

BIOREFINING OF SUGAR-BEET PROCESSING WASTES
BY
ANAEROBIC BIOTECHNOLOGY:
WASTE STABILIZATION AND BIOPRODUCT FORMATION

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EMRAH ALKAYA

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BY
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WASTE STABILIZATION AND BIOPRODUCT FORMATION**

submitted by **EMRAH ALKAYA** in partial fulfillment of the requirements for the degree of **Master of Science in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Göksel N. Demirer
Head of Department, **Environmental Engineering**

Prof. Dr. Göksel N. Demirer
Supervisor, **Environmental Engineering Dept., METU**

Examining Committee Members:

Prof. Dr. Celal F. Gökçay
Environmental Engineering Dept., METU

Prof. Dr. Göksel N. Demirer
Environmental Engineering Dept., METU

Prof. Dr. F. Dilek Sanin
Environmental Engineering Dept., METU

Prof. Dr. Ayşenur Uğurlu
Environmental Engineering Dept., HU

Assoc. Prof. Dr. İpek İmamoğlu
Environmental Engineering Dept., METU

Date: 01.08.2008

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : Emrah Alkaya

Signature :

ABSTRACT

BIOREFINING OF SUGAR-BEET PROCESSING WASTES BY ANAEROBIC BIOTECHNOLOGY: WASTE STABILIZATION AND BIOPRODUCT FORMATION

Alkaya, Emrah

M.Sc., Department of Environmental Engineering

Supervisor: Prof. Dr. Göksel N. Demirer

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The main objective of this study was to investigate two of the possible exploitation routes of anaerobic digestion (acid-phase and methane-phase) for the treatment of sugar-beet processing wastes, while producing valuable biobased products. For this purpose, four sets of laboratory experiments were carried out in a stepwise fashion:

First, in the biochemical methane potential (BMP) assay (Set-up 1) wastewater and beet-pulp were efficiently digested (63.7–87.3% COD removal and 69.6–89.3% VS reduction) in batch anaerobic reactors. Secondly, wastewater and beet-pulp could simultaneously be converted to VFAs in acidogenic anaerobic reactors with considerable acidification degrees (43.8–52.9%), optimizing the operational conditions (Set-up 2). Then, the produced VFAs were recovered by liquid-liquid extraction (Set-up 3), in which highest VFA recoveries (60.7–97.6%) were observed at 20% trioctylphosphine oxide (TOPO) in kerosene with K_D values ranging between 1.54 and 40.79 at pH 2.5. Finally, methane-phase anaerobic

digestion was evaluated in two different reactor configurations, namely fed-batch continuously mixed reactor (FCMR) and anaerobic sequencing batch reactor (ASBR) (Set-up 4). Methane production yield of 255 ± 11 mL/g COD-added was increased to 337 ± 15 mL/g COD-added (32.2% increase in methane yield) when configuration was changed from FCMR to ASBR. In addition, tCOD removal was increased from 68.7 ± 2.2 to $79.7 \pm 1.1\%$.

Based on the result obtained in this study, it is postulated that, biorefining of sugar-beet processing wastes by anaerobic digestion can not only be a solution for environmental related problems, but also contribute to resource conservation and sustainable production via valuable bio-based product formation.

Keywords: Biorefining, Anaerobic Digestion, Sugar Industry Wastes, Treatment, Bioproduct Formation.

ÖZ

ŞEKER ENDÜSTRİSİ ATIKLARININ ANAEROBİK BİYOTEKNOLOJİ UYGULAMALARIYLA BİYORAFİNASYONU: ATIK STABİLİZASYONU VE BİYOÜRÜN ELDESİ

Alkaya, Emrah

Yüksek Lisans, Çevre Mühendisliği Bölümü
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Çalışmanın temel amacı, şeker endüstrisi atıksuyu ve pancar küspesinin arıtılmasında, değerli biyoürün üretimine de olanak tanıyan, anaerobik bozundurma var olan işletme yöntemlerinden ikisini (asit-fazı ve metan-fazı) araştırmaktır. Bu amaçla, dört set laboratuvar deneyi gerçekleştirilmiştir.

İlk olarak, biyokimyasal metan potansiyeli (BMP) deneyleri (1. Set) ile kesikli anaerobik reaktörlerde atıksu ve pancar küspesi verimli bir şekilde (%63,7–87,3 KOİ ve %69,6–89,3 UKM giderimi) bozundurulmuştur. İkinci olarak, atıksu ve pancar küspesi birlikte asidojenik anaerobik reaktörlerde (2. Set) önemli ölçüde gerçekleşen asidifikasyon dereceleriyle (%43,8–52,9) UYA'lara dönüştürülmüştür. Daha sonra, üretilen UYA'lar, en yüksek kazanımların (%60,7–97,6) pH 2,5'de kerosene içerisinde %20'lik TOPO kullanılarak 1,54 ve 40,79 K_D değerleri ile gerçekleştirildiği sıvı-sıvı ekstraksiyon ile geri kazanılmıştır (3. Set). Son olarak metan-faz anaerobik bozundurma, sürekli karıştırmalı yarı-kesikli reaktör (YKR) ve

anaerobik ardışık zamanlı kesikli reaktör (AKR) olmak üzere iki farklı reaktörde araştırılmıştır (4. Set). YKR düzeneğinden AKR düzeneğine geçildiğinde, metan üretim verimi 255 ± 11 mL/g eklenen-KOİ'den 337 ± 15 mL/g eklenen-KOİ mertebesine yükselmiştir (metan üretim veriminde %32,2 artış). Bununla birlikte toplam KOİ giderimi $\%68,7 \pm 2,2$ 'den $79,7 \pm 1,1$ seviyesine yükseltilmiştir.

Bu çalışmada elde edilen sonuçlar temel alındığında, şeker endüstrisi atıklarının anaerobik biyoteknoloji uygulamalarıyla biyorafınasyonunun, çevre problemlerini çözmekle kalmayıp, değerli biyoürün eldesi ile de kaynak korunmasına ve sürdürülebilir üretime olanak sağlayacağı savunulmaktadır.

Anahtar Kelimeler: Biyorafınasyon, Anaerobik Bozundurma, Şeker Endüstrisi Atıkları, Arıtım, Biyoürün Eldesi.

To ece...

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ABBREVIATIONS

AD	: Anaerobic Digestion
ASBR	: Anaerobic Sequencing Batch Reactor
BM	: Basal Medium
BMP	: Biochemical Methane Potential
COD	: Chemical Oxygen Demand
FCMR	: Fed-batch Continuously Mixed Reactor
GC	: Gas Chromatograph
H-Ac	: Acetic Acid
H-Bu	: n-Butyric Acid
H-Pr	: Propionic Acid
HRT	: Hydraulic Retention Time
H-Va	: n-Valeric Acid
MLSS	: Mixed Liquor Suspended Solids
MLVSS	: Mixed Liquor Volatile Suspended Solids
OLR	: Organic Loading Rate
ORP	: Oxidation-Reduction Potential
sCOD	: Soluble Chemical Oxygen Demand
SRT	: Solids Retention Time
tCOD	: Total Chemical Oxygen Demand
TKN	: Total Kjeldahl Nitrogen
TOPO	: Trioctylphosphine Oxide
TS	: Total Solids
TSS	: Total Suspended Solids
tVFA	: Total Volatile Fatty Acid
TVS	: Total Volatile Solids
VFA	: Volatile Fatty Acid

CHAPTER 1

INTRODUCTION

1.1. Background Information

Today's excessive resource use and devastating change in the global environment are becoming so obvious that communities have no other choice but to take certain measures on both local and global scale. It is now widely accepted that, the traditional "consumption based" development should be shifted towards a "sustainable" one, which ensures the continuity of the life as we know it without impairing the well-being of future generations. So, there is a growing speculation on the concept "development", which has to be changed into "sustainable development" parallel to the change in the production and consumption trends of the societies. Without a doubt, to achieve this objective, major transition has to be made in the supply of energy and raw materials in the direction from non-renewable resources towards renewables, including "biomass". As many others stated:

"A major step for the development of a sustainable, industrial society will be the shift from our dependence on petroleum to the use of renewable resources." (Sauer et al. 2008).

"Shifting society's dependence away from petroleum to renewable biomass resources is generally viewed as an important contributor to the development of a sustainable industrial society and effective management of greenhouse gas emissions." (Ragauskas et al. 2006).

“We are now at the beginning of an era where new, renewable sources of energy are sought with increasing vigour; biomass, renewable carbon, is guaranteed a place in the new energy portfolio for the foreseeable future.” (Clark et al. 2006).

Biomass has been considered as one of the main alternatives to fossil resources, being a renewable source for value-added product (biofuels, biochemicals etc.) formation throughout the years (Compere and Griffith 1975; Levy et al. 1981, Vandak et al. 1997; Wackett 2008). As a result, the approach, known as “biorefining”, was established. With the advances in biotechnology, a variety of chemicals such as alcohols, ketones, and organic acids, as well as biofuels like biodiesel, ethanol, methane and hydrogen can be manufactured by biological transformations. Low value plants, grass, heathers and energy crops are some of the feedstocks suitable for the purpose of biorefining (Clark et al. 2006).

Taking one step forward, it is possible to combine resource conservation, biomass exploitation and waste management via “environmental biotechnology”, a branch of environmental science and technology. Which makes environmental biotechnology unique is that, it uses the biotechnology for the conservation and/or remediation of environment. Englande and Jin (2006) advocate that, there exist significant opportunities for value-added product formation from by-products/wastes through biotechnological approaches. A number of wastes, including food crop’s by-products, marine resource wastes, food wastes (Clark et al. 2006), industrial and agricultural wastewaters (Angenent et al. 2004) are ideal candidates for bioprocessing.

“Anaerobic digestion” is regarded as one of the several biological processing strategies which produce bioenergy or biochemicals while treating industrial and agricultural wastes (Angenent et al. 2004). During the digestion process, anaerobic bacteria produce valuable bio-based products (methane, hydrogen, organic acids,

alcohols, bio-fertilizers, etc.) either as intermediate or end products, which makes it more attractive from the economical point of view. So, anaerobic digestion, alone or in combination with other downstream processes, has been evaluated as a viable option for the management of wastes of polluting industries for decades.

Sugar-beet processing industry, which is a polluting industry, devotes increasing attention to environmental problems associated with high energy consumption and production of large amounts of wastes. Recently, sugar plants take measures to reduce energy consumption, recycle materials and energy and optimize the operation of manufacturing process in order to achieve waste minimization and sustainable production (Krajnc et al. 2007). Sugar industry wastes, with high content of biodegradable organics, can be considered a suitable source of renewable energy and bioproducts via anaerobic biological digestion. Parallel to the advances in genetics, biology and environmental biotechnology, now anaerobic digestion of sugar-beet processing wastes, has the potential for achieving dual-goal of waste management and value-added product generation.

1.2. Aim and Scope of the Study

The main objective of this study was to investigate two of the possible exploitation routes of anaerobic digestion (acid-phase and methane-phase) for the treatment of sugar-beet processing wastes, while producing valuable biobased products. In the literature, most of the studies regarding the anaerobic digestion of sugar-beet processing wastes targeted to the biomethanation and waste stabilization of each individual waste stream, resulted from different processing lines (Stoppok and Buchholz 1985; Iza et al. 1990; Hutnan et al. 2000, 2001; Farhadian et al. 2007). However, wastewater, as the primary source of sugar industry related environmental problems, and beet-pulp, a by-product generated in vast amounts, can be managed in an integrated manner. Although anaerobic treatment of wastewater is rather established, bioprocessing of beet-pulp is developing with a

significant potential as alternative to conventional animal feeding practices. In order to fill a gap in the literature, this study aimed both at the anaerobic acid-phase and methane-phase co-digestion of sugar industry wastewater and beet-pulp, as well as the recovery of produced organic acids as valuable bioproducts.

For this purpose, four sets of laboratory experiments were carried out in a stepwise fashion (Figure 1.1). First, biochemical methane potential (BMP) assay (Set-up 1) was conducted to investigate the effect of waste mixing and F/M ratio on the co-digestion of wastewater and beet-pulp, in addition to the digestion of the wastes separately. As a result of this part of the study, optimum waste mixing ratio was set and is used in the subsequent experimental set-ups.

After determination of optimum waste mixing ratio in BMP assay, two different routes were followed. In the first route (Set-up 2 and 3) the main focus was on the generation and recovery of volatile fatty acids through acid-phase anaerobic digestion followed by liquid-liquid extraction. In the second route (Set-up 4) methane-phase anaerobic digestion was evaluated in two different reactor configurations, namely fed-batch continuously mixed reactor (FCMR) and anaerobic sequencing batch reactor (ASBR).

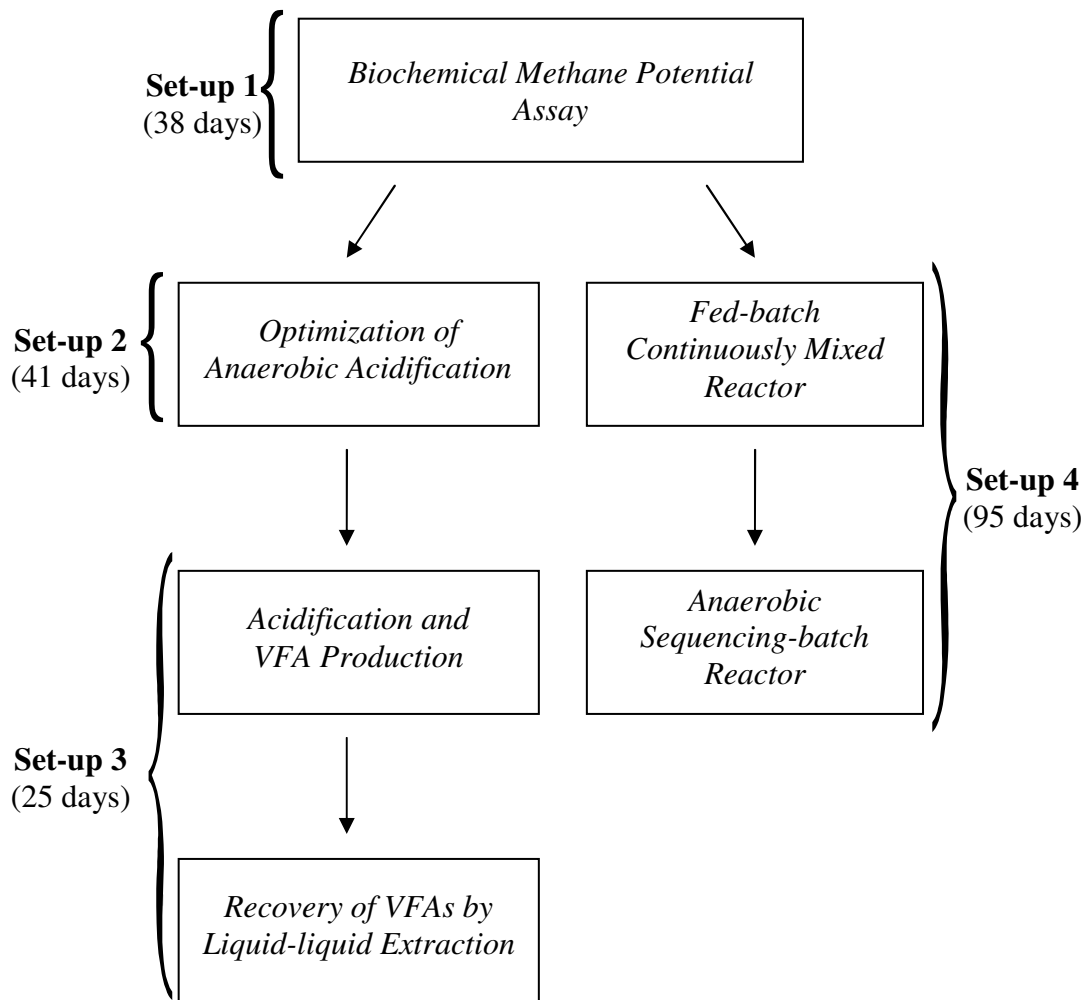


Figure 1.1. Schematic representation of the experimental set-ups

CHAPTER 2

LITERATURE REVIEW

2.1. Anaerobic Digestion

2.1.1. Process Description

Anaerobic digestion is a biological process in which a group of microorganisms biodegrade the organic matter (substrate) in the absence of free molecular oxygen (O_2). As a result of this complex biological process, organic matter is mainly converted into a mixture of methane (CH_4) and carbon dioxide (CO_2) as well as new bacterial cells (Romano and Zhang 2008). Throughout the process, complete bioconversion of organic matter into stable end products is accomplished by a series of interdependent metabolic reactions in which different classes of microorganisms take part.

Being one of the earliest biological waste treatment methods, anaerobic digestion was evolved as an established technology and now is being used for the treatment of wide variety of organic wastes originated from domestic, industrial and agricultural activities. Organic fraction of municipal solid waste (Nguyen et al. 2007; Hartmann and Ahring 2005), domestic wastewater (van Haandel et al. 2006), waste activated sludge (Bolzonella et al. 2005; Romano and Zhang 2008), fruit and vegetable wastes (Bouallagui et al. 2005), animal manure (Gungor-Demirci and Demirer 2004; Demirer and Chen 2004; Hartmann and Ahring 2005; Karim et al. 2005; Maranon et al. 2008), sugar industry wastes (Hutnan et al. 2001; Farhadian et al. 2007; Koppar and Pullammanappallil 2007) pharmaceutical wastewater (Oktem

et al. 2006) and brewery effluents (Connaughton et al. 2006) can be pronounced among numerous waste types, suitable for anaerobic biological treatment. Besides the ability of bio-product formation, anaerobic treatment has some other advantages over aerobic treatment, such as very little excess sludge production, no or little nutrient requirement, high-strength waste treatment ability, seasonal operation flexibility and lower operational costs (Gavrilescu 2002).

2.1.2. Phases of Anaerobic Digestion

The biochemistry of anaerobic digestion involves stepwise reactions in four major stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 2.1). In each of these stages, different groups of bacteria are responsible for the bioconversion of intermediate metabolites into substrates for subsequent stages. These well-organized groups of microorganisms and established balance between consumption and production of intermediate metabolites ensure the efficient digestion process.

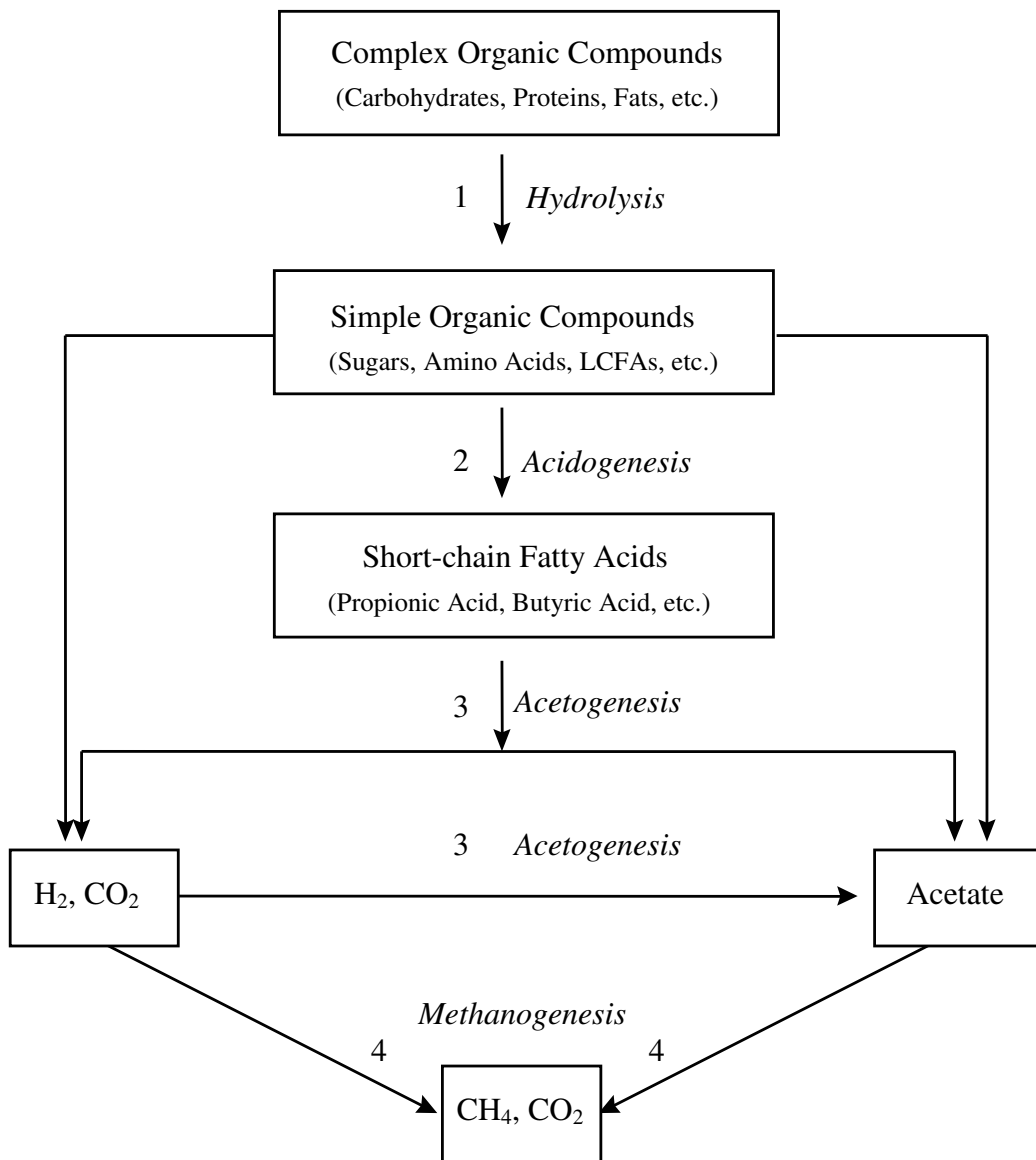


Figure 2.1. Phases of anaerobic digestion (Gerardi 2003)

The first stage in anaerobic digestion is the hydrolysis and solubilization of complex organic compounds (carbohydrates, proteins, fats, etc.) into simpler organics such as sugars, amino acids and long chain fatty acids (LCFAs). In this stage, hydrolytic bacteria produce extracellular enzymes for disintegration of

complex organic compounds which can not penetrate through bacterial cells due to their polymeric structures. This group of hydrolytic bacteria is composed of obligate and facultative anaerobes which are also responsible for the removal of oxygen, introduced in small amounts when feeding the digester (Parawira 2004a).

The next stage is referred to as acidogenesis since the major metabolites are short-chain fatty acids like, acetic, propionic and butyric acids as well as alcohols, hydrogen (H₂) and carbon dioxide (CO₂). Through acidogenic anaerobic metabolism, soluble organic compounds are degraded by wide variety of obligate and facultative anaerobic microorganisms in different fermentative pathways (Gerardi 2003). Among these degradation pathways, the one which gives a higher energy yield via acetate, carbondioxide and hydrogen is more common (Schink 1997).

