MICROBIAL POPULATION DYNAMICS IN AN ANAEROBIC COMPLETELY STIRRED TANK REACTOR TREATING A PHARMACEUTICAL WASTEWATER

by

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ABSTRACT

In this study, effects of a chemical synthesis based pharmaceutical wastewater on performance of an anaerobic completely stirred tank reactor (CSTR) and activity of acetoclastic methanogens, number and composition of methanogens and non-methanogens were evaluated. The CSTR was initially fed with glucose. After that, it was fed with pre-aerated pharmaceutical wastewater diluted by glucose at different dilution ratios and then with raw pharmaceutical wastewater diluted with pre-aerated wastewater to enable acclimization of acetoclastic methanogens to the wastewater which contains non-biodegradable/toxic compounds for the anaerobic treatment.

At initial study with glucose, 92% soluble COD removal efficiency was achieved with the CSTR at an organic loading rate (OLR) of 6 kgCOD/m^3.d corresponding to an F/M ratio of 0.43 with a HRT of 2.5 days. Methane yield was 0.32 m^3CH_4/kgCOD and specific methanogenic activity (SMA) was found to be 336 $mlCH_4$/gTVS.d.

After initial study with glucose, the CSTR was fed with several dilutions of pre-aerated pharmaceutical wastewater with glucose (10%, 30% and 70%) and then 100% pre-aerated pharmaceutical wastewater. Due to the deteriation in the performance and decrease in the activity of acetoclastic methanogens after feeding with 100% pre-aerated pharmaceutical wastewater, HRT was increased from 2.5 days to 3.5 days. 71% soluble COD removal efficiency was obtained with a HRT of 3.5 days where methane yield was 0.28 m^3CH_4/kgCOD. However, SMA value was found to be 166 $mlCH_4$/gTVS.d indicating approximately 47% activity loss of the acetoclastic methanogens compared with the results of feeding with glucose.

Finally, raw pharmaceutical wastewater diluted with pre-aerated wastewater was fed into the CSTR in increasing ratios of 10%, 30% and 60%. Although a slight decrease in the performance was seen at a dilution ratio of 10% raw wastewater, there was a decrease in all parameters including soluble COD removal efficiency, methane yield, activity test results and an increase in total VFA concentration at a dilution ratio of 60% raw

pharmaceutical wastewater. According to the results of the SMA test indicating poor activity of acetoclastic methanogens, the study was discontinued at this ratio.

According to the microbiological studies, there were variations in the dominant species and the ratio of acetoclastic methanogens to total bacteria during the operation. M*ethanococcus* like species and short rods were dominant species after the operation with glucose. Short rods and medium rods were dominant at the end of the operation with 100% pre-aerated wastewater while short rods remained most dominant species until the end of the study. The ratio of acetoclastic methanogens to the total bacteria decreased at the end of the operation with glucose from 38% to 30% when 60% raw pharmaceutical wastewater was introduced to the CSTR.

ÖZET

Bu çalışmada, kimyasal sentez bazlı bir ilaç atıksuyunun tam karışımlı bir anaerobik reaktörün performansına ve asetoklastik metanojenlerin aktivitesine, sayılarına ve kompozisyonuna etkisi farklı giriş kompozisyonlarında değerlendirilmiştir. Tam karışımlı reaktör öncelikle glikoz ile beslenmiştir. Daha sonra reaktör, asetoklastik metanojenlerin, anaerobik arıtmada zor ayrışabilen ve/veya toksik maddeler içeren atıksuya alışabilmesi için öncelikle değişik oranlarda glikoz ile seyreltilmiş havalandırılmış ilaç atıksuyu ile daha sonra ham ilaç atıksuyu ile seyreltilmiş havalandırılmış atıksu ile beslenmiştir.

Glikozla yapılan çalışmanın sonunda, 6 kgKOİ/m^3.gün organik yüklemede ve 2.5 gün hidrolik bekletme süresinde (HBS) 92% çözünmüş kimyasal oksijen ihtiyacı (KOİ) giderim verimi elde edilmiştir. Metan verimi 0.32 m^3CH_4/kgKOİ ve spesifik metanojenik aktivite (SMA) 336 $mlCH_4$/gTUM olarak bulunmuştur.

Glikozla yapılan çalışmadan sonra, tam karışımlı reaktör öncelikle glikoz ile çeşitli oranlarda (10%, 30% and 70%) seyreltilmiş havalandırılmış ilaç atıksuyu ile, sonra da 100% havalandırılmış ilaç atıksuyu ile beslenmiştir. 100% ilaç atıksuyu reaktöre beslendikten sonra, performansdaki bozulma ve otofloresan metanojenik aktivitedeki düşüş nedeniyle HBS 2.5 günden 3.5 güne arttırılmıştır.

HBS 3.5 günde, 71% KOİ giderimi elde edilirken metan verimi 0.28 m^3CH_4/kgCOD olarak bulunmuştur. Bununla birlikte, SMA 166 $mlCH_4$/gTUM olarak bulunmuş ve bu değer glikozla besleme sonunda elde edilen sonuçla karşılaştırıldığında yaklaşık 47% aktivite kaybı olduğunu göstermiştir.

Son olarak ham ilaç atıksuyu havalandırılmış atıksuyu ile artan oranlarda seyreltilerek (10%, 30% ve 60%) tam karışımlı reaktöre beslenmiştir. Reaktör 10% ham atıksuyu ile beslendiğinde performansta küçük bir düşüş olmasına rağmen, 60% ham atıksu seyrelme

oranında KOİ giderim verimi, metan verimi, SMA dahil bütün parametrelerde dramatik bir düşüş meydana gelmiştir. SMA testinin sonuçlarına göre asetoklastik metanojenlerin aktivitesinde meydana gelen düşüş nedeniyle çalışma bu oranda sona erdirilmiştir.

Mikrobiyolojik çalışmaların sonuçlarına göre, dominant otofloresan metan türleri ve otofloresan metanojenlerin toplam bakteri içindeki oranı çalışma boyunca değişim göstermiştir. Glikoz ile yapılan çalışmanın sonunda dominant türler *Methanococcus* ve kısa çubuklar olarak bulunmuştur. 100% havalandırılmış atıksu ile beslemeden sonra kısa ve orta çubuklar dominant iken çalışma sonuna kadar dominant tür kısa çubuk olarak gözlenmiştir. Otofloresan metanojenlerin toplam bakteri içindeki oranı glikozla beslemenin sonunda %38 olmasına rağmen, %60 ham ilaç atıksuyu reaktöre beslendikten sonra bu oran %30'a düşmüştür.

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AMP	Actual Methane Production
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
CSTR	Completely Stirred Tank Reactor
d	Day
h	Hour
HRT	Hydraulic Retention Time
l	Liter(s)
mg	Miligram(s)
mL	Militer(s)
MLSS	Mixed Liquour Suspended Solids
MLVSS	Mixed Liguour Volatile Suspended Solids
OLR	Organic Loading Rate
PMP	Potential Methane Production
SMA	Specific Methanogenic Activity
SRT	Solid Retention Time
SS	Suspended Solids
TS	Total Solids
TVS	Total Volatile Solids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids

1. INTRODUCTION

The possible treatment alternatives for wastewaters have been a growing concern to prevent adverse effects of the pollutants on natural resources. The use of anaerobic treatment plants has increased due to the advantages such as high treatment capability of high strength wastewaters with low biomass production and the energy production of methane gas. However, anaerobic treatment systems require a careful operation since methanogens, which are involved in the removal of organic acids to end products such as carbon dioxide and methane, are affected by the fluctuations in the environmental conditions such as pH, temperature and toxic or inhibitory compounds.

Pharmaceutical industry generates strong wastewaters which cause problems in treatment plants. Especially chemical synthesis based pharmaceutical industry produce problematic wastewaters to treat since the processes of the industry use a wide variety of the priority pollutants including solvents which have a toxic effect on biological treatment. Although anaerobic systems can be acclimatized to the toxic or inhibitory compounds, some wastes may be too concentrated or toxic for the microbial population.

The study attempts to evaluate the effects of the wastewaters of a chemical synthesis based pharmaceutical industry on the performance of the anaerobic treatment systems by using a completely stirred tank reactor under various influent compositions. The changes caused by wastewater composition in both activity and bacterial composition in terms of number and composition of acetoclastic methanogens are also evaluated during the study by using specific methanogenic activity test (SMA) and direct microscopic count since treatment capacity of anaerobic systems is primarily determined by the concentration and activity of microbial population within the reactor. It is important to recognize the changes caused by wastewater composition and operating conditions during the treatment in order to take precautions.

It has been stated that the amount of active methanogenic population in an anaerobic reactor is

of great importance to achieve efficient wastewater treatment since an acceptable removal of organic compounds depends on the presence of an adequate level of methanogenic activity (Ince *et. al.*, 1994). Different techniques have been developed by a number of researchers in order to determine specific methanogenic activity (Monteggia, 1991; Reynolds, 1986; Valcke and Verstrate, 1983, Van den Berg *et. al.*, 1974). In this study, the SMA test developed by Monteggia (1991) was used in order to determine acetoclastic methanogenic activity of the seed sludge, organic loading rate during initial study with glucose and losses in the activity of the acetoclastic methanogens caused by the wastewater composition.

2. LITERATURE REVIEW

2.1. FUNDAMENTALS OF ANAEROBIC DIGESTION

2.1.1. Process Description

Anaerobic digestion is a multistage biochemical process of both series and parallel reactions in which complex organics are stabilized into mainly methane (CH_4) and carbon dioxide (CO_2) with trace amounts of hydrogen in the absence of oxygen (Figure 2.1.1.). Many specific groups of bacteria play role in the conversion of complex organic materials to the end products. There are also some approaches stated as models consisting of different number of steps namely:

- Four-stage Model
- Six-stage Model (Stronach *et. al.*, 1986)
- Nine-stage Model (Harper and Pohland, 1986)

Figure 2.1.2 illustrates the nine-stage model of anaerobic digestion established by Harper and Pohland (1986). These stages are:

1. Hydrolysis of organic polymers to intermediate organic monomers
2. Fermentation of organic monomers
3. Oxidation of propionic and butyric acids and alcohols by obligate H_2 producing acetogens (OHPA)
4. Acetogenic respiration of bicarbonate by homoacetogens
5. Oxidation of propionic and butyric acid and alcohols by sulfate reducing bacteria (SRB) and nitrate reducing bacteria (NRB)
6. Oxidation of acetic acid by SRB and NRB

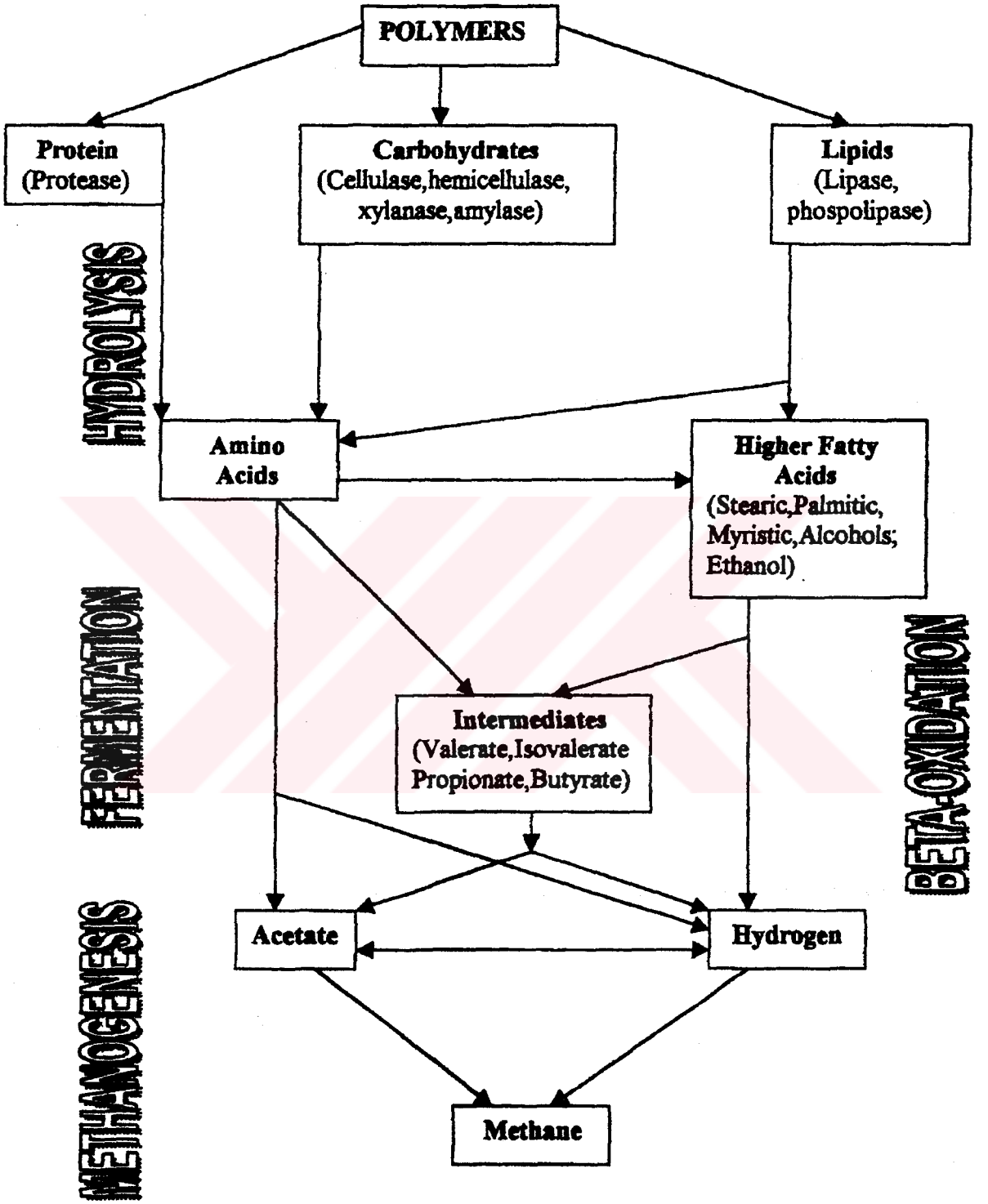


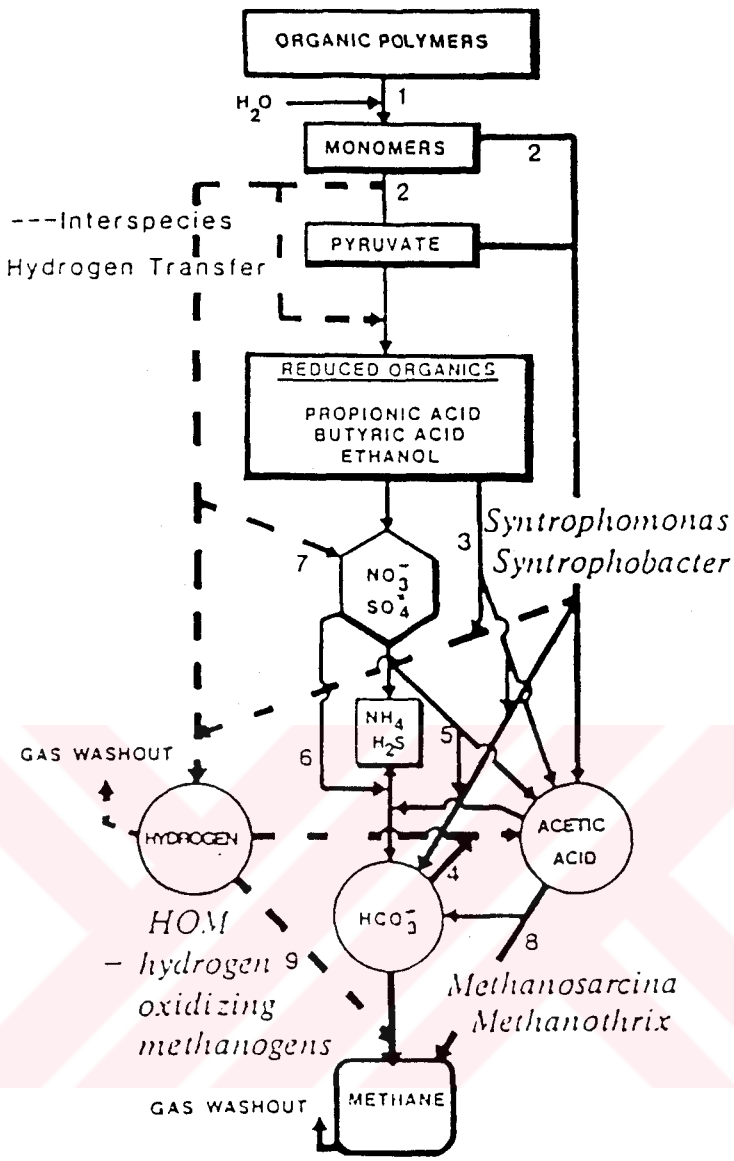
Figure 2.1.1. The Breakdown of Organic Polymers (Stronach *et al.*, 1986)

7. Oxidation of hydrogen by SRB and NRB
8. Acetoclastic methane formation
9. Methanogenic respiration of bicarbonate

Although the whole process has a very complex structure, the major stages of anaerobic digestion mechanism can be summarized as hydrolysis, acidogenesis, acetogenesis and methanogenesis. Complex organic compounds such as cellulose, proteins, lipids and carbohydrates are broken down into soluble organic compounds in the first phase of anaerobic digestion, called hydrolysis. The step is accomplished by extracellular enzymes of facultative anaerobic bacteria, the reaction rates of which are influenced by pH, cell residence time and the waste constituents (Payton and Haddock, 1986). Hydrolysis may not be observed in all anaerobic treatment systems. However, the success of anaerobic digestion is strongly related to this step for certain wastes because particulate organic material cannot pass through the bacterial cell membrane and cannot be utilized for the growth of bacteria. The step may be a rate-limiting step for the wastes such as pharmaceutical, some food wastes (Corbitt, 1990).

Acidogenesis is the fermentation process of hydrolyzed soluble organic compounds such as amino acids, sugars, and long chain fatty acids into carbon dioxide, hydrogen gas and volatile fatty acids (VFA). Acetic acid, butyric acid and propionic acid are major products of the step. However, higher fatty acids namely valeric acid, caproic acid, iso-butyric acid, iso-valeric acid and iso-caproic acid can also be produced at lower concentrations. The step is accomplished by acidogens.

In acetogenesis, all VFAs having more than two carbons are oxidized to acetic acid by the acetogenic bacteria. The oxidation process performed by the obligate hydrogen producing acetogenic bacteria is named as β oxidation. An acetate molecule is removed from fatty acids at each reaction until all fatty acids are converted to acetate by the oxidation process.



Legend:

1) hydrolysis of organic polymers
2) fermentation of organic monomers
3) oxidation of propionic and butyric acids and alcohols by OPRA
4) acetogenic respiration of bicarbonate
5) oxidation of propionic and butyric acids and alcohols by SRB and NRB
6) oxidation of acetic acid by SRB and NRB
7) oxidation of hydrogen by SRB and NRB
8) acetoclastic methane formation
9) methanogenic respiration of bicarbonate

Figure 2.1.2. Substrate Conversion Patterns Associated with the Anaerobic Digestion (Harper and Pohland, 1986)

In the last step, end products of the previous stage are converted into methane and carbon dioxide by an Archea called methanogens. Methanogenesis is the slowest and the most sensitive step of the anaerobic digestion process since specific environmental conditions are required for the growth of methanogens.

2.1.2. Microbiology and Biochemistry of Anaerobic Digestion

2.1.2.1. Microbiology of Anaerobic Digestion

In anaerobic treatment process the production of methane from the degradation of organic matter depends on the complex interaction of different groups of bacteria. Figure 2.1.3 shows bacterial types and substrate utilization in anaerobic digestion. The major groups of bacteria and the reactions taking place in anaerobic digestion are as follows:

1.Hydrolytic fermentative bacteria
2.Acidogenic (acid forming) bacteria
3.Hydrogen-producing acetogenic bacteria
4.Hydrogen-utilizing acetogenic bacteria
5.Carbondioxide-reducing methanogens
6.Acetoclastic methanogens

2.1.2.1.1. Hydrolysis

Complex wastes are required to be degraded or hydrolyzed into units as a first step to be taken up by the microbial cell. The hydrolysis of macromolecules such as lipids, proteins and carbohydrates under anaerobic conditions is carried out by specific extracellular enzymes, the reaction rates of which are influenced by pH, cell residence time and the waste constituents in the digester produced by hydrolytic bacteria.

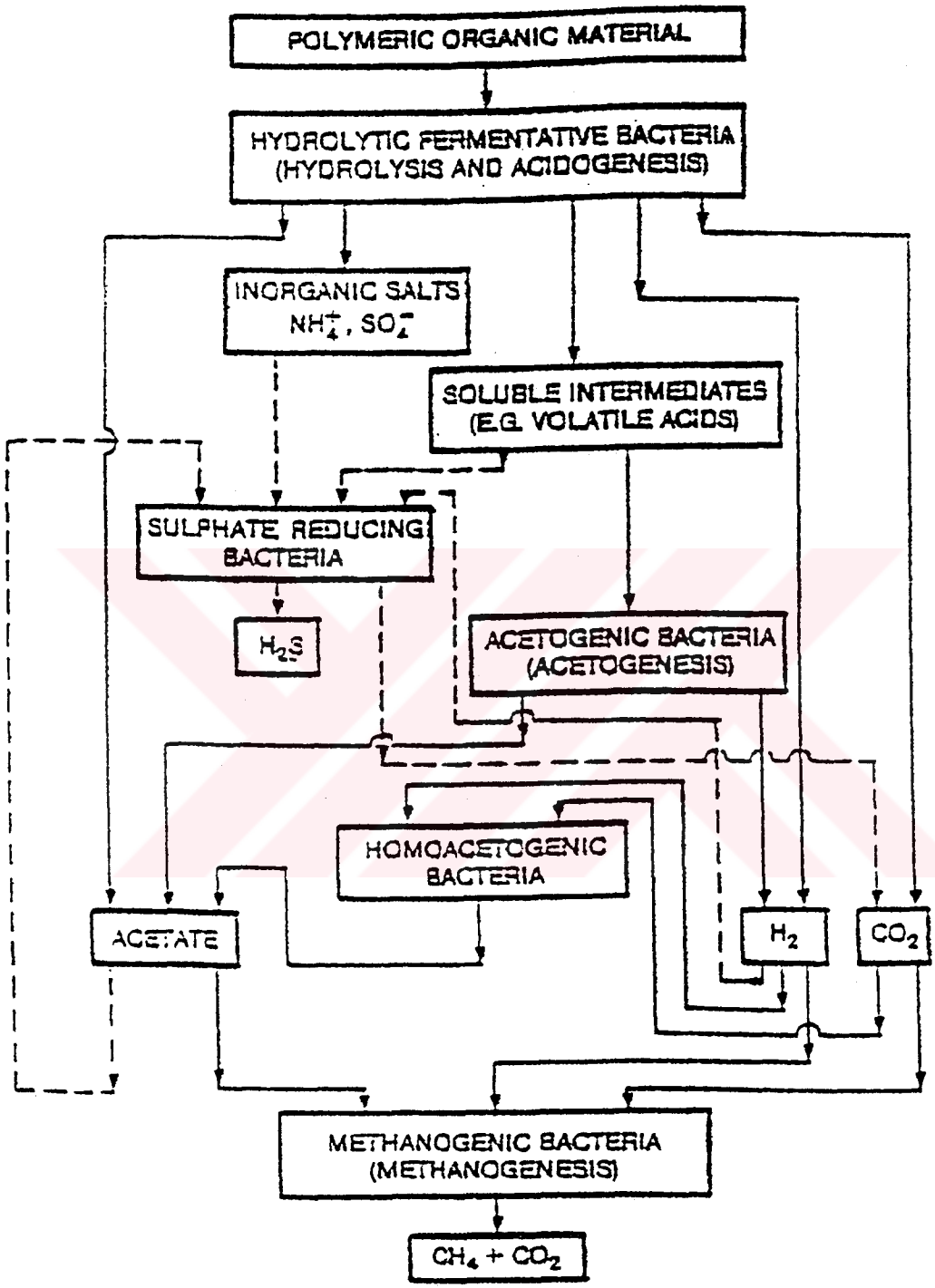


Figure 2.1.3. Schematic Diagram Showing Bacterial Types and Substrate Utilization in Anaerobic Digestion (Energy Technology Support Unit, 1992)

In an anaerobic digestion process where a substantial portion of the waste stream contains complex organic compounds, the hydrolytic bacteria and their enzymes are of paramount importance since their activity produces the simpler substrates for the succeeding steps in the degradation sequence (Stronach *et. al.*, 1986). It was stated that Clostridium is responsible for degradation of compounds containing cellulose and starch while Bacillus play role in the degradation of proteins and fats (Lema *et. al.*, 1991; Noike *et. al.*, 1985). The types of hydrolytic microorganisms are reported namely as, the cellulytic (*Clostridium thermocellum*), proteoytic (*Clostridium bifermentas, Peptococcus*), lipolytic (genera of clostridia and micrococci) and aminolytic (Clostridium butyricum, *Bacillus subtilis*) bacteria (Hungate, 1982; Payton and Haddock, 1986).

The hydrolytic bacteria may also break down the some intermediate products to simple volatile fatty acids, carbon dioxide, hydrogen, ethanol (Eastman and Ferguson, 1981). In the hydrolysis of organic matter, pH and cell residence time are important factors with respect to the reaction rate.

2.1.2.1.2. Acidogenesis and Acetogenesis

Acidogenesis

The breakdown products such as amino acids, sugars and long chain fatty acids of the hydrolysis phase are converted to the intermediary products acetate, carbon dioxide and hydrogen by acid forming bacteria. It was reported that acetate is the most important compound produced in the fermentation of organic substrates with propionate production of secondary consequence (Sorensen *et al.*, 1981).

There are two groups of acid producing bacteria. The first group is acidogens or fermentative bacteria which metabolizes amino acids and sugars to the intermediary products, acetate or hydrogen. Temperature, pH and the composition of the influent feed are important parameters affecting the formation of end product. The catabolism of these organic compounds is carried

out by a large number of both obligatory and facultatively anaerobic microorganisms and the process utilizes single amino acids, pairs of amino acids or a single amino acid with a non-nitrogenous compound. Single amino acids are converted under anaerobic conditions by clostridia, mycoplasmas and streptococci while butanol, butyric acid, acetone and iso-propanol are generally produced by the bacteria of the genera *Clostridum* and *Butyribacterium* , for example *Cl.butyricum* produces butyrate, *Cl. acetobutylicum* mainly acetone and butanol and *Cl. butylicum* produces butanol in addition to hydrogen, carbondioxide and iso-proponol.

Acetogenesis

The second group of acid forming bacteria is obligate hydrogen producing acetogenic bacteria (OHPA) which produce acetic acid, carbondioxide and hydrogen from propionate, butyrate and other higher fatty acids by the β-oxidation process. A molecule is removed from fatty acids having more than two carbons at each reaction until all fatty acids are converted to acetate molecules. (Bacteria producing acetic acid are *Methanobacterium bryantii, Desulfovibrio Syntrophobacter wolinii* (responsible for acetic acid production from propionic acid (Malina et. al. 1992; Stronach et. al., 1986), *Syntrophomonas wofei* (responsible for acetic acid production from butyric, caproic and valeric acids) (Malina *et. al.* 1992; Gujer *et.al.*, 1983), *Syntrophus buswellii.*

There is a syntrophic association between the OHPA and the hydrogen-consuming methanogens. Both *homoacetogens* and *methanogens* are strictly anaerobic prokaryotes using CO_2 as an electron acceptor in energy metabolism and use H_2 as a major electron donor. These processes result in the generation of ion gradient, either of H^+ or Na^+, which drives ATPases in the membrane. Acetogenesis also involves energy conservation via substrate-level phosphorylation. Besides H_2, electron donors for acetogenesis include C_1 compounds, sugars, organic acids, alcohols, amino acids and certain nitrogen bases, depending on the organisms. When a substrate conversion sequence was dominated with a particular metabolic pathway, it is frequently regulated by the intensity of the hydrogen production and its potential for

accumulation to inhibiting levels. Therefore, a lack of syntrophy between these bacteria can cause excessive accumulation of hydrogen or intermediate conversion products.

Most homoacetogenic bacteria are gram positive and many are classified in the genus *Clostridium*. Homoacetogens convert CO_2 to acetate by the acetyl-CoA pathway. Organisms producing acetate or oxidizing acetate via the acetyl-CoA pathway are listed in Table 2.1.1. The reaction (2.1) carried out by homoacetogens:

$$4H_2+H^-+2HCO_3^- \rightarrow CH_3COO^-+4H_2O \qquad (2.1)$$

Organisms such as *Acetobacterium woodii* and *Clostridium aceticum* can grow through two mechanisms, either chemoorganotrophically by fermentation of sugars (reaction 2.2) or chemolithotrophically through the reduction of CO_2 to acetate with H_2 as electron donor (reaction 2.3). Acetate is the major product produced via two mechanisms.

$$C_6H_{12}O_6 \rightarrow 3CH_3COO^- + 3H^+ \qquad (2.2)$$

$$2HCO_3^-+4H_2 + H^- \rightarrow CH_3COO^- + 4H_2O \qquad (2.3)$$

Glucose is converted to two molecules of pyruvate and two molecules of NADH (the equivalent of 4H) through the glycolytic pathway by homoacetogens and two molecules of acetate are produced as follows:

$$2 \text{ pyruvate}^- \rightarrow 2 \text{ acetate}^- + 2CO_2 + 4H \qquad (2.4)$$

Two molecules of CO_2 generated in reaction 2.4 are reduced to acetate by the homoacetate fermentation using the four electrons generated from glycosis plus the four electrons produced from the oxidation of two pyruvates to two acetates (reaction 2.4). Starting from pyruvate, the overall production of acetate (reaction 2.5) can be written as:

$$2 \text{ pyruvate}^- + 4H \rightarrow 3 \text{ acetate}^- + H \qquad (2.5)$$

Table 2.1.1. Organisms Employing the Acetyl-CoA Pathway of CO_2 Fixation (Madigan *et. al.*, 2000)

I. Acetate synthesis the result of energy metabolism
Acetoanaerobium noterae
Acetobacterium woodii
Acetobacterium wieringae
Acetogenium kivui
Acetitomaculum ruminis
Clostridium aceticum
Clostridium thermoaceticum
Clostridium formicoaceticum
Desulfotomaculum orientis
Sporomusa paucivorans
*Eubacterium limosum(*also produces butyrate)
Treponema sp. strains ZAS-1 and ZAS-2
II. Acetate synthesis in autotrophic metabolism
Autotrophic homoacetogenic bacteria
Autotrophic methanogens
Autotrophic sulfate-reducing bacteria
III. Acetate oxidation in energy metabolism
Reaction: Acetate+$2H_2O \rightarrow 2CO_2+8H$
Group II sulfate reducers (other than *Desulfobacter*)
Reaction: Acetate$\rightarrow CO_2+CH_4$
Acetotrophic methanogens (*Methanosarcina, Methanothrix)*

The acetyl-CoA pathway can be used in autotrophic growth for certain sulfate-reducing bacteria and also used by methanogens which can grow autotrophically on H_2+CO_2. By contrast, certain bacteria such as acetotrophic methanogens and sulfate-reducing bacteria employ the reactions of the acetyl-CoA pathway primarily in the reverse direction as a means of oxidizing acetate to CO_2. *Carbon monoxide* (CO) *dehydrogenase* which is a complex

enzyme containing the metals Ni, Zn, and Fe as metal cofactors is a key enzyme of the acetyl-CoA pathway and catalyzes the following reaction:

$$CO_2+H_2 \rightarrow CO+ H_2O \qquad (2.6)$$

The CO produced through the reaction ends up in the carbonyl position of acetate. The methyl group (CH_3) of acetate is generated from the reduction of CO_2 by a series of reactions involving the coenzyme *tetrahydrofolate* and then, it is transferred from *tetrahydrofolate* to an enzyme containing vitamin B_{12} as cofactor. The CH_3 group is combined with CO in CO dehydrogenase to form acetate in the final step of the pathway. The mechanism contains the methyl group which is attached to an atom of nickel in the enzyme, combining with CO, which is bound to an atom of Fe in the enzyme, along with coenzyme A to form acetyl-CoA.

2.1.2.1.3. Methanogenesis

The performance of the anaerobic reactor and the quality of the effluent depend on the activity of methanogens. Methanogenesis is defined as a rate-limiting step in the whole anaerobic digestion process due to the slow growth rate of the methanogens comparing with acidogens. (Malina *et. al.*,1992; Noike *et. al.*, 1985; Speece, 1983; Ghosh *et. al.*, 1975).

Methane production in the anaerobic degradation is carried out by a group of strictly anaerobic Archea called the methanogens which are structurally prokaryotic cells that show a diversity of cell wall chemistries. For example, walls of *Methanobacterium* species and relatives include pseudomurein while *Methanococcus* and *Methanoplanus* species include the protein or glycoprotein walls. *Methanosarcina* and relatives contain the methanochondroitin (so named because of its structural resemblance to chondroitin sulfate). Table 2.1.2 illustrates taxonomy of methanogens based on both phenotypic as well as phylogenetic (comparative 16S rRNA sequencing) analyses (Madigan *et. al.*, 2000).

Methanogens convert the end products of the previous step into methane and carbon dioxide via two conversion mechanisms including decarboxylation of acetic acid and reduction of

carbon dioxide.

