# ENHANCING THE PERFORMANCE OF ANAEROBIC DIGESTION OF DAIRY MANURE THROUGH PHASE-SEPARATION

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

VEDAT YILMAZ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
ENVIRONMENTAL ENGINEERING

JUNE 2007

Approval of the Graduate School of Natural and Applied Sciences

_____

Prof. Dr. Canan Özgen
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Doctor of Philosophy.

_____

Prof. Dr. Göksel N. Demirer
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

_____

Prof. Dr. Göksel N. Demirer
Supervisor

**Examining Committee Members**

| | | |
|---|---|---|
| Prof. Dr. Celal F. Gökçay | (METU, ENVE) | _____ |
| Prof. Dr. Göksel N. Demirer | (METU, ENVE) | _____ |
| Prof. Dr. Ülkü Yetiş | (METU, ENVE) | _____ |
| Prof. Dr. Filiz B. Dilek | (METU, ENVE) | _____ |
| Prof. Dr.Ayşenur Uğurlu | (Hacettepe Unv, ENVE) | _____ |

**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.**

Vedat YILMAZ

## ABSTRACT

ENHANCING THE PERFORMANCE OF ANAEROBIC DIGESTION OF DAIRY MANURE THROUGH PHASE-SEPARATION

Yılmaz, Vedat

Ph.D., Department of Environmental Engineering

Supervisor: Prof. Dr. Göksel N.Demirer

June 2007, 138 pages

Anaerobic digestion (AD) is an effective way to convert animal manures into profitable byproducts as well as to reduce the pollution of water, air, and soil caused by these wastes. Conventional high-rate anaerobic reactors cannot effectively process high-solids containing animal manures. The two-phase configuration for AD has several advantages over conventional one-phase processes such as increased stability of the process, smaller and cost efficient process configurations, etc.

This study investigated the two-phase AD of dairy manure with particular emphasis on the effects of solids retention time (SRT), organic loading rate (OLR) and pH on anaerobic acidification of unscreened dairy manure; the effects of temperature on biogas production and the comparison of one-phase and two-phase system performance of AD. The results revealed that pre-acidification of dairy manure in daily-fed continuously-mixed reactors with no recycle led to substantial volatile fatty acids production. The optimum operational conditions for anaerobic acidification were determined as SRT and OLR of 2 days and 15 g VS/L.day. The pH control at a range of 5.0-5.5 was not

found to be necessary for optimum acidification. Molecular analysis indicated that acidogenic bacteria population increased whilst the aerobic bacteria population decreased as time passed in acidogenic phase. The effect of temperature was clearly observed on biogas production efficiency. Two-phase configuration was determined more efficient than one-phase system. The biogas production in two-phase system was calculated to be 41% higher than that of the one-phase for the same OLR of 3.5 g VS/L.day. This translates into significant performance improvement and reduced volume requirement. This finding represents a further step in the achievement of wider use of simple anaerobic reactor configurations in rural areas.

Keywords: Dairy manure, anaerobic digestion, two-phase, methane

# ÖZ

SIĞIR ATIKLARININ ANAEROBİK BOZUNDURMA PERFORMASININ FAZ AYRIMI İLE GELİŞTİRİLMESİ

YILMAZ, Vedat

Doktora, Çevre Muhendisliği Bölümü
Tez Yöneticisi: Prof. Dr. Göksel N. Demirer

Haziran 2007, 138 sayfa

Hayvansal gübrenin hem yararlı ürünlere dönüşmesinde hem de bu atıklar kaynaklı su, toprak ve hava kirliliğinin azaltılmasında anaerobik bozundurma (AB) etkin bir yöntemdir. Konvansiyonel yüksek-hızlı anaerobik reaktörler yüksek katı içeriği olan hayvan gübrelerinin bozundurulmasinda etkili olamamaktadır. İki-fazlı AB proses kararlılığının artması, daha küçük ve düşük maliyetli reaktörlerin işletilebilmesi, vb. birçok nedenlerle tek-fazlı sistemlere göre avantajlıdır. Yüksek katı-içerikli atıklar ilk fazda asidifikasyon ile sıvılaşmakta, bu da ikinci fazda daha yüksek metan oluşumlarına yol açarak, faz ayrımlı AB'yı çok daha etkin kılmaktadır.

Bu çalışma ile elenmemiş mandıra gübresinin iki fazlı AB'da hidrolik bekletme suresi, yükleme hızı ve pH nın asidifikasyondaki etkileri özelinde, sıcaklığın biyogaz üretimine etkisi ile iki faz ve tek faz sistemlerin AB üzerindeki perfomans etkileri araştırılmıştır. Sonuçlar göstermektidir ki geri dönüşümsüz asidifikasyon reaktörlerinde yuksek duzeyde uçucu yağ asidi olusmaktadir. Bu reaktör için optimum hidrolik bekletme suresi,ve yükleme hızı sırasıyla 2 gün ve 15 g KM/L.gün olarak seçilmiştir. Optimum asidifikasyon için 5.0-5.5

aralığındaki pH kontrolüne gerek olmadığı bulunmuştur. Moleküler analiz sonuclarina gore zamanla asidogenik bakteri popülasyonları artarken aerobik bakteri populasyonlari azalmıştır. Sıcaklığın biyogaz üretimi uzerinde onemli bir etkisinin oldugu gözlenmistir. İki fazlı sitem tek fazlıdan daha etkili bulunmuştur. Alıkonma süreleri dikkate alınarak yapılan hesaplama ile 3.5 g KM/L.gün yükleme oranındaki iki fazlı sistemin tek faza göre %41 daha fazla biyogaz üretimi sağladığı belirlenmiştir. Böylece sistem performansında önemli iyileşme ve gerekli olan hacimde azalma gerçekleşmektedir. Bu bulgular ile kırsal alandaki basit anaerobik bozundurma reaktörlerinin yaygın olarak kullanılmasında kazanımlar sunmaktadır.

Anahtar Kelimeler: Mandıra atığı, anaerobik bozundurma, iki-faz, metan.

to Ramazan DEMİR

"...zincirlerinden başka kaybedecek şeyleri yoktur..." NHR

## ACKNOWLEDGMENTS

I wish to express my deepest gratitude to my supervisor Prof. Dr. Göksel N. Demirer for his guidance, advice, trust and encouragement throughout this research.

My special thanks goes to Z33 roommates Hande Yükseler and Meltem Ünlü for making life full of joy. I would also like to express my gratitude to my friends, C.Burak Bural, Emrah Alkaya, Erkan Şahinkaya, Eylem Doğan, Hakan Moral, Kadir Gedik, Murat Taşdemir, Nimet Uzal, Nuray Ateş, Recep Özdemir, Sibel Uludağ-Demirer, Seçil Türkmenoğlu, and Tuba Ergüder for their support. I am also thankful to the Environmental Engineering Department Staff, Aynur Yıldırım, Cemalettin Akın, Gizem Uğurlu, Güldane Kalkan, Gülşen Erdem, Kemal Demirtaş, Ramazan Demir, and Yakup Pınarbaşı.

I also like to thank Hasan Güney from my elementary school for his teaching, mentoring and tutoring.

My warmest thanks are dedicated to my wife Ebru for inspiration and being by my side all the time, and my family for all the patience, encouragement and love.

To Ilgın, I look forward to watching you grow and succeed in your life. I am truly blessed and thankful to be your father, and I hope I'm able to provide guidance for you throughout your life.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## ABBREVIATIONS

| | |
|---|---|
| ABR | : Anaerobic baffled reactor |
| AD | : Anaerobic digestion |
| CGP | : Cumulative gas production |
| CHP | : Combined heat and power |
| COD | : Chemical oxygen demand |
| CSTR | : Completely stirred tank reactor |
| CTAB | : Hexadecyltrimethylammonium bromide |
| DNA | : Deoxyribonucleic acid |
| EDTA | : Disodium ethylenediamine tetraacetic acid |
| GC | : Gas chromatograph |
| GHG | :Greenhouse gas |
| HAc | : Acetic acid |
| HRT | : Hydraulic retention time |
| HSW | : Household solid waste |
| OLR | : Organic loading rate |
| ORP | : Oxidation reduction potential |
| RT | : Retention time |
| sCOD | : Soluble chemical oxygen demand |
| SRT | : Solids retention time |
| TPAD | : Temperature-Phased Anaerobic Digestion |
| TS | : Total solids |
| tVFA | : Total volatile fatty acid |
| VFA | : Volatile fatty acid |
| VS | : Volatile solids |
| VSS | : Volatile suspended solids |

# CHAPTER 1

# INTRODUCTION

## 1.1. Background Information

The production of farm animals in large scale units has considerably increased in the world. Manure residues from livestock industries in the World have been identified as major sources of environmental pollution. A typical 635 kg cow, for example generates 22,805 kg/year of raw wet manure, containing 2,208 kg/year of volatile solids which is equivalent to 2,429 kg/year of chemical oxygen demand. Traditionally, manure residues from cattle operations have been disposed off directly or after compositing as soil amendments in the agricultural industry. Since this practice has resulted in the degradation of air, soil, and water resources, new regulations for protecting the environment have been promulgated to control land application of manure (US EPA, 1995). As such, livestock industries and regulatory agencies are seeking the alternate technologies to manage manure residues in an environment-friendly manner.

Biotechnology has the potential to manage this problem in a cost-effective and sustainable manner. Cattle manure residues can not only serve as substrates for microorganisms to grow, but also serve as substratum for attached microbial communities to thrive, providing essential nutrients and micro-nutrients. Anaerobic digestion (AD) has been recognized as a preferred biotechnology for treating such complex wastes and the same time regenerating useful chemicals and/or generating energy and reducing the volume for disposal (Burke, 2001).

The utilisation of energy in the form of biogas is one of the environmentally sound alternatives available using renewable energy sources. Biogas is formed

by anaerobic degradation of organic material, the main constituent being energy-rich methane. The organic waste produced by municipalities, industry and agriculture have an energy value that is today not fully utilised. In the future, the energy potential of waste should be exploited in a more efficient way. Anaerobic digestion may well be the organic waste treatment of the future, as the potentially valuable methane could ensure the economic viability of the process. Further benefits include a reduction in waste volume and the production of a biofertiliser retaining all the nutrients of the original material. Also, if methane is allowed to form under uncontrolled conditions in anaerobic environments it may be released to the atmosphere, and it is believed that 18% of the global warming effect is due to methane emission (Ghosh, 1997; Green Matters, 2003).

Although animal manure may have tremendous potential for biogas production, there must be a need for energy or other economic benefit to justify its pursuit. The production of biogas through anaerobic fermentation on small farms, however, is difficult to justify because of the size of reactor needed, the amount of dilution water required and the limited availability of capital and operational skills.

AD is an effective way to convert animal manure into profitable by-products as well as to reduce the pollution of water, air, and soil caused by these wastes. Extensive research has been conducted on AD of animal manure. There are, though, several areas of research that must be pursued to make AD technology more advantageous such as improving AD relatively low digestion rates. Conventional high-rate anaerobic reactors cannot effectively process high-solids containing animal manures. Furthermore, one-phase AD of high-solids containing wastes requires the waste to be diluted. This, in turn, results in a significant increase in digester volumes. Thus, demonstrating an innovative anaerobic process configuration that can process animal manure at relatively short retention times (RT) will be a significant step towards achieving effective exploitation of AD for animal manure.

The two-phase configuration for AD has several advantages over conventional one-phase processes such as increased stability of the process, smaller and cost efficient process configurations, etc. One relevant feature of the two-phase

AD is that when a high solids-containing waste is introduced to the first phase it is liquefied along with acidification. This translates into less liquid addition and, thus, less energy requirements for heating, storing, etc. Even though several aspects of two-phase configuration including liquefaction might be very significant for efficient AD of dairy manure, its application on animal manure is very limited. Finally, the process can be smaller and more cost effective (Demirer and Chen, 2005). The results of several studies have clearly demonstrated the applicability and efficiency of two-phase AD for high solids containing wastes.

Two-phase AD have been applied in the biogasification of: wastewater treatment sludge (Ghosh, 1991; Elefsiniotis and Oldham, 1994) organic fractions of municipal solid wastes (Ghosh, 1985; Chanakya et al., 1992), industrial wastes and sludge (Ghosh et al., 1985), olive mill solid waste and olive pomace (Borja et al., 2005), grass (Raposo et al., 2004), coffee pulp juice (Calzada et al., 1984), food waste (Koster, 1984), cane–molasses alcohol stillage (Wang et al., 2003), spent tea leaves (Goel et al., 2001), brewery wastewater (Ahn et al., 2001), dairy wastewater (Ince, 1998; Yu and Fang, 2001), abattoir wastes (Wang and Banks, 2003) as well as some studies focusing on improving reactor design, control and operational parameters.

Conventional one-phase slurry digestion is not an effective system for wastes containing high solids (>10%), since they require the manure to be capable of being pumped which in itself necessitates a concentration below 10% solids. This, in turn, results in a significant increase in fluid and digester volume which results in increased capital and operating costs. Although most animal wastes are produced as slurry, the housing methods, bedding, and collection methods used produce a material of much higher solids content. For example, cattle housed in sheds and bedded on straw produce a farmyard manure of approximately 26% solids. The significance of high solid content animal manure in relation to the performance of AD in terms of reactor volumes, pumping, handling, mixing, and clogging are emphasized in several studies. The associated investment costs for large-size reactors, as well as the heating, handling, dewatering, and disposal of the digested residue decrease the benefits of conventional slurry AD of high solids containing wastes (Demirer and Chen, 2004).

Furthermore, it is a well known fact that increase of temperature enhances the rate of hydrolysis, acidification and methanogenesis stages contributing to accelerate methane production. From the critical analysis of literature it is inferred that systematic studies are lacking on biomethanation at psychrophilic conditions. As a result, the majority of the researches proposed in the literature to enhance biogas production are aimed at increasing the digester temperature to mesophilic or thermophilic range. Even though mesophilic/thermophilic operation of anaerobic digesters elevates the digestion rate, it also increases the operational cost especially at small–scale farm level operations. Thus, a novel process configuration for the AD of animal manure at psychrophilic conditions without compromising the process performance or an elevated performance at mesophilic conditions would be a significant contribution to the field.

## 1.2. Objectives of This Study

Even though several aspects of two-phase configuration including liquefaction might be very significant for efficient AD of dairy manure, its application has been limited to screened dairy manure only. Burke (2001) also pointed out the fact that phased digestion has not been applied to dairy waste. In recognition of these facts and in support of their needed application to high solids containing dairy manure, the aim of this study is to investigate and optimize the application of phase-separation for AD of unscreened dairy manure both at low temperature and mesophilic conditions.

The objectives of the thesis are given below:

- To investigate the effects of retention time and organic loading rate on anaerobic acidification and biogasification of unscreened dairy manure in daily-fed continuously-mixed reactors with no recycle.
- To investigate the effects of pH on the acidification stage of two-phase system.
- To investigate the effects of temperature on the of biogas production in a conventional one-phase and two-phase anaerobic reactor configurations.
- To investigate the advantages of two-phase AD for unscreened dairy manure.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Anaerobic Treatment of Animal Manure

Anaerobic digestion (also known as fermentation) is one of the most important treatment techniques available for animal manures and other organic wastes. It is a common technology for the purification of municipal and industrial wastewaters which not only reduces their environmental impact but which also produces a useful by-product in the form of methane. The use of anaerobic fermentation for waste treatment is widely demonstrated in Asia with several million small scale biogas plants in China and India. However, in Europe and USA, the use of this promising technique is relatively limited.

Until now, many options have been proposed for the treatment and disposal of animal manure. Land application (Sommer and Hutchings, 2001; Araji et al., 2001), pond systems (Wang et al., 1996), composting (Tiqua and Tam, 1998; Guerra-Rodriquez et al., 2001), constructed wetlands (Knight et al., 2000; Clarke and Baldwin, 2002), anaerobic treatment (Huang and Shih, 1981; Lo and Liao, 1985; Shyam, 2001) are examples of these alternatives. Among these options, anaerobic treatment offers the best solution in terms of pollution reduction and energy production which contributes to the resource conservation by reducing conventional energy consumption. Also it improves the fertilize value of the manure.

Anaerobic treatment is an established and proven technology for the treatment of animal manure and has been widely studied by many researchers considering different aspects to optimize the process efficiency like,

temeperature (Hammad et al., 1999; Sanchez et al., 2000), solids concentration (Bujoczek et al., 2000) retention time (Aubart and Fauchille, 1983), reactor types (Ong et al., 2000), and phase separation (Demirer and Chen, 2005; Lo and Liao, 1985).

## 2.2 Management of Dairy Cattle Manure

Methods of collection, storage, and use of dairy cattle manure have undergone increased scrutiny during the last 15 to 20 years. This is in response to local increases in manure quantities (from increases in herd size) and to heightened environmental awareness concerning adverse effects of manure on the quality of surface water and groundwater. Dairy cattle manure contains significant amounts of the primary nutrients (N, P, and K) as well as other essential plant nutrients and hence is an excellent nutrient source for crop growth. However, if excess amounts of manure are applied beyond the use capacity of the crops and soil or if manure is improperly applied, losses by surface runoff and leaching can contribute to eutrophication of surface water bodies or contamination of groundwater. The primary issue with dairy cattle manure, both now and for the future, is development of management systems that use the resource without adverse environmental impacts. In a number of regions, the amount of dairy cattle manure produced exceeds loading capacity of soils available for manure application.

As dairy farm size has increased, so has the quantity of dairy cattle manure handled per dairy farm (Morgan and Keller, 1987). The increased manure production plus heightened environmental awareness of associated soil and water quality problems has exacerbated the need for management systems that can use the biomass and nutrients in the manure without creating unacceptable air, soil, or water pollution.

## 2.3 Manure Production and Composition

Because dairy cattle normally spend a large portion of their time in the feeding and lounging barn, milking parlor, and pasture areas, they deposit a large portion of their manure in those areas (Westerman and Overcash, 1980). Manure dropped in pasture areas may or may not be of environmental concern, depending on herd size, pasture area and location, and amount of time the

animals spend in the area. The major source area for dairy cattle manure, which must be handled, stored, and treated or used, is the building complex containing the feeding barn, lounging barn, and milking parlor. The daily manure production (feces and urine) per 454 kg of body weight for Holstein cows is approximately 34 kg, of which about 70 percent, or 24 kg, is solids, and 30 percent, or 10 kg, is liquid (North Carolina Agricultural Extension Service, 1973). On this basis, the daily manure production of a mature Holstein cow weighing 636 kg is about 48 kg. The properties of dairy cattle manure depend on several factors, including the digestibility and protein and fiber contents of the feed, and the animal's age, environment, and productivity. Table 2.1 shows estimates of daily manure production and manure properties for a range of animal sizes.

The actual composition of any particular batch of dairy cattle manure as removed from the milking parlor, feeding, or lounging areas depends on the amount of moisture, the amount of bedding material present, and the rations fed. Bedding incorporated into the manure increases the total solids content, while water added during washing dilutes the material.

Manure collected by either scraping or flushing generally goes to a storage area. In some systems manure is immediately spread on land without storage, but this is not appealing to many dairy farmers primarily because of frequency of disposal. Transport of manure from the storage areas is dependent on the flow characteristics of the material. Dairy cattle manure can be classified as semisolid, semiliquid, or liquid (Sobel and Muck, 1983). Semisolid manure will not flow with perceptible movement unless given mechanical assistance. Most fresh manure is in this category and, unless flushed, must be manually or mechanically transported. Semiliquid manure is material that has undergone dilution. This type of manure will slowly flow without mechanical assistance and contains between 5 and 15 percent total solids (Merkel, 1981). Liquid manure generally contains less than 5 percent total solids (wet basis), flows freely without mechanical assistance, and is associated with feedlot runoff and effluents from milking parlors and treatment systems.

**Table 2.1.** Dairy cattle manure production and characteristics (Midwest Plan Service, 1993)

| Animal size (kg) | Total manure production (kg/d) | (L/d) | Water (%) | Density ($kg/m^3$) * | TS† (kg/d) | VS‡ (kg/d) | $BOD_5$ § (kg/d) | Nutrient content (kg/d) N | P | K |
|---|---|---|---|---|---|---|---|---|---|---|
| 68 | 5.9 | 6.0 | 87.3 | 994 | 0.8 | 0.7 | 0.12 | 0.03 | 0.006 | 0.019 |
| 114 | 10.0 | 9.8 | — | — | 1.4 | 1.1 | 0.20 | 0.04 | 0.008 | 0.034 |
| 227 | 19.5 | 19.6 | — | — | 2.7 | 2.3 | 0.39 | 0.10 | 0.018 | 0.064 |
| 454 | 39.0 | 39.3 | — | — | 5.4 | 4.5 | 0.77 | 0.19 | 0.034 | 0.128 |
| 636 | 54.5 | 54.8 | — | — | 7.6 | 6.4 | 1.08 | 0.27 | 0.048 | 0.181 |

* Density best estimate, not ASAE data
† TS total solids
‡ VS volatile solids
§ $BOD_5$ biochemical oxidation demand

In Turkey alone, there are an estimated of over 13 million of cows. As the calorific value of biogas is about 6 $kWh/m^3$ (this corresponds to half a liter of diesel oil), this process would lead to saving of an enormous amount of fuel per year. This huge amount of animal wastes produces 11 million-ton of dry solid per year. The estimated available biogas production is calculated as 2.1 billion $m^3$ by using some rough assumptions; this biogas yields 48 million joule of energy (Başçetinçelik et al., 2005).

## 2.4. The Microbiological Processes in Anaerobic Digestion

The anaerobic degradation of organic substances to its most reduced form of methane, is a purely microbial process. The energy released during the degradation steps which was originally stored in the substrate is predominantly recovered by the methane formed:

33g organic material ($C_xH_yO_z$) = 22g $CO_2$ + 8g $CH_4$ + 3g biomass …….. (1)

This is a gross oversimplification of a complex process. The conversion of biodegradable organic material to $CH_4$ and $CO_2$ is facilitated by three major groups of bacteria (Fig. 2.1). The fermenting bacteria (group I) converts the organic material to short-chain fatty acids (especially acetic acid) through hydrolysis by extracellular enzymes and subsequent fermentation of the

hydrolyzed products. Other products of the fermentation process are alcohols, $CO_2$ and $H_2$. The short-chain fatty acids that are longer than acetate are oxidized by the hydrogen producing, acidogenic bacteria (group II) under production of $H_2$, formic acid, acetic acid and $CO_2$. The end products from the fermenting and the acidogenic bacteria (formic acid, acetic acid, and $H_2$) are converted to $CH_4$ and $CO_2$ by the methane producing bacteria (group III). Two additional groups of microorganisms are active in the conversion processes. One is the homoacetogens (group IV) who ferments a broad range of components under production of acetic acid. Acetic acid oxidizers (group V) oxidize acetic acid to $H_2$ and $CO_2$ if the $H_2$ is removed at the same time by other processes. The homoactogens can reverse their action and produce other types of fatty acids than acetate if the concentration of acetate, hydrogen or ethanol is high.

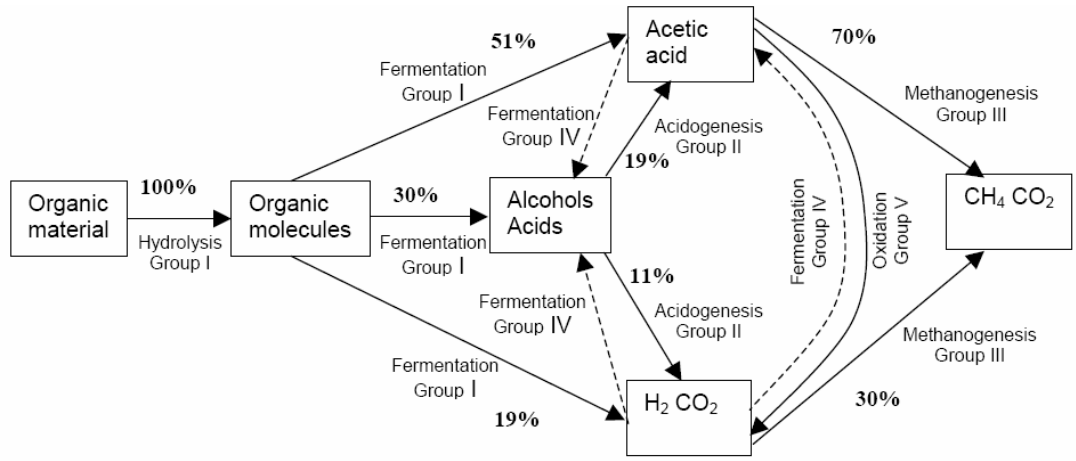


**Figure 2.1**. Schematic of the processes and microorganisms responsible for conversion of complex organic material to $CH_4$ and $CO_2$ Percentages indicate relative quantity of organic matter converted by the different processes (Poulsen, 2004)

*Step 1: Hydrolysis*

The first stage of anaerobic digestion is carried out by a mixture of fermentative bacteria, (also called acid formers), which hydrolyse the complex substrate and convert it to simpler soluble compounds. The first step in this degradation is enzymatic hydrolysis which occurs in the substrate solution, outside of the cells, by the action of exocellular enzymes produced by the bacteria cells. The hydrolysis results in the formation of sugars from carbohydrates, amino acids from proteins, and fatty acids from lipids. Some of these initial processes can be slow and often determine the overall rate of

anaerobic digestion process. The intermediate compounds produced are further broken down to soluble organic end products such as formate, acetate, propionate, butyrate, lactate, and ethanol as well as carbon dioxide gas (Gunaseelan, 1997).

*Step 2: Acetogenesis*

The products of the hydrolysis are further degraded by fermentative bacteria predominantly to volatile fatty acids (VFA's) and mainly acetate along with $CO_2$. Under anaerobic conditions, a fast growth of acetogenic bacteria also occurs. These are active in a wide temperature range of 3 to 70$^{o}$C, with an optimum at around 30$^{o}$C. They need an intensive contact with the substrates, meaning, that agitation of the substrate has positive effects. Hydrogen is produced as a bi-product from this stage (Gunaseelan, 1997).

*Step 3: Methane formation*

The third step involves the production of methane by methanogenic bacteria. They are living in colonies and are very specific for the temperature ranges, being classified as psychrophilic (<20°C), mesophilic (20-40°C) and thermophilic (> 40°C). They convert the intermediate products to methane and carbon dioxide via one of two routes. Approximately 70% of the methane is formed by acidotrophic methane bacteria from VFA's (and especially, acetic acid). The remaining 30% are obtained by the utilisation of hydrogen and carbon dioxide by hydrogenotrophic bacteria. This second pathway is important to the entire digestion process, since it is responsible for removing hydrogen and maintaining the low hydrogen partial pressure required for the production of acetate. If hydrogen concentration increases above a minimal level ($10^{-4}$ atm), the fermentative bacteria will change to the production of acids other than acetic, and the conversion to acetate by the acetogens will be reduced. Since the primary pathway for methane production is by cleavage of the acetate molecule, a decreased rate of biogas production will result (Vandevivere et al., 2002).

Because methanogenic bacteria are both sensitive and slow growing, it is important to maintain optimum environmental conditions, such as temperature and pH. They are also inhibited by excessive agitation. Methanogenic bacteria are strictly anaerobic, so the presence of molecular oxygen is toxic for them,

and even inorganic sources of oxygen, (e.g. nitrates) may inhibit their growth. Thus, the successful digester operation results in excluding oxygen from the reaction vessel. This is also important from the safety point of view, since the introduction of air could result in an explosive gas mixture (Dohanyos and Zabranska, 2001). But for desulfurisation it is accepted that a small bleed of oxygen is introduced at the top of the fermenter gas dome to enhance the growth of sulphur bacteria which produce elementary sulphur from hydrogen sulphide $H_2S$.

## 2.5. The bio-chemistry of Anaerobic Digestion

### 2.5.1. pH and buffer capacity

Most microorganisms grow best under neutral pH conditions; acid or alkaline media can adversely affect metabolism by altering the chemical equilibrium of enzymatic reactions, or by actually destroying the enzymes. The methanogenic bacteria are the most pH sensitive. If the pH decreases below 6, an inhibition of the methane-forming bacteria can be observed, as the volatile acids accumulate in the digester (Gerardi, 2003).

The equilibrium of carbon dioxide and bicarbonate ion with ammonium ion as a major cation, exert substantial resistance to pH change, known as the buffer capacity. In aqueous systems carbon dioxide is in equilibrium with carbonic acid, which dissociates to give hydrogen and bicarbonate ions. Anaerobic reactors also contain other weak acid-base systems, mainly ammonia and orthophosphoric and volatile acids, but the carbonic acid system remains the most important in determining pH. Both carbon dioxide and ammonia are products of the anaerobic digestion. The breakdown of organic acids produces carbon dioxide, which reacts with water to form carbonic acid. Ionisation of the carbonic acid produces bicarbonate and hydrogen ions (Equation 2).

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO^-_3 + H^+ \quad \text{..... (2)}$$

Volatile fatty acids decrease the buffering capacity of the bicarbonate ions (Equation 3):

$$RCOO\text{-}H + NH_4HCO_3 \rightarrow RCOO\text{-}NH_4 + H_2CO_3 \quad \text{..... (3)}$$

while the addition of ammonia will increase the bicarbonate by forming an

ammonium salt with bicarbonate taken from the $CO_2$ pool (Equation 4):

$$NH_3 + CO_2 + H_2O \rightarrow NH^+_4 + HCO_3 \quad \text{..... (4)}$$

The higher the concentration of bicarbonate in the solution, the greater the buffering capacity and resistance to changes in pH. However, a change in pH can occur, for example, if the feed rate is suddenly increased significantly and the system is overloaded. Since fermentative bacteria grow faster than the methanogens, acids will accumulate. Other effects, such as a sudden temperature change or introduction of a toxin, can also lead to poor conditions which manifest as low pH in the reactor contents.

There are two main operational strategies for correcting a low pH condition in a digester. The first approach is to stop the feed and allow the methanogenic population time to reduce the concentration of fatty acids and thus raise the pH to an acceptable level of at least 6.8. Once the pH returns to normal, the feeding can be resumed at a reduced level and than increased gradually as stability returns. The second method involves an addition of chemicals to directly raise the pH and provide additional buffer capacity. Reducing the feed rate in combination with chemical additives may be necessary in some cases. An advantage of the chemical neutralisation is that the pH can be adjusted immediately by addition of strong bases, bicarbonates, and carbonates to the liquid phase, or removal of carbon dioxide from the gas. If a strong base (such as NaOH or $NH_4OH$) or a carbonate salt (such as $Na_2CO_3$) is added, ionic equilibrium occurs very rapidly and carbon dioxide is removed from the gas space to form the required bicarbonate alkalinity (Capri and Marais, 1975).

Chemicals for pH control fall into two major categories: those that add bicarbonate alkalinity directly (bicarbonates), and those that trap carbon dioxide and convert it to bicarbonate (strong bases and carbonate salts). Control by chemicals, which trap carbon dioxide, require that their addition be in small steps to allow time for gas equilibrium to occur between each addition. The direct addition of bicarbonate, on the other hand, has no such effects on the system and thus can be done more precisely.

If lime ($Ca(OH)_2$) is added to a digester, it traps carbon dioxide and converts it

to bicarbonate. Consequently, when the bicarbonate concentration reaches 500 to 1,000 mg/L, the addition of more lime causes forming of insoluble calcium carbonate. This removes carbon dioxide from the gas space, but does not increase alkalinity. The drop in carbon dioxide partial pressure will cause the pH to rise rapidly, but since the alkalinity has not been increased, this pH is unstable so that as soon as biological activity increases the pH drops rapidly. Consequently, lime should only be added when the pH is below 6.5, and then only in sufficient quantity to raise the pH to about 6.8 (Chynoweth and Pullammanappallil, 1996).

These problems with calcium-containing chemicals suggest that better pH control can be obtained with sodium-containing chemicals especially with direct addition of sodium bicarbonate. However, the use of sodium should be done with caution, when the effluents are to be spread on to cropland. An alternative chemical for pH control, without any risk for the soil, is liquid (diluted) ammonia, which has been successfully tried in the anaerobic digestion of olive oil mill wastes (Georgakakis and Dalis, 1993).

### 2.5.2. Volatile acids

Observations of the effect of volatile fatty acid concentration on the micro-organisms in anaerobic reactors are complicated by the fact that the acids will also affect the pH. When the pH is held constant near neutrality, the volatile acids have no significant toxic effects upon methanogenic bacteria at concentrations up to 10,000 mg/L. Among these acids, inhibitory effects have been demonstrated only for propionate, and that only at relatively high concentrations of 1,000 mg/L or more (McCarty and McKinney, 1961).

### 2.5.3. Ammonia

Ammonia is rapidly formed in a digester (by the breakdown of proteins present in the waste) where it can act as a potent inhibitor of methanogenesis. A number of publications suggest that the free ammonia form ($NH_3$) rather than ammonium ($NH_4^+$) is the real inhibitor. This implies that the pH and temperature (by its effect on pH) will have a strong effect on the inhibitory concentration of ammonia by influencing the equilibrium. Thus ammonia toxicity thresholds are very sensitive to pH above 7. Free ammonia levels

should be maintained below 80 mg/l while ammonium ion can generally be tolerated up to 1,500 mg/L as ($NH_4^+$) with acclimation, stable operation has been demonstrated for ammonia nitrogen concentration up to 8,000 mg/L (Van Velsen, 1979). Higher nitrogen contents in the substrates slow down the process. The highest methane contents are obtained with C:N ratios between 13:1 and 28:1. In agricultural biogas plants, ammonia concentrations are of concern when protein rich co-substrate is digested such as slaughterhouse waste. A widespread sign of protein overloading - besides reduced methane formation – is an increase in VFA-concentrations and large amounts of foam.

#### 2.5.4. Sulphides

Sulphides are produced in anaerobic reactors by the reduction of sulphates present in the influent and by the degradation of proteins. If the concentration of soluble sulphides exceeds 200 mg/L, then the metabolic activity of methanogenic bacteria will be strongly inhibited, leading to the failure of the process (Lawrence and McCarty, 1967). Because heavy metals form highly insoluble precipitates with sulphide, the addition of a metal, such as iron, provides a simple means of reducing the soluble sulphide concentration.

#### 2.5.5. Heavy metals and toxins

Heavy metals are toxic to both major anaerobic populations at very low concentrations. Nonetheless, they need not cause a problem in anaerobic reactors because only *soluble* metals have an effect and their concentrations can be reduced to non-toxic values by precipitation with sulphides. If the naturally-occurring sulphides are not sufficient to prevent heavy metal toxicity, then they should be supplemented by an addition of ferrous sulphate. Other noxious substances such as antibiotics from feed or veterinary treatments or farm disinfectants can slow down the process.

### 2.6. Biodegradability and Potential Biogas Yield

The term "biodegradability" is usually expressed either as a percentage of the ultimate chemical oxygen demand (COD) removal or that of the destruction of volatile solids (VS) during anaerobic digestion; it varies considerably from waste to waste even if taken from similar sources. In most cases, the experimental determination of biodegradability is preferred to the use of

published values. It can be carried out by the batch bioassay method, Biochemical Methane Potential (Owen et al., 1979) based on the batch incubations of the substrates under standard conditions. To determine the refractory fraction of substrate which is not available for anaerobic digestion, it is assumed that the degradable portion is completely converted to $CH_4$ and $CO_2$ products over a very long reaction time; the remaining COD is deemed refractory.

In many cases, VS can be related to the COD value and thus used in predicting biogas production. However, the relationship between COD and VS is very empirical and varies varying considerably from waste to waste. For example, the COD/VS ratio for carbohydrate is about 1.1 whereas that for a lipid about 2.9 and for a protein 1.5. Therefore the prediction of biogas production is more precise when it is calculated from the mass balance in terms of COD (Vandevivere et al., 2002).

In practice, the methane potential in manure is assessed on the basis of the content of VS in the manure and empirical standards for the production of methane per kg of VS. This has been 290 L $CH_4$/kg of VS in pig manure and 210 litres of $CH_4$/kg of VS in cattle manure (Martinez and Burton, 2003). Ranges for typical gas yields for some other organic substrates are depicted in Table 2.2. The co-fermentation of cattle slurry with different amounts of fodder sugar beet silage resulted in extremely high biogas and methane yields, due to the high content of the easily fermentable organic matter. This substrate and other similar plant materials are ideal co-ferments for animal farms causing no additional hygienic risks.

**Table 2.2.** Typical biogas yields from various agricultural biomass (Martinez and Burton, 2003; Abdel-Hadi et al., 2002)

| Substrate | Range of biogas yield (L/kg VS) | Mean biogas yield (L/kg VS) |
|---|---|---|
| Pig manure | 340-550 | 450 |
| Cattle manure | 150-350 | 250 |
| Poultry manure | 310-620 | 460 |
| Horse manure | 200-350 | 250 |

**Table 2.2.** Typical biogas yields from various agricultural biomass (Martinez and Burton, 2003; Abdel-Hadi et al., 2002)

| Substrate | Range of biogas yield (L/kg VS) | Mean biogas yield (L/kg VS) |
|---|---|---|
| Sheep manure | 100-310 | 200 |
| Straw from cereals | 180-320 | 250 |
| Corn (maize) straw | 350-480 | 410 |
| Fodder sugar beets | 344-982 | 810 (thermophilic)<br>690 (mesophilic) |
| Grass | 280-550 | 410 |
| Vegetable residues | 300-400 | 350 |
| Sewage sludge | 310-640 | 450 |

## 2.7. The Effect of Physical Parameters on Anaerobic Digestion

### 2.7.1 Temperature

Methane is formed in nature over a wide temperature range from close to freezing in the arctic tundra, to 100°C such as in the steam of natural geysers. However, micro-organisms exhibit optimal growth and metabolic rates within a well-defined range of temperatures which is specific for each species (particularly the upper limit which depends on the thermostability of the protein molecules). Different bacteria will respond to changes in temperature in different ways. Consequently, a biomass developed in a reactor at one temperature is likely to have a different microbial composition to that a reactor operating at another temperature. It is known that a long time is required to adapt and stabilize the reactor's microbial flora, even when small changes had been made in its temperature. And methanogenic bacteria are more sensitive to changes in temperature than other organisms in AD digesters. This is due to the faster growth rates of the other groups, which can achieve substantial metabolism even at low temperatures. In technical applications three different temperature ranges are distinguished:

- psychrophilic temperature (or cryophilic) from 10°C to 20°C
- mesophilic temperature from 20° to 40°C

- thermophilic temperature from 40° to 60°C

A number of mesophilic and thermophilic anaerobic bacteria are described in literature but, so far, no anaerobic psychrophilic bacteria have been found with a relative temperature maximum below 20°C. The work of Zeemann et al. (1988) and Wellinger and Sutter (1988) rather suggest a slow adaptation of mesophilic bacteria to lower temperatures. The methanogenic bacteria seem to be ubiquitous in anaerobic environments and obviously do survive a wide temperature range. It is therefore not surprising to find that the change from mesophilic to thermophilic temperatures or vice versa is not a problem in animal waste digesters as long as the change occurs smoothly and slowly. However, it might take months before some mesophilic cultures are adapted to psychrophilic temperatures.

Once the adaptation to low temperatures is complete, the system reacts very well to stress situations. The ultimate gas yield of psychrophilic digestion is on average significantly lower than at mesophilic temperatures. Within practical time limits (up to 100 days) it was found that the degradation at 22°C of sewage sludge, cattle manure and swine manure takes twice as long as at 35°C.