Third stage is acetogenesis, which involves the degradation of higher organic acids to acetate, carbondioxide and hydrogen. This stage of anaerobic digestion process is crucial since the pronounced products of acetogenesis are major substrates for methane fermentation in final stage. Acetogens are slow growing microorganisms which are sensitive to environmental changes and fluctuations in organic load (Parawira 2004a). In addition, they are obligate hydrogen producers and their metabolic activities depend on low partial pressures of hydrogen. This special feature makes them prefer “syntrophic associations” with hydrogen consuming methanogens.

In the final stage of anaerobic digestion process, acetate, hydrogen and carbondioxide as well as other substrates such as, methanol, methylamines and formate are chiefly converted into methane and carbondioxide (Parawira 2004a). Slow-growing methanogenic microorganisms are obligate anaerobes and are sensitive to changes in environmental and operational conditions which make this stage “rate-limiting” in most of the cases.

2.1.3. Operational Conditions

In order to maintain a well balanced and effective anaerobic digestion process, a number of operational and environmental conditions must be satisfied. Among numerous operational conditions, the most influential ones can be listed as; temperature, solids retention time (SRT), organic loading rate (OLR), pH, alkalinity and nutrients.

Temperature

Anaerobic microorganisms, especially methanogens, are strongly influenced by temperature which makes digestion process preferable at mesophilic (30–35 °C) and thermophilic (50–60 °C) temperatures (Gerardi 2003). On the other hand, the use of new or modified bioreactors enable the use of psychrophilic (<20 °C) temperatures for anaerobic treatment of different effluents since they sustain required residence times for methane producers to grow (Connaughton et al. 2006).

Solids Retention Time

Solids retention time (SRT) is the average time that bacteria (solids) are retained in the digester. SRT must be kept long enough (higher than 15 days) to ensure sufficient residence especially for slow-growing methanogens to mature (Gerardi 2003). Depending on the required treatment efficiencies and/or operational conditions (temperature, waste characteristics, mixing, etc.) different SRT values may suit specific treatment needs. In some low-rate reactors, such as suspended growth continuously mixed reactors with no recycle, SRT is equal to hydraulic retention time (HRT). Whereas in high-rate reactors, it is much higher (as high as 100 days) than HRT, leading to smaller digester volumes through improved biomass immobilization.

Organic Loading Rate

Organic loading rate (OLR) is the amount of substrate, fed per unit volume of digester in a unit period of time and is very crucial for digester performance (Rajeshwari et al. 2000). OLR is closely linked to removal of organics (COD) in the form of methane and the number of methanogens retained in the digester (SRT). In other words, high methanogenic activity by biomass immobilization ensures efficient removal of organics and enables high OLRs. Romano and Zhang (2008) claim that, optimal OLRs are dependent on various operational parameters including the substrate, type of reactor, HRT, nutrients and alkalinity. In suspended and attached growth reactors, typical OLR values are reported as 0.25–3 and 10–100 g COD/L-day respectively (Rajeshwari et al. 2000).

pH and Alkalinity

Anaerobic digestion is a strongly pH dependent process. Although each of the microbial groups prefers specific pH ranges, most of them perform well near neutral pH conditions. Methanogens operate optimum at a range of 6.5 to 8.2 while acidogens prefer between 4 and 6.5 (Speece 1996). In a well-operating anaerobic digester, deviations of pH from desired ranges are prevented by the alkalinity present. In order to maintain the pH at or near neutral, alkalinity concentrations from 2000 to 4000 mg/L (as CaCO₃) are usually required (Tchobanoglous et al. 2003).

Nutrients

As it is common for most of the biological processes, nutrients are essential for anaerobic microbial growth. Besides main carbon source, nutrients like, nitrogen, phosphorus, sulphur and trace metals (Fe, Co, Ni, etc.) must be present in the digestion medium, in adequate amounts. Although, low biomass (sludge) yield,

guarantees reduced nutrient requirements, compared to aerobic processes, nutrient supplementation may be necessary in some cases. According to Speece (1996) effects of nutrients on methanogenesis are multiplicative.

2.1.4. Anaerobic Digesters

Throughout the history, anaerobic digesters evolved from low rate reactors like, simple septic tanks and anaerobic ponds, to modern and sophisticated high rate reactors such as, anaerobic filters and upflow anaerobic sludge blankets (van Haandel et al. 2006).

Septic Tanks and Anaerobic Ponds

Being the oldest low rate anaerobic treatment systems, septic tanks and anaerobic ponds have been used for years. In these simplest forms of digesters neither mechanical mixing nor heating is applied (Gijzen 2002). Though the construction materials and dimensions are different for both of the reactor types, the removal mechanisms of organic matter are the same: During the passage of the liquid, settleable materials accumulate at the bottom of the reactor where biodegradable fractions are simultaneously decomposed by anaerobic microorganisms (van Haandel et al. 2006). Volume of these low rate reactors are designed to set HRTs between 6-30 days. In order to prevent the decrease in this effective volume, accumulated sludge is removed periodically from the bottom of these reactors.

Completely Mixed Digester

Completely mixed, conventional tanks are simple, low rate reactors without biomass recycle. In these systems, during the biodegradation process, anaerobic bacteria are allowed to contact with the substrate in suspension through continuous or intermittent mixing. Since no specific method is applied for concentration and

retention of microorganisms, biomass is wasted continuously with the treated effluent (Malina and Pohland 1992). As a result of this situation, HRT is equaled to SRT, necessitating higher reactor volumes compared to high rate reactors where biomass immobilization occupied. When operated at mesophilic temperatures, this type of reactors requires detention times of 15–25 days (Rittmann and McCarty 2001). With their unsophisticated mechanics, completely mixed digesters are preferable for the treatment of wastes with high solids content.

Anaerobic Contact Process

Anaerobic contact process is a system composed of a completely mixed digester, followed by a settling tank, designed for biomass concentration and recycle. After biological degradation and gravity settling, clear supernatant is wasted as effluent while concentrated biomass is recycled back to the digester (Gavrilescu 2002). This way of biomass immobilization permits the use of lower HRTs and smaller reactor volumes compared to conventional digesters without recycle. As, Malina and Pohland (1992) mentioned, in this configuration, treatment efficiency is highly dependent on settling characteristics of bioflocs and up to 90–95% COD removal is achievable when sludge bulking is not experienced. For this kind of treatment systems, typical OLR and HRT values are reported as 1.0–8.0 kg COD/m³-day and 0.5–5 days respectively (Tchobanoglous et al. 2003).

Anaerobic Sequencing Batch Reactor (ASBR)

In this suspended growth process, after a sufficient biodegradation period, biomass is settled in the same vessel for solid-liquid separation and biomass immobilization. The operation of ASBR involves four steps: (1) feed, (2) react, (3) settle and (4) withdraw. As alternatives to continuous systems, anaerobic batch reactors have been extensively studied due to their superior process controls and biomass retentions (Zaiat et al. 2001). Depending on the used temperature (9–23 °C) and

HRT (6–46 h) average COD removal efficiencies of 56–88% is reported for a bench scale ASBR (Bodik et al. 2002).

Anaerobic Filter (AF)

Anaerobic filter is an attached growth system, composed of an anaerobic tank filled with support media such as stones, gravels, plastic particles, etc. The wastewater flows either upwards or downwards through the porous material (Hobson and Wheatley 1993). Microorganisms, attached on the surface of the support material, biodegrade the organics, during the passage of the wastewater. In AFs, high concentrations of biomass tolerate higher OLRs (2–10 kg COD/m³-d) and HRTs as low as hours (10–50 h), for effective treatment of organic wastes (Hobson and Wheatley 1993). The major drawback of the system is its tendency to accumulate suspended solids (SS) which negatively impact reactor hydraulics and internal mass transfer (Malina and Pohland 1992). Because of this reason, AFs are not suitable for the wastewaters with high SS content.

Upflow Anaerobic Sludge Blanket Reactor (UASB)

Development of UASB process is widely accepted as a milestone for anaerobic digestion and the process is used in hundreds of operating plants and applications for a wide range of effluents (Tchobanoglous et al. 2003). The success of the UASB process extensively relies on the “granule formation”. Sludge granules are rigid, high-density forms, generated as result of the aggregation of anaerobic bacteria. In this treatment system, wastewater is distributed to the granular sludge blanket at the bottom of the reactor (Gavrilescu 2002). The treated effluent, biogas and biomass are separated at the top of the reactor by the help of a three phase separator. Owing to the high settling velocity of granular sludge, UASB reactors operate hydraulically similar to upflow anaerobic filters, without the use of support media. Avoidance of the support media usage makes this process space efficient when

compared to fixed film reactors. With this high-rate anaerobic process, COD removal efficiencies of 90–95% were achieved at OLRs ranging from 12 to 20 kg COD/m³-d (Tchobanoglous et al. 2003).

2.1.5. Anaerobic Co-digestion of Wastes

Efficiency of anaerobic digestion highly depends on waste characteristics in addition to reactor configurations and other operational parameters. Temperature, organic strength, buffering capacity, solids and nutrient content can be stated as crucial waste characteristics, affecting the anaerobic biodegradation. If the characteristics of a waste are inappropriate for targeted treatment efficiency, some measures can be taken to improve its digestibility. Co-digestion is one of the options used for the enhancement of anaerobic degradation of wastes with different characteristics.

Anaerobic co-digestion is the simultaneous biodegradation of different wastes in a reactor to establish positive synergism in the digestion medium (Mata-Alvarez et al. 2004). Merits of co-digestion include: balancing suitable ratio between required nutrients, diluting potential toxic compounds (Sosnowski et al. 2003), supplying buffering capacity (Mshandete et al. 2004), sharing the equipments, establishing required moisture content, and easing the handling of wastes (Mata-Alvarez et al. 2004). In addition, anaerobic co-digestion is advantageous, if the amount of a single waste generated at a particular site is not sufficient to make anaerobic digestion cost effective (Parawira et al. 2004c).

There are numerous studies in the literature regarding the anaerobic co-digestion of various wastes which covers; food industry wastes (Murto et al. 2004; Carucci et al. 2005), animal manure (Gungor-Demirci and Demirer 2004; Umetsu et al. 2006), municipal solid waste (Hartmann and Ahring 2005; Zupancic et al. 2008),

wastewater sludge (Romano and Zhang, 2008), fish wastes (Mshandete et al. 2004) and algal sludge (Yen and Brune 2007).

In most of these studies, remarkable improvements were observed in both treatment efficiencies and biogas productions. Yen and Brune (2007) reported that the simultaneous digestion of algal sludge and waste paper increased the methane production rate by 104%, as compared to algal sludge digestion alone. The research proved that the limitation of low C/N ratio of algal sludge can be overcome by addition of waste paper. During anaerobic mesophilic batch digestion of solid potato waste, Parawira et al. (2004c) observed that the sugar beet leave addition improved the accumulated methane production and methane yield by 31–62%. In a full-scale experiment Zupancic et al. (2008) studied the effect of organic waste addition into two conventional digesters with a combined volume of 2000 m³, fed by waste sludge from wastewater treatment plants. Results showed that, the synergistic effect of organic waste of domestic refuse increased the specific biogas production and volatile suspended solids degradation efficiency (VSS) by 54% and 14% respectively.

2.2. Volatile Fatty Acids (VFAs)

2.2.1. The Importance and Production of VFAs

In chemistry, especially biochemistry, a fatty acid is a carboxylic acid or organic acid, often with a long aliphatic tail (long chains), either saturated or unsaturated. Carboxylic acids are organic acids characterized by the presence of a carboxyl group, which has the formula -C(=O)-OH , usually written as -COOH . In general, the salts and anions of carboxylic acids are called carboxylates. Volatile fatty acids are fatty acids with a carbon chain of six carbons or fewer.

They are short-chain fatty acids (formic, acetic, propionic etc.) which are produced either synthetically from fossil resources or as metabolic intermediates in acidification (fermentation) step of anaerobic digestion process. As it is the case for most of other commodity chemicals, they are usually derived from fossil fuels through chemical synthesis (Eggeman and Verser 2005). However fermentation, using renewable resources, is more preferable from the viewpoint of sustainable development and human health (Huang et al. 2007).

2.2.2. VFA Production through Anaerobic Acidification

Anaerobic biodegradation can be separated into two phases in order to enhance treatment efficiencies and/or produce bio-products. The first phase, in which hydrolysis and acidification takes place, is referred to as “acidification phase” since organic acids are major intermediates. These intermediates are further converted to methane and carbondioxide in the second phase, named as “methane fermentation”.

Two-phase anaerobic systems have been extensively studied and numerous advantages of phase separation over conventional anaerobic digestion have been demonstrated (Pohland and Ghosh 1971; Massey and Pohland 1978; Cohen et al. 1980, 1982; Demirer and Chen 2004; Yilmaz and Demirer 2008; Demirer and Othman 2008). Some of these advantages include, increased process stability and control, need of smaller reactor volumes and high tolerance to toxicity and shock loads. These advantages enable the two-phase anaerobic systems be used to treat many kinds of wastes from following sources: distillery, landfill leachate, coffee, cheese whey and dairy, starch, fruit and vegetable solid, food, pulp and paper, olive mill, abattoir, dye, primary and activated sludge and solid (Ke et al. 2005).

Along with its applications as the first step of a phase-separated anaerobic waste treatment system, anaerobic acidification can be exploited separately for bio-product formation. As Parawira et al. (2004b) stated anaerobic acidification could

be useful for the production of organic acids (e.g. VFAs) which have variety of industrial uses.

Anaerobic acidification process relies on the establishment of environmental and operational conditions which favor acidogenic microbial growth, while preventing methanogenic activity. The major practical method for selectively enrich acidogenic microorganisms is to set a low retention time, short enough to repress methanogenic activity (Hobson and Wheatley 1993; Guerrero et al. 1999). For anaerobic digesters, SRTs of 2 hours to 2 days are reported to be suitable for the accomplishment of an efficient acidification process (Speece 1996). In this situation VFAs tend to accumulate, which lowers the pH values to the levels, inappropriate for methanogenic activity (Hobson and Wheatley 1993).

Throughout the years, acid-phase anaerobic digestion received considerable attention and relevant studies covered a wide range of operational parameters including; pH (Horiuchi et al. 2002; Yu and Fang 2002), wastewater strength (Yu and Fang 2001), HRT (Maharaj and Elefsiniotis 2001; Cha and Noike 1997; Demirer and Chen 2004), temperature (Maharaj and Elefsiniotis 2001; Cha and Noike 1997) and mixing of wastes (Banerjee et al. 1999).

2.2.3. Recovery of VFAs from Fermentation Broth by Extraction

The major barrier in the use of fermentation process for VFA production is the technical difficulty associated with their recovery from the fermentation broths (Weier et al. 1992; Eggeman and Verser 2005). In addition, separation and purification of the organic acids from bulk liquids represents the majority of the production cost (Angenent et al. 2004; Gluszczyk et al. 2004). Therefore topics about the effective recovery of these fermentation products receive considerable attention.

There are various techniques, applied for the recovery of organic acids from fermentation broths, including; electrodialysis (Wang et al. 2006; Huang et al. 2007), ion-exchange (Gluszcz et al. 2004), adsorption (Joglekar et al. 2006) and liquid-liquid extraction (Mostafa 1999; Matsumoto et al. 2001; Senol and Dramur 2004). Among these methods, liquid-liquid extraction is accepted as an efficient, economical and environmentally friendly method for separation of carboxylic acids (Eyal and Canari 1995).

Liquid-liquid extraction is one of the oldest and well-established chemical operations. It is based on the transfer of a solute (organic acid in this case) in a liquid solution (fermentation broth in this case) by contacting with another liquid solvent (extractant) which is relatively immiscible with the solution. Efficiency of organic acid extraction depends highly on the nature of the acid extracted, the concentration of the extractant, the type of diluent (Tamada et al. 1990) and pH (Yang et al. 1991). Alcohols, ketones, ethers and aliphatic hydrocarbons can be used as solvents for the extraction of organic acids from fermentation broths. However, organophosphates such as trioctylphosphine oxide (TOPO), tri-n-butyl phosphate (TBP) and aliphatic amines are found to be more efficient for the purpose (Yang et al. 1991). Wardell and King (1978) used triisooctylamine in various dilutes to extract 0.5 wt % acetic acid solution, which resulted in 12–81 % acid removal. In another study propionic and butyric acids were extracted using Alamine 336 and Aliquat 336 with distribution coefficients (K_D) ranging between 1.94 and 15.50 (Yang et al. 1991).

2.2.4. Industrial Uses of VFAs

Being valuable chemical products, VFAs have diverse uses in the market. They are utilized for the manufacture of various organic compounds including alcohols, aldehydes, ketones, esters and olefins (Eggeman and Verser 2005). Esters of acetic acids have commercial value as solvents for plastics, lacquers, resins and gums as

well as included in cosmetic formulations (Saha et al. 2000). Propionic acid is used as animal feed, grain preservation, antifungal agents, plasticizers and herbicides (Gu et al. 1999) while butyric acid has applications in the foodstuff and beverage industry (Vandak et al. 1997). These organic acids are also treated as substrates for the biological synthesis of polyhydroxyalkanoates (PAH), biopolymers used for the production of biodegradable plastics (Bengtsson 2008). In addition, VFAs play instrumental roles as carbon sources in the biological nutrient removal (BNR) processes for wastewater treatment (Maharaj and Elefsiniotis 2001).

2.3. Beet-sugar Industry

2.3.1. Sugar-beet Processing

Edible sugar (sucrose) is produced from two major raw materials, which are sugar beet and sugar cane (European Commission 2006). More than 60% of the world's sugar requirement is met by sugar cane processing while the balance is by sugar beet (World Bank Group 1998). Although raw material is different, the objectives of both practices are the same: to extract the sucrose from the raw material and to transform it into sugar crystals.

The production of sugar from sugar beet is composed of five major steps: Beet preparation, sugar extraction, juice purification, juice concentration/evaporation, and crystallization (Barjol and Chavanes 2003).

First, sugar-beets are delivered by conveyor belts or flume water (continuous stream of water) to the place where washing is performed. In this part of the process, beets are separated from soils, taps and leaves. Then, clean sugar beets are cut into slices, known as cosettes, in order to increase surface area for an effective extraction process. In the next step, cosettes are passed into diffusers where countercurrent extraction of sucrose takes place at 68 to 72 °C (European

Commision 2006). As a result of extraction, impure sugar juice and beet pulp (exhausted pulp) is generated. After that, produced sugar juice is removed from impurities, through lime addition, followed by precipitation and filtration. This purification step results in thin juice, needs to be concentrated and crystallized. Prior to crystallization, concentration of thin juice is performed by successive evaporating vessels, producing syrup with a solid content around 70% (Barjol and Chavanes 2003). Resulting thick juice is further evaporated in special vacuum pans to form sugar crystals (European Commision 2006). At the end of the process, crystals are separated from liquid phase by centrifugation and dried for storage.

2.3.2. Characteristics and Management of Wastes

Traditional sugar-beet processing practices have adverse environmental impacts due to production of large amounts of wastes and by-products, as well as high consumption of energy, water and lime (Vaccari et al. 2005). Besides major product, edible crystal sugar, production process ends up with wastewater, exhausted beet-pulp, molasses, lime sludge, soil sludge, sand and vegetable matter (Krajnc et al. 2007).

The wastewater is the primary cause of sugar industry related pollution. Flume water, representing about 70% of the total wastewater volume, is the major contributor (Iza et al. 1990; Dilek et al. 2003), although different wastewater streams, such as wash water and excess condensate water, are present. It contains high concentrations of hydrocarbons and soluble organic matter, mainly sugars, as a result of leaching from cut and damaged surfaces of the beets (Shore et al. 1984; Iza et al. 1990). COD content of this high strength wastewater is reported as 5000–20000 mg/L (European Commision 2006). Management of the wastewater varies from factory to factory but sedimentation followed by biological treatment is most common (Shore et al. 1984).

One of the by-products of sugar industry is the beet-pulp, a solid residue generated after sucrose diffusion/extraction process. Approximately 250 kg pressed pulp (25% dry matter) is produced per ton of beet processed (Spagnuolo et al. 1997). This cellulosic by-product composed mainly of cellulose (21–27%), araban (20–22%), galactan (5.5–7%), pectin (17–18%), protein (9%), sucrose (1–2%) and ash (5%) (Arntz et al. 1985). Contrary to wastewater, beet-pulp is utilized for further use instead of treatment and disposal. It is mostly used as animal feed where cattle-raising industry is developed (Hutnan et al. 2000). For this purpose, beet-pulp may be used directly or could be dried alone or in combination with molasses (Krajnc et al. 2007). On the other hand, in the countries where, cattle-raising industry is underdeveloped, it is dumped in landfills (Voragen et al. 1997).

Molasses, a valuable by-product, is the final syrup from the crystallization step and has variety of industrial uses (Barjol and Chavanes 2003). Fermentation and alcohol distillation industries are two of various market applications of molasses. Moreover, molasses is also used as supplement for animal feed with high carbohydrate and protein content (Krajnc et al. 2007).

Sugar industries use enormous amounts of lime, primarily for the removal of impurities in sugar juice. This practice results in high amounts of lime sludge to be managed. Although it has some uses in soil amendment and cement industries, it remains as a waste which is difficult to dispose (Vaccari et al. 2005).

2.3.3. Anaerobic Digestion of Wastewater and Beet-pulp

As a viable option for waste management in sugar-beet processing facilities, anaerobic digestion was broadly evaluated. Most of the studies focused on the anaerobic treatment and biogasification of wastewater and beet-pulp which are two major waste streams of beet processing plants.

Wastewater from the beet-sugar mill contains high concentrations of hydrocarbons which makes it suitable for anaerobic biological degradation. It is also characterized by extremely low concentrations of phosphorus and nitrogen. Since this situation (low C/N and C/P ratios) is unfavorable for aerobic biological treatment, anaerobic digestion is preferable for effective treatment of the wastewater (Wang et al. 1986). Consequently, there have been several studies on anaerobic digestion of beet processing wastewater (Shore et al. 1984; Wang et al. 1986; Iza et al. 1990; Farhadian et al. 2007). According to Farhadian et al. (2007), there has been considerable attention on anaerobic digestion of sugar beet processing wastewaters, mainly by UASB processes.

Koppar and Pullammanappallil (2007) advocates that, the cost of the beet-pulp drying is increasing due to the increase in fuel prices and more profitable applications need to be developed. In some studies, anaerobic digestion, producing energy-rich methane gas, is proven to be a feasible alternative for the utilization of beet-pulp (Lane 1984; Weiland 1993; Koppar and Pullammanappallil 2007). Moreover, studies which cover two-phase anaerobic digestion of beet-pulp were conducted and merits of phase separation were indicated for biogasification and methane production (Stoppok and Buchholz 1985; Hutnan et al. 2000, 2001). Hutnan et al. (2001) states that, the energy, produced from beet-pulp through anaerobic digestion can meet 30.4% of the daily energy requirement of a sugar factory, which processes 2000 tons of sugar beet per day. In fact, there is a common belief among researches that more profitable and sustainable applications must be developed for the utilization of beet-pulp, other than animal feeding.

