IMPACT OF RICE HUSK AS LITTER MATERIAL ON BIOGAS GENERATION FROM CHICKEN MANURE

by

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B.S. in Chemistry, Marmara University, 2007

Submitted to the Institute of Environmental Sciences in partial fulfillment of
the requirements for the degree of
Master of Science
in
Environmental Sciences

Bogazici University

2011

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Prof. Dr. Turgut T. Onay and Assoc. Prof. Dr. Burak Demirel for their valuable, guidance, support, encouragement and patience during this study.

I am also thankful to Gülhan Özkösemen, Elif Hot, Filiz Ayılmaz and Mehmet Ali Küçüker for their support and suggestions during my laboratory studies and measurements.

I would like to thank Berkin İmer from Pales Engineering Construction Mining and Consulting Industrial Commercial Limited Company for his support and throughout the study.

I would like to thank Birgül Aras from AKSA Akrilik Kimya A.Ş. for her support tolerance in order for me to complete this study.

I would like to thank Heinrich Böll Stiftung Foundation for their support and valuable comments.

This study was supported by Boğaziçi University Research Fund with project number 5067.

I would like to thank to my family and my friends for their love and encouragement during this thesis.

On a personal note, I would also express my special thanks to Meriç Bahar. He was always with me giving his patience, tremendous support, understanding and constant encouragement.

ABSTRACT

Last decades energy required in almost all fields of everyday life is provided mostly by expensive imported energy sources in Turkey. In order to meet the increasing demand of energy, it is compulsory to find alternative sources. Research activities on the biogas production by using animal and agricultural residues are becoming attractive research area due to abundant availability of them in Turkey.

The increase in production and concentration of intensive livestock activities are producing an important amount of organic wastes. Poor management of these wastes could cause serious environmental and health problems. It is necessary to implement proper management options to minimize the risks. In addition, agriculture industry that generates a big amount of wastes (rice husk, straw, grass) is also producing a surplus of residues that is necessary to treat. In Turkey there are approximately 17.000 poultry farms, with a total capacity reaching 235 million poultry. According to these statistics, in Turkey, poultry manure potential is approximately 5.5 million ton/year.

In this study the impact of different amount of rice husk as a litter material on biogas generation from anaerobic digestion of chicken manure was investigated using batch reactors. For this purpose chicken manure and different amount of rice husk were mixed and digestate under mesophilic anaerobic conditions. Within the scope of this study, it was found that cumulative biogas generation of the reactors was affected negatively with increasing concentrations of rice husk. On the other hand, composition of the biogas, in terms of methane and carbon dioxide contents, methane concentration in biogas increased with increasing RH ratio.

ÖZET

Türkiye'de her alanda gereksinim duyulan enerji, son yıllarda çogunlukla dış ülkelerden ithal edilmesi yolu ile pahalı bir sekilde temin edilmektedir. Artmakta olan enerji ihtiyacını karsılamak amacı ile alternatif enerji kaynaklarının tespit edilmesi kaçınılmaz olmustur. Türkiye'de bol olarak bulunması nedeni ile tarım artıkları ve hayvan gübresi ile biyogaz üretimi üzerine araştırma çalışmaları önem kazanmıştır.

Canlı hayvan üretiminin artması ile önemli miktarda organik atık oluşmaktadır. Bu atıkların yönetiminin düzgün yapılmaması ciddi çevre ve sağlık problemlerine neden olabilmektedir. Riskleri minimuma indirmek için bu atıkların doğru yönetimi çok önemlidir. Ek olarak, tarım endüstrisi de, işlenmesi gereken büyük miktarlarda atık (kavuz, saman, çimen) üretmektedir. Türkiye' de 235 milyon kanatlı hayvan kapasitesi ile yaklaşık 17.000 kanatlı çiftliği bulunmaktadır. Bu veriler ışığında, Türkiye' de kanatlı gübresi potansiyeli yaklaşık 5.5 milyon ton/yıl' dır.

Bu çalışmada, altlık olarak kullanılan kavuzun farklı oranlarının, tavuk gübresinin anaerobik parçalanmasından oluşan biyogaz oranına etkisi araştırılmıştır. Bu amaç ile tavuk gübresi ve farklı oranlarda ki kavuz mezofilik anaerobik şartlar altında karıştırılmıştır. Çalışmanın amacı doğrultusunda, kümülatif biyogaz oluşumunun, artan kavuz oranı ile azaldığı gözlemlenmiştir. Diğer taraftan, metan ve karbondioksit bileşimi anlamında biyogaz kompozisyonunun, metan konsantrasyonunun artan kavuz oranı ile arttığı gözlemlenmiştir.

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Symbol	Explanation	Units used
ATP	Adenosine Triphosphate	
Ca	Calcium	
Cd	Cadmium	
CM	Chicken Manure	
COD	Chemical Oxygen Demand	mg L^{-1}
Cu	Copper	
DNA	Deoxyribonucleic acid	
Fe	Ferrous	
HRT	Hydraulic Retention Time	hour
K	Potassium	
Mg	Magnesium	
Mn	Manganese	
Na	Sodium	
Ni	Nickel	
NH_4^+-N	Ammonium nitrogen	mg L^{-1}
NRB	Nitrogen Reducing Bacteria	
PO_4^{3-}	Orthophosphate	mg L^{-1}
RH	Rice Husk	
RNA	Riboxynucleic Acid	
SRB	Sulphate Reducing Bacteria	
TS	Total Solids	%
VS	Volatile Solid	%
TKN	Total Kjeldahl Nitrogen	
TOC	Total Organic Carbon	mg L^{-1}
TP	Total Phosphorus	%
Pb	Lead	
USEPA	United States Environmental Protection Agency	
Zn	Zinc	

1. INTRODUCTION

Since the 1970s, due to the oil price crisis and the increase in the need for the limited resources of fossil fuels, countries have focused on the development of technologies that use renewable energy sources like biomass, geothermal, hydropower, wind and solar power. The renewable energy resources are attractive, since they are unlimited and help preventing pollution and emission of greenhouse gases (Demirbas et al., 2006; Celiktas et al., 2009).

Biomass is a renewable energy source and as a national energy policy its importance will increase in the near future. Among the other technologies to convert biomass into energy; biogas production through anaerobic digestion is one of the oldest and most promising technologies to gain energy (Chynoweth et al., 2001). Biogas, which is omit a mixture of methane and carbon dioxide, organic slurry as digested residue and other inorganic products are generated as a result of the breakdown of organic material by microbial population that lives in an oxygen free environment. The biogas can be used to produce both electrical power and heat and the digested residue can be re-circulated as bio-fertilizer to the fields (Björnsson et al., 2001)

Among the renewable energy resources, especially biomass is an attractive source for Turkey. Agricultural production generates biomass as wastes (e.g., animal manure and crop residues), which have disposal costs as well as adverse environmental impacts. The use of agricultural residues for fuels is not only beneficial as a sustainable energy source, but solves disposal problems and reduces greenhouse gases (Kheshgi et al., 2000). There are approximately 17.000 poultry farms, with a total capacity reaching 235 million poultry (YUM-BIR, 2011). According to these statistics, in Turkey, poultry manure potential is approximately 5.5 million ton/year (EIE, 2011). This huge amount of manure causes environmental problems such as nutrient leaching and odor when it is poorly managed. On the other hand if animal manure is managed properly; it can be a valuable resource for renewable energy production as biogas and a source of nutrients for agriculture (Holm-Nielsen et al., 2009). From this point of view, this study on the biogas production by

anaerobic digestion of chicken manure is becoming an attractive research area due to environmental awareness, disposal problems due to abundant availability of the waste. Especially in Turkey, high proportion (163.5 million) of 235 million poultry is broiler chickens. For broiler production litter material is very important in controlling of the room conditions and providing comfortable medium for animals. Rice husk as litter material has been rapidly gaining importance and is now being commonly used because of its cost and proper physical properties make it a preferable litter base (Embury, 2004). On the other hand, rice husk has relatively low biodegradability due to its high lignin content (crude fiber, 32-50%; cellulose 34-44%; lignin 21-47%) (Iyagba et al., 2009). Anaerobic digestion is typically a proper option for treatment of chicken manure (0.35-0.60 m^3 kg^{-1} VS biogas yield; 60-80% CH_4 content) (Steffen et al., 1998). In regards to the base bedding materials rice husk is relatively poor (Kalra et al., 1986).

The objective of this study is to investigate the impact of different amount of rice husk as a litter material on biogas generation from anaerobic digestion of chicken manure. For this purpose chicken manure and different amount of rice husk were mixed run under mesophilic anaerobic conditions.

There is a need for such study in Turkey, since there is a high chicken manure potential that must be managed together with rice husk which is widely used as litter material. As a renewable energy source, biogas production from chicken manure is a useful management option, energy independence and environmentally friendly.

2. THEORETICAL BACKGROUND

2.1. Fundamentals of Anaerobic Degradation

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 treatment systems can operate at different temperatures and convert a broad variety of wastes, such as wastewater, food and beverage, pharmaceutical, pulp and paper, petrochemical, alcohol distilleries, dairy, textile and leachate (Macarie, 2000). Anaerobic digestion is also used for municipal wastewaters, solid wastes, agricultural wastes and manures. Anaerobic treatment process is a useful technique ability to convert highly objectionable wastes into useful products (McCarty, 2001). 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).

Anaerobic degradation process was reported by some authors as a Nine-stage Model (Harper and Pohland, 1986) which has been listed as follows and given in Figure 2.1.

- Hydrolysis of organic polymers to intermediate organic monomers,
- Fermentation of organic monomers,
- Oxidation of propionic and butyric acids and alcohols by obligate H_2 producing acetogens,
- Acetogenic respiration of bicarbonate by homoacetogens,
- Oxidation of propionic and butyric acids and alcohols by sulfate reducing bacteria (SRB) and nitrate reducing bacteria (NRB),
- Oxidation of acetic acid by SRB and NRB,
- Oxidation of hydrogen by SRB and NRB,
- Acetoclastic methane formation,

- Methanogenic respiration of bicarbonate.

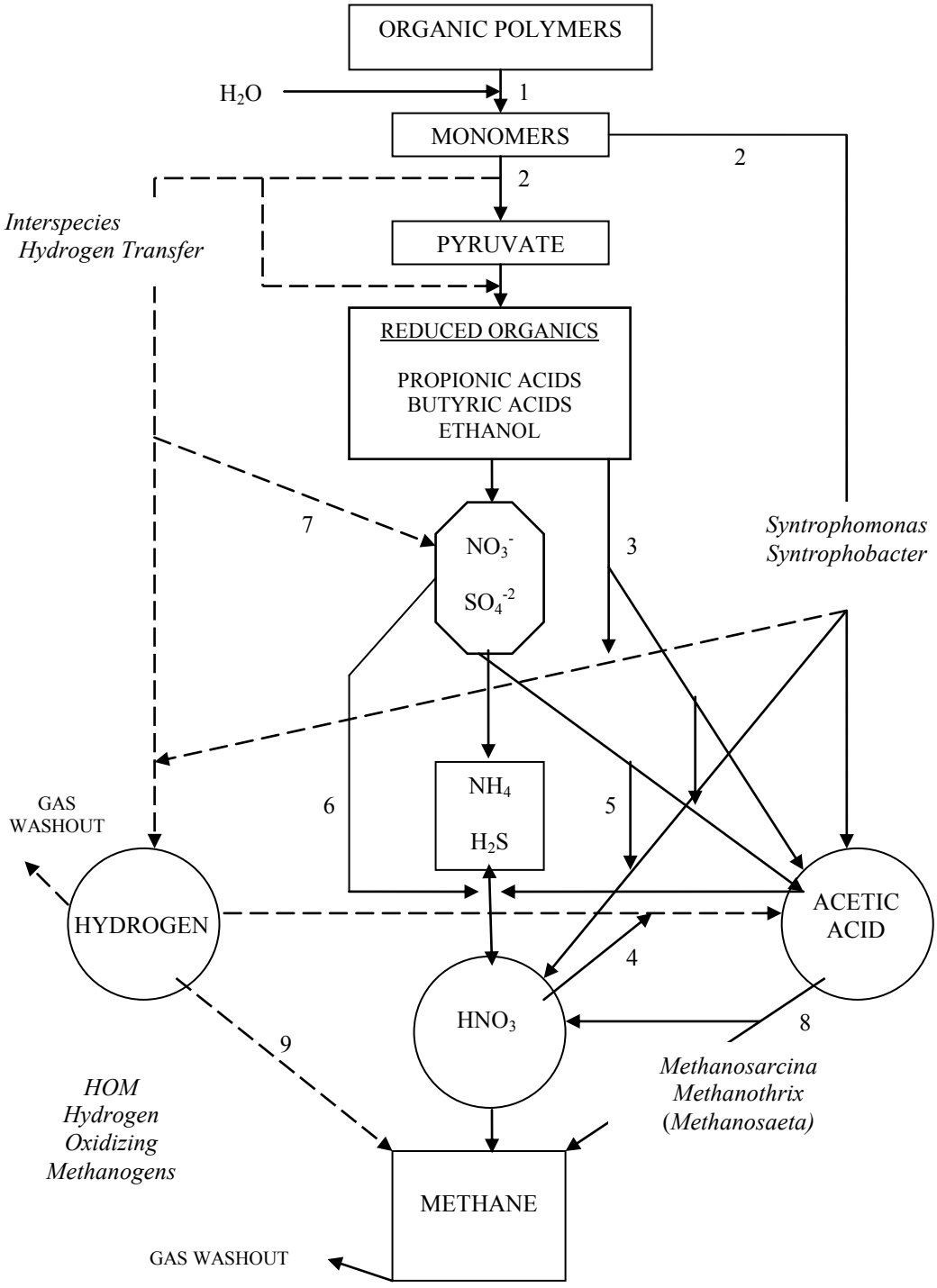

Figure 2.1. Substrate conversion patterns associated with the anaerobic digestion (Harper and Pohland, 1986).