On the other hand, there is less difference between mesophilic and thermophilic digestion. There is a faster degradation at higher temperatures (Maly and Fadrus, 1971; Baserga et al., 1995), but ultimate gas yields are similar (Beck and Abdel-Hadi et al., 2002). The main difference is the higher volumetric methane yield per day which can be reached with thermophilic digestion, thus allowing higher specific methane yields from a given volume of a biogas reactor. Thermophilic AD also offers other advantages over mesophilic digestion: increased rates of volumetric methane production per day, lower viscosity, less biomass formation, increased conversion rate of organic matter from waste to biogas, and more effective and faster pathogen inactivation (Dohanyos and Zabranska, 2001).
Anaerobic digestion will take place at usable rates across a broad temperature range of 15-65°C. The mesophilic range, from about 25-40°C is generally considered as optimum for heated digesters. Table 2.3 shows some effects of temperature in the mesophilic range. It is the rate than the extent of the

reactions that is affected by temperature, a lower digester temperature can be compensated for by a longer retention time (Chen, 1983). The data in Table 2.3 were obtained from a CSTR with a 10-12 day RT (retention time). Most of the small climatically heated Indian and Chinese digesters do run at less than 25°C for much of time, but with retention time of about 50 days. Similar amounts of gas per cow can be obtained as from a digester running at 35°C and 20 day RT (Hobson, 1990).

**Table 2.3.** The effects of temperature on anaerobic digestion of piggery wastes in CSTRs

| | Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 15 | 25 | 30 | 35 | 40 | 44 |
| Gas (L/kg TS) | 0 | 260 | 300 | 300 | 360 | 420 |
| Solids degradation (%) | 0 | - | 33 | 36 | 37 | 38 |

### 2.7.2. Hydraulic retention time

The hydraulic retention time (HRT) describes the average time the substrate remains in a digester. It is defined as the reactor working volume divided by the mean volume flowrate. If the HRT is too short the organic material will not be fully degraded resulting in low gas yields and possible inhibition of the process. Short retention time can also result in washout of the methanogenic bacteria if the retention time is shorter than their rate of multiplication. Accumulation of sludge or sand in the reactor or poor mixing efficiency can reduce the active reactor volume and thereby lower the HRT in an otherwise well-designed system. In a continuous-flow digester, the HRT has to be longer than the doubling time of the bacteria to prevent the wash-out problem. The minimal HRT is dependent on the type of material to be digested. The rate of degradation of the main organic compounds increase in the following order:

- cellulose
- hemicellulose
- proteins
- fat
- carbohydrates

As a result, the digestion of pig manure with its high fat content requires lower HRTs than cattle manure which contains comparably high cellulose and hemicellulose concentrations. Average HRTs for mesophilic digestion are:

- cattle manure 12 to 25 days

- cattle manure with straw bedding 15 to 35 days
- pig manure 10 to 20 days

The optimum choice of HRT is mostly dependent on the temperature and to some degree on the type of material being digested. The amount of data available for characterizing HRT as a function of temperature is, at present, limited. A general trend for the HRT-temperature relationship for reactors treating animal manure or biodegradable municipal waste is shown in Fig. 2.2 (solid line). Measured HRT for 20 biogas plants in operation (mainly Danish plants) treating various mixtures of animal manure and organic wastes from industry and households are shown for comparison. In general, HRT decreases with increasing temperature up to about 60°C. Increasing the temperature above 60°C results in an increase in optimum HRT. It is noted that the HRT-temperature relationship is still not well understood and in most cases the optimum HRT is found by fine-tuning the system during the initial stages of the operation (Wheatley, 1990).

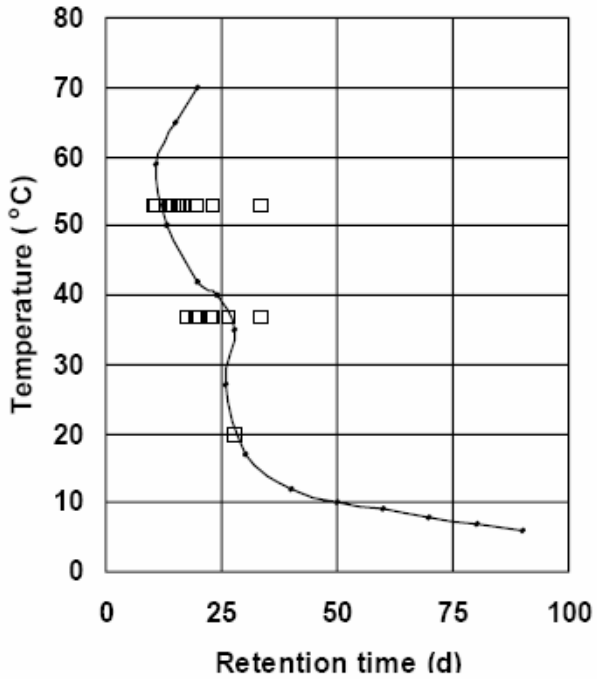


**Figure 2.2.** Relationship between hydraulic retention time and temperature in anaerobic digestors treating;
i) animal manure and biodegradable wastes (solid line),
ii) data for 20 Danish plants (box)
(Danish Energy Agency, 1996).

### 2.7.3. Organic loading rate

The organic loading rate (OLR) describes the amount of organic material (expressed as chemical oxygen demand-COD or volatile solids-VS) which is fed

daily per $m^3$ of digester working volume. For agricultural digesters it is usually defined as:

OLR ($kg/m^3.day$) = daily flow ($m^3/day$) x VS($kg/m^3$)/reactor volume ($m^3$) (5)
= VS/HRT

The optimal OLR for mesophilic reactors falls in the ranges:

- cattle manure 2.5 - 3.5 kg VS/m³.d
- cattle manure with co-substrates 5.0 - 7.0 kg VS/m³.d
- pig manure 3.0 - 3.5 kg VS/m³.d

If heavy loads of co-substrates are occasionally fed into the digester it is advisable to decrease the basic OLR to lower values than indicated.

If there is an excess of easy degradable nutrients fed to the fermentor, the process may be affected, because the first step, acidification, produces more end products than the second step can utilize. Such an overload leads to a drop in the pH-value and inhibition of the methanogenic activity. Some of the degradation steps will not yield energy unless their products are efficiently removed by the next group of bacteria.

## 2.8. Process Design

A well-designed anaerobic digester involves more than just a gas tight manure pit or a digestion vessel. A farm-type operation usually is built up of four elements:

- the production unit itself, which includes feed and product tanks as well as the anaerobic digester and control equipment;
- gas monitoring and upgrading equipment along with safety devices
- the gas storage facilities;
- the equipment for gas (and treated manure) utilisation.

A variety of AD systems have been developed for the digestion of a wide range of organic residues but there is no single design which can digest all the organic waste fractions in an optimal way. The main design criteria are the operation mode (batch-fed, semi-batch fed and continuous-flow systems), substrate

characteristics, mixing systems and the use of pre-treatment options (Gunaseelan, 1997).

### 2.8.1 Operation type

*Batch systems*

In batch-systems, the fresh substrate is fed together with an inoculum (approx. 10%) of digested sludge from the former batch into a reaction vessel. During the first couple of days of the cycle, the material can be aerated in order to increase the batch temperature. Alternatively the vessel contents are heated from external sources, possibly using waste heat from a related CHP (combined heat and power) process. During the following three to four weeks the substrate is anaerobically degraded, at first with an increasing daily gas production. After reaching a maximum (after approximately 10 to 14 days), depending on the microbial availability of the nutrients in the waste material, methane production decreases to reach a steady rate of about half maximum production.

To compensate the unsteady gas formation at least three to four batch digesters ought to be operated together with filling at different times. This system is mainly used for the digestion of fibrous substrates with limited microbial availability such as straw-rich solid waste. In order to maintain digestion temperature (and to wet the solid waste), a part of the clarified liquid in the digester is drawn off, pumped through a heat exchanger and recycled where it is sprayed on top of the substrate (Gunaseelan, 1997).

The main attraction of batch processing is the simplicity and low cost implied and indeed, this is a common type to be found throughout parts of Asia. Potentially, the system also allows for changes in conditions to accommodate the progress of the process and the dominant microbial activity at any stage. In reality, this is rarely done owing to the difficulty in monitoring the process yet it remains an option for operators with a great deal of experience with AD.

*Semi-batch systems*

The dividing line between batch and semi-batch (or semi-continuous) is not always clear as a total emptying of a reactor is not necessarily carried out at regular intervals marking the end of a cycle. Not uncommonly, quantities of

waste are periodically added and removed to a digester leading to a *de facto* semi-continuous system. One system that uses this approach is the accumulation continuous-flow (ACF). These are batch-fed processes where the reactor serves at the same time as the manure storage pit. The fresh manure flows into the digester as it is produced from the farm. The digested manure is removed occasionally when it is needed for fertilisation. In times when no fertilizer is needed (winter) the full digester overflows into a holding tank which is often covered by a rubber membrane serving as a gas storage. The system was originally designed for farms which had to increase their manure storage capacity.

*Continuous systems*

The most common type of medium and large-scale AD system in Europe is now the continuous-flow tank reactor. The raw waste is fed regularly into a digester, displacing an equal volume of digested material. The working volume in the digester remains constant. The vessel content is homogenized regularly. Most of the smaller systems are fed once or twice a day, but the frequency of input is increased with the use of easily fermentable substrates. The larger digesters are operated closer to true continuous with feeding intervals of less than one hour. However, even a single daily addition will still approximate to a continuous process in a system with a long residence time running to 10-30 days; the consequence is steady conditions within the vessel which is the key requirement of any steady state system.

### 2.8.2 Substrate composition

The design of a digester is strongly influenced by the make up of the substrate fed to it, i.e. the composition, homogeneity and the dry matter content of the waste. Wastewater from food factories is low in total and suspended solids and needs to be handled in high-rate digesters such as up-flow anaerobic sludge blankets (UASB) or anaerobic packed-bed filters (AF). The idea behind high-rate systems is to increase the biomass (bacteria) in the digester to maintain a high degradation rate. This is achieved in packed bed reactors by offering a large surface where the bacterial colonies can attach. The alternative UASB design make use of the tendency that anaerobic bacteria tend to develop small clumps (granules) which can easily be settled out an retained in a similar way to the activated sludge process used for municipal waste water treatment

works.

For agricultural substrates and wastes, rich in solid material, the high-rate reactors are not suitable: granule formation is hindered and packed beds will clog immediately. Livestock manures which are the predominant waste material in agricultural AD are heterogeneous materials with total solid concentrations varying between 2% and 10%. The required digester is therefore a simple continuously stirred tank reactor (CSTR) with the provision for co-digestion, often combined with an influent holding tank. Blending of several waste materials to achieve the optimal composition is an important feature of co-digestion; it also allows for a more consistent feed which itself is an aid to a better run process.

### 2.8.3 Mixing

The substrate in an agricultural anaerobic digester is usually mixed intermittently ranging from several times a day to several times per hour. The power applied for mixing varies in function of size and form of the digester, the composition of the substrate and the type of agitator being used. It covers the range from 10 to 100 $Wh/m^3$ per day; usually a mixing value of more than 30 $Wh/m^3.d$ is recommended. There are several reasons for mixing:

- incorporation of the fresh feed with digested substrate;
- to break up large particles in the substrate;
- exchange and distribution of heat to warm up the substrate and to achieve an even temperature distribution throughout the digester;
- avoid or disrupt surface scum layers and sediment formation;
- ensure an even release of biogas bubbles trapped in the substrate.

If the substrate is not mixed, it tends to separate into a sediment and a scum layer; this is especially a problem with manure from layer houses and blends including kitchen wastes and grass clippings. The scum is particularly difficult to remove after it has dried out through continuous gas production. As long as the particles floating to the top are incorporated into the liquid phase, they remain wet and soft and can easily be removed or reintegrated into the substrate volume.

In larger digesters usually two to three mixers are applied in different depths of

the digester. In small-size family plants only one stirrer is installed for economical reasons. It is therefore important that it is adjustable for the mixing of a possible scum and sediment formation. For all the mixing purposes mentioned, the speed of rotation is not necessarily important. Large, slow-rotating mixers can run as low as 15-50 rpm.

Hydraulic and pneumatic stirrers are restricted to dilute substrates such as pig manure with little potential for scum formation. A horizontal paddle stirrer, on the other hand, is especially well designed for straw-rich cattle manure. However, it can also handle more dilute substrates.

The most widely used stirrers are the propeller mixers. They allow a very flexible application with respect to the substrate composition and the form and size of the digester. The only limit is the digester temperature which can be a problem for submerged motors; temperatures above 40$^{o}$C require special a cooling provision.

## 2.9. Digestion Systems for Solid Manure

Systems for the digestion of solid waste material have been less successful than for the liquid systems owing to problems linked to methane losses and the necessary batch process implied. Nonetheless, in the Czech Republic four biogas plants for the treatment of solid cattle manure were constructed during the eighties and nineties (Pastorek and Kara, 1998) with typical operational parameters given in Table 2.4. The quantity of solid manure produced per livestock unit (LU) was 35-40 kg/d with 20-25% of dry solids, and depends on the amount of straw used for bedding. In the fresh manure the ratio of faeces to straw was about 1:1 in terms of dry solids. Analysis of the fresh manure revealed a proportion of about 90% total volatile solids.

The fermentation unit consists of a special cylindrical cage, which is placed on the concrete surface in a typical the plant configuration. Manure is collected directly on the platform and from there it is filled to the cage. The filling time should be not longer than 20 days to avoid excess oxidation of organic mater but some activity is desirable to preheat the substrate. The temperature of the manure increases to 50-60°C during which time about 10% of organic matter is degraded. After heating it up to the desired temperature, the full cage is then

covered by an insulated gas tight cover. Residual oxygen is removed by the action of facultative bacteria and conditions then become anaerobic. During the next 2-4 days biogas production starts.

The maximum biogas production rate is achieved during 3-5 days when completely anaerobic conditions are achieved with fermentation temperatures of 35-42°C. The rate of biogas production then slowly decreases, and after 30 days of operation it represents only 20-30% of the maximum daily value. During this time the temperature drops down to around 30°C. When maturation of manure is finished, the fermentation unit is disconnected from the biogas system and the gas tight cover is removed from the "mature" cage and transferred to a "fresh" one. Depending on the daily amount of manure produced and on the rate of fermentation it is necessary to operate 5-8 units to get a continuous methane production.

**Table 2.4.** Operational parameters of four Czech biogas plants for anaerobic fermentation of solid cattle manure (Pastorek and Kara, 1998)

| | Hustope.e | Jindåichov | Výšovice | Agroklas Slavkov |
|---|---|---|---|---|
| Quantity of manure (t/d) | 44 | 21 | 11 | 2,5-4 |
| Reactor volume ($m^3$) | 169 | 85 | 110 | 30 |
| Number of reactors | 8 | 6 | 6 | 6 |
| Retention time (d) | 32 | 28 | 30 | 30 |
| Fermentation temperature (°C) | 35-40 | 35-40 | 35-40 | 35-40 |
| Construction material | steel | steel | steel | steel |
| Total biogas production ($m^3$/d) | 1000-1200 | 600 | 350-400 | 90-125 |
| Investment cost (1000 €) | ~240 | ~153 | ~97 | ~156 |
| Start of operation | 1986 | 1989 | 1987 | 1998 |

Specific biogas production varies from 0.8 to 1.6 $m^3$ per LU per day, with a mean value of 1.2 and methane concentration of 60%. Performance depends on the amount of straw in the manure and on the time of fermentation. Biogas is stored in a gas-holder and is used in cogeneration units or for heating. From 1 tone of manure with 20 % TSS and 50 % of straw, 20 to 25 $m^3$ of biogas can be produced with a total energy value of 100 to 125 kWh. By the utilization of

this biogas in cogeneration units 35 to 40 kWh of electricity and 55 to 75 kWh of heat energy can be generated (Dohanyos and Zabranska, 2001).

*Co-processing and centralized facilities*

The limitations of a small farm based digester can be overcome in larger operations that include co-processing with other organic materials enabling:

- more efficient digestion of some biomass materials;
- easier handling of blended wastes ;
- improved nutrient balance and utilization;
- additional income by charging gate fees to take external wastes.

These benefits can be greatly increased with the large scale production approach of centralized plants serving several farms along with the local community and food industry as well. This approach has been followed in several parts of Europe, in particular in Denmark where annual biogas production from such installations exceeded 2 million $m^3$ in 1994. The concept of centralized biogas plants in Denmark was partly a reaction to relatively disappointing results obtained with small scale, single-farm plants in the 1980's and more stringent environmental legislation concerning storage and land application of animal manure. Environmental and agricultural benefits include: savings for farmers, improved fertilisation efficiency, less greenhouse gas emission, and cheap, environmentally sound waste recycling. The main disadvantage of centralized plants (compared to single-farm plants) is the cost of manure transportation and the possible risk for spreading noxious substances originating from the industrial or municipal wastes used. The cost of transportation to and from the farms to the processing units can add up to 50% of the total operating costs of the plants. Gas purification may also be needed for the (removal of sulphur.

## 2.10. Environmental Impacts of Anaerobic Digestion

The environmental impacts of on-farm anaerobic digestion depend on the manure management system that the digester amends or replaces as well as the actual use of the biogas produced. Typically, the anaerobic digestion of dairy manure followed by flaring of biogas, combustion of biogas for electricity,

or production and use of bio-methane as fuel can provide a number of direct environmental benefits. These include:

- Reduction of greenhouse gases
- Destruction of pathogens
- Potential reduction of VOC emissions
- Odor control
- Improved water quality

One potentially negative environmental impact of anaerobic digesters that combust the biogas is the creation of nitrogen oxides ($NO_x$), which are regulated air pollutants and an ozone precursor. Nitrogen oxides are created by combustion of fuel with air. Combustion of dairy biogas or any other methane containing gas (whether in a flare, reciprocating or gas turbine engine, or a boiler) will emit $NO_x$. The emission rate varies but is generally lowest for properly engineered flares and highest for rich burn reciprocating (piston) engines. $NO_x$ emissions are controlled by using lean burn engines, catalytic controls or microturbines. The latter two methods are fouled by the high sulfur content of biogas, and the $H_2S$ must be scrubbed to prevent the swift corrosion of these devices (Krich et al., 2005).

### 2.10.1 Reduction of greenhouse gases

The atmospheric concentration of $CH_4$ and $N_2O$ is increasing at annual rates of 0.3% and 1%, respectively. Within the EU, agriculture is estimated to contribute nearly half of the $CH_4$ emissions and more than half of the $N_2O$ emissions. The main sources of $CH_4$ are animal digestion and stores with animal manure, while $N_2O$ mainly originates from the turnover of mineral fertilizers and animal manure applied to arable soils, and from the decomposition of crop residues.

Anaerobic digestion of liquid manure (slurry) and organic waste can reduce the emission of both gases, due to the removal of organic matter. Furthermore, energy from biogas production will substitute fossil fuels, thereby reducing new $CO_2$ emissions. Fermentation of animal manure and waste in biogas digesters will reduce the level of volatile solids. Since VS drives the microbial processes

that may lead to $CH_4$ production during anaerobic storage, the removal of VS in biogas digesters prior to storage will also reduce the potential for $CH_4$ emissions into the atmosphere.

Sommer and Hutchings (2001) have developed a model designed to estimate the total reduction in greenhouse gas emissions, resulting from co-digestion of animal slurry and organic waste in biogas plants. The fundamental principle of the model is to estimate the reduction of VS in slurry and organic waste during fermentation in biogas digesters and stores. Volatile solids are used as the main driving variable to predict $CH_4$ and $N_2O$ emissions during digestion, storage and field application of untreated and digested manure and waste. Methane emissions from slurry channels inside animal houses and during storage are further related to temperature, while $N_2O$ emissions from field-applied slurry are related to nitrogen input, soil moisture and the application methods used.

Predictions from this model indicate that digestion of pig manure can reduce greenhouse gas emissions from 1.4 kg of $CO_2$ (per kg of VS in the manure), to between 0.4 and 0.8 kg. Digestion of cattle manure reduced emissions of greenhouse gases from 1.3 kg (per kg of VS) to between 0.2 and 1.0 kg.

### 2.10.2. Destruction of pathogens

Bicudo et al. (1999) observed that following treatment in an anaerobic lagoon, there is only a small reduction in the number of bacterial indicators (about 1 log unit) and a high concentration of microorganisms remain in the final effluent (e.g., 105 per 100 mL for both Faecal *coliforms* and Fecal *streptococci* and 104 per 100 mL for *Clostridia*). A combination of the abundance of nutrients, the low light intensity and the reduced competition between bacterial, phytoplankton and zooplankton species within the lagoons create conditions for the growth of such bacteria. Humenik and Overcash (1976) concluded from laboratory experiments that the time needed for bacteria to double was between 0.5 to 1 hour and that the microbial population maintained a steady viability throughout the duration of the experiments. Possibly mass transfer and direct liberation of breakdown products from the sludge helped to maintain a large viable population in the reactor supernatant.

Mesophilic and especially thermophilic anaerobic digestion will ensure a more effective reduction of pathogens by means of sanitation. The most important parameters related to sanitation are temperature and guaranteed minimum retention time. With mesophilic fermentation at 30°C pathogens will be reduced significantly within a period of about 14 days, which is the process time chosen for mesophilic reactors; but one can not guarantee, that the treatment will always lead to a significant inactivation of all pathogens. Martens et al. (1998) reported that at 30°C Faecal *Streptococci* were reduced by only 1-2 $\log_{10}$ units: the treatment has an effect, but it will not always lead to a complete hygienisation of the slurry. It is therefore recommended that mesophilic fermentation should be carried out at temperatures towards the upper end of the normal range (35-40°C).

In thermophilic bioreactors the slurry is normally fermented at temperatures above 50°C. The process temperature is the decisive element: the higher the temperature, the faster the inactivation of microorganisms. A study by Martens et al. (1998) indicated that Faecal *Streptococci*, Salmonella Senftenberg and Enteritis, Bovine Enterovirus Equine Rhinovirus and Faecal *Streptococci* were inactivated with more than four $\log_{10}$ units within 24 hours. Bovine Parvovirus was reduced from 7-8 $\log_{10}$ units to less than 3.5 within 35 days. Thermophilic anaerobic digestion, which traditionally has a reactor retention time of 10-12 days, has therefore proved to be a very efficient treatment for the reduction of pathogens (Bendixen, 1994).

### 2.10.3. Reduced volatile organic compound emissions

Volatile organic compounds (VOC), in combination with $NO_x$ and sunlight, produce ozone, the primary element in smog and a criteria air pollutant. Thus VOCs are an ozone precursor and are regulated by law. VOCs are an intermediate product generated by methanogenic bacteria during the transformation of manure into biogas. It is expected that the total volume of VOCs generated is related to the total volume of $CH_4$ produced, but the more effective the methanogenic decomposition, the lower the VOCs as a percentage of the biogas. VOCs are created by enteric fermentation (the digestion process of the cow) and released primarily through the breath of the cow. They are also produced by the anaerobic decomposition of manure. A well designed and managed anaerobic digester may reduce VOCs by more completely

transforming them into $CH_4$. Some fraction of the remaining VOCs in the biogas should be eliminated through the combustion of the biogas.

### 2.10.4. Increased nitrogen oxide emissions

When biogas or any fuel is combusted in an internal combustion engine it produces $NO_x$, a criteria air pollutant as well as a precursor to ozone and smog. For reciprocating engines the main $NO_x$ production route is thermal, and is strongly temperature dependent. Internal combustion engines can produce a significant amount of $NO_x$. Maximum $NO_x$ formation occurs when the fuel mixture is slightly lean, i.e. when there is not quite enough oxygen to burn all the fuel. Lean-burn engines typically have lower $NO_x$ formation than stoichiometric or rich-burn engines because more air dilutes the combustion gases, keeping peak flame temperature lower. Gas turbines and microturbines also produce a very low level of $NO_x$ because peak flame temperatures are low compared to reciprocating engines. A system to flare gas, if properly engineered, will generate a substantially lower level of $NO_x$ than an uncontrolled reciprocating engine (Krich et al., 2005).

Dairy anaerobic digesters that burn biogas for electricity typically use reciprocating internal combustion engines; microturbines have not been used successfully because impurities in the biogas corrode the engines. When there is enough biogas to support a lean-burn engine, $NO_x$ can be kept relatively low.

There are several catalytic conversion technologies for reducing $NO_x$ emissions which can be used on rich- and lean-burn engines that use natural gas, but the impurities in dairy biogas will substantially shorten the life of the catalytic $NO_x$ controls. If the $H_2S$ content of the biogas is reduced to a very low level before introduction to the engine, the emissions from the scrubbed dairy biogas will not degrade catalytic controls or microturbines as quickly. If biogas is upgraded to biomethane, the selective catalytic reduction technologies used for natural gas engines can be used to keep $NO_x$ formation at acceptable levels. Biomethane will not corrode microturbines and electricity generated in microturbines from biomethane has a very low accompanying NO formation.

### 2.10.5. Control of Unpleasant Odors

Most of the approximately 100 anaerobic digesters processing animal manure in the USA were built to address odor complaints from neighbors. Most of the odor problem comes from $H_2S$, VOC, and ammonia ($NH_3$-N) emissions from dairy manure. While hard to measure objectively, these odors are perceived as a serious environmental problem by residents in proximity to dairy farms. Fortunately, anaerobic digestion is a good method for controlling these odors, particularly if used in conjunction with a system that will scrub the $H_2S$ from the biogas.

### 2.10.6. Improved water quality

An anaerobic digester will have minimal effect on the total nutrient content of the digested manure. However, the chemical form of some of the nutrients will be changed. A digester decomposes organic materials, converting approximately half or more of the organic nitrogen (org-N) into $NH_3$-N. Some phosphorus (P) and potassium (K) are released into solution by decomposing material. A minimal amount of the P and K will settle as sludge in plug flow and complete mix digesters. However 30% to 40% of the P and K are retained in covered-lagoon digesters in the accumulated sludge. Dissolved and suspended nutrients are of lesser concern as they will flow through the digester.

The anaerobic digestion process is an effective way to reduce high BOD in the effluent. Biological oxygen demand is a measure of the amount of oxygen used by microorganisms in the biochemical oxidation of organic matter; BOD concentrations in dairy wastewater are often 25 to 40 times greater than those in domestic wastewater. Anaerobic processes can remove 70% to 90% of the BOD in high-strength wastewater at a lower cost, in terms of both land and energy inputs, than aerated systems.

### 2.10.7. Motivation for realizing environmental benefits on dairy farms

Many of the environmental benefits discussed above also can be realized by capturing the biogas produced at a dairy and flaring it. In fact, flaring typically produces less $NO_x$ than combustion of the biogas for generating electricity.
Whether used to generate electricity, or upgraded to biomethane and used for vehicular or engine fuel, biogas is a renewable energy product. Like other renewable energy sources, such as solar and wind-generated power, biogas can

be substituted for greenhouse-gas-emitting fossil fuels, producing a net decrease in GHG emissions. On those dairy farms where manure is stored under anaerobic conditions, i.e., where it is not stored in piles that decompose aerobically over time, there is an added benefit. Using biogas as a fuel results in the reduction of $CH_4$ emissions that would otherwise be released into the atmosphere, e.g., through storage in uncovered lagoons. However, without financial or regulatory motivations, farmers will have little motivation to capture and use dairy biogas (Krich et.al., 2005).

## 2.11. Two-phase Anaerobic Digestion

Two-phase anaerobic digestion processes have been extensively studied and in a few cases also applied in practice. In such processes, two bioreactors are operated in series, with the initial reactor operated at a much shorter hydraulic retention time (HRT), as little as one tenth or less of the HRT used in a typical single-stage reactor. The second reactor is operated at typical anaerobic digestion HRT, generally over 15 days. Thus, the first reactor is much smaller than the second reactor, in which nearly all conversion to methane occurs.

The essential concept of two-phase digestion is to separate the two main microbiological processes of anaerobic digestion, acidogenesis (production of volatile fatty acids, $H_2$ and $CO_2$) and methanogenesis (production of methane from the fatty acids, $H_2$ and $CO_2$). These two reactions are carried out by distinct bacterial species and populations, and the two-phase anaerobic digestion process is based on the concept that the operational characteristics of each stage can be adjusted to favor the bacteria: very short HRTs and solids retention times (SRTs), with resulting organic-acid formation and low pH in the first stage; longer HRTs and conversion of the acids to methane (and $CO_2$) at neutral pH in the second. Low sludge ages in the first-phase reactor wash out slower-growing methanogenic organisms while the acidifiying organisms remain. With efficient acidification of complex substrate in the first-phase reactor, there is insufficient substrate to support a significant population of acidifiying organisms in the second-phase reactor. Thus the aim is to provide an optimal environment for each of these distinct microbial populations, thus allowing an overall faster reaction (e.g., reducing the reactor size of the combined first and second stage compared to conventional systems). Two-phase digestion is also claimed to result in a greater overall yield of methane,

as a larger fraction of the substrates will be metabolized and converted to biogas, presumably by action of the more vigorous acidogenic bacteria.

A detailed research was undertaken by Ghosh (1987) to study the relative efficacies of two-phase and single-stage anaerobic digestion processes under the same organic loading rate, fermentation temperature (mesophilic and thermophilic), and culture-dilution rates, and to ascertain the effects of three important control variables (pH, temperature, and HRT) on such response variables as gas production, volatile acids production, and major organic components reduction during separate acidogenic and methanogenic digestions. Ghosh (1987) demonstrated that two-phase anaerobic digestion stabilized municipal sludge at higher efficiencies and rates than those achieved by conventional single-stage CSTR digestion at mesophilic as well as thermophilic temperatures, and at several levels of HRT, loading rate, and feed VS concentration. The analysis indicated that the two-phase process is less vulnerable to upsets due to unbalances acidogenic-methanogenic fermentation and the consequent accumulation of acids and prevalence of acidic pH. In contrast, in single-stage CSTR digestion, the VA production rate is higher than the VA conversion rate at lower HRT's and higher loading rates; thus, reliable system operation can be expected only at high HRT's, where the rates of acids production and conversion are balanced. Almost all two-phase systems performed better than the single-stage process in terms of gas and methane yields and production rates. The methane phase digester of the two-phase generated significantly higher ammonium bicarbonate alkalinity and buffer capacity than the single-stage process; this buffer-capacity differential increased as the system HRT was decreased. Two-phase digestion was, therefore, more stable than single-stage digestion, and this relatively stability increased at the shorter HRT's.

The different microbial groups involved in anaerobic digestion do not have the same requirements regarding reactor conditions. The growth rate and pH optima are different for acidogenic and methanogenic organisms. In a one-phase digester, the pH and organic loading rate are adjusted to suit the slow-growing methanogenic organisms at the expense of the relatively fast-growing acidogens and the process efficiency as a whole (Massey and Pohland, 1978). The one-phase process is a compromise, and the conditions for the different

microbial groups can better be optimized if the process is divided into two separate stages in separate reactors. The hydrolysis and the conversion into acids then take place in one reactor and the effluent from this step is used as a feed stream to the methane-producing reactor. Instability or failure of single-phase anaerobic digesters due to the imbalance between the rates of production and consumption of volatile fatty acids has been widely reported for a variety of wastewaters (Cohen et al., 1979; Ghosh, 1991; Fox and Pohland, 1994). Therefore, it has been proposed that the two phases be physically separated by using two reactors in series; one for VFA production and another for methane production (Pohland and Ghosh, 1971). The two-phase approach has been successfully applied in several cases for the digestion of organic fraction of municipal wastes and wastewaters (Chanakya et al., 1992; Ghosh, 1991; Pohland and Ghosh, 1971; Dinopoulou et al., 1988). One important application is when the substrate is in a solid form and the first phase includes both liquefaction and acidification. A conventional one-phase digester can contain up to 95% water. It is energetically and economically wasteful to treat solid waste (20-40% dry matter) in liquid phase slurry digesters. The less liquid that is added, the less energy is required for heating, storing and spreading (Hawkes and Hawkes, 1987). Studies have been carried out in which straw, manure and other types of agricultural waste are enclosed in a simple tank and which is used as a percolating filter with leachate recirculation. The liquid containing the dissolved organic compounds is then transferred to a methanogenic reactor (Weiland, 1993).

In order to optimize the two phases of anaerobic digestion, it is necessary to engineer the operation of the first methanogenesis reactor towards those acids which are preferable substrates for methanogenesis. It is now recognized the methanogens can use directly only acetic acid, formic acid, and hydrogen, while butyric and propionic acids need to be converted to the later compounds by a special group of microorganisms, the obligatory hydrogen producing acetogenesis bacteria. The rate of butyric acid removal has been found to be higher than that of the rest of the VFA.
Two variations of the two-phase idea are shown in Figure 2.3 (Fox and Pohland, 1994). Some of the advantages and disadvantages of the two-phase approach are summarized in Table 2.5 (Fox and Pohland, 1994; Ghosh et al., 1975; Pohland and Ghosh, 1971). Of particular significance among the advantages is

that the methanogens in the second phase could be effectively protected by close monitoring of the effluent from the acidogenic phase, and potential problems could be eliminated before the sensitive methanogens were subjected to stress.

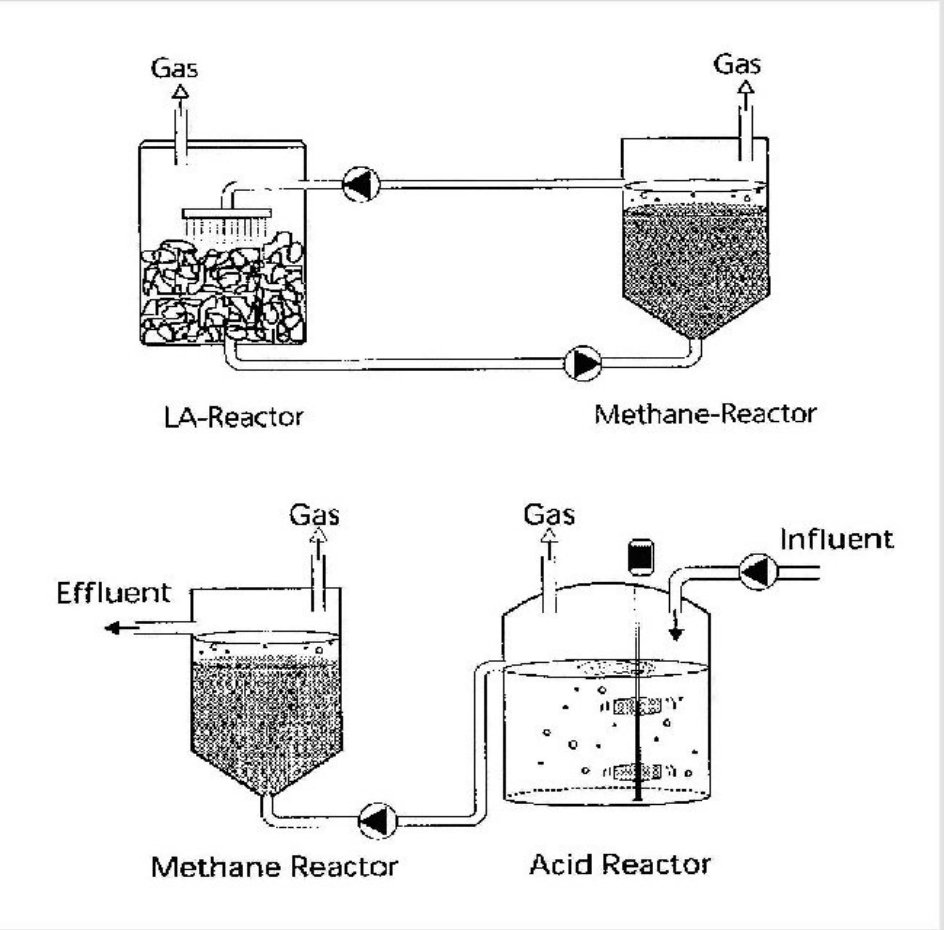


**Figure 2.3**. Variations of two-phase approach
(Fox and Pohland, 1994) (LA: liquefaction/acidification)

Applications of two-phase AD have occurred in the biogasification of: wastewater treatment sludge, organic fractions of municipal solid wastes, industrial wastes and sludge, olive mill solid waste and olive pomace, grass, coffee pulp juice, food waste, cane–molasses alcohol stillage, spent tea leaves , brewery wastewater , dairy wastewater, abattoir wastes as well as some studies focusing on improving reactor design, control and operational parameters.

**Table. 2.5.** Advantages and disadvantages of two-phase processes (Björnsson, 2000)

| Advantages of two-phase processes | Disadvantages of two-phase processes |
|---|---|
| ✓ Optimized process conditions for the individual groups of microorganisms | ✓ Demand for more elaborate process design and monitoring |
| ✓ Allows better monitoring of the liquid reaching the methanogens | ✓ Loss of inert-species hydrogen transfer |
| ✓ Methanogens protected from overloaded and toxic shocks | ✓ Loss of potential methane formation from $H_2$ and $CO_2$ from acidogenic step |
| ✓ Process can be smaller and more cost efficient | |

The main advantage of the two-stage system is the greater biological stability it affords for very rapidly degradable wastes like fruits and vegetables (Pavan et al., 1999). In the practice, however, the greater reliability of two-stage systems has indeed at times been observed, at least in discontinuously-fed laboratory set-ups. For example, Pavan et al. (1999) compared the performances of the one- and two-stage systems, using pilot complete mix reactors fed with very rapidly hydrolysable biowastes from fruit and vegetable markets. While the one-stage system failed at 3.3 kg VS/$m^3$.d, the performance of the two-stage plant remained stable at an overall system OLR of 7 kg VS/$m^3$.d. This departure from theoretical predictions can be explained by the fact that actually applied OLR vary a great deal with time and space due to the heterogeneity of wastes and due to the discontinuous working of the feeding pump (feeding occurred only four times daily in the Pavan's study).

The two-stage process was demonstrated by Liu et al. (2006) as an optimal way which combined hydrogen (1st stage) and methane (2nd stage) production in this study. The short HRT in the first stage (2 d) resulted in effective separation of hydrogen production from methane production (15 d), without the need of external additions. Experimental results demonstrate that two-stage process in this study worked very well. The stable hydrogen production yield was 43mL $H_2$/g $VS_{added}$ or 250mL $H_2$/g $VS_{removed}$. It was higher than 165mL $H_2$/g $VS_{removed}$ which was also produced from household solid waste at 37°C by Valdez-Vazquez et al. (2005). It fell in the hydrogen potential range (26.3–96mL $H_2$/g VS added) of HSW (household solid waste) reported by Okamoto et al. (2000). It was shown that, two-stage process generated 7500mL $CH_4$/d (or 500mL $CH_4$/g VS), which was 21% higher than the methane (6200mL $CH_4$/d or

413mL $CH_4$/g VS) from one-stage process. This was consistent with VFA data. Total VFA value in the second-stage process (1.8mM) was much lower than that of the one-stage process (3.5 mM). It shows that more VFAs were converted to methane in two-stage process. It shall be also noticed that HRT was 17 days in total for two-stage process while 15 days for one-stage process. Mata-Alverez et al. (1993) found 510mL $CH_4$/g VS was achieved in two-stage process for HSW fermentation while 428mL $CH_4$/g VS in one-stage process, resulting in 19% methane increase but without hydrogen production.