CHAPTER 3

MATERIALS AND METHODS

3.1. Waste Characteristics

Sugar industry wastes (wastewater and pressed beet-pulp) were obtained from a private beet-sugar factory located near Amasya, during the beet-campaign period. Characterizations of the wastes were carried out and the results were tabulated (Table 3.1 and 3.2). After the characterization, wastes were kept frozen at $-20\text{ }^{\circ}\text{C}$ in order to inhibit biological activity prior to the use in the experimental studies.

Before the characterization and the use in the study, wastewater was settled for 1-hour to remove the suspended materials (mostly inorganic) which are very common for sugar-beet processing wastewater. The time period of 1-hour was chosen to represent the typical hydraulic retention time of primary sedimentation before the secondary treatment systems.

In order to achieve physical homogeneity, beet-pulp was processed as follows: First the frozen beet-pulp was thawed at room temperature and further dried at $105\text{ }^{\circ}\text{C}$ for 24 hours. Then, the dried pulp particles were grinded by the help of a pestle and the homogenized powdered pulp was used for reactor feeding.

Table 3.1. Wastewater characteristics (1 hr settled)

Parameter	Value (mg/L)
tCOD	6621 ± 113.2
sCOD	6165 ± 517.1
TS	6062 ± 53.0
VS	2832 ± 25
TSS	665 ± 21.2
VSS	335 ± 7.1
pH	6.82
Alkalinity (as CaCO ₃)	1760
TKN	10
P _{Total}	2.7
tVFA (as H-Ac)	1115 ± 20
H-Ac	394 ± 5
H-Pr	610 ± 12
H-Bu	46 ± 1
Calcium (Ca ²⁺)	378 ± 5.7

Table 3.2. Pressed beet-pulp characteristics

Parameter	Value
Moisture (%)	85 ± 0.1
TS (%)	15 ± 0.1
VS (%TS)	94 ± 0.01
COD (g/g dry weight)	1.22 ± 0.15
TKN (%TS)	7.28
P _{Total} (%TS)	1.0 ± 0.28

3.2. Inoculum

The mixed anaerobic cultures were obtained from the anaerobic sludge digesters of the municipal wastewater treatment plant of Ankara to be used as seed in the study. Prior to the use as inoculum, it was concentrated by gravity settling for 24 hours. By this way, seed VSS concentration was increased and then used in the experiments.

3.3. Basal Medium

In order to supply adequate nutrients for an optimum microbial growth, reactors were fed by basal medium (BM) which contains the following constituents (concentrations are given in parentheses as mg/L): NH_4Cl (1200), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (400), KCl (400), $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (300), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (50), $(\text{NH}_4)_2\text{HPO}_4$ (80), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (40), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (10), KI (10), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.5), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.5), ZnCl_2 (0.5), $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (0.5), $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.5), H_3BO_3 (0.5), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.5), $\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$ (0.5), Cysteine (10), (Gungor-Demirci and Demirer 2004). In BMP assay (Part 3.5.1) and first period (alkalinity added period) of the optimization of anaerobic acidification study (Part 3.5.2), NaHCO_3 (6000) was also included in BM.

3.4. Analytical Methods

Total Chemical Oxygen Demand

tCOD determination for beet-pulp characterization was carried out as described in standard methods (5220 B. Open Reflux Method) (APHA 2005). All other tCOD determinations were carried out by EPA approved reactor digestion method (for COD range of 0-1500 mg/L) and spectrophotometric determinations were performed by using a spectrophotometer (SN 05827, PC Multidirect).

Soluble Chemical Oxygen Demand

Prior to analyses, samples were filtered through 0,45 µm pore sized filters (Millipore). Then, sCOD determinations were carried out by EPA approved reactor digestion method (for COD range of 0-1500 mg/L) and spectrophotometric determinations were performed by using a spectrophotometer (SN 05827, PC Multidirect).

Biogas Production

Biogas productions were measured with water replacement devices. In BMP assay, a device consisting of a 50 mL burette connected to a 500 mL water reservoir was used. So, a needle connected to the burette via latex tubing was inserted through the rubber stoppers of the serum bottles to determine produced biogas amount in headspace of 250-mL reactors. In all other reactors, biogas productions were measured by using a graduated water reservoir (1000 mL) connected directly to the reactor headspace. Acid brine (10% NaCl w/v, 2% H₂SO₄ v/v) was used as displaced water, in order to eliminate the solubilization of the biogas (Tezel et al. 2007).

Biogas Composition

Biogas compositions were determined with a gas chromatograph (Thermo Electron Co.) equipped with a thermal conductivity detector (TCD). Produced biogases were separated as hydrogen (H₂), carbon dioxide (CO₂), oxygen (O₂), methane (CH₄) and nitrogen (N₂) by using parallel connected columns (CP-Moliseve 5A and CP-Porabond Q) at a fixed oven temperature of 45 °C. Helium was used as carrier gas at 100 kPa constant pressure. The inlet and detector temperatures were set to 50 °C and 80 °C respectively.

Volatile Fatty Acids

The gas chromatograph (Thermo Electron Co.), used for biogas composition determinations, was also used for the periodical VFA measurements. However the column, the detector and the operational conditions were different: Nukol column (Model 25326, 15 m × 0.53 mm) was used to separate VFAs (acetic, propionic, n-butyric, iso-butyric, n-valeric, iso-valeric, n-caproic, iso-caproic and n-heptanoic acids). Flame ionization detector (FID) was used for this purpose which was adjusted to 280 °C as operating temperature. Helium was used as carrier gas with a constant flow rate of 6 mL/min and the inlet temperature was kept at 250 °C. Oven temperature was initially set to 100 °C with 2 min holding time and then increased up to 200 °C with 8 °C/min ramping.

Prior to the gas chromatography injections, series of pretreatments were conducted for VFA measurements: First, samples were filtered through 0.22 µm pore-sized filters. Then the samples were diluted with deionized water to assure the VFA concentration of the sample to be in the range of pure VFA calibration of gas chromatograph. After filtering and dilution, the samples were acidified with 98% formic acid to a pH less than 2.5, in order to convert the fatty acids to their undissociated forms (i.e. acid forms).

Total Solids and Volatile Solids

TS and VS determinations were carried out as described in standard methods (2540 B. Total Solids Dried at 103–105 °C, 2540 E. Fixed and Volatile Solids Ignited at 550 °C) (APHA 2005).

Suspended Solids and Volatile Suspended Solids

SS and VSS determinations were carried out as described in standard methods (2540 D. Total Suspended Solids Dried at 103–105 °C) (APHA 2005).

Total Kjeldahl Nitrogen

TKN was measured according to the procedure described in standard methods (4500-N_{org} B. Macro-Kjeldahl Method) (APHA 2005).

Total Phosphorus

P_{Total} determinations were carried out as described in standard methods (4500-P) (APHA 2005).

Calcium Ion

Ca²⁺ determinations were carried out as described in standard methods (3500-Ca B. EDTA Titrimetric Method) (APHA 2005).

Alkalinity

Alkalinity was measured according to standard methods (2320-B Titration Method) (APHA 2005).

pH

pH values were measured with a pH meter (HI 8314, Hanna Instruments) and a pH probe (HI 1230, Hanna Instruments).

Oxidation-Reduction Potential

ORP values of the reactors were measured with a pH meter (PH 510, Eutech) equipped with an ORP electrode (Recorder S-500C, Sensorex).

Sludge Volume Index

SVI determinations were carried out as described in standard methods (2710 D. Sludge Volume Index) (APHA 2005).

3.5. Experimental Set-ups and Procedures

3.5.1. Set-up 1: Biochemical Methane Potential Assay

Anaerobic batch reactors were used in order to determine the anaerobic biodegradation and biogas generation potential (Owen et al. 1979) of beet-sugar industry wastes. Separate and co-digestion of wastewater and beet-pulp were studied in 250-ml serum bottles with effective volume of 150 ml. All reactors were inoculated with anaerobic seed sludge, establishing a VSS concentration of 8800 mg/L. Then the wastes were added to the reactors with different amounts to give F/M ratios (mg waste-COD/mg seed-VSS) in the range of 0.51–2.56 (Table 3.3). BM was also added into the reactors to supply adequate macro- and micro-nutrients. Control reactors, containing only anaerobic seed sludge (8800 mg VSS/L) and BM, were also incubated to determine the background gas production. All reactors were run in duplicates and presented data composed of the averaged values.

Prior to incubation, totally 20 reactors were purged with 75% N₂ and 25% CO₂ for 3–4 minutes in order to maintain anaerobic conditions with proper pH. Then the reactors were sealed with natural rubber stoppers and plastic screw-caps. Prepared

reactors were incubated in a temperature controlled room at 35 ± 1 °C. Continual mixing was applied at 175 rpm by using a mechanical shaker for 38 days of operation.

During digestion period, biogas productions were daily, biogas compositions were periodically recorded. After the digestion period was ended, all reactors were subjected to pH, VS, and COD determinations, in order to analyze the treatment efficiencies.

Table 3.3. Initial conditions inside the reactors

Reactor	pH*	F/M (g COD/g VSS)	Wastewater COD (mg/L)	Beet-pulp COD (mg/L)	Total COD (mg/L)
R1	8.13	0.51	4500	-	4500
R2	7.59	0.26	-	2250	2250
R3	8.01	0.51	-	4500	4500
R4	7.96	1.02	-	9000	9000
R5	7.97	2.05	-	18000	18000
R6	7.88	0.77	4500	2250	6750
R7	7.70	1.02	4500	4500	9000
R8	7.71	1.54	4500	9000	13500
R9	7.67	2.56	4500	18000	22500
Control	7.48	-	-	-	-

*Prior to purging with 75% N₂ + 25% CO₂ gas mixture.

3.5.2. Set-up 2: Optimization of Anaerobic Acidification

Since the objective of this study was the optimization of the operational parameters (HRT, waste mixing ratio and pH) on anaerobic acidification, 6 reactors were operated with different combinations of the corresponding parameters (Table 3.4). Reactors were run as duplicates (totally 12 reactors) to attain statistical reliability and the presentation of the data involves the averaged values of duplicate reactors.

250 mL serum bottles with effective volume of 150 mL were operated by daily fed-batch feeding strategy as continuously mixed acidogenic reactors. Reactors were kept continuously mixing at 175 rpm by using a mechanical shaker in a temperature controlled room (35 ± 1 °C) for 41 days.

In order to prevent uncontrolled pH drops and to investigate the effect of higher operational pH (6.9–7.5) on the acidification, first 20 days of operation was carried out by adding external alkalinity in the form of NaHCO_3 . In this period, NaHCO_3 concentrations were fixed at 6000 mg/L in the reactors by daily additions. Then, the reactors were operated 21 more days without adding any external alkalinity to observe the natural pH drop, as a result of acidification, and to investigate the effect of lower operational pH (5.7–7.4) on acidification.

For the purpose of reactor feeding, three different stock solutions were prepared (Table 3.5). In order to investigate the effect of waste mixing ratio on acidification, prepared stock solutions involved same concentrations of BM but different mixing ratio of wastewater (WW) and pulp (P) in terms of tCOD.

As the control parameters of reactor operations, pH, ORP, biogas production, biogas composition, VFA and sCOD concentrations were measured. Among these parameters, pH, ORP and biogas production were daily, VFAs, sCOD, and biogas compositions were periodically measured.

Table 3.4. Operational parameters of the reactors

Reactor	Feed	HRT (Days)	Waste mixing ratio (WW:P)	OLR (g COD/L-day)
R1	Stock #1	2	1:0	2.7
R2	Stock #2	2	1:0.5	4
R3	Stock #3	2	1:1	5.4
R4	Stock #1	4	1:0	1.35
R5	Stock #2	4	1:0.5	2
R6	Stock #3	4	1:1	2.7

Table 3.5. Compositions of stock solutions

Composition	Stock #1	Stock #2	Stock #3
Wastewater (mg/L as tCOD)	5300	5300	5300
Pulp (mg/L as tCOD)	–	2650	5300
tCOD (mg/L)	5300	7950	10600
sCOD (mg/L)	4932 ± 72	5211 ± 421	5318 ± 288
pH	8.02	7.99	8.08

3.5.3. Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

In the optimization study for anaerobic acidification (Set-up 2), optimum operational conditions were already set for VFA production. These optimum conditions were applied for the operation of a daily-fed semi-continuous

acidification reactor in third experimental set-up. Then the fermentation broth was subjected to subsequent extraction experiments (Set-up 3).

Acidification/VFA Production

Simultaneous acidification of sugar industry wastewater and beet-pulp were achieved in an anaerobic reactor composed of a 550 mL flask with an effective volume of 500 mL. Reactor was daily fed by wastewater, pulp and BM mixture. Feed of the reactor included same amounts of wastewater and pulp in terms of COD concentrations (1:1 waste mixing ratio). Operational conditions were fixed at 2 day and 5.4 g COD/L-day for HRT and OLR values respectively. Without adding any external alkalinity, reactor was continuously mixed (175 rpm) by the help of a magnetic stirrer at 35 ± 1 °C for 20 days of operation.

To be able to assess the acidification performance of the reactor; pH was daily, VFA, solids, COD concentrations were periodically monitored as control parameters. After 13–15 days of operation, control parameters were stabilized indicating that steady-state was reached. So as to express the steady-state effluent characteristics and acidification performance of the reactor, averaged values of control parameters are calculated in the last 5 days of operation (day 16–20).

Effluents of five consecutive days (day 16–20) were also collected together in a flask and kept at +4 °C, to be used further in extraction experiments. Prior to its use as aqueous phase in the extraction experiments, fermentation broth was filtered through a 0.45 µm pore sized filter.

Liquid-Liquid Extraction of VFAs

The aim of extraction experiments was to assess the influence of pH and extractant (TOPO in kerosene) concentrations on the recovery of VFAs from fermentation broth.

Extractant solvents were prepared at 30 °C by dissolving TOPO (98.5% purity, Fluka-00676) in kerosene (Fluka-60710) with different ratios (5, 10, and 20% wt) using a magnetic stirrer. Then the prepared solvents were used for the extraction in two different pH conditions (2.5 and 5.5). In the first run, prior to extraction, pH of the aqueous phase was decreased to 2.5, which is smaller than pK_a values of VFAs ($pK_a = 4.8$) (Kanicky and Shah, 2003), by using 0.1 N H_2SO_4 solution, to ensure the predominance of undissociated forms (i.e. acid forms) of VFAs. Then, in the second run, the aqueous phase (filtered fermentation broth) was subjected to extraction without any pH adjustments (at pH 5.5) to observe the effect of pH.

For the extraction of VFAs, 10 mL of each aqueous and organic phase were mixed in a 30 mL separatory funnel, shaking at room temperature for 5 minutes. In this equilibrium condition, the extraction mixture was allowed to settle for 1–3 minutes. After two phases were separated by gravity, aqueous phase was subjected to VFA and COD analyses. Then, organic phase VFA concentrations ($[HA]_{org}$) were determined by mass balance (Eq. 3.1).

$$([HA]_{org})_{eq} = \frac{([HA]_{aq} \times V_{aq})_{initial} - ([HA]_{aq} \times V_{aq})_{eq}}{(V_{org})_{eq}} \quad (3.1)$$

Where; V_{aq} : Volume of aqueous phase (mL)

V_{org} : Volume of organic phase (mL)

The percent weight of acid, transferred from the aqueous phase into organic phase was expressed as the percentage recovery of corresponding acid (Eqn 3.2). So as to compare total extraction efficiencies of different runs, all VFAs were expressed as acetic acid concentration (mg/L as H-Ac) and summation of these concentrations were expressed as total VFA concentration (tVFA) as well as separate VFAs (acetic, propionic, n-butyric, and n-valeric) for recovery calculations.

$$\% \text{ Recovery} = \frac{([\text{HA}]_{\text{org}} \times V_{\text{org}})_{\text{eq}}}{([\text{HA}]_{\text{aq}} \times V_{\text{aq}})_{\text{eq}}} \quad (3.2)$$

After the extraction, the ratio of the acid concentration of organic phase ($[\text{HA}]_{\text{org}}$) to that of aqueous phase ($[\text{HA}]_{\text{aq}}$) (Eqn 3.3) was defined as distribution ratio (K_D) at equilibrium (Wardell and King 1978; Yang et al. 1991).

$$K_D = \left(\frac{[\text{HA}]_{\text{org}}}{[\text{HA}]_{\text{aq}}} \right)_{\text{eq}} \quad (3.3)$$

3.5.4. Set-up 4: Evaluation and Comparison of Fed-batch and Sequencing-batch Reactors in terms of Biomethanation

The aim of this part of the study is to compare a fed-batch continuously mixed anaerobic reactor (FCMR) to anaerobic sequencing batch reactor (ASBR) in terms of waste stabilization and methane production. For this purpose, a reactor, having 1150 mL effective volume and 50 mL headspace, was operated as FCMR, which was then operated as an ASBR by changing operational conditions, after steady-state is reached. During both operational periods, a stock solution, containing BM together with equal amounts of pulp and wastewater in terms of COD was daily fed to the reactor as influent. Characteristics of influent are given in Table 3.6.

Table 3.6. Influent characteristics

Parameter	Value (mg/L)
tCOD	10600
sCOD	5318 ± 288
TS	9193
VS	6348
SS	4832
VSS	4268
tVFA (as H-Ac)	892 ± 16
pH	7.65

Fed-Batch Continuously Mixed Reactor (FCMR)

Reactor was operated by daily feeding-wasting at an HRT of 15 days. Since, biomass recycle was not practiced, SRT was equal to HRT. Reactor operation was carried out in a temperature controlled room (35 ± 1 °C), while continual mixing was applied at a rate of 200 rpm by a magnetic stirrer. During this period of digestion, OLR was fixed at 0.71 g COD/L-day. Before being converted to ASBR configuration, reactor was operated for 50 days, in which last 10 days of period was concluded as steady-state.

As the control parameters; pH and gas production were daily, solids, COD, alkalinity, VFA, and gas compositions were periodically measured.

Anaerobic Sequencing Batch Reactor (ASBR)

Beginning with 51.day, reactor was operated as an ASBR, applying the operation scheme, depicted in Figure 3.1. The feed-waste cycle of 24 hours was composed of four steps: (1) feed, (2) react, (3) settle, and (4) withdraw. The whole operation of the configuration was carried out manually without using any automation.

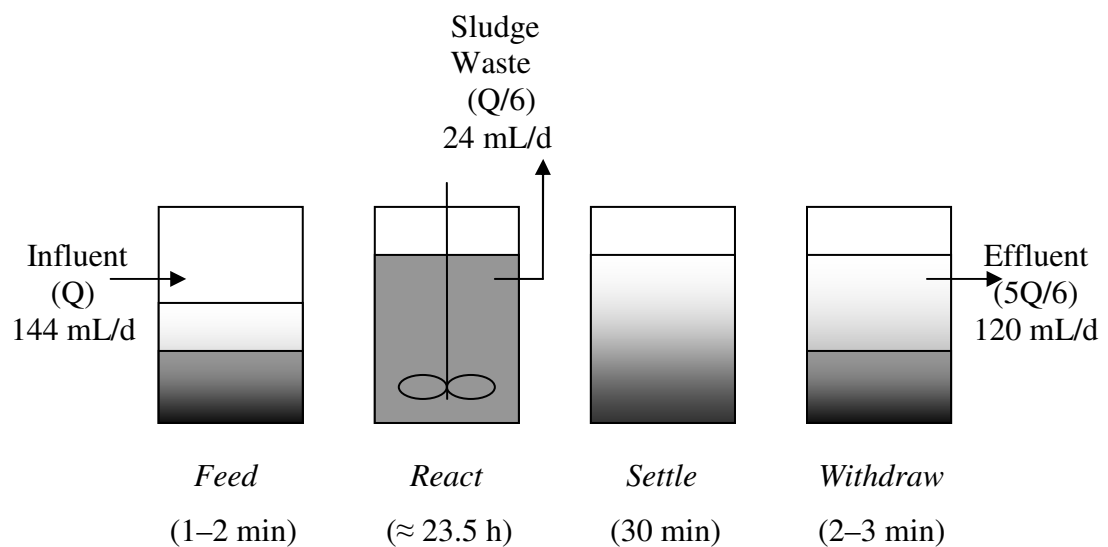


Figure 3.1. Operation scheme of the ASBR configuration

When compared to FCMR operation, daily feed volume was higher to result in an HRT of 8 days. Correspondingly, OLR value was increased to 1.33 g COD/L-day. After feeding, reactor was continuously mixed at 200 rpm, during which biodegradation occurs. When reaction was over (prior to sedimentation), volume of mixed liquor, equal to one sixth of feed volume ($Q/6$), was wasted deliberately to prevent the accumulation of non-biodegradable fraction of influent waste. Since daily sludge wasting was performed prior to settling, mixed-liquor solids

concentrations represented the waste-sludge characteristics. As a consequence of periodical waste of sludge, SRT was kept between 24-30 days, which was computed as (Tchobanoglous et al. 2003):

$$\text{Biomass Retention (d)} = \frac{\text{Total Biomass in the Reactor (mg)}}{\text{Biomass Leaving the Reactor (mg/d)}} \quad (3.4)$$

$$\text{SRT} = \frac{X \times V}{(X \times Q_w) + (X_e \times Q_e)} \quad (3.5)$$

Where; X : Biomass concentration in the reactor (mg/L MLVSS),
V : Effective volume of the reactor (L),
Q_w : Daily wasted volume of the mixed liquor (L/day),
X_e : Biomass concentration in the effluent of the reactor (mg/L VSS),
Q_e : Daily wasted volume of the effluent (L/day).

During the operation of ASBR, tCOD and solid concentrations were periodically determined for both mixed liquor and effluent, in order to use in the mass balance calculations. In addition, pH and biogas production was daily, sCOD, VFA, gas composition, alkalinity, and SVI, was periodically measured as the other control parameters.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Biochemical Methane Potential Assay

4.1.1. Anaerobic Biodegradability of Wastewater and Beet-Pulp

Reactors were operated until no significant biogas production was detected. So, cumulative biogas productions are depicted in Figure 4.1 for 38 days of operation. Initially in all of the reactors relatively high biogas production rates were observed. Sung and Dague (1995) stated that, high F/M ratio, established right after feeding of a batch reactor, provides a high driving force for methanogenic activity and elevated biogas production rates. So, as biodegradation proceeded with time, depletion of available substrates resulted in the decrease in biogas production rates, which was an expected result. During the first 10 days of operation, reactors produced 57.0–85.1% of their total biogas productions (as of day 38), without any indication of a significant inhibition (Table 4.1). It is clear from Figure 4.1 that, total biogas production was proportional to initial feed concentration. Accordingly, highest biogas production was calculated as 1725 mL in R9 which incubated with a COD concentration of 22500 mg/L.

