PRODUCTION OF BIOGAS THROUGH ANAEROBIC CO-DIGESTION OF ICE CREAM PLANT WASTEWATER AND ICE CREAM PRODUCTION RESIDUE

by
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to my family

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ABSTRACT

PRODUCTION OF BIOGAS THROUGH ANAEROBIC CO-DIGESTION OF ICE CREAM PLANT WASTEWATER AND ICE CREAM PRODUCTION RESIDUE

The primary objective of this study is to determine whether it is possible to dispose of ice-cream production residue (off-spec) and ice-cream wastewater together and to recover energy by anaerobic co-digestion process. Mesophilic batch anaerobic digestion tests were performed under laboratory conditions to evaluate production of methane (CH_4) from these substrates. It was found out that it was possible to obtain a high methane yield of 0.338 L CH_4 g^{-1} $COD_{removed}$ from anaerobic digestion of wastewater alone, with almost 70 % of methane in biogas, while anaerobic digestion of off-spec alone did not seem feasible, due to particular characteristics of the off-spec. When wastewater and off-spec were mixed at a ratio of 9:1 (WW:O) by weight, a methane yield of 0.131 L CH_4 g^{-1} $COD_{removed}$ could be observed. Lower methane yields for anaerobic co-digestion of wastewater and off-spec could be attributed to high concentrations of sulfate and oil & grease coming from off-spec. Since both ice-cream wastewater and off-spec had variable pH ranges and a poor buffering capacity, adequate amount of buffer must always be present in the anaerobic system to sustain anaerobic degradation.

ÖZET

DONDURMA FABRİKASI ATIKSUYU VE DONDURMA PROSES ATIĞININ OKSİJENSİZ ORTAMDA BERABER PARÇALANMASI YOLUYLA BİYOGAZ ÜRETİMİ

Bu çalışmanın öncelikli amacı, dondurma fabrikası atıksuyu ile dondurma proses atığının oksijensiz ortamda beraber parçalanması sonucunda enerji geri kazanımının mümkün olup olmadığının belirlenmesidir. Bahsedilen substratlar kullanılarak metan (CH_4) gazı üretiminin değerlendirilmesi amacıyla mezofilik batch reaktörlerde oksijensiz parçalanma testleri laboratuvar şartlarında gerçekleştirilmiştir. Dondurma atıksuyunun tek başına anaerobik parçalanması yoluyla 0.338 L CH_4 g^{-1} $KOİ_{giderilen}$ gibi yüksek bir metan veriminin gerçekleştirilebileceği sonucuna varılmıştır Bu şekilde üretilen biyogazın metan içeriği yaklaşık %70’dir. Dondurma proses atığının tek başına oksijensiz ortamda parçalanması belirli özellikleri nedeniyle uygulanabilir bulunmamıştır. Ağırlık bazında 9:1 oranında karışımı hazırlanan dondurma atıksuyu ve dondurma proses atığının ise, 0.131 L CH_4 g^{-1} $KOİ_{giderilen}$ değerinde metan verimi verdiği gözlenmiştir. Hazırlanan karışımdan oksijensiz parçalanma sonucu elde edilen düşük metan verimi, dondurma proses atığının ihtiva ettiği yüksek sülfat ve yağ/gres konsantrasyonlarına dayandırılabilinir. Dondurma fabrikası atıksuyunun ve dondurma proses atığının değişken pH aralığına ve zayıf tamponlama kapasitesine sahip olması nedeniyle, yeterli miktarda tampon çözelti oksijensiz sistemin sağlıklı ve devamlı bir şekilde çalışabilmesi için gereklidir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Units Used
atm	Atmospheric Pressure	
CH_4	Methane	(mL & %)
CO_2	Carbon Dioxide	(%)
CO	Carbon Monoxide	
COD	Chemical Oxygen Demand	(mg L^{-1})
Cu	Copper	(mg g^{-1})
Fe	Iron	(mg g^{-1})
H	Hydrogen	
H_2S	Hydrogen Sulphide	
HRT	Hydraulic Retention Time	(hour)
K	Potassium	(mg g^{-1})
Mg	Magnesium	(mg g^{-1})
Mn	Manganese	(mg g^{-1})
N_2	Nitrogen	
Na	Sodium	(mg g^{-1})
$NaHCO_3$	Sodium Bicarbonate	
Ni	Nickel	(mg g^{-1})
NH_3	Ammonia	
NH_4^+-N	Ammonium Nitrogen	(mg L^{-1})
NRB	Nitrogen Reducing Bacteria	
O_2	Oxygen	
SRB	Sulphate Reducing Bacteria	
TS	Total Solids	(%)
VFA	Volatile Fatty Acid	(mg L^{-1})
VS	Volatile Solids	(%)
TKN	Total Kjeldahl Nitrogen	(mg L^{-1})
TP	Total Phosphorus	(mg L^{-1})

1. INTRODUCTION

The social and economic requirements for low-cost, low-technology wastewater treatment technologies have accelerated the research on more advanced levels of wastewater treatment. Biological treatment has been routinely applied to the treatment of domestic, industrial, commercial and agricultural wastes. Among the biological treatment alternatives, anaerobic processes offer several significant advantages such as including high degree of conversion of available organic carbon to gaseous end products, low production of biological solids as a result of minimal energy consumption for microbial growth, and generation of product gases rich in recoverable methane content (Demirel, 2003).

Turkey is facing rapid a population growth. As a result of this growth, domestic and industrial power demand is also increasing gradually. Turkey is meeting its energy requirement mostly from fossil fuel sources provided from foreign countries. Therefore, utilization of domestic renewable energy sources is economically and environmentally very important. Biomass is a renewable energy source that has important advantages in terms of sustainability, accessibility and not posing adverse effects on the environment. Because of these features, biomass energy should play an important role for Turkey (Topal et al., 2008).

Anaerobic treatment processes possess a number of advantages over comparable aerobic processes when used for the on-site treatment of strong industrial wastes. The cost of plant aeration, which may be considerable with some aerobic systems, is eliminated, and the methane gas produced by anaerobic digestion can be used to generate heat or electricity, thus reducing plant running costs. The optimization of anaerobic treatment processes may, therefore, be of considerable economic significance (Goodwin et al., 1989).

Algida Çorlu Plant generates industrial wastewater and ice cream production residues (off-spec) from ice cream production. The characteristics of wastewater and off-spec are very variable and depend on the production cycles. The wastewater generated from ice cream production contains carbohydrates, proteins and fats. Therefore, this type of wastewater has a high amount of organic content that can expressed in terms of its high biological oxygen demand (BOD) and chemical oxygen demand (COD) values. It is a well known fact that this type of wastewater has a great potential for anaerobic treatment processes due to its high organic content (Demirel et al., 2005).

The aim of this experimental study is to investigate the potential of biogas (methane) generation yields and disposal of the ice cream wastewater and off-spec taken from Algida Çorlu plant by anaerobic co-digestion process.

2. ANAEROBIC DIGESTION PROCESS

2.1. Fundamentals of Anaerobic Digestion Process

Anaerobic wastewater treatment is considered to be the most effective solution for organically polluted industrial waste streams (van Lier et al., 2001), and has gained interest due to increasing energy prices and more stringent legislation for the discharge of industrial wastewater since 1970's (Lettinga et al., 1995). Anaerobic degradation is a process whereby a portion of organic carbon is biologically converted to methane and carbon dioxide in an oxygen-free environment. The conversion of organic material into CH_4 and CO_2 is brought about by the cooperation of specific groups of microorganisms. Anaerobic wastewater treatment systems can be operated at different temperatures and convert a broad variety of wastes, such as food and beverage, pharmaceutical, pulp and paper, petrochemical, alcohol distilleries, dairy, textile, leachates (Macarie, 2000). Anaerobic digestion is also used for treatment of municipal wastewaters, solid wastes, agricultural wastes and manures. Anaerobic treatment processes are known for the unique ability to convert highly objectionable wastes into useful products (McCarty, 2001).

The process was initially applied to complex feedstocks such as municipal wastewater sludges which contained a variety of nutrients and alkalinity sources, food processing wastewaters (such as the effluent from meat packing plants), and sugar beet operations (Speece, 1983). Since around 1970, the process has also been applied to a wider scale for wastewater purification. Today, thousands of full scale anaerobic wastewater treatment plants are operational all over the world (van Starkenburg, 1997).

Anaerobic digestion is a biochemical technology used for the treatment of various organic wastes and the final product is biogas, which can be used as a fuel for heating or co-generation of electricity and heat (Hamed et al., 2009). Biogas consists of methane (CH_4), carbon dioxide (CO_2) and trace amounts of hydrogen (H_2), nitrogen (N_2), hydrogen sulfide (H_2S), ammonia (NH_3), and water vapor (H_2O). Methane (CH_4) is the constituent that has energy value in this mixture and the quality of the biogas is measured by the methane amount it contains (Walsh et al., 1989). The typical composition of biogas is given in Table 2.1.

Table 2.1. Typical composition of biogas (Karellas et al., 2009).

Gas	**Unit**	**Composition**
Methane (CH_4)	%	55-75
Carbon dioxide (CO_2)	%	25-45
Carbon monoxide (CO)	%	0-0.3
Nitrogen (N_2)	%	1-5
Hydrogen (H_2)	%	0-3
Hydrogen Sulphide (H_2S)	%	0.1-0.5
Oxygen (O_2)	%	Trace

2.1.1. Phases of Anaerobic Digestion

Methane fermentation is a complex process. Several models have been proposed to explain the biochemical steps in anaerobic digestion such as Three-stage Model (Gerardi, 2003), Six stage Model (Lester et al., 1986), and Nine-stage Model (Harper and Pohland, 1986).

Phases of Nine-stage Model (Harper and Pohland, 1986) have been listed as follows and given in Figure 2.1.

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,

4. Acetogenic respiration of bicarbonate by homoacetogens,
5. Oxidation of propionic and butyric acids and alcohols by sulfate reducing bacteria (SRB) and nitrate reducing bacteria (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.

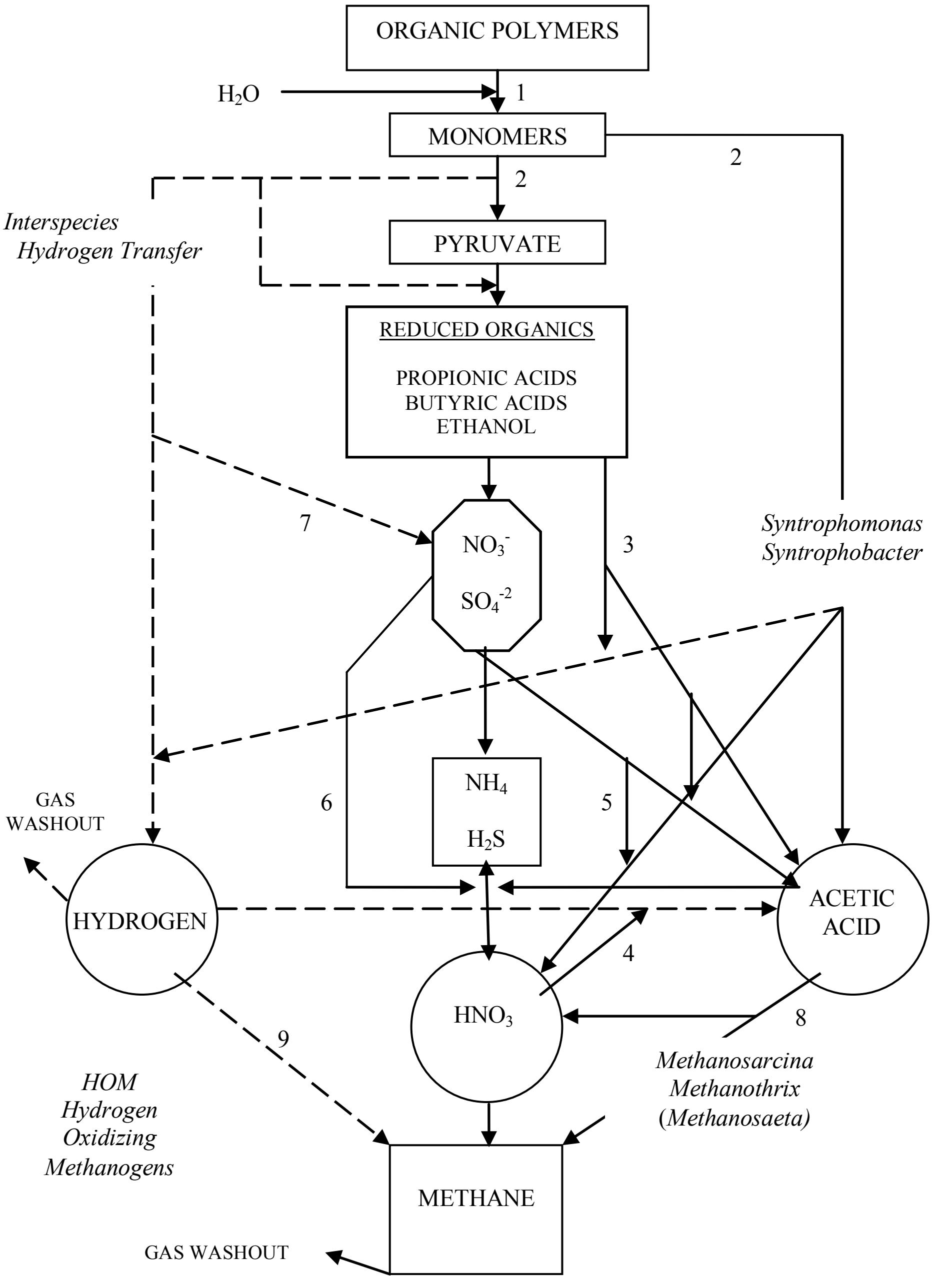

Figure 2.1. Substrate conversion patterns associated with the anaerobic digestion (Harper and Pohland, 1986).

In anaerobic digestion process there are numerous interactions between four major metabolic groups that are generally accepted as present in anaerobic digesters; hydrolytic-fermentative bacteria, proton-reducing acetogenic bacteria, hydrogenotophic methanogens, and acetolastic methanogens (Zinder et al., 1984). Therefore, the steps of anaerobic digestion process can be divided up into four phases of degradation, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The simplified process of anaerobic digestion process is shown in Figure 2.2 (Teodorita et al. 2008).

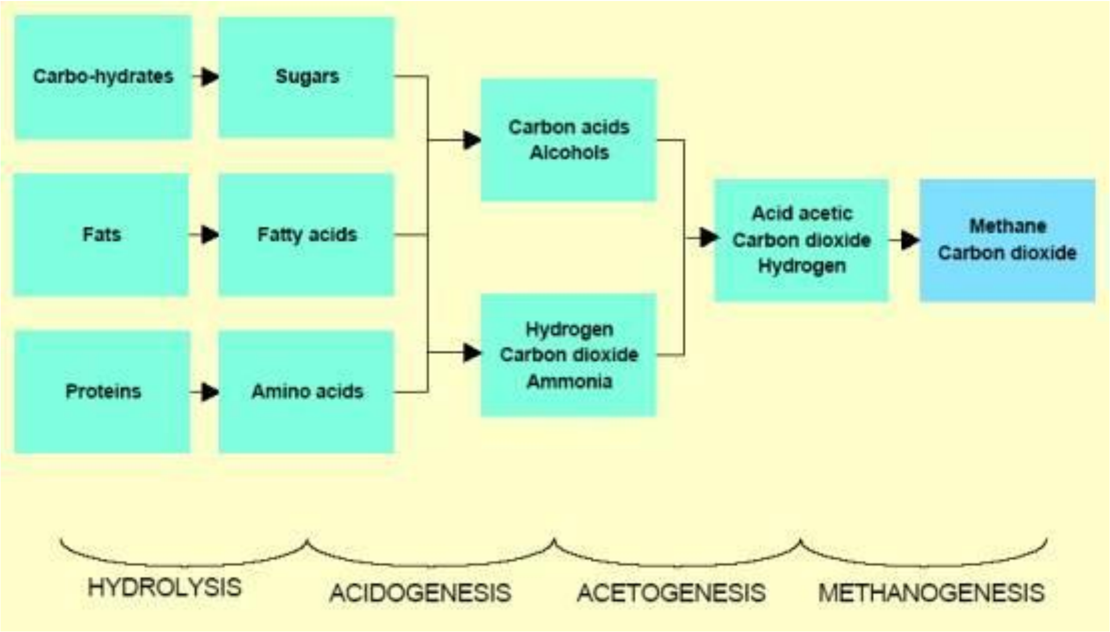

Figure 2.2. Main steps of anaerobic digestion process (Teodorita et al. 2008).

<u>2.1.1.1. Hydrolysis.</u> Hydrolysis is the first step in anaerobic degradation process. Complex wastes are required to be hydrolyzed into units as a first step to be taken up by the microbial cells. The hydrolysis of macromolecules such as lipids, proteins and carbohydrates under anaerobic conditions is carried out by specific extracellular enzymes. The activity of these extracellular enzymes during this phase is influenced mainly by the waste content of the digester and pH (Demirel, 2003).

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). Hydrolysis can be the rate limiting step in overall conversion of complex substrates to methane (Pavlostathis and Giraldo-Gomez, 1991).

2.1.1.2. Acidogenesis. The breakdown products such as amino acids, sugars and long chain fatty acids of the hydrolysis phase are converted to the intermediate products such as acetate, carbon dioxide and hydrogen by acid forming bacteria. It was reported that acetate was the most important compound produced in the fermentation of organic substrates with propionate production of secondary consequence (Sorensen et al., 1982).

Particularly temperature, pH, alkalinity and the composition of the feed are main factors that are crucial during this phase of the anaerobic digestion process. In addition, the hydrogen concentration is also significant, because under lower H_2 partial pressure (lower than 10^{-4} atm), acetic and butyric acids are produced together with H_2 and CO_2. Under higher H_2 partial pressure (higher than 10^{-4} atm), acetic, propionic and butyric acids are generated together with H_2 and CO_2 (Demirel, 2003). Common volatile acids, their formulas and COD equivalents are given in the Table 2.2.

Table 2.2. Common volatile organic acids (Demirel, 2003).

Volatile Acid	Chemical Formula	COD Equivalent
Acetic Acid	CH_3COOH	1.066
Propionic Acid	CH_3CH_2COOH	1.512
Butyric Acid	$CH_3CH_2CH_2COOH$	1.816
Valeric Acid	$CH_3CH_2CH_2CH_2COOH$	2.036
Caproic Acid	$CH_3CH_2CH_2CH_2CH_2COOH$	2.204

2.1.1.3. Acetogenesis. The short chain fatty acids other than acetate, namely propionic and butyric acids, are converted into acetate, H_2, and CO_2 in this step by the obligate hydrogen producing bacteria. The activity of acetogens is of paramount importance, since fatty acids cannot be consumed in their original form by mathanogens in the following step of anaerobic degradation. Degradation of the products of the acidogenesis in this phase is a slow and energetically unfavorable process which has always been considered as the rate limiting step of the soluble part of anaerobic digestion. Conversion of both butyric and propionic acids need energy input and only proceed at very low hydrogen partial pressure. Acetic acid formation from butyric (1) and propionic (2) acids are as follows:

$$(1)\ CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + H_2 \qquad (2.1)$$

$$(2)\ CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2 \qquad (2.2)$$

Hydrogen, which is produced during acetogenesis along with CO_2, is utilized by hydrogen consuming organisms to form methane. A mutualistic relation is therefore necessary between acetogens and methanogens to remove hydrogen from the system (Demirel, 2003).

2.1.1.4. Methanogenesis. In the final stage, methanogenesis takes place from CO_2 reduction by H_2 and from acetate. In this phase of anaerobic digestion process, H_2 and the acetic acid are converted to CH_4 gas and CO_2. Strict anaerobic microorganisms called methanogens or methane formers carry out this final step of the process. Since methanogens are not able to consume complex organics directly, the substrate must be converted to mainly acetate in the previous stages of the process. The stabilization of the waste is accomplished when CH_4 and CO_2 are produced. Methane gas is highly insoluble, and its departure from solution represents actual waste stabilization (Metcalf & Eddy, 1991).

It is estimated that approximately 70 to 75 % of the methane formed comes from acetate (acetoclastic methanogenesis); therefore acetate is the primary precursor for methane production (Gujer and Zehnder, 1983). According to McCarty and Mosey (1991), this is the most important reaction in the anaerobic digestion process. All the other VFAs need to be converted to acetate before they can be converted to methane. The bacteria, which ferment acetate to biogas grow slowly and the kinetics of their growth often dominates the overall rate of reaction (McCarty and Mosey, 1991). The remaining 20 to 25 % of the methane comes from H_2 and CO_2. Methane formation from acetic acid (1) and from carbon dioxide and hydrogen (2) are as follows:

$$(1)\ CH_3COOH \rightarrow CH_4 + CO_2 \qquad (2.3)$$

$$(2)\ CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (2.4)$$

Since both actual waste stabilization and effective COD removal take place in this final phase of the process, the performance of methanogenesis is of paramount importance in anaerobic digestion (Demirel, 2003).

2.2. Environmental and Operational Factors Effecting Anaerobic Digestion Process

With all biological processes, the constancy of the living conditions is of importance. A temperature change or changes in the substrates or the substrate concentration can lead to shutdown of the gas production. The microbial metabolism processes are dependent on many parameters, so that, for an optimum fermenting process, numerous parameters must be taken into consideration and be controlled. Furthermore, the environmental requirements of the fermentative bacteria, by which the hydrolysis and acidification of the substrates occur, differ from the requirements of the methane forming microorganisms (Deublein & Steinhauser, 2008).

Main environmental and operational factors are listed below (Demirel, 2003).

- Temperature
- pH
- Alkalinity (Buffering capacity)
- Nutrients
- Inhibition and toxicity
- Mixing

2.2.1. Temperature

The anaerobic digestion process can take place at different temperatures, divided into three temperature ranges: psychrophilic (below 25°C), mesophilic (25°C – 45°C), and thermophilic (45°C – 70°C). There is a direct relation between the process temperature and the hydraulic retention time (HRT) (Biogas Handbook, 2008).

