EFFECT OF TRACE ELEMENTS ON BIOGAS PRODUCTION: MAIZE SILAGE

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ABSTRACT

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In the last decade, the energy requirement in the world has been increasing drastically. Turkey has been the second country in respect of natural gas and electricity requirement growth after China. The growing energy demand has resulted in dependency on energy imports in Turkey. In order to decrease the energy import, alternative energy sources should be developed. Biogas is a type of renewable energy that is generated by using organic matter like food waste, animal waste and energy crops. Biomass capacity is very high in Turkey, and biogas, as an alternative energy source, has a very grand potential to contribute to meet the energy demand in Turkey. The purpose of this experimental work was to analyze the effect of trace elements on biogas generation from maize silage as substrate. Co, Ni and Mo were selected as trace elements, and these elements were added to the reactors separately or in combination at varied doses. The biogas volume, biogas composition, biogas yield and methane yield were monitored in order to investigate the impact of trace element supplementation. Ni and Co were put to the reactors at a rate of 0.1 mg/L and 0.5 mg/L, respectively. The concentration to be added to the reactors for Mo were selected as 0.05 mg/L and 0.25 mg/L. As a result of the experiments, the trace element addition by Ni, Co and Mo increased the biogas volume, biogas composition, biogas yields and methane yields for anaerobic digestion of maize silage.

ÖZET

ESER ELEMENTLERİN BİYOGAZ ÜRETİM ÜZERİNDEKİ ETKİSİ: MISIR SİLAJI

Son on yıllık zaman zarfında, dünyada enerji ihtiyacı şiddetli bir şekilde atmaktadır. Türkiye Çin'den sonra doğalgaz ve elektrik ihtiyacı artışı konusunda ikinci sıradaki ülkedir. Türkiye'de bu büyüyen enerji ihtiyacı enerji ithaline bağımlı hale getirmiştir. Bu enerji ithalini azaltmak için alternatif enerji kaynakları geliştirilmelidir. Biyogaz gıda atığı, hayvan atığı ve enerji bitkileri gibi organik maddeler kullanılarak üretilen bir alternatif enerji çeşididir. Türkiye'de biyokütle kapasitesi oldukça yüksektir ve biyogaz alternatif bir enerji kaynağı olarak Türkiye'de ki enerji ihtiyacını karşılamak için büyük bir potansiyele sahiptir. Bu projenin amacı eser elementlerin mısır silajından biyogaz üretimi üzerine etkilerini belirlemektir. Eser element olarak Co, Ni ve Mo seçilmiştir ve reaktörlere farklı dozlarda tek tek ya da birlikte eklenmiştir. Eklenen eser elementlerin etkisini belirlemek için biyogaz hacmi, biyogaz kompozisyonu, biyogaz verimi ve metan verimi ölçülmüştür. Ni ve Co reaktörlere sırasıyla 0.1 mg/L ve 0.5 mg/L miktarlarında eklenmiştir. Reaktörlere eklenen Mo konsantrasyonu ise 0.05 mg/L ve 0.25 mg/L olarak seçilmiştir. Çalışma sonuçlarına göre; eser element eklenmesi (Ni, Co ve Mo) mısır silajının anaerobik çürütülmesinde biyogaz hacmini, biyogaz kompozisyonunu, biyogaz verimini ve metan verimini arttırmıştır.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	**Explanation**
Fe	Iron
Al	Aluminum
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Cu	Copper
Hg	Mercury
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Ni	Nickel
P	Phosphorus
Pb	Lead
S	Sulphur
Zn	Zinc

Abbreviation	**Explanation**	**Unit**
AD	Anaerobic Digestion	
COD	Chemical Oxygen Demand	mg O_2/L
DEC	Dedicated Energy Crops	
DM	Dry Matter	
FID	Flame Ionization Detector	
HRT	Hydraulic Retention Time	Hour
ICP-MS	Inductively Coupled Plasma Mass Spectrometry	
MGC	Milligascounter	
OLR	Organic Loading Rate	
ORP	Oxidation Reduction Potential	mV
SRT	Sludge Retention Time	Hour
TAC	Total Alkaline Carbonate	