Conventional one-phase slurry digestion is not an effective system for wastes containing high solids (>10%), since they require the manure to be capable of being pumped which in itself necessitates a concentration below 10% solids. This, in turn, results in a significant increase in fluid and digester volume which results in increased capital and operating costs. Although most animal wastes are produced as slurry, the housing methods, bedding, and collection methods used produce a material of much higher solids content. For example, cattle housed in sheds and bedded on straw produce a farmyard manure of approximately 26% solids. The significance of high solid content of animal manure in relation to the performance of AD in terms of reactor volumes, pumping, handling, mixing, and clogging are emphasized in several studies. The associated investment costs for large-size reactors, as well as the heating, handling, dewatering, and disposal of the digested residue decrease the benefits of conventional slurry anaerobic digestion of high solids containing wastes.

One relevant feature of the two-phase approach is that when a high solid containing waste is introduced to the first phase it is liquefied along with acidification. This translates into less liquid addition and, thus, less energy requirements for heating, storing, and spreading for two-phase AD systems. The results of several studies have clearly demonstrated the applicability and efficiency of two-phase AD for high solids containing wastes.

Even though several aspects of two-phase configuration including liquefaction might be very significant for efficient AD of dairy manure, its application has been limited to screened dairy manure only (Lo and Liao, 1985). Burke (2001) also pointed out the fact that phased digestion has not been applied to dairy

waste. In recognition of this fact and in support of its needed application to high solids waste, the objective of this study was to exploit the advantages of two-phase AD for unscreened dairy manure.

Serious disadvantages of the two-phase digestion are the possible loss of syntrophic interspecies hydrogen transfer and the loss of methane potential by $H_2$ and $CO_2$ production in the acidogenic phase. Fox and Pohland (1994) found that the two-phase system was suitable when the substrate contained carbohydrates and proteins, but when treating fatty acids and aromatics the one-phase degradation was preferable due to the important syntrophic relations between acetogens and hydrogenotrophic methanogens during anaerobic oxidation. It has been suggested that the performance of the process could be improved by directing the hydrolysis towards ethanol and lactate formation, thus providing the syntrophic bacteria with more potentially available energy (Pipyn and Verstraete, 1981).

Another disadvantage of two-phased systems was accepted as their operational difficulty, since the operation of two different reactors is more complicated than one reactor. However, the anaerobic baffled reactor (ABR) is one of the effective options to deal with this disadvantage. ABR consists of a simple rectangular tank divided into several compartments by means of movable baffles. The liquid flows upwards and downwards between the baffles. On its upward passage, the waste flows through an anaerobic sludge blanket. As waste leaves each sludge blanket chamber at the top, it is directed by a baffle to the bottom of the next chamber (Ritmann and McCarty, 2001). This type of reactor appears to be able to treat manure with high solid content, such as animal manure. The design offers the advantages of reactors in series, i.e. high efficiency, low bypass, resistance to shock loading together with high biomass retention capacity. Furthermore, a two-phase reactor configuration can easily be set-up by using a ABR without constructing two separate reactors. This offsets the commonly stated disadvantage of two-phase reactors in terms of construction and operation of two separate reactors.

## 2.12. Molecular Ecology of Anaerobic Reactor Systems

Anaerobic reactor systems are essential for the treatment of solid and liquid wastes and constitute a core facility in many waste treatment plants. Although much is known about the basic metabolism in different types of anaerobic reactors, little is known about the microbes responsible for these processes. Only a few percent of *Bacteria* and *Archaea* have so far been isolated, and almost nothing is known about the dynamics and interactions between these and other microorganisms. This lack of knowledge is most clearly exemplified by the sometimes unpredictable and unexplainable failures and malfunctions of anaerobic digesters occasionally experienced, leading to sub-optimal methane production and wastewater treatment.

Most anaerobic microbial processes are characterized by close association of numerous functional groups of microorganisms. The understanding of anaerobic processes has improved greatly during recent decades with advances made in microbial physiology, biochemistry, ecology, kinetics, and mathematical modeling. These contributions have led to an expansion of anaerobic processes by introducing better designs and operational controls. However, the understanding of anaerobic processes is far from complete. Understanding the microbial ecology in anaerobic reactor systems requires;
(1) identification and classification of microorganisms,
(2) quantification of microbial abundance, and
(3) quantification and identification of activity.

Morphology and other microbial traits have previously been used for identification and quantification of microbial populations. Grotenhuis et al. (1991) microscopically counted cell numbers of methanogens and identified aceticlastic methanogens based on morphology, and hydrogenotrophic methanogens by visualizing autofluorescence at 420 nm. Morphology and ultrastructure have also been used extensively in scanning or transmission electron microscopy studies to show the location of certain microorganisms in anaerobic granules. Information gained from morphology-based techniques is, however, ambiguous and limited since most microorganisms are small in size, and simple in morphology and ultrastructure. In the absence of special morphological features or autofluorescence, physiological and biochemical traits

have been used for identification. Furthermore, enrichments on defined substrates have been helpful to identify prevalent species in anaerobic granules, and Most Probable Number (MPN) estimates have been used frequently for quantification of different trophic groups of anaerobic microorganisms. These methods are, however, cultivation dependent and therefore limited by the ability of microorganisms to grow under laboratory conditions. It is well known that only a very small fraction of the microorganisms in nature is culturable by present cultivation techniques, because of unrecognized nutrient and growth conditions, or the interruption of intrinsic interdependencies such as syntrophic interactions.

During the last years, bacterial identification based on molecular methods, especially those including the sequencing of genes coding for ribosomal 16S rRNA, has become a very important tool to study bacterial communities in environmental samples (Giovannoni et al., 1999; Ward et al., 1990; Muyzer et al., 1993; Ludwig and Schleifer, 1994; Amann et al., 1995 and Head et al., 1998). The application of these methods on art objects revealed the presence of microorganisms, which had never been identified in these environments before. By applying these methods, the potential of such methods to investigate biodeterioration processes was demonstrated and it was suggested that such techniques should be integrated as a part in restoration strategies (Gurtner et al., 2001). Hence, the applications of molecular investigation strategies to study biodiversity have to undergo permanent improvement to overcome any inherent limitations.

Using both 16S rDNA phylogenetic analysis and enrichment culture techniques, it is possible to characterise the microbial diversity and culture characteristics of the isolated microorganisms in different environments, allowing a more complete picture. The phylogenetic information obtained by using molecular techniques about the identity of the members of a bacterial community can be a very useful tool for the specific design of appropriate culture media.

The ribosomal sequences are present in all organisms and they contain variable and highly conserved regions which allow distinguishing between organisms on all phylogenetic levels. In addition, a lot of data exist in the databases (Maidak et al., 1999), which can be used to compare the DNA-sequences of unknown

microorganisms and allow a phylogenetic identification. To identify bacteria in sample material, ribosomal sequences are analysed by transcribing ribosomal RNA into cDNA, which can then be cloned (Ward et al., 1990). Alternatively, extracted DNA can be used as a template to amplify ribosomal gene fragments with primers for universal sequences by PCR (Polymerase Chain Reaction). The PCR amplified fragments can be cloned as well. The result of both strategies is a clone library, containing ribosomal sequences as inserts. By sequencing individual inserts and comparing the obtained sequences with sequences present in databases, it is possible to identify the phylogenetic position of the corresponding bacteria without their cultivation. An alternative to this approach is the Denaturing Gradient Gel Electrophoresis (DGGE) of PCR-amplified gene fragments coding for rRNA (Muyzer et al., 1993). This technique allows the separation of partial 16S rDNA amplified fragments of identical length but different sequence due to their different melting behaviour in a gel system containing a gradient of denaturants. As a result, a band pattern is obtained, which reflects the complexity of the microbial community. The reliability of the technique is very high; all species present in the community that are over 1% of the total population can be detected by DGGE analysis. This percent is much higher than cultivation.

By excising individual DGGE bands from the gel and re-amplifying the DNA, it is possible to get sequence information of single community members (Muyzer et al., 1993; Muyzer and Smalla, 1998). However, phylogenetic analyses of sequences obtained directly from DGGE patterns are often difficult. Sequence information obtained by direct sequencing of manually excised bands does not always allow reliable phylogenetic analyses due to the short sequence length (200-500 bp). Furthermore, co-migration of several different 16S rDNA sequences, which have the same melting behaviour and therefore the same position in the gel, leads to overlapping DGGE bands which cannot be sequenced directly. The various approaches and tools used in these analyses are outlined in Figure 2.4.

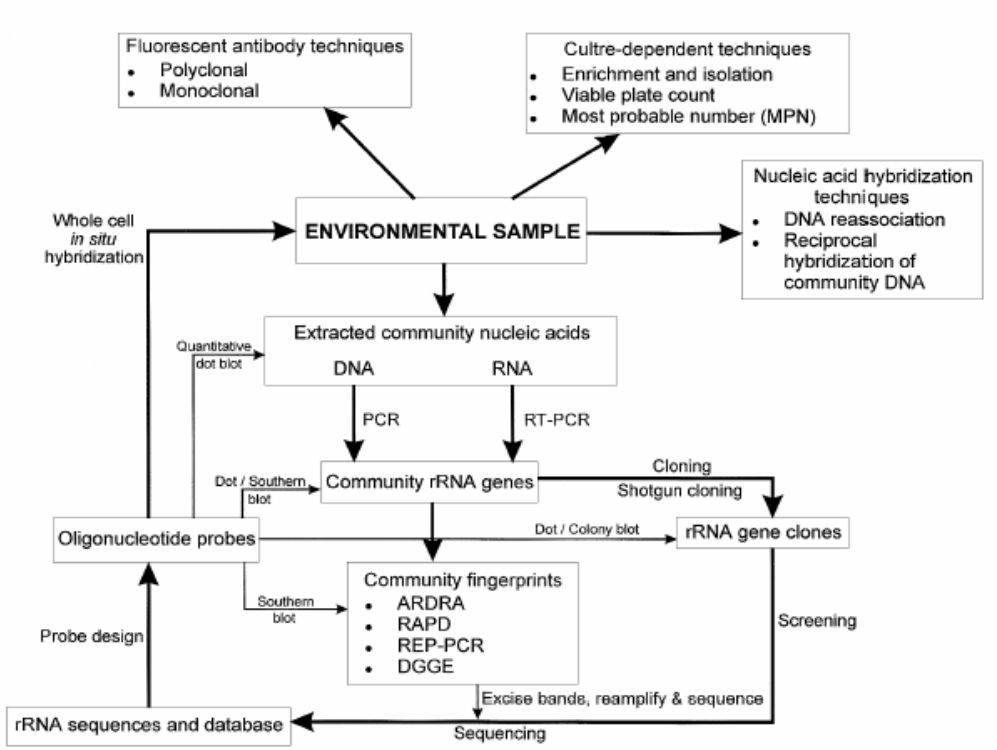


**Figure 2.4.** Commonly used molecular approaches in microbial ecology (Theron and Cloete, 2000)

### 2.12.1.*The molecular approach to study microbial communities*

*Analyses of naturally ocurring rRNA and rDNA*

The starting point for the molecular approach and related procedures is the extraction of nucleic acids of sufficient quality to permit activity of the enzymes used in subsequent procedures, as Polymerase Chain Reaction (PCR). There are two strategies based on rRNA and rDNA to identify bacteria in sample material. The first approach is based on the recovery of rRNA that is transcribed into cDNA, cloned and sequenced (Ward et al., 1990). The alternative approach is based on the recovery of high molecular weight DNA directly from sample material, followed by the amplification of rDNA by PCR (Polymerase Chain Reaction), cloning and sequencing. The result of both strategies is a clone library, containing ribosomal sequences as inserts.

*The PCR-clone-sequence approach*

The extracted DNA is subjected to PCR amplification using "universal" primers or primers designed to amplify rRNA genes from particular group of organisms. The broad-range amplification of 16S rDNA genes with universal 16S rDNA

primers allows the unselective detection of unexpected or hitherto unknown bacteria in medical and environmental samples. Various methods are available for the extraction and purification of nucleic acids from a wide range of environmental samples. These are usually based on chemical and/or physical disruption of cells combined with treatments to remove contaminating materials, such as humic acids and metals that can inhibit the efficiency of subsequent enzymatic reactions. Any one of three basic approaches can then be used to obtain rRNA gene clones from the "total community" nucleic acids.

The simplest and currently the most widely adopted method to obtain 16S rRNA genes from the environment is through the use of PCR. rRNA genes can be amplified directly from the total community DNA using rRNA specific primers and then cloned using standard methods. By taking advantage of the highly conserved nature of rRNA, universal primers capable of annealing to rRNA genes from all three domains (*Archaea*, *Bacteria*, *Eukarya*) or primers designed to amplify rRNA genes from a particular group of organisms can be used (Ward et al, 1993; Amann, 1995; Amann et al., 1997).

*Sequencing of specific clones*

Automated DNA sequencing systems have greatly facilitated the rapid screening and analysis of large gene libraries. Initial screening of rRNA gene-containing clones by different methods such as restriction fragments length polymorphism (RFLP) analysis of purified plasmid DNA or insert DNA obtained by colony PCR for the presence of near identical sequences, can greatly reduce the number of clones that require complete sequencing. However, RFLP is of limited use for demonstrating the presence of specific phylogenetic groups and is a time-consuming method. By sequencing individual clones and comparing the obtained sequences with sequences present in databases, it is possible to identify the phylogenetic position of the corresponding bacteria without their cultivation.

*Denaturing Gradient Gel Electrophoresis (DGGE)*

An alternative to this approach is the Denaturing Gradient Gel Electrophoresis (DGGE) of PCR-amplified gene fragments coding for rRNA (Muyzer et al., 1993). DGGE is a method by which fragments of DNA of identical or near identical length but different in sequence composition can be resolved

electrophoretically. DGGE has been extended to the analysis of PCR-amplified 16S rRNA genes from environmental samples. In DGGE analysis, separation is based on changes in electrophoretic mobility of DNA fragments migrating in a vertical polyacrylamide gel containing a linearly increasing concentration of DNA denaturants (formamide and/or urea). As the DNA fragments are subjected to electrophoresis, partial melting of the double-stranded DNA occurs in discrete regions, the so-called melting domains, at a denaturant concentration specific for the nucleotide sequence of the DNA. The migration of the fragment therefore is severely retarded. Sequence variation within such domains alters their melting behavior, and sequence variants of the different amplification products stop migrating at different positions in the denaturing gradient.

Although DGGE analysis of PCR-amplified 16S rDNA fragments provide a rapid method to characterize community population structure, more specific information of population composition can be obtained by secondary analysis of the DGGE banding pattern. Individual bands (fragments) may be excised from the gel, subjected to a second round of PCR amplification and sequenced. Alternatively, the DNA can be transferred to nylon membranes and then challenged with group- and species-specific oligonucleotide probes to identify specific populations within the microbial community.

As DGGE is relatively rapid to perform and many samples can be electrophoresed simultaneously, the method is particularly useful when examining time series and population dynamics. Once the identity of an organism associated with any particular band has been determined, fluctuations in individual components of a microbial population, due to environmental perturbations, can be rapidly assessed. This method has been applied to the analysis of 16S rRNA genes from environmental samples (Muyzer *et al*., 1993). As a result, a band pattern is obtained, which reflects the complexity of the microbial community. By excising individual DGGE bands from the gel and reamplifying the DNA, it is possible to get sequence information of single community members (Muyzer et al., 1993; Muyzer and Smalla, 1998). DGGE is relatively rapid to perform, and many samples can be run simultaneously. The method is, therefore, particularly useful when examining time series and population dynamics. Once the identity of an organism associated with any

particular band has been determined, fluctuations in individual components of a microbial population, due to environmental perturbations, can be rapidly assessed. DGGE represents a powerful tool for monitoring microbial communities.

*Whole-cell hybridisation*

This approach is *Fluorescent In Situ Hybridisation* (FISH). End-labeled oligonucleotides are sufficiently sensitive to allow the specific detection of individual microbial cells directly in sample materials. Fluorescent rRNA-targeted oligonucleotide probes confer fluorescent stain specifically to cells of a phylogenetically coherent group on various taxonomic levels from species up to the kingdom level. They can be applied to samples without prior cultivation and determine the cell morphology and identity of microorganisms, their abundance and the spatial distribution in situ (Amann et al. 1995). Cells showing specific hybridisation with the fluorochrome-labelled probe can be identified and enumerated. There are also some limitations associated with the technique. These can be divided in four main categories: cell permeability problems, target site accessibility, target site specificity and sensitivity.

## 2.13. Studies on Anaerobic Manure Treatment

Farm animal manure is characterized by high total solids and organic content, $NH_3$-N concentration and pathogens. Because of insufficient or uncontrolled handling and disposal, they represent a danger to public health and the environment. Anaerobic systems offer an option for the safe treatment of these wastes, mainly due to their special advantages such as low energy requirement, less waste biomass generation, a useful and economically valuable end-product, suitability for seasonal operations and the elevated organic loading rates achievable. Thus, anaerobic treatment is an attractive option for farm animal manure that has high organic content. There are many studies about the digestion and co-digestion of these wastes in the literature. In this section, a review of these studies is given.

Anaerobic treatability of cattle waste was studied on farm scale plant by Hammad et al. (1999). A cubical digester was constructed under the ground surface and the produced methane gas was used as an energy source for

heating and for producing electricity for domestic use. The results showed that among various types of manure (cattle, poultry, sheep, and horse) cattle manure gave the highest rate of biogas production even at low temperatures (at 15, 17, 18, 21, and 23°C), in second place came the poultry manure. For example, at 18°C, 0.24 $m^3$ biogas/$m^3$.day was produced. Methane content of this gas was 58%. As the unit was not heated, the ambient temperature had a controlling effect on all performance parameters, such as biogas quantity, methane percentage. These parameters were enhanced by the increase of ambient temperature.

The effect of temperature and retention time (RT) on the rate of methane production from waste of beef cattle was investigated by using continuously mixed anaerobic fermentors by Varel et al. (1980). This study compared the efficiencies of methane production at mesophilic and thermophilic temperatures and at long and short RTs. The results indicated that there was little difference in rates of methane production between 40 and 60°C at RT of 6 days or longer. However, there was kinetic advantage at thermophilic temperatures and short RT (<6 days).

Nozhevnikova et al. (1999) worked on the anaerobic manure treatment under psychrophilic conditions (5-20°C) and extreme thermophilic (55-82°C) conditions. The results of this study showed the possibility of the development of a low-temperature methanogenic community in a system previously not subjected to psychrophilic conditions; however this type of microbial community was sensitive to temperature increase. On the other hand, anaerobic digestion of manure under hyper-thermophilic conditions resulted in a development of a thermophilic acidogenic microbial population producing volatile fatty acids. Thus, a two-step anaerobic manure treatment was proposed in which the sanitation of manure and saving energy present with i) acidogenic fermentation at high temperature, ii) separation for solid and liquid fractions, iii) treatment of liquid manure fraction under low temperature conditions. In a two-year survey, 8 farm digesters were monitored fermenting either cattle, pig or chicken manure or mixtures of to find the effect of physical and chemical parameters on methane production (Wellinger, 1999). This work confirmed that temperature was extremely important parameter also in full scale installations. Any change in digestion temperature was expressed by gas

production within the following 24 hours, independent of other parameters in the system. For cattle manure with a high content of straw bedding, the optimal gas production was achieved at a high HRT of 25 days.

In the quiescent state, cattle manure slurry stratifies into three distinct layers; a floating scum layer, a bottom sludge layer and a watery middle layer, with most of the biologically degradable component of the slurry being contributed by the particulate matter in these layers. By using this fact, Ong et al. (2000) treated the whole slurry in an unmixed digester. To enhance bio-methanation of the whole slurry in an unmixed digester, the retention times of three layers were independently varied, by manipulating the discharge outlet position, such that the more degradable fractions were retained longer. The results showed that when effluent was discharged from the bottom, gas production was consistently less then when effluent was discharged from the middle, or when slurry was mixed evenly and discharged. This was attributed to the gradual accumulation of solids at the bottom which were being detained longer by moving the discharge position to the middle.

Anaerobic digestion of cattle waste at mesophilic and thermophilic temperatures in a continuously stirred reactor was studied by Mackie and Bryant (1985). Digestion was carried out at 40$^{o}$C and 60$^{o}$C. $CH_4$ production was higher in the thermophilic than the mesophilic digester. $CH_4$ production decreased more rapidly with each increase in VS loading rate and decrease in RT in the mesophilic than the thermophilic digester. The biological efficiency of thermophilic methane production from the cattle waste at long to very short RTs and from low to high feed concentrations by Varel et al. (1980). Results indicated that methane fermentation of cattle feedlot waste at thermophilic temperature is maximum at about 60$^{o}$C and is very easily and rapidly initiated. It is highly stable to temperature changes between 55 and 60$^{o}$C, to changes in RT between 15 and 3 days, and to increases in the amount of VS in the feed from 2% to about 8 to 12%, depending on the RT and loading rate.

A two-stage 68°C/55°C anaerobic degradation process for treatment of cattle manure was studied by Nielsen et al. (2004). The results of this study demonstrated that it is possible to improve the anaerobic degradation of cattle manure when applying 68$^{o}$C pretreatment before a traditional 55°C digestion.

In batch experiments, an increase in the specific methane yield, ranging from 24% to 56%, was obtained when cattle manure and its fractions were pretreated at 68°C for periods of 36, 108, and 168 h and subsequently digested at 55°C.

When compared with a conventional single-stage reactor operated at 55°C with 15-day HRT, enhanced methane yield and increased VS reduction was obtained when a pretreatment reactor operated at 68°C with a 3-day HRT was connected to a 55°C reactor with a 12-day HRT. Both systems were operated at an organic loading rate of 3 g VS/L.day. The improved digestion in the two-stage reactor was obviously caused by improved hydrolysis. The pretreatment reactor was characterized by a stable performance generating 7% to 9% of the total methane production of the two-stage system, but the operational temperature of 68°C was found to severely affect the aceticlastic methanogens and syntrophic consortia converting VFA into methane. Microbial population analysis revealed that the pretreatment reactor harbored a smaller population of cultivable anaerobes than the 55°C reactors.

Although it is recommended to keep the temperature of the thermophilic digestion process below 60°C to ensure that the fluctuation in the operational temperature would have not fatal impact on the microbial activity, increased demand for pathogen kill during anaerobic digestion could increase the interest for digestion at high temperatures than 60°C. In the light of this information, Ahring et al. (2001) investigated the effect of temperature increase from 55 to 65°C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. This study documented that it was possible to treat cattle manure in an anaerobic reactor at 65°C. However, the consequence of the temperature shift from 55 to 65°C was a lower methane yield and an increased amount of the VFA in the effluent. Hydrolysis and fermentation at 65°C were not significantly affected, but seemed to be carried out by populations of extreme thermophiles. The activity and amounts of methanogens, with exception of hydrogenotrophic methanogens were significantly reduced at 65°C and an establishment of new populations active at 65°C was indicated.

Like temperature, the total solids (TS) content of the feedstock has been shown to be one of the most important factors governing the net energy production of anaerobic digesters (Hall et al., 1985). The effects of TS concentrations of cattle waste slurries on biogas yield was investigated by Itodo and Awulu (1999). Cattle manure with TS concentrations of 5%, 10%, 15%, and 20% was fed to the batch digesters and gas yield were measured. Experiments were undertaken in the mesophilic temperature range. The results indicated that biogas yield decreased with increasing TS. Higher gas yield was obtained from TS with lower concentrations because at the higher TS, the slurry became too thick.

Angelidaki and Ahring (1993) examined the effects of addition of different ammonia concentrations and the possibility of adaptation to ammonia during anaerobic thermophilic digestion of cattle manure in continuously-fed lab scale reactors. The methane yield decreased to 25%, with both 4 and 6 g N/L added compared to controls with 1.5 g N/L ammonia. When ammonia was introduced gradually, the process was unaffected up to 3 g N/L and only slightly affected 4 g N/L, with signs of recovery after 1 RT. At a concentration of 5 g N/L, process performance was seriously affected. Same authors presented another study in 1994 and examined the combined effect of temperature and ammonia using continuously fed reactors. Results of this study showed that the biogas yield was not influenced by temperature in the range of 40-55°C when ammonia concentration was low. The results also clearly demonstrated a higher sensitivity to increased temperatures at higher ammonia loads.

Shyam (2001) carried out simple modifications on the common fixed-dome type family-size biogas plant for digestion of fresh undiluted cattle manure. No (very little) water was required for mixing with the manure. The modified plant generated approximately 50% more biogas than the common fixed-dome type biogas plant and made the handling of both input slurry as well as the digested slurry easier. Hall et al. (1985) reported a study, which investigated the batch digestion of cattle manure-straw mixture through which liquor was continuously recirculated. To improve the process, digesters were linked in series to form a semi-continuous process, which was self-inoculating. At the end of the study, the operation of two digesters in series to form a semi-continuous process was

found to function successfully and to have increased solids reduction and gas production when compared with the results of batch experiments.

Wellinger et al. (1992) developed a continuous flow digester called ANCOM (Anaerobic Compositing of Manure) which should fulfill the following premises. First, the digester should handle the manure without pretreatment, i.e. the straw should not be chopped either before or after bedding. Second, the consistency of the digested manure should still allow its field application by a conventional manure spreader, i.e. the total solids should be around 15% or higher. The experiments demonstrated that the gas yield increases with increasing quantities of liquids. Best results were achieved with TS values equivalent to 13% or lower. Results also showed that the amount of gas produced from the batch experiments reduced when the larger amounts of straw added.

Work by Hills (1980) indicates that for fresh dairy manure gas production per unit volume of digester increases linearly with the loading rate. By doubling the loading rate (by doubling the solids concentration of the feed and holding the retention time constant), the gas output also nearly doubles. Hills' investigation (1980) also indicates that the high solids content within the digester tends to suppress the separation of floatable solids thereby restricting the formation of a scum layer. The phenomenon suggests that for high solids fresh dairy manure digesters the mixing requirement may be lessened or, in fact, completely eliminated

Summers et al. (1987) studied AD of the fattening-cattle manure in mesophilic CSTR. Maximum gas production from fattening-cattle waste was 289 litres per kilogram of TS fed (including gas from VFA) at 20 or more days RT. A number of studies have been made on mesophilic digestion of cattle wastes. The solids degradation and biogas productions have generally been similar to those found here, but, because of differences in the feeds of the animals and in compositions of the wastes, exact comparison of results is not possible. For example, Varel et al. (1980) studied methane production from waste of beef cattle fed mainly on corn where the waste contained appreciable amounts of starch. They found maximum methane production of about 260 litres per kilogram of VS fed to the digester at 18 days RT at 35°C. This figure is higher

than that found in the present experiments but could be accounted for by the difference in animal feeds. The methane content of their biogas was about the same as here, 46-55% at different RT and temperatures. The fattening-cattle wastes in their experiments gave higher biogas productions than the dairy-cattle wastes, probably because the fattening cattle were fed ad libitum and partially degraded feed passed to the faeces, while the feed of the dairy cattle was more strictly controlled and was more completely degraded in the animal. The various feeds also differed in composition.

In another study by Pain et al. (1984) for a full size digester plant, they reported biogas yields of 204 litres per kilogram of Digestion of whole and separated cattle wastes 6% TS fed for unseparated slurry at 20 days RT, with a TS reduction of 26%. The biogas averaged 54% methane. For waste separated by a commercial separator with a brushed-screen followed by roller-pressing against a screen with 3 mm perforations, gas production averaged 279 and 251 litres per kilogram of TS fed at 20 and 15 days RT, with gas of 55% methane. TS reduction averaged 31% with feed slurry of about 4% TS. These results should be compared with the present results calculated for slurry with gas from acids included.

Lo et al. (1983, 1984) studied digestion of screened and unscreened dairy cattle wastes in laboratory stirred-tank digesters from 16 to 1 days RT. Their results were generally similar to the present ones, with a biogas of maximum 63.8% methane from screened slurry and 54% methane from whole slurry. They found the optimum RT for biogas production per unit of VS fed was 8-10 days with screened slurry of 3.3% VS content. Gas yields per kg TS fed to the digester were greater for separated than for unseparated slurries, as might be expected if larger and less-degradable fibres were being removed in the separation. However, separation does remove some potentially methanogenic material and thus separation decreases total gas available from a particular volume of slurry, as calculated by Pain et al. (1984). On the other hand, this potentially available gas in the whole slurry can be obtained only by running the digester at about 20 days RT, while the solids in the separated waste can be digested at about 10 days RT. The separated slurry is also easier to handle in pumps and pipelines, and the solids removed can be composted to give a soil conditioner and fertiliser.

Thermophilic digestion at 55°C is a biological process in which microorganisms convert waste while producing methane, carbon dioxide and traces of other gases. High methane production rate and good stability using completely mixed thermophilic digesters to digest screened dairy manure have been previously observed by others. For example, Liao and Lo (1985) studied single phase and two-phase mesophilic and thermophilic digesters and found no advantage in terms of biogas production in separating the acid and methane forming phases for digestion of dairy manure. Using screened dairy manure as the substrate, there was no indication that a two-phase system would be superior to the one-phase system under thermophilic conditions (Liao and Lo, 1985). It should be noted that Liao and Lo (1985) screened the raw manure using a No. 10-mesh. The manure fed to the digester had a volatile solids content of only 3%.

Hydraulically flushed manure may present problems of high volume and low degradable solids concentrations. Liao and Lo (1987) operated a mesophilic fixed-film digester treating hydraulically flushed dairy manure. Three influent manures were prepared; the first was screened with No. 10-mesh (10 openings per inch), the second was the supernatant from settled manure, and the third was supernatant from settled and screened manure. All influents were fed to digesters with 1 day HRT values. The VS destruction was low, 22% for screened manure, 4.4% for settled manure, and 14.3% for screened-settled manure. The methane production was very good, 1.23 L $CH_4$/L.day for screened manure, 1.17 L $CH_4$/L.day for settled manure, 1.06 L $CH_4$/L.day for screened-settled manure (Liao and Lo, 1987).

Two-phase AD for unscreened dairy manure was investigated for possible exploitation of the advantages for the first time by Demirer and Chen (2005). The results indicated that the use of a two-phase reactor at a SRT/HRT of 10 days (2 days acidogenic and 8 days methanogenic) for AD of dairy manure; resulted in 50 and 67% higher biogas production or volume reduction at OLRs of 5 and 6 g VS/L.day, respectively, relative to a conventional one-phase configuration with SRT/HRT of 20 days, and also an elevated OLR of 12.6 g VS/L.day possible which was not achievable for conventional one-phase configuration. Consequently, the new configuration translates into significant cost savings due to both superior performance and reduced volume requirements.

Table 2.6 is compiled to evaluate the methane production and the total VS reduction obtained in this study by comparing it with the performance data for different anaerobic reactors treating dairy and cattle manure. When comparing the performance reported in different studies, it must be kept in mind that different reactor types, operating temperatures, loading rates, hydraulic retention times, etc. were used in these studies. Therefore, such a comparison may lead to erroneous outcomes unless all the operating conditions are considered. Even though this was not possible due to missing experimental details, unclear operational descriptions, etc. Table 2.6 may still serve as a basis of comparison of the performance level obtained in this study versus similar studies in the literature.

**Table 2.6.** Performance data for different anaerobic reactors treating dairy or cattle manure

| Reactor configuration | OLR (g VS/L.day) | HRT (days) | Temp (°C) | $CH_4$ (mL/g VS.d) | VS reduction (%) | Reference |
|---|---|---|---|---|---|---|
| CSTR | 3.3 | 18 | 35 | 260 | 52 | Varel et al., 1980 |
| CSTR | 5 | 12 | 35 | 235 | 55 | " |
| CSTR | 6,7 | 9 | 35 | 218 | 52 | " |
| CSTR | 10 | 6 | 35 | 160 | 50 | " |
| CSTR | 2 | 16.2 | 35 | 270 | 50-63 | Karim et al, 2005 |
| CSTR | 3 | 15 | 37 | 224 | 37 | Mladenovska et al, 2003 |
| Plug flow | 9 | 15 | 35 | 78 | 24 | Hills and Mehlschau, 1984 |
| CSTR | 11.6 | 15.1 | 35 | 90 | 25 | Hill 1980 |
| CSTR | 3 | 15 | 55 | 241 | 43 | Nielsen et al., 2004 |
| TPAD | 3 | 3+12 | 65+55 | 260 | 47 | " |
| CSTR | 0.91 | 20 | 36 | 65 | 20 | Qasim et al, 1984, |
| Two-phase CSTR | 2 | 10 | 35 | 65 | 68 | Demirer and Chen, 2005 |
| Two-phase CSTR | 6,3 | 10 | " | 112 | 33-40 | " |
| CSTR | 2 | 20 | " | 130 | 48-50 | " |
| CSTR | 6.3 | 20 | " | 135 | 42-52 | " |
| CSTR | 2.79 | 25 | | 250 | 38,3 | Singh et al., 1988 |
| TPAD | 2.84 | 4+10 | 58+38 | 250 | 39 (27+16) | Harikishan and Sung, 2003 |

| Reactor configuration | OLR (g VS/L.day) | HRT (days) | Temp (°C) | $CH_4$ (mL/g VS.d) | VS reduction (%) | Reference |
|---|---|---|---|---|---|---|
| TPAD | 4.5 | 4+10 | 58+38 | 240 | 40 (28+16) | " |
| CSTR | 3 | 13 | 40 | 210 | | Mackie and Bryant, 1995 |
| CSTR | 2,90 | 10 | 30 | 133 | | Lo et al.,1984 |
| Batch | | 20 | 30 | 213 | | Hawkes et al., 1984 |
| Batch | | | 35 | 148 | | Moller et al., 2004 |

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1. Dairy Manure and Anaerobic Seed Cultures

Wet manure was collected from a private dairy near Gölbaşı, Ankara, and stored at 4°C prior to use. Table 3.1 depicts the characterization of the dairy manure used in the experiments.

**Table 3.1.** Characteristics of dairy manure

| Characteristics | Concentration (g/L) |
|---|---|
| TS | 202 ± 8.6 |
| VS | 135 ± 19.8 |
| COD | 165 ± 24.2 |
| Density | 1042 ± 45 |
| pH | 7-8 – 8.1 |

The mixed anaerobic culture used as seed was obtained from the anaerobic sludge digesters at the Ankara wastewater treatment plant, which has a SRT of 14 days. The mixed anaerobic culture was concentrated by settling before being used as inoculum. The volatile suspended solids (VSS) concentration of the concentrated seed cultures used was 23930 ± 3162 mg/L.

Before the characterization and the experiments, a reliable COD determination was investigated due to the high solid content and heterogeneity of the manure used. For this reason different manure concentrations were prepared and then the diluted samples were tested by the Reflux Method (A.P.H.A, 1995) for COD. The relationship between manure concentration, VS, and corresponding COD values are given in Figures 3.1 and 3.2. Therefore, a good correlation was

obtained, the COD content of manure can be easily estimated with respect to its VS content.

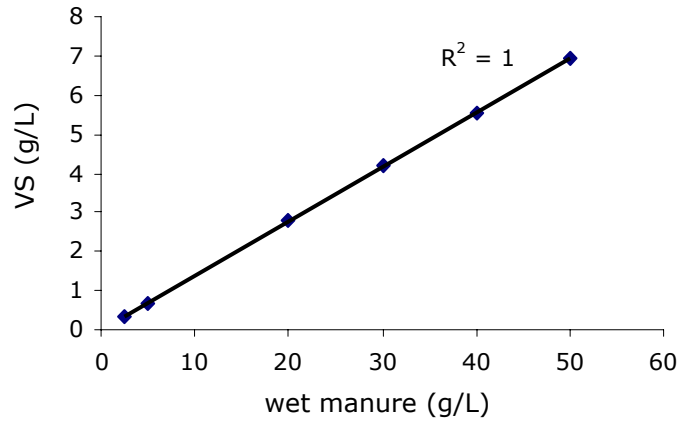


**Figure 3.1** The relationship between VS content of manure and wet manure concentration

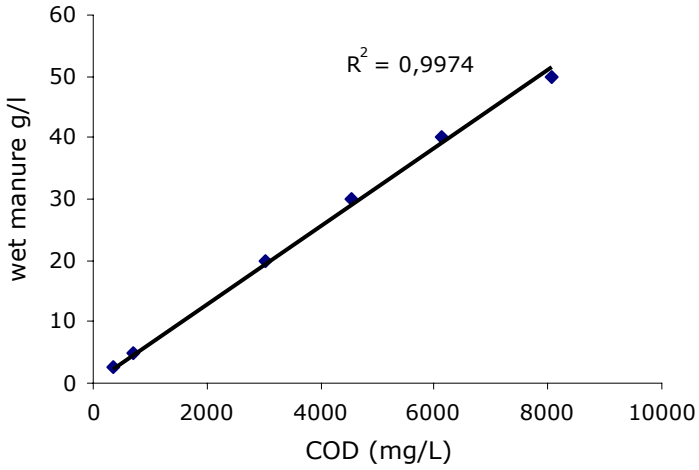


**Figure 3.2.** The relationship between the manure concentration and COD

## 3.2. Experimental Set-up

Four different experimental set-ups were envisaged. In the first part (Set I) of the study, the optimum retention time and organic loading rate (OLR) values leading to maximum acidification and VS reduction were investigated. Thus, nine daily-fed continuously-mixed acidogenic anaerobic reactors with no recycle were operated as duplicates. The total volume of reactors was 250 mL. Reactor

operation involved daily feeding of wet dairy manure and wasting of corresponding reactor contents (Table 3.2). Solids and hydraulic retention times (SRT/HRT) applied to each reactor was the same since no recycle of the effluent was practiced. Initially 100 mL of concentrated anaerobic seed was added to each reactor and then, the reactors were flushed with $N_2$ gas for 3 min and maintained in an incubator shaker at 35±2°C and 165 rpm. The next day dairy manure (25 mL to reactors 1–4, 50 mL to reactors 5–8, and 80 mL to reactors 9–12) were added to each reactor. Daily feeding and wasting were conducted as seen in Table 3.2.

**Table 3.2.** Daily feeding and wasting used for acidogenic reactors (Set I)

| Reactor | SRT (days) | OLR (g VS/L.day) | Volume of feeding/wasting (mL) |
|---|---|---|---|
| 1 | 4 | 5 | 25 |
| 2 | 4 | 10 | 25 |
| 3 | 4 | 15 | 25 |
| 4 | 2 | 5 | 50 |
| 5 | 2 | 10 | 50 |
| 6 | 2 | 15 | 50 |
| 7 | 1.25 | 5 | 80 |
| 8 | 1.25 | 10 | 80 |
| 9 | 1.25 | 15 | 80 |

In the second part (Set II) of the study, the optimum pH value for acidification of dairy manure was investigated. Two identical reactors with a working volume of 800 mL were inoculated with 400 mL mixed anaerobic culture and 400 mL of wet dairy manure. Both reactors were operated at an HRT/SRT of 2 days and OLR of 15 g VS/L.day. To determine pH effect, pH of one of the reactors was not controlled, while the other was set to a constant value in the range of 5.0-5.5 using a pH-stat unit (Takashima and Speece, 1989; Demirer and Speece, 1999). In this part of the study, gas measurement was conducted by using a U-tube displacement made from an inverted buret. The U-tube made from an inverted buret consisted of a 50 ml buret on the one side a plastic tube having the same internal diameter of buret on the other side. The tip of the buret was connected small tubing attached on a syringe needle. The plastic tube was connected to a 3 way plastic connector to allow water over flow resulting from

an increase of the water level on the other side during measurements of gas (Fig.3.3).

In third set of experiment (Set III), two conventional semi-continuous reactors were operated at a SRT of 20 days. These reactors were run with different temperature to compare the efficiencies of biogas production but the same OLRs. Two semi-continuous reactors of 3 L volume used for this experiment were fed daily at 25°C (representing low temperature) and at 35°C (mesophilic temperature). Each reactor was sealed with a silicone rubber stopper connected to silicone rubber gas tubing leading to the respective gas holder. Gas was collected by the displacement of water in calibrated gas holders (Fig.3.4). Initially, it was filled with water up to the top and the volume of biogas was measured daily by taking the water level readings in the cylinder. Gas sampling points provided via three-way luer plastic. The gas holders were connected to adjustable reservoirs so that the gas volumes could be measured at atmospheric pressure.