2.1.1. Phases of Anaerobic Digestion

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 classified into four major phases including hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

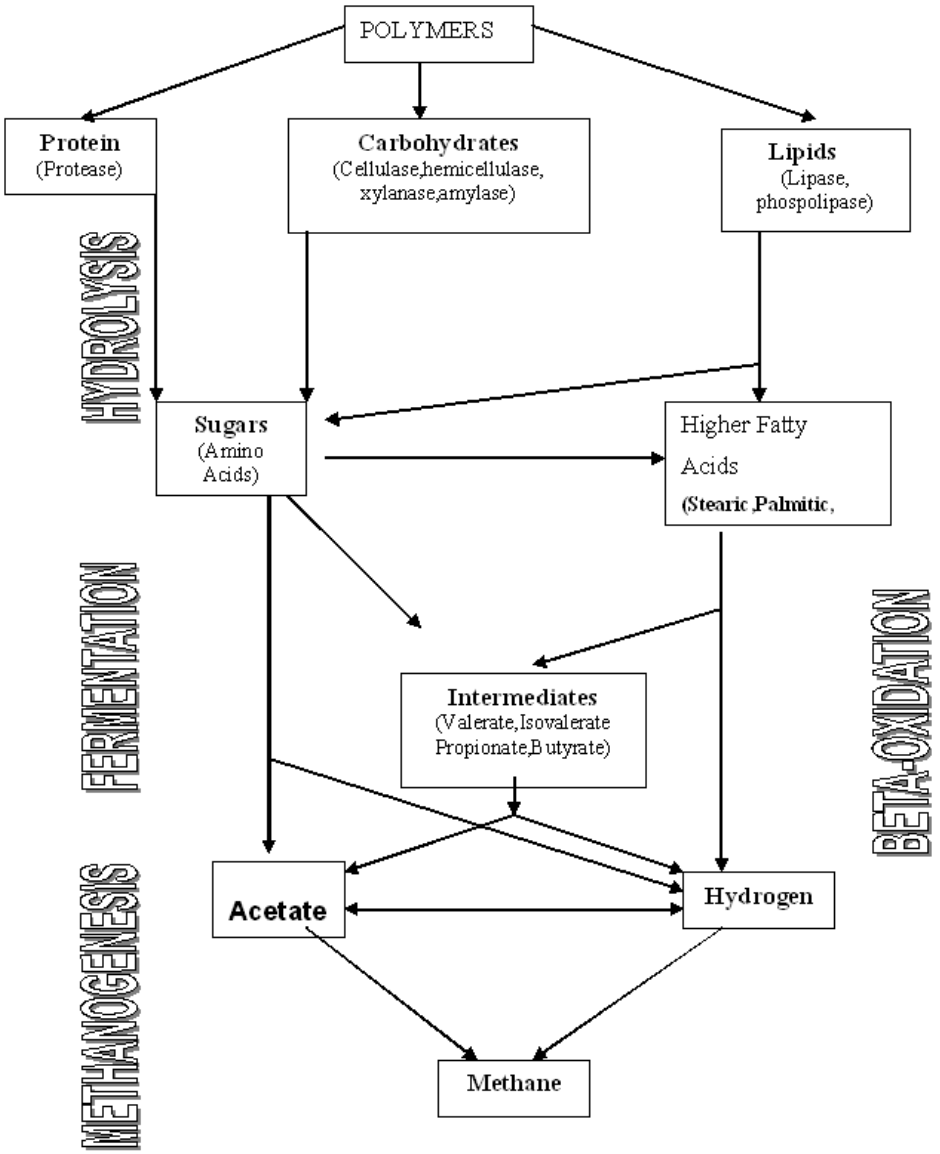

Figure 2.2. The breakdown of organic polymers (Stronach et al., 1986)

2.1.1.1. Hydrolysis. Hydrolysis is the first step in anaerobic degradation process. Complex organic material (i.e. carbohydrates, cellulose, lipids, and proteins, etc.) is catalyzed and chemically converted into simpler compounds (i.e. sugars, glycerine and fatty acids, and amino-acids) through the function of hydrolytic enzymes, such as cellulose, protease, and lipase, which split the long molecular chains into monomer units (Parkin and Owen 1986; Speece 1996). Subsequently, these simple compounds are absorbed into a variety of micro-organisms for use in cellular metabolism and catabolism. This stage is relatively slow and may be limiting in anaerobic digestion of the organics recalcitrant to biodegradation, such as lignin. Stabilization does not occur during hydrolysis; the organic matter is simply converted into a soluble form that can be utilized by a future consortium of bacteria (Parkin and Owen 1986). The activity of these extracellular enzymes is influenced by waste character and pH.

2.1.1.2. Acidogenesis. The acid-forming or acidogenesis stage involves acid-forming fermenters, hydrogen producers and acetogens (acetic acid-forming). Once complex organics are hydrolyzed, these various bacteria convert sugars, amino acids and fatty acids to low molecular weight organic acids, hydrogen, and carbon dioxide. The products formed vary with the types of bacteria as well as environmental conditions. It was reported that acetate is the most vital compound produced in the fermentation of organic substrates with propionate production of secondary consequence (Sorensen et al., 1981). The community of bacteria responsible for acid production may include facultative anaerobic bacteria, strict anaerobic bacteria, or both (i.e. *Bacteroides, Bifidobacterium, Clostridium, Lactobacillus, Streptococcus,* etc.). Single amino acids are converted by *Clostridia, Mycoplasmas* and *Streptococci* while butanol, butyric acid, acetone and iso-propanol are generally produced by the bacteria of the genera *Clostridum* and *Butyribacterium* under anaerobic conditions (i.e *Clostridium butyricum* produces butyrate, *Costridium acetobutylicum)* mainly produces acetone and butanol and *Clostridium butylicum* produces butanol in addition to hydrogen, carbondioxide and iso-propanol (Novaes, 1986).

2.1.1.3. Acetogenesis. The short chain fatty acids other than acetate namely, propionic acid and butyric acid, are converted to acetate, hydrogen (H_2), and carbon dioxide (CO_2) by the obligate hydrogen-producing bacteria. They produce acetic acid, carbondioxide and

hydrogen from propionate, butyrate and other higher fatty acids by the β-oxidation process. Fatty acids having more than two carbons lose one molecule at each reaction till all fatty acids are converted to acetate molecules. Acetic acid producing bacteria are *Methanobacterium bryantii, Desulfovibrio Syntrophobacter wolinii* (responsible for acetic acid production from propionic acid) (Stronach et al., 1986; Malina et al., 1992), *Syntrophomonas wofei* (responsible for acetic acid production from butyric, caproic and valeric acids) and *Syntrophus buswellii* (Gujer et al., 1983; Malina et al., 1992).

2.1.1.4. Methanogenesis. In the final stage, the end products of the previous step are converted in to methane and carbon dioxide by methanogens via two conversion mechanisms including decarboxylation of acetic acid and reduction of carbon dioxide in the absence of other electron acceptors such as oxygen nitrate, and sulfate except bicarbonate and protons as terminal electron acceptors (Garcia et al., 2000; De Bok et al., 2004; Stams et al., 2006).

Formic acid, acetic acid, methanol, and hydrogen can all be used as energy sources by the various methanogens with the major types of methanogens utilizing these various sources described as acetotrophic, methyltrophic, or hydrogenotrophic, respectively. The primary route is the fermentation of the major product of the acid forming phase, acetic acid, to methane and carbon dioxide, and is accomplished by the acetotrophs. So far, only the *Methanosarcinales*, specifically *Methanosarcina* and *Methanosaeta*, have been shown to be capable of utilizing acetate with *Methanasarcina* being uniquely capable of utilizing all three forms of substrate for methane production and as such is not an obligatory acetotroph (Madigan et al. 2003a). It was also verified that *Methanosarcina* will preferentially utilize H_2/CO_2 when both H_2/CO_2 and acetate are present in culture, and not until the depletion of H_2/CO_2 can *Methanosarcina* start to uptake acetate (Ferguson and Mah 1983a). About two thirds of methane gas is derived from acetate conversion while the remaining 1/3 is primarily a result of hydrogenotrophs (Novaes 1986; Morgan *et al.* 1991). The presence of hydrogen in solution inhibits oxidation, thereby requiring that hydrogenotrophic bacteria are present to ensure more complete conversion (Novaes 1986; Parkin and Owen 1986).

It has been reported that at least ten substrates can be converted to methane by pure cultures of methanogens. Three classes of compounds including CO_2-type substrates, methyl substrates and acetate are listed in Table 2.1.

Table 2.1. Substrates converted to methane by various methanogenic *Archaea* (Madigan et al., 2002).

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

CO_2-type substrates including CO_2, formate and carbon monoxide are reduced to methane by bacteria. Although the reduction of carbon dioxide to methane is generally hydrogen dependent, other substrates in this class can provide the electrons for CO_2 reduction.

$$CO_2+4H_2 \rightarrow CH_4+2H_2O \qquad \Delta G^\circ=-131kJ \qquad (2.1)$$

Methyl group substances which are listed above as the second class of methanogenic substrates are converted to methane by two mechanisms. The formation of

methane by reducing methyl group substances using an external electron donor such as H_2 is the first mechanism. In the conversion equations methanol (CH_3OH) is used as a model methyl substrate.

$$CH_3OH+H_2 \rightarrow CH_4+H_2O \qquad \Delta G°= -113 \text{ kJ} \qquad (2.2)$$

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

$$4CH_3OH \rightarrow 3CH_4+CO_2+2H_2O \qquad \Delta G°= -319 \text{ kJ} \qquad (2.3)$$

Acetate is the final methanogenic substrate. The conversion mechanism of acetate to methane and carbon dioxide called the acetotrophic reaction (Pavlostathis and Gomez, 1991).

$$CH_3COO^-+H_2O \rightarrow CH_4+HCO_3^- \qquad \Delta G°= -31 \text{ kJ} \qquad (2.4)$$

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

Characteristics and Taxonomy of Methanogens. Methanogens are microorganisms that produce methane as the end-product of their anaerobic respiration. All methanogens are strictly anaerobic *Archaea* belonging to *Euryarchaeota*. They are a large and diverse group, all of which are obligate methane producers that obtain most of their energy from methanogenesis (Table 2.2.).

Methanogens have been cultivated from a wide variety of anaerobic environments. In addition to temperate habitats, they are also common in environments of extreme

temperatures, salinity and pH. The common methanogenic habitats include marine sediments, freshwater sediments, flooded soils, human and animal gastrointestinal tracts, termites, anaerobic digesters, landfill, geothermal systems and heartwood of trees.

Table 2.2. Characteristics of methanogenic *Archaea* (Madigan et al., 2002).

Order	**Morphology**	**Substrate for methanogenesis**
Methanobacteriales		
Methanobacterium	Long rods	H_2+CO_2, formate
Methanobrevibacter	Short rods	H_2+CO_2, formate
Methanosphaera	Cocci	Methanol+H_2
Methanothermus	Rods	H_2+CO_2, can also reduce S^0; hyperthermophile
Methanococcales		
Methanococcus	Irregular cocci	H_2+CO_2, pyruvate+CO_2, formate
Methanomicrobiales		
Methanomicrobium	Short rods	H_2+CO_2, formate
Methanogenium	Irregular cocci	H_2+CO_2, formate
Methanospirillum	Spirilla	H_2+CO_2, formate
Methanoplanus	Plate-shaped cells	H_2+CO_2, formate
Methanocorpusculum	Irregular cocci	H_2+CO_2, formate, alcohols
Methanoculleus		H_2+CO_2, alcohols, formate
Methanosarcinales		
Methanosarcina	Large irregular cocci in packets	H_2+CO_2, methanol, methylamines, acetate
Methanolobus	Irregular cocci in aggregates	Methanol, methylamines
Methanohalobium	Irregular cocci	Methanol, methylamines;halophilic
Methanococcoides	Irregular cocci	Methanol, methylamines
Methanohalophilus	Irregular cocci	Methanol, methylamines, methyl sulfides; halophile
Methanosaeta	Long rods to filaments	Acetate
Methanopyrales		
Methanopyrus	Rods in chains	CO_2, hyperthermophile, growth at 110°C

Morphologically, the methanogens exhibit a wide variety of shapes and sizes, including rods, regular and irregular cocci, long-chained rods, spirilla, sarcina and irregular unusual flattened plates. Motility is sometimes present. Some species can aggregate in clusters. Several species of *Methanosarcina* and *Methanosaeta* contain gas vacuoles. The gram reaction can be positive or negative even within members of the same genus (Garcia et al., 2000).

Methanogens have unique cell membrane lipid and lack a rigid cell wall. They are capable of degrading substrates such as organic wastes and produce methane by their specialized coenzymes. Coenzymes that are unique to methane forming microorganisms are coenzyme M and the nickel containing coenzymes. Coenzyme-M is used to reduce CO_2 to methane. The nickel-containing coenzymes are important hydrogen carriers in methanogens (Thauer and Shima, 2006).

Even though, methanogens are very diverse, they are only capable of utilizing a small number of substrates. The substrates are limited to three major types including CO_2, methyl-group containing compounds, and acetate. Most organic substances, i.e, carbohydrates and long-chain fatty acids and alcohols, are not appropriate substrates for methanogens (Table 2.3.). These compounds must first be processed by anaerobic bacteria or eukaryotes to produce the substrates used by methanogens. Thus, in most methanogenic environments, most of the energy available for growth is utilized by these nonmethanogenic organisms (Thauer and Shima, 2006).

Table 2.3. Methanogenic orders (Karakashev et al., 2005).

Order	**Physiology**
Methanopyrales	Hydrogenotrophic; hyperthermophilic
Methanobacteriales	Hydrogenotrophic; mesophilic or thermophilic
Methanococcales	Hydrogenotrophic; mesophilic or thermophilic
Methanomicrobiales	Hydrogenotrophic; mesophilic
Methanosarcinales	Strict aceticlastic (*Methanosaetaceae*), aceticlastic or hydrogenotrophic (*Methanosarcinaceae*); mesophilic or thermophilic

Most methanogens are hydrogenotrophs that can reduce CO_2 to methane with H_2 as the primary electron donor. Many hydrogenotrophic methanogens are also able to use formate as the major electron donor. Besides, some hydrogenotrophic methanogens can also use secondary alcohols, such as 2-propanol, 2-butanol, and cyclopentanol, as electron donors. A small number of methanogens can also use ethanol.