There are numerous studies carried out to determine kinetic coefficients in pure cultures of methanogens. Kinetic coefficients of the microorganisms involved in the conversion processes are given in Table 2.1.3 and Table 2.1.4. Some of the microorganisms involved in the process of the methane production from acetate are the genera of *Methanosarcina* and *Methanothrix*, (Malina *et. al.*, 1992; Noike *et. al.*, 1985; Zehnder *et. al.*, 1982) whereas Methano*thrix soehngenii, Methanosarcina barkeri, Methanobacterium sp., Methanococcus mazei* are the examples of the most common species defined in the literature. (Malina *et. al.*, 1992; Fannin *et. al.*, 1983)

Methanobrevibacterium and *Methanobacterium* are the most common genera involved in the process of the methane production via carbondioxide reduction by the hydrogen-utilizing methane bacteria. The formation of volatile acids are regulated by the bacteria. Some of the species reported in the literature are *Methanospirillum hungatei, Methanobrevibacter snithii, Methanobacterium formicicum, Methanosarcina barkeri.*

Table 2.1.2. Characteristics of Methanogenic Archea (Madigan *et. al.*, 2000)

Genus	**Morphology**	**Number of Species**	**Substrate for methanogenesis**	**DNA (mol %GC)**
Methanobacteriales				
Methanobacterium	Long rods	19	H_2+CO_2, formate	29-61
Methanobrevibacter	Short rods	7	H_2+CO_2, formate	27-31
Methanosphaera	Cocci	2	Methanol+H_2	26
Methanothermus	Rods	2	H_2+CO_2, can also reduce S^0; hyperthermophile	33

Table 2.1.2. Characteristics of Methanogenic Archea (continued)

Genus	Morphology	Number of Species	Substrate for methanogenesis	DNA (mol %GC)
Methanococcales				
Methanococcus	Irregular cocci	11	H_2+CO_2, pyruvate+CO_2, formate	29-34
Methanomicrobiales				
Methanomicrobium	Short rods	2	H_2+CO_2, formate	45-49
Methanogenium	Irregular cocci	11	H_2+CO_2, formate	51-61
Methanospirillum	Spirilla	1	H_2+CO_2, formate	46-50
Methanoplanus	Plate-shaped	3	H_2+CO_2, formate	38-47
Methanocorpusculum	cells	5	H_2+CO_2, formate, alcohols	48-52
Methanoculleus	Irregular cocci	6	H_2+CO_2, alcohols, formate	54-62
	Irregular cocci			
Methanosarcinales				
Methanosarcina	Large irregular cocci in packets	8	H_2+CO_2, methanol, methylamines, acetate	41-43
Methanolobus	Irregular cocci in aggregates	5	Methanol, methylamines	38-42
Methanohalobium	Irregular cocci	1	Methanol, methylamines;halophilic	44
Methanococcoides	Irregular cocci	2	Methanol, methylamines	42
Methanohalophilus	Irregular cocci	3	Methanol, methylamines, methyl sulfides; halophile	41
Methanothrix	Long rods to filaments	4	Acetate	52-61
Methanopyrales				
Methanopyrus	Rods in chains	1	H_2+CO_2, hyperthermophile, growth at 110 °C	60

Table 2.1.3. Kinetic Coefficients of Methanogens in Mesophilic Phase (Ince, 1993)

Substrate	Species	Kinetic Coefficient			Reference
		K_s mM/l	Y kgVSS/kgCOD	μ_{max} l/d	
Acetate	*M.thrix soehngenii*	0.4-0.6	0.023	0.11	Huster *et al.*, 1982
	M.sarcina barkeri	4-5	0.024	0.21	Wandrey, Aivasidis, 1983
	M.bacterium sp.	0.17	0.01	0.26	Cappenberg, 1975
	M.coccus mazei	-	-	0.53	De Zeeuw, 1984
H_2/CO_2	*M.spirillum hungatei*	0.002	0.021	0.05	Robinson&Tiedje, 1984
	M.brevibacter snithii	0.001	0.045	4.02	Pavlostathis *et. al.*, 1991
	M.bacterium formicicum	0.002	0.051	0.29	Schonheit *et. al.*, 1980
	M.sarcina barkeri	-	0.087	3.02	Weimer, Zeikus, 1978

Table 2.1.4. Kinetic Coefficients of Mixed-Culture Anaerobic Bacteria in Digesters (Henze and Harremoes, 1982)

Bacterial Group	Kinetic Coefficient			
	μ_{max} l/d	Y kgVSS/kgCOD	K_s mgCOD/l	K kgCOD/kgVSS.d
Acetate Producing Bacteria	2.0	0.15	200	13
Methane Producing Bacteria	0.4	0.03	50	13
Overall	0.4	0.18	-	2

2.1.2.1.3.1. Substrates for Methanogenesis

It has been reported that at least ten substrates can be converted to methane by pure cultures of methanogens. Three classes of compounds including CO_2-type substrates, methyl substrates and acetate are listed in Table 2.1.5.

Table 2.1.5. Substrates Converted to Methane by Various Methanogenic Archea (Madigan *et. al.*, 2000)

I.CO_2-type substrates
Carbon dioxide (with electrons derived from H_2, certain alcohols, or pyruvate)
Formate
Carbon monoxide
II.Methyl substrates
Methanol
Methylamine
Dimethylamine
Trimethylamine
Methylmercaptan
Dimethylsulfide
III.Acetotrophic substrate
Acetate

In the first class of substrate, CO_2-type substrates including CO_2, formate and carbon monoxide are reduced to methane. Although the reduction of CO_2 to CH_4 is generally H_2 dependent, other substrates in this class can supply the electrons for CO_2 reduction.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^\circ = -131 \text{ kJ} \qquad (2.7)$$

The second class of methanogenic substrates are methyl group substances which are converted to methane via two conversion mechanisms. First mechanism is the formation of methane by reducing methyl group substances using an external electron donor such as H_2. In the conversion equations methanol (CH_3OH) is used as a model methyl substrate.

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \quad \Delta G^\circ = -113 \text{ kJ} \tag{2.8}$$

Alternatively, the methyl group substances can be oxidized to CO_2 in order to generate the electrons needed to reduce other molecules of CH_3OH to CH_4 in the absence of H_2.

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad \Delta G^\circ = -319 \text{ kJ} \tag{2.9}$$

The final methanogenic substrate is acetate. The conversion mechanism of acetate to methane and carbondioxide called the acetotrophic reaction. It has been stated that 70% of the methane produced is derived from the acetic acid and the remaining 30% is produced from the reduction of CO_2 (Pavlostathis and Gomez, 1991).

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad \Delta G^\circ = -31 \text{ kJ} \tag{2.10}$$

Each of the above reactions are exergonic and can be used to synthesize ATP. Concerning carbon for cellular biosynthesis, CO_2 is the precursor for all cellular components when growing on CO_2+H_2. If methanogenic substrates are acetate or methylated compounds, these compounds are also used in the organic cell components with the fixation of some CO_2.

2.1.2.2. Biochemistry of Methanogenesis

The coenzymes of methanogenesis can be divided into two classes. Coenzymes in the first class function in carrying the C_1 unit from the initial substrate, CO_2, to the final product, CH_4

and the second class is involved in redox reactions to supply the electrons necessary for the reduction of CO_2 to CH_4.

C_1 carriers: Methanofuran containing a five-membered furan ring and an amino nitrogen atom that binds CO_2 is involved in the first step of methanogenesis. Methanoprotein which is a methanogenic coenzyme resembling the vitamin folic acid is the C_1 carrier in the intermediate steps of CO_2 reduction to CH_4. Coenzyme M (CoM) is involved in the terminal step of the methanogenesis. It function in the conversion of a methyl group to CH_4.

Redox Coenzymes: Electron donors playing role in methanogenesis are the coenzymes F_{420} and 7-mercaptoheptanoyl-threonine phosphate, or coenzyme B (CoB). Coenzyme F_{420} which is an electron donor in several steps of CO_2 reduction is a flavin derivative, structurally resembling the common flavin coenzyme (FMN). The coenzyme absorbs light at 420 nm and fluorescences blue-green. This specification enables their distinction and possible identification of an organism as a methanogen within anaerobic sludges using fluorescence microscopy (Mink and Dugan, 1977). The structure of CoB resembles the vitamin pantothenic acid which is a part of acetyl-CoA.

2.1.2.2.1. Biochemistry of CO_2 Reduction to CH_4

The reduction of CO_2 to CH_4 is generally H_2 dependent. However formate, carbon monoxide and some certain organic compounds such as alcohols can supply the electrons for CO_2 reduction. The steps in the CO_2 reduction shown in Figure 2.1.5 are:

*A methanofuran-containing enzyme activates CO_2 and reduce it to the formly level.
*The formly group is transferred from methanofuran to an enzyme containing methanoprotein and then reduced in two separate steps to the methylene and methyl levels.
*The methyl group is transferred from methanoprotein to an enzyme which contains CoM.
*The methyl reductase system in which F_{430} and CoB are intimately involved reduces the methyl-CoM to methane. The CH_3 group is removed from CH_3-CoM by Coenzyme F_{430}. It

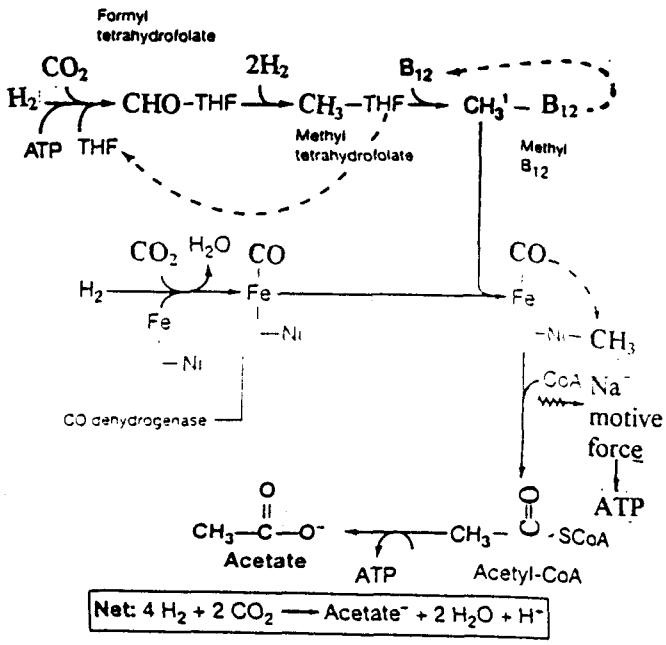


Figure 2.1.4. Reactions of the Acetyl-CoA Pathway (Madigan et. al., 2000)

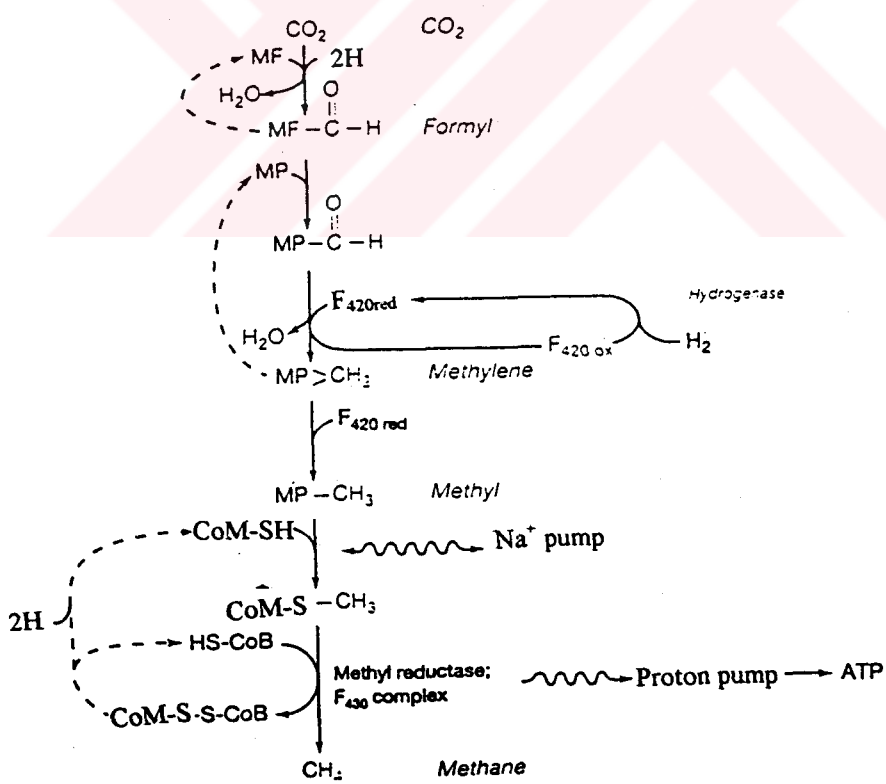


Figure 2.1.5. Pathway of Methanogenesis from CO_2 (Madigan *et. al.*, 2000)

forms a Ni^{2+}-CH_3 complex at the end of the reaction. This is reduced by electrons from CoB generating methane and a disulfide complex of CoM and CoB. When the complex reduced by the H_2, free CoM and CoB are generated and this reaction allows from energy conservation.

2.1.2.2.2. Methanogenesis from Methyl Compounds and Acetate

Methyl compounds are catabolized by donating methyl groups to a corrinoid protein, contain a porphyrin- like corrin ring with a central cobalt atom, to form CH_3-corrinoid complex. The methyl group is donated with the CH_3-corrinoid to CoM to give CH_3-CoM from which methane is formed in the same way as in the terminal step. If reducing power (such as H_2) is not present to drive the terminal step, some of the methanol must be oxidized to CO_2 to yield electrons. Figure 2.1.6 shows the reactions of the acetyl-CoA pathway during growth on methanol or acetate by methanogenic Archea.

Acetate is firstly activated to acetyl-CoA which can interact with carbon monoxide dehydrogenase of the acetyl-CoA pathway when it is the substrate for methanogenesis and then, the methyl group of acetate is transferred to the corrinoid enzyme to yield CH_3-corrinoid, and from there it goes through the Co-M-mediated terminal step of methanogenesis.

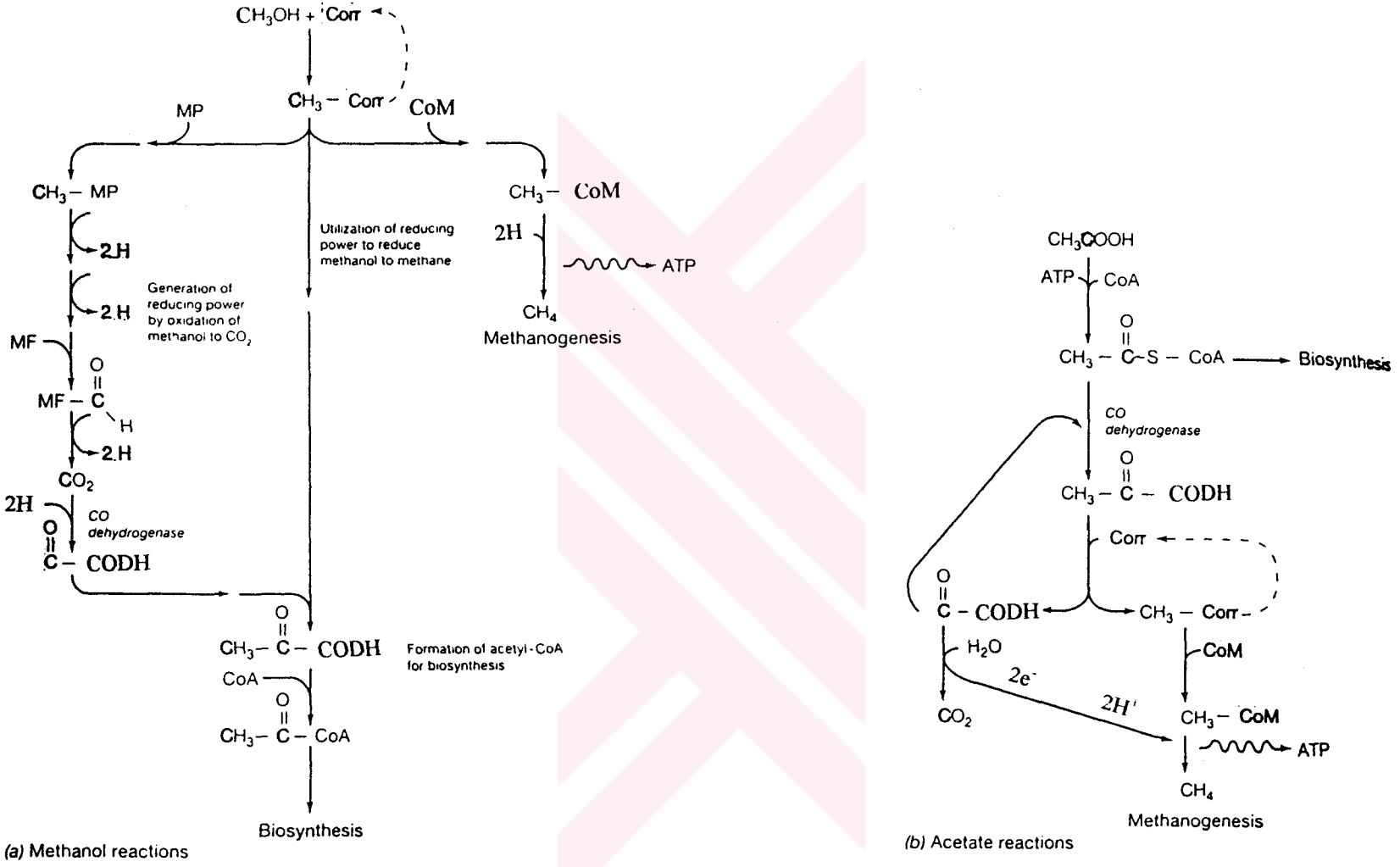


Figure 2.1.6. Utilization of Reactions of the Acetyl-CoA Pathway During Growth on Methanol (a) or Acetate (b) by Methanogenic Archea (Madigan *et. al.*, 2000)

2.1.3. Environmental Factors

2.1.3.1. pH

pH is an important parameter which affects the solubility of substances and the reaction behavior of microorganisms thereby influencing performance of anaerobic digestion. Near neutral pH conditions, a generally accepted optimum range of approximately 6.5 to 8.2 for methanogens, are preferred for operation of most anaerobic digestion. Deviations from this optimum may result in excess production and accumulation of acidic or basic conversion products such as organic fatty acids or ammonia respectively. It is reported that pH below 6.0 are inhibitory to methanogenic bacteria whereas acid forming bacteria can live at this pH and continue to produce volatile fatty acids despite low pH, therefore aggravating the environmental conditions further (Pohland, 1987; Malina *et. al.*, 1992; Jeris *et. al.*, 1985). Methanogenesis continue at lower pH values at reduced rates but instability is observed due to the destruction of the bicarbonate buffer system under the excess production and accumulation of organic fatty acids.

2.1.3.2. Temperature

The temperature is a significant parameter affecting microbial systems in several ways including ionization equilibrium, solubility of substrates, substrate removal rate and other constants such as specific growth rate (k), decay biomass yield, and Ks. Anaerobic processes have been shown to be strongly affected by the temperature variations. Especially methane conversion of acetate to CH_4 is known as more sensitive to temperature than the acetate forming process (Stover *et al.*, 1994). A sudden alteration in reactor temperature of even a few degrees may result in a corresponding increase in VFA and COD in the effluent because of a marked upset in microbial metabolism. Temperature fluctuations become more important in high loading rates. The degradation of propionate has also demonstrated temperature sensitivity (Van Lier *et. al.*, 1996).

Mesophilic (35-37 °C) and thermophilic (50-60 °C) are defined as optimum temperature ranges in anaerobic digestion. The mesophilic range has been studied widely since thermophilic range requires additional heat which increases the operational cost (McCarty, 1964). Since the performance of methane-forming bacteria is affected adversely by the sharp and frequent variations in temperature, it is important to maintain an operating temperature as constant as possible within the mesophilic and thermophilic ranges.

2.1.3.3. Nutrients

Methanogens need trace amounts of elements called as micronutrients besides nitrogen and phosphorus for their fundamental requirements of bacterial metabolism (Speece *et al.*, 1983). The most significant micronutrients considered as necessary for various conditions of active methanogenesis are iron, nickel, magnesium, calcium, sodium, barium, tungstate, molybdate, selenium and cobalt (Henze *et. al.*, 1983). Some of the elements such as selenium, tungsten and nickel are important in the enzyme systems of acetogenic and methanogenic bacteria (Stronach *et. al.*, 1986).

2.1.3.4. Toxic Substances in Anaerobic Digestion and Inhibition

Anaerobic digestion is known as a sensitive process to inhibitory or toxic substances which affect the activities of anaerobic bacteria. These substances may result from either influent waste stream or the metabolic activities of the digester bacteria themselves. Toxic compounds influence anaerobic digestion either by slowing down the rate of metabolism at low concentrations or killing the organism. Studies on toxicity revealed that some toxicants exhibit a reversible effect on the methanogens at the low concentrations.

Methanogenesis is generally the most sensitive step to these materials although all groups involved in process can be affected. The sensitivity of the bacteria increases with the effects of

undesirable environmental conditions. However, methanogens can be acclimatized to these compounds (Speece and Parkin, 1983). LC_{50} concentrations of a variety of organic chemicals for unacclimated methanogens (Blum and Speece, 1991) are shown in Table 2.1.6.

Table 2.1.6. LC_{50} Concentrations of Some Organic Chemicals for Unacclimated Methanogens (Blum and Speece, 1991).

Toxicant	**LC_{50} concentration (mg/L)**
Benzene	1200
Toluene	580
Chlorobenzene	270
1,2-Dichlorobenzene	150
Phenol	2100
Chloromethane	50
Methylene chloride	7.2
Chloroform	0.9
Methanol	22000
Ethanol	43000
Acetone	50000

The manifestations of inhibition are generally increase in VFA concentration and in the carbon dioxide percentage in the final gaseous product and decrease in pH, gas production rate and methane content.

2.1.3.4.1. Volatile Acids Inhibition

The most common inhibition that causes instabilities in the anaerobic reactor is known as the accumulation of volatile acids produced by the acidogenic culture. The instability is generally noticed with a rapid and marked increase in VFA concentrations which is an indication of the failure of the methanogenic population due to the undesirable environmental conditions such as shock loading, nutrient depletion or infiltration of inhibitory substances.

High concentrations of the volatile acids such as acetic acids, butyric acids and propionic acids cause toxicity. However, it was stated that acetate was the least toxic of the VFAs (Ianotti and Fischer 1984), whilst propionate was the major cause of the system failure in anaerobic digesters (McCarty and Brosseau, 1963). Ianotti and Fischer (1984) found that inhibition of the microbial growth was observed at 35 g/l acetic acid and in excess of 3 g/l propionic acid concentrations. They also stated that the minimum toxic concentration of butyrate was significant at 10 g/l.

The conversion-rate inhibition by VFAs at acidic pH values can be attributed to the existence of unionized VFAs in significant quantities in the system (Andrews 1969). The following pH-dependent equilibrium exists between the ionized and unionized components of VFAs:

$$CH_3COOH \leftrightarrow CH_3COO^- + H^+ \qquad (2.11)$$

As the pH value drops, the equilibrium shifts to the left resulting in an increase in the concentration of unionized VFAs. Krocker et al. (1979) found that when the concentration of the unionized acid rises to above 10 mg/l, digester failure can be generally expected.

2.1.3.4.2. Sulfide Inhibition

Sulfides may result from the introduction of the waste stream and/or the biological production in the anaerobic digestion via reduction of the sulfates or other sulphur-containing inorganic compounds. Many industrial processes such as pulp and paper, palm oil, wine distillery, petroleum refineries produce sulfates containing wastes. Sulphates and other oxidized forms of sulphur can be reduced under anaerobic conditions by the sulphur reducing bacteria (SRB).

Anderson et al. (1986) found out that sulphate in the influent of an anaerobic digester could inhibit methanogenesis due to both the competition for acetate and hydrogen by SRBs and the production of sulfide from sulphate reduction by SRBs. While soluble sulfide concentrations between 50 and 100 mg/l can be tolerated in anaerobic treatment with little or no acclimation, concentrations up to 200 mg/l of soluble sulfides does not show a significant inhibition effect after some acclimation. It was stated that sulfide concentrations in excess of 200 mg/l had a direct toxic effect on anaerobic systems and caused inhibition including complete failure of gas production (Stronach *et. al.*, 1986). However, there is a relationship between the toxicity level of sulfide and the free hydrogen sulfide concentration.

2.1.3.4.3. Ammonia-Nitrogen Inhibition

High concentrations of ammonia are formed in the digester during the degradation of the protein and urea found in the wastewaters. Although ammonia is an important buffer in anaerobic treatment, high concentrations of ammonia can cause failure in the operation.

Ammonia can be present in the form of ammonium ion (NH_4^+) or dissolved ammonium gas (NH_3). There is an equilibrium between two forms of ammonia. The equilibrium shifts to the ammonia gas at higher pH levels. The ammonia gas is inhibitory at a much lower concentration than the ammonium ion. Although ammonia nitrogen concentrations up to 1000 mg/l have no adverse effect on methanogens, ammonia nitrogen concentration in the range of 1500 and 3000 mg/l may have inhibitory effect on methanogens especially at higher pH

values. When the ammonia-nitrogen concentration is in excess of 3000 mg/l, the ammonium ion itself is toxic regardless of pH and may cause failure in the process (McCarty, 1964).

Table 2.1.7. Effect of Ammonia-Nitrogen on Anaerobic Treatment (McCarty, 1964)

Ammonia Nitrogen Concentration (mg/l)	**Effect on Anaerobic Treatment**
50-200	Beneficial
200-1000	No adverse effect
1500-3000	Inhibitory at higher pH values
>3000	Toxic

2.1.3.4.4. Heavy Metals Inhibition

Heavy metal toxicity is known as the most common cause of failure of anaerobic microbial conversion processes which are influenced by the oxidation-reduction potential, pH and ionic strength and the resultant speciation of the metals or metal complexes. Certain heavy metals are reported as toxic to anaerobic organisms, even at low concentrations. Low but soluble concentrations of copper, zinc and nickel salts are associated with the problems of heavy metal toxicity in anaerobic treatment.

It has been stated that heavy metal ions inhibit metabolism and kill organisms by inactivating the sulfhydryl groups of their enzymes in forming mercaptides (Mosey *et. al*, 1975). Heavy metals in the soluble form are regarded as more toxic than insoluble forms (Hayes, 1978). Although the existence of heavy metals in trace amounts is essential for bacterial activity, the free concentrations of heavy metals exceeding a particular threshold concentration cause the failure of a digester system.

Many heavy metals form insoluble sulfides or hydroxides under pH conditions in the range of

those found in digesters. The precipitation of heavy metals as salts which is a highly efficient removal mechanism carried out in some systems is not inhibitory to bacterial metabolism. Therefore, one way to avoid heavy metal toxicity is to add chemicals such as sulphates, which will form non-toxic complexes or insoluble precipitates.

2.1.3.4.5. Anthropogenic and Recalcitrant Compounds Inhibition

Most of the industries produce aromatic organic chemicals that are known to persist unchanged for many years in the environment. The effluents of pharmaceutical industry, dye manufacturing and dyeing tannery and pulp-mill industries contain high levels of aromatic and other complex organic compounds. These compounds include insecticides, surfactants and polymers such as polyethylene and polyvinyl alcohol. The chlorinated hydrocarbons are generally considered to be the most persistent of the organic pesticides. However, some of the chlorinated organic pesticides such as aldrin, lindane and dieldrin can be degraded.

Solvents in extensive use in industry and commerce are frequently chlorinated C_1 and C_2 hydrocarbons including trichloroethylene, perchloroethylene, 1,1,1 -trichloroethane and dichloromethane.

It has been found that hydrogenophilic methanogens oxidize alcohols, such as isopropanol and sec-butanol to acetone and sec-butone, respectively, during growth on H_2/CO_2. They also reported that homoacetogenic bacteria is capable of metabolizing isopropanol to acetate and higher fatty acids (Henry *et al.*, 1996). Simple alcohols such as methanol and ethanol can readily be degraded in anaerobic systems. Anaerobic microorganisms which are responsible for complex aromatic hydrocarbon biodegradation can produce methanol in nature (Heijthuijsen and Hansen, 1990; Oremland *et. al.*, 1982). Therefore, methanol is ubiquitous in nature and bioavailable to microorganisms since it can be utilized as a source of carbon and energy due to methanol's high solubility in water. It has been stated that at least 11 species of methanogens can grow on methanol (Madigan *et. al.*, 2000).

It has been found that in a previous study, anaerobic hybrid reactors started up on C_1 and C_2 alcohols and VFA adapted readily (within 11 days) to the successive introduction of a range of C_3 and C_4 aliphatic solvents characteristic of many pharmaceutical wastewaters and of the solvents tested, only tert-butanol was shown to be recalcitrant to anaerobic digestion (Henry *et. al.*, 1996).

2.1.4. Advantages and Disadvantages of Anaerobic Treatment

Advantages of anaerobic treatment

1. Methane production as a potential source of energy
2. Low production of waste biological sludge
3. Seasonal operation is possible due to capability of operation on a stop/start basis
4. High organic loading rates possible
5. Medium and high concentrated wastewaters can be treated with high treatment efficiency.
6. Lower nutrient requirements
7. Low operating costs due to low power consumption, little nutrient requirements, low excess sludge
8. No offensive odor exists
9. Low maintenance costs

Disadvantages of anaerobic treatment

1. Long start-up period is required for development of biomass inventory due to the slow growth rate.
2. Inefficient mixing causes the settlement of digester contents in the base of the reactor and accumulation of scum on the surface
3. No significant N- or P- removal
4. Low stability because of sensitivity to some compounds and changes in pH, temperature, wastewater characteristics etc.

2.2. ANAEROBIC REACTOR CONFIGURATIONS

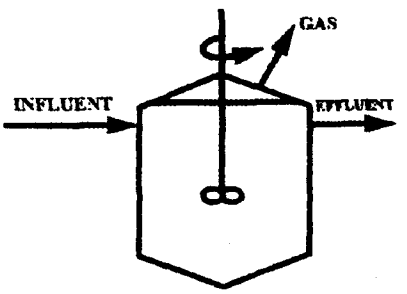
2.2.1. Suspended Growth Systems

2.2.1.1. Completely Mixed Digester

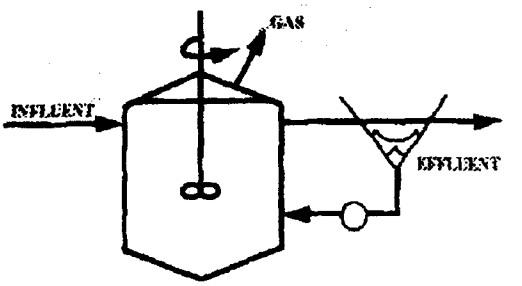
The conventional anaerobic digester is the simplest anaerobic reactor design application of the conventional flow-through tank without any biomass recycle. These systems are suitable for wastewaters containing high concentrations of particulates or extremely high concentrations of soluble biodegradable organic materials. (Figure 2.2.1). The average retention time of anaerobic microorganisms in the reactor (SRT) is equal to hydraulic retention time (HRT). Due to the slow growth of methanogens, process stability can be limited by the short SRTs and large reactor volumes are required to maintain necessary SRTs. Because of the relatively low biomass concentrations and short operating SRTs, loading rates are typically low (1-10 kgCOD/m^3.day). If the internal mixing devices used are adequate, it provides uniform conditions such as substrate, temperature and pH throughout the reactor and minimizes dead volume accumulation and flow channeling.

2.2.1.2. Anaerobic Contact Processes

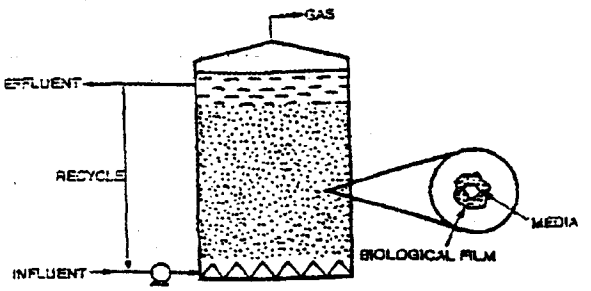
The anaerobic contact process that overcomes the disadvantages of the conventional digester process by separating and recycling biomass back to the anaerobic reactor is consisted of a completely stirred digester, a settling tank and a sludge-recycling unit (Figure 2.2.1). The system SRT can be controlled independently from the HRT with the sludge recycle. Therefore, high treatment efficiency can be achieved by using short HRTs and smaller digesters due to the longer SRTs obtained with sludge recycle. Organic loading rates of 0.5 to 10 kgCOD/m^3.day can be applied to the reactor with HRTs of range between 0.5 and 5 days.



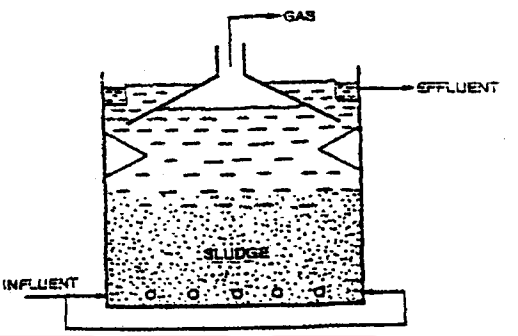
Completely Mixed Digester



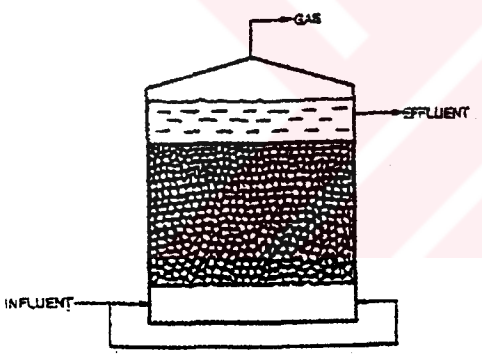
Anaerobic Contact Process



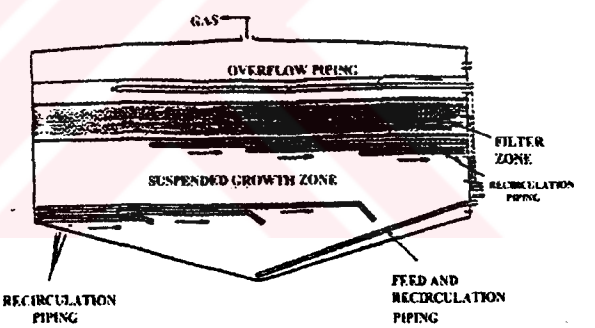
Anaerobic Expanded / Fluidized Bed Reactor



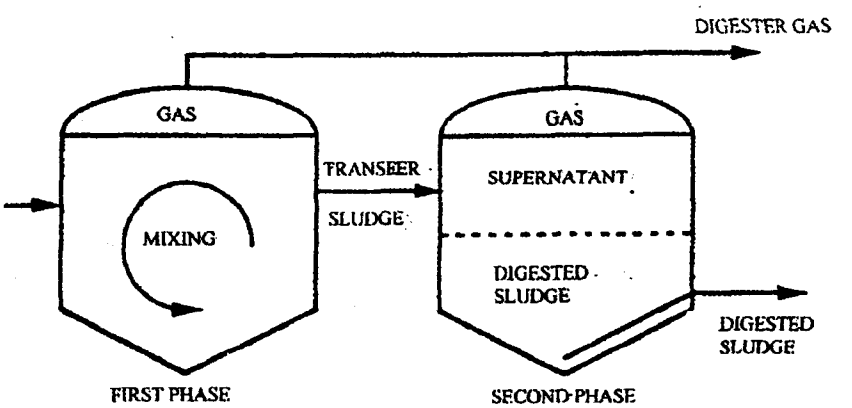
UASB(upflow anaerobic sludge blanket)



Anaerobic Packed Bed Reactor(Downflow or Upflow)



Hybrid Reactor



Two-Phase Anaerobic System

Figure 2.2.1. Typical Reactor Configurations Used in Anaerobic Treatment (Metcalf&Eddy, 1991)

2.2.1.3. Upflow Anaerobic Sludge Blanket (UASB) Reactor

In the upflow anaerobic sludge blanket reactor (Figure 2.2.1), the wastewater passing through an expanded bed (blanket) of highly active biomass which is kept in suspension is degraded. The biomass present in the reactors are dense granules or flocs of 1-5 mm with highly settleable. The upper part of the reactor is designed for the purpose of gas-solids separation, thus allowing biogas collection and internal sludge recycling. Rising gas during the degradation process provides the mixing force to maintain contact between the biomass and the wastewater. Therefore, the reactor can be operated without any internal mixing device. High treatment efficiency can be obtained with short hydraulic retention times and energy demand is low in the process. However, there are difficulties on the control of the granular sludge.

2.2.2. Attached Growth Systems

2.2.2.1. Fixed Bed Processes

Fixed bed processes contain a flooded bed of inert filter medium which is used for the development of high biomass concentrations required for efficient anaerobic treatment of wastewaters. While wastewater is passing through the medium, soluble organic compounds in the feed diffuse in surfaces of the attached biomass where the organics are converted to intermediate and final products namely methane and carbondioxide.

Fixed bed processes can be used for almost all types of industrial wastewaters with low (COD<1000 mg/l) to intermediate (COD>20000 mg/l) concentrations (Figure 2.2.1).