TCD	Thermal Conductivity Detector	
TKN	Total Kjeldahl Nitrogen	g/kg
TOC	Total Organic Carbon	
TP	Total Phosphorus	mg/L
TS	Total Solids	%
TSS	Total Suspended Solids	g/kg
TVA	Total Volatile Acids-	
VFA	Volatile Fatty Acids	mg/L
VS	Volatile Solids	%
VSS	Volatile Suspended Solids	

1. INTRODUCTION

Energy is one of the most important and vital problem all over the world in the 21th century. As a result of this, energy prices are increasing continuously. In addition to the rising energy prices, fossil energy sources are also limited, and when these energy sources are depleted, the best alternative is renewable energy sources like solar energy, biomass or geothermal energy (Deublein and Steinhauser, 2008).

Many countries have prepared their own scenarios and projections about energy consumption. In Sweden, the government aimed at reducing emissions from city busses. For this reason, a biogas plant in Linköping is established to use produced methane as fuel for city busses instead of diesel. Also, in Denmark, the government planned to provide their energy requirement from renewable energy sources, and finish their fossil-fuel dependency by 2020 (Moestedt et al., 2016; Boldrin et al., 2016; Hagos et al., 2017). However, choosing the type of renewable energy is very important. Because, some of renewable energy sources like hydro energy are limited sources, so they could not meet the increasing energy requirement of the world (Budzianowski and Postawa, 2017).

Today, other problem of the society is the removal and reduction of wastes. Organic wastes are being produced at an increasing rate, and the elimination of such wastes is one of the most significant environmental problems. Not only sustainable waste management but also waste prevention and reduction have become priorities in many countries all over the world. According to the sustainable waste management practices, uncontrolled waste dumping is not an acceptable method to dispose of wastes. Since recycling of nutrients and organic wastes and energy recovery cannot be provided by sanitary landfill or incineration, these techniques cannot be considered as the optimum methods for disposal of organic wastes (Deublein and Steinhauser, 2008; Rutz et al., 2008).

Anaerobic digestion is a multi-stage biological technology during complex organic matters are converted to methane-containing biogas. Digestible organic wastes, animal manure and slurries are converted into renewable energy through anaerobic digestion (Rutz et al., 2008; Sun et al., 2016).

Anaerobic digestion is a mostly utilized technique to generate energy, especially in Asian countries. Actually, studies about anaerobic digestion process were started in 18th century. In 1776, the relationship between gas generation and organic loading was discovered by Alessandro Volta. In

1804-1808, methane gas, which is a combustible gas produced as a result of anaerobic digestion process, was found out by John Dalton and Humphrey Davy (Neshat et al., 2017).

Anaerobic digestion has many advantages. Thanks to anaerobic digestion process, not only energy supply problem can be solved but also greenhouse gases problem can also be solved. Climate change problem occurs because of anthropogenic greenhouse gas emissions. Along with the utilization of renewable energy resources substituted for fossil-based energy sources, it is possible to decrease the greenhouse gas emissions. In 2015, the requirement of clean energy production and decarbonisation were explained by the European Union ‘Framework Strategy for a Resilient Energy Union with a Forward-Looking Climate Change Policy’ (Sun et al., 2016; Munk and Lebuhn, 2014; Martin et al., 2017; Kakuk et al., 2017; Budzianowski and Postawa, 2017).

Biogas holds upper hand against the other renewable energy sources like solar energy and wind energy in terms of continuity, since wind and solar energy are dependent on weather conditions. So, they are discontinuous and fluctuating renewable energy sources (Terboven et al., 2017).

In addition, another advantages of biogas production is the production of carbon dioxide. Carbon dioxide can be utilized in lots of sectors like medicine industry, food industry, chemical industry etc. The utilization of the by-product of biogas production process also gained advantage materially (Kalinichenko et al., 2016).

Biogas production process also provides a natural fertilizer (digestate) for cultivation. In addition, the organic fraction from the overall waste streams is removed by anaerobic digestion process. In this way, the biochemical stability of landfill sites and the effectiveness of energy conversion by incineration of the residual wastes are increased (Moestedt et al., 2016; Bharathiraja et al., 2016; Rutz et al., 2008).