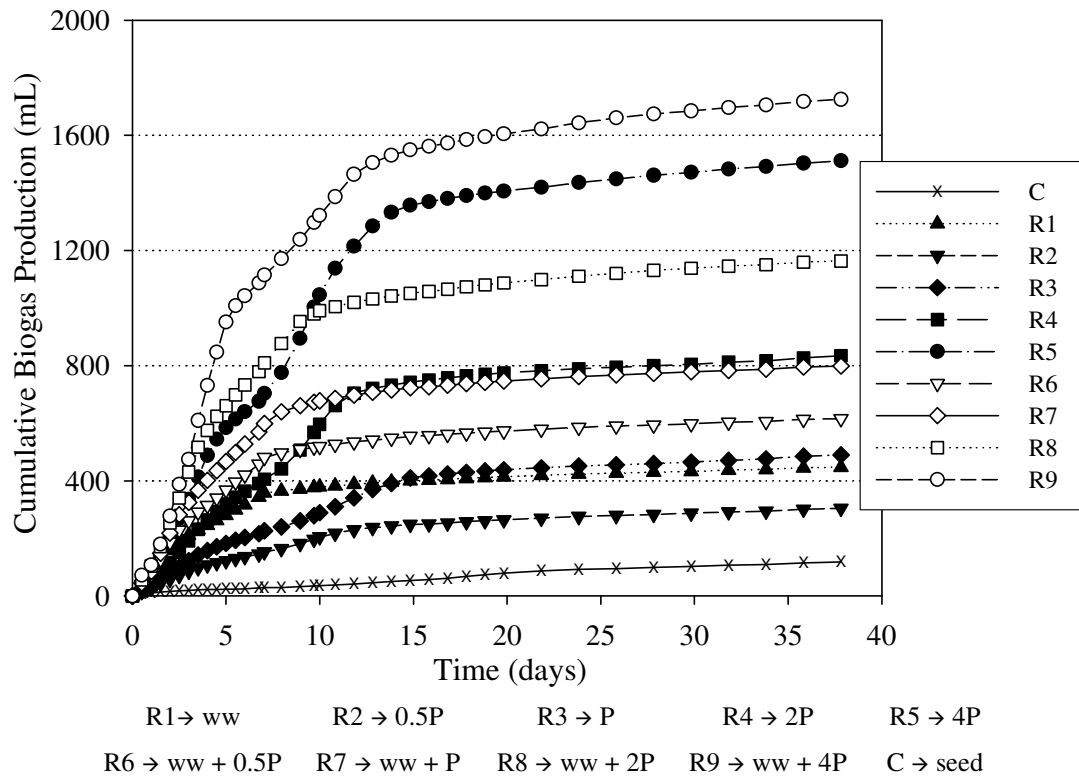


Figure 4.1. Cumulative biogas production data obtained in BMP assay

In order to compute methane generations, mass balance evaluations were carried out (Eqn 4.1, 4.2). Calculations were performed periodically using two consecutive analyses of biogas compositions (P_1 and P_2) and total biogas production data (V_b) obtained between these analyses. During the calculations, methane, generated in control reactor was subtracted from that of reactor of interest, to be able to determine the net methane generation (Eqn 4.3).

$$\text{Generation} = \text{Accumulation in headspace of the reactor} + \text{Output} \quad (4.1)$$

$$G_{\text{Generation}} = \left[\frac{(P_2 - P_1) \times V_h}{100} \right] + \left[\frac{(P_1 + P_2) \times V_b}{100} \right] \quad (4.2)$$

$$G_{\text{net}} = G_r - G_c \quad (4.3)$$

- Where; G : Methane generation (mL),
 G_r : Methane generation in reactor (mL),
 G_c : Methane generation in the control reactor (mL),
 G_{net} : Net methane generation in reactor (mL),
 P₁ : Initial methane content in biogas (%),
 P₂ : Final methane content in biogas (%),
 V_h : Volume of headspace (mL),
 V_b : Volume of total biogas produced (mL),

Figure 4.2 illustrates the cumulative net methane generations of reactors. As it was observed in biogas productions, higher net methane generations were reported for the reactors with higher substrate concentrations. Even so, reactors, containing wastewater (R1 and R7), were superior to that of fed by only pulp (R3 and R4), although COD concentrations and F/M ratios were the same. This finding was the first evidence of a higher biodegradability for wastewater, compared to beet-pulp. It is also supported by the records of methane yield and COD removal data (Table 4.1).

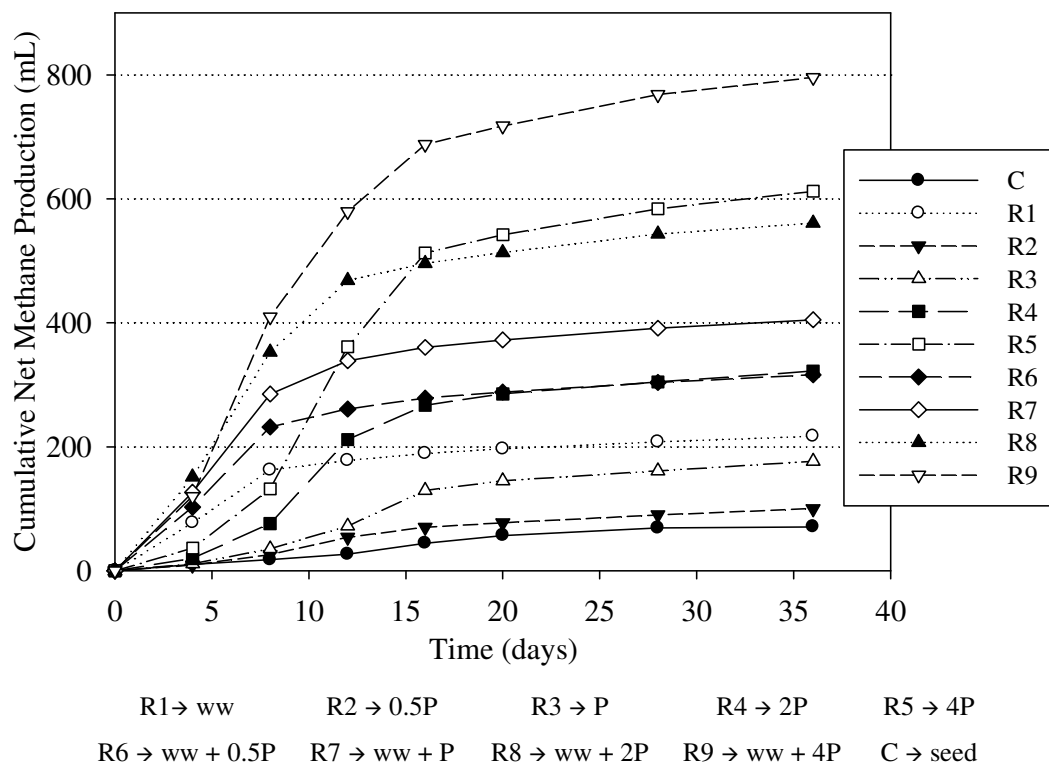


Figure 4.2. Cumulative net methane production data obtained by mass balance

Results point that the highest values of methane yield (321.6 mL/g COD added), COD removal (87.3%) and VS reduction (89.3%) were observed at R1, which was fed only by wastewater. In fact, it is widely accepted that, sugar industry wastewater is highly biodegradable with its soluble carbohydrates, mainly sucrose (Shore et al. 1984; Iza et al. 1990). Having the same F/M ratio (0.51), R1 and R3 can be evaluated, in terms of treatment efficiencies, to compare relative biodegradabilities of wastewater and pulp. Lower methane yield (261.8 mL/g COD added), COD removal (79.8%) and VS reduction (82.5%) was observed at R3. This observation can be considered as a direct result of ligno-cellulosic composition of

beet-pulp, which causes a slight difficulty for degradation when compared to wastewater.

Table 4.1. Comparison of the reactors in terms of treatment performances

Reactor	Initial F/M (g COD/g VSS)	Final pH	COD removal (%)	VS reduction (%)	CH ₄ yield (mL/g COD added)	% of total biogas (produced in first 10 days)
Control	-	7.53	-	28.4	-	29.3
R1	0.51	7.58	87.3	89.3	321.6	85.1
R2	0.26	7.43	79.6	82.2	296.4	64.8
R3	0.51	7.48	79.8	82.5	261.8	57.0
R4	1.02	7.46	63.7	69.6	238.6	68.1
R5	2.05	7.45	66.6	73.1	226.7	66.4
R6	0.77	7.48	84.2	84.4	311.9	83.8
R7	1.02	7.47	81.5	80.2	299.9	84.2
R8	1.54	7.47	72.3	73.0	276.9	83.6
R9	2.56	7.48	64.1	75.1	235.8	75.2

Other than the type of waste, F/M was influential on the treatment performance of the reactors (Table 4.1). For a particular type of waste (only pulp in R2, R3, R4, R5 or wastewater+pulp mixture in R6, R7, R8, R9) as F/M were increased, treatment efficiencies and methane yields were decreased. This finding is in accordance with the literature, where high value of F/M accepted to be toxic (Prashanth et al. 2006). Product inhibition or inadequate nutrient amounts could also be the other reasons

for the decreasing biodegradabilities, with increasing F/M values. Still, in all F/M values, discussed treatment efficiencies (63.7–87.3% COD removal and 69.6–89.3% VS reduction) are indications of high biodegradability for both wastewater and beet-pulp.

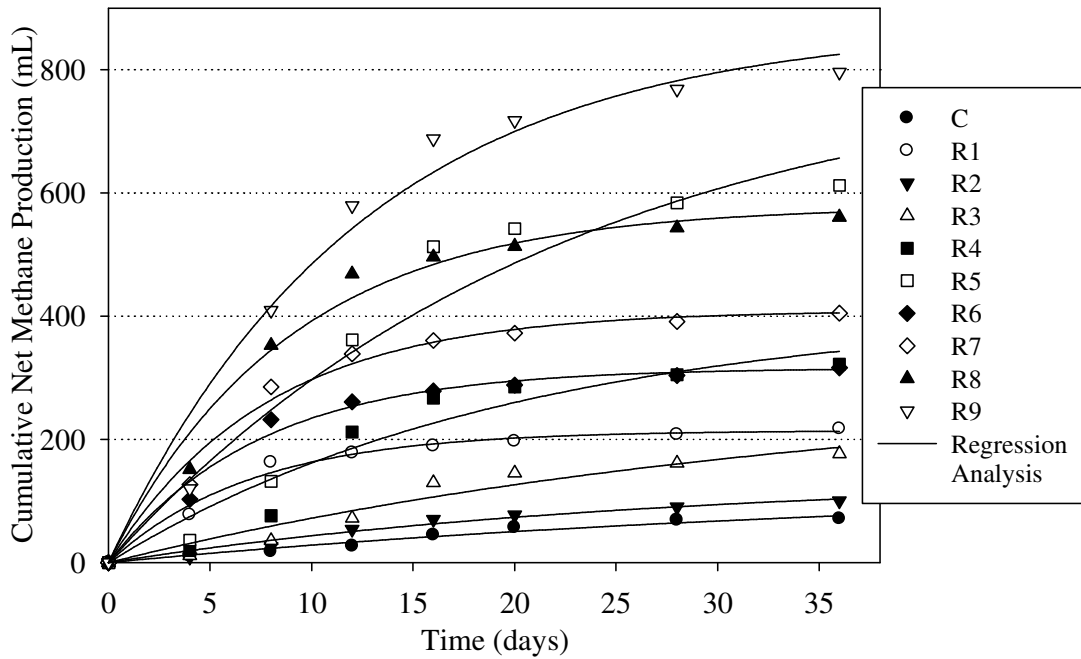
4.1.2. Methane Production/Waste Stabilization Rate

In anaerobic treatment systems, waste stabilization is achieved by methane production (Speece 1996). On account of this information, rate of methane production directly reflects the rate of stabilization process, crucial for design and operation of anaerobic treatment systems. So, determining the rate limiting step, as well as analyzing the overall biodegradation rate is of fundamental importance.

Pavlostathis and Giraldo-Gomez (1991) claimed that, as the initial step in anaerobic biochemical reactions, hydrolysis follows first-order kinetics with respect to the concentration of biodegradable particulate matter. Since, “hydrolysis” is the overall rate controlling step in anaerobic digestion of beet-pulp (Arntz et al. 1985), in this study methane production data was analyzed by first-order kinetics:

$$G_t = G_f (1 - e^{-kt}) \quad (4.4)$$

Where; G_t : Cumulative methane generation at time t (mL),
 G_f : Ultimate methane generation (mL),
 k : First-order rate constant (day^{-1})
 t : Time (days).



R1 → ww R2 → 0.5P R3 → P R4 → 2P R5 → 4P
R6 → ww + 0.5P R7 → ww + P R8 → ww + 2P R9 → ww + 4P C → seed

Figure 4.3. Representation of non-linear regression analysis

Figure 4.3 indicates the experimentally recorded cumulative net methane productions and modeled first-order rate functions (SigmaPlot version 10.0, Systat Software, Inc.). It is visualized from the figure that, the reactors, containing wastewater (R1, R6, R7, R8, and R9) were in better agreement with the modeled functions than were the reactors containing only pulp (R2, R3, R4, and R5). This finding is also supported by the tabulated R^2 data (Table 4.2). Addition of wastewater tended to decrease lag periods, which resulted in relatively “smooth” functions in terms of methane productions (Figure 4.2). As a consequence, better regression results (R^2 ranging between 0.9685–0.9854) were observed in the corresponding reactors.

Table 4.2. Computed G_f and k values with 95% confidence limits

Reactor	R^2	Produced total CH_4 (mL)	G_f (mL)	k (day ⁻¹)
Control	0.9687	71.1	128.0 ± 41.3	0.025 ± 0.011
R1	0.9854	217.1	214.3 ± 6.9	0.143 ± 0.016
R2	0.9702	100.2	139.7 ± 27.4	0.037 ± 0.012
R3	0.9392	176.7	293.8 ± 120.5	0.028 ± 0.017
R4	0.9196	322.1	410.6 ± 97.8	0.050 ± 0.022
R5	0.9184	612.0	818.72 ± 24.1	0.045 ± 0.022
R6	0.9829	316.2	316.0 ± 11.6	0.135 ± 0.016
R7	0.9845	404.8	409.3 ± 14.8	0.129 ± 0.015
R8	0.9816	560.7	578.4 ± 25.5	0.113 ± 0.015
R9	0.9685	795.8	872.2 ± 70.9	0.081 ± 0.016

Wastewater, containing easily degradable carbohydrates, might have initiated the enrichment of bacteria as well as enabling them to rapidly acclimate to the substrates. Accordingly, major outcome of wastewater addition, was to increase methane production rate, rather than increasing the ultimate biodegradability. Indeed, it was difficult to differentiate between methane production yields, which are 226.7–311.9, and 235.8–321.6 mL/g COD-added respectively for wastewater added and non-added reactors (Table 4.1). However, there was a remarkable difference, in terms of k values, between wastewater added (0.081–0.143 day⁻¹) and non-added reactors (0.028–0.050 day⁻¹). Highest k value was observed in R1 (0.143) and decreased with increasing F/M values, computed G_f values were in good agreement with experimentally determined total methane productions, except, control, R3 and R5 reactors (Table 4.2).

Results, obtained in this study are in accordance with related literature. Prashanth et al. (2006) observed in batch bioassay that, at all F/M, k value was decreased linearly with increase in particulate fraction of COD to total COD. In that study, depending on the synthetic constituents of the reactors, dominated either by particulate (cellulose) or soluble (sucrose and peptone) COD, k values ranged between 0.0346 and 0.1827 day⁻¹. On the other hand, Sanchez et al. (2000), computed k values of 0.012–0.086, when fitting the cumulative methane accumulation data to first-order kinetics, for batch anaerobic digestion of cattle manure.

To the best of our knowledge anaerobic co-digestion of beet-sugar industry wastes (wastewater and beet-pulp) were examined for the first time in the literature. As a result of the biochemical methane potential assay, it was concluded that the anaerobic co-digestion of wastewater and beet-pulp is promising since wastewater addition significantly increases the rate of biomethanation of beet-pulp. Another potential advantage of co-digestion is that, the wastewater replaces the fresh water, used as diluent for anaerobic digestion of pulp alone. So, in the rest of the study, co-digestion of these wastes was evaluated for optimization of anaerobic acidification, and recovery of produced VFAs, as well as effective biomethanation in fed-batch and sequencing-batch reactors.

4.2. Optimization of Anaerobic Acidification

4.2.1. pH Profiles of the Reactors

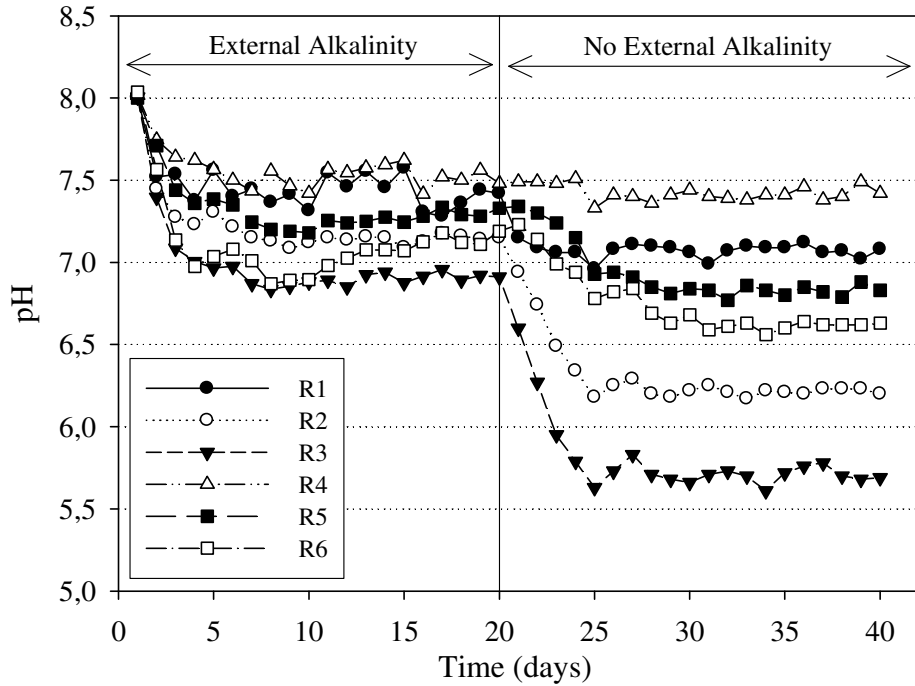
pH drops were observed with varying rates and extents for all reactors (Figure 4.4). Organic acid productions were initiated from the beginning of the reactor operations which resulted in these expected pH drops (Figure 4.5). For the same

substrate (stock solution), 2 days of HRT led to lower pH values than 4 days. The noticeable differences between the operational pHs were mainly due to the differences of the methanogenic activities occurring in the reactors. Since, growth rates of methanogenic microorganisms are lower than the other members of anaerobic consortia, they require more time to survive in anaerobic systems (Speece 1996). So, in this study, 4 days of HRT served as a better growing condition for methanogens than 2 days. As a result, methanogenic microorganisms led to VFA consumption and corresponding buffering effect that prevents further pH drops at 4-day HRT (R4, R5, R6). This situation was the reason of higher pH values, observed in these reactors. Indeed, VFA concentration profiles (Figure 4.5) and biogas compositions (Table 4.3) of the reactors supported this speculation.

After external alkalinity addition was ceased, pH values were further decreased and stabilized at lower levels (Figure 4.4). The observed pH decreases were steeper (down to 5.7–7.1) at 2-day HRT since the VFA accumulations (Figure 4.5) were too high for the inherent alkalinity of wastewater to buffer. In this period, steady-state pH values were higher at 4-day HRT (6.6–7.4). The figure would be different if the operation of the reactors were initiated without adding external alkalinity. In the presence of alkalinity microorganisms could have acclimate to the substrate, which then possibly result in the controlled pH drop (down to 5.7–7.1) and not below 5.7.

Additionally, pH patterns of the reactors differed from each other when they are compared in terms of waste mixing ratios. For the same HRT, increased OLRs, resulting from pulp addition, naturally increased the amount of acidification products (VFAs) which lead to low operational pHs (5.7–6.8). It was approved that high OLRs result in higher VFA accumulations and related pH decreases (Ghosh 1987). In this study, having 2 days of HRT and pulp addition along with wastewater, R2 and R3 were operated in the pH range of 5.6–6.2 without external alkalinity addition. Since the pH range of 4–6.5 is accepted as optimum for the

growth of anaerobic acidogenic microorganisms (Speece 1996), proper pH conditions could be established in R2 and R3.



R1 → ww (2days) R2 → ww + 0.5P (2 days) R3 → ww + P (2 days)
R4 → ww (4 days) R5 → ww + 0.5P (4 days) R6 → ww + P (4 days)

Figure 4.4. Temporal variations of pH values of the reactors

4.2.2. Volatile Fatty Acid Productions

Figure 4.5 illustrates the inverse relationship between VFA accumulation and operational HRT of the reactors. 2 days of HRT yielded higher tVFA concentrations (2159–3635 mg/L as H-Ac), which is the indication of higher acidogenic activity and/or lower methanogenic activity. Since the acidogenic

microorganisms grow much faster than the methanogens, they could successively survive in HRTs as low as 2 days. This also means that at 4-day HRT (R4, R5, R6) methanogens could be retained long enough to metabolize VFAs for methane production, causing to lower VFA concentrations (1814–2640 mg/L as H-Ac). Produced biogas compositions, which indicate higher methane percentages at 4-day HRT (Table 4.3), were strong evidences of this claim.

Lowest VFA concentrations (1814–2244 mg/L as H-Ac) were recorded in the reactors, which were fed only by wastewater (R1 and R4). As expected, VFA concentrations were proportionally increased with the increase in amount of pulp added and the highest concentrations were observed in 2-day-HRT reactor (R3) which was fed by wastewater and pulp in equal amounts in terms of COD (1:1). These results indicated that pulp was successfully acidified together with wastewater, which led to the increase of the VFA concentrations.

After the cessation of alkalinity addition, VFA concentrations were not changed noticeably. This observation indicated that, the change in narrow operating pH values Figure 4.4 did not cause a major change in the acidification trend of sugar industry wastes, even in R3 which indicated the steepest decrease from 6.9 to 5.7.

