EVALUATION OF BIOGAS PRODUCTION AS A SOURCE OF RENEWABLE ENERGY THROUGH CO-DIGESTION OF AGRICULTURAL RESIDUES AND POULTRY MANURE

by

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ABSTRACT

The need for alternative energy sources for Turkey is obvious. Biogas production via agricultural residues and manure seems to be an advantageous option for Turkey, since the main income is generally provided from the agro industry. Through anaerobic digestion of agricultural residues and poultry manure, wastes will be properly disposed and biogas, which is a clean and sustainable source of energy, will be continuously recovered. As a renewable energy source, biogas production from agro industrial wastes is a useful management option, energy independent and environmentally friendly. Biogas can be used for production of heat, electricity, as fuel in vehicles and as a substitute of natural gas.

The objective of this study was to investigate the potential of biogas generation via anaerobic co-digestion of the chosen agricultural residue -sunflower residues- and poultry manure. Mixtures composed of different amounts of substrates were prepared and digested under anaerobic conditions with the addition of inoculum culture (seed sludge), using batch tests under mesophilic laboratory conditions.

If the potential of biogas production from co-digestion of poultry manure and agricultural residue can be determined by batch-tests, then it will be possible to calculate the theoretical amount of energy that can be produced from these substrates, since the production amounts of agricultural residue and manure are known. According to the results obtained, it will be possible to use these substrates for the continuous lab scale studies or larger scale commercial agricultural biogas plants.

Within the scope of this study, several compositions of sunflower residue, poultry manure and inoculum mixtures were elaborated and digested under mesophilic conditions. However reliable methane production could be only achieved in the first two trials. This outcome is mainly attributed to the low biodegradability of the sunflower residues thus further studies, including pretreatment methods are recommended.

ÖZET

Türkiye'de alternatif enerji kaynaklarına olan ihtiyaç gözle görülmektedir. Başlıca gelir kaynağının genellikle tarım endüstrisinden sağlandığı düşünüldüğünde, tarımsal kökenli atıklardan ve gübreden biyogaz üretimi Türkiye için avantajlı bir seçenek olarak görülmektedir. Tarımsal kökenli atıklar ve tavuk gübresi anaerobik çürütme yoluyla uygun bir şekilde bertaraf edilirken; temiz ve sürdürülebilir bir enerji kaynağı olan biyogaz sürekli olarak geri kazanılabilir. Tarım sanayi atıklarından yenilenebilir enerji kaynağı olarak biyogaz üretimi; dışa bağımsız, çevre dostu, faydalı bir atık yönetim biçimidir. Biyogaz ısı eldesinde, elektrik olarak, araçlara yakıt olarak ve doğal gaz yerine kullanılabilmektedir.

Bu çalışmanın amacı, birleşik çürütme yoluyla tarımsal kökenli atıklardan seçilen ayçiçeği kabuğu ve tavuk gübresinin biyogaz üretim potansiyelinin incelenmesidir. Farklı miktarlardaki hammaddelerle oluşturulan karışımlar hazırlanarak anaerobik koşullarda aşı çamuru ilavesi ile mezofilik laboratuar koşullarında çürütülmüştür.

Eğer tavuk gübresi ve tarımsal kökenli atıklarla oluşturulacak birleşik çürütmede biyogaz üretim potansiyeli karışım deneyleri ile belirlenebilirse, tarımsal kökenli atık ve gübre miktarları bilindiğinden bu hammaddelerden üretilecek teorik enerji miktarını hesaplamak mümkün olabilecektir. Elde edilen sonuçlara göre bu hammaddeleri laboratuar ölçekli çalışmalarda ya da daha büyük ölçekli ticari sürekli tarımsal biyogaz tesislerinde kullanmak mümkün olabilecektir.

Bu çalışma kapsamında, çeşitli oranlardaki ayçiçeği kabukları, tavuk gübresi ve aşı karışımları değerlendirilerek, mezofilik şartlar altında çürütülmüştür. Ancak, güvenilir metan üretimi yalnızca ilk iki denemede elde edilebilmiştir. Bu sonuç büyük ölçüde ayçiçeği kabuklarının düşük orandaki biyolojik parçalanmasına bağlanmaktadır. Bu nedenle ön hazırlık yöntemlerini de içeren ileri çalışmalar önerilmektedir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	**Explanation**	**Units used**
Ca	Calcium	(mg/L)
Cd	Cadmium	(mg/L)
Co	Cobalt	(mg/L)
Cr	Chromium	(mg/L)
Cu	Copper	(mg/L)
Fe	Iron	(mg/L)
Hg	Mercury	(mg/L)
K	Potassium	(mg/L)
Mg	Magnesium	(mg/L)
Mn	Manganese	(mg/L)
Na	Sodium	(mg/L)
Ni	Nickel	mg/L
TS	Total Solids	(%)
VS	Volatile Solids	(%)
TKN	Total Kjeldahl Nitrogen	(g/kg)
Pb	Lead	(mg/L)
Pm	Poultry Manure	-
WWTS	Waste Water Treatment Sludge	-
Zn	Zinc	(mg/L)
VFA	Volatile Fatty Acid	(mg/L)
TP	Total Phosphorous	(g/kg)
TS	Total Sulphide	(g/kg)

AD	Anaerobic Digestion	-
FID	Flame Ionization Detector	-
TCD	Thermal Conductivity Detector	-
STP	Standard Conditions For Temperature and Pressure	-

1. INTRODUCTION

Turkey’s recent developments in industrial and agricultural sectors are obviously promising. As industrial development increases, energy demand of Turkey increases consequently. Turkey’s energy consumption has been growing much faster than its energy generation, making Turkey an energy importer (Gokcol et al., 2008).

There is no doubt that the increase of foreign dependence on energy also results in significant political and economical concerns. If we consider the current potential of Turkey’s renewable energy sources, Turkey can easily work on to solve this foreign dependency issue. Furthermore, the whole world focuses on energy alternatives due to global warming concerns and future limitation of fossil fuels, particularly limitation of CO_2.

Turkey has a remarkable amount of renewable energy sources, which should be turned into a benefit. Apart from renewable energy sources, such as hydro, wind, solar and geothermal power potential, biomass is an another option that can be used as a great alternative to fossil fuels, since energy generated via biomass lessens air emissions compared to fossil fuels, reduces the amount of waste sent to landfills and at the same time decreasing dependence on foreign oil (Gokcol et al., 2008).

There are several technology options to generate energy via biomass sources. Combustion, gasification, pyrolysis and anaerobic digestion can be listed as the most commonly used technology options. For the treatment of agricultural resources, anaerobic digestion is the most common and efficient energy generation process. Biogas can be used for production of heat, electricity, as fuel in vehicles and as a substitute of natural gas.

The production of biogas through anaerobic digestion has advantages compared to other biogas generation options. Many resources; such as crops, grass, leaves, manure, fruit and vegetable waste or algae can be used for the anaerobic digestion. While minimizing the survival of pathogens, anaerobic digestion forms an opportunity to utilize the digestate from anaerobic fermentation and the mentioned digestate is a valuable fertilizer due to the increased availability of nitrogen and short term fertilization efficiency. Anaerobic digestion also results in a significant reduction of odors and forms a positive change in the composition of odors as well as providing sanitation (Weiland, 2009). The anaerobic digestion process is able to inactivate viruses, bacteria and the parasites in the feedstock which is of great importance if the digestate is used as fertilizer in agricultural purposes (Holm-Nielsen et al., 2009).

Since one of the main income sources for Turkey is agriculture, agricultural residues form another concern for the waste management system. Various agricultural residues, such as grain dust, wheat straw, hazelnut shell, etc. are mostly observed by products of agricultural processes. Besides, in addition to crop and agricultural residues, animal waste forms another waste management problem of the agro industry. Therefore, in order to produce renewable energy and to dispose of agricultural and animal wastes properly, biogas technology seems to be an important alternative for Turkey. 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, biogas production from anaerobic co-digestion of animal manure and agricultural residues is a beneficial process, which provides waste stabilization, odor control, energy production, pathogen reduction, nutrient conservation and mineralization while preventing greenhouse gas emissions (Wilkie, 2005).

The objective of this experimental study was to investigate the potential of biogas generation via anaerobic co-digestion of the chosen agricultural crop -sun flower residues- and poultry manure. Through anaerobic digestion of agricultural residues and poultry manure, wastes can be properly disposed and biogas, which is a clean and sustainable source of energy, can be continuously recovered. Another objective is to investigate whether the digestate, which is another important product of AD, can be applied to the land for agricultural purposes. The digestate has been also analyzed and the results are cross checked with the limit values of the regulations enforced in Turkey.

2. ANAEROBIC DIGESTION PROCESS

2.1. Fundamentals of Anaerobic Digestion

Anaerobic digestion is a multi-step biological process during which the organic carbon is converted to its most oxidized (CO_2) and most reduced (CH_4) state without the presence of oxygen (O_2). The product of the process is biogas, which is a mixture of methane and carbon dioxide, as well as trace gases such as hydrogen sulfide (H_2S) and hydrogen (H_2). The typical composition of biogas is given in Table 2.1 (Holm-Nielsen et al., 2009).

Table 2.1. Typical composition of biogas (Holm-Nielsen et al., 2009).

Gas	Percentage in Biogas (%)
Methane (CH_4)	55-75
Carbon dioxide (CO_2)	25-45
Carbon monoxide (CO)	0-0.3
Nitrogen (N_2)	1-5
Hydrogen (H_2)	0-3
Hydrogen sulphide (H_2S)	0.1-0.5
Oxygen (O_2)	Trace

The biogas production process is complex and sensitive, since several groups of microorganisms are involved. The important stages in anaerobic digestion are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These steps have been simply illustrated in Figure 2.1 as a flow chart and explained in the following sections. In addition, Figure 2.2 summarizes the steps of the anaerobic digestion process.

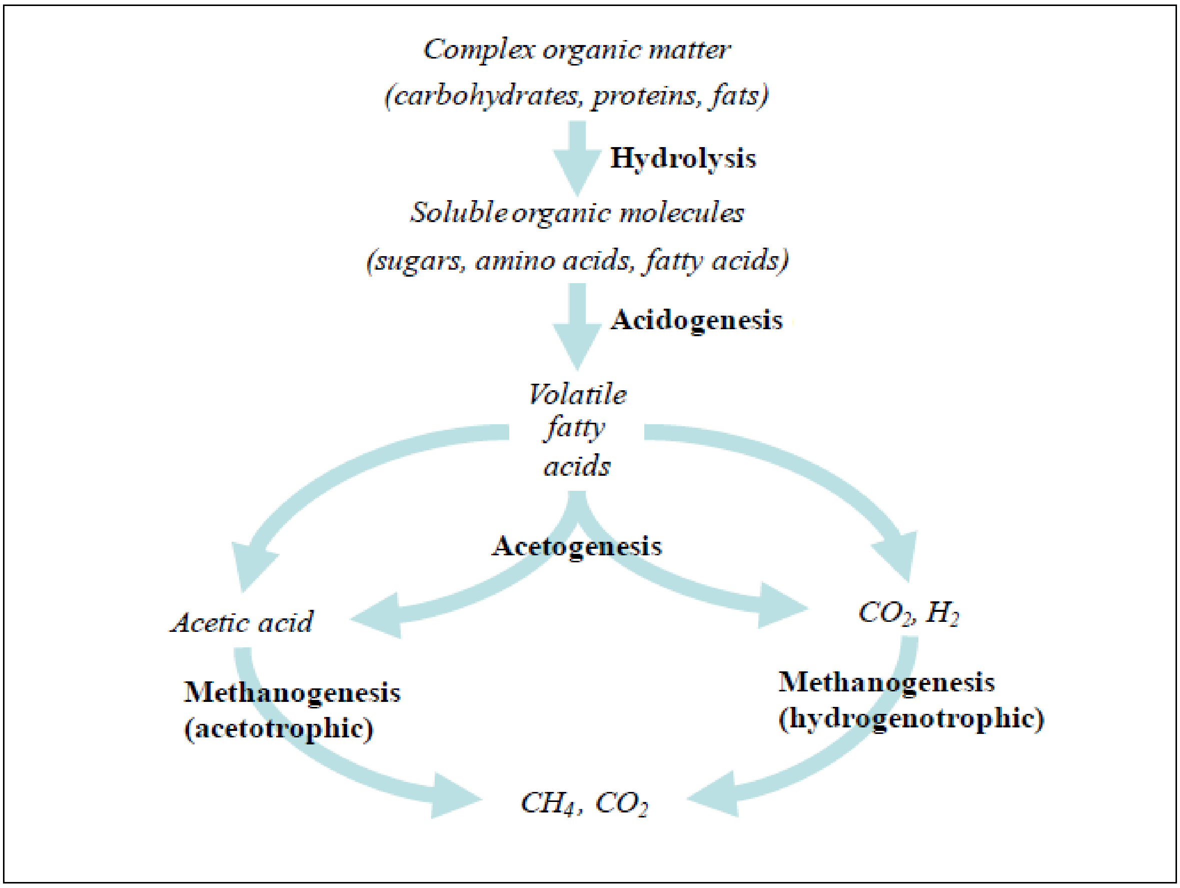


Figure 2.1. Flow chart of the anaerobic digestion process (Joshua et al., 2008).

Anaerobic digestion process can be used to purify different types of wastewater such as food and beverage, alcohol distillery, pharmaceutical, dairy, yeast-potato-starch processing, pulp and paper, textile and leachate. A wide range of biomass types can be used as substrates for the production of biogas from anaerobic digestion process. The most common used feed stocks are listed below (Teodorita et al., 2008);

- animal manure and slurry,
- agricultural residues and by products
- digestible organic wastes from food and agro industries,
- organic fraction of municipal waste and from catering,
- sewage sludge,
- energy crops (e.g. maize, miscanthus, sorghum, clover).

The substrates for anaerobic digestion process can be classified according to their dry matter content. Substrates with dry matter content lower than 20% are utilized in wet digestion (wet fermentation). This category is mainly composed of animal manure and wet organic wastes from food industries. Substrates with dry matter content as 35% is called dry digestion (dry fermentation), and it is typical for energy crops and silages.

The low methane yield and process instability are sometimes observed in anaerobic digestion processes. A wide variety of inhibitory substances are the primary cause of anaerobic digester upset or failure. In literature, there exist studies to identify the minimization and to wipe out the back arrivals of inhibitors. Co-digestion with another waste, adaptation of microorganisms to inhibitory substances, and incorporation of methods to remove inhibitors before anaerobic digestion can considerably improve the waste treatment efficiency and increase methane production (Chen et al., 2008).

2.1.1. Stages of Anaerobic Digestion

Major interactions occur between four metabolic groups. Therefore, the important stages in anaerobic digestion are classified into four major phases, which are hydrolysis, acidogenesis, acetogenesis and methanogenesis, respectively.

Hydrolysis	During this stage, carbohydrates, fats and proteins are broken down into sugars, fatty acids and amino acids, respectively.
Acidogenesis	Next, sugars, fatty acids and amino acids are broken down to form carbonic acids, alcohols, hydrogen, carbon dioxide and ammonia.
Acetogenesis	The products of the above reactions are further converted to produce more hydrogen and carbon dioxide, along with acetic acid.
Methanogenesis	Methanogens convert products from the intermediate processes into methane, carbon dioxide and water.

Figure 2.2. Steps of the anaerobic digestion process (Joshua et al., 2008; Holm-Nielsen et al., 2009).

2.1.1.1. Hydrolysis Hydrolysis is the first phase in which the complex organics (carbohydrates, proteins, lipids) are hydrolyzed to monomeric organics (sugars, amino acids, fatty acids). Since the cell membrane is semi permeable and unable to intake complex organic material, hydrolysis is the first step in which the complex organics are liquefied so that their sizes can become small enough to pass through cell membrane. The activity during this phase is influenced mainly by the substrate character and the pH (Demirel, 2003). The hydrolysis phase is relatively slow and it can be rate limiting in anaerobic digestion of biomass which contain lignin (Mshandate et al., 2006). Since lignin is tightly attached to the hemicelluloses, it covers the cellulose and creates a physical barrier for the hydrolytic enzymes. Thus, large particles have relatively small surface area and limit the microorganisms to degrade the complex structure of the substrate. Several methods have been investigated in order to improve the biodegradability of such materials by increasing the substrate surface area and accessibility to bacterial attack (Bayır, 2009). Pretreatment methods to increase biodegradability of the cellulose and lignin containing materials will be mentioned in Section 2.4.