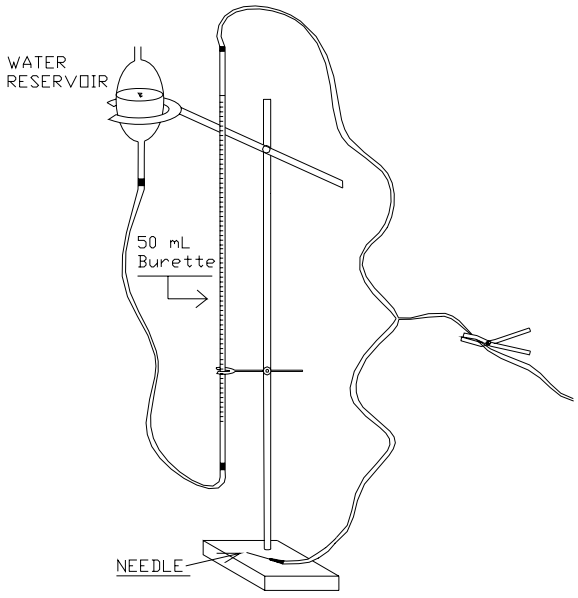


**Figure 3.3**. Schematic illustration of water replacement device

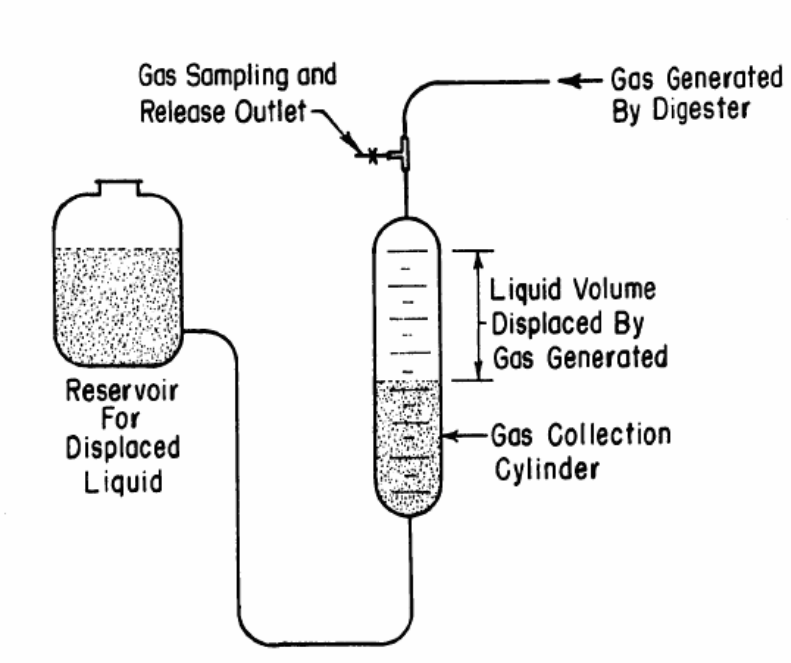


**Figure 3.4.** Gas volume measurement system

A schematic representation of the laboratory-scale, one-phase and two-phase anaerobic digestion system used in the experimental system in Set IV is depicted in Fig 3.5. Even though several aspects of two-phase configuration might be very significant for efficient AD of dairy manure, its application has been limited to screened dairy manure only. Therefore, this study investigated possible exploitation of the advantages of two-phase AD for unscreened dairy manure. The one-phase conventional configuration (R1) was run as the control for the two-phase configuration (R2). The effective volumes of R1, R21, and R22 were 1000, 400, and 1000 mL, respectively. The two-phase configuration contained R21 and R22 as the first (acidogenic) and second (methanogenic) phases. The SRT/HRT values of R1, R21, R22 and the overall two-phase configuration were 20, 2, 8.6, and 10.6 days, respectively. All the reactors were fed daily. The gas production in R1, R21 and R22 were monitored by a water replacement device (Fig.3.4) was used to monitor the gas production. Two sets of reactors were maintained at 25°C in a temperature-controlled water bath and 35°C (±2) in a controlled room, and were shaken manually once a day after gas production. R1, R21, and R22 were seeded with 1000, 400, and 1000 mL of mixed anaerobic seed culture.

The performance of the reactors was monitored by measuring biogas production and soluble COD, VS, volatile fatty acid (VFA), pH, and oxidation reduction potential (ORP).

The experiments performed in this study are summarized in Table 3.3 with their objectives.

**Table 3.3.** Set-specific experiment targets

| Experiments | Objective of experiments |
|---|---|
| Set I | Selection of optimum OLR and SRT for the acidogenic reactors |
| Set II | Investigating the effect of pH control in acidogenic phase |
| Set III | Effect of temperature on biogas production |
| Set IV | Comparison of two-phase and one-phase systems on the efficiency of AD of dairy manure |

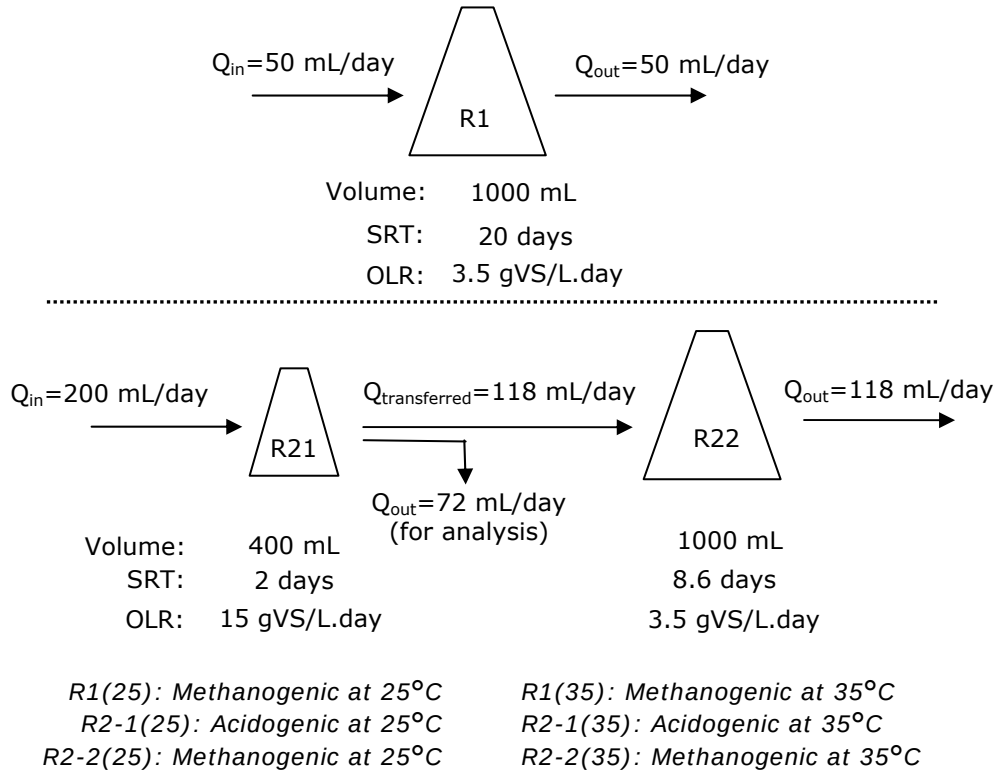


**Figure 3.5.** Experimental set-up used in Set IV

## 3.3 Analytical Methods

The pH, daily gas production , total solids (TS), volatile solids (VS), methane percentage, total volatile fatty acids (tVFA) and effluent soluble COD (sCOD) were monitored in each reactor. pH, TS, VS analysis were performed using Standard Methods (A.P.H.A. 1995). sCOD was measured using Hach COD vials according to the EPA approved digestion method (HACH Water Analysis Handbook, 1992). Accordingly, after 2 h digestion, sample sCOD were directly read using Hach 45 600-02 spectrophotometer (Hach Co. Loveland, Co., USA).

*Total Volatile Fatty Acids*

A gas chromatograph (GC) (Thermo Electron Co.) equipped with a flame ionization detector and a 30 m column was used for VFA analyses. The column temperature was started at 100°C with 2 min holding time and then increased to 250°C with 8°C/min ramping, and the injector/detector temperature was kept at 200/350°C with nitrogen as the carrier gas and a flow rate of 30 mL/min. The gas flow rates were gauged at 350 mL/min for air and 35 mL/min for hydrogen. Liquid samples were prepared by centrifuging for 15 minutes at 3,000–4,000 rpm and by filtering 5 mL of the supernatant through a 0.22 mm glass fiber filter (Whatman Co.). The filtered samples were acidified with 99% formic acid to a pH less than 3 to convert the fatty acids to their undissociated forms (i.e., acetic acid, propionic acid, butyric acid, etc.) before injecting 1 µL of the acidified samples into the GC.

*Gas Analysis*

Total gas volume produced in the reactors was measured by connecting the reactor headspace to a water displacement column filled with distilled water and recording the volume of displaced solution. Gas samples for gas composition analysis were taken by a 100 µL Hamilton gas-tight glass syringe from gas sampling port. The gas composition was determined by a (GC) unit (Thermo Electron Co.) equipped with thermal conductivity detector. Methane, nitrogen and carbon dioxide were separated through a 15 m Porapak Q, 5 mm I.D.column. Column was operated with helium as the carrier gas at a constant pressure of 20 kPa at 40°C. The injector was maintained at 100°C, and the detector temperature was set to 100$^{o}$C. The calibration was carried out by using an individual standard gas for each of the gas measured.

***Molecular Analysis***

Overall performance of anaerobic treatment systems is totally dependent on the composition of microbial populations in the anaerobic reactors. Determination of changes in microbial populations and its effect on performance at various operating conditions of a two-stage anaerobic digestion system would be of considerable interest. This part, therefore, examined microbiological aspects, including changes in the number and composition of the acidogenic bacteria in acid phase. The strategy used is summarized in Figure 3.6. This molecular approach was already successfully used to describe various microbial consortia such as soil, blanket bog peat, marine microbial community, hydrothermal vent, human colonic biota and termite gut (Godon et al., 1997a).

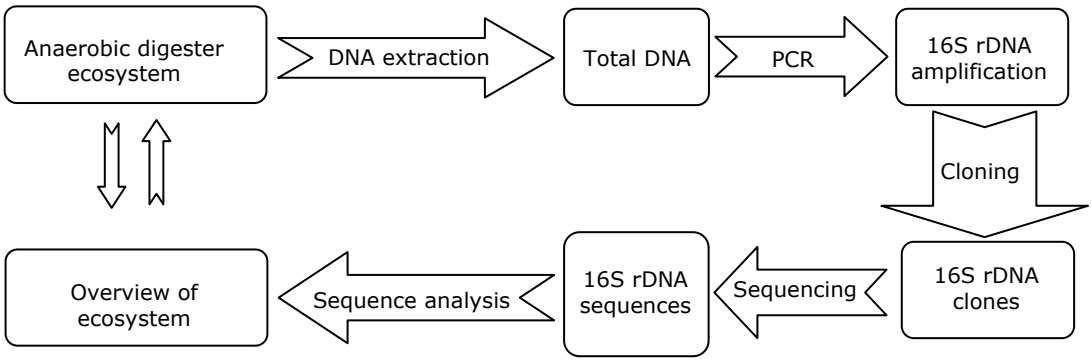


**Figure 3.6.** Strategy applied in determination of microbial consortia (Godon et al.,1997a)

**Extraction and purification of total genomic DNA**

Fifty milliliters were collected from the reactors after completely mixed and DNA (Deoxyribonucleic acid) was extracted by using a modified of the protocol developed by Zhou et al. (1996). Since hexadecyltrimethylammonium bromide (CTAB) performed better in reducing humic contamination, it was used in the buffer for sodium dodecyl sulfate (SDS)-based DNA extraction. Dairy manure aliquots were washed serially in PBS and 0.85% KCl. The 3 mL samples were mixed with 7mL of DNA extraction buffer (100 mM Tris-HCl [pH 8.0], 100 mM sodium EDTA (disodium ethylenediamine tetraacetic acid) [pH 8.0], 100 mM sodium phosphate [pH 8.0], 1.5 M NaCl, 1% CTAB, 5mg/ml lysosyme) and 50 µl of proteinase K (10 mg/mL) by horizontal shaking at 225 rpm for 30 min at 37°C. After the shaking treatment, 1 ml of 10% SDS was added, and the

samples were incubated in a 65°C water bath for 2.5 h with gentle end-over-end inversions every 15 to 20 min. The supernatants were collected after centrifugation at 6,000x *g* for 10 min at room temperature and transferred into 50-mL centrifuge tubes. Supernatants of extractions were mixed with an equal volume of chloroformisoamyl alcohol (24:1, vol/vol). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h. The pellet of crude nucleic acids was obtained by centrifugation at 16,000 x *g* for 20 min at room temperature, washed with cold 70% ethanol, and resuspended in sterile deionized water, to give a final volume of 250 µL.

**Purification of crude DNA extracts**

One-fifth of the crude DNA extract from 3 ml samples was processed in gel plus minicolumn. The extracts were was subjected to gel electrophoresis, and the DNA band was excised, melted and purified by following the rapid protocol of the manufacturer (Genemark gel extraction kit).

**Amplification, cloning, screening, and sequencing of SSU rDNA**

Amplification of SSU (small subunit) rDNA genes from purified genomic DNA from a sample was carried out with primers for conserved domains. PCR was performed by using the protocol adopted by Godon et al (1997b). Three bacterial rDNA gene libraries were prepared (E.coli position 8 to 1509, primers w01-w02) and named R21(35)-1, 2, 3. The primers used are listed in Table 3.4. Each reaction tube contained 0.2 mg of each primer (Table 3.4), 0.2 mg of purified template DNA, 1x Taq reaction buffer (Fermentas), 2.5 mM MgCl2, 22 mM (each) deoxynucleoside triphosphate, and 1 U of *Taq* DNA polymerase (Fermentas), adjusted to a total volume of 50 µl. The reaction mixture was prepared on ice, covered with mineral oil, and placed in a thermocycler (TECHNE, Cambridge, UK). After an initial denaturation at 94°C for 2 min, 25 temperature cycles were performed at 50°C for 1 min, 72°C for 1 min, and 94°C for 1 min and final extension at 72°C 10 min. The PCR products were electrophoresed on a 0.9% agarose gel and viewed by ethidium bromide staining. Bands of the proper size range (ca. 1,500 bases) were excised and eluted with gel extraction kit (Genemark). The purified products were ligated into the pGEMt plasmid (Promega, Madison, Wis.). The ligation products were transformed into *Escherichia coli* DH5-□ competent cells with ampicillin

selection and blue/white screening (Sambrook et al., 1989). Plasmid preparations for DNA sequencing were made with microcolumns as specified by the manufacturer (Genemark). The nucleotide sequences of plasmid inserts were determined by automated DNA sequencing by using the dideoxy chain-termination method (Sanger et al.,1977) and the ABI model 373A sequencer (Applied Biosystems, Perkin-Elmer). Plasmid DNAs were sequenced with the w015 SSU rDNA primer (Table 3.4). A partial sequence of at least 500 bp was performed for each clone.

**Table 3.4.** Sequence and target positions of primers used in this study.

| Name | Sequence | Target | Position* |
|---|---|---|---|
| w01 | AGAGTTTGATCMTGGCTC | 16S rRNA bacteria | F8 |
| w02 | GNTACCTTGTTACGACTT | 16S rRNAuniversal | R1509 |
| w15 | AGCRAACAGGATTAGATAC | 16S rRNA bacteria | F777 |

* The position corresponds to the primer 5' end, using E.coli SSU (small subunit) rRNA as a reference (Brosius et al., 1981); F and R correspond to forward and reverse primer, respectively.

**Sequence analysis**

An equal portion (about 500 bp) of SSU rDNA (*E. coli* positions 812 to 1307) (Brosius et al., 1981), was used for sequence analysis. Homology searches based on the Blast algorithm (Altschul et al., 1990) were performed online (http://www.ncbi.nlm.nih.gov/blast) to identify the closest relatives to the obtained sequences.

Three samples were examined from acidogenic reactor - R21(35) on day 4, 12, and 38 were subjected to molecular analysis. All sample preparations, extractions, clonings, sequencings were done in Department of Biology at Dicle University, and sequence analysis were done by a certified commercial laboratory in İstanbul.

**Nucleotide sequence accesion numbers**

The nucleotid sequence data reported in this study will appear in the GenBank nucleotide sequence database under accession no. EF681621 to EF681748.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1. Optimization of Acidification Conditions

### 4.1.1. The effect of SRT and OLR on acidification

The products of the anaerobic acid-phase digestion may be markedly affected by the specific characteristics of wastewater, operational parameters such as HRT, SRT, and environmental factors such as pH, temperature, reactor configuration, oxidation-reaction potential (ORP), and available trace minerals (Andrews and Pearson, 1965; Ghosh et al., 1975).

An important operational variable, which can be easily controlled, is the HRT. It governs the amount and type of substrate being used by the cells. Since anaerobic digestion is a two-phase process, HRT can act as a selection parameter for the acidogenic phase only if it encourages the growth of acid formers and concurrently suppresses the growth of methane producers.

The degree of acidification indicated a general tendency to vary proportionally with HRT and inversely with organic loading rate and initial substrate concentration (Dinopoulou et al., 1988).

In order to examine the influence of SRT and organic loading rate on the performance of the acidogenic phase of the anaerobic digestion, the degree of acidification, the product formation and finally the effluent composition of the effluent were investigated.

Nine acidogenic anaerobic reactors were operated for 57 days to determine the optimum SRT and OLR values resulting in maximum acidification and in turn VS reduction. Three different OLRs (5, 10 and 15 g VS/L.day) were applied to the reactors. For each OLR value, three SRTs (1.25, 2 and 4 days) were studied. The reactors were observed for different parameters (pH, cumulative gas production (CGP), TS, VS, gas composition, tVFA and sCOD). The data obtained in the experiments were presented in Figures 4.1-4.9. The summary of results are given in Figure 4.10 in terms of the change in the operating parameters (pH, tVFA, VS, CGP, methane content and sCOD) with respect to the combination of OLR and SRT values. The data used in Figure 4.10 covers the data beyond the average steady-state conditions for each reactor. In other words, the data within the first "3xSRT" days (12 days for R1-R3, 6 days for R4-R6, and 4 days for R7-R9) were not included.

The pH values in all 9 reactors showed the same trends (Fig.4.1-4.9). At the beginning of the reactor operation, pH values sharply decreased within 6 to 8 days; afterwards a slight increase was observed, and finally they reached a steady pH range.

Daily gas production revealed a variation with respect to OLR and SRT; increased upon the increase of OLR and SRT. After reaching the steady state conditions, daily gas productions of R1-R9 were 35, 90, 120, 25, 45, 60, 10, 25, and 25 mL/day, respectively (data were not shown). The maximum daily gas production was observed at an OLR and SRT of 15 g VS/L.day and SRT of 4 days, respectively.

It was observed that $N_2$ concentrations decreased with increasing SRT and decreasing OLR. On the other hand, $CH_4$ and $CO_2$ concentrations depicted a proportional trend with variations in SRT and OLR. For example, at an OLR of 15 g VS/L.day, upon increase of SRT in the order 1.25, 2 and 4 days, the $N_2$ content in the corresponding reactors (R9, R6, R3) decreased as 71%-53% and 36%, whilst, $CH_4$ and $CO_2$ contents were 14%-22%-30% and 15%-25%-30%, respectively.