Methyl-group containing compounds, including methanol, methylated amines (monomethylamine, dimethylamine, trimethylamine, and tetramethylammonium), and methylated sulfides (methanethiol and dimethylsulfide) are other types of substrates that are used by methanogenic *Archaea*. Methanogens that are able to use methylated compounds, or methylotrophic methanogens, are limited to the order *Methanosarcinales*, except for *Methanosphaera* species, which belong to the order *Methanobacteriales* (Fricke et al, 2006).

The third type of substrate that is used by methanogens is acetate. Acetate is a major intermediate in the anaerobic food chain, and as much as 70% of the biologically generated methane is derived from acetate. Surprisingly, only two genera are known to use acetate for methanogenesis: *Methanosarcina* and *Methanosaeta*. They carry out an aceticlastic reaction that splits acetate, oxidizing the carboxyl-group to CO_2 and reducing the methyl group to CH_4. *Methanosarcina* is a relative generalist that prefers methanol and methylamine to acetate, and many species also utilize H_2. *Methanosaeta*, which is thought to use only acetate although recent studies revealed that *Methanosaeta* might be metabolically more diverse than previously thought (Smith and Smith, 2007), and it is a superior acetate utilizer. It can use acetate at concentrations as low as 5–20 μM, while *Methanosarcina* requires a minimum concentration of about 1.0 mM. The difference of acetate affinity is probably due to differences in the first step of acetate metabolism. *Methanosarcina* uses the low-affinity acetate kinase (AK)-phosphotransacetylase (PTA) system to activate acetate to acetyl-CoA, while *Methanosaeta* uses the high-affinity adenosine monophosphate (AMP) – forming acetyl-CoA synthetase. Moreover, based on their genome sequences, these two genera probably have different modes of electron transfer and energy conservation, even though the main steps in the methanogenesis pathway are likely to be similar (Liu and Whitman, 2008).

Table 2.4. Typical organisms of methanogenesis reactions (Zinder et al., 1990; Liu and Whitman, 2008).

Reaction	Organisms
I. CO_2-type	
$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	Most methanogens
$4\ HCOOH \rightarrow CH_4 + 3\ CO_2 + 2\ H_2O$	Many hydrogenotrophic
$CO_2 + 4$ isopropanol$\rightarrow CH4 + 4$ acetone $+ 2\ H_2O$	methanogens
$4\ CO + 2H_2O \rightarrow CH_4 + 3\ CO_2$	Some hydrogenotrophic
	methanogens
	Methanothermobacter and
	Methanosarcina
II. Methylated C1 compounds	
$4\ CH_3OH \rightarrow 3\ CH_4 + CO_2 + 2\ H_2O$	*Methanosarcina* and other
	Methanogens
$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	methylotrophic
	Methanomicrococcus blatticola and
$2\ (CH_3)_2\text{-}S + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 2\ H_2S$	*Methanosphaera*
$4\ CH_3\text{-}NH_2 + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 4\ NH_3$	Some methylotrophic methanogens
$2\ (CH_3)_2\text{-}NH + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 2\ NH_3$	Some methylotrophic methanogens
$4\ (CH_3)_3\text{-}N + 6\ H_2O \rightarrow 9\ CH_4 + 3\ CO_2 + 4\ NH_3$	Some methylotrophic methanogens
$4\ CH_3NH_3Cl + 2\ H_2O \rightarrow 3\ CH_4 + CO_2 + 4\ NH_4Cl$	Some methylotrophic methanogens
	Some methylotrophic methanogens
III. Acetate	
$CH_3COOH \rightarrow CH_4 + CO_2$	*Methanosarcina* and *Methanosaeta*

2.2. Environmental and Operational Factors Affecting Anaerobic Processes

2.2.1. Temperature

Temperature is an important parameter for microbial systems. It affects the system in several ways including ionization equilibrium, solubility of substrates, substrate removal rate and other constants such as specific growth rate, decay biomass yield, and half saturation constant. Anaerobic processes are proven to be strongly affected by the temperature variations. Especially conversion of acetate to CH_4 is known as more sensitive to temperature than the acetate forming process (Stover et al., 1994). Methane production has been documented under a wide range of temperatures. Differentiation is generally made between three temperature ranges:

- the psychrophilic temperature range lies below 20°C,
- the mesophilic temperature range between 20°C and 40°C and
- the thermophilic temperature range above 40°C.

Thermophilic digestion generally operates at temperature ranges of 50–65°C. It allows higher loading rates and is also conductive to greater destruction of pathogens. One drawback of thermophilic digestion is its higher sensitivity to toxicants. Because of their slower growth as compared with acidogenic bacteria, methanogenic bacteria are very sensitive to small changes in temperature, which leads to a decrease of the maximum specific growth rate while the half-saturation constant increases (Noike et al., 1985; Speece, 1983).

2.2.2. pH

pH is also a significant parameter that affects the solubility of substances and the reaction behaviour of microorganisms. As a consequence it influences performance of anaerobic digestion. Most methanogenic bacteria function in a pH range between 6.1 and 7.5. Deviations from this optimum may result in excess production and accumulation of acidic or basic conversion products such as organic fatty acids or ammonia. It has been

shown that pH below 6.0 are inhibitory to methanogenic bacteria while acid forming bacteria can live at this pH and keep producing volatile fatty acids despite low pH, therefore making the environmental conditions worse (Pohland and Suidan, 1987). Acidogenic bacteria produce organic acids, which tend to lower the pH of the bioreactor (Malina and Pohland, 1992). Under normal conditions, this pH reduction by the acidogenic bacteria is buffered by the bicarbonate which is produced by methanogens. Under adverse environmental conditions, the buffering capacity of the system can be upset, eventually stopping the production of methane. An increase in volatile acid level serves as an early indicator of system upset.

2.2.3. Nutrients

In addition to macronutrients N, P, and S trace amounts of elements called micronutrients besides nitrogen and phosphorus are also required for methanogen's fundamental bacterial metabolism (Speece and Parkin, 1983). Iron, nickel, magnesium, calcium, sodium, barium, tungstate, molybdate, selenium and cobalt are considered as necessary for various conditions of active methanogenesis (Henze and Harremoes, 1983). Some of the elements such as selenium, tungsten and nickel are significant in the enzyme systems of acetogenic and methanogenic bacteria (Stronach, 1986).

2.2.4. Mixing

Mixing allows the complete contact between the reactor contents and the biomass. It also reduces the inhibitory effects of local build-up of VFAs and other digestion products. Moreover, mixing prevents settling which could cause reduction of substrate and microorganism contact.

2.2.5. Alkalinity

Alkalinity is the ability of a system to buffer the undesired effects of volatile and other acids which tend to depress the pH below desired level. High alkalinity concentration indicates that the system is safeguarded against pH fluctuations, while low alkalinity

indicates that the high concentrations of acids may lower the pH so that the biological activity may cease. In the literature, it was reported that the alkalinity should be above 1200 mg $CaCO_3$ L^{-1} for stable operation. (Parawira, 2004). Additionally, researchers stated that alkalinity should not be less than 1500 mg $CaCO_3$ L^{-1} for balanced digestion (Gunaseelan, 1997).

2.2.6. 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 complexing may affect the phenomenon of inhibition (Chen et al., 2007).

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 concentration 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.

$$NH_4^+ + OH^- \longrightarrow NH_3 + H_2O \qquad (2.5)$$

All substrates contain nitrogen. Table 2.5. lists the nitrogen content of various organic substances and the C/N ratio. 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) in substrate. Nonetheless, given enough time, the methanogens are capable of adapting to NH_4-N concentrations in the range of 5000-7000 mg L^{-1} substrate, the main prerequisite being that the ammonia level (NH_3) does not exceed 200-300 mg NH_3-N L^{-1} substrate. 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.

Table 2.5. Nitrogen-content and C/N-ratio data for a selection of substrates, compiled from various sources (Hoerz et al. 2011)

Biodegradable Material	**N %**	**C/N**
Animal Dung		
Hog	2.8	13.7
Cow	1.8	19.9
Chicken	3.7	9.65
Duck	0.8	27.4
Household Wastes		
Nightsoil	7.1	6.72
Kitchen Waste	1.9	28.6
Crop Residues (air-dry)		
Corn Stalks	1.2	56.6
Rice Straw	0.7	51
Corn Cobs	1.0	49.9
Peanut Hulls	1.7	31
Bagasse	0.4	-
Others		
Water Lily	2.9	11.4

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 sulphure-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.

Volatile Fatty Acids (VFA) 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 its 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 (i.e.; 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 (Ionnati and Fisher, 1983). The same researchers indicated that butyrate has a toxic effect at 1000 mg L^{-1} concentrations minimum. 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.

Heavy Metal Inhibition. Heavy metal 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).

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.

2.3. Biomass and Biogas

Biomass is a term for all organic material that stems from plants (including algae, trees and crops). Biomass is produced by green plants converting sunlight into plant material through photosynthesis and includes all land- and water-based vegetation, as well as all organic wastes. The biomass resource can be considered as organic matter, in which the energy of sunlight is stored in chemical bonds. When the bonds between adjacent carbon, hydrogen and oxygen molecules are broken by digestion, combustion, or decomposition, these substances release their stored, chemical energy (Klass, 2004). Biomass is characterised in different ways but one simple method is to define four main types, namely;

- woody plants,
- herbaceous plants/grasses,
- aquatic plants,
- manures

The three major components of waste biomass from agricultural and animal residues are cellulose, hemicellulose, and lignin. Cellulose is the most abundant component of biomass and is found almost exclusively in plant cell walls. In contrast to cellulose, hemicellulose is a heterogeneous branched polysaccharide that binds tightly, but non-covalently, to the surface of each cellulose microfibril. Hemicellulose differs from cellulose, in consisting primarily of xylose and other five-carbon monosaccharides (Lynd et al., 2002). Lignin is a three dimensional phenyl-propane polymer with phenylpropane units held together by ether and carbon-carbon bonds. It has a high molecular weight and is amorphous in nature. Lignin gives structural rigidity and its hydrophobic nature prevents water loss from plant vascular systems. Lignin is difficult to degrade enzymatically and its presence with plant tissue significantly impedes cellulose degradation. It is comprised of *trans*-coniferyl, *trans*-sinapyl, and *trans-p*-coumaryl alcohol monomers and acts as the ‘net’ holding the fibrous structure together (Frear, 2009).

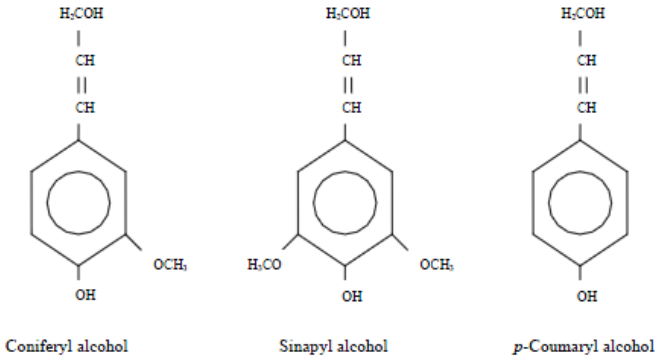

Figure 2.3. Lignin monomers

Rice husk has relatively low biodegradability due to its high lignin content (crude fiber, 31.71-49.92%; cellulose 34.34-43.80%; lignin 21.40-46.97%) (Iyagba et al., 2009). Kalra and Panwer (1986) showed that anaerobic digestion of rice husk alone and the mixture of cattle dung relatively showed low biogas yield. They mentioned that the main reason for a low biogas yield from rice husk could be the high C/N ratio. They also mentioned that the low gas yield from rice husk could also be due to high lignin content. On the other hand, Thipwimon et al. (2002) indicated that rice husk has a high potential for use as a feedstock by combustion method for electricity production. Its calorific value is 16.2 MJ kg^{-1}. It performs better than fossil fuels (especially coal and oil) from the point of view of environmental emissions.

Chicken manure has a higher fraction of biodegradable organic matter than other livestock wastes (Hill, 1983; Morris et al., 1975). Yet this substrate, rich in organic nitrogen, when anaerobically digested, can cause a reduction of process performance caused by ammonia accumulation.

Among the types of animal manure, poultry manure has a higher biogas yield compared to other animal manures (Table 2.9). This substrate, rich in organic nitrogen, when anaerobically digested at its original solids content of 20-25%, can cause a reduction of process performance caused by ammonia accumulation (Bujoczek et. al., 2000). Consequently, ammonium toxicity presents a major problem during the anaerobic treatment of protein-rich wastes. A range of ammonia-nitrogen concentration between 50

mg dm^{-3} and 30 g dm^{-3} was shown to be the most typical for methanogenic digestion of poultry manure (Krylova et al., 1997).

Although anaerobic treatment is an established method for treating animal manure and has been widely studied by many researchers, very few studies have been conducted on the anaerobic treatment of CM (Huang et al., 1981; Magbanua et al., 2001; Demirci et al., 2004). Many studies have focused on reducing the effect of ammonia inhibition and improving the process of fermentation of CM for the production of methane. In one of these studies, CM was diluted with water to decrease the total percentage of solid (Bujoczek et al., 2000; Converse et al, 1981; Webb et al., 1985). Demirci and Demirer have strongly recommended preacclimation in order to increase the efficiency of the digestion process for a mixture of cattle manure and chicken manure.

Table 2.6. Biogas yield of animal manure (EIE, 2011).

Resource	**Biogas yield (liter/kg)**	**CH_4 (methane) (vol%)**
Cattle manure	90-130	65
Poultry manure	310-620	60
Pig manure	340-550	65-70

Hills and Roberts (1983) showed fermented mixtures of dairy cattle manure combined with barley straw, rice straw, or rice hulls. They reported an increase in CH_4 yield as the C/N ratio increased, with maximum CH_4 yields between C/N ratios of 25 to 32, and a decrease in CH_4 yield as the C/N ratio increased further. Depending upon the C/N ratio, several conditions can exist when crop residue-manure mixtures are fermented. At low C/N ratio, carbon addition stimulates the CH_4 yield by reducing ammonia inhibition. At high C/N ratios, carbon addition decreases the CH_4 yield as nitrogen becomes a limiting nutrient. On the other hand, rice husk has relatively low biodegradability (10-20%) due to its inorganic matter component silica (20-51%) (Rohatgi et al., 1987; Daud et al., 2007). Daud et al., (2007) analyzed content of rice husk by using SEM-ESD micrograph (scanning electron microscope) and investigated that its silica content was %51.70 (Figure 2.4). Biogas production from chicken manure with mixed rice husk can negatively be

affected by litter material due to rice husk's high lignin and inorganic silica content (Chastain et al., 2011).

