BIOGAS PRODUCTION FROM SUGAR BEET AND MAIZE AT VARIOUS TOTAL SOLID CONTENTS

by

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ABSTRACT

Nowadays, energy required in almost all fields of everyday life is provided mostly by expensive imported energy sources in Turkey. In order to meet the increasing demand of energy, it is indispensable to find alternative sources. Research activities on the biogas production by using energy crops are becoming attractive research area due to abundant availability of the crops in Turkey in addition to the reasons mentioned above.

The objective of this study was to evaluate the performance of biogas production system using sugar beet and maize as substrates. Additionally, the optimum total solid content at which the most efficient biogas production took place was investigated for each crop. For this purpose, eleven different lab-scale digesters which were loaded with energy crops were monitored regularly. Anaerobically digested granular sludge was also added to the reactors and the digesters were placed in a temperature controlled water bath. During the experiments which were carried out by using maize as a substrate, the effect of inoculum to substrate ratio on the biogas production efficiency of the system was also investigated.

The results confirmed that the digester including maize and seeding sludge at 12 % total solid content with an inoculum to substrate ratio (I/S) of 1.5 performed the most efficient digestion process with regard to its biogas production, methane yield and biogas yield. On the other hand, methane generation could not be observed for the reactor including the same amount of total solid content but using the lower inoculum to substrate ratio of 1. This conclusion was attributed to high levels of VFA generated as a result of biological decomposition. The amount of seeding sludge was found to be insufficient to neutralize the negative effects of organic acids which were formed by degradation of high concentrations of organic matter contained in the reactor.

ÖZET

Türkiye'de her alanda gereksinim duyulan enerji, günümüzde çoğunlukla dış ülkelerden ithal edilmesi yolu ile pahalı bir şekilde temin edilmektedir. Artmakta olan enerji ihtiyacını karşılamak amacı ile yeni enerji kaynaklarının tespit edilmesi kaçınılmaz olmuştur. Bahsedilen bu sebeplere ilave olarak, Türkiye'de bol olarak bulunması nedeni ile enerji bitkilerinden biyogaz üretimi üzerine araştırma çalışmaları önem kazanmıştır.

Bu çalışmanın amacı şeker pancarı ve mısırı kullanarak biyogaz üretimi sağlayan sistemlerin performansını değerlendirmektir. Ayrıca, en etkili biyogaz üretiminin gerçekleştiği en uygun toplam katı madde oranı her bitki için incelenmiştir. Bu amaç doğrultusunda, enerji bitkisi içeren 11 adet laboratuvar ölçekli reaktör düzenli olarak gözlemlenmiştir. Anaerobik ortamda çürütülmüş granül çamur, reaktörlere ilave edilmiş ve reaktörler sıcaklık kontrolü bulunan su banyolarına yerleştirilmiştir. Substrat kaynağı olarak mısır ile yürütülen deneyler sırasında ayrıca I/S oranının sistemin biyogaz üretimi üzerindeki etkisi incelenmiştir.

Çalışmanın sonucunda, %12 toplam katı madde oranına sahip, mısır ile aşı çamuru içeren ve I/S oranı 1.5 olan reaktörün; biyogaz üretimi, biyogaz verimi ve metan verimi açısından en etkili reaktör olduğu belirlenmiştir. Diğer taraftan, aynı oranda toplam katı madde oranına sahip ancak I/S oranı 1 olan reaktörde metan oluşumu gözlenememiştir. Bu sonuç, biyolojik parçalanma sonucunda meydana gelen yüksek miktardaki uçucu yağ asitleri konsantrasyonuna dayandırılmıştır. Aşı çamurunun, reaktör içinde yüksek miktarda bulunan organik maddelerin parçalanması sonucu oluşan organik asitleri nötralize edecek miktardan az olduğu belirlenmiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	**Explanation**	**Units used**
IS ratio	Inoculum to Substrate ratio	$\frac{\text{g inoculum as TS}}{\text{g biomass as TS}}$
TS	Total Solid	%
VS	Volatile Solid	%
VFAs	Volatile Fatty Acids	(mg L^{-1})
TKN	Total Kjeldahl Nitrogen	%
TP	Total Phosphorus	%
COD	Chemical Oxygen Demand	(mg L^{-1})
TOC	Total Organic Carbon	(mg L^{-1})
NH_4^+-N	Ammonium nitrogen	(mg NH_4^+-N L^{-1})
PO_4^{3-}	Orthophosphate	(mg PO_4^{3-} L^{-1})
SB	Sugar Beet	-

1. INTRODUCTION

Since the adverse environmental effects of greenhouse gases have reached critical levels and the energy requirement increased, it is necessary to find alternative and cost effective sources of renewable energy. As a result of the consumption of nonrenewable fossil fuels such as coal, gasoline and natural gas; air and water pollution, global warming and adverse effects on animal and plant life take place. However, renewable energy resources can provide many environmental benefits by avoiding these impacts and risks while producing electrical, thermal or mechanical energy from natural sources [1].

Although fossil fuels will be depleted in the near future, the majority of energy consumption in Turkey is based on petroleum (40 %), coal (25 %), natural gas (21 %), and others (14 %) [2]. Turkey is heavily dependent on expensive imported energy resources, which more than half of the energy consumption is being supplied with this manner [3]. This situation creates a big burden on the national economy and air pollution is becoming a significant problem which should be considered. Nowadays, renewable energy consumption is gaining importance in order to meet the continuously increasing energy requirement without causing environmental pollution in our country.

Biomass is a renewable energy source and as a national energy policy its importance will increase in the near future. Among the other technologies to convert biomass into energy; biogas production through anaerobic digestion is one of the oldest and most promising technologies to gain energy [4]. Biogas, which is a mixture of methane and carbon dioxide gas, organic slurry as digested residue and other inorganic products are generated as a result of the breakdown of organic material by microbial population that lives in an oxygen free environment. The biogas can be used to produce both electrical power and heat and the digested residue can be re-circulated as bio-fertilizer to the fields [5]. Waste vegetable oil, animal manure, municipal solid wastes and agricultural crops which are grown specifically to produce energy (energy crops) can be utilized as biomass.

Recently, energy crops are considered as potential energy sources for anaerobic digestion systems due to their feasibility and high amount of organic matter content. Varieties with high protein, fat, cellulose, hemi-cellulose, starch content and with high potential for biomass production are especially suitable for anaerobic digestion. Maize, cereals and sugar beet are some of these energy crops owing to their high starch and low lignin contents [6].

There are various studies on biogas production from energy crops in the literature. However, harvesting time, inoculum to substrate ratio (I/S) , temperature and total solid content (TS %) are some of the parameters that should be investigated for each crop in order to be able to determine the most suitable and cost effective energy crop for anaerobic digestion.

Main objective of this research was to develop and study the feasibility of anaerobic digestion of sugar beet and maize by using anaerobic granular sludge as a seeding material. The other aim is to investigate the optimum total solid concentration at which sugar beet and maize generates the most efficient biogas at constant temperature. For this purpose, anaerobic digestion experiments were conducted under mesophilic temperature by using batch reactors containing various amounts of crop (sugar beet or maize) and anaerobic sludge. Reactors having different total solid contents were filled with shredded crop and seed sludge.

The results obtained from this experimental setups were compared to evaluate biogas production of sugar beet and maize at two different total solid contents (10 % TS, 12 % TS). The optimum concentration for each crop was determined under constant temperature.

2. LITERATURE REVIEW

Nowadays, the world's energy markets rely heavily on the fossil fuels although it is projected that this type of energy resources will be depleted in the near future. Renewable energy has gained importance in terms of sustainable development, not only for exhausting probability of fossil fuels but also because of environmental pollution, global heating and dependence on importing.

In Turkey, there is a growing demand for energy by 8 % per annum, whereas the world average is 1.8 % [2]. Turkey is heavily dependent on expensive imported energy resources that cause a big burden on the economy because domestic energy production is insufficient to meet rapidly increasing demand. Additionally, air pollution is becoming a great environmental concern in our country since fossil make up the majority of energy consumption in Turkey [2]. Due to its geographical location, Turkey has several advantages for extensive use of renewable energy resources.

The energy sources are divided into three categories; fossil fuels, renewable energy sources and nuclear energy sources [7]. Main renewable energy resources are composed of biomass energy, hydro energy, geothermal energy, solar energy and wind energy [8]. There is a rising interest for biomass, since it represents a large potential renewable energy source which could benefit society with a clean fuel in many forms.

2.1. Biomass

Biomass refers to living and recently dead biological material that can be used as fuel or for industrial production [9]. Biomass is formed through the process of photosynthesis, which is depicted by the equation below, by capturing solar energy in living plants and it is primarily consisted of carbohydrates and lignin. This energy which is stored in chemical bonds of biomass can be released in many forms by digestion, combustion, or decomposition.

$$CO_2 + H_2O + \text{light} + \text{chlorophyll} \rightarrow (CH_2O) + O_2 \qquad (2.1)$$

The energy content of different biomass materials determines their calorific value which depends heavily on the percentage of carbon and hydrogen. Carbohydrate is the primary organic product which is represented by (CH_2O) in the equation. For each gram mole of carbon fixed, about 470 kJ (112 kcal) is absorbed [10]. With increasing carbon content, structures of biomass become more hydrocarbon-like and the heating value increases.

The energy needed in many fields of modern life can be obtained from biomass materials such as wastes, forest products, energy crops and aquatic plants which are listed in detail below [7].

Wastes. This type of biomass includes agricultural production and processing wastes, crop residues, mill and urban wood wastes, solid wastes.

Forest Products. Wood; logging residues; trees and wood residues; sawdust, etc. from forest clearings which accounts for about 65 percent of biomass energy used in the world, constitutes another type of biomass [11].

Energy Crops. Energy crops are plant materials which are grown specifically to produce energy and it includes;

- Woody crops such as willows, poplars and eucalyptus
- Starch Crops; corn, wheat, barley
- Sugar crops; sugar cane and sugar beet
- Forage crops; grasses, alfalfa and clover
- Liquid Bio-fuel Energy Crops for example soybeans, sunflowers, rapeseed

Aquatic Plants. Algae, waterweed and water hyacinth are also used for the energy production.

2.2. Bio-Energy (Bio-Fuel)

Bio-energy (bio-fuel) which is derived from biomass is used to meet a variety of energy needs by means of generating electricity, fuelling vehicles, heating homes, and providing process heat for industrial facilities. Biomass does not contribute to the build up of CO_2 in the atmosphere when it is harvested and processed by sustainable means. Because it emits roughly the same amount of carbon during its conversion as is taken up during its growth [12]. Thus, biomass is considered as carbon neutral. The renewed interest for biomass as an alternative energy source is apparent in most of the countries not only because it mitigates the effect of greenhouse gases but also it decreases our reliance on foreign oil.

The oldest and most common type of bio-fuel is wood heat because of its abundant availability and simplicity while processing as an energy source. The other bio energy sources including alcohol, biogas and biodiesel are listed below;

2.2.1. Liquid Bio-Fuels

Large quantities of liquid bio-fuels are presently used in many countries due to its potential to replace or supplement traditional petroleum based transportation fuels and its ability to be used in existing vehicles with little or no modification to engines and fueling systems. They can also be used for the purpose of heating and electricity production. The two most common types of liquid bio-fuels are ethanol and biodiesel. A specific plant or substance used for bio-energy is called feedstock.

2.2.1.1. Ethanol. Ethanol is a highly flammable alcohol which is formed by fermentation of sugar or starch which exists in agricultural raw materials. Main sources of sugar required to produce ethanol can be derived from energy crops; such as maize and wheat. Ethanol or ethyl alcohol (C_2H_5OH) is a biodegradable and colorless liquid [13]. When ethanol is blended with gasoline, the existing fuels burn more completely and air pollution reduces.

2.2.1.2. Biodiesel . Biodiesel is a form of diesel fuel produced through transesterification process by using vegetable oils, animal fats, or recycled restaurant greases as feedstock. Biodiesel is simple to use, nontoxic, biodegradable, and it produces less air pollutants than petroleum-based diesel. It can be used in its pure form or blended with petroleum diesel.

2.2.2. Solid Bio-fuels

2.2.2.1. Wood. The energy trapped in the chemical bonds of wood biomass can be used with combustion, gasification and cogeneration. Wood fuel is not a threat to acid rain pollution and particulate emissions are controllable since it contains minimal amounts of sulfur and heavy metals.

2.2.2.2. Charcoal. Charcoal is usually produced by heating wood or other substances in the absence of oxygen. The soft, black, porous material contains 85 % to 98 % carbon with the remainder consisting of volatile chemicals and ash.

2.2.3. Gaseous Bio-fuels

2.2.3.1. Syngas. Syngas, or synthesis gas is a gas mixture that contains different amounts of carbon monoxide, carbon dioxide and hydrogen [14]. It is produced from gasification of biomass or fossil fuels and can be burned to produce heat and power.

2.2.3.2. Biogas. Biogas refers to a gas produced by the breakdown of organic matter by bacteria in an oxygen-free environment. Depending on the design of the system and feedstock used for this process, biogas typically contains 55-75 % pure methane. Energy crops can be digested by using animal manure or anaerobic sludge as a seeding material in order to generate biogas. Co-generation of energy crop and animal manure is illustrated in the Figure 2.1 [15].

Landfills are the other sources of biogas in which large amounts of solid waste is buried and decomposed. Biogas which is generated after the breakdown of organic matter in the garbage can be collected and burned to produce energy.

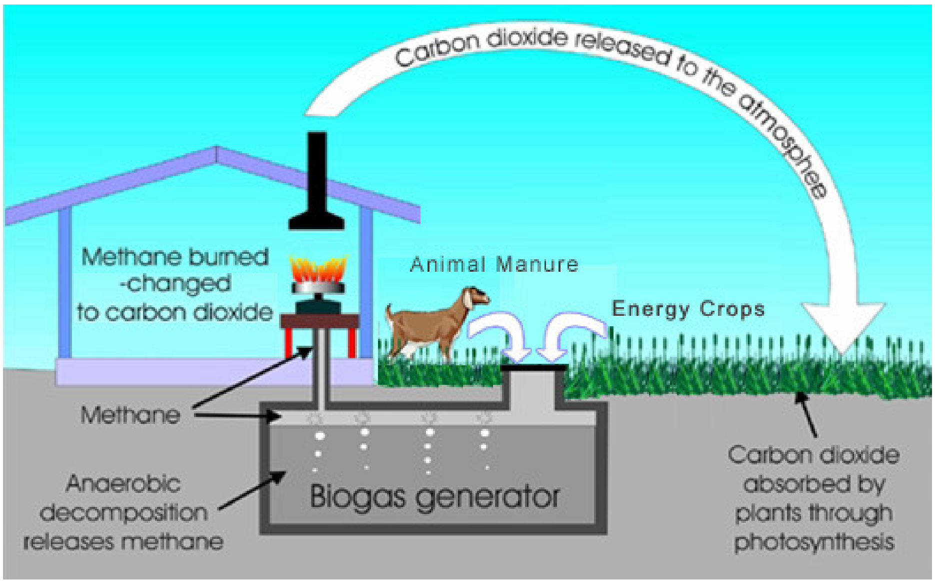


Figure 2.1. Biogas generation by using Animal Manure and Energy Crops [15]

2.3. Processing Technologies of Biomass Fuels

Conversion of biomass into useful forms of energy can be conducted on three pathways including thermal, physical and biochemical processing. Main features of how biomass is used as a source of energy are schematically illustrated in Figure 2.2 [16]. Conventionally, biomass can be burnt directly or converted into solid, liquid and gaseous fuels by using various technologies which are explained within this chapter.

Thermal conversion process has some advantages over other technologies with regard to its faster conversion rate and its possibility to be used in already existing fossil fuel based technologies. However, economic application of this kind of processes is only possible with feeds which have low water content (<50 %) and those have potential to be dewatered inexpensively [4]. Therefore, determination of the suitable energy conversion process is achieved by considering the properties of the biomass such as its moisture content. Various types of thermal processing methods of biomass are listed in detail below;

2.3.1. Combustion

Complete combustion is accomplished by the chemical reaction of biomass and oxygen. As a consequence of it, chemical energy is released; CO_2 and H_2O are formed as ultimate products of organic matter. In ideal conditions, CO_2 and water are the only products those are supposed to be generated during this process. However, under normal conditions this type of combustion does not occur with most solid fuels including biomass.

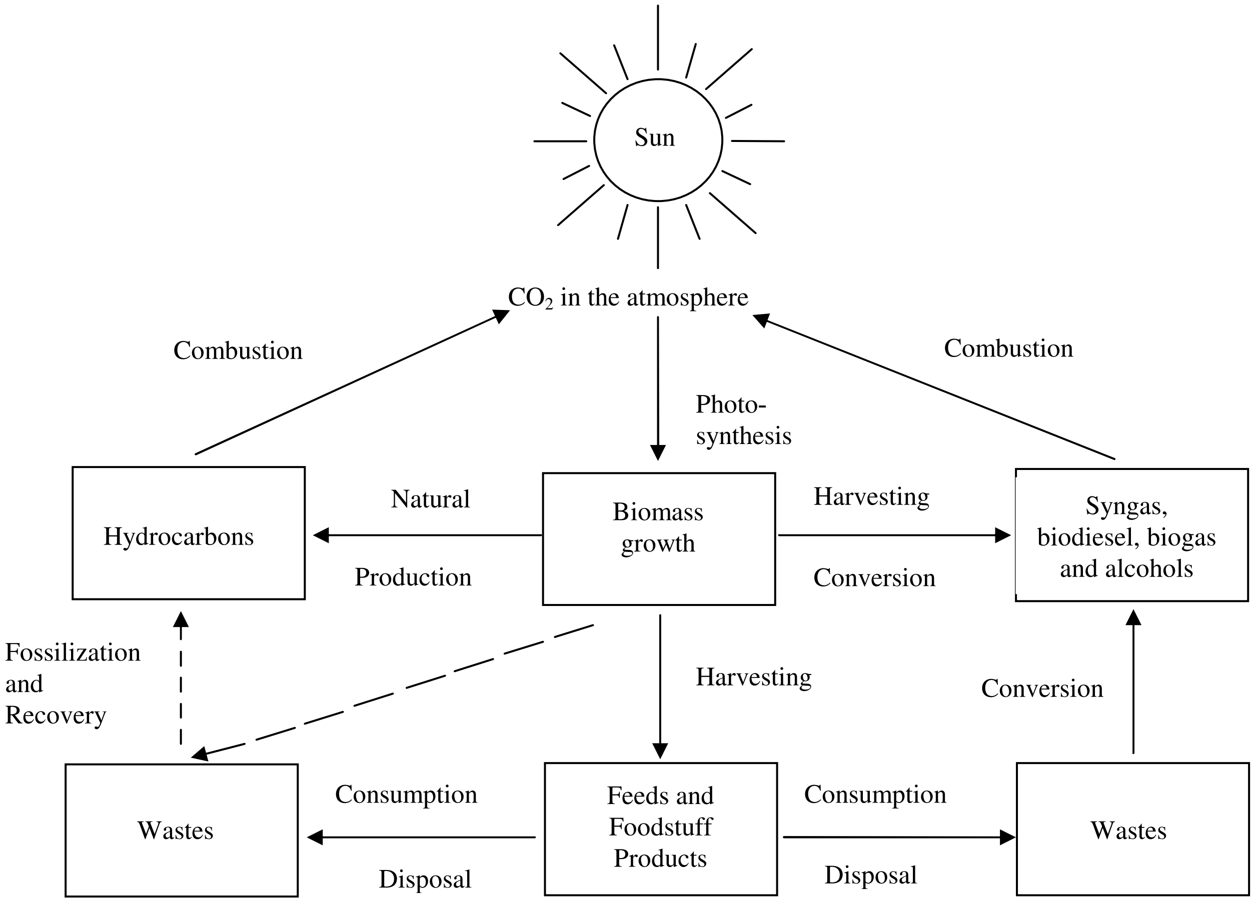


Figure 2.2. Main features of biomass energy technology [16]

Transportation of low-density biomass feedstock is a barrier to implement the combustion as a versatile technology. Other challenges include low energy content and alkali metal slagging [17]. Co-firing of biomass with fossil fuels is considered as a solution to improve efficiency and reduce costs.

2.3.2. Gasification

Thermal gasification is an advanced process that converts carbonaceous materials into carbon monoxide and hydrogen by reacting the raw material with a controlled amount of oxygen and/or steam at high temperatures (>700 °C) [18]. The resulting gas, known as synthesis gas or syngas, can be burned to produce heat and steam or used in gas turbines to produce electricity. Gasification is a very efficient method for extracting energy from biomass since it offers a method of power generation with higher efficiencies than combustion-based systems.

2.3.3. Pyrolysis

Pyrolysis refers to chemical decomposition induced in organic materials in the absence of oxygen at elevated temperatures, in order to produce a hydrocarbon rich gas mixture, an oil-like liquid and a carbon rich solid residue. This process also occurs as an intermediate step in combustion and gasification.