2.1.1.2. Acidogenesis In acidogenesis phase, the products of hydrolysis are converted into methanogenic substrates by acidogenic bacteria. This stage involves acid forming fermenters, hydrogen producers and acetogens. Simple sugars, amino acids and fatty acids are degraded into acetate, carbon dioxide and hydrogen as well as into volatile fatty acids (VFA) and alcohols. VFAs are the byproducts during anaerobic digestion process (Tajarudin, 2006). Common volatile organic acids and their chemical formulas are presented in Table 2.2.

Table 2.2. Common volatile fatty acids.

Acid	Chemical Formula
Acetic acid	CH_3COOH
Propionic acid	CH_3CH_2COOH
Butryric acid	$CH_3CH_2CH_2COOH$
Valeric acid	$CH_3CH_2CH_2CH_2COOH$
Caproic acid	$CH_3CH_2CH_2CH_2CH_2COOH$

The end products produced in acidogenesis phase mainly depend on the characteristics of the substrate as well as types of the bacteria and the environmental conditions. The activity during that phase is mainly influenced by temperature, pH, alkalinity and the composition of the substrate.

2.1.1.3. Acetogenesis In acetogenesis stage, products from acidogenesis phase, which can not be directly converted to methane by methanogenic bacteria, are converted into methanogenic substrates. Degradation of the products of the acidogenesis is relatively slow and energy consuming. Conversion of both butyric and propionic acids need energy input and low hydrogen partial pressure (Demirel, 2003). Therefore, acetogenesis is considered as the rate limiting step of the soluble part. Volatile acids formed in the second stage during acidogenesis are broken down to acetic acid, carbon dioxide and hydrogen by acetogenic bacteria. Acetic acid formation from butyric and propionic acids are illustrated below.

$$CH_3CH_2CH_2COOH + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ H_2$$

$$CH_3CH_2COOH + 2\ H_2O \rightarrow CH_3COOH + 3\ H_2 + CO_2$$

The end products of above illustrated equations are consumed in the methanogenesis phase.

2.1.1.4. Methanogenesis In methanogenesis stage; the end products, acetic acid, hydrogen and carbon dioxide are converted to methane and carbon dioxide. Acetogenesis and methanogenesis usually run in parallel as the conversion of volatile acids occur and methanogenic bacteria start to convert the byproducts to methane and carbon dioxide.

Since the methanogens are not able to consume complex organics directly, the substrate must be converted to mainly acetate in the previous phases (Demirel, 2003). All of the VFAs need to be converted to acetate so that anaerobic digestion process can reach maximum efficiency.

Methanogenic bacteria are divided into two categories; acetotrophic and hydrogenotrophic, in which it varies according to the usage of acetate (CH_3COOH) or carbon dioxide (CO_2) for the formation of methane (Wulf, 2005). It has been reported that about two thirds of methane gas is derived from acetate conversion, while the remaining one third is derived from carbon dioxide and hydrogen (Novaes, 2006; Morgan et al., 1991). The equations given below illustrate the conversion of by products to end products;

Hydrogenotrophic methanogens

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$$

Acetotrophic methanogens

$$CH_3COOH \rightarrow CH_4 + CO_2$$

The methanogenic bacteria are very sensitive to the accumulation of the hydrogen as well as the presence of electron acceptors such as nitrate and sulfate. According to the characteristics of the substrate, H_2S and N_2 gases can be formed with the main end products, CH_4 and CO_2 (Karatas, 2006). The stabilization of waste is completed when methane (CH_4) and carbon dioxide (CO_2) are observed as the final products.

2.2. Environmental and Operational Factors Effecting Anaerobic Digestion Process

Environmental and operational factors are directly in correspondence with the specific growth rate, gas production and substrate utilization rate. If optimum conditions are not provided, these factors may adversely affect the anaerobic digestion system. Environmental and operational factors are listed below and these factors will be explained in this section.

- Temperature
- pH
- Buffering capacity (alkalinity)
- Nutrient requirements
- Presence of inhibitory and toxic substances
- Mixing effect

2.2.1. Temperature

Anaerobic digestion system can work under a wide range of temperatures; the psychrophilic temperature range (below 20°C), the mesophilic temperature range (between 20°C and 40°C), the thermophilic temperature range (above 40°C). The optimum ranges of temperature for anaerobic digestion has been reported as the mesophilic range (30 to 38°C) and the thermophilic range (49 to 57°C) (Metcalf and Eddy, 2003).

Anaerobic digestion systems are commonly employed at mesophilic temperatures. However, thermophilic range is more efficient than mesophilic range and thermophilic reactors can accept higher organic loading rates while producing lower quantities of sludge. In addition, studies state that hydraulic retention time of mesophilic reactors are longer than the thermophilic reactors. Figure 2.3 illustrates the hydraulic retention times of the three temperature ranges (Calli, 2011). On the other hand, mesophilic reactors are preferred more, since mesophilic reactors are stable compared to thermophilic reactors and from the economical point of view, commercial thermophilic reactors require more energy for heating. Besides, studies show that thermophilic reactors produce high concentrations of volatile fatty acids in the effluent and this is an undesired inhibitory condition.

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

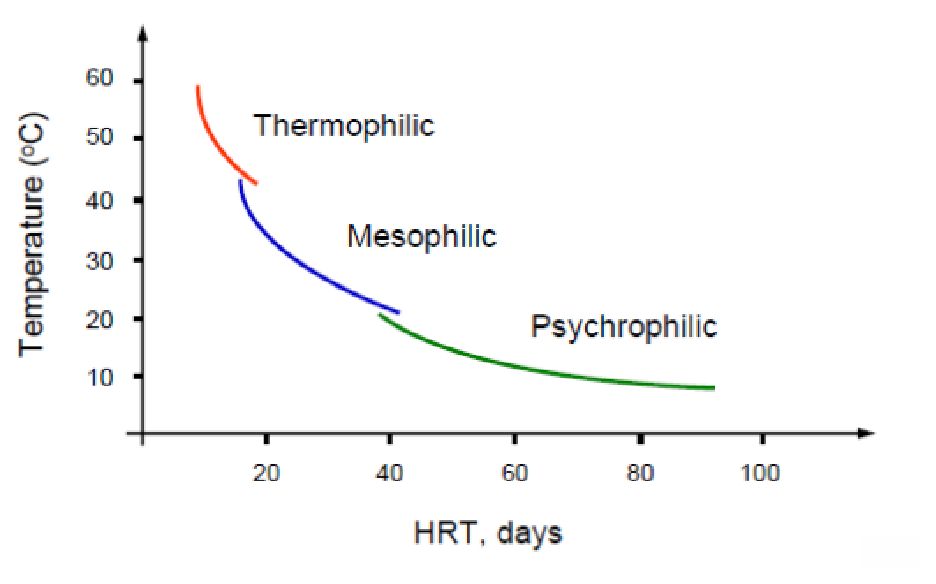


Figure 2.3. Hydraulic retention time (HRT) versus temperature (Calli, 2011).

Temperature changes greater than 1°C per day affect process performance thus, changes less than 0.5°C per day can be tolerated (Metcalf and Eddy, 2003). If microorganisms are adversely affected by temperature fluctuations, it may need several days or weeks to ameliorate and provide a healthy population.

2.2.2. pH

pH is an effective parameter which has a significant influence on the AD microorganisms, alkalinity and solubility rate of the substrates. It is generally reported that optimum pH ranges for anaerobic digestion process vary between 6.8 and 8.0. In addition, it is well known that a slight change in pH may result in reduction of gas production or gas production may even cease. Thus, pH is considered as one of the most effective factors of anaerobic digestion process.

Under normal conditions pH reduction by acidogenic bacteria is buffered by the bicarbonate produced by methanogens. By-products like carbon dioxide and volatile fatty acids affect the pH of the reactor. Excess volatile fatty acid accumulation cause pH values to be lower. The poor buffering capacity may result in toxicity and inhibition of methanogenic bacteria and consequently may prevent methane production.

Low pH and excessive acid production and accumulation are more inhibitory to methane forming bacteria than to acid formers. Methanogenic bacteria survive in a pH range between 6.0 and 7.2. The pH below 6.0 is toxic to methanogenic bacteria. However, acid forming bacteria have a better tolerance and can live at that lower pH range and keep producing volatile fatty acids. Therefore, increase in volatile fatty acid levels can be an indicator of system failure.

2.2.3. Alkalinity

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

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

$$N_2 + 3H_2 \rightarrow 2NH_3$$

$$NH_3 + H_2O + CO_2 \rightarrow NH_4HCO_3$$

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

2.2.4. Nutrient Requirements

If a wastewater has a balanced composition, in terms of macro and micro nutrients, the growth rate of the microorganisms will be higher than those of in an unbalanced wastewater. The most important nutrients are nitrogen and phosphorus, which are mainly named as macronutrients. In addition to nitrogen and phosphorus, many other elements are required in trace amounts. Microelements like iron, nickel, cobalt, selenium, molybdenum or tungsten are important for the growth and survival of the anaerobic digestion microorganisms as sodium, potassium, magnesium, sulfur.

Both macro and micro nutrients have to be in an available form for the microorganisms. Although these elements are needed in low concentrations, the lack of these nutrients has an adverse effect upon the microbial growth and performance. If the performance of an anaerobic treatment plant is poor without any obvious reason, firstly trace elements should be checked and the wastewater has to be supplemented with the trace elements prior to treatment. These requirements can be inferred from the elemental composition of the methanogens since methanogens have relatively high concentrations of some micronutrients (Rajeshwari et al., 2000). Table 2.3 presents the elemental composition of the methanogens (Rajeshwari et al., 2000).

In the same study it is also advised that the nutrient concentration in the influent should be adjusted to a value equal to twice the minimal nutrient concentration required so that small excess in the needed nutrient concentration can be guaranteed as safety factor (Rajeshwari et al., 2000).

Table 2.3. Typical elemental composition of methanogens (Rajeshwari et al., 2000).

Macronutrients		Micronutrients	
Element	Concentration (mg/kg)	Element	Concentration (mg/kg)
N	65.000	Fe	1800
P	15.000	Ni	100
K	10.000	Co	75
S	10.000	Mo	60
Ca	4.000	Zn	60
Mg	3.000	Mn	20
		Cu	10

2.2.5. Inhibition and Toxicity

Another important factor which influences the activity of anaerobic microorganisms is the presence of toxic and inhibitory compounds. They can be brought into the AD system together with the feedstock or are generated during the process. A substance is inhibitory when it causes an adverse effect in the microbial community or inhibition of bacterial growth. The inhibitors commonly present in anaerobic digesters include volatile fatty acids, ammonia, sulfide, alkali metal ions, heavy metals, and organics. The inhibition levels of these substances vary in the literature. These variations are due to the different characteristics of the waste composition and environmental conditions. Inhibition and toxicity can be prevented by getting precautions such as co-digestion with other waste, adaptation of microorganisms to inhibitory substances and addition of removals.

In addition to below outlined inhibitors some organic substances can also react in inhibitory or toxic behavior. Besides, absence of oxygen is an obligatory for anaerobic digestion systems. The presence of oxygen may affect the system adversely.

2.2.5.1. Volatile Fatty Acids (VFA) The most common inhibition in anaerobic process is caused by the accumulation of volatile fatty acids. Volatile fatty acid accumulation should be considered together with the pH and the alkalinity of the system. Especially under low pH conditions, the volatile fatty acids exist mostly in the free form, so diffusion in the cell will be higher and consequently the inhibition will increase. It is essential that the anaerobic digestion system has enough buffer capacity to neutralize potential VFA accumulation (Rajeshwari et al., 2000). Besides, studies state that proper mixing of the digester can avoid local VFA accumulation in the system.

In literature, it is stated that VFA level up to 6000 mg/L do not inhibit anaerobic systems. A well balanced anaerobic digestion system can tolerate VFA concentrations of up to 4000 mg/L (Tajarudin, 2006).

2.2.5.2. Ammonia Inhibition All substances contain nitrogen and microorganisms need both nitrogen and carbon for the integration of cell structure. However at high pH ranges even a low nitrogen concentration may act as an inhibitor. As the nitrogen in the substrates decomposes, ammonium (NH_4^+) is produced. If pH in the anaerobic digester increases, ammonium will be converted to ammonia (NH_3) as illustrated in the below equation.

$$NH_4^+ + OH^- \rightarrow NH_3 + H_2O$$

At high pH, the above given equation shifts to right. As a result, free ammonia (NH_3) which has toxic effect on the growth of the microorganism is formed. Concentrations between 50 mg/L and 200 mg/L are reported to be favorable (Bhattacharya, 1989). However, severe inhibition of free ammonia nitrogen for unadapted bacteria was reported to be 700 mg/L and 1100 mg/L in literature (Kaparaju, 2005). Table 2.4 shows the behaviors of ammonia nitrogen concentrations in anaerobic treatment systems (Stronach et al., 1986). In addition, studies also confirm the importance of acclimation; microorganisms should have enough time to adapt the inhibitory substance since a relatively small increase in ammonia concentration results in a crucial failure from no effect condition (Bhattacharya, 1989).

Table 2.4. The effects of ammonia nitrogen concentrations on anaerobic treatment (Stronach et al., 1986).

Ammonia nitrogen concentration (mg/L)	Effects on anaerobic treatment
50-200	Beneficial
200-1000	No adverse effect
1500-3000	Inhibitory at high pH ranges
Above 3000	Toxic

Although ammonia nitrogen concentrations above 1500 mg/L is considered as inhibitory, studies on the effects of ammonia nitrogen concentrations show variations about limit values (Bhattacharya, 1989). Thus, more research is needed to focus on ammonia toxicity. However, these variations are mainly attributed with the outcome of acclimation characteristics, solids retention time and kinetics of anaerobic systems exposed to ammonia (Bhattacharya, 1989).

2.2.5.3. Heavy Metal Inhibition Heavy metals such as chromium, iron, cobalt, copper, zinc, cadmium and nickel and also alkali metals like sodium, potassium, magnesium and calcium are also important in anaerobic systems, since they are required for microbial growth and they affect specific growth rate. However, excessive amounts of heavy metal concentrations slow down the growth and can cause inhibition and toxicity. Not only excessive amounts but also low concentrations of cooper, zinc, nickel and iron can adversely affect anaerobic treatment. Especially, industrial wastewaters may contain different heavy and alkali metals at different concentrations. Excess concentrations of alkali metals; sodium, potassium, calcium and magnesium can be strongly toxic for anaerobic treatment systems.

In literature, there are several reports which study the inhibitory effects of heavy metals in anaerobic digestion systems. The toxicity of heavy metals depends on their concentration in the soluble or ionic form and not on the total concentration in the digester. Thus, even for the same metal, reported toxic concentrations have varied widely. The presence or addition of sulfide to a digester containing heavy metals results in the formation of the corresponding metal sulfides. Thus, heavy metal toxicity can be prevented by the addition of sulfide (Lawrence and McCarty, 1965).

<u>2.2.5.4. Sulfide Inhibition</u> Even though sulfide can be employed effectively to control heavy metal toxicity, higher concentrations cannot be tolerated and sulfide inhibition may occur. Sulfide inhibition is mainly observed in methanogenesis, since the methanogens are more vulnerable to hydrogen sulfide (H_2S) toxicity. Soluble sulfide concentrations between 50 to 100 mg/L can be tolerated without adaptation of anaerobic microbial community, but sulfide concentrations in the form of H_2S are found to be toxic above 200 mg/L (Stronach et al., 1986). However, it is indicated in the literature that hydrogen sulfide (H_2S) toxicity is observed at the near end of range 50 mg/L (Parkin et al., 1990).

2.2.6. Mixing

Mixing enhances the distribution of microorganisms, substrate and nutrients in the reactor. Adequate mixing provides the whole reactor working volume available for use, since it provides sufficient contact between the bacteria and the substrate. In anaerobic digestion process mixing can be provided by mechanical equipments, biogas recirculation or by feed recycle.

The advantages of mixing are reported as in the following (Callı, 2011):

- Mixing reduces scum build-up and prevents grit deposition.
- Mixing equalizes the temperature within the digester.
- Mixing provides uniformity throughout the digester both chemically and physically.

- Mixing minimizes toxicity as dispersing toxic materials entering the tank.
- Mixing provides rapid hydrolysis of wastes by allowing the hydrolytic bacteria to attack a much larger surface area.

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

2.3. Biomass and Biogas Potential of Turkey

Turkey's energy demand has generally been met by fossil fuels via foreign sources. Turkey is an energy importer, although it has a great potential of renewable energy sources. Besides, due to the oil price crisis and the increase in the need for the limited resources of fossil fuels, developed and developing countries have focused on the alternative sources of energy that can stand for fossil fuels.

Despite the potential of various renewable energy sources such as solar, wind and even geothermal potential, mainly hydro power plants are installed and used in Turkey. Among others; geothermal energy, solar and biomass applications are rarely utilized and installed up to a small scale plants. The installed capacity of power plants by resources in Turkey is presented in Table 2.5 (EIE, 2011).