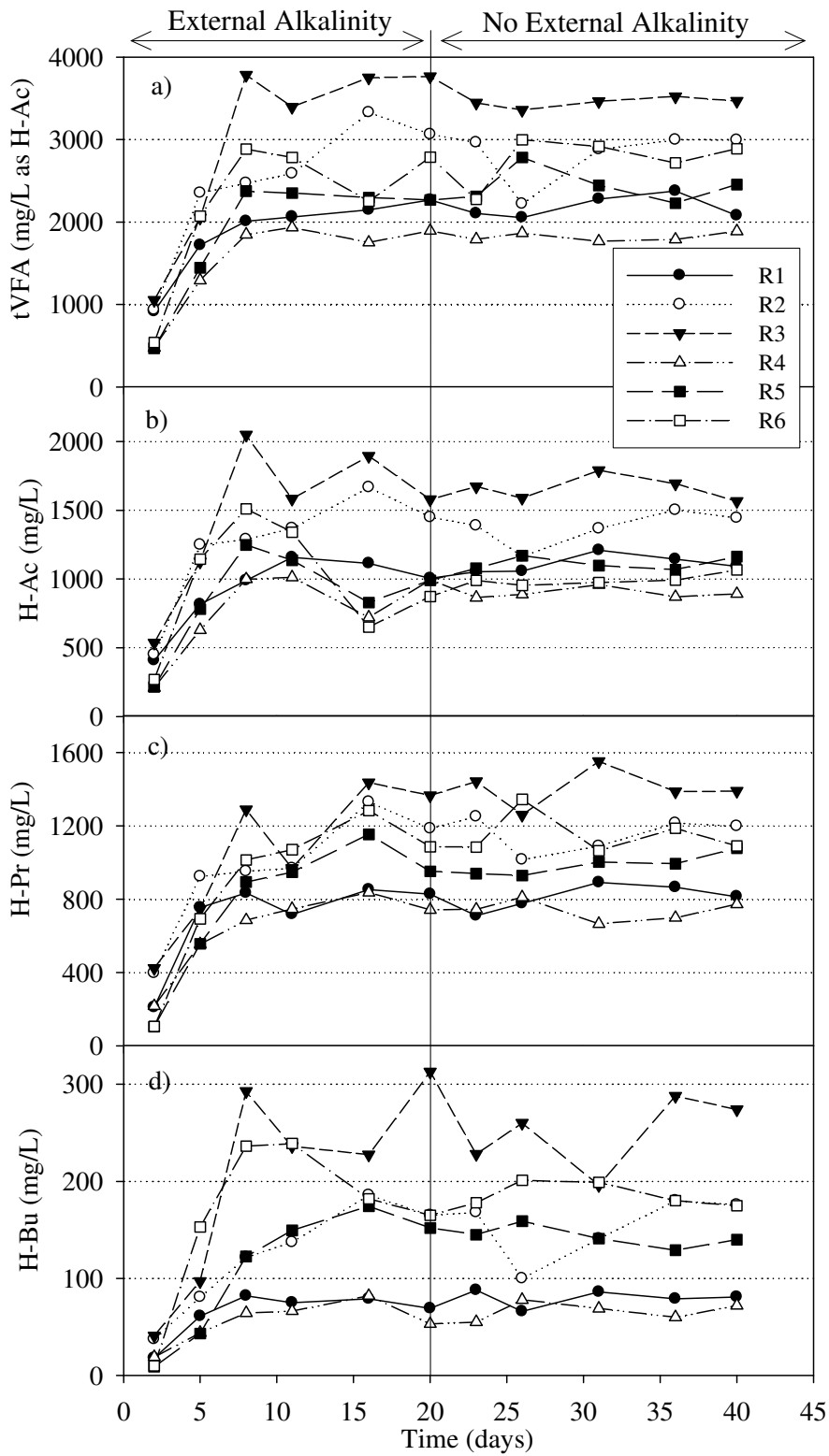


Figure 4.5. VFA concentration profiles of the reactors: (a) Total Volatile Fatty Acids ; (b) Acetic Acid; (c) Propionic Acid; (d) Butyric Acid

One of the main interests of this study was to compare the proportions of the substrates converted to VFAs. For this purpose, “acidification degree” was calculated for each of the reactors in order to express the acidification efficiency (Figure 4.6). Dinopoulou et al. (1988) stated that, acidification degree can be determined by calculating the proportion of the initial substrate which is converted to VFAs as end products. So, in this study, organic content of initial substrate and VFAs were based on COD concentrations. By this way, it was possible to present the related conversion efficiencies for all operated reactors as:

$$\text{Degree of acidification (\%)} = \frac{S_f^*}{S_i} \times 100 \quad (4.5)$$

Where; S_i : Initial substrate concentration, measured in COD (mg/L),

S_f^* : Produced VFAs, expressed as theoretical equivalents of COD concentrations (mg/L).

* The COD equivalents of each VFA : Acetic acid, 1.066; Propionic acid, 1.512; Butyric acid, 1.816; Valeric, 2.036; Caproic acid, 2.204. (Yilmaz and Demirer 2008).

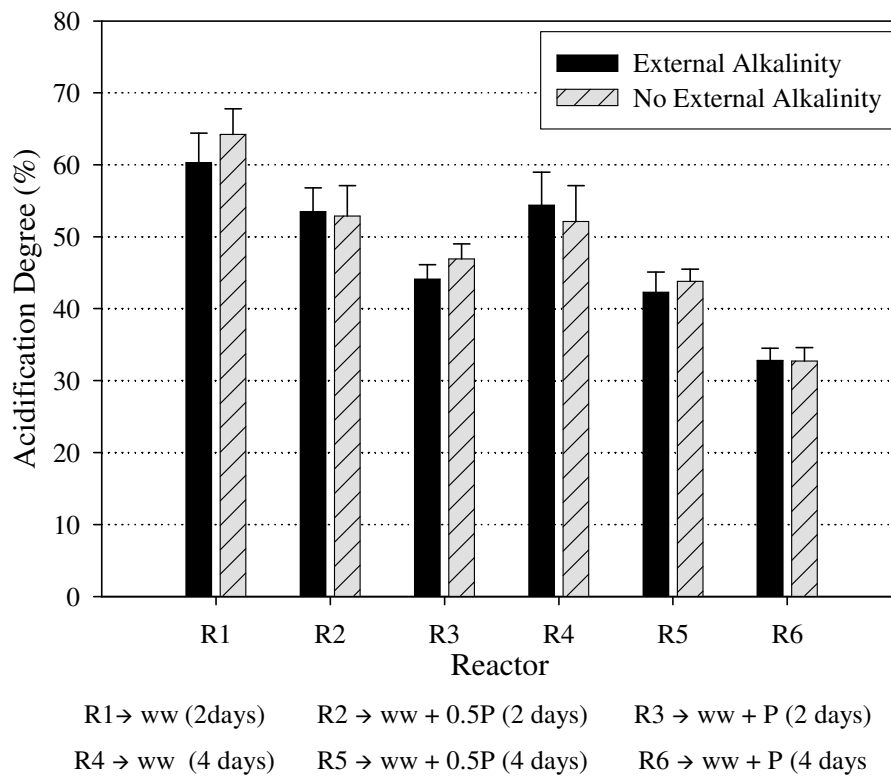


Figure 4.6. Observed acidification degrees in the reactors

The highest acidification degrees (60.3–64.2%) were observed in R1, which was only fed by wastewater and operated at 2 days of HRT. The increase in pulp addition caused a decrease in acidification degree which was accepted as the direct result of increasing OLR. Similarly, in a complex wastewater acidification study, the degree of acidification was found to diminish with increasing OLR (Dinopoulou et al. 1988). Still, the reactors which were fed by wastewater and pulp with HRT of 2 days (R2, R3) denoted substantial degrees of acidification (44.1–53.5%) when compared with other studies: 15–60% for complex wastewater (Dinopoulou et al. 1988); 56% for dairy wastewater (Demirel et al. 2004); 10.3–43.4% for fish meal processing wastewater (Guerrero et al. 1999).

In all of the reactors, main acidification products were H-Ac (40.3–49.2% w/w of tVFA), H-Pr (36.3–42.6% w/w of tVFA) and H-Bu (3.6–7.5% w/w of tVFA) comprising 88.3–96.2% of tVFAs. The higher molecular weight VFAs (valeric, caproic, heptanoic etc.) were produced with insignificant amounts. On the other hand, alcohols (e.g. ethanol, methanol), other major metabolites of anaerobic acidification, was not detected at all. It is a well-known fact that substrate characteristics and operational conditions play a major role on product distribution in an acidification reactor (Dinopoulou et al. 1988; Yu and Fang 2002; Horiuchi et al. 2002). In this study, the dominance of H-Ac, H-Pr and H-Bu can be associated with the carbohydrate degradation, since both wastewater (Wang et al. 1986) and pulp (Hutnan et al. 2000) contains high amounts of sugars. This is relevant with the literature in which these short-chain fatty acids were found to be dominant in acidogenic reactors (Dinopoulou et al. 1988; Yu and Fang 2002). Parawira et al. (2004b) stated that higher molecular weight VFAs are generally found in protein fermentation.

4.2.3. Soluble Chemical Oxygen Demand Concentrations

sCOD concentration distributions of the reactors were depicted in Figure 4.7. The highest sCOD concentrations (5422–5826 mg/L) were observed in R3 which was operated at an HRT of 2 days. 4-day HRT led to lower sCOD concentrations resulting from the consumption of VFAs by methanogens. It can be seen from Figure 4.5 and 4.7 that, there is a direct relationship between VFAs and sCOD concentrations. Since, hydrolysis of particulate organic matter occurs simultaneously during the acidification of soluble organics, sCOD concentrations were higher (3490–5826 mg/L) in the reactors which have pulp addition together with wastewater.

The effects of operational pH values on sCOD concentrations were negligible when the alkalinity-added period was compared with the period without alkalinity

addition. This result indicated that the trend of hydrolysis was not significantly altered with the decreases in operational pH values, within the studied range of 5.7–7.4.

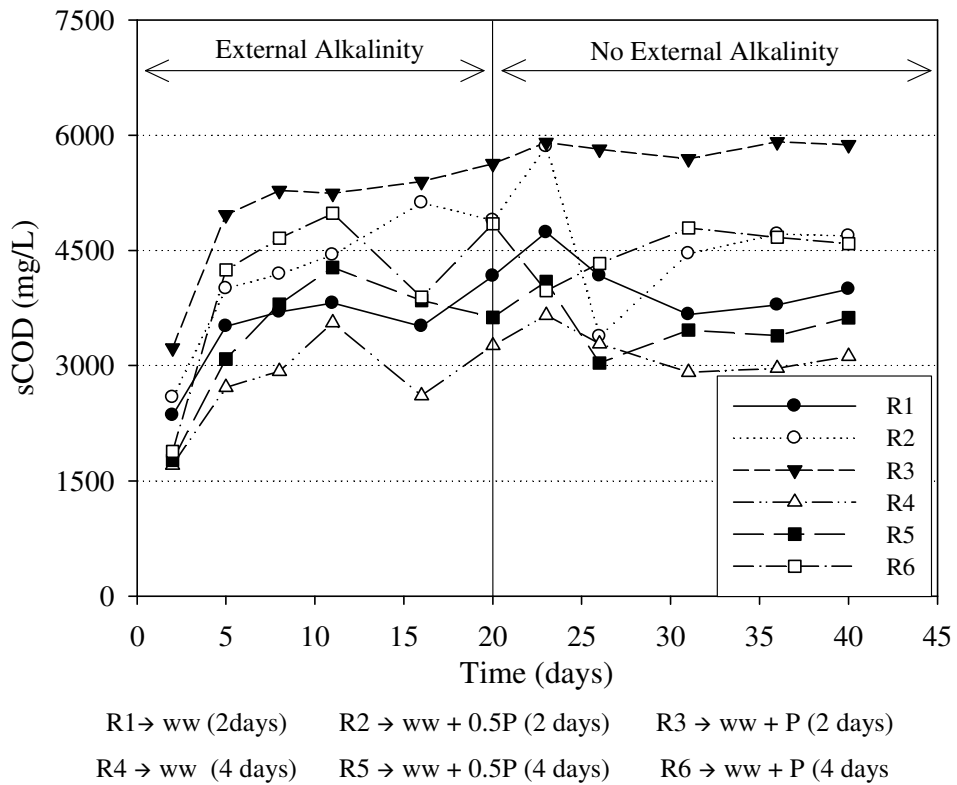


Figure 4.7. Temporal variations of sCOD concentrations in the reactors

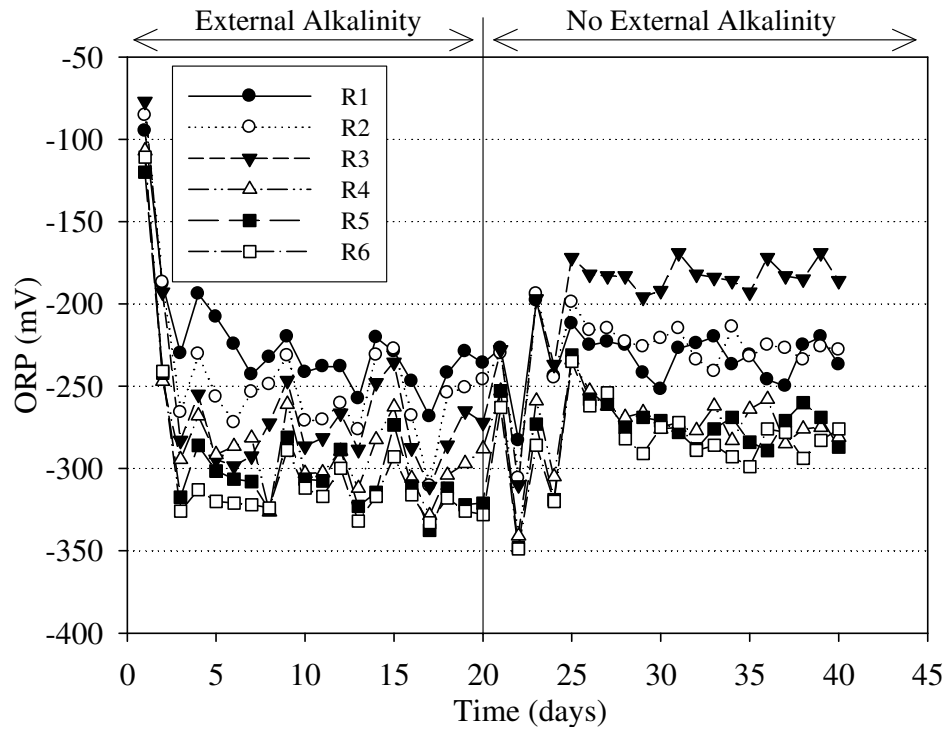
4.2.4. Oxidation-Reduction Potentials

ORP values of anaerobic reactors are inspected to detect the relative amounts of oxidized materials such as nitrate ions (NO_3^-) and sulfate ions (SO_4^{2-}), and reduced materials, such as ammonium ions (NH_4^+), which describes the conditions of the

reactors whether they are oxic or anaerobic. At ORP values between -100 mV and -300 mV, degradation of organic compounds proceeds primarily as fermentation and acid formation. On the other hand, ORP values lower than -300 mV indicates considerable methanogenic activity in anaerobic conditions (Gerardi 2003).

Figure 4.8 illustrates that, ORPs of reactors were diminished to steady-state values after the operation of 3–5 days. ORP values of the reactors were between -226 mV and -350 mV in the period of alkalinity addition. After alkalinity addition was stopped, ORP values started to increase and attained steady-state between -162 mV and -300 mV. This observation indicates that, the pH drops resulted from the cessation of alkalinity addition slightly altered the circumstances in favor of fermentation and mixed-acid formation, inhibiting the methanogenic activities. This idea was supported by the results of biogas compositions (Table 4.3) which indicate that methane percentages were slightly decreased along with decreasing pH values.

Moreover, 2-day HRT yielded higher ORP values (-183 mV – -234 mV) than 4-day HRT especially in the period, without alkalinity addition. In 4-day-HRT reactors, the observed ORP values were between -273 mV and -318 mV which indicated higher methanogenic activities than 2-day-HRT reactors.



R1 → ww (2days) R2 → ww + 0.5P (2 days) R3 → ww + P (2 days)
 R4 → ww (4 days) R5 → ww + 0.5P (4 days) R6 → ww + P (4 days)

Figure 4.8. Temporal variations of ORP values of the reactors

4.2.5. Biogas Productions and Compositions

The direct relationship between the operational HRT and the amount of daily produced biogas can be observed in Figure 4.9. Reactors, which were operated with an HRT of 4 days (R4, R5, R6) were producing higher amounts of biogas (27–72 mL/day) compared to those operated with an HRT of 2 days (19–25 mL/day). When the biogas productions are interpreted in combination with their composition (Table 4.3), it can be stated that, higher methanogenic activity was responsible for the higher biogas productions in 4-day-HRT reactors although OLR was lower (1.35–2.7 g COD/L-day).

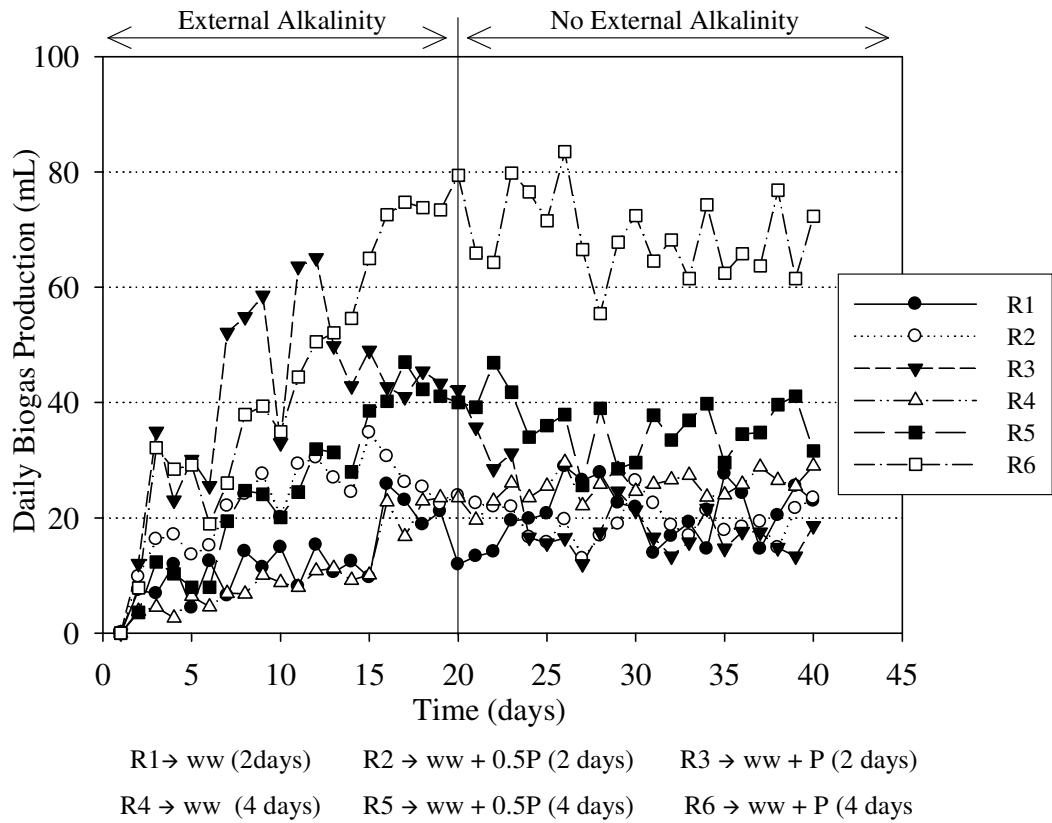


Figure 4.9. Daily biogas production data

Since, the daily biogas productions were primarily affected by methane productions, conditions, influencing methanogenic activity, also influenced the total biogas productions. So, this explains why lowest biogas productions (19–21 mL/day) were observed in R2 and R3, in which pH values were low enough (5.7–6.2) to inhibit methane productions to some extent. Thus, pulp addition increased the substrate concentrations and highest biogas productions (31–72 mL/day) were recorded at R5 and R6 which were operated with 4 days of HRT.

In acidogenic anaerobic reactors, methanogenic activity must be restrained to be able to reach high VFA accumulations. Methanogenic activities and related methane productions could be suppressed to some extent with 2 days of HRT (R1,

R2, and R3). As a result, lower methane percentages (5.6–25.3%) were detected within the produced biogases of these reactors when compared with the percentages appeared in the 4-day-HRT reactors (39.5–53.4%).

Additionally, Table 4.3 indicates the inverse relationship between substrate concentrations and methane productions. It can be observed that, increased substrate concentrations resulting from pulp additions caused significant decreases in methane productions; especially at 2-day HRT. The main reason was the increased VFA accumulation in corresponding reactors, resulting in the lower operational pHs (5.7–6.9). So, it was evident that, pulp addition, causing to VFA accumulation, suppressed the methane production.

When the biogas compositions of 4-day-HRT reactors were inspected, it was claimed that neither HRTs nor the operational pH values (6.6–7.5) were acceptable for the targeted methanogenic activity inhibition. In fact, Yu and Fang (2002) stated that the pH values of acidogenic reactors must be kept below 5.5 to inhibit methanogenic microorganisms after observing high methane concentrations (31%) even at pH values as low as 6.5.

After cessation of alkalinity addition, methane percentages of the reactors were slightly decreased following the decreases in pH values. The lowest methane percentage (5.6%) was observed at R3 in the period without alkalinity addition. This result indicates that, the optimum conditions for inhibition of methanogenic activity were 2 days of HRT and 5.7–6.2 range of pH in this study.

Table 4.3. Biogas compositions, recorded at corresponding steady-state period

Reactor	External Alkalinity		No External Alkalinity	
	CH ₄ (%)	CO ₂ (%)	CH ₄ (%)	CO ₂ (%)
R1	25.3 ± 1.7	74.7 ± 1.7	23.2 ± 5.9	76.8 ± 5.9
R2	16.3 ± 2.1	83.7 ± 2.1	16.7 ± 3.3	83.3 ± 3.3
R3	6.4 ± 1.5	93.7 ± 1.5	5.6 ± 0.5	94.4 ± 0.5
R4	53.4 ± 0.4	46.6 ± 0.4	49.8 ± 2.1	50.2 ± 2.1
R5	51.6 ± 3.3	48.4 ± 3.3	45.1 ± 2.6	54.9 ± 2.6
R6	44.5 ± 2.7	55.5 ± 2.7	39.5 ± 2.2	60.5 ± 2.2

When all available data are analyzed, optimum operational conditions for anaerobic acidification were selected as 2-day HRT and 1:1 waste mixing ratio (in terms of COD) without external alkalinity addition. These operational conditions lead to the highest tVFA concentrations (3635 ± 209 mg/L as H-Ac) with an acidification degree of 46.9 % at the highest OLR of 5.4 g COD/L-d. So, these operational conditions were set for the production of VFAs in an acidification reactor (Part 4.3), which then subjected to the subsequent extraction experiments, composing the third experimental set-up of the study.

4.3. Recovery of VFAs by Liquid-liquid Extraction

4.3.1. Acidification / VFA Production

As it is depicted in Figure 4.10, VFA production performance of the acidification reactor was inspected by the control parameters of pH, VFA, solids and COD concentrations. As a result of acidogenic activity, pH value of the reactor was decreased sharply from the pH value of seed sludge, which is higher than 7.5, to

around 5.5. As discussed in part 4.2.5, this pH value was critical for the inhibition of methanogenic activity, favoring anaerobic acidogenic growth.

While pH was decreasing, sCOD and VFA concentrations were increasing on account of simultaneous hydrolysis and acidification of organic matter. Figure 4.10 shows that, steady-state condition was reached in 10–13 days, at tVFA concentrations of 2700-3000 mg/L as H-Ac. Beginning from the reactor start-up, solid (TS, VS, MLSS and MLVSS) concentrations were decreased till the steady-state is reached. At steady-state, through the hydrolysis of particulate matter, significant removal of SS (37.5%) and VSS (40.5%) were achieved with corresponding effluent concentrations of 3020 and 2540 mg/L respectively (Table 4.4).