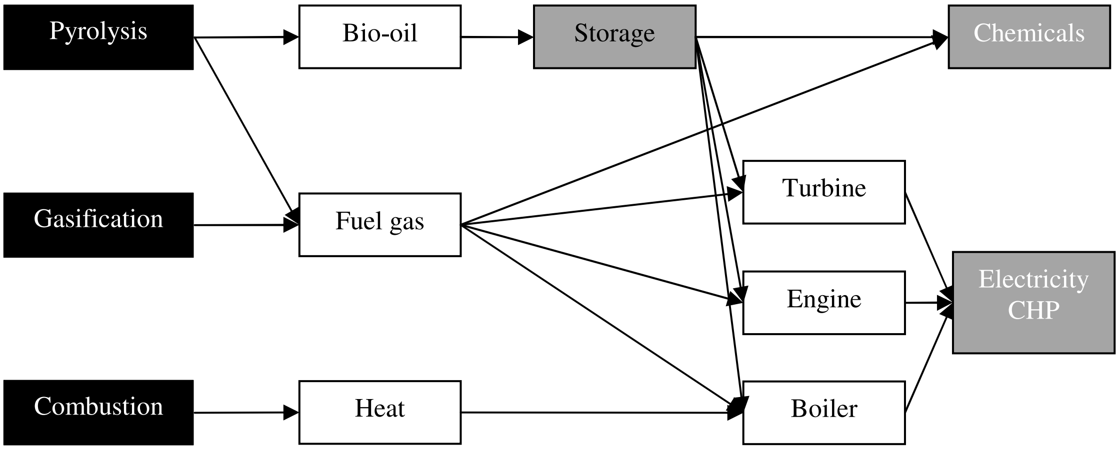


Figure 2.3. Products of thermal biomass conversion processes [14]

Applied heating rates determines the type of pyrolysis ranging from slow pyrolysis which yield up to 80 % bio-oil to flash pyrolysis that forms mostly char [19]. The products of three different thermal conversion techniques are illustrated in Figure 2.3.

Physical processing technologies are commonly used during preparation of the feedstocks prior to energy production. Types of physical operations are ;

2.3.4. Dewatering and Drying

During dewatering process, all or part of the moisture trapped in the biomass is removed as vapor. In case of biomass is processed thermally, it is usually necessary to partially dry the feedstock prior to conversion. Otherwise, the energy consumed by the conversion process would be more than produced in the form of energy or fuel. Open-air solar drying or industrial dryers such as spray dryers, drum dryers and convection ovens can be used for this purpose.

2.3.5. Size Reduction

Generally, it is favorable to reduce particle size of biomass before it is used as a feedstock for the conversion process. Because, size-reduction influences the rate of conversion, operating conditions of the process and product yields. Reduction in physical size is often achieved by grinding, cutting or impact mechanisms.

2.3.6. Densification

Biomass densification is the process whereby biomass in the form of small particles, like straw, sawdust or chips, is concentrated by machines into small pellets or briquettes. This process increases the bulk density of biomass by about 10 to 12 times of its original bulk density depending on the particular machine used [10]. Densification improves the convenience and accessibility of biomass by reducing the bulkiness of biomass products and therefore increasing their transportability.

Biochemical processing is also used as another technology to convert biomass into usable energy by using various energy crops. Biochemical conversion methods are explained in detail below;

2.3.7. Alcoholic Fermentation

Ethanol production through fermentation involves conversion of biomass materials, which contain sugar, starch or cellulose, by microorganisms and yeasts in the absence of oxygen [17]. The most common type of feedstock for fermentation is sugar cane, but other materials can be used, including wheat and other cereals, sugar beet and wood.

This process begins by grinding up the feedstock, so it is more quickly and easily processed and then it is mixed with water and yeast, kept warm in large tanks called fermenters. As a result of the breakdown of sugar by yeasts, ethanol is produced. In order to remove water and other impurities in the diluted alcohol product (10-15 % ethanol), distillation is necessary. Subsequent to this step, the concentrated ethanol (95 % by volume) is drawn off and condensed to a liquid form [7].

2.3.8. Anaerobic Digestion

Anaerobic digestion is a versatile biochemical process, whereby mixed populations of bacteria decompose organic matter into an energy-rich biogas consisting of methane, carbon dioxide and trace amounts of H_2, NH_3 and H_2S under strict anaerobic conditions. Compared to the other biomass conversion technologies, anaerobic digestion process has many advantages. Ethanol is becoming a popular biomass-derived fuel because of its availability for storage and transport. However, fermentation process requires extensive feedstock pretreatment and pure culture maintenance during its production. Besides, it results in overall low process efficiencies due to the energy requirements associated with feed processing and product separation. Besides, methanol and hydrogen are not well developed processes for the commercial and everyday use and these types of bio-fuels are more difficult to produce. In despite of their rapid conversion rates, thermal processes are economically feasible only with the feed stocks having low water content, owing to the high energy requirement during the evaporation.

2.3.8.1. Phases of Anaerobic Digestion Process. Anaerobic digestion is a biochemical process that involves various stages in order to decompose complex organic compounds. According to previous studies, these stages change between two and nine steps [20, 21, 22, 23]. However, four-stage process consisting of hydrolysis, acidogenesis, acetogenesis and methanogenesis is used widely. This decomposition process is illustrated in the Figure 2.4 [24].

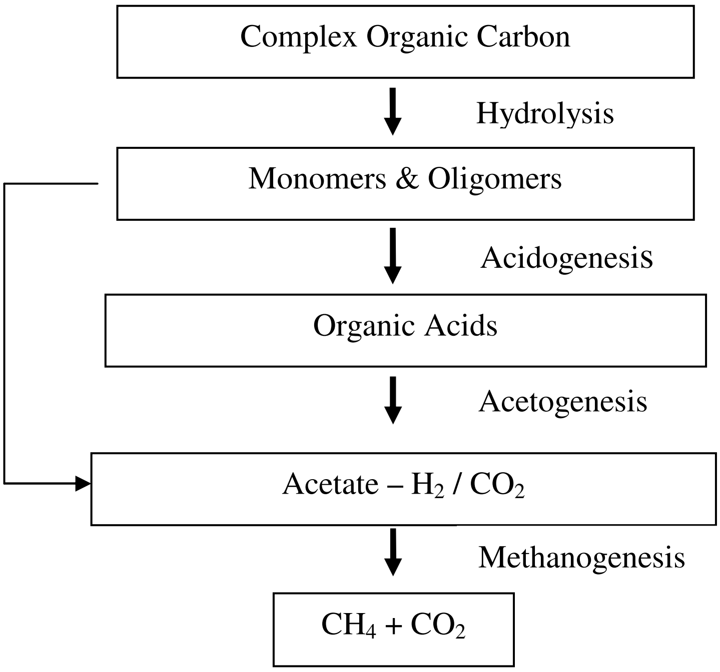


Figure 2.4. Anaerobic pathway [24]

The hydrolysis process initiates the decomposition of the biomass by reducing complex organics such as cellulose, proteins, lipids and carbohydrates to dissolved organics, primarily sugars, alcohols, amino acids and higher fatty acids by means of extracellular enzymes of facultative anaerobic bacteria. In the second stage, acidogenesis, the products of hydrolysis are fermented into volatile organic acids, carbon dioxide and hydrogen gas. The third stage involves the oxidation of alcohols and volatile acids longer than two carbons to acetic acid, carbon dioxide and hydrogen by acetogenic bacteria. As the final stage, these acetic acid, hydrogen and carbon dioxide are converted into the final products of anaerobic decomposition that are methane and carbon dioxide by specific group of microorganism called as methanogenesis [25].

2.3.8.2. Microbiology of Anaerobic Digestion. The conversion of biomass to methane is a complex anaerobic degradation process that requires the concerted action of many microorganisms which are classified with respect to their physiology, nutritional requirements, growth and metabolic characteristics. Within this concept, three groups of bacteria are involved throughout this process; hydrolytic bacteria, transitional bacteria and methanogenic bacteria which are described in detail below.

Hydrolytic bacteria are responsible for the break down of complex organic molecules (proteins, cellulose, lignin, and lipids) into soluble monomer molecules such as amino acids, glucose, fatty acids, and glycerol which are directly available to the next group of bacteria. This step of anaerobic digestion is catalyzed by extracellular enzymes such as cellulases, proteases, and lipases [26].

The hydrolysis phase is relatively slow and it can be rate limiting in anaerobic digestion of biomass which contain lignin. In the literature, it was stated that due to the chemical and physical construction of lignocelluloses, hydrolysis phase plays a significant role in the process of biochemical methane generation from the biomass having such kind of structure [27]. Since lignin is tightly attached to the hemicelluloses, it covers the cellulose and creates a physical barrier for the hydrolytic enzymes. Under this circumstance, large particles have relatively small surface area and limit the microorganisms to attack fibers and breakdown the structure of the biomass. 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.

The soluble organic matters which are produced as a result of the hydrolysis stage are converted to methanogenic substrates by transitional bacteria through the acidogenesis and acetogenesis stages.

The methanogenic (methane- producing) bacteria carry-out the major final step in the production of methane by the decomposition of biomass [26]. This kind of bacteria is doubly useful in the degradation process compared to aerobic bacteria since they produce less cell material while they form an energy-rich product. Methanogenic bacteria is divided into two categories; acetotrophic and hydrogenotrophic, in which it varies according to the usage of acetate (CH_3COOH) or CO_2 for the formation of methane [28]. About two-thirds of methane is derived from acetate and one third from carbon dioxide. The methanogens can also convert formic acid and methanol. The related equations are illustrated with Eq.2.2 and Eq.2.3.

Acetotrophic methanogens

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad (2.2)$$

Hydrogenotrophic methanogens

$$4\,H_2 + CO_2 \longrightarrow CH_4 + 2\,H_20 \qquad (2.3)$$

The methanogens are very sensitive to the accumulation of the hydrogen as well as the presence of electron acceptors such as nitrate and sulfate. Additionally, this group of bacteria has a number of requirements for growth, such as absence of oxygen and pH of 6-8.

2.3.8.3. Types of Anerobic Digesters. During the anaerobic degradation process; warmed, sealed, airless containers (digesters) are utilized in order to provide ideal conditions for the bacteria to convert the organic material to energy in an oxygen-free environment [29]. There are various kinds of anaerobic digesters which are classified with regard to their feeding and working principle.

Conventional Single Stage Digesters

Batch Digesters. Batch anaerobic digesters are the simplest type of reactors which are simple to setup and operate. Their operation consists of loading the digester with organic materials together with inoculums and allowing the mixture to degrade. When the gas production is ceased or becomes negligible, the effluent is removed and the process is repeated [30]. This type of digester is commonly used at the household and farm scale. In spite of its simple usage, main disadvantage is that it is unstable and uncontrollable during the fermentation and this situation may cause digester failure and variations in the quantity and composition of the product gas [26].

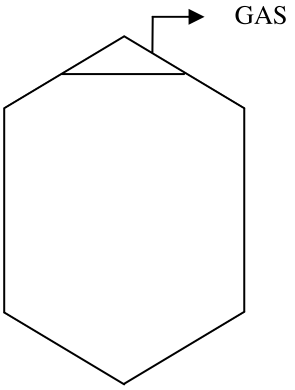
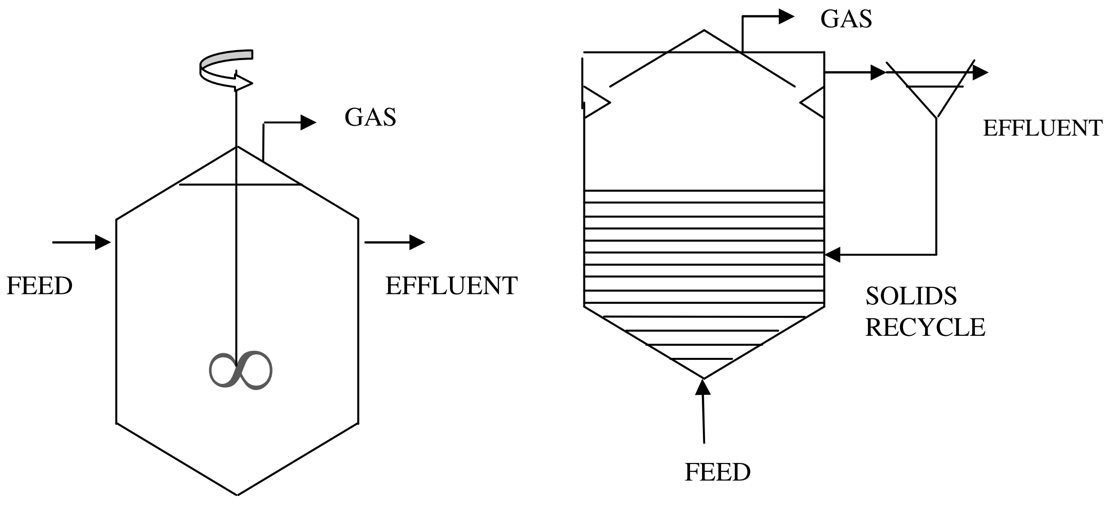


Figure 2.5. Batch Digester

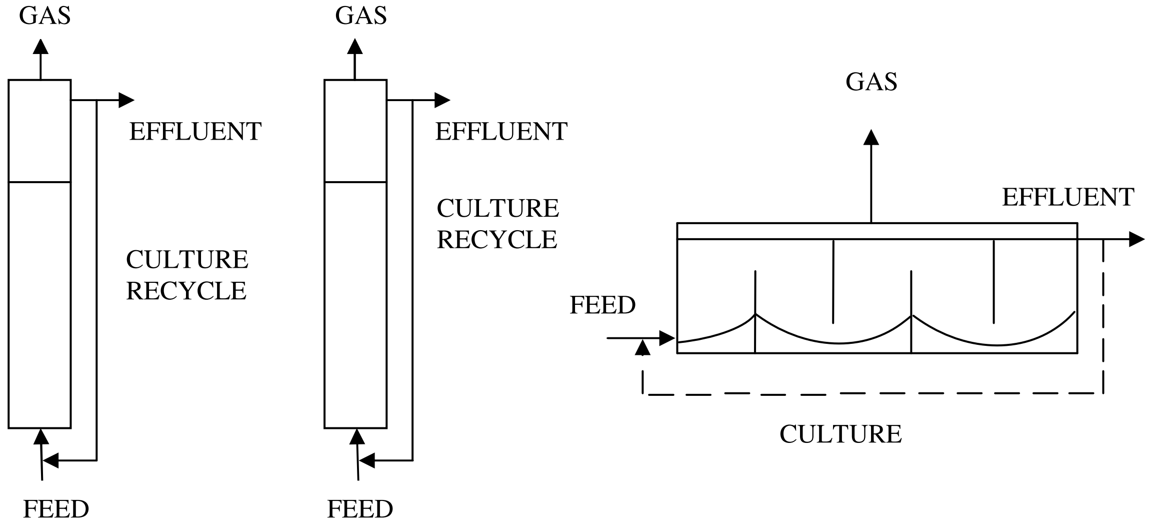
Continuously Fed Digesters. In these digesters, organic material is constantly or regularly fed into the digester and the material moves through it either mechanically or by the force of the new feed pushing out the residue. Compared to batch-type digesters, continuous digesters produce biogas without the interruption of loading material and unloading effluent. They may be better suited for large-scale operations since proper design, operation, and maintenance of continuous digesters produce a steady and predictable supply of usable biogas. There are various kinds of continuously fed digesters; continuously stirred tank reactor (CSTR), continuously stirred tank reactor with solids recycle (CSTR / SR), plug- flow reactor, up-flow anaerobic sludge blanket and attached film reactor which are illustrated in the Figure 2.6. [26].



Continuously Stirred Tank Reactor

Up-flow Anaerobic Sludge Blanket Reactor with Solids Recycle

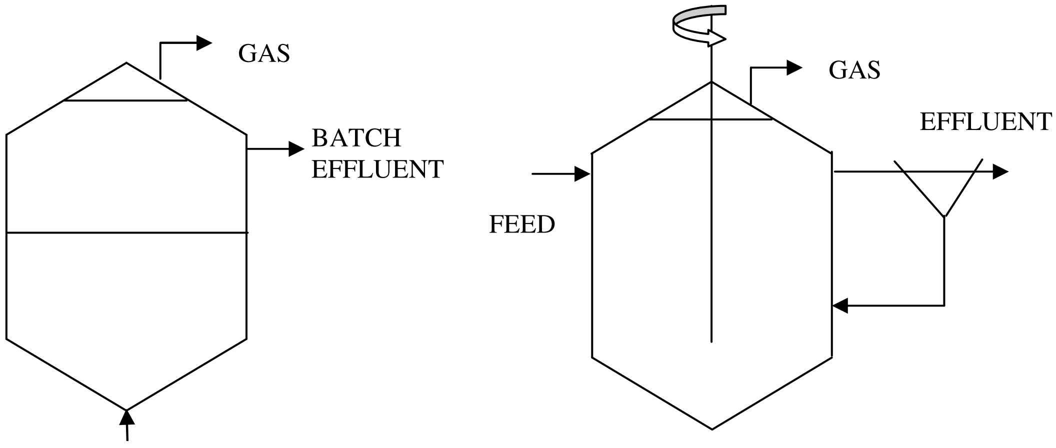
Figure 2.6. Single Phase Continuously Fed Anaerobic Digester Schemes



Fluidized-Bed
Reactor

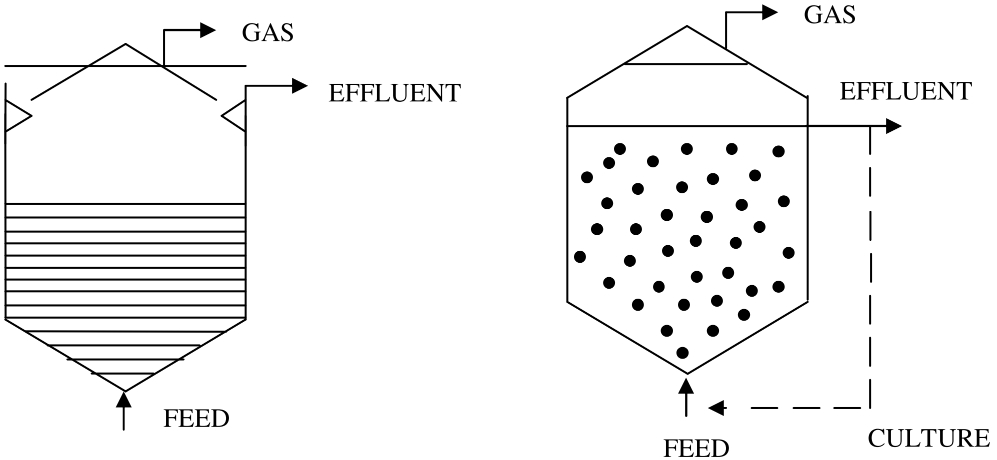
Expanded- Bed
Reactor

Baffle Flow Reactor
(Plug Flow Reactor)



Upflow Solids Reactor

Continuously Stirred Tank Reactor
With Solids Recycle



Up-flow Anaerobic
Sludge Blanket Reactor

Up-flow Anaerobic
Filter Reactor

Figure 2.6. Single Phase Continuously Fed Anaerobic Digester Schemes (continued)

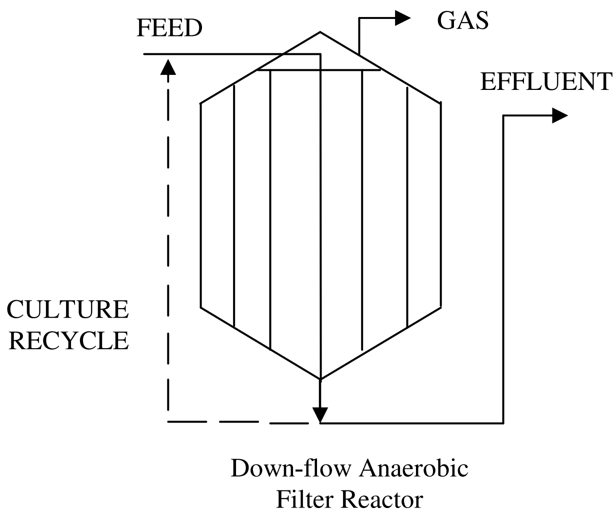


Figure 2.6. Single Phase Continuously Fed Anaerobic Digester Schemes (continued)

Two Phase Digesters. In a two-stage digester, the residue generated during the first stage of this process is reduced at the second stage digester in which the same reactions take place with different retention times. With the help of this kind of system, it is possible to separate methanogenic and non-methanogenic phases and hereby reduce the instability of performance caused by fluctuation in feedstock loading, pH and toxic feed components. Reactors, such as plug-flow and up-flow solids digesters, work with the same principle and provide phase separation in single reactor while; other two phase reactors enhance the phase separation in different reactors [26].

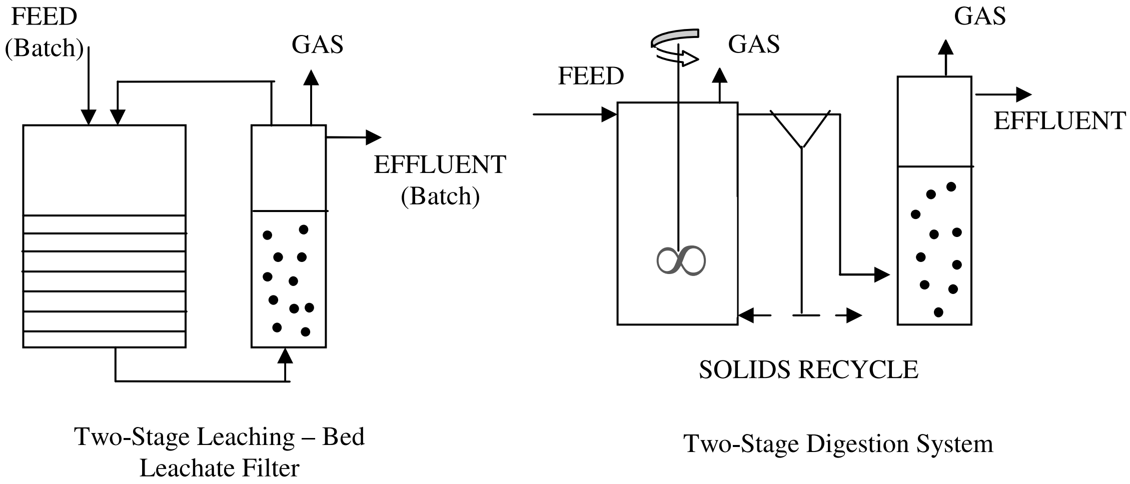


Figure 2.7. Two-stage digester schemes

2.3.8.4. Factors Affecting Anaerobic Digestion of Energy Crops. There are various factors affecting anaerobic digestion of energy crops which are: temperature, pH, TS content, I/S ratio, harvesting time, type of inoculum and input energy crop characteristics.