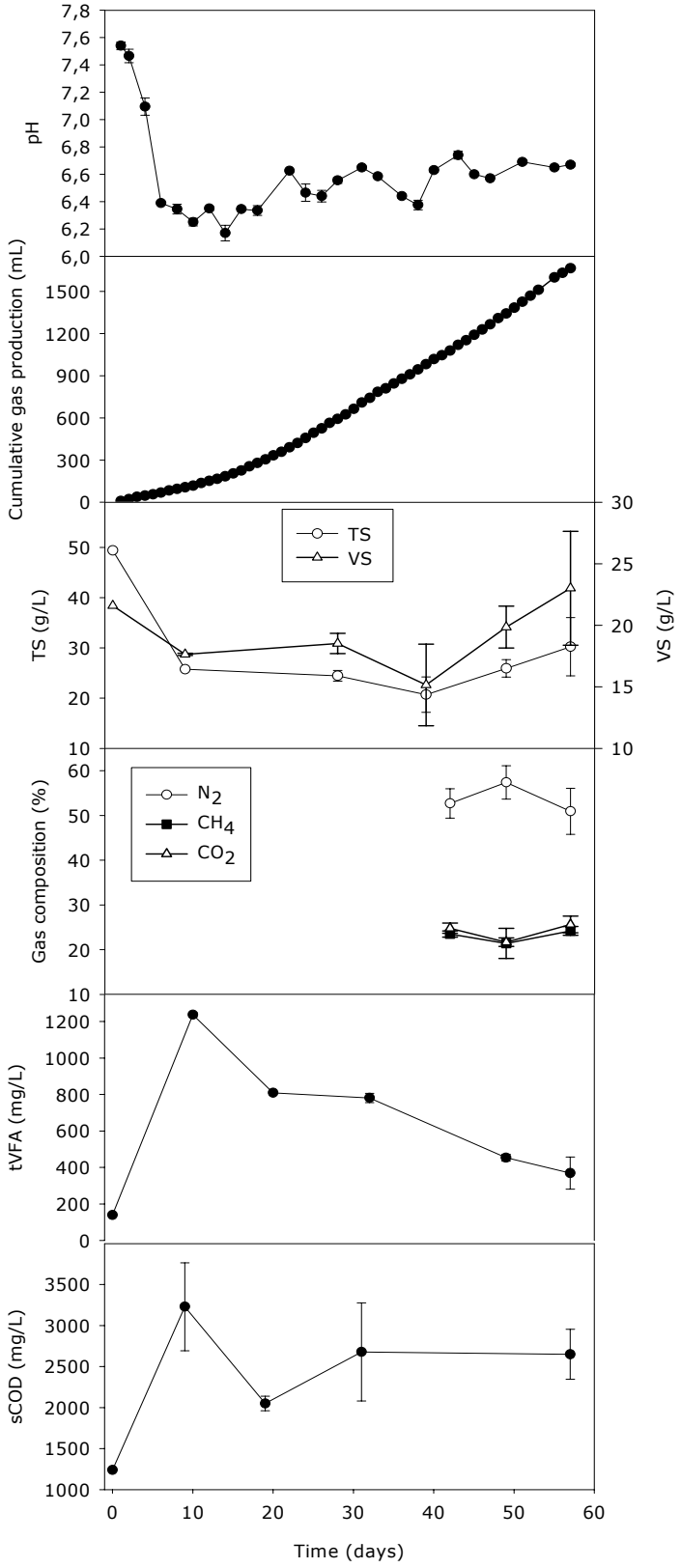


**Figure 4.1.** The observed parameters for Reactor 1 (SRT: 4 days, OLR: 5 g VS/L.day)

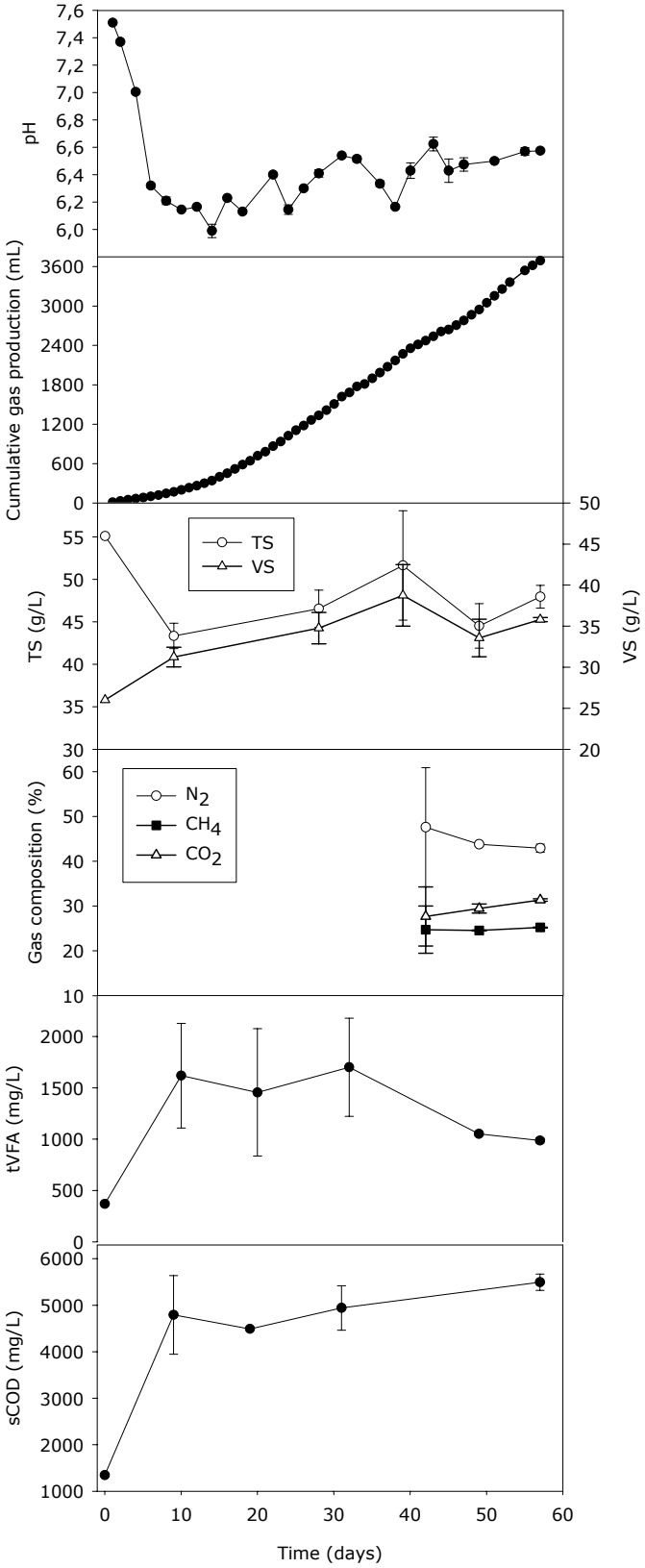


**Figure 4.2.** The observed parameters for Reactor 2 (SRT: 4 days, OLR: 10 g VS/L.day)

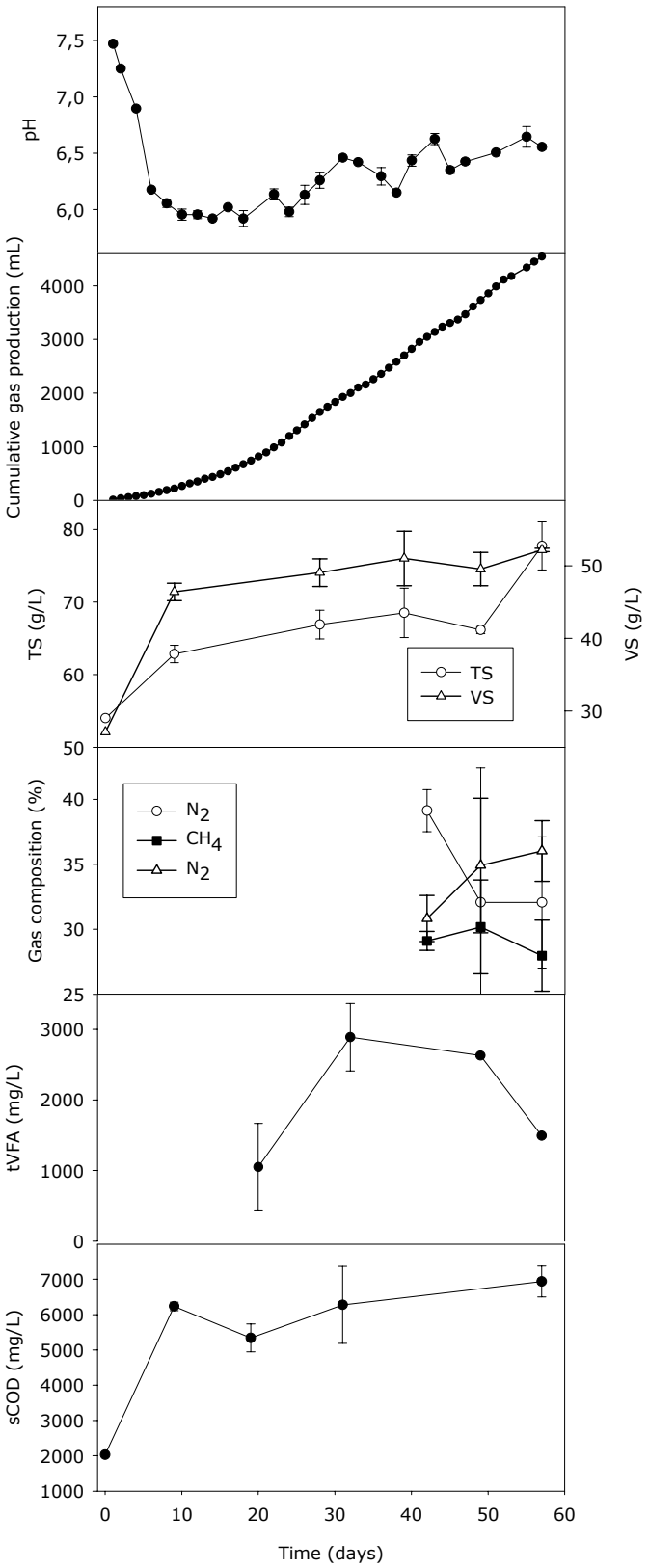


**Figure 4.3.** The observed parameters for Reactor 3 (SRT: 4 days, OLR: 15 g VS/L.day)

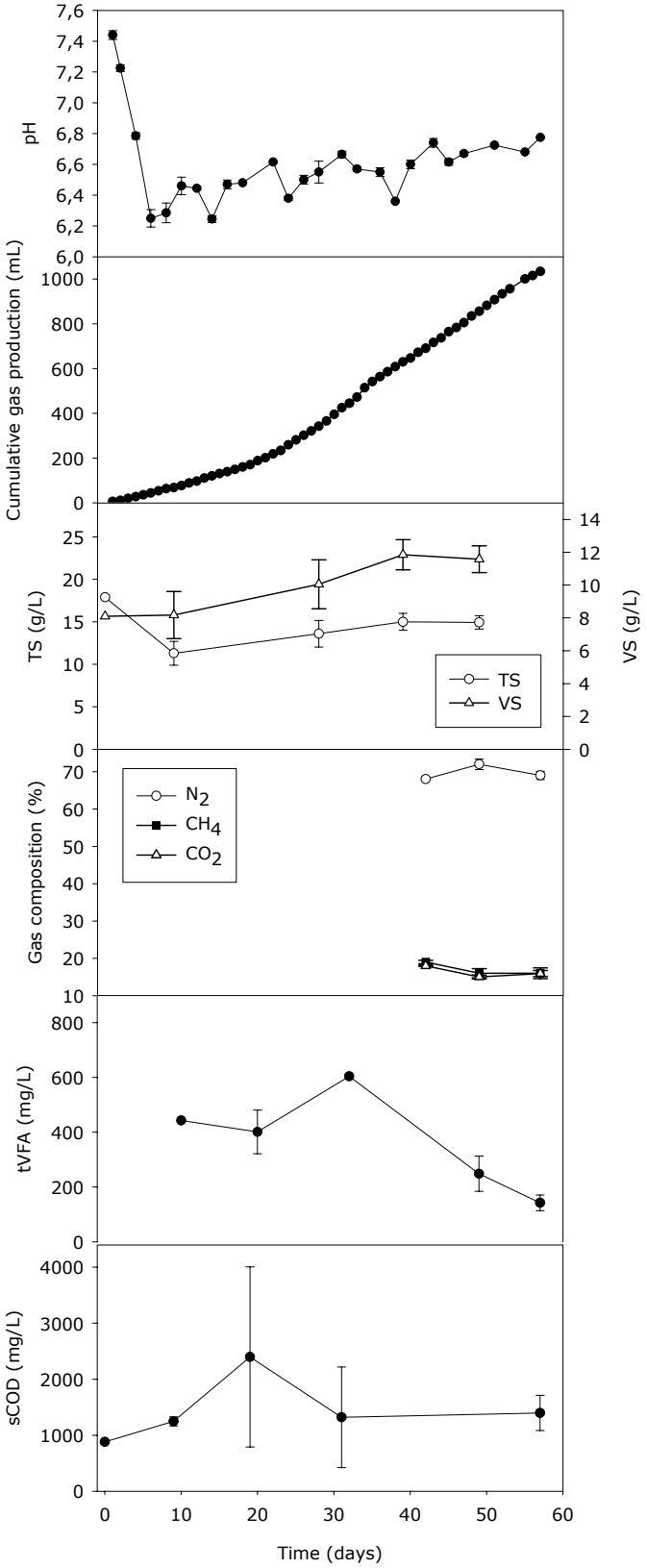


**Figure 4.4.** The observed parameters for Reactor 4 (SRT: 2 days, OLR: 5 g VS/L.day)

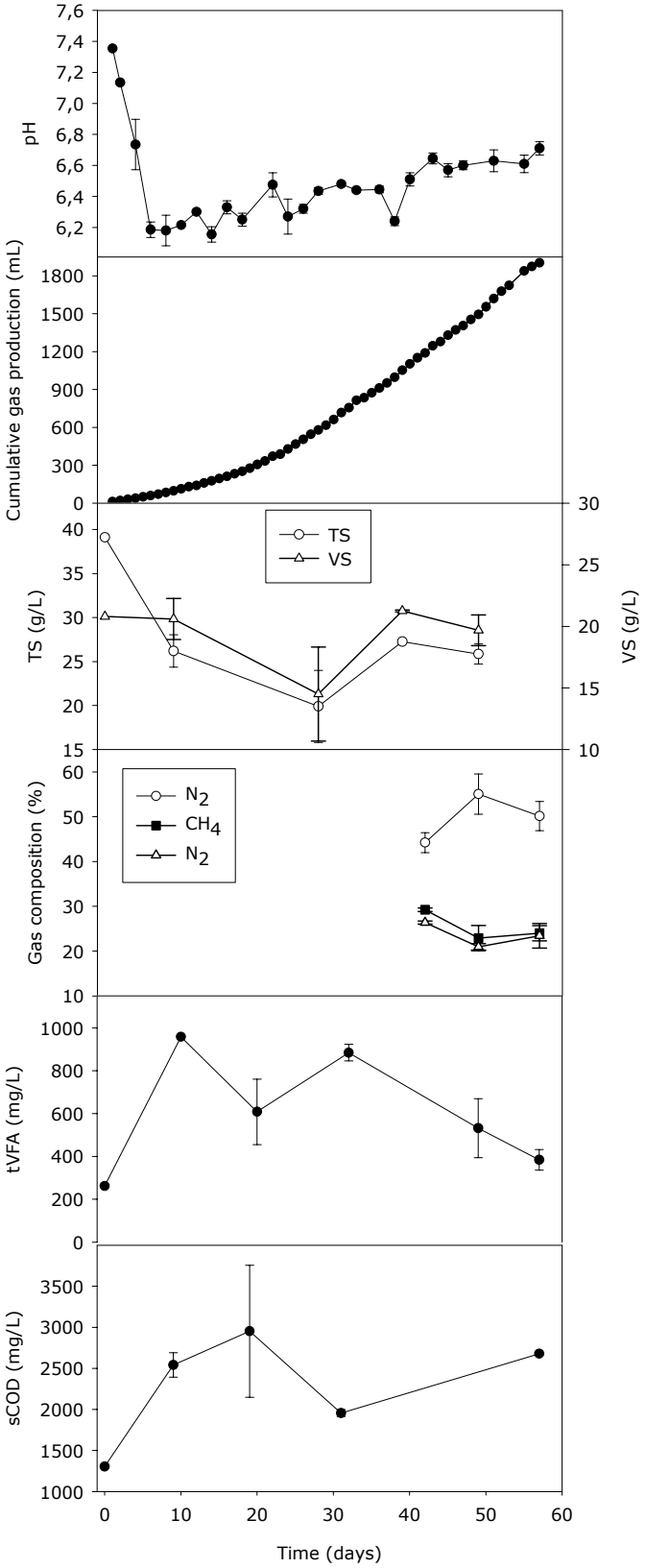


**Figure 4.5.** The observed parameters for Reactor 5 (SRT: 2 days, OLR: 10 g VS/L.day)

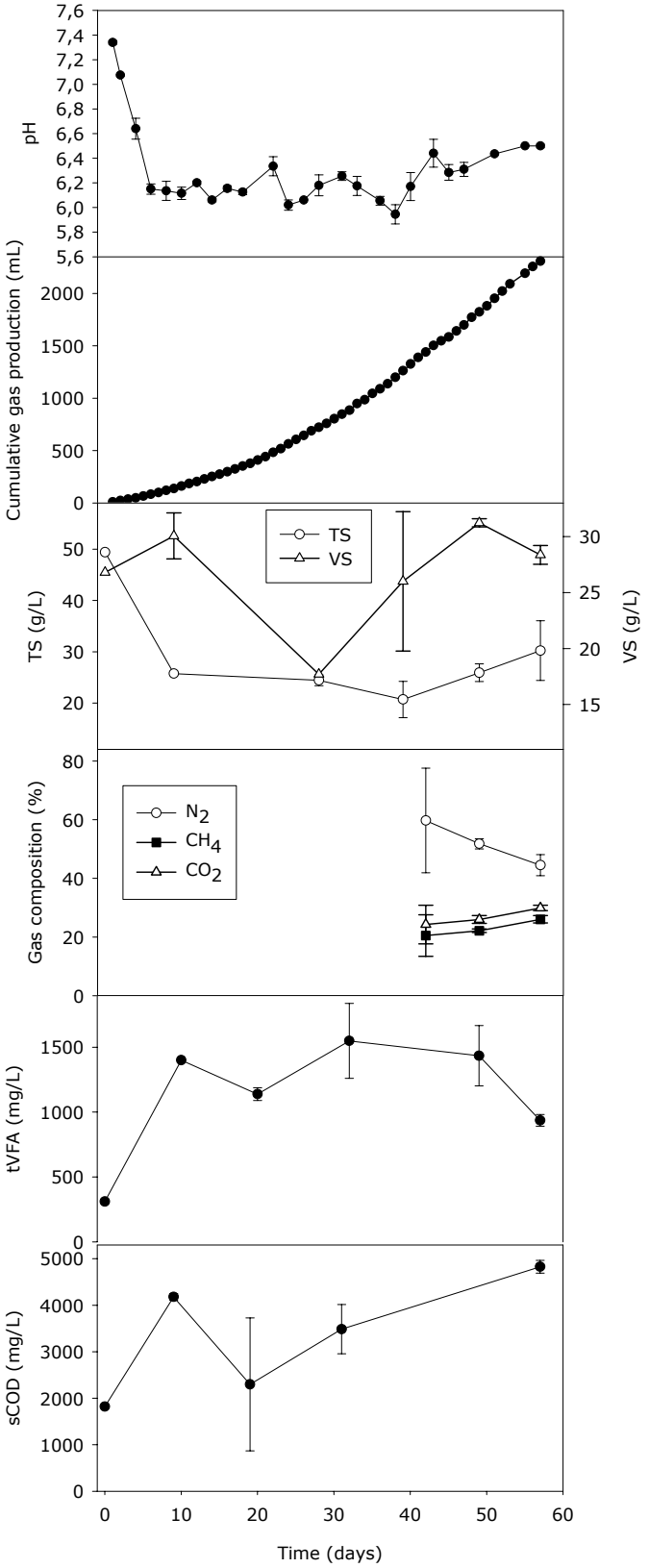


**Figure 4.6.** The observed parameters for Reactor 6 (SRT: 2 days, OLR: 15 g VS/L.day)

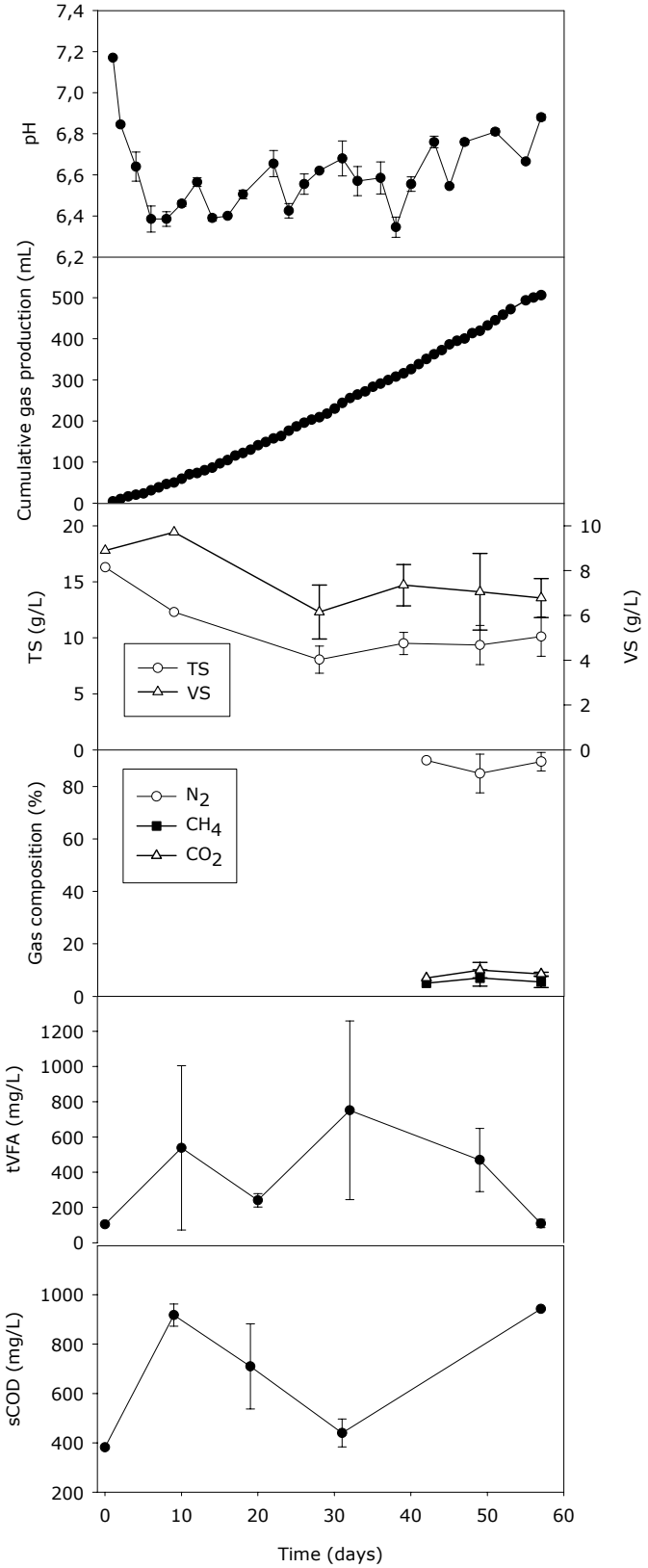


**Figure 4.7.** The observed parameters for Reactor 7 (SRT: 1,25 days, OLR: 5 g VS/L.day)

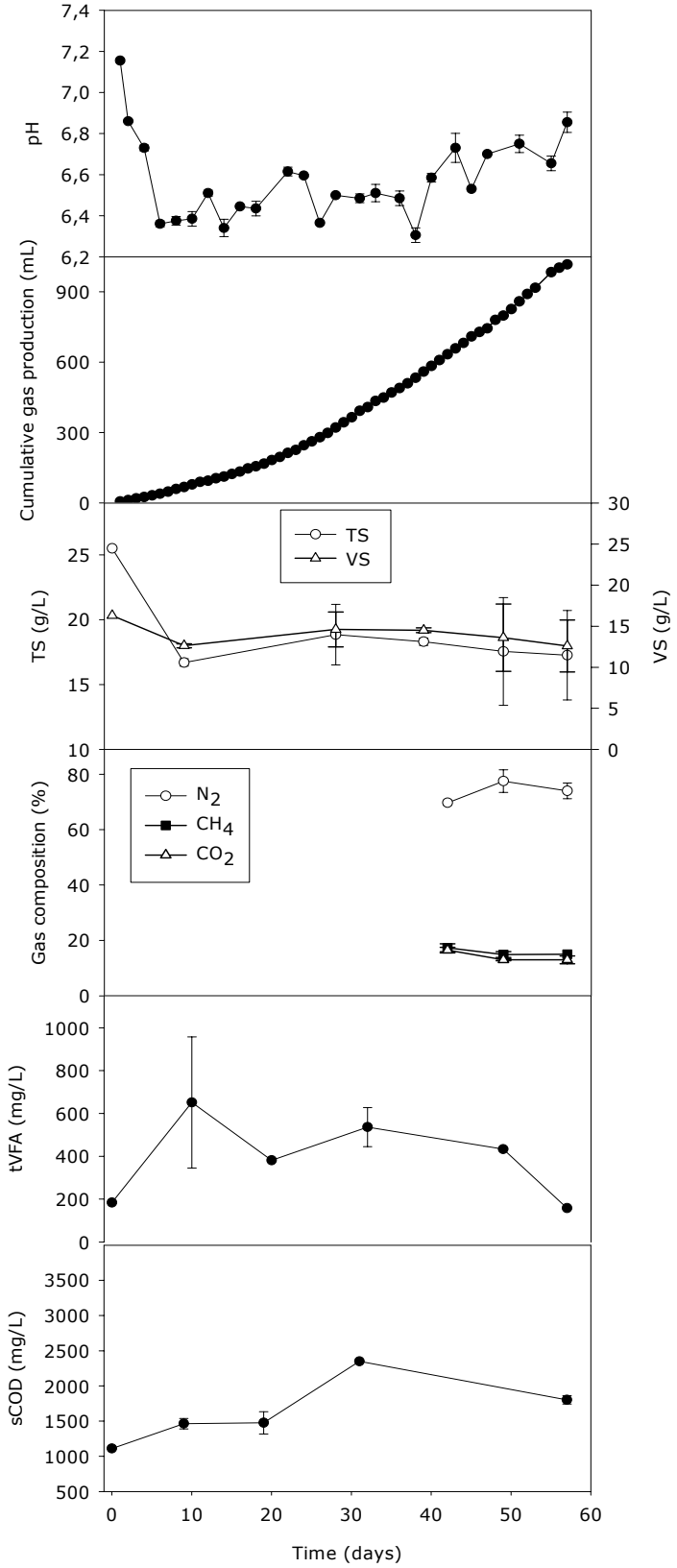


**Figure 4.8.** The observed parameters for Reactor 8 (SRT: 1,25 days, OLR: 10 g VS/L.day)

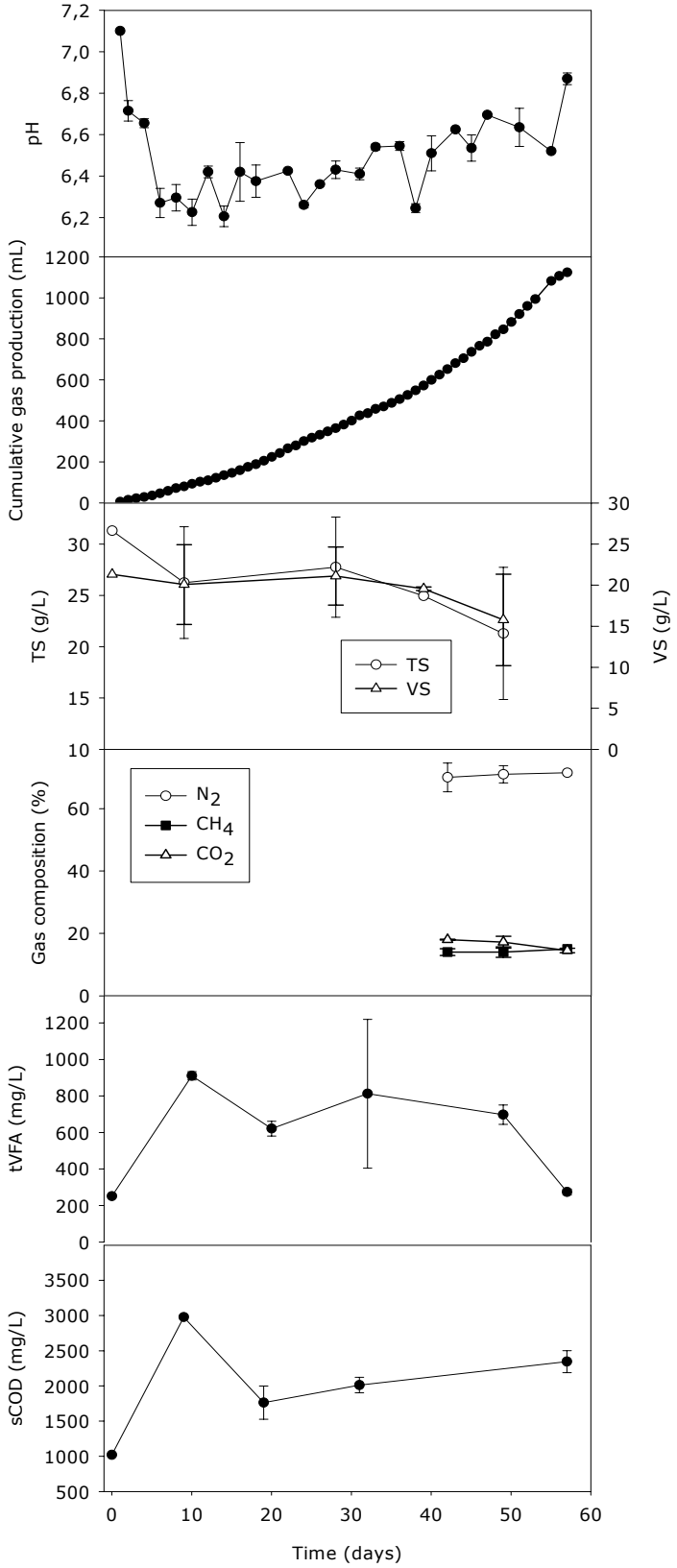


**Figure 4.9.** The observed parameters for Reactor 9 (SRT: 1,25 days, OLR: 15 g VS/L.day)

The tVFA and sCOD profiles indicated similar trends. At the beginning of the reactor operation, a rapid increase was observed in the concentration of both of the parameters. Afterwards, sCOD values were nearly constant, while tVFA concentrations showed a declining trend towards the end of the experiments in all reactors. This declining trend could be acceptable since these acidogenic reactors were not completely run as in acidification phase. Therefore as time passing some methanogens grow and started to consume the produced VFA in acidification phase. This decreasing in tVFA concentrations were consisted with methane content increasing in reactors. The tVFA concentrations increase as OLR increases for the same SRTs in all reactors.

As seen in Figure 4.10.a, pH drop was inversely proportional with the increase in the SRT for each OLR studied. Similarly, for each SRT studied, as the OLR increased, pH decreased. It was observed that the extent of pH drop increased with the increase in the OLR being smallest for the lowest OLR of 5 g VS/L.day. Besides, it should be noted that the extent of pH drop was also affected by the SRT. For all the OLRs studied, the extent of pH drop for the SRT increase from 1.25 to 2 days was greater than that observed for SRT of 2 to 4 days. It is a well known fact that low retention times and high loading rates lead to higher acidification in two-phase systems. However, as seen in Figure 4.10.a, average pH values observed in the reactors were within 6.2-6.6 and the extent of pH drops was lower relative to acidification of other high solid substrates such as organic fraction of municipal solid wastes. Han et al. (2002) operated the MUSTAC (multi-step sequential batch two-phase anaerobic composting) process to recover methane and composted material from food waste, where the pH ranged between 6.5 and 7.0 during acidogenic fermentation step. In another research, Kübler and Schertler (1994) demonstrated that favourable pH condition was 6.7 in the three-phase anaerobic degradation of solid waste. Verrier et al. (1987) stated that both mesophilic and thermophilic liquefaction and acidogenesis of vegetable solid wastes were found to be maximal when the pH was maintained at approximately 6.5 in the hydrolysis reactor. The relatively high pH values observed in this study can be explained by the alkalinity generated by the anaerobic biodegradation of nitrogenous organic compounds contained in the dairy manure used in this study (Ghosh, 1987; Speece, 1996; Wang et al., 2003). The similar self-buffering capacity of the

manure was also observed in other anaerobic acidification studies (Demirer and Chen, 2004/2005).

As expected, the increase in the OLR resulted in the increase in the tVFA production (Fig. 4.10.b). In addition, the extent of tVFA production for the SRT increase from 1.25 to 2 days was greater than that observed for SRT increase from 2 to 4 days especially for OLRs of 10 and 15 g VS/L.day. This observation was also verified by the extent of pH drop (being greater for SRT increase from 1.25 to 2 days). These tVFA production trends for all reactors coincided with the sCOD productions (Fig. 4.10.c) which increased with the increased OLR and SRT.

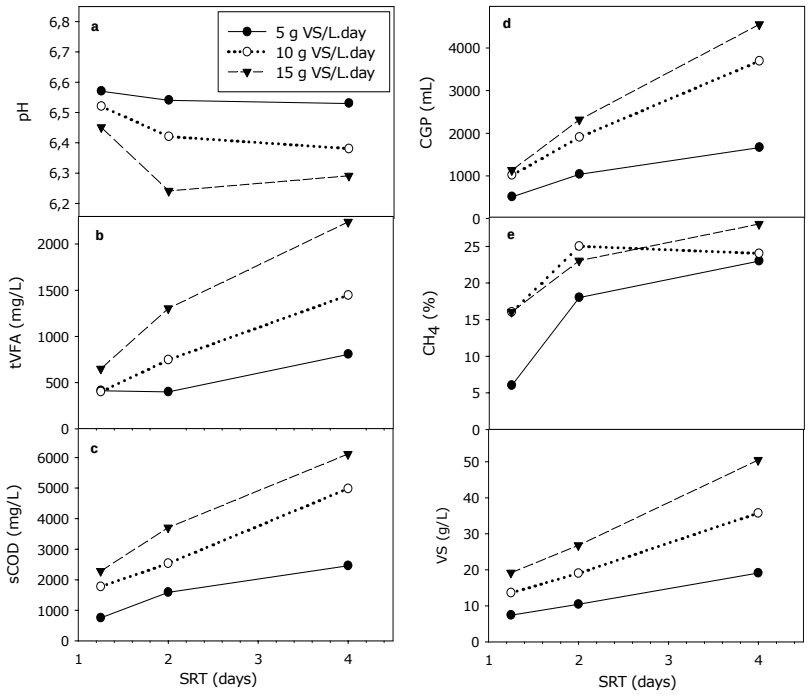


**Figure 4.10.** The monitored parameters for all reactors with respect to their SRT's
(● : 5 g VS/L.day, ▼: g VS/L.day, ○: g VS/L.day)

Gas production enhancement is the ultimate goal in the two-phase anaerobic digestion process. The acidification phase is generally characterized by a very low gas production, mostly in the form of $CO_2$, $N_2$, and $H_2$, which are by-products of many pathways followed for substrate metabolism.

The effect of SRT and OLR was also observed for cumulative gas production (CGP) data. As the OLR and SRT increased the CGP in the reactors increased (Fig. 4.10.d). It is well known that in addition to VFAs and alcohols both $H_2$ and $CO_2$ are produced through anaerobic acidification. However, GC analyses unexpectedly indicated that methane was produced in all of the reactors studied at varied OLRs and SRTs (Fig. 4.10.e). Ideally, the methane content in gas produced in reactors should be negligible. In practice, however, varied amounts of methane have been detected in acid-phase digesters (Eastman et al., 1981; Ghosh, 1987). This may be due to either incomplete separation of the two phases, which results in the coexistence of heterotrophic methane producers, or the presence of certain fast-growing autotrophic methanogenic organisms such as Methanobacterium, or both. Especially, methane percent of the biogas increased from 5 to 15-27% when SRTs and OLRs were increased to greater values than 1.25 days and 5 g VS/L.day, respectively. Although the pH conditions were close to the optimum operating conditions of highly organic wastes required for acetogenesis. The applied SRT values (1.25 to 4 days) were not favorable for the most sensitive anaerobic bacteria type known as methanogens. The methane production at such low SRTs could be explained by unintentional extended retention times of microorganisms in the reactors due to very high solids concentration and thus lack of homogeneity during daily wasting of sludge (Ghosh, 1985, 1987). GC analyses also indicated a significant amount of $N_2$ in the biogas of all reactors changing from 35 to 90 % (data was not shown). As expected, denitrification was more dominant at the higher oxidation-reduction potential at the beginning of the experiment. Denitrification might occur during the acidogenic phase, so as to achieve simultaneous VFA production and nitrate elimination, a system could be applied to organic carbon and nitrogen removal from the wastes (Rustrian et al., 1998; Vigneron et al., 2007). In the experiments of Set IV, the $CH_4$:$CO_2$:$N_2$ percentage was 30:35:35 (by volume, see Section 4.3), which indicates similarity with the range reported in the literature for the acid-phase step (Ghosh et al., 1975; Fongsatitkul, 1992). Although the importance of the separation of the acidogenic and methanogenic phases is well known, only a few studies were carried out for the investigation of the acidogenic phase of AD with manure and they have not focused on the formation of nitrogen in the biogas.

Better hydrolysis in acidification process means higher VS reduction. Therefore, in addition to pH and tVFA production, VS is among the critical parameters in the determination of the acidification extent of dairy manure known with its high solids content. The average VS concentrations observed in the reactors at varied SRT and OLR combinations were given in Figure 4.10.f. It was observed that increasing the OLR and SRT resulted in the VS accumulation. However, due to the continuous feeding and wasting process, such an accumulation may not clearly indicate the possible VS reduction in the reactors. Therefore, a completely stirred tank reactor (CSTR) model was used to observe the change in the VS content of the reactors at steady-state conditions. In this CSTR model, each reactor was assumed as operating under feeding and wasting process without any destruction/degradation of the substrate (Fig. 4.11). Therefore, the comparison of the model output and the experimental data for each reactor would yield the corresponding VS reduction.

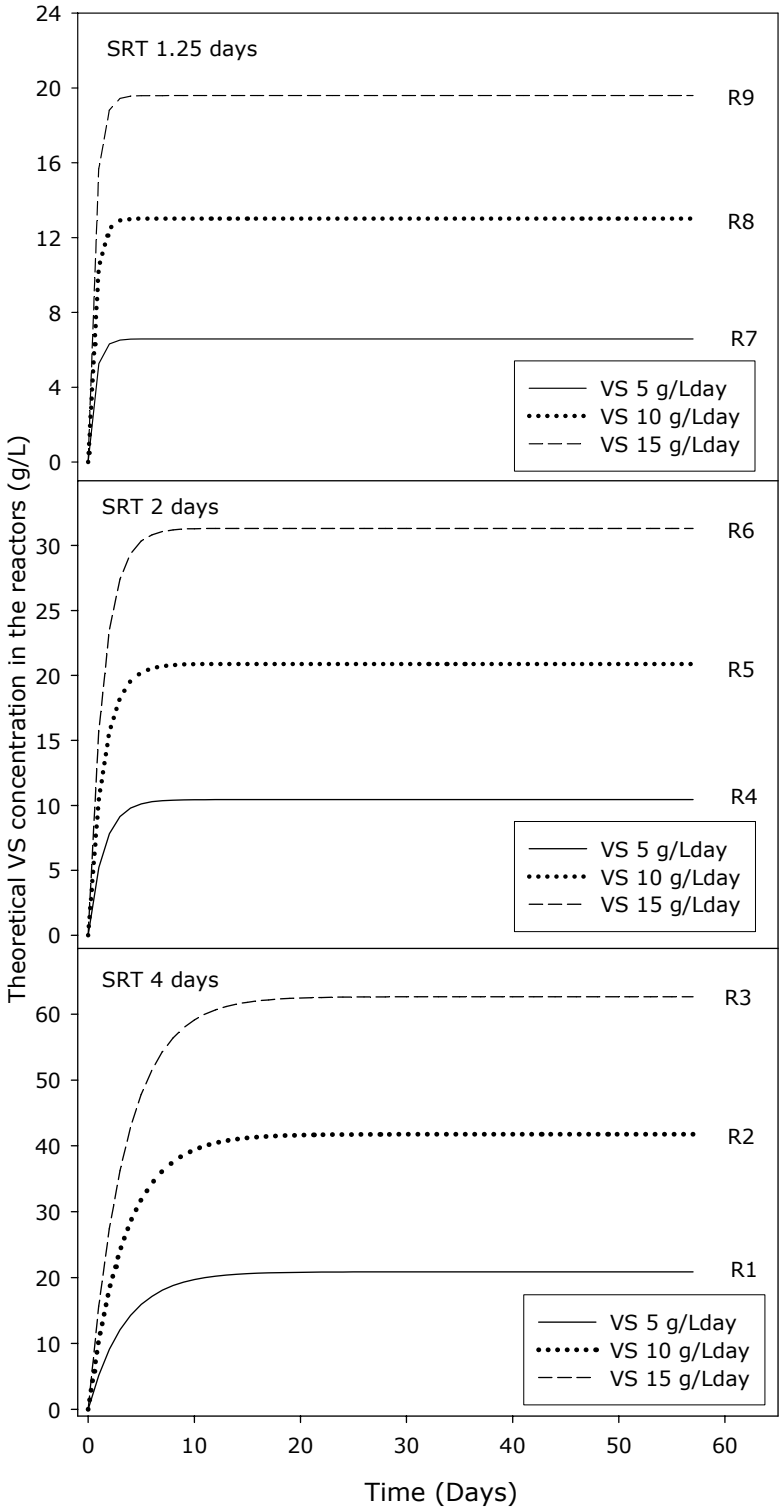


**Figure 4.11.** Theoretical VS accumulation in the reactors assuming there is no VS reduction

Experimentally determined average VS concentrations (Fig. 4.10.f) and theoretically calculated VS concentrations (Fig. 4.11) of the reactors were used in Figure 4.12 to assess how much VS reduction at each OLR and corresponding SRT value were obtained. For better comparison, percent VS reductions in each reactor were calculated by considering the theoretical and experimental VS concentrations and given in Table 4.1.

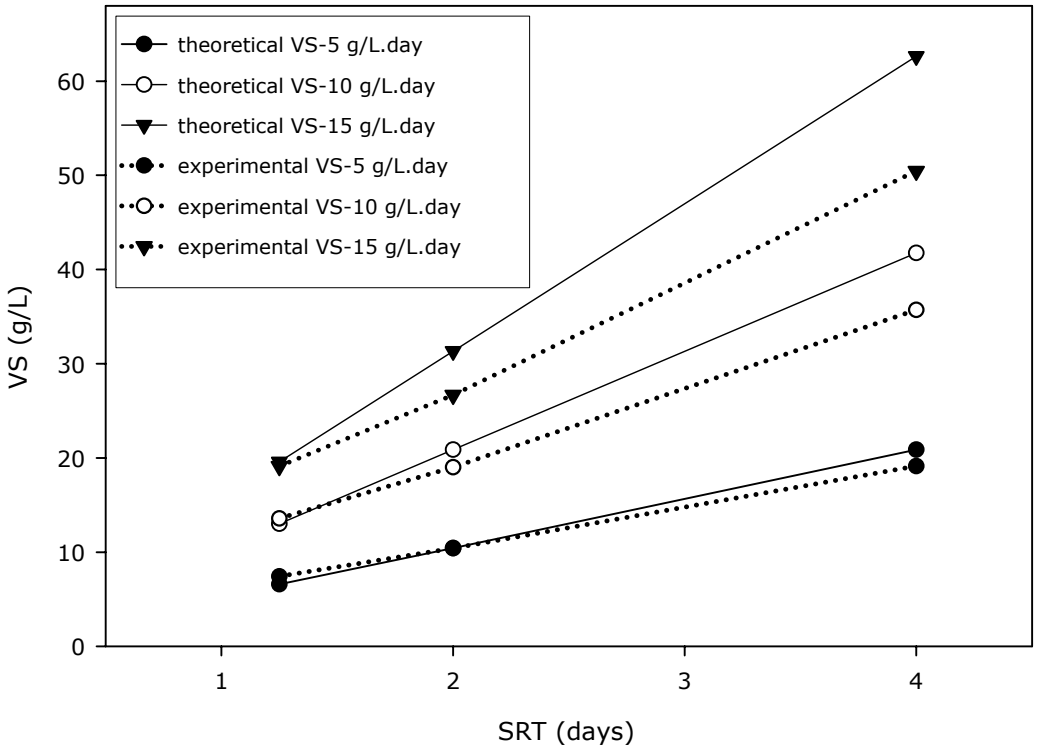


**Figure 4.12.** Theoretical no VS reduction and experimental VS concentrations in the reactors at steady-state

As can be seen in Figure 4.12 and Table 4.1, as OLR and SRT increased as the percent VS reduction increased. The highest VS removal was seen at R3 with 19.5 %, which is followed by R6 with 14.8%. The third highest VS removal was observed in R2 with 14.5%. The rest of the reactors did not display a significant VS reduction performance. Especially in the reactors operated at SRT of 1.25 days (R7-R9) almost no VS destruction was observed (Table 4.1). There are limited investigations on the anaerobic acidification of manure in the literature. The performance of a novel high-rate anaerobic process, the anaerobic digestion elutriated phased treatment (ADEPT) process, for treating a slurry-type piggery waste (55 g COD/L and 37 g TS/L) was investigated by Ahn et

al.(2004). VS reduction by the hydrolysis was found as 10%. An another ADEPT process with SHARON (Single reactor system High Ammonium Removal Over Nitrite) and ANAMMOX (Anaerobic Ammonium Oxidation) processes were operated for the purpose of resource recovery and nitrogen removal from slurry-type piggery waste. The ADEPT operated at acidogenic loading rates of 3.95 g sCOD/L.day, VS reduction was determined as 13%. Ghosh (1991) determined 25% VS reduction by using pilot-scale two-phase anaerobic digestion of waste activated sludge with HRT of 3.1 days and OLR of 18.9 kg VS/$m^3$.d. The high fiber levels contained in the dairy manure digestion temperature, mixing intensity, etc. may contribute to lower VS reductions in acidification stage. The VS conversion data in related literature are very consistent with this study results.

**Table 4.1.** The comparison of the reactors operated in Set I with selected parameters

| Reactor | VS Reduction (%) | tVFA (mg/L as HAc) | pH |
|---|---|---|---|
| 1 | 8.4 | 806 | 6.53 |
| 2 | 14.5 | 1444 | 6.38 |
| 3 | 19.5 | 2236 | 6.29 |
| 4 | 0 | 399 | 6.54 |
| 5 | 8.9 | 476 | 6.42 |
| 6 | 14.8 | 1300 | 6.24 |
| 7 | 0 | 412 | 6.57 |
| 8 | 0 | 400 | 6.52 |
| 9 | 2.3 | 647 | 6.45 |

***Degree of acidification***

Reactors and their acidification performances were also compared in terms of acidification extent and the rate of product formation. The degree of acidification can be quantified using the percentage of the initial substrate concentration converted to VFAs (Dinopoulou et al., 1988). The initial substrate concentration (*S*i) was measured in mg total COD/L and the quantity of VFAs was converted to the theoretical equivalent in mg COD/L (*S*p), using the COD equivalents for each VFA (Demirel and Yenigun, 2004). The following formula was used to express the degree of acidification in this work:

Degree of acidification *(%)* = *(Sp/Si)* × 100 ……..……(5)

The COD equivalents of each volatile acids for the conversion were taken as follows: acetic acid, 1.066; propionic acid, 1.512; butyric acid, 1.816; valeric, 2.036; caproic acid, 2.204 (Demirel and Yenigun, 2004).

Higher organic loadings and shorter SRTs were previously reported to provide the optimum conditions for the acid-forming bacteria (Gosh et al., 1985). According to previous studies, HRT was reported to greatly affect the VFA production and distribution (Elefsiniotis and Oldham, 1994). It is seen that the OLR was not affected the production of VFA (Fig. 4.13). Hydrolysis is reported to be the rate-limiting step during anaerobic digestion of complex organic waste. The rate of hydrolysis depends on the extracellular enzymes produced by fermentative acidogens, the biomass concentration, the substrate concentration and the specific surface area of the particulate (Eastman and Ferguson, 1981). SRT influenced the degree of acidification rather than OLR in this work. Parawira et al. (2004) researched the production of volatile fatty acids by anaerobic digestion of solid potato waste was investigated using a batch solid waste reactor. The ratio of VFA to COD was found as 0.5 independent of OLR. As the biodegradibility of potato waste is much higher than manure the ratio of VFA production is also higher. The degree of acidification was higher at low SRTs, but when OLR increased to 15 g VS/L.day the acidification degree was also increased (Figure 4.14). Bouallagui et al. (2004) indicated that the two-phase anaerobic digestion of a mixture of fruit and vegetable wastes using two coupled anaerobic sequencing batch reactors operated at mesophilic temperature. The acidification reactor was operated at a constant HRT of 3 days and fed with different dilutions of wastes to change the OLR. Acidification yields were obtained as 40.3, 38.9, and 44.4% for each different OLRs. These yields were 25-30% higher than our results. This difference was mainly caused by the characteristics of wastes as explained in the above sentence. The highest acidification degree was observed in R6, except R7. This is one of the parameters to choice better conditions for acidification phase, and R6 was the better than the other reactors. More detailed discussion on acidification is given on Section 4.3.

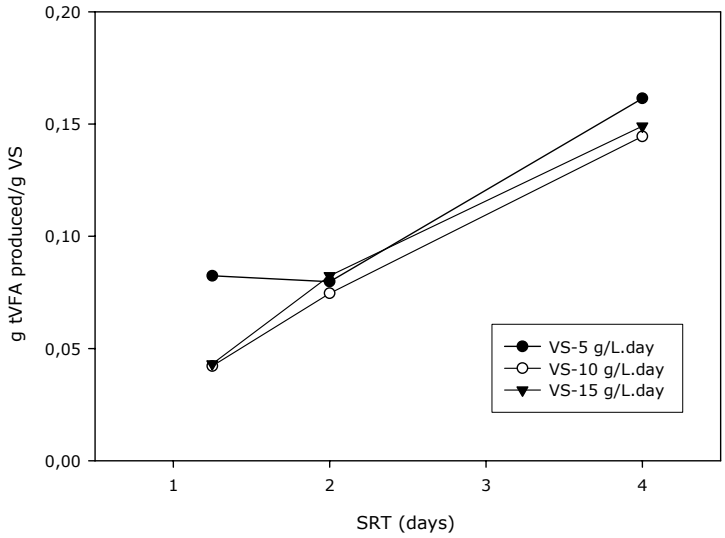


**Figure 4.13.** The rate of tVFA produced per gram VS feed and at different SRTs

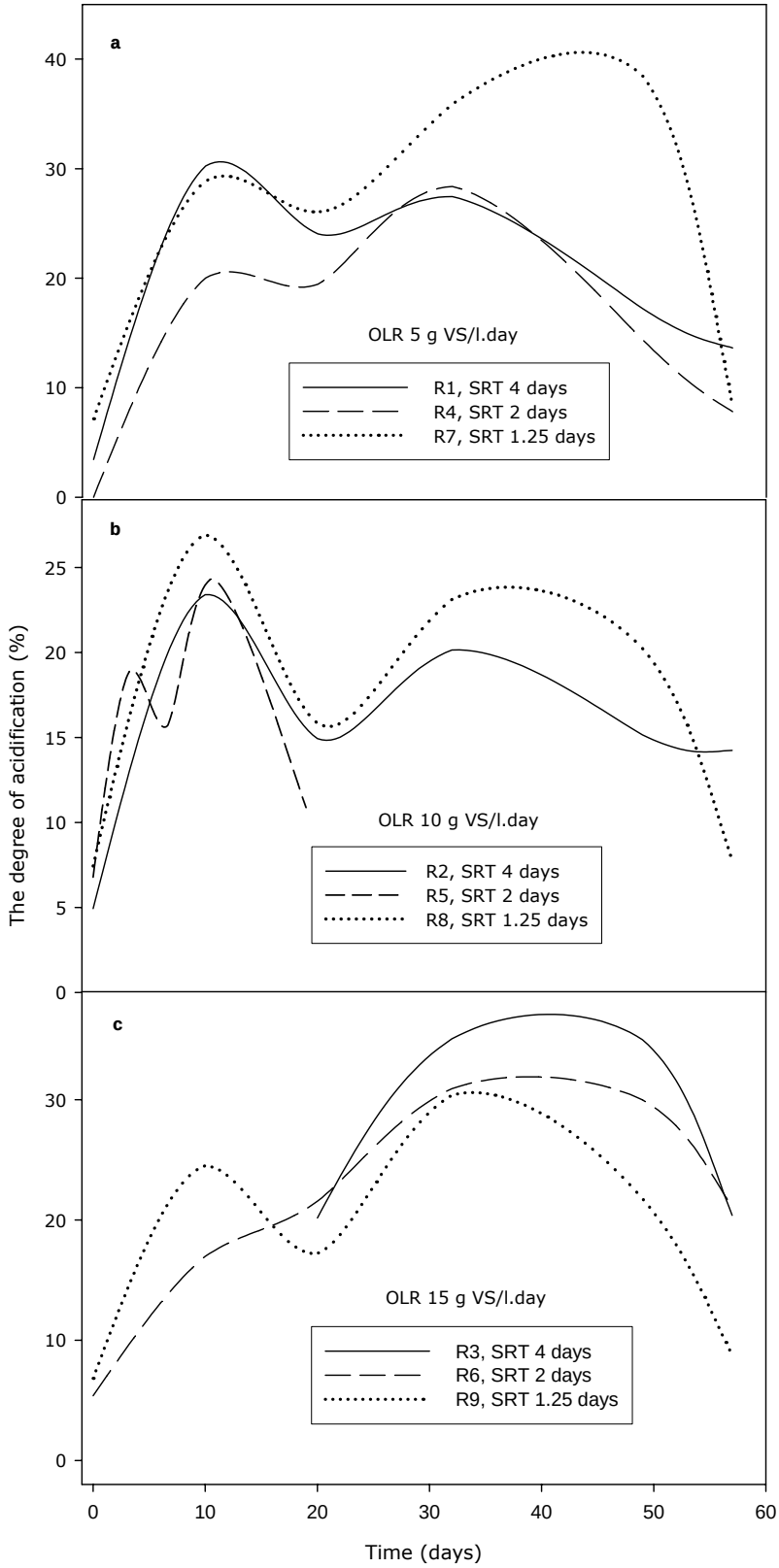


**Figure 4.14.** The degree of acidification at different OLRs

### 4.1.2. The effect of pH control on acidification

As previously mentioned, R6 in Set I yielded the second highest VS reduction (14.8%) and the lowest pH among all the reactors operated (Table 4.1). Moreover, tVFA concentration and degree of acidification in R6 was greater than most of the reactors (Fig. 4.10.b and 4.14). It is known that the increase in SRT (or HRT) value results in the increase in the investment and capital costs of the treatment systems. Therefore, considering both from the economical point of view and acidification performance in terms of tVFA production and VS reduction, SRT of 2 days and OLR of 15 g VS/L.day were selected as the optimum operational conditions for acidification of dairy manure (R*). The selected conditions were used in the second part of the study where the optimum pH or pH effect on acidification of dairy manure was investigated in two reactors (Set II). The selected operating parameters were also harmonious with the relevant literature (Andrews and Pearson, 1965; Ghosh, 1987; Demirer and Chen, 2005). SRT (or HRT) is an important parameter in the process as it controls the contact time between the bacteria and the substrate. In general, researches have indicated that the net VFA production increases with an increase in the SRT, with certain limitations. Andrews and Person (1965) have indicated that the increasing trend follows till a HRT of 2.4 days. In an another research to study the relative efficiency of two-phase and single-stage, high-rate anaerobic sludge digestion conducted by Ghosh (1987) selected a HRT of 2 days. The advantages of two-phase AD for unscreened dairy manure were investigated by Demirer and Chen (2005). In their two-phase configuration, 10 days of SRT was selected which consisted of an acidogenic first phase with a SRT of 2 days and methanogenic second phase with a HRT of 8 days.

In order to investigate the effect of pH control, pH was set to a constant value between 5.3-5.5 using a pH-stat unit in one of the reactors (R*-pH). In the other reactor (R*), pH was not controlled. Both reactors were operated for 42 days. During this period, pH varied between 6.2-6.4 in R* (pH-uncontrolled reactor) while it was 5.3-5.5 in R*-pH. The optimum pH range of acidogenic bacteria is 5.2-6.5 while it is 6.6-8.5 for acetogenic/methanogenic bacteria (Demirer and Chen, 2004). Thus, it was thought that methanogenesis which was experienced in the acidogenic reactors in Set I of the study could be eliminated and optimum acidification conditions and in turn maximum

acidification could be achieved in R*-pH at a lower pH of 5.3-5.5. As expected, methanogenesis was inhibited in R*-pH, while methane percent of the biogas content varied around 5-7% in R* (Fig. 4.15.a). However, acidification efficiency was just the opposite of the expectations. In terms of tVFA production, uncontrolled reactor (R*) displayed a better performance. Peak tVFA concentration observed in R* reached up to 2300 mg/L (as HAc), while it was only 980 mg/L in R*-pH (Fig. 4.15.b-c). The effect of pH on hydrolysis and acidogenesis of suspended organic materials in terms of volatile suspended solid (VSS) solubilization, specific acid production, and soluble COD production were evaluated by Kim et al. (2003). The greatest degree of solubilization was observed at pH of 6.5 in terms of VSS removal and sCOD production. Dinopoulou et al. (1987) ascertained the influence of operational parameters, such as HRT, OLR, pH, and temperature on the performance of the first phase AD. The optimum pH in order to achieve both a high degree of acidification and a high rate of acid production was found to be 7.0.

Better hydrolysis and in turn acidification conditions were also verified by the sCOD analyses where the concentrations of R* were equal and greater than that of R*-pH most of the time (Fig. 4.15.d). In both of the reactors, acetic and propionic acids were the main VFA products, whereas butyric, *i*-butyric, valeric, i-valeric and caproic acids were also present, but in substantially lower quantities. The main fermentation pathway was found as acetic acid fermentation, which was mainly suppressed in the R*-pH, thus resulted in lower tVFA production. This is relevant with the literature reporting the favourable pH conditions for acetic acid production as 6.0-6.5 (Yu and Fang, 2001).