Turkey has a remarkable amount of renewable energy sources, which should be turned into a benefit. Apart from other renewable energy sources, biomass is an another option that can be used as an alternative to fossil fuels, since energy generated via biomass lessens air emissions compared to fossil fuels, reduces the amount of waste sent to landfills and at the same time decreasing dependence on foreign oil.

Table 2.5. Breakdown of installed capacity by resources in Turkey (EIE, 2011).

Resources	Installed capacity (%)	Installed capacity (MW)
Coal	24	10.095
Natural gas	32	13.612
Oil	4	1.793
Hydraulic	33	14.083
Renewables	7	2.809
Total Installed Capacity		42.394

Biomass can be a preferable renewable energy source in Turkey, because there is a huge potential of biomass resources, which are listed below. Turkey's annual biomass energy potential is given in Figure 2.4 (Demirbas, 2008).

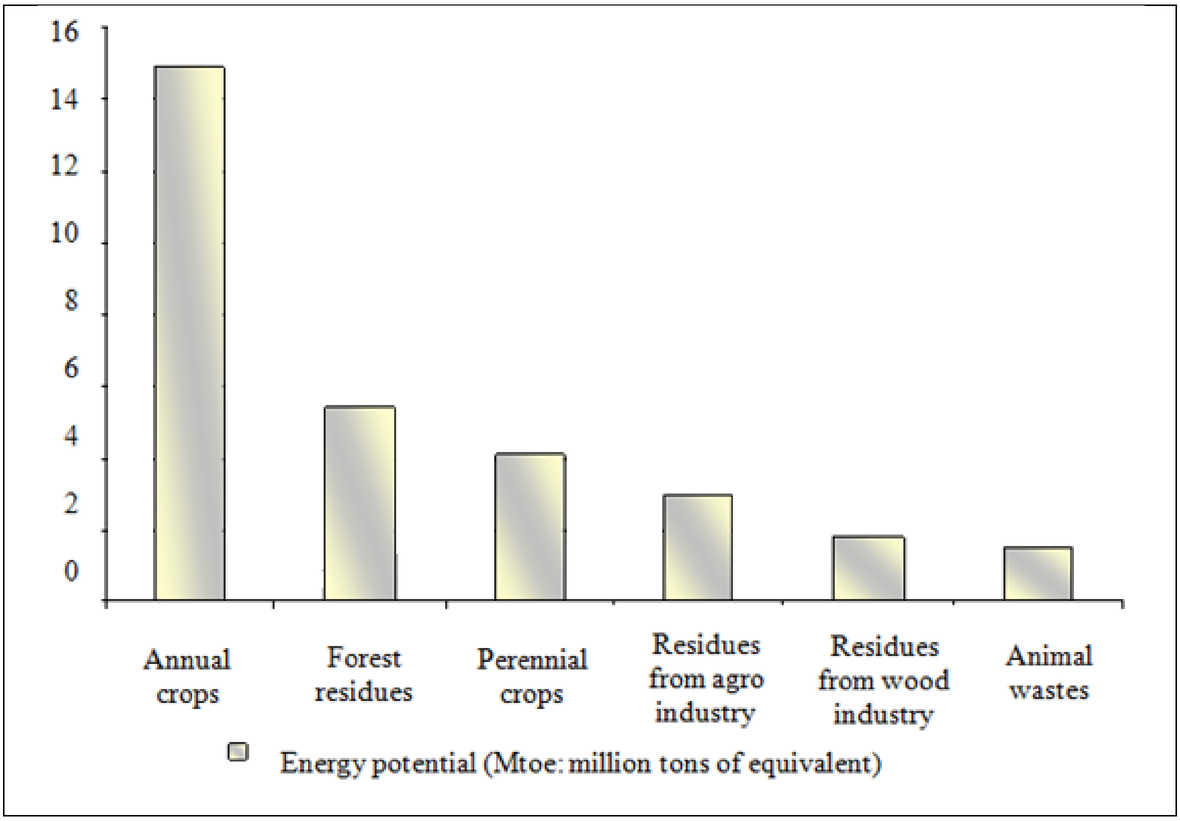


Figure 2.4. Turkey's annual biomass energy potential (Demirbas, 2008).

The available agricultural and animal residues in Turkey have been estimated to be roughly equal to 22–27% of energy consumption (Ozturk and Bascetincelik, 2006). The potential of animal waste in Turkey is also given in Table 2.6 (EIE, 2011).

Table 2.6. Annually 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

As the quantities of residues from the annual and perennial crops cultivated in Turkey were calculated and estimated using data from local authorities of Ministry of Agriculture and Rural Affairs, the total annual amount of agricultural residues in Turkey is reported to be about 50–65 Mtons (Ozturk and Bascetincelik, 2006).

As mentioned before, Turkey has highly developed in terms of agriculture and animal husbandry. For the agro industry, residues of the harvested crops and other agricultural remains make another concern as waste management problem. Ozturk and Bascetincelik (2006) classified the agricultural residues in three categories;

- Annual crop residues that remain in the field after the crops are harvested. The main annual crops in Turkey are cereals, maize, cotton, rice, sunflower, groundnuts and soybeans;
- Perennial residues in Turkey that remain in the field after pruning of trees,
- Agro-industrial residues such as; cotton-ginning, seed oil industries, olive oil industries, rice industries, corn industries, wine and seed factories.

In general after harvesting, the residues are left over the field. Cereal straw is used for various purposes such as animal feeding and animal bedding. However, most of the agricultural residues cannot be utilized as animal feeding and left available in order to supply the villagers' combustion need. Thus, apart from combustion, more environment friendly recovery option should be found and utilized immediately.

2.4. Poultry Manure and Sunflower Residues as Substrates for Anaerobic Digestion Process

In literature, generally, anaerobic digestion has mainly been associated with the treatment of animal manure and sewage sludge from aerobic wastewater treatment. Several trials were carried out with the additions of different co-substrates to manure rendered from pigs, cows and chickens. The mentioned co-substrates include agricultural residues as well as organic residues from industries, municipal bio-waste, food waste etc. Anaerobic digestion is typically a proper option for treatment of chicken manure (0.35-0.60 m^3/kg VS biogas yield; 60-80% CH_4 content) (Pola, 2011), whereas agricultural crops may need pretreatment techniques due to their low biodegradability.

The low biodegradable organic compounds of agricultural yields are mainly composed of cellulose, hemicellulose and lignin. An effective pretreatment should break the strong link of lignocelluloses, thus increasing the access of bacteria. Related studies point out that physical pretreatment of agricultural crops such as shredding does not provide a better surface area contact between microorganisms and substrates (Batstone et al., 2005). However, methane potential of sunflower stalks was increased by several thermo-chemical pretreatments. In one of the studies regarding to lignin removal; oxidative and alkaline pretreatment were found more effective compared to acidic pretreatment of hemicelluloses removal (Monlau et al., 2011).

During this experimental work, one of the agricultural residues generated from a factory located in Uzunköprü, Edirne, was selected and used to evaluate its biogas production potential. Current utilization approach of the selected residue, along with other residues generated from the same factory, is summarized in Table 2.7.

Apart from rice and wheat processing, the mentioned factory produces sunflower oil. The head sections of the sunflower plant are collected from the fields, the wastes are left for the villagers so that they can collect and can use the residues as heating stuff. When the villagers collect the residues the agricultural terrain is cleaned from the residues and prepared for the future harvesting period.

When the head sections of the sunflower residue reach the factory, mainly three different wastes are produced. Firstly, the seeds are separated from the plant and head sections, these residues are also utilized for heating stuff and distributed to the villagers. Secondly, the seeds are separated from their hulls. Unfortunately, these hulls cannot be reused. They neither have a burning potential nor quality to be used as feeding material. Finally, the third waste is generated during the oil producing phase as a muddy paste, which is rich in protein and other nutrients. The mentioned residues are utilized as pulps and the villagers buy them for the cattle feeding.

Table 2.7. Current utilization approach of agricultural residues.

Name of the Plant	Sun flower	Rice	Wheat
Part of the plant, separated as waste	Mainly hulls, soon after harvesting firstly stalks are separated	Thin and rough rice hulls including stalks	Mainly hulls; including bran and middlings
Utilization approach of the wasted sections - if any	Feed stuff as pulps	Rough rice hulls are not used and they separate them as waste. Thinner rice hulls are used as litter material on chicken yards and for isolation purposes	Utilized as feed stuff

Since the second waste, sunflower hulls, cannot be reused and they have no other applications, sunflower hulls were chosen as the agricultural residue to investigate whether they can be used in an AD process for waste disposal and biogas generation.

Other co-substrate, poultry manure has been provided from a broiler chicken yard located in Bolu region, which provides meat for a well known chicken brand in the general market.

There are several studies which put forward the advantage of poultry manure compared to the other animal manures. Among the types of animal manure, chicken manure has a higher fraction of biodegradable organic matter (Wulf, 2005; Weiland, 2009; Gokcol et al., 2008). Biogas yield of different variations of manure types are illustrated in Table 2.8 and it is reported that biogas yield rendered via excreta from chicken varies between 0.3 and 0.8 m^3/kg TS (Deublein and Steinhauser, 2008). Therefore, chicken manure is considered as an attractive co-substrate for anaerobic digestion process.

Table 2.8. Biogas yield of animal manure (EIE, 2011).

Manure type	Dry matter (%)	Organic dry matter in dry matter content (%)	Biogas yield (m^3/kg TS)
Liquid manure from cattle	6-11	68-85	0.1-0.8
Excreta from cattle (fresh)	25-30	80	0.6-0.8
Liquid manure from pigs	3-10	77-85	0.3-08
Excreta from pigs	20-25	75-80	0.27-0.45
Excreta from chicken	10-29	67-77	0.3-0.8
Excreta from sheep (fresh)	18-25	80-85	0.3-0.4
Excreta from horses (fresh)	28	25	0.4-0.6

In Turkey, poultry industry has been developing fast in the last decades. There are approximately 17.000 poultry farms, with a total capacity reaching 235 million poultry (Yum-Bir, 2011). According to these statistics, in Turkey, chicken manure potential is approximately 5.5 million ton/year (EIE, 2011). Especially in Marmara region, there is a rapid growth in poultry sector. Marmara region and the vicinity is the location where more than 50 breeding companies and most of the reputable chicken companies are located. Utilization of chicken manure as a substrate will be an efficient way in terms of both acting as an alternative to waste disposal approach and for providing the required nutrients to the AD process. Apart from the potential input of biogas generation, as chicken manure is very rich in terms of nitrogen and phosphorous, waters coming from chicken yards causes deterioration in the small creeks (Koc, 2002).

As mentioned before, agro industry takes a significant place in Turkey's economy. Therefore, there is a significant amount of agricultural wastes produced. However, especially in rural areas, where the locals are mainly busy with agriculture and animal husbandry, agricultural wastes are mixed with animal wastes and are used as fuel via direct combustion. From this point of view anaerobic digestion is an option to recover the wastes and to lower the carbon monoxide portions rendered from direct combustion.

2.5. Literature Survey

The need for alternative energy sources for Turkey is obvious. As mentioned before in terms of energy generation, there is a high foreign oil dependency. Therefore, in order to dispose agricultural residues and poultry manure and to recover bio-energy from these wastes, it is an important alternative to evaluate for producing renewable energy by the sources of the country. In literature, there exist studies on anaerobic digestion of agricultural residues and manure for production of biogas. However, no literature was encountered on anaerobic co-digestion of agricultural residues and animal waste (especially poultry manure) for Turkey. A recent literature review summarizing the related studies is given in Table 2.9.

A number of studies are available to determine the potential of biogas via animal wastes. For Turkey, Ekinci et al. (2010) prepared a detailed feasibility report on the potential biogas plants which utilize animal manure. The study has been prepared nationwide, as much as possible. 10% of the collectable animal manure from each province of Turkey were analyzed (Ekinci et al., 2010). In this economical feasibility study, the capacities and the number of possible biogas plants, the maximum amount of methane that could be generated, electrical and heat energy, digestate production, amount of CO_2

reduction, and revenues were calculated. The collectable animal manure includes wastes of dairy cattle, beef cattle, sheep, goats, horses, broiler chickens, poultry chickens, turkeys, ducks and geese.

There exist several thesis studies conducted in Turkey, which investigated the optimum conditions for the anaerobic digestion of poultry manure (Ekinci, 2009; Karatas, 2006). Since poultry farms are highly developed in Turkey and make a significant market, there also studies regarding to the environmental effects of poultry farming (Koc, 2002). Apart from the economic point of view, Koc (2002) also focuses on the surface waters which are highly affected by the process effluents of the poultry farms.

However, there are not many studies for the co-digestion of agricultural residues, especially with poultry manure. Since, dairy cattle is more common in Turkey, studies generally focus on co-digestion of cattle manure. In one of the case studies in Turkey, effect of operational parameters on anaerobic co-digestion of dairy cattle manure and agricultural residues was studied (Demirer et al., 2010). In this study, clover, grass and wheat straw were used as agricultural residues and semi-continuous reactors fed with/without agricultural residues, were operated under varied temperatures (10°C, 20°C and 35°C). The reactors were fed with same feedstock type, but operated under different operational conditions (same HRT but different temperature, or, same temperature but different HRTs). Demirer et al. (2010) indicated that the effect of agricultural residue addition did not influence the rate and extent of biomethanation of cattle manure, however, the effect of temperature was clearly observed on reactor performance.

Ozturk and Bascetincelik (2006) studied agricultural biomass potential in Turkey on a regional basis and considered agricultural waste management alternatives such as combustion, gasification and anaerobic digestion. Leconte et al. (2009) investigated the effect of different proportions of rice hulls and/or saw dust with poultry manure on co-composting efficiency and final compost quality. Excluding the co-composting technique on rice hulls and sawdust, the study takes interest on the results over the biodegradability of rice hulls and sawdust. Since rice hulls are covered by a waxed surface and high silica contents that makes an inhibitory effect on the process efficiency.

Table 2.9. Literature review of recent studies.

Title of the article	Biogas percentage or highest methane yield	Conditions	Application type
Biogas and CH_4 productivity by co-digesting swine manure with three crop residues as an external carbon source (Miller et al., 2010)	0.68	mesophilic	batch study
Substituting energy crops with organic wastes and agro-industrial residues for biogas production (Schievano et al., 2009)	65± 5%	mesophilic	batch study
Dry anaerobic ammonia–methane production from chicken manure (Kitamura et al., 2009)	103.5 mL/g VS	thermophilic	batch study
Dry mesophilic fermentation of chicken manure for production of methane by repeated batch culture (Abouelenien et al., 2009)	31 mL/g VS	mesophilic	batch study
Optimization of biogas production by co-digesting whey with diluted poultry manure (Gelegenisa et al., 2007)	40%	mesophilic	continuously stirred tank reactors
Biogas production from blends of powdered rice husk with some agro industrial wastes (Uzodinma et al., 2007)	70-78%	mesophilic	Chinese model biodigestor
Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations (Thomas et al., 2006)	7500–10200 m^3/N ha	mesophilic	batch study
Effects of acidic pretreatmet on biogas production from chicken manure (Taner and Ardıc, 2003)	55-59%	mesophilic	batch study
High solid anaerobic digestion of chicken manure (Bujoczek et al., 2000)	60-70%	mesophilic	batch study

A more detailed study of Amon et al. (2007) reported methane production through anaerobic digestion of various energy crops in Austria. The study investigates the optimum time of harvesting and its effects on methane yield. The results put forward the importance of harvesting period. For example, the highest methane yield was achieved from maize harvested at maximum ripeness (full maturity). However, cereals should be harvested when the grain is in the milk stage. Sunflowers were also analyzed within the scope of the study and significant differences between the results were found. Sunflower varieties differed in their oil composition. First harvesting was performed when the leaves were still undergrowth. The second was done at full flowering but methane yield was on a much lower level. However, the maximum methane yield was observed at the fourth harvesting period. Thus, the study suggests further research to clarify if difference results of methane production depend on the oil composition of the sunflower at different harvesting periods.

3. MATERIAL AND METHODS

3.1. Batch Reactor Set up Ratios and Loading

To accomplish the objectives of the study outlined above, in each test, different amount of the chosen agricultural crop, namely the sun flower residues, were mixed with the poultry manure, and sufficient amounts of microbial seed as inoculum were added to the mixture, in order to provide the required microbial culture. The anaerobic digestion process takes place at mesophilic (35 - 42^0C) or thermophilic (45 - 60^0C) temperature conditions. In this study, the anaerobic batch reactors were run under mesophilic conditions at 35^0C.