Temperature. Biogas production through the process of anaerobic digestion is strongly affected by temperature in the reactor. Anaerobic degradation of energy crops can be carried out at various temperature ranges: psycrophilic (<30 °C), mesophilic (30-40 ºC) and thermophilic (50-60 ºC) [25]. However, microbial reaction rates vary with different temperature conditions, ceasing at very low or very high temperatures and anaerobic microorganisms are most active in the two temperature ranges; mesophilic and thermophilic. Many degradation processes are carried out faster with increasing temperature but thermophilic systems are not economical techniques for conversion of biomass because of higher energy requirement.

The impacts of the temperature increasing from mesophilic to thermophilic ranges on the stability of the anaerobic digestion of energy crops were investigated by researchers and it was found that the digester performance was negatively affected with increasing temperature [31]. As a result of another study which was conducted in order to evaluate the effect of various temperatures; such as 8 °C, 16 °C, 24 °C and 37 °C on the performance of anaerobic digestion process, researchers stated that the methanogenic activity of the system at 16 °C and 8 °C was significantly lower than the process taking place at 24 °C and 37 °C due to higher VFA concentrations [32].

In the literature it was reported that the optimal temperature for the anaerobic digestion process is mesophilic temperature [25]. They also indicated that the temperature in the anaerobic digesters in which the organic content of the crops is converted into methane; should be kept constant since methanogens are very sensitive to sudden thermal changes.

It is possible to produce methane from energy crops under psycrophilic and thermophilic conditions. However, it is necessary to use microbial communities those are acclimated to these temperature ranges prior to digestion process.

pH and Alkalinity. pH is a significant parameter which directly affects the growth and the performance of the microbial population. Very low pH values which can be observed owing to the excessive production of organic acids have negative effects on the methanogenic bacteria utilizing organic portion of the crops. As a consequence, the environmental conditions in the system will be destabilized. Therefore, it is crucial to maintain optimal pH value for the success of the anaerobic digestion process.

Some researchers stated that the optimum pH range for the biogas production process is between 6.8-7.5, while another group of scientists reported that pH of the digester should be kept within a desired range of 7.0-8.5 in order to provide optimum conditions for the methanogenic activity [27, 33]. Besides, optimum pH for anaerobic digestion was also expressed as 6.8-7.2 in another study [25].

Alkalinity is the ability of a system to buffer the undesired effects of volatile and other acids which tend to depress the pH below desired level. High alkalinity concentration indicates that the system is safeguarded against pH fluctuations, while low alkalinity indicates that the high concentrations of acids may lower the pH so that the biological activity may cease. In the literature, it was reported that the alkalinity should be above 1200 mg $CaCO_3$ L^{-1} for stable operation [30]. Additionally, researchers stated that alkalinity should not be less than 1500 mg $CaCO_3$ L^{-1} for balanced digestion [34].

Nutrients. Efficiency of the anaerobic digestion process is dependent on the adequate supply of the nutrients. In addition to the necessary macronutrients; nitrogen, carbon, and phosphorus, bacterial population also need trace elements such as nickel, cobalt, molybdenum, iron etc. [28].

Total Solid Concentration (TS %). The amount of fermentable material of feed is defined as solid concentration and it is one of the most important parameters which affects the rate and nature of the process during the anaerobic batch digestion of energy crops. Some researchers reported that the total solid concentration ranging between 7-9 % of the agricultural waste was best suited for digestion and it was unstable below a total solids level of 7 % (of manure) while a level of 10 % caused an overloading of the fermenter [25]. As a consequence of a study which was conducted by using solid potato waste alone and in combination with sugar beet leaves as substrates, the maximum methane yield of 0.32 L CH_4 g VS $^{-1}{}_{degraded}$ was achieved by using a mixture of inoculum and potato waste having 8 % TS content [30].

Inoculum to Substrate Ratio (I/S). Inoculum to substrate ratio is another significant parameter which shows the effect of substrate concentration during anaerobic digestion as well as the effect of inoculum concentration on anaerobic degradability and methane productivity. Together with total solid content, they have considerable effects on the cost and the performance of the system. It will not be possible to provide a successful digestion process unless a balanced correlation is obtained between the microorganisms and the substrate. Additionally, it should be considered with great care that excessive amount of substrate causes inhibition and failure of the system. Thus, it is very crucial for the digestion process to keep I/S ratio within the desired range.

As a result of batch fermentation experiments which were conducted by a group of scientists, it was reported that methane yield was lower at I/S ratios below 0.25 and it increased at a decreasing rate up to an I/S ratio of 2, after which it remained relatively constant [34]. During another research, the effect of various I/S ratios (3, 2, 1.5 and 1) on the methane production process of maize was investigated and the maximum specific methane production rate of 23 mL CH_4 g VSS $^{-1}$ day $^{-1}$ was obtained for I/S ratio of 1 [35]. Additionally, cumulative biogas and methane production that was observed throughout the system was maximum (29819 and 17451 mL) again for I/S ratio of 1.

Harvesting time of the energy crop. Methane production during the anaerobic digestion of energy crops is directly affected by the chemical composition of the plant which changes as the plant matures. Thus, the time and frequency of harvest are very important factors which should be considered in order to optimize the biomass yield and feedstock quality [36]. Laboratory scale batch experiments were conducted by a group of researchers in order to investigate the effects of different plant species and growth stages on the biogas production [37]. As a result of this study, the maximum biogas yield (987 L kg VS $^{-1}$), methane content (68 %) and methane yield (658 L kg VS $^{-1}$) was observed for barley at the milk stage (the period after the grain flowered before it reaches the full ripeness). During another study, thirteen early to late ripening maize varieties were investigated in order to determine the most suitable type of crop for methane production [38]. As a consequence, it was stated that the methane yield declined from 312 - 365 NL CH_4 kg VS $^{-1}$ to 268–286 NL CH_4 kg VS $^{-1}$ as the crop approaches full ripeness.

Plant Characteristics. Composition of the biomass digested is one of the significant factors influencing methane production rate. Biomass contains varying amounts of cellulose, hemicellulose, lignin, protein, sugar and starch. Cellulose generally represents the largest fraction of the biomass with a range of 40-50 % and its biodegradability is greater than lignin [12]. Lignins are phenolic polymers which contribute to structural rigidity of plant tissues towards chemical breakdown by the microorganisms and enzymes that are used to digest solid biomass [17]. Thus, the relative proportions of cellulose and lignin is one of the determining factors in identifying the suitability of biomass plant species for processing as energy crops.

The physical state of a crop, especially its size and surface area is likely to influence its decomposition rate [25]. Utilization of feedstocks having too large particle size would result in clogging of the digester and it causes difficulties for microorganisms carrying out the digestion process.

Toxic Substances. The methane producing bacteria are known to be very sensitive mainly to free ammonia and volatile acids.

Ammonium (NH_4^+) is produced as a result of decomposition of crops containing nitrogen. In the reactors having high concentration of ammonium at high pH ranges, the equilibrium between ammonia and ammonium shifts to the right as it is illustrated with the chemical reaction below. As a result of it, free ammonia (NH_3), which has toxic effects on the growth and mechanism of the microorganisms responsible for the biogas production, is generated.

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_20 \qquad (2.4)$$

In order to evaluate the effect of temperature, ammonia, and their interconnectivity on the methane yield of anaerobic processes for animal waste treatment, four anaerobic sequencing batch reactors (ASBRs) were conducted and operated during an experimental study [39]. As a result of this study, it was reported that the methane yield decreased by 45% when total ammonium-N and ammonia-N were increased in two of the four ASBRs to levels > 4,000 mg NH_4^+ -N L^{-1} and > 80 mg NH_3-N L^{-1}, respectively. However, this relative inhibition was reduced from 45% to 13% compared to the low-ammonia control reactors when the operating temperature was increased from 25°C to 35°C (while the free ammonia levels increased from ~ 100 to ~ 250 mg NH_3-N L^{-1}). Thus, the operator may prevent ammonia toxicity by increasing the operating temperature within the mesophilic range.

Major forms of volatile fatty acids those are produced during the anaerobic digestion of energy crops are acetic, propionic and butyric acids. Although they serve as a food to methanogens, they are inhibitory to the digestion process at high concentrations. As a result of an experimental study which was conducted by some researchers, it was reported that high concentrations of VFA were found in experiments with mesophilic sludge and beet tops at 16 °C and 8 °C [32]. At 16 °C, concentrations of acetic acid, propionic acid and butyric acid were 1670 mg L^{-1}, 657 mg L^{-1} and 72 mg L^{-1} respectively while they were 2370 mg L^{-1} 567 mg L^{-1} and 87 mg L^{-1} for 8 °C. For experiments at 24 °C and 37 °C no VFA's occurred in concentrations higher than 50 mg L^{-1}. Therefore, VFA may cause the inhibition of the digestion system unless it is buffered by inoculum or some other additional chemicals.

2.3.8.5. Enhancement of Biogas Production. Anaerobic digestion of energy crops may be accelerated if microbiological activity in the reactors could be enhanced by providing a suitable environment for the growth and reproduction. It is possible to increase biogas production by using the following techniques; use of additives and pretreatment of the substrates. By following the procedures mentioned above, the degradation of fermentable material will be improved and gas production will be enhanced with higher methane concentrations.

Pretreatment of the substrate can be executed by various techniques and most common types of these methods are; particle size reduction, alkali treatment, and ensilage.

Particle size reduction is considered as a technique for the enhancement of biogas production, since this physical pretreatment method increases the surface area where the microorganisms can attack and break down the structures of the substrate. However, cutting of the feedstock into too small particles would be uneconomical. Some researchers reported that, the biogas produced by using wheat straw and paddy straw with particle sizes of 0.088 and 0.40 mm was almost equal, thus, grinding below 0.40 mm would not be economical [40].

Alkali treatment of the feedstock was considered as an effective method for the improvement of the biogas production [41]. It was also reported that methane production increased to 222 L CH_4 (STP) kg^{-1} $VS_{initial}$ when the barley waste was subjected to alkaline hydrolysis pre-treatment before co-digestion with activated sludge.

Ensilage or silaging is the process of preserving green food in airtight conditions, either in a storage silo or in plastic wrapping. Application of this technique to the feedstocks of anaerobic digestion process improves the availability of nutrients for the methanogenic metabolism. Biogas production capacity of thirteen maize varieties was investigated through an experimental study and it was stated that fresh, non-conserved maize produced 225 NL CH_4 kg VS^{-1} which was 25% less than silaged maize [38]. It was also reported that lactic acid, acetic acid, methanol, alcohols, formic acid, H^+ and CO_2, which are formed during the silaging process, are important precursors for methane formation.

Use of additives involves the supplementation of some chemicals for the purpose of improving the conditions in which the anaerobic digestion process takes place. In the literature, it was reported that the plant which includes higher content of heavy metals (Cr, Cu, Ni and Zn) had a higher CH_4 yield than the control reactor [42]. Besides, it was stated that the addition of iron salts at various concentrations [$FeSO_4$ (50 mM), $FeCl_3$ (70 μM)] enhanced the gas production rate. As a result of an experimental study which was conducted and operated by using mature Napier grass as substrate, it was stated that the biogas production was enhanced by 40 % with the help of daily addition of a solution containing nickel, cobalt, molybdenum, selenium and sulphate [43]. In order to determine the optimum amount and the most suitable type of the additives (nutrients, iron salts or heavy metals) for the enhancement of biogas production, each crop should be investigated with regard to its methane production rate by using various chemicals.

3. MATERIALS AND METHODS

3.1. Reactor Experiments

3.1.1. Anaerobic Batch Digestion Experiments

Batch reactors in which anaerobic digestion experiments take place were conducted in the laboratory with features presented in Figure 3.1.

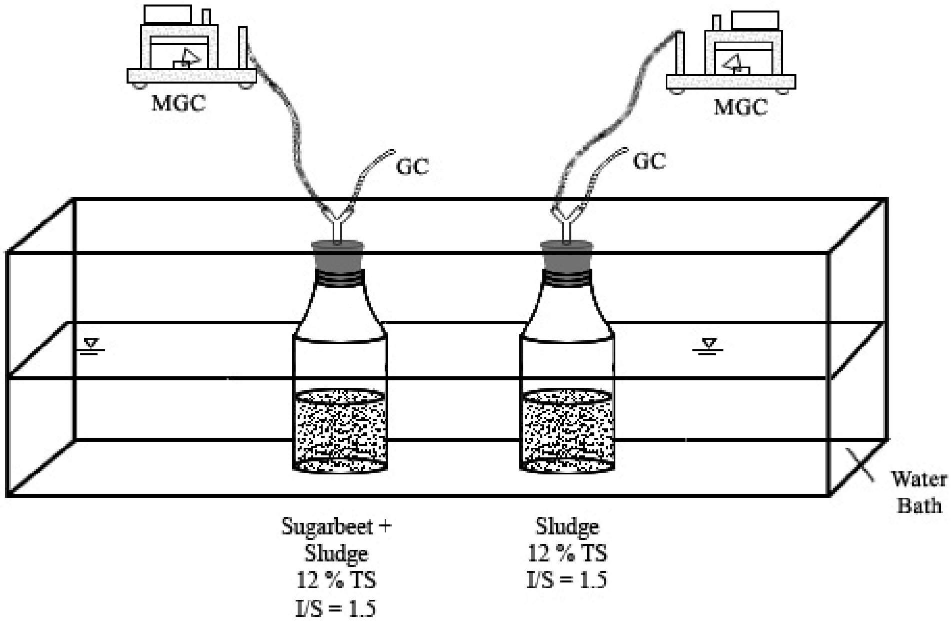
All the experiments were conducted by using 1 L borosilicate glass bottles working as reactors each with 800 mL active volume. The reactors were capped with rubber stopper with 3 cm diameter and equipped with a V shape gas collection port at the top. One opening of the port was connected to Miligascounter ® (MGC) by a PVC hose with 7 mm inlet and 10 mm outlet diameter in order to measure the volume of biogas produced. The other opening was capped by a rubber stopper and functioned as a gas sampling port.

MGC's were placed on a trestle which was constructed for the purpose of laboratory usage and it was kept lower than the bottles in order to prevent flow of the siliconic liquid from MGC into the bottles.

Reactors were placed in a water bath (Nüve, BM 402) at an average temperature of 37 °C and the temperature was kept constant by an automatic heat controller. All purpose thermometer was used to be sure that the mesophilic condition was maintained in the bottles which will enhance the digestion process. Besides, water level in the water bath was controlled on a regular basis and filled with distilled water as it was evaporated.

To commence and enhance the rate of anaerobic degradation with methane production, each reactor was seeded with anaerobically digested sludge which was collected from wastewater treatment plant of Kent Gıda factory in Gebze (Istanbul). Total solid concentration of anaerobic sludge was between 7-9 %.

First Set-up



Second Set-up

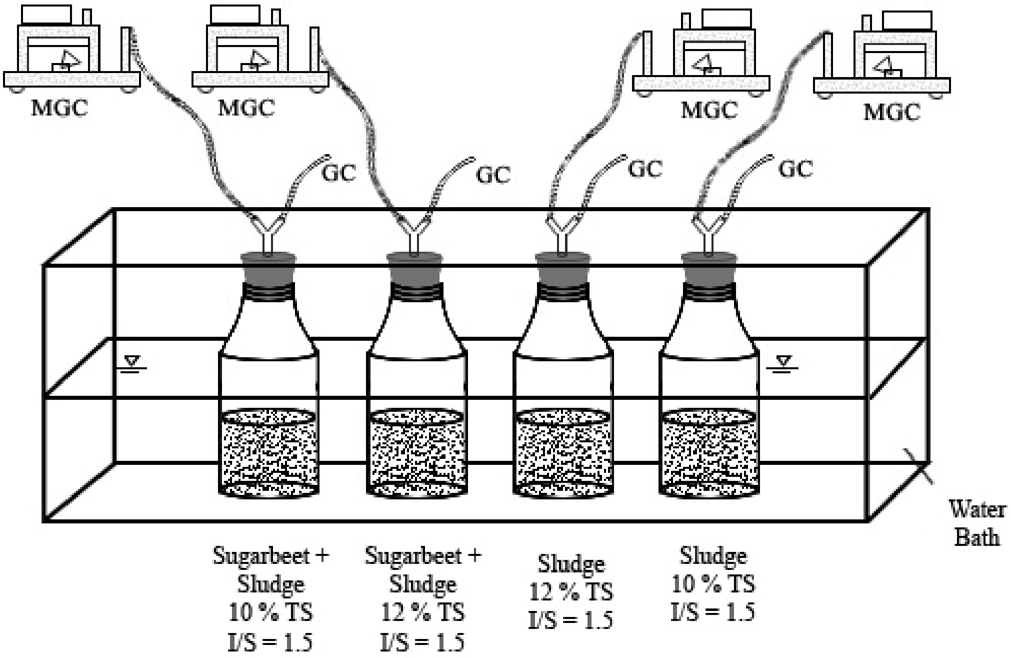


Figure 3.1. Configuration of the system for anaerobic digestion of crops

Third Set-up

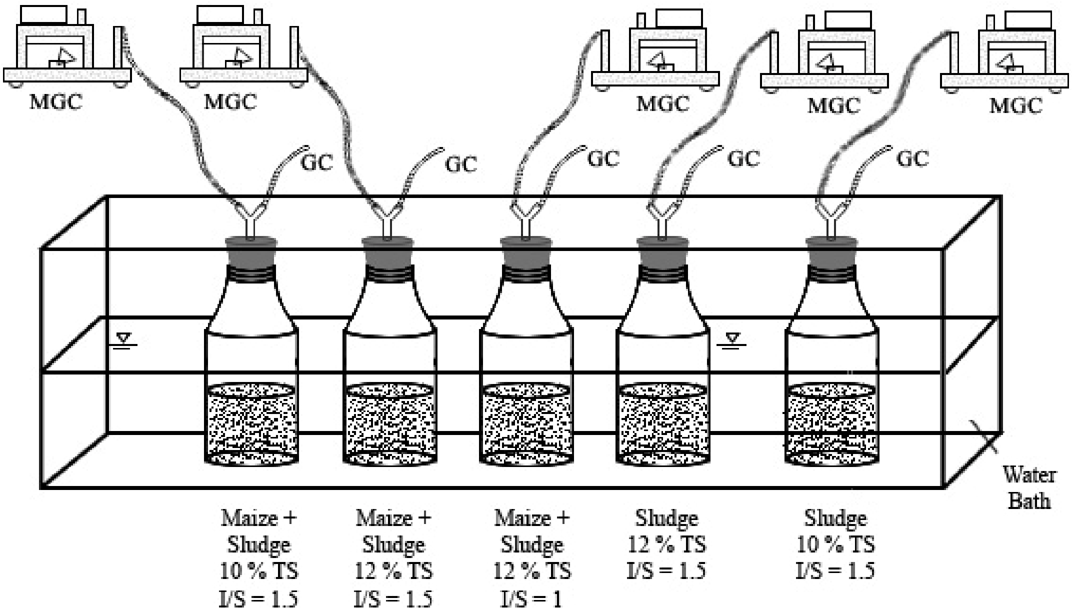


Figure 3.1. Configuration of the system for anaerobic digestion of crops (continued)

The bottles were filled with energy crop (sugar beet or maize) as a substrate and anaerobic granular sludge as a seeding material. Sugar beet and maize are suitable energy crops for anaerobic digestion due to their high biomass yield and biogas production capacity. Moreover, these crops were selected due to their abundant availability in Turkey. Crops and granular sludge were stored in the cold room at 4°C. Prior to addition to the bottles, crops and sludge were heated to the room temperature and analyzed for their total solid (TS) and volatile solid (VS) contents. Subsequent to these analyses, weight of crops and seed sludge which should be added to the bottles was calculated depending on their TS contents.

Two different concentrations of crop (maize or sugar beet) and seed sludge mixture (10 % TS and 12 % TS) were prepared for batch experiments. The inoculum (seed sludge) to substrate (crop) ratio was arranged to 1.5. During batch experiments of maize, I/S ratio of 1.0 was also investigated in respect to its effect on anaerobic digestion. A picture of experimental set-up is illustrated in the Figure 3.2.

Figure 3.2. Experimental Set-up Layout

Preliminary studies suggested that physical pretreatment such as grinding provide large surface area and enhance the contact between microorganisms and substrates [25]. Therefore, sugar beet was shredded using a kitchen blender and maize was grounded with mill in a village of Çanakkale. The seed sludge was thoroughly mixed and filtered through a screen with a pore size of 3 mm and concentrated through centrifugation by using Nüve - NF 615 and Nüve - NF 1200. Subsequently, homogenized crop and sludge was added to the bottles and the mixture was diluted to 800 mL with deonized water. Same procedure was followed and equal amounts of crop and sludge was used for the preparation of samples.

Control reactors including seed sludge without any addition of crop were also conducted in the laboratory for each crop and sludge mixture which has a definite TS content.

Preliminary studies indicated that methane production can be observed in the pH range of 7.0–8.5 [12]. Therefore, the pH of the reactors was adjusted to 7.4 by using 1N

KOH and H_2SO_4 in order to provide suitable conditions for the growth of methanogens. Afterwards, the bottles were capped with stoppers and a coating of silicon was applied to all connections and joints to ensure that the units are gas tight. Reactors were purged with nitrogen gas for 2 minutes in order to displace oxygen from the system and directly establish the anaerobic conditions.