During the acidification, no remarkable difference was observed between influent and effluent tCOD and TS concentrations. When these findings were evaluated together with produced biogas compositions at steady-state (100% CO₂, 0% CH₄), it was claimed that methanogenic activity was successfully inhibited. On the other hand, 8.4 % reduction of tCOD can be associated with the waste stabilization during facultative hydrolysis and acidogenesis as a result of small oxygen penetration with air during fed-batch reactor feeding.

Main acidification products were recorded as acetic (48.4% w/w of tVFA) propionic (38.1% w/w of tVFA), n-butyric (5.9 % w/w of tVFA) and n-valeric (3.2% w/w of tVFA) acids, corresponding 95.6% of tVFA. Acidification degree was calculated as discussed in part 4.2.2 and it was found as 37.8%.

Table 4.4. Influent and effluent characteristics of acidification reactor

Parameter	Influent (mg/L)	Effluent (mg/L)	Change (%)
tCOD	10600	9708 ± 555	-8.4
sCOD	5318 ± 288	5755 ± 30	+8.2
TS	9193	9041 ± 189	-1.7
VS	6348	5495 ± 389	-13.4
SS	4832	3020 ± 57	-37.5
VSS	4268	2540 ± 85	-40.5
tVFA (as H-Ac)	892 ± 16	2913 ± 152	+37.8*
pH	7.65	5.5	-

*Degree of acidification, computed as described in Part 4.2.2.

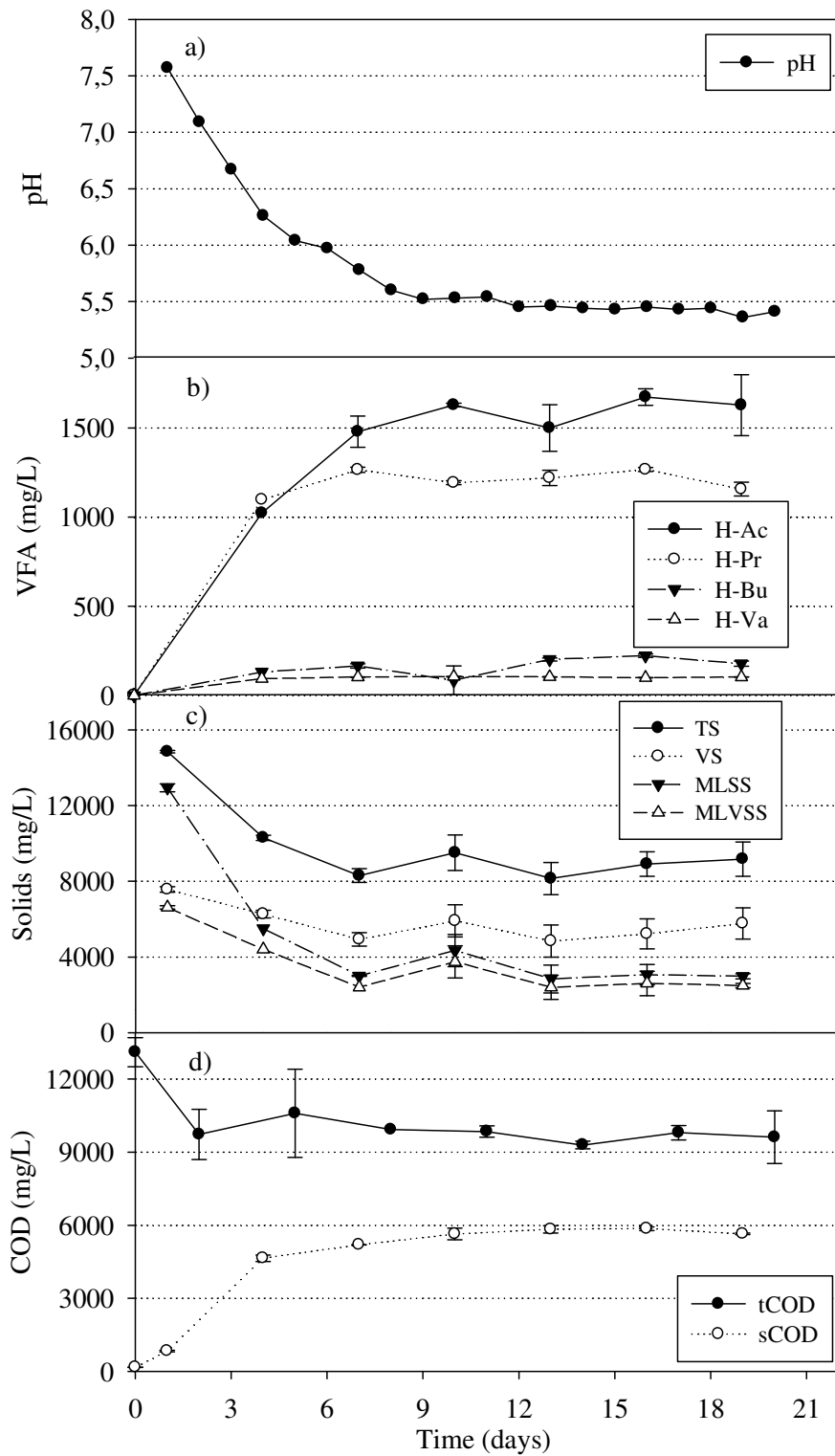


Figure 4.10. Temporal variations of control parameters of acidification reactor:
 (a) pH; (b) VFA; (c) Solids; (d) COD

Table 4.5. Characteristics of aqueous phase, used in extraction experiments

Parameter	Value (mg/L)
sCOD	5693 ± 414
H-Ac	1645 ± 91
H-Pr	1283 ± 43
H-Bu	202 ± 8
H-Va	111 ± 0
tVFA (as H-Ac)	2955 ± 73
tVFA (as COD)	4286 ± 177
pH	5.5

4.3.2. Extraction Efficiencies at pH 2.5

It is a well-known fact that pH is an important parameter for the extraction of carboxylic acids, controlling the equilibrium concentrations between aqueous and organic phases. In addition, carboxylic acids are extracted more effectively at low pH values, generally lower than the pK_a of the corresponding acids, where they are present at their undissociated forms (Yang et al. 1991; Vandak et al. 1997; Malmay et al. 2001). As it is depicted in Figure 4.11, at pH 2.5, percent recoveries of VFAs were changed from 43.3 to 97.6%, depending on the type of the acid extracted and the concentration of TOPO in kerosene. As the concentration of TOPO in kerosene was increased, efficiency of extraction was increased. As a result, highest VFA recoveries (60.7–97.6%) were observed at 20% TOPO in kerosene with K_D values ranging between 1.54 and 40.79 (Table 4.6). This result was parallel to the other studies regarding the use of TOPO in kerosene as extractant solvent (Golob et al. 1981; Mostafa 1999).

Apart from extractant concentration, nature of the acid was highly influential on the extraction efficiency. Eyal and Canari (1995) advocated that, the extraction of carboxylic acids, which have similar pK_a values, is determined by many parameters including presence of functional groups and steric hindrance. On the other hand, according to Tamada and King (1990), hydrophobicity is another important parameter and acids with higher hydrophobicity are more suitable for liquid-liquid extraction. When this information is combined with the fact that longer chain carboxylic acids are more hydrophobic, it can be proposed that extraction affinity of an organic acid is increased parallel to its chain length (Yang et al. 1991). Our findings confirm the related literature, indicating a gradual increase in extraction efficiency parallel to the increase in the chain length of the VFA. So, showing the highest recovery percentages (94.2–97.6%) of all acids, valeric acid was very suitable for extraction even in 5% TOPO in kerosene concentration at pH 2.5. Still, considerable degree of recovery (43.3–60.6%) was computed for acetic acid with K_D values ranging between 0.76 and 1.54.

The obtained results of extraction experiments were in good agreement with early studies in the literature regarding the use of TOPO as extractant. Wardell and King (1978) determined K_D values ranging between 0.8 and 4.8 in the extraction of 0.5 wt % acetic acid solution, depending on the diluent used. Golob et al. (1981) calculated K_D values in the range 0.055–1.165, changing with initial acetic acid concentration and increasing with the percentage of TOPO in kerosene. On the other hand, use of commercial extractants (Aliquat 336 and Alamine 336) yield K_D values of 1.94–15.50, depending on the diluent used and acid (propionic/butyric acid) extracted (Yang et al. 1991).

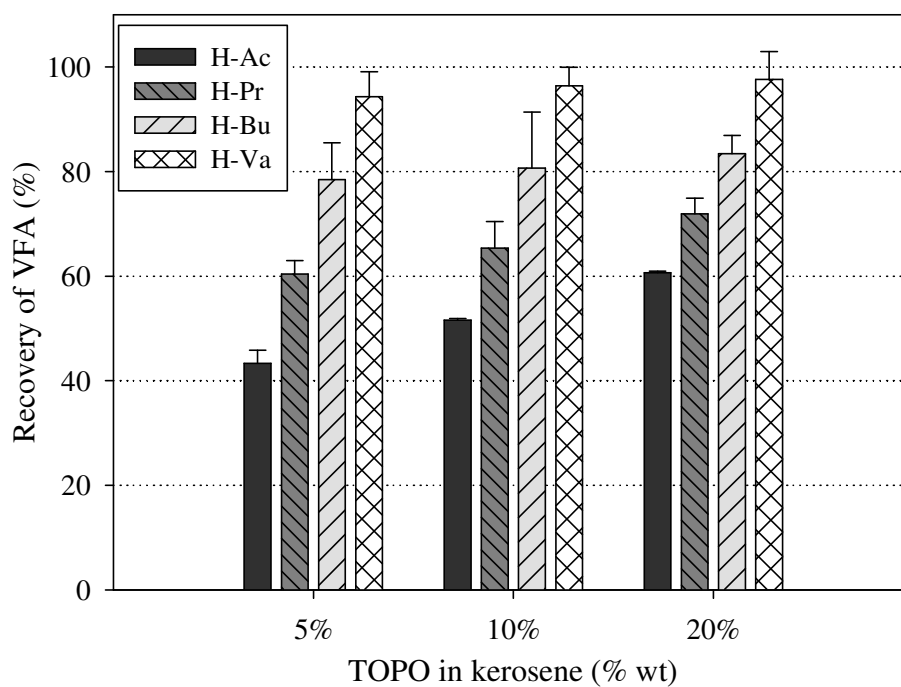


Figure 4.11. Efficiency of recovery of VFAs at pH 2.5

Table 4.6. K_D values at pH 2.5

Acid	TOPO concentration in kerosene (% wt)		
	5	10	20
Acetic	0.76 ± 0.04	1.07 ± 0.01	1.54 ± 0.01
Propionic	1.52 ± 0.06	1.89 ± 0.15	2.56 ± 0.11
n-Butyric	3.64 ± 0.33	4.16 ± 0.55	5.03 ± 0.21
n-Valeric	16.47 ± 0.84	26.35 ± 0.99	40.79 ± 2.21

4.3.3. Extraction Efficiencies at pH 5.5

Although challenging, it is important to find a way to extract carboxylic acids at relatively high pH values, between 5 and 7, in which acidogenic anaerobic microorganisms function well. In the literature, there are numerous studies, focusing on the extraction of carboxylic acids at high pH values, where simultaneous fermentation and extraction is possible (Vandak et al. 1997; Gu et al. 1999, Wu and Yang 2003). As it is illustrated in Figure 4.13, at pH 5.5, it was possible to recover VFAs to some extent (23.4–73.3%). However, the effect of TOPO concentration in kerosene was insignificant on extraction efficiency. In addition, recovery percentages of acetic and propionic acids, which are two major contributors to tVFA concentration, are remained as low as 29.3–30.1% even at 20% TOPO in kerosene. Consequently, when compared to pH 2.5, lower K_D values were recorded for all VFAs, with the values ranging from 0.31 to 2.75, valeric acid being the highest. Similar to the case at pH 2.5, higher chain-length VFAs were tended to be extracted more, in each TOPO concentrations at pH 5.5. Yet, there was not a clear differentiation between the extraction efficiencies of acetic and propionic acids, with K_D values of 0.31–0.39 and 0.32–0.41 respectively (Table 4.7).

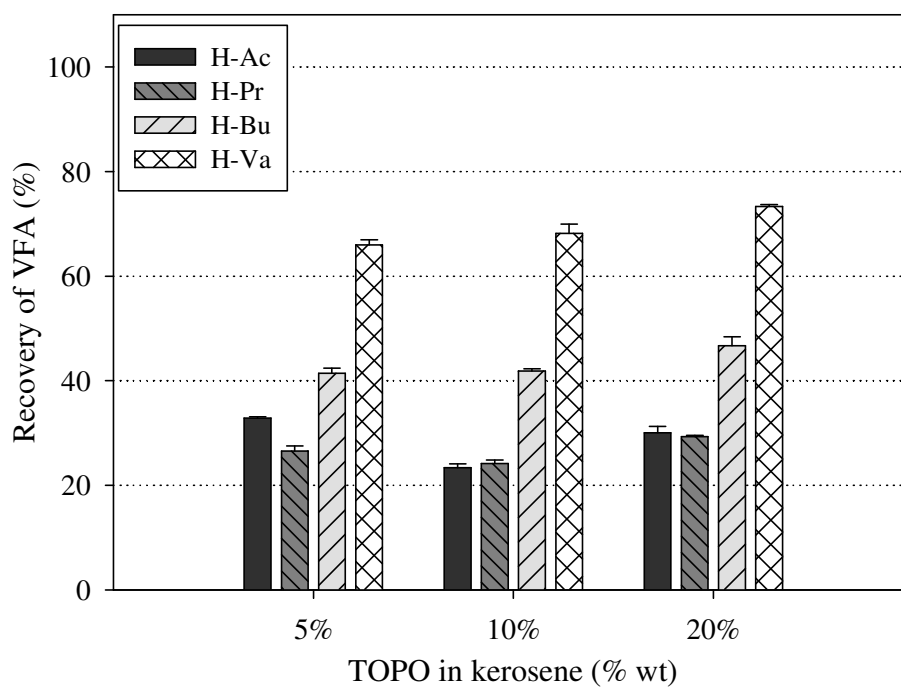


Figure 4.13. Efficiency of recovery of VFAs at pH 5.5

Table 4.7. K_D values at pH 5.5

Acid	TOPO concentration in kerosene (% wt)		
	5	10	20
Acetic	0.49 ± 0.00	0.31 ± 0.01	0.43 ± 0.02
Propionic	0.36 ± 0.01	0.32 ± 0.01	0.41 ± 0.00
n-Butyric	0.71 ± 0.02	0.72 ± 0.01	0.88 ± 0.03
n-Valeric	1.94 ± 0.03	2.14 ± 0.06	2.75 ± 0.01

4.3.4. tVFA Extractions and COD Removal Efficiencies

By means of VFA recovery, not only economical advantages are acquired, but also contribution to the environmental protection is achieved. Indeed, removal of VFAs, which contribute to the majority of sCOD in acidified waste streams, can be accepted as a mean of wastewater treatment.

As, each of the acids were found with different concentrations and their removal efficiencies were different, there was a need to compare total extraction efficiencies of different runs on a common basis. Thus, tVFA recoveries were computed and depicted in Figure 4.14. It is clear from the figure that, the effect of acid dissociation, influenced by pH, is extremely important on tVFA recovery. At pH 2.5, where the VFAs are present in their undissociated forms, recovery efficiencies were recorded as 53.4–67.4%. On the other hand, low extraction efficiencies lead to reduced recovery percentages (26.2–32.4%) at pH 5.5, when compared to 2.5.

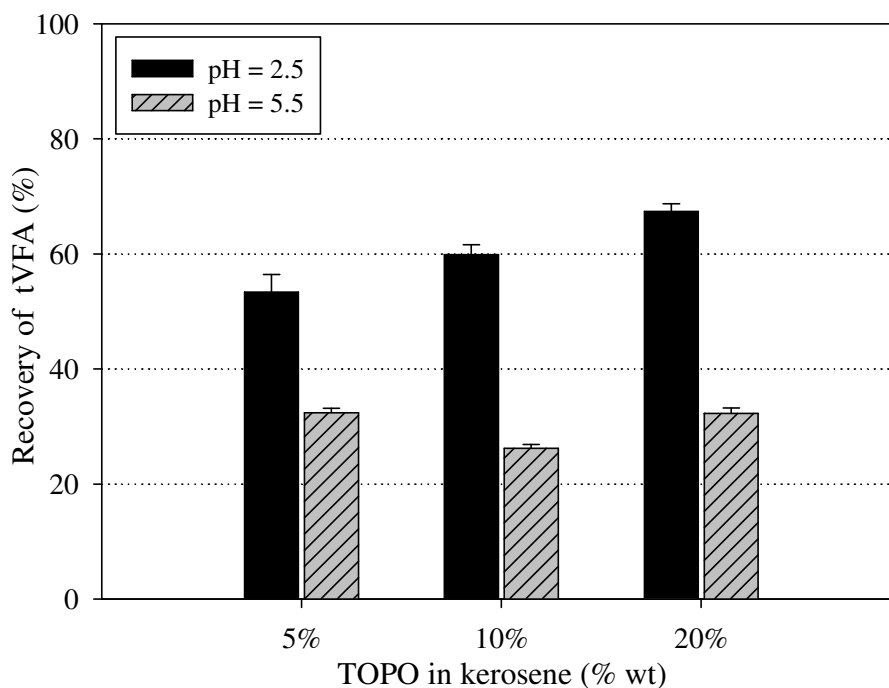


Figure 4.14. Effect of pH and TOPO in kerosene concentration on tVFA recovery

Table 4.8. K_D values of tVFAs

pH	TOPO concentration in kerosene (% wt)		
	5	10	20
2.5	1.14 ± 0.07	1.49 ± 0.04	2.07 ± 0.04
5.5	0.48 ± 0.01	0.35 ± 0.01	0.48 ± 0.01

This situation was also confirmed by the results of COD experiments (Figure 4.15). Up to 71.8% COD removals were achieved, at 20% TOPO in kerosene at pH 2.5, while the removal efficiencies remained between 19.1–22.3% at pH 5.5. In addition, at pH 2.5, the increase in TOPO concentration directly increased the COD removal efficiencies, as it does for tVFA recovery. Such a relation could not be realized at pH 5.5, due to poor transfer of VFAs from aqueous to organic phase.

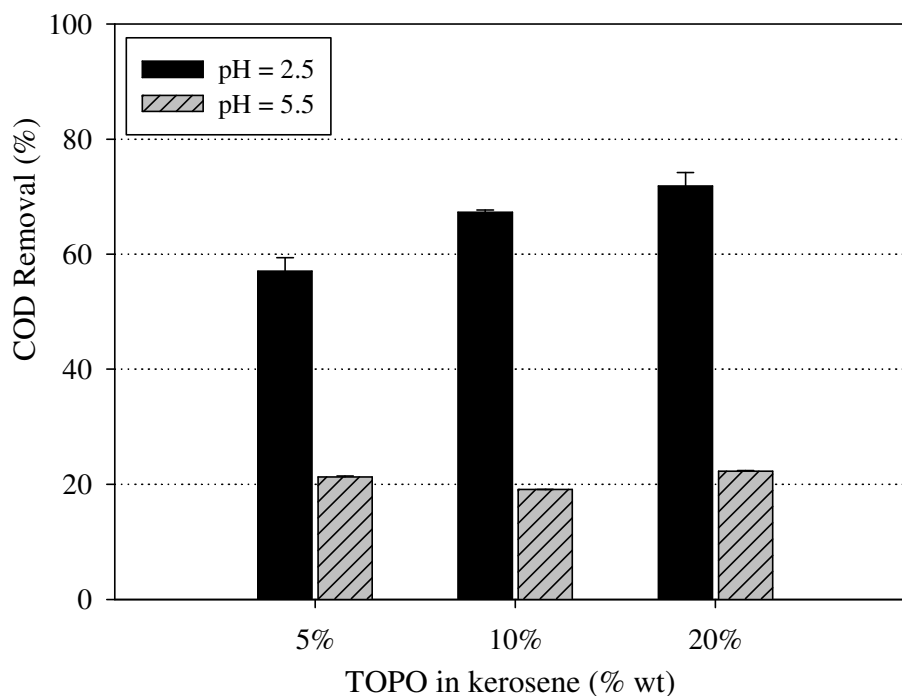


Figure 4.15. Effect of pH and TOPO in kerosene concentration on COD removal

As discussed previously in this study, higher chain-length VFAs are more susceptible to extraction. This tendency, can lead to promising results in terms of waste stabilization, since their contribution to COD is higher than shorter chain-length VFAs (Table 4.9). In our case of acidification, acetic and propionic acids, were major products, and their extraction mechanisms dominate the COD removal efficiencies. However, it would be possible to achieve much higher COD removal percentages when waste characteristics and/or operational conditions were set to selectively produce higher chain-length VFAs, prior to extraction.

Table 4.9. Theoretical COD equivalence of VFAs (Yilmaz and Demirer 2008).

VFA	COD* equivalent (g/g Acid)
Acetic	1.066
Propionic	1.512
n-Butyric	1.816
n-Valeric	2.036

Volatile fatty acid (VFA) production followed by recovery was correlated with simultaneous COD removal to fill a gap in the literature. Investigating the COD contribution of each individual acid and their recovery as waste stabilization was introduced for the first time. It was clear from the results that, the effect of TOPO in kerosene concentration was as crucial as the effect of pH on the recovery of VFAs via liquid-liquid extraction. Consequently, optimum conditions for extraction were determined as 20% TOPO in kerosene at pH 2.5. When this extraction process is followed by distillation (Golob et al. 1981) or vacuum distillation (Gu et al. 1998),

regeneration of extractant solvents and production of pure organic acids can be achieved, which is another topic of research, needs further investigations.

4.4. Evaluation and Comparison of Fed-batch and Sequencing-batch Reactors in terms of Biomethanation

4.4.1. Fed-batch Continuously Mixed Reactor

As described early in this study (Part 3.5.4), a fed-batch continuously mixed anaerobic reactor, having 15 days of HRT (or SRT), was operated for 50 days (Figure 4.16.a). As it is depicted in Figure 4.16.b, daily biogas production was increased up to 248 ± 9 mL/day, where a steady daily production was attained. Following the start-up of the reactor, pH value fluctuated between 7.2 and 7.4, prior to the stabilization at near pH 7.25 (Figure 4.16.c). This pH value can be regarded as a proper value for anaerobic microbial growth, since the range of 6.5–8.2 is accepted as optimum (Speece 1996). Initial high alkalinity values (4900 mg/L as CaCO_3) were associated with the inherent conditions of seed sludge, which fully occupied the reactor content during the start-up. Parallel to the decrease in pH values, alkalinity of the reactor was decreased to around 2750 mg/L as CaCO_3 and sustained until the end of operation (Figure 4.16.c). Malina and Pohland (1992) stated that 1500–3000 mg/L alkalinity (as CaCO_3) is the range, where sufficient buffering capacity is present for an efficient anaerobic digestion process.