Reactor configurations in fixed bed processes are:

1.Upflow anaerobic filters (UAF)
2.Downflow anaerobic filters

Although UAF can handle high organic loadings (Wetland and Rozzi, 1991; Young and Young, 1991), there are some drawbacks of the reactor type such as dead zones and channeling (Iza et. al., 1991; Wetland and Rozzi, 1991) due to the accumulation of the solids within the reactor.

2.2.2.2. Anaerobic Expanded/Fluidized Bed Processes

The biomass is attached to the surface of small particles having low specific gravity particles that are kept in suspension by the upward velocity of the flow of the feed and recycle. The particles which are generally in 0.45-0.7 mm diameter and made of materials such as porous alumina, high-density plastic beads and quartzite sand provide a very large specific surface for biological growth as a thin film. Therefore, high biomass concentrations that are not subject to diffusional limitations can be developed on the surface of the particles. Biomass retains longer in the reactor because particles increase the settling velocity of the attached biofilm.

2.2.3. Hybrid Systems

The hybrid systems shown in Figure 2.2.1 have simple design and require no special gas or sludge separation device. While UASB reactors are limited by the settling properties of the granular sludge, anaerobic filters are restricted with channeling and plugging due to the accumulation of suspended biomass in the bottom. The hybrid systems combine a UASB and an anaerobic filter in the top part of the reactor and overcome the disadvantages of both of the configurations.

2.2.4. Two-phase Systems

Different groups of bacteria which can show variations with respect to physiology, nutritional requirements, growth, metabolic characteristics and sensitivity to environmental conditions

play role in the anaerobic biodegradation of organic matter (Ghosh *et al*, 1975; Ince Kasapgil *et. al.*, 1995; Ince *et. al*, 1995; Ince Kasapgil *et. al.*, 1996; Ince *et. al.*, 1996; Ince Kasapgil *et. al*, 1997; Ince Kasapgil *et. al.*, 2000, Anderson *et. al.*, 1994). Environmental conditions can be optimized for the acid and methane-forming bacteria by using two completely mixed biochemical reactors in series in two-phase systems. Although there are numerous chemical and physical separation techniques, it is generally accepted that the most appropriate method to achieve this is by means of kinetic control which provides the required growth rates of each in separate reactors (Pohland and Ghosh, 1971; Ghosh *et al*, 1975; Kasapgil *et. al.*, 1995).

The advantages of two-phase system:

1. Optimization of environmental conditions for the acidogenic bacteria as well as methanogenic population.

2. Increased stability of the entire system since
-Over loading can be prevented by controlling acidification,
-Toxic or inhibitory material to the methanogenic bacteria can be removed in the acidogenesis phase.

3. In the disposal of relatively acidification reactor, there is insignificant methane producing biomass loss.

4. Biogas may contain higher methane content.

5. The faster start-up of high rate systems may be possible in case of the wastewater is pre-hydrolyzed and acidified.

6. A possible reduction in the required digester volume and consequently savings in the investment and operating cost.

2.3. PHARMACEUTICAL INDUSTRY PROFILE

2.3.1. Description of the Industrial Processes of Pharmaceutical Industry

Pharmaceutical products are grouped into four categories based on the Standard Industrial Classification (SIC) system. These are;

a) Medical chemicals and botanical products
b) Pharmaceutical preparations
c) Diagnostic substances
d) Biological products except diagnostic substances

2.3.2. Process Descriptions

2.3.2.1. Chemical Synthesis

A wide variety of drugs are produced by chemical synthesis using numerous types of chemical reactions, recovery processes and chemicals including organic and inorganic reactants and catalysts. In addition, some of these chemicals are solvents and listed as priority pollutants (USEPA, 1983). The primary pollutants that are utilized in the processes are benzene, toluene, xlene, cyclohexan, pyridine etc (USEPA, 1982). Table 2.3.1 shows the solvents used in the chemical synthesis process.

Each pharmaceutical is usually manufactured in a "campaign" which may last a few weeks or a few months depending on the demand for the product on the market. At the end of the campaign, process equipments are cleaned and then used to make a completely different product utilizing different raw materials, executing a different recipe and creating different wastes.

Generally, a few number of batch reactors are used in sequencing reactions during chemical

Table 2.3.1. Solvents Used in the Chemical Synthesis Process (USEPA Office of Compliance Sector Notebook Project: Profile of the Pharmaceutical Manufacturing Industry, 1997)

Chemical	Priority Pollutant Under the Clean Water Act	Hazardous Air Pollutant under the Clean Air Act	Chemical	Priority Pollutant Under the Clean Water Act	Hazardous Air Pollutant under the Clean Air Act
Acetone			Ethylene glycol		X
Acetonitrile		X	Formaldehyde		X
Ammonia (aqueous)			Formamide		
n-Amyl acetate			Furfural		
Amyl Alcohol			n-Heptane		
Aniline		X	n-Hexane		X
Benzene	X	X	Isobutyraldehyde		
2-Butanone (MEK)		X	Isopropanol		
n-Butyl acetate			Isopropyl acetate		
n-Butyl alcohol			Isopropyl ether		
Chlorobenzene	X	X	Methanol		X
Chloroform	X	X	Methylamine		
Chloromethane	X	X	Methyl cellulose		
Cyanide	X		Methylene chloride	X	X
Cyclohexane			Methyl formate		
o-Dichlorobenzene (1,2-Dichlorobenzene)	X		Methyl isobutyl ketone (MiBK)		X
1,2-Dichlorobenzene	X		2-Methylpyridine		
Diethylamie			Petroleum naphtha		
Diethyl Ether			Phenol	X	X
N,N-Dimethyl acetamide			Polyethylene glycol 600		
Diethylamine			n-Propanol		
N,N-Dimethylaniline		X	Pyridine		
N,N-Dimethylformamide		X	Tetrahydrofuran		
Dimethyl sulfoxide			Toluene	X	X
1,4-Dioxane		X	Trichlorofloromethane		
Ethanol			Triethylamine		X
Ethyl acetate			Xylenes		X

synthesis with addition of different equipments for the applications of mixing, boiling, cooling, crystallization etc in order to obtain desired production. Solvent extraction can also be carried out in the reactors.

Solvents are used for product recovery and purification as well as reaction media. Materials such as gaseous, solid and high viscous are dissolved and the molecules are forced to approach each other by using solvents. They have the ability to control the heat of reactions by acting as rate limiters on the reactions to prevent undesirable heating coming out from high departing rates of molecules. Generally in all systems the solvents can be separated and recovered from mother liquor and product solution by using distillation column units and other solvents (USEPA Guide, 1991). Aqueous wastes of the chemical synthesis processes are mainly resulted from such operations. Figure 2.3.1 shows a simplified process flow diagram for chemical synthesis.

Waste Streams in Chemical Synthesis Process

Waste streams generated in the chemical synthesis processes are both numerous and complex due to the varied operations, reactions employed and the raw materials. A mother liquor that consisted of unconverted reactants, reaction byproducts and residual product in the organic solvent base is generated in the almost every step of the organic synthesis. The process may also generate acids, bases, cyanide and metals. The spent solvents can be recovered on-site by distillation or extraction. The operation can also generate solvent recovery wastes such as still bottom tars. The use of volatile solvents may also cause air emissions, which may be reduced by employing scrubbers or condensers to reclaim the solvent vapors.

The wastewater of the industry contains miscible solvents, filtrates, concentrates, equipment cleaning, wet scrubbers and spills. Some of these waste streams can cause inhibition in biological treatment systems (EPA guide 1991). Pretreatment may be necessary prior to the discharge due to the concentration or toxicity of the waste stream. Wastewaters of the chemical synthesis processes have high concentrations of biological oxygen demand (BOD),

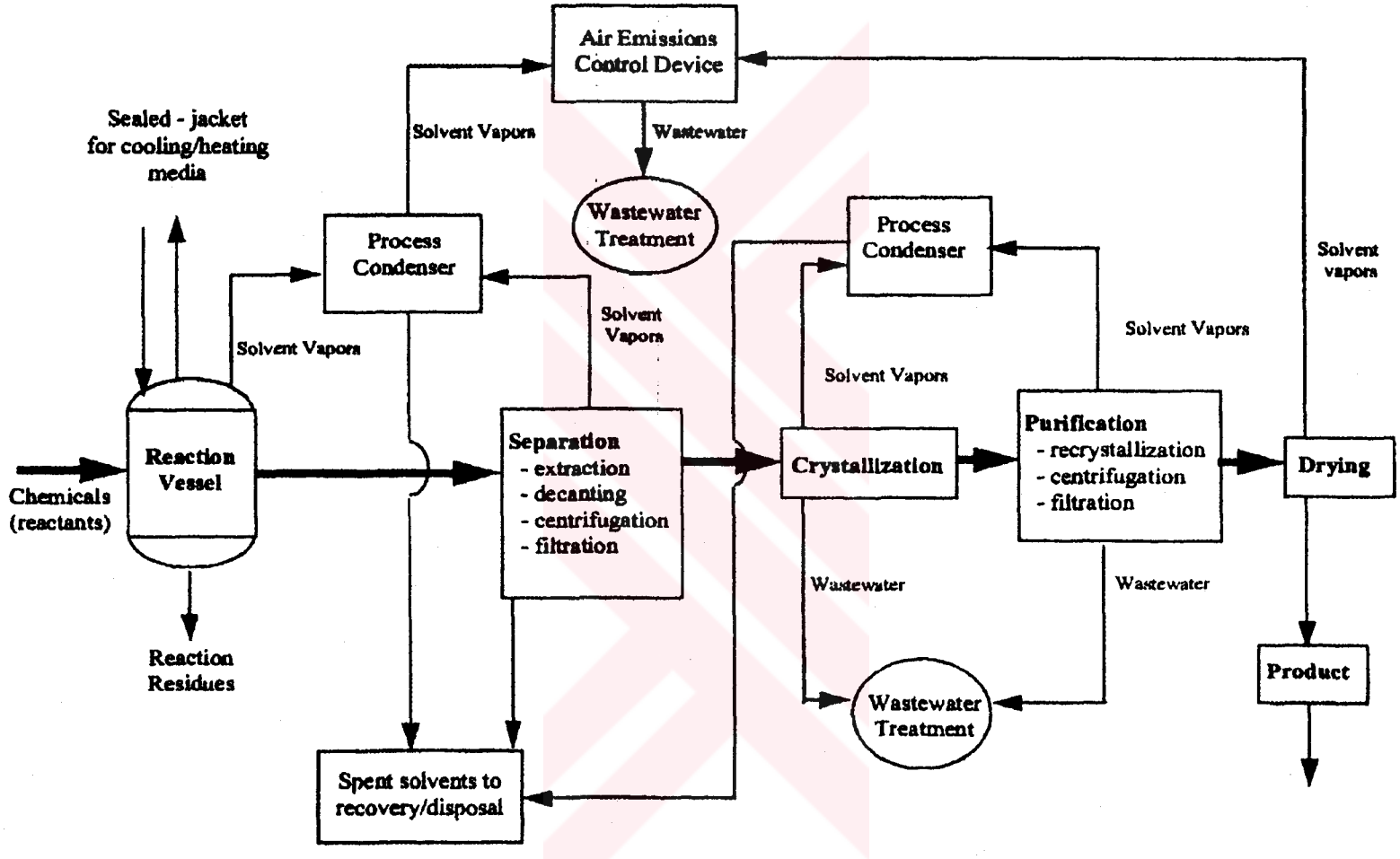


Figure 2.3.1. Simplified Process Flow Diagram for Chemical Synthesis (Economic Impact and Regulatory Flexibility Analysis of Proposed Effluent Guidelines for the Pharmaceutical Manufacturing Industry, 1995).

chemical oxygen demand (COD), suspended solids (SS) with a pH range of 1.0-11.0. Wastewater characterization of an industry using chemical synthesis process in Turkey is illustrated in Table 2.3.2.

Table 2.3.2. Wastewater Characterization of an Industry Using Chemical Synthesis in Turkey (Sahin, 1984).

Parameter	Wastewater Characterization
BOD, mg/l	3370
COD, mg/l	16000
TKN, mg/l	540
Phosphorus, mg/l	-
BOD_5/COD	0.21

2.3.2.2. Fermentation

Fermentation process is used to produce antibiotics such as penicillin, steroids and Vitamin B_{12}. The applications of the process consist of the inoculum and seed culture preparation, fermentation, product recovery and purification.

Sterile inoculum is prepared with a microbial strain population maintained under suitable lab conditions. A portion of this culture is matured into a dense media through a series of test tubes, agar slants and shaker flasks and then transferred to a seed tank that operates like a full-scale fermenter and is designed for maximum cell growth. The nutrient material is conveyed from the seed tank to the sterilized fermenter through a series of sterilized lines and valves and fermentation commences after the transfer. During fermentation, agitation and aeration are carried out to the vessel contents and parameters such as dissolved oxygen content, pH, temperature are carefully monitored.

Following the step, the fermenter broth is usually filtered to remove the solid residues resulting from the fermentation process. The product is recovered by using several techniques such as solvent extraction, precipitation, ion exchange and carbon adsorption.

1) Solvent extraction: The fermentation product is transferred into the solvent phase by contacting aqueous filtrate with an organic solvent, such as methylene chloride, butyl acetate, chloroform benzene. The product is recovered by applying further extraction processes, precipitation or crystallization.

2) Precipitation: Product is recovered directly from the treated fermenter broth in precipitation processes.

3) Ion exchange or carbon adsorption: An ion exchanger resin, adsorption resin or activated carbon are used to recover the product in the fermenter broth.

Waste Streams in Fermentation Process:

Large volumes of wastes such as the spent aqueous fermentation medium and solid debris are generated in the fermentation process. Spent fermentation medium contains unconsumed raw materials such as sugar, starch, fat, protein, nitrogen, phosphorus etc.

During filtration process, large quantities of wastes are generated which are solids in the form of filter cake including solid remains of the cell, filter aid, some residual product and liquids as a result of product recovery operation, equipment cleaning and fermenter vent gas scrubbing. Wastewaters of the process have high BOD, COD and TSS levels with a pH range of 4 to 8 (USEPA, 1983). Table 2.3.3 shows wastewater characterization of a fermentation process used in antibiotic production.

Table 2.3.3. Wastewater Characterization of a Fermentation Process Used in Antibiotic Production (Şahin, 1984).

Parameter	Value
Flow rate, m^3/d	265
COD, mg/l	4000
BOD, mg/l	1900-2000
Total Solids, mg/l	5600-6000
Suspended Solids, mg/l	85-1230
PO_4^{-3}, mg/l	2-3
pH	6.9-7.7

2.3.2.3. Formulation

In formulation processes, pharmaceutical active compounds produced in bulk form are prepared in dosage forms such as tablets, capsules, liquids, ointments and creams.

Waste Generation In Formulation

The formulation manufacturing is the least wasteful operations in the pharmaceutical industry since the necessary production steps have typically small wastewater flows. A few of the unit operations use water in a way that would cause wastewater generation. However, the main use of water in the operation is for cooling water in the chilling units and for equipment and floor wash. The wastewaters have typically low BOD, COD, and TSS concentration; relatively small flow; and pH values of 6.0 to 8.0.

Wastewater sources of formulation operations are:

*Cooling, sterilization, cleaning and equipment wash waters
*Wet scrubbers
*Chemical spills
*Laboratory wastes

The use of water to clean out mixing tanks causes unusual quantity and concentration into the plant sewer system. Wastewater may also be contaminated by dust and fumes from scrubbers either in building ventilation systems or on specific equipment. However, these wastewaters are readily treatable by biological systems. Wastewater characterizations of some of the industries using formulation are shown in Table 2.3.4.

Table 2.3.4. Wastewater Characterizations of the Industries Using Formulation (USEPA, 1982)

Industry	Flow rate (m^3/day)	BOD (mg/l)	COD (mg/l)	SS (mg/l)
1	0.38	230	550	120
2	0.12	500	2100	1615
3	0.42	300	475	-
4	2.80	1000	1100	40
5	0.19	70	300	60

2.3.2.4. Natural Product Extraction

Pharmaceuticals including allergy relief medicines, insulin, morphine, alkaloids, and papaverine are produced using natural material sources such as roots, leaves and animal glands in the natural product extraction.

Although large volumes of natural source material were used in the process, the amount of finished product is too much smaller. Due to this volume reduction, sequencing systems are used instead of the continuous and conventional batch processes in the natural product extraction. Product recovery operations utilize a wide variety of solvents including ketones and alcohols in order to dissolve the fats and oils that would contaminate the product. Table 2.3.5 shows wastewater characterization of an industry using natural product extraction.

Table 2.3.5. Wastewater Characterization of an Industry Using Natural Product Extraction (Struzeski, 1975)

Parameter	Wastewater characterization
Flow rate (m^3/d)	57
BOD (mg/l)	1000-1700
Total Solids (mg/l)	4000-8500
Suspended Solids (mg/l)	200-800
pH	7.3-7.6

2.3.3. Wastewater Treatment Techniques

2.3.3.1. In-site Treatment

In-site treatment technologies are carried out to remove pollutants especially toxic and primary pollutants from the waste stream. Summary of raw material inputs and pollution outputs in the pharmaceutical industry is shown in Table 2.3.6. Pharmaceutical industry contains in large amounts the primary pollutants as solvents. In-site treatment alternatives in a pharmaceutical plant are split into three groups (Ozturk *et. al.*, 1996) namely solvent recycle and recovery, steam stripping and activated carbon adsorption.

Table 2.3.6. Summary of Raw Material Inputs and Pollution Outputs in the Pharmaceutical Industry (USEPA, 1995)

Process	Chemical Inputs	Air Emissions	Wastewater	Residual Wastes
Chemical Synthesis	Catalysts,solvents, reactants,e.g. *benzene, chloroform, methylene chloride, toluene, methanol, xylenes etc.*	VOC emissions from reactor vents, acid gases (*halogen acids, sulfur dioxide, nitrous oxide*), fugitive emissions from pumps, sample collections, valves, and tanks	Wastewater contains the spent solvents, catalysts, reactants; pump seal waters, wet scrubber wastewater; equipment cleaning wastewater; wastewater contain high BOD, COD, TSS with pH of 1-11.	Reactor wastes, reaction residues
-Separation	Solvents used in separation and extraction, e.g.*methanol, toluene, hexanes* etc.	Emissions of VOC from filtering systems fugitive emissions from pumps, tanks and centrifuges	Spills, leaks, equipment cleaning wash waters, spent separation solvents	
-Purification	Purification Solvents e.g. *methanol, toluene, hexanes* etc.	Solvent vapors from purification tanks; fugitive emissions	Spills, leaks, equipment cleaning wash waters, spent purification solvents	
-Drying	Finished active intermediates	VOC emissions from manual loading and unloading of dryers	Spills, leaks, equipment cleaning wash waters,	
Natural Product Extraction	Roots, plants, extraction solvents, animal tissues, e.g. *ammonia, chloroform, phenol, toluene*, etc.	VOCs from extraction chemicals and solvent vapors	Equipment cleaning wash waters, spent solvents, natural product extraction wastewater, Wastewaters have low BOD, COD, TSS.	Spent raw materials
Fermentation	Inoculum, sugars, starches, fermentation solvents, nutrients, e.g. *ethanol, methanol* etc.	Extraction solvent vapors, particulates, odoriferous gases	Spent fermentor broth, wastewater containing sugars, starches, nutrients, etc.: Wastewaters have high BOD, COD, TSS and pH of 4-8.	Fermentation residues, waste filter cake
Formulation	Active drug, sugar, syrups, binders, etc.	Particulates, tablet dusts	Equipment cleaning wash waters, spent solvents, spills, Wastewaters have low BOD, COD, TSS and pH of 6-8.	Particulates, waste packaging, rejected tablets, capsules.

Solvent Recycle and Recovery

Solvents are used in large amounts for a wide range of applications, from synthesis, extraction, and purification of active ingredients to cleaning process equipment. The types of solvent recovery techniques are distillation, evaporation, decantation, centrifugation, and filtration. However, there are some limitations in on and off-site recycling and recovery since several types of solvents (including water), reactants, and other contaminants may be present. If the recycling is not economical, the spent solvents may be incinerated, buried, injected to a deep well or sent to hazardous waste landfills.

Solvent content of the wastewater generated during the recycling processes is removed with steam stripping and activated carbon adsorption.

Steam Stripping

Steam is used both in heating and evaporation of volatile organics in steam stripping which is a distillation method. The method contains a counter current packed column where steam is given from the bottom and water with the spent solvent from upper layers. The method removes many types of volatile organics as benzene, chloroform, methylene chloride, toluene and etc.

Activated Carbon Adsorption

The removal of volatile organic compounds with a steam stripping method is more economical than with an activated carbon adsorption. Some organics can be biodegraded in biological treatment processes, so generally additional activated carbon systems are not required if advanced treatment is not required.

2.3.3.2. External Treatment

Wastewaters of the pharmaceutical industry are generally high strength wastewaters which may contain toxic materials. Primary, secondary or advanced treatment may be used according to the characteristics of wastewater, discharge standards and treatment requirements. Table 2.3.7 shows wastewater treatment technology trends in pharmaceutical industry.

The processes in the treatment plants for pharmaceutical industry wastewater are based on:
-Equalization/Neutralization
-Primary Sedimentation
-Biological Treatment
-Advanced Treatment

According to the characteristics of the pharmaceutical wastewater, one or various combinations of these treatment methods can be used. As can be seen from Table 2.3.7, the use of neutralization, equalization, activated sludge, clarification, multimedia filtration, steam stripping, granular activated carbon, and oxidation have all increased after 1986. Especially, stripping by vapor or air is very important for the pharmaceutical wastewaters containing solvents.

Table 2.3.7. Wastewater Treatment Technology Trends in Pharmaceutical Industry (USEPA, 1997)

Treatment Technology	Percentage of Facilities Using Technology prior to 1986	Percentage of Facilities Using Technology in 1989/1990
Neutralization	26.0	44.3
Equalization	20.1	28.6
Activated sludge	16.9	20.5
Settleable solids removal	13.3	NA
Primary sedimentation	12.0	NA
Aerated lagoon	7.5	4.9
Primary clarification	3.9	9.8
Chlorination	3.6	2.5
Polishing ponds	3.2	NA
Waste stabilization pond	2.9	2.5
Trickling filter	2.9	2.0
Multimedia filtration	2.3	6.1
Stream stripping	1.9	5.7
Evaporation	1.9	NA
Secondary clarification	1.6	20.9
Granular activated carbon	1.3	3.3
Oxidation	1.0	2.0
Dissolved air flotation	1.0	NA
pH adjustment	NA	50.0
Phase separation	NA	12.3

NA: not available

3. AIM

To evaluate the effects of a chemical synthesis based pharmaceutical wastewater on:

- the performance of an anaerobic completely stirred tank reactor (CSTR),
- the activity of acetoclastic methanogens,
- number and composition of autofluorescent methanogens and non-methanogens during different operating conditions.

4. MATERIALS AND METHODS

4.1. Description of Completely Mixed Anaerobic Reactor

The CSTR was made of plexyglass material with a 7.5 litre of volume. The reactor consisted of a sampling port, a gas collection unit, an influent and effluent line. A schematic diagram of the CSTR is shown in Figure 4.1. The reactor was placed in a water bath where the temperature was maintained as constant as possible using a thermostat. The temperature of the system was kept between 35±2 °C which is optimum for anaerobic bacteria and mixing was maintained at 90 rpm throughout the study. pH was one of the most important control parameters and kept between 6.8-7.2. pH of the reactor was adjusted to the range by using 1N HCl or 1 N NaOH.

The CSTR was controlled for any liquid or gas leakage before the operation. Ports were closed with air-tightening material such as slicone and grease in order to avoid air-leakage into the reactor.The reactor was flushed with inert nitrogen gas for 10 minutes from the effluent line in order to provide anaerobic conditions before the operation.

4.2. Seed Sludge

The seed sludge used in this study was initially obtained from the treatment plant of a bakery yeast factory and then was inoculated with the anaerobic granular sludge taken from an UASB reactor of an alcohol industry wastewater treatment plant because of the poor performance of the initial sludge.

In the anaerobic sludge taken from alcohol industry, TS and TVS experiments were conducted instead of the SS and VSS due to the granular characteristics since filtration can not be accomplished. The characteristics of the seed sludge taken from the bakery yeast and alcohol industry are given in Table 4.1.

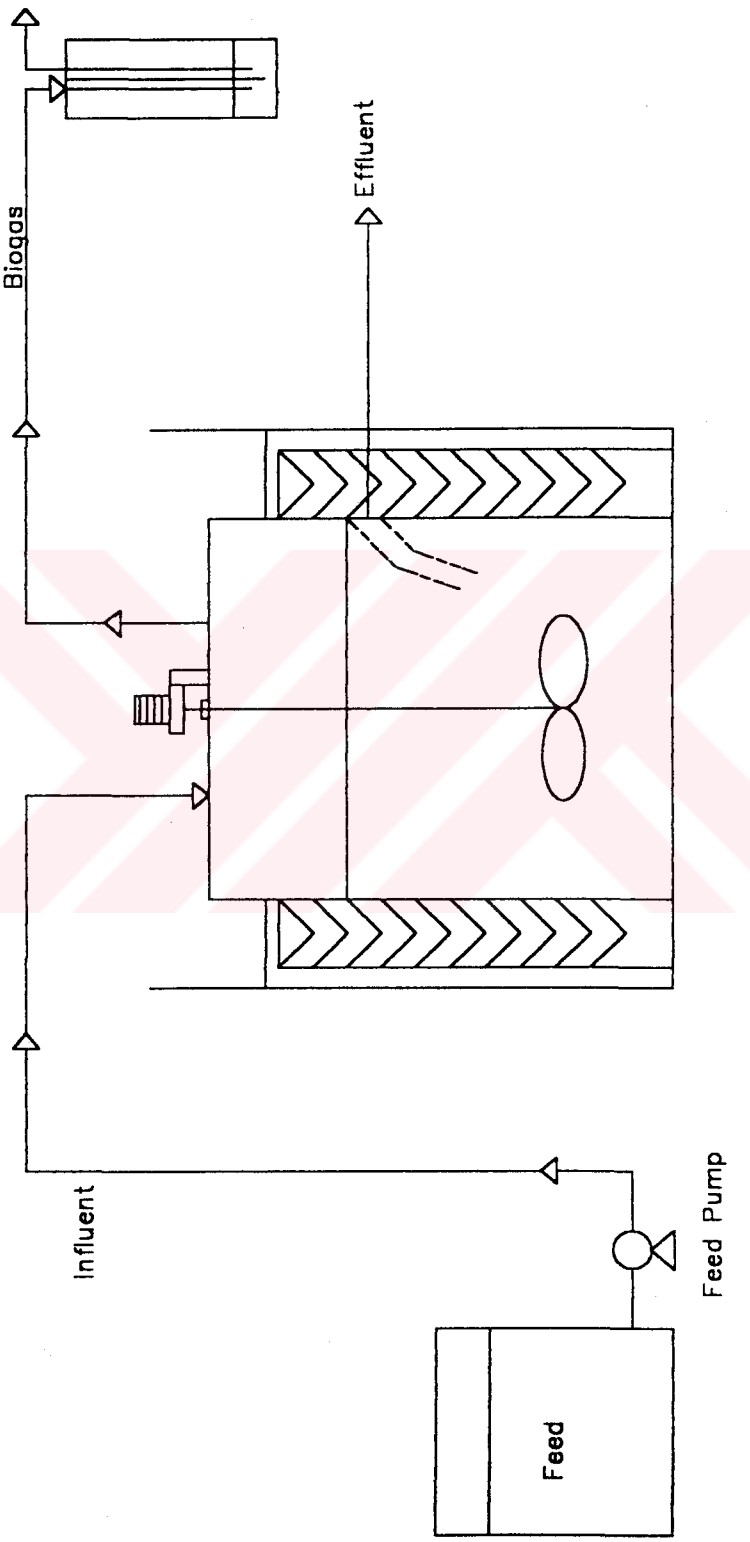


Figure 4.1. Schematic Configuration of the Anaerobic CSTR

Table 4.1. Characteristics of the Seed Sludge.

Sludge	Suspended Solids (mg/l)	Volatile Suspended Solids (mg/l)	Total Solids (mg/l)	Total Volatile Solids (mg/l)	VSS/SS (%)	TVS/TS (%)
Bakery Yeast Industry	10740*	3020*	-	-	28	
Alcohol Distilling Industry	-	-	154740*	145200*	-	94

*represents average values

4.3. Treatment Plant of the Bakery Yeast Industry

The treatment plant of the bakery yeast industry consists of a two-staged treatment plant as an anaerobic and aerobic stages. The wastewater is mainly produced during the centrifugation and filtration facilities. The high strength wastewaters are initially collected in one stream and sent into the anaerobic stage. The anaerobic reactors were designed as an Upflow Anaerobic Sludge Blanket (UASB) reactors with internal and external sludge recirculation units. Effluent of the anaerobic stage combined with the low strength wastewaters are fed into aerobic stage.

4.4. Treatment Plant of Alcohol Distilling Industry

The wastewaters originated from the alcohol distilling industry have been treated in a two-stage treatment plant. Initially, wastewater containing anise seeds have been treated in physical&chemical treatment units. In the second stage, an Upflow Anaerobic Sludge Blanket (UASB) reactor has been used to treat the high-strength wastewater. Finally, wastewaters from the UASB reactor and domestic origin are fed into an activated sludge unit.

4.5. Substrate

The CSTR was fed with only glucose in the first part of the study. Nutrients (nitrogen and phosphorus as $(NH_2)_2CO$ and KH_2PO_4 respectively) were added to the glucose solution according to the C:N:P ratio of 400:5:1. Then pre-aerated and raw pharmaceutical wastewater were used as feed during different stages of operation of the reactor. The wastewater was taken from a chemical synthesis based pharmaceutical industry. Before the pre-aerated pharmaceutical wastewater was fed to the CSTR, optimum aeration time was determined. The characteristics of the pharmaceutical wastewater used in this study are given in Table 4.2.

Table 4.2. Characteristics of the Chemical Synthesis Based Pharmaceutical Wastewater

Parameter	Concentration (mg/l)
COD	39000-60000
COD (after 48 hours of aeration)	25000-30000
TKN	1000-1575
PO_4-P	3-6
Suspended Solids	800-1000
Volatile Suspended Solids	500-690
pH	7-8

4.6. Determination of Optimum Aeration Time

The pharmaceutical wastewater was aerated and optimum aeration time was found to be approximately 43 hours in order to remove all solvents from the wastewater. COD concentration decreased from 48750 mg/l to 24000 mg/l at the end of the aeration period. More than 50% of the COD can be removed by aeration indicating high solvent concentration in the pharmaceutical wastewater.

4.7. Description of the Pharmaceutical Industry

The wastewater used in this study was taken a chemical synthesis based pharmaceutical industry. Raw materials used in the industry are ampicillin trihydrate, ampicillin anhydrous, amoxicillin trihydrate, bacampicillin, sultamicillin base, sultamicillin tosylate, cephalexin monohydrate, cephradine, cefadroxil monohydrate, cefuroxime axetil, paracetamol, nifedipine, amlodipine besylate, azithromycin dihydrate, erythromycin base, erythromycin stearate. The main productions of the industry are bacampicilline and sultamicilline tosylate and flow diagrams of the productions are shown in Figure 4.2 and Figure 4.3, respectively.

The wastewater treatment plants of the industry consists of an equalization tank, a chemical treatment unit, a secondary equalization tank, a nitrification-denitrification unit and an aerobic activated sludge system. There is also a filter-pres unit for the sludges produced in the chemical and biological treatment units.

4.8. Analytical Methods

All experiments were carried out according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1992). Table 4.3 and 4.4 list the monitoring schedule and analytical methods used in this study, respectively.