Biogas usage has also many environmental advantages. For example, soil erosion and deforestation could be decreased by biogas usage (Bozym et al., 2015).

The content of generated biogas is dependent on the properties of the substrate. A wide range of materials such as municipal solid waste, fruit and vegetable waste, industrial waste, animal waste, energy crops and residues of them could be utilized as substrate for biogas generation. In EU, many studies were conducted about the type of biogas substrates. According to these studies, maize silage was selected as one of the most efficient substrates for biogas generation (Senghor et al., 2017; Kalinichenko et al., 2016).

However, biogas production process, which occurs via microorganisms, is affected from many factors such as environmental and operational conditions, since it is a complex and sensitive process (Hagos et al., 2017).

In addition to many parameters such as environmental conditions and operational conditions, substrate characteristic also plays an important role on anaerobic digestion process. Some methods such as trace element addition are also being used to enhance biogas production process. Trace element addition has beneficial impacts on biogas generation. However, the effect of trace element addition is not always obvious as a result of the complexity of biogas production process, Also the optimum doses required and the impacts of trace elements have not been understood entirely. It is very essential to specify the optimum doses and combinations to benefit the positive effects of trace elements addition for biogas production (Cai et al., 2018; Choong et al., 2016). Ni, Co and Mo are one of the most important microelements for biogas production process.

The main objective of this experiment is to analyze the impacts of Ni, Co and Mo on biogas production from maize silage. For this purpose, anaerobic digestion experiments were performed under mesophilic temperature by using batch reactors containing maize silage and anaerobic seed sludge. Reactors having different trace elements concentrations were filled with maize silage and anaerobic sludge. Also control reactors without any trace element addition were operated.

The results obtained from reactors with trace element supplementation were compared to the results obtained from control reactors and, the effect of trace element on biogas production from maize silage was determined.

2. THEORETICAL BACKGROUND

2.1. Fundamentals of Anaerobic Digestion Process

Anaerobic digestion process is the decomposition of the organic matter in the absence of free oxygen by a mixed culture of microorganisms. Anaerobic digestion process is prevalent in lots of natural environments, for instance; the peat bogs or the ruminant stomachs. Anaerobic digestion is largely used today to produce renewable energy. Digesters, which are airproof tanks, are used as reactors. Biogas and digestate are the two major outputs. Biogas is a gas which is a combination of methane, carbon dioxide, and trace amount of other gases. Digestate is the decomposed substrate including micro and macro nutrients, so it is an appropriate substance to be used as bio-fertilizer for agricultural purpose (Rutz et al., 2008; Bharathiraja et al., 2016).

In 1895, the biogas production via a biological system was documented for the first time in United Kingdom. From then on, the anaerobic digestion process was improved and used to treat wastewater and stabilize sludge. Because of the energy crisis in the early ‘70s and later due to global climate change, the attention of the society to the utilization of renewable energy sources greatly increased. Global efforts of replacing the fossil fuels with renewable energy sources energy generation and the requirement of finding environmentally sustainable methods for the treatment of animal manure and organic wastes have increased the attention in biogas technology today (Metcalf and Eddy, 1979; Rutz et al., 2008).

Today, some of the most significant implementations of anaerobic digestion are biogas production and usage of agricultural-based substrates. In some Asian countries such as China, India, and Vietnam, energy production is supplied by millions of small scale reactors. These small scale digesters are family owned and they are used to supply daily life necessities, such as heating, cooking and lighting. In addition, they are utilizing simple technologies, which are practical to install and operate. For instance; the number of household biogas reactors were up to 18 million in 2006 in China, and it was considered that Chinese has 145 billion cubic meters of biogas potential. Figure 2.1 shows the growth of biogas plants in China. At present, about 5 million small-scale biogas digesters are also in running in India (Deublein and Steinhauser, 2008; Rutz et al., 2008).