A similar approach to Set I was used in Set II to compare the VS removal efficiency of the reactors. A CSTR model was used to predict the theoretical VS concentrations in the reactors under the operational case of feeding/wasting but no degradation/destruction. The theoretical VS and experimental VS concentration of the reactors is depicted in Figure 4.15.e. The effect of pH on the degree of acidification is shown in Figure 4.16. It can be easily seen that uncontrolled reactor performed better acidification than the pH-controlled reactor. The degree of acidification achieved during the current study varied between 10 and 25%, 4 and 12% with uncontrolled and controlled reactors,

respectively. The pH controlled reactor was operated under the range of 5.3-5.5. This pH interval might not be favorable for the highest acidification degree achievable, so new pH ranges should be investigated. For example, as Yu and Fang (2001) indicated the optimum pH was 6.0-6.5. The influence of operational parameters, such as hydraulic retention time, organic loading rate, influent substrate concentration, pH, and temperature, on the performance of the first phase of anaerobic digestion has been investigated by Dinopoulou et al. (1988). A complex substrate based on beef extract was used in their experiments. The predominant fermentation products were always acetic and propionic acid, independent of the values of the operational parameters. The optimum pH and temperature were 7 and 40°C, respectively.

The pH control did not result better acidification in terms of VFA production and VS reduction in this study. sCOD concentrations were similar in both pH-controlled and uncontrolled reactors. The only main difference was observed in the gas compositions. While the pH-uncontrolled reactor produced methane, pH-controlled reactor did not. But as mentioned above, the methane from acidification phase could be transmitted to methanogenic phase of the system to increase the overall system efficiency (Ghosh, 1987). Mixing is also another important operational parameter, as the temperature, pH, etc. Therefore, the reactors in Set I and Set II experiments were operated under mixing conditions. But in Set IV experiments, acidogenic phase was operated without mixing, and their results was comparable higher than in this part. As a result, there is no need to mix and pH control the acidogenic reactors in terms of increasing the efficiency for unscreened dairy manure.

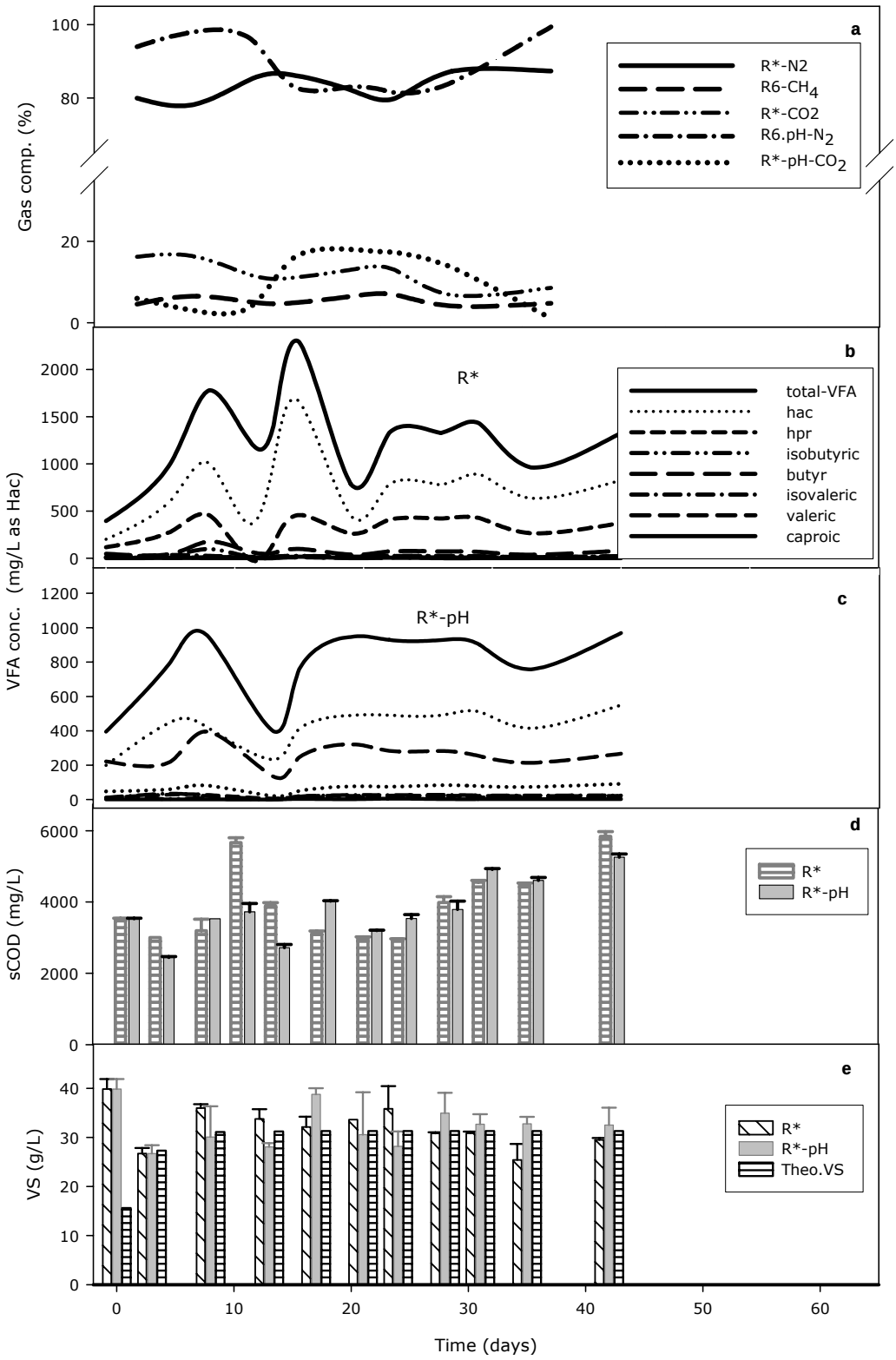


**Figure 4.15.** tVFA, VS, sCOD, and gas composition values observed in Set II experiments

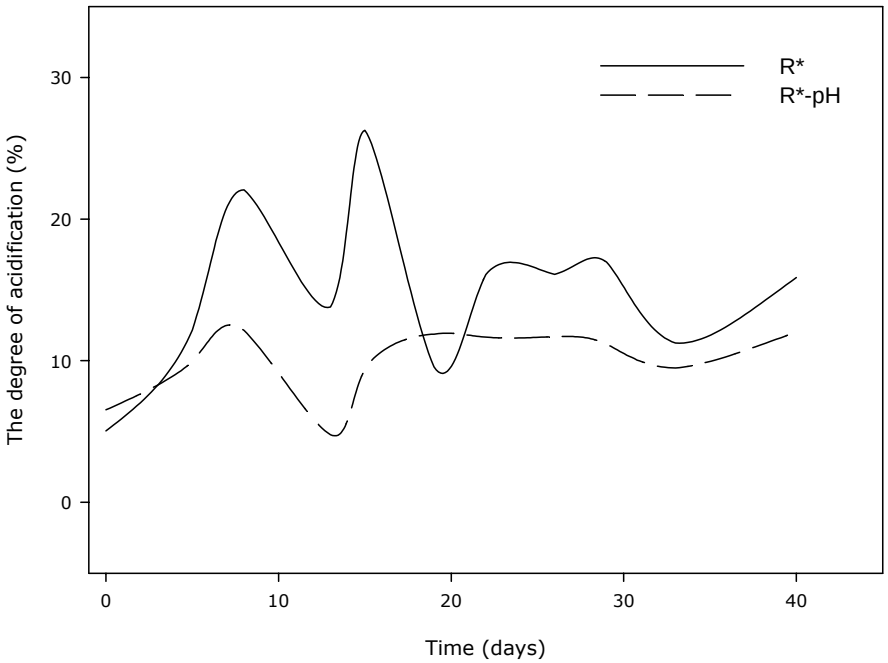


**Figure 4.16.** The degree of acidification in pH controlled and uncontrolled reactors

## 4.2. The Effect of Temperature on Biogas Production

The methane yield depends on the origin of the manure, fiber content, time and conditions of storage, pretreatments and the amount of seeding employed in methanogenic production assays. Temperature is one of the most important physical factors affecting microbial activity within an anaerobic digester, and methane production is strongly temperature dependent. Fluctuations in temperature affect the activity of methane-forming bacteria to a greater extent than the operating temperature. Temperature influences not only methane-forming bacteria but also volatile acid-forming bacteria. Therefore, fluctuations in temperature may be advantageous to certain groups and disadvantageous to other groups. Although methane production can occur over a wide range of temperatures, anaerobic digestion of wastes is applied generally in the mesophilic range, with an optimum temperature of approximately 35°C. Bacterial activity and growth decrease by one half for every 10°C decrease in temperature below 35°C (Hulshoff-Pol, 1998). The rate of anaerobic digestion of waste and methane production is proportional to digester temperature, that

is, the higher the temperature the greater the destruction rate of volatile solids and the production of methane. But in rural areas these optimum conditions could not be easily achieved and the operation might be ineffective in terms of biogas production. In case the AD system produces satisfying amounts of biogas at low temperature conditions, the farmers will be willing to handle and manage their manures with the AD process. But the temperature is an important parameter as explained above and the performance of AD of manure is poor at low temperature. Application of two-phase configuration may be promising to increase this lowered performance efficiency.

The experiments of Set III were carried out to compare the efficiencies of biogas production at two different temperature levels at same OLRs. Two daily-fed CSTR reactors of 3 L volume were operated at 25°C (low temperature - R25) and at 35°C (mesophilic temperature - R35). Mesophilic conditions resulted more than twice gas production than low temperature conditions (Fig. 4.17). And also it is clearly seen that, daily gas production increase was well-proportional to OLR increase from 1 to 3.5 g VS/L.day. Average biogas yields were calculated as 130 and 300 mL biogas/g VS added, for OLR of 1 and 3.5 g/L.d, respectively (Fig. 4.18).

For whole dairy-cattle slurries, digestion at 35°C was maximal at 20 or more days HRT with 170 L of biogas (58% CH4) per kilogram of TS fed from in a slurry of 5-7.5% TS. At 25°C gas production was 130 L per kilogram of TS fed, from solids alone, at 20 days HRT (Summers et al., 1987). Kim et al. (2006) investigated the effects of temperature on anaerobic digestion of food waste in a methanogenic batch type reactor. The amount of biogas produced from the reactors at 40°C, 45°C, and 50°Cs were found as 7.3, 8.7, and 10.4 L/d, respectively. Varel et al. (1980) investigated the effect of temperature and retention time on the rate of methane production from waste of beef cattle fed a finishing diet by using continuously mixed 3 L working volume anaerobic fermentors. The highest methane yield at that rate (liters/gram of volatile solids) was 0.19 at HRT of 9 days and 30°C, 0.16 at HRT of 6 days and 35°C, 0.23 at HRT of 6 days and 40°C. Digestion temperature greatly affects methane reactor size for identical animal live weight product on facilities. Operation at 60°C requires approximately half of the detention time that 35°C operation requires for the same methane productivity (Hill 1994).

The experiments depicted that even though the efficiency of biogas production is significantly lower relative to mesophilic conditions, AD of dairy manure could be operated at low temperature conditions.

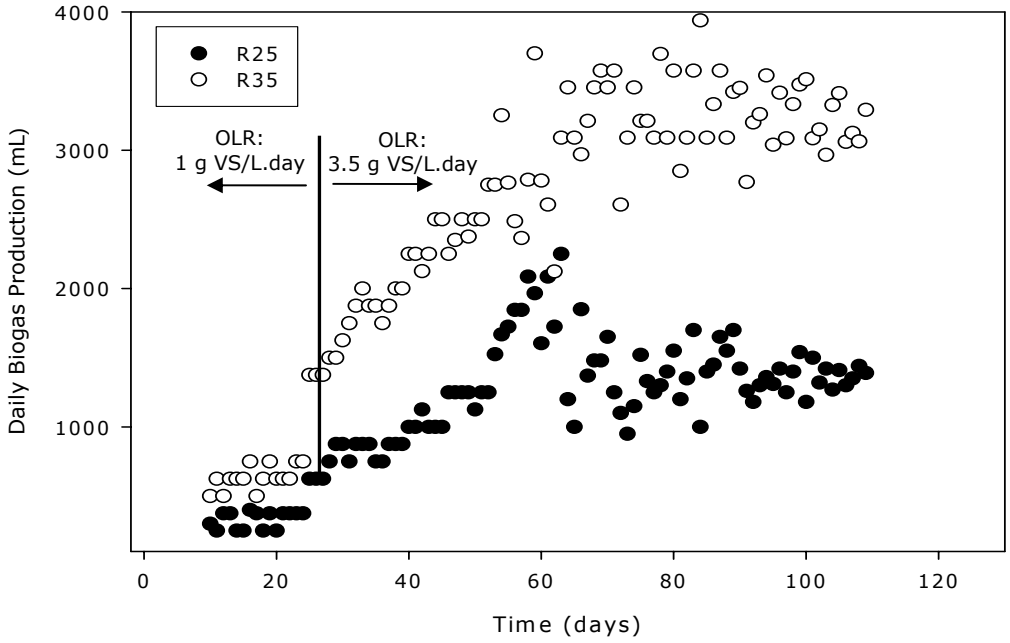


**Figure 4.17.** Daily gas productions at 25°C and 35°C temperatures

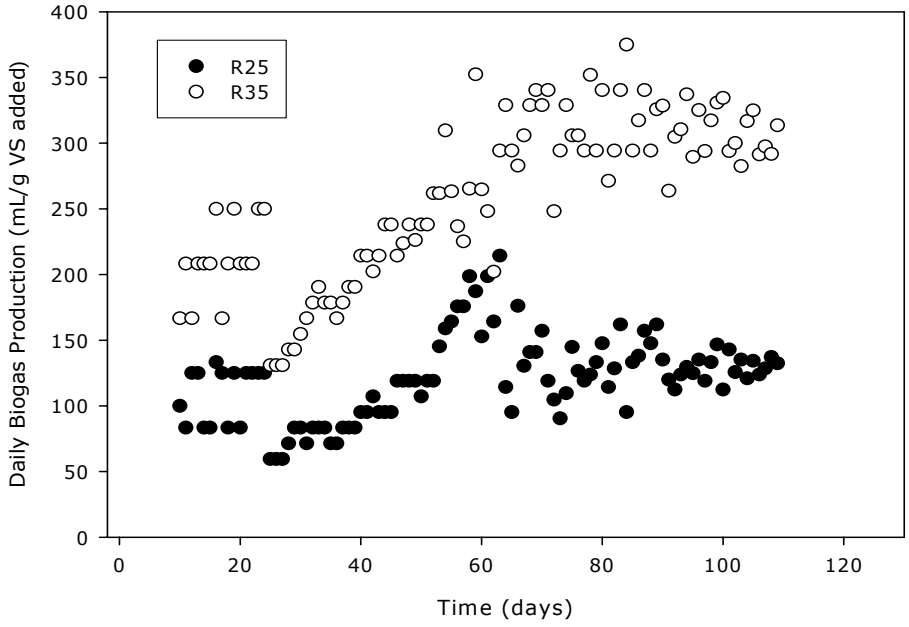


**Figure 4.18**. Daily gas production yields at 25°C and 35°C temperatures

### 4.3. Two-phase Configuration

Considerable progress has been made since Borchardt (1971) and Pohland and Ghosh (1971) published their research to suggest that two-phase fermentation affords an opportunity to optimize the major fermentation steps of anaerobic digestion to effect substrate conversion at higher rates and stabilities than those of conventional single-stage digestion. The two-phase digestion process has been applied in pilot and commercial scales for the stabilization of high strength industrial liquid wastes to demonstrate the benefits of phase separation and optimization (Ghosh et al., 1985). Applications of two-phase AD have occurred in the biogasification of: wastewater treatment sludge (Ghosh, 1991), organic fractions of municipal solid wastes (Chanakya et al., 1992), dairy wastewater (Ince, 1998), as well as some studies focusing on improving reactor design, control and operational parameters (von Sachs et al., 2003; Fox and Pohland, 1994). These researches showed that, two-phase configuration has some advantages over one-phase system.

A difficult biochemical reaction step that is of little concern in liquid waste digestion, but is a potential bottleneck in case of particulate slurry or solid feeds is hydrolysis. A heterogeneous particulate feed that is difficult to dispose of and is constituted of the complex polymeric compounds of lignocellulosics, proteins, and lipids (Ghosh, 1987).

Advanced digestion utilizes process configurations that could overcome the aforementioned limitations of conventional digestion, and permits process operation at much higher loading rates and shorter HRT than those of the latter. The natural response of an anaerobic digester to high-loading short-HRT operation is separation of the acid-forming phase, it appears reasonable to assist this process and develop a phased system in which conversion of the feed to fatty acids is optimized in the first phase. Because conditions promoting optimum substrate-to-acids conversion are not conductive to stable and efficient acid-to-methane conversion, acidic effluents from the first-phase acid digester must be methaneted in a separate methane- phase digester operated in tandem with the first-phase acid digester. Many researches evolves naturally when anaerobic digestion is conducted at high loading rates and short HRT in the interest of enhanced substrate conversion rate, reduced plant cost, and

increased net energy production efficiency. Thus, two-phase digestion is an advanced generic multi-stage process in which the acid-forming and methane-forming bacterial phases are optimized in separate reactors to substantially enhance the overall process kinetics and reduce plant capital cost (Cyhnoweth and Pullammanappallil, 1996).

Many researches have shown that two-phase system more effective than one – phase system in terms of increasing the stability of the process, higher organic loading rates, shorter HRT and increasing the biogas production. But in some studies this acquiescence was not found as satisfactory. Lo et al. (1986) studied both completely-mixed and fixed-film reactors using screened dairy manure as feed material. In terms of overall systems performance the two-phase systems were not superior to the one-phase systems. In another research, Liao and Lo (1985) studied thermophilic AD using screened dairy manure as feed substrate. The results indicated that satisfactory high-rate thermophilic digestions could be obtained at short hydraulic retention times for both one- and two-phase systems. There was no marked difference in performance between mesophilic and thermophilic temperatures in the acid-phase reactor. There was no indication that a two-phase system would be superior to one-phase thermophilic digestion of screened dairy manure.

For livestock wastes, the ultimate methane yield depends on species, ration, age of manure, method of collection and storage, and the amount of foreign material in the manure. The methane yield also depends on the origin of the manure, time and conditions of storage, pretreatments and the amount of seeding employed in methanogenic production assays. Increasing active biomass seeded increases the organic matter employed in biochemical methanogenic reactions, lessening that used in cell synthesis processes. In this way, methanogenic productivity will rise.

A number of studies have been conducted on mesophilic digestion of cattle wastes. A detailed comparison of performance data for different anaerobic reactors treating dairy or cattle manure is given in Table 2.6.

The biodegradability (g biodegradable volatile solid/g volatile solids) of swine, beef and dairy manure was calculated as 0.90, 0.60 and 0.36, respectively. These theoretical values indicated the available biogas production changing with different manure types (Husain, 1998). For different samples of dairy manure and changing storage times, the values for specific methanogenic productivity found at 35°C were the following: 0.193–0.321, 0.287–0.378, and 0.462–0.635 L $CH_4$/g VS added for raw dairy manure, screened manure, for liquid fraction, respectively (Rico et al., 2007).

Even though several aspects of two-phase configuration might be very significant for efficient AD of dairy manure, its application has been limited to screened dairy manure only. Therefore, this study investigated possible exploitation of the advantages of two-phase AD for unscreened dairy manure. A schematic representation of the laboratory-scale, one-phase and two-phase anaerobic digestion system used in Set IV experimental system is depicted in Fig 3.5. The one-phase conventional configuration (R1) was run as the control for the two-phase configuration (R2). The effective volumes of R1, R21, and R22 were 1000, 400, and 1000 mL, respectively. The two-phase configuration contained R21 and R22 as the first (acidogenic) and second (methanogenic) phases. The SRT/HRT values of R1, R21, R22 and the overall two-phase configuration were 20, 2, 8.6, and 10.6 days, respectively. The HRT of the one-phase system (20 days) represents a typical value which is commonly used in conventional AD of animal manure. The total HRT of the two-phase configuration (10.6 days) was adjusted in a way to observe the effect of reducing the HRT by half relative to one-phase system. All the reactors were fed daily. One of the reactor systems was maintained at 25°C in a temperature-controlled water bath and the other was at 35°C (±2) in a temperature controlled room, and both were shaken manually once a day after gas production. The gas production started in the first week of the reactor operation in all reactors. Gas volumes were measured daily. The results are shown in Fig.4.19. The average biogas production values of R1(35), R22(35), R1(25), R22(25) were obtained as 1230±180, 1000±90, 770±70, 290±50 mL/day, respectively. Also, a noteworthy gas production of 130 mL was seen in the mesophilic acidogenic reactor (R21(35)).There were three different gas production trends in Figure 4.19. This could be explained by the heterogeneous

characteristics of the different manure samples collected at different times. This difference resulted in different biodegradability yields.

It is clearly seen that temperature affects the performance of the biogas production (Figure 4.19). The biogas production increased 56% when the temperature increased from 25°C to 35°C in one-phase reactor. These results are very consistent with literature as discussed in the Section of 4.2 (Summers et al., 1987; Varel et., 1980).

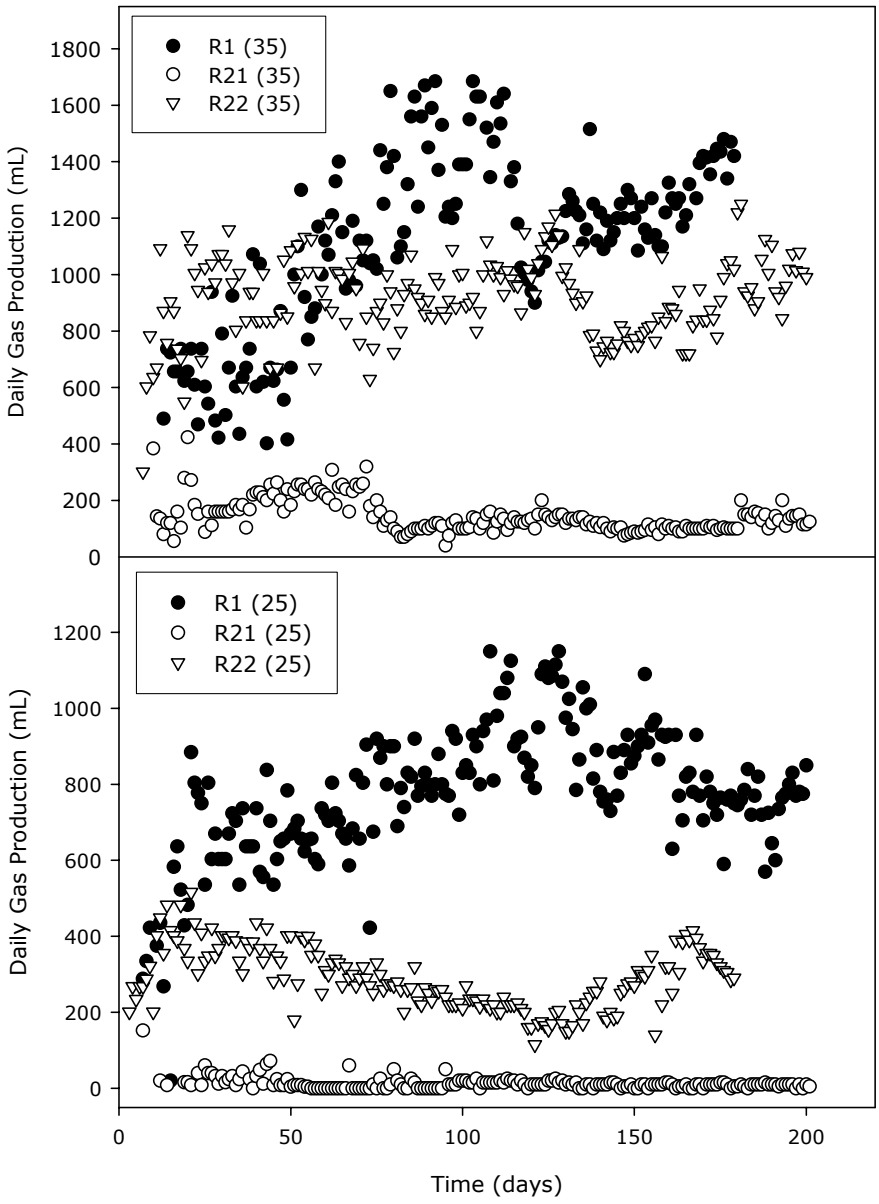


**Figure 4.19.** Daily gas productions at 35°C and 25°C

The biogas yields except acidogenic steps of the reactors were plotted in Figure 4.20. The average methane content of R1(35), R22(35), R1(25), and R22(25) were determined as 63, 65, 63, and 43 %, respectively (Figure 4.21). The methane yields of these reactors calculated as 221, 216, 132, 43 mL $CH_4$/g VS added, respectively. The performances of the reactors in terms of biogas yield could be easily comparable with literature values except R22(25). Varel et al. (1980) found maximum methane production of about 260 litres per kilogram of VS fed to the digester at 18 days RT at 35°C. This figure is higher than that was found in this study but could be accounted for the usage of beef manure as animal feeds. Because the biodegradability beef manure was calculated as 67% higher than dairy manure (Husain, 1998).

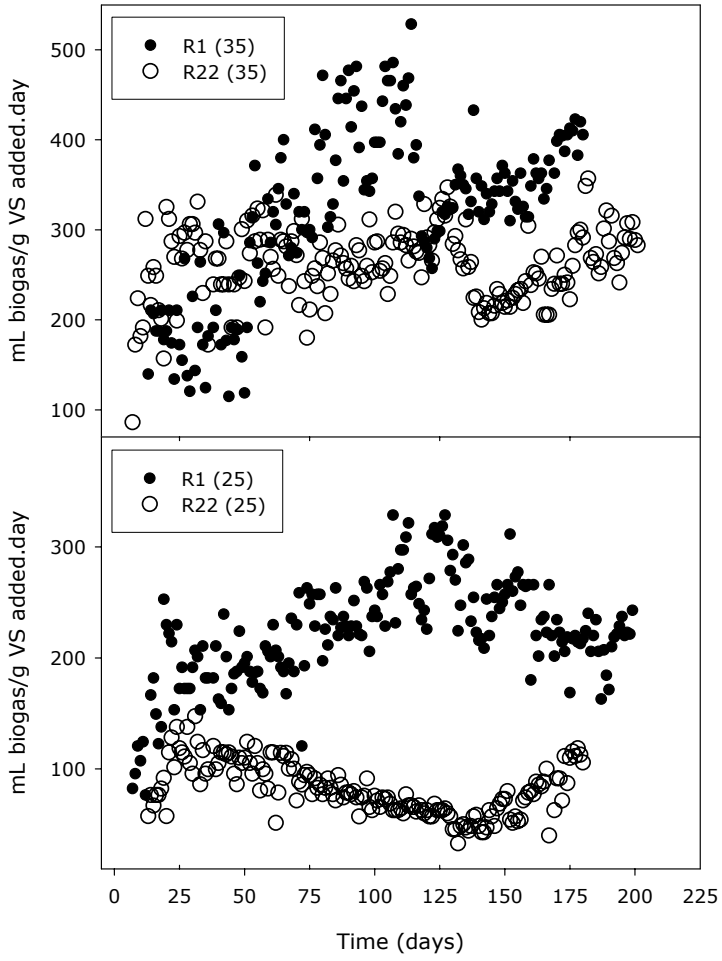


**Figure 4.20.** Biogas production yields at 35°C and 25°C

When the biogas production yields are compared at mesophilic temperature, the performance of two-phase system (216 mL $CH_4$/g VS) is slightly lower than one-phase system (221 mL $CH_4$/g VS) in this study. The earlier experiments with fattening-cattle waste had suggested that a HRT of about 20 days was required at 35°C for optimum methanogenic anaerobic digestion and that gas production was reduced significantly at 10 days of SRT (Bousfield et al., 1979 cited in Summers at al., 1987). Demirer and Chen (2005) demonstrated that a conventional one-phase reactor for unscreened dairy manure at a HRT of 20 days produced 0.235 L biogas/g VS. When HRT reduced to 10 days, initially an increased was seen in gas production but a few days later an abrupt decline in biogas production were observed, then biogas production was reduced by 90%. It must also be noted that the two-phase configuration could perform fairly well at an elevated OLR of 12.6 g VS/L day which was not possible for conventional one-phase configuration. Hobson and Wheatley (1993) concluded that the effect of retention time on the percentage degradation of the biodegradable portions of the solids in cattle wastes decreased from 86 to 62 % when HRT reduced from 20 to 10 days at 35°C. In an another work by Wellinger (1999) gas yield of straw-rich solid cattle waste was found as 270 and 190 mL/g VS at HRT of 20 and 10 days, respectively.

From this above discussion, it is obvious that, the HRT is directly affecting the biogas production. A simple calculation could be reveal which system is preferable in terms of higher biogas production yield. When the HRT of two-phase system is increased from 8.6 to 20 days, the system would produce 313 mL $CH_4$/g VS instead of 221 mL $CH_4$/g VS by using the literature data (Figure 4.21, and Figure 4.22) for the same substrate (Hobson and Wheatley, 1993; Wellinger, 1999). Thus, gas production in two-phase system (R22(35)) would be 41% higher than that of the one-phase system (R1(35)). Moreover, a small amount of produced methane from the acid phase (R21) may also be delivered to R22 or directly collected; it is for sure that methane generation of R2 will also increase.

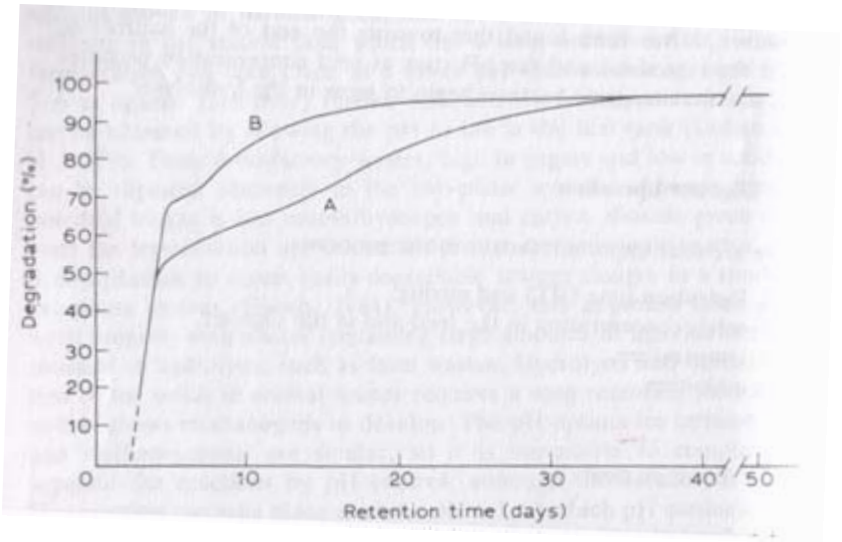


**Figure 4.21.** The effect of the retention time on the percentage degradation of the biodegradable portions of the solids in cattle – (A) and pig – (B) waste slurries at about 35°C

(Hobson and Wheatley, 1993)

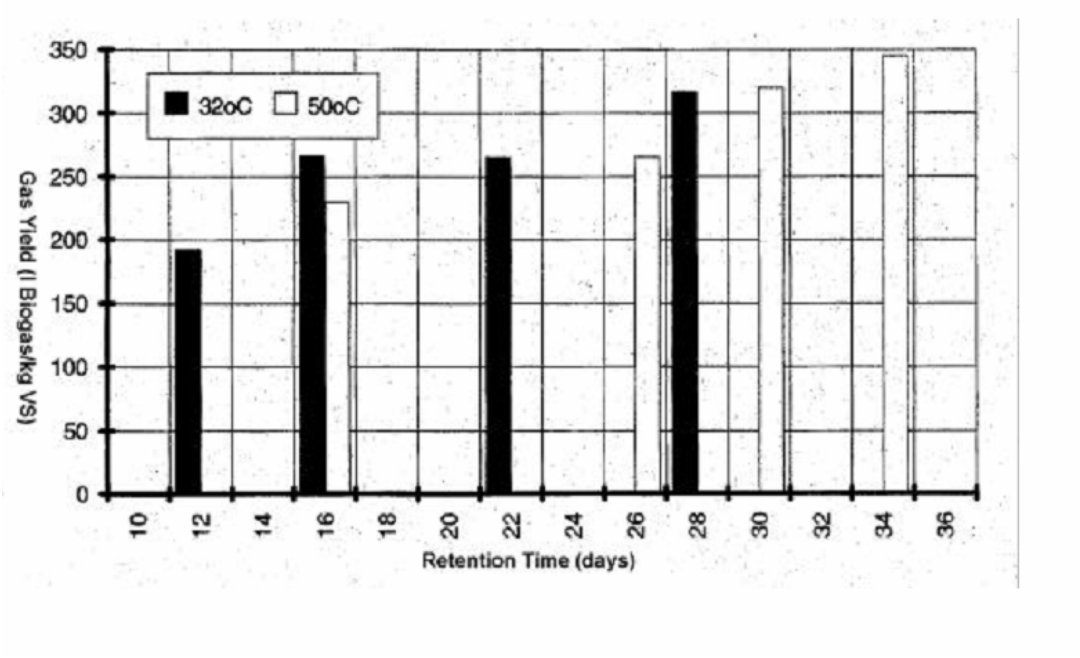


**Figure 4.22.** Gas yield of straw-rich solid cattle waste in function of HRT and Temperature

(Wellinger, 1999)

Nearly all reactors used in AD experiments in literature were operated with mixing of the feeds. But the reactors used in this study were mixed manually only for a minute after daily feeding. The idea for that, the system should be very simple and cheap so that farmers could easily operate the reactors without any experience. The information available in the literature on the role of mixing in anaerobic digesters is contradictory. Most of the literature on anaerobic digestion, for both low and high solids applications, emphasizes the importance of adequate mixing to improve the distribution of enzymes and microorganisms throughout the digester. Intermittent mixing in the anaerobic digestion of livestock waste under mesophilic temperature conditions has been recommended by Mills (1977) and Smith et al. (1988). Hashimoto (1982) found higher biogas production from beef cattle wastes under both continuous mixing and vacuum than under intermittent mixing and normal pressure conditions. Karima et al. (2005) showed that the unmixed and mixed digesters performed quite similarly when fed with 5% manure slurry and the methane yield was found to be 0.26–0.28 l $CH_4$/g volatile solids loaded. However, the effect of mixing and the mode of mixing became important when the digesters were fed thick manure slurry feeds (10% and 15%). Digesters fed with 10% and 15% manure slurry and equipped with external mixing produced about 10–30% more biogas than the unmixed digester. It is obviously seen that mixing is promoting the biogas production. In case the AD system in this study operated under mixing conditions the system would provide higher efficiency.

The two-stage systems provide higher efficiencies, a more stable design, a higher throughput, smaller tank sizes by 40-60%, higher methane content in the biogas (65- 75% methane vs. 50-55% for conventional technologies), (Weiland, 1993). The biogas mainly consists of methane, carbon dioxide, and nitrogen (Figure 4.23). The methane content of the reactors R1(35), R22(35), and R1(25) have nearly constitute 63-65% of the biogas produced. These three reactors had similarities regarding the percentages of $CO_2$ and $N_2$. Carbon dioxide and nitrogen contents averaged 30-35 and 1-2 percent by volume, respectively. R22(25) did not provide the similar results and its biogas production efficiency was fairly lower than the others. It mainly consists of 40-45% of $CH_4$, 30-35% of $CO_2$, and 25-30% of $N_2$. This low efficiency might be explained as the effect of low temperature conditions. Further investigations must be performed on acid phase to increase the efficiency of overall system.

The acid-phase digestion products may be markedly affected by the specific characteristics of wastes, operational parameters such as HRT, SRT, and environmental factors such as pH, temperature, reactor configuration, oxidation-reaction potential (ORP), and available trace minerals (Andrews et al. 1965; Ghosh et al. 1975). Moreover, a small amount of produced methane from the acid phase may also be delivered to the methane phase for later conveyance to collection means.

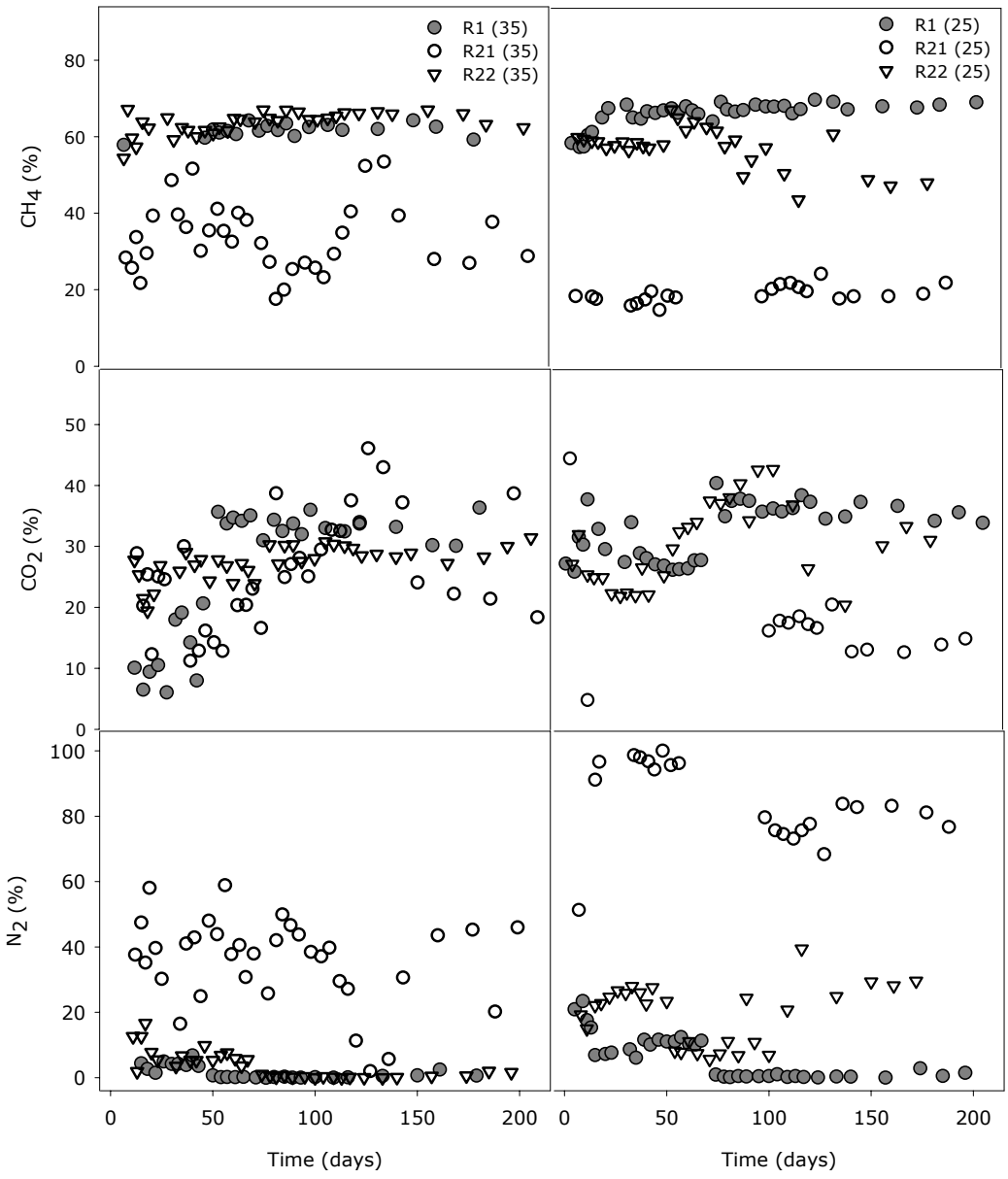


**Figure 4.23.** $CH_4$, $CO_2$, and $N_2$ (%) contents in the reactors

Mackie and Bryant (1995) studied AD of cattle fed using CSTR. Methane production rate was found as 210 mL/g VS fed with a OLR of 3 g/VS.d. The biogas produced in this mesophilic operation contained had $CH_4$ content of 59%. Pain et al. (1984) studied a full-size digester plant. They reported biogas yields of 204 L/kg of TS fed for unseparated slurry at 20 days RT, with a TS reduction of 26%. The biogas had 54% of methane.