1 L borosilicate glass bottles were used as batch reactors and they were filled with mixtures composed of different amounts of the sun flower residues, poultry manure and inoculum culture. Two parallel reactors were run without sun flower addition as control reactors. Four reactors were run as test reactors. All the reactors were loaded depending on their mass. Table 3.1 illustrates the ratios and the percentages loaded in each set.

Firstly, it was decided to prepare descending percentages of sunflower residues and increasing percentages poultry manure (in terms of weight). However, in the further studies, it was impossible to observe biogas generation. According to the literature data, this consequence could be attributed to the low biodegradability of the sunflower residues. Due to huge volume and low weight of the sunflower residues, it was not able to achieve a proper TS ratio in batch reactors. The bottles could only get maximum 75 grams of sunflower residue (maximum amount to cover the volume of the bottle). In one case, shredding has also been applied in order to lower the volume of sunflower residues. However this could only help to lower the total volume 50% and had no effect on the biogas production. Similar studies also indicate that physical pretreatment such as grinding does not provide a better surface area contact between microorganisms and substrates (Hill, 1983). Therefore, it was decided to dominate poultry manure instead of sun flower.

Considering the inoculum to substrate ratio, new sets were prepared, but the TS ratio was so high that the system achieved dry digestion conditions, by reaching TS ratio more than 20%. Thus, the substrate proportion as well as inoculum was divided to half. Still a proper TS ratio could not be provided, therefore the active volume was also increased at set 6.

Table 3.1. Loading ratios of the experimental sets.

Sets and Working Volumes	Weight (gr)			Substrate/Active Volume (gr/mL)	
	Sun Flower	Poultry Manure	Inoculum	Sun Flower	Poultry Manure
Set 1 (500 mL)	75	15	150	0.15	0.03
Set 2 (500 mL)	60	30	150	0.12	0.06
Set 3 (500 mL)	10	190	100	0.02	0.38
Set 4 (500 mL)	20	180	100	0.04	0.36
Set 5 (500 mL)	20	80	50	0.04	0.16
Set 6 (800 mL)	5	95	50	0.006	0.12

Homogenized substrate mixture and inoculum were added to the bottles and the overall mixture was diluted to active volume limit with tap water. All the analytical analyzes were conducted using deionised water. However, the reactors are considered as trial of the commercial digester tanks and it is not feasible to fill the big digesters with deionised water. Therefore, the reactors were filled with tap water.

Each reactor was seeded with anaerobic digested sludge. The inoculum to substrate ratio was set to I/S: 1:2 (w/w). The sludge was initially collected from Pakmaya, İzmit Plant and later from Fritolay, Kandıra Factory. For Pakmaya İzmit Plant, the total solid (TS) concentration of the anaerobic sludge was 3.36% as average and the volatile solid (VS) concentration was 47.40%. For Fritolay Factory, the total solid (TS) concentration of the anaerobic sludge was 3.01% as average and the volatile solid (VS) concentration was 54.47%. The characteristics of the inoculum sludge and substrates are analyzed and presented in Section 3.3.

Both substrates 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 total solid (TS), volatile solid (VS) contents and pH of the mixtures were analyzed. Although poultry manure has a solid physical characteristic, it easily solvable with the addition of water. The images of raw inlet substrates are presented in Figure 3.1 and Figure 3.2, respectively.

Figure 3.1. Original sample-poultry manure.

Poultry manure samples were like a thick muddy paste composed of dung and some feather. As presented in Figure 3.2, sun flower residues were bulky and only included the hulls of the sun flower seeds. Even the weight of the sunflower residues were low, they had covered a big volume of space.

Figure 3.2. Original sample-sun flower residue.

The optimum methane production can be achieved at pH range of 7.0–8.5 (McKendry, 2002). Therefore, the pH of the batch reactors was adjusted to 7.0-7.2 by using 6 N NaOH and 6 N H_2SO_4 so as to maintain optimum pH conditions.

Ports of the batch reactors were closed with rubber stopper and the connections were well sealed with silicone. The reactors were placed in a water bath at an average temperature (35^0C) under mesophilic conditions. The experimental set up is also shown in Figure 3.3. It is important to keep the temperature constant during the digestion process, since the temperature changes or fluctuations will adversely affect biogas production. Therefore, the temperature was managed to be kept constant by an automatic heat controller. The temperature of the set up area was monitored daily so as to provide corrections according to Standard Conditions for Temperature and Pressure (STP). The water level of the water bath was controlled and since it evaporated, the water bath was filled regularly with water.

Figure 3.3. Experimental set up layout.

So as to provide the homogenous media during the anaerobic digestion phase, reactors were gently mixed daily. Reactors were purged with nitrogen gas so as to remove oxygen from the system so that this degasification process can assist to provide anaerobic conditions. In addition, this application also visualizes the air permeable loops and helps to control whether the system is gas tight or not. Each set was composed of 4 parallels and two control mixtures.

There are several techniques for the laboratory gas measurement methods (Petrozzi and Dunn, 1991). In this study, in order to determine the biogas yield, cumulative biogas production was monitored daily via inverted graduated cylinders and gas composition (CH_4/CO_2) was analyzed regularly with a gas chromatograph (GC, Agilent). A schematic diagram for inverted graduated cylinder utilization is given in Figure 3.4. (Olson, 2010). Acidified water ($pH<2$) was used to minimize the dissolution of carbon dioxide in water (Monlau et al., 2011).

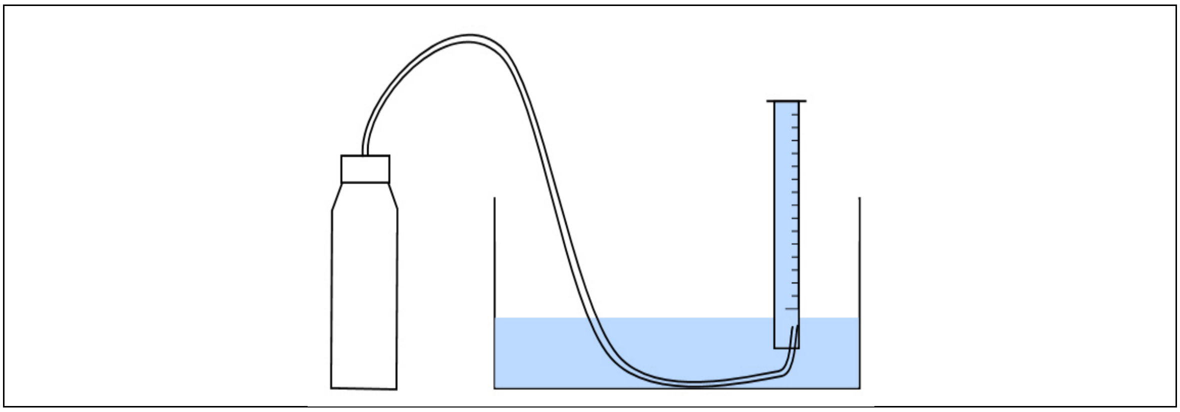


Figure 3.4. Schematic diagram of inverted graduated cylinder utilization (Olson, 2010).

Observed cumulative biogas production values were corrected according to Standard Conditions for Temperature and Pressure (STP). In order to evaluate the biogas potential of the substrates used, the results are expressed as (mL biogas/gr VS added) and (mL biogas/gr VS degraded).

The biogas composition with regard to CH_4 and CO_2 content was analyzed using a 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). 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.

3.2. Analytical Procedure and Analyze Techniques

All the raw stocks, mixtures before loading and mixtures after digestion were analyzed for total solids (TS), volatile solids (VS), pH and alkalinity. Analyzed parameters throughout the study and the analytical protocol for the whole experimental work are summarized in Table 3.2. Parallel samples were used for the measurements. Total Kjeldahl Nitrogen (TKN), total phosphorus (TP), total suphide (TS), ammonium-N, alkalinity, total solids (TS), total volatile solids (TVS), suspended solids (SS) and volatile suspended solids (VSS) analyses were performed according to the procedures outlined in Standard Methods for the Examination of Water and Wastewaters (APHA/AWWA/WPCF, 1998).

Alkali and heavy metals analyzes (calcium-Ca, potassium-K, magnesium-Mg, sodium-Na, iron-Fe, nickel-Ni, lead-Pb, copper-Cu, manganese-Mn, zinc-Zn and mercury-Hg) were performed with a Perkin Elmer Analyst 300 AAS and Optima 2100 DV ICP-MS via liquid extracts of the substrates. Liquid extracts were prepared as a result of the total solids concentration. 1 L extract preparation was considered throughout extract preparation and calculations were realized accordingly. All the mixtures were hydro extracted via centrifuge system and the top solution was filtered with 0.45 mm filter paper.

Monitoring of pH was carried out offline, using a WTW pH 330 pH-meter with a WTW Sen Tix probe. The volatile fatty acid (VFA) concentrations were measured using Perkin Elmer Clarus 600 Gas Chromatograph with a flame ionization detector (FID). Maximum operational temperature of the FID detector is 260ºC; oven temperature set point is 100ºC and after inlet the temperature increases up to 240ºC. Injection volume is 2.0 µL and helium gas (He) is used as carrier gas and consumes 0.8ml/min. Hydrogen gas (H_2) per 45.0 ml/min and air per 450.0 ml/min are also utilized in FID detector. Column of the Perkin Elmer Clarus 600 Gas Chromatograph FID detector has 30 meter length and 0.32 mm inner diameter.

Table 3.2. Analytical protocol of the study.

Parameter	Agricultural Crop (sun flower residue)	Chicken manure	Inoculum (sludge)	Substrate + Inoculum	
				Before digestion (influent)	After digestion (effluent)
pH	√	√	√	√	√
Alkalinity		√		√	√
Total solids (TS)	√	√	√	√	√
Volatile solids (VS)	√	√	√	√	√
Suspended solids (SS)	√	√	√	√	√
Volatile suspended solids (VSS)	√	√	√	√	√
Volatile fatty acids (VFA)					√
Total Kjeldahl nitrogen (TKN)	√	√			
Total phosphorous (TP)	√	√			
Alkali and Heavy metals K, Mg, Na, Ca, Fe, Ni, Zn, Cu, Pb, Co, Cr, Cd, Hg	√	√			√

4. RESULTS AND DISCUSSION

4.1. Characteristics of Substrate and Inoculum

4.1.1. Characteristics of Inoculum

Experimental sets number 1 and 2 have been prepared with the seed sludge obtained from Pakmaya Izmit Plant. The methane production was observed in the first two experiments. However, for the following couple of trials, biogas production could not be achieved. These trials are not reported here. The seed sludge was then changed for further trials. Therefore, sets 3, 4, 5 and 6 have been run with the anaerobic seed sludge taken from Fritolay Izmit Plant. For all the sludge samples, total solids, volatile solids and pH were measured before each set. The results are presented below in Table 3.3.

Table 3.3. Characteristics of the seed sludge.

Parameters	Sludge obtained from Pakmaya	Sludge obtained from Pakmaya	Sludge obtained from Fritolay	Sludge obtained from Fritolay
	Sludge 1	Sludge 2	Sludge 3	Sludge 4
pH	7.05	7.36	7.18	7.16
Total Solids (TS) (%)	2.23	4.49	3.65	2.36
Volatile Solids (VS) (%/TS)	44.15	50.65	56.76	52.19

4.1.2. Characteristics of Sun Flower Residue

During the experimental work, two different stocks of sun flower residue were used. The characteristics of the sun flower residue are given in Table 3.4 below. First two sets have been prepared with the first stock and for the final 4 batch sets, the second sunflower residue stock were used.

Sun flower residues were analyzed for pH, total solid (TS), volatile solid content (VS%/TS), Total Kjeldahl Nitrogen (TKN), total phosphate (TP), heavy and alkali metals. The pH of sunflower residues ranged from 5.09 to 5.37, and on average, the pH was around 5.23. As explained in section 3.2, analyzes were conducted via the extract prepared from the sunflower residues. Each analyze were conducted with parallel samples. Lead (Pb), cobalt (Co), cadmium (Cd) and mercury (Hg) were not determined in the sunflower extracts, however, nickel (Ni), copper (Cu) and chromium (Cr) were analyzed.

The total solid (TS) concentration of the sun flower residues ranged from 91 to 92%. The volatile solids (VS) concentration ranged between 97 and 98%/TS. The moisture content ranged between 8 and 9%. The moisture content of the sunflower residues was very low compared to that of poultry manure. The pH of sun flower residues ranged between 5.09 and 5.37, with an average value of 5.23. The pH of the sun flower residues were analyzed via liquid extracts of the substrate. Extract preparation is explained in Section 3.2.

The two sun flower stocks were quite different in terms of TKN results. The first sun flower stock consist of 2.60 g/kg, whereas the second feed stock was relatively high; 11 g/kg. On average, TKN was observed as 6.80 g/kg. The concentration of phosphate ranged between 4 to 9 g/kg with an average value of 6.50 g/kg.

Potassium concentration was relatively high when compared with other alkali metals. On average, the potassium concentration was determined as 522 mg/L. Magnesium concentration of the sunflower residues ranged between 27 to 39 mg/L with an average value of 33 mg/L. Sodium concentration of the sunflower residues ranged between 5 to 8 mg/L with an average value of 6.50 mg/L. Calcium concentration of the sunflower residues ranged between 41 to 45 mg/L with an average value of 43 mg/L.

As mentioned previously, the heavy metal measurements were also conducted for sun flower residues. Lead (Pb), cobalt (Co), cadmium (Cd) and mercury (Hg) were not found in the samples analyzed. However, iron (Fe), nickel (Ni), zinc (Zn), copper (Cu) and chromium (Cr) were detected. Iron concentration of the sunflower residues ranged between 0.02 to 2.80 mg/L with an average value of 1.40 mg/L. Nickel concentration of the sunflower residues ranged between 0.04 to 0.05 mg/L with an average value of 0.045 mg/L. Zinc concentration of the sunflower residues was observed as 0.09 mg/L in two different sun flower feed stocks. Copper and chromium were only observed in the second sun flower stock sample. The copper concentration was observed as 0.08 mg/L and chromium concentration was 0.04 mg/L. The heavy metal presence might be attributed to the composition of the fertilizers that are extensively used in agricultural activities.

Table 3.4. Characteristics of sun flower residue.

Parameters	Average value	Sun flower 1st Stock	Sun flower 2nd Stock
pH	5.23	5.09	5.37
Total Solids (TS) (%)	91.50	91	92
Volatile Solids (VS) (%/TS)	97.50	97	98
TKN (g/kg)	6.80	2.60	11
Total phosphate (g/kg)	6.50	4	9
Moisture content (%)	8.50	9	8
Potassium (K) (mg/L)	522	361	682
Magnesium (Mg) (mg/L)	33	27	39
Sodium (Na) (mg/L)	6.50	5	8
Calcium (Ca) (mg/L)	43	41	45
Iron (Fe) (mg/L)	1.40	0.02	2.80
Nickel (Ni) (mg/L)	0.045	0.04	0.05
Zinc (Zn) (mg/L)	0.09	0.09	0.09
Copper (Cu) (mg/L)	0.08	ND	0.08
Lead (Pb) (mg/L)	ND	ND	ND
Cobalt (Co) (mg/L)	ND	ND	ND
Chromium (Cr) (mg/L)	0.004	ND	0.004
Cadmium (Cd) (mg/L)	ND	ND	ND
Mercury (Hg) (mg/L)	ND	ND	ND

4.1.3. Characteristics of Poultry Manure

During the experimental work, four different stocks of poultry manure were used. The characteristics of the poultry manure are given in Table 3.5 below.

Poultry manure were analyzed for pH, total solid (TS), volatile solid content (VS%/TS), Total Kjeldahl Nitrogen (TKN), total phosphate (TP), heavy and alkali metals. As explained in section 3.2 analyzes were conducted via the extract prepared from the poultry manure. Each measurement was conducted with parallel samples. When compared to the sunflower residues, heavy metal parameters were all available in poultry manure extracts. Even mercury (Hg) was measured as 0.02 mg/L.

The total solid (TS) concentration of the poultry manure ranged from 49 to 74%, with an average value of 65%. The volatile solids (VS) concentration ranged between 84 and 92%/TS, with an average value of 99%/TS. The moisture content ranged between 26 and 51%. The moisture content of the poultry manure was very high compared to that of sunflower residues.

The pH analyzes were conducted via the extract as mentioned before and described in section 3.2. The pH of poultry manure samples ranged between 5.23 to 5.96 and the average pH value was 5.56.

TKN results ranged between 14 to 31 g/kg with an average value of 22 g/kg. The phosphate concentration ranged between 48 to 81 g/kg with an average value of 64 g/kg. The phosphate concentration of poultry manure was very high compared to that of the sunflower residues.

