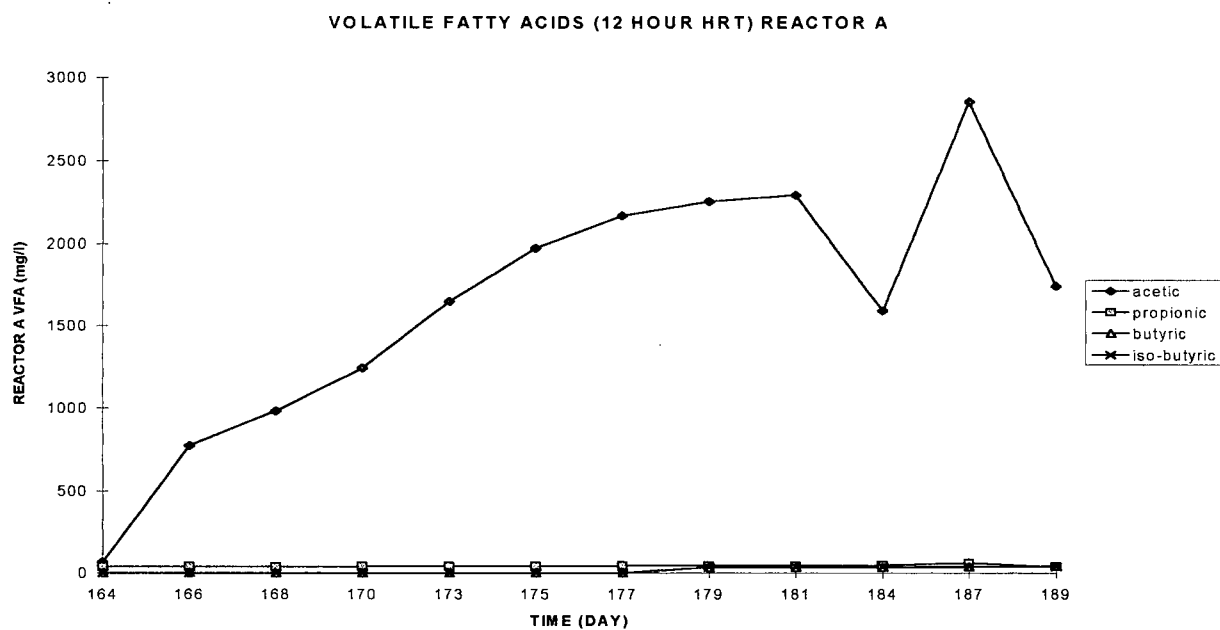


Figure E.12 VFA concentration vs Time (Reactor A : 12 hour HRT)

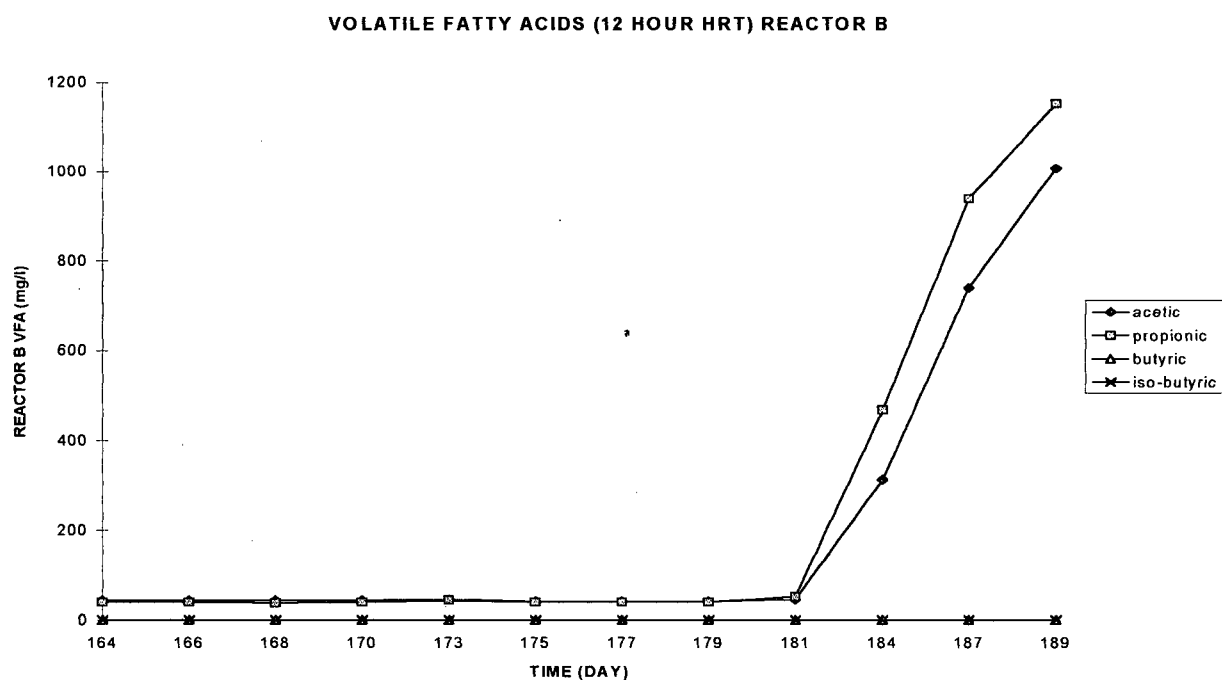


Figure E.13 VFA concentration vs Time (Reactor B : 12 hour HRT)