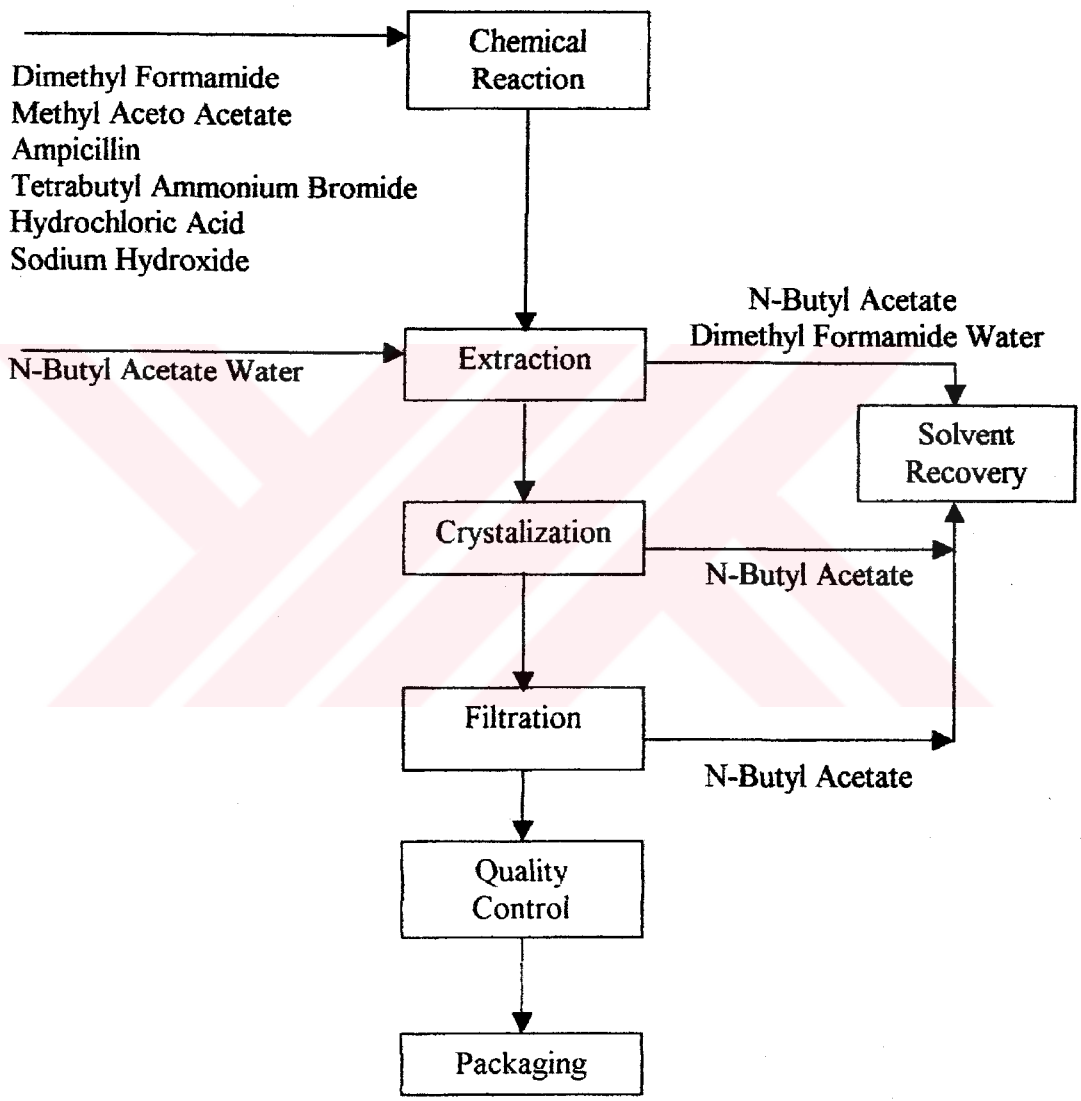


Figure 4.2. Process Flow Diagram For Bacampicilline Production

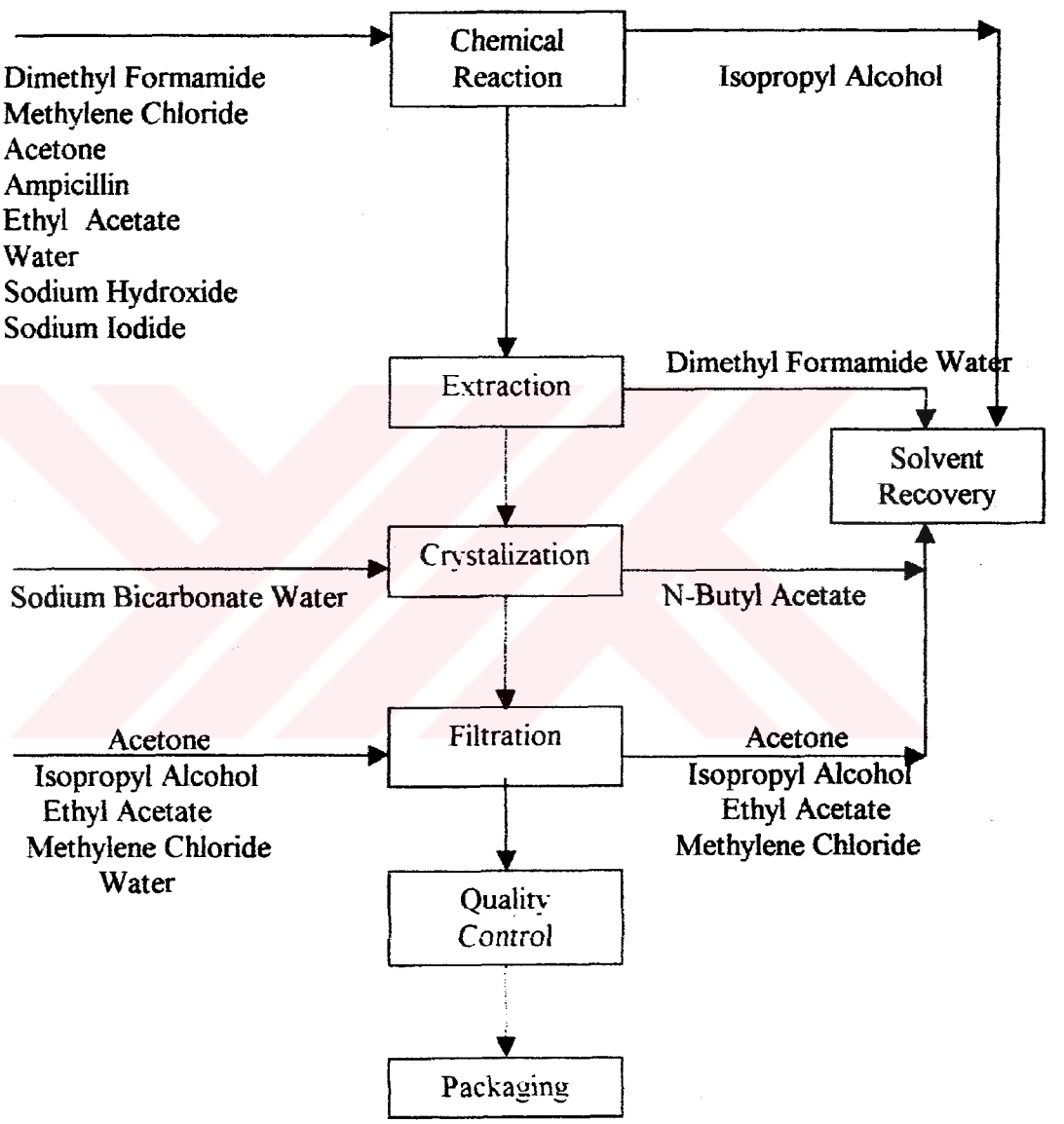


Figure 4.3. Process Flow Diagram For Sultamicilline Production

Table 4.3. Monitoring Schedule

Parameter	Frequency	Sampling Location
COD:Influent: Effluent:	Daily 3×/week	Feed line Effluent line
Gas: Production Composition	Daily 1×/week	Drescher Bottle Gas line
Solids: Effluent SS/VSS Reactor TS/TVS	1×/week	Effluent line Reactor
Temperature	Continuous	Water Bath
pH	Daily	Reactor Sampling Port
Volatile Fatty Acids	Steady-state	Effluent line
TKN	Wastewater Characterization	
PO_4-P	Wastewater Characterization	

Table 4.4. Analytical Methods and Instrumentation

Parameter	Method	Instrument/Reference
COD	Dichromate closed reflux	Standard Methods (1985)
Gas: Production Composition	Water Displacement Gas Chromatography	Gas Measurement Bottle HP Gas Chromatograph
Solids:	Gravimetric	Standard Methods (1985)
Temperature	Probe	Water resistance
pH	pH meter	WTW pH 320/SET Mikro-processor pH-meter
Alkalinity	Titration	Standard Methods (1985)
Volatile Fatty Acids	Gas liquid Chromatography	HP 5890 Model II
TKN	Distillation and titration	Standard Methods (1985)
PO_4-P	Ascorbic acid	Standard Methods (1985)

4.9. Description of the SMA Test Equipment

The SMA test unit was developed by Monteggia (1991). This current procedure used in this study was modified by Ince *et. al.*,1994. The SMA test equipment consisted of eight 1-liter reactors (digestion flasks). They are submerged in a water bath which had a temperature control within a range of 35 ± 0.5 °C. pH is adjusted by using 1N NaOH and 2N HCl. A schematic diagram of the specific methanogenic activity (SMA) test is shown in Figure 4.4. Continuos mixing of the anaerobic sludge was maintained at a speed of 90 rpm by magnetic stirrers throughout the test periods. At the beginning of each test all connections of the SMA test unit were greased for avoiding air leakage to the anaerobic reactor so that anaerobic conditions were well established during the tests.

In the gas metering system used in the test equipment consists of a three-way solenoid valve set before. The valve is controlled with a pressure measurement device (monometer or pressure transducer). There is a gas bulb for temporary storage of the gases and a line for interconnection anaerobic reactor and the units of the system. Normally, two ports of the valve are open (1 and 2) and communicated with the pressure measurement device and the gas bulb. The pressure in the reactor increases gradually because of biological activity. When the pressure inside the system reached a set value, the control system sends an electrical signal to a control interface that activates the three-way solenoid valve, simultaneously closing the second port and open the third port to the atmosphere. Thus, excess gas in the gas valve is released to the atmosphere. The complete releasing of the gases takes three seconds. A cycle is completed.

A PC connected to the gas metering system which has an eight channel analog input board model DAS 800 supplied by Metrabyte corporation. Eight independent digesters can be connected to the eight channels simultaneously and results can be monitored.

Sensitive gas metering system should be occasionally calibrated by injecting a known volume of gas to the system.

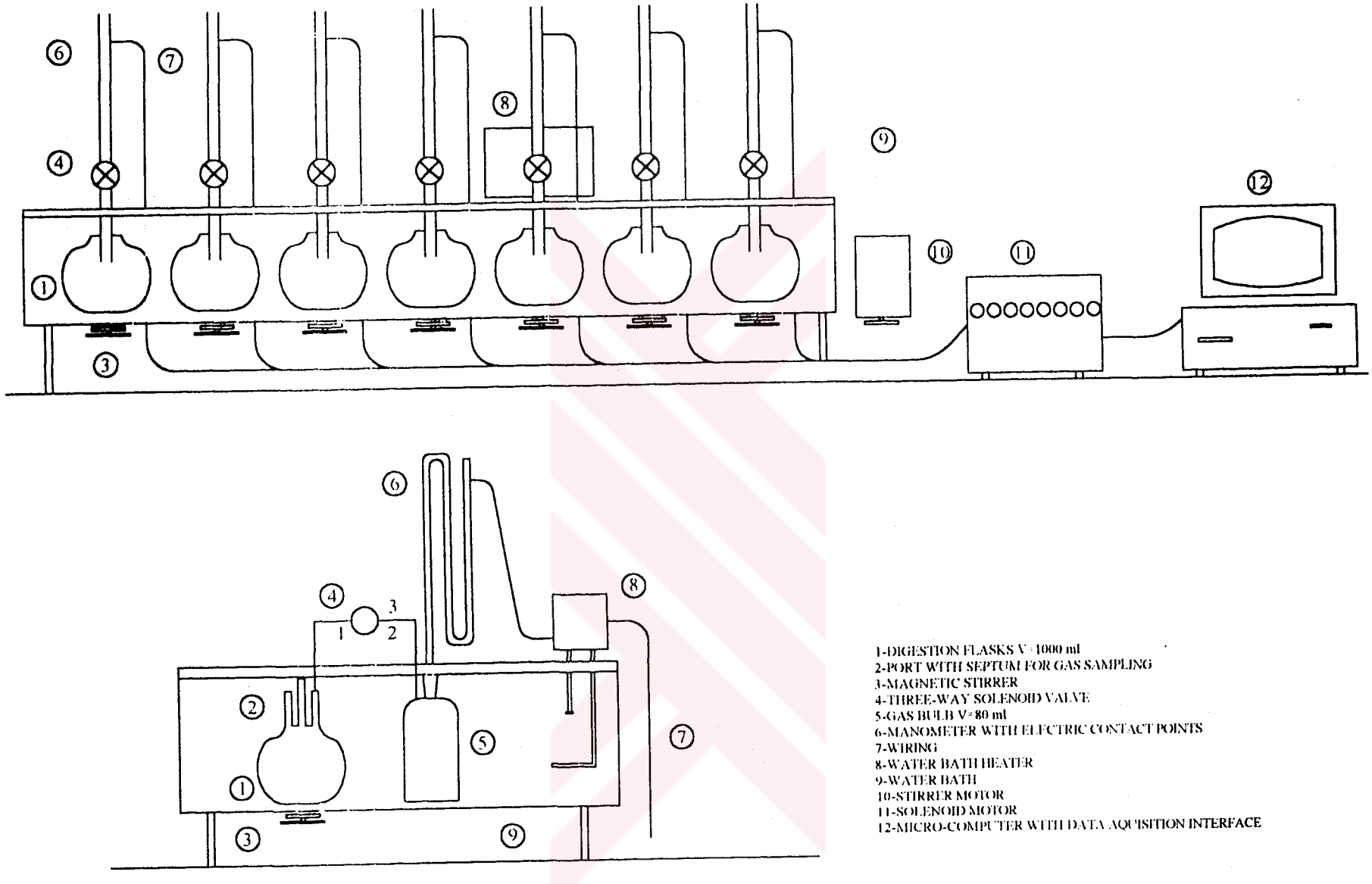


Figure 4.4. Illustration of Experimental Set-up for Specific Methanogenic Activity Test

4.9.1. Experimental Procedure of Specific Methanogenic Activity Test

1. The volatile suspended solid content (VSS) of the sludge sample to be analyzed must be determined before the test is started (preferably 12 hour in advance).
2. The concentration of volatile suspended solid (VSS) in the reactors is brought about 2000 mg/l by diluting sludge sample with a mineral stock solution (Table 4.5).
3. The pH of the reactors should be adjusted to 6.8.
4. Water level in monometer is adjusted by using respirometer.
5. Reactors should be flushed with nitrogen gas (5-10 PSI) about a period of 10 minutes to maintain anaerobic conditions in the reactor. In the study, the test reactors were flushed with nitrogen gas about 5 minutes. The taps of the reactors must be closed immediately after flushing and all connections of the SMA test unit must be greased in order to prevent air leakage.
6. Temperature of the reactor content should be maintained 35 ± 0.5 °C by heating water bath.
7. Acclimatize the test sample for 12-16 hours. Gas production during the time can be neglected.
8. Substrate is introduced to the SMA reactor. In this experiment, 3000 mg/l acetate was used as substrate.
9. Mixing system (magnetic stirrer) should be opened and data collection system should be reset. Biogas production is saved automatically for every hour.
10. Methane concentration is determined at regular intervals by taking 0.4ml gas with 1 ml volume syringe from each reactor.
11. If a suddenly fall in biogas concentrations occurs in spite of enough substrate, test is ended.
12. The volume of methane produced per unit of time is calculated by using following equation.

$$G = A \times B \times C \times 24$$

$$SMA = G / (E \times F)$$

A: Biogas amount per unit of time

B: methane percent of biogas produced (%)

C: valve factor

E: concentration of biomass in the SMA reactor (gVSS/l)

F: active volume of the SMA test reactor

G: methane gas produced per day (ml CH_4 / day)

SMA: Specific methane activity (ml CH_4 / gVSS.day)

Table 4.5. Mineral Stock Solution (Valcke and Verstraete, 1983)

Chemical Composition	Concentration (mg/l)
KH_2PO_4	2500
K_2HPO_4	1000
NH_4Cl	1000
$MgCl_2$	100
$Na_2S.7H_2O$	100
Yeast Extract	200

4.9.2. Feed and Seed Sludge for SMA Tests

Acetate was used as feed during SMA tests since approximately 72% of methane formed during anaerobic digestion of a complex substrate results from acetic acid (McCarty, 1964). 2000 mg/l acetate concentration was used for the sludge sample of the bakery yeast industry in the SMA test. Four different acetate concentrations were used for the sludge sample taken from alcohol distilling industry in order to determine the maximum acetoclastic methane production rate. 3000 mg/l acetate concentration was found to be the most favorable substrate and the substrate concentration was used for the SMA tests carried out each steady-state.

4.10. Microbiological Studies

4.10.1. Bacterial Enumeration

Sample Preparation

At the each steady-state of the operation during the study, 100 ml sludge sample was taken from the CSTR and then SMA of the sludge sample was tested. After the SMA test ended, 5 ml sludge sample was taken from the SMA test reactor for the microbiologic studies. The samples of each steady-state were diluted 200 times to give total count of 200 in one field of view. Strict anaerobic techniques described by Hungate (1969) and modified by Bryant (1972) were used throughout all media preparations and sample handling. In the sampling, 1 ml sterile pipettes were used in order to transfer the media between the tubes and the samples were homogenized with sterile glass beads before the each dilution. Homogenization was carried out with a Nova test-tube shaker, at 2500 rpm for ten minutes. A method described by Pike *et al.* (1972) was used for homogenization

4.10.2. Direct Microscopic Count

Enumeration of the total bacteria and total autofluorescent methanogen in the samples was made using an Olympus BX-50 Model Epiflourescence Microscope fitted with a 100 W high-pressure mercury lamp. Magnification of 600 was used with Olympus×60 water immersion lenses with a ×10 eyepiece.

The samples were counted with a Neubauer Chamber which had a depth of 0.1mm and an area of 1 mm^2. The samples were diluted to give counts of approximately between 100 and 400 per field of view. There are 5×5 squares and each of the square contains 16 small squares (4×4) in the chamber. Volumes of the squares are known (4.3). One drop of sample was placed on to the Improved Neubauer Chamber. 5 among 25 squares (each of them 4×4) were chosen randomly for count and an average was taken. Bacteria were counted only on the top and the left graduation lines of the each small cube in order to avoid

duplicate errors. The following formula (4.1) was used to calculate the numbers of the total bacteria and methanogenes per milliliter (Ince *et. al.*, 1995; Ince and Ince, 2000).

$$N=M*DF/V \quad (4.1)$$

N=number of organisms per unit volume
M=mean count per square
DF=dilution factor
V=volume of the area

Area of each small square chamber=1/400 mm^2
Depth of the chamber=0.1 mm

$$\text{Area}=16*1/400=0.04\ mm^2 \quad (4.2)$$

$$\text{Volume}=0.04\ mm^2\times 0.1\ mm=4\times 10^{-6}\ ml \quad (4.3)$$

5. RESULTS AND DISCUSSION

In this study, the effects of a chemical synthesis based pharmaceutical wastewater on the performance of an anaerobic CSTR, acetoclastic methanogenic activity, number and composition of autofluorescent methanogens were studied in order to determine the overall performance of the CSTR under various influent compositions.

5.1. Performance of the Anaerobic Completely Stirred Tank Reactor (CSTR)

The performance of a CSTR was evaluated for 182 days during which the reactor was operated at stages of initial study with glucose (104 days) and steady-state conditions. The study was summarized in Table 5.1.

5.1.1. Initial Study with Glucose

Ten liters anaerobic sludge was taken from a full-scale anaerobic treatment plant of a bakery yeast industry. Initially, a part of the sludge was used to carry out SS, VSS and SMA tests. The sludge was left at 4 °C for 24 hours in order to obtain the settled part of the sludge. A 3.5 litre settled sludge was introduced to the CSTR. The rest of the reactor was filled with tap water to a level of 7.5 liters. The reactor was flushed with nitrogen gas for 10 minutes in order to maintain anaerobic conditions inside the reactor. Finally, the temperature of the reactor was gradually increased from room temperature to 36 °C over a period of 24 hours without feeding.

In the first part of the study, the system was fed with only glucose which is known as one of the most readily degradeble carbonhydrates and quite soluble material which do not limit the rate of biodegradation (Noike *et. al.*, 1985, Zoetemeyer *et. al.*, 1981). Glucose produces a large intermediatery metabolite in anaerobic digestion, as it is the main product of hydrolysis of cellulosic materials which are found as large organic compounds in wastes. Therefore, in many experimental studies it was commonly used as a representative substrate (Endo *et. al.*, 1986). It was recommended that start-up periods for pharmaceutical wastewater treatment should be carried out by using with gradual replacement of readily

degradable substrate (Stronach et. al., 1986). Therefore, it was used as a substrate during start-up period in order to apply further loadings with pharmaceutical wastewater after reaching 6 kg COD/m^3.d and find out the maximum performance and PMP capacity of the system at this OLR.

Table 5.1 Summary of Operational Schedule with Feeding Strategy Applied to the CSTR

Operation	**Time (Days)**	**Feeding Strategy**
Initial studies (Sludge of Bakery Yeast)	1-44	Glucose (HRT=2.5)
Initial studies (Seeding of sludge taken from an Alcohol Distilling Industry)	46-104	Glucose (HRT=5) (HRT=2.5)
Steady-State	105-112	10% pre-aerated wastewater 90% glucose (HRT=2.5)
Steady-State	113-119	30% pre-aerated wastewater 70% glucose (HRT=2.5)
Steady-State	120-128	70% pre-aerated wastewater 30% glucose (HRT=2.5)
Steady-State	129-152	100% pre-aerated wastewater (HRT=2.5)
Steady-State	157-162	100% pre-aerated wastewater (HRT=3.5)
Steady-State	163-169	10% raw pharmaceutical wastewater 90% pre-aerated wastewater (HRT=3.5)
Steady-State	170-176	30% raw pharmaceutical wastewater 70% pre-aerated wastewater (HRT=3.5)
Steady-State	177-181	60% raw pharmaceutical wastewater 40% pre-aerated wastewater (HRT=3.5)

A specific methanogenic activity (SMA) test of the inoculation sludge was carried out in order to determine the most acceptable initial organic loading rate (De Zeeuv, 1984; Ince *et. al.*, 1995). SMA value of the seed sludge indicating poor methanogenic activity was

78.43 ml CH_4/gVSS.d (VSS 3060 mg/l). It was stated that the initial loading rate should be chosen according to the biomass concentration and its specific methanogenic activity (Ince *et. al.*, 1994). According to the results of SMA test carried out for the initial inoculation sludge taken from the bakery yeast fermentation industry, the CSTR system was firstly fed with glucose at an OLR of 1 kg COD/m^3.d with a HRT of 2.5 days and then OLR was planned to be increased in a stepwise mode up to 6 kg COD/m^3.d in the start-up period. OLR and HRT were used as control parameters throughout the study.

The pH of the reactor was kept at 6.8-7.2 and the temperature was maintained at the optimum values for the mesophilic bacteria within a range of 35±2 °C. Alkalinity of the system was maintained around 3000 mg/l as $CaCO_3$ by using $NaHCO_3$ to prevent fluctuations in pH.

The COD:N:P ratio of the system was maintained constant as 400:5:1 during the operation of the CSTR system. Urea and KH_2PO_4 were used in order to satisfy nitrogen and phosphorus requirement according to this ratio.

The CSTR performed well at an organic loading rate of 1 kg COD /m^3.d at which point 75% COD removal efficiency was achieved. It was decided to increase OLR to 1.5 kg COD/m^3.d. This treatment efficiency did not continue at this OLR and the CSTR system was then operated at lower OLRs for the rest of the study. However, the system did not show a good performance with initial inoculation sludge after this point. The average COD removal efficiency could hardly exceed 50% even at an OLR of 1kg COD/ m^3.d. The SMA test was carried out to determine the specific methanogenic activity of the sludge from the CSTR after deterioration in the performance. SMA value of the sludge taken from the CSTR was 15 mlCH_4/gVSS.d and the results confirmed that potential acetoclastic methanogenic capacity of the sludge was very low which resulted in a very poor COD removal efficiency even at an OLR of 1 kg COD/m^3.d.

It shows that one of the most important things is to determine acetoclastic methanogenic activity of the sludge when selecting a sludge as inoculum before start-up and steady-state operation in order to obtain high treatment efficiency. Both the quality and quantity of the acetoclastic methanogens are very important and should be taken into consideration when

designing and operating anaerobic reactors besides VSS parameter used in the design of anaerobic reactors.

Therefore, it was decided to seed the reactor with a sludge having a high acetoclastic methanogenic activity to obtain a better performance for the further organic loadings. 3.5 litre mixed liqour was drawn from the reactor and 3.5 litre anaerobic granular sludge taken from an alcohol distilling industry was inoculated to the CSTR system. SMA value of the seed sludge was 446 ml CH_4/gTVS.d which indicated highly active anaerobic granular sludge.

The reactor was fed with glucose at an OLR of 1 kg COD/m^3.d with a HRT of 5 days and COD removal efficiency was found to be 89%. COD removal efficiency with respect to OLR during initial study with glucose is shown in Figure 5.1.1. Then, OLR was increased in a stepwise mode to 6 kg COD/m^3.d. Figure 5.1.2 shows the COD removal efficiency with respect to influent and effluent COD concentrations against operating time during start-up. 99% COD removal efficiency was found both at OLRs of 1.5 and 3 kg COD/m^3.d, respectively. HRT was decreased to 2.5 days at an OLR of 3 kg COD/m^3.d (day 56). A SMA test was carried out at an OLR of 3 kg COD/m^3.d. SMA value was 140 ml CH_4/gTVS.d for 3000 mg/l acetate. According to the results, it was decided that the CSTR system could be loaded at higher OLRs and OLR was increased to 5 kg COD/m^3.d. The CST reactor was very effective in responding to this sudden increase at this organic loading rate at which point 99% COD removal efficiency was obtained. Then OLR was increased to 6 kg COD/m^3.d. This high treatment efficiency also continued at this OLR. An SMA test was carried out on day 75. SMA test results yielding an AMP/PMP ratio of 0.1, it was found that the CSTR system was operated under its potential. Concerning COD removal efficiency corresponding to an F/M ratio of 0.13, it was decided to draw sludge from the CST reactor in order to increase F/M ratio to a suitable value for the anaerobic reactors. 300 ml sludge was drawn from the reactor every day between days 76 and 90 (totally 3 l). This resulted in a F/M ratio of 0.54 with the withdrawal of granular sludge from the reactor and COD removal efficiency decreased to 82% at this point. The VFA concentration in the CST reactor was 1623 mg/l as acetic acid. Therefore, only for once 300 ml sludge was replaced into the CSTR in order to maintain sufficient quality of active acetoclastic methanogenic population. This responded an increase in COD removal

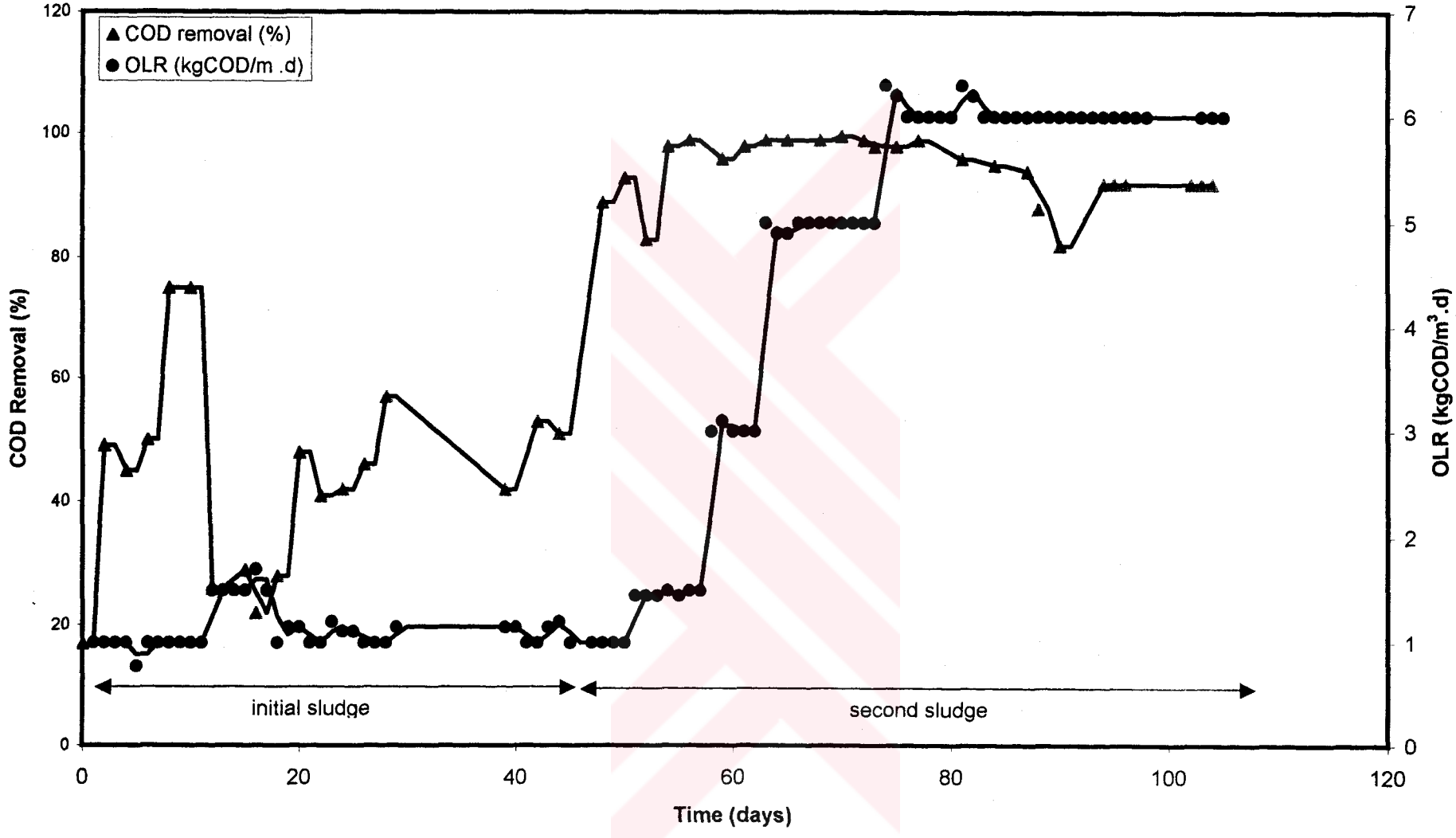


Figure 5.1.1. COD Removal and OLR During Initial Study with Glucose

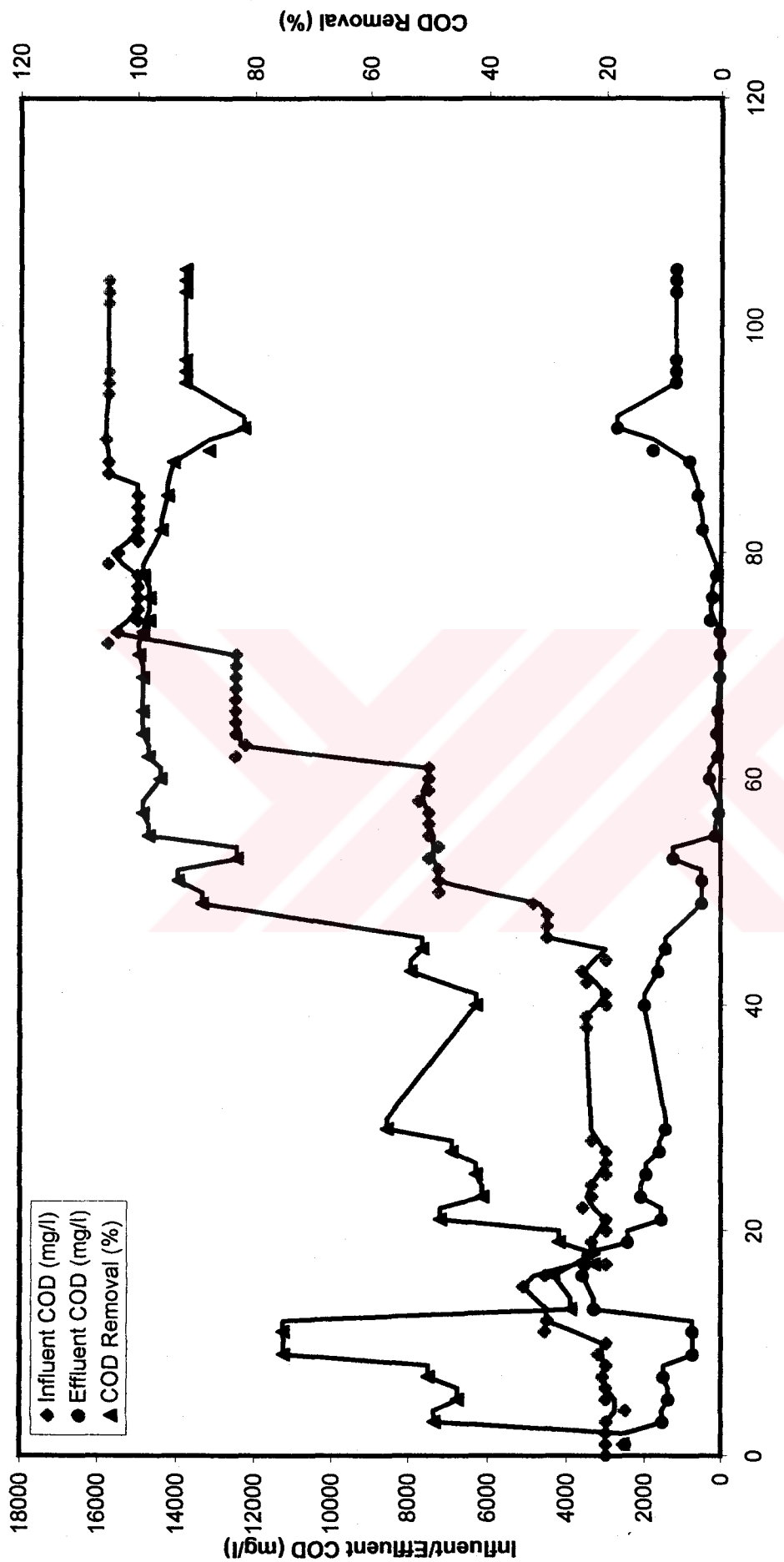


Figure 5.1.2.COD Removal Efficiency with Respect to Influent/Effluent COD Concentrations Against Operating Time When CSTR Was Fed with Glucose

efficiency to 92%. The VFA concentration decreased to 53 mg/l as acetic acid. The methane yield was 0.32 m^3CH_4/kg COD removed at this point. SMA test was then carried out at an F/M ratio of approximately 0.45 and SMA value was found to be 336 ml CH_4/gTVS.d.

5.1.2. Steady-State Operation and Results

After the start-up, the CST reactor was firstly fed with pre-aerated pharmaceutical wastewater with several dilutions made by glucose and then with raw pharmaceutical wastewater diluted with pre-aerated wastewater.

Operation of the CSTR system are summarized as followings:

1.Performance of the CSTR with pre-aerated pharmaceutical wastewater diluted with glucose

- 10% pre-aerated pharmaceutical wastewater and 90% glucose
- 30% pre-aerated pharmaceutical wastewater and 70% glucose
- 70% pre-aerated pharmaceutical wastewater and 30% glucose

2.Performance of CSTR with %100 pre-aerated pharmaceutical wastewater

3.Performance of CSTR raw pharmaceutical wastewater diluted with pre-aerated pharmaceutical wastewater

- 10% raw and 90% pre-aerated pharmaceutical wastewater
- 30% raw and 70% pre-aerated pharmaceutical wastewater
- 60% raw and 40% pre-aerated pharmaceutical wastewater

This feeding strategy was used in order to obtain a better reactor performance and to allow microorganisms to acclimatize themselves to the pre-aerated wastewater and finally to the raw wastewater. An initial experiment carried out to determine the optimum aeration time for raw pharmaceutical wastewater, it was found that the wastewater should be aerated for a minimum 48 hours in order to strip solvents (methylene chloride, ethyl acetate, isopropyl

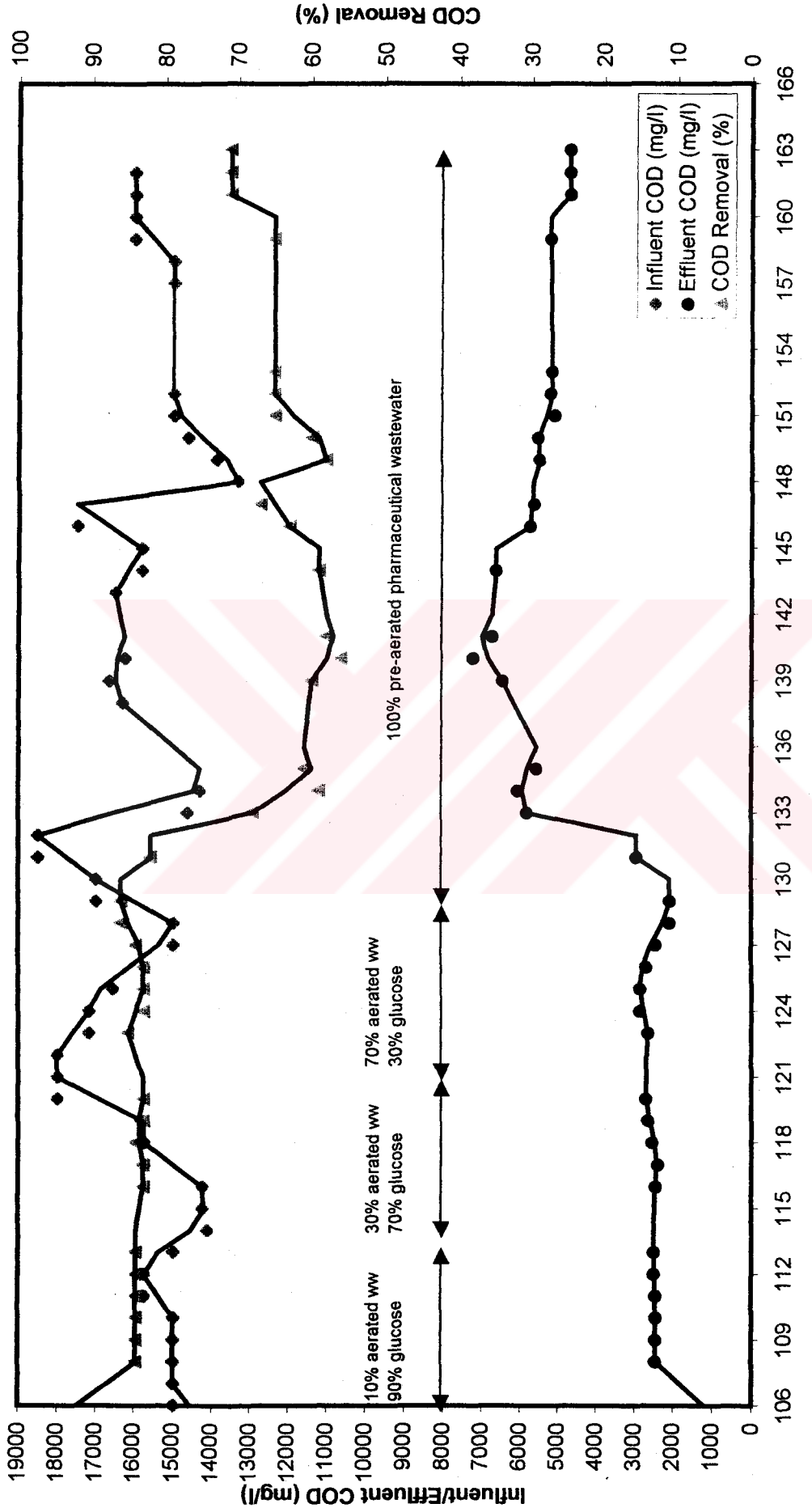


Figure 5.1.3. COD Removal Efficiency with Respect to Influent and Effluent COD Concentrations Against Operating Time

alcohol,etc.). The COD of the raw pharmaceutical wastewater was decreased from a range of 39000-60000 mg/l to 25000-30000 mg/l after 48 hours of aeration.