Potassium concentrations were relatively high when compared with other alkali metals. The potassium concentration of the poultry manure ranged between 1206 to 2018 mg/L, and on average, potassium was determined as 1592 mg/L. Magnesium concentration of the poultry manure ranged between 120 to 194 mg/L with an average value of 120 mg/L. Sodium concentration of the poultry manure ranged between 131 to 240 mg/L with an average value of 170 mg/L. Calcium concentration of the poultry manure ranged between 270 to 397 mg/L with an average value of 356 mg/L. Alkali metal concentrations of poultry manure were very high compared to the alkali metal concentrations of the sunflower residues.

Heavy metal analyzes were also conducted with poultry manure samples. Iron concentration of the poultry manure ranged between 0.20 to 20 mg/L, with an average value of 8 mg/L. Nickel concentration of the poultry manure ranged between 0.10 to 0.30 mg/L, with an average value of 0.20 mg/L. Zinc concentration of the poultry manure ranged between 4 to 20 mg/L with an average value of 11 mg/L. Copper concentration of the poultry manure ranged between 0.10 to 0.60 mg/L with an average value of 0.30 mg/L. Lead was only measured in the first sample as 0.07 mg/L. Cobalt concentration of the poultry manure ranged between 0.02 to 0.15 mg/L with an average value of 0.10 mg/L. Cadmium was only observed in the third sample as 0.04 mg/L. Chromium concentration of the poultry manure ranged between 0.02 to 0.15 mg/L with an average value of 0.10 mg/L. Mercury was observed in the first and the third samples as 0.02 mg/L.

The heavy metal presence in the poultry manure might be attributed to bioaccumulation. There are several studies in literature, which investigated the presence of these elements in the commercial feedstuffs and their accumulation (Mantovi, et al., 2003; Sager, 2007). Commercial feedstuffs are frequently enriched with essential elements such as copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), cobalt (Co), molybdenum (Mo), and selenium (Se), to promote optimum nutrient supply and optimum growth rates. A related study is presented in section 4.9 as comparison.

Table 3.5. Characteristics of poultry manure.

Parameters	Average value	Poultry manure sample 1	Poultry manure sample 2	Poultry manure sample 3	Poultry manure sample 4
pH	5.56	5.23	5.96	5.58	5.46
Total Solids (TS) (%)	65	64	49	74	71
Volatile Solids (VS) (%/TS)	89	90	89	84	92
TKN (g/kg)	22	14	31	22	21
Total phosphate (g/kg)	64	81	48	65	61
Moisture content (%)	35	36	51	26	29
Potassium (K) (mg/L)	1592	1206	1499	2018	1644
Magnesium (Mg) (mg/L)	149	194	121	120	159
Sodium (Na) (mg/L)	170	142	131	167	240
Calcium (Ca) (mg/L)	356	386	371	397	270
Iron (Fe) (mg/L)	8	4	0.20	20	9
Nickel (Ni) (mg/L)	0.20	0.30	0.20	0.10	0.30
Zinc (Zn) (mg/L)	11	4	4	20	16
Copper (Cu) (mg/L)	0.30	0.10	0.20	0.60	0.30
Lead (Pb) (mg/L)	0.07	0.07	ND	ND	ND
Cobalt (Co) (mg/L)	0.10	0.15	0.02	0.10	0.15
Chromium (Cr) (mg/L)	0.10	0.15	0.02	0.15	0.10
Cadmium (Cd) (mg/L)	0.04	ND	ND	0.04	ND
Mercury (Hg) (mg/L)	0.02	0.02	ND	0.02	ND

After the sets were finalized, TKN, Ammonium-N and sulfide analyzes have been conducted to a new poultry manure stock. The purpose of these re-measurements was to evaluate the inhibition in the system and cessation of methane generation.

The total solid (TS) concentration of the poultry manure was calculated as 66% on average and the volatile solids (VS) concentration was calculated as 86%/TS on average. TKN analyze results showed an average value of 41.43 g/kg and the ammonium-N result was calculated as 12.35 g/kg. The phosphate concentration was 81 g/kg and the total sulfide concentration was measured as 88 g/kg.

4.2. Results and Related Data for Set 1

The first experiment was carried out using 75 gr of sunflower residues and 15 gr of poultry manure with the addition of 150 gr inoculum. The active volume was set to 500 mL. Before and after the digestion, pH, total solids, volatile solids, total Kjeldahl nitrogen and total phosphorous were analyzed. The results are presented in Table 4.2, under Section 4.1.2. Cumulative biogas generation versus time for Set 1 is illustrated in Figure 4.1 and Table 4.1 presents the methane generation results of Set 1. Since this experimental work also aims to investigate the potential use of the digestate for agricultural purposes, alkali and heavy metal content of the digestate was measured. The results are also compared to the limit values of the regulations in law and digestate utilization was mentioned in Section 4.8.

4.2.1. Cumulative Biogas Generation

Cumulative biogas generation versus time for Set 1 is illustrated in Figure 4.1 and Table 4.1 presents the methane generation yields of Set 1. The first experiment lasted for 39 days and the cumulative biogas production was 2154 mL. Produced biogas result was corrected according to standard conditions for temperature and pressure (STP) and the average biogas production was determined to be 1934 mL. The average methane produced in Set 1 was calculated as 518 mL.

Table 4.1. Summary of results for Set 1.

Biogas produced at STP (mL)	Cumulative methane produced (mL)	Biogas produced /VS degraded (L/kg)	Biogas produced /VS added (L/kg)	Methane produced/VS degraded (L/kg)	Methane produced/VS added (L/kg)
1934	518	23.00	22.00	6.00	5.90

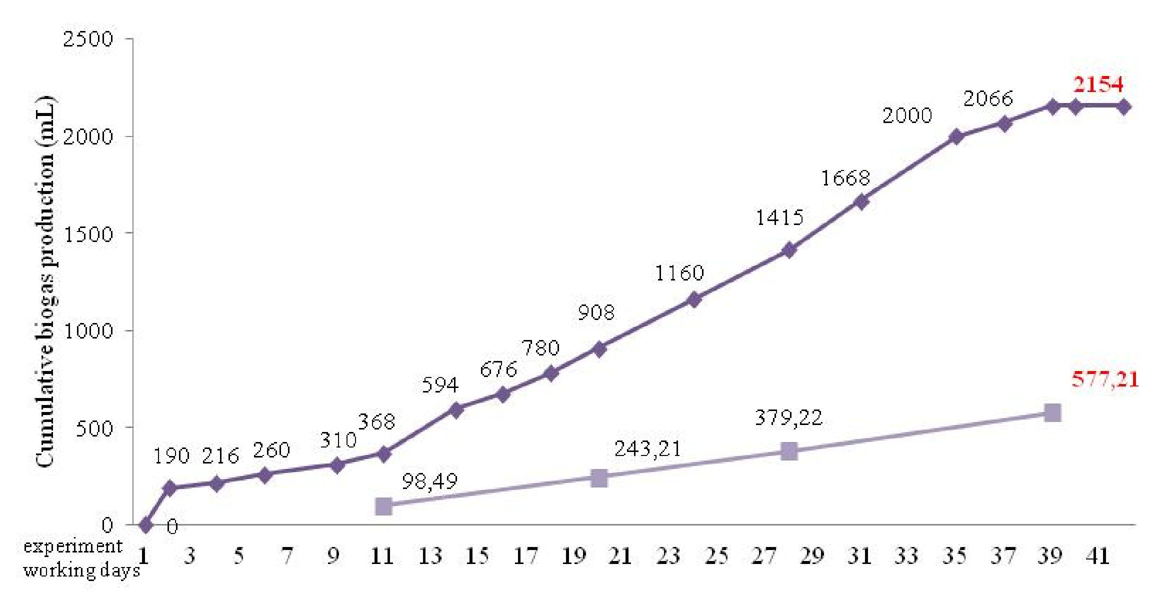


Figure 4.1. Cumulative biogas production versus time for Set 1.

Biogas composition measurements were conducted for four times during this set. The highest amount of methane composition in biogas was around 26%.

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

Total solids concentration of the sludge and substrate mixture before digestion was calculated as 58% and decreased to 21% at the end of digestion process. The high change in the TS concentration of the sample might have been due to the mixture which was not homogenized. The volatile solids (VS) concentration of the sludge and substrate mixture before digestion was observed as 98%. However, there was not much reduction in VS concentration. After the digestion, the VS concentration was 95%. This outcome might be attributed to the low biodegradable characteristic of the sun flower residues (Set 1 included sunflower dominant mixture with 75 gr sun flower and 15 gr poultry manure).

The initial pH of the sludge and substrate mixture before the digestion was measured as 6.63. At the end of the digestion, the pH was 7.67. The TKN value of the sludge and substrate mixture before digestion was calculated as 34 g/kg and increased to 44 g/kg at the end of digestion process. The concentration of phosphate in the sludge and substrate mixture before digestion was calculated as 95 g/kg and increased to 122 g/kg at the end of digestion process.

Alkali and heavy metal analyzes were conducted for the digestate. Potassium and sodium results were relatively high when compared with other alkali metal analyzes. Potassium concentration of the sludge and substrate mixture after the digestion was observed as 1133 mg/L and sodium concentration of the sludge and substrate mixture after the digestion was observed as 940 mg/L. Magnesium concentration of the sludge and substrate mixture after the digestion was observed as 93 mg/L, while calcium concentration of the sludge and substrate mixture after the digestion was 157 mg/L.

Compared to the alkali metal results, heavy metal concentrations were very low in the digestate. Iron concentration of the sludge and substrate mixture after the digestion was observed as 2 mg/L, while nickel concentration was 0.09 mg/L. The concentrations of zinc and copper were 0.25 and 0.05 mg/L, respectively. Lead was not found in the sludge and substrate mixture. Cobalt concentration of the sludge and substrate mixture after the digestion was observed as 0.02 mg/L. Chromium concentration of the sludge and substrate mixture after the digestion was observed as 0.05 mg/L. Cadmium was not detected, but mercury was measured in the sample. The mercury concentration was 0.25 mg/L.

The results of the heavy and alkali metal analyzes are further explained in Section 4.8 and compared with the related regulations in force to see whether the digestate can be used for agricultural applications.

Table 4.2. Analyze results of Set 1, before and after digestion.

Parameters	Sludge and substrate mixture before digestion	Sludge and substrate mixture after digestion
pH	6.63	7.67
Total Solids (TS) (%)	58	21
Volatile solids (VS) (%/TS)	98	95
Total Kjeldahl Nitrogen (TKN) (g/kg)	34	44
Total P (g/kg)	95	122
Potassium (K) (mg/L)	_	1133
Magnesium (Mg) (mg/L)	_	93
Sodium (Na) (mg/L)	_	940
Calcium (Ca) (mg/L)	_	157
Iron (Fe) (mg/L)	_	2
Nickel (Ni) (mg/L)	_	0.09
Zinc (Zn) (mg/L)	_	0.25
Cooper (Cu) (mg/L)	_	0.05
Lead (Pb) (mg/L)	_	ND
Cobalt (Co) (mg/L)	_	0.02
Chromium (Cr) (mg/L)	_	0.05
Cadmium (Cd) (mg/L)	_	ND
Mercury (Hg) (mg/L)	_	0.25

4.3. Results and Related Data for Set 2

The second experiment was carried out using 60 gr of sunflower residues and 30 gr of poultry manure with the addition of 150 gr inoculum. The active volume was set to 500 mL. Before and after the digestion, pH, total solids, volatile solids, total Kjeldahl nitrogen and total phosphorous were analyzed. The results are presented in Table 4.4, under Section 4.2.2. Cumulative biogas generation versus time for Set 2 is illustrated in Figure 4.2 and Table 4.3 presents the methane generation results of Set 2. Since this experimental work also aims to investigate the potential use of the digestate for agricultural purposes, alkali and heavy metal content of the digestate was measured. The results are also compared to the limit values of the regulations in law and digestate utilization was mentioned in Section 4.8.

4.3.1. Cumulative Biogas Generation

The cumulative biogas generation versus time for Set 2 is illustrated in Figure 4.2 and Table 4.3 presents the methane generation results of Set 2. The second experiment lasted for 30 days and the cumulative biogas production was 1746 mL. Produced biogas result was corrected according to standard conditions for temperature and pressure (STP), and the average biogas was calculated as 1568 mL. Average methane produced in Set 2 was calculated as 804 mL.

Table 4.3. Summary of results for Set 2.

Biogas produced at STP (mL)	Cumulative Methane produced (mL)	Biogas produced /VS degraded (L/kg)	Biogas produced /VS added (L/kg)	Methane produced/VS degraded (L/kg)	Methane produced/VS added (L/kg)
1568	804	18.00	17.90	9.00	9.20

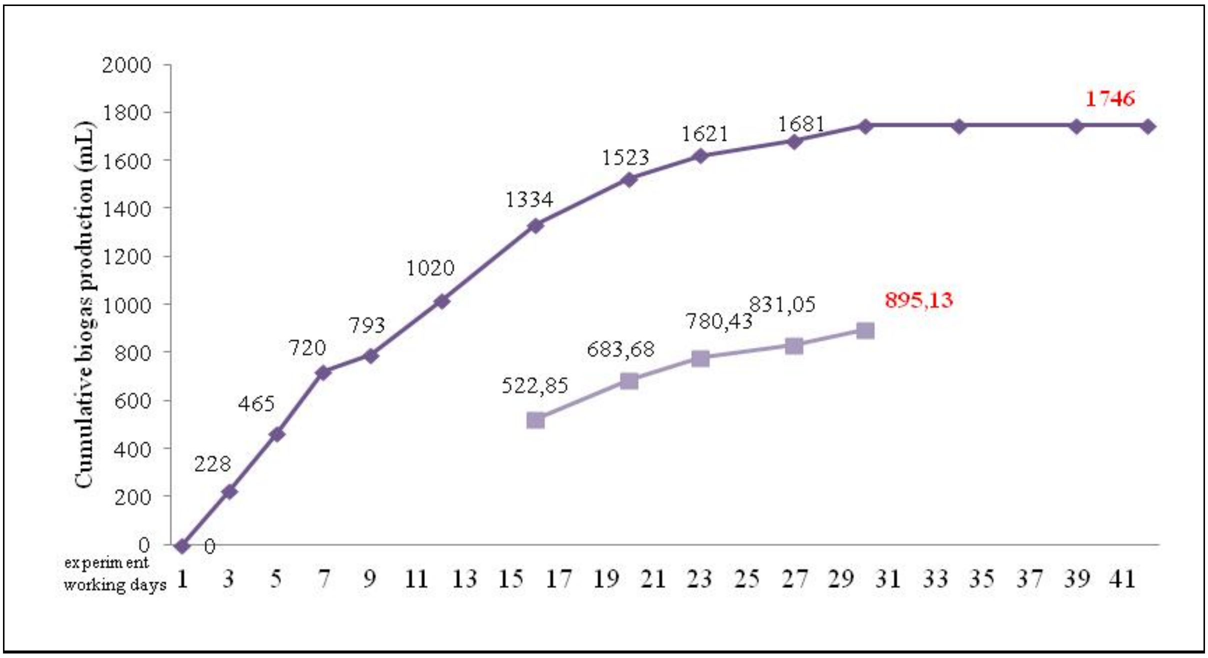


Figure 4.2. Cumulative biogas production versus time for Set 2.

Biogas composition was analyzed for five times. The highest amount of methane in biogas composition was 51%, however, the methane yields were low.

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

Total solids concentration of the sludge and substrate mixture before digestion was calculated as 44% and decreased to 43% at the end of digestion process. There was not much change in total solids concentration. The volatile solids (VS) concentration of the sludge and substrate mixture before digestion was observed as 97%/TS. After the digestion, volatile solids concentration was decreased to 95%. This outcome might be attributed to the low biodegradable characteristic of the sun flower residues which were relatively dominant in Set 2. Therefore, the VS destruction was quite low.

TKN values of the sludge and substrate mixture before digestion was calculated as 15 g/kg and increased to 20 g/kg at the end of digestion process. The concentration of phosphate in the sludge and substrate mixture before digestion was calculated as 39 g/kg and decreased to 21 g/kg at the end of digestion process.

Alkali and heavy metal analyzes were conducted for the digestate. Potassium and sodium results were relatively high when compared with other alkali metal analyzes. Potassium concentration of the sludge and substrate mixture after the digestion was observed as 1315 mg/L and sodium concentration of the sludge and substrate mixture after the digestion was observed as 704 mg/L. Magnesium concentration of the sludge and substrate mixture after the digestion was observed as 87 mg/L. Calcium concentration of the sludge and substrate mixture after the digestion was observed as 152 mg/L.