The temperature stability is decisive for anaerobic digestion. In practice, the operation temperature is chosen with consideration to the feedstock used and the necessary process temperature is usually provided by floor or wall heating systems, inside the digester. Figure 2.3 shows the rates of relative biogas yields depending on temperature and retention time (Biogas Handbook, 2008).

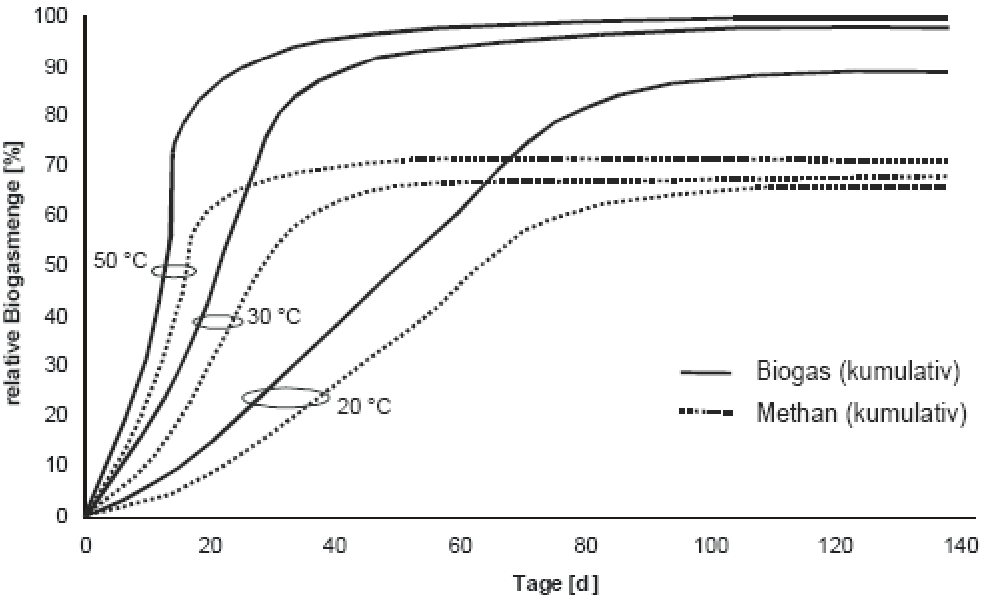

Figure 2.3. Relative biogas yields, depending on temperature and retention time (LfU, 2007).

Temperature is one of the most effective limiting factors of an anaerobic digestion system, since the microbial system is directly and easily vulnerable to temperature fluctuations. A relatively constant temperature level should be maintained during anaerobic digestion process. A rapid rise in temperature may denature the proteins and structural components of the cell. Faster growing acidogens can be adjusted to environmental changes more rapidly, than to slow growing methanogens.

In addition, the solubility of various compounds (NH_3, H_2, CH_4, H_2S and VFA) also depend on temperature. This can be of great significance for materials which have an inhibiting effect on the process (Biogas Handbook, 2008).

2.2.2. pH

Anaerobic conversion process operate best mostly at near neutral pH (Malina and Pohland, 1992). Frostell (1995) reported that the optimum pH range for anaerobic treatment varied from 6.8 to 8.0. According to Metcalf and Eddy (1991), the pH of the aqueous environment in the reactor should range from 6.6 to 7.6. Deviations from this optimum may occur due to excess production and accumulation of acidic or basic conversion products (such as organic fatty acids and ammonia) or due to the characteristics of the influent substrate. The intensity of pH also affects the solubility and reaction behavior of other substances, both organic and inorganic species (Malina and Pohland, 1992).

Low pH and excessive acid production and accumulation are definitely more inhibitory to methane formers than they are to acid formers. The optimum pH range is from 6.8 to 8.0 for methane formers, and if the pH drops below 6.2, methane production rate decreases sharply. Acidogenic bacteria have a better tolerance for low pH values and despite decreases in pH, acid production may proceed. If pH control is not implemented, the ultimate result is inhibition of methanogenesis and complete process failure (Frostell, 1985). However, it is known that methanogenesis occurs both in acidic and alkaline environments. Consequently, methanogenesis is not limited to neutral pH level.

2.2.3. Alkalinity

Alkalinity is an important parameter in anaerobic treatment process since alkalinity stands as the ability of a system to buffer the undesired effects of volatile acids that can pull the pH below desired level. In other words, high alkalinity concentrations show that the system is balanced against low tending pH ranges and work as a safety margin against sharp decreases of pH and VFA accumulation.

In case of pH balance arrangements, sodium bicarbonate ($NaHCO_3$) addition is the mainly consulted alternative in anaerobic digestion systems. The bicarbonate alkalinity in anaerobic digestion buffers the system and prevents the drop in pH due to VFA accumulation. Bicarbonate alkalinity is generally present in the system and comes mainly from the biodegradation of organics containing nitrogen. The equation given below illustrates the reaction of ammonia with carbon dioxide to form ammonium bicarbonate which acts as a natural buffering system (Tajarudin, 2006).

$$N_2 + 3H_2 \rightarrow 2NH_3 \tag{2.5}$$

$$NH_3 + H_2O + CO_2 \rightarrow NH_4HCO_3 \tag{2.6}$$

A typical anaerobic reactor should have an alkalinity of 2000 to 3000 mg L^{-1} as $CaCO_3$ (Calli, 2011). This amount is considered as a safety factor for the pH changes rendered from loading. Studies also state that in a balanced anaerobic digestion system alkalinity should not be less than 1500 mg L^{-1} as $CaCO_3$ (Gunaseelan, 1997).

2.2.4. Nutrients

The growth rate of microorganisms is greatly influenced by the composition of the wastewater. If a wastewater has a balanced composition, in terms of macro and micro nutrients, in addition to carbon source, the growth rate of the microorganisms in this wastewater will be definitely much higher than those of in an unbalanced wastewater. This factor has a substantial effect on the performance of anaerobic treatment (Frostell, 1985).

The most important nutrients are nitrogen and phosphorus, which are called as macronutrients. Domestic wastewater contains high concentrations of both, but many industrial effluents are deficient. The ratio between biodegradable COD and available nitrogen (N) and phosphorus (P) should be kept at 500/6.7/1 during the start up. For complex type wastes, particularly if they contain high concentrations of carbohydrate, the ratio should be 300/6.7/1. If nutrients must be added, the amounts used should be lowered when the system attains steady-state conditions (Frostell, 1985).

In addition to nitrogen and phosphorus, many other elements are required in trace amounts for optimum anaerobic treatment. Such elements are summarized briefly in Table 2.3 (Frostell, 1985). Approximate levels for some trace elements demand of low and high strength wastewaters are also outlined in Table 2.4 (Weiland and Rozzi, 1991).

Table 2.3. Elements required in anaerobic treatment process (Adapted from Frostell, 1985).

Element	Reference
Sodium (Na)	Sprott and Jarrel, 1981; Perski et al., 1981
Potassium (K)	Sprott and Jarrel, 1981; Perski et al., 1981
Magnesium (Mg)	Sprott and Jarrel, 1981; Perski et al., 1981
Iron (Fe)	Speece and McCarty, 1964; Callander and Barford, 1983
Sulfur (S)	Diekart et al., 1980; Gunnarson 1982
Nickel (Ni)	Schonheit et al., 1980; Murray and van der Berg, 1981
Cobalt (Co)	Schonheit et al., 1980; Murray and van der Berg, 1981
Molybdenum (Mo)	Schonheit et al., 1980; Murray and van der Berg, 1981
Selenium (Se)	Jones and Stadtman, 1977; Zehnder et al., 1980
Tungten (W)	Jones and Stadtman, 1977; Zehnder et al., 1980

Table 2.4. Trace element demand of different concentrated wastewaters (Weiland and Rozzi, 1991).

Trace Element	Concentration Range 10 g COD L^{-1}	Concentration Range 50 g COD L^{-1}
Iron (Fe)	0.5 – 20	3 – 100
Nickel (Ni)	0.05 – 3	0.3 – 15
Molybdenum (Mo)	0.01 – 0.05	0.05 – 0.2
Cobalt (Co)	0.05 – 2	0.3 – 10

If performance of an anaerobic treatment plant is poor without any obvious reason, deficiency of trace elements may be the problem and should be checked at first (Frostell, 1985).

2.2.5. Inhibition and Toxicity

Inhibitory substances are usually the main cause of anaerobic reactor failures, since they are found in substantial concentrations in wastewaters and sludges (Chen et al., 2007). A wide range of inhibitors cause the occasional failure of anaerobic digesters. A substance may be called inhibitory when it causes an adverse shift in the microbial community or inhibition of bacterial growth. A decrease of the steady-state rate of methane gas production and accumulation of organic acids usually point out the inhibition (Kroeker et al., 1979). The inhibition levels reported for most substances on anaerobic digestion vary in the literature. These variations are caused by the complexity of the anaerobic digestion process, where mechanisms such as antagonism, synergism, acclimation and complexity may affect the phenomenon of inhibition (Chen et al., 2007).

2.2.5.1. Ammonia Inhibition. The methane producing bacteria are known to be very sensitive mainly to free ammonia and volatile acids. Ammonium (NH^+_4) is produced as a result of decomposition of crops containing nitrogen. In the reactors having high concentrations of ammonium at high pH ranges, the equilibrium between ammonia and ammonium shifts to the right as it is illustrated with the chemical reaction below. As a result of this reaction free ammonia (NH_3), which has toxic effects on the growth and mechanism of the microorganisms responsible for the biogas production, is generated.

$$NH_4^{\,+} + OH^- \rightarrow NH_3 + H_2O \tag{2.7}$$

All substrates contain nitrogen. For higher pH values, even a relatively low nitrogen concentration may inhibit the process of fermentation. Noticeable inhibition occurs at a nitrogen concentration of roughly 1700 mg L^{-1} ammonium-nitrogen (NH_4-N). Nonetheless, given enough time, the methanogens are capable of adapting to NH_4-N concentrations in the range of 5000-7000 mg L^{-1}, the main prerequisite being that the ammonia level (NH_3) does not exceed 200-300 mg NH_3-N L^{-1}. The rate of ammonia dissociation in water depends on the process temperature and pH value of the substrate slurry.

Microorganisms need both nitrogen and carbon for assimilation into their cell structures. Various experiments have shown that the metabolic activity of methanogenic bacteria can be optimized at a C/N ratio of approximately 8-20, whereby the optimum point varies from case to case, depending on the nature of the substrate.

2.2.5.2. Sulfide Inhibition. Sulfate is a common constituent of many industrial wastewaters (O'Flaherty et al., 1998). In anaerobic reactors, sulfate is reduced to sulfide by the sulfate reducing bacteria (SRB) (Koster et al., 1986; Hilton and Oleszkiewicz, 1988). Introduction of the waste streams and/or the biological production in the anaerobic digestion may cause the sulfides via reduction of sulfates or other sulfure-containing inorganic compounds. Anderson et al. (1986) found that sulfate 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 sulfate reduction by SRBs. While soluble sulfide concentrations between 50 and 100 mg L^{-1} can be tolerated in anaerobic treatment with slightly or no acclimation, higher than 200 mg L^{-1} soluble sulfides does not show a significant inhibitory effect after acclimation. Stronach et al. (1986) stated that sulfate concentrations in excess of 200 mg L^{-1} had a direct toxic effect on anaerobic systems.

2.2.5.3. Volatile Fatty Acids (VFAs) Inhibition. Anaerobic reactor effluent contains low concentrations of higher fatty acids, however it contains higher concentrations of mainly acetic acid, propionic and butyric acids. Studies show that two important fermentation types occur complementary to each other; butyric and propionic acid. During butyric acid fermentation, butyrate, acetate, hydrogen and CO_2 are produced, while propionic acid type fermentation produces propionate, acetate and some valerate, with no significant gas production (Dinopolou et al., 1988). The most common inhibition in anaerobic processes is the accumulation of VFA produced by acidogenic bacteria. Inhibition is identified by high accumulation of VFA in the system, which is an indicator of failure of methanogenic population. This failure might be caused by negative impact of adverse environment conditions including shock loading, nutrient depletion or infiltration of inhibitory substances. High concentrations of VFA (like butyric and propionic acid) in a system is making toxic impact on the microorganisms in the reactor. It is reported that inhibition of microbial growth was observed at 35 mg L^{-1} acetic acid and excess of 3000 mg L^{-1} propionic acid concentrations. The same researchers indicated that butyrate has a toxic effect at 1000 mg L^{-1}. The inhibition of VFA at acidic medium can be attributed to the existence of unionized VFA in significant quantities in the system. When the pH value drops, the equilibrium goes to the left causing the increasing of unionized VFAs. Kroeker et al. (1979) reported that reactor failure can be generally expected at the concentrations above 10 mg L^{-1} of unionized acids.

2.2.5.4. Heavy Metal Inhibition. Heavy metals may cause toxic effect on anaerobic processes, which are influenced by the oxidation – reduction potential, pH and ionic strength and the resultant speciation of the metals or metal complexes. The heavy metals which have a particular concern include chromium, iron, cobalt, copper, zinc, cadmium, and nickel (Jin et al., 1998). Heavy metals are not biodegradable and can accumulate to potentially toxic concentrations (Sterritt and Lester, 1980).

Sodium, potassium, magnesium and calcium are also important light metal ions in anaerobic systems. They are required for microbial growth and affect specific growth rate like any other nutrient (Chen et al., 2007). Moderate concentrations of these ions are stimulating microbial growth in anaerobic systems. On the other hand, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity (Soto et al., 1993).

2.2.5.5. Organic Inhibitors. Many organic chemicals, that are sources of food for anaerobic microorganisms at low concentrations, can show inhibitory effects at higher concentrations. A wide range of organic compounds can inhibit anaerobic degradation. Organic chemicals which are poorly soluble in water or adsorbed to surfaces of sludge solids may accumulate to high levels in anaerobic digesters. The accumulation of apolar pollutants in bacterial membranes causes the membrane to swell and leak, disrupting ion gradients and eventually causing cell lysis (Heipieper et al., 1994; Sikkema et al., 1994). The parameters that affect the toxicity of organic compounds include toxicant concentration, biomass concentration, toxicant exposure time, cell age, feeding pattern, acclimation, and temperature (Yang and Speece, 1986). The inhibition concentration ranges vary widely for specific toxicants. Blum and Speece (1991) conducted a comparative analysis of the toxicity of a large number of organic compounds to unacclimated mixed cultures. Since the cultures were not acclimated, meaning they are not given time to adapt to inhibition, the compounds probably were not degraded following addition.

2.2.6. Mixing

Mixing ensures the absolute contact between the reactor contents and the biomass. The advantage of mixing is that it avoids settling which could lead to reduction of substrate and microorganism contact. The advantages of mixing are reported as follows (Calli, 2011):

- Mixing reduces scum build-up and prevents grit deposition,
- Mixing equalizes the temperature within the digester,
- Mixing provides uniformity throughout the digester both chemically and physically,
- Mixing minimizes toxicity as dispersing toxic materials entering the tank,
- Mixing provides rapid hydrolysis of wastes by allowing the hydrolytic bacteria to attack a much larger surface area.

Mixing also reduces the inhibitory effects of local build up of VFAs and other digestion products while preventing settling of the substrate. However, researches state that mixing does not contribute to the biogas generation efficiency at high levels (Ozdemir, 2009).

2.3. Literature Survey

Co-digestion process of different substrates has received significant attention in recent years. Therefore, several studies are carried out about co-digestion process of different co-substrates.

Borja et al. (1994) carried out a laboratory study was to verify the use of Monod kinetics for the modeling of continuous up flow anaerobic sludge blanket (UASB) reactors treating ice-cream wastewater. They investigated eight different hydraulic retention times (HRT) between 5 and 0.4 days at an average influent COD concentration of 520 mg L^{-1}. The maximum substrate utilization rate (k) and half saturation coefficient (K_L) were determined to be 1.39 kg COD removed kg^{-1} VSS day^{-1} and 0.29 kg COD kg^{-1} VSS day^{-1}, respectively. The yield coefficient (Y) and sludge decay rate coefficient (K_d) were also determined to be 0.16 kg VSS kg^{-1} COD removed and 0.028 day^{-1}, respectively. Methane production was maximum (1.61 L day^{-1}) at a 0.8 day HRT corresponding to a biomass loading rate of 0.106 kg COD kg^{-1} VSS day^{-1}. Biogas yield ranged between 0.52 L g^{-1} COD (HRT = 2.0 days) and 0.31 L g^{-1} COD (HRT = 0.4 days). In addition, the methane percentage in the biogas varied between 71.0 (HRT = 5.0 days) and 48.2 % (HRT = 0.4 days) (Borja et al., 1994).

During the study of Hawkes et al. (1994), pilot-scale anaerobic digesters were operated using ice-cream wastewater for over three years. The performances of four reactor designs, an anaerobic filter, a contact process and an UASB of capacity 5 m^3, and a 0.5 m^3 fluidized bed, were compared. The anaerobic filter, with a 3.3 m^3 Pall ring bed, operated at organic loading rates (B_y) around 6 kg COD m^{-3} d^{-1}, provided a total COD removal around 67 %. The contact process gave consistently good total COD removals of 80 %. Mixed liquor suspended solids (MLSS) did not rise above 3 kg m^{-3}, and contact process was operated at B_y of 1 kg COD m^{-3}d^{-1}. The fluidized bed reactor, operated on sand or granular activated carbon (GAC) support media at B_y of 4 and 2 kg COD m^{-3} d^{-1} respectively, gave about 60 % total COD removal, but difficulty was experienced with GAC break-up. The UASB reactor gave the poorest performance, (approximately 50 % total COD removal at a B_y of 2 kg COD m^{-3} d^{-1}) since successful granulation was not achieved. The anaerobic filter was also operated for 9 months after removing half of its packing material, but the former loading rate could not be achieved without instability. Poor biomass retention in all reactors, possibly related to the fat content of the wastewater, was the limiting factor in performance. Using experience gained at pilot scale, a full-scale up flow anaerobic filter was subsequently installed on an ice-cream factory site. This reactor showed similar performance to the pilot-scale filter (Freda et al., 1994).

Borja et al. (1995) used an anaerobic fluidized bed reactor, operating at 35°C, to treat ice-cream wastewater with an influent COD of 5.2 g L^{-1}. Start-up of the reactor was achieved within 30 days by use of a regime that included stepped increases in influent COD and methanol substitution of the substrate. A steady state was achieved with 94.4 % COD reduction at an organic loading rate of 15.6 g L^{-1} d^{-1} at HRT of 8h. This rate was somewhat faster than those achieved in previously reported studies. The reactor was subjected to transient changes in temperature, pH, influent flow-rate and COD for 6 and 12 h periods. The quality of the effluent deteriorated under these shock load conditions but returned to steady-state values within 6-16 h upon restoring normal conditions (Borja et al., 1995).

Ince et al. (1998) studied the performance of a pilot-scale upflow anaerobic filter (UFAF) treating a dairy wastewater for a period of 3 months. Results showed that the UFAF system achieved over 85 % chemical oxygen demand (COD) and 90 % biological oxygen demand (BOD_5) removal efficiencies at an organic loading rate (OLR) of 6 kg COD $m^{-3}d^{-1}$ with a hydraulic retention time (HRT) of 20 hours. Percent methane in the biogas produced in the UFAF was found to be in a range of 75 % - 85 % with the corresponding methane yield of 0.32-0.34 m^3 CH_4 kg^{-1} COD removed. The system produced approximately 770 liters CH_4 d^{-1}. This would maintain all energy requirements of the feed and recirculation pumps and mixing. Approximately 35 % of the energy requirement for maintaining the desired temperature of the pilot-scale anaerobic filter column would also be recovered (Ince et al., 1998).

Fifteen milk processing plants in the Upper Midwest of the United States participated in a study carried out by Danalewich et al. (1998) to obtain information on general process operation, waste generation and treatment practices, chemical usage, and wastewater characteristics. Long term data on wastewater characteristics were obtained for 8 of the 15 dairy plants, and a 24-h composite wastewater sample was characterized in detail for each plant. Wastewater flow rates and characteristics varied greatly among and within plants and were not easily predictable even when detailed information on processing operations was available. In addition, the contribution of milk and milk products to the waste streams was underestimated by plant operators. The use of caustic soda, phosphoric acid, and nitric acid for cleaning had a significant impact on wastewater characteristics, despite the implementation of changes in chemical usage practices during recent years. In particular, the use of phosphoric acid based cleaning products has been reduced to eliminate or decrease discharge fines. It was determined that most of the onsite treatment facilities required renovations and/or operational changes to comply with current and future discharge regulations, especially with respect to nutrient (nitrogen and phosphorus) levels in their waste streams. It was concluded that biological nutrient removal of dairy wastewaters should feasible given the relatively high concentrations of easily degradable organics, the generally favorable organic matter to total phosphorus ratio, and the very favorable organic matter to nitrogen ratio (Danalewich et al., 1998).

Hu et al. (2001) investigated the process kinetics of the mesophilic (35°C) anaerobic digestion of ice-cream wastewater. The Monod equation and the Contois equation were used to develop two basic steady-state models. The kinetic parameters required for the application of the steady-state models were determined using a laboratory-scale continuously stirred tank reactor (5L) fed with a synthetic ice-cream wastewater at a range of hydraulic retention times (2.99–7.45 days). Both models were evaluated with a set of routine analytical data obtained from a pilot-scale ($5m^3$) anaerobic contact digester treating the ice-cream wastewater at a commercial site. The steady-state model obtained with the Contois equation was more suitable than that obtained with the Monod equation for describing the process kinetics of the pilot-scale anaerobic contact process. The main reason for this is that the kinetic model based on the Contois equation considers the effect of any changes in influent substrate concentration in its prediction (Hu et al., 2001).

Demirel et al. (2005) published a paper to summarize recent research efforts and case studies in anaerobic treatment of dairy wastewaters. The main characteristics of industrial dairy waste streams were identified and the anaerobic degradation mechanisms of the primary constituents in dairy wastewaters, namely carbohydrates (mainly lactose), proteins and lipids were described. Primary attention was then focused on bench–pilot–full scale anaerobic treatment efforts for dairy waste effluents. Combined (anaerobic–aerobic) treatment methods were also discussed. Finally, areas where further research and attention are required are identified (Demirel et al., 2005).