After initial study with glucose, the CSTR was fed with pre-aerated pharmaceutical wastewater diluted with glucose with a ratio of 10% at an OLR of 6 kg COD/m^3.d. COD removal efficiency decreased to 84% while methane yield decreased to 0.27 m^3CH_4/kg COD removed. The VFA concentration increased to 63 mg/l as acetic acid at the end of the steady-state of the ratio. The CSTR system was affected by the pre-aerated wastewater even if low concentrations of the pre-aerated wastewater. It was intended to strip solvents out from the wastewater by aeration. However, there may be still some organics which are not easily biodegradable in the anaerobic treatment. The SMA test confirmed that the PMP rate of the CST reactor sludge showed a significant decrease at this ratio. SMA value decreased from 336 mlCH_4/gTVS.d to 244 mlCH_4/gTVS.d. COD removal efficiency with respect to influent and effluent COD concentrations can be seen at Figure 5.1.3 when the CSTR was fed with pre-aerated pharmaceutical wastewater at different dilutions with glucose.

At a ratio of 30% pre-aerated wastewater, 83% COD removal efficiency was achieved. Methane yield was found to be 0.29 m^3CH_4/kg COD removed. Methane yield and percentages during operation is shown Figure 5.1.5. The VFA concentration was 45 mg/l.

When 70% dilution ratio of pre-aerated pharmaceutical wastewater was used, 86% COD removal efficiency was achieved. Both COD removal efficiency and SMA value increased indicating acclimation of the biomass to the pre-aerated wastewater. However, VFA concentration was increased to 62 mg/l. PMP rate of the anaerobic sludge was found to be 305 mlCH_4/gTVS.d and methane yield increased to 0.30 m^3CH_4/kg COD removed.

It was decided to feed the system with 100% pre-aerated pharmaceutical wastewater. However, a sudden decrease was observed in the COD removal efficiency compared with the previous performance. It was decreased from 86% to 65%. SMA value was found to be 163 mlCH_4/gTVS.d. Methane yield also decreased to 0.20 m^3CH_4/kg COD removed. HRT was increased from 2.5 days to 3.5 days in order to improve the performance of the CSTR system. COD removal efficiency and PMP rate of the sludge showed an increase to 71%

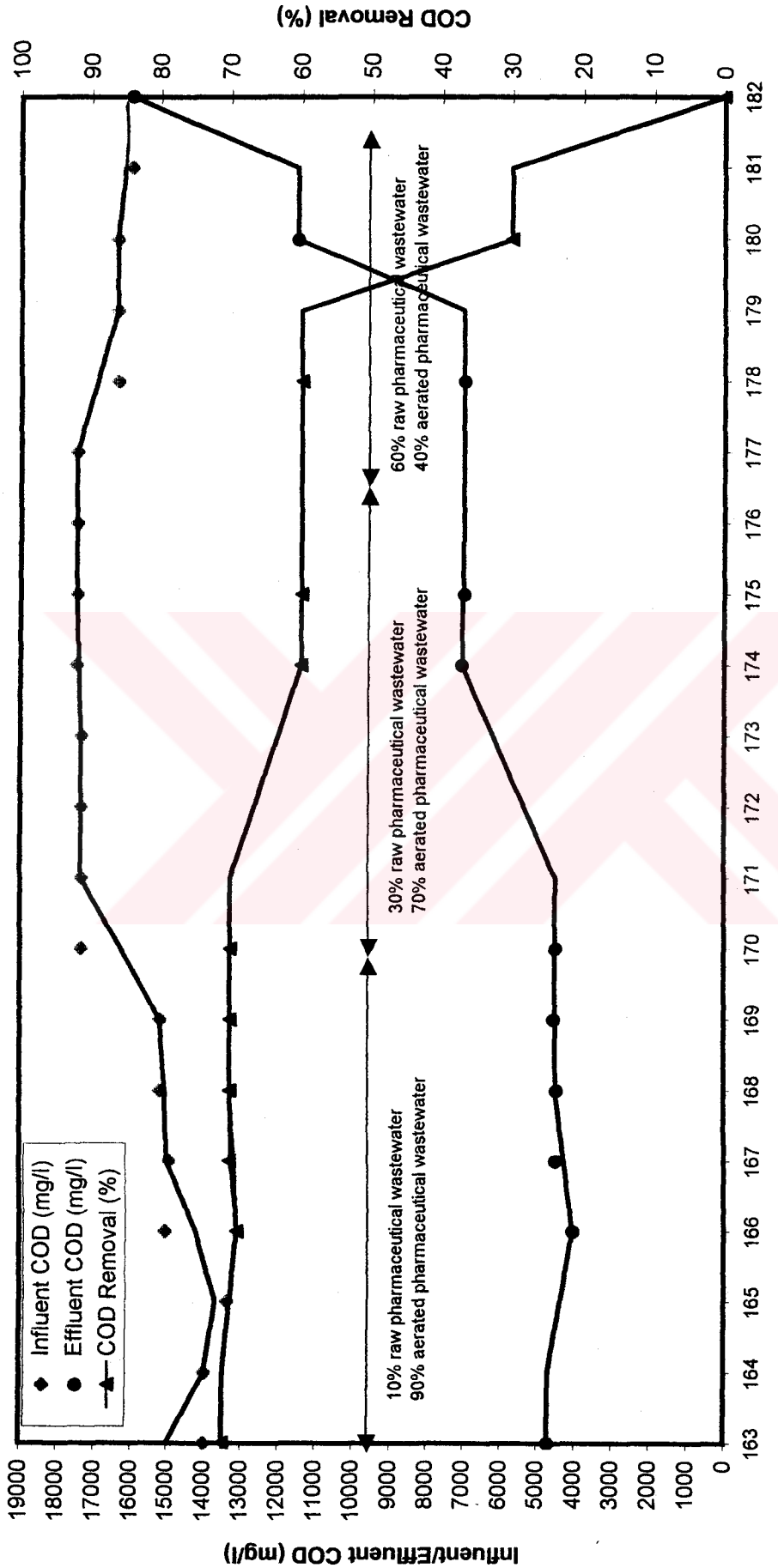


Figure 5.1.4. COD Removal Efficiency with Respect to Influent and Effluent COD Concentrations Against Operating Time

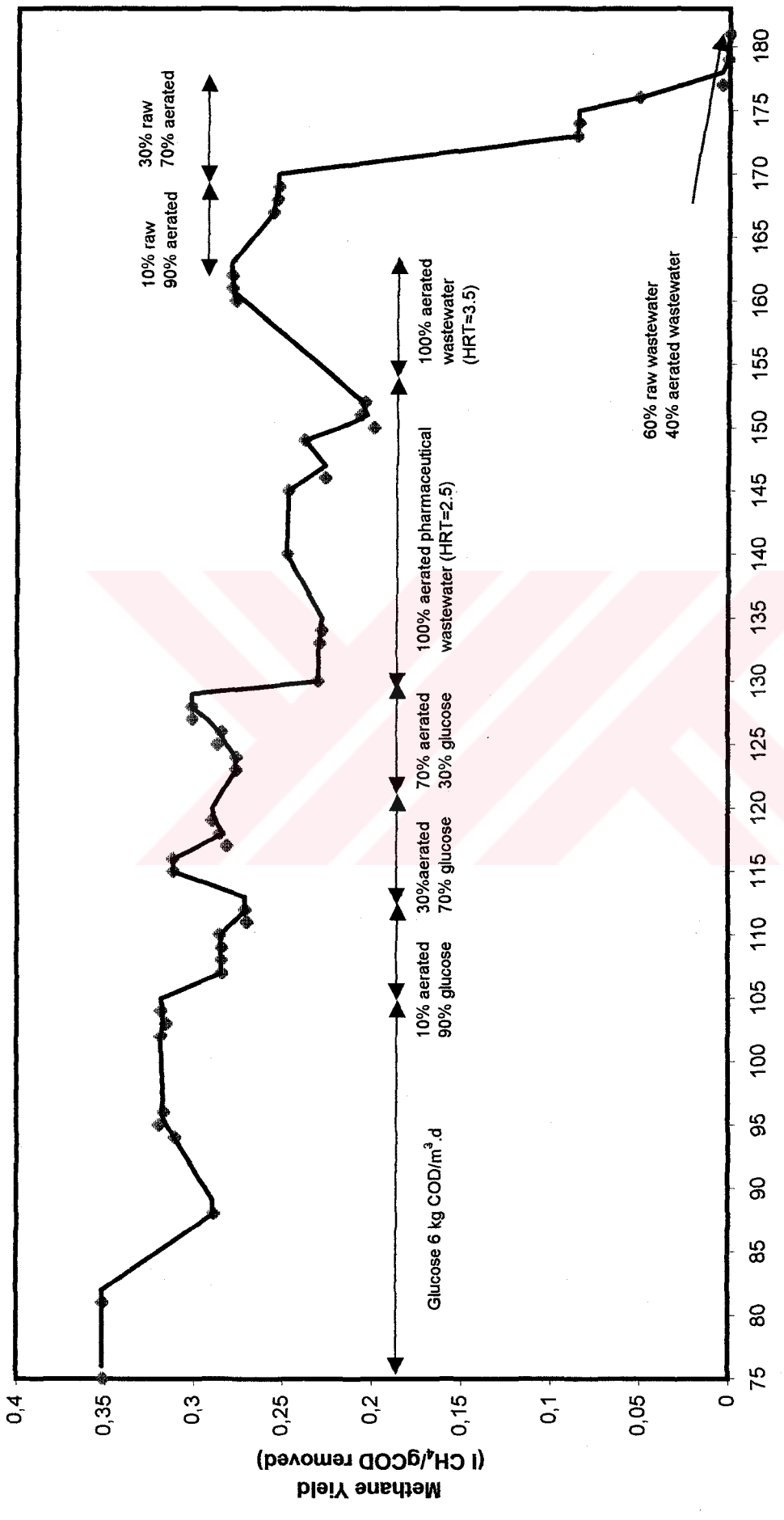


Figure 5.1.5. Methane Yield Against Operating Time

and 166 mlCH_4/gTVS.d. Methane yield increased again to 0.28 m^3CH_4/kg COD removed. However, VFA concentration in the CSTR is dramatically increased to 1474 mg/l at the end of the operation with 100% pre-aerated wastewater indicating that the system was adversly affected by the chemical composition of the wastewater.

In case of applying 10% raw and 90% pre-aerated pharmaceutical wastewater, a slight decrease was observed in COD removal efficiency. At this ratio, 70% COD removal efficiency was achieved corresponding to an OLR of 4.3 kgCOD/m^3.d. In spite of this slight decrease in COD removal efficiency, SMA value decreased to 113 mlCH_4/gTVS.d indicating that the potential acetoclastic methanogenic activity was highly affected by the solvents found in the raw pharmaceutical wastewater. VFA concentration was 1175 mg/l at this ratio. Methane yield was 0.25 m^3CH_4/kgCOD removed. COD removal efficiency is shown in Figure 5.1.4 during this feeding strategy.

At a dilution ratio of 30% raw wastewater, COD removal efficiency was achieved as 60% at an OLR of 5 kgCOD/m^3.d. A sharp decrease was observed in the methane yield which was found as approximately 0.05 m^3CH_4/kgCOD removed and the VFA concentration increased to 1417 mg/l.

At a dilution ratio of 60% raw pharmaceutical wastewater, COD removal efficiency showed a dramatic decrease to 9%. The VFA concentration was increased to 9370 mg/l. Figure 5.1.7 and Figure 5.1.8 show changes in methane yield and VFA as acetic acid at the steady-states of the operation, respectively. An SMA test was carried out and found to be 0 ml CH_4/gTVS.d confirming that the biomass was completely inhibited from the chemical composition of the raw wastewater. The study was, therefore, operated for an additional period of approximately 10 days at a ratio of 60% raw wastewater and no improvement was observed in the system performance. Hence, the operation was discontinued.

Figure 5.1.6 illustrates effluent MLSS and MLVSS concentrations during the different stages of the operation period. As seen, when 100% aerated pharmaceutical wastewater was introduced, a dramatic increase in VSS concentration in the effluent was observed. It still increased when raw wastewater was fed to the reactor and after that point granular characteristics of the sludge was disturbed in the CSTR.

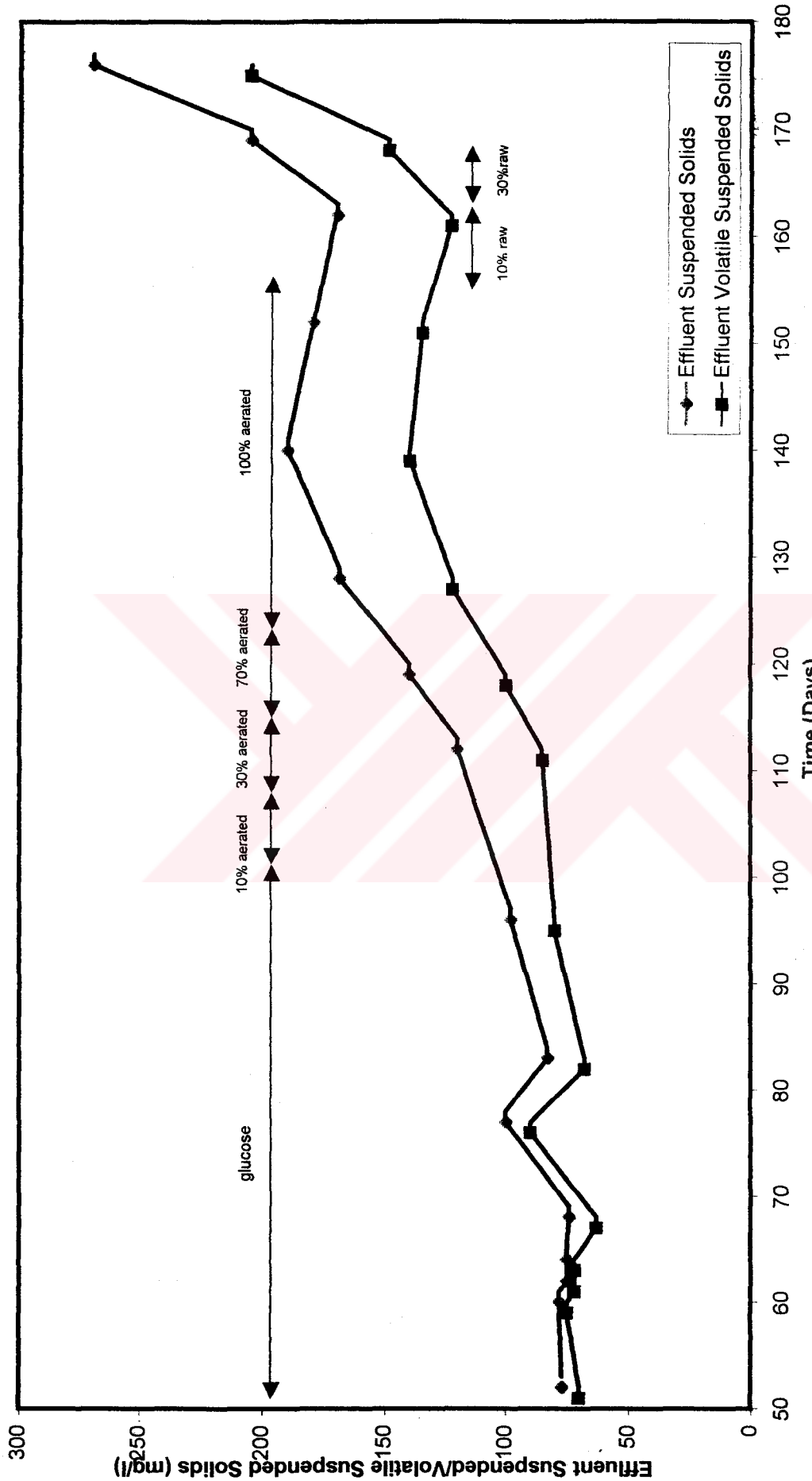


Figure 5.1.6. Changes in Suspended Solids/Volatile Suspended Solids Concentration from Effluent of CSTR During Operation Period

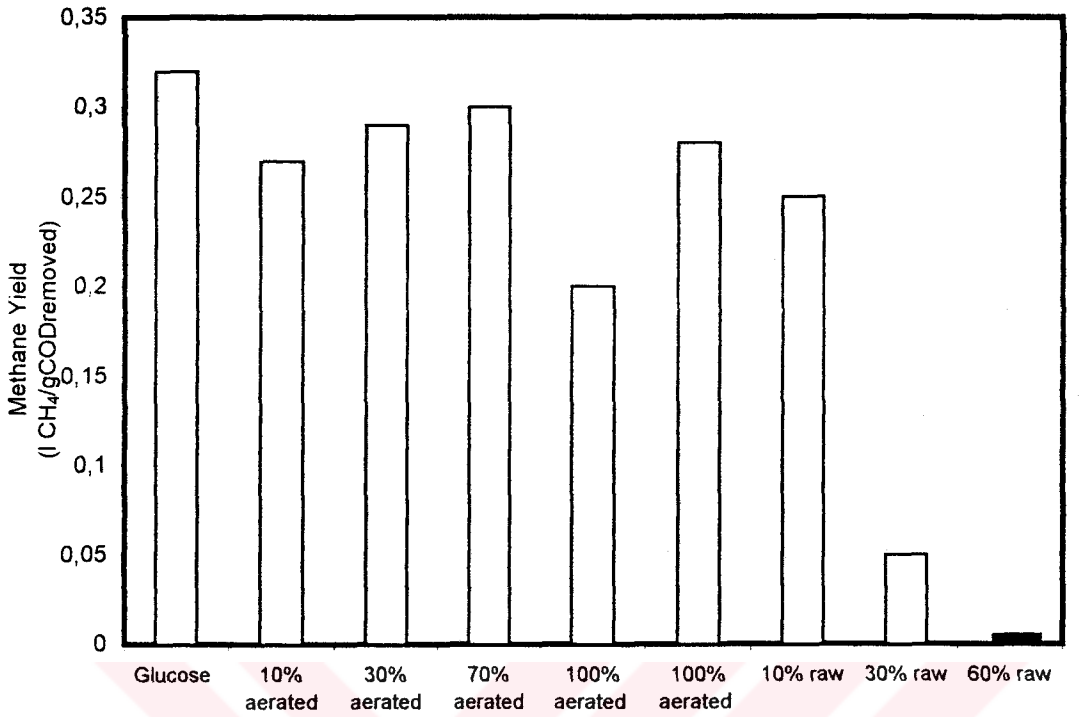


Figure 5.1.7. Methane Yield During Operation of the CSTR

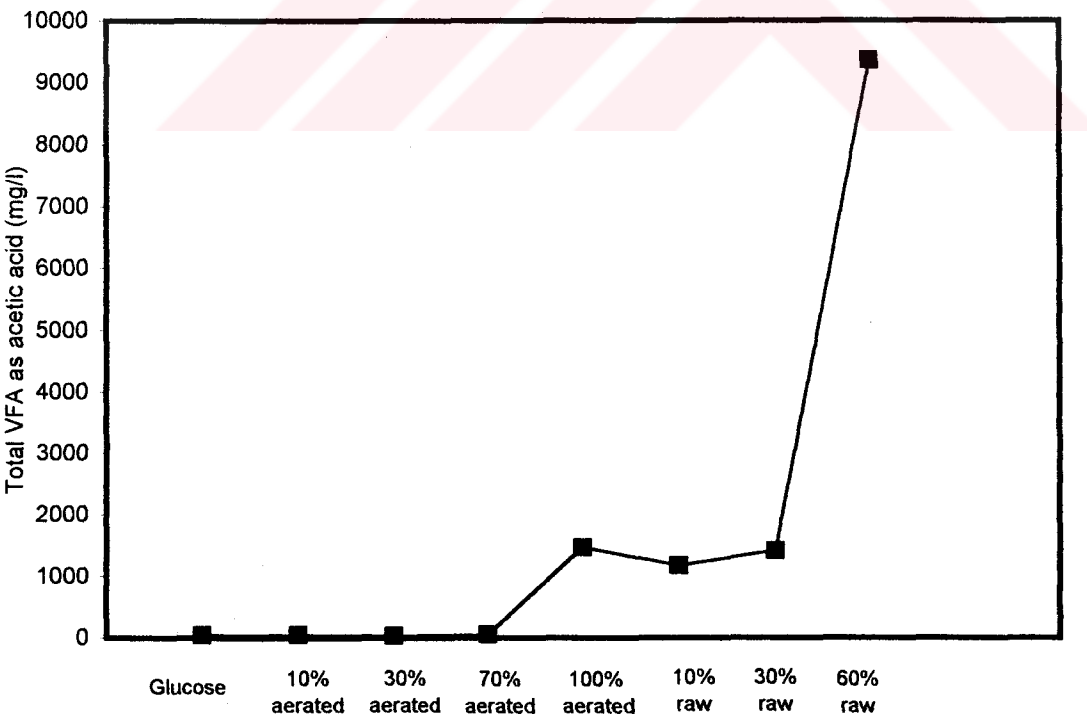


Figure 5.1.8. Total VFA As Acetic Acid During Operation

It has been stated that the rate limiting step depends on the nature of the substrate, process configuration, temperature and loading rate (Speece, 1983). It was found that the system configuration has a direct effect on the performance when substrate complexity is in question. While substrate complexity becomes an important factor affecting single-stage CSTR process efficiency, it is less important in the other process configurations such as two-stage and UASB (Azbar *et. al.*, 2000). In the study performed with raw pharmaceutical wastewater, a gradual decrease to a significant degree was seen in the performance due to the complex nature of the raw pharmaceutical wastewater and their immediate dispersion in the anaerobic CSTR system. Because of these reasons, while low concentrations of raw pharmaceutical wastewater at a dilution ratio of 10% and 30% was less significant on the performance of the CSTR, the system was significantly affected by the high concentrations of the wastewater at a dilution ratio of 60%.

5.2. Results of Specific Methanogenic Activity Tests

SMA test can be defined as a direct measurement of the rate of the methane production per unit microbial biomass per unit of time. Determining acetoclastic methanogenic activity of the sludge makes possible to select a proper sludge as inoculum and operate the anaerobic systems effectively during the start-up and steady-state operations. It is possible to decide the suitable OLRs to be applied into the reactor, to observe the changes in the activity of acetoclastic methanogenes and subsequently to predict failing conditions and take precautions using SMA test. Generally, VSS parameter is used as an indication of the biomass concentration however, the parameter can not distinguish the microbial biomass and any other particulate organic material which may be present in the anaerobic reactor. Moreover, it does not provide any information about the potential acetoclastic methanogenic activity of the reactor sludge (Reynolds,1986). SMA test helps to recognize the amount of active methanogenic population in the anaerobic sludge.

5.2.1. Initial Studies

Determination of potential methane production (PMP) rate of the sludge taken from an anaerobic wastewater treatment plant of a bakery yeast industry

An SMA test of the anaerobic sludge taken from a treatment plant of a bakery yeast industry was carried out in order to determine initial organic loading rate which should be applied to CSTR system. Temperature and pH were maintained at 35 ± 0.5 °C and 7.0 ± 0.2 respectively throughout the SMA tests. The data collected over 28 hours are shown in Figure 5.2.1. The maximum potential methanogenic activity was measured after 11 hours as 78 ml CH_4/gVSS.d at an acetate concentration of 2000 mg/l. Compared with previous SMA test of the same sludge carried out in 1996, it is seen that there was a decrease in the PMP rate of the sludge from 154 ml CH_4/gVSS.d to 78 ml CH_4/gVSS.d (Ince *et.al.*, 1996). The decrease in the PMP rate of anaerobic sludge might have been due to infavorable operating conditions and/or changes in wastewater composition resulting in a decrease in potential methanogenic activity of biomass.

According to the results of the previous studies carried out with enriched cultures cultivated on acetate, the maximum PMP rate was reported to be approximately 1000 ml CH_4/gVSS.d if all the biomass measured as VSS consists of acetoclastic methanogens (Valcke and Verstraete, 1983). Based on the statement, it can be said that the acetoclastic methanogens of the sludge from the bakery yeast treatment plant constituted approximately 7-8% of the VSS which indicates very low methanogenic activity in this study while that of was 33% for rettery wastewater, 63% for sugar beat wastewater using a UASB reactor, 35% for distilling wastewater using a UASB reactor (Ince *et. al.*, 2001), 15% for fermentation industry wastewater using a CSTR (Ince, 1996), 17% for brewery wastewater with CUMAR system and 13% for piggery manure wastewater using a completely mixed anaerobic reactor have been found (Ince, 1993). When taking into consideration characterizations of the sludge (VSS/SS ratio of 0.28, sludge volume index 110 ml/g SS) and comparing PMP rates of the sludges (>200 ml CH_4/gVSS.d) treating similar wastewaters in literature with the anaerobic sludge used in the study, it can be said that the inoculation sludge of the anaerobic treatment plant of the bakery yeast factory is low

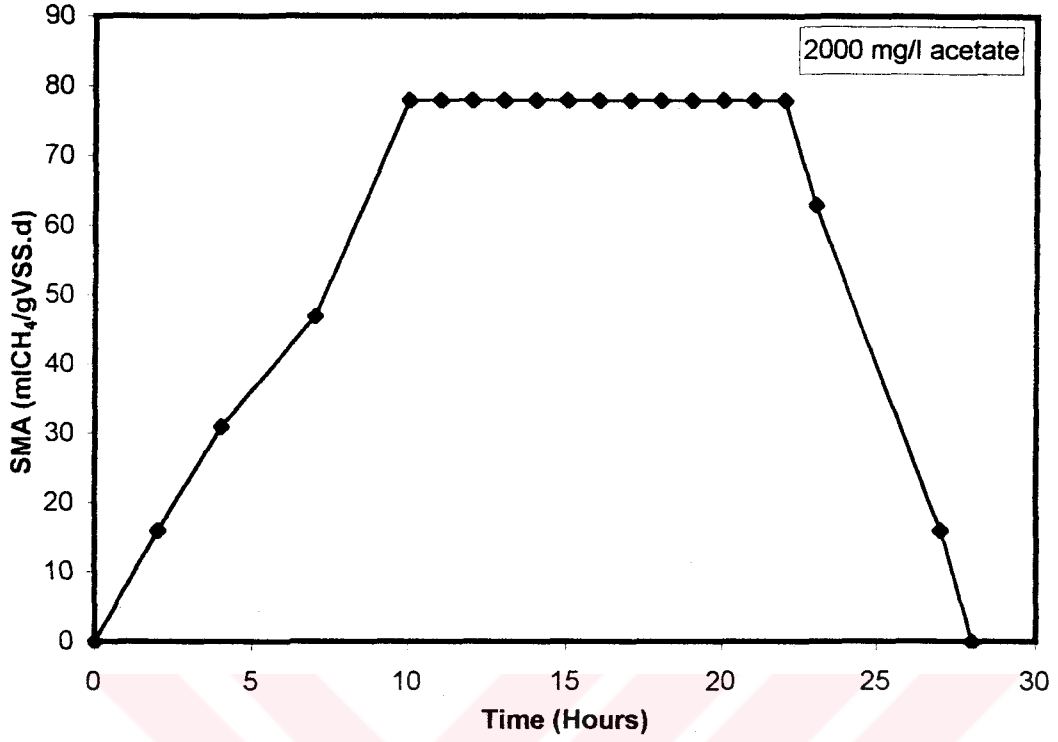


Figure 5.2.1. Plot of SMA of the Sludge Sample Taken from a Bakery Yeast Factory

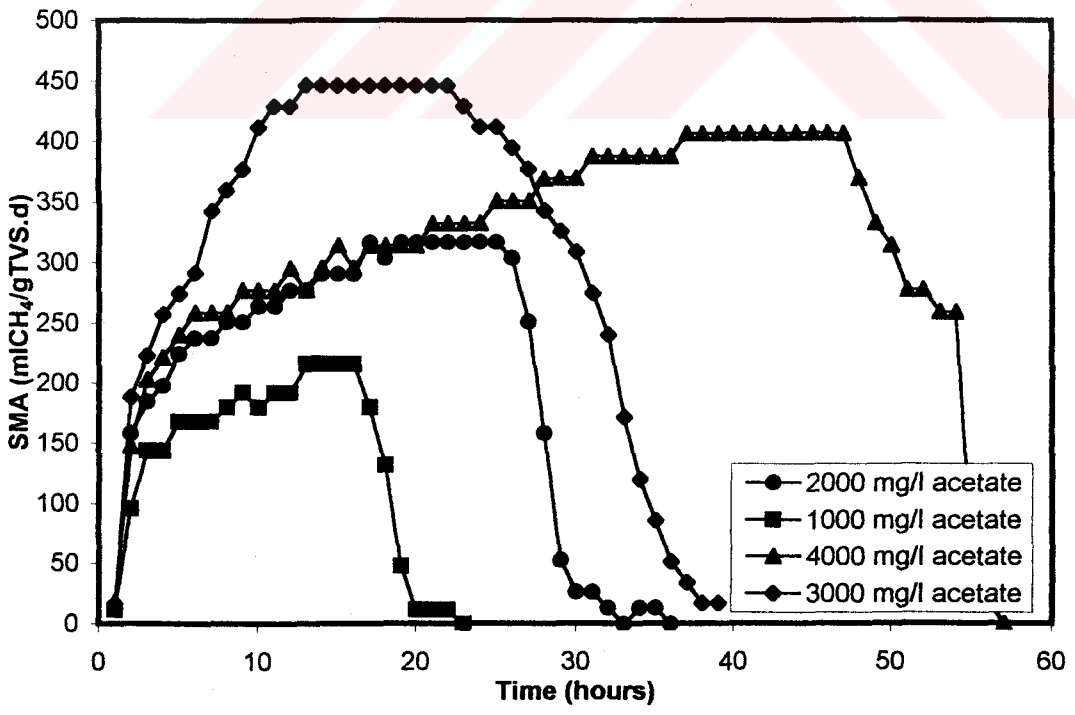


Figure 5.2.2. Plot of SMA of the Sludge Sample Taken from an Alcohol Distilling Factory at Different Acetate Concentrations

quality. Therefore, a new anaerobic sludge from a UASB reactor at a wastewater treatment plant of an alcohol distilling industry was used for the further studies.

Determination of Potential Methane Production Rate of the sludge taken from an UASB reactor of an alcohol distilling factory

Acetate concentrations of 1000 mg/l, 2000 mg/l, 3000 mg/l, 4000 mg/l were initially used in order to reach maximum methane production rate of the sludge taken from UASB reactor. The sludge sample was diluted with the mineral stock solution to a concentration of approximately 2000 mg/l TVS in the SMA test reactor and its pH was adjusted to 6.8-7.0 adding 1 N NaOH. As can be seen Figure 5.2.2, the maximum PMP rates observed in the tests were 216 ml CH_4/gTVS.d, 317 CH_4/gTVS.d, 446 CH_4/gTVS.d, 407 CH_4/gTVS.d for 1000 mg/l, 2000 mg/l, 3000 mg/l, 4000 mg acetate /l respectively.

For acetate concentrations of 1000 mg/l and 2000 mg/l, maximum acetoclastic methane production rates were obtained after 13 and 17 hours of feeding with acetate respectively and reached their maximum values for a period of only 3-4 hours and then decreased sharply. This might have been due to the rate limiting factor of substrate.

Acetate concentrations of 3000 mg/l and 4000 mg/l were maintained in the SMA test reactors in order to make sure that the substrate concentration was not limiting the acetoclastic methanogenic activity. As can be seen Figure 5.2.2, at an acetate concentration of 3000 mg/l, the PMP rate gradually increased in the first 12 hours and remained almost stable for the next 9 hours at its maximum value of 446 ml CH_4/gTVS.d and then a gradual decrease in gas production followed. A maximum PMP rate of 407 mlCH_4/gTVS.d at 4000 mg/l acetate concentration was observed in first 36 hours. According to the results of the SMA tests, the most favorable substrate concentration was found to be 3000 mg/l as acetate yielding a maximum PMP value of 446 ml CH_4/gTVS.d. Compared with pure culture studies, it can be interpreted that the acetoclastic methanogens constituted approximately 45% of the anaerobic sludge taken from the UASB reactor of the alcohol distilling factory. When taking into consideration characterizations of the sludge (TVS/TS ratio of 0.96, sludge volume index 15 ml/g TVS) and comparing PMP rates of the sludges

(>200 ml CH_4/gVSS.d) treating similar wastewaters in literature with the sludge used in the study, it can be said that the sludge of the anaerobic treatment plant of the alcohol distilling factory is good quality.

5.2.2. Methanogenic Activity Changes During Initial Study with Glucose

It has been reported that initial organic loading rate should be determined depending on the biomass concentration and its specific methanogenic activity (Ince et. al., 1994). A specific methanogenic activity (SMA) test was carried out to the sludge in order to determine the most acceptable initial organic loading rate. (De Zeeuv, 1984) SMA value of the seed sludge which indicated potential acetoclastic methanogenic activity was 78 ml CH_4/gVSS.d (VSS 3060 mg/l). According to the result of the SMA test of the seed sludge, the CSTR system was initially fed with glucose at an OLR of 1 kg COD/m^3.d corresponding to an F/M ratio of approximately 0.128 kg COD/kgVSS.d and then OLR was stepwise increased to 6 kg COD/m^3.d step by step.

Because the SMA test only measures the methane production from acetic acid called as the acetoclastic methanogenic activity and can not detect methane produced by hydrogen utilizing methanogenic bacteria, AMP rates evaluated from the reactors were corrected by a multiplying factor of 0.7.