3.1.2. Sampling and Analytical Methods

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

Figure 3.3. Miligascounter ® (MGC) used for the measurement of gas volume

Mixtures of energy crop and anaerobic sludge were analyzed for Total Solid (TS), Volatile Solid (VS), pH, Alkalinity, Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), Volatile Fatty Acid (VFA), Ammonium nitrogen (NH_4^+-N), Orthophosphate (PO_4^{3-}), Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC). The volume of gas production was monitored daily and biogas composition was analyzed once a week throughout the study. All these analyses were performed according to Standard Methods for the Examination of Water and Wastewaters [26]. Analytical protocol which was followed during the digestion experiments was listed in the Table 3.1.

Table 3.1. Analytical Protocol of the study

Parameters	Crop	Sludge	Mixture of crop and sludge before digestion	Mixture of crop and sludge after digestion	Biogas	Frequency
TS	x	x	x	x		once/study
VS	x	x	x	x		once/study
pH			x	x		once/study
Alkalinity			x	x		once/study
TKN			x	x		once/study
Total P			x	x		once/study
VFA			x	x		once/study
NH_4^+ - N			x	x		once/study
PO_4^{3-}			x	x		once/study
COD			x	x		once/study
TOC			x	x		once/study
Gas Volume					x	Daily
Gas Content					x	once/week

The determination of Total Solid (TS) content of energy crop and anaerobic granular sludge solely and as a mixture was performed according to Standard Methods [26]. Homogenized samples were weighed in tared clean ceramic dishes and evaporated on the steam bath (Julabo Ecotemp TW 12). Afterwards, the samples were kept at 105 °C in the Nüve-FN 500 drying oven. As a final step, the samples in the dishes were cooled in the desiccator and weighed.

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

Due to its significance as indicative parameter in anaerobic digestion, the pH of the mixtures was monitored at the beginning and at the end of the experiment. pH of samples was measured by a pH probe attached to a ORION SA 520 pH meter after calibration with pH 4, pH 7 and pH 10.

The alkalinity of a sample is its acid neutralizing capacity and it is another significant parameter for the growth of methanogens those are effective in biogas production. Alkalinity was monitored according to the Titration method (2320 B), outlined in the Standard Methods [26].

Total Kjeldahl Nitrogen (TKN) is the sum of the organic nitrogen and ammonia nitrogen and this parameter was determined by using Nessler Method subsequent to digestion of the sample. This analysis was performed by the following procedure in HACH/DR 2010 Spectrophotometer Handbook [44]. Sample was digested with concentrated sulfuric acid at 440 °C in Digesdahl Digestion Apparatus and Hydrogen Peroxide was added. One drop of TKN indicator and 8N KOH solution were added to the sample until the first permanent blue color was observed. The volume of the sample was completed to 25 mL and then mineral stabilizer, polyvinyl alcohol dispersing agent were added. Same procedure was followed by using deionized water as the blank. The TKN of the sample was read as mg L^{-1} at 460 nm by using HACH DR / 2010 Spectrophotometer.

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

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

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

The concentrations of Volatile fatty acids (VFAs) were determined using a HP 5890 Series II Gas Chromatograph with a flame ionization detector (FID) and an HP FFAP column (10 m x 530 µm x 1 µm). Injection port and detector were operated at 260 °C and 260 °C, respectively. The oven temperature was programmed to start at 80 °C and was gradually increased 25 °C per minute until final temperature of 260 °C was reached. The amount of sample injected was 1µL.

COD is commonly used to characterize organic compounds in liquid mixtures. It is the predominant parameter for most of the wastewater treatment processes [45]. Although organic matter is predominantly described in terms of VS for digestion of energy crops, COD concentration is also determined in order to have more information on the characteristics of crop and sludge mixture. This analysis was made by closed reflux, colorimetric method. Firstly, 2.5 mL samples were placed into HACH vials. Afterwards, 1.5 mL potassium dichromate and 3.5 mL of acid digestion mixture were added into the vials respectively. The vials were placed into HACH COD digester and digested for two hours at 150°C. Finally, the digested samples were measured colorimetrically at 600 nm by using HACH DR / 2010 Spectrophotometer. Potassium hydrogen phthalate (KHP) solutions were used for preparing calibration curves (0-800 ppm).

Total organic carbon (TOC) was monitored with a Shimadzu TOC-V CSH analyzer operating in the non-purgeable organic carbon (NPOC) mode. The instrument was calibrated by standard solutions of KHP (1-40 ppm). Prior to determination of the TOC content, the samples were analyzed for their COD concentration and the theoretical TOC content was calculated. Afterwards, samples were diluted not to exceed the limits which can be detected by the TOC analyzer.

4. RESULTS AND DISCUSSION

4.1. Preliminary Analysis

4.1.1. Energy Crop Analysis

Two parallel samples were obtained for each energy crop (sugar beet, maize) and analyzed for their total solid (TS %) and volatile solid content (VS %). The amount of substrates which should be added into the reactors was calculated according to the results of TS analyses. Additionally, these two significant parameters were also investigated in order to determine the organic matter content of the crops.

The samples were placed in a dish, weighted and kept at 105 ^{o}C in the oven for 15-16 hours. After the samples were re-weighted, the TS content of the crop was calculated. Subsequent to the determination of the TS content, the dried and cooled samples were placed in the oven working at 550-600 ºC and ignited for about 6-7 hours. Afterwards, the samples were cooled and re-weighted for the calculation of VS content. The sugar beet was determined to contain approximately 16 percent total solid of which 92 percent was volatile solids. The information presented above may be tabulated as follows:

Table 4.1. TS and VS contents of the energy crops

	1st SET-UP		**2nd SET-UP**		**3rd SET-UP**	
SAMPLE	TS %	VS %	TS %	VS %	TS %	VS %
Sugar beet	16.38	91.50	16.30	92.00	-	-
Maize	-	-	-	-	85.82	97.62

4.1.2. Anaerobic Granular Sludge Analysis

In order to determine percentages of solid and volatile solids in the seeding sludge, parallel samples of approximately 20 g were analyzed. The previously homogenized and concentrated samples were poured in the dishes, weighted, evaporated and dried at 105 °C. After the samples were re-weighted, the percentage of solid in the sludge was computed. The samples were ignited at 550 °C for 24 hours to determine the volatile solids percentage of the seeding sludge. The results are illustrated in the Table 4.2.

Table 4.2. TS and VS contents of the anaerobic granular sludge

	1st SET-UP		**2nd SET-UP**		**3rd SET-UP**	
SAMPLE	TS %	VS %	TS %	VS %	TS %	VS %
Sludge	13.32	84.36	13.30	84.95	11.50	85.34

4.2. Reactor Experiment

4.2.1. Reactor Loading

Subsequent to these analyses, the amount of crops and anaerobic granular sludge which were added to the reactors was calculated by considering their TS contents and I/S ratios (Eq. 4.1, Eq. 4.2 and Eq. 4.3). Since the total volume of the ingredient was set to 800 mL, volume of 100 g mixture was measured by using a graduated cylinder in order to determine the density of it. Afterwards, the final weight of the crop, granular sludge and deonized water was calculated by using the equations illustrated below. An example for this calculation is outlined in the Appendix A.

$$\text{Weight of the Crop (g)} = \left[\frac{F * A * 100}{(B+1) * C * E}\right] \tag{4.1}$$

$$\text{Weight of the Sludge (g)} = \left[\frac{F * \left(A - \frac{A}{B+1} \right) * 100}{D * E} \right] \tag{4.2}$$

$$\text{Weight of the Water (g)} = \left[\frac{F}{E} * \left(100 - \left(100 * \left[\left(\frac{A}{C * (B+1)} \right) + \frac{A - \frac{A}{B+1}}{D} \right] \right) \right) \right] \tag{4.3}$$

where;

A = Total Solid content of the mixture (10 %-12 %)
B = Inoculum to Substrate Ratio (I/S)
C = Total Solid content of the crop (%)
D = Total Solid content of the sludge (%)
E = Volume of the 100 g mixture (mL)
F = Final volume of the mixture (800 mL)

4.2.2. Digestate Analysis

The main factors affecting the anaerobic digestion of biomass are total solid, volatile solid, pH, alkalinity, volatile fatty acids, total Kjeldahl nitrogen, ammonium nitrogen, total phosphorus, orthophosphate, chemical oxygen demand and total organic carbon. In this study, these parameters were monitored to detect and describe the conditions at which anaerobic digestion of energy crop takes place.

4.2.2.1. TS and VS content. In order to determine the organic matter contained in the reactors prior to digestion and the consumption rate as a result of the degradation, total solid and volatile solid content were analyzed for each set-up. It was not possible to maintain the projected amount of total solid content in the first and the second set-up due to the high humidity of the sugar beet. However, the specified percentages were achieved during the preparation of the last set-up, since solid content of the maize was much higher than the sugar beet. Findings of these analyses are given in the Table 4.3 and 4.4.

Table 4.3. TS contents of the reactors at the beginning and at the end of the experiments

TS (%)	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	8.80	5.60	-	-
12 % TS (SB) I/S=1.5	10.00	7.30	10.50	7.77	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	9.95	5.16
12 % TS (Maize) I/S=1.5	-	-	-	-	11.74	7.38
12 % TS (Maize) I/S=1	-	-	-	-	11.84	9.79
Blank 10 % TS I/S=1.5	-	-	9.37	7.89	10.36	5.71
Blank 12 % TS I/S=1.5	10.27	7.19	12.18	9.65	11.33	6.19

TS and VS contents of all of the reactors decreased through to the end of the experiment as a result of biological degradation. The highest VS degradation rate was observed for the reactor including maize and granular sludge at a ratio of 1.5 and having a final total solid content of 12 %. It was followed by the reactor having the same amount of total solid content during the second set-up.

Table 4.4. VS contents of the reactors at the beginning and at the end of the experiments

VS (%)	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	85.50	72.62	-	-
12 % TS (SB) I/S=1.5	85.98	67.02	85.20	72.30	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	90.36	82.64
12 % TS (Maize) I/S=1.5	-	-	-	-	90.81	81.36
12 % TS (Maize) I/S=1	-	-	-	-	91.95	90.59
Blank 10 % TS I/S=1.5	-	-	85.00	79.63	85.65	78.41
Blank 12 % TS I/S=1.5	82.65	80.79	82.27	76.43	85.89	79.42

However, VS degradation should be considered together with biogas yield and methane yield which are the most significant parameters, in order to evaluate the performance of an anaerobic digestion system which is carried out by using energy crop.

4.2.2.2. pH. Since a slight change in pH of the reactor could result in reduction of gas production, this parameter is considered as one of the most significant factors which is effective during anaerobic digestion process. The amount of carbon dioxide and volatile fatty acids which are produced during anaerobic digestion process affects the pH of the reactor content. During the acid formation phase of anaerobic digestion, excessive production of volatile fatty acids and their accumulation cause pH values to be lower. Sugar beets are readily degradable crops and as a result of fermentation to volatile fatty acids (VFAs) in the initial stages of anaerobic digestion, the pH falls drastically, which hinders the methane production unless sufficient buffering source is provided to the system. As the anaerobic process enters the methane fermentation phase, pH values increases to more neutral values owing to the conversion of volatile fatty acids and hydrogen to methane and carbon dioxide. The measured pH values of the reactors at the beginning and at the end of the digestion process are presented in the Table 4.5.

Table 4.5. pH of the reactors at the beginning and at the end of the experiments

pH	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	7.25	7.91	-	-
12 % TS (SB) I/S=1.5	7.68	8.20	7.47	7.93	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	7.23	7.53
12 % TS (Maize) I/S=1.5	-	-	-	-	7.49	7.71
12 % TS (Maize) I/S=1					6.86	4.85
Blank 10 % TS I/S=1.5	-	-	7.86	7.60	7.86	7.71
Blank 12 % TS I/S=1.5	7.97	8.14	8.40	8.07	7.80	7.74

Due to high levels of alkalinity in the anaerobic granular sludge, pH of the mixtures prepared was mostly above the desired level which was a good indicator of a potentially well-balanced anaerobic degradation process. However, during the last experimental setup which was carried out by using maize as a substrate; the reactor having an I/S ratio of 1 had much lower pH than the others owing to the higher amount of substrate contained in it. Due to the formation of acidic conditions in this reactor, pH of 4.85 was observed in the output of the reactor and it was resulted in cease of gas formation at the 37th day of the experiment. This may be attributed to the insufficient buffering capacity that would neutralize the VFAs produced by the maize.

4.2.2.3. Alkalinity and VFA. Volatile fatty acids are produced as a result of the degradation of organic matter in the biomass and it may cause inhibition of the system resulting with depletion of biogas production. Since the negative effect of this parameter is neutralized and the acidic condition is buffered with high alkalinity in well balanced systems, the relationship between these parameters is a crucial point which needs consideration. The measured alkalinity and VFA concentrations for the batch reactors are given in the Table 4.6 and Table 4.7.

Table 4.6. Alkalinity concentration analyzed at the beginning and at the end of the experiments

ALKALINITY (mg L^{-1} $CaCO_3$)	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	1965.5	2418.3	-	-
12 % TS (SB) I/S=1.5	1590	1970	1858.6	2376.8	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	1717	2230.5
12 % TS (Maize) I/S=1.5	-	-	-	-	2091.8	2186.7
12 % TS (Maize) I/S=1	-	-	-	-	1736.8	1240
Blank 10 % TS I/S=1.5	-	-	3331.5	5510.2	16667	4252.7
Blank 12 % TS I/S=1.5	2851	3786	2433.3	3826.5	1805.6	5850.3

Initial alkalinity concentrations of the reactors in this study were above the threshold level which was reported to be 1200 mg L^{-1} in literature [30, 34]. The conditions for the biogas production were proved to be suitable reflected by high pH values and alkalinity concentrations together with some other factors which are mentioned in this section. Through to the end of the experiment, the initial alkalinity increased due to the contribution of soluble CO_2 which is not completely removed from the reactor as gas and the high level of bicarbonate.

Table 4.7. VFA concentrations analyzed at the beginning and at the end of the experiments

VFA (mg L^{-1})	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	1723.05	590.58	-	-
12 % TS (SB) I/S=1.5	4821	310	2561.76	254.42	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	228.89	126.48
12 % TS (Maize) I/S=1.5	-	-	-	-	458.91	97.24
12 % TS (Maize) I/S=1	-	-	-	-	487.89	128.32
Blank 10 % TS I/S=1.5	-	-	724.83	143.20	149.74	876.59
Blank 12 % TS I/S=1.5	131,7	1066,727	101.68	2307.52	182.16	958.75

Since sugar beet has high quantities of soluble organic matter which was rapidly converted into acids, high levels of VFAs were observed at the beginning of the first and the second experimental set-up. No significant accumulation of soluble acids had occurred to cause stress in the digesters. This is confirmed by the VFA concentrations observed in the final contents of the digesters.

4.2.2.4. TKN and NH_4^+-N.

Adequate amounts of nutrients are essential for supporting the growth and maintenance of microbial population, as well as the efficient operation of anaerobic degradation. Nitrogen is needed for the production of protein, enzymes, ribonucleic acid (RNA), and deoxyribonucleic acid (RNA).

Ammonium nitrogen is produced from the decomposition of organic material containing nitrogen. If the reactor contains high ammonium nitrogen at elevated pH ranges, free ammonia nitrogen is produced and it has toxic effects on microorganisms. The toxic level of free ammonia nitrogen was reported to be 700 mg L^{-1} and 1100 mg L^{-1} in the literature [46]. When the results obtained from three experimental set-ups were evaluated, it was found that free ammonia concentrations in the output of the reactors ranged between 101.6 mg L^{-1} - 342 mg L^{-1} which were lower than the toxic level. Free ammonia concentrations were calculated by using the equation below.

$[NH_3] = [NH_4]. 10^{(pKb - pOH)}$ (4.4)

where;

$[NH_3]$ = Molarity of free ammonia (mol L^{1-})

$[NH_4]$ = Molarity of ammonium (mol L^{1-})

pKb = ionisation constant of free ammonia

pOH = 14 - pH

TKN, which is another significant parameter, represents the sum of the organic nitrogen and ammonia nitrogen. The findings of the TKN and NH_4^+-N in all reactors are given in Table 4.8 and Table 4.9.

Table 4.8. NH_4^+-N conc. analyzed at the beginning and at the end of the experiments

NH_4^+-N (mg L^{-1})	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	312	2225	-	-
12 % TS (SB) I/S=1.5	182	2285	792	2725	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	123	2070
12 % TS (Maize) I/S=1.5	-	-	-	-	126	1930
12 % TS (Maize) I/S=1	-	-	-	-	119	810
Blank 10 % TS I/S=1.5	-	-	98	836	19	1640
Blank 12 % TS I/S=1.5	133	1790	51	1745	18.5	1720

Initial concentrations of ammonium nitrogen and TKN in the second run of %12 TS reactor was higher than the findings of previous run conducted with the same crop. This result may be contributed to the difference in the harvesting time of the sugar beet which was used during both of the experiments. The concentration of ammonium nitrogen analyzed prior to digestion was the lowest for the last run using maize as an energy crop.

NH_4^+-N concentrations in the three set-ups increased through to the end of the experiments to as high as 2725 mg L^{-1}. Increasing trend of ammonium nitrogen in all reactors during batch experiments is the result of the rapid decomposition of organic material containing nitrogen. On the other hand, TKN content of the reactors was reduced at the end of the experiments.

Table 4.9. TKN concentrations analyzed at the beginning and at the end of the experiments

TKN (%)	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	6.1	4.9	-	-
12 % TS (SB) I/S=1.5	7.76	6.5	9.6	6.2	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	8.9	4.1
12 % TS (Maize) I/S=1.5	-	-	-	-	9.2	5.3
12 % TS (Maize) I/S=1	-	-	-	-	5.4	4.3
Blank 10 % TS I/S=1.5	-	-	3.5	3.16	1.6	1.2
Blank 12 % TS I/S=1.5	5.5	5.28	5.44	4.87	3.19	3.1

4.2.2.5. TP and Orthophosphate. Another nutrient necessary for growth and performance of the microbial population is phosphorus. It is used to synthesize energy-storage compounds (adenosine triphosphate-ATP) as well as RNA and DNA. Total phosphorus (TP) and orthophosphates (PO_4^{-3}) were monitored as one of the major nutrients in anaerobic batch degradation. The findings of orthophosphate and TP concentrations for the anaerobic batch reactors are presented in Table 4.10 and 4.11.

Table 4.10. PO_4^{-3} concentrations analyzed at the beginning and at the end of experiments

PO_4^{-3} (mg L^{-1})	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	333	49	-	-
12 % TS (SB) I/S=1.5	157.5	18.7	360	39	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	326	95.2
12 % TS (Maize) I/S=1.5	-	-	-	-	482	32
12 % TS (Maize) I/S=1	-	-	-	-	315.78	296.78
Blank 10 % TS I/S=1.5	-	-	5.5	1.5	4.8	2.89
Blank 12 % TS I/S=1.5	7	1.6	13.5	1	4.4	2.3

Within the same amount of TS content (12 % TS), two different experimental set-ups conducted by using sugar beet and findings showed us that the second run had higher initial PO_4^{-3} concentration than the first one. The highest concentration was observed in the last run which was carried with maize including 12 % TS content. When these results are evaluated together with ammonium nitrogen, TKN and TP concentrations, it may be concluded that the amount and the depletion rate of nutrients may vary depending on the type and the harvesting time of the crop.

Table 4.11. TP concentrations analyzed at the beginning and at the end of the experiments

TP (%)	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	12.5	8.95	-	-
12 % TS (SB) I/S=1.5	16.13	12.75	17.47	11.74	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	15.73	10.49
12 % TS (Maize) I/S=1.5	-	-	-	-	19.48	12.75
12 % TS (Maize) I/S=1	-	-	-	-	18.24	15.48
Blank 10 % TS I/S=1.5	-	-	8.36	6.24	6.72	5.74
Blank 12 % TS I/S=1.5	9.25	8.76	9	7.73	6.73	6

Total phosphorus and orthophosphate concentrations in the reactors followed similar attenuation trend throughout the experimental period as a result of biological utilization. The highest depletion of orthophosphate was observed for the reactor having 12 % TS content with a I/S ratio of 1.5 during the last set-up. On the other hand, the lowest consumption was detected for the reactor which includes the same amount of TS but with a lower I/S ratio (1) owing to the inhibition of the system occurred at the 37th day of the experiment.

Blank reactors which were conducted without addition of energy crop had the lowest TP content of 6 - 9.25 %. The highest consumption of total phosphorus was observed in the reactor containing 12 % total solid during the last run. A similar decline was achieved in the 10 % TS reactor in the same run.

4.2.2.6. Chemical Oxygen Demand (COD). COD is a chemical parameter which is assessed as an indication of the relative biodegradability of the biomass and it is commonly used to characterize organic compounds contained in the substrate. Although organic matter is predominantly described in terms of VS for digestion of energy crops, COD concentration is also determined in order to have more information on the characteristics of crop and sludge mixture. COD concentrations of the batch reactors are given in Table 4.12.

Table 4.12. COD concentrations analyzed before and after the digestion

COD (mg L^{-1})	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	27875	5000	-	-
12 % TS (SB) I/S=1.5	19825	5875	54000	6000	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	6250	1826,3
12 % TS (Maize) I/S=1.5	-	-	-	-	8800	2338.52
12 % TS (Maize) I/S=1	-	-	-	-	7700	6186.78
Blank 10 % TS I/S=1.5	-	-	7762.5	5100	1730	1129
Blank 12 % TS I/S=1.5	6625	3675	8000	4450	1900	958.45

Similar with the results of ammonium nitrogen and phosphate which were mentioned above, initial COD concentrations of the reactors including 12 % TS content were found to be different in the first and the second setup although the same crop was used as a substrate. Findings of these analyses indicated that the most promising reactor was the one having 12 % TS at the second run with the highest COD concentration contained in it. The reactors which were evaluated throughout the last run had the lowest COD concentrations and they were expected to produce the lowest amount of biogas. Nevertheless, all of the parameters were considered as a whole in order to evaluate the performance of the system with regard to its biogas production. In the second and the third run, the reactors which contain 10 % TS had lower COD concentrations than the ones having 12 % TS content as it was supposed to be.