3. MATERIALS AND METHODS

3.1. Wastewater and Off-spec Samples

For this experimental work, composite wastewater and off-spec samples were collected by ALGIDA staff and then the samples were delivered to Boğaziçi University. These samples were received on 02.02.2012, 15.02.2012 and 08.03.2012, respectively. Due to variable characteristics of both wastewater and off-spec, three different samples were requested at different dates from ALGIDA plant, in order to obtain reproducible results. The wastewater samples were only composed of wastewater generated from ice cream manufacturing process and contained no domestic wastewater.

In the experimental work, firstly, the wastewater and off-spec characteristics were determined. Then, three different sets of reactors were operated under the same experimental conditions in order to determine the biogas and methane production potential of the wastewater and off-spec. Samples were kept at + 4°C to preserve their structure during the study.

3.2. The Experimental Work

The duration of the experimental work is given in Table 3.1. The experimental set-up used during the experiments is shown in Figure 3.1.

Table 3.1. The duration of the experimental work.

	Beginning of the experiment	**End of the experiment**	**Operation time (days)**
Set I	07.02.2012	27.02.2012	17
Set II	16.02.2012	12.03.2012	25
Set III	09.03.2012	02.04.2012	24

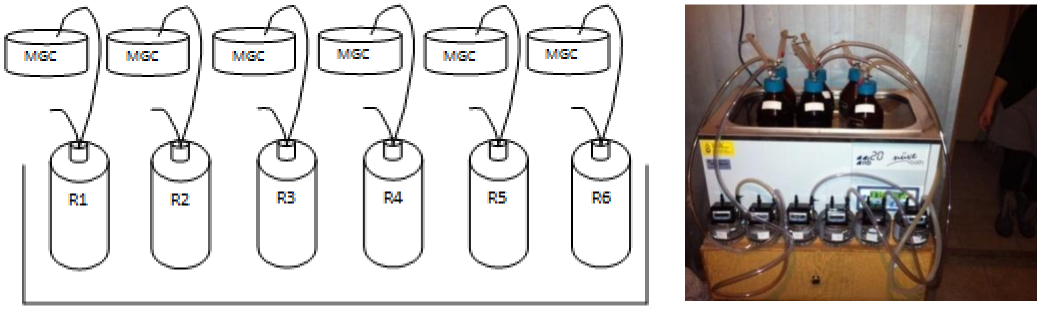

Figure 3.1. The schematic configuration of the experimental set-up.

In total, three different set of experiments were conducted in order to compare the reproducibility of the experimental findings, because the characteristics of both wastewater and off-spec samples were quite different for each sampling. Due to variable characteristics of both wastewater and off-spec, duration time of experiments differed in each set as well (Table 3.1). Various mixture ratios were studied during the experiments, in order to promote production of methane from anaerobic co-digestion of ice cream wastewater and off-spec.

Before the experiments were started, specific set-up ratios for each set were determined. The reactors were loaded with wastewater, off-spec and seed sludge using various pre-determined mixture ratios. Glass reactors of 1 L capacity were used in the experiments. In order to provide alkalinity, differing amounts buffer solutions were also added to the reactors in each set of experiments, since both wastewater and off-spec samples did not have an adequate buffering capacity to sustain anaerobic degradation. As buffer, 1 N potassium bicarbonate ($KHCO_3$) buffer solution was used. Reactors were flushed with nitrogen (N_2) gas in order to provide an anaerobic environment. The reactors were operated under mesophilic conditions (approximately at 37°C). The reactor loadings are given in Table 3.2. Control reactors for each set were also run. Duplicate reactors were operated for each set and mixture ratios.

In Set I, wastewater, off-spec and wastewater & off-spec mixture samples were operated for 17 days. It was observed that, buffer addition to the reactors stimulated the degradation and thus providing a good environment for methane production. For this reason, reactors with increased amount of buffer were loaded in order to obtain better methane production rates in Set II. In Set II, wastewater, off spec and different configuration of the mixtures by weight (WW:O=1:1; 3:1; 9:1) were loaded to the reactors and operated for 25 days. After discovering that different ratios of wastewater and off-spec demonstrated different methane yields (due to variable wastewater and off-spec properties) a third set of reactors were run to examine the dilution effect of the wastewater on the wastewater & off-spec mixture (Set III). In order to find the best ratio for production of methane, different mixture ratios were studied in Set III (WW:O=3:1; 6:1; 9:1). The reactors were operated for 24 days.

In addition, an inoculum to substrate (I:S) ratio value of 1:2 is kept constant for all reactors except control reactors.

Table 3.2. Reactor loadings (SET I, II and III).

	Ratio		**Seed Sludge (ml)**	**Wastewater (WW) (ml)**	**Off-spec (O) (ml)**	**Buffer (ml)**
	(W/W)	**(I:S)**				
SET I						
R1 & R2	-	1.96	250	500	-	24
R3 & R4	-	0.06	250	-	500	24
R5 & R6	1:1	0.11	250	250	250	24
SET II						
R1(Control)	-	-	250	-	-	-
R2 & R3	-	1.30	250	500	-	100
R4 & R5	-	0.08	250	-	500	150
R6 & R7	1:1	0.15	250	250	250	150
R8 & R9	3:1	0.27	250	375	125	150
R10 & R11	9:1	0.51	250	450	50	150
SET III						
R1(Control)	-	-	250	-	-	-
R2 & R3	-	2.83	250	500	-	100
R4 & R5	-	0.09	250	-	500	150
R6 & R7	3:1	0.35	250	375	125	150
R8 & R9	6:1	0.56	250	430	70	150
R10 & R11	9:1	0.73	250	450	50	150

3.3. Analytical Methods

All of the analytical measurements were carried out according to the procedures outline in Standard Methods for the Examination of Water and Wastewaters (APHA/AWWA/WPCF., 1998). The parameters measured for characterization of wastewater, off-spec and seed sludge are summarized in Table 3.3. In order to determine biogas and methane yields of the batch reactors, biogas was measured by Milligascounter (Ritter, Bochum, Germany) on a daily basis and then the correction was made by taking into account the standard temperature and pressure (STP). The biogas composition (methane-CH_4 and carbon dioxide-CO_2) of the reactors was measured with the Gas Chromatograph (GC- Agilent) four times a week.

Table 3.3. Parameters measured for wastewater and off-spec characterization.

Parameter	**Substrate (ice cream wastewater)**	**Substrate (off-spec)**	**Inoculum (Sludge)**	**Substrate + Inoculum (before digestion)**	**Substrate + Inoculum (after digestion)**
pH	✓	✓	✓	✓	✓
Oil & Grease	✓	✓			
Sulfate (SO_4^{2-})	✓	✓			
Chloride (Cl^-)	✓				
Total Solids (TS)	✓	✓	✓	✓	✓
Volatile Solids (VS)	✓	✓	✓	✓	✓
Total Kjeldahl Nitrogen (TKN)	✓	✓			
Total Phosphorous (TP)	✓	✓			
Chemical Oxygen Demand (COD)	✓	✓		✓	✓
Volatile Fatty Acids (VFA)					✓
Metal Analysis (Ca, Mg, K, Na, Zn, Mn, Fe, Cu, Co, Ni)	✓				

3.3.1. pH

Since it has a significant role on anaerobic digestion, the pH of the mixtures was monitored at the beginning and at the end of the experiment. pH of samples was measured by WTW 330 pH-meter with a WTW Sen Tix probe after it has calibrated with standard solutions which have pH 4, pH 7 and pH 10 values.

3.3.2. Oil and Grease

Oil and greases are made up primarily of fatty matter of animal and/or vegetable origins and also hydrocarbons of petrogenic (petroleum) origin. Thus, oils and greases are distinguished on the basis of its solubility characteristics, not its chemical composition. However, they are characterized as having similar physical characteristics, not its chemical composition (Saçan, 2010).

5 mL of HCl is added to 1L of sample to set the acidity to pH 2 or lower and it is transferred to a separatory funnel. Later, sample bottle is rinsed with 30 mL hexane and it is added to separatory funnel. The mixture is shaken vigorously for 2 minutes. After layers are separated, hexane layer is drained through a funnel containing solvent-moistened filter paper into a clean, tared distilled flask. At the same time, 1 g of Na_2SO_4 is added to filter paper to maintain a clear solvent.

This operation is applied two times more, but sample container is rinsed with each solvent portion. Extracts are combined in the tared distilling flask and filter paper is washed with additional 10 mL hexane. The hexane is distilled from the extraction in a water bath at 80-85°C. Finally, it is cooled in a desiccator for 30 minutes and weighed.

3.3.3. Sulfate

Sulfate determinations are made by using HACH DR / 2010 Spectrophotometer SulfaVer 4 Sulfate Reagent is used for the method.

3.3.4. Chloride

When the samples are preparing, a 100-mL sample or a suitable portion diluted to 100 mL. If the sample is highly colored, 3 mL $Al(OH)_3$ suspension should be added, mixed, let settle, and filtered. If sulfide, sulfite, or thiosulfate is present, 1 mL H_2O_2 should be added and stirred for 1 min.

Samples are directly titrated in the pH range 7 to 10. Sample pH was adjusted to 7 to 10 with H_2SO_4 or NaOH if it was not in this range. For adjustment, a pH meter was used with a nonchloride-type reference electrode (If only a chloride-type electrode is available, determine amount of acid or alkali needed for adjustment and discard this sample portion). Treat a separate portion with required acid or alkali and continue analysis.) 1.0 mL K_2CrO_4 was added as an indicator solution. Samples were titrated with standard $AgNO_3$ titrant until a pinkish yellow end point was observed.

3.3.5. Total Solids

To determine total solids, firstly, evaporating dish is heated at 550±50°C for one hour in a muffle furnace. Then, it is cooled, desiccated and weighed, respectively. Next, 50 mL sample is transferred into evaporating dish to evaporate the sample on a steam bath. After all of the sample evaporated, dish is dried at least one hour at 103 – 105°C. Finally, it is cooled in desiccator and weighed until a constant weight is obtained.

3.3.6. Volatile Solids

Residues produced from “Total Solids” determination are ignited in a muffle furnace at 550±50°C for 20 minutes. Then, it is cooled and desiccated. Finally, it is weighed until a constant weigh is obtained.

3.3.7. Total Kjeldahl Nitrogen

Total Kjeldahl Nitrogen (TKN) is the sum of the organic nitrogen and ammonia nitrogen and this parameter was determined by using Nessler Method subsequent to digestion of the sample. This analysis was performed by the following procedure in HACH/DR 2010 Spectrophotometer Handbook. Sample was digested with concentrated sulphuric acid at 440 °C in Digesdahl Digestion Apparatus and Hydrogen Peroxide was added. One drop of TKN indicator and 8N KOH solution were added to the sample until the first permanent blue color was observed. The volume of the sample was completed to 25 mL and then mineral stabilizer, polyvinyl alcohol dispersing agent were added. Same procedure was followed by using deionized water as the blank. The TKN of the sample was read as mg L^{-1} at 460 nm by using HACH DR / 2010 Spectrophotometer.

3.3.8. Total Phosphorus

Phosphorus is an essential element to the growth of algae and other biological organisms. Because of noxious algal blooms that occur in surface waters, there is presently much interest in controlling the amount of phosphorus compounds that enter surface waters in domestic and industrial waste discharges and natural runoff. Municipal wastewaters, for example, may contain from 4 to 16 mg L^{-1} of phosphorus as P (Metcalf & Eddy, 2004).

Total Phosphorus content of the sample was determined by using Phosver 3 (Ascorbic Acid) Method after the samples which were digested in Digesdahl Digestion Apparatus. Dry sample was digested with concentrated sulfuric acid at 440°C in Digesdahl Digestion Apparatus and Hydrogen Peroxide solution was added. The contents of one Phosver 3 phosphate powder pillow was poured into 25 mL of digested sample and total phosphorus content of the sample was measured using HACH DR/2010 Spectrophotometer at 880 nm. One Phosver 3 phosphate powder pillow were poured into 25 mL of digested sample and allowed 2 minutes to develop color.

3.3.9. Chemical Oxygen Demand

The chemical oxygen demand (COD) test is used to measure the oxygen equivalent of the organic material in wastewater that can be oxidized chemically using dichromate in an acid solution, as illustrated in the following equation, when the organic nitrogen is in the reduced state (oxidation number = -3) (Metcalf & Eddy, 2004).

$$C_nH_aO_bN_c + dCr_2O_7^{2-} + (8d+c)\,H^+ \rightarrow nCO_2 + (a+8d-3c/2)\,H_2O + cNH_4^+ + 2d\,Cr^{3+} \tag{3.1}$$

Where;

$$d = (2n/3) + (a/6) + (b/3) - (c/2) \tag{3.2}$$

COD results are reported in terms of milligrams of oxygen. Since the equivalent weight of oxygen is 8, it would seem logical to use an N/8 or 0.125N solution of oxidizing agent in the determination. Experience with the test has shown that it has sufficient sensitivity to allow the use of a stronger solution of dichromate, and an N/4 or 0.25 N solutions are recommended. This allows the use of larger samples by doubling the range of COD that can be measured in the test procedure, since each milliliter of a 0.25 N solution of dichromate is equivalent to 2 mg of oxygen (Sawyer C. N.).

Firstly, 2.5 mL samples were placed into HACH vials. Afterwards, 1.5 mL potassium dichromate and 3.5 mL of acid digestion mixture were added into the vials respectively. The vials were placed into HACH COD digester and digested for two hours at 150°C. Finally, the digested samples were measured colorimetrically at 600 nm by using HACH DR / 2010 Spectrophotometer. Potassium hydrogen phthalate (KHP) solutions were used for preparing calibration curves.

3.4. Calculation of Biogas Production Potential

According to the cumulative methane generation, methane yields of each reactor were calculated by taking into account seed sludge correction. Specific methane yield calculations were executed to find the methane potential per g COD removed, g COD added, g VS removed and g VS added. The calculations made with sludge corrections are shown below;

$$VS_{removed} = VS_{in} - VS_{out} \tag{3.3}$$

$$COD_{removed} = COD_{in} - COD_{out} \tag{3.4}$$

$$\text{Specific methane yield} = \text{Total amount of methane produced}/VS_{removed} \tag{3.5}$$

$$\text{Specific methane yield} = \text{Total amount of methane produced}/COD_{removed} \tag{3.6}$$

Although a control reactor was not operated during Set I, the control reactors were operated in the Set II and Set III. Since the same inoculum used for Set I, II and III, which has the same performance throughout the Set II and III, values of Set II and III were used for the sludge correction of Set I.

4. RESULTS AND DISCUSSION

4.1. Results

4.1.1. The Characterization of Wastewater and Off-spec Samples

The characterization of wastewater (WW) and off-spec (O) samples, which were received on three different dates, are given in Table 4.1. It can be seen that the characteristics of both wastewater and off-spec are quite variable for each set of samples.

Table 4.1. Characterization of wastewater (WW) and off-spec (O) samples.

Parameters	**Unit**	**Set 1 (02.02.2012)**		**Set 2 (15.02.2012)**		**Set 3 (08.03.2012)**	
		WW	**O**	**WW**	**O**	**WW**	**O**
pH		4.22	3.97	5.31	3.47	9.79	4.28
Total Solids (TS)	%	0.76	25.07	1.21	19.19	0.60	15.81
Volatile Solids (VS)	%	93.98	94.01	89.08	90.61	82.68	92.59
Chemical Oxygen Demand (COD)	mg L^{-1}	7312	255182	10418	303097	8636	249960
Total Kjeldahl Nitrogen (TKN)	mg kg^{-1}		26627		144231		114504
	mg L^{-1}	165		145		150	
Total Phosphorus (TP)	mg kg^{-1}		5983		9119		10120
	mg L^{-1}	32		38		43	
Sulfate (SO_4^{-2})	mg kg^{-1}		31910		10422		101201
	mg L^{-1}	200		350		200	
Chloride (Cl^{-})	mg L^{-1}	-	-	550	-	400	-
Oil & Grease	mg L^{-1}	2815	47365	2424	7028	2427	15100

Since pH values of both wastewater and off-spec samples were very low, except wastewater sample of Set III, they have low buffering capacity. Only the pH of wastewater in Set III was relatively high. It seemed that the pH of the ice cream wastewater fluctuated depending on the process cycle used. When the TS results were compared, the off-spec samples had high TS values. Because of this reason, off-spec samples were diluted via preparing mixtures with wastewater samples during the experiments. Both wastewater and off-spec contained high concentrations of sulfate (SO_4^{-2}) and oil & grease. The samples contained sufficient amounts of both nitrogen and phosphorus as macro-nutrients, which were vital for microorganisms during anaerobic digestion process. Wastewater samples of Set II and Set III contained chloride ($Cl^{\overline{\ }}$), which might have resulted probably from the cleaning activities throughout the ice cream production process.

4.1.2. Initial and Final Analysis for Batch Reactors

During the initial and final analysis of all reactors, chemical oxygen demand (COD) removal parameter was taken into account to evaluate wastewater reactors' performance and volatile solids (VS) destruction parameter was taken into account to evaluate the performance of off-spec and off-spec & wastewater mixture reactors.

4.1.2.1. Analysis for Set I. The results of initial and final analysis for Set I are given in the Table 4.2 and the results of methane yield calculations are given in Table 4.3.

Table 4.2. The results of initial and final analysis for Set I.

		R1 (WW)		**R2 (WW)**		**R3 (O)**	
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		7.56	7.93	7.56	7.90	6.82	3.76
TS	%	1.19	0.90	1.19	0.97	17.66	10.34
VS	%	73.13	34.70	73.13	33.80	92.83	67.61
COD	mg L^{-1}	4618	2879	4618	2390	-	-
		R4 (O)		**R5 (1:1)**		**R6 (1:1)**	
		Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		6.82	3.67	6.86	3.97	6.86	3.92
TS	%	17.66	10.90	9.75	8.60	9.75	8.77
VS	%	92.83	67.34	88.61	79.91	88.61	79.90
COD	mg L^{-1}	-	-	-	-	-	-

It can be inferred according to Table 4.2 that the pH values of wastewater reactors were nearly kept constant during the anaerobic digestion process, but the pH values in off-spec and mixture reactors decreased significantly. The decrease of pH in off-spec and off-spec & wastewater mixture indicated that the anaerobic digestion process could not have been carried out properly, resulting in acidification. The removal of COD in reactors containing wastewater resulted in formation of methane in Set I.

Table 4.3. The results of the methane yield calculations for Set I.

Reactor	**VS Removed ($g\ L^{-1}$)**	**COD Removed ($g\ L^{-1}$)**	**COD Removal (%)**
R1 (WW)	4.757	1.739	37.650
R2 (WW)	4.777	2.228	48.252
R3 (O)	42.702	-	-
R4 (O)	43.857	-	-
R5 (1:1)	15.243	-	-
R6 (1:1)	15.383	-	-
	Cumulative Methane (L)	**L Methane g^{-1} VS Removed**	**L Methane g^{-1} COD Removed**
R1 (WW)	0.548	0.115	0.315
R2 (WW)	0.433	0.091	0.194
R3 (O)	0.000	0.000	0.000
R4 (O)	0.000	0.000	0.000
R5 (1:1)	0.000	0.000	0.000
R6 (1:1)	0.000	0.000	0.000

As it can be seen in Table 4.3 that the methane generation was not observed in off-spec (R3 and R4) and off-spec & wastewater mixture reactors (R5 and R6). Because of this reason, methane yield calculations of these reactors were not displayed in the table.

4.1.2.2. Analysis for Set II. The results of initial and final analysis for Set II are given in the Table 4.4 and the results of methane yield calculations are given in Table 4.5.

According to the results given in Table 4.4, the pH values of control reactor, wastewater reactors and 9:1 (WW:O) ratio mixture reactors did not change significantly in terms of initial and final values. However, pH values of off-spec reactors, 1:1 (WW:O) mixture reactors and 3:1 (WW:O) mixture reactors decreased indicating acidification.

Table 4.4. The results of initial and final analysis for Set II.

		R1 (S)		**R2 (WW)**		**R3 (WW)**	
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		7.1	7.83	7.5	7.62	7.5	7.63
TS	%	3.5	2.47	2.18	1.70	2.18	1.67
VS	%	70	63.47	80.2	31.57	80.2	30.84
COD	mg L^{-1}	13142	9956	17095	11658	17095	6958
		R4 (O)		**R5 (O)**		**R6 (1:1)**	
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		6.99	3.76	6.99	3.73	7.27	4.32
TS	%	11.03	8.04	11.03	10.92	6.55	5.73
VS	%	76.57	66.14	76.57	71.62	88.26	64.18
COD	mg L^{-1}	-	-	-	-	-	-
		R7 (1:1)		**R8 (3:1)**		**R9 (3:1)**	
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		7.27	4.13	7.5	6.61	7.5	6.23
TS	%	6.55	5.05	4.59	3.52	4.59	3.15
VS	%	88.26	65.16	77.19	47.19	77.19	52.59
COD	mg L^{-1}	-	-	-	-	-	-
		R10 (9:1)		**R11 (9:1)**			
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**		
pH		7.59	7.06	7.59	7.03		
TS	%	2.8	2.66	2.8	2.31		
VS	%	82.22	42.52	82.22	40.29		
COD	mg L^{-1}	-	-	-	-		

According to Table 4.5, COD removal was observed in wastewater reactors and VS removal was observed in off-spec and off-spec & wastewater mixture reactors. Methane generation occurred in all reactors. When the results of mixture recators are compared, it can be observed that the removals of VS and the methane yields increased parallelly to the increase of wastewater ratio in mixture.

Table 4.5. The results of methane yield calculations for Set II.