First SMA test was carried out at an OLR of 3 kg COD/m^3.d. PMP rate of anaerobic sludge taken from the CSTR reactor was 140 ml CH_4/gTVS.d at which point 99% COD removal efficiency with an HRT of 2.5 days was achieved. Results of SMA tests carried out during start-up are shown in Figures 5.2.3-5.2.5. According to the SMA test results and COD removal efficiency, it was decided to increase OLR to 5 kg COD/m^3.d. The same COD removal efficiency was obtained at this OLR and the CSTR system was fed with glucose at an OLR of 6 kg COD/m^3.d. An SMA test was immediately carried out at this OLR and PMP rate was found to be 403 ml CH_4/gTVS.d. When the PMP rate compared with the actual methane production rate (AMP) of 43ml CH_4/gTVS.d obtained from the CSTR , the AMP/PMP ratio was found to be 0.1 indicating that the CSTR was operated under its potential loading capacity of the system. The results obtained from the both reactor and the SMA tests at OLR of 6 kgCOD/m^3.d indicated that AMP/PMP ratio was

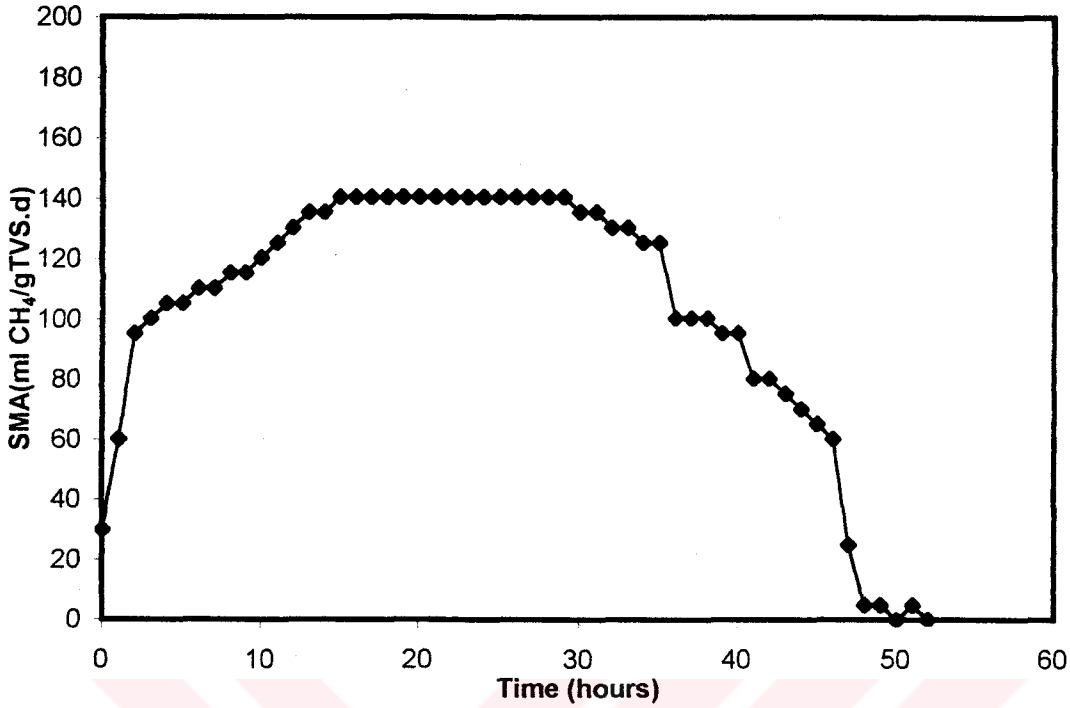


Figure 5.2.3. Plot of Specific Methanogenic Activity at an Organic Loading Rate of 3 kgCOD/m^3.d (on day 60)

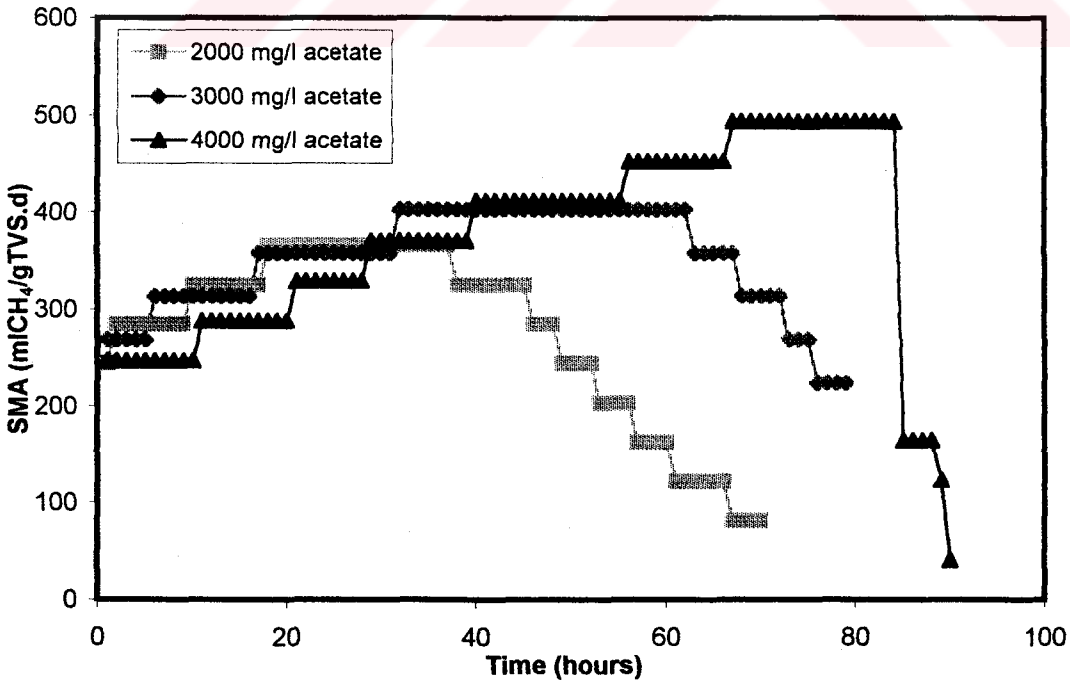


Figure 5.2.4. Plot of Specific Methanogenic Activity at an Organic Loading Rate of 6 kgCOD/m^3.d (on day 75)

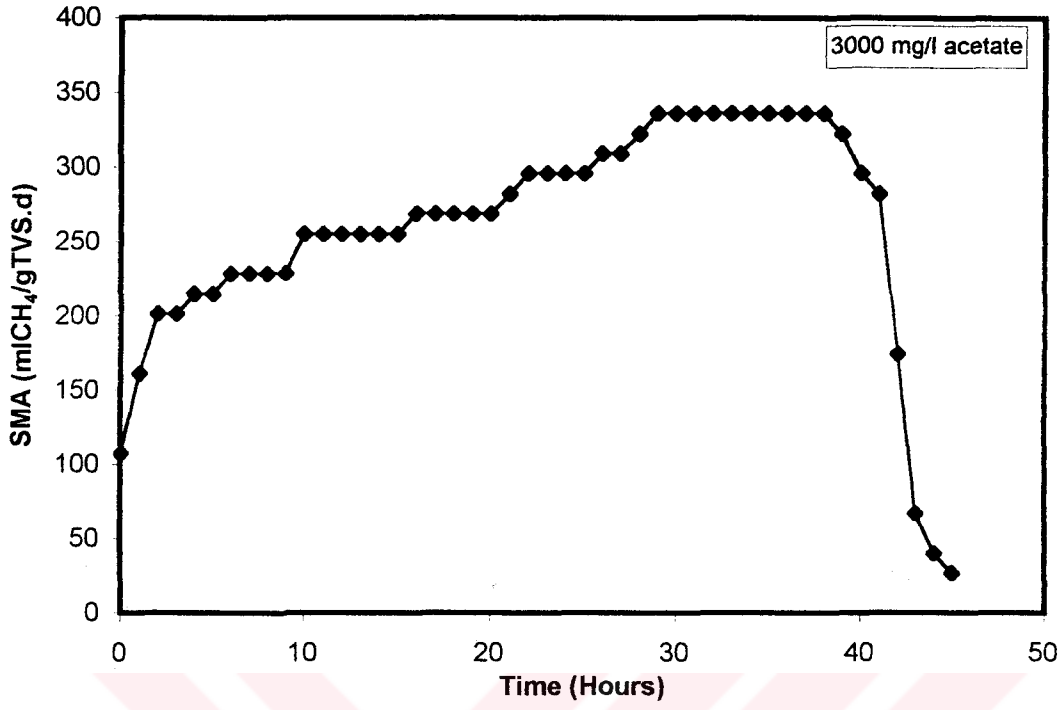


Figure 5.2.5. Plot of SMA at an Organic Loading Rate of 6 kgCOD/m^3.d (day 104)

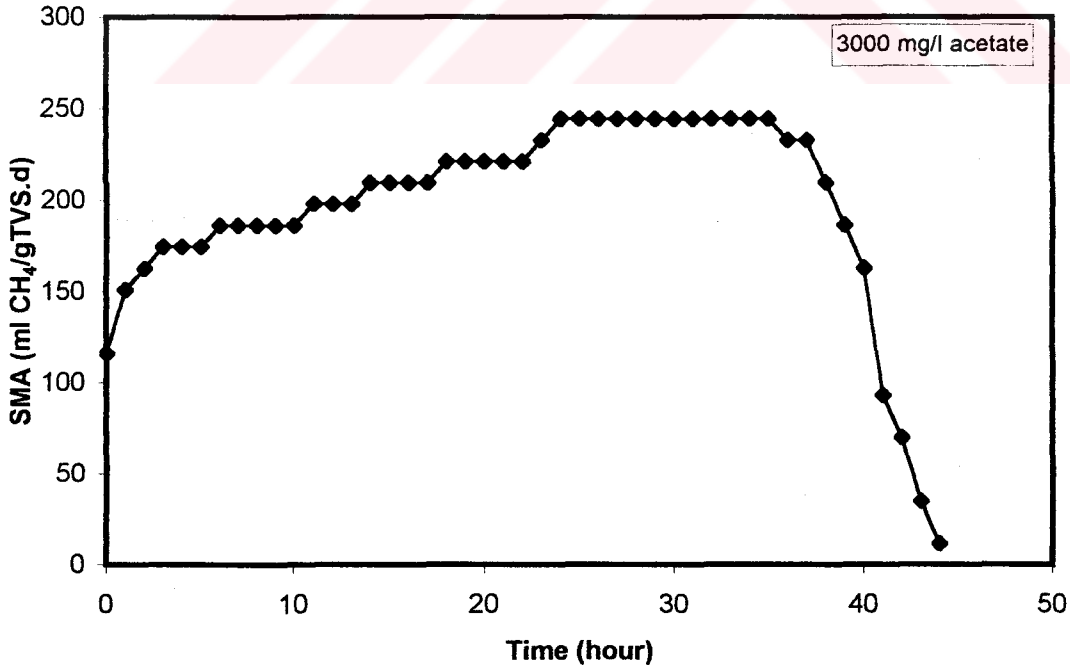


Figure 5.2.6. Plot of SMA When the CSTR Was Fed with 10% Pre-Aerated Wastewater (day 112)

very low. It was, therefore, decided to draw sludge from the CSTR in order to increase F/M ratio and consequently AMP/PMP ratio to a desired level. 300 ml sludge was drawn from the reactor on days between 76 and 90 days (totally 3 l). COD removal efficiency decreased to 82% corresponding to an F/M ratio of 0.54. 300 ml sludge was replaced into the reactor in order to increase COD removal efficiency. On day 104, an SMA test was again carried out at this OLR at which point F/M ratio was 0.43 and 92% COD removal efficiency. The SMA value was found to be 336 ml CH_4/gTVS.d indicating an AMP/PMP ratio of 0.26.

5.2.3. Methanogenic Activity Changes During Steady-States Operation

After start-up, CSTR was fed with pre-aerated pharmaceutical wastewater mixed with glucose at a dilution ratio of 10%. OLR and HRT were maintained same as start-up period. A SMA test was carried out after feeding with 10% pre-aerated pharmaceutical wastewater when reached at steady-state at which point COD removal was 84%. As can be seen in Figure 5.2.6, SMA value was found to be 244 ml CH_4/gTVS.d (2340 mg/l TVS). Compared this result with the value of 336 ml CH_4/gTVS.d which is obtained at a steady-state operation with glucose, 27 % decrease was observed in the PMP rate of the anaerobic granular sludge. There was a decrease in COD removal efficiency and methane yield.

30% dilution ratio of pre-aerated wastewater was given to the anaerobic reactor. At the steady-state, SMA value was 287 ml CH4/gTVS.d. The result of the SMA test is shown in Figure 5.2.7. An increase can be seen compared to 10% dilution ratio in the PMP rate of the sludge indicating acclimatization of the biomass to the pre-aerated pharmaceutical wastewater. However, there was still 15% decrease at this ratio compared with the value achieved at the end of the initial study with glucose.

At 70% dilution ratio of pre-aerated wastewater, SMA value was increased to 305 ml CH_4/gTVS.d. Acclimatization of the biomass to the wastewater continued at this ratio. Activity loss was 9% at this ratio compared with the value obtained at the end of the feeding with glucose. The reactor was very effective in responding to this ratio and COD removal was 86%. Figure 5.2.8 shows the SMA test carried out at the end of the period at 70% dilution ratio. At this point, 100% pre-aerated pharmaceutical wastewater was fed to

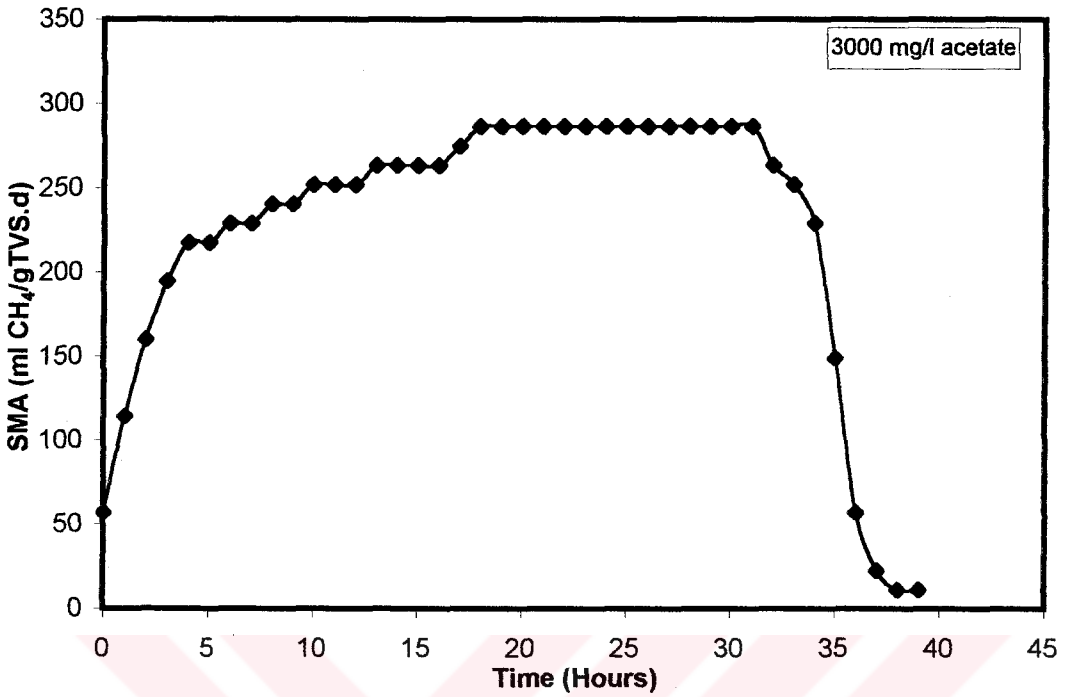


Figure 5.2.7. Plot of SMA When the CSTR Was Fed with 30% Pre-Aerated Wastewater (day 119)

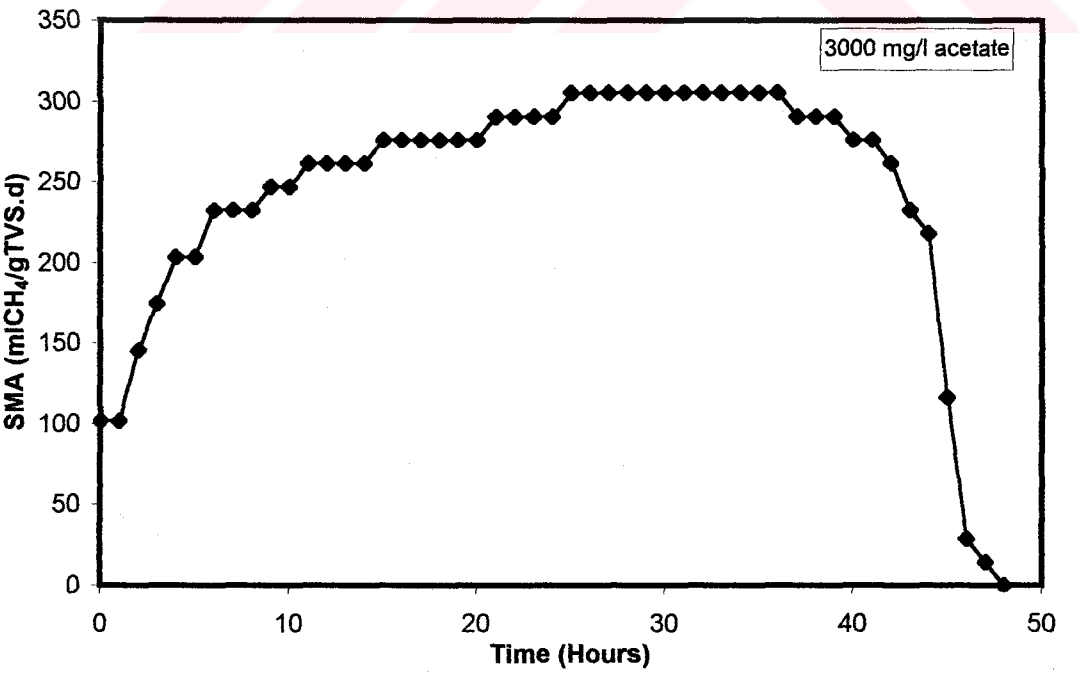


Figure 5.2.8. Plot of SMA When the CSTR Was Fed with 70% Pre-Aerated Wastewater (day 128)

the CSTR. COD removal efficiency decreased to 65% at this steady-state. SMA value was found to be 163 mlCH_4/gTVS.d indicating 50% decrease in PMP rate. Therefore, HRT was increased from 2.5 days to 3.5 days in order to find out whether any improvement in the performance of the system could be achieved. This resulted in an increase in COD removal efficiency to 71% at an OLR of 4.5 kgCOD/m^3.d. PMP rate of the anaerobic sludge slightly increased compared with the previous value obtained at the steady-state of 100% pre-aerated pharmaceutical wastewater. It increased from 163 ml CH_4/gTVS.d to 166 ml CH_4/gTVS.d. Figure 5.2.9 and 5.2.10 show the specific methanogenic activity when the CSTR was fed with 100% pre-aerated wastewater. As can be seen from the figures, although a slight increase is observed in PMP rate at 100% pre-aerated pharmaceutical wastewater when HRT was increased from 2.5 to 3.5 days, time required to reach the maximum activity decreased significantly indicating imrovement in the activity of acetoclastic methanogens due to the effect of the increase in HRT to 3.5 days.

Finally, the CSTR was fed with raw pharmaceutical wastewater diluted with pre-aerated wastewater at a dilution ratio of 10% at an OLR of 4.3 kgCOD/m^3.d. At the steady-state, an SMA test was carried out and a PMP rate of 113 mlCH_4/gTVS.d was found. The result of the SMA test is shown in Figure 5.2.11. Although COD removal efficiency was 70% at the steady-state of this dilution ratio, according to the SMA test performed at this point there was a decrease to a significant degree in the activity of acetoclastic methanogenes showing the biomass was adversely affected by the raw wastewater because of the chemical composition of the pharmaceutical wastewater. It shows that conventional parameters like COD, pH, biogas flow rate provide information about just current conditions inside the reactor but the activity of methanogens give better responses due to the changes in the operating conditions, wastewater composition and toxic materials. However, the variations in the acetoclastic methanogenic activity should be considered carefully in order to protect the anaerobic reactors against undesirable operating conditions.

As can be seen from Figure 5.2.12, when the raw wastewater was introduced to the CSTR at a dilution ratio of 30%, a sharp decrease was observed in the activity of acetoclastic methanogenes. PMP rate of the system decreased to 20 ml CH_4/gTVS.d. Time required to

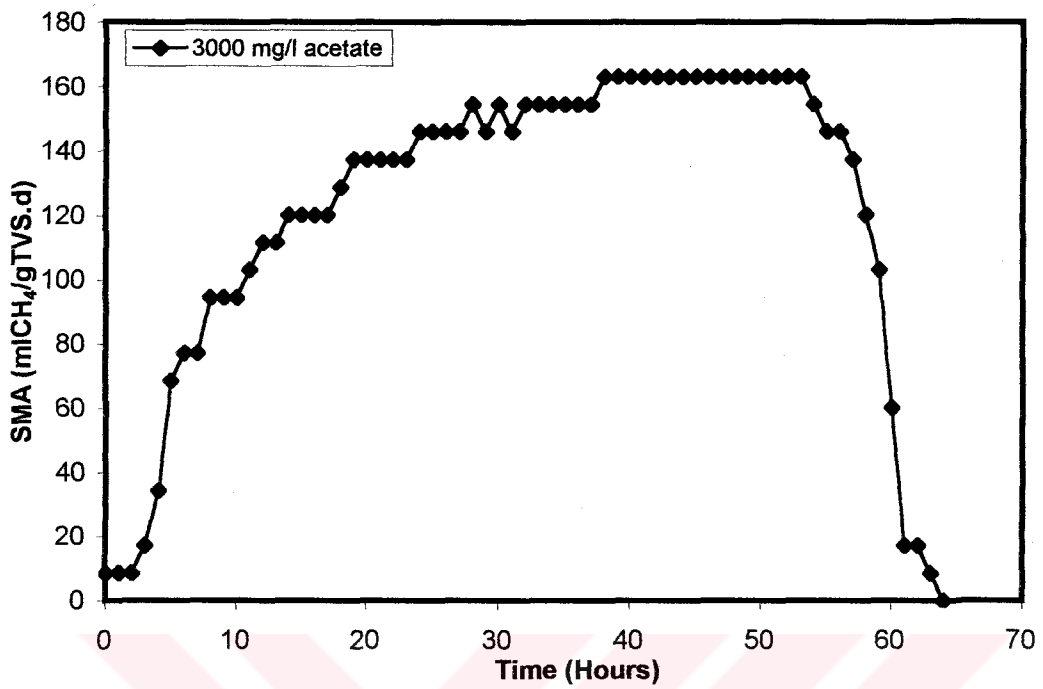


Figure 5.2.9. Plot of SMA When the CSTR Was Fed with 100% Pre-Aerated Wastewater at HRT=2.5 (day 152)

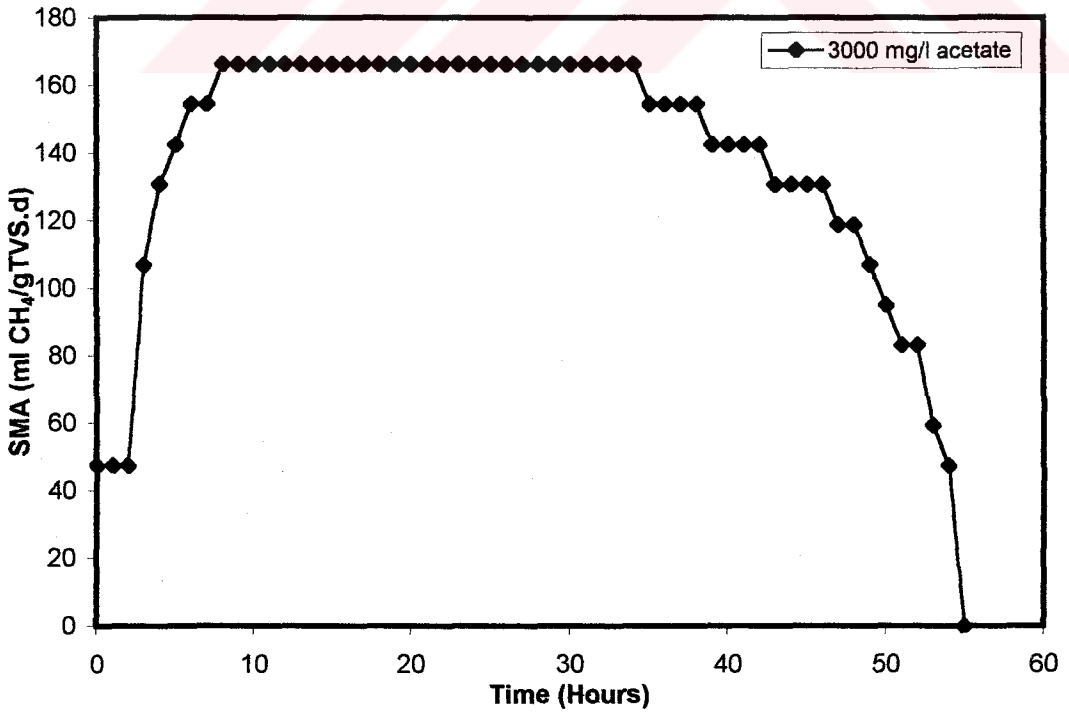


Figure 5.2.10. Plot of SMA When the CSTR Was Fed with 100% Pre-Aerated Wastewater at HRT=3.5 (day162)

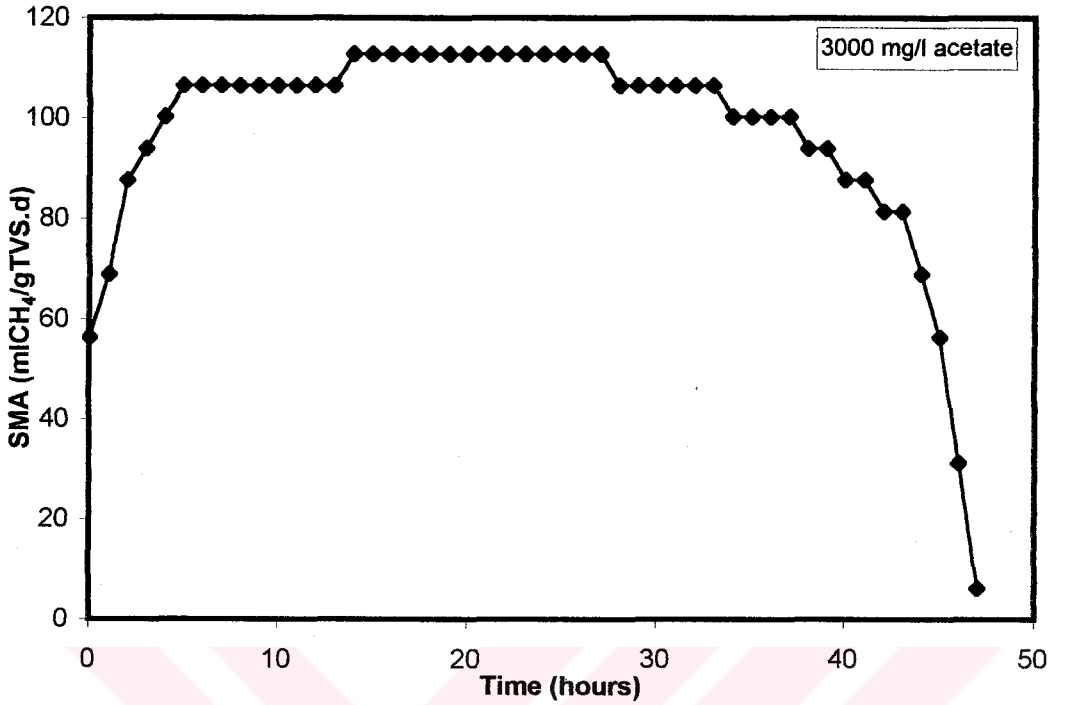


Figure 5.2.11. Plot of SMA When the CSTR Was Fed with 10% Raw Pharmaceutical Wastewater (day 169)

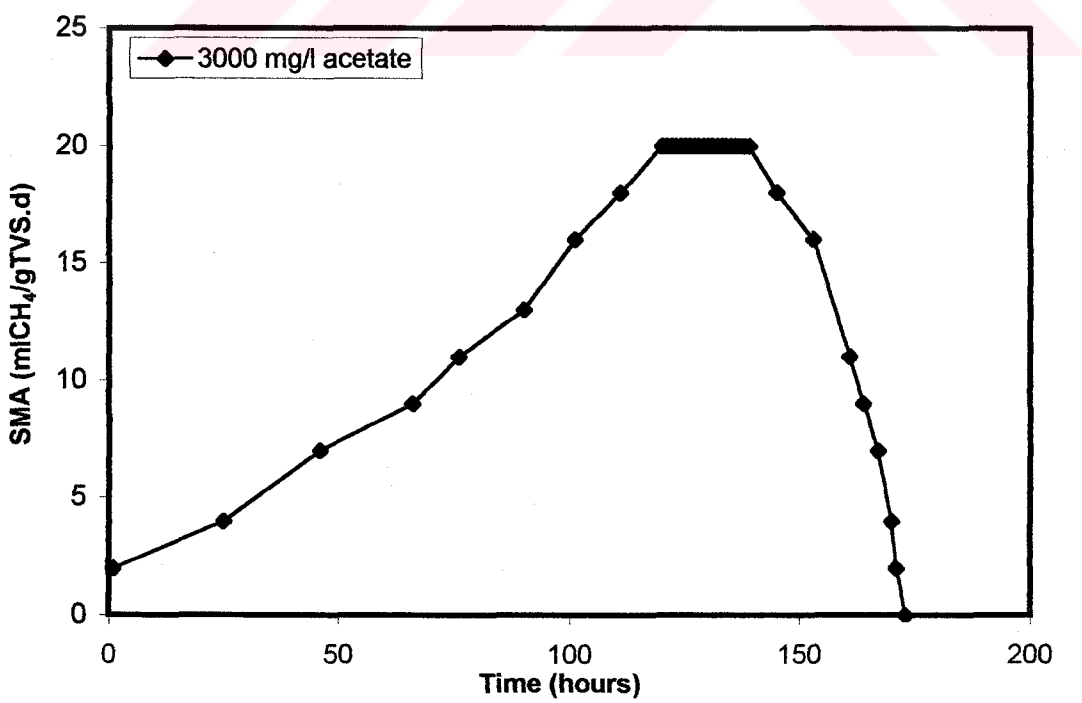


Figure 5.2.12. Plot of SMA When the CSTR Was Fed with 30% Raw Pharmaceutical Wastewater (day 176)

reach the maximum acetoclastic methanogenic activity dramatically increased to 120 hours. Methane yield also showed a dramatic decrease to 0.05 m^3CH_4/kgCOD removed.

The CSTR reactor was fed with raw pharmaceutical wastewater at a dilution ratio of 60%. According to the SMA test performed at the end of the operating period, a PMP value of 0 ml CH_4/gTVS.d was found which confirmed that there was not any potential acetoclastic methanogenic capacity of the sludge remained. Therefore, a poor anaerobic reactor performance in terms of COD removal efficiency, biogas flow rate, VFA were observed.

5.2.4. AMP/PMP Ratio

It has been stated that when the anaerobic reactors were operated approximately at an AMP/PMP ratio of 0.6 during start-up period, the anaerobic systems can be efficiently run in terms of COD removal and methane yield (Monteggia, 1991; Ince *et. al*, 1994). It has been also reported that SMA test can be used a reliable method in order to predict OLRs which could be applied to the anaerobic digestion reactors since it shows potential acetoclastic methanogenic activity (Ince *et. al*, 1994).

Figure 5.2.13 shows the SMA test results at the end of the steady-states of the different feeding strategy. PMP rate shows a sudden decrease when the CSTR was fed with 10% pre-aerated pharmaceutical wastewater and then an increase gradually at ratios of 30% and 70% indicating acclimization. However, a dramatic decrease was observed at a ratio of 100% pre-aerated wastewater and the decrease continued gradually at ratios of 10% and 30% raw wastewater. At a ratio of 60% raw wastewater, PMP rate found to be as 0 mlCH_4/gTVS.d indicating that acetoclastic methanogens were inhibited from the wastewater composition.

AMP values obtained from the CST reactor were compared with PMP values obtained from the SMA test during different operating conditions in order to monitor the effects of the feeding strategy used in this study on the ratio of AMP/PMP. Both of the parameters are tabulated in Table 5.2. At the end of the operation with glucose an AMP/PMP ratio of 0.26 was obtained indicating that the CST reactor could be loaded at higher organic

loading rates. Figure 5.2.14 shows the AMP values obtained at the end of the steady-states of the different feeding strategies during the operation.

At the end of the operation with 10% pre-aerated wastewater mixed with glucose, there was a slight decrease in the AMP value of the CST reactor, a sharp decrease in the PMP rate of the anaerobic sludge taken from the anaerobic reactor. AMP/PMP ratio increased to 0.28. However, increase in the ratio is due to the sharp decrease in the PMP value of the sludge indicating that acetoclastic methanogens was adversely affected by the pre-aerated wastewater.

The AMP/PMP ratio decreased to 0.25 at the end of the operation period with pre-aerated pharmaceutical wastewater at a dilution ratio of 30%. As can be seen from Table 5.2., slight increases in both AMP and PMP ratios were observed. At dilution ratios of 30%, 70%, 100% pre-aerated pharmaceutical wastewater, the ratio showed a slight change. However, when evaluating the AMP and PMP values for the ratio of 100% aerated pharmaceutical wastewater, the ratio did not change due to the decreases in both values. At 100% pre-aerated wastewater, the ratio showed a slight increase to 0.26 due to the increase in HRT from 2.5 days to 3.5 days.