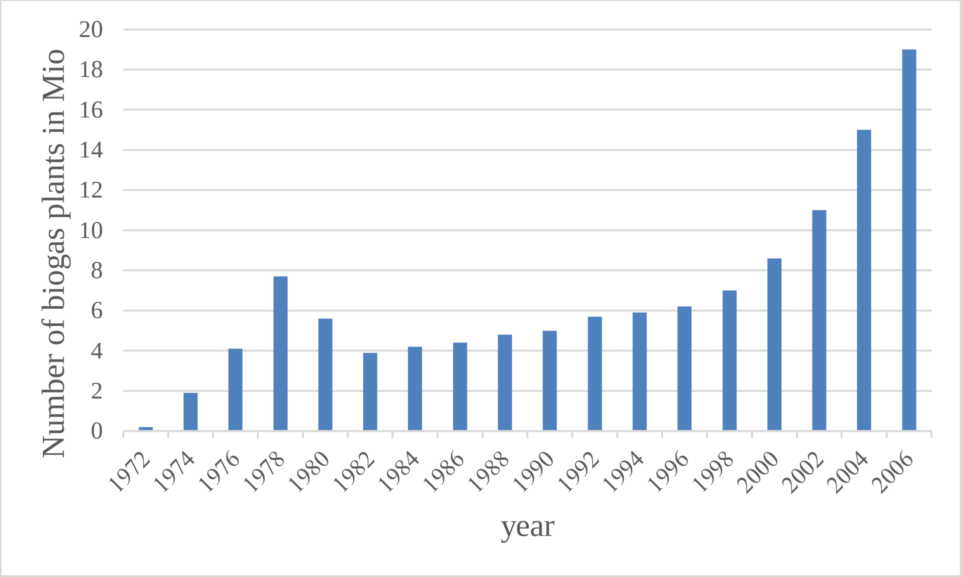

Figure 2.1. Growth of biogas plants in China (Deublein and Steinhauser, 2008).

In Europe and North America, there are thousands of active agricultural biogas facilities and, the number of biogas plant is continuously increasing. In addition, the most advanced technologies are used in the most of these biogas plants. The technical outrunners can be listed as Germany, Austria, Denmark and Sweden. The number and total amount of digested matter of large fermentation and co-fermentation biogas facilities of organic waste are shown in Table 2.1. Biogas plant inventory in Germany in 2015 is given in Table 2.2. In 2000, the law of “Renewable Energies” became efficient in Germany. This law stated the rules for the subsidization of the power supplied by the biogas plants. In 2005, a biogas facility was built to feed 10 m^3/h crude biogas (giving to 6 m^3/h clean biogas) into the natural gas network; (equal to 400 MWh/a^-) in Austria. It was reported that in 2007, more than 3700 agricultural biogas facilities were in operation in Germany (Deublein and Steinhauser, 2008). According to the Figure 2.2, 17.2% of the produced renewable-based electricity is generated from biogas in Germany in 2016 (Liebetrau et al., 2017). Figure 2.3 shows the expansion of biogas production in Germany (Simet, 2016). Besides, USA, Canada and Latin American countries are investigating developed biogas technologies (Rutz et al., 2008).

Table 2.1. Number and total amount of digested matter of large fermentation and co-fermentation biogas facilities of organic waste (>2500mg/a) in Europe in 1997 (Deublein and Steinhauser, 2008).

	Number of biogas plants	Mg of digested waste per year
Austria	10	90000
Belgium	2	47000
Denmark	22	1396000
Finland	1	15000
France	1	85000
Germany	39	1081700
Italy	6	772000
Netherlands	4	122000
Poland	1	50000
Spain	1	113500
Sweden	9	341000
Switzerland	10	76500
England	1	40000
Ukraine	1	12000
Total	108	4241700

Table 2.2. Biogas facility inventory in Germany in 2015 (https://mediathek.fnr.de/media/downloadable/files/samples/b/r/brosch.biogas-2013-en-web-pdf.pdf).