Reactor	**VS Removed (g L^{-1})**	**COD Removed (g L^{-1})**	**COD Removal (%)**
R1 (S)	2.762	3.190	24.24
R2 (WW)	11.088	2.250	31.800
R3 (WW)	7.558	6.950	59.300
R4 (O)	24.848	-	-
R5 (O)	21.518	-	-
R6 (1:1)	13.308	-	-
R7 (1:1)	21.668	-	-
R8 (3:1)	18.268	-	-
R9 (3:1)	13.208	-	-
R10 (9:1)	9.358	-	-
R11 (9:1)	6.838	-	-
	Cumulative Methane (L)	**L Methane g^{-1} VS Removed**	**L Methane g^{-1} COD Removed**
R1 (S)	0.010	0.004	0.003
R2 (WW)	0.285	0.026	0.129
R3 (WW)	1.110	0.147	0.160
R4 (O)	0.009	0.000	-
R5 (O)	0.011	0.001	-
R6 (1:1)	0.021	0.002	-
R7 (1:1)	0.028	0.001	-
R8 (3:1)	0.116	0.006	-
R9 (3:1)	0.018	0.001	-
R10 (9:1)	0.316	0.034	-
R11 (9:1)	0.336	0.049	-

4.1.2.3. Analysis for Set III. The results of initial and final analysis for Set III are given in the Table 4.6. and the results of methane yield calculations are given in Table 4.7.

According to the results given in Table 4.6, pH values of control reactor, wastewater reactors and 9:1 (WW:O) ratio mixture reactors did not change significantly in terms of initial and final values. However, pH values of off-spec reactors, 3:1 (WW:O) mixture reactors and 6:1 (WW:O) mixture reactors decreased according to the initial values that indicated acidification. Initial TS values showed that wet digestion conditions were valid for the reactors. When the initial and final COD and VS results were compared it could be observed that the removals of these parameters were maintained.

Table 4.6. The results of initial and final analysis for Set III.

		R1 (S)		**R2 (WW)**		**R3(WW)**	
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		7.04	7.51	7.97	7.61	7.97	7.74
TS	%	3.02	2.60	2.37	1.81	2.37	1.76
VS	%	66.91	62.36	38.28	24.06	38.28	32.39
COD	mg L^{-1}	16042	12819	10501	4939	10501	5924
		R4 (O)		**R5 (O)**		**R6 (3:1)**	
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		6.94	3.91	6.94	3.81	7.53	5.97
TS	%	9.70	8.29	9.70	8.20	4.24	3.45
VS	%	62.64	58.42	62.64	61.97	55.12	46.50
COD	mg L^{-1}	-	-	-	-	-	-
		R7 (3:1)		**R8 (6:1)**		**R9 (6:1)**	
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
pH		7.53	6.10	7.76	6.38	7.76	6.86
TS	%	4.24	3.57	3.49	2.94	3.49	3.14
VS	%	55.12	52.68	38.01	27.74	38.01	27.46
COD	mg L^{-1}	-	-	-	-	-	-
		R10 (9:1)		**R11 (9:1)**			
Parameter	**Unit**	**Initial**	**Final**	**Initial**	**Final**		
pH		7.80	7.81	7.80	7.84		
TS	%	3.19	2.24	3.19	2.40		
VS	%	46.20	27.57	46.20	29.37		
COD	mg L^{-1}	-	-	-	-		

Table 4.7. The results of methane yield calculations for Set III.

Reactor	**VS Removed ($g\ L^{-1}$)**	**COD Removed ($g\ L^{-1}$)**	**COD Removal (%)**
R1 (S)	3.12	3.223	20
R2 (W)	3.57	2.339	53
R3 (W)	2.22	1.357	44
R4 (O)	15.92	-	-
R5 (O)	9.65	-	-
R6 (3:1)	4.44	-	-
R7 (3:1)	1.38	-	-
R8 (6:1)	4.09	-	-
R9 (6:1)	3.25	-	-
R10 (9:1)	5.74	-	-
R11 (9:1)	4.92	-	-
	Cumulative Methane (L)	**L Methane g^{-1} VS Removed**	**L Methane g^{-1} COD Removed**
R1 (S)	0.07	0.022	0.022
R2 (W)	0.79	0.222	0.338
R3 (W)	0.45	0.202	0.332
R4 (O)	0.00	0.00	-
R5 (O)	0.76	0.079	-
R6 (3:1)	0.12	0.027	-
R7 (3:1)	0.17	0.121	-
R8 (6:1)	0.71	0.175	-
R9 (6:1)	0.56	0.172	-
R10 (9:1)	2.118	0.369	-
R11 (9:1)	1.558	0.317	-

According to Table 4.7, VS and COD removals were observed in all reactors. Best removal efficiency and the best methane yield values were observed in reactors that contained only wastewater and 9:1 (WW:O) ratio mixture in terms of L Methane g^{-1} VS Removed. Off-spec reactors had the lowest VS removal values. When the results of mixture recators were compared, it could be inferred that the removals of VS and the methane yields were increased proportionally to the increase of wastewater ratio in mixture.

4.1.3. Batch Test Results for Biogas Generation

The cumulative biogas and cumulative methane (CH_4) production and the composition of biogas (v/v) for each set of experiments are reported in this section. In each set of experiments, the tests were conducted in duplicate. The best result from each duplicate was selected and reported here. The results of all experiments for each set are also given in the Appendix.

4.1.3.1. The Results of Set I. The cumulative biogas and cumulative methane (CH_4) production and the composition of biogas (v/v) for Set I are shown in Figures 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6, respectively.

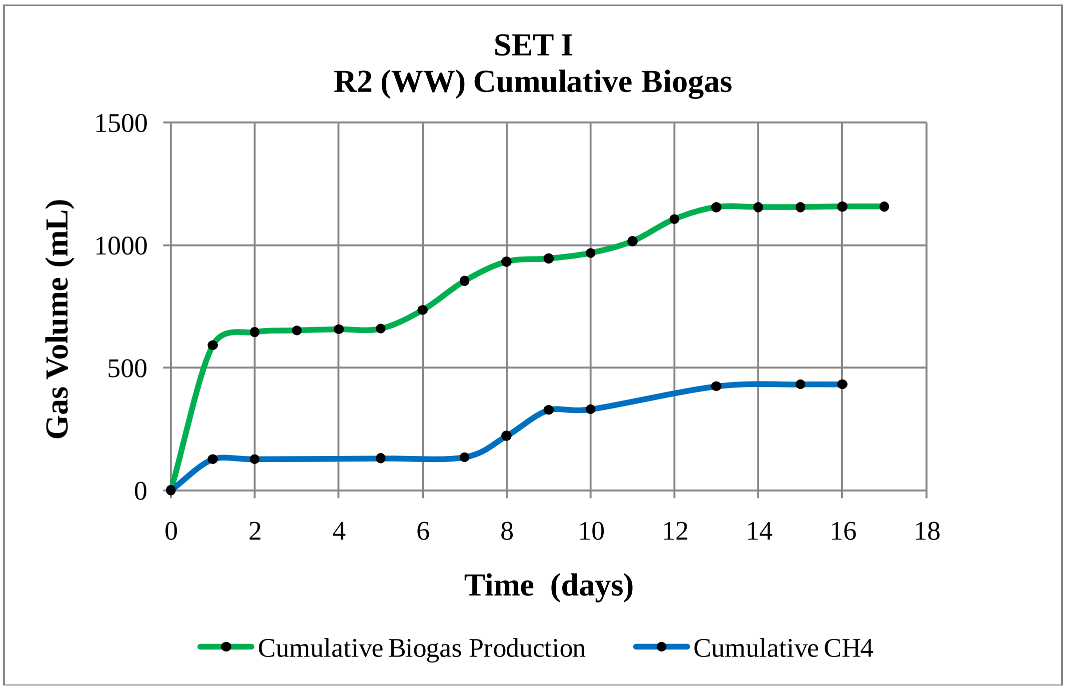

Figure 4.1. The cumulative biogas and methane for R2 containing wastewater (WW) in Set I.

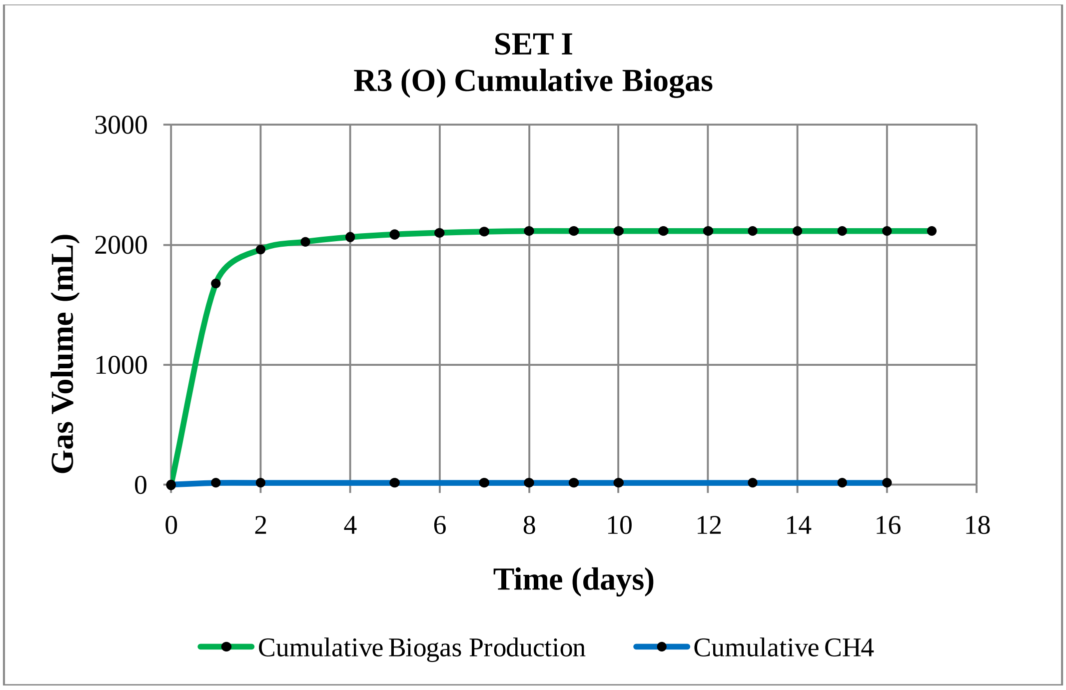

Figure 4.2. The cumulative biogas and methane for R3 containing off-spec (O) in Set I.

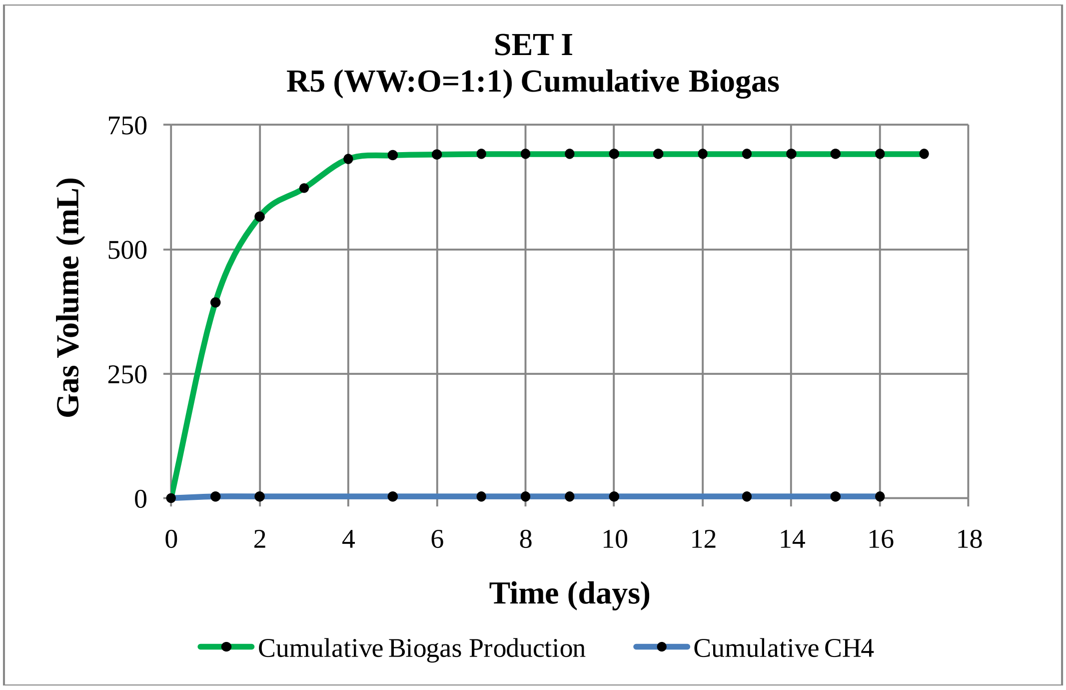

Figure 4.3. The cumulative biogas and methane for R5; containing a mixture of wastewater and off-spec in Set I.

In Set I, cumulative methane production was measured only from anaerobic digestion of wastewater alone (Figure 4.1.). Even though there was biogas production observed both from anaerobic digestion of off-spec (Figure 4.2) and mixture of wastewater and off-spec (Figure 4.3), there was no methane measured in the biogas. Biogas measurements are given in the Appendix.

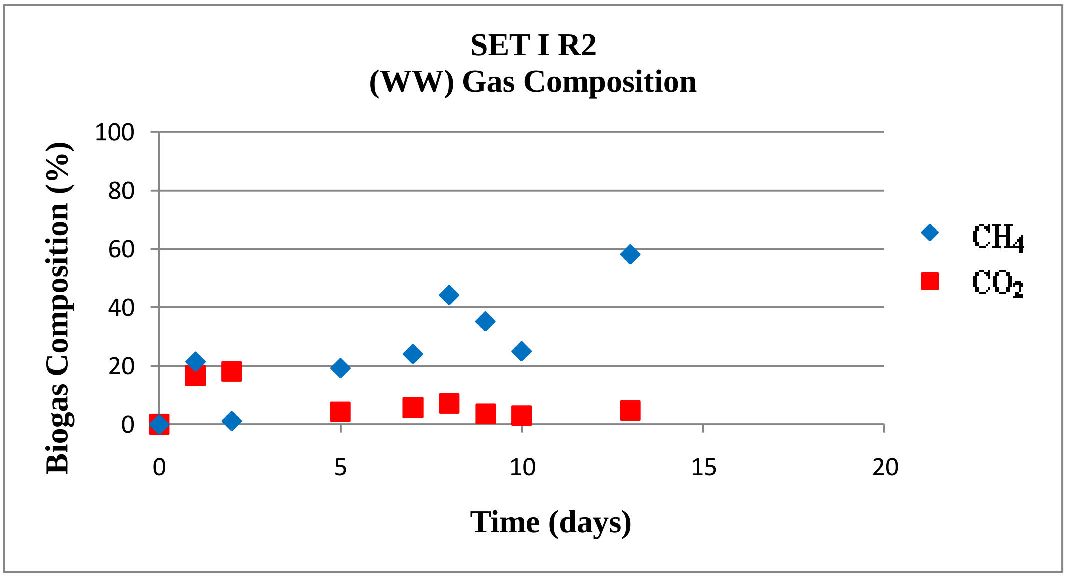

Figure 4.4. Biogas composition (CH_4/CO_2) for reactor R2 containing wastewater in Set I.

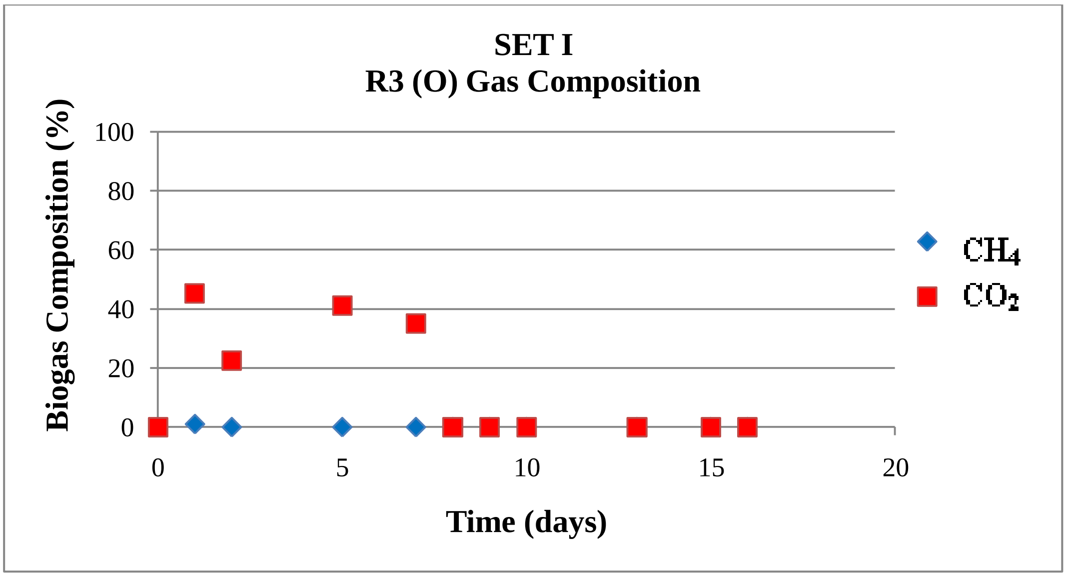

Figure 4.5. Biogas composition (CH_4/CO_2) for reactor R3 containing off-spec in Set I.

Regarding to the composition of biogas in Set I, the percentage of methane in biogas produced from anaerobic digestion of wastewater alone ranged between 20 and 60 % (Figure 4.4). However, no methane was measured in biogas generated from anaerobic digestion of off-spec (Figure 4.5) and mixture of wastewater and off-spec (Figure 4.6), indicating that the anaerobic digestion could not be accomplished in these trials. Since the biogas production in mixture reactors stopped, GC analysis could not been carried out after the 7th day.

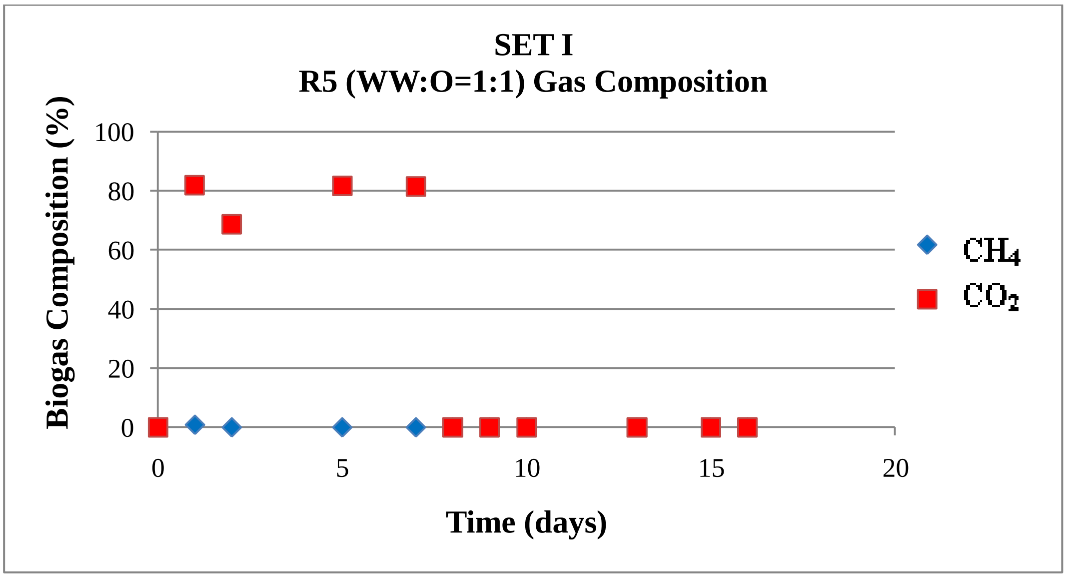

Figure 4.6. Biogas composition (CH_4/CO_2) for reactor R5 containing a mixture of wastewater and off-spec in Set I.

<u>4.1.3.2. The Results of Set II.</u> The cumulative biogas and cumulative methane (CH_4) production and the composition of biogas (v/v) for Set II are shown in Figures 4.7, 4.8, 4.9, 4.10, 4.11, 4.12, 4.13, 4.14, 4.15 and 4.16, respectively.

In Set II, methane production was observed from anaerobic digestion of wastewater alone (Figure 4.7). No methane production was observed from anaerobic digestion of off-spec alone (Figure 4.8), and from the mixture of wastewater and off-spec at a ratio of 1:1 (Figure 4.9). A low methane generation could be detected from the mixture of wastewater and off-spec at a ratio of 3:1 (Figure 4.10).

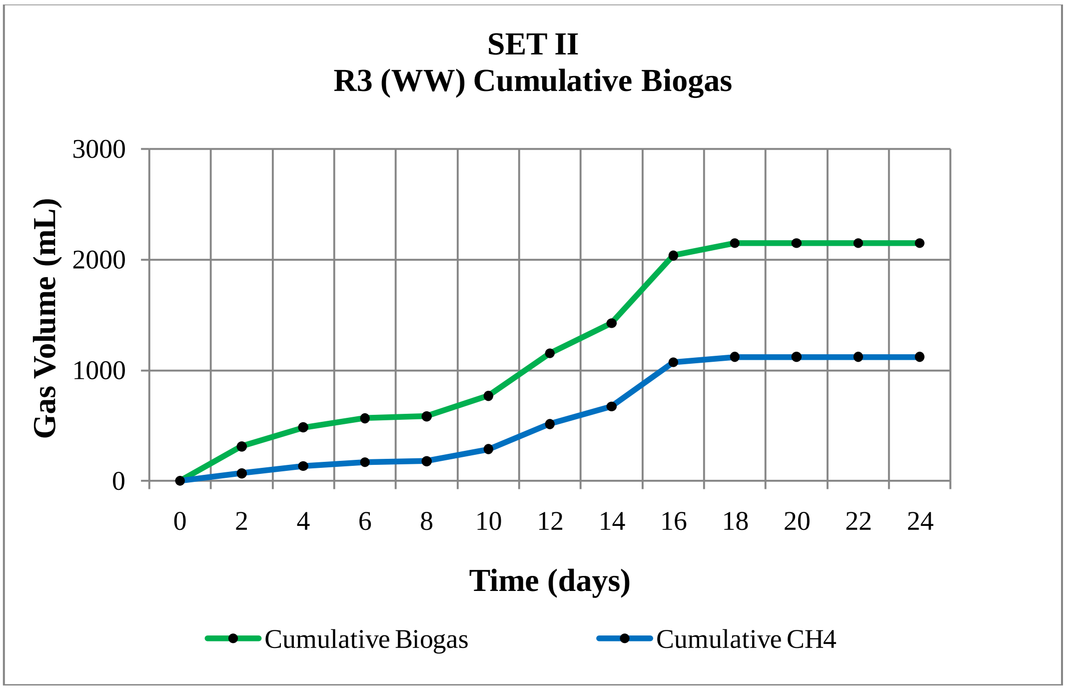

Figure 4.7. The cumulative biogas and methane for reactor R3 containing only wastewater in Set II.