Figure 4.17.a indicates that tCOD concentrations of the reactor were stabilized at 3322 ± 235 mg/L, after a decreasing period of 35–40 days. When this information was combined with the data obtained from the other operational parameters (biogas production, pH, alkalinity, solids and VFA concentrations), it was claimed that steady-state was achieved in 40 days. This time period is in agreement with the theoretical time of $3 \times \text{HRT}$ needed for reaching steady-state conditions. At steady-state conditions, influent tCOD concentration of 10600 mg/L was decreased by

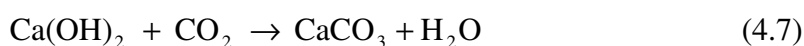
biodegradation to 3322 ± 235 mg/L as effluent, which correspond to a $68.7 \pm 2.2\%$ tCOD removal. Obtained tCOD removal data was evaluated/compared with steady-state methane production via mass balance calculations (Eq 4.8, 4.9, 4.10), and discussed accordingly in the following parts of the study. On the other hand, sCOD concentration was determined as 484 ± 40 mg/L. This value was achieved after an initial increase, with a peak value of 1804 mg/L (at day 10), followed by subsequent decrease. Pronounced increase in sCOD concentration was a direct result of VFA accumulation (Figure 4.17.d).

Since biomass separation was not practiced, mixed liquor of the reactor represents the effluent solids concentrations as well (Figure 4.17.b). After 40 days of operation, TS and VS concentrations were recorded as 6504 ± 197 and 2878 ± 379 mg/L respectively at steady-state. When influent concentration of VS (6348 mg/L) was taken into consideration, 54.7% reduction was computed. Figure 4.17.b also depicts that MLSS and MLVSS concentrations were stabilized at 2847 ± 194 and 1980 ± 173 mg/L, respectively.

During early days of operation (0–20 days), VFAs (acetic, propionic and iso-butyric acids) were accumulated in the system (Figure 4.17.d). At their peak concentration values, acetic, propionic and butyric acids were measured as 475, 649 and 48 mg/L. It was an expected result, since highest concentrations of VFAs are usually observed in the form of acetic, propionic, butyric and iso-butyric acids during the start-up of anaerobic systems (Rittmann and McCarty 2001). It is considered that, the main reason of this situation was higher growth rates of acidogenic microorganisms compared to methanogens. It took time (10–30 days) for methanogens to mature in the system and start converting VFAs into methane. As a result, acetic, propionic and iso-butyric acids were diminished below 50 mg/L after 30 days of operation, which was sustained until the end of operation.

Biogas compositions of the reactor was analyzed frequently and steady-state values were tabulated (Table 4.10). A remarkable observation was the considerably high

methane percentages in biogas ($81.9 \pm 4.7\%$) when compared to typical values of 65–70% (Gerardi 2003). The reason behind was the calcium ion (Ca^{2+}), which is commonly found in sugar-beet processing wastewaters, due to the usage of lime (Iza et al. 1990). In beet-sugar factories lime is added to flume/wash water for adjusting its pH and improving the settling characteristics. As expected, the wastewater used in this study includes considerable amounts of Ca^{2+} (378 mg/L) (Table 3.1.). It is a known fact that, lime reacts with soluble carbondioxide to form bicarbonate alkalinity ($\text{Ca}(\text{HCO}_3)_2$) as well as precipitates (CaCO_3) (Eq 4.6, 4.7). This information is supported by Gerardi (2003), who claimed that, the carbondioxide in biogas can replace the amount lost in the sludge due to discussed reactions. This situation clearly explains the lower carbondioxide ($18.1 \pm 1.3\%$) and relatively higher methane percentages ($81.9 \pm 4.7\%$) in biogas.



Steady-state daily methane production was used to calculate methane production yield which was determined as 255 ± 11 ml/g COD added. As expected, this value was lower than the value (299 ml/g COD added) obtained in BMP assay with the reactor (R7) fed by the same substrate (1:1 waste mixing ratio in terms of COD), which was discussed in Part 4.1.1. This situation indicates that there is still a significant opportunity to increase the methane yield through increasing biomass retention. Still this methane yield was higher than the yield (210 mL/g COD added) calculated by Weiland (1993), who obtained the result during conventional anaerobic digestion of beet-pulp alone. This difference can directly be associated with the addition of wastewater which is more biodegradable than beet-pulp as discussed in Part 4.1.1. Another reason of this difference might be the addition of BM in this study which supplies adequate amounts of nutrients.

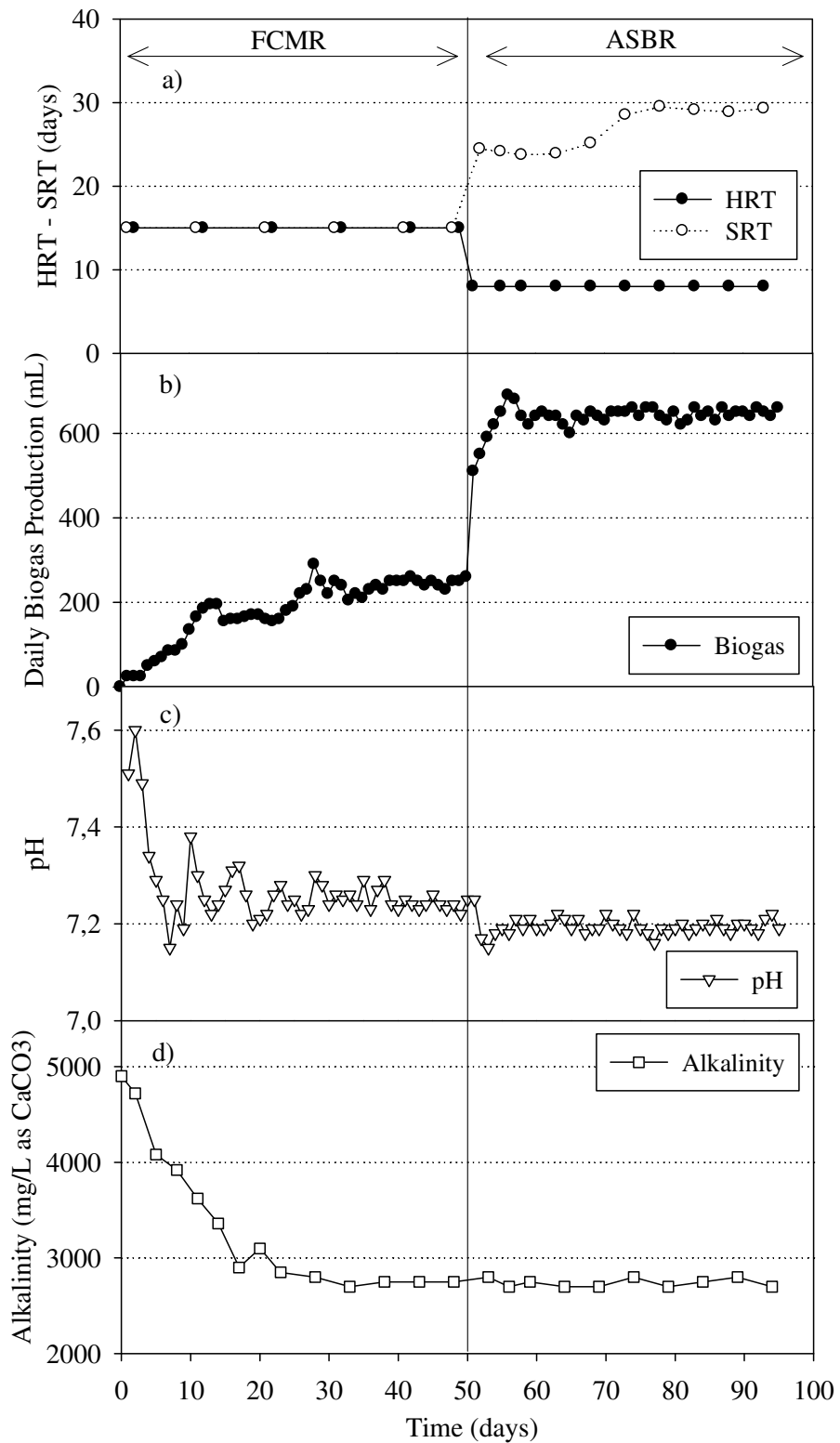


Figure 4.16. Temporal variations of control parameters of reactors:
 (a) HRT-SRT; (b) Biogas Production; (c) pH; (d) Alkalinity

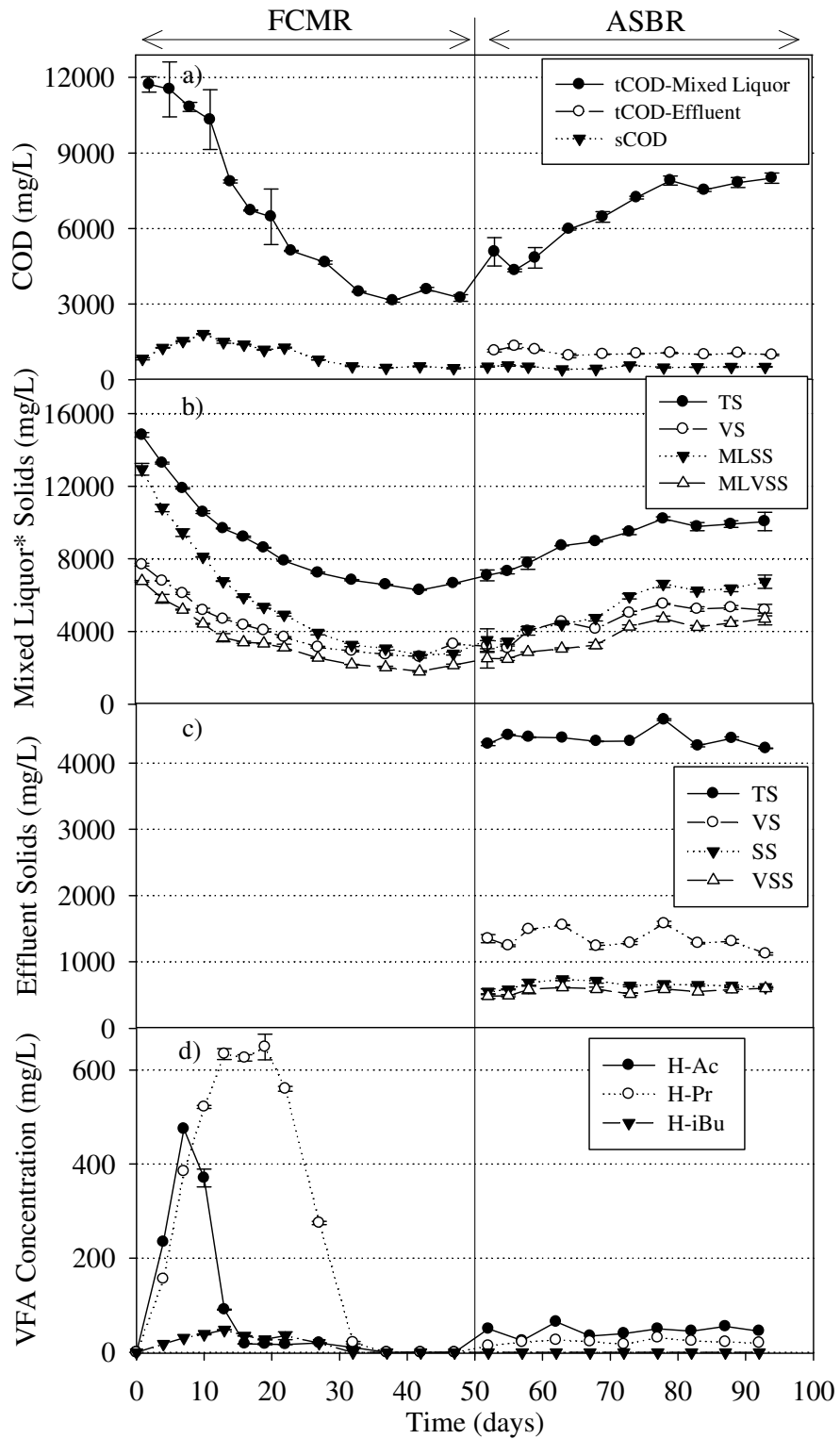


Figure 4.17. Temporal variations of control parameters of reactors: (a) COD; (b) Mixed Liquor* Solids; (c) Effluent Solids; (d) VFA

* For FCMR system, the mixed liquor also represents the effluent

Table 4.10. Biogas compositions at steady-state

System	CH ₄	CO ₂
FCMR	81.9 ± 4.7	18.1 ± 4.7
ASBR	79.7 ± 3.3	21.3 ± 3.3

COD Balance for FCMR system (at steady-state)

A mass balance calculation was carried out including methane production (as COD removal), to evaluate the COD removal data, calculated by influent and effluent COD concentrations (Eq 4.8, 4.9, 4.10). All parameters were calculated by using steady-state values and details are given in Appendix A. On a daily basis, input-COD (0.795 g) was compared with output-COD (methane-COD as 0.514 ± 0.035 g + effluent COD as 0.249 ± 0.018 g), which was computed as 0.763 ± 0.039 g. This result indicates that input-COD and output-COD were nearly equal. Coefficient of variation of 2.9% (from input to output) indicated that the data was sufficiently reliable. Consequently, percentage of COD removal, calculated by methane production ($64.6 \pm 2.8\%$), matches the percentage calculated by influent and effluent COD concentrations ($68.7 \pm 2.2\%$). This double-check also indicates the reliability of the experimental results in terms of total COD removal.

$$\text{Input-COD (g/day)} = \text{Output-COD (g/day)} \quad (4.8)$$

$$\text{COD}_i = \text{COD}_e + \text{COD}_m \quad (4.9)$$

$$\frac{Q}{4.243} \times C_i = \left(\frac{Q}{4.243} \times C_e \right) + \left(\frac{Q_m}{4.243} \times T_{43} \right) \quad (4.10)$$

$$\text{COD}_i = \text{COD}_e + \text{COD}_m$$

Where; COD_i : Influent COD (g/day),
 COD_e : Effluent COD (g/day),
 COD_m : COD equivalent of daily produced methane (g/day),
 Q : Daily waste-feed volume (L/day),
 Q_m : Daily methane production (L/day),
 C_i : Influent COD concentration (g/L),
 C_e : Effluent COD concentration (g/L),
 T_m : Theoretical COD equivalence of methane at 35 °C and 1 atm
(2.53 g/L methane) (Speece 1996).

4.4.2. Anaerobic Sequencing Batch Reactor

When the operational scheme of the reactor was shifted to the sequencing-batch mode (ASBR), a quick response was noticed in daily biogas productions (Figure 4.16.b). Following an increase for 5–7 days, a steady production was reached. At steady-state, daily biogas production was averaged to give 648 ± 10 ml/day. This significant rise in biogas production, when compared to FCMR data, was a result of higher methanogenic activity, in addition to the increase in OLR value from 0.71 to 1.33 g COD/L-day. During the change from FCMR to ASBR system, a slight decrease was observed in pH values (Figure 4.16.c), which can be associated with the slight increase in VFA concentrations (Figure 4.17.d). Meanwhile, alkalinity value was maintained between 2700–2800 mg/L as $CaCO_3$ (Figure 4.16.d). It can be postulated that the optimum conditions for an efficient anaerobic digestion process was sustained in terms of pH and alkalinity values as discussed in Part 4.4.1.

One of the evidences of biomass immobilization in ASBR configuration was the increasing tCOD concentrations of the mixed-liquor (Figure 4.17.a). Before it reaches to steady-state value of 7785 ± 239 mg/L, tCOD concentration was increased for 30 days. On the other hand, tCOD concentration of the effluent

(supernatant, remained after settling) was maintained between 1329 and 952 mg/L, with the steady-state value of 1008 ± 38 mg/L. The notable difference between tCOD-reactor (mixed-liquor) and tCOD-effluent indicates the remarkable settling performance of biosolids, which is discussed later in this part of the study. When enhanced bimethanation was assisted with biomass separation via gravity settling, influent tCOD concentration of 10600 mg/L was able to diminish to 1008 ± 38 mg/L, a decrease of $90.5 \pm 3.6\%$. In addition to that, by COD balance calculations, tCOD removal was computed as $79.7 \pm 1.1\%$, taking into account the daily wasted sludge-COD (Eq 4.11, 4.12, 4.13). On the other hand sCOD was sustained at 503 ± 10 mg/L at steady-state condition.

Figure 4.17.b depicts that, the mixed-liquor solids concentrations (TS, VS, MLSS, and MLVSS) were in an increasing trend, which initiated with the start-up of ASBR operation. The observed increase in solids concentrations, which lasted for 25–30 days, were absolute evidences of biomass immobilization. Particularly, MLVSS concentrations were crucial, representing biomass retention. At steady-state conditions, MLVSS concentration was detected as high as 4470 ± 222 mg/L. Different from the case of mixed-liquor, effluent solids concentrations were not deviated from the start-up till the end of operation (Figure 4.17.b). By using MLVSS (mixed-liquor) and VSS (effluent), SRT was determined periodically as described in Part 3.5.4. Manipulation of SRT value, through sludge wasting is a common practice for ASBR operations (Timur and Öztürk 1999; Cheong and Hansen 2008). In this study, by means of deliberate wasting of sludge, SRT was stabilized at 29.1 ± 0.2 days as steady-state value (Figure 4.16.a).

VFA concentrations were slightly increased (from 10–30 mg/L to 20–70 mg/L) after changing reactor configuration from FCMR to ASBR. Ghosh (1987) claims that, in an anaerobic treatment system increase in OLR value usually causes to VFA accumulation and corresponding pH decrease. In this study, accumulation of VFAs was insignificant (20–70 mg/L), although OLR value was increased almost twofold (from 0.71 to 1.33 g COD/L-day). This situation was due to the increased retention

of methanogenic microorganisms in the system. At SRT value of 29.1 ± 0.2 days, methanogens were able to enrich in the system and effectively convert produced VFAs into methane.

As it was the case for FCMR configuration, produced biogas consists of high percentages of methane ($79.7 \pm 3.3\%$) (Table 4.10), because of the reasons discussed in Part 4.4.1.

COD Balance for ASBR system (at steady-state)

As it was the case for FCMR system, COD balance calculations were carried out for ASBR configuration, in order to express tCOD removal performance, and associate it with methane production (Eq 4.11, 4.12, 4.13). Details of the calculations were given in Appendix A. Results prove that, 1.53 g daily COD input, was removed from the reactor as methane (1.31 ± 0.06 g COD/day), waste-sludge (0.19 ± 0.01 g COD/day) and effluent of the reactor (0.12 ± 0.00 g COD/day) at steady-state conditions. So, 1.53 g COD/day input was close to the value of the output which was 1.62 ± 0.06 g COD/day. Coefficient of variation of 4.0 % (from input to output) shows the high reliability of the gathered data. So, tCOD removal, calculated by methane production (85.6 ± 3.9 %), was slightly higher than the removal percent (79.7 ± 1.1 %) calculated by COD concentrations of waste-sludge and effluent.

$$\text{Input mass of COD (g/day)} = \text{Output mass of COD (g/day)} \quad (4.11)$$

$$\text{COD}_i = \text{COD}_e + \text{COD}_w + \text{COD}_m \quad (4.12)$$

$$\frac{Q_i \times C_i}{COD_i} = \left(\frac{Q_e \times C_e}{COD_e} \right) + \left(\frac{Q_w \times C_w}{COD_w} \right) + \left(\frac{Q_m \times T}{COD_m} \right) \quad (4.13)$$

Where; COD_i : Influent COD (g/day),
 COD_e : Effluent COD (g/day),
 COD_w : Waste-sludge COD (g/day),
 COD_m : COD equivalent of daily produced methane (g/day),
 Q_i : Daily feed volume (L/day),
 C_i : Influent COD concentration (g/L),
 Q_e : Daily effluent volume (L/day),
 C_e : Effluent COD concentration (g/L),
 Q_w : Daily waste-sludge volume (L/day),
 C_w : Daily waste-sludge COD concentration (g/L),
 Q_m : Daily methane production (L/day),
 T_m : Theoretical COD equivalence of methane at 35 °C and 1 atm
(2.53 g COD/L methane) (Speece 1996).

Methane Production Rate in ASBR System

One of the major characteristics of ASBR system is the high initial food concentrations (F/M), a driving force for metabolic activity, which result in increased substrate utilization rate immediately after feeding (Dague et al. 1992). Substrate utilization rate (in other words methane production rate) has paramount importance, since it determines the extent of COD removal, as well as settling characteristics of sludge at the end of cycle. Figure 4.18 depicts the methane production rate of ASBR system at reaction step during steady-state condition. Since F/M ratio was decreased with time, methane production rate, the major barrier for the settling of sludge, decreased as well. This situation favors biomass settling and sludge retention, at the end of reaction step of ASBR system (Dague et al. 1998).

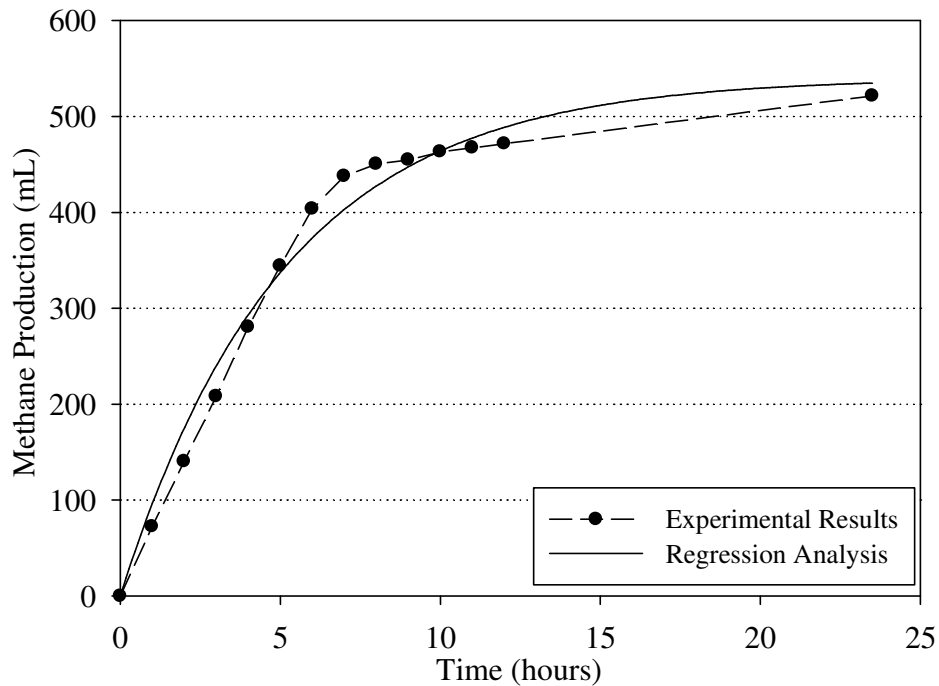


Figure 4.18. Methane production of ASBR configuration during 24-h cycle

Owing to the concerns discussed early in this study (Part 4.1.2), methane production rate of the reactor was modeled using first-order rate equation (Eq 4.14). Regression analysis indicated that, experimental results were in good agreement with modeled function ($R^2 = 0.9830$) with 95% confidence limits. Ultimate methane production (G_f) was computed as 540.2 ± 8.8 mL, while k was determined as 0.196 ± 0.012 hour⁻¹ (Table 4.11). When this k value is compared to the range of values obtained in Part 4.1.3 (0.028 – 0.143 day⁻¹), a significant difference is observed. As expected, elevated concentrations of microorganisms, already acclimated to the substrate, yielded this remarkable enhancement of biomethanation rate. On the other hand, experimentally found total methane production was very close to the computed G_f value. This result indicated that the methane production

rate was lowered at the end of cycle, which was a very suitable condition for an efficient biomass settling.

$$G_t = G_f (1 - e^{-kt}) \quad (4.14)$$

Where; G_t : Cumulative methane generation at time t (mL),

G_f : Ultimate methane generation (mL),

k : First-order rate constant (day^{-1})

t : Time (days).