Facility Type	**Number of Facilities**	**Electricity Generation [GWh_{el}/annual]**	**Heat Generation [GWh_{th}/annual]**
Sewage Sludge	1,252	1,389	2,022
Organic waste	333	1,294	551
Agriculture	8,000	31,097	13,225
Biomethane	187	2,599	2,924
Landfills	440	396	129
Total	**10,212**	**36,775**	**18,851**

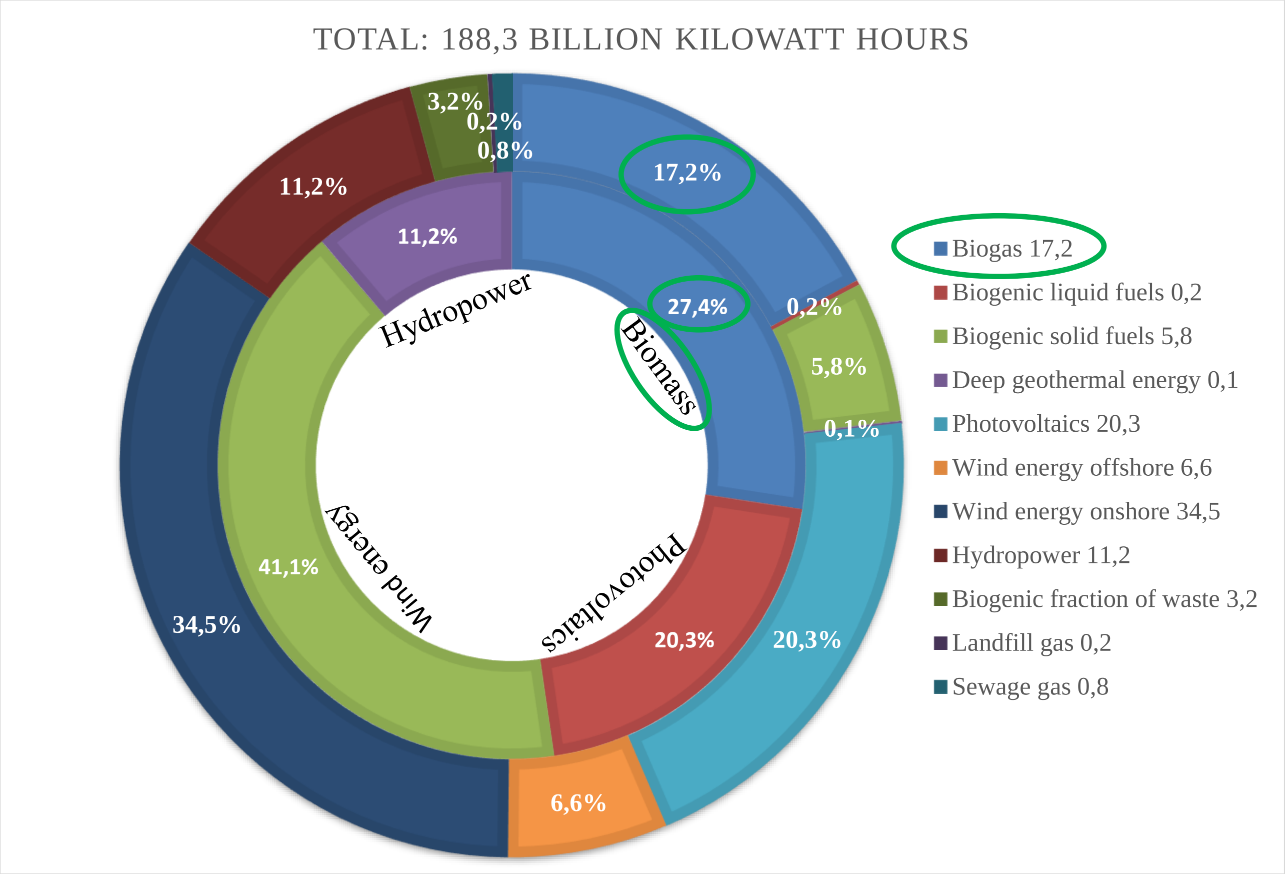

Figure 2.2. Renewables-based electricity generation in Germany in 2016 (https://mediathek.fnr.de/media/downloadable/files/samples/b/r/brosch.biogas-2013-en-web-pdf.pdf).