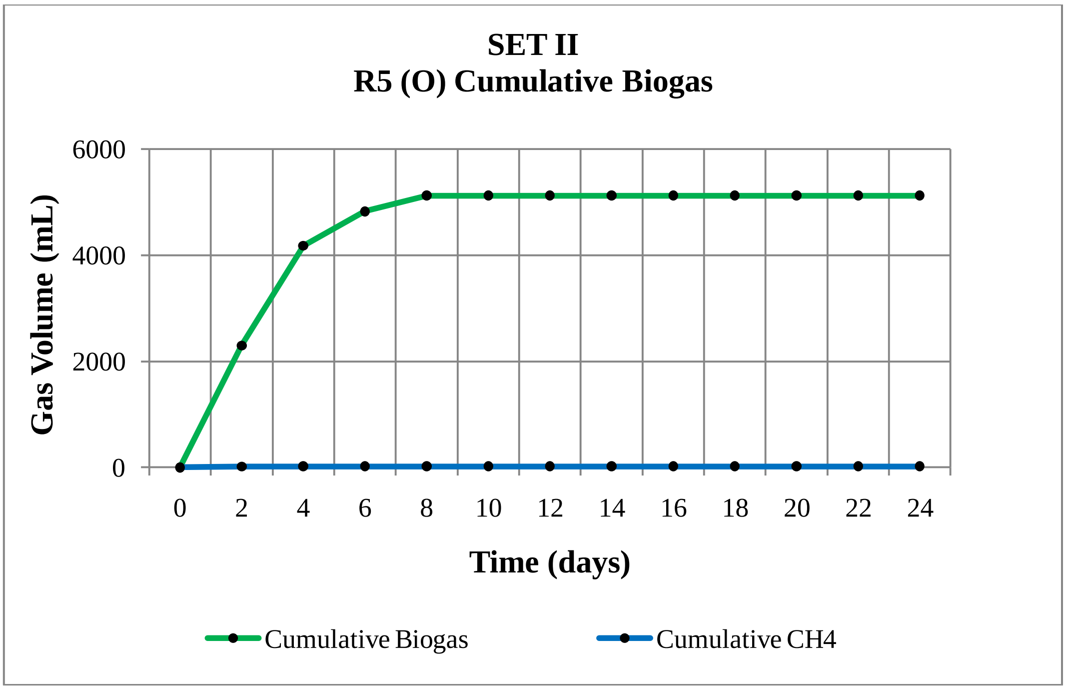

Figure 4.8. The cumulative biogas and methane for reactor R5 containing off-spec in Set II.

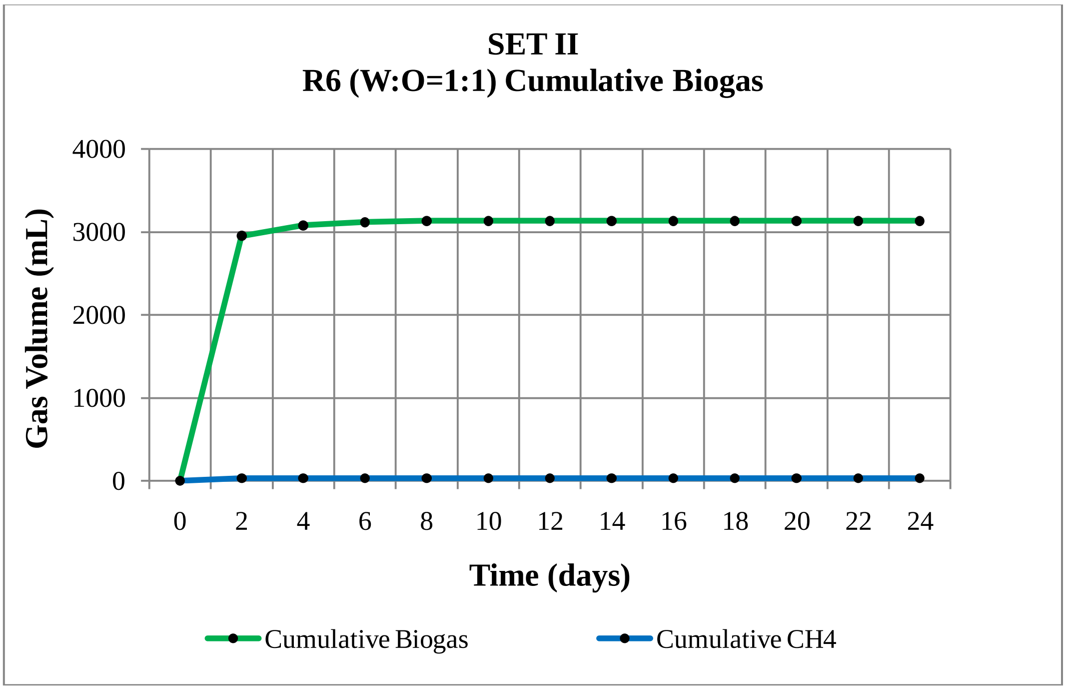

Figure 4.9. The cumulative biogas and methane for reactor R6 containing a mixture of wastewater and off-spec (WW:O=1:1) in Set II.

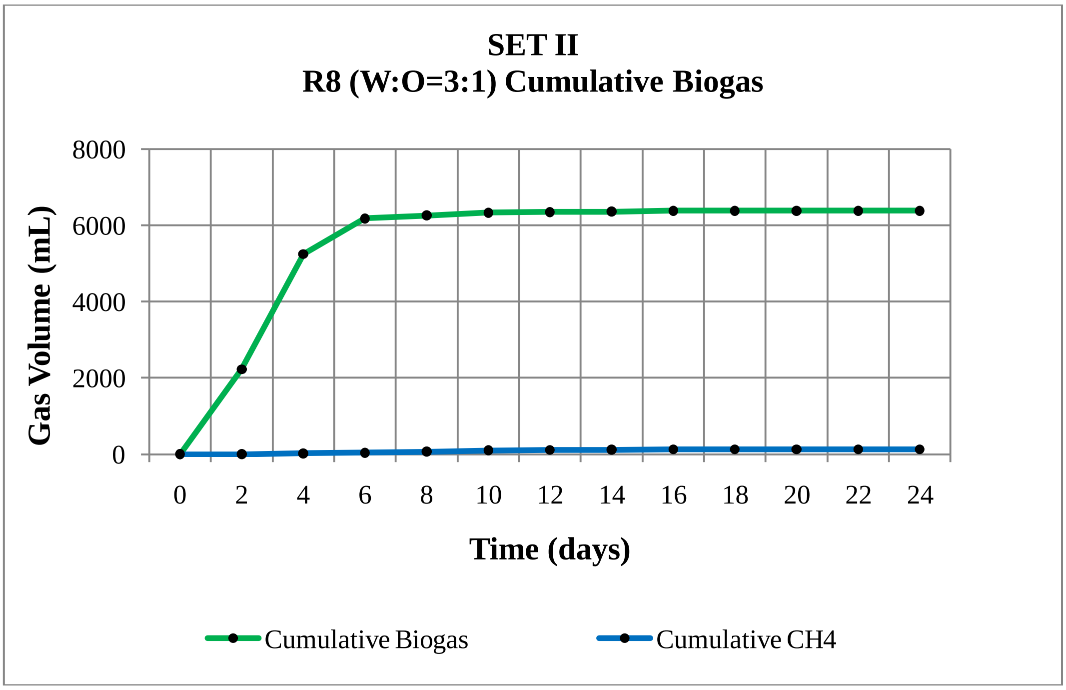

Figure 4.10. The cumulative biogas and methane for reactor R8 containing a mixture of wastewater and off-spec in Set II.

However, the volume of methane produced was small. When the wastewater and off- spec was mixed at a ratio of 9:1, methane could be produced from anaerobic co-digestion of wastewater and off-spec (Figure 4.11).

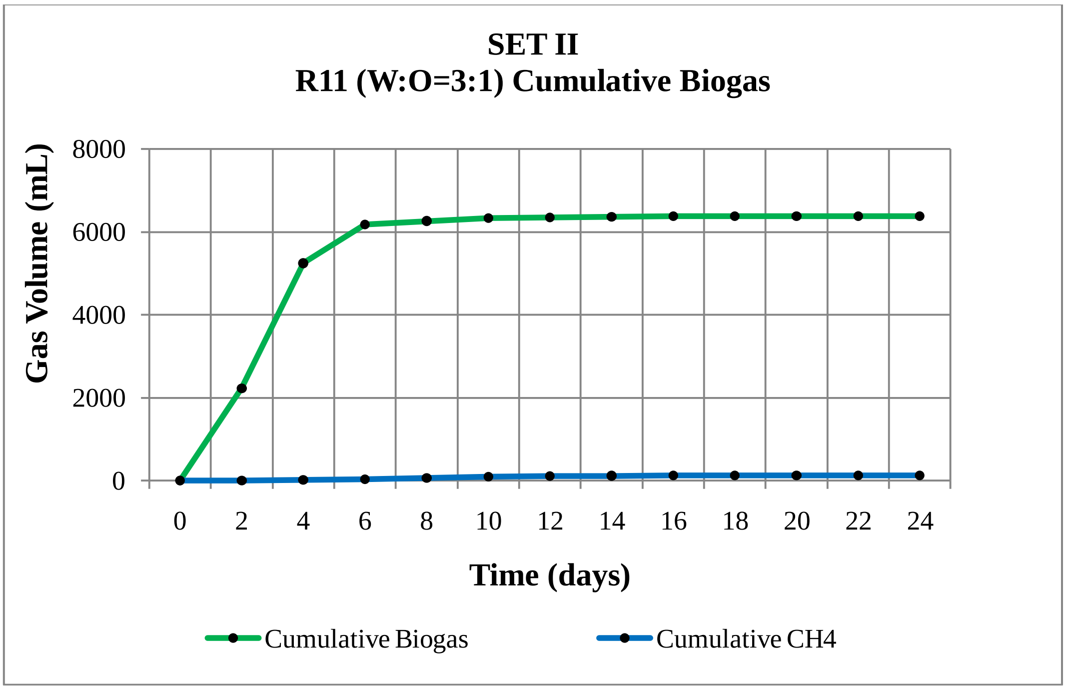

Figure 4.11. The cumulative biogas and methane for reactor R11 containing a mixture of wastewater and off-spec in Set II.

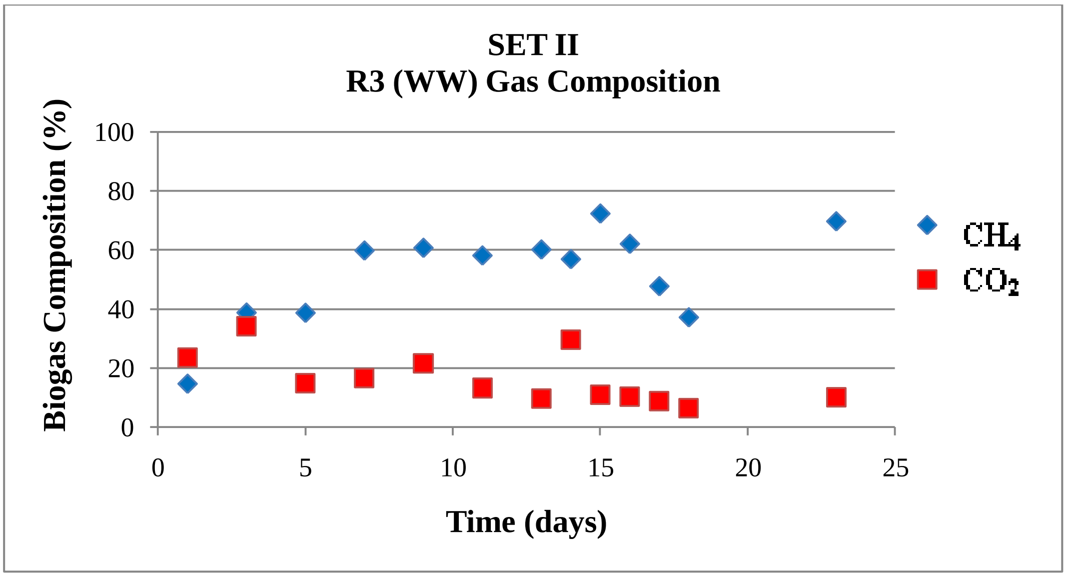

Figure 4.12. Biogas composition (CH_4/CO_2) for reactor R3 containing only wastewater (WW) in Set II.

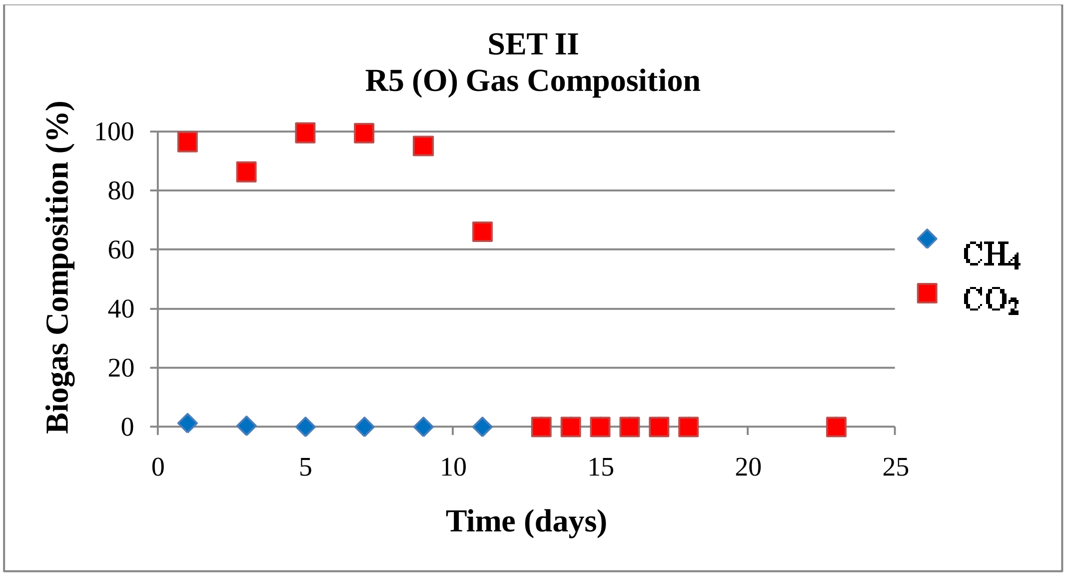

Figure 4.13. Biogas composition (CH_4/CO_2) for reactor R5 containing off-spec (O) in Set II.

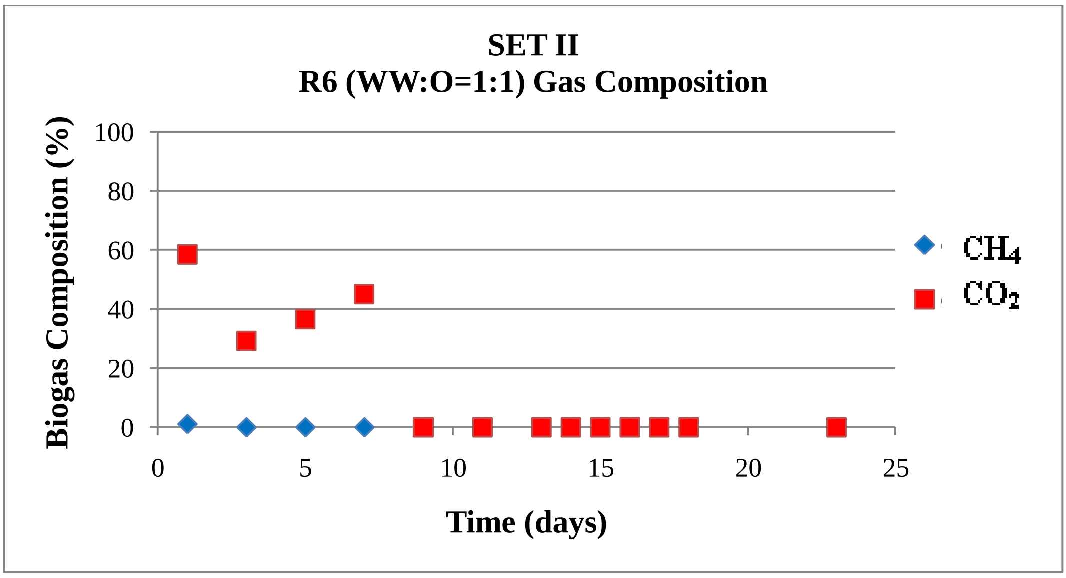

Figure 4.14. Biogas composition (CH_4/CO_2) for reactor R6 containing mixture of wastewater and off-spec (WW:O=1:1) in Set II.

In Set II, regarding the composition of biogas, the percentage of methane ranged from 40 to 80 %, and on average it was about 60 % (Figure 4.12) from anaerobic digestion of wastewater alone. Anaerobic digestion of off-spec alone did not produce methane again in this set (Figure 4.13).

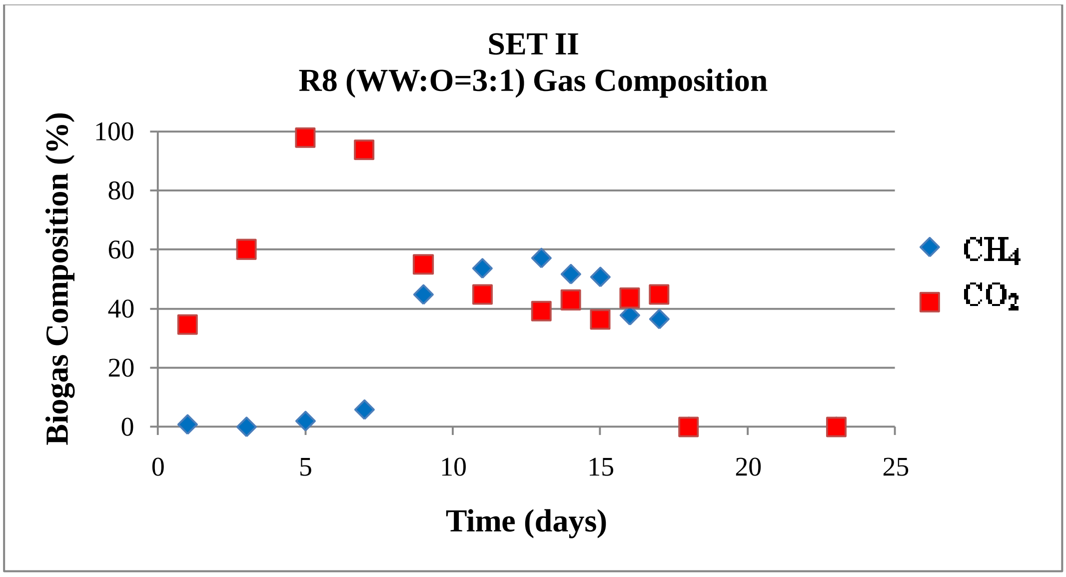

Figure 4.15. Biogas composition (CH_4/CO_2) for reactor R8 containing mixture of wastewater and off-spec (WW:O=3:1) in Set II.

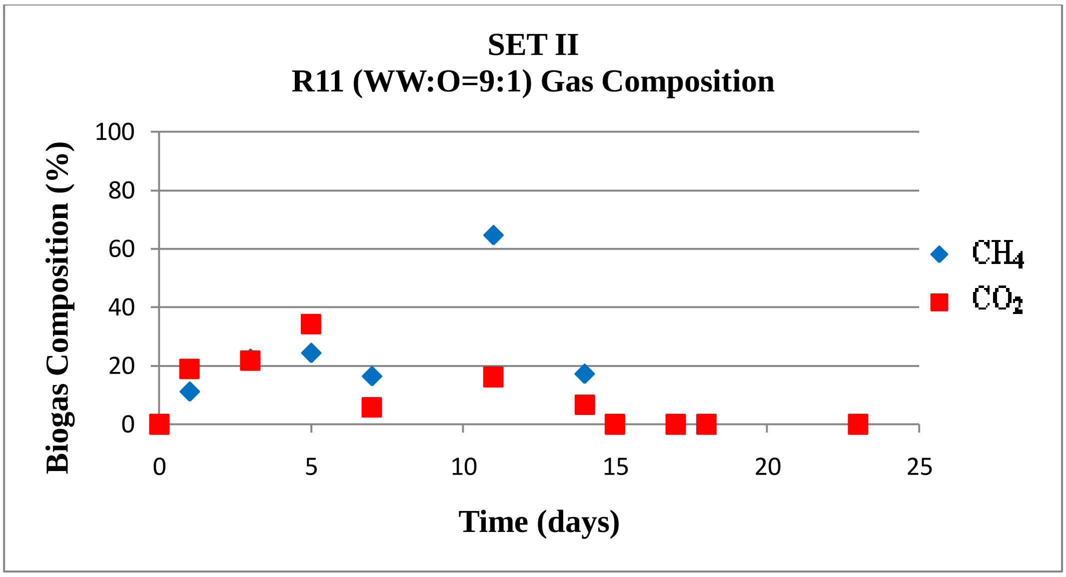

Figure 4.16. Biogas composition (CH_4/CO_2) for reactor R11 containing mixture of wastewater and off-spec (WW:O=9:1) in Set II.