Heavy metal analyzes were also conducted with the sludge and substrate mixture after the digestion. Compared to the alkali metal analyzes, heavy metal results were relatively very low. Iron concentration of the sludge and substrate mixture after the digestion was observed as 0.8 mg/L. Nickel concentration of the sludge and substrate mixture after the digestion was observed as 0.15 mg/L. Zinc concentration of the sludge and substrate mixture after the digestion was observed as 0.15 mg/L. Copper concentration of the sludge and substrate mixture after the digestion was observed as 0.02 mg/L. Lead was not found in the digested sludge and substrate mixture. Cobalt concentration of the sludge and substrate mixture after the digestion was observed as 0.02 mg/L. Chromium concentration of the sludge and substrate mixture after the digestion was observed as 0.01 mg/L. Cadmium was not determined in the digested sludge and substrate mixture. However, 0.03 mg/L mercury concentration was observed.

Results of the heavy and alkali metal analyzes are further explained in Section 4.8 and compared with the related regulations in force.

Table 4.4. Analyze results of Set 2, before digestion and after digestion.

Parameters	Sludge and substrate mixture before digestion	Sludge and substrate mixture after digestion
pH	7.04	8.62
Total Solids (TS) (%)	44	43
Volatile solids (VS) (%/TS)	97	95
Total Kjeldahl Nitrogen (TKN) (g/kg)	15	20
Total P (g/kg)	39	21
Potassium (K) (mg/L)	_	1315
Magnesium (Mg) (mg/L)	_	87
Sodium (Na) (mg/L)	_	704
Calcium (Ca) (mg/L)	_	152
Iron (Fe) (mg/L)	_	0.8
Nickel (Ni) (mg/L)	_	0.15
Zinc (Zn) (mg/L)	_	0.15
Cooper (Cu) (mg/L)	_	0.02
Lead (Pb) (mg/L)	_	ND
Cobalt (Co) (mg/L)	_	0.02
Chromium (Cr) (mg/L)	_	0.01
Cadmium (Cd) (mg/L)	_	ND
Mercury (Hg) (mg/L)	_	0.03

4.4. Results and Related Data of Set 3

After Sets 1 and 2 were completed, there were some batch tests conducted. However, these trials did not provide biogas production properly. Therefore, these trials are not reported in this thesis. In one of these trials, mechanical shredding was applied to the sunflower hulls as a pretreatment option. Despite this, there was no biogas production. Thus, in order to promote biogas production, starting from Set 3, a different source of seed sludge and higher amounts of poultry manure were used in batch tests. In addition, lower TS concentrations were also used to see the impact on biogas generation.

The third experiment was carried out using 10 gr of sunflower residues and 190 gr of poultry manure with the addition of 100 gr inoculum. So as to provide the ratio of Inoculum:Substrate=1:2 (w/w), 100 gr inoculum was added. The active volume was set to 500 mL. Before and after the digestion, pH, total solids, volatile solids, total Kjeldahl nitrogen and total phosphorous were analyzed. The analyze results are presented in Table 4.5, under Section 4.3.2. Cumulative biogas generation versus time for Set 3 is illustrated in Figure 4.3. Since this experimental work also aims to investigate the potential use of the digestate for agricultural purposes, alkali and heavy metal content of the digestate was measured. The results are also compared to the limit values of the regulations in law and digestate utilization was mentioned in Section 4.8.

4.4.1. Cumulative Biogas Generation

Cumulative biogas generation versus time for Set 3 is illustrated in Figure 4.3. The third experiment lasted for 8 days, very short compared to previous sets, and the cumulative biogas production was 1278 mL. Produced biogas result was corrected according to standard conditions for temperature and pressure and biogas production was calculated as 1147 mL. However, methane generation was almost none. According to the measurements, the biogas was mainly composed of carbon dioxide (CO_2) and nitrogen gas (N_2). The highest methane observed in biogas composition was around 2%.

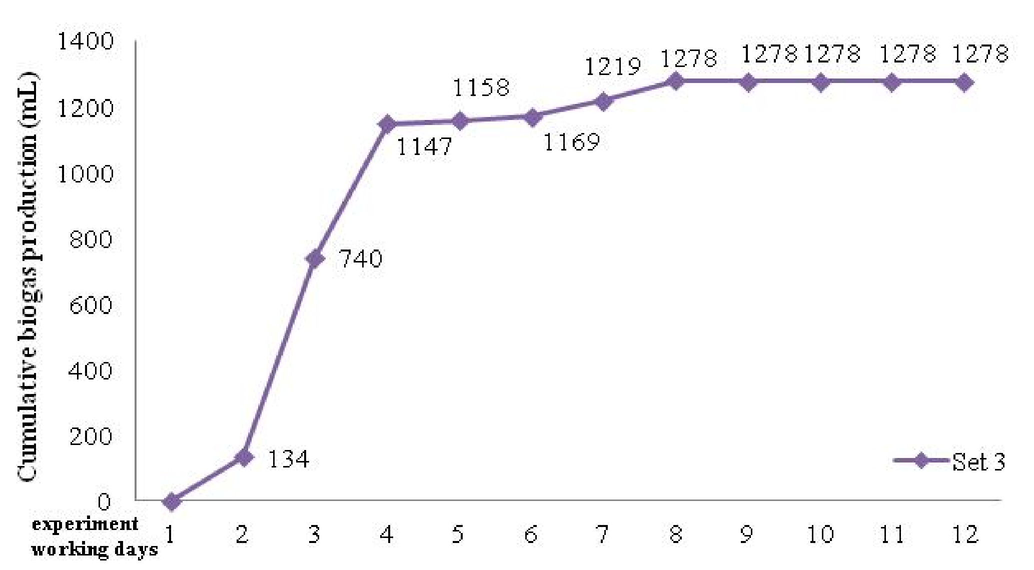


Figure 4.3. Cumulative biogas production versus time for Set 3.

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

The initial pH of the mixture was measured as 7.00. At the end of the digestion, the pH was measured to be 5.53. The decrease in pH can be considered as a prior indicator of VFA accumulation. Total solids concentration of the sludge and substrate mixture before digestion was calculated as 66% and decreased to 49% at the end of digestion process. The volatile solids (VS) concentration of the sludge and substrate mixture before digestion was observed as 97%/TS. After the digestion, volatile solids concentration decreased to 96%/TS.

TKN values of the sludge and substrate mixture before digestion was calculated as 19 g/kg and decreased to 15 g/kg at the end of digestion process. The phosphate concentration of the sludge and substrate mixture before digestion was calculated as 51 g/kg and decreased to 19 g/kg at the end of digestion process.

Alkali and heavy metal analyzes were conducted for the digestate. Potassium and sodium results were relatively high when compared with other alkali metal analyzes. Potassium concentration of the sludge and substrate mixture after the digestion was observed as 2634 mg/L and sodium concentration of the sludge and substrate mixture after the digestion was observed as 1808 mg/L. Magnesium concentration of the sludge and substrate mixture after the digestion was observed as 50 mg/L, while the calcium concentration of the sludge and substrate mixture was 85 mg/L.

Compared to the alkali metals, the concentrations of heavy metal results were very low. Iron concentration of the sludge and substrate mixture after the digestion was observed as 10.3 mg/L and nickel concentration was 0.5 mg/L. Zinc concentration of the sludge and substrate mixture after the digestion was observed as 7 mg/L, while the copper concentration was 0.06 mg/L. Lead was found in the digested sludge and substrate mixture, and its concentration was 0.02 mg/L. Cobalt concentration of the sludge and substrate mixture after the digestion was observed as 0.25 mg/L. Chromium concentration of the sludge and substrate mixture after the digestion was observed as 0.40 mg/L. Cadmium was not determined in the digested sludge and substrate mixture. However, 0.24 mg/L mercury concentration was observed.

Results of the heavy and alkali metal analyzes are further explained in Section 4.8 and compared with the related regulations in force.

Table 4.5. Analyze results of Set 3, before digestion and after digestion.

Parameters	Sludge and substrate mixture before digestion	Sludge and substrate mixture after digestion
pH	7.00	5.53
Total Solids (TS) (%)	66	49
Volatile solids (VS) (%/TS)	97	96
Total Kjeldahl Nitrogen (TKN) (g/kg)	19	15
Total P (g/kg)	51	19
Potassium (K) (mg/L)	_	2634
Magnesium (Mg) (mg/L)	_	50
Sodium (Na) (mg/L)	_	1808
Calcium (Ca) (mg/L)	_	85
Iron (Fe) (mg/L)	_	10.3
Nickel (Ni) (mg/L)	_	0.5
Zinc (Zn) (mg/L)	_	7
Cooper (Cu) (mg/L)	_	0.06
Lead (Pb) (mg/L)	_	0.02
Cobalt (Co) (mg/L)	_	0.25
Chromium (Cr) (mg/L)	_	0.40
Cadmium (Cd) (mg/L)	_	ND
Mercury (Hg) (mg/L)	_	0.24

4.5. Results and Related Data of Set 4

The fourth experiment was run using 20 gr of sunflower residues and 180 gr of poultry manure with the addition of 100 gr inoculum. The active volume was set to 500 mL. Before and after the digestion, pH, total solids, volatile solids, total Kjeldahl nitrogen and total phosphorous were analyzed. The results are presented in Table 4.6, under Section 4.4.2. Cumulative biogas generation versus time for Set 4 is illustrated in Figure 4.4. Since this experimental work also aimed to investigate the potential use of the digestate for agricultural purposes, alkali and heavy metal content of the digestate was measured. The results are also compared to the limit values of the regulations in law and digestate utilization was mentioned in Section 4.8.

4.5.1. Cumulative Biogas Generation

The cumulative biogas generation versus time for Set 4 is illustrated in Figure 4.4. The fourth experiment lasted for 8 days and the cumulative biogas production was 1152 mL. Like in Set 3, the experiment did not last long. Produced biogas result was corrected according to standard conditions for temperature and pressure, and biogas production was calculated as 1034 mL. However, methane generation could not be achieved in Set 4 again. The biogas was mainly composed of carbon dioxide (CO_2) and nitrogen gas (N_2), and less than 1% of methane composition was observed.

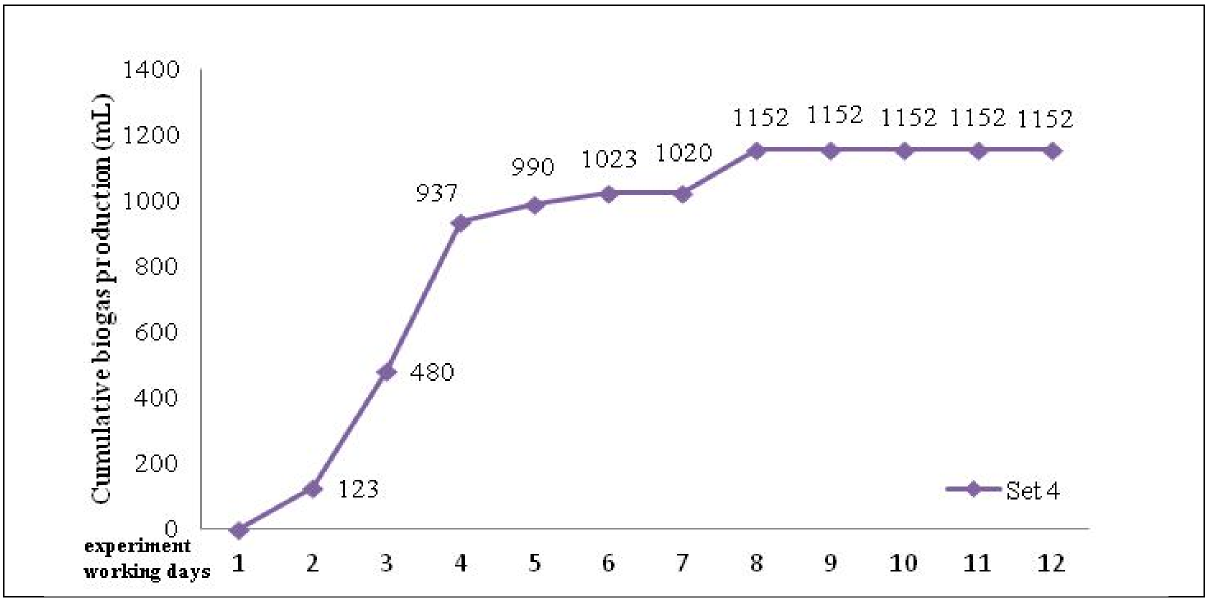


Figure 4.4. Cumulative biogas production versus time for Set 4.

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

The initial pH of the mixture was measured as 6.91. At the end of the digestion pH was measured as 5.95. Decrease in pH can be considered as a prior indicator of VFA accumulation. Total solids concentration of the sludge and substrate mixture before digestion was calculated as 65% and decreased to 24% at the end of digestion process. The volatile solids (VS) concentration of the sludge and substrate mixture before digestion was observed as 94%/TS. After the digestion, volatile solids concentration decreased to 92%/TS.

TKN results of the sludge and substrate mixture before digestion was calculated as 27 g/kg and increased to 33 g/kg at the end of digestion process. The concentration of phosphate in the sludge and substrate mixture before digestion was calculated as 87 g/kg and decreased to 53 g/kg at the end of digestion process.

Alkali and heavy metal analyzes were conducted for the digestate. Potassium and sodium results were relatively high when compared with other alkali metal analyzes. Potassium concentration of the sludge and substrate mixture after the digestion was observed as 1175 mg/L and sodium concentration of the sludge and substrate mixture after the digestion was observed as 2850 mg/L. Magnesium concentration of the sludge and

substrate mixture after the digestion was observed as 33 mg/L. Calcium concentration of the sludge and substrate mixture after the digestion was observed as 170 mg/L.

In terms of heavy metal content, the iron concentration of the sludge and substrate mixture after the digestion was observed as 7.7 mg/L, while nickel concentration was 0.4 mg/L. Zinc concentration of the sludge and substrate mixture after the digestion was observed as 2 mg/L. Copper concentration of the sludge and substrate mixture after the digestion was observed as 0.07 mg/L. Lead was determined in the poultry manure sample and lead was also found in the digested sludge and substrate mixture at a concentration of 0.01 mg/L. Cobalt concentration of the sludge and substrate mixture after the digestion was observed as 0.15 mg/L. The concentrations of chromium and cadmium were 0.45 and 0.02 mg/L, respectively. In addition, 0.17 mg/L of mercury was also detected in the digestate.

Results of the heavy and alkali metal analyzes are further explained in Section 4.8 and compared with the related regulations in force.

Table 4.6. Analyze results of Set 4, before digestion and after digestion.

Parameters	Sludge and substrate mixture before digestion	Sludge and substrate mixture after digestion
pH	6.91	5.95
Total Solids (TS) (%)	65	24
Volatile solids (VS) (%/TS)	94	92
Total Kjeldahl Nitrogen (TKN) (g/kg)	27	33
Total P (g/kg)	87	53
Potassium (K) (mg/L)	_	1175
Magnesium (Mg) (mg/L)	_	33
Sodium (Na) (mg/L)	_	2850
Calcium (Ca) (mg/L)	_	170
Iron (Fe) (mg/L)	_	7.7
Nickel (Ni) (mg/L)	_	0.4
Zinc (Zn) (mg/L)	_	2
Cooper (Cu) (mg/L)	_	0.07
Lead (Pb) (mg/L)	_	0.01
Cobalt (Co) (mg/L)	_	0.15
Chromium (Cr) (mg/L)	_	0.45
Cadmium (Cd) (mg/L)	_	0.02
Mercury (Hg) (mg/L)	_	0.17

4.6. Results and Related Data of Set 5

The fifth experiment was carried out using 20 gr of sunflower residues and 80 gr of poultry manure with the addition of 50 gr inoculum. The active volume was set to 500 mL. As the methane generation could not be achieved in the previous experiments, decreasing of the TS ratio was considered to be a good idea which may promote methane generation. Therefore, a less amount of substrate and inoculum (by weight) was used in this trial.

Before and after the digestion, pH, total solids, volatile solids, total Kjeldahl nitrogen and total phosphorous were also analyzed. The analyze results are presented in Table 4.7, under Section 4.5.2. Cumulative biogas generation versus time for Set 5 is illustrated in Figure 4.5. Since this experimental work also aims to investigate the potential use of the digestate for agricultural purposes, alkali and heavy metal content of the digestate was measured. The results are also compared to the limit values of the regulations in law and digestate utilization was mentioned in Section 4.8.

4.6.1. Cumulative Biogas Generation

Cumulative biogas generation versus time for Set 5 is illustrated in Figure 4.5. The fifth experiment lasted for 10 days and the cumulative biogas production was 1800 mL. Produced biogas result was corrected according to standard conditions for temperature and pressure and biogas production was calculated as 1616 mL. According to the gas measurements, the biogas was mainly composed of carbon dioxide (CO_2) and nitrogen gas (N_2). Methane generation could not be achieved successfully, and the observed methane concentration ranged between 0.4 and 2.9%.