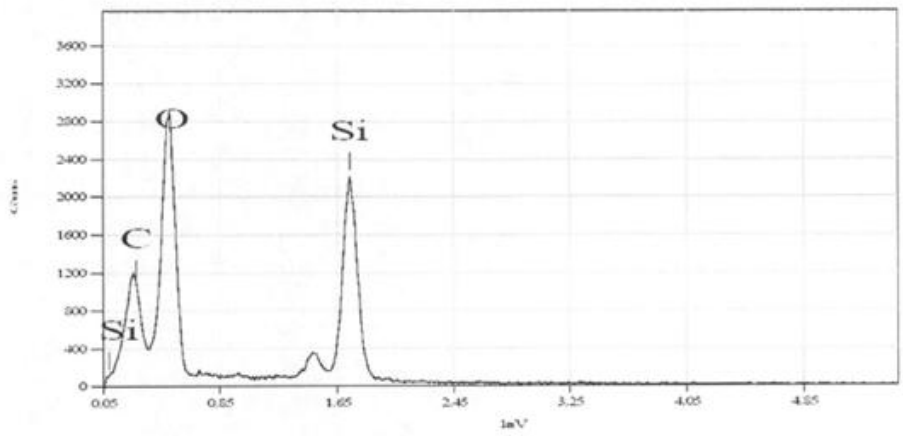

Figure 2.4. SEM-ESD micrograph of rice husk.

Biogas is an end product, which is produced by anaerobic digestion of biomass. Anaerobic digestion is a biochemical process in which organic matter is degraded to methane through microbiological action in the absence of oxygen. Biogas may be used for electricity production, for heating or as a fuel. Typical biogas composition is given in Table 2.6. After upgrading biogas can also be used as fuel in vehicles or as a subside for natural gas (feeding into natural gas grid).

Table 2.7.Typical biogas composition (Jensen et al., 2000).

Composition	**Unit**	**Biogas**
CH_4 (methane)	Vol%	55-70
CO_2 (carbon dioxide)	Vol%	30-45
N_2 (nitrogen)	Vol%	0-2
H_2S (hydrogen sulphide)	ppm	~500
Properties		
Density	kg/m^3	1.13
Ignition Temperature	°C	650-750
Energy	MJ/m³ Methane	35.9

2.4. Biomass in Turkey

The energy generation in Turkey is dominated by fossil fuels. The share of fossil fuels in total generation has been steadily increasing for last two decades and reached to the peak share of 82,5% (TEIAS, 2011). Being a net energy importer and high fossil fuel dependent for energy generation, Turkey is in need of decreasing its dependency on fossil fuels in order to secure energy supply as well as to decrease foreign trade deficit and to reduce carbon emissions. This can only be possible with transition to low carbon economy. The effective measures in place have been promoting increase in renewable energy capacities and end-use energy efficiency.

Turkey has significant hydro, wind, solar, biomass and geothermal power potential. Among them, only hydro potential is utilized to some extent with dam type HEPPs. There is also growing number of small-scale hydro, wind and geothermal power plant investments but their share is very small comparing potentials of these resources. On the other hand, solar and biomass power applications hardly exist.

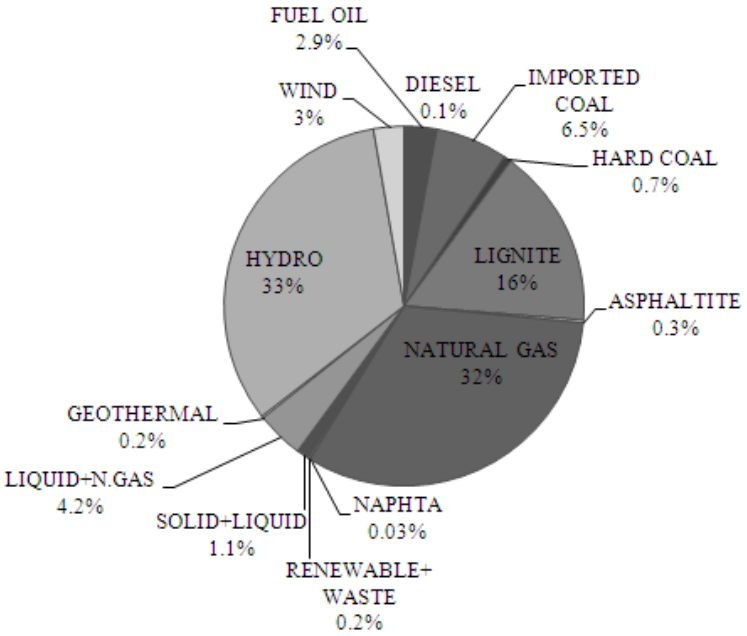

Figure 2.5. % Breakdown of installed capacity by resources in Turkey (TEIAS, 2011).

According to the energy security strategy paper of the Ministry of Energy and Natural Resources, the share of Turkey's renewable energy is aimed to be increased to 30% in the overall energy generation by 2023. In order to reach this target, the entire technical and economic potential of hydro and economic potential of geothermal power will be utilized, the installed wind power capacity will be increased to 20.000 MW and possible potential for other sources (as biomass and solar) are also planned to be utilized till 2023. Breakdown of installed capacity by resources in Turkey is given in Figure 2.5. (TEIAS:Turkish Electricity Transmission Company).

Table 2.8. Breakdown of installed capacity by resources in Turkey (TEIAS, 2011).

THE DISTRIBUTION OF INSTALLED CAPACITY BY PRIMARY ENERGY RESOURCES	
Primary Energy Resources	**MW**
FUEL OIL	1446,7
DIESEL	26,5
IMPORTED COAL	3281,0
HARD COAL	335,0
LIGNITE	8139,7
ASPHALTITE	135,0
NATURAL GAS	16269,0
NAPHTA	16,9
RENEWABLE+WASTE	96,9
SOLID+LIQUID	551,5
LIQUID+N.GAS	2140,3
GEOTHERMAL	94,2
HYDRO	16512,7
WIND	1377,5
TOTAL	**50422,7**

Turkey has annually 8.6 million toe (tonne of oil equivalent) biomass potential, of which only 11.000 toe was used for energy production in 2007 (Durmus et al., 2009). Total recoverable bio-energy potential in Turkey is given in Table 2.8.

Table 2.9. Total recoverable bio-energy potential in Turkey (Cicek et al., 2009).

Type of biomass	**Energy potential (ktoe)**
Dry agriculture residue	4.560
Moist agriculture residue	250
Animal waste	2.350
Forestry and wood processing residues	4.300
Municipality wastes and human extra	1.300
Firewood	4.160
Total bio-energy	16.920

After reviewing the energy structure of Turkey, it seems necessary to take measures toward the optimum utilization of biomass as an energy source. Since animal husbandry and agriculture are highly developed in Turkey, a substantial amount of animal wastes and agricultural animal residues are produced each year. Organic wastes are of vital importance for the soil, but in Turkey most of these organic wastes are used as fuel through direct combustion. Animal wastes are mixed with agricultural residues to increase the calorific value, and are then dried for use. This is the principal fuel of many villages in rural region of Turkey, especially in mountainous regions.

Anaerobic digestion for methane production is a possible solution to recover the wastes as fertilizers and produce energy. In Turkey, much effort has been put into biogas research and projects were developed since the 1960s (Kaygusuz ve Turker, 2002). Many studies on biogas have been performed between 1980–1986 in Central TOPRAKSU Research Institute (Ankara Research Institute of Rural Services) in Turkey. Although some research was conducted by the efforts of a few individuals from universities and governmental organizations, any institution addresses to council have not been seen since 1986. For this reason, biomass technology could not be commonly applied so far in Turkey (Bilgin et al., 2002). Studies on energy forests began in 1980 with the Fourth Five-Year Development Plan in Turkey. Although universities, national research institutes, companies and international organizations have actively been involved with the subject,

due to lack of collaboration and organization between these different projects, further development was not achieved.

2.5. Chicken Husbandry and Chicken Manure Management

Commercial chicken production is an important industry in Turkey. Chicken husbandry is mainly located in Marmara Region of Turkey. Broiler production has the big portion of the chicken husbandry. Broiler is a young bird of either sex bred and grown specifically for highly efficient meat production. Broilers are kept 42-47 days in a special closed house (Figure 2.6). Broilers are usually killed at 5 to 7 weeks of age (alternative term – meat chicken) (Bilgic, 2008).

Figure 2.6. Typical broiler housing

In poultry farms, litter material is important in controlling of the room conditions and providing comfortable medium for animals. It should be capable of drying quickly and be soft compressible and absorbent. As collected chicken manure may contain feces, urine, and especially litter material which may affect the biogas production. Typical litter materials include (Sarica et al., 1998; Ipek et al., 2002);

- Barley straw,
- wheat straw,
- rice husk,
- timber shavings,
- sawdust,
- wood chips,
- chopped straw,
- shredded paper,
- corn cobs,
- peanut hulls

Rice husk has been rapidly gaining importance and is now being commonly used as a litter material. Its cost, size, freedom from dust, density, thermal conductivity, drying rate and compressibility make it a preferable litter base (Emburry, 2009).

Table 2.10. Annual animal waste potential in Turkey (EIE, 2011).

Animal type	**Number**	**Amount of wet manure (ton/year)**	**Biogas amount (m^3/year)**
Big-cattle	11.054.000	39.794.400	1.313.215.200
Small-cattle	38.030.000	26.621.000	1.544.018.000
Poultry	243.510.453	5.357.230	417.863.937
TOTAL	292.594.453	71.772.630	3.275.097.137

In Turkey, poultry industry has been developing for the last decades. There are approximately 10.000 chicken farms, with a total capacity reaching 250 million poultry (Table 2.9.) (YUM-BIR, 2011). According to these statistics, in Turkey, chicken manure potential is approximately 5.5 million ton/year.

Animal manure causes environmental problems when it is poorly managed. Nutrient leaching, especially nitrogen and phosphorous are main pollutants which lead to

air and water pollution. This problem of inappropriate use of livestock wastes can be solved by applying the anaerobic digestion process. The animal production sector is responsible for 18% of the overall green house gas emissions. If animal manure is managed properly, it can be a valuable resource for renewable energy production as biogas and a source of nutrients for agriculture (Holm-Nielsen, 2009). From point of this view, biogas production from anaerobic digestion of animal manure is a beneficial process which provides waste stabilization, odor control, energy production, pathogen reduction, nutrient conservation and mineralization and prevents greenhouse gases emissions (Wilkie, 2005). Biogas is an extremely useful source of renewable energy, whilst digestate is a highly valuable biofertiliser (Lukehurst 2010). The application of anaerobic digestion for animal manure is given in Figure 2.7.

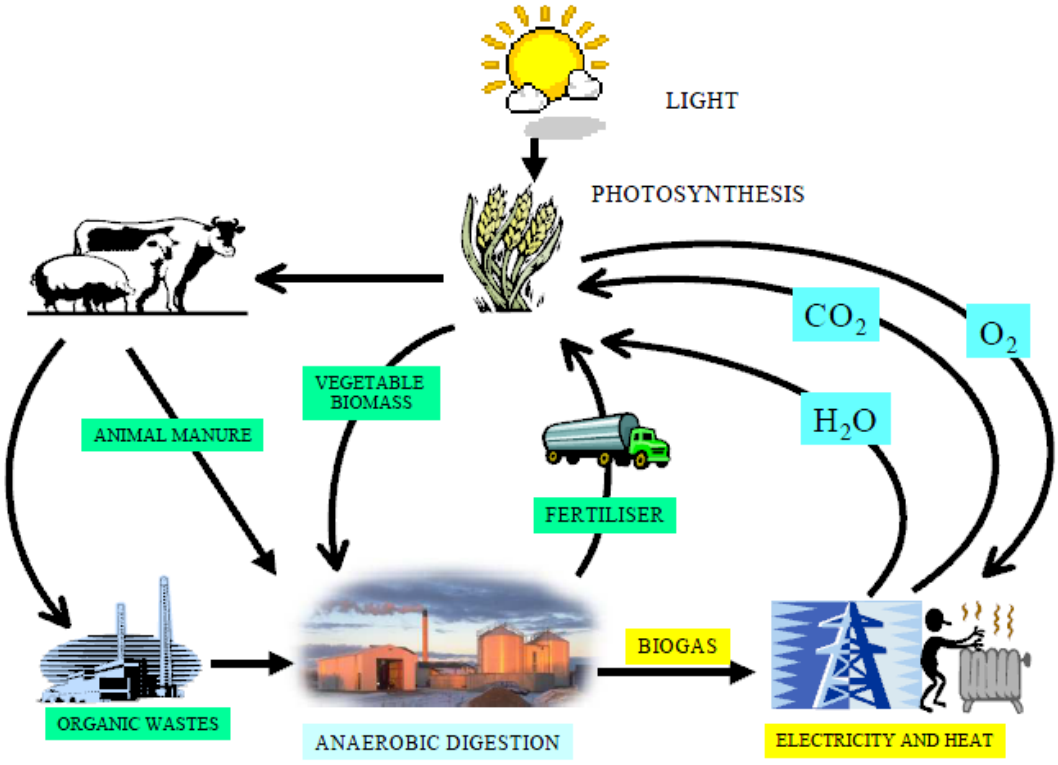

Figure 2.7. The sustainable cycle of biogas (Al Seadi, 2001)

Organic waste can be divided into two groups: carbon-rich such as grass and crop straws, and nitrogen-rich such as urine, human feces, and chicken manure. The carbon-rich waste contains a lot of carbon cellulose, which promotes biogas production, and the nitrogen-rich waste provides nutrients which promote the growth and reproduction of

anaerobic bacteria. The fuel is used for cooking, to heat the living rooms, and to drive electrical generators. At the same time, the process in the biogas plant kills the germs in the feces, leaving a hygienic residue for use as fertilizer. The people suffer less from parasitic infections and the nutritional value of the soils is improved, yielding large crops. In addition the direct environmental benefits from use of digestate as a fertiliser, such practices will result in lower gaseous emission into the atmosphere as well as in less diffuse pollution from surface run off and leaching. These direct benefits will help governments meet targets for reducing GHGs along with meeting the requirements of countries.