COD concentrations which were analyzed prior to digestion decreased through to the end of the experiment as a result of decomposition process. The highest degradation rate of

COD with a value of 88 % was observed in the second run conducted with sugar beet and sludge mixture having final TS content of 12 %. On the other hand, the reactor which had a final solid content of 12 % in the last run showed the lowest rate of 19,65 % due to the inhibition of the system. This was confirmed by the ceased biogas production at the 37th day of the experiment.

The properties of the anaerobic granular sludge which were used as a seeding material in each run differed from one to another due to the variation in the operation of the wastewater treatment plant. The percentage of the COD degraded throughout the runs were between 45 and 50 for the blank reactor with a final TS content of 12 % and 34 for the blank reactor having 10 % TS. The results obtained related to the COD concentrations showed that the increase in the TS content of the reactor caused higher COD concentrations. However, COD degradation in the reactors showed difference with the type of energy crop used.

4.2.2.7. Total Organic Carbon (TOC). TOC includes a variety of organic compounds, including humic acids, fulvic acids, VOAs and carbohydrates. The results of TOC concentrations detected within the batch reactors, which were conducted under anaerobic conditions, are illustrated in the Table 4.13. TOC exhibited a similar trend with COD removal, except from 12 % TS reactor which was studied in the last run. Depletion of the organic carbon could not be obtained owing to the inhibition occurred within this reactor.

Table 4.13. TOC concentrations of the reactors analyzed before and after the digestion

TOC (mg L^{-1})	**1st SET-UP**		**2nd SET-UP**		**3rd SET-UP**	
Reactor	Beg.	End	Beg.	End	Beg.	End
10 % TS (SB) I/S=1.5	-	-	9615	976	-	-
12 % TS (SB) I/S=1.5	5342	1022	19960	853.5	-	-
10 % TS (Maize) I/S=1.5	-	-	-	-	1254	557.8
12 % TS (Maize) I/S=1.5	-	-	-	-	2117	879.5
12 % TS (Maize) I/S=1	-	-	-	-	1047	1856
Blank 10 % TS I/S=1.5	-	-	1349	1176	663,5	476.32
Blank 12 % TS I/S=1.5	1846	1221.8	1736	1458	986	755.2

Initial TOC concentration in the reactor including 12 % TS content in the last run was determined as 19960 mg L^{-1} which was the highest value observed throughout the experimental study. Degradation of organic carbon (TOC) was observed at the end of the experiments in the all reactors having I/S ratio of 1.5. The process efficiency were in the range of 55 – 95.7 % in terms of TOC reduction and the highest degradation rate was achieved for the reactor including 12 % solid content in it which was studied during the second run. Success of methanogenic activity was confirmed by the gas production with high methane content in addition to the decline in TOC concentrations.

4.2.3. Gas Analysis

The analyses covered a wide range of parameters; biogas volume, biogas quality (significantly in terms of methane and carbon dioxide content), biogas yield and specific methane yield. Biogas volume was determined as the major indicator of a successful anaerobic digestion process while the quality of it was investigated since methane and carbon dioxide are the major products of anaerobic conversion of biomass. In order to evaluate if the biogas production system is economically viable, two other parameters were investigated including biogas yield and methane yield. The results of daily gas production, cumulative gas production, gas composition, biogas yield and methane yield are presented in Figures 4.1 through 4.15.

4.2.3.1. Gas Production. The amount of biogas production was monitored every day and daily gas production was determined in the anaerobic batch reactors by recording the total amount of gas produced in 24 hours. The daily gas volumes produced in the batch reactors through three set-ups are presented in Figures 4.1, 4.2 and 4.3. These results can be utilized in order to characterize the performance of the system regarding the microbial activity within the reactors.

Daily biogas production patterns resembled each other and similar values have been reported for anaerobic batch degradation of sugar beet including 12 % TS content during the first and the second set-up. Daily biogas production values of these reactors were at its highest level between 0-20 days with a value ranging from 4000 to 4500 mL.

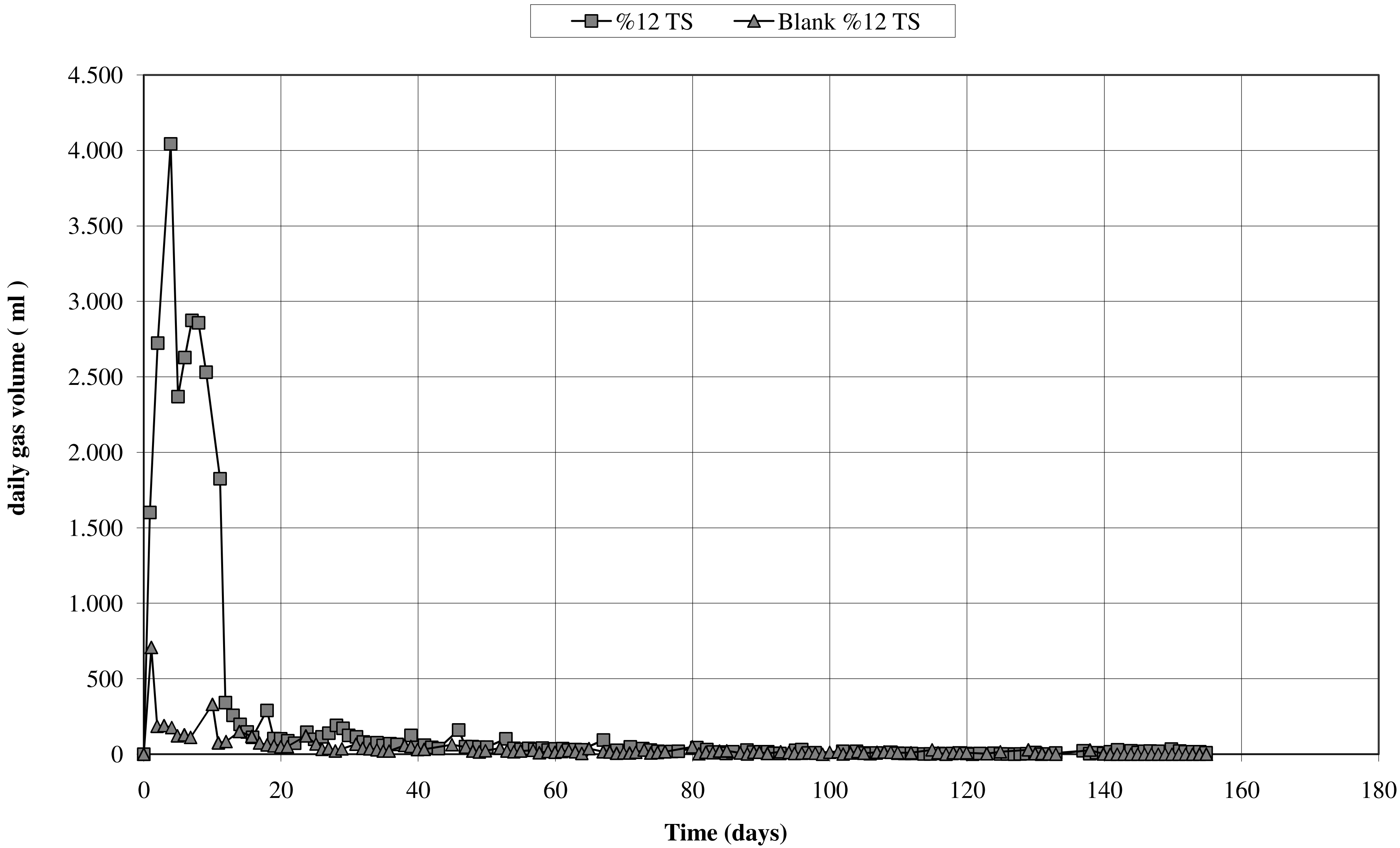


Figure 4.1. Daily gas volume produced in the reactors at the 1st set-up

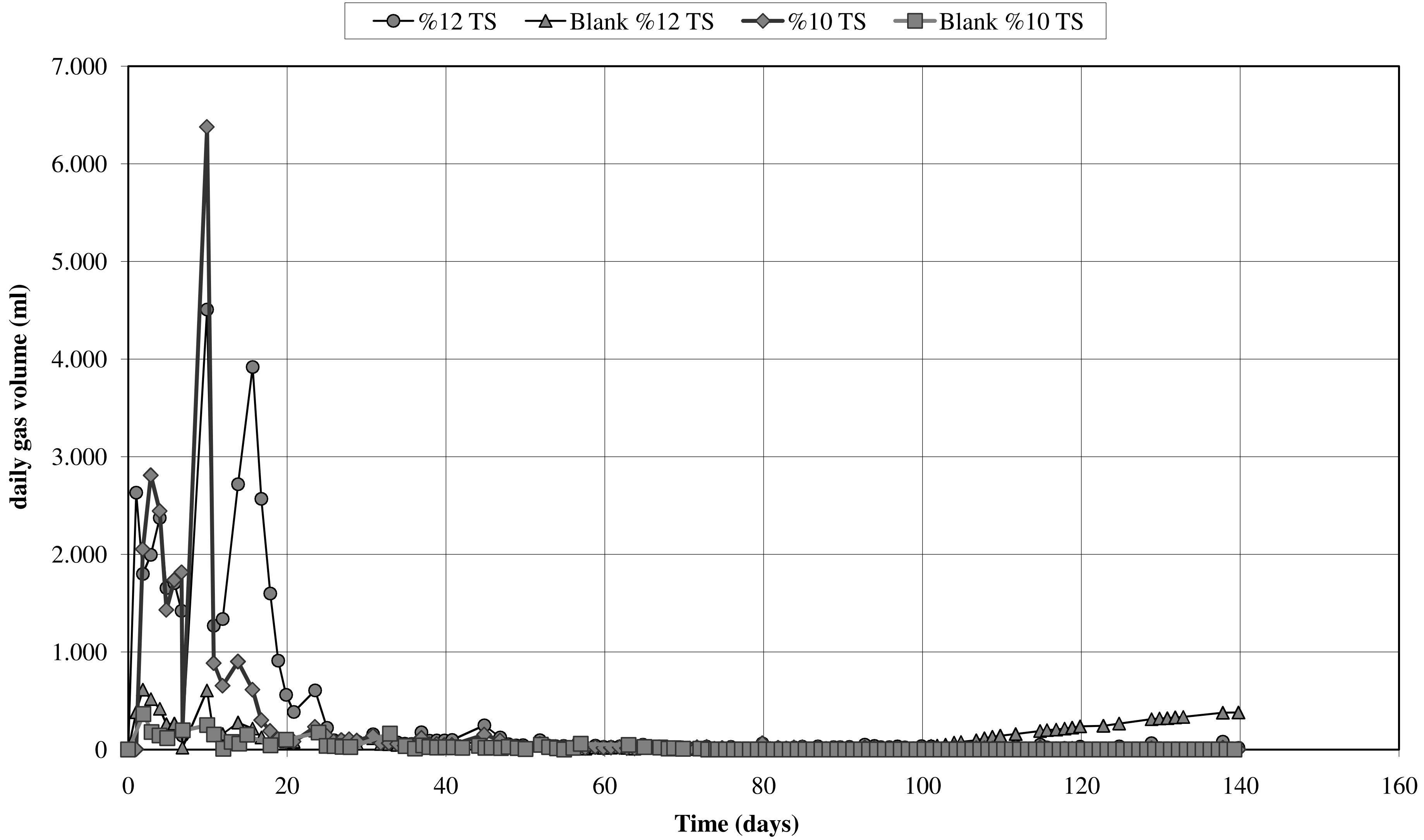


Figure 4.2. Daily gas volume produced in the reactors at the 2nd set-up

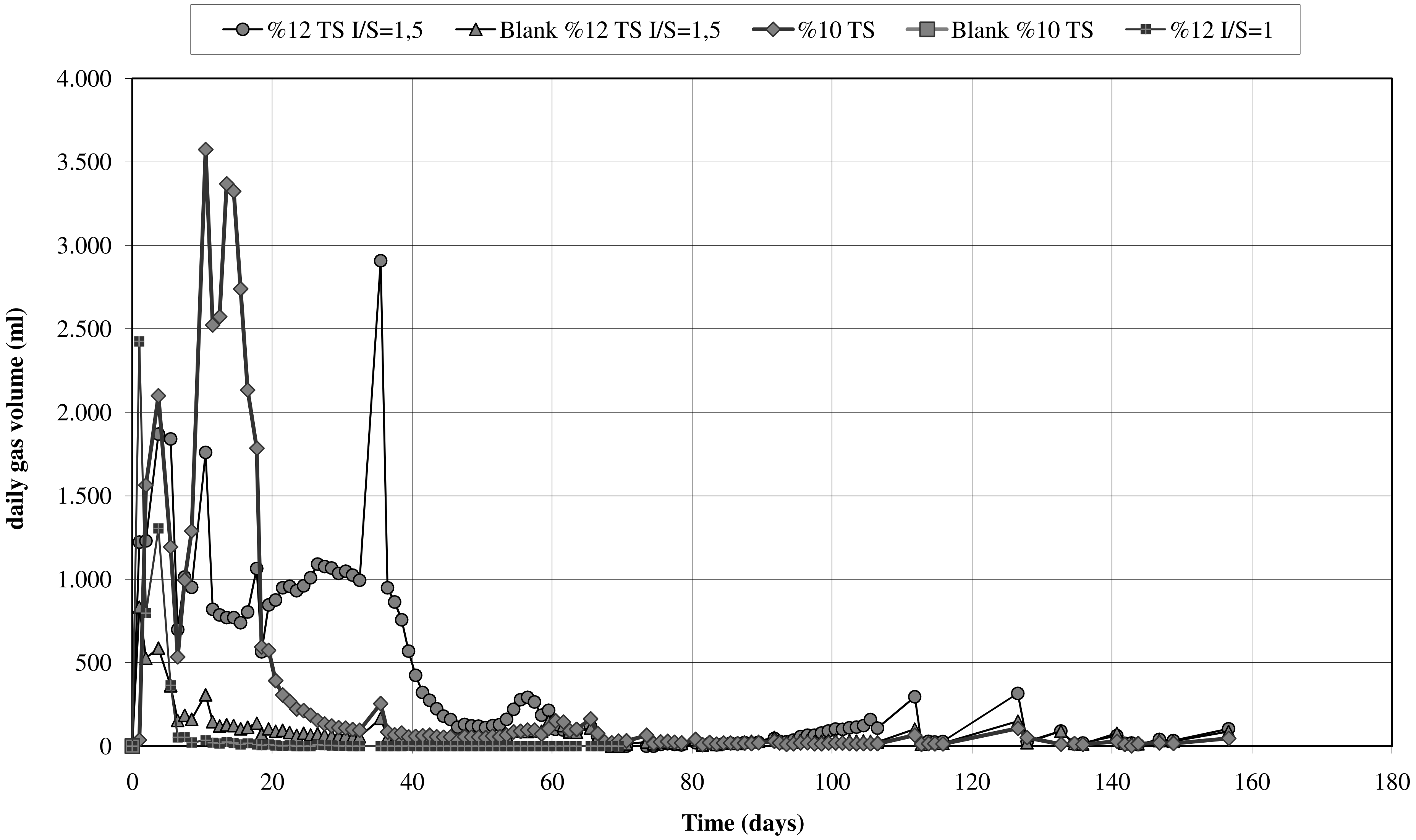


Figure 4.3. Daily gas volume produced in the reactors at the 3rd set-up

The highest daily biogas volume of 6377 mL was monitored for the reactor containing 10 % TS during the second run at the 10th day of the set-up. Another sharp increase of daily biogas production was not observed in the reactors in which sugar beet was digested after the 20th day of the experiments. The daily biogas production of the blank reactor which had 12 % TS content increased through to the end of the experiment owing to the existence of oxygen in the reactor as a result of a failure that was done during gas sampling.

When the last set-up was evaluated in terms of daily biogas production, the highest volume of 3573 mL was detected in the reactor including 10 % TS at the 10th day of the last run which was conducted in order to digest maize as an energy crop. The reactor containing 12 % TS maize and sludge mixture with I/S ratio of 1.5 showed a sharp increase at the 35th day with a value of 2908.12 mL. The lowest values were monitored for the mixture which had 12% final solid content with I/S ratio of 1 due to the inhibition of the system. The observed inhibition of the reactor was supported by methane yield and biogas yield which will be mentioned in the following sections.

The cumulative biogas production rates from reactors at various TS contents are illustrated in the Figures 4.4, 4.5 and 4.6. The values of cumulative biogas after 140-180 days of digestion time for three different set-ups were respectively; 24455 mL (12 %TS; 1st run), 22637 mL (10 % TS; 2nd run), 31223 mL (12 %TS;2nd run) and finally 29624 mL (10 % TS; I/S ratio 1,5; 3rd run), 35262 mL (12 % TS; I/S ratio 1,5;3rd run), 5225 mL (12 % TS; I/S ratio 1; 3rd run). Biogas production from inoculum alone was measured as well and subtracted from the biogas production that was measured in the digesters that contained inoculum and crop. However, biogas production was not examined for the blank reactor of 12 %TS with I/S ratio of 1 due to the inhibition that was took place in it. Thus, the value of this reactor is the total biogas production of both crop and sludge. Additionally, the cumulative biogas value of the reactor containing 12 %TS at the second run was calculated by disregarding the last data which were monitored for the blank reactor due to unexpected increase of production as a result of a failure that was mentioned above.