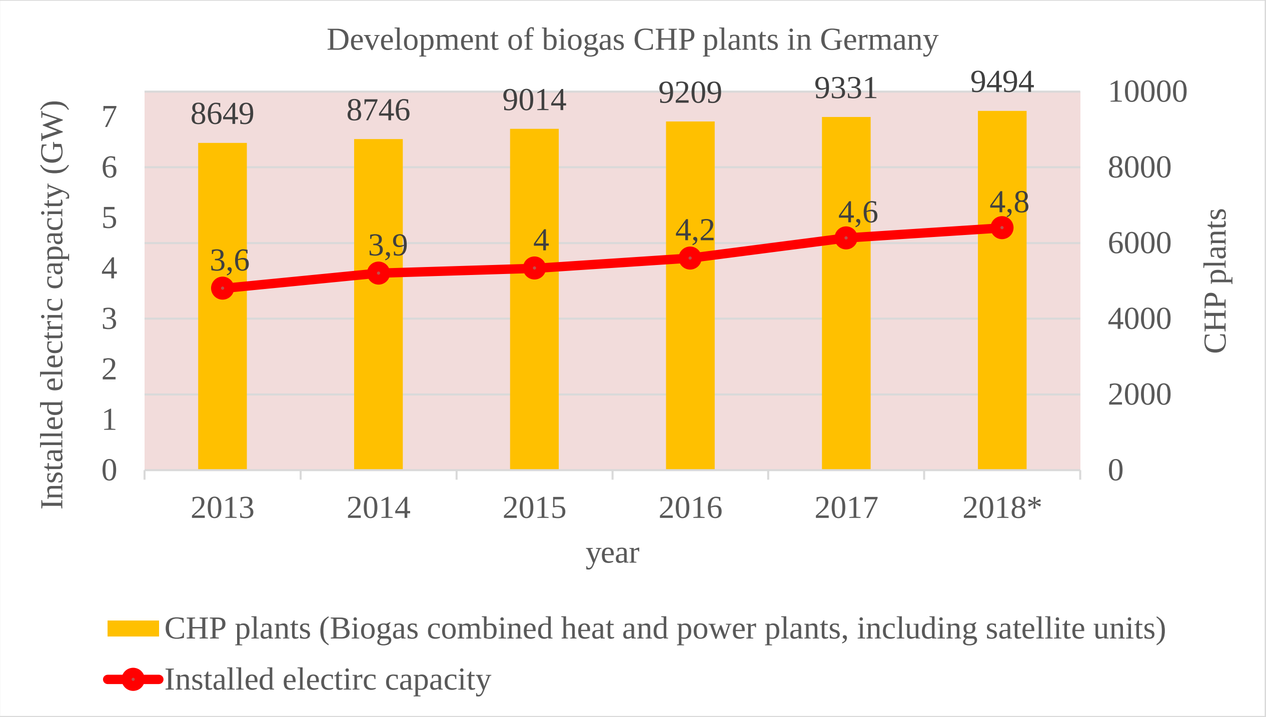

Figure 2.3. Expansion of biogas production in Germany (http://www.fnr.de/fileadmin/allgemein/pdf/broschueren/broschuere_basisdaten_bioenergie_2018_engl_neu.pdf).

Important researches about anaerobic digestion technologies are performed around the world to enhance digestion technologies, process stability and performance. New anaerobic digestion technologies, new digesters, feeding systems, new combination of anaerobic digestion substrates, storage plants, and other equipment are consistently developed and tested (Rutz et al., 2008).

Biogas, which composed of primarily of methane and carbon dioxide, is a low-cost and CO_2-neutral source of clean and renewable energy. Therefore, biogas is a significant priority of the European transportation and energy policy. Biogas also has many socio-economic advantages for the society as well as for the involved stakeholders. General properties of biogas are listed in Table 2.3 (Deublein and Steinhauser, 2008; Rutz et al., 2008; Hagos et al., 2017).

Table 2.3. General properties of biogas (Deublein and Steinhauser, 2008).

Composition	55-70% methane (CH_4) 30-45% carbon dioxide (CO_2) Traces of other gases
Energy content	6.0-6.5 kWh/m^3
Fuel equivalent	0.60-0.65 L oil/m^3 biogas
Explosion limits	6-12% biogas in air
Ignition temperature	650-750 ^{o}C (with the above mentioned methane content)
Critical pressure	75-89 bar
Critical temperature	-82.5 ^{o}C
Normal density	1.2 kg/m^3
Smell	Bad eggs (the smell of desulfurized biogas is hardly noticeable)
Molar Mass	16.043 kg/kmol

For effective biogas production, there are some requirements, such as; optimum feedstock composition, optimum nutrient quantity and composition and optimum mixing (Bozym et al., 2015).