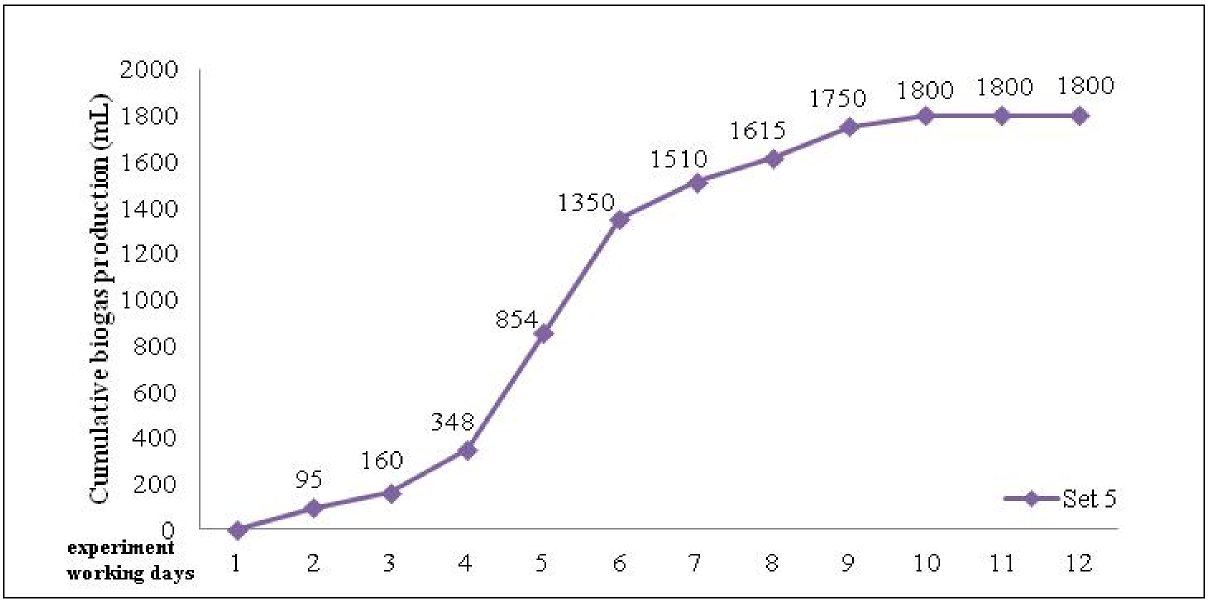


Figure 4.5. Cumulative biogas production versus time for Set 5.

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

The initial pH of the mixture was measured as 7.08. At the end of the digestion, the pH was measured to be 5.58. Total solids concentration of the sludge and substrate mixture before digestion was calculated as 57% and decreased to 48% at the end of digestion. The volatile solids (VS) concentration of the sludge and substrate mixture before digestion was observed as 99%/TS. After the digestion, volatile solids concentration decreased to 97%/TS.

TKN results of the sludge and substrate mixture before digestion was calculated as 26 g/kg and decreased to 23 g/kg at the end of digestion process. Phosphate results of the sludge and substrate mixture before digestion was calculated as 99 g/kg and decreased to 44 g/kg at the end of digestion process.

Alkali and heavy metal analyzes were conducted for the digestate. Potassium and sodium results were relatively high when compared with other alkali metal analyzes. Potassium concentration of the sludge and substrate mixture after the digestion was observed as 2642 mg/L and sodium concentration of the sludge and substrate mixture after the digestion was observed as 2292 mg/L. Magnesium concentration of the sludge and

substrate mixture after the digestion was observed as 140 mg/L. Calcium concentration of the sludge and substrate mixture after the digestion was observed as 510 mg/L.

As mentioned before, heavy metal analyzes were also conducted for the sludge and substrate mixture after the digestion. Compared to the alkali metal analyzes, heavy metal results were very low, relatively. Iron concentration of the sludge and substrate mixture after the digestion was observed as 5 mg/L. Nickel concentration of the sludge and substrate mixture after the digestion was observed as 0.2 mg/L. Zinc concentration of the sludge and substrate mixture after the digestion was observed as 4.30 mg/L. Copper concentration of the sludge and substrate mixture after the digestion was observed as 0.06 mg/L. Since lead was determined in the poultry manure sample and lead was found in the digested sludge and substrate mixture, as 0.02 mg/L. Cobalt concentration of the sludge and substrate mixture after the digestion was observed as 0.1 mg/L. Chromium concentration of the sludge and substrate mixture after the digestion was observed as 0.2 mg/L. Cadmium concentration of the sludge and substrate mixture after the digestion was observed as 0.01 mg/L, while 0.1 mg/L of mercury concentration was measured.

Table 4.7. Analyze results of Set 5, before digestion and after digestion.

Parameters	Sludge and substrate mixture before digestion	Sludge and substrate mixture after digestion
pH	7.08	5.58
Total Solids (TS) (%)	57	48
Volatile solids (VS) (%/TS)	99	97
Total Kjeldahl Nitrogen (TKN) (g/kg)	26	23
Total P (g/kg)	99	44
Potassium (K) (mg/L)	_	2642
Magnesium (Mg) (mg/L)	_	140
Sodium (Na) (mg/L)	_	2292
Calcium (Ca) (mg/L)	_	510
Iron (Fe) (mg/L)	_	5
Nickel (Ni) (mg/L)	_	0.20
Zinc (Zn) (mg/L)	_	4.30
Cooper (Cu) (mg/L)	_	0.06
Lead (Pb) (mg/L)	_	0.02
Cobalt (Co) (mg/L)	_	0.1
Chromium (Cr) (mg/L)	_	0.2
Cadmium (Cd) (mg/L)	_	0.01
Mercury (Hg) (mg/L)	_	0.1

Results of the heavy and alkali metal analyzes are further explained in Section 4.8 and compared with the related regulations in force.

4.7. Results and Related Data of Set 6

The last experiment was carried out using 5 gr of sunflower residues and 95 gr of poultry manure with the addition of 50 gr inoculum. The active volume was set to 800 mL. Since methane generation could not be achieved in the previous experiments, wet digestion conditions were tried. So as to lower the TS ratio, the mixture prepared for Set 6 was diluted. Thus, less amount of substrate was fed to the batch reactor and the active volume was increased up to 800 mL. Before and after the digestion, pH, total solids, volatile solids, total Kjeldahl nitrogen and total phosphorous were analyzed. The analyze results are presented in Table 4.8, under Section 4.6.2. Cumulative biogas generation versus time for Set 6 is illustrated in Figure 4.6. Since this experimental work also aims to investigate the potential use of the digestate for agricultural purposes, alkali and heavy metal content of the digestate was measured. The results are also compared to the limit values of the regulations in law and digestate utilization was mentioned in Section 4.8.

4.7.1. Cumulative Biogas Generation

Cumulative biogas generation versus time for Set 6 is illustrated in Figure 4.6. The sixth experiment lasted for 10 days and the cumulative biogas production was 1180 mL. Produced biogas result was corrected according to standard conditions for temperature and pressure and average biogas was observed as 1059 mL. However, methane generation could not be achieved in Set 6 again properly. According to the gas measurements, the produced biogas was mainly composed of carbon dioxide (CO_2) and nitrogen gas (N_2). Only 1 to 2.4% of methane was observed in biogas composition from batch digesters.

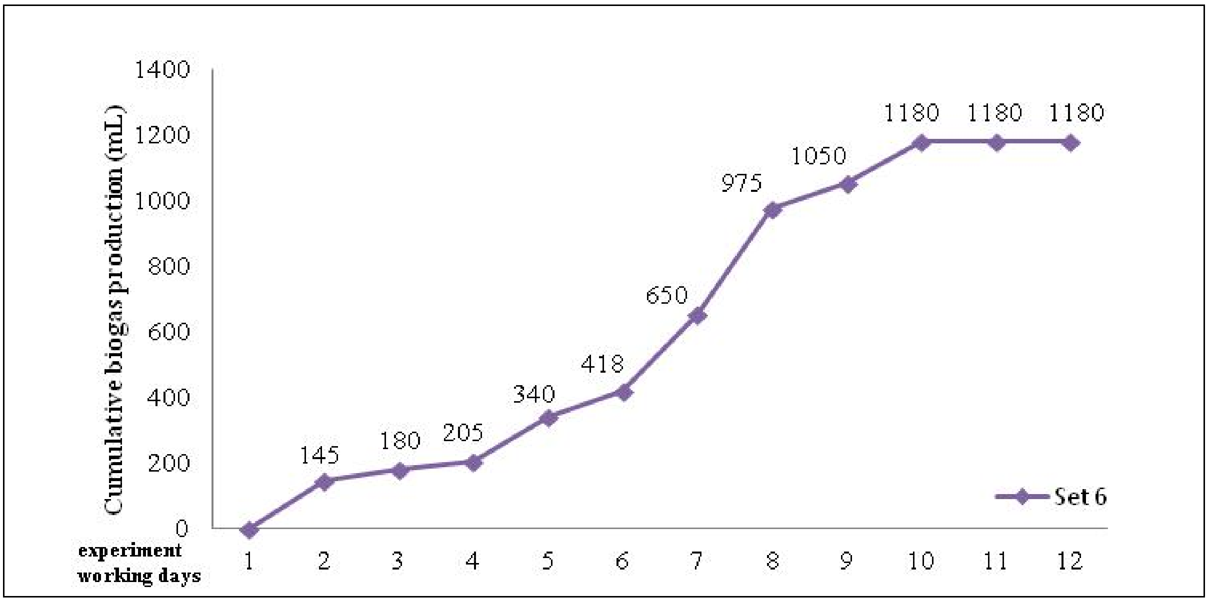


Figure 4.6. Cumulative biogas production versus time for Set 6.

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

The initial pH of the mixture was measured as 7.02. At the end of the digestion pH was measured as 6.08. Total solid concentration of the sludge and substrate mixture before digestion was calculated as 18% and lowered to 17% at the end of digestion process. The volatile solids (VS) concentration of the sludge and substrate mixture before digestion was observed as 95%/TS. After the digestion, volatile solids concentration decreased to 92%/TS.

TKN results of the sludge and substrate mixture before digestion was calculated as 32 g/kg and decreased to 29 g/kg at the end of digestion process. Phosphate results of the sludge and substrate mixture before digestion was calculated as 147 g/kg and decreased to 63 g/kg at the end of digestion process.

Alkali and heavy metal analyzes were conducted to the digestate. Potassium and sodium results were relatively high when compared with other alkali metal analyzes. Potassium concentration of the sludge and substrate mixture after the digestion was observed as 1883 mg/L and sodium concentration of the sludge and substrate mixture after the digestion was observed as 1417 mg/L. Magnesium concentration of the sludge and

substrate mixture after the digestion was observed as 165 mg/L. Calcium concentration of the sludge and substrate mixture after the digestion was observed as 264 mg/L.

Heavy metal analyzes were also conducted with the sludge and substrate mixture after the digestion. Compared to the alkali metal analyzes, heavy metal results were low. Iron concentration of the sludge and substrate mixture after the digestion was observed as 7 mg/L. Nickel concentration of the sludge and substrate mixture after the digestion was observed as 0.15 mg/L. Zinc concentration of the sludge and substrate mixture after the digestion was observed as 0.65 mg/L. Copper concentration of the sludge and substrate mixture after the digestion was observed as 0.05 mg/L. Lead was also found in the digested sludge and substrate mixture, at a concentration of 0.03 mg/L. Cobalt concentration of the sludge and substrate mixture after the digestion was observed as 0.06 mg/L. Chromium concentration of the sludge and substrate mixture after the digestion was observed as 0.1 mg/L. Cadmium was not determined in the sludge and substrate mixture after the digestion, however, 0.005 mg/L of mercury was detected.

Results of the heavy and alkali metal analyzes are further explained in Section 4.8 and compared with the related regulations in force.

Table 4.8. Analyze results of Set 6, before digestion and after digestion.

Parameters	Sludge and substrate mixture before digestion	Sludge and substrate mixture after digestion
pH	7.02	5.69
Total Solids (TS) (%)	18	17
Volatile solids (VS) (%/TS)	95	92
Total Kjeldahl Nitrogen (TKN) (g/kg)	32	29
Total P (g/kg)	147	63
Potassium (K) (mg/L)	_	1883
Magnesium (Mg) (mg/L)	_	165
Sodium (Na) (mg/L)	_	1417
Calcium (Ca) (mg/L)	_	264
Iron (Fe) (mg/L)	_	7
Nickel (Ni) (mg/L)	_	0.15
Zinc (Zn) (mg/L)	_	0.65
Cooper (Cu) (mg/L)	_	0.05
Lead (Pb) (mg/L)	_	0.03
Cobalt (Co) (mg/L)	_	0.06
Chromium (Cr) (mg/L)	_	0.1
Cadmium (Cd) (mg/L)	_	ND
Mercury (Hg) (mg/L)	_	0.005

4.8. Results of Volatile Fatty Acids (VFA) Measurements

Since methane production has been observed only in the first two sets, VFA analyzes were not conducted for Set 1 and 2. However, the final 4 sets could not achieve proper methane production. Since cessation of methane production is mainly attributed with total VFA, the concentration of total VFA is an important parameter as this can be the first indicator that digestion is not progressing normally. Thus, VFA analyzes were also applied to determine whether VFA accumulation resulted in a perturbation in the system. Table 4.9 presents the results for VFA measurements. Figure 4.7 illustrates these results.

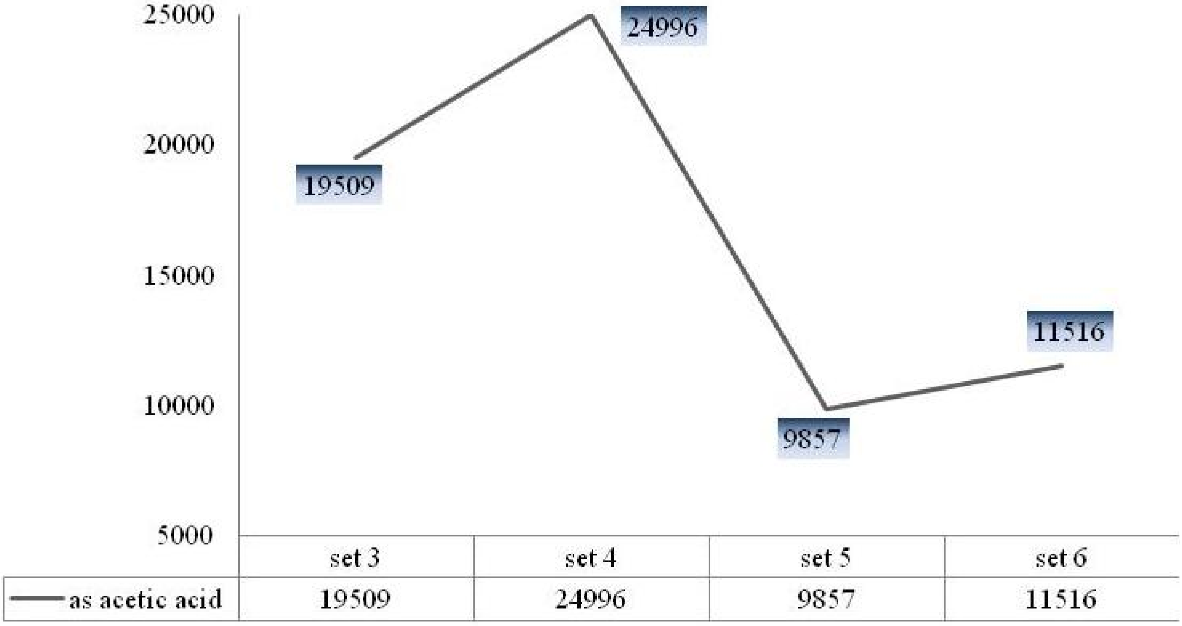
Table 4.9. VFA results for Sets 3, 4, 5 and 6.

VFA	SET 3	SET 4	SET 5	SET 6
Acetic (mg/L)	8626	6788	6613	4066
Propionic (mg/L)	4140	5662	1450	2482
Isobutyric (mg/L)	537	582	349	244
Butyric (mg/L)	3182	4304	160	2274
Isovaleric (mg/L)	549	779	430	390
Valeric (mg/L)	741	3711	139	791
Isocaproic (mg/L)	443	51	213	_
Caproic (mg/L)	79	1002	_	466
Heptanoic (mg/L)	_	497	_	104

If the results are considered, acetic acid, propionic acid and butyric acid are dominant in all the sets. The highest acetic acid presence was observed in set 3, whereas the highest propionic acid was observed in set 4. Heptanoic acid was also observed in sets

4 and 6. Set 4 differentiates from the others; as propionic, isobutyric, butyric, isovaleric and valeric acid results were comparatively high from the other sets. It has been suggested that the propionic acid to acetic acid ratio can be used as an indicator of digester imbalance (Marchaim and Krause, 1993). Propionic acid to acetic acid ratio greater than 1.4 indicated digester failure (Hill et al., 1987).