For mixtures of wastewater and off-spec, the highest methane composition in biogas was 60 % and it was obtained at a ratio of 9:1. When the biogas composition was analyzed, it was observed that the biogas contained high amount of nitrogen in addition to CH_4 and CO_2 (Figure 4.16). Even though methane could be measured in biogas from reactor R8 (Figure 4.15), the cumulative methane production was very low. Therefore, mixing ratio of wastewater and off-spec at 9:1 seemed to provide better methane yield in this set.

4.1.3.3. The Results of Set III. The cumulative biogas and cumulative methane (CH_4) production and the composition of biogas (v/v) for Set III are shown in Figures 4.17, 4.18, 4.19, 4.20, 4.21, 4.22, 4.23, 4.24, 4.25 and 4.26, respectively.

In terms of cumulative methane production in Set III, generally the best results were obtained with respect to Set I and Set II, most probably due to characteristics of both wastewater and off-spec. The methane production could be observed in all of the trials in Set III. Among mixtures of wastewater and off-spec, the best results were obtained again from the mixture ratio of 9:1. In addition, it can be inferred that methane production ability increases when the wastewater ratio increases in the wastewater and off-spec mixture reactors.

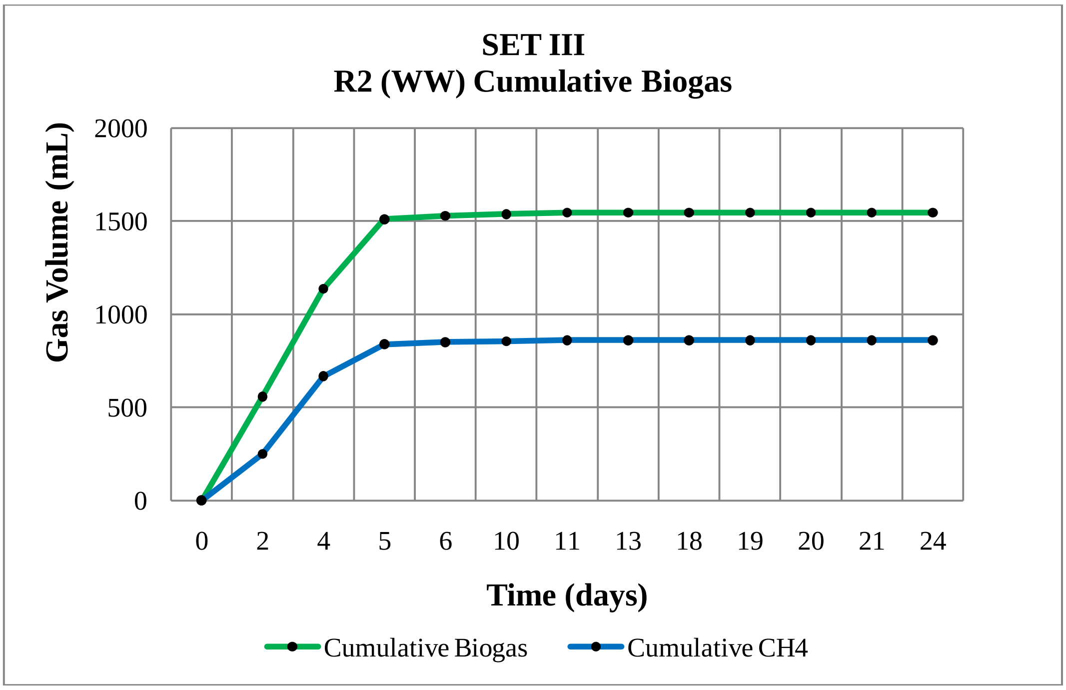

Figure 4.17. The cumulative biogas and methane for reactor R2 containing wastewater in Set III.

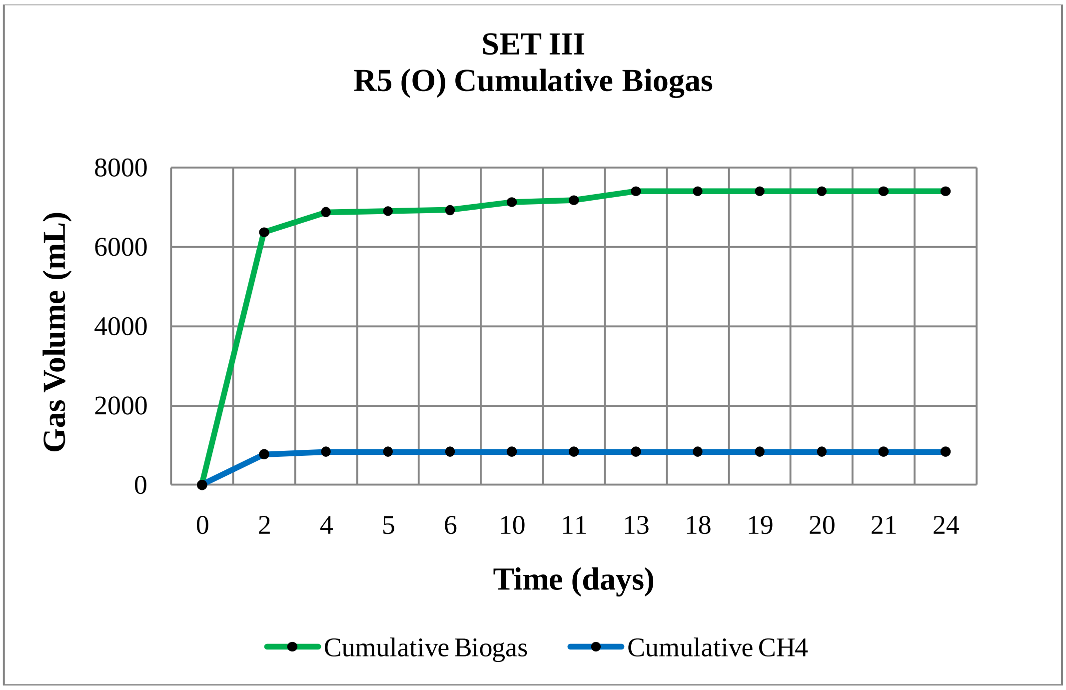

Figure 4.18. The cumulative biogas and methane for reactor R5 containing off-spec in Set III.

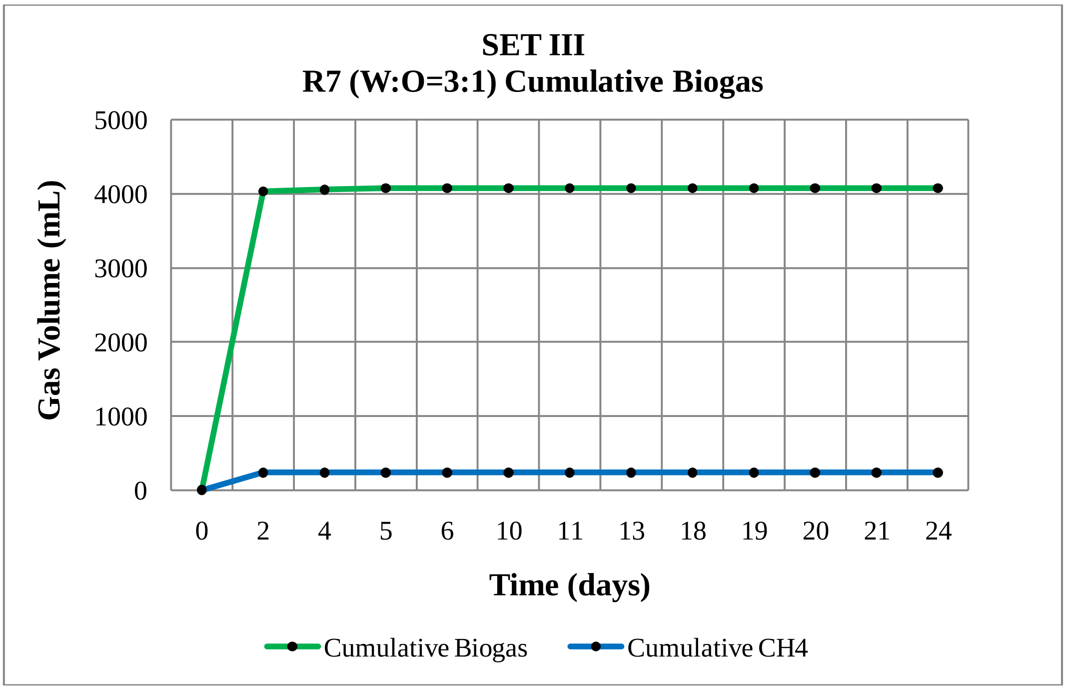

Figure 4.19. The cumulative biogas and methane for reactor R7 containing a mixture of wastewater and off-spec in Set III.

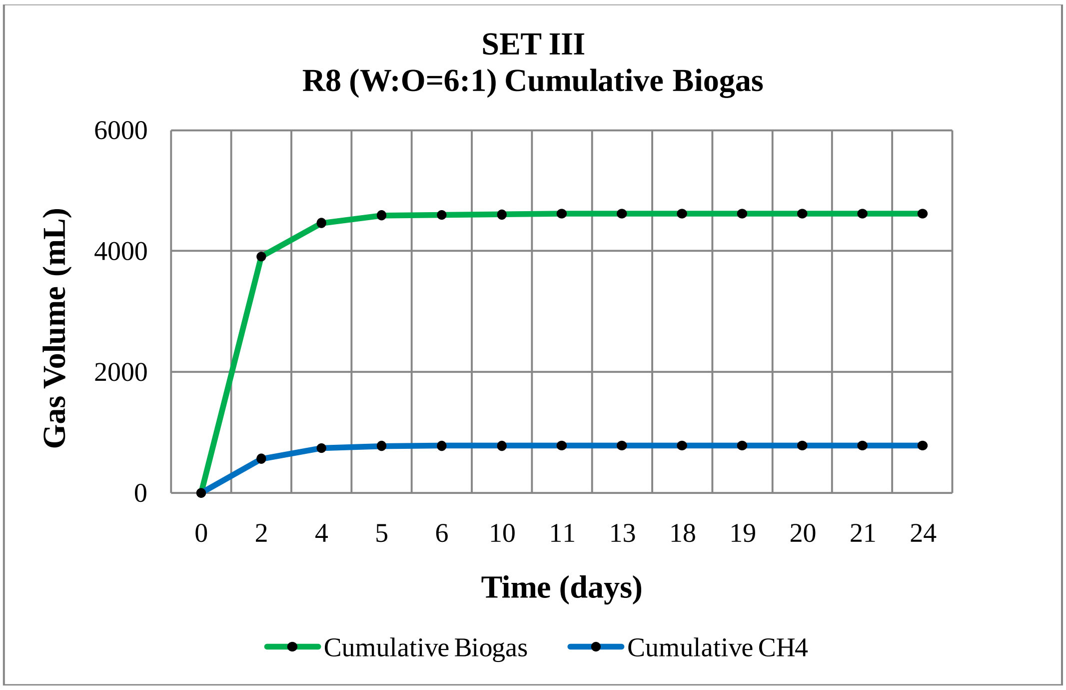

Figure 4.20. The cumulative biogas and methane for reactor R8 containing a mixture of wastewater and off-spec in Set III.

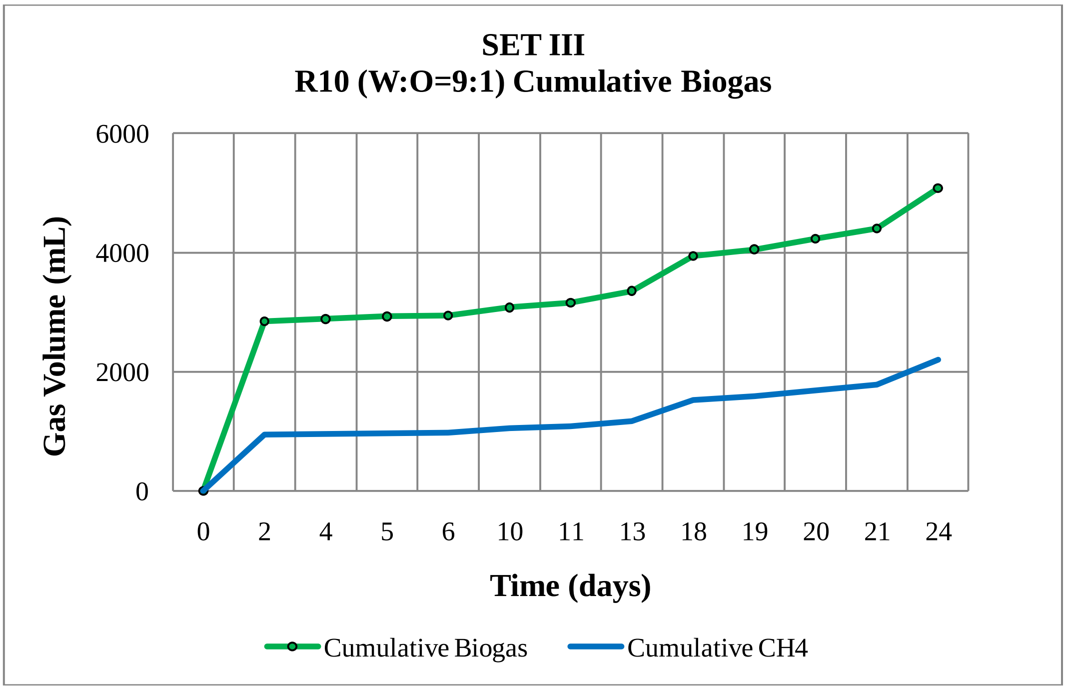

Figure 4.21. The cumulative biogas and methane for reactor R10 containing a mixture of wastewater and off-spec in Set III.

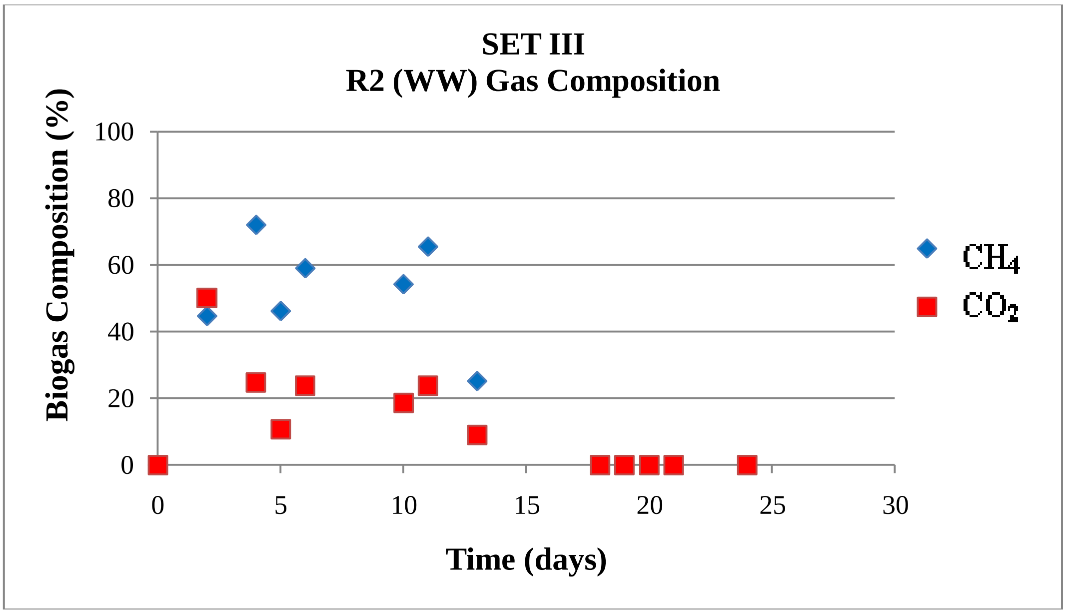

Figure 4.22. Biogas composition (CH_4/CO_2) for reactor R2 containing only wastewater in Set III.

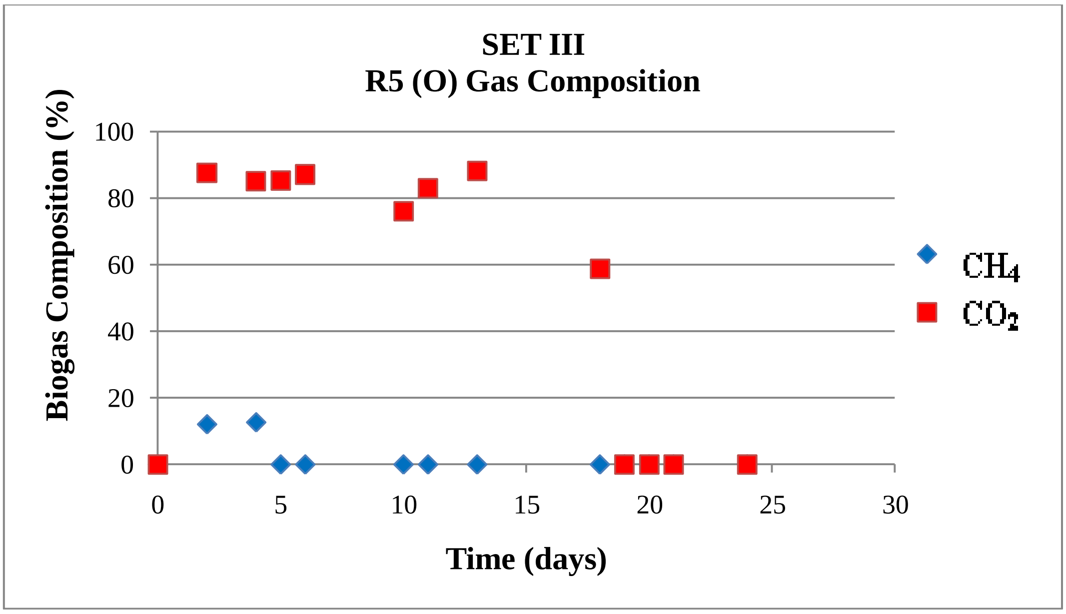

Figure 4.23. Biogas composition (CH_4/CO_2) for reactor R5 containing only off-spec (O) in Set III.

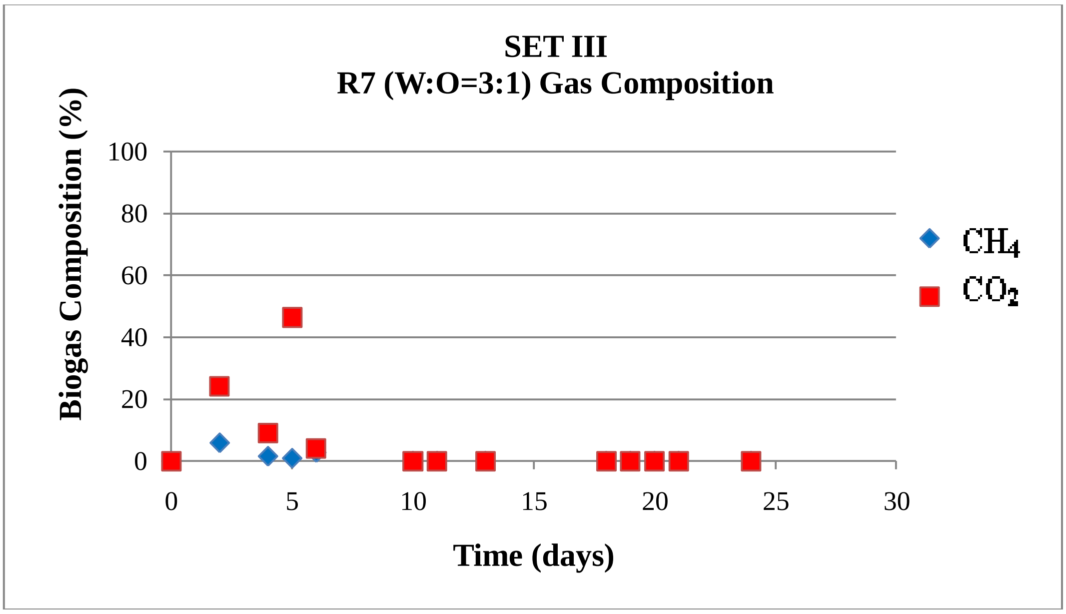

Figure 4.24. Biogas composition (CH_4/CO_2) for reactor R7 containing mixture of wastewater and off-spec (WW:O=3:1) in Set III.

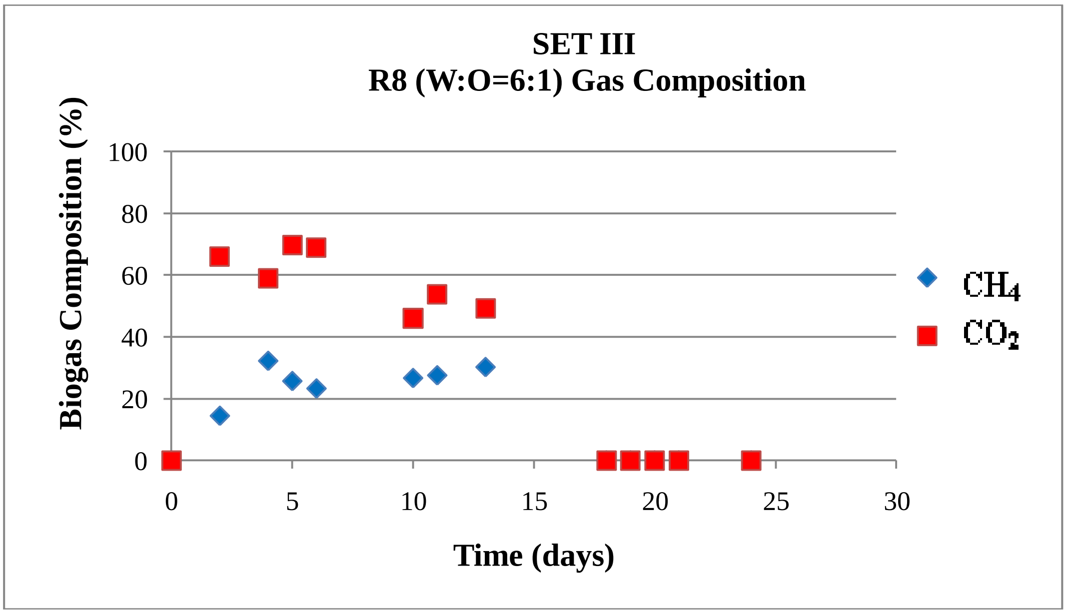

Figure 4.25. Biogas composition (CH_4/CO_2) for reactor R8 containing mixture of wastewater and off-spec (WW:O=6:1) in Set III.