Figure 2.8. Digestate injection into soil

Moreover application of raw manure slurry as fertiliser can cause burning of plant leaves, this is the effect of low-density fatty acids, such as acetic acid. When fertilising with digestate, plant burns are avoided, as most fatty acids have been broken down by the AD process. Digestate flows more easily off the plants vegetable parts compared to raw slurry, which reduces the time of direct contact between digestate and the aerial parts of the plants, reducing the risk of leaf damage. Digestate is more homogenous, compared to raw slurry, with an improved N-P balance. It has a declared content of plant nutrients, allowing

accurate dosage and integration in fertilization plans of farms. Digestate contains more inorganic nitrogen, easier accessible to the plants, than untreated slurry. N-efficiency will increase considerably and nutrient losses by leaching and evaporation will be minimized if digestate is used as fertilizer in conformity with good agricultural practice (Figure 2.8). Compared to compost and to untreated slurry application, digestate supplies larger portions of carbon, available for the reproduction of organic substances in soils. During AD, decomposable organic bounds such as cellulose and fatty acids are broken down. The lignin bounds, valuable for formation of humus, remain. Methane bacteria themselves produce a whole series of amino acids, which are available for plants and other living organisms in the soil (Al Seadi et al., 2008).

3. MATERIALS AND METHODS

3.1. Reactor Experiments

3.1.1. Anaerobic Batch Digestion Experiments

Anaerobic digestion experiments were conducted using laboratory equipments consisting of digesters operated in batch mode presented in Figure 3.1.

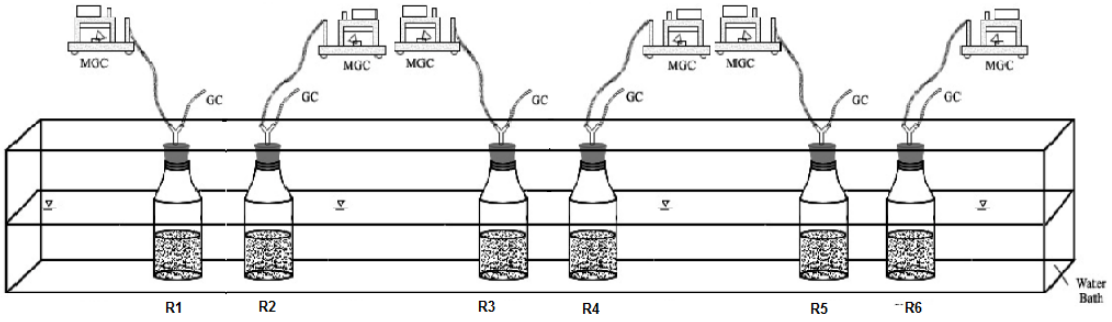

Figure 3.1. Configuration of the system for anaerobic digestion of CM and RH mixtures

Table 3.1. Legend of the reactors

Reactor	Substrate
R1	Control**
R2	30% Chicken Manure + 70% Rice Husk
R3	40% Chicken Manure + 60% Rice Husk
R4	50% Chicken Manure + 50% Rice Husk
R5	60% Chicken Manure + 40% Rice Husk
R6	70% Chicken Manure + 30% Rice Husk

*RH: Rice Husk, CM: Chicken Manure

**Only CM+I (without rice husk)

All the experiments were conducted by using 1 L borosilicate glass bottles working as reactors each with 800 mL active volume. The reactors were capped with rubber stopper

and equipped with a V shape gas collection port at the top. One opening of the port was connected to Milligascounter ® (MGC-1) by a PVC hose with in order to measure the volume of biogas produced. The other opening was capped by a rubber stopper and functioned as a gas sampling port for GC measurement. Reactors were placed in a water bath at an average mesophilic temperature of 37 °C and the temperature was kept constant by an automatic heat controller. All purpose thermometer was used to be sure that the mesophilic condition was maintained in the water bath which will enhance the digestion process. Besides, water level in the water bath was controlled on a regular basis and filled with water as it was evaporated. To allow the complete contact between the microorganisms and digestate, reactors were mixed gently every day.

To commence and enhance the rate of anaerobic degradation with methane production, each reactor was seeded with anaerobically digested sludge which was collected from Pakmaya İzmit Plant. The inoculum to substrate (CM+RH) ratio was I/S: 1:1 (w/w). Total solid concentration of anaerobic sludge was 3.5 % and volatile solid concentration was 54%.

The bottles were filled with chicken manure and rice husk mixture as substrates and anaerobic granular sludge as inoculum. Chicken manure is a suitable substrate for anaerobic digestion due to its high biomass yield and biogas production capacity. Broiler litter, chicken manure and rice husk were supplied from a commercial chicken husbandry in Bandırma, Balıkesir. Substrates and inoculum were stored in the cold room at 4°C. Prior to addition to the bottles, substrates and inoculum were allowed to reach the room temperature and analyzed for their total solid (TS) and volatile solid (VS) contents.

To investigate the CM: RH ratio of the original sample, broiler litter, was separated by physical methods (Figure 3.2). Broiler litter was diluted in water and, CM and RH were separated based on their gravimetric differences. In the mixture RH was floating on the surface because of its lower density. RH was separated from the mixture by filtration and kept 24 hours in oven at 105^0C for humidity and then weighted. It was found that original sample contained 50%CM + 50%RH on the weight basis. According to this rate, five different mixtures were prepared for batch experiments (Table 3.1.). On the other hand R1

control reactor was also run to investigate RH ratio on biogas generation of CM. R1 was loaded only CM and inoculum (without rice husk). All reactors were loaded depending on their mass. Reactors were run with their parallel. A picture of experimental set-up is given in the Figure 3.5.

To consider appropriate loading option 3 different set up were conducted for this study. Before the studying reactor loading on weight basis, 2 different set up were conducted to investigate the suitable reactor loading parameters on basis volume and TS% of substrate. For the first set up (Set up 1st) reactors were filled according to their volume basis (800 mL working volume). RH is a bulky crop residue therefore reactors were filled RH, very little weight could be used. Between the Control reactor (without RH) and the other reactors, there were high VS fed differences. This loading style was not a proper comparison biogas production from Control and CM+RH mixtures. For the second set up (Set up 2nd) same weights were used for loading of the reactors with the same I/S ratio 10%. While reaching these values volume of the reactors was different and this loading style led to different head spaces for the reactors. Different reactor head space caused failure of the measurement methane content of the biogas produced from the reactors. As a result of these tests reactor loadings was done on the weight basis with the same volume and different I/S ratio (Set up 3rd).

Figure 3.2. Chicken Manure

Figure 3.3. Rice Husk

Figure 3.4. Original sample - Broiler litter

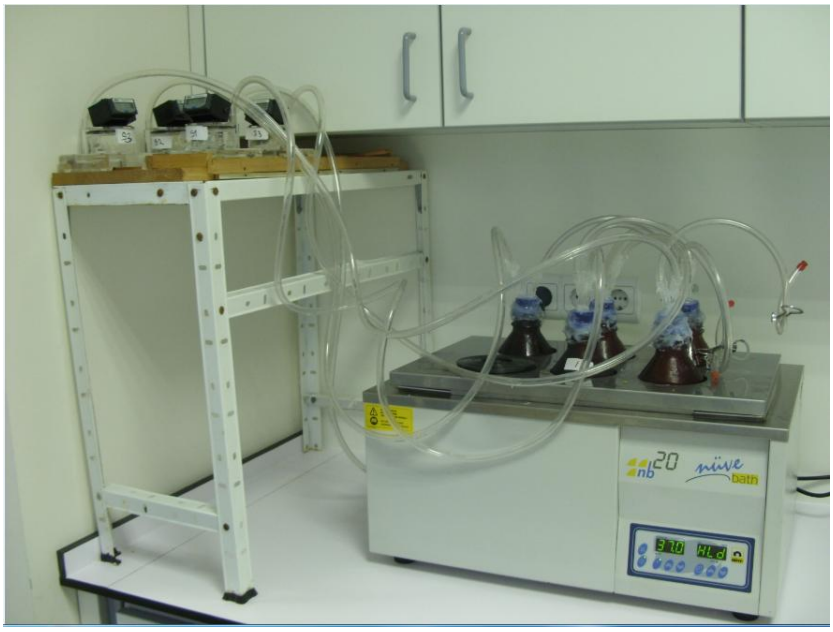

Figure 3.5. Experimental Set-up Layout

Preliminary studies showed that physical pretreatment of chicken manure and rice husk such as grinding does not influence the process in order to enhance the surface area contact between microorganisms and substrates (Batstone et al., 2008). Homogenized substrate (CM+RH) and inoculum were added to the bottles and the mixture was diluted to 800 mL with deionised water. Same procedure was followed for different ratios of substrate and inoculum mixtures preparation. Control reactors, Control (R1), including chicken manure and inoculum without any addition of rice husk were also conducted in the laboratory for investigation of what is impact of rice husk as litter material on biogas generation from chicken manure.

In literature, it was indicated that methane production can be observed in the pH range of 7.0–8.5 (McKendry, 2002). Therefore, the pH of the reactors was adjusted to 7.0-7.2 by using 6 N NaOH and 6N H_2SO_4 in order to provide suitable conditions for the growth of methanogens. Afterwards, the bottles were capped with stoppers and a coating of silicon was applied to all connections and joints to ensure that the units were gas tight. Reactors were purged with nitrogen gas for 5 minutes in order to displace oxygen from the system and directly establish the anaerobic conditions. The biogas produced in the bottles was collected and analyzed on a regular basis for quantity and composition. The volume of

gas produced was determined daily by Milligascounter® type MGC-1 (Ritter, Bochum, Germany) (Figure 3.6).

Figure 3.6. Milligascounter® (MGC-1) used for the measurement of biogas volume

The biogas composition with regard to CH_4 and CO_2 content was analyzed using HP 6850 gas chromatograph (Carboxen 1010 plot column 30 m x 0.53 mm) equipped with a thermal conductivity detector (TCD). Helium gas was used as the carrier gas (2 mL min^{1-}). Calibration was made using 99.99 % Supelco methane and carbon dioxide standards and 5% gas mixture. Injection port and detector were operated at 150 °C and 160 °C respectively. The oven temperature was programmed to start at 70 °C and was gradually increased 5 °C per minute until final temperature of 150 °C was reached. Pure carbon dioxide, methane and gas mixture standards were used to calibrate gas chromatograph and obtain calibration curves.

3.1.2. Analytical Techniques

Reactor Loading. All the feedstocks were prepared and placed into the reactors. Each reactor was fed depending on their suitable weight. Since the total volume of the ingredient was set to 800 mL.

Before the loading of the reactors; CM, RH, inoculum and their mixtures were analyzed for Total Solid (TS), Volatile Solid (VS), pH, Alkalinity, Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), Ammonium Nitrogen (NH_4^+-N), Orthophosphate (PO_4^{3-}), Chemical Oxygen Demand (COD), C/N ratio, Total Organic Carbon (TOC) and Heavy Metals. Each parameter was studied triplets. The volume of gas production was monitored daily and biogas composition was analyzed once a week throughout the study. For the gas measurements double samples were used from each parallel reactor. All these analyses were performed according to Standard Methods for the Examination of Water and Wastewaters (APHA, 1998). Analytical protocol which was followed during the experiments was listed in the Table 3.2.

Table 3.2. Analytical protocol of the study

Parameter	**Chicken manure**	**Rice husk**	**Inoculum (sludge)**	**substrate + inoculum before digestion**	**substrate + inoculum after digestion**
pH	✓	✓	✓	✓	✓
Alkalinity	✓	✓	✓	✓	✓
Total solids (TS)	✓	✓	✓	✓	✓
Volatile solids (VS)	✓	✓	✓	✓	✓
Total organic carbon (TOC)	✓	✓	✓	✓	✓
C/N	✓	✓	✓	✓	✓
Ammonium- nitrogen (NH_4^+-N)	✓	✓	✓	✓	✓
Total Kjeldahl nitrogen (TKN)	✓	✓	✓	✓	✓
Total phosphorous	✓	✓	✓	✓	✓
Orthophosphate (PO_4^{3-})	✓	✓	✓	✓	✓
COD	✓	✓	✓	✓	✓
Heavy metals	✓				

Total Solids (TS). The determination of Total Solid (TS) content of substrate and inoculum solely and as a mixture was performed according to Standard Methods. Homogenized samples were weighed in tared clean ceramic dishes and evaporated on the steam bath (Julabo Ecotemp TW 12). Afterwards, the samples were kept at 105^0C in the Nüve-FN 500 drying oven. As a final step, the samples in the dishes were cooled in the desiccator and weighed.

Volatile Solids (VS). Volatile solids (VS) are rough approximation of organic matter present in the solid part of the sample. After the determination of total solid content, the dried samples were ignited to constant weight at 550 ± 50 °C in the Nüve – MF 120 oven. Solids remaining after ignition are fixed solids while the weight of lost on ignition represents the volatile solids.

pH. Due to its significance as indicative parameter in 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 pH meter after calibration with pH 4, pH 7 and pH 10.

Alkalinity. The alkalinity of a sample is its acid neutralizing capacity and it is another significant parameter for the growth of methanogens those are effective in biogas production. Alkalinity was monitored according to the potentiometric method (2320 B), outlined in the Standard Methods.

Total Kjeldahl Nitrogen (TKN). 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 colour 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 control.

The TKN of the sample was read as mg L^{-1} at 460 nm by using HACH DR / 2010 Spectrophotometer.

Total Phosphorus (TP). 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 colour.

Phosphate (PO_4^{3-}). Phosphate (PO_4^{3-}) present in the samples was determined by the Ascorbic Acid Method which was described above. Unlike the Total Phosphorus analysis, samples analyzed for their phosphate concentration were not digested and they were used directly.

Ammonium nitrogen (NH_4^+-N). Ammonium nitrogen (NH_4^+-N) content of the digestate samples was monitored by using Nessler Method. As a first step, 1 g dried sample was poured in 25 mL mixing graduated cylinder. Then, three drops of mineral stabilizer and polyvinyl alcohol dispersing agent were added and 1 mL of Nessler Reagent was poured into each cylinder. Ammonium concentration as mg L^{-1} NH_4^+-N read at 425 nm by using HACH DR / 2010 Spectrophotometer.