Total volatile fatty acid ratios are also calculated as acetic acid equivalents; the molar equivalent sum of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic and heptanoic acid. Figure 4.7 illustrates the acetic acid equivalents of VFA results. As illustrated in Figure 4.7, the presence of volatile fatty acids was observed in Sets 3, 4, 5 and 6. According to literature, the observed concentrations were relatively high and they were above the inhibitory levels reported. Under low pH conditions, VFA accumulation takes place. The outcome of VFA accumulation is the cessation of methane production since the system could not accomplish methanogenesis phase.



	set 3	set 4	set 5	set 6
as acetic acid	19509	24996	9857	11516

Figure 4.7. Acetic acid equivalent of VFA concentrations.

In literature, it is stated that inhibition rendered from VFA presence has been observed above the concentration of 4000 mg/L (Tajarudin, 2006) or even a less concentration of 800 mg/L acetic acid level was reported to imbalance the system (Hill et al.1987). Other researchers also stated that the volatile fatty acid concentrations should be lower than 1000-1500 mg/L, exceeding acetic acid presence result in system failure. As total volatile fatty acids as acetic acid equivalent are presented in Figure 4.7, all the values are above the limits given in the literature. The highest concentration was measured with Set 4 as 24996 mg/L VFA as acetic acid equivalent. The high values of VFA measurements can be considered as the main cause of the cessation of methane production.

4.9. Overall Evaluation of Experimental Findings

When the results of this experimental work are evaluated, it seems that anaerobic co-digestion of sun flower hulls (the agricultural residue) and poultry manure does not seem feasible, particularly in terms of methane production. Actually, the main objective of this work was to evaluate whether the anaerobic digestion process could offer an alternative option for disposal of both sun flower hulls and poultry manure. As states in previous sections, sun flower hulls have no more use and they are regarded as waste. Management of poultry manure is a very common and great problem, especially in Marmara region, where the poultry farms are commonly located. Poultry manure has traditionally been land spread on soil. However, over-application of this material can result in eutrophication of water bodies, spread of various pathogens, air pollution and emission of greenhouse gases (GHG) into the atmosphere (Kelleher et al., 2002). Animal manure is an important source of man-made GHG leading to methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions at huge quantities (Steed and Hashimoto, 1994; Moller et al., 2004). Therefore, it is obligatory to implement proper management strategies to minimize these risks.

During the experiments, in Set 1 and 2, methane production from anaerobic batch co-digestion of sun flower hulls and poultry manure could be obtained. However, methane yields were both quite low in both of these trials when compared with the results reported in literature using similar types of substrates (Section 2.5). The volatile solids (VS) destruction was also very low in both trials, indicating that the substrate, particularly the sun flower hulls, was resistant to anaerobic biodegradation. Like rice husk, which is a commonly used bedding material in poultry farms, probably the high lignin content of sun flower hulls made it a difficult substrate for anaerobic digestion (Tait et al., 2009). Therefore, it can be stated that the lignocellulosic structure of the sun flower hulls seemed to result in an overall relatively low VS conversion efficiency in batch digestion tests providing lower methane yields.

In literature, it has been reported that there are various pre-treatment techniques available in order to increase the biodegradability of agricultural residues for anaerobic digestion process. Among these techniques, wet explosion (Biswas et al., 2011), bioaugmentation and thermochemical pre-treatment (Costa et al., 2011), solid-liquid separation (Moller et al., 2002) were applied to improve biogas and methane yields. As stated previously, no pre-treatment technique was applied in this experimental work to simulate real case conditions. Only in one trial, which is not reported in this thesis, mechanical shredding was applied to the sun flower hulls, in order to reduce its particle size. However, the batch test using this pre-treated sun flower hulls was not successful.

In Sets 3, 4, 5 and 6, due to low biodegradability of the sun flower hulls, higher amounts of poultry manure and seed sludge from a different source were used. However, the methane generation was very low, almost none, in all of these trials even though there was biogas production. Like sun flower hulls, poultry manure was also reported to be a difficult substrate to handle for anaerobic digestion process in some cases. Animal manure contains more readily degradable organic materials than other agricultural by-products, however, it also contains a high amount of lignocellulose fibers as well (Bruni et al., 2010). The low biodegradability of lignocelloluse in biogas digesters results from the presence of lignin that is non-degradable in an anaerobic environment (Triolo et al., 2011), which makes manure sometimes a difficult substrate for anaerobic digestion. In addition, manure contains proteins and urea, and ammonia is released from them through degradation, which is known to be a potential inhibitor for aceticlastic methanogens (Heinrichs et al., 1990). Actually, it is the unionized form of ammonia that causes problems for the methanogens in anaerobic digestion process (Angelidaki and Ahring, 1993). It has been reported that the higher nitrogen content of poultry manure makes it a difficult substrate when compared with other types of manure from other animal farms (Salminen and Rintala, 2002; Bujoczek et al., 2000). Since methane generation could not have been achieved in sets 3, 4, 5 and 6, in addition to VFA measurement, sulfide and ammonium-N analyzes were also conducted for the poultry manure stock. In literature, the inhibitory effects of ammonia, that directly affected the methanogenesis, was reported to be in the range above 3000 mg/L and even above pH 7.4 conditions, 1500-3000 mg/L presence could slow down the methanogenesis phase (Calli et al., 2005). Chen (2008) reported that 50% reduction on the methane production occurred between ammonia concentrations of 1.7 and 14 g/L. It was also reported that the excess concentrations of sulphide around 200 mg/L at neutral pH might result in dissolved hydrogen sulphide toxicity as well (Calli, 2011). On the other hand, it was also reported that methanogenesis was not inhibited by sulfate concentrations up to 5000 mg/L (Karhadkar et al., 1987). According to measurements in this work, TKN concentrations were at an average value of 41.43 g/kg and the ammonium-N result was calculated as 12.35 g/kg in the poultry manure used. The concentration of sulphide was around 88 g/kg (on average). These results indicated that high concentrations of both ammonia and sulphide coming from the poultry manure might have caused inhibition in the system, resulting in the cessation of biogas production and methane generation.

Heavy metals concentrations were also measured after batch tests were completed. According to the results obtained, heavy metals such as iron (Fe), nickel (Ni), zinc (Zn), copper (Cu), lead (Pb), cobalt (Co), chromium (Cr), cadmium (Cd), and mercury (Hg) could be determined in the samples. However, the concentrations of these heavy metals were quite low. Therefore, they did not seem to cause any perturbation in the system. Seed sludge and poultry manure seemed to be the potential sources of these heavy metals. The inhibitors in anaerobic digestion include ammonia, sulphide, heavy metals and organics (Chen et al., 2008). In addition to ammonia, the presence of organic compounds might also have played a negative role during batch digestion.

High concentrations of VFAs accumulated in Sets 3, 4, 5 and 6, and eventually, the methane composition of biogas was almost none in all of these trials. These results indicated that the methanogenesis did not take place in batch tests. In batch anaerobic reactors, VFA concentrations around 6000 mg/L were reported to cause inhibition (Siegert and Banks, 2005). Higher VFA concentrations were measured in Sets 3 to 6.

Based on these preliminary findings from this experimental work, it seems that an alternative option, such as combustion, can be considered for disposal of sun flower hulls. If anaerobic co-digestion of sun flower hulls and poultry manure is to be realized, then the most suitable pre-treatment techniques should be determined and applied, to improve biogas and methane yields, and to prevent perturbation of present inhibitory compounds in anaerobic digestion.

4.10. Analyses of Digestate

As a recovery approach, this study also focused on the reuse alternatives of the digestate. Since poultry manure is not managed properly, this approach aims to provide an environmental friendly solution to PM management in poultry farms. Therefore, the digestate has been analyzed after batch digestion tests were completed. Even though methane generation could not have been achieved in sets 3, 4, 5 and 6, measurements were carried out for all of the sets so that heavy metal presence could be determined beforehand. According to the measurements, the concentrations of the heavy metals were found to be low. Thus, heavy metal inhibition is not considered to cease methane production in the system. If methane generation could have been achieved efficiently, using digestate as agricultural purposes could have offered an environmental friendly option.

The analyzed parameters included both alkali and heavy metals; such as calcium-Ca, potassium-K, magnesium-Mg, sodium-Na, iron-Fe, nickel-Ni, lead-Pb, copper-Cu, manganese-Mn, zinc-Zn and mercury-Hg. According to Regulation on Agricultural Utilization of Domestic and Municipal Wastewater Treatment Sludge, limit values and the analyze results are presented in the below given table (online Legislation Information System). The mentioned regulation considers limit values of the following elements; Nickel-Ni, Zinc-Zn,Copper-Cu, Lead-Pb, Chromium-Cr, Cadmium-Cd, Mercury-Hg. In all the sets, analyze results remain within the limit values so the digestate can be considered as a proper fertilizer.

Table 4.10. Limit outlet values according to the regulation on agricultural utilization of domestic and municipal WWT sludge.

Heavy metals	Limit value (mg/kg oven dried material)	Results of SET-1 (mg/kg)	Results of SET-2 (mg/kg)	Results of SET-3 (mg/kg)	Results of SET-4 (mg/kg)	Results of SET-5 (mg/kg)	Results of SET-6 (mg/kg)
Nickel (Ni)	300	0.51	0.84	1.22	1.13	2.01	1.29
Zinc (Zn)	2500	1.37	0.81	18.42	5.28	19.11	5.47
Copper (Cu)	1000	0.25	0.12	0.17	0.20	0.55	0.44
Lead (Pb)	750	ND	ND	0.04	0.02	0.16	0.24
Chromium (Cr)	1000	0.27	0.06	1.05	1.23	1.66	0.87
Cadmium (Cd)	10	ND	ND	ND	0.05	0.06	ND
Mercury (Hg)	10	1.34	0.16	ND	ND	ND	ND

According to the regulation on the production, import, marketing and agricultural utilization of organic, organomineral fertilizers and microbial enzyme containing soil conditioners (online Legislation Information System) limit values are presented in the below given table. The mentioned regulation considers limit values of the following elements; Nickel (Ni), Zinc (Zn), Copper (Cu), Lead (Pb), Chromium (Cr), Cadmium (Cd), Mercury (Hg). In both cases, outlet analyze results remain within the limit values. Therefore, the digestate can be utilized for agricultural purposes.

Table 4.11. Limit outlet values according to the regulation on the production, import, marketing and agricultural utilization of organic, organo-mineral fertilizers and microbial enzyme containing soil conditioners.

Heavy metals	Limit value (mg/kg oven dried material)	Results of SET-1 (mg/kg)	Results of SET-2 (mg/kg)	Results of SET-3 (mg/kg)	Results of SET-4 (mg/kg)	Results of SET-5 (mg/kg)	Results of SET-6 (mg/kg)
Nickel (Ni)	120	0.51	0.84	1.22	1.13	2.01	1.29
Zinc (Zn)	1100	1.37	0.81	18.42	5.28	19.11	5.47
Copper (Cu)	450	0.25	0.12	0.17	0.20	0.55	0.44
Lead (Pb)	150	ND	ND	0.04	0.02	0.16	0.24
Chromium (Cr)	350	0.27	0.06	1.05	1.23	1.66	0.87
Cadmium (Cd)	3	ND	ND	ND	0.05	0.06	ND
Mercury (Hg)	5	1.34	0.16	ND	ND	ND	ND

There are several studies in literature, which investigated the elements in the commercial feedstuffs and their accumulation (Mantovi et al., 2003; Sager, 2007). Commercial feedstuffs are frequently enriched with essential elements Copper (Cu), Manganese (Mn), Iron (Fe), Zinc (Zn), Cobalt (Co), Molybdenum (Mo), and Selenium (Se) to promote optimum nutrient supply and optimum growth rates. As stated in literature, heavy metals and other elements found in the manures are a result of bioaccumulation. For comparison, respective data is quoted from a study which researches variety of manures and the feedstuffs (Sager, 2007). Within the others, poultry dung is selected and compared to the digestate results. Table 4.12 presents this comparison.

Table 4.12. Comparison of analyze results with another relative study.

Heavy metals	Poultry dung (mg/kg)	Results of SET-1 (mg/kg)	Results of SET-2 (mg/kg)	Results of SET-3 (mg/kg)	Results of SET-4 (mg/kg)	Results of SET-5 (mg/kg)	Results of SET-6 (mg/kg)
Nickel (Ni)	8.5	0.51	0.84	1.22	1.13	2.01	1.29
Zinc (Zn)	314	1.37	0.81	18.42	5.28	19.11	5.47
Copper (Cu)	66	0.25	0.12	0.17	0.20	0.55	0.44
Lead (Pb)	5.4	ND	ND	0.04	0.02	0.16	0.24
Chromium (Cr)	10.7	0.27	0.06	1.05	1.23	1.66	0.87
Cadmium (Cd)	0.43	ND	ND	ND	0.05	0.06	ND
Mercury (Hg)	ND	1.34	0.16	ND	ND	ND	ND

4. CONCLUSIONS

The aim of this study was to investigate and to evaluate the biogas production potential of sunflower residues and poultry manure through anaerobic co-digestion process. Both sun flower residues and poultry manure constitute a waste management problem in rural areas of Turkey. Therefore, a feasible and environmentally friendly option should be found to dispose of these materials properly and to recover energy.

In order to evaluate biogas production potential from both substrates, anaerobic batch digestion tests were run under mesophilic conditions. During the experiments, methane production could only be achieved in the first two trials. However, the methane yields observed were low. The highest methane composition of biogas was 51%. The low biodegradability of the sunflower residues and the characteristics of manure did not enable a feasible methane production in these trials. Thus, further studies, including pretreatment of the sunflower residues, are recommended to increase biogas yields for anaerobic co-digestion of sunflower residues and poultry manure. In addition, alternative options, such as combustion or gasification, should also be evaluated, to handle sun flower hull residues.

Since proper methane generation could not be achieved in all of the experiments, volatile fatty acids were also analyzed to determine whether VFA accumulation caused a perturbation in the system. High concentrations of VFA were measured in sets from 3 to 6, when there was almost no methane generation in batch digesters. High VFA production can be ascribed with the cease of the methane production in these trials. Heavy metals coming mostly from poultry manure did not seem to play a role in poor methane generation values. In addition to VFA, the high concentrations of ammonia and sulphide in poultry manure might have also caused inhibition in batch systems in sets 3 to 6.

This study also focused on the reuse alternatives of the digestate. Therefore, after batch tests, the digestate was analyzed to provide further utilization of the digestate for agricultural purposes. The results were compared with the limit values of the related regulations in force. Since, the values remain within the limit values, agricultural usage of the digestate can be considered as a potential waste management option as long as high methane yields can be obtained from anaerobic co-digestion of these substrates.

As a summary, within the scope of the laboratory studies conducted under this thesis, proper methane generation could not have been achieved. Based on the experimental findings of this work, it can be concluded that the sunflower residues are not proper substrates for anaerobic digestion process. Pretreatment of sunflower residues may increase the potential of methane generation. On the other hand, without any pretreatment poultry manure can be used for anaerobic digestion. However, it is essential to monitor the concentrations of ammonia and sulphide in poultry manure. Generally, it can be stated that both sunflower residues and poultry manure are difficult substrates to handle in anaerobic digestion process together. The lignin content of sunflower is the major problem, while for poultry manure, high ammonia concentration can play an important role in an anaerobic digestion system.

5. RECOMMENDATIONS

As one of the main income sources, animal husbandry and agriculture are highly developed in Turkey. Significant amount of animal wastes and agricultural residues are produced every day, forming another waste management problem of the agro industry. Through anaerobic digestion of agricultural residues and poultry manure, wastes can be properly disposed and biogas, which is a clean and sustainable source of energy, can be continuously recovered.

Due to low biodegradability of the sunflower residues it can be stated that without any prior process sunflower residues are not proper for anaerobic digestion process. However, both physical and chemical pretreatment methods can be applied to increase the biodegradability of the sunflower residues. Thus, methane yield from anaerobic digestion of the sunflower residues can be increased and sun flower residues can be co-digested together with other type of substrates. For this purpose, further studies should be conducted with sun flower residues.

According to the current status, the poultry manure wastes remain uncontrolled. An environment friendly solution to the unmanaged poultry manure wastes should be elaborated and immediately applied. Using poultry manure as a substrate for anaerobic digestion can be a reasonable alternative and agricultural usage of the digestate can be considered as a wise waste management option.

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