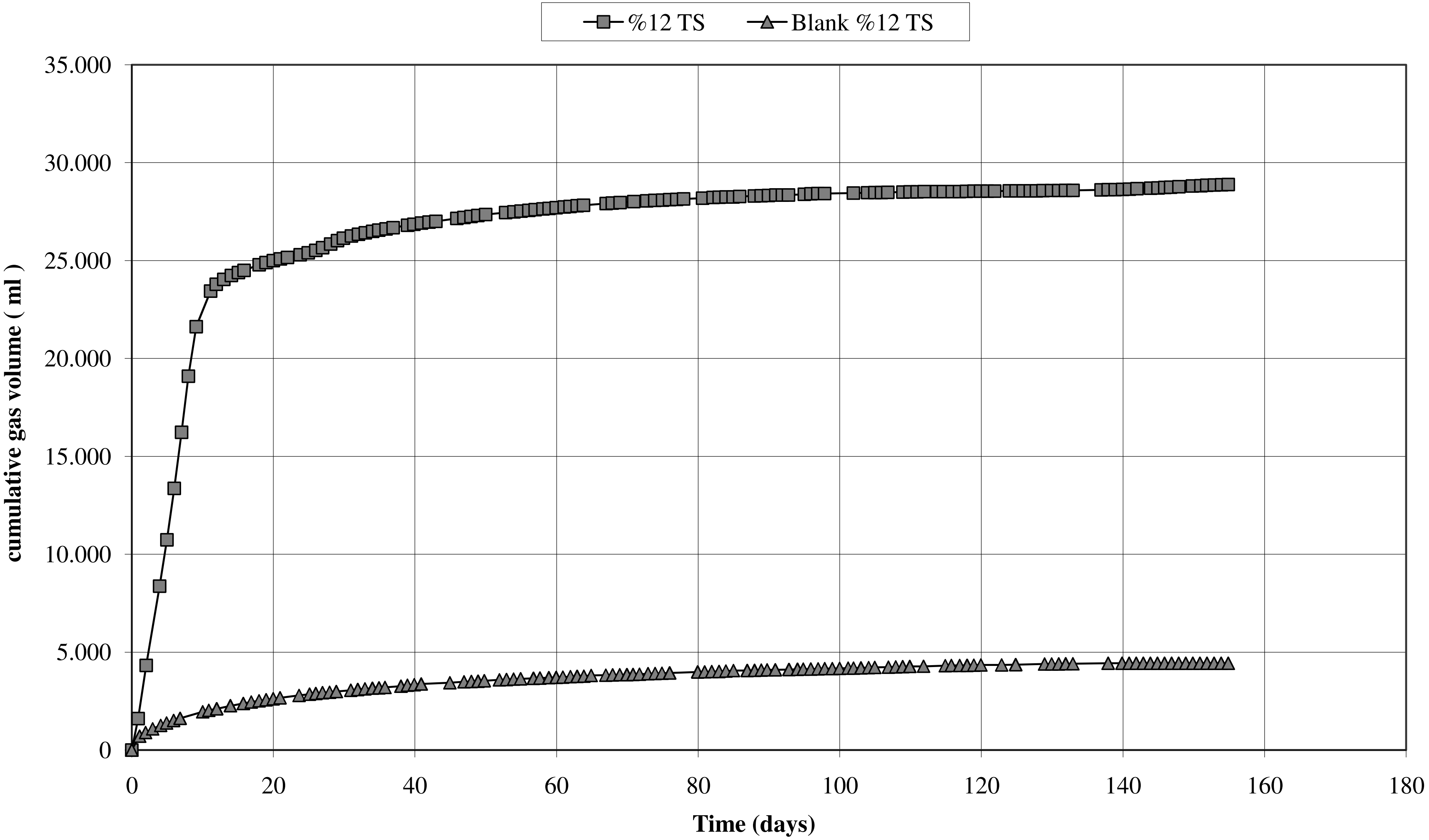


Figure 4.4. Cumulative gas production in the reactors at the 1st set-up

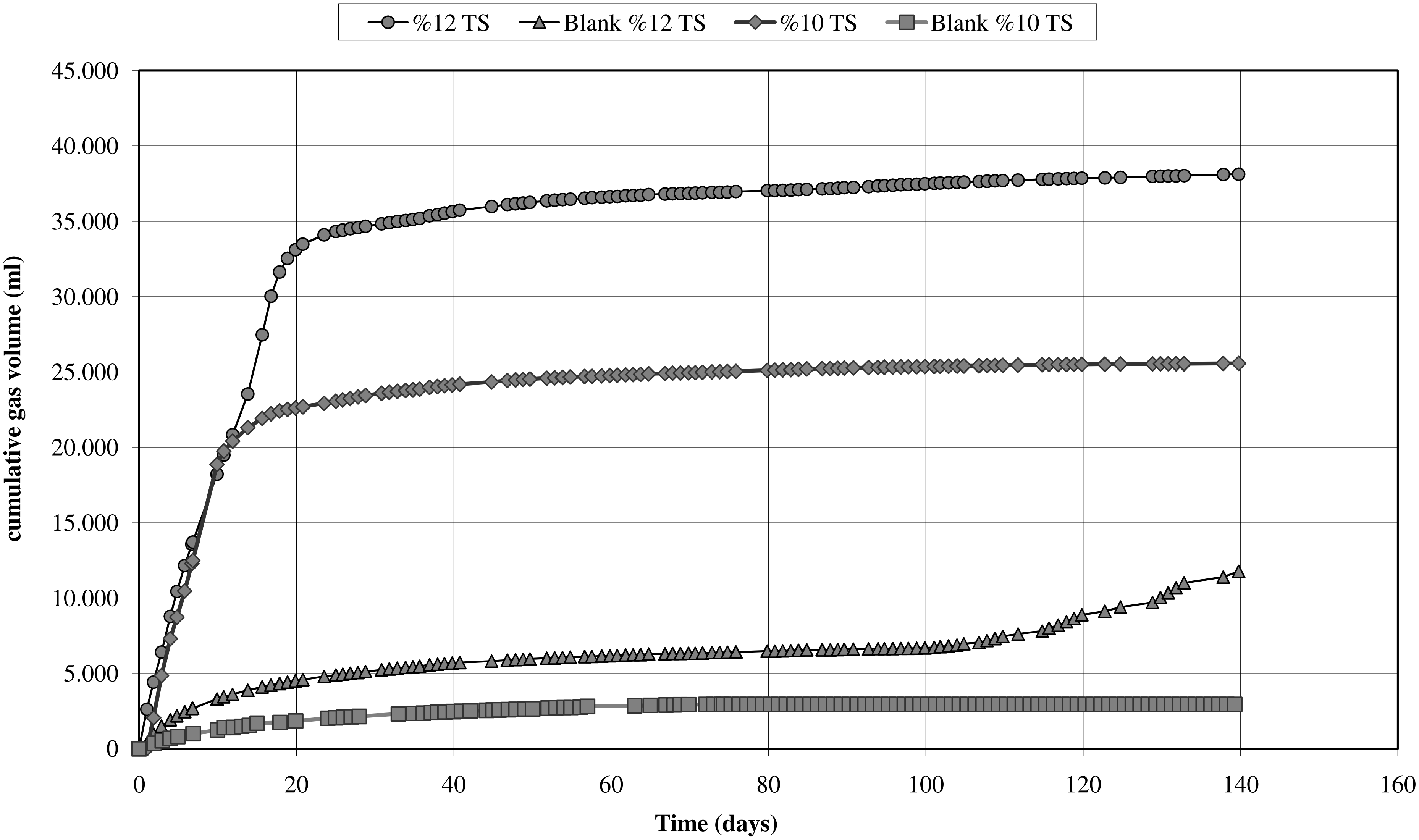


Figure 4.5. Cumulative gas production in the reactors at the 2nd set-up

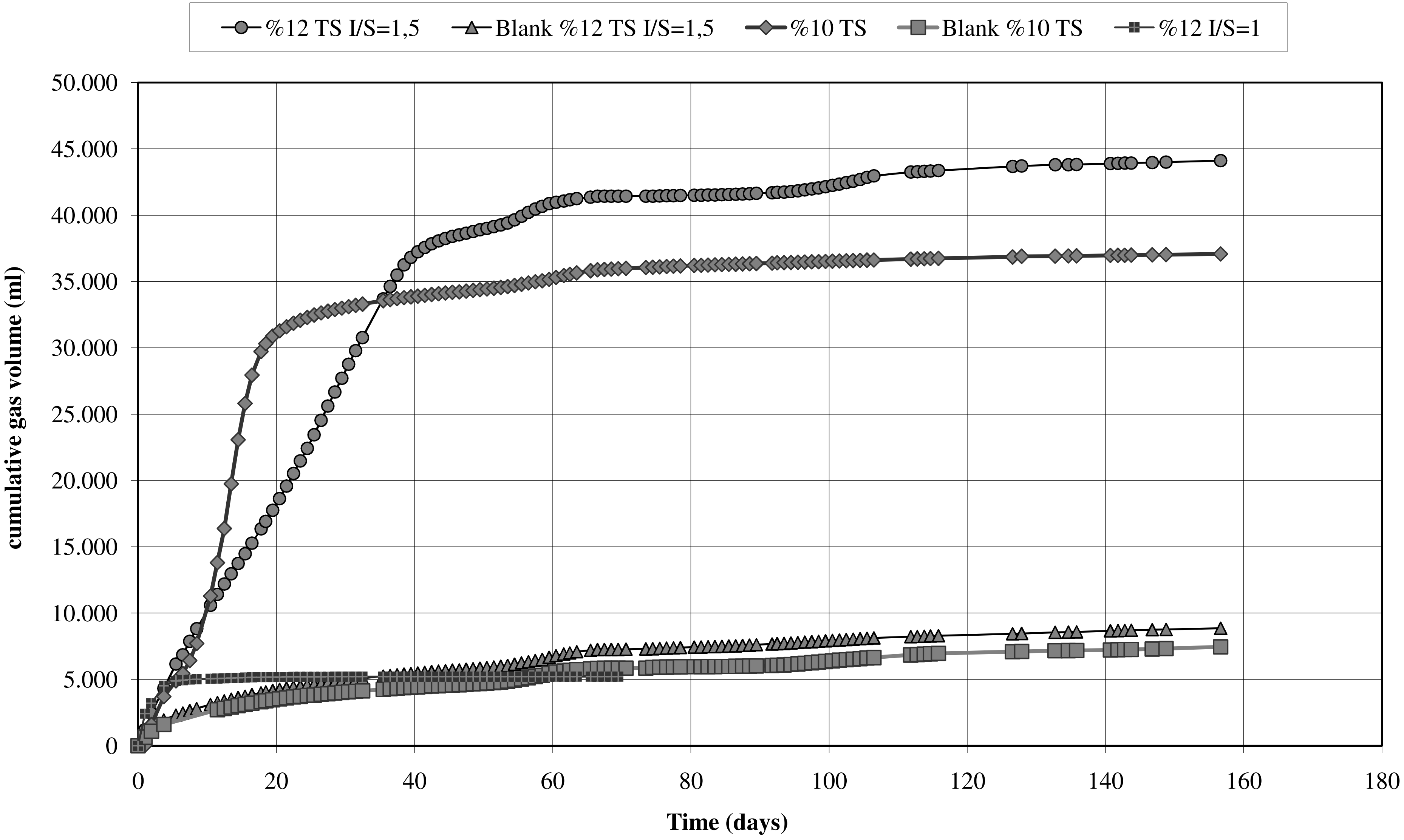


Figure 4.6. Cumulative gas production in the reactors at the 3rd set-up

The inhibition which occurred in the reactor including 12 % TS maize and sludge with I/S ratio of 1 was reproved with the stable cumulative biogas production values after 37 days. This was caused by high amounts of sub-products generated resulting from natural hydrolysis process that were not suitable for the methanogenic population.

From these figures, it was obvious that the total gas produced was the highest for the reactor with 12 % TS content and I/S ratio of 1.5 which was studied during the last run. Nevertheless, daily and cumulative biogas production values can be used for qualitative characterization of reactors related to the microbial activity within the reactors meanwhile evaluated together with the biogas composition.

4.2.3.2. Biogas Composition. Composition of the biogas in terms of methane and carbon dioxide reflects the biological activity and organic material conversion in the reactors. Thus, this parameter is another significant point that should be considered while determining the performance of an anaerobic digestion process. Biogas quality (CH_4, CO_2) for the batch reactors was analyzed 15-20 times in course of the 4-5 month digestion period. The cumulative methane production values obtained from digestion of different substrates at various TS contents are illustrated in the Figures 4.7, 4.8 and 4.9.

As shown in the Figure 4.7 and 4.8, rapid production of the methane was observed due to high humidity and organic matter content of the substrate and high levels of alkalinity which was provided by the existence of the anaerobic granular sludge. The highest methane content values for the reactors containing 12 % TS during the first and the second set-up were 66 % and 71 % respectively. Additionally, the average methane contents detected throughout these two runs resembled each other with values of 59 % for the first and 63 % for the second set-up. However, the results of the blank reactors were not similar since the anaerobic granular sludge used as seeding material was different in the second set-up due to change in the operation of the wastewater treatment plant. Methane content of the biogas which was produced in the batch reactor with 10%TS content was at its highest level (69 %) at the 66th day of the second run. Besides, average percentage of the methane in this digester (60.8 %) was lower than the reactor having 12 %TS content as it was expected to be.

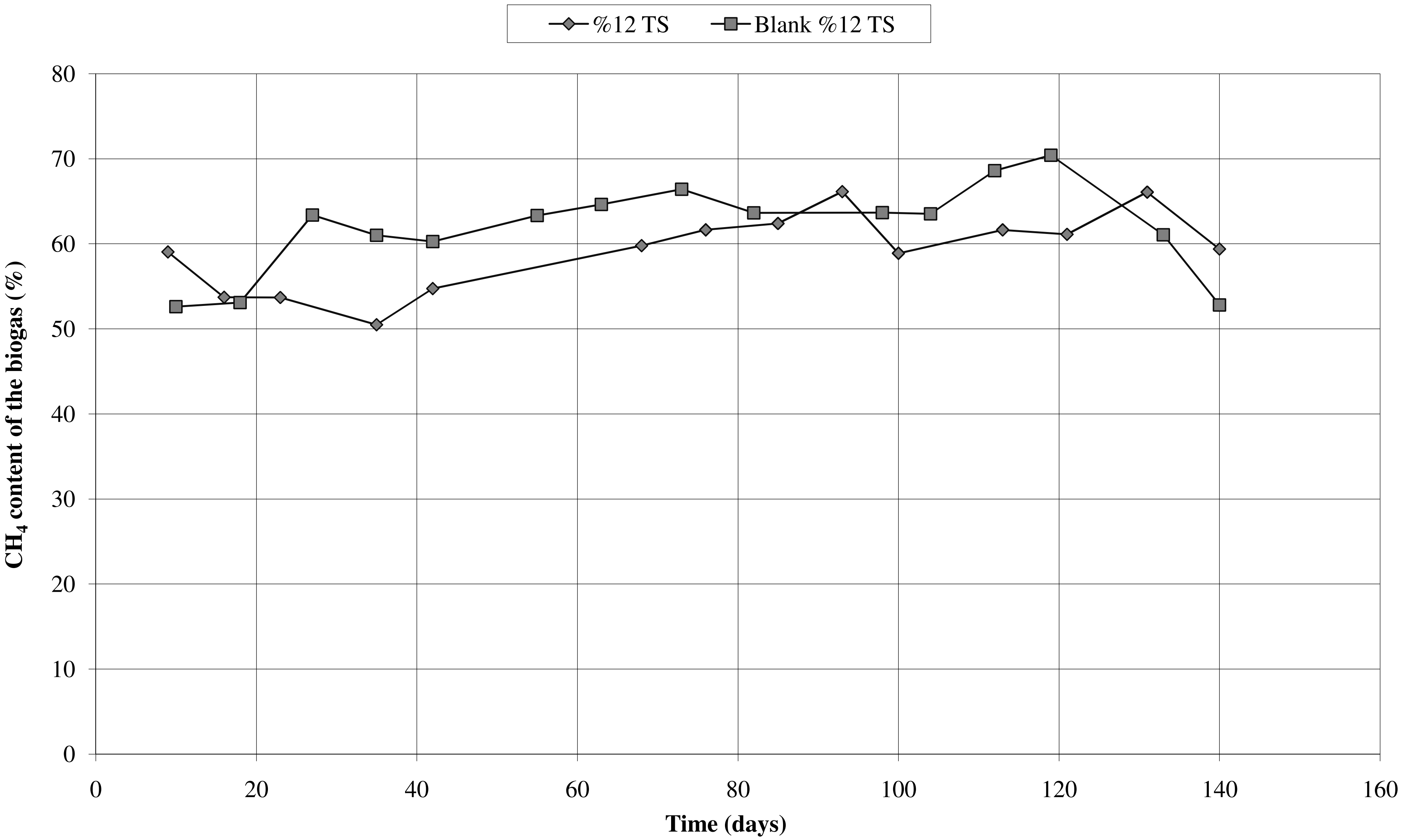


Figure 4.7. Methane Content of the Biogas Produced in the reactors at the 1st set-up

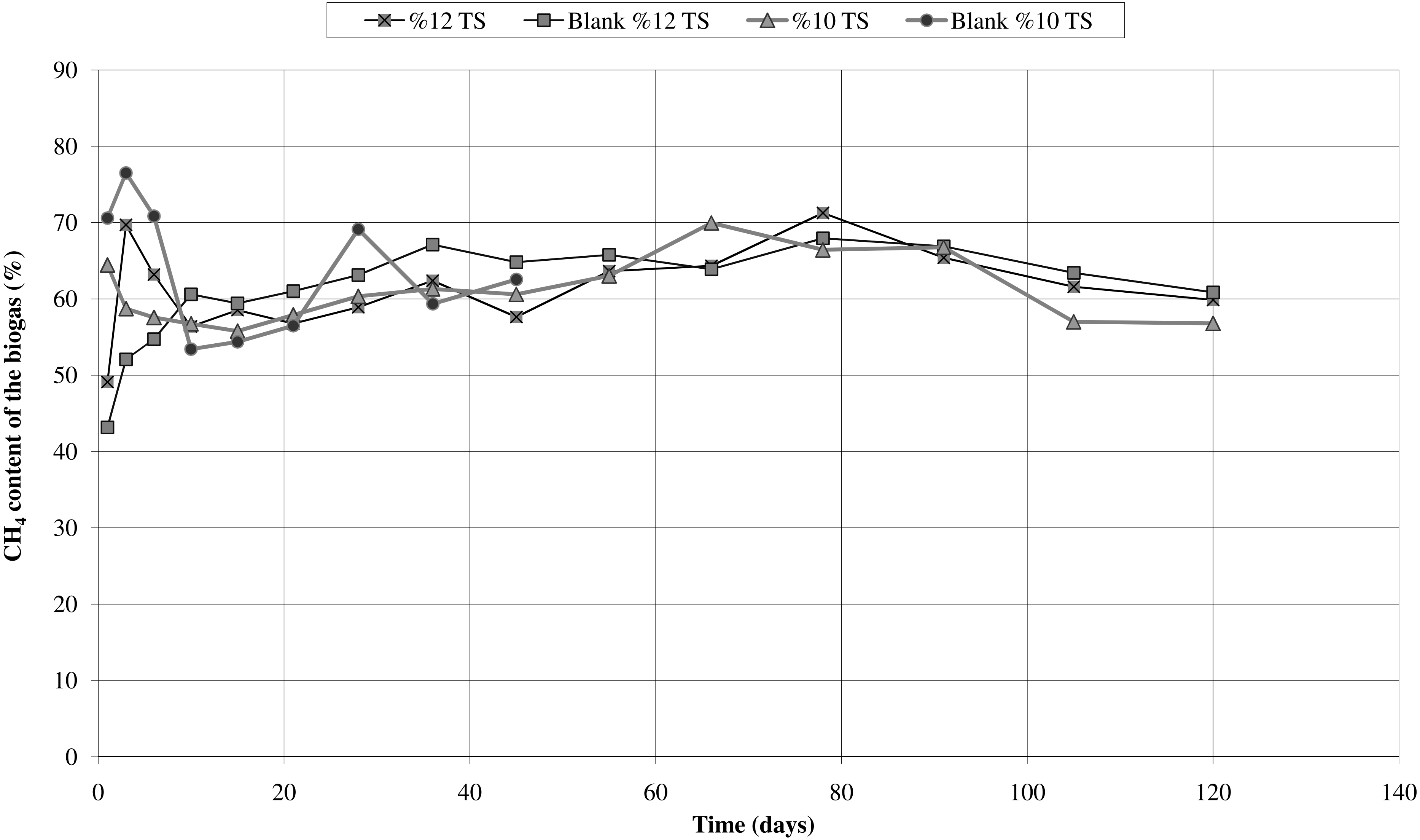


Figure 4.8. Methane Content of the Biogas Produced in the reactors at the 2nd set-up

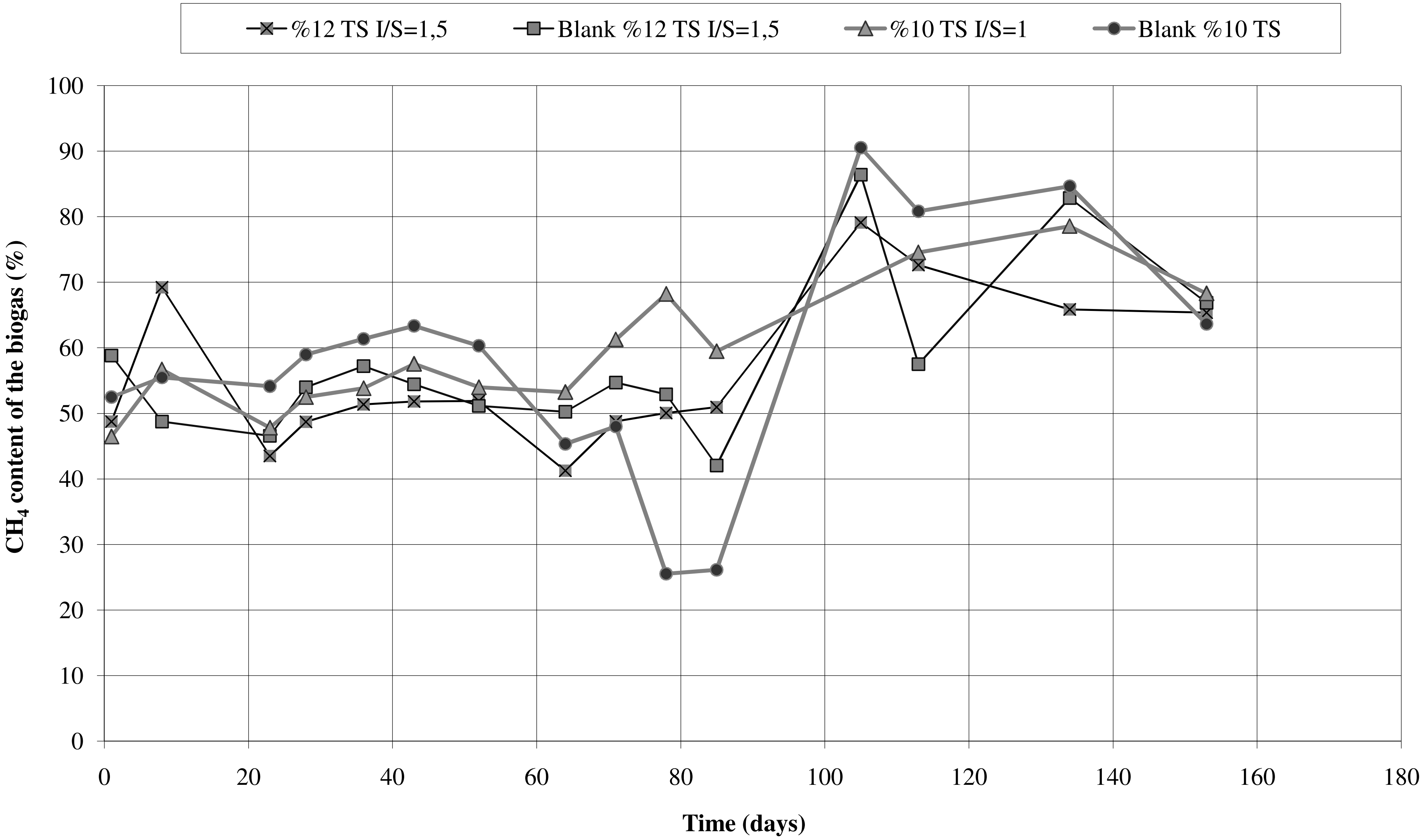


Figure 4.9. Methane Content of the Biogas Produced in the reactors at the 3rd set-up

The average methane content values of the digesters during the last run were 59.5 % for the reactor including 10 %TS with I/S ratio of 1.5 and 55.9 % for the batch digester having 12 % TS with I/S ratio of 1.5. The highest (90.53 %) and the lowest (25.5 %) methane content values were observed for the blank reactor including final TS content of 10 %. In almost all of the reactors the highest level of methane production was detected at the 105th day of the experiment. These values were much higher than the standard composition of the biogas. This may be contributed to a failure which was done during the calibration of the GC.