Chemical Oxygen Demand s(COD). COD is commonly used to characterize organic compounds in liquid mixtures. It is the predominant parameter for most of the wastewater treatment processes. Although organic matter is predominantly described in terms of VS for digestion of chicken manure and rice husk, soluble s(COD) concentration is also determined in order to have more information on the characteristics of substrate and sludge mixture. This analysis was made by closed reflux colorimetric method. 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 (0-800 ppm).

Total organic carbon (TOC). Total organic carbon (TOC) analysis was done according to DIN EN 13137 TOC determination in waste, sludges and sediments (European Committee for Standardization, 2001). In this procedure the carbonates present in sample are previously removed by treating the sample with acid. The carbon dioxide released by the following combustion step is measured. In this study Method B (direct procedure) was applied by first soaking the sample in acid to remove inorganic carbon, and then determining the remaining carbon with Costech Instruments ECS 4010 elemental analyses system.

C/N Ratio. C/N ratios of the samples were determined according to total organic carbon and total nitrogen content of the substrates. Results from the elemental analyses system were used to calculate the C/N ratio.

Metal Concentrations. Heavy metal concentrations of manure samples were determined for all of the sampling points within the scope of this study. Measurement of the pollutants becomes possible when all the desired metals are extracted from the soil media to aqueous phase. For this reason, to determine the heavy metal concentration of samples they were digested by strong acids in order to set metals free into a certain amount of liquid solution. During the digestion, organic part of the soil is completely destroyed, releasing the metal ions bound to it (Guney, 2006). Total metal concentrations in soils were determined on filtered liquids extracts with Perkin Elmer instruments, Optima 2100 DV ICP-MS after microwave digestion based on EPA Method 3052 ‘Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices’ (USEPA, 1996).

3.1.3. Characteristics of Substrate and Inoculum

Substrate CM and RH Analysis. Two parallel samples were obtained for each sample and analyzed for their pH, total solid (TS %), volatile solid content (VS %), alkalinity, COD, C/N, TOC, TKN, TP, ortaphosphate, ammonium-N and heavy metals. The characteristics of CM and RH are given in Table 3.3.

Table 3.3. Characterization of CM and RH

Parameter	CM	RH
pH	6.1	6.2
TS %	20	91
VS %	63	82
Alkalinity, mg $CaCO_3$ L^{-1}	8218	104
s(COD), mg O_2 L^{-1}	25512	1700
C/N	13	85
TOC %	20	38
TKN, g kg^{-1} dry matter	41	5.1
TP, g kg^{-1} dry matter	21	3.2
Ortaphosphate, g kg^{-1} dry matter	233	0.3
Ammonium-N, g kg^{-1} dry matter	12.4	0.7
Heavy Metals		
Zn^{2+} ppm	239	-
Cu^{2+} ppm	36	-
Mn^{2+} ppm	292	-
Fe^{2+} ppm	614	-
Pb^{2+} ppm	ND	-
Ni^{2+} ppm	ND	-
Cd^{2+} ppm	ND	-
K^{+} ppm	2180	-
Ca^{2+} g kg^{-1} dry matter	4670	-
Mg^{2+} g kg^{-1} dry matter	858	-
Na^{+} g kg^{-1} dry matter	2657	-

Inoculum Analysis. In order to determine characteristics of anaerobic sludge, pH, total solid, volatile solid content, alkalinity, COD, C/N, TOC, TKN, TP, ortaphosphate, ammonium-N parameters were analyzed. The results are given in Table 3.4.

Table 3.4. Characterization of inoculums

Parameter	**Inoculum**
pH	7.6
TS %	3.5
VS %	54
Alkalinity mg, $CaCO_3$ L^{-1}	4940
COD, mg O_2 L^{-1}	45212
C/N	8
TOC %	30
TKN, g kg^{-1} dry matter	18.5
TP, g kg^{-1} dry matter	52.6
Ortaphosphate, g kg^{-1} dry matter	112
Ammonium-N, g kg^{-1} dry matter	3.7

4. RESULTS AND DISCUSSION

4.1. Substrate Analysis at the Beginning and End of the Digestion

The main factors affecting the anaerobic digestion of biomass are total solid, volatile solid, pH, alkalinity, volatile fatty acids, total Kjeldahl nitrogen, ammonium nitrogen, total phosphorus, orthophosphate, chemical oxygen demand and total organic carbon. These parameters were monitored to detect and describe the conditions at which anaerobic digestion of chicken manure and rice husk took place.

4.1.1. Total Solids (TS%) and Volatile Solids (VS%) Content

In order to determine the organic matter contained in the reactors prior to digestion and the consumption rate as a result of the degradation, total solid and volatile solid content were analyzed for each reactor.

Table 4.1. TS and VS contents of the reactors at the beginning and at the end of the experiment

		TS (%)		VS (%TS)		
Reactor	**Substrate**	**Before Digestion**	**After Digestion**	**Before Digestion**	**After Digestion**	**VS% Conversion Rate**
R1	Control	3.8	3.2	57	48	16%
R2	30% CM+ 70% RH	10	9.7	82	80	2%
R3	40% CM+ 60% RH	10	9.5	78	75	4%
R4	50% CM+ 50% RH	9	8.1	63	59	6%
R5	60% CM+ 40% RH	7.3	6.5	61	57	7%
R6	70% CM+ 30% RH	6.7	5.9	59	55	7%

TS and VS contents of the reactors decreased through to the end of the experiment as a result of biological degradation. The highest VS content degradation rate was observed 16% for the reactor R1 Control including CM and inoculum mixture without RH. On the

other hand the lowest VS degradation rate was observed 2% for the reactor R2 which was including the most RH ratio. However, VS degradation should be considered together with biogas yield and methane yield which are the most significant parameters, in order to evaluate the performance of an anaerobic digestion system.

4.1.2. pH and Alkalinity

Since a slight change in pH of the reactor could result in reduction of gas production, this parameter is considered as one of the most significant factors which is effective during anaerobic digestion process. The amount of carbon dioxide and volatile fatty acids which are produced during anaerobic digestion process affects the pH of the reactor. During the acid formation phase of anaerobic digestion, excessive production of volatile fatty acids and their accumulation cause pH values to be lower.

Since the negative effect of pH is neutralized and the acidic condition is buffered with high alkalinity in well balanced systems, the relationship between these parameters is a crucial point which needs consideration. The measured alkalinity concentrations for the batch reactors are given in Table 4.2. Initial alkalinity concentrations of the reactors were above the threshold level which was reported to be 1200 mg $CaCO_3$ L^{-1} in literature (Parawira et al., 2004). The conditions for the biogas production were suitable as reflected by high pH values and alkalinity concentrations. Through to the end of the experiment, the initial alkalinity increased due to the contribution of soluble CO_2 which was generated by anaerobic conversion process.

Due to high levels of alkalinity in the anaerobic granular sludge, pH of the mixtures prepared was mostly above the desired level which was a good indicator of a potentially well-balanced anaerobic degradation process. pH 7.3-8.1 was observed in the output of the reactor and these values showed that the process were well-balanced in the reactors.

Table 4.2. The pH and alkalinity values of the reactors at the beginning and at the end of the digestion experiment

		pH		Alkalinity mg $CaCO_3 L^{-1}$	
Reactor	**Substrate**	**Before Digestion**	**After Digestion**	**Before Digestion**	**After Digestion**
R1	Control	7.2	7.9	4494	5223
R2	30% CM+ 70% RH	7	7.3	2064	2167
R3	40% CM+ 60% RH	7.1	7.5	1763	2012
R4	50% CM+ 50% RH	7.2	7.7	2107	2877
R5	60% CM+ 40% RH	7.1	7.8	1677	2356
R6	70% CM+ 30% RH	7.1	8.1	1116	2752

4.1.3. TKN and NH_4^+-N

Adequate amounts of nutrients are essential for supporting the growth and maintenance of microbial population, as well as the efficient operation of anaerobic degradation. Nitrogen is needed for the production of protein, enzymes, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA). Ammonium nitrogen is produced from the decomposition of organic material containing nitrogen.

If the reactor contains high ammonium nitrogen at elevated pH ranges, free ammonia nitrogen is produced and it has toxic effects on microorganisms. The toxic level of free ammonia nitrogen was reported to be 700 mg L^{-1} and 1100 mg L^{-1} in literature (Kaparaju, 2005). Chicken manure (CM) generated from farms contains 20% or more dry matter, which is rich in nitrogen.

The production of endogenous ammonia-nitrogen rises considerably during anaerobic digestion of chicken manure. It was found that ammonium concentrations in the output of the reactors ranged between 17- 38 g kg^{-1} dry matter.

TKN, which is another significant parameter, represents the sum of the organic nitrogen and ammonia nitrogen. Initial and final concentrations of ammonium nitrogen and TKN in R1 which have no RH were higher than the findings of other reactors. This result

was attributed to the difference portion of CM. The concentration of ammonium nitrogen analyzed prior to digestion was indicated the lowest for the R2. Increasing trend of ammonium nitrogen in all reactors during batch experiments is the result of the rapid decomposition of organic material containing nitrogen. On the other hand, TKN content of the reactors was reduced at the end of the experiments. TKN and NH_4^+-N results from all reactors are given in Table 4.3.

Table 4.3. TKN and NH_4^+-N concentrations analyzed at the beginning and at the end of the experiment

		TKN (g kg^{-1} dry matter)		**Ammonium-N (g kg^{-1}dry matter)**	
Reactor	**Substrate**	**Before Digestion**	**After Digestion**	**Before Digestion**	**After Digestion**
R1	Control	37	45	11.7	34
R2	30% CM+ 70% RH	15	17	4.8	11
R3	40% CM+ 60% RH	19	21	5.4	17
R4	50% CM+ 50% RH	28	32	7.1	24
R5	60% CM+ 40% RH	26	32	8.6	23
R6	70% CM+ 30% RH	32	38	10.6	28

4.1.4. TP and Orthophosphate (PO_4^{-3})

Another nutrient necessary for growth and performance of the microbial population is phosphorus. It is used to synthesize energy-storage compounds (adenosine triphosphate-ATP) as well as RNA and DNA. Total phosphorus (TP) and orthophosphates (PO_4^{-3}) were monitored as one of the major nutrients in anaerobic batch degradation. Orthophosphate and TP concentrations for the anaerobic batch reactors are presented in Table 4.4. Total phosphorus and orthophosphate concentrations in the reactors followed similar attenuation trend throughout the experimental period as a result of microbial assimilation. Due to high CM and inoculum weight reactor R1 had the highest TP and PO_4^{-3} concentrations.

Table 4.4. TP and PO_4^{-3} concentrations analyzed at the beginning and at the end of experiment

		TP (g kg^{-1}dry matter)		PO_4^{3-} (g kg^{-1}dry matter)	
Reactor	**Substrate**	**Before Digestion**	**After Digestion**	**Before Digestion**	**After Digestion**
R1	Control	47.6	22	202	123
R2	30% CM+ 70% RH	18.1	7.8	32.4	12
R3	40% CM+ 60% RH	19.4	9.3	45	27
R4	50% CM+ 50% RH	23.2	12.4	86.5	56
R5	60% CM+ 40% RH	27	15	109	89.3
R6	70% CM+ 30% RH	33	18	124	105

4.1.5. s(COD) and TOC

COD is a chemical parameter which is represented as an indication of the relative biodegradability of the biomass and it is commonly used to characterize organic compounds contained in the substrate. Although organic matter is predominantly described in terms of VS degradation for digestion of chicken manure and rice husk, COD concentration is also determined in order to have more information on the characteristics of substrate and sludge mixture.

Findings of these analyses indicated that the most promising reactor was the R1. Control reactor R1 with the highest s(COD) concentration contained in it and the highest COD removal occurred in it. The reactors which contained lower RH had the lowest s(COD) concentrations and they were expected to produce the lowest amount of biogas. Nevertheless, all of the parameters were considered as a whole in order to evaluate the performance of the system with regard to its biogas production.

s(COD) concentrations which were analyzed prior to digestion decreased through to the end of the experiment as a result of decomposition process. The highest degradation rate of s(COD) with a value of 81 % was observed in the reactor R1.

On the other hand, the reactor R6 which had lowest RH ratio showed the lowest COD degradation rate of 51%. While the lowest COD degradation occurred in R6, conversely the highest TOC conversion appeared in the same reactor. This may be highest CM ratio led to highest ammonia content may caused inhibition of the system. COD degradation in the reactors showed differences with the ratio of CM.

TOC includes a variety of organic compounds, including humic acids, fulvic acids, VOAs and carbohydrates. Success of methanogenic activity was confirmed by the gas production with high methane content in addition to the decline in TOC concentrations. The results of s(COD) and TOC concentrations detected within the batch reactors, which were conducted under anaerobic conditions, are illustrated in the Table 4.5.

Table 4.5. TOC and s(COD) concentrations of the reactors analyzed before and after the digestion

		TOC (%)		**s(COD) (mg O_2 L^{-1})**	
Reactor	**Substrate**	**Before Digestion**	**After Digestion**	**Before Digestion**	**After Digestion**
R1	Control	28	26	11850	2305
R2	30% CM+ 70% RH	35	34	3870	1223
R3	40% CM+ 60% RH	33	33	4187	1308
R4	50% CM+ 50% RH	32	30	7022	2674
R5	60% CM+ 40% RH	30	27	6562	2894
R6	70% CM+ 30% RH	30	25	6025	2965

4.1.6. C/N ratio:

The carbon: nitrogen (C/N) ratio expresses the relationship between the quantity of carbon and nitrogen present in organic materials. Materials with different C/N ratios differ widely in their yield of biogas. If C/N ratio is higher than that range, biogas production will be low, because the nitrogen content of the feed material will be consumed rapidly by methanogenic bacteria for meeting their protein requirements rather than reacting on the carbon in the material. Conversely if C/N ratio is very low, that is outside the ideal range, nitrogen will be liberated and will accumulate in the form of ammonia, which raises the pH value of the slurry in the digester.