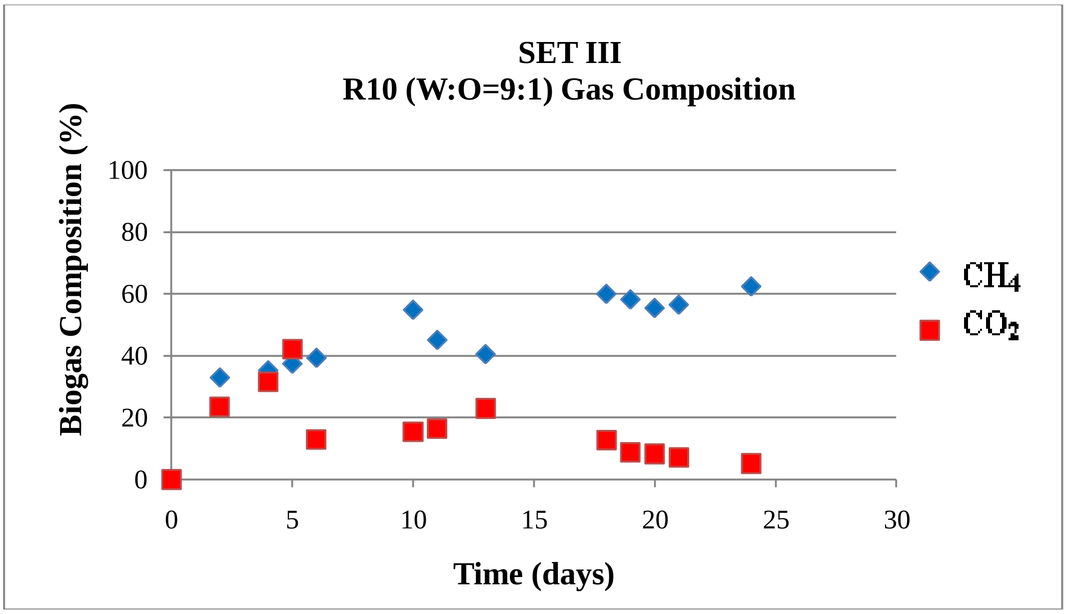

Figure 4.26. Biogas composition (CH_4/CO_2) for reactor R10 containing mixture of wastewater and off-spec (WW:O=9:1) in Set III.

Regarding biogas composition in Set III, the highest methane composition was measured to be around 70 % for anaerobic digestion of wastewater alone. The low CH_4 and CO_2 compositions between 10th and 15th days were due to the high nitrogen content in biogas (Figure 4.22). For the first time among all sets, the methane composition ranged between 0 and 20 % for anaerobic digestion of off-spec alone (Figure 4.23). The highest methane composition for mixtures of wastewater and off-spec reached to 60 % at a mixture ratio of 9:1 (Figure 4.26). The methane composition ranged between 20 and 40 % at a mixture ratio of 6:1 (Figure 4.25). The methane and carbon dioxide compositions were very low in R7 (WW:O=3:1) (Figure 4.24). This low content resulted due to the oxygen content that varied between 19 and 22 % and nitrogen content varied between 50 and 70 %. Because of this reason, it can be inferred that this reactor was not sealed well.

4.1.4. Volatile Fatty Acid (VFA) Concentrations

The concentrations of VFAs are given in Table 4.8. In set I, the highest VFA concentration was observed in batch reactors (R3 & R4) containing only off-spec. In batch reactors containing wastewater and off-spec mixture (R5 & R6), a lower VFA

Table 4.8. Volatile fatty acid concentrations in each set for batch anaerobic digestion tests.

		Concentration (mg L^{-1})								
		Acetic	**Propionic**	**Isobutyric**	**Butyric**	**Isovaleric**	**Valeric**	**Isocaproic**	**Caproic**	**Heptanoic**
SET I	**WW**	693	10	0	4	0	0	25	0	0
	O	974	62	0	88	0	0	43	0	0
	W:O=1:1	567	22	0	29	0	0	0	0	0
SET II	**WW**	875	58	0	26	0	0	0	0	0
	O	273	10	0	14	0	0	0	0	0
	W:O=1:1	261	13	0	7	0	0	6	0	0
	W:O=3:1	191	118	0	137	4	63	0	62	20
	W:O=9:1	775	12	0	0	0	0	0	0	0
SET III	**WW**	9	0	0	0	0	0	0	0	8
	O	1621	83	3	9	1	29	0	3	0
	W:O=3:1	385	332	5	104	10	110	0	33	29
	W:O=6:1	470	339	9	69	17	58	0	11	7
	W:O=9:1	240	312	9	0	11	0	0	0	0

concentration was measured than those of reactors containing only wastewater (R1 & R2). However, a negligible methane yield was calculated for (R5 & R6) (Table 4.3). In set II, the highest VFA concentration was measured in reactors containing only wastewater (R2 & R3), while except for reactor R 11 containing wastewater and off-spec mixture, all the other reactors had lower VFA content. In set III, the VFA amount detected in reactors containing only wastewater (R2 & R3) was almost negligible, indicating that all of the VFAs were successfully consumed and converted to CH_4. All the other reactors contained high amount of VFAs.

4.1.5. Metal Concentrations

Metal analysis results of the off-spec samples which were received on three different dates are given in the Table 4.9. Sodium, potassium, magnesium and calcium are important light metal ions in anaerobic systems. They are required for microbial growth and affect specific growth rate like any other nutrient (Chen et al., 2007). Moderate concentrations of these ions are stimulating microbial growth in anaerobic systems. On the other hand, excessive amounts slow down the growth, and even higher concentrations can cause severe inhibition or toxicity (Soto et al., 1993). As it can be seen in Table 4.9, the concentrations of these light metals were within the optimum range for a healthy anaerobic digestion process. Therefore, the presence of these metals in off-spec was not likely to affect the process adversely.

Table 4.9. Results for metal analysis of off-spec samples.

Metal	**Concentration (mg g^{-1})**		
	Set I	**Set II**	**Set III**
Zinc (Zn)	0.063	0.000	0.000
Manganese Mn)	0.000	0.006	0.002
Nickel (Ni)	0.000	0.027	0.004
Copper (Cu)	0.020	0.002	0.014
Cobalt (Co)	0.000	0.000	0.000
Iron (Fe)	0.136	0.140	0.296
Sodium (Na)	1.129	0.917	1.836
Potassium (K)	1.289	0.613	1.761
Calcium (Ca)	1.345	0.598	0.884
Magnesium (Mg)	0.361	1.958	0.363

4.2. Discussion

4.2.1. Methane Production Potential of Ice Cream Wastewater

The comparison of methane yields for each set of experiments is given in Table 4.10. According to the experimental findings obtained, batch anaerobic digestion of ice cream wastewater alone can produce high methane yield. In Set III, the methane yield was calculated to be 0.338 L CH_4 g^{-1} $COD_{removed}$. In literature, the methane yield from anaerobic digestion of dairy industry wastewater was reported to vary between 0.32 to 0.34 m^3 CH_4 kg^{-1} $COD_{removed}$ (Ince, 1998). The methane content of biogas generated from anaerobic digestion of ice cream wastewater can reach more than 70 % (Set III). Typical methane percentages reported for anaerobic digestion of dairy industry effluents range from 60 to 85 % (Ince O., 1998; Rao et al., 2008; Demirel et al., 2005). Thus, the methane percentage in the biogas produced was also in agreement with the results reported in literature. Due to high COD concentration content, ice cream wastewater seems suitable for anaerobic treatment in order to produce biogas as a source of renewable energy.

Since three different samples received on different dates were studied, reported methane yields also varied depending on the characteristics of the wastewater samples. The wastewater pH was quite variable, and the buffering capacity (alkalinity) of the wastewater was also quite low. In addition, the wastewater contained high concentrations of sulfate and oil & grease. Low buffering capacity, and high concentrations of sulfate and oil & grease are bottlenecks of anaerobic digestion process. During batch tests, external buffer solutions were used to sustain anaerobic degradation. Therefore, if this wastewater is going to be treated in an anaerobic wastewater treatment plant, measures should be taken to provide enough buffering capacity to influent wastewater and to cope with high concentrations of sulfate and oil & grease.

4.2.2. Methane Production Potential of Off-spec

The comparison of methane yields for each set of experiments is given in Table 4.10. According to the experimental findings obtained, batch anaerobic digestion of off-spec alone was not feasible, since the methane yields obtained were almost negligible. The off-spec also has differing characteristics, most probably due to production cycles, and it has a very low pH and no buffering capacity. In addition, the off-spec contains very high concentrations and sulfate and oil & grease, both of which make the anaerobic digestion process quite unfavorable. Therefore, in spite of its high COD content, batch anaerobic digestion of off-spec alone did not produce satisfactory results during this experimental work.

4.2.3. Methane Production Potential of Ice Cream Wastewater and Off-spec Mixture

The comparison of methane yields for each set of experiments is given in Table 4.9. Different mixture ratios by weight were pre-determined in order to provide methane production from anaerobic co-digestion of ice cream wastewater and off-spec. External buffer solution was also used to provide alkalinity so that anaerobic digestion could be sustained in wastewater and off-spec mixtures. Like wastewater, the off-spec samples always had a very low pH value and no buffering capacity. Both of these factors can easily result in acidification in an anaerobic digestion system. Therefore, it can be stated that the mixture of ice cream wastewater and off-spec contains high concentrations of both sulfate and oil & grease, and it has a quite low buffering capacity. These factors eventually affect methane yields adversely in an anaerobic treatment process.

According to the results obtained, for anaerobic co-digestion of ice cream wastewater and off-spec, the methane yields generally increased when the dilution ratio between the wastewater and off-spec also increased. When the dilution ratios were low, like 1:1 or 3:1, lower methane yields were obtained, which did not favour biogas production.

Among all sets of experiments, the best results were obtained in Set III. Namely, the highest methane yields were obtained. In addition, the highest methane percentage in biogas reached 60 % in WW:O=9:1 mixture in Set III (Figure 4.26). These results indicate that the off-spec has to be mixed with wastewater at high dilution ratios so that methane production from anaerobic co-digestion of wastewater and off-spec can be realized.

4.2.4. Overall Evaluation of Results

An overall comparison of the methane yields for all sets of experiments are given in Table 4.10. In general, the methane yields from anaerobic digestion of wastewater alone were always within acceptable values, while anaerobic digestion of off-spec alone was not suitable. Among wastewater and off-spec mixtures, the highest dilution of off-spec with wastewater (WW:O=9:1) provided the highest methane yield. In order to promote methane generation from anaerobic digestion of ice cream wastewater and off-spec, it is very important to provide adequate buffering capacity.

Table 4.10. Comparison of methane yields for each set of experiment.

	SET	**Time Duration (Day)**	**Methane Yield (L Methane produced g^{-1} COD removed)**	**Methane Yield (L Methane produced g^{-1} VS destructed)**	**Cumulative Biogas Production (mL)**	**Cumulative Methane Production (mL)**
WW	**SET I**	17	0.194	0.091	1269	491
	SET II	20	0.160	0.147	2198	931
	SET III	11	**0.338**	**0.222**	1334	791
O	**SET I**	8	-	0.000	1984	21
	SET II	13	-	0.001	5126	30
	SET III	18	-	0.079	7141	765
Mixture (WW & O)	**SET I (1:1)**	7	-	0.000	694	5
	SET II (1:1)	6	-	0.001	2829	77
	SET II (3:1)	18	-	0.006	6386	127
	SET II (9:1)	11	**-**	**0.049**	2629	589
	SET III (3:1)	10	-	0.121	3665	167
	SET III (6:1)	11	-	0.175	4207	713
	SET III (9:1)	24	**-**	**0.369**	4540	2118

The initial COD/VS ratios of wastewater samples of Set I, Set II and Set III were calculated as 0.57, 0.99 and 1.23, respectively. When this values are compered with the other studies, they can be accepted normal except Set I. For instance, Winter et al. (2010) calculated this ratio to be between 1.67 and 1.85 for press water of food waste.

According to experimental findings obtained in this work, batch mesophilic anaerobic digestion of ice cream wastewater alone (without co-substrate addition) could produce a high CH_4 yield. Particularly in Set III, the CH_4 yield was 0.338 L CH_4 g^{-1} $COD_{removed}$. The CH_4 yield from mesophilic anaerobic treatment of a dairy industry wastewater by a pilot-scale upflow anaerobic filter was reported to vary between 0.32 to 0.34 m^3 CH_4 kg^{-1} $COD_{removed}$ with a CH_4 composition ranging between 75 and 85 % in biogas (Ince, 1998). In this work, the CH_4 content of biogas generated from anaerobic digestion of ice-cream wastewater could also reach more than 70 %. Typical CH_4 generation values reported for anaerobic digestion of dairy industry effluents range from 60 to 85 % (Demirel et al., 2005; Ince, 1998; Rao et al., 2008). Thus, the CH_4 content of biogas from anaerobic digestion of ice-cream wastewater was in agreement with those results reported. Obviously, particularly due to its high COD concentration, ice cream wastewater seemed suitable for anaerobic treatment, particularly for recovery of energy from this wastewater type by an anaerobic wastewater treatment process. However, the CH_4 yields seemed to vary much depending on the characteristics of the influent wastewater. Dairy wastewater flow rates and characteristics vary greatly and they are quite difficult to predict even when detailed information on processing operations is available (Demirel et al., 2005; Danalewich et al., 1998). In this work, the wastewater pH was quite variable, and some batch tests performed without buffer solution indicated that the buffering capacity was also quite low. The use of acid and alkaline cleaning agents, in addition to various process operations, influences the wastewater characteristics, particularly the pH (Danalewich et al., 1998; Kasapgil et al., 1994). The alkalinity of dairy wastewaters was also reported to be less than 1000 mg $CaCO_3$ L^{-1} in most cases (Kasapgil et al., 1994; Demirel and Yenigun, 2004; Demirel and Yenigun, 2006), often not high enough to sustain an anaerobic treatment process successfully. Therefore, external use of a buffering agent became a very important and essential tool to accomplish anaerobic batch digestion tests in this work. In addition to pH instability and low buffering capacity, the ice cream wastewater also contained high concentrations of SO_4^{-2}, oil & grease and Cl^- in each trial. Variable pH, inadequate buffering capacity along with high SO_4^{-2} and oil & grease concentrations are bottlenecks of an anaerobic digestion process. The SO_4^{-2} and oil & grease concentrations in ice cream wastewater ranged between 200 – 350 and 2424 – 2815 mg L^{-1}, respectively, while Cl^- concentration was between 400 and 550 mg L^{-1}. Since Cl^- concentrations above 5000 mg L^{-1} were reported to cause inhibition in anaerobic treatment processes (Fusuwankaya et al., 2009; Jiao et al., 2010), the adverse impacts of Cl^- in this work could be ignored. It has already been reported that the presence of high oil & grease content in dairy waste streams caused problems due to their low biodegradability in anaerobic treatment (Vidal et al., 2000; Cammarota and Freire, 2006). Actually, it is the long chain fatty acids (LCFAs) that cause problems in anaerobic treatment of fatty materials by adsorption onto the cell wall/membrane, interference with the transport or protective function (Rinzema et al., 1994). Sulfate is reduced to sulfide by the sulfate reducing bacteria, and therefore, two stages of inhibition can take place suppressing CH_4 production (Hulshoff Pol et al., 1998; Chen et al., 2008). The presence of sulphur compounds in a wastewater must be considered in case of an anaerobic treatment system option for purification (Hulshoff Pol et al., 1998). On the other hand, if reactors were acclimated, then sulfate concentrations up to 5000 mg L^{-1} did not pose any adverse affects on methanogenesis (Isa et al., 1986). Therefore, SO_4^{-2} concentration in this work also seemed to have no significant adverse impact. Furthermore, ammonia produced from the degradation of milk protein can also inhibit anaerobic treatment of dairy waste streams (Vidal et al., 2000). It was reported that an anaerobic treatment system could tolerate total ammonia concentrations ranging from 1000 to 6000 mg L^{-1} (Callı et al., 2005). Ammonia was not monitored in batch reactors in this work, but the amount of TKN in the ice-cream wastewater varied from 145 to 165 mg L^{-1}, while the off-spec contained a high TKN concentration that might have affected the anaerobic degradation adversely.

Anaerobic batch digestion of off-spec alone did not seem to be feasible, since the observed CH_4 yields were almost negligible. The off-spec also had differing characteristics in each trial, most probably due to production cycles, and it had a very low, acidic pH and no buffering capacity. Even external supplementation of buffering solutions did not improve degradation. In addition, the off-spec contained very high concentrations of SO_4^{-2} and oil & grease, both of which made the anaerobic digestion process quite unfavorable. Therefore, in spite of its high COD content, batch anaerobic digestion of off-spec alone did not produce satisfactory results.

Different mixture ratios by weight were employed to achieve high CH_4 yields from anaerobic co-digestion of ice cream wastewater and off-spec. The mixture of ice cream wastewater and off-spec contained high concentrations of both SO_4^{-2} and oil & grease, and it also had a quite low buffering capacity. As summarized above, both of these factors could easily result in acidification of an anaerobic digestion system. These factors eventually affected the CH_4 yields adversely. On the other hand, 60 % of CH_4 in biogas could be observed during co-digestion in Set III. According to the results obtained, the CH_4 yields generally increased when the dilution ratio between the wastewater and off-spec increased. When the dilution ratios were low, like 1:1 or 3:1, by weight, lower CH_4 yields were obtained. These results indicated that the off-spec had to be mixed with wastewater at high dilution ratios so that CH_4 production from anaerobic co-digestion of ice-cream wastewater and off-spec could be realized. The dilution effect of wastewater seemed to decrease the concentrations of inhibitory components in the off-spec and thereby, promoting CH_4 production, along with adequate buffering capacity. The SO_4^{-2} and oil & grease concentrations in off-spec ranged between 200 – 1600 and 7028 – 47365 mg L^{-1}, respectively, much more higher than those of wastewater, probably causing perturbation. Among wastewater and off-spec mixtures, the highest dilution of off-spec with wastewater (WW:O=9:1) provided the highest CH_4 yield.

The VFA measurements indicated that, particularly in set III, the VFAs were effectively consumed and converted to CH_4 in reactors only with ice-cream wastewater, providing the highest methane yield observed (0.338 L CH_4 g^{-1} $COD_{removed}$) in this study. VFA concentrations above 2000 mg L^{-1} were reported to cause inhibition in batch anaerobic digestion tests (Siegert and Banks, 2005).

Milk fat was found to be a major cause for reduction of the methanogenic activity for anaerobic degradation of dairy streams (Perle et al., 1995). Especially, the oil & grease content of the off-spec, which was much above the inhibitory concentrations reported, might have adversely affected the process. In set I and II, even though higher VFA content in reactors containing only wastewater was observed, these reactors could provide higher methane yields than those of the reactors with only off-spec and wastewater/off-spec mixtures. It seemed that in set I and II, acidogenesis and acetogenesis phases could not have been properly accomplished in reactors with off-spec and wastewater/off-spec mixtures. Therefore, the particular characteristics of the off-spec, in addition to ice-cream wastewater, was most probably quite influential on the process stages in set I and II. In addition, in reactors only with ice-cream wastewater, the VFAs could not be consumed completely both in set I and II, resulting in lower methane yields than those of reactors in set III. In this case, it can be speculated that methanogenesis was the rate-limiting step in anaerobic process for reactors with wastewater.

Dairy industry wastewaters are generated in an intermittent way, and the flow and characteristics of wastewater change from one plant to another depending on the systems and the methods of operation (Rico et al., 1991). No information was encountered in literature about management of ice cream production residues. However, this residue, which has a high TS content around 16 to 25 %, has to be disposed of properly as well, since it has no commercial use anymore. Anaerobic treatment has always been a common and a favorable option for purification dairy waste effluents (Perle et al., 1995), and in this particular case, the high COD content ranging from 7312 to 10418 mg L^{-1} makes the ice cream wastewater a favorable substrate for recovery of CH_4, in spite of high concentrations of oil & grease, SO_4^{-2}, and variable pH values in the ice cream wastewater. On the other hand, even though co-digestion of various types of industrial wastewaters with solid organic materials is quite common for energy recovery (Boullagui et al., 2009; Weiland, 2000; Hedegaard and Jaensch, 1999; Bjornsson et al., 2000), anaerobic co-digestion of off-spec with ice cream wastewater did not seem to be feasible due to lower methane yields observed.

5. CONCLUSIONS AND RECOMMENDATIONS

The following conclusions and recommendations are drawn based on the findings of this experimental work.

Depending on the production cycles, both ice cream wastewater and off-spec have variable characteristics. Ice cream wastewater has usually low pH values and very little buffering capacity. The wastewater also contains high concentrations of sulfate and oil & grease. Off-spec has low pH values and it has no buffering capacity. It also contains very high concentrations of sulfate and oil & grease.

Batch anaerobic digestion of ice cream wastewater alone produced high methane yields. Methane generation could be observed in each trial for ice cream wastewater. Therefore, it can be concluded that ice cream wastewater can be treated anaerobically to produce biogas as a source of renewable energy. However, adequate buffering capacity must always be provided in the wastewater for anaerobic treatment.

Batch anaerobic digestion of off-spec alone produced a very little methane yield only in one trial, while generally no methane production was observed in all other trials. Therefore, anaerobic digestion of off-spec alone does not seem favorable and is not recommended.

When they are mixed at a proper ratio and as long as adequate buffering capacity is provided, batch anaerobic digestion of ice cream wastewater and off-spec together can produce biogas, namely methane. Among all trials, the best results were obtained when the ice cream wastewater and off-spec were mixed at a ratio of 9:1 (WW:O) by weight.