Lo and Liao (1985) investigated the AD of screened dairy manure with a two-phase digestion system consists of a completely mixed reactor for the acidogenic reaction and a fixed-film reactor for the methanogenic reaction. The methane content of the biogas generated in the first-stage reactor ranged from 40.2% to 55.1%, while methane content of the biogas in the second-phase fixed-film reactor ranged from 61.5% to 72.3%. The acidogenic reactor in the first-stage reaction was confirmed by the low methane content of the biogas.

The methanogenic digestion of unscreened and unmixed manure in this study revealed that a slightly higher methane percentage (63-65%) was obtained relative to the similar investigations in the literature (Lo and Liao, 1985; Summers et al., 1987; Weiland, 1993; Mackie and Bryant, 1995).

Acidogenic reactors had different biogas compositions and productions than the methanogenic reactors. Gas production in R21(25) was not considerable while approximately 130 mL gas production was observed in R21(35). The biogas produced in R21(35) contained 30-35% of $CH_4$, 25-30% of $CO_2$, and 40-45% of $N_2$. This also indicated a similarity with Set I experiments, since the percentages of biogas composition were nearly the same (Figure 4.6). The selected 2 days of SRT was not favorable for the most sensitive anaerobic bacteria type known as methanogens. The methane production at such low SRTs could be explained by unintentional extended retention times of microorganisms in the reactors due to very high solids concentration and thus lack of homogeneity during daily wasting of sludge (Ghosh, 1985/1987). A well operated anaerobic acidification reactor should ideally contain few methanogens. Optimum conditions for acidification severely retard methanogenic activity but do not eliminate all methanogens, which are sensitive to the operating conditions but may persist in a dormant or semi-

dormant state. Denitrification might occur during the acidogenic phase, so as to achieve simultaneous VFA production and nitrate elimination, a system could be applied to organic carbon and nitrogen removal from the wastes (Rustrian et al., 1998; Vigneron et al., 2007). Also, the $CH_4$:$CO_2$:$N_2$ percentages indicated similarity with the ranges reported in the literature for the acidification phase step (Ghosh et al., 1975; Fongsatitkul, 1992). Although the importance of the separation of the acidogenic and methanogenic phases is well known, only a few studies were carried out for the investigation of the acidogenic phase of AD with manure and they have not focused on the formation of nitrogen. During the hydrolytic and acetogenic steps (initial stages of the anaerobic digestion) organic nitrogen compounds are degraded and ammonia nitrogen is released at different rates depending on the molecular complexity of the compounds (Henze and Harremoes, 1983). Both acidogenesis and denitrification were observed in an acidogenic reactor of two-phase anaerobic digestion fed with synthetic substrate containing glucose and nitrate by Noike et al. (2002).

Volatile solid content often used as a measure of the biodegradability of the organic fraction of waste. The influent and effluent VS concentrations in the reactors are plotted in Figure 4.24. The effluent concentrations revealed a stable trend especially in mesophilic reactors. This stable trend presented that a constant VS reduction occurred throughout the operation.

The VS reductions illustrated some changes with operation period (Figure 4.25). The highest VS conversion was observed with 35-40% in R1(35) between days 10 and 100, but during days 100-200 R2(35) had the highest VS reduction with 30-35%. A 20-30% VS conversion resulted a wide range in R1(25), this was mainly caused by the operation of this reactor didn't show stability. The VS reduction observed in R2(25) and R21(35) was under 20% parallel to their gas production and they were very fluctuating. Although both of the systems had the same OLR relative to their inlet concentrations, the inlet concentration of R22 was the effluent concentration of R21 in which there was an average VS reduction of 17%. Therefore, the OLR in R22 was calculated as 2.9 g VS/L.day. R22(35) had 10-50% higher VS reduction than R22(25), since the performance of R22(25) was low. The VS reduction in R21(25) was nearly below 10% at all times. R21(35) represents the R6 in Set I experiments, and

had 5-50% higher VS conversion than R6. This is mainly resulted from the heterogeneity of manure.

Hill (1980) studied the anaerobic digestion of dairy manure (average TS of 20%) in feed batch reactors, which were manually mixed once daily. Approximately 25 % of the volatile solids were destroyed after a period of 13 weeks. Ahring (2001) reported 28% volatile solids conversion in a thermophilic digester operated at a loading of 3 kg/$m^3$/d. Ghaly and Pyke (1992) operated a dairy waste completely mixed mesophilic digester at a loading of 3.6 kg/$m^3$/d. They achieved a 46 percent conversion of volatile solids to gas. Qasim et al. (1984) operated a completely mixed mesophilic digester at an organic loading rate of 3.2 kg/$m^3$/d and achieved a 52.9 percent volatile solids conversion to gas. Echiegu et al. (1992) operated a completely mixed dairy waste digester at an organic loading rate of 2 kg/$m^3$/d but only achieved a 40 percent conversion. Robbins et al. (1983) also operated a completely mixed mesophilic digester at an organic loading rate of 2.6 kg per cubic meter per day that achieved a 30 percent conversion of volatile solids to gas. Hills and Kayhanian (1985) operated a completely mixed mesophilic digester at a 1.8 kg/$m^3$/d loading that achieved a 31 percent volatile solids destruction and a 38 percent conversion at 1.0 kg/$m^3$/d. When these findings are compared with the experimental results obtained in this study, it is seen than these values are very similar to our findings.

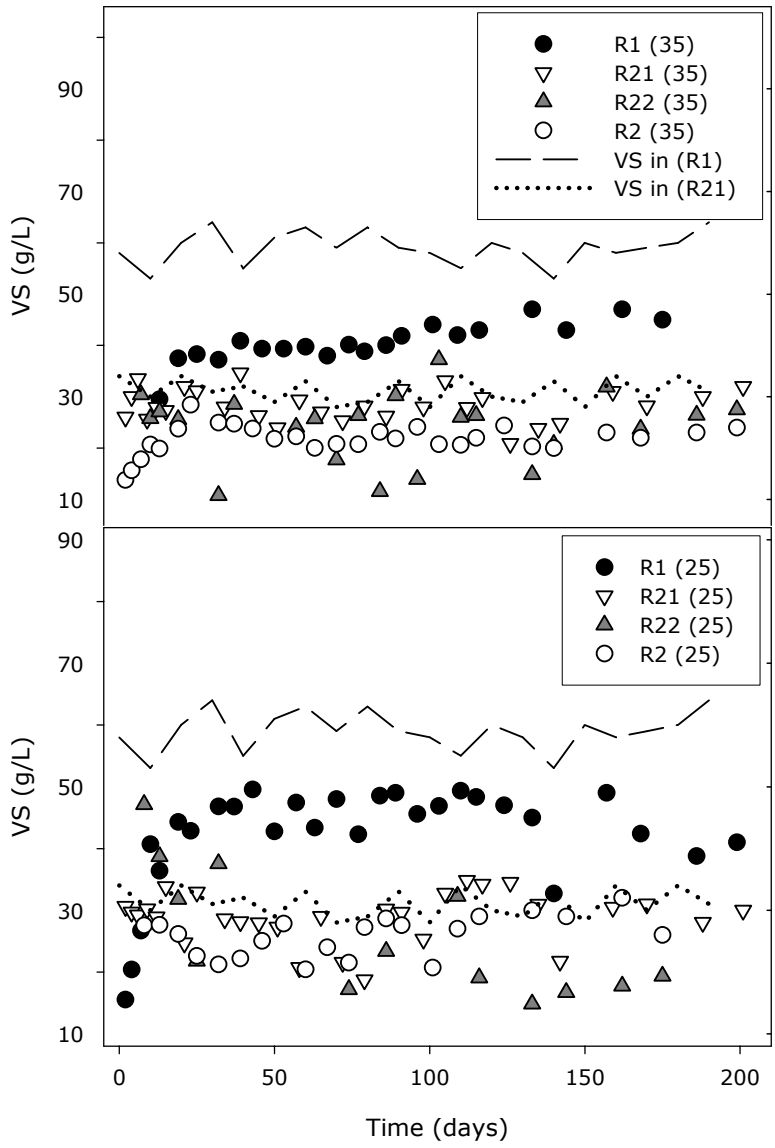


**Figure 4.24.** VS concentrations in the reactors

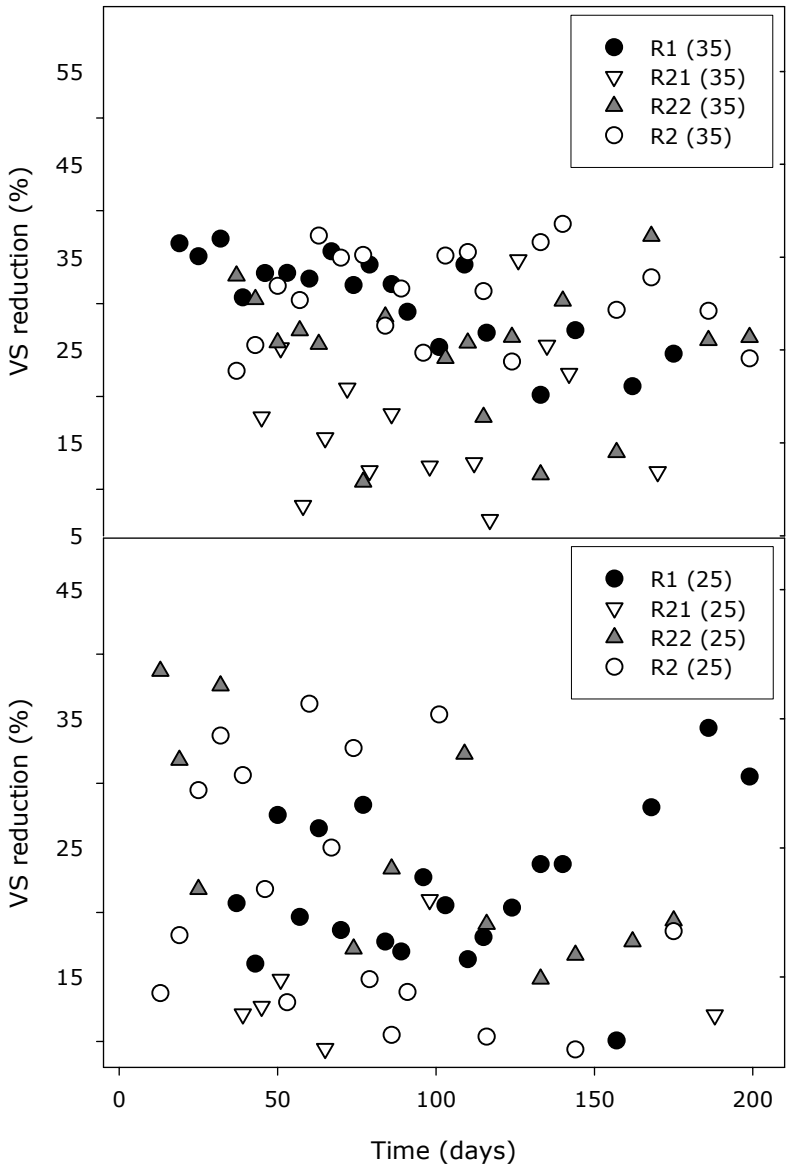


**Figure 4.25.** VS reductions in the reactors

When the results from this study are compared with the relevant literature (Hill, 1980; Qasim et al., 1984; Hills and Kayhanian, 1985) VS reductions were nearly in the same range (26-37%) except R2(25). But exact comparison of results is not possible because of differences in the feeds of the animals and in compositions of the wastes. Two-phase system in the mesophilic temperature showed the same reduction with one-phase reactor up to day 100. After that period, R2(35) illustrated 25% higher conversion than R1(35).

In a well-balanced anaerobic digestion process, all products of a previous metabolic stage are converted into the next one without significant build up of intermediary products. In general, hydrolysis is the rate-limiting step if the substrate is in particulate form (Ghosh and Klass, 1978; Eastman and Ferguson, 1981; Arntz et al., 1985; Noike et al., 1985). The rate of hydrolysis is a function of factors such as pH, temperature, composition and particle size of the substrate, and high concentrations of intermediate products (Veeken and Hamelers, 1999). The pH is the primary process variable in controlling the hydrolysis rate of the anaerobic solid state fermentation process, not the VFA concentration. Of course, the VFA concentration via chemical equilibrium influences the pH in the waste and, for a specific waste composition; the VFA concentration and pH can be related to each other. However, this relationship depends on the composition of the waste, which may differ from waste to waste and may even change during the process (Veeken et al., 2000).

As VS conversion percentages, effluent sCOD concentrations had also the same trend (Figure 4.26). Since the biogas production was due to the degradation of organic compounds. VS and COD parameters could be considered in the same manner as the characteristics of the biodegradability. So, the reduction trends should be similar in terms of VS and COD. The removal of soluble COD concentrations decreased significantly with decreasing temperature. The sCOD reductions of R1(35), R2(35), R1(25) were found as 45, 40, and 55%, respectively. The amount of sCOD in R21(35), R21(25), and R22(25) were increased 65, 25, and 35%, respectively. The hydrolysis and solubilization of complex materials is the main mechanism in that phase, so that the amount of sCOD increased except R22(25).

The degree of acidification was found to increase with hydraulic retention time and temperature and decrease with increasing substrate concentration and organic loading rate. Dinopoulou et al. (1988) had similar observations and further observed that temperature also effected the acidogenic phase following the Arrhenius equation.

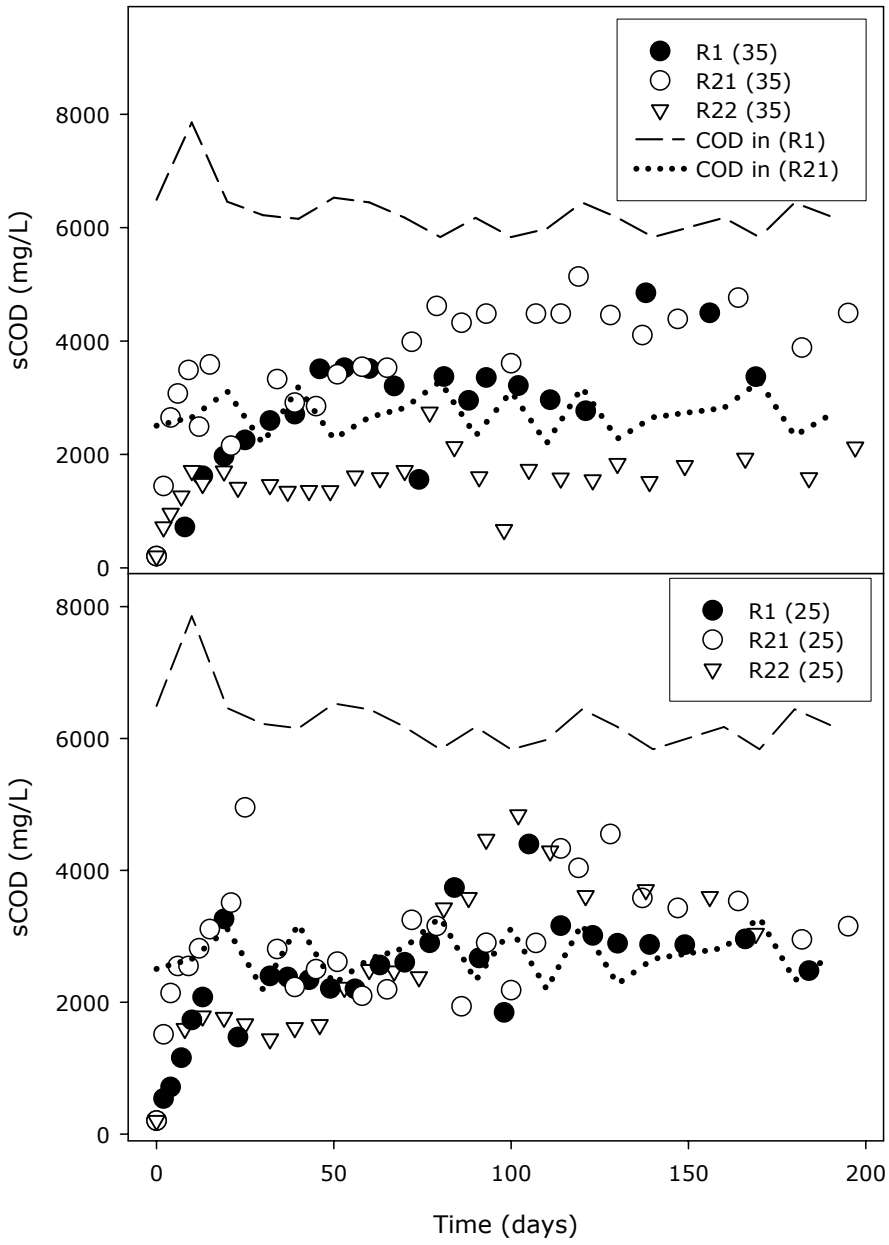


**Figure 4.26**. sCOD concentrations in the reactors

The total volatile fatty acids (as HAc) for runs are displayed in Figure 4.27. Acetic acid was the dominating VFA in reactors. The effluents of reactors contained mainly acetic acid, propionic and butyric acids, although higher fatty acids were found at lower concentrations. Acetate has been shown to be the

main precursor of methane produced in the thermophilic anaerobic bioreactors treating lignocellulosic waste or cattle manure.

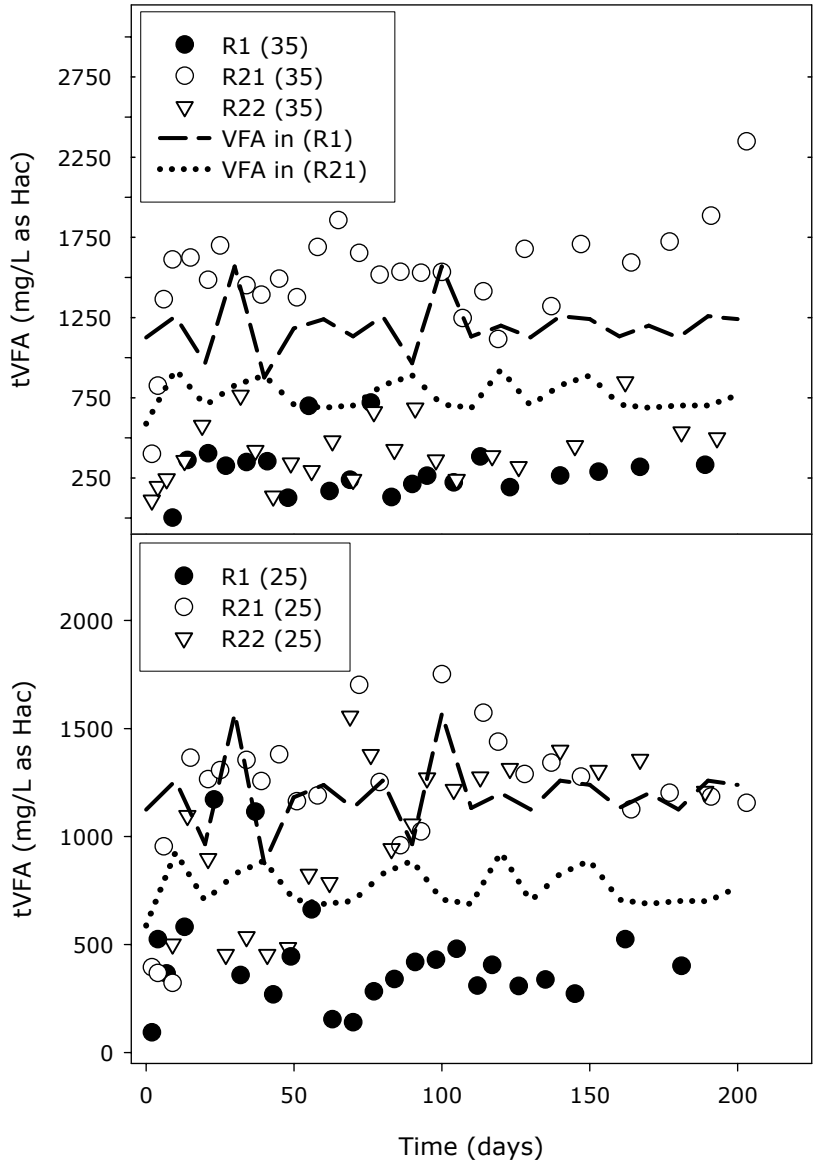


**Figure 4.27.** tVFA concentrations in the reactors

The effluent tVFA concentrations of the first-phase reactor at mesophilic and low temperature operated at 2 day HRT increased to 1700 and 1300 mg/litre (as acetic acid), more than 100 and 60% increase over that of the influent of R21(35) and R21(25), respectively. The effluent VFA concentration of the second-stage reactor in mesophilic temperature decreased to 350 mg/litre (as acetic acid), but the effluent concentration of R22(25) remained the same as

expected. The tVFA concentration of R1(25) was much lower than R22(25), since biogas production in R1(25) was more than double of R22(25). This resulted more VFA consumption in R1(25). Acetic acid was also the predominant VFA in the effluent. These results could be explained with together the experiments in Set I. The acidogenic efficiency could be increased without mixing and pH control, but temperature was an important parameter.

Total effluent tVFA value in one-phase reactor was lower than that of the two-phase reactor at mesophilic temperature. It does not mean that more VFAs were converted to methane in one-phase reactor, since more VFA transferred from R21(35) to R22(35). Consequently, higher VFA concentration was converted to biogas in two-phase system. In other words the efficiency of two-phase system was higher than one-phase system in terms of VFA consumption.

The VFA:COD ratio is a measure of the degree of success of acidogenesis, representing the amount of solubilized matter which has been converted to VFAs (Maharaj, 1999). The degree of acidification is presented in Figure 4.26. The data were obtained from experiments using constant influent concentration, constant HRT, no pH control and variable temperature. As can be seen from Figure 4.28, the degree of acidification increased with increasing temperature and using two-phase configuration. The one-phase reactors resulted the lowest acidification formation with 4-5% in R1(35) and R1(25) reactors. R21(35) and R22(35) revealed that a nearly stable acidification degree of 30 and 8-10%, respectively. R21(25) and R22(25) showed almost the same trend and a little bit lower than R21(35).

Bouallagui et al. (2004) investigated the two-phase anaerobic digestion of a mixture of fruit and vegetable wastes (FVW) using two coupled anaerobic sequencing batch reactors (ASBR) operated at mesophilic temperature. The acidification reactor was operated at a constant HRT of 3 days and fed with different dilutions of FVW to change the OLR. The whole experiment was carried out over three runs (Run 1: OLR = 3.7 g COD/L.d; Run 2: OLR = 7.5 g COD/L.d and Run 3: OLR = 10.1 g COD/L.d). Acidification yields were obtained as 40.3, 38.9, 44.4%, respectively. These are approximately 25% higher than

our results, since the biodegradability of fruit and vegetables are higher than manure.

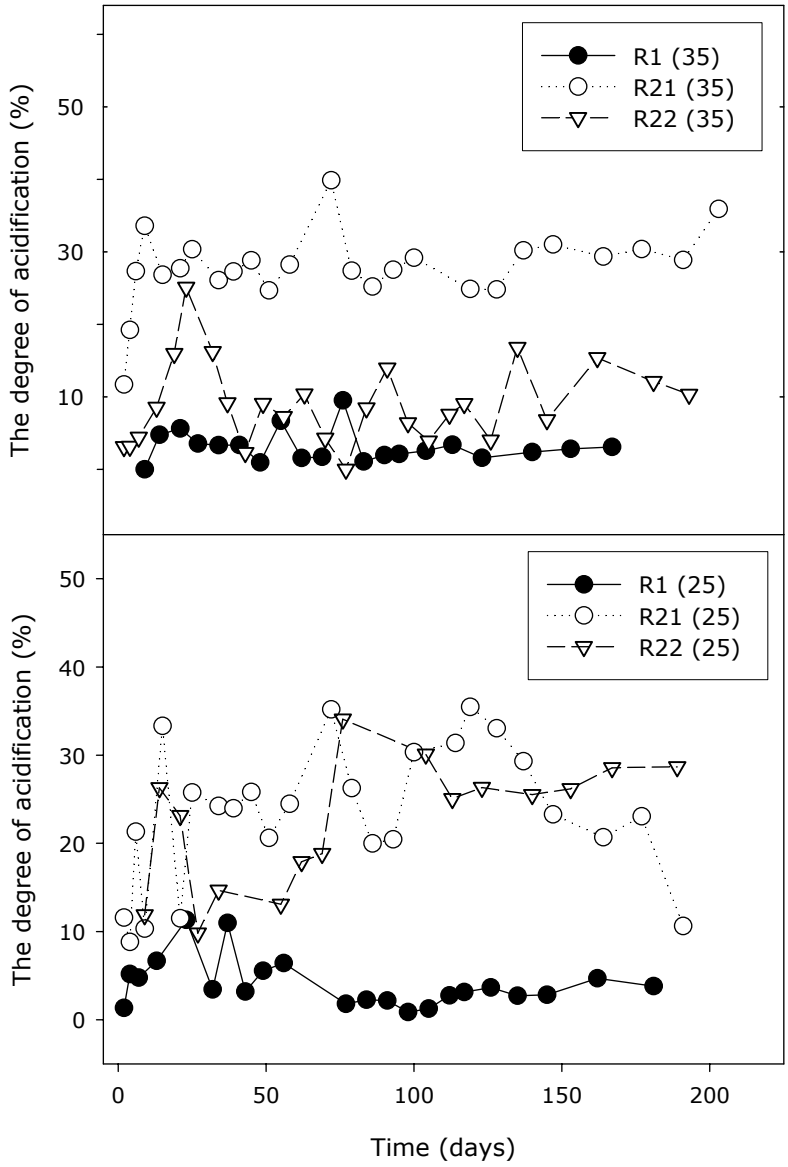


**Figure 4.28.** Degree of acidification in the reactors

The influence of various operational parameters, on the conversion of the substrate to volatile acids, on the rate of acid production per unit of reactor, and on the composition of the reactor effluent were investigated with a complex medium as substrate, which was based on beef extract (Dinopoulou et al., 1988). The initial COD concentrations and hydraulic retention times identified

as 3 g/L and 6 h, respectively, the degree of acidification achieved was between 30 and 60%. The degree of acidification was found to increase with the hydraulic retention time and decrease with the influent substrate concentration and organic loading rate, while the opposite held true for the rate of product formation. Furthermore, it has been demonstrated that acidification is primarily determined by the hydraulic retention time and the rate of product formation by the influent substrate concentration.

The anaerobic hydrolysis and acidification of wastewaters rich in organic suspended solids and protein was studied in continuous stirred reactors by Guerrero et al. (1999). The acidification efficiencies obtained as 44 and 23% at 55 and 37°C , respectively, operating at a HRT of 24 h.

The measured pH values are given in Figure 4.29. The lowest pH measurements were obtained around 6.5-6.8 in R21(35), while 7.0-7.2 in R21(25). The pH values of R22(35), R22(25), R1(35), and R1(25) were 7.0-7.2, 6.8-7.0, 7.3-7.5, and 7.2-7.5, respectively. As the low performance for the biogas production, R22(25) also indicated the lowest pH values of all reactors, except R21(35). The pH in an anaerobic digester initially will decrease with the production of volatile acids. However, as methane-forming bacteria consume the volatile acids and alkalinity is produced, the pH of the digester increases and then stabilizes. The methane production remained at low levels, so the pH of R22(25) could not increase. On the other hand, VFA production in R21(35) was higher than R21(25) (Figure 4.27), so pH level of R21(35) was lower than R21(25). Cattle manure is a complex substrate containing undissolved and dissolved organic matter such as polysaccharides, lipids, proteins and inorganic compounds of importance for the chemical environment. Therefore, pH levels did not expected lower than 6.5 in acidogenic phase. Dinopoulou et al. (1988) ascertained the influence of operational parameters, such as HRT, OLR, pH, and temperature on the performance of the first phase AD. The optimum pH in order to achieve both a high degree of acidification and a high rate of acid production was found to be 7. The apparent kinetic constants of the biomethanization process increased 2.3 times when the initial pH of the influent was increased from 7.0 to 7.6 at mesophilic temperature (Sanchez et al., 2000). The stability of pH values in mesophilic reactors indicated the stable performances of the reactors.

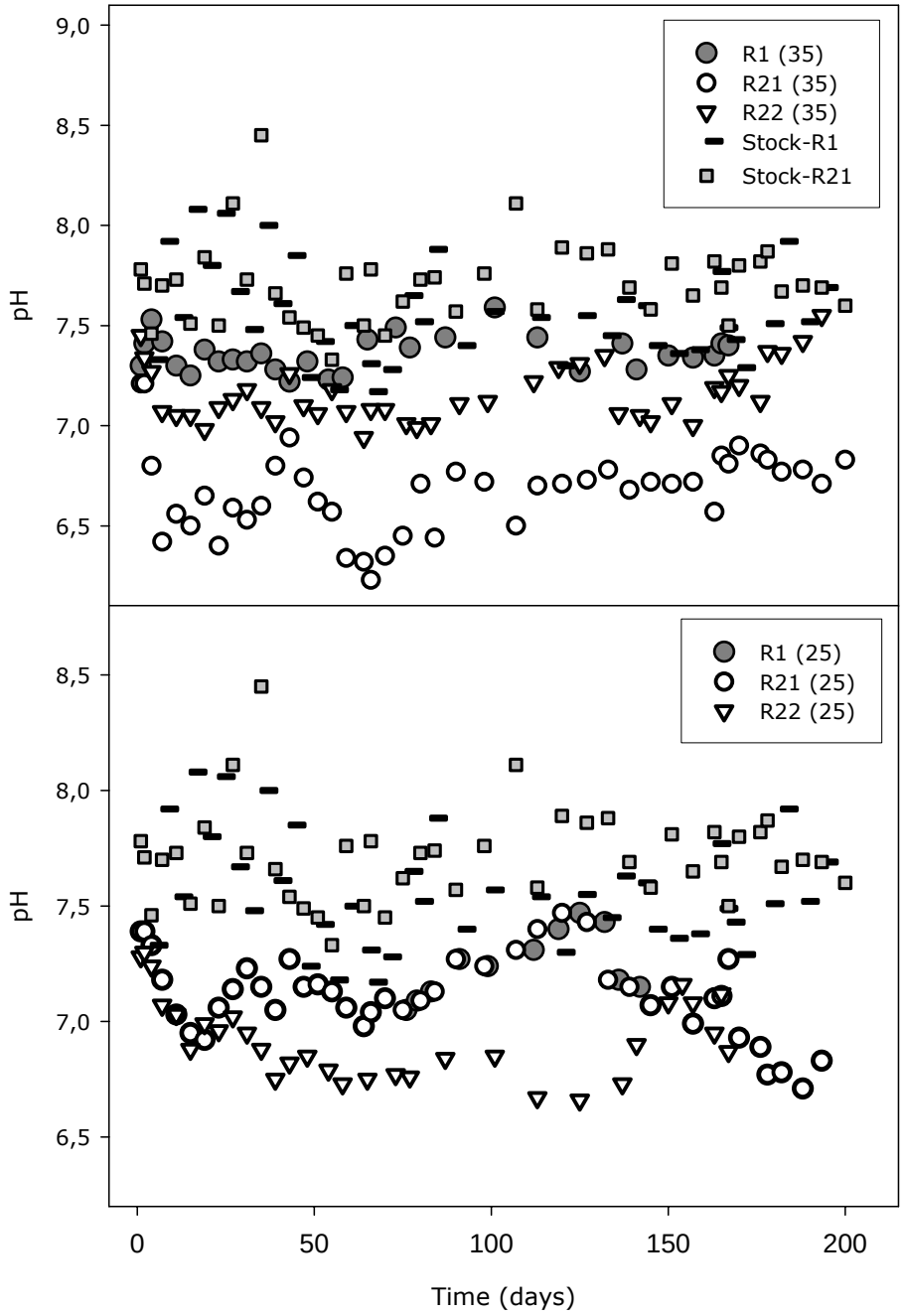


**Figure 4.29**. pH profiles in the reactors

## 4.4. Characterization of Bacterial Communities

Overall performance of anaerobic treatment systems is totally dependent on the composition of microbial populations in the anaerobic reactors. Determination of changes in microbial populations and its effect on performance at various operating conditions of a two-stage anaerobic digestion system would be of considerable interest. This section, therefore, examined microbiological aspects, including changes in the number and composition of the microbial populations of acidogenic reactor using molecular identification techniques.

### 4.4.1. Evaluation of DNA extraction

The bacterial population structure of bioreactors was monitored using 16S rDNA sequence libraries. All periods sampled had unique effects on the bacterial population structure of cattle manure. In the optimized DNA extraction method, the DNA fragments were larger than 23 kb and similar in size to DNA isolated from pure cultures. These results suggest that the extraction protocol did not cause severe shearing of DNA (Zhou et al., 1996). Repeated washing of cattle manure in PBS/KCl (phosphate saline buffer and 0.85%KCl) prior to extraction greatly improved the quality of the DNA extract, probably by removing humic compounds which are inhibitory to the PCR process (Lebuhn et al., 2003). Chloroform-isoamylalchol extraction of samples did not yield PCR amplifiable DNA. The best results were obtained with the gel-plus-minicolumn as Zhou et al., determined in their study (Zhou et al., 1996). This was probably due to a better removal of PCR inhibitory compounds, as evidenced by the presence of yellow-brown color in some (non-amplifiable) extracts. Gel-plus-minicolumn method resulted in complete removal of the dark color from crude DNA solutions. No PCR products were observed with DNA from crude extracts.

### 4.4.2. Microbial communities

Three samples were analyzed from mesophilic acidogenic reactor – R21(35) on the 4th, 12th and 38th day. Over the whole study, the bacterial 16S rDNA patterns showed at least 5 different major species (actinobacteria, firmicutes, gamma-proteobacteria, bacteroidetes, alpha-proteobacteria) which represented the most abundant bacterial sequences of the digester community at the different times of sampling (Figure 4.30). During the fourth days of the reactor the most frequently encountered microbial group were the *Gamma-*

*Proteobacteria* (36% of analyzed sequences), all the represented by *Pseudomonas spp*. followed by the *Actinobacteria* (14%) and *Firmicutes* (12%), represented mostly by members of the Clostridiales. The *Clostridium* spp. sharply increased by 25% and *Gamma-Proteobacteria* (dominantly *Pseudomonas* and *Acinetobacter* species) decreased to 14% on twelfth day. Other bacterial groups detected in the reactor were the *Actinobacteria* (18%) and *Bacteroidetes* (8%) at the same period. *Clostridium* spp. are acetogenic microorganisms known to demethylate aromatic compounds and gain energy by the conversion of *o*-methyl groups to acetic acid (Heider and Fuchs, 1997; Mechichi et al., 1999). Therefore, this microbial group might hold a critical role in the anaerobic digestion specifically on the production of acetic acid, an essential step for methane production by acetoclastic methanogenic microorganisms. While there were significant decrease to 7% in the phylum *Actinobacteria*, *Gamma-Proteobacteria* (19%) and *Firmicutes* (17%) were still significant components of the microbial communities during 38th day. In this period, the members of *Bacteroidetes* and *Alpha-Proteobacteria* were also found in bioreactor with 10% and 7%, respectively. The *Gamma-Proteobacteria*, *Actinobacteria* are mainly known as aerobic (Cirne et al., 2007, Lynd et al., 2002) and *Firmicutes* and *Bacteroidetes* are significant components of the microbial communities during the anaerobic decomposition. Toerien and Hattingh (1969) reported that species belonging to the genera of Bacteroides, Clostridium, Butyrivibrio, Eubacterium, Bifidobacterium and Lactobacillus are dominantly found in hydrolysis process. As a summary, *Gamma-Proteobacteria* and *Actinobacteria* were decreased, while *Firmicutes*, *Bacteroidetes* and *Alpha-Proteobacteria* were increased during the 38 days of mesophilic acidogenic reactor.

It is apparent that many of the sequences obtained in this study were related closely only to uncultured clones for which physiological and other properties remain unknown. This illustrates the incomplete nature of the microbial database and also the need for further basic research in this area. Although fermentative bacteria are very important in anaerobic digestion, the conditions required for their growth as well as cell metabolism and ecology are not completely understood.

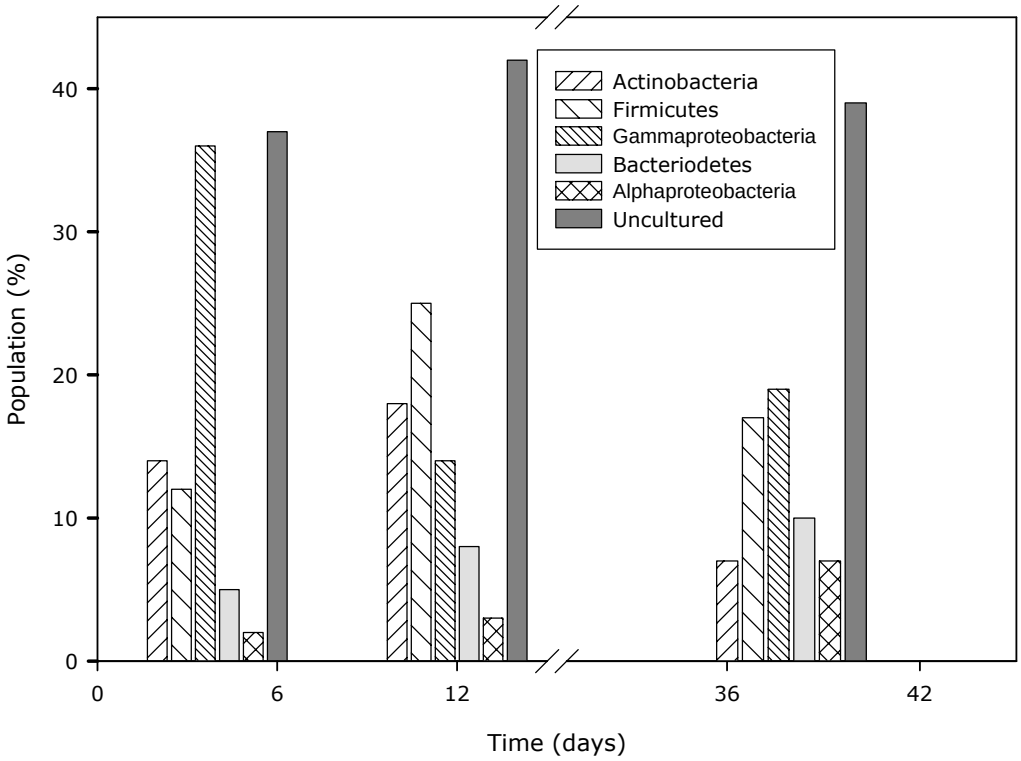


**Figure 4.30**. Distributions of bacterial populations in mesophilic acidogenic reactor

# CHAPTER 5

# CONCLUSIONS

Even though several aspects of two-phase AD such as increased stability, lower retention time requirements, liquefaction, etc. are very significant for enhanced AD of manure until now, its application has been limited to a few studies. However, efficient application of two-phase AD to high solids containing animal manure could reduce the required volumes as well as pumping, handling and mixing costs. This would constitute a further step in achieving a wider use of AD.