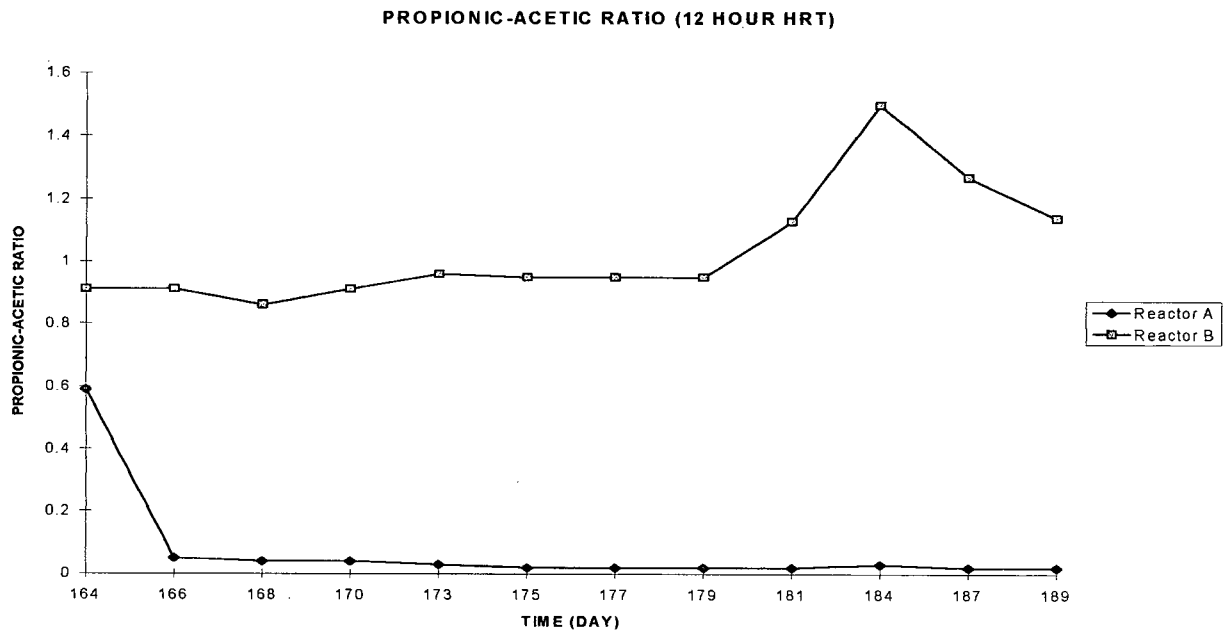


Figure E.14 Propionic-acetic acid Ratio vs Time (12 hour HRT)

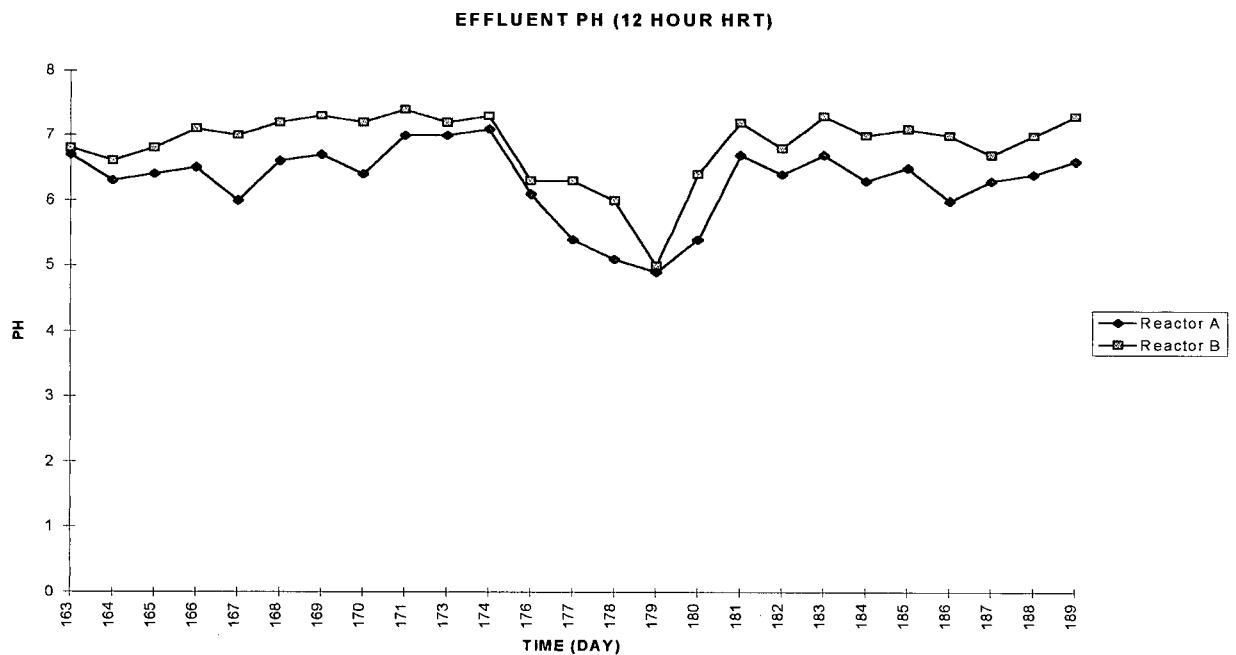


Figure E.15 Effluent pH vs Time (12 hour HRT)