Table 5.2. Changes in AMP/PMP Ratio During Operation

Feeding Strategy	**Time**	**CH_4 production (ml/day)**	**AMP ($mlCH_4/gTVS.d$)**	**AMP*0.7**	**PMP ($mlCH_4/gTVS.d$)**	**AMP/PMP**
Glucose	104	13924	127	89	336	0.26
10%	112	10800	98	69	244	0.28
30%	119	11400	104	73	287	0.25
70%	128	11660	106	74	305	0.24
100%	152	6026	55	38	163	0.24
100% (HRT=3.5)	162	6762	61	43	166	0.26
10%	169	5796	53	37	113	0.33
30%	176	1920	17	12	30	0.41
60%	181	0	-	-	0	-

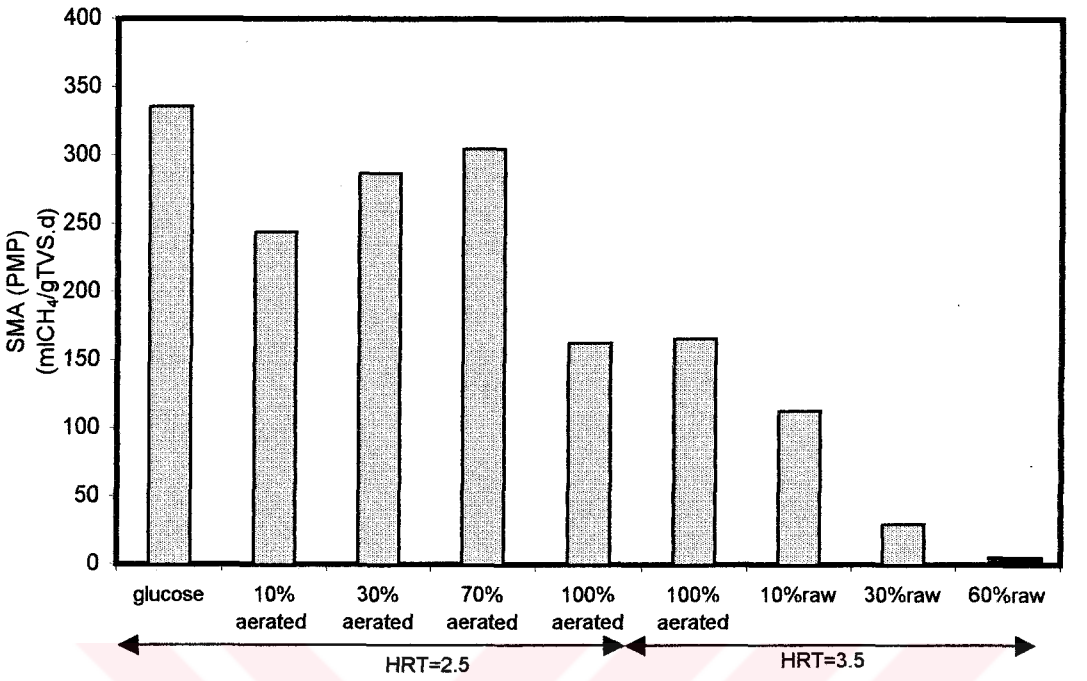


Figure 5.2.13. Specific Methanogenic Activity Test Results During Operation

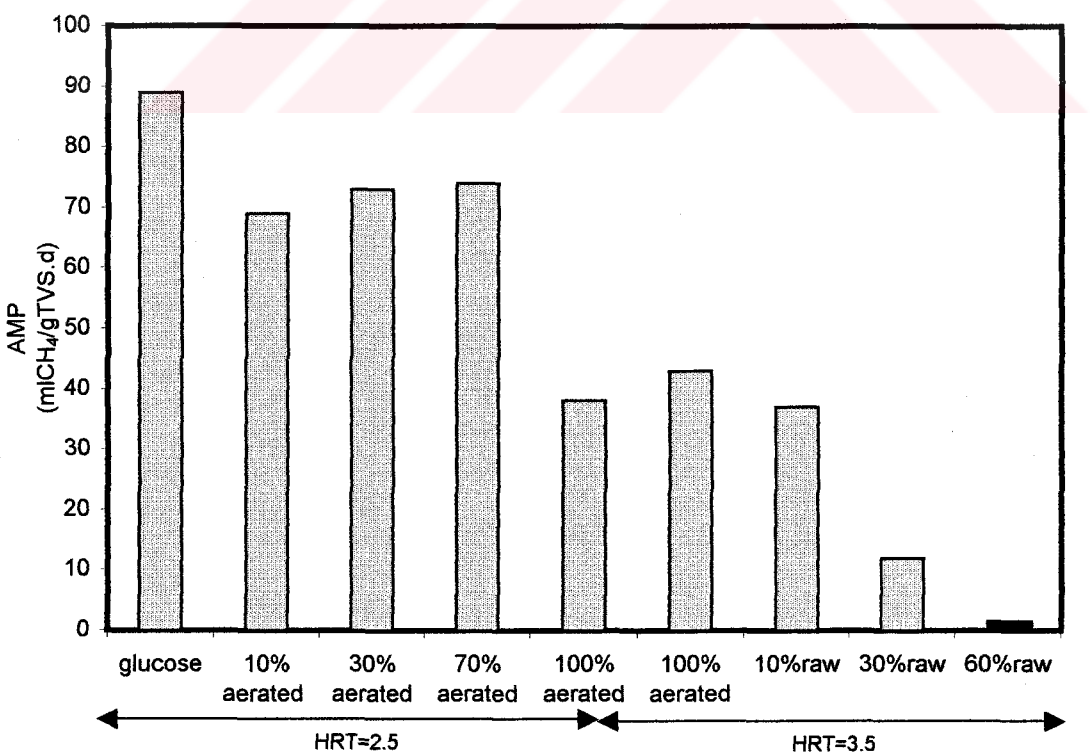


Figure 5.2.14. Overall AMP Results During Operation

At a ratio of 10% raw pharmaceutical wastewater, AMP/PMP ratio increased to 0.33 due to the significant decrease in PMP rate which is a sign of influence of the wastewater composition on the activity of the acetoclastic methanogens. However, despite the significant decrease in PMP rate, AMP showed a slight decrease. When CSTR was fed with the raw wastewater at a ratio of 30%, AMP/PMP value increased to 0.41 due to the significant decrease in both AMP and PMP. Therefore, it is not possible to take precautions with conventional parameters since AMP gives a late response to PMP which can show the changes in the activity of the acetoclastic methanogens during operation period.

AMP/PMP ratio of 0.6 could not have been achieved at initial study with glucose and AMP value was much lower than PMP indicating that the system was operated under its potential. Although AMP/PMP ratio increased to 0.33 and 0.41 when raw pharmaceutical wastewater was fed into the anaerobic reactor at dilution ratios of 10% and 30% respectively, the increase in the ratio is caused by the significant decrease in PMP rate due to the composition of the pharmaceutical wastewater. Therefore, AMP and PMP values should be taken into consideration separately when wastewaters like chemical synthesis based pharmaceutical wastewater which may affect on the PMP capacity of the acetoclastic methanogens due to the wastewater composition was used.

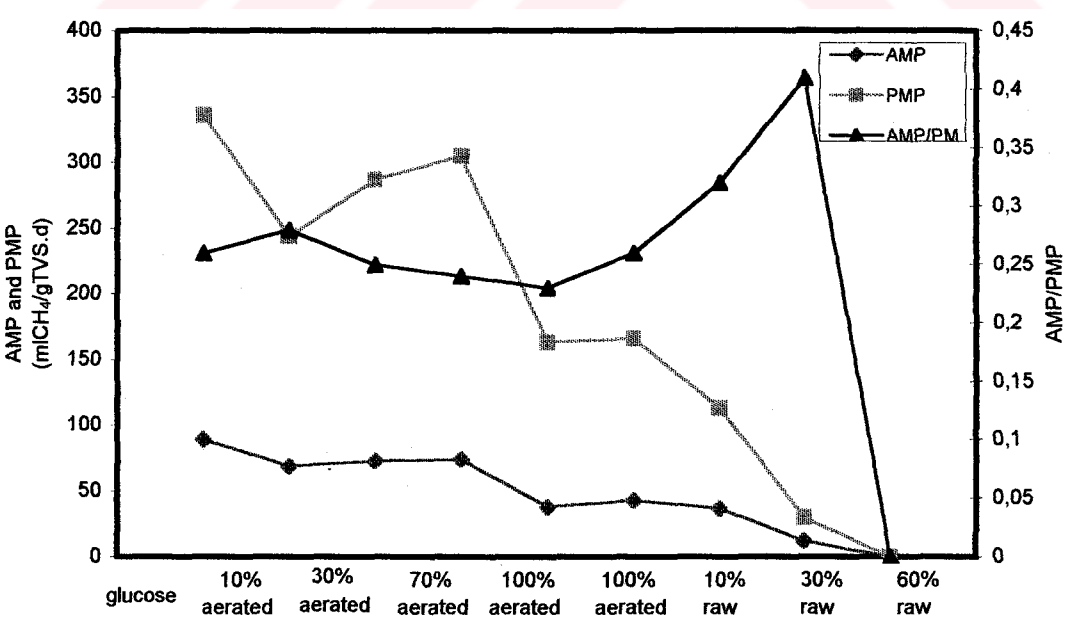


Figure 5.2.15. AMP and PMP Values with Respect to AMP/PMP Ratio at Each Steady-State

5.3. Microbiological Results

In this study, microscopic examination was carried out both for the seed sludge taken from an UASB reactor of an Alcohol Distilling Industry and for the samples taken from each steady-state. All bacterial counts were expressed per mg TVS instead of counts per ml in order to avoid the effect of changes in the concentration of biomass in the CSTR.

The technique of direct microscopic count is a rapid method in order to determine numbers and composition of autofluorescent methanogens in a sludge sample. However,the viable and non-viable methanogens can not be distinguished from each other by the method which can not also give any information about the activity of the viable methanogens which performs removal of organic material. Furthermore, it has been stated that 20-30% of methanogens, such as *Methanothrix*, do not exhibit fluorescence and therefore, it causes errors (Dolfing *et al.*, 1985). Despite all restrictions, direct microscopic counts provide general information about the numbers and morphology of the methanogenic species and their changes due to the operating conditions.

Performance of anaerobic treatment systems is related to the composition and amount of the methanogens in anaerobic reactors. Microbial population is affected by wastewater composition, system configuration and operation of anaerobic reactor. It has been stated that changes in the species composition of a microbial population would result in changes in methanogenic activity since each methanogenic bacterial group have their own specific activity of which directly affect the performance of an anaerobic reactor in terms of effluent quality and methane yield (Ince *et.al.*, 1995b, 1997). Microscopic examinations have been carried out in order to determine the effects of the different feeding strategy on the morphology and numbers of methanogens at each steady-state. Total bacteria were also counted in order to find out the proportion of the methanogens in microbial populations.

5.3.1. Proportion of Methanogens

An initial microscopic examination was carried out before the addition of the seed sludge in order to find out the properties of the sludge in terms of numbers of autofluorescent

methanogens and their morphology. The percentage of methanogens in the seed sludge taken from Alcohol Distilling Industry was found to be 29.7%. Numbers of autofluorescent methanogens and the ratio of total to autofluorescent methanogens are tabulated in Table 5.3.1.

After the CST reactor was fed with glucose, the numbers of autofluorescent methanogens increased to a range of 38% of the total bacterial population. During the operation, the proportion of methanogens did not show a regular variation. However, the percentage of methanogenic species in the total bacteria decreased to 30% at the end of the operation period with raw pharmaceutical wastewater at a ratio of 60%.

Figure 5.3.1 and 5.3.2 illustrate changes in the number of total autofluorescent methanogens and the proportion of autofluorescent methanogens to total bacteria, respectively. As can be seen from Figure 5.3.1, numbers of total autofluorescent methanogens increased during the operation period with 10%, 30%, 70% pre-aerated pharmaceutical wastewater mixed with glucose and then showed a decrease after 100% pre-aerated wastewater was fed into the CSTR. The decrease gradually continued during the operation with raw wastewater at a ratio of 10%, 30%, 60%.

5.3.2. Morphological Composition

The examination of the seed sludge taken from UASB reactor in an alcohol distilling industry showed that methanogenic species such as *Methanococcus* like as well as rod-shaped species such as short, medium and long rod shaped methanogens were present.

When the CSTR was fed with glucose at an OLR of 6 kgCOD/m^3.d, dominant groups were *Methanococcus* like species and short rods. Although *Methanococcus* like species were observed when the CST reactor was fed with only glucose during the start-up and 10% pre-aerated pharmaceutical wastewater mixed with glucose, their numbers were insignificant during the other ratios throughout the operation. Figure 5.3.4-5.3.6 show the variations of the rod-shaped methanogens such as short, medium and long rods.

Table 5.3.1. Results of Microbiological Assessments Carried Out for the Seed Sludge and Sludge Taken from the CSTR

Time	Examined Sludge	Number of Coccus #/mgTVS	Number of Short Rods #/mgTVS	Number of Medium Rods #/mgTVS	Number of Long rods #/mgTVS	Number of Saccina #/mgTVS	Number of Autofluor. Methanogens #/mgTVS	Number of Total Bacteria #/mgTVS	Autofluoresc. Methanogens/ Total Bacteria (%)	Dominant Species
	Alcohol Distillery	8.42×10^{7}	5.23×10^{7}	4.36×10^{7}	1.74×10^{7}	2.9×10^{6}	2×10^{8}	6.74×10^{8}	30	Short rods Medium rods Long rods
104	**Glucose**	7.34×10^{8}	1.16×10^{8}	5.79×10^{7}	3.9×10^{7}	none	9.46×10^{8}	2.51×10^{9}	38	Coccus Short rods
112	**10% aerated**	3.21×10^{8}	4.49×10^{8}	2.53×10^{8}	2.1×10^{7}	none	1.03×10^{9}	2.99×10^{9}	34	Coccus Short rods Medium rods
119	**30% aerated**	none	1.23×10^{9}	2.98×10^{8}	6.4×10^{7}	none	1.6×10^{9}	3.64×10^{9}	44	Short rods Medium rods
128	**70% aerated**	none	1.08×10^{9}	4.31×10^{8}	6.5×10^{7}	none	1.57×10^{9}	3.45×10^{9}	45	Short rods
162	**100% aerated**	none	9.36×10^{8}	3.88×10^{8}	4.6×10^{7}	none	1.37×10^{9}	3.63×10^{9}	38	Short rods Medium rods
169	**10% raw**	none	8.49×10^{8}	1.92×10^{8}	2.7×10^{7}	none	1.07×10^{9}	2.53×10^{9}	42	Short rods Medium rods
176	**30% raw**	none	6.23×10^{8}	1.48×10^{7}	1.5×10^{7}	none	8.6×10^{8}	2.16×10^{9}	40	Short rods
182	**60% raw**	none	5.27×10^{8}	6.58×10^{7}	none	none	5.93×10^{8}	1.98×10^{9}	30	Short Rods

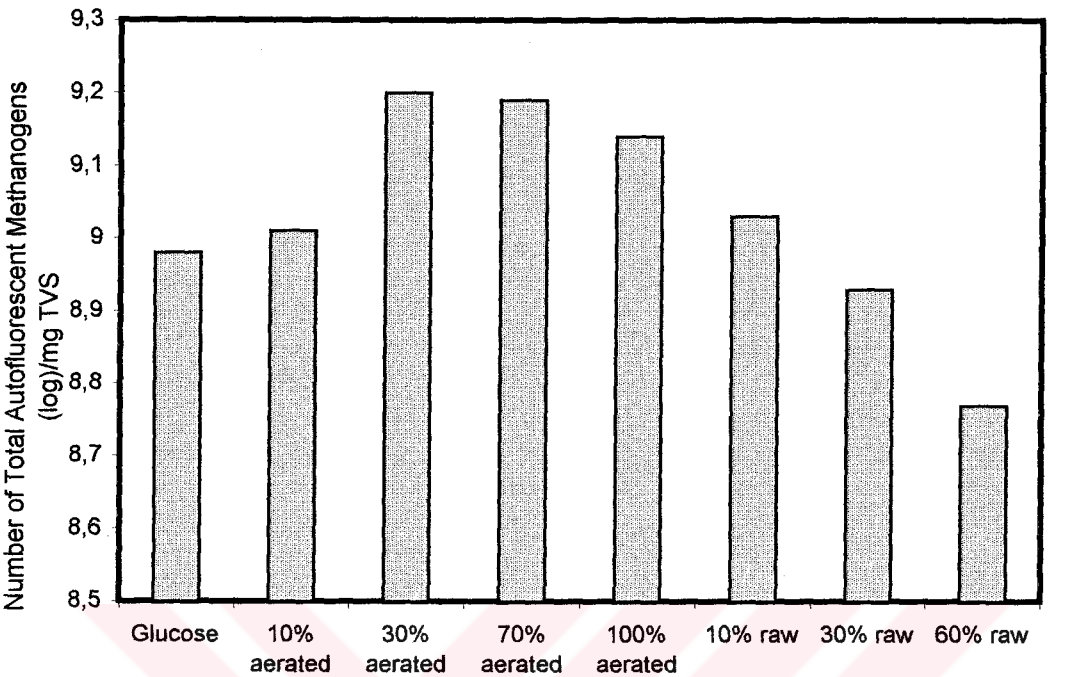


Figure 5.3.1. Changes in Number of Total Autofluorescent Methanogens

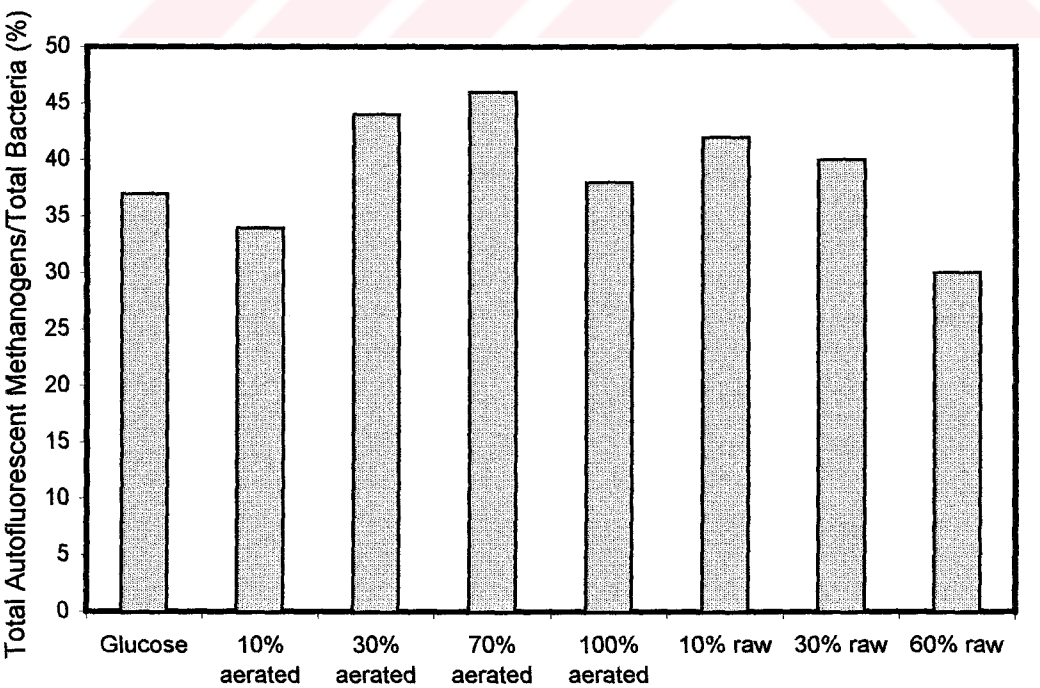


Figure 5.3.2. Changes in Ratio of Total Autofluorescent Methanogens to Total Bacteria (%)

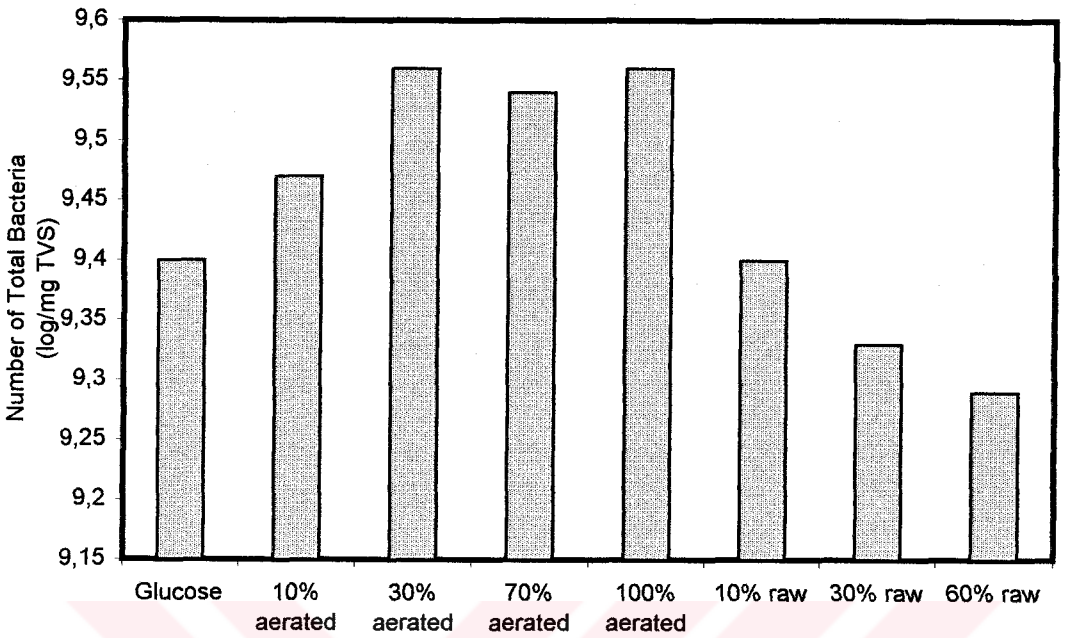


Figure 5.3.3. Changes in Number of Total Bacteria

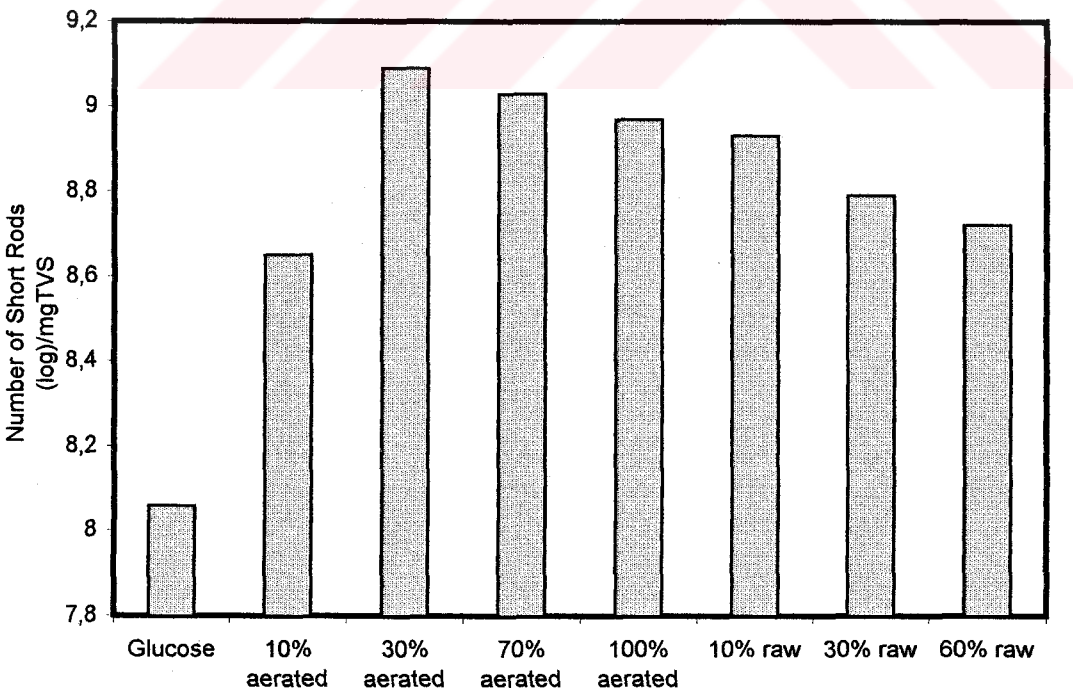


Figure 5.3.4. Changes in Number of Short Rods

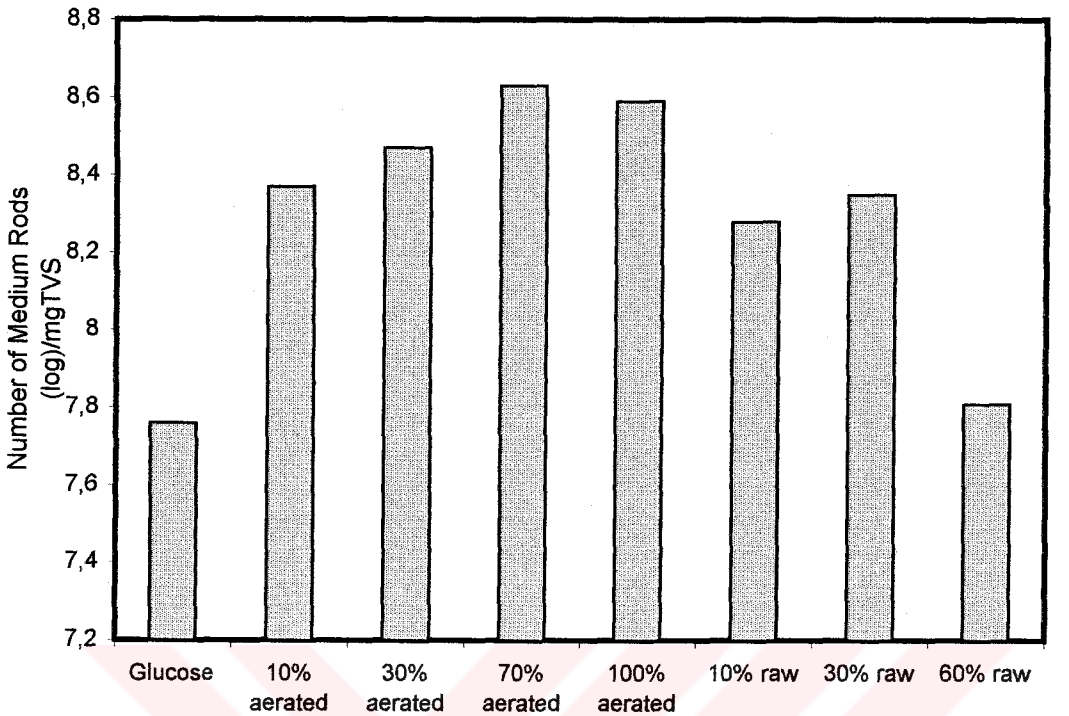


Figure 5.3.5. Changes in Number of Medium Rods

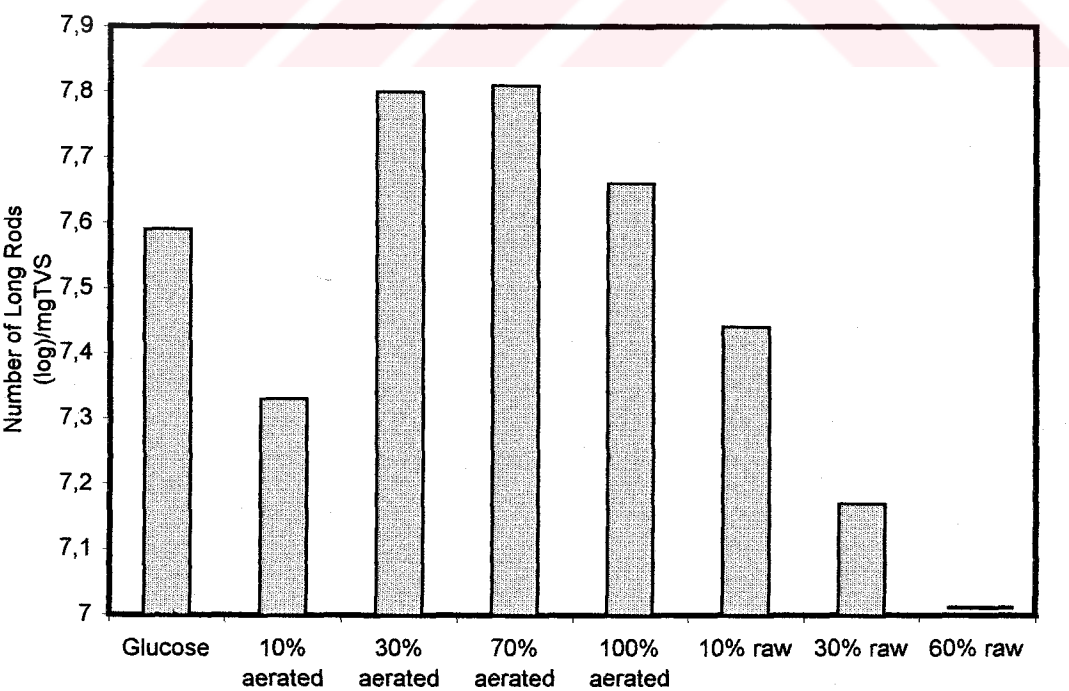


Figure 5.3.6. Changes in Number of Long Rods

As can be seen from Figure 5.3.5 and 5.3.6, the numbers of medium and long rods gradually increased until a dilution ratio of 100% pre-aerated pharmaceutical wastewater was fed to the anaerobic reactor. After that ratio, a significant decrease was observed. Although the numbers of short rods gradually decreased after a dilution ratio of 70%, the species was the most dominant methanogens even at a dilution ratio of 30% and 60% raw pharmaceutical wastewater.

Long rods compared with other rod-shaped methanogens were not observed in significant numbers. Their numbers decreased when 10% pre-aerated wastewater was introduced to the anaerobic reactor, and then increased at dilution ratios of 30% and 70% pre-aerated wastewater. After dilution ratios of 100% pre-aerated, 10% raw and 30% raw pharmaceutical wastewater, their numbers decreased significantly and they were not observed after a ratio of 60% raw wastewater.

Figure 5.3.7 shows the changes in numbers of total autofluorescent methanogens with respect to COD removal efficiency during operating time. A decrease was seen in COD removal efficiency with the decrease in number of total autofluorescent methanogens. The change in metabolic activity of the sludge during steady-state operation is illustrated in Figure 5.3.8. As seen, maximum metabolic activity was observed when the CSTR was fed with glucose and there was a decrease in metabolic activity during the study after pre-aerated pharmaceutical wastewater and raw wastewater was introduced to the anaerobic reactor. The decrease was dramatic after feeding with 30% raw pharmaceutical into the reactor.

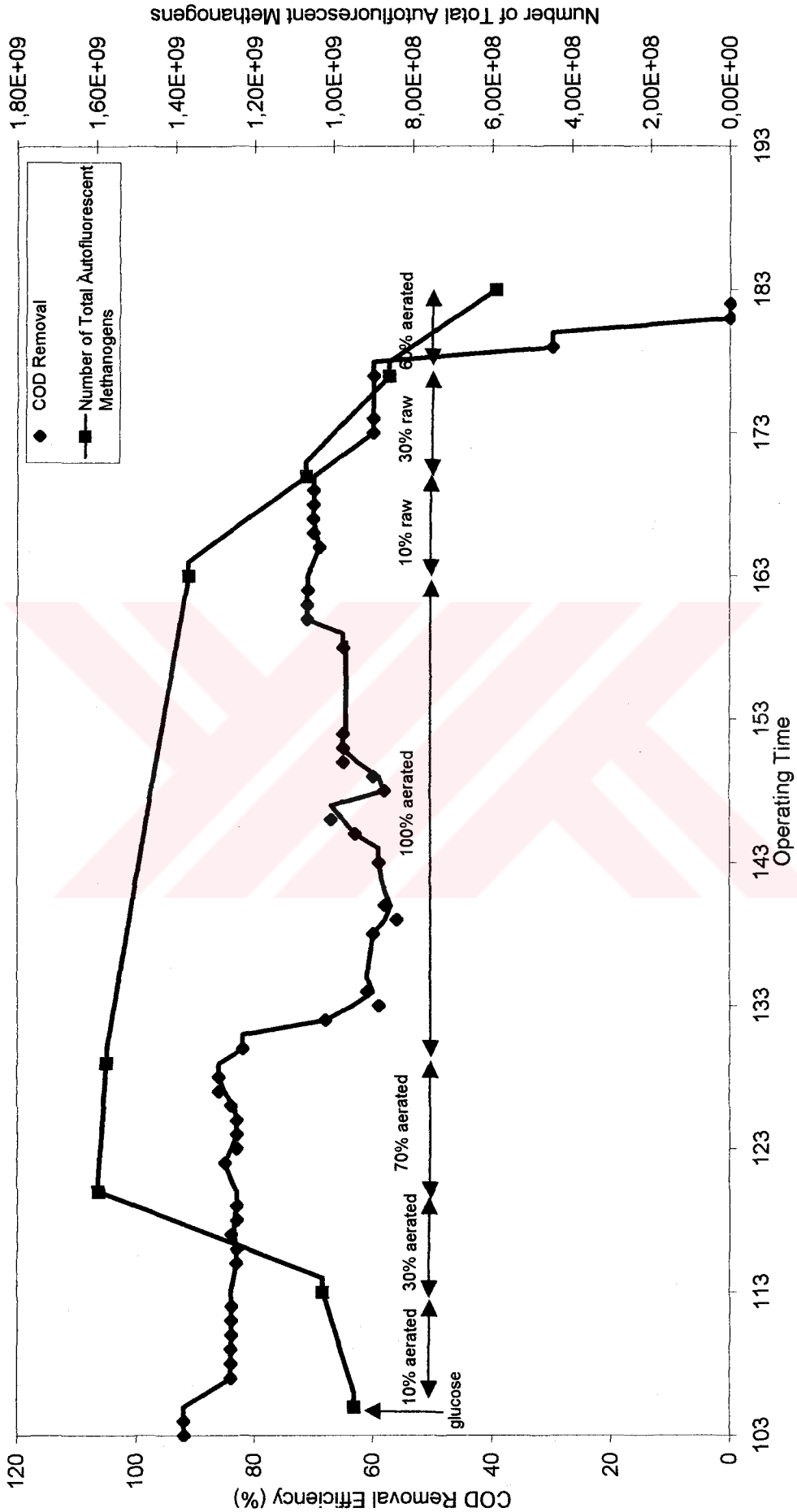


Figure 5.3.7. COD Removal Efficiency and Number of Total Autofluorescent Methanogens Against Operating Time

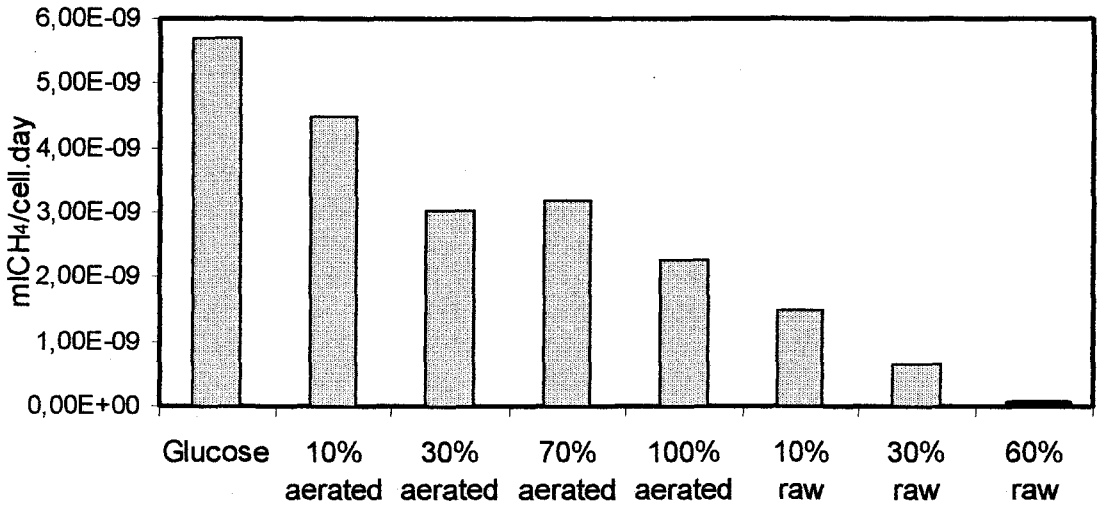


Figure 5.3.8. Changes in Metabolic Activity of Autofluorescent Methanogens During Steady-State Operation

Results of microbiological studies did not show significant variations. However, results of SMA test and metabolic activity of autofluorescent methanogens indicated very significant decreases which reflected the poor performance of the anaerobic reactor particularly after feeding with 100% pre-aerated pharmaceutical wastewater.

6. CONCLUSIONS

In this study, performance, specific methanogenic activity tests (SMA) and microbiological studies were carried out in order to determine overall performance of anaerobic CSTR when treating chemical synthesis based pharmaceutical wastewater. The CSTR was fed with only glucose during the initial study and the anaerobic reactor was firstly fed with pre-aerated pharmaceutical wastewater with several dilutions made by glucose and then with raw pharmaceutical wastewater diluted with pre-aerated wastewater.