Similar to previous two set-ups, methane was observed from the beginning of the last run due to high organic matter contained in the reactors with respect to volatile solid content. The VS of the mixtures were 85 g, 80 g and 71 g in the batch digesters including 12 %TS (I/S ratio 1.5), 12 % TS (I/S ratio 1) and 10 %TS (I/S ratio 1.5) respectively. Additionally, the anaerobic granular sludge provided the suitable conditions in this run with the help of its high buffering capacity. Methane production could not be performed in the reactor containing final TS content of 12 % with I/S ratio of 1 due to inhibition. When the methane contents of the reactors at three set-ups were evaluated, it was obvious that the values for the blank reactors were higher than the digesters including crops and seed, since the content of these reactors were acclimated to the anaerobic conditions.

4.2.3.3. Biogas Yield and Methane Yield. Prior to considering biogas production system as a potential alternative energy source two other parameters; biogas yield and methane yield should be considered.

Figure 4.10, 4.11 and 4.12 shows the cumulative biogas yield as a function of time at different TS contents. As it can be seen from these figures below, all three set-ups followed a similar pathway. The results obtained from the experiments show us that the digester containing 12 % TS with an I/S ratio of 1.5 at the end of the first and the second run were 0.62 L g VS $^{-1}$ $_{\text{degraded}}$ and 0.61 L g VS $^{-1}$ $_{\text{degraded}}$ respectively. As it was expected, the biogas yield of the digester including 10 % TS was much lower than the 12 % TS reactor with a value of 0.482 L g VS $^{-1}$ $_{\text{degraded}}$.

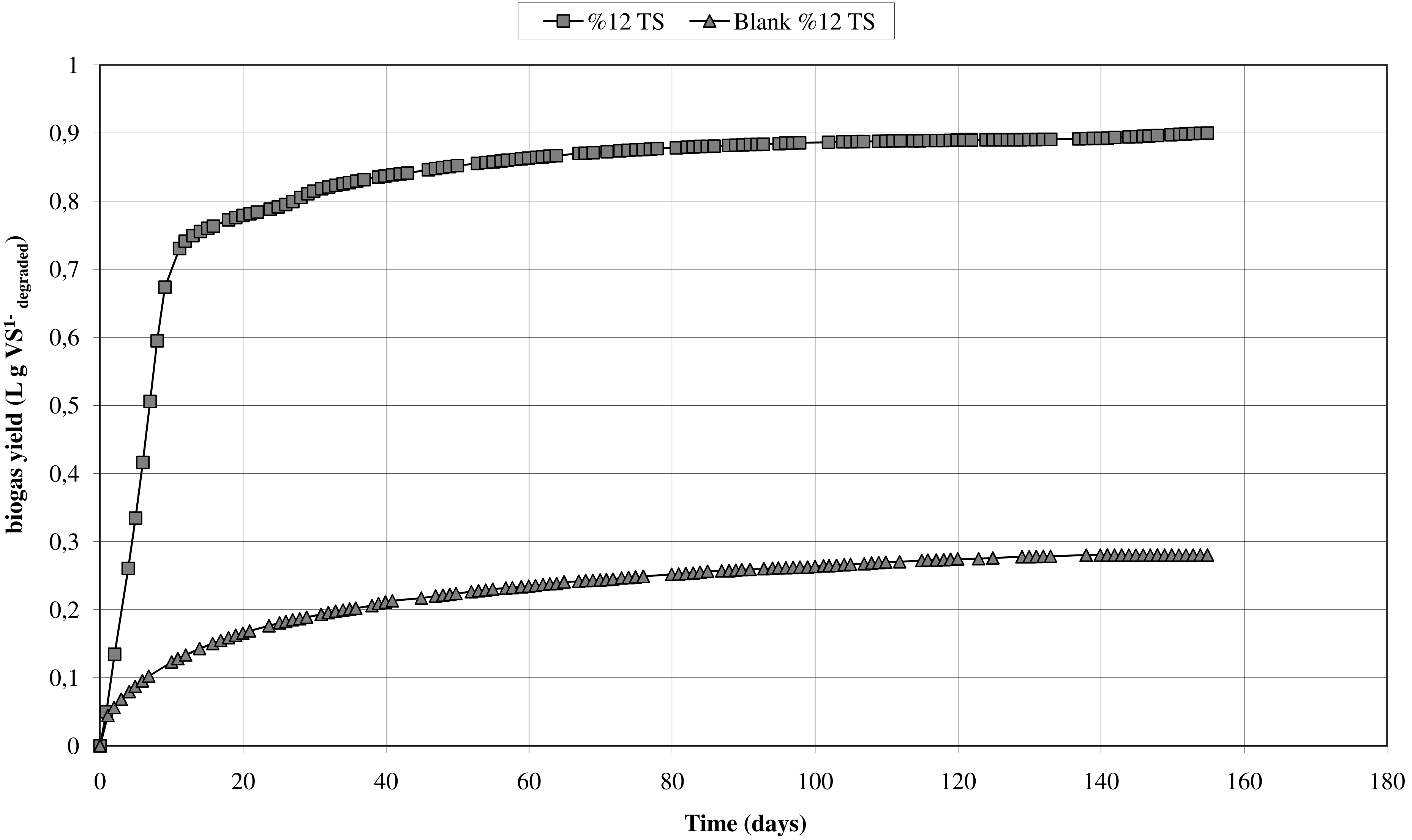


Figure 4.10. Biogas Yield Obtained from Anaerobic Digestion of Sugar Beet at the 1st set-up

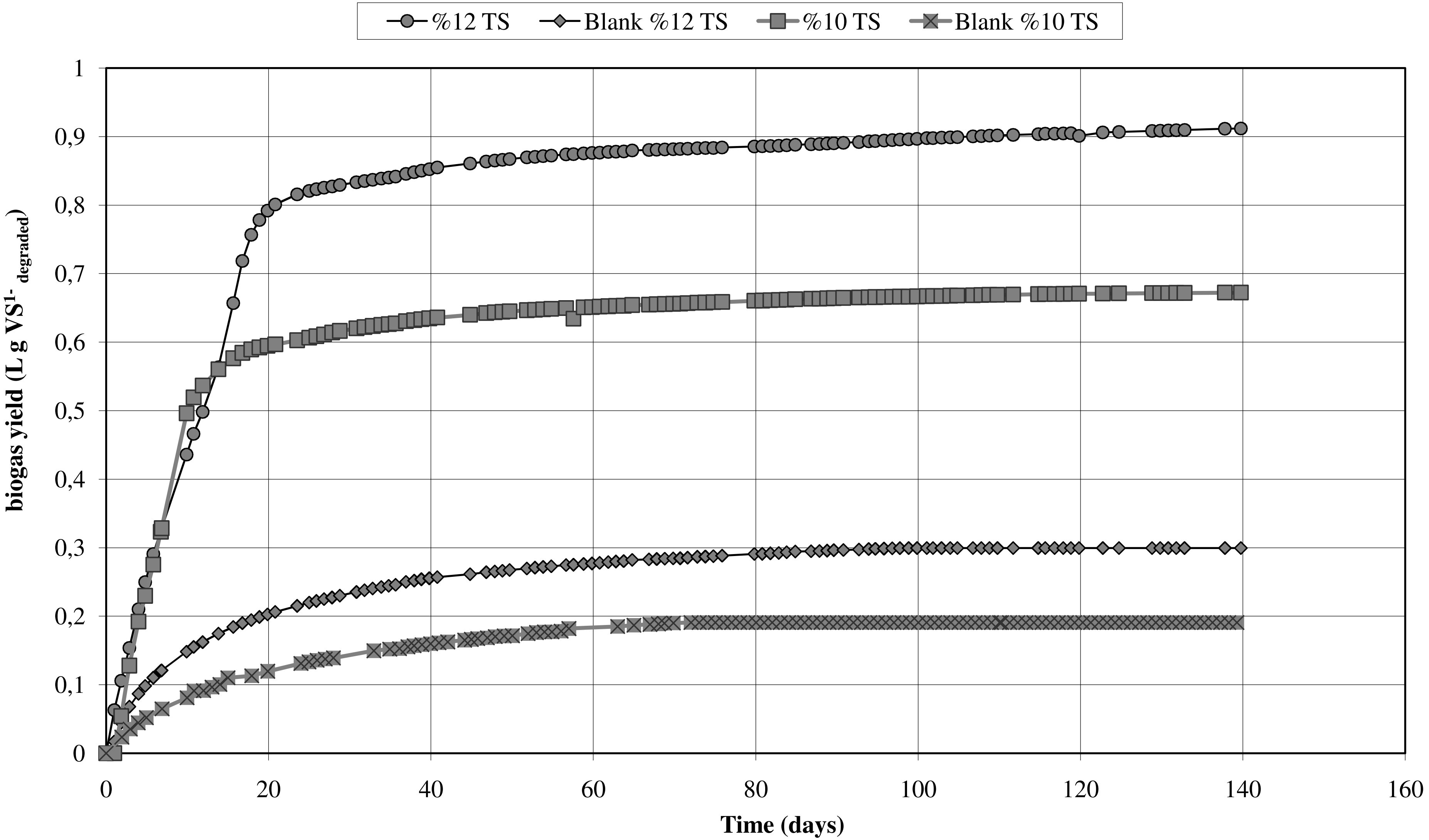


Figure 4.11. Biogas Yield Obtained from Anaerobic Digestion of Sugar Beet at the 2nd set-up

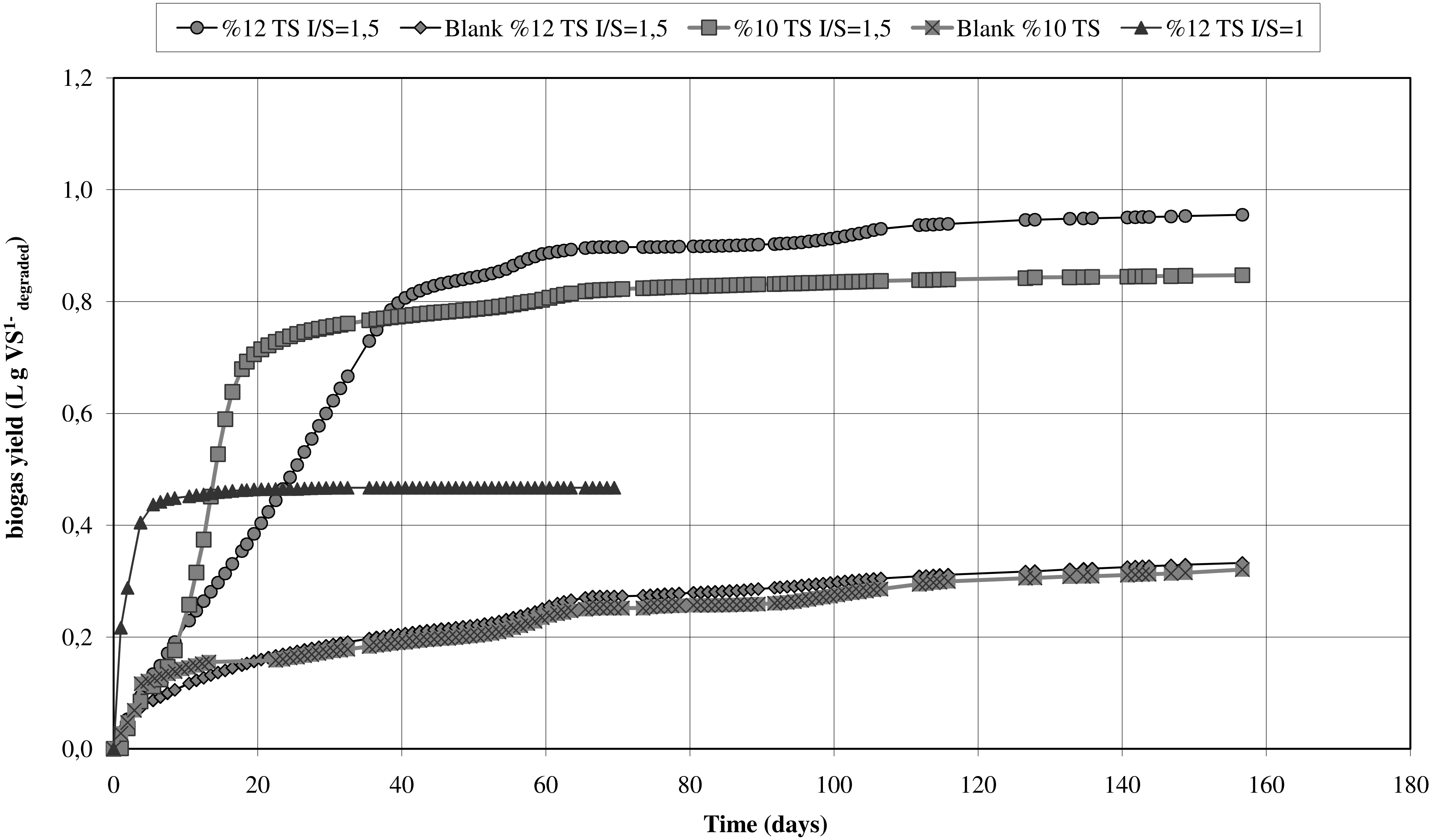


Figure 4.12. Biogas Yield Obtained from Anaerobic Digestion of Maize at the 3rd set-up

During the last run, the reactor which included a final TS content of 12 % with I/S ratio of 1.5 had a similar result with the first and the second set-up (0.622 L g VS $^{-1}$ $_{\text{degraded}}$). However, the biogas yield calculated for 10 % TS reactor was 0.526 L g VS $^{-1}$ $_{\text{degraded}}$ which was higher than the second set-up. The lowest value was obtained during the last run in the reactor having 12 % TS content with I/S ratio of 1. These values were calculated by subtracting the amount of biogas produced by the control from the biogas production of each reactors and dividing the difference by the mass of volatile solids contained in the mixture of crop and sludge. In addition, biogas yield values were also corrected at standard temperature and pressure conditions (STP) to be able to compare these results with further studies in the literature. These values were; 0.5118 L g VS $^{-1}$ $_{\text{degraded}}$ (12 % TS; I/S ratio 1.5; 1st set-up), 0.506 L g VS $^{-1}$ $_{\text{degraded}}$ (12 % TS; I/S ratio 1.5; 2nd set-up), 0.398 L g VS $^{-1}$ $_{\text{degraded}}$ (10 % TS; I/S ratio 1.5; 2nd set-up), 0.514 L g VS $^{-1}$ $_{\text{degraded}}$ (12 % TS; I/S ratio 1.5; 3rd set-up) and 0.435 L g VS $^{-1}$ $_{\text{degraded}}$ (10 % TS; I/S ratio 1.5; 3rd set-up).

In order to investigate the effects of different plant species and growth stages on the biogas production, an experimental set-up was conducted by a group of researchers. The highest biogas yield of 0.987 L g VS $^{-1}$ $_{\text{degraded}}$ was achieved by using barley as an energy crop which is appreciably greater than the biogas yield values mentioned above [37]. In contrary, biogas yield obtained during another experimental study was between 0.208–0.268 L g VS $^{-1}$ $_{\text{degraded}}$ [38].

The results from the biomethanation process were expressed also in terms of methane yield (L CH_4 g VS $^{-1}{}_{\text{degraded}}$) and the findings were illustrated in the Figure 4.13, 4.14 and 4.15. In these figures, the methane yield of the reactors including both energy crops and sludge were not corrected by taking into account the values of blank reactors since the methane produced in the blank reactors and the others were monitored at different period of time. Methane production from energy crops depends on their composition, the inoculum/substrate ratio, and also the experimental conditions employed. Economic biogas production requires high methane yields. Key factors for a maximum methane yield are species and variety of energy crops, time of harvesting, mode of conservation and pretreatment of the biomass prior to the digestion process but also the nutrient composition of the energy crop [31].

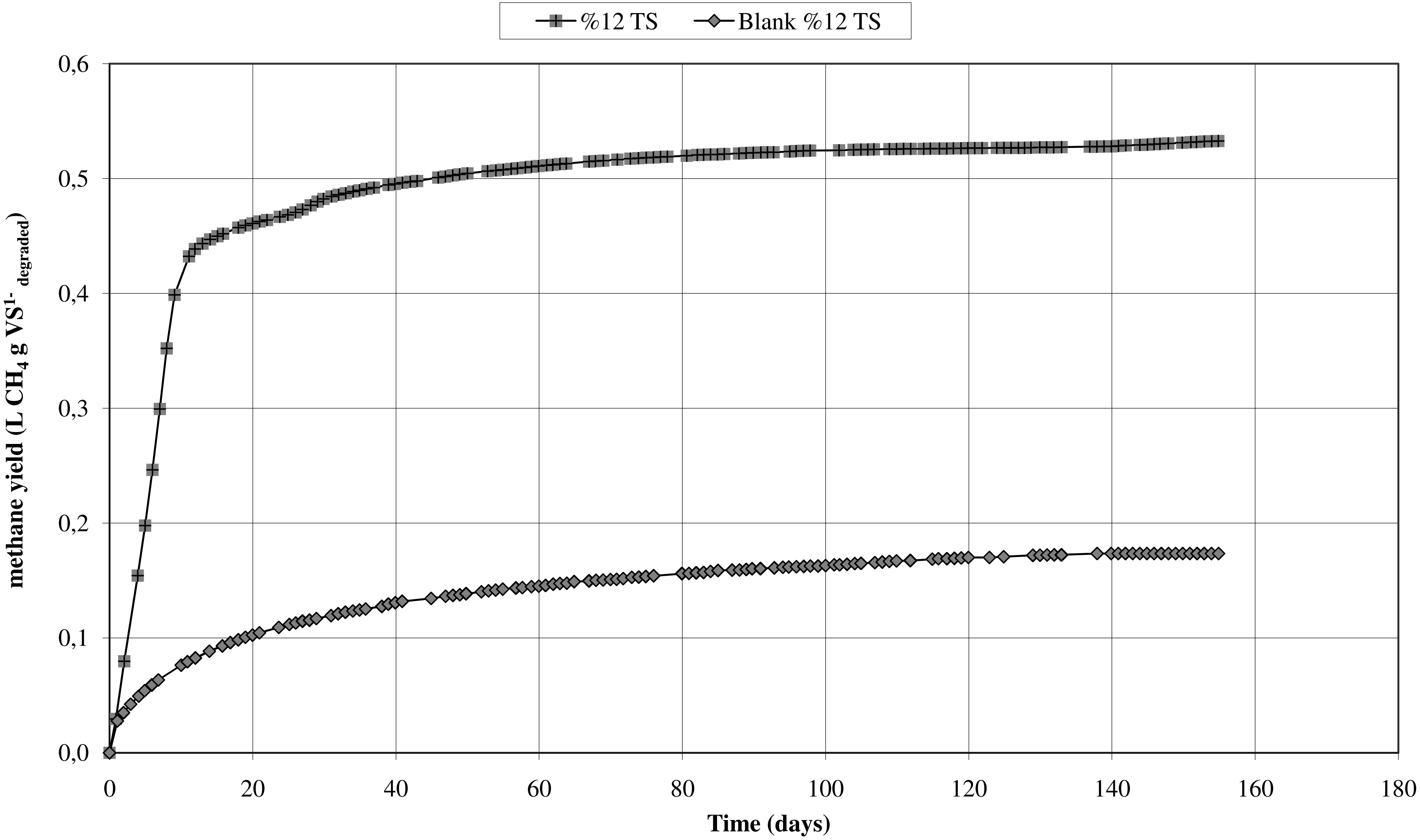


Figure 4.13. Methane Yield Obtained from Anaerobic Digestion of Sugar Beet at the 1st set-up

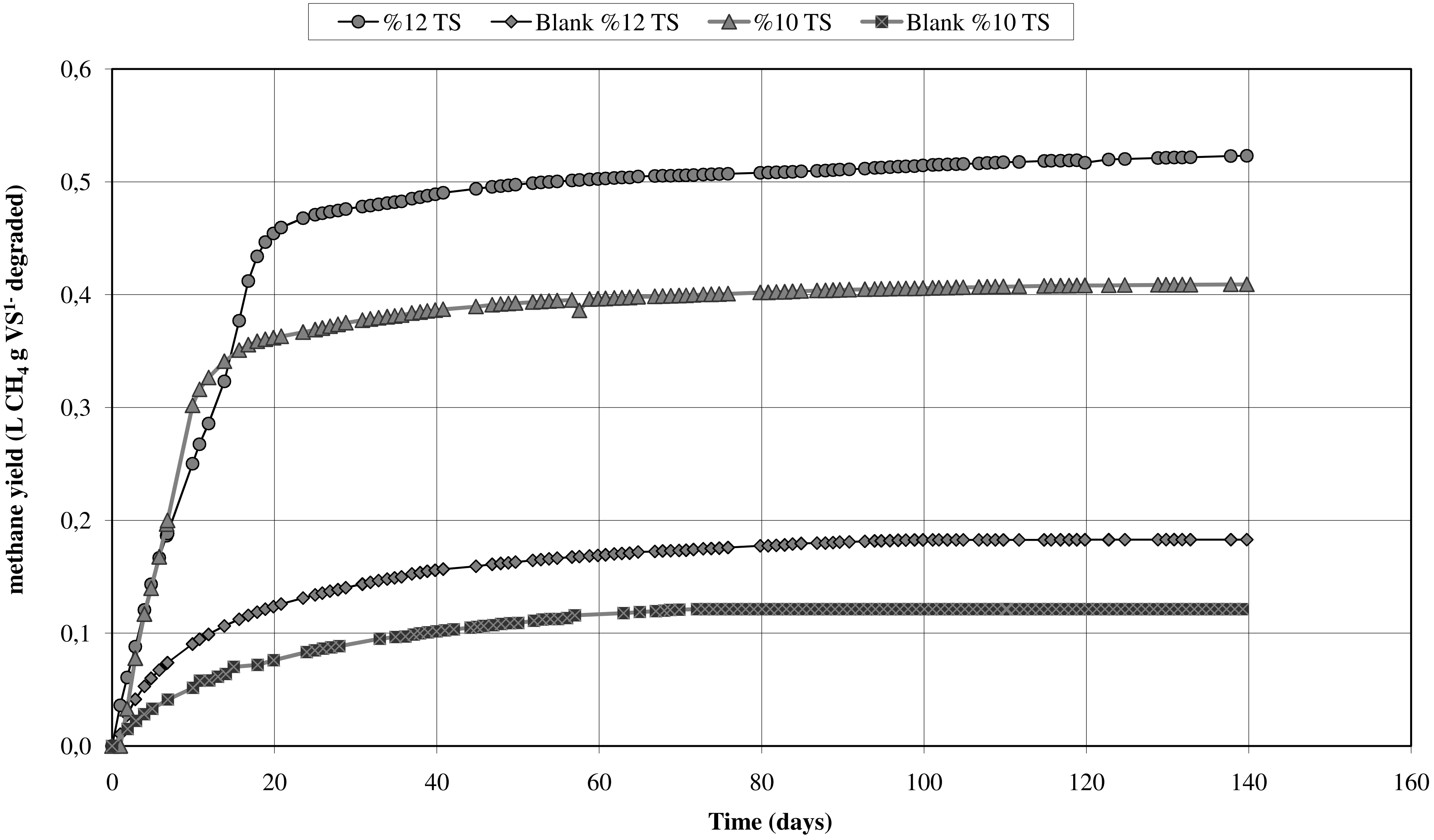


Figure 4.14. Methane Yield Obtained from Anaerobic Digestion of Sugar Beet at the 2nd set-up