It is possible to digest both ice cream wastewater and off-spec together anaerobically to produce biogas. The methane yields will significantly depend on the characteristics of both wastewater and off-spec.

The amountof the wastewater in the reactors containing mixtures must be increased to get higher methane yields via anaerobic co-digestion of wastewater and off-spec.

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APPENDIX:

CUMULATIVE BIOGAS GENERATION AND COMPOSITION

Table A1. Daily biogas production for Set I.

	Biogas Production (mL)					
Time (day)	**R1 (WW)**	**R2 (WW)**	**R3 (O)**	**R4 (O)**	**R5 (1:1)**	**R6 (1:1)**
1	583.56	591.63	1677.34	1430.99	393.59	685.12
2	76.12	53.29	284.21	334.91	172.40	2.71
3	10.13	7.59	64.55	2.54	56.92	8.12
4	38.06	4.32	38.32	43.62	59.01	0.00
5	68.50	2.53	22.83	35.63	7.32	0.00
6	101.49	75.87	13.69	2.55	1.63	0.00
7	74.28	118.87	9.14	2.55	0.81	0.00
8	174.37	78.37	5.07	0.00	0.00	0.00
9	25.37	12.72	0.00	0.00	0.00	0.00
10	15.22	22.72	0.00	0.00	0.00	0.00
11	2.54	48.05	0.00	0.00	0.00	0.00
12	10.73	90.13	0.00	0.00	0.00	0.00
13	83.15	48.94	0.00	0.00	0.00	0.00
14	0.00	0.03	0.00	0.00	0.00	0.00
15	2.54	0.00	0.00	0.00	0.00	0.00
16	0.00	2.53	0.00	0.00	0.00	0.00
17	2.54	0.00	0.00	0.00	0.00	0.00

Table A2. Cumulative biogas production for Set I.

	Biogas Production (mL)					
Time (day)	**R1 (WW)**	**R2 (WW)**	**R3 (O)**	**R4 (O)**	**R5 (1:1)**	**R6 (1:1)**
1	583.56	591.63	1677.34	1430.99	393.59	685.12
2	659.68	644.92	1961.55	1765.90	565.99	687.83
3	669.81	652.50	2026.10	1768.44	622.92	695.95
4	707.87	656.82	2064.42	1812.06	681.93	695.95
5	776.37	659.35	2087.25	1847.70	689.25	695.95
6	877.86	735.22	2100.95	1850.24	690.88	695.95
7	952.14	854.09	2110.09	1852.79	691.69	695.95
8	1126.51	932.46	2115.16	1852.79	691.69	695.95
9	1151.88	945.18	2115.16	1852.79	691.69	695.95
10	1167.10	967.90	2115.16	1852.79	691.69	695.95
11	1169.64	1015.95	2115.16	1852.79	691.69	695.95
12	1180.37	1106.08	2115.16	1852.79	691.69	695.95
13	1263.52	1155.02	2115.16	1852.79	691.69	695.95
14	1263.52	1155.05	2115.16	1852.79	691.69	695.95
15	1266.06	1155.05	2115.16	1852.79	691.69	695.95
16	1266.06	1157.58	2115.16	1852.79	691.69	695.95
17	1268.59	1157.58	2115.16	1852.79	691.69	695.95

Table A3. Daily gas production for Set II.

	Biogas Production (mL)										
Time (day)	**R1 (S)**	**R2 (WW)**	**R3 (WW)**	**R4 (O)**	**R5 (O)**	**R6 (1:1)**	**R7 (1:1)**	**R8 (3:1)**	**R9 (3:1)**	**R10 (9:1)**	**R11 (9:1)**
1	85	170	216	1441	759	2851	2686	127	2989	1892	2163
2	3	22	43	1426	1718	132	72	2124	215	374	356
3	3	40	149	3	1353	72	70	2081	3	686	1
4	3	3	45	23	692	2	1	1718	0	81	7
5	7	22	107	124	3	47	1	111	34	113	52
6	16	11	5	36	582	29	0	37	517	94	39
7	11	11	8	38	19	0	0	0	1071	16	8
8	13	11	5	30	0	0	0	122	812	3	0
9	24	8	16	3	0	0	0	5	812	2	0
10	24	50	175	3	0	0	0	6	42	11	0
11	24	50	175	3	0	0	0	6	42	11	0
12	0	50	224	0	0	0	0	5	7	0	0
13	0	103	195	8	0	0	0	15	16	0	1
14	0	5	112	0	0	0	0	5	9	0	0
15	0	13	195	2	0	0	0	5	0	0	0
16	0	5	139	0	0	0	0	6	0	0	0
17	0	4	139	0	0	0	0	6	0	0	0
18	0	4	139	0	0	0	0	6	16	0	0
19	0	4	51	0	0	0	0	0	0	0	0
20	0	0	61	0	0	0	0	0	0	0	0
21	0	52	0	0	0	0	0	0	0	0	0
22	0	52	0	0	0	0	0	0	0	0	0
23	0	2	0	0	0	0	0	0	0	0	0
24	0	2	0	0	0	0	0	0	0	0	0
25	0	8	0	0	0	0	0	0	0	0	0

Table A4. Cumulative biogas production for Set II.

	Biogas Production (mL)										
Time (day)	**R1 (S)**	**R2 (WW)**	**R3 (WW)**	**R4 (O)**	**R5 (O)**	**R6(1:1)**	**R7(1:1)**	**R8(3:1)**	**R9(3:1)**	**R10(9:1)**	**R11(9:1)**
1	85.34	170.09	216.05	1441.14	758.82	2850.86	2685.53	127.25	2988.74	1892.28	2163.16
2	87.92	191.69	258.73	2867.06	2476.43	2982.60	2757.11	2250.76	3203.92	2266.43	2519.04
3	90.51	232.19	408.10	2869.59	3829.61	3054.22	2826.64	4331.84	3206.55	2952.82	2520.51
4	93.10	234.89	453.45	2892.43	4521.69	3056.37	2827.66	6049.73	3206.55	3033.58	2527.74
5	100.43	256.49	560.14	3016.75	4524.40	3103.80	2828.69	6161.08	3240.81	3146.63	2579.95
6	116.29	267.29	565.47	3052.27	5106.62	3132.78	2828.69	6198.19	3758.11	3240.84	2618.58
7	126.86	278.09	573.48	3090.33	5125.58	3132.78	2828.69	6198.19	4828.71	3256.99	2626.94
8	140.08	288.88	578.81	3120.78	5125.58	3132.78	2828.69	6320.14	5640.67	3259.68	2626.94
9	163.86	296.98	594.81	3123.31	5125.58	3132.78	2828.69	6325.22	6452.63	3261.89	2626.94
10	187.65	347.38	769.52	3125.85	5125.58	3132.78	2828.69	6331.54	6494.98	3272.90	2627.37
11	211.43	397.77	944.24	3128.39	5125.58	3132.78	2828.69	6337.87	6537.33	3283.91	2627.81
12	211.43	448.17	1168.29	3128.39	5125.58	3132.78	2828.69	6342.94	6543.97	3283.91	2627.81
13	211.43	550.76	1363.01	3136.00	5125.58	3132.78	2828.69	6358.17	6559.78	3283.91	2628.68
14	211.43	556.16	1475.03	3136.00	5125.58	3132.78	2828.69	6363.24	6568.89	3283.91	2628.68
15	211.43	569.66	1669.75	3137.72	5125.58	3132.78	2828.69	6368.32	6568.89	3283.91	2628.68
16	211.43	575.06	1808.45	3137.72	5125.58	3132.78	2828.69	6374.03	6568.89	3283.91	2628.68
17	211.43	578.66	1947.15	3137.72	5125.58	3132.78	2828.69	6379.75	6568.89	3283.91	2628.68
18	211.43	582.27	2085.85	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68
19	211.43	585.87	2136.53	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68
20	211.43	585.87	2197.88	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68
21	211.43	638.27	2197.88	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68
22	211.43	690.68	2197.88	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68
23	211.43	692.66	2197.88	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68
24	211.43	694.64	2197.88	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68
25	211.43	702.22	2197.88	3137.72	5125.58	3132.78	2828.69	6385.47	6584.70	3283.91	2628.68

Table A5. Daily gas production for Set III.

	Biogas Production (mL)										
Time (day)	**R1 (S)**	**R2 (WW)**	**R3 (WW)**	**R4 (O)**	**R5 (O)**	**R6 (3:1)**	**R7 (3:1)**	**R8 (6:1)**	**R9 (6:1)**	**R10 (9:1)**	**R11 (9:1)**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	196.90	279.09	243.08	1952.39	3185.95	2609.40	2017.46	1955.84	2077.93	1422.58	1587.47
2	196.90	279.09	243.08	1952.39	3185.95	2609.40	2017.46	1955.84	2077.93	1422.58	1587.47
3	18.50	289.63	232.22	2.54	491.76	1315.59	2.65	397.66	144.43	2.69	6.16
4	0.00	289.63	208.53	2.54	13.17	16.25	18.67	154.05	46.04	37.68	13.00
5	0.00	372.97	190.90	5.07	27.08	9.73	21.21	128.62	14.08	43.07	29.86
6	0.00	17.74	5.09	0.00	24.37	12.43	0.00	8.40	5.78	13.46	6.15
7	0.00	2.64	2.00	0.00	49.35	4.43	0.00	1.32	6.32	34.32	12.73
8	0.00	1.99	3.29	0.00	49.35	4.43	0.00	1.32	6.32	34.32	12.73
9	0.00	1.97	3.68	0.00	49.35	4.43	0.00	1.32	6.32	34.32	12.73
10	0.00	3.68	2.90	0.00	49.35	4.43	0.00	1.32	6.32	34.32	12.73
11	0.00	7.68	4.88	0.00	54.16	0.00	0.00	13.17	0.00	80.75	8.78
12	0.00	0.00	0.00	0.00	132.72	0.00	0.00	0.00	0.00	94.21	54.45
13	0.00	0.00	0.00	0.00	89.33	0.00	0.00	0.00	0.00	99.59	74.65
14	0.00	0.00	0.00	0.00	37.01	0.00	0.00	0.00	0.00	63.60	56.68
15	0.00	0.00	0.00	0.00	40.92	0.00	0.00	0.00	0.00	105.98	80.04
16	0.00	0.00	0.00	0.00	33.10	0.00	0.00	0.00	0.00	155.05	146.64
17	0.00	0.00	0.00	0.00	40.65	0.00	0.00	0.00	0.00	184.10	126.80
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	80.75	60.60
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	110.36	35.13
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	177.65	121.20
21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	172.27	129.11
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	197.11	169.49
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	180.58	161.42
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	171.42	145.11

Table A6. Cumulative biogas production for Set III.

	Biogas Production (mL)										
Time (day)	**R1 (S)**	**R2 (WW)**	**R3 (WW)**	**R4 (O)**	**R5 (O)**	**R6 (3:1)**	**R7 (3:1)**	**R8 (6:1)**	**R9 (6:1)**	**R10 (9:1)**	**R11 (9:1)**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	196.90	279.09	243.08	1952.39	3185.95	2609.40	2017.46	1955.84	2077.93	1422.58	1587.47
2	393.80	558.19	486.16	3904.78	6371.90	5218.80	4034.92	3911.68	4155.86	2845.16	3174.94
3	412.30	847.82	718.38	3907.31	6863.66	6534.38	4037.57	4309.34	4300.29	2847.85	3181.10
4	412.30	1137.45	926.91	3909.85	6876.83	6550.63	4056.25	4463.39	4346.33	2885.53	3194.10
5	412.30	1510.42	1117.81	3914.93	6903.91	6560.36	4077.45	4592.00	4360.40	2928.60	3223.96
6	412.30	1528.17	1122.91	3914.93	6928.29	6572.79	4077.45	4600.41	4366.18	2942.06	3230.10
7	412.30	1530.81	1124.91	3914.93	6977.63	6577.22	4077.45	4601.72	4372.50	2976.38	3242.84
8	412.30	1532.80	1128.20	3914.93	7026.98	6581.65	4077.45	4603.04	4378.82	3010.70	3255.57
9	412.30	1534.77	1131.88	3914.93	7076.33	6586.09	4077.45	4604.35	4385.14	3045.02	3268.31
10	412.30	1538.45	1134.77	3914.93	7125.68	6590.52	4077.45	4605.67	4391.46	3079.34	3281.04
11	412.30	1546.12	1139.65	3914.93	7179.84	6590.52	4077.45	4618.84	4391.46	3160.09	3289.83
12	412.30	1546.12	1139.65	3914.93	7312.56	6590.52	4077.45	4618.84	4391.46	3254.30	3344.28
13	412.30	1546.12	1139.65	3914.93	7401.89	6590.52	4077.45	4618.84	4391.46	3353.89	3418.93
14	412.30	1546.12	1139.65	3914.93	7438.90	6590.52	4077.45	4618.84	4391.46	3417.49	3475.61
15	412.30	1546.12	1139.65	3914.93	7479.82	6590.52	4077.45	4618.84	4391.46	3523.47	3555.65
16	412.30	1546.12	1139.65	3914.93	7512.92	6590.52	4077.45	4618.84	4391.46	3678.53	3702.29
17	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	3862.63	3829.08
18	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	3943.38	3889.68
19	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	4053.74	3924.81
20	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	4231.39	4046.02
21	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	4403.67	4175.12
22	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	4600.77	4344.61
23	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	4781.35	4506.03
24	412.30	1546.12	1139.65	3914.93	7553.57	6590.52	4077.45	4618.84	4391.46	4952.78	4651.14

Table A7. Biogas composition (CH_4 and CO_2) for Set I.

Date		**R1 (WW)**	**R2 (WW)**	**R3 (O)**	**R4 (O)**	**R5 (1:1)**	**R6 (1:1)**
08.02.2012	**CH_4 (%)**	42.10	21.49	1.02	1.68	0.86	0.90
	CO_2 (%)	19.22	16.64	45.29	57.51	81.92	63.83
09.02.2012	**CH_4 (%)**	27.30	1.17	0.00	0.00	0.00	0.00
	CO_2 (%)	18.57	18.07	22.46	59.50	68.71	0.00
12.02.2012	**CH_4 (%)**	9.14	19.30	0.00	0.00	0.00	0.00
	CO_2 (%)	2.17	4.21	41.18	54.92	81.66	65.97
14.02.2012	**CH_4 (%)**	47.80	24.11	0.00	0.00	0.00	0.00
	CO_2 (%)	13.88	5.68	35.05	60.47	81.48	0.00
15.02.2012	**CH_4 (%)**	51.18	44.29	0.00	0.00	0.00	0.00
	CO_2 (%)	26.01	7.07	0.00	0.00	0.00	0.00
16.02.2012	**CH_4 (%)**	60.83	35.26	0.00	0.00	0.00	0.00
	CO_2 (%)	8.07	3.70	0.00	0.00	0.00	0.00
17.02.2012	**CH_4 (%)**	51.69	25.05	0.00	0.00	0.00	0.00
	CO_2 (%)	6.62	2.90	0.00	0.00	0.00	0.00
20.02.2012	**CH_4 (%)**	48.81	58.21	0.00	0.00	0.00	0.00
	CO_2 (%)	17.91	4.63	0.00	0.00	0.00	0.00
22.02.2012	**CH_4 (%)**	42.17	-	0.00	0.00	0.00	0.00
	CO_2 (%)	3.37	-	0.00	0.00	0.00	0.00
23.02.2012	**CH_4 (%)**	64.37	-	0.00	0.00	0.00	0.00
	CO_2 (%)	12.66	-	0.00	0.00	0.00	0.00

Table A8. Biogas composition (CH_4 and CO_2) for Set II.

Date		R1 (S)	R2 (WW)	R3 (WW)	R4 (O)	R5 (O)	R6 (1:1)	R7 (1:1)	R8 (3:1)	R9 (3:1)	R10 (9:1)	R11 (9:1)
17.02.2012	**CH_4 (%)**	2.01	21.49	14.65	0.45	1.26	1.10	1.43	0.81	0.00	4.81	11.23
	CO_2 (%)	1.96	28.05	23.52	23.61	96.48	58.45	51.93	34.54	3.34	18.30	18.99
19.02.2012	**CH_4 (%)**	5.37	41.42	38.77	0.88	0.36	0.00	0.00	0.00	0.00	17.43	22.40
	CO_2 (%)	2.61	35.07	34.11	50.23	86.31	29.12	19.92	60.07	9.60	42.86	21.82
21.02.2012	**CH_4 (%)**	5.60	1.63	38.68	0.00	0.00	0.00	0.00	1.97	0.00	16.79	24.40
	CO_2 (%)	2.83	50.62	14.86	30.59	99.54	36.59	0.00	97.83	16.56	47.48	34.21
23.02.2012	**CH_4 (%)**	1.99	52.12	59.80	0.00	0.00	0.00	0.00	5.83	0.00	12.31	16.47
	CO_2 (%)	0.90	11.17	16.56	45.46	99.42	45.08	0.00	93.70	53.46	12.55	5.88
25.02.2012	**CH_4 (%)**	9.18	59.93	60.72	0.00	0.00	0.00	0.00	44.72	0.00	30.74	-
	CO_2 (%)	2.70	12.80	-35.38	69.41	0.46	0.00	0.00	-48.38	83.44	9.46	-
27.02.2012	**CH_4 (%)**	4.52	20.82	58.13	0.00	0.00	0.00	0.00	53.57	15.47	9.40	64.58
	CO_2 (%)	15.67	2.87	-35.41	54.54	0.58	0.00	0.00	-91.99	31.07	47.31	16.20
29.02.2012	**CH_4 (%)**	8.80	63.05	60.16	0.00	0.00	0.00	0.00	57.05	27.45	9.29	-
	CO_2 (%)	24.38	11.01	17.09	30.59	0.00	0.00	0.00	37.76	-26.36	71.84	-
01.03.2012	**CH_4 (%)**	5.99	79.96	56.90	0.00	0.00	0.00	0.00	51.61	30.96	39.61	17.27
	CO_2 (%)	9.33	13.25	29.67	34.38	0.00	0.00	0.00	42.95	47.48	43.04	6.62
02.03.2012	**CH_4 (%)**	0.69	68.72	72.34	0.00	0.00	0.00	0.00	50.65	27.93	0.00	0.00
	CO_2 (%)	4.80	23.76	10.91	24.22	0.00	0.00	0.00	36.27	35.10	0.00	0.00
05.03.2012	**CH_4 (%)**	-	74.00	62.08	0.00	0.00	0.00	0.00	37.73	27.94	34.71	-
	CO_2 (%)	3.28	10.94	10.33	0.00	0.00	0.00	0.00	43.62	35.98	15.43	-
06.03.2012	**CH_4 (%)**	0.94	55.53	47.71	0.00	0.00	0.00	0.00	36.43	20.10	0.00	0.00
	CO_2 (%)	4.11	9.61	8.81	0.00	0.00	0.00	0.00	44.88	32.68	0.00	0.00
07.03.2012	**CH_4 (%)**	0.00	52.68	37.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CO_2 (%)	0.00	23.28	6.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.03.2012	**CH_4 (%)**	0.00	61.20	69.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	CO_2 (%)	0.00	8.45	10.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table A9. Biogas composition (CH_4 and CO_2) for Set III.

Date		**R1 (S)**	**R2 (WW)**	**R3 (WW)**	**R4 (O)**	**R5 (O)**	**R6 (3:1)**	**R7 (3:1)**	**R8 (6:1)**	**R9 (6:1)**	**R10 (9:1)**	**R11 (9:1)**
11.03.2012	**CH_4 (%)**	17.13	44.73	45.99	0.00	12.09	0.00	5.85	14.46	14.38	33.03	33.06
	CO_2 (%)	7.18	50.18	9.41	12.51	87.57	70.89	24.17	65.92	42.56	23.53	21.29
13.03.2012	**CH_4 (%)**	12.96	72.07	54.45	0.00	12.68	13.93	1.56	32.21	13.68	35.35	11.37
	CO_2 (%)	5.14	24.74	9.50	6.94	85.10	80.55	9.14	58.92	45.83	31.52	40.56
14.03.2012	**CH_4 (%)**	6.92	46.22	25.92	0.00	0.00	12.10	0.92	25.69	21.66	37.51	9.15
	CO_2 (%)	2.26	10.73	44.23	51.77	85.21	83.44	46.40	69.66	51.69	42.18	40.72
15.03.2012	**CH_4 (%)**	3.45	59.06	16.35	0.00	0.00	11.47	2.77	23.29	7.34	39.41	9.90
	CO_2 (%)	1.28	23.83	52.91	49.33	87.08	85.21	4.08	68.90	3.13	13.02	2.76
19.03.2012	**CH_4 (%)**	0.00	54.27	20.17	0.00	0.00	18.19	0.00	26.66	11.52	54.92	43.61
	CO_2 (%)	0.00	18.66	6.86	0.00	76.08	72.96	0.00	45.98	65.92	15.45	12.35
20.03.2012	**CH_4 (%)**	0.00	65.54	11.73	0.00	0.00	31.37	0.00	27.54	16.35	45.19	34.95
	CO_2 (%)	0.00	23.80	4.94	0.00	82.92	51.86	0.00	53.81	70.21	16.54	20.53
22.03.2012	**CH_4 (%)**	0.00	25.20	3.47	0.00	0.00	24.20	0.00	30.22	28.59	40.60	24.92
	CO_2 (%)	0.00	9.00	16.94	0.00	88.11	62.55	0.00	49.28	46.28	22.97	6.20
27.03.2012	**CH_4 (%)**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	60.07	30.23
	CO_2 (%)	0.00	0.00	0.00	0.00	58.81	0.00	0.00	0.00	0.00	12.75	7.68
28.03.2012	**CH_4 (%)**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	58.27	46.57
	CO_2 (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.79	7.08
29.03.2012	**CH_4 (%)**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	55.50	29.19
	CO_2 (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.28	15.43
30.03.2012	**CH_4 (%)**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	56.59	41.69
	CO_2 (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.19	5.18
02.04.2012	**CH_4 (%)**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	62.49	46.15
	CO_2 (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.17	3.39