Table 4.11. Computed G_f and k values with 95% confidence limits

System	R^2	Produced total CH_4 (mL)	G_f (mL)	k (hour^{-1})
ASBR	0.9830	521.4	540.2 ± 8.8	0.196 ± 0.012

Sludge Settling Characteristics of ASBR system

In operating cycle of an ASBR system, sludge settling step is critical, since it determines the effluent characteristics as well as enabling biomass retention. Indeed there are numerous studies in the literature, regarding the improvements in biomass settling in ASBR configuration, including biomass granulation (Zaiat et al. 2001; Wirtz and Dague 1997). As Dague et al. (1992) stated low substrate concentrations and resulting low methane production, achieved at the end of reaction step create ideal conditions for biomass flocculation and separation.

SVI assay is widely used to determine the settleability of treatment sludge. Sludge with an SVI less than 100 is regarded as very well-settled sludge, while the value, greater than 100 indicates settling problems (Vesilind 1980). In this study, SVI experiments were carried out to inspect sludge settling throughout the 45 days of operation of ASBR. Results indicated that sludge was highly settleable (SVI of 57.4 ± 4.9) from the beginning of the operation till the end. Vesilind (1980) stated that sludge settling adversely affected by the increase in sludge concentration. In this study, fluctuation of SVI value was insignificant, although MLSS concentration was increased from 2847 ± 194 mg/L to steady-state value of 6450 ± 257 mg/L. This information supported the idea that the settling characteristics of produced sludge were desirable for an effective biomass separation.

In fact, sugar industry wastewater is well-known with the ability to produce highly settleable sludge when using anaerobic contact process, which relies on biomass sedimentation and recycle (Hobson and Wheatley 1993). This high settleability is mainly because of the inherent Ca^{2+} content of the wastewater caused by lime addition as coagulant, discussed in Part 4.4.1. Effect of calcium concentrations on bioflocculation and granulation in anaerobic digestion was studied by some researchers (Langerak et al. 1998; Yu et al. 2001). Yu et al. (2001) advocated that calcium concentrations from 150 to 300 mg/L enhance the biomass accumulation and granulation during the start-up of a UASB reactor. This information explains the reason behind the determined high settleability, when the Ca^{2+} concentration of used wastewater (378 ± 5.7 mg/L) was taken into consideration. Metal cations present in BM (Part 3.3), could also stimulate the coagulation process, which needs to be further investigated.

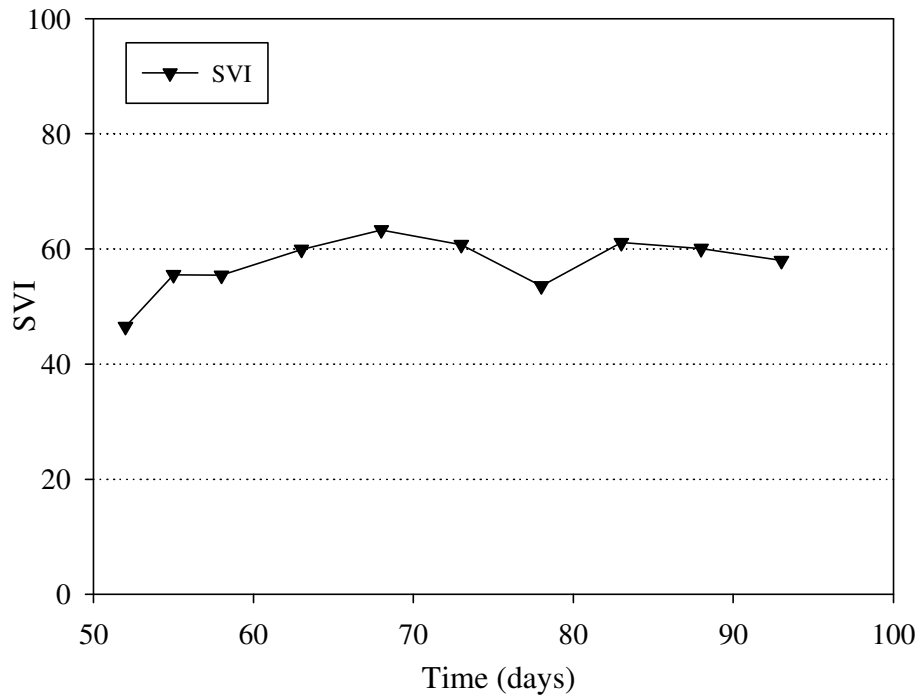


Figure 4.19. Temporal variations of SVI during ASBR operation

4.4.3. Comparison of the Treatment Efficiencies

Although influents were the same, there was a remarkable difference between effluents of FCMR and ASBR systems (Table 4.1). This difference was a direct result of the enhanced biomethanation, followed by efficient sludge sedimentation in ASBR configuration, discussed in Part 4.4.2. As it is depicted in Figure 4.16.a SRT values were determined as 15 and 29.1 ± 0.2 days respectively for FCMR and ASBR systems. Although HRT value of ASBR configuration was lower (8 days) than that of FCMR (15 days), and corresponding OLR was higher (from 0.71 to 1.33 g COD/L-day) discussed biomass retention enabled significant increase in biomethanation for ASBR. So, methane production yield of 255 ± 11 was increased to 337 ± 15 mL/g COD-added (32.2% increase in methane yield) when

configuration was changed into ASBR (Table 4.12). In addition, tCOD removal was increased from 68.7 ± 2.2 to $79.7 \pm 1.1\%$. VS reductions were also calculated as described in Appendix B. VS reduction in ASBR system was computed as $70.0 \pm 1.3\%$, while this value remained as $54.7 \pm 6.0\%$ in FCMR configuration. This difference corresponds to an increase in VS reduction with an order of $22.0 \pm 0.1\%$.

Although ASBR was proved to be an efficient system for the treatment of various wastes like, swine manure (Droste and Masse 1995), landfill leachate (Timur and Öztürk 1999), dairy manure (Dugba and Zhang 1999), cocking wastewater (Li et al. 2005) and brewery wastewater (Xiangwen et al. 2008), it was applied to beet-sugar processing wastes for the first time. The experimental results indicate that, in terms of treatment performances ASBR system was competitive with other reactor configurations, which was used for anaerobic digestion of beet-pulp (Table 4.13).

Table 4.12. Influent and effluent concentrations at steady-state

Parameter	Influent (mg/L)	FCMR effluent* (mg/L)	ASBR effluent (mg/L)
tCOD	10600	3322 ± 235	1008 ± 38
sCOD	5318 ± 288	484 ± 40	503 ± 10
TS	9193	6504 ± 197	4286 ± 79
VS	6348	2878 ± 379	1238 ± 101
SS	4832	2847 ± 194	637 ± 15
VSS	4268	1980 ± 173	578 ± 26
tVFA (as H-Ac)	892 ± 16	10–30	20–70
pH	7.65	7.25	7.20

* In FCMR system, effluent represents the mixed liquor, since biomass separation was not practiced.

Table 4.13. Comparison of the anaerobic treatment systems, adapted from Koppar and Pullammanappallil (2007)

Reactor type	HRT (days)	OLR (g COD/L-d)	Methane yield (mL g VS added)	VS reduction (%)	Reference
CSTR	27 ± 8	5.7 ± 1.7	0.358	81 ± 2	Frostell et al. (1984)
CSTR	2.4–7	0.9–2.7	0.346–0.355	NA**	Stoppok and Bucholz (1985)
Non-stirred tank	1–17	2.5–6.7	0.352	NA	Hutnan et al. (2000)
CSTR-UASB	13	2	0.235	92	Hutnan et al. (2001)
Leach-bed	7	4	0.336***	96	Koppar and Pullammanappallil (2007)
FCMR	15	0.71	0.426 ± 0.018	54.7 ± 6.0*	Present Study
ASBR	8	1.33	0.563 ± 0.025	70.0 ± 1.3*	Present Study

* Calculated as described in Appendix B.

** NA : Not available

*** Reported at standard temperature and pressure.

CHAPTER 5

CONCLUSIONS

The main objective of this study was to investigate two of the possible exploitation routes of anaerobic digestion (acid-phase and methane-phase) for the treatment of sugar-beet processing wastes, while producing valuable biobased products. From the obtained results of experimental setups (Set-up 1, 2, 3, 4) the following conclusions can be drawn:

Set-up 1: Biochemical Methane Potential Assay

- In the studied F/M range (0.51–2.56 g COD/g VSS), observed treatment efficiencies (63.7–87.3% COD removal and 69.6–89.3% VS reduction) were indications of high biodegradability for both wastewater and beet-pulp, which decreased with increasing F/M.
- When experimentally recorded cumulative net methane productions and modeled first-order rate functions were evaluated, remarkable difference was noticed between reactors in terms of rate constants (k values). k values differentiated in the ranges between 0.081–0.143 day⁻¹ and 0.028–0.050 day⁻¹ respectively for wastewater added and non-added reactors.
- These results indicated that anaerobic co-digestion of wastewater and beet-pulp is promising since wastewater addition significantly increases the rate of biomethanation of beet-pulp.

Set-up 2: Optimization of Anaerobic Acidification

- Sugar industry wastewater and beet-pulp can simultaneously be converted to VFAs in acidogenic anaerobic reactors with considerable acidification degrees (43.8–52.9%).
- Increased OLRs, resulting from pulp addition, increased the amount of acidification products (VFAs) which led to relatively low operational pH values (5.7–6.8). In this pH range, methanogenic activity was inhibited and lowest methane percentages (5.6–16.3%) were observed in biogas compositions.
- Optimum operational conditions for anaerobic acidification were selected as 2-day HRT and 1:1 waste mixing ratio (in terms of COD) without external alkalinity addition. These operational conditions led to the highest tVFA concentrations (3635 ± 209 mg/L as H-Ac) with an acidification degree of 46.9 % at the highest OLR of 5.4 g COD/L-d.

Set-up 3: Recovery of VFAs by Liquid-Liquid Extraction

- The effect of TOPO in kerosene concentration was as crucial as the effect of pH on the recovery of VFAs via liquid-liquid extraction. Consequently, pH 2.5 was determined as optimum. At this pH, percent recoveries of VFAs were changed from 43.3 to 97.6%, depending on the type of the acid extracted and the concentration of TOPO in kerosene. As the concentration of TOPO in kerosene was increased, efficiency of extraction was increased. As a result, highest VFA recoveries (60.7–97.6%) were observed at 20% TOPO in kerosene with K_D values ranging between 1.54 and 40.79.

- At pH 2.5, the increase in TOPO concentration directly increased the COD removal efficiencies, as it does for tVFA recovery. Up to 71.8% COD removals were achieved, at 20% TOPO in kerosene at pH 2.5, while the removal efficiencies remained between 19.1–22.3% at pH 5.5.

Set-up 4: Evaluation and Comparison of Fed-batch and Sequencing-batch Reactors in terms of Biomethanation

- Although HRT value of ASBR configuration was lower (8 days) than that of FCMR (15 days), and corresponding OLR was higher (from 0.71 to 1.33 g COD/L-day) increased biomass retention enabled significant increase in biomethanation for ASBR. So methane production yield of 255 ± 11 mL/g COD-added was increased to 337 ± 15 mL/g COD-added (32.2% increase in methane yield) when configuration was changed from FCMR to ASBR. In addition, tCOD removal was increased from 68.7 ± 2.2 to $79.7 \pm 1.1\%$.
- The experimental results indicate that, in terms of treatment performances ASBR system was competitive with other reactor configurations, which was used for anaerobic digestion of beet-pulp (Table 4.13).

Based on these conclusions, it is postulated that, biorefining of sugar-beet processing wastes by anaerobic digestion can not only be a solution for environmental related problems, but also contribute to resource conservation and sustainable production via valuable bio-based product formation.

Future Work

In order to establish an integrated approach for the management of sugar-beet processing wastes by anaerobic digestion, further research is needed in:

- (i) Evaluation of the effect of operational parameters on anaerobic acid-phase and methane-phase digestion like, nutrient availability (anaerobic digestion without BM) and physical pretreatment of beet-pulp (anaerobic digestion without drying and grinding)
- (ii) Investigating the potential benefits of anaerobic co-digestion, targeted to bio-product formation, in pilot-scale and demonstration studies on-site,
- (iii) Developing a basis for the comparison of discussed processing routes (anaerobic acid-phase and methane-phase digestion), taking into account the economical and technical concerns via comprehensive feasibility studies.

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APPENDIX A

COD BALANCE CALCULATIONS AT STEADY-STATE

Calculations for FCMR System

$$\text{Input-COD (g/day)} = \text{Output-COD (g/day)} \quad (\text{A.1})$$

$$\text{COD}_i = \text{COD}_e + \text{COD}_m \quad (\text{A.2})$$

$$\frac{Q}{1} \times \frac{C_i}{2} = \left(\frac{Q}{14} \times \frac{C_e}{2} \right) + \left(\frac{Q_m}{14m} \times \frac{T_m}{43} \right) \quad (\text{A.3})$$
$$\text{COD}_i = \text{COD}_e + \text{COD}_m$$

- Where; COD_i : Influent COD (g/day),
 COD_e : Effluent COD (g/day),
 COD_m : COD equivalent of daily produced methane (g/day),
 Q : Daily waste-feed volume (L/day),
 Q_m : Daily methane production (L/day),
 C_i : Influent COD concentration (g/L),
 C_e : Effluent COD concentration (g/L),
 T_m : Theoretical COD equivalence of methane at 35 °C and 1 atm (2.53 g/L methane) (Speece 1996).

Daily Methane Production (Q_m) = Methane Content of Biogas x Biogas Production

$$Q_m = \left[\frac{(81.9 \pm 4.7)}{100} \right] \times (248 \pm 9) \text{ mL/day}$$

$$Q_m = 203 \pm 14 \text{ mL} = 0.203 \pm 0.014 \text{ L/day}$$

$$\begin{aligned} \underline{\text{COD}}_m &= 0.203 \pm 0.014 \text{ L/day} \times 2.53 \text{ g/L Methane} \\ &= \mathbf{0.514 \pm 0.035} \text{ g COD/day} \end{aligned}$$

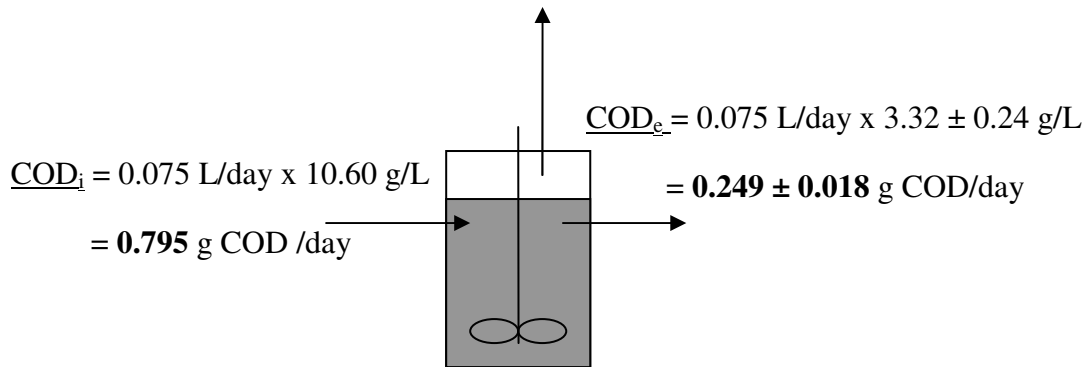


Figure A.1: Schematic representation of COD balance of FCMR

$$\underline{\text{Input-COD}} = \mathbf{0.795 \text{ g/day}}$$

$$\begin{aligned} \underline{\text{Output-COD}} &= \mathbf{0.539 \pm 0.035} + \mathbf{0.249 \pm 0.018} \text{ g/day} \\ &= \mathbf{0.763 \pm 0.039} \text{ g/day} \end{aligned}$$

Coefficient of variation between 0.795 g/day and 0.763 g/day = **2.9%**

Calculations for ASBR System

$$\text{Input mass of COD (g/day)} = \text{Output mass of COD (g/day)} \quad (\text{A.4})$$

$$\text{COD}_i = \text{COD}_e + \text{COD}_w + \text{COD}_m \quad (\text{A.5})$$

$$\frac{Q_i \times C_i}{\text{COD}_i} = \frac{(Q_e \times C_e)}{\text{COD}_e} + \frac{(Q_w \times C_w)}{\text{COD}_w} + \frac{(Q_m \times T_m)}{\text{COD}_m} \quad (\text{A.6})$$

- Where; COD_i : Influent COD (g/day),
 COD_e : Effluent COD (g/day),
 COD_w : Waste-sludge COD (g/day),
 COD_m : COD equivalent of daily produced methane (g/day),
 Q_i : Daily feed volume (L/day),
 C_i : Influent COD concentration (g/L),
 Q_e : Daily effluent volume (L/day),
 C_e : Effluent COD concentration (g/L),
 Q_w : Daily waste-sludge volume (L/day),
 C_w : Daily waste-sludge COD concentration (g/L),
 Q_m : Daily methane production (L/day),
 T_m : Theoretical COD equivalence of methane at 35 °C and 1 atm
(2.53 g COD/L methane) (Speece 1996).

Daily Methane Production (Q_m) = Methane Content of Biogas x Biogas Production

$$Q_m = \left[\frac{(79.9 \pm 3.3)}{100} \right] \times (648 \pm 10) \text{ mL/day}$$

$$Q_m = 518 \pm 22 \text{ mL} = 0.518 \pm 0.022 \text{ L/day}$$

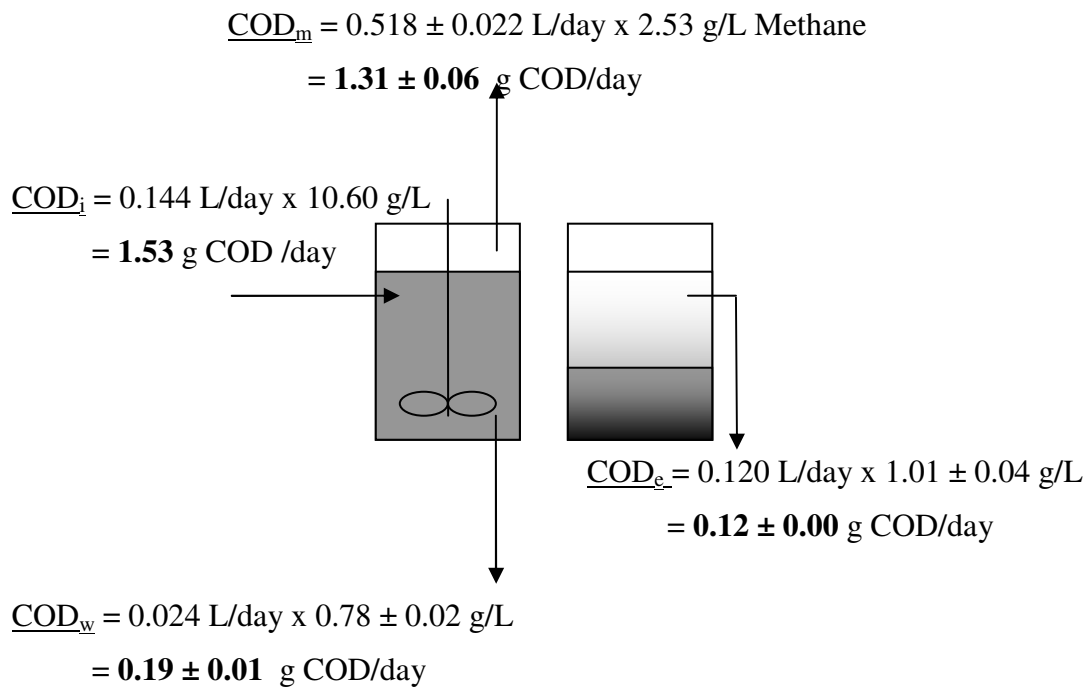


Figure B.1: Schematic representation of COD balance of ASBR

$$\underline{\text{Input-COD}} = \boxed{1.53 \text{ g/day}}$$

$$\underline{\text{Output-COD}} = (0.12 \pm 0.00) + (0.19 \pm 0.01) + (1.31 \pm 0.06)$$

$$= \boxed{1.62 \pm 0.06 \text{ g/day}}$$

Coefficient of variation between 1.53 g/day and 1.62 g/day = 4%

APPENDIX B

VS REDUCTION CALCULATIONS AT STEADY-STATE

Calculations for FCMR System

$$\%VS \text{ Reduction} = \frac{\text{Input VS} - \text{Output VS}}{\text{Input VS}} \times 100 \quad (\text{B.1})$$

$$\%VS \text{ Reduction} = \frac{\frac{\text{Input VS}}{647.48} - \frac{\text{Output VS}}{647.48}}{\frac{\text{Input VS}}{142.43}} \times 100 \quad (\text{B.2})$$

Where; VS_i : Influent VS concentration (mg/L),
 VS_e : Effluent VS concentration (mg/L),
 Q : Daily waste-feed volume (L),

$$\%VS \text{ Reduction} = \frac{(6348 \text{ mg/L} \times 0.075 \text{ L}) - (2878 \pm 379 \times 0.075 \text{ L})}{(6348 \text{ mg/L} \times 0.075 \text{ L})} \times 100$$

$$\%VS \text{ Reduction} = \mathbf{54.7 \pm 6.0}$$

Calculations for ASBR System

$$\% \text{VS Reduction} = \frac{\text{Input VS} - \text{Output VS}}{\text{Input VS}} \times 100 \quad (\text{B.3})$$

$$\% \text{VS Reduction} = \frac{\text{Input VS} - [(\text{VS}_e \times Q_e) + (\text{VS}_w \times Q_w)]}{\text{Input VS}} \times 100 \quad (\text{B.4})$$

- Where; VS_i : Influent VS concentration (mg/L),
 Q_i : Daily influent volume (L),
 VS_e : Effluent VS concentration (mg/L),
 Q_e : Daily effluent volume (L),
 VS_w : Waste-sludge VS concentration (mg/L),
 Q_w : Daily waste-sludge volume (L),

$\% \text{VS Reduction} =$

$$\frac{(6348 \text{ mg/L} \times 0.144 \text{ L}) - (1238 \pm 101 \times 0.12 \text{ L}) + (5243 \pm 70 \times 0.024 \text{ L})}{(6348 \text{ mg/L} \times 0.144 \text{ L})} \times 100$$

$\% \text{VS Reduction} = 70.0 \pm 1.3 \%$