Related to the high C/N ratio of RH, mixtures in the reactors showed higher C/N ratio with the increasing weight of RH. Reactor R2 had the highest C/N ratio 56. C/N ratio differences at the beginning and end of the study, according to carbon and nitrogen degradation showed similar trend.

Table 4.6. C/N ratio of the reactors analyzed before and after the digestion

		C/ N	
Reactor	**Substrate**	**Before Digestion**	**After Digestion**
R1	Control	12	8
R2	30% CM+ 70% RH	56	52
R3	40% CM+ 60% RH	45	40
R4	50% CM+ 50% RH	30	25
R5	60% CM+ 40% RH	26	22
R6	70% CM+ 30% RH	18	15

4.2. Gas Analysis

The following parameters were obtained by gas analysis; biogas volume, biogas quality (significantly in terms of methane and carbon dioxide content), biogas yield and specific methane yield. Biogas volume was determined as the major indicator of an ongoing anaerobic digestion process, while its quality was investigated since methane and carbon dioxide are the major products of anaerobic conversion of biomass. In order to evaluate the economical viability of the system, two other parameters: biogas yield and methane yield were calculated. The results of daily gas production, cumulative gas production, gas composition, biogas yield and methane yield are given in the following section.

4.2.1. Daily Gas Production

The amount of biogas production was monitored every day and daily gas production was determined in the anaerobic batch reactors by recording the total amount of gas produced in 24 hours. The daily gas volumes produced in the batch reactors are

presented in Figure 4.1. These results were utilized in order to evaluate performance of the system regarding to manure decomposition within the reactors.

The experimental study was conducted for 30 days. Daily biogas production patterns were similar for each reactor during the experiment. Reactor R1 showed the highest daily biogas production with a value 2556 mL.

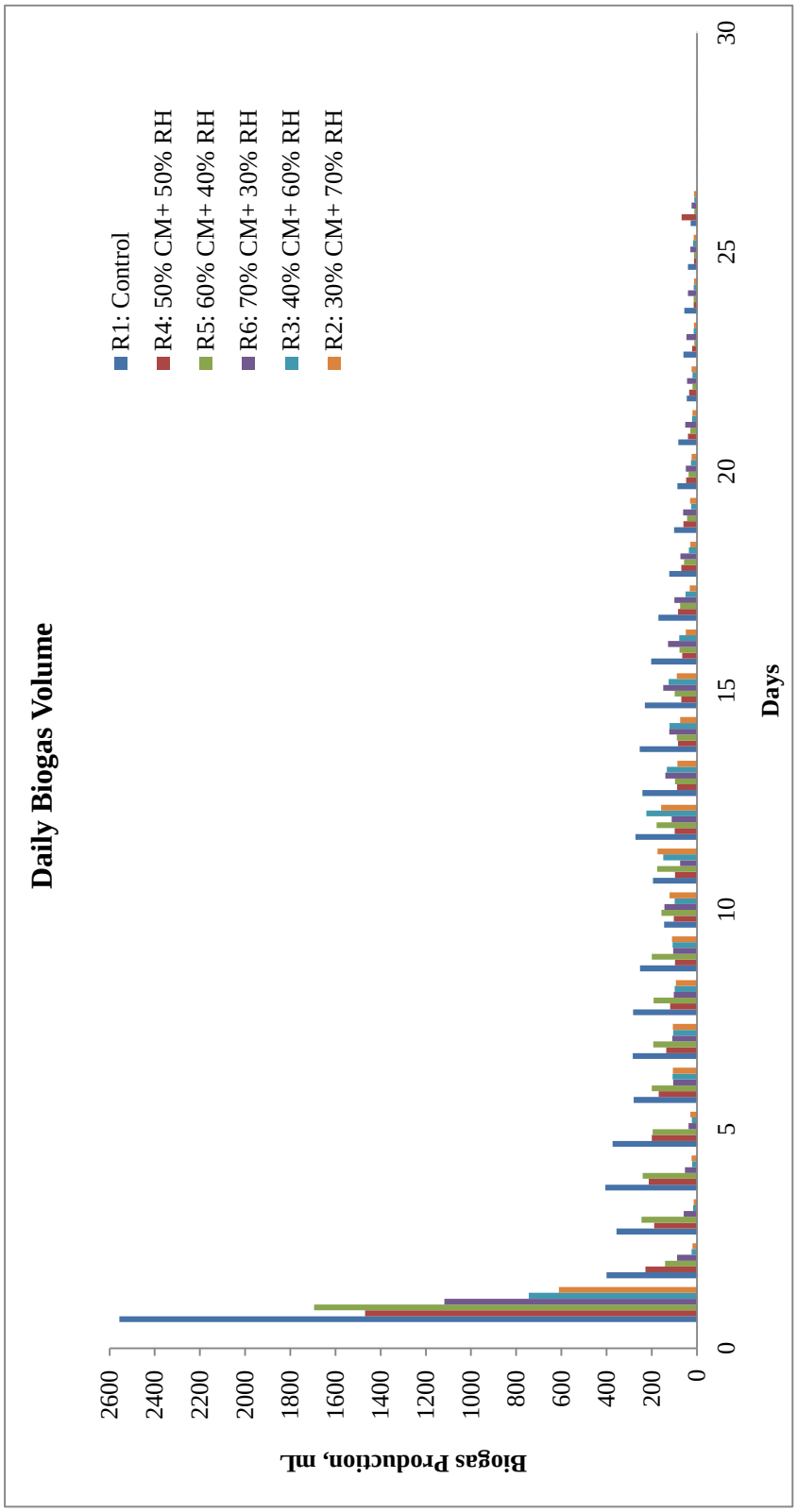

Figure 4.1. Daily biogas generation in the reactors

4.2.2. Cumulative Biogas Production

Biogas production from inoculum alone was monitored as a control and subtracted from the biogas production that was measured in the reactors that contained inoculum and CM and RH. Control (R1) reactor without rice husk addition showed the highest biogas production 7518 mL at the end of the 30 days. This result was expected due to low biodegradability of rice husk and its high lignin content (Kalra, 1986; Corrêa, 2009). In a similar experimental work, Tait et. al (2009) investigated anaerobic digestion of three different wheat straw, barley straw and rice husk spent litter from deep litter piggery housing. The authors showed that the wheat straw was generally more degradable (approximately 60%) compared to barley straw (40–50%). On the other hand, rice husk was relatively poorly degradable (<20%), but its degradability was improved by weathering in a pig shed, perhaps partly due to breakdown in microstructure observed for rice husk.

Rice husk has higher organic carbon content as shown in Table 4.5, which indicates its high cellulose, hemicelluloses, pectin, lignin and plant wax content. Lignin and plant wax are difficult to be degraded my microbial activities and can be a major rate determining step in anaerobic digestion process. R1 control reactor gave the highest mean and cumulative volume of biogas. On the other hand, reactor R2 gave the lowest mean and cumulative biogas volume 2075 mL, though it had relatively low biodegradable organic content (70% RH). Reactor R3 also showed low biogas production (2406 mL). Adequate chemical properties (volatile solids, nutrients, pH and C/ N ratio) are known to favour biogas production. These results may be attributed to the chemical composition of the RH. On the other hand, reactor R5 produced highest biogas compared to other reactors (4483 mL). Reactor R6 with the highest manure proportion produced lower biogas (3168 mL) and this was comparable to R4 (3877 mL).

There was not a net trend for biogas production from the reactors as shown in Figure 4.2. Except the control reactor R1, the other reactors, which contained RH, showed different trends. R2 and R3 reactors showed much lower cumulative biogas production

compared to the other reactors. On the other hand, Reactor R5 (60%CM + 40%RH) showed higher biogas production compared to R4 (50%CM + 50%RH).

Uzodinma et al. (2007) investigated co-digestion of RH with waste streams such as brewer's spent grain (SG), cassava waste water (CW) and carbonated soft drink sludge (SL). The pure RH system produced flammable biogas after 15 days post charging period with low cumulative biogas yield of 137.6. Rice husk has higher carbon content, which indicates that rice husk contains a lot of cellulose, hemicelluloses, pectin, lignin and plant wax. Lignin and plant wax are difficult to degrade and can be a major rate determining step in anaerobic digestion process.

Iyagba et al. (2009) investigated co-digestion of cow dung (CD) with rice husk for biogas production at laboratory-scale under room temperature (26-29°C) and reported that (50% CD+50%RH: w/w) showed a cumulative biogas production of around 162 ml at the end of 38 days. When the ratio of RH was increased in the mixture to 75 and 100 %, respectively, cumulative biogas production was not significant. In this experimental work, the cumulative biogas production generally decreased with the increase in RH when compared to control reactor.

However, much higher cumulative biogas production was observed in this study when compared to the findings of Iyagba et al. (2009). Mesophilic temperature used in this work, along with inoculum and substrate characteristics might have caused this difference.

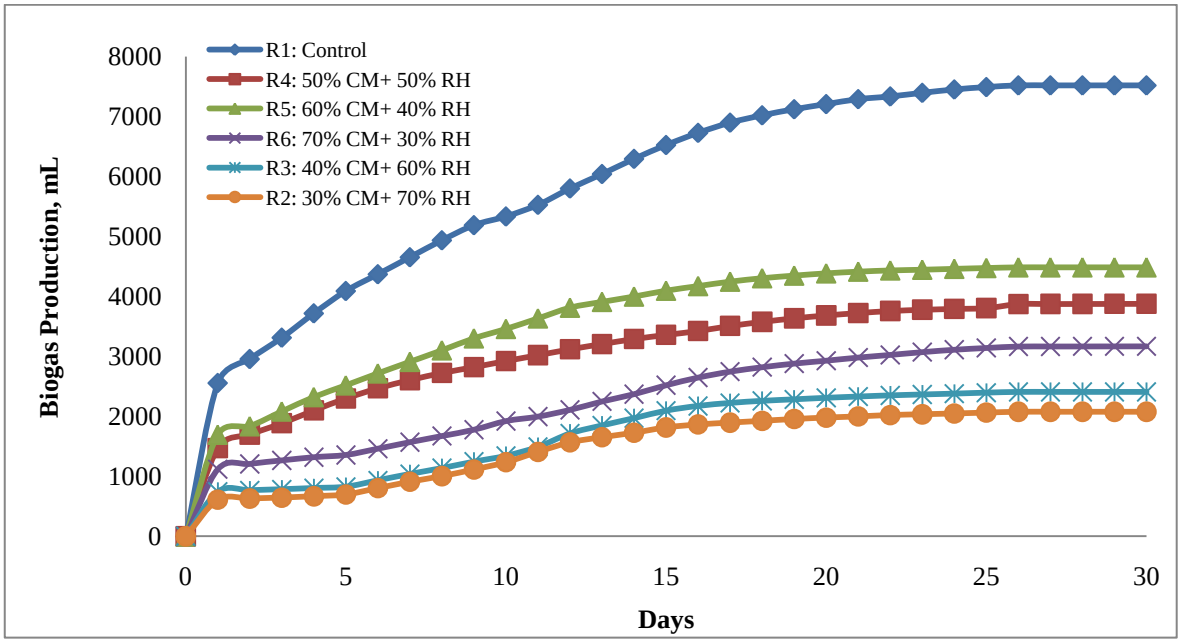

Figure 4.2. Cumulative biogas volume produced in the reactors

4.2.3. Biogas Composition

Composition of the biogas in terms of methane (CH_4) and carbon dioxide (CO_2) reflects the biological activity and the degree of organic material conversion in the reactors. Thus, this parameter is another significant point that should be considered while determining the performance of an anaerobic digestion process. The methane content of the reactors showed different trends compared to biogas production data. Reactor R2 which contained the highest methane concentration (82%) produced lowest biogas volume during the study. This result did not observed before in the literature. However, in this study, from the parallel reactors with double measurements CH_4 content of the biogas produced in R2 was 82%. Under suitable conditions methane content of the biogas produced from glucose is 80-90%. To compare other substrates this value was unexpected. Surprisingly, reactor R1 (the control reactor) produced the lowest methane concentration (57%) in biogas. Other reactors CH_4 production was R3: 34-71%, R4: 35-68%, R5: 36-64%, R6: 37-62%, In addition, the methane concentration in biogas increased with increasing RH ratio.

Biogas composition (CH_4, CO_2) for the batch reactors was analyzed once a week. The methane composition of the biogas is given in Figure 4.3.

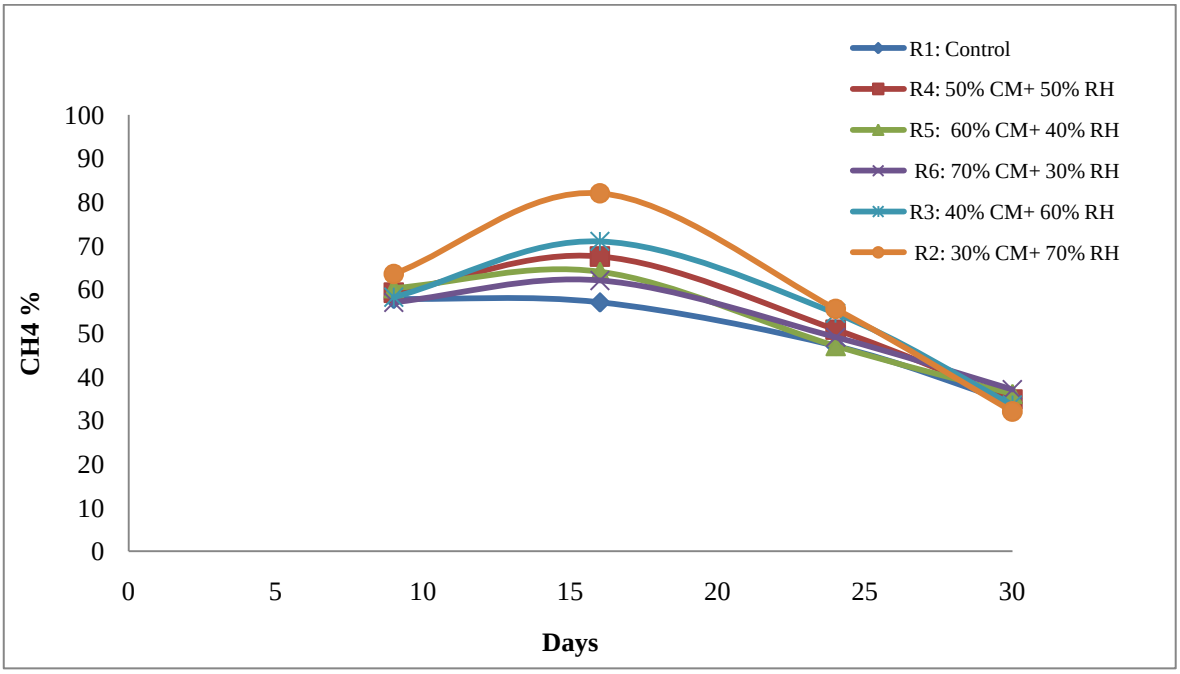

Figure 4.3. The methane (CH_4) content of the biogas produced in the batch reactors

When the characteristics of both CM and RH are compared (Table 3.3), RH has a higher VS and TOC content, and more balanced in terms of C/N ratio. Addition of RH to CM seemed to balance the C/N ratio of the mixture, producing a better microbial activity that resulted in a higher CH_4 percentage in the biogas produced.