This study, in recognition of all these facts, investigated the application of two-phase AD for unscreened dairy manure.

Based on the results of this study, the following conclusions could be drawn:

- Three different organic loading rates (5, 10 and 15 g VS/L.day) and hydraulic retention times (1.25, 2, and 4 days) were applied to acidogenic reactors. It was observed that, as the SRT decreased as the degree of acidification increased. The optimum operational conditions leading to the highest degree of acidification (28%) were selected as SRT of 2 days and OLR of 15 g VS/L.day.

- Acidification of dairy manure in daily-fed continuously-mixed reactors with no recycle at OLR and HRT of 2 g VS/L.day, 15 days, respectively, led to tVFA production of 1420 mg HAc/L. The acidification products were mainly acetate (~ 60%), and propionate (~ 30%) corresponding to the carbohydrate acidification. VFA production per mass of VS feeding ratio was dependent on SRT rather than OLR.

- The pH control at a range of 5.0-5.5 did not improve the acidification relative to uncontrolled pH case.

- Acidification extent is higher at mesophilic temperatures (30%) than at low temperature (25%).

- The concentrations of *Gamma-Proteobacteria* and *Actinobacteria* were decreased, while *Firmicutes*, *Bacteroidetes* and *Alpha-Proteobacteria* were increased during the 38 days of mesophilic acidogenic reactor. This indicated the system adaptation started after 10 days, aerobic bacteria concentration was started to decrease while, and the majority of bacteria were composed of acidogenic bacteria.

- Based on the high $N_2$ content of the biogas from the acidogenic reactors, and with the supporting literature, denitrification might be responsible from simultaneous VFA production and nitrate elimination. Further study is needed to achieve concurrent organic carbon and nitrogen removal from wastes through anaerobic acidification.

- The tVFA concentrations was reduced from 1700 to 400 mg HAc/L and 1300 to 300 mg HAc/L in R22(35) and R1(35), respectively. This means that the performance of two-phase system was higher than one-phase in terms of VFA conversion.

- The daily biogas productions were 1300 mL and 800 mL at mesophilic temperature and at low temperature (25°C) in the one-phase reactor.

- The use of a two-phase reactor at a HRT of 10.6 days (2 days acidogenic and 8.6 days methanogenic) for AD of dairy manure would result in 41% higher biogas production (based on the experimental results and literature data) relative to a conventional one-phase configuration with HRT of 20 days.

- It is obvious that more research should be conducted at different reactor operational conditions to widen the applicability of two-phase systems for animal manure.

# REFERENCES


Abdel-Hadi, M., Beck, J. and Jungbluth, T., 2002. Methane yields from co-fermentation of liquid fodder sugar beet silage. *Landtechnik,* 2 (57).

Ahn, Y.H., Min, K.S. and Speece, R.E., 2001. Pre-acidification in anaerobic sludge bed process treating brewery wastewater. *Water Res.*, 35(18), 4267-4276.

Ahn, Y.-H., Bae, J.-Y., Park, S.-M. and Min, K.S., 2004. Anaerobic digestion elutriated phased treatment of piggery waste. *Water Sci. Technol.*, 49(5-6), 181–189.

Ahring B., Sandberg M. and Angelidaki I., 1995. Volatile fatty acids as indicators of process imbalance in anaerobic digesters. *Appl. Microbiol. Biotechnol*., 3, 559-565.

Ahring, B.,K., Ibrahim, A.A. and Mladenovska, Z., 2001. Effect of temperature increase from 55°C to 65°C on performance and microbial population dynamics of an anaerobic reactor treating cattle manure. *Water Res.*;35(10),2446–2452.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman,D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.

Amann, R. I., 1995. In situ identification of microorganisms by whole cell hybridization with rRNA-targeted nucleic acid probes, in Molecular Microbial Ecology Manual, Akkermans, A. D. L., van Elsas, J. D., and de Bruijn, F. J., Eds., Kluwer Academic Publishers, Dordrecht..

Amann, R.I., Ludwig, W., and Schleifer, K.H., 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbial Rev.*, 59, 143–169.

Amann, R., Glockner, F.O. and Neef, A., 1997. Modern methods in subsurface microbiology: in situ identification of microorganisms with nucleic acid probes, FEMS Microbiol. Rev., 20, 191.

Andrews, J.F. and Pearson, E.A., 1965. Kinetics and characteristics of volatile acid production in anaerobic fermentation process. *Internat. J. Air Wat. Poll.*, 9, 439-469.

Angelidaki, I. and Ahring, B.K., 1993. Thermophilic anaerobic digestion of livestock waste: the e€ect of ammonia. *Appl. Microbiol. Biotechnol.*, 38, 560-564.

APHA (American Public Health Association), 1995. Standard methods for the examination of water and wastewater. 19th Ed., Washington, DC.

Araji, A.A., Abdo, Z.O. and Joyce, P., 2001. Efficient use of animal manure on cropland-economic analysis. *Biosource Technol.*, 79, 179-191.

Arntz, H.J., Stoppok, E. and Buchholz, K., 1985. Anaerobic hydrolysis of beet pulp Discontinuous experiments. *Biotechnol.Lett.*,7, 113-118.

Aubart, C.H. and Fauchiller, S., 1983. Anaerobic digestion poultry wastes-PartI. Biogas production and pollution decrease in terms of retention time and total solids content. *Process Biocehmistry*, March/April, 31-34.

Baserga, U., Egger, K. and Wellinger, A., 1995. Biogas Produktion aus Festmist. FAT Bericht No. 451. 1-12.

Başçetinçelik, C., Karaca, H.H., Öztürk, M. and Kacıra, K., 2005. Agricultural Biomass Potential in Turkey. Proceedings of the 9th International Congress on Mechanization and Energy in Agriculture & 27th International Conference of CIGR Section IV: The Efficient Use of Electricity and Renewable Energy Sources in Agriculture, Sep.27-29, 2005, İzmir-TURKEY.

Bendixen, H.J., 1994. Safe guards against pathogens in Danish biogas plants. *Water Sci. Technol.*, 30(12), 171-180.

Bicudo, J.R., Safley, L.M. and Westerman, P. W., 1999. Nutrient content and sludge volumes in single-cell recycle anaerobic swine lagoons in north Carolina. *Trans. ASAE*, 42(4), 1087-1094.

Björnsson L., 2000. Intensification of the biogas process by improved process monitoring and biomass retention. PhD Dissertation, Dept. of Biotechnology, Lund University, Sweden. ISBN 91-7874-075-4.

Borchardt, J.A., 1971. Anaerobic phase separation by dialysis technique. *Anaerobic Bio.Treatment Processes*, 108-125.

Borja, R., Rincon, B., Raposo, F., Alba, J. and Martin, A., 2002. A study of anaerobic digestibility of two-phases olive mill solid waste (OMSW) at mesophilic temperature. *Process Biochem*., 38(5), 733–42.

Borja, R., Sánchez, E., Rincón, B., Raposo, F., Martin, M.A. and Martin, A., 2005. Study and optimisation of the anaerobic acidogenic fermentation of two-phase olive pomace, *Process Biochem.*, 40(1), 281-291.

Bouallagui, H., Torrijos, M., Godonc, J.J., Moletta, R., Ben Cheikh, R., Touhami, Y., Delgenes, P. and Hamdi, M., 2004. Two-phases anaerobic digestion of fruit and vegetable wastes: bioreactors performance. *Biochemical Engineering Journal*, 21, 193–197.

Brosius J., Dull T.J., Sleeter D.D. and Noller H.F., 1981. Gene organization and primary structure of a ribosomal RNA operon from E. coli. *J. Mol. Biol*., 148, 107-127.

Budiastuti H., 2004. Intensification of single stage continuously stirred tank anaerobic digestion process using carriers. PhD. Dissertation. Murdoch University, Canning Vale DC, Australia.

Bujoczek, G., Oleszkiewicz, J., Sparling, R. and Cenkowski, S., 2000. High solid digestion of chicken manure. *J. Agricultural Engineering*, 76, 51-60.

Burke, DA., 2001. Dairy waste anaerobic digestion handbook. Options for recovering beneficial products from dairy manure. Environmental Energy Company, Olympia, WA.

Calzada, J.F., de Porres, E., Yurrita, A., de Arriola, M.C., de Micheo, F., Rolz, C. and MenchúA Cabello, J.F., 1984. Biogas production from coffee pulp juice: One- and two-phase systems, *Agr. Wastes*, 9(3), 217-230.

Capri, M. and Marais, G.vR., 1975. pH adjustment in anaerobic digestion. *Water Res.*, 9(3), 307-314.

Chanakya, H.N., Borgaonkar, S., Rajan, M.G.C. and Wahi, M., 1992. Two-phase anaerobic digestion of water hyacinth or urban garbage. *Bioresource Technol.*, 42 (2), 123–31.

Chen, Y.R., 1983. Kinetic analysis of anaerobic digestion pig manure and its design implications. *Agr. Wastes*, 8, 65-81.

Chynoweth, D. P. and Pullammanappallil, P., 1996. Anaerobic digestion of municipal solid waste, CRC Press, 71-113.

Cirne, D.G., Lehtomaki, A.D., Bjornsson, L. and Blacka,L., 2007. Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *J. Applied Microbiology*, online.

Clarke, E. and Baldwin, A.H., 2002. Responses of wetland plants to ammonia and water level. *Ecological Engineering*, 18, 257-264.

Cohen, A., Breure, A.M., Van Andel, J.G. and Van Deursen, A., 1979. Anaerobic digestion of glucose with separated acid production and methane formation. Water Res., 13 (4), 571-580.

Danish Energy Agency, 1999. Centralized biogas plants, Danish Institute of agricultural and fisheries economics.

Demirel, B. and Yenigun, O., 2004. Anaerobic acidogenesis of dairy wastewater: the effects of variations in hydraulic retention time with no pH control, *J. Chemical Technol Biotechnol.*, 79 (7), 755–760.

Demirer, G.N. and Speece, R.E., 1999. Inhibitory effects and biotransformation of acrylic acid in computer-controlled pH-Stat reactors, *Biotechnol. and Bioeng.*, 62, 200-207.

Demirer, G.N. and Chen, S., 2004. Effect of retention time and organic loading rate on anaerobic acidification and biogasification of dairy manure. *J. Chemical Technol. Biotechnol*., 79 (12), 1381-1387.

Demirer, G.N. and Chen, S., 2005. Two-phase anaerobic digestion of unscreened dairy manure. *Process Biochemistry*, 40 (11), 3542-3549.

Dinopoulou, G., Rudd, T. and Lester, J.N., 1988. Anaerobic acidogenesis of a complex wastewater: I. The influence of operational parameters on reactor performance. *Biotechnol. Bioeng.*, 31 (6), 958-968.

Dohanyos M. and Zabranska J., 2001. Anaerobic Digestion, chap. 13, In: Spinosa L., Vesilind P.A. Edit. Sludge into Biosolids, IWA Publishing, ISBN 1-900222-08-6.

Eastman, J.A. and Ferguson, J.F., 1981. Solubilisation of organic carbon during the acid phase anaerobic digestion. *J. WPCF*, 53, 352–366.

Elefsiniotis, P. and Oldham, W.K., 1994. Anaerobic acidogenesis of primary sludge: The role of solids retention time. *Biotechnol Bioeng.*, 44, 7–13.

Elefsiniotis, P. and Oldham, W.K., 1994. Effect of HRT on acidogenic digestion of primary sludge. *J. Env. Eng.*, 120, 645-660.

Fongsatitkul, P., 1992. A Modified Two-Stage Anaerobic Digestion of Sewage Sludge. Ph.D.,The University Of British Columbia.

Fox, P. and Pohland, F.G., 1994. Anaerobic treatment applications and fundamentals: substrate specifity during phase separation. *Water Environ. Res.*, 66 (5), 716-723.

General Bioenergy Inc., 2000. Optimized Anaerobic Treatment Process *Bioenergy Update*. 2(1).

Georgakakis, D. and Dalis, D., 1993. Controlled anaerobic digestion of settled olive oil wastewater. *Bioresource Technol.*, 46, 221-226.

Gerardi, M.H.. 2003. The Microbiology of Anaerobic Digesters, John Wiley & Sons. Inc., Hoboken, New Jersey.

Ghaly, A. E. and Pyke, J. B., 1992. Biogas Production From Cheese Whey/Dairy Manure. Halifax, Nova Scotia, Agricultural Engineering Department Technical University of Nova Scotia.

Ghosh, S., 1985. Solid-phase methane fermentation of solid wastes. Trans ASME, 107, 402–405.

Ghosh, S., 1987. Improved Sludge Gasification by Two-Phase Anaerobic Digestion, *J. Env. Eng.*, 113 (6), 1265-1284.

Ghosh, S., 1991. Pilot-scale demonstration of two-phase anaerobic digestion of activated sludge. *Water Sci. Technol.*, 23 (7), 1179–1188.

Ghosh, S., 1997 Anaerobic digestion for renewable energy and environmental restoration, Pp. 9-16, In: Proceedings of the 8th International Conference on Anaerobic digestion, Sendai International Center, Sendai, Japan, May 25-29.

Ghosh, S. and Klass, D.L. 1978 Two-Phase Anaerobic Digestion. *Process Biochemistry*, 13, 15-24.

Ghosh, S., Conrad, J.R. and Klass, D.L., 1975. Anaerobic acidogenesis of wastewater sludge. J WPCF, 47, 30–45.

Ghosh, S., Ombregt, J.P. and Pipyn, P., 1985. Methane production from industrial wastes by two-phase anaerobic digestion, *Water Res.*, 19(9), 1083-1088.

Giovannoni, S. J., Britschgi, T. B., Moyer C.L. and Field, K.G., 1999. Genetic diversity in Sargasso Sea bacterioplankton. *Nature* (London). 60–63.

Godon, J.J., Zumstein, E., Dabert, P., Habouzit, F. and Moletta, R., 1997a. Microbial 16S rRNA diversity in an anaerobic digester. *Wat. Sci. Tech.*, 36 (6-7), 49-55.

Godon, J.J., Zumstein, E., Dabert, P., Habouzit, F. and Moletta, R., 1997b. Molecular microbial diversity of an aneorobic digestor as determined by small-subunit rDNA sequence analysis. *Appl. Environ. Microbiol*., 63 (7), 2802-2813.

Goel, B., Pant, D.C. and Kishore, V.V.N., 2001. Two-phase anaerobic digestion of spent tea leaves for biogas and manure generation, *Bioresource Technol.*, 80(2), 153-156.

Green Matters, 2003. Harvesting manure for energy, nutrients and more. Green Matters, Issue No. 15, Alberta Environmentally Sustainable Agriculture (AESA) Council, Edmonton, Canada.

Grotenhuis, J-T.C., Smit, M., Plugge, C.M., Yuansheng, X., van Lammeren, A-A.M., and Stams, A-J.M., 1991. Bacteriological composition and structure of granular sludge adapted to different substrates. *Appl Environ Microbiol*., 57,1942-1949.

Guerra-Rodriguez, E., Diaz-Ravina, M. and Vazquez, M., 2001. Co-composting of chestnut burr nad leaf litter with solid poultry manure. *Biosource Technol.*, 78, 107-109.

Guerrero L., Omil F., Mendez R. and Lema, J.M., 1999. Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. *Water Research*, 33(10), 3281-3290.

Gunaseelan, V. N., 1997. Anaerobic digestion of biomass for methane production: a review, *Biomass and Bioenergy*, 13(1-2), 83-114.

Gurtner, C.S., Pinar, G., Lubitz, W. and Rölleke, S., 2001. An advanced molecular strategy to identify bacterial communities on art objects. *J. Microbiological Methods*, 45(2), 77-87.

Hammad, M., Badarneh, D. and Tahboub, K., 1999. Evaluating variable organic waste to produce methane. *Energy Conservation and Management*, 40, 1463-1470.

Hashimoto, A.G., 1982. Effect of mixing duration and vacuum on methane production rate from beef cattle waste. *Biotechnol. Bioeng.*, 24, 9–23.

Hawkes, F.R. and Hawkes, D.L., 1987. Anaerobic Digestion, In Bu'lock, J. and B. Kristiansen (eds.), Basic Biotechnology. Academic press, London, 337-358.

Head, I. M., Saunders, J. R. and Pickup, R.W., 1998. Microbial evolution, diversity, and ecology: a decade of ribosomal analysis of uncultivated microorganisms. *Microb. Ecol.* 35 (1998), pp. 1–21

Heider, J. and Fuchs, G., 1997. Microbial anaerobic aromatic metabolism, *Anaerobe*, 3, 1–22.

Henze, M. and Harremoes, P., 1983. Anaerobic treatment of wastewater in fixed film reactors. A literature review. *Water Sci. Technol*., 15 (1), 101.

Hill, D. T., 1984. Economically optimized design of methane fermentation systems for swine production facilities. *Trans. ASAE*, 27(2), 525-529.

Hills, D.T., 1980. Methane gas production from dairy manure at high solids concentration. *Trans. ASAE*, 23(1), 122-126.

Hills, D.T., 1982. A comprehensive dynamic model for animal waste methanogenisis. *Trans. ASAE*, 25(5), 1374-1380.

Hills, D.T., 1984. Methane productivity of the major animal waste types. *Trans. ASAE*, 27, 530-534.

Hills, D.J. and Kayhanian, M., 1985. Methane from settled and filtered flushed dairy wastes. *Trans. ASAE,* 28(3), 865-869.

Hobson, P.N., 1990. The treatment of agricultural wastes. In Anaerobic digestion: a waste treatment technology. Ed. Wheatley, A., 93-138. ISBN: 1851665269. Elsevier Applied Science, London.

Hobson, P.N. and Wheatley, A.D., 1993. Anaerobic digestion: modern theory and practice. 256 pages. ISBN: 1851669582. Elsevier Applied Science, London.

Huang, J.J.H. and Shih, J.C.H., 1981. The potential of biological methane generation from chicken manure. *Biotechnology and Bioengineering*, 23, 2307-2314.

Huang, G.F., Fang, M., Wu, Q.T., Zhou, L.X., Liae, X.D. and Wong, J.W.C., 2001, Composting of pig manure with leaves. *Environmental Technol.,* 22(10), 1203-1212.

Humenik, F.J., and M.R. Overcash. 1976. Design Criteria for Swine Treatment Systems. EPA Report 600/2-76-233, 291 pp.

Husain, A., 1998. Mathematical models of the kinetics of anaerobic digestion a selected review. *Biomass Bioenergy*, 14(5/6), 561–571.

Hwang, I.S., Min, K.S., Choi, E. and Yun, Z., 2006. Resource recovery and nitrogen removal from piggery waste using the combined anaerobic processes. *Water Sci. Technol.*, 54(8), 229–236

Ince O., 1998. Performance of a two-phase anaerobic digestion system when treating dairy wastewater, *Water Res.*, 32(9), 2707-2713.

Itodo, I. and Awulu, J.O., 1999. Effects of total solids concentrations of poultry, cattle and piggery waste slurries on biogas yield. *Trans. ASAE.,* 42, 1853–1855.

John, H.M., 2005. An evaluation of a mesophilic, modified plug flow anaerobic digester for dairy cattle manure. *AgSTAR Program*, EPA, USA.

Jukes, T.H. and Cantor, C.R., 1969. Evolution of protein molecules. In: Mammalian Protein Metabolism, H. N. Munro (Ed), pp.21-132. Academic Press, New York.

Karima, K., Hoffmanna, R., Klassonb, K.T. and Al-Dahhan, M.H., 2005. Anaerobic digestion of animal waste: Effect of mode of mixing, *Water Res*., 39, 3597–3606.

Krich, K., Augenstein, D., Batmale, J.P., Benemann, J., Rutledge, B. and Salour, D., 2005. Biomethane from Dairy Waste Sourcebook for the Production and Use of Renewable Natural Gas in California Prepared for Western United Dairymen.

Knight, R.L., Payne, Jr.V.W.E., Borer, R.E., Clarke, Jr.R.A. and Pries, J.H., 2000. Constructed wetlands for livestock wastewater management, *Ecological Engineering*, 15, 41-55.

Koster I.W., 1984. Liquefaction and acidogenesis of tomatoes in an anaerobic two-phase solid-waste treatment system, *Agr. Wastes*, 11(4), 241-252.

Kubler, H., and Schertler, C., 1994. Three-phase anaerobic digestion of organic wastes, *Water Sci. Tech.,* 30(12), 367-374.

Lawrence, A. W. and McCarty, P.L., 1967. Kinetics of methane fermentation in anaerobic waste treatment. Department of Civil Engineering, Stanford University, PaloAlto, California.

Lebuhn, M., Effenberger, M., Gronauer, A., Wilderer, P.A. and Wuertz S. (2003) Using quantitative real-time PCR to determine the hygienic status of cattle manure. *Wat. Sci. Tech.*, 48 (4), 97-103.

Liu, D., Liu, D., Zeng, R.J. and Angelidaki, I., 2006. Hydrogen and methane production from household solid waste in the two-stage fermentation process. *Water Res.*, 40, 2230-2236.

Lo K.V. and Liao P.H., 1985. Two-Phase Anaerobic Digestion of Screened Dairy Manure, *Biomass*, 8, 81-90.

Lo K.V., Bulley, N.R., Liao P.H. and Whitehead, A.J., 1983. The effect of solids separation pretreatment on biogas production from dairy manure. *Agric. Wastes*, 8, 346-350.

Lo, K.V., Liao, P.H., Whitehead, A.J. and Bulley, N.R., 1984. Mesophilic anaerobic digestion of screened and unscreened dairy manure. *Agricultural Wastes*, 11, 269-283.

Ludwig, W. and Schleifer, K.H., 1994. Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS Microbiol. Rev*., 15, 155-173.

Lynd, L.R., Weimer, P.J., vanZyl, W.H. and Pretorius, I.S., 2002. Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiol Mol. Biol. Rev.*, 66, 506–577.

Mackie, R.I. and Bryant, M.P., 1995. Anaerobc digestion of cattle waste at mesophilic and thermophilic temperatures. *App. Microbial. Biotechnol.*, 43, 346-350.

Maharaj, I., 1999. Acid-phase anaerobic digestion of primary sludge: the role of hydraulic retention time (HRT), temperature and a starch-rich industrial wastewater, M.Sc. thesis. The University of Manitoba, Winnipeg, Manitoba, Canada.

Maidak, B.L., Larsen, L., McCaughey, M.J., Overbeek, R., Olsen, G.J., Fogel, K., Blandy, J. and Woese, J.R., 1994. The Ribosomal Database Project. *Nucleic Acids Res*., 22, 3485-3487.

Maidak, B.L., Cole, J.R., Parker Jr., C.T., Garrity, G.M., Larsen, N., Li, B., Lilburn, T.G., McCaughey, M.J., Olsen, G.J., Overbeek, R., Pramanik, S., Schmidt, T.M., Tiedje, J.M. and Woese, C.R., 1999. A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Res.*, 27, 171-173.

Maly, J. and Fadrus, H., 1971. Influence of temperature on anaerobic digestion. *J. Water Poll. Control Fed.* 43: 64.

Martinez, J. and Hao, X., 1996. A field treatment plant for pig slurry. *Water Sci. Technol.*, 34(11), 87-92.

Martinez, J. and Burton, C., 2003. Manure management and treatment: an overview of the european situation. XI International Congress ISAH 2003 23 - 27 February 2003, Mexico City

Massey, M.L. and Pohland, F.G., 1978. Phase separation of anaerobic stabilization by kinetic control. *J. WPCF*, 50, 2204–2221.

Mata-Alvarez, J., Mtzviturtia, A., Llabresluengo, P. and Cecchi, F., 1993. Kinetic and performance study of a batch 2-phase anaerobicdigestion of fruit and vegetable wastes. *Biomass Bioenergy*, 5(6), 481–488.

Mata-Alvarez, J, Mace, S., Llabre, S. P., 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Biores Technol., 74 (1), 3–16.

McCarty, P.L. and McKinney, R.E., 1961. Volatile acid toxicity in anaerobic digestion. *J. WPCF, 33,* 223-232.

McCarty, P.L. and Smith D.P., 1986. Anaerobic wastewater treatment. *Environ. Sci. Technol.*, 1200-1206.

Mechichi, T., Labat, M., Patel, B.K.C., Woo, T.H.S., Thomas, P. and Garcia, J.L., 1999. *Clostridium methoxybenzovorans* sp. nov., a new aromatic degrading homoacetogen from olive oil mill wastewater treatment digester, *International Journal of Systematic Bacteriology*, 49, 1201–1209.

Midwest Plan Service. 1993. Livestock waste facilities handbook. MWPS-18. Third Edition. Iowa State University, Ames, IA.

Mills, J.P., 1977. A comparison of an anaerobic digester and an aeration system treating piggery waste from the same source. *Food, Fertilizer, and Agricultural Residues - Cornell Agricultural Waste Management Conference Proceedings*, 1977. *Ann Arbor Science*. 415-422.

Miyahara, T., Mizuno, O. And Noike, T., 2000. Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes. *Water Sci. Technol.*, 41 (3), 25–32.

Merkel, J.A., 1981. Managing livestock wastes. The AVI Publishing Company, Inc., Westport, CN.

Morgan, R.M., and Keller. L.H., 1987. Economic comparisons of alternative waste management systems on Tennessee Dairy Farms. University of Tennessee Agricultural Experiment Station Bulletin 656.

Morken, J. and Sakshaug, S., 1998. Direct ground injection of livestock waste slurry to avoid ammonia emission. *Nutrient cycling in agroecosystems*, 51, 59-63.

Moyer, C.L., Dobbs, F.C. and Karl, D.M., 1995. Phylogenetic diversity of the bacterial community from a microbial mat at an active hydrothermal vent system, Loihi Seamount, Hawaii. *Appl. Environ. Microbiol*., 61, 1555-1562.

Muyzer, G., de Waal, E. And Uitterlinden, A., 1993. Profiling of complex microbial populations by denaturing gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16SrRNA. *Appl. Environ. Microbiol.*, 59, 695–700.

Muyzer, G. and Smalla, K., 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology, *Antonie Van Leeuwenhoek*, 73, 127–141

Nielsen, H.B, Mladenovska, Z., Westermann, P. and Ahring, B.K., 2004. Comparison of Two-Stage Thermophilic (68°C/55°C) Anaerobic Digestion With One-Stage Thermophilic (55°C) Digestion of Cattle Manure, *Biotech. Bioeng.*, 86 (3), 291-301.

Noike, T., Takabatake, H., Mizuno, O. and Ohba, M., 2002. Inhibition of hydrogen fermentation of organic wastes by lactic acid bacteria. *Int. J. Hydrogen Energy*, 27, 1367-1371.

Nozhevnikova, A.N., Simankova, M.V. and Kotsyurbenko, O.R., 2001. Temperetaure characteristics of methanogenic archea and acetogenic bacteria isolated from col environments. *Water Sci. and Technol.*, 44, 41-48.

North Carolina Agricultural Extension Service. 1973. Dairy waste management alternatives. North Carolina State University Circular, 568.

O'Keefe, D.M. and Chynoweth, D.P., 2000. Influence of phase separation, leachate recycle and aeration on treatment of municipal solid waste in simulated landfill cells, *Biores. Technol.*, 72(1), 55-66.

Ong, H.K., Greenfield, P.F., Pullammanappallil, P.C., 2000. An operational strategy for improved biomethanation of cattle-manure slurry in an unmixed, single-stage digester. *Biores. Technol.*, 73, 87-89.

Owen, W. F., Stuckey, D. C., Healey, J. B., Young, L. Y. and McCarty, P. L., 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity, *Wafer Res., 13, 485-492.*

Pain, B.F., West, R., Oliver, B. and Hawkes, D.L., 1984. Mesophilic anaerobic digestion of dairy cow slurry on a farm scale. First comparisons between digestion before and after solids separation. *J. Agric. Engng. Res.*, 29, 249-56.

Parawira, W., Murto, M., Read, J.S. and Mattiasson, B., 2004. Volatile fatty acid production during anaerobic mesophilic digestion of solid potato waste. *J. Chemical Technol. Biotechnol.*, 79(7), 673–677.

Pavan, P., Battistoni, P., Cecchi, F., Mata-Alvarez, J., 1999. Two-phase anaerobic digestion of source-sorted OFMSW: performance and kinetic study. In *II Int. Symp. Anaerobic Dig. Solid Waste*, held in Barcelona, June 15-17, 1999 (eds. J. Mata- Alvarez, A. Tilche and F. Cecchi), Vol. 1, 91-98, Int. Assoc. Wat. Qual.

Pipyn, P. and Verstraete, W., 1981. Lactate and ethanol as intermediates in two-phase anaerobic digestion. *Biotechnol. Bioeng.*, 23, 1145-1154.

Pohland, F.G. and Ghosh, S., 1971. Developments in anaerobic stabilization of organic wastes the two-phase concept. *Environ. Lett.*, 1, 255–66.

Poulsen, T., 2004. Solid waste management, Aalborg University, Denmark, Section for Environmental Engineering, AAU.

Raposo, F., Borja, R., Sánchez, E., Martín, M. A. and Martín, A., 2004. Performance and kinetic evaluation of the anaerobic digestion of two-phase olive mill effluents in reactors with suspended and immobilized biomass, *Water Res.*, 38(8), 2017-2026.

Qasim, R. S., Warren, K., and Udomsinrot, K. 1984. Methane gas production from anaerobic digestion of cattle manure. *Energy Sources*, 7(4), 319–341.

Rico, J.L., Garcia, H., Rico, C. and Tejero, I., 2007. Characterisation of solid and liquid fractions of dairy manure with regard to their component distribution and methane production. *Bioresource Technology*, 98, 971–979.

Ritmann, B.E. and McCarty, P.L., 2001. Environmental biotechnology: princeples and applications. McGraw Hill, New York.

Robbins, J.E., Armold, M.T. and Weiel, J.E., 1983. Anaerobic Digestion of Cellulose-Dairy Cattle Manure Mixtures. *Agricultural Wastes*, 8, 105-118.

Rustrian, E., Delgenes, J.P., Bernet, N. and Moletta, R., 1998. Simultaneous removal of carbon, nitrogen and phosphorus from wastewater by coupling two-

step anaerobic digestion with a sequencing batch reactor *J. Chem. Technol. Biotechnol.*, 73, 421-431

Saitou, N. and Nei, M., 1987. The neighbor-Joining, a new method for constructing phylogenetic trees. *Mol. Biol. Evol.*, 4, 406-425.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular cloning, A laboratory manual. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press.

Sanchez, E., Borja, R., Weiland, P., Travieso, L. and Martin, A., 2000. Efeect of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal in the batch anaerobic digestion process of cattle manure. *Bioprocess Engineering*, 22, 247-252.

Sanger, F., Nicklen, S and Coulson, A.R., 1977. DNA sequencing with chain termination inhibitors. Proc.Natl.Acad. Sci. USA 74, 5463-5467.

Sharma, V.K., Testa, C., Castelluccio, G., 1999. Anaerobic treatmnet of semi-solid organic waste. *Energy conservation and management*, 40, 369-384

Shyam, M. 2001. A biogas plant for the digestion of fresh undiluted cattle dung, *Boiling point*, No.47, 33-35.

Sobel, A.T. and Muck, R.E., 1983. Energy in animal manures. *Energy in Agriculture*, 2, 161-176.

Sommer, S.G. and Hutchings, N.J., 2001. Ammonia emission from field applied manure and its reduction. European journal of agronomy, 15, 1-15.

Smith, P.H., Bordeaux, F.M., Goto, M., Shiralipour, A., Wilkie, A., Andrews, J.F., Ide, S. and Barnett, M.W., 1988. Biological production of methane from biomass, p. 291-334, In W.H. Smith and J.R. Frank (ed.), *Methane from Biomass: A Systems Approach*. Elsevier Applied Science, Ltd., London.

Speece R.E., 1996. Anaerobic biotechnology for industrial wastewaters. Vanderbilt University, Arachae Press.

Summers, R., Hobson, N., Harries, C.R. and Richardson, A.J., 1987. Stirred-tank, mesophilic, anaerobic digestion of fattening-cattle wastes and of whole and separated dairy-cattle wastes, *Biological Wastes*, 20(1), 43-62.

Takashima, M. and Speece, R.E., 1989. Mineral nutrient requirements for high-rate methane fermentation of acetate at low SRT. *J. Water Pollut Control Fed.*, 61, 1645-1651.

Theron, J. and Cloete, T. E., 2000. Molecular Techniques for Determining Microbial Diversity and Community Structure in Natural Environments. *Critical Reviews in Microbiology*, 26(1), 37–57.

Thörneby, L., Persson, K., and Tragardh, G., 1999. Treatment of liquid effluents from dairy cattle and pigs using reverse osmosis. *J Agricultural Eng. Res.*, 73,(2), 159-170.

Tiquia, S.M. and Tam, N.F.Y., 1998. Composting of spent pig litter in turned and forced-aerated piles. *Env. Pollution*, 99, 329-337.

Toerien, D.F., and Hattingh, W.H.J., 1969. The micobology of anaerobic digestion. *Wat. Res.*, 3, 385-414.

US EPA, 1995. Guide manual on NPDES regulations for concentrated animal feeding operations, EPA 833-B-95-001.

Valdez-Vazquez, I., Rios-Leal, E., Esparza-Garcia, F., Cecchi, F. and Poggi-Varaldo, H.A., 2005. Semi-continuous solid substrate anaerobic reactors for H-2 production from organic waste: mesophilic versus thermophilic regime. *Int. J. Hydrogen Energy*, 30 (13–14), 1383–1391.

Veeken, A. and Hamelers, B., 1999. Effect of temperature on hydrolysis rates of selected biowaste components, *Bioresource Technology*, 69, 249–254.

Veeken, A., Kalyuzhnyi, S., Scharff, H. and Hamelers, B., 2000. Effect of pH and VFA on Hydrolysis of Organic Solid Waste, *J. Environmental Engineering*, 126(12), 1076-1081.

Velsen A.F.M. van, 1979. Adaptation of methanogenic sludge to high ammonia-nitrogen concentrations. *Water Res*., 13, 996-999.

Vandevivere, P., De Baere, L. and Verstraete, W., 2002. Types of anaerobic digesters for solid wastes, taken from the book 'Biomethanization of the Organic Fraction of Municipal Solid Wastes', IWA Publishing.

Varel, V.H., Hashimoto, A.G. and Chen, Y.R., 1980. Effect of temperature and retention time on methane production from beef cattle waste. *App. Environ. Microbiol.*, 40(2), 217-222.

Verrier, D., Roy, F. and Albagnac, G., 1987. Two-phase methanization of solid vegetable waste. *Biological Wastes, 22, 163-177.*

Vigneron V, Ponthieu M, Barina G, Audic JM, Duquennoi C, Mazéas L, Bernet N. and Bouchez, T., 2007. Nitrate and nitrite injection during municipal solid waste anaerobic biodegradation. *Waste Manag.*, 27(6), 778-791.

Von Sachs, J., Meyer, U., Rys, P. and Feitkenhauer, H., 2003. New approach to control the methanogenic reactor of a two-phase anaerobic digestion system. *Water Res*., 37(5), 973-982.

Yılmazer, G. and Yenigün, O., 2004. Two-phase anaerobic treatment of cheese whey, *Water Sci. Technol.*, 40(1), 289–295.

Yu, H.Q. and Fang, H.H.P., 2001. Acidification of mid- and high-strength dairy wastewaters. *Water Research*, 35(15), 3697-3705.

Wang, B., Dong, W., Zhang, J. and Cao, X., 1996. Experimental study of high rate pond system treating piggery wastewater. *Water Sci. Technol.*, 41(3), 231-238.

Wang, J.Y., Xu, H.L., Zhang, H. and Tay, J.H., 2003. Semi-continuous anaerobic digestion of food waste using a hybrid anaerobic solid–liquid bioreactor. *Water Sci. Technol.,* 48(4), 169–174.

Ward, D., Weller, R., and Bateson, M.M., 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature,* 345, 63–65.

Ward, D. M., Bateson, M. M., Weller, R., and Ruff-Roberts, A. L., 1992. Ribosomal RNA analysis of microorganisms as they occur in nature, *Adv. Microb. Ecol.*, 12, 219.

Wang, Z. and Banks, C.J., 2003. Evaluation of a two stage anaerobic digester for the treatment of mixed abattoir wastes. *Process Biochem.*, 38(9), 1267–73.

Wang, J.Y., Xu, H.L., Zhang, H. and Tay, J.H., 2003. Semi-continuous anaerobic digestion of food waste using a hybrid anaerobic solid-liquid bioreactor. *Wat. Sci. Technol.*, 48(4), 169-174.

Weiland, P., 1993. One and two-step anaerobic digestion from the organic fraction of municipal solid waste, *Water Sci. Technol.*, 27, 145-151.

Wellinger, A. and Sutter, K., 1988. Biogas production at low temperatures. Proceedings of Energy from Biomass and Wastes XII, New Orleans, Institute of Gas Technology, Chicago, IL, 21 pp.

Wellinger, R.J., Wolf, A.J. and Zakian, V.A., 1992. Use of non-denaturing Southern hybridization and two dimensional agarose gels to detect putative intermediates in telomere replication in Saccharomyces cerevisiae. *Chromosoma,* 102(1), 150-156

Wellinger, A., 1999. Process design of agricultural digesters. Nova Energie GmbH Elggerstrasse 36 8356 Ettenhausen, Switzerland. Retrieved from: http://homepage2.nifty.com/biogas/cnt/refdoc/whrefdoc/d14prdgn.pdf

Westerman, P.W., and M.R. Overcash. 1980. Dairy open lot and lagoon-irrigated pasture runoff quantity and quality. *Trans. ASAE*, 23, 1157-1164.

Wheatley, A., 1990. Anaerobic Digestion: A Waste Treatment Technology, *Applied Science*, 31, 2-31.

Woese, C.R., 1987. Bacterial evolution. *Microbiol. Rev*., 51, 221-271.

Zeeman, G., Sutter, K., Vens, T., Koster, M. and Wellinger, A., 1998. Psychrophilic digestion of dairy cattle and pig manure: start-up procedures of batch, fed-batch and CSTR-type digesters. *Biological Wastes*, 26, 15-31.

Zhou, J., Bruns, M.A. and Tiedje, J.M., 1996. DNA recovery from soils of diverse composition. *Appl. Environ. Microbiol*., 62(2), 316-322.

## CURRICULUM VITAE

### PERSONAL INFORMATION

Surname, Name: Yılmaz, Vedat
Nationality: Turkish (TC)
Date and Place of Birth: 01 Feb. 1970 , Sivas
Marital Status: Married
Phone: +90 312 210 58 80
Fax: +90 312 210 26 46
email: yvedat@odtu.edu.tr

### EDUCATION

| Degree | Institution | Year of Graduation |
|---|---|---|
| MS | Çukurova University, Graduate School of Natural and Applied Sciences, Soil Department | 99 |
| BS | METU Environmental Engineering | 93 |
| High School | Sivas Lisesi | 87 |

### WORK EXPERIENCE

| Year | Place | Enrollment |
|---|---|---|
| 2001-2007 | METU Department of Environmental Engineering | Research Assistant |
| 2000-2001 | Akdeniz University, Department of Environmental Engineering, Antalya | Research Assistant |
| 1995-2000 | Dicle University, Environmental Research Center, Diyarbakır | Research Assistant |
| 1993-1995 | GAP Administration, Reg. Direc., Şanlıurfa | Engineer |