At the end of the initial study with glucose, 92% soluble COD removal efficiency was achieved with the CSTR at an HRT of 2.5 days and methane yield was 0.32 m^3 CH_4/kg COD removed.

A maximum 70% COD removal efficiency was achieved with 100% pre-aerated pharmaceutical wastewater with an HRT of 3.5 days while methane yield was 0.28 m^3 CH_4/kg COD removed.

All parameters such as COD removal efficiency, methane yield, SMA values, VFA concentration, numbers of autofluorescent methanogens were adversly affected after feeding the CSTR with raw pharmaceutical wastewater. A maximum 60% COD removal efficiency was observed with 30% raw pharmaceutical wastewater and due to the very poor performance of the CSTR with a dilution ratio of 60% raw pharmaceutical wastewater, further loadings could not have been applied. The VFA concentration showed a dramatic increase to 9370 mg/l as acetic acids.

SMA value was found to be 336 ml CH_4/gTVS.d when CSTR was fed with glucose at an OLR of 6 kgCOD/m^3.d. with a HRT of 2.5 days, the value decreased to 166 mlCH_4/gTVS.d indicating approximately 50% loss in the activity of acetoclastic methanogens when 100% pre-aerated pharmaceutical wastewater was introduced to the anaerobic reactor with a HRT of 3.5 days. PMP rate of the sludge taken from the CSTR gradually decreased after raw pharmaceutical wastewater was fed and it was found out that no acetoclastic methanogenic activity remained at the end of the operation with 60% raw

wastewater confirming that the biomass was completely inhibited from the chemical composition of the raw wastewater.

Instead of the AMP/PMP ratio, AMP and PMP rates should be taken into consideration separately since PMP capacity of acetoclastic methanogens can be adversly affected from wastewaters like chemical synthesis based pharmaceutical wastewaters containing inhibitory or toxic compounds for microbial population.

Microscopic examination revealed that seed sludge taken from an alcohol distilling industry contain high amount of autofluorescent methabogens including short rods, medium rods, long rods, *Methanococcus* like species and insignificant methanosarcina. Dominant methanogens were short rods and *Methanococcus* like species.

Numbers of autofluorescent methanogens in the seed sludge was 30% and then, increased to 38% after feeding with glucose and then decreased again to 30% at the end of the study. However, it should be noted that specific methanogenic activity of the seed sludge showed a dramatic decrease from 446 $mlCH_4/gTVS.d$ to 0 indicating that activity of the methanogens is more important than the numbers. Metabolic activity of methanogens showed also decrease during operation.

Although *Methanococcus* and short rods were dominant species in the sludge taken from CST reactor after operation with only glucose and 10% pre-aerated pharmaceutical wastewater, numbers of *Methanococcus* were insignificant during the operation with other feeding strategies.

Short rods remained the most dominant species throughout the operation followed by the medium rods.

Methanosarcina and filamentous species were not observed in significant numbers at any stage of the operation.

Although methanococcus and short rods were dominant species in the sludge taken from CST reactor after operation with only glucose and 10% pre-aerated pharmaceutical

wastewater, numbers of *Methanococcus* were insignificant during the operation with other feeding strategies.

7. RECOMMENDATIONS

Since reactor configuration has a direct effect on the system performance, the study may be performed in a two-phase anaerobic treatment system or in another anaerobic reactor configuration such as fixed film systems instead of suspended growth anaerobic systems which are more sensitive to toxic or inhibitory compounds.

A study should be carried out to determine the solvents which have the most adverse effect on the acetoclastic methanogens and their inhibitory concentrations.

Acetoclastic methanogenic activity of the reactor sludge should be determined to operate the anaerobic systems effectively during start-up periods and steady-state operations and to decide the most suitable OLRs to be applied into anaerobic reactors. It is also possible to observe the changes in the activity of acetoclastic methanogenes during the operation due to the wastewater composition and operating conditions and subsequently to predict possible unsuitable conditions for anaerobic reactors.

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APPENDIX A: Performance of the CSTR

Time	HRT	OLR	COD inf.(mg/l)	CODeff.(mg/l)	COD rem.(%)	**Feeding Strategy**
0	3	1	3000	2500	17	Glucose
1	3	1	3000			Glucose
2	3	1	3000	1531	49	Glucose
3	3	1	3000			Glucose
4	3	0,77	2500	1375	45	Glucose
5	3	1	3000			Glucose
6	3	1	3000	1500	50	Glucose
7	3	1	3100			Glucose
8	3	1	3000	750	75	Glucose
9	3	1	3200			Glucose
10	3	1	3000	750	75	Glucose
11	3	1,5	4563			Glucose
12	3	1,5	4500	3300	26	Glucose
13	3	1,5				Glucose
14	3	1,5				Glucose
15	3	1,7	5125	3600	29	Glucose
16	3	1,5	4563	3543	22	Glucose
17	3	1	3000			Glucose
18	3	1,15	3375	2425	28	Glucose
19	3	1,15	3375			Glucose
20	3	1	3000	1560	48	Glucose
21	3	1	3000			Glucose
22	3	1,2	3600	2100	41	Glucose
23	3	1,11	3375			Glucose
24	3	1,11	3375	1950	42	Glucose
25	3	1	3000			Glucose
26	3	1	3000	1600	46	Glucose
27	3	1	3000			Glucose
28	3	1,15	3375	1450	57	Glucose
38	3	1,15	3500			Glucose
39	3	1,15	3500	2000	42	Glucose
40	3	1	3000			Glucose
41	3	1	3000			Glucose
42	3	1,15	3500	1650	53	Glucose
43	3	1,2	3626			Glucose
44	3	1	3000	1450	51	Glucose
45						Glucose
46	5	1	4500			Glucose
47	5	1	4500			Glucose
48	5	1	4500	506	89	Glucose
49	5	1	4875			Glucose
50	5	1,45	7250	500	93	Glucose
51	5	1,45	7250			Glucose

Time	HRT	OLR	COD inf.	CODeff.	COD rem.(%)	Biogas(l)	CH_4%	CH_4 yield	SS (mg/l)	VSS (mg/l)	TS	TVS	F/M	**Feeding Strategy**
52	5	1,45	7250	1250	83				77	70	54913	50380	0,03	Glucose
53	5	1,5	7500											Glucose
54	5	1,45	7250	150	98									Glucose
55	5	1,5	7500											Glucose
56	2,5	1,5	7500	63	99									Glucose
57	2,5	3	7500											Glucose
58	2,5	3,1	7750											Glucose
59	2,5	3	7500	300	96									Glucose
60	2,5	3	7500						78	75				Glucose
61	2,5	3	7500	80	98									Glucose
62	2,5	5	12500						75	72				Glucose
63	2,5	4,9	12250	113	99									Glucose
64	2,5	4,9	12500						75	72				Glucose
65	2,5	5	12500	75	99									Glucose
66	2,5	5	12500											Glucose
67	2,5	5	12500											Glucose
68	2,5	5	12500	38	99				74	63				Glucose
69	2,5	5	12500											Glucose
70	2,5	5	12500	38	99,7									Glucose
71	2,5	5	12500											Glucose
72	2,5	5	15750	38	99						54913	50380	0,13	Glucose
73	2,5	6,3	15500	290	98						53500	48637	0,13	Glucose
74	2,5	6,2	15000											Glucose
75	2,5	6	15000	238	98	26,4	0,59	0,35171			52400	46790	0,13	Glucose
76	2,5	6	15000				0,59							Glucose
77	2,5	6	15000	138	99		0,59		100	90	48981	43962	0,14	Glucose
78	2,5	6	15000				0,59							Glucose
79	2,5	6	15750				0,59							Glucose
80	2,5	6,3	15500				0,59				40200	30600	0,2	Glucose
81	2,5	6,2	15000	510	96	25,92	0,59	0,3518						Glucose
82	2,5	6	15000				0,59				26136	22800	0,26	Glucose
83	2,5	6	15000				0,59		83	68	25996	22426	0,26	Glucose
84	2,5	6	15000	630	95		0,59				24330	19090	0,31	Glucose

Time	HRT	OLR	COD inf.	CODeff.	COD rem.(%)	Biogas(l)	CH_4%	CH_4 yield	SS (mg/l)	VSS (mg/l)	TS	TVS	F/M	**Feeding Strategy**
85	2,5	6	15000				0,59							Glucose
86	2,5	6					0,59							Glucose
87	2,5	6	15750	843	94		0,59							Glucose
88	2,5	6	15750	1800	88	20,52	0,59	0,28929						Glucose
89	2,5	6					0,59							Glucose
90	2,5	6	15825	2740	82		0,59				16784	11640	0,54	Glucose
91	2,5	6					0,59							Glucose
92	2,5	6					0,59							Glucose
93	2,5	6					0,59							Glucose
94	2,5	6	15750	1200	92	23,04	0,59	0,31142			20443	13704	0,46	Glucose
95	2,5	6	15750	1200	92	23,657	0,59	0,31976						Glucose
96	2,5	6	15750	1200	92	23,49	0,59	0,31751	98	80				Glucose
97		6					0,59							Glucose
98							0,59							Glucose
99							0,59							Glucose
100							0,59							Glucose
101							0,59							Glucose
102	2,5	6	15750	1200	92	23,6	0,59	0,31899			21030	14666	0,43	Glucose
103	2,5	6	15750	1200	92	23,4	0,59	0,31629						Glucose
104	2,5	6	15750	1200	92	23,6	0,59	0,31899						Glucose
105	2,5	5,65	14125				0,52							10% pre-aerated
106	2,5	6	15000				0,52							10% pre-aerated
107	2,5	6	15000	2450	84	20,6	0,52	0,28452						10% pre-aerated
108	2,5	6	15000	2450	84	20,6	0,52	0,28452						10% pre-aerated
109	2,5	6	15000	2450	84	20,6	0,52	0,28452						10% pre-aerated
110	2,5	6	15000	2450	84	20,7	0,52	0,2859						10% pre-aerated
111	2,5	6,3	15750	2500	84	20,7	0,52	0,27079						10% pre-aerated
112	2,5	6,3	15750	2500	84	20,77	0,52	0,27171	120	85				10% pre-aerated
113	2,5	6	15000				0,6							30% pre-aerated
114	2,5	5,65	14125				0,6							30% pre-aerated
115	2,5	5,7	14250	2450	83	18,4	0,6	0,31186						30% pre-aerated
116	2,5	5,7	14250	2400	83	18,514	0,6	0,31247						30% pre-aerated
117	2,5	6,3	15750	2550	84	18,6	0,6	0,28182						30% pre-aerated

Time	HRT	OLR	COD inf.	CODeff.	COD rem.(%)	Biogas(l)	CH_4%	CH_4 yield	SS (mg/l)	VSS (mg/l)	TS	TVS	F/M	**Feeding Strategy**
118	2,5	6,3	15750	2650	83	18,72	0,6	0,2858						30% pre-aerated
119	2,5	6,3	15812	2700	83	19	0,6	0,28981	140	100				30% pre-aerated
120	2,5	7,2	18000				0,55							70% pre-aerated
121	2,5	7,2	18000			20,57	0,55							70% pre-aerated
122	2,5	7,2	18000	2650	85	21,12	0,55							70% pre-aerated
123	2,5	6,8	17180	2860	83	21,6	0,55	0,27654						70% pre-aerated
124	2,5	6,8	17180	2862	83	21,6	0,55	0,27657						70% pre-aerated
125	2,5	6,6	16560	2700	83	21,72	0,55	0,2873						70% pre-aerated
126	2,5	6,3	15750	2450	84	20,64	0,55	0,28451						70% pre-aerated
127	2,5	6	15000	2100	86	21,2	0,55	0,30129						70% pre-aerated
128	2,5	6	15000	2100	86	21,2	0,55	0,30129	169	122				70% pre-aerated
129	2,5	6,8	17000			21,12	0,46							100% pre-aerated
130	2,5	6,8	17000	2950	82	21,1	0,46	0,23027						100% pre-aerated
131	2,5	7,4	18500				0,46							100% pre-aerated
132	2,5	7,4	18500	5825	68	13,9	0,46							100% pre-aerated
133	2,5	5,8	14625	6075	59	12,8	0,46	0,22955						100% pre-aerated
134	2,5	5,7	14312	5575	61	13	0,46	0,22815						100% pre-aerated
138	2,5	6,5	16312	6475	60		0,46							100% pre-aerated
139	2,5	6,6	16662	7250	56	16,662	0,46							100% pre-aerated
140	2,5	6,5	16250	6750	58	15,36	0,46	0,24792	190	140				100% pre-aerated
141	2,5						0,46							100% pre-aerated
142	2,5						0,46							100% pre-aerated
143	2,5	6,6	16500	6650	59	18,48	0,46							100% pre-aerated
144	2,5	6,3	15813				0,46							100% pre-aerated
145	2,5	6,3	15813	5750	63	16,2	0,46	0,24684						100% pre-aerated
146	2,5	6,9	17488	5650	67	17,485	0,46	0,22648						100% pre-aerated
147	2,5						0,46							100% pre-aerated
148	2,5	5,3	13312	5500	58	12,78	0,46							100% pre-aerated
149	2,5	5,5	13875	5537	60	12,96	0,46	0,23833						100% pre-aerated
150	2,5	5,9	14625	5100	65	12,384	0,46	0,19936						100% pre-aerated
151	2,5	6	15000	5200	65	13,2	0,46	0,20653						100% pre-aerated
152	2,5	6	15000	5170	65	13,1	0,46	0,20434	180	135				100% pre-aerated
157	3,5	4,3	15000											100% pre-aerated

Time	HRT	OLR	COD inf.	CODeff.	COD rem.(%)	Biogas(l)	CH_4%	CH_4 yield	SS (mg/l)	VSS (mg/l)	TS	TVS	F/M	**Feeding Strategy**
158	3,5	4,3	15000	5200	65	13,2								100% pre-aerated
159	3,5	4,6	16000											100% pre-aerated
160	3,5	4,6	16000	4700	71	13,7	0,49	0,2776						100% pre-aerated
161	3,5	4,6	16000	4700	71	13,8	0,49	0,27963						100% pre-aerated
162	3,5	4,6	16000	4700	71	13,8	0,49	0,27963	170	123				100% pre-aerated
163	3,5	4	14000											10% raw
164	3,5	4	14000											10% raw
165	3,5	3,8	13375	4050	69									10% raw
166	3,5	4,3	15062	4500	70	10,944								10% raw
167	3,5	4,3	14940	4475	70	12,48	0,46	0,25634						10% raw
168	3,5	4,3	15200	4550	70	12,6	0,46	0,25431						10% raw
169	3,5	4,3	15200	4500	70	12,6	0,46	0,25312	205	149				10% raw
170	3,5	5	17375			10,8								30% raw
171	3,5	5	17375											30% raw
172	3,5	5	17375											30% raw
173	3,5	5	17375	7050	60	9,5	0,2	0,08599						30% raw
174	3,5	5	17500	7000	60	9,6	0,2	0,08545						30% raw
175	3,5	5	17500											30% raw
176	3,5	5	17500			9,6	0,2	0,05127	270	205				30% raw
177	3,5	5	17500	7000	60	9,6	0,01	0,00427						60% raw
178	3,5	4,7	16400											60% raw
179	3,5	4,7	16400	11500	30	0,96	0,01	0,00092						60% raw
180	3,5	4,7	16400											60% raw
181	3,5	4,7	16000	16000	0	0		0						60% raw
182	3,5	4,7	16000	17500	0	0	0	0						60% raw

APPENDIX A: Results of VFA

Feeding Strategy	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	valeric	n-caproic	Total
Glucose	1026	250	78	109	109	52	0	1624
Glucose steady-state	10	15	0	0	0	28	0	53
10% aerated+ 90% glucose	15	17	0	10	0	21	0	63
30% aerated+ 70% glucose	0	17	0	9	0	19	0	45
70%aerated+ 30% glucose	0	31	0	10	0	21	0	62
100% aerated wastewater	1252	0	14	48	126	34	0	1474
10% raw+ 90% aerated	959	33	65	0	90	29	0	1176
30% raw+ 60% aerated	1163	41	56	17	111	29	0	1417
60% raw+ 40% aerated	4187	45	66	3152	52	40	1825	9367

APPENDIX B:Results of Specific Methanogenic Activity Test

SMA test result of sludge taken from fermentation industry

Time	SMA ($mlCH_4/gTVS.d$)
0	0
2	16
4	31
7	47
10	78
11	78
12	78
13	78
14	78
15	78
16	78
17	78
18	78
19	78
20	78
21	78
22	78
23	63
27	16
28	0

SMA test result of sludge taken from an alchol distilling industry

1000 mg/l

Time	Pulse	SMA ($mlCH_4/gTVS.d$)
0	1	12
1	8	96
2	12	144
3	12	144
4	14	168
5	14	168
6	14	168
7	15	180
8	16	192
9	15	180
10	16	192
11	16	192
12	18	216
13	18	216
14	18	216
15	18	216
16	15	180
17	11	132
18	4	48
19	1	12
20	1	12
21	1	12
22		0

2000 mg/l acetate

Time	Pulse	SMA ($mlCH_4/gTVS.d$)
0	1	13
1	12	158
2	14	185
3	15	198
4	17	224
5	18	238
6	18	238
7	19	251
8	19	251
9	20	264
10	20	264
11	21	277
12	21	277
13	22	290
14	22	290
15	22	290
16	24	317
17	23	304
18	24	317
19	24	317
20	24	317
21	24	317
22	24	317
23	24	317
24	24	317
25	23	304
26	19	251
27	12	158
28	4	53
29	2	26
30	2	26
31	1	13
32	0	0
33	1	13
34	1	13
35	0	0

SMA test result of sludge taken from an alchol distilling industry

3000 mg/l

Time	Pulse	Time	SMA ($mlCH_4/gTVS.d$)
0	1	0	17
1	11	1	189
2	13	2	223
3	15	3	257
4	16	4	275
5	17	5	292
6	20	6	343
7	21	7	360
8	22	8	378
9	24	9	412
10	25	10	429
11	25	11	429
12	26	12	446
13	26	13	446
14	26	14	446
15	26	15	446
16	26	16	446
17	26	17	446
18	26	18	446
19	26	19	446
20	26	20	446
21	26	21	446
22	25	22	429
23	24	23	412
24	24	24	412
25	23	25	395
26	22	26	378
27	20	27	343
28	19	28	326
29	18	29	309
30	16	30	275
31	14	31	240
32	10	32	172
33	7	33	120
34	5	34	86
35	3	35	51
36	2	36	34
37	1	37	17
38	1	38	17

Results of SMA test when the CSTR was fed with glucose at an OLR 3 kgCOD/m^3.d

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4$/gTVS.d)
0	6	0	30
1	12	1	60
2	19	2	95
3	20	3	100
4	21	4	105
5	21	5	105
6	22	6	110
7	22	7	110
8	23	8	115
9	23	9	115
10	24	10	121
11	25	11	126
12	26	12	131
13	27	13	136
14	27	14	136
15	28	15	141
16	28	16	141
17	28	17	141
18	28	18	141
19	28	19	141
20	28	20	141
21	28	21	141
22	28	22	141
23	28	23	141
24	28	24	141
25	28	25	141
26	28	26	141
27	28	27	141
28	28	28	141
29	28	29	141
30	27	30	136
31	27	31	136
32	26	32	131
33	26	33	131
34	25	34	126
35	25	35	126
36	20	36	100
37	20	37	100
38	20	38	100
39	19	39	95
40	19	40	95
41	16	41	80
42	16	42	80
43	15	43	75
44	14	44	70
45	13	45	65
46	12	46	60
47	5	47	25
48	1	48	5

Results of SMA test when the CSTR was fed with glucose at an OLR 6 kgCOD/m^3.d (day 75)

	Pulse	Pulse	Pulse	SMA	SMA	SMA
	Acetate concentrations					
Time	2000 mg/l	3000 mg/l	4000 mg/l	2000 mg/l	3000 mg/l	4000 mg/l
1	6	6	6	244	269	247
2	7	6	6	285	269	247
3	7	6	6	285	269	247
4	7	6	6	285	269	247
5	7	6	6	285	269	247
6	7	7	6	285	313	247
7	7	7	6	285	313	247
8	7	7	6	285	313	247
9	7	7	6	285	313	247
10	8	7	6	325	313	247
11	8	7	7	325	313	288
12	8	7	7	325	313	288
13	8	7	7	325	313	288
14	8	7	7	325	313	288
15	8	7	7	325	313	288
16	8	7	7	325	313	288
17	8	8	7	325	358	288
18	9	8	7	366	358	288
19	9	8	7	366	358	288
20	9	8	7	366	358	288
21	9	8	8	366	358	329
22	9	8	8	366	358	329
23	9	8	8	366	358	329
24	9	8	8	366	358	329
25	9	8	8	366	358	329
26	9	8	8	366	358	329
27	9	8	8	366	358	329
28	9	8	8	366	358	329
29	9	8	9	366	358	371
30	9	8	9	366	358	371
31	9	8	9	366	358	371
32	9	9	9	366	403	371
33	9	9	9	366	403	371
34	9	9	9	366	403	371
35	9	9	9	366	403	371
36	9	9	9	366	403	371
37	9	9	9	366	403	371
38	8	9	9	325	403	371
39	8	9	9	325	403	371
40	8	9	10	325	403	412
41	8	9	10	325	403	412
42	8	9	10	325	403	412
43	8	9	10	325	403	412
44	8	9	10	325	403	412
45	8	9	10	325	403	412
46	7	9	10	285	403	412
47	7	9	10	285	403	412
48	7	9	10	285	403	412

Results of SMA test when the CSTR was fed with glucose at an OLR 6 kgCOD/m^3.d (day 75) (continued)

	Pulse	Pulse	Pulse	SMA	SMA	SMA
Time	2000 mg/l	3000 mg/l	4000 mg/l	2000 mg/l	3000 mg/l	4000 mg/l
50	6	9	10	244	403	412
51	6	9	10	244	403	412
52	6	9	10	244	403	412
53	5	9	10	203	403	412
54	5	9	10	203	403	412
55	5	9	10	203	403	412
56	5	9	11	203	403	453
57	4	9	11	163	403	453
58	4	9	11	163	403	453
59	4	9	11	163	403	453
60	4	9	11	163	403	453
61	3	9	11	122	403	453
62	3	9	11	122	403	453
63	3	8	11	122	358	453
64	3	8	11	122	358	453
65	3	8	11	122	358	453
66	3	8	11	122	358	453
67	2	8	12	81	358	494
68	2	7	12	81	313	494
69	2	7	12	81	313	494
70	2	7	12	81	313	494
71		7	12		313	494
72		7	12		313	494
73		6	12		269	494
74		6	12		269	494
75		6	12		269	494
76		5	12		224	494
77		5	12		224	494
78		5	12		224	494
79		5	12		224	494
80			12			494
81			12			494
82			12			494
83			12			494
84			12			494
85			4			165
86			4			165
87			4			165
88			4			165
89			3			124
90			1			41

valve factor=	1,3	1,43	1,3
TVS=	450mg/l	470 mg/l	470 mg/l

Results of SMA test when the CSTR was fed with glucose at an OLR 6 kgCOD/m^3.d (day 104)

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4$/gTVS.d)
0	8	0	108
1	12	1	161
2	15	2	202
3	15	3	202
4	16	4	215
5	16	5	215
6	17	6	229
7	17	7	229
8	17	8	229
9	17	9	229
10	19	10	256
11	19	11	256
12	19	12	256
13	19	13	256
14	19	14	256
15	19	15	256
16	20	16	269
17	20	17	269
18	20	18	269
19	20	19	269
20	20	20	269
21	21	21	282
22	22	22	296
23	22	23	296
24	22	24	296
25	22	25	296
26	23	26	309
27	23	27	309
28	24	28	323
29	25	29	336
30	25	30	336
31	25	31	336
32	25	32	336
33	25	33	336
34	25	34	336
35	25	35	336
36	25	36	336
37	25	37	336
38	25	38	336
39	24	39	323
40	22	40	296

Results of SMA test when the CSTR was fed with glucose at an OLR 6 kgCOD/m^3.d (day 104) (continued)

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4$/gTVS.d)
41	21	41	282
42	13	42	175
43	5	43	67
44	3	44	40
45	2	45	27
46	2	46	27
47	1	47	13
48	1	48	13

valve factor=1.42
$\%CH_4$=0.92
TVS=2590 mg/l

Results of SMA test when the CSTR was fed with 10% pre-aerated wastewater

Time (hour)	Pulse	Time (hour)	SMA ($mlCH_4/gTVS.d$)
0	10	0	116
1	13	1	151
2	14	2	163
3	15	3	175
4	15	4	175
5	15	5	175
6	16	6	186
7	16	7	186
8	16	8	186
9	16	9	186
10	16	10	186
11	17	11	198
12	17	12	198
13	17	13	198
14	18	14	209
15	18	15	209
16	18	16	209
17	18	17	209
18	19	18	221
19	19	19	221
20	19	20	221
21	19	21	221
22	19	22	221
23	20	23	233
24	21	24	244
25	21	25	244
26	21	26	244
27	21	27	244
28	21	28	244
29	21	29	244
30	21	30	244
31	21	31	244
32	21	32	244
33	21	33	244
34	21	34	244
35	21	35	244
36	20	36	233
37	20	37	233
38	18	38	209
39	16	39	186
40	14	40	163
41	8	41	93
42	6	42	70
43	3	43	35
44	1	44	12
45	1	45	12

valve factor=1.11
Methane percantage=0,92
TVS=2340 mg/l

Results of SMA test when the CSTR was fed with 30% pre-aerated wastewater

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4/gTVS.d$)
0	5	0	57
1	10	1	115
2	14	2	160
3	17	3	195
4	19	4	218
5	19	5	218
6	20	6	229
7	20	7	229
8	21	8	241
9	21	9	241
10	22	10	252
11	22	11	252
12	22	12	252
13	23	13	264
14	23	14	264
15	23	15	264
16	23	16	264
17	24	17	275
18	25	18	287
19	25	19	287
20	25	20	287
21	25	21	287
22	25	22	287
23	25	23	287
24	25	24	287
25	25	25	287
26	25	26	287
27	25	27	287
28	25	28	287
29	25	29	287
30	25	30	287
31	25	31	287
32	23	32	264
33	22	33	252
34	20	34	229
35	13	35	149
36	5	36	57
37	2	37	23
38	1	38	11
39	1	39	11

valve factor=1.11
Methane percantage=0,91
TVS=2350 mg/l

Results of SMA test when the CSTR was fed with 70% pre-aerated wastewater

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4/gTVS.d$)
0	7	0	102
1	7	1	102
2	10	2	145
3	12	3	174
4	14	4	204
5	14	5	204
6	16	6	233
7	16	7	233
8	16	8	233
9	17	9	247
10	17	10	247
11	18	11	262
12	18	12	262
13	18	13	262
14	18	14	262
15	19	15	276
16	19	16	276
17	19	17	276
18	19	18	276
19	19	19	276
20	19	20	276
21	20	21	291
22	20	22	291
23	20	23	291
24	20	24	291
25	21	25	305
26	21	26	305
27	21	27	305
28	21	28	305
29	21	29	305
30	21	30	305
31	21	31	305
32	21	32	305
33	21	33	305
34	21	34	305
35	21	35	305
36	21	36	305
37	20	37	291
38	20	38	291
39	20	39	291
40	19	40	276
41	19	41	276
42	18	42	262
43	16	43	233
44	15	44	218
45	8	45	116
46	2	46	29
47	1	47	15
48	0	48	0

valve factor=1.42, TVS=2318 mg/l, methane percentage=0.89

Results of SMA test when the CSTR was fed with 100% pre-aerated wastewater (HRT=2.5)

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4/gTVS.d$)
0	1	0	9
1	1	1	9
2	1	2	9
3	2	3	17
4	4	4	34
5	8	5	69
6	9	6	77
7	9	7	77
8	11	8	94
9	11	9	94
10	11	10	94
11	12	11	103
12	13	12	112
13	13	13	112
14	14	14	120
15	14	15	120
16	14	16	120
17	14	17	120
18	15	18	129
19	16	19	137
20	16	20	137
21	16	21	137
22	16	22	137
23	16	23	137
24	17	24	146
25	17	25	146
26	17	26	146
27	17	27	146
28	18	28	155
29	17	29	146
30	18	30	155
31	17	31	146
32	18	32	155
33	18	33	155
34	18	34	155
35	18	35	155
36	18	36	155
37	18	37	155
38	19	38	163
39	19	39	163
40	19	40	163
41	19	41	163
42	19	42	163
43	19	43	163
44	19	44	163
45	19	45	163
46	19	46	163
47	19	47	163
48	19	48	163

Results of SMA test when the CSTR was fed with 100% pre-aerated wastewater HRT=2.5 (continued)

Time (hour)	Pulse	Time (hour)	SMA ($mlCH_4/gTVS.d$)
49	19	49	163
50	19	50	163
51	19	51	163
52	19	52	163
53	19	53	163
54	18	54	155
55	17	55	146
56	17	56	146
57	16	57	137
58	14	58	120
59	12	59	103
60	7	60	60
61	2	61	17
62	2	62	17
63	1	63	9

valve factor=1
Methane percantage=0,80
TVS=2485 mg/l

Results of SMA test when the CSTR was fed with 100% pre-aerated wastewater (HRT=3.5)

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4/gTVS.d$)
0	4	0	48
1	4	1	48
2	4	2	48
3	9	3	107
4	11	4	131
5	12	5	143
6	13	6	154
7	13	7	154
8	14	8	166
9	14	9	166
10	14	10	166
11	14	11	166
12	14	12	166
13	14	13	166
14	14	14	166
15	14	15	166
16	14	16	166
17	14	17	166
18	14	18	166
19	14	19	166
20	14	20	166
21	14	21	166
22	14	22	166
23	14	23	166
24	14	24	166
25	14	25	166
26	14	26	166
27	14	27	166
28	14	28	166
29	14	29	166
30	14	30	166
31	14	31	166
32	14	32	166
33	14	33	166
34	14	34	166
35	13	35	154
36	13	36	154
37	13	37	154
38	13	38	154
39	12	39	143
40	12	40	143
41	12	41	143
42	12	42	143
43	11	43	131
44	11	44	131
45	11	45	131
46	11	46	131
47	10	47	119
48	10	48	119

Results of SMA test when the CSTR was fed with 100% pre-aerated wastewater (HRT=3.5) (continued)

Time(hour)	Pulse	Time(hour)	SMA ($mlCH_4/gTVS.d$)
49	9	49	107
50	8	50	95
51	7	51	83
52	7	52	83
53	5	53	59
54	4	54	48
55	0	55	0

valve factor=1.42
TVS=2550 mg/l
CH_4%=80%

Results of SMA test when the CSTR was fed with 10% raw wastewater

Time (hour)	Pulse	Time (hour)	SMA ($mlCH_4/gTVS.d$)
0	9	0	56
1	11	1	69
2	14	2	88
3	15	3	94
4	16	4	100
5	17	5	107
6	17	6	107
7	17	7	107
8	17	8	107
9	17	9	107
10	17	10	107
11	17	11	107
12	17	12	107
13	17	13	107
14	18	14	113
15	18	15	113
16	18	16	113
17	18	17	113
18	18	18	113
19	18	19	113
20	18	20	113
21	18	21	113
22	18	22	113
23	18	23	113
24	18	24	113
25	18	25	113
26	18	26	113
27	18	27	113
28	17	28	107
29	17	29	107
30	17	30	107
31	17	31	107
32	17	32	107
33	17	33	107
34	16	34	100
35	16	35	100
36	16	36	100
37	16	37	100
38	15	38	94
39	15	39	94
40	14	40	88
41	14	41	88
42	13	42	81
43	13	43	81
44	11	44	69
45	9	45	56
46	5	46	31
47	1	47	6

Methane percantage=0.78 TVS=3650 mg/l valve factor=1.1

Results of SMA test when the CSTR was fed with 30% raw wastewater

Time (hour)	SMA ($mlCH_4/gTVS.d$)
1	2
25	4
46	7
66	9
76	11
90	13
101	16
111	18
120	20
121	20
122	20
123	20
124	20
125	20
126	20
127	20
128	20
129	20
130	20
131	20
132	20
133	20
134	20
135	20
136	20
137	20
138	20
139	20
145	18
153	16
161	11
164	9
167	7
170	4
171	2
173	0

APPENDIX C=Results of Microscopic Examination

Morphology	Number	Number	log (#)
Sludge taken from an alcohol distilling industry			
total bacteria	232	1,35E+09	9,13
coccus	29	1,68E+08	8,23
small rod	18	1,05E+08	8,02
medium rod	15	87108014	7,94
long rod	6	34843206	7,54
sachina	1	5807201	6,76
total autofluorescent	69	4,01E+08	8,6
Glucose			
total	130	2,51E+09	9,4
coccus	38	7,34E+08	8,86
small rod	6	1,16E+08	8,06
medium rod	3	57915058	7,76
long rod	2	38610039	7,59
total autofluorescent	49	9,46E+08	8,98
10% aerated+ 90% glucose			
total	140	2,99E+09	9,47
coccus	15	3,21E+08	8,5
small rod	21	4,49E+08	8,65
medium rod	11	2,35E+08	8,37
long rod	1	21367521	7,33
total autofluorescent	48	1,03E+09	9,01
30% aerated+ 70% glucose			
total	171	3,64E+09	9,56
small rod	58	1,23E+09	9,09
medium rod	14	2,98E+08	8,47
long rod	3	63829787	7,8
total autofluorescent	75	1,6E+09	9,2
70% aerated+ 30% glucose			
total	160	3,45E+09	9,54
small rod	50	1,08E+09	9,03
medium rod	20	4,31E+08	8,63
long rod	3	64710958	7,81
total autofluorescent	73	1,57E+09	9,19
100% aerated			
total	159	3,63E+09	9,56
small rod	41	9,36E+08	8,97

medium rod	17	3,88E+08	8,59
long rod	2	45641260	7,66
total autofluorescent	60	1,37E+09	9,14
10% raw+ 90% aerated			
total	185	2,53E+09	9,4
small rod	62	8,49E+08	8,93
medium rod	14	1,92E+08	8,28
long rod	2	27397260	7,44
total autofluorescent	78	1,07E+09	9,03
30% raw+ 70% aerated			
total	146	2,16E+09	9,33
small rod	42	6,23E+08	8,79
medium rod	15	2,22E+08	8,35
long rod	1	14823599	7,17
total autofluorescent	58	8,6E+08	8,93
60% raw+ 40% aerated			
total	30	1,98E+09	9,29
small rod	8	5,27E+08	8,72
medium rod	1	65876153	7,81
long rod	0	0	0
total autofluorescent	9	5,93E+08	8,77

APPENDIX C: Metabolic Activity ($mlCH_4$/cell.day)

Feeding	Methanogens	TVS(mg/l)	Methanogenic Population	Methane production rate(ml/d)	Metabolic activity
Glucose	945945946	2590	2,45E+12	13924	5,68327E-09
10% aerated	1025641026	2340	2,4E+12	10800	4,5E-09
30% aerated	1595744681	2350	3,75E+12	11400	3,04E-09
70% aerated	1574633305	2318	3,65E+12	11660	3,19452E-09
100% aerated	1369237791	2191	3E+12	6762	2,254E-09
10% raw	1068493151	3650	3,9E+12	5796	1,48615E-09
30% raw	859768752	3373	2,9E+12	1920	6,62069E-10
60% raw	593000000	7590	4,50087E+12	0	0