Uzodinma et al. (2007) also investigated that methane content of the biogas produced from brewer's spent grain (SG): Rice Husk (RH) was 68.3%, cassava waste water (CW): RH was 77.8%, soft drink sludge (SL): RH was 73.5%.

The carbon dioxide concentration of the reactors showed similar trend as depicted in Figure 4.4. CH_4 and CO_2 concentrations were balanced in the reactors. CO_2 concentration of the biogas changed between 20-38%. Between 15^{th} -20^{th} days, while CH_4 concentration showed a peak value, CO_2 concentrations decreased.

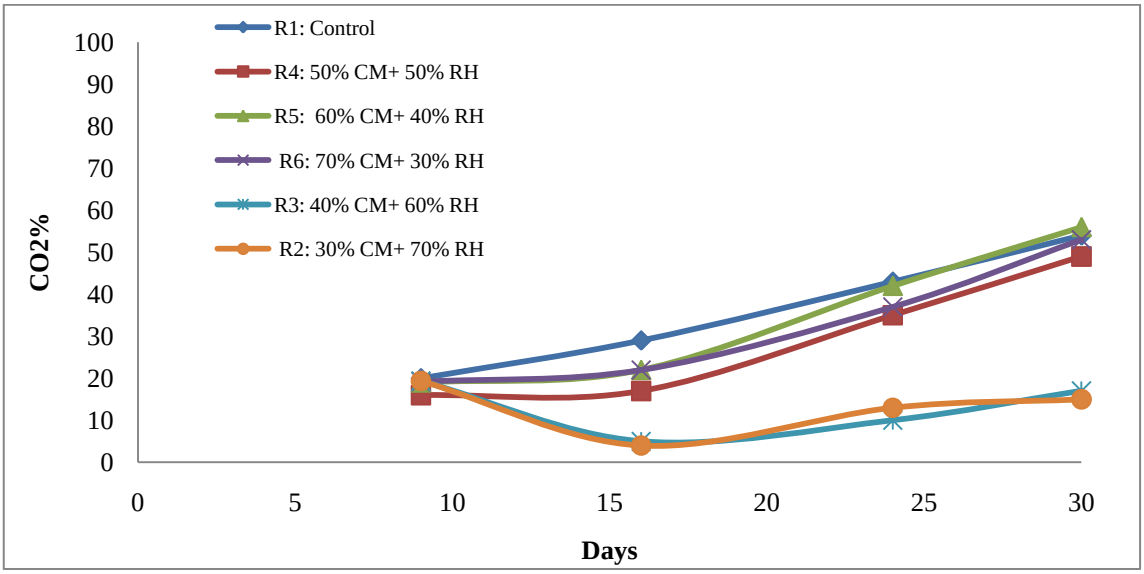

Figure 4.4. The carbon dioxide (CO_2) content of the biogas produced in batch reactors

The cumulative methane production obtained from digestion of different substrates mixtures are illustrated in Figure 4.5. The highest cumulative methane production was indicated for reactor R1. Reactor without RH content produced higher cumulative methane. Reactor R6 showed highest methane content (82%). On the other hand, because of the lowest cumulative biogas production from reactor R6, its cumulative methane production was also lower compared to other reactors. Probably, the biodegradable fraction of the mixture was rapidly converted to methane and when this fraction was no longer available for the microbial activity, the cumulative biogas production declined.

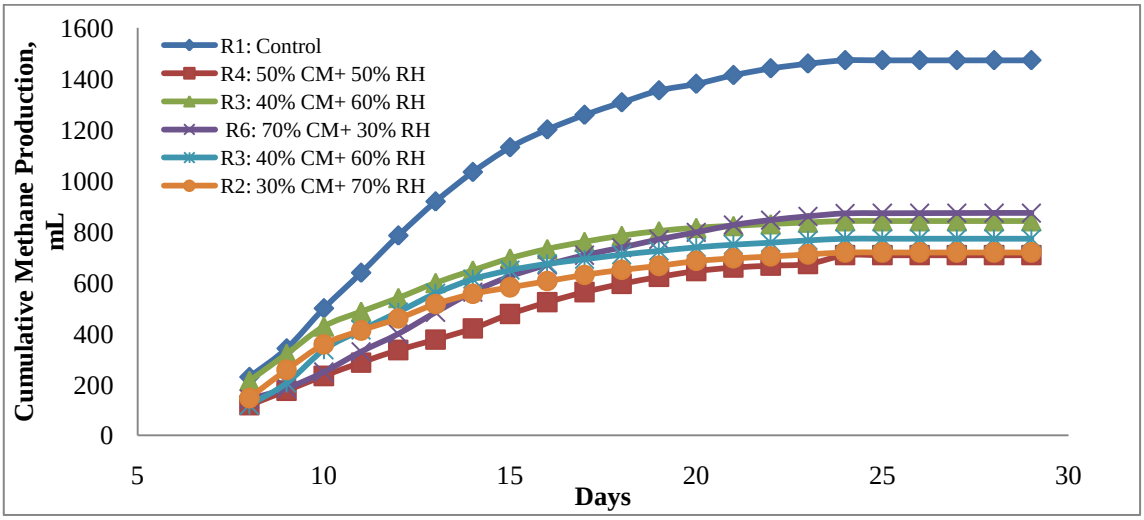

Figure 4.5. Cumulative methane production in the reactors

4.2.4. Biogas Yield and Methane Yield

The results from the anaerobic digestion process were also expressed in terms of biogas and methane yields (L CH_4 g^{-1} VS_{deg}) and the findings are given in Table 4.7. The methane yield of the reactors including both substrate and sludge were not corrected by taking into account the values of control reactors since the methane produced in the control reactors and the others were monitored at different period of time.

Prior to considering biogas production system as a potential alternative energy source, two parameters; biogas yield and methane yield should also be considered. In Table 4.7., the biogas and methane yields based on g VS degraded are shown. For reactor R1 chicken manure and inoculum mixture showed similar biogas yields value mentioned the literature (Steffen et al., 1998; Al Seadi, 2008). Also, Reactor R1, which did not contain rice husk , showed slightly different biogas yield with a value 1369 mL g^{-1} VS_{deg} and a methane yield 780.4 mL g^{-1} VS_{deg}.

Maximum methane yield was calculated for each set-up by subtracting the final volume of methane produced by the control from the methane production of each reactors and dividing the difference by the weight of substrate (in VS) which was degraded as a result of anaerobic digestion. A maximum methane yield is especially important with the

digestion of chicken manure as these have production costs that have to be covered by the methane production.

According to the experimental findings obtained, Reactor R5 showed the highest biogas and methane yields compared to the other reactors. In addition, it also showed the highest cumulative biogas production. On the other hand similar to cumulative biogas production trend, reactors R2 and R3 showed lower biogas and methane yields. Reactors R2 and R3, which contained the highest amount of RH percentages of 70 and 60 %, respectively, produced the lowest yields, indicating the adverse effect of increasing RH addition to the mixture above a certain percentage.

Table 4.7. Biogas and Methane Yields based on VS_{deg}

		Biogas Yield mL g^{-1} VS_{deg}	Methane Yield mL g^{-1} VS_{deg}
R1	Control	1369	780.4
R2	30% CM+ 70% RH	283.1	191.1
R3	40% CM+ 60% RH	266.3	170.4
R4	50% CM+ 50% RH	410	254.2
R5	60% CM+ 40% RH	526.8	374.1
R6	70% CM+ 30% RH	414.9	340.2

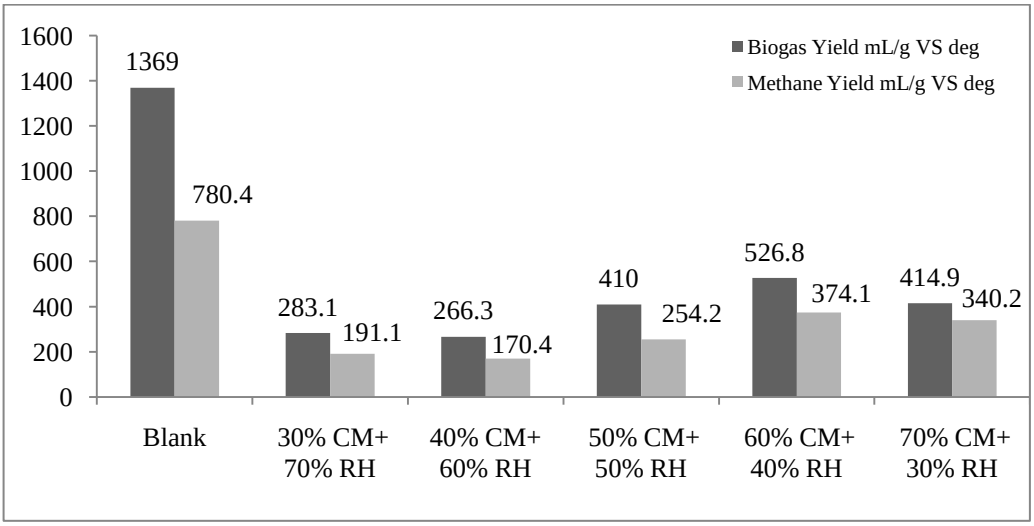

Figure 4.6. Biogas and Methane Yields based on VS_{deg}

5. CONCLUSION

This project was carried out in order to understand the potential of biogas generation from chicken residues. For this purpose different ratios of chicken manure and rice husk were used and the effects of rice husk on biogas and methane generation were investigated. This study was supported by Boğaziçi University Research Fund with project number 5067.

In this study, biogas production potential of chicken manure and rice husk was investigated by anaerobic digestion of these two substrates with the addition of anaerobically digested sludge as inoculum. According to CM and RH ratio in the original sample (broiler litter) five different ratios [(30%CM + 70%RH), (40%CM + 60%RH), (50%CM + 50%RH), (60%CM + 40%RH), (70%CM + 30%RH)] were prepared for batch experiments, that were conducted at mesophilic temperature ($37\ ^{0}C$).

According to the experimental findings, Reactor R1 (control reactor without RH content) showed the highest cumulative biogas production with a value 7518 mL. Among the reactors which contained RH, reactor R5 showed the highest cumulative biogas production with a value 4483 mL. On the other hand there was not a net biogas production trend observed between reactors R2, R3, R4, R5, and R6. Cumulative biogas volume produced in the reactors are in the order of R5>R4>R6>R3>R2. Addition of RH decreased the cumulative biogas production in the batch reactors.

Quantitative analysis of biogas composition for the reactors indicates that methane content was high for all the blends whereas CH_4 and CO_2 were found in variable proportion according to the ratio of RH. The composition of the biogas in terms of CH_4 and CO_2 content, reactor R2 contained the highest methane concentration (82%) during the study. Surprisingly reactor R2 with highest RH ratio (70%) showed lowest cumulative biogas production, but conversely generated highest methane concentration in biogas. Different from cumulative biogas volume produced from the reactors, biogas composition showed a net trend. Methane concentration in biogas produced from the reactors are in the

order of R2>R3>R4>R5>R6>R1. Reactor R1 (control reactor) had the lowest methane concentration in biogas (57%). It can be concluded that the methane concentration in biogas rises with increasing RH ratio.

Reactor R5 showed the highest biogas yield 526.8 mL g^{-1} VS_{deg} and methane yield 374.1 mL g^{-1} VS_{deg} compared to the other reactors. In addition, it also showed the highest cumulative biogas production. On the other hand, similar to cumulative biogas production trend, reactors R3 showed lower biogas yield 266.3 mL g^{-1} VS_{deg} and methane yield 170.4 mL g^{-1} VS_{deg}. Addition of RH to the CM above a certain percentage seemed to influence biogas and methane yields adversely. This can be attributed to the chemical properties of rice husk.

It can be concluded that the rice husk is not a suitable substrate for anaerobic digestion because of its chemical properties. The chemical properties of rice husk seem to affect biogas generation adversely. Pre-treatment of rice husk prior to anaerobic digestion process can be an alternative in order to increase biogas and methane yields.

6. RECOMMENDATIONS

After reviewing the energy policy of Turkey, it seems necessary to take measures toward the optimum utilization of biomass as an energy source. Since animal husbandry and agriculture are highly developed in Turkey, a substantial amount of animal wastes and agricultural animal residues are produced each year. Anaerobic digestion for methane production is a possible solution to recover the chicken manure as fertilizers and produce energy.

Compared to the other residues, due to the low biodegradability of rice husk used in this experiment, rice husk may be converted to energy using different methods (such as combustion, gasification). For this purpose, further studies should be done with broiler litter. On the other hand, in order to increase the methane yield from anaerobic digestion of rice husk, pre-treatment of rice husk prior to anaerobic digestion should also be investigated in further studies. Pre-treatment of rice husk by chemical/physical methods can be employed to increase its biodegradability so that it can be co-digested together with other type of agricultural substrates.

Furthermore, projects should be developed for sustainable management of animal and agricultural residues. Biogas is a renewable energy source and as a national energy policy its importance will increase in the near future, since the revised version of the new renewable energy act issued in January 2011 in Turkey supports electricity production from biomass with a feed-in-tariff of 13,3 USD cent/kWh. From this point of view, anaerobic digestion of poultry wastes should be improved and encouraged by the Ministry of Energy and Natural Resources for a sustainable and secure source of energy and green house gas emission reduction.

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