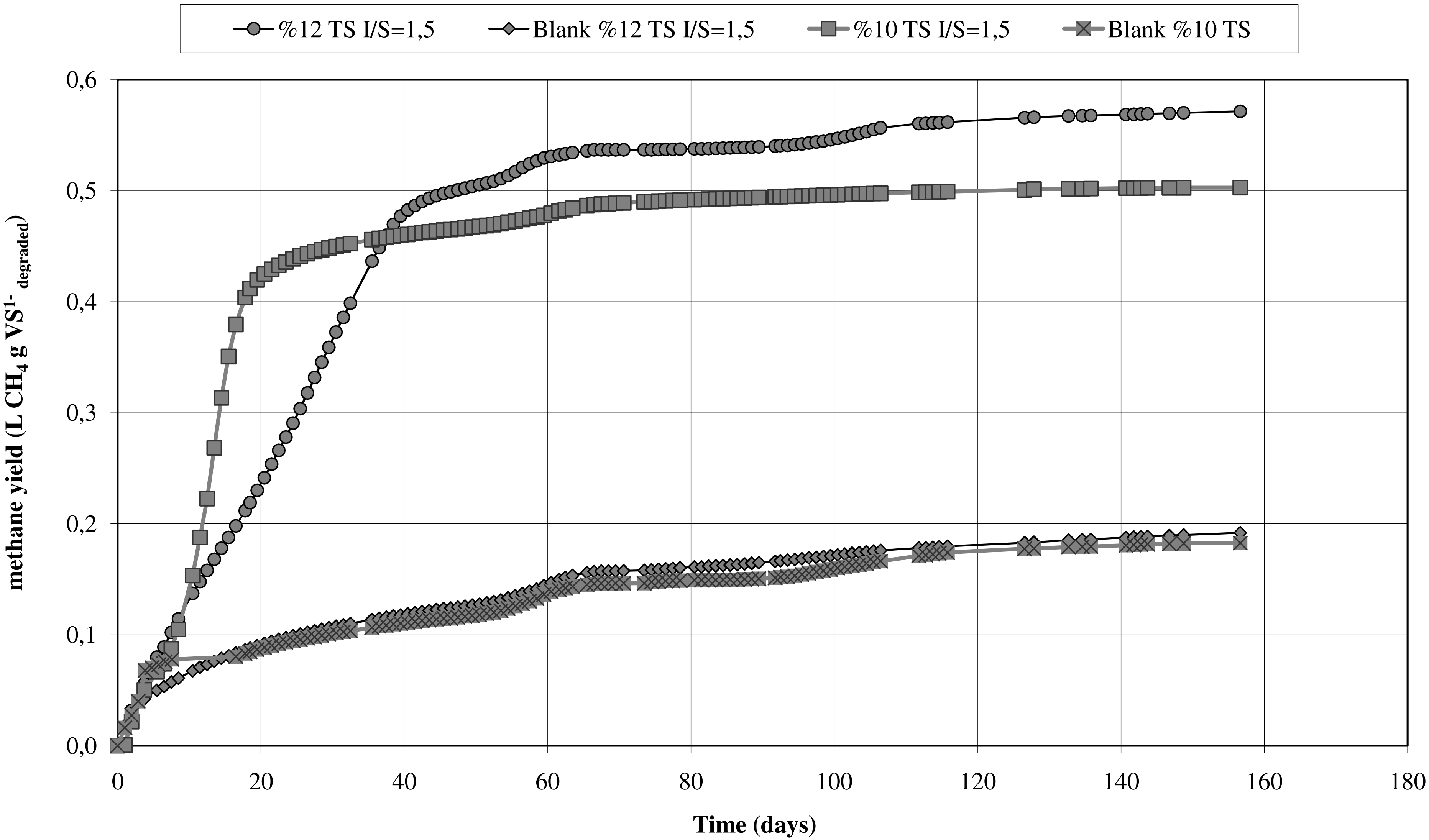


Figure 4.15. Methane Yield Obtained from Anaerobic Digestion of Maize at the 3rd set-up

Maximum methane yield was calculated for each set-up by subtracting the final volume of methane produced by the control from the methane production of each reactors and dividing the difference by the weight of substrate (in VS) which was degraded as a result of anaerobic digestion. A maximum methane yield is especially important with the digestion of energy crops as these have production costs that have to be covered by the methane production. The values obtained were 0.359 L CH_4 g VS $^{-1}{}_{degraded}$ (12 % TS; I/S ratio 1.5; 1st set-up), 0.34 L CH_4 g VS $^{-1}{}_{degraded}$ (12 % TS; I/S ratio 1.5; 2nd set-up), 0.288 L CH_4 g VS $^{-1}{}_{degraded}$ (10 % TS; I/S ratio 1.5; 2nd set-up), 0.376 L CH_4 g VS $^{-1}{}_{degraded}$ (12 % TS; I/S ratio 1.5; 3rd set-up) and 0.318 L CH_4 g VS $^{-1}{}_{degraded}$ (10 % TS; I/S ratio 1.5; 3rd set-up). The highest value corresponded to the TS of 12 % at the second set-up.

Batch experiments were conducted by using digesters having 8 % TS (with I/S ratio of 1.5) within the scope of a study operated by a group of researchers. As a result of this study, the methane yield of 0.32 L CH_4 g VS $^{-1}{}_{degraded}$ was achieved which was lower than the results mentioned above [30].

In addition, methane yield values were also corrected at standard temperature and pressure conditions (STP) to be able to compare these results with further studies in the literature. The highest methane yield of 0.28 L CH_4 g VS $^{-1}{}_{degraded}$ was obtained at the reactor including 12 % TS sugar beet during the last run. This was followed by the digester having the same amount of final solid content at the second run with a value of 0.271 L CH_4 g VS $^{-1}{}_{degraded}$.

When the methane yield values of the reactors with 10 %TS were compared, it was seen that efficiency of the last run was higher than the second run. The findings for the second and the last set-up were; 0.238 L CH_4 g VS $^{-1}{}_{degraded}$ and 0.27 L CH_4 g VS $^{-1}{}_{degraded}$ respectively.

The anaerobic digestion experiments of maize and dairy cattle manure was carried out by considering the harvesting time of the crop and resulted in the methane yields ranging from 0.312-0.365 L CH_4 g VS $^{-1}{}_{degraded}$ to 0.268 – 0.286 L CH_4 g VS $^{-1}{}_{degraded}$ [38]. These values were similar with the methane yields of the maize which were mentioned above.

Comparisons of methane yields reported in the literature cannot be precise because of differences in the feedstock and in the experimental conditions. Methane yields from energy crops can vary depending on their time of harvesting and pretreatment methods. Additionally the digestion process may yield different methane yields depending on whether the whole crop or rejects are used as feedstock. Acclimatization of the inoculum to the feedstock is also important for optimum yields, as well as temperature, solid content and I/S ratio.

4.3. Evaluation of Biogas as an Alternative Energy Source in Turkey

This section covers the investigation of the biogas production system in order to determine whether or not it would be an alternative energy source in Turkey. Since this thesis evaluated the potential of sugar beet and maize to produce biogas with anaerobic granular sludge and developed a system for optimum conditions, it will be appropriate to complete the effort with an investigation of the contribution of biogas produced from sugar beet and maize to the energy requirement of Turkey.

For this purpose, total energy produced for each crop at various TS contents was calculated by considering the statistical values given in the Appendix A and the energy content of methane which was reported to be 35 MJ/m^3 CH_4 in the literature [18]. As a result, about 4.5% of the energy requirement in Turkey would be met by the production of biogas from sugar beet including 12 %TS and I/S ratio of 1.5. The highest contribution would be achieved by the utilization of maize as an energy crop with a value of 7 %.

Consequently, results from this study suggest that sugar beet and maize are potential substrates for the production of biogas and could provide additional benefits to the energy requirement of Turkey. However, these types of feed-stocks which were used during this study have also nutritional value and hereby residues of these crops should also be investigated with regard to their biogas production potential.

5. SUMMARY AND CONCLUSIONS

In this study, biogas production potential of sugar beet and maize were investigated by the anaerobic digestion of these two energy crops with the addition of anaerobically digested sludge. Different concentrations of TS and I/S ratio values were used and the effects of them on methane yield and productivity were also evaluated. For this purpose, three experimental set-ups were conducted in which the reactors were placed in temperature controlled water bath and loaded with different crops at various total solid contents in order to determine the biogas production potential. Related to these stated objectives, the results obtained can be summarized as follows;

1. In order to reach the objectives, the following experiments were performed throughout the study. Mixtures of energy crop and seeding sludge were analyzed for TS, VS, pH, Alkalinity, TKN, TP, VFA, ammonium nitrogen, orthophosphate, COD and TOC. The volume of daily gas production and biogas composition were the other parameters monitored. The experimental results are as follows;

    a) Initial pH values of the mixtures were between 7.23 and 8.4 disregarding the data observed for the reactor having 12% TS with I/S ratio of 1 at the last run. These values were in the range for methanogenic activity given as 7.0 – 8.5 [27] which was a good indicator of a potentially well-balanced anaerobic degradation process. This was attributed to high levels of alkalinity which was provided by the existence of anaerobically digested granular sludge. The pH values of almost all of the reactors exhibited a similar trend through to the end of the study. At the end of the experiments, it was obvious that the pH of the reactors increased except the decline observed for the reactor having I/S ratio of 1 at the last run. Due to the formation of acidic conditions in this reactor, pH of 4.85 was observed and it resulted in cease of gas formation. This may be attributed to the insufficient buffering capacity that would neutralize the VFAs produced by the maize. However, in the other reactors pH values stayed in the 7 – 8.5.

b) Alkalinity was another parameter monitored in the mixtures of energy crop and anaerobic sludge. An increasing trend was observed in the alkalinity findings of the three set-ups. This was due to the decrease in the volatile fatty acid concentrations in the reactors as it was also supported by the formation of ammonium as a result of organic matter depletion. This may also attributed to the existence of CO_2 in the samples which was not completely removed from the reactor as gas. Initial alkalinity concentrations of the reactors were above the desired level (1200 mg L^{-1}) which is necessary for the growth and proceeding of microbial population.

c) At the beginning of the first and the second run, high levels of VFAs were observed owing to the high quantities of soluble and easily degradable organic matter contained in the sugar beet. During all three set-ups VFAs were depleted to the end of the experiment and there was no accumulation of soluble acids which would cause inhibition except from the reactor including 12 % TS with I/S ratio of 1 at the last run. Since the acidic conditions could not be buffered, the system was inhibited and methane production could not be observed in this reactor. However, in the other digesters the conditions for biogas production were proved to be suitable with the help of high pH values and alkalinity concentrations together with low VFAs.

d) During this study, for the reactors including same amount of final solid content (12%) at the first and the second run had different initial TKN and ammonium nitrogen concentrations. This difference was caused by utilization of various sugar beets which were harvested at different period of time of the year. NH_4^+-N concentrations in the three set-ups increased through to the end of the experiments. Rapid decomposition of organic material containing nitrogen resulted in the increasing trend of ammonium nitrogen in all reactors during batch experiments. On the other hand, TKN content of the reactors was reduced at the end of the experiments.

e) Total phosphorus (TP) and orthophosphate (PO_4^{-3}) concentrations were also analyzed in order to investigate the nutrient availability in the batch reactors. First two experimental set-ups were operated by using the same TS content (12 % TS) and the results showed that the digester conducted at the second run had higher initial orthophosphate concentration than the one which was studied during the first run. Results of ammonium nitrogen, TKN and TP concentrations indicated that the parameters analyzed in the same substrate may vary depending on the harvesting time of the crop. It was also observed that total phosphorus and orthophosphate concentrations in the reactors were depleted through to the end of the experiments as a result of biological utilization. The highest depletion of ortophosphate was observed for the reactor having 12 % TS content with I/S ratio of 1.5 during the last set-up. On the other hand, the lowest consumption was detected for the reactor which includes the same amount of TS but with I/S ratio of 1 owing to the inhibition of the system occurred at the 37th day of the experiment.

f) Organic matter contained in the batch reactors are mostly expressed as VS for the anaerobic degradation processes which are carried out by using energy crops. However, COD was also determined during this study in order to have more information on the batch digestion of sugar beet and maize which was carried out under anaerobic conditions. Similar with the results of orthophosphate, ammonium nitrogen, TKN and TP, initial COD concentrations of the reactors having 12 % TS content were different in the first and second set-up. According to these findings, the reactor including 12 % TS at the second setup was predicted that it would be the most promising reactor in terms of methane production. However, anaerobic degradation of the reactor with 12% TS content during the last run produced much more biogas than the reactors studied at the previous two set-ups despite of its very low COD content. This indicated that COD should be considered together with the other parameters while evaluating the performance of a digestion system with regard to biogas production. COD concentrations of almost all of the reactors followed a similar depletion trend as a result of biological decomposition of organic matter. The highest degradation (88 %) was observed in the second run conducted with sugar beet and sludge mixture having final TS content of 12 %. On the other hand, the reactor which had a final solid content of 12 % in the last run showed the lowest rate of 19,65 % due to the inhibition of the system.

g) Total organic carbon was also analyzed in order to evaluate the efficiency of the anaerobic degradation process in this study. Initial TOC concentration of the reactor containing 12 % TS at the last run was the highest with a value of 19960 mg L^{-1}. At the end of the experiments, degradation of organic carbon was observed in all reactors disregarding the one having 12 % TS which was used at the last run. The digester which was conducted at this run by using I/S ratio of 1 and having a final solid content of 12 % failed due to the accumulation of VFAs in the reactor. The efficiency of the systems with regard to TOC degradation was in the range of 55 – 95.7 %.

h) When daily gas production values of the reactors were compared, it was seen that the maximum values were observed between the 0-20th days of the first and the second experimental set-ups. In addition, the highest daily gas production values for the reactors having 12 % TS was in the range of 4000 – 4500 mL at the first and the second run respectively. Unexpectedly, monitored daily gas production was 6377 mL in the reactor including 10 % TS sugar beet and sludge mixture which was much higher than the values achieved by reactors containing 12 % TS. Through to the end of the experiments, a sharp increase of gas production was detected in the control reactor (12 % TS; 2nd set-up) due to presence of oxygen which entered accidentally to the system while sampling. The last set-up was also evaluated with regard to its daily gas production rates and the highest value of this run, which was 3573 mL, was observed for the reactor with a final solid content of 10 %. The lowest value was monitored for the mixture which had 12 % final solid content with I/S ratio of 1 and it was ceased after 37th days due to the inhibition of the system.

i) The cumulative biogas volumes produced after 140-180 days of digestion time at three different set-ups were in the range of 5225 – 35262 mL. The lowest value was monitored during the anaerobic digestion of maize and sludge with a final TS content of 12 % and I/S ratio of 1. The highest production was obtained at the same run, in the digester including same amount of solid content but having I/S ratio of 1.5.

j) Gas composition was also monitored in the generated gas. The data showed that all reactors except from the one with 12 % TS and I/S ratio of 1 were found to be in the methane formation stage because the first readings were above 40 % for these reactors, in a range which was typical for methane formation phase. The reactor which digested sugar beet and sludge mixture at I/S ratio of 1 and with TS content of 12 % could not produce methane due to the inhibition which occurred in the reactor. However, in the other digesters a rapid methane generation was observed owing to the high humidity and organic matter content of the substrate. The average methane contents monitored throughout the first and the second runs were 59-63% (12 % TS reactors). The average methane content values of the digesters during the last run were 59.5 % for the reactor including 10 % TS with I/S ratio of 1.5 and 55.9 % for the batch digester having 12 % TS with I/S ratio of 1.5. Similar to previous two set-ups, methane production in the reactors which were operated at the last run was observed at the beginning of the experiments due to high organic matter content which was expressed as VS.

k) Biogas yield and methane yield values were also evaluated to determine the performance of the anaerobic digestion experiments. Results obtained at the end of the three set-ups confirmed that the biogas yield values were nearly same in the digesters having 12 % TS with I/S ratio of 1.5. The data were in the range of 0.61 – 0.622 L g VS $^{-1}{}_{\text{degraded}}$. Biogas yield calculated for 10 % TS reactor during the last run was 0.526 L g VS $^{-1}{}_{\text{degraded}}$ which was higher than the second set-up. The lowest value was obtained during the last run in the reactor having 12 % TS content with I/S ratio of 1.

The highest methane yield of 0.376 L CH_4 g VS $^{-1}{}_{\text{degraded}}$ was achieved at the reactor including 12 % TS sugar beet with I/S ratio of 1.5 during 3rd set-up. The lowest value was obtained as a result of the digestion of sugar beet and sludge mixture at a ratio of 1.5 and having a final solid content of 10 % at the second run. In addition, biogas yield and methane yield values were also corrected at standard temperature and pressure conditions (STP) to be able to compare these results with further studies in the literature.

2. Based upon experimental results obtained during the investigation, the following conclusions are provided.

    a) Sugar beet and maize were found to be suitable energy crops for biogas production through the process of anaerobic degradation. Batch digestion of these two types of substrates resulted in methane production which is an indicator of a successful system. When effectiveness of all reactors was compared according to experimental results taken throughout the study, it was noticed that the reactor including 12 % TS (I/S ratio 1.5) during the last run was the most successful one with regard to its cumulative biogas production, biogas yield and methane yield. Additionally, the efficiency of the system was reproved by the depletion of phosphate, TKN and TP. The lowest VFA values which were detected in the reactors studied during this set-up was also another indicator of a successful system.

    b) The difference in some parameters such as COD, phosphate and ammonium were observed in the digesters including same total solid content of sugar beet and sludge mixture (12 %). This was attributed to the difference in type and harvesting time of the crop. Therefore, it was decided that prior to determination of the most suitable energy crop for biogas production, each plant should be investigated for its biogas production potential by using various types which will be collected at different period of time of the year.

    c) According to the results of COD concentrations monitored in the reactors, it was detected that the initial COD content and the depletion rate of the digester is not an indicator of a successful system by itself. Although the batch reactor including 12 % TS at the second run had the highest COD depletion rate, it was not the most effective system with regard to biogas yield and methane yield values.

d) Utilization of anaerobic granular sludge as a seeding material resulted in high levels of alkalinity which provided suitable conditions for degradation process with its high buffering capacity. However, optimum ratio between the substrate and the seeding material (I/S ratio) should be maintained in order to prevent inhibition.

6. RECOMMENDATIONS

In order to determine the most suitable energy crop for biogas production, further investigations should be performed. Since the performance of the system improved with increasing TS content of the mixture in this study, reactors including solid contents higher than 12 % are recommended to be studied. Additionally, effects of various inoculums to substrate ratios on the biogas production potential of the reactors should be evaluated to determine the optimum conditions at which the highest biogas production rate is observed. Various harvesting time, temperature ranges and pretreatment methods (silaging) are the other parameters which should be studied for each type of energy crop. Finally, investigation of anaerobic digestion potential of crop residues is recommended since energy crops which were studied during this project has also nutritional value.

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APPENDIX A

Sample calculation for correction of the biogas yield and methane yield values at standard temperature and pressure

$$\text{B} = A * \frac{273.15}{T + 273.15} * \left(1 - \frac{p}{101.325}\right)$$

where;

A = biogas yield and methane yield values calculated according to results of experiments

B = the values corrected at standard conditions

T = temperature at which the reactors were operated (37 °C)

P = water vapor partial pressure at 37 °C = 6.27 kPa

APPENDIX B

Loading Conditions of the reactor including 12% TS with ISR of 1.5 at the second set-up – a sample calculation for a reactor loading

Total solid content of sugar beet: 16%

Total solid content of sludge: 13%

Total solid content of the mixture: 12%

Weight of the crop which was added to the reactor $= \left[\frac{800ml*12*100}{(1.5+1)*16*100}\right]$

$= 240$ g

Weight of the sludge which was added to the reactor $= \left[\frac{800*\left(12-\frac{12}{1.5+1}\right)*100}{13*100}\right]$

$= 443$ g

Weight of the water $= \left[\frac{800}{100}*\left(100-\left(100*\left[\left(\frac{12}{16*(1.5+1)}\right)+\frac{12-\frac{12}{1.5+1}}{13}\right]\right)\right)\right]$

Weight of the water $= 116.92$ g