Substrate composition is a significant criterion for anaerobic digestion process, because, it has an impact on the viscosity of the process liquids. Mixing problems, breakdown of stirrers or foaming could occur because of the viscosity of process liquids (Moestedt et al., 2016).

Co-digestion is a process when the substrate consist of a homogenous mix of two or more kinds of feedstock types such as animal slurries and organic wastes, and this is a very common method commonly used today. Many different types of wastes can be utilized as substrate for anaerobic digestion process. The most common substrates are summarized in Table 2.4. (Deublein and Steinhauser, 2008; Rutz et al., 2008)

Dedicated energy crops (DEC), which is a new type of substrate for anaerobic digestion, is grown specially for producing energy. They can be herbaceous such as grass or maize, also woody crops such as willow or oak (Rutz et al., 2008).

Table 2.4. Biowastes, suitable for biological treatment (Rutz et al., 2008).

Waste Code	Waste description	
02 00 00[1]	Waste from agriculture, horticulture, aquaculture, forestry, hunting and fishing, food preparation and processing	Waste from agriculture, horticulture, aquaculture, forestry, hunting and fishing Waste from the preparation and processing of meat, fish and other foods of animal origin Wastes from the fruit, vegetables, cereals, edible oils, cocoa, tea and tobacco preparation and processing: conserve production; yeast and yeast extract production, molasses preparation and fermentation Wastes from sugar processing Wastes from the dairy products industry Wastes from the baking and confectionery industry Wastes from the production of alcoholic and non-alcoholic beverages (except coffee, tea and cocoa)
03 00 00	Wastes form wood processing and the production of panels and furniture, pulp, paper and cardboard	Wastes from wood processing and the production of panels and furniture Wastes from pulp, paper and cardboard production and processing
04 00 00	Waste from the leather, fur and textile industries	Wastes from the leather and fur industry Wastes from the textile industry
15 00 00	Waste packing; absorbents, wiping cloths, filter materials and protective clothing not otherwise specified	Packaging (including separately collected municipal packaging waste)
19 00 00	Waste from waste management facilities, off-site waste water treatment plants and the preparation of water intended for human consumption and water for industrial use	Wastes from anaerobic treatment of waste Wastes from waste water treatment plants not otherwise specified Wastes from the preparation of water intended for human consumption or water for industrial use
20 00 00	Municipal wastes (household waste and similar commercial, industrial and institutional wastes) including separately collected fractions	Separately collected fractions (except 15 01) Garden and park wastes (including cemetery waste) Other municipal wastes

[1] The six-digit code identifies the correspondent entry in the European Waste Catalogue (EWC) adopted by the European Commissions.

Some important factors should be thought carefully while substrate is selected for the anaerobic digestion process. These factors are listed below (Deublein and Steinhauser, 2008);

- According to the determined anaerobic process, the content of the substrate should be proper,
- The substrate which have a maximum nutritional value should be selected,
- There should be no pathogenic microorganism in the substrate for anaerobic digestion process,
- The substrate should include harmful substances and trash as low as possible for a successful and effective anaerobic digestion process,
- For the further processes, the biogas composition should be -proper,
- The composition of the fermentation residue should be usable.

Some features of the substrate such as origin, and dry matter (DM) content play a significant role on the selection of the substrates for AD. For wet digestion process, if the DM content of substrates are lower than 20 %, they can be considered as suitable materials. Manure and animal slurries along with several wet bio wastes from food industries can be considered as the examples of this category. If the DM content of the substrate is as high as 35%, such substrates are used for dry digestion, and silages or energy crops can be considered as the example of such substrates. The DM, sugar, lipid and protein content of substrates play an important role on the choosing of types and quantities of feedstock for the mixture of AD substrate (Rutz et al., 2008).

Substrates which have high amounts of lignin, cellulose and hemicelluloses are suitable for co-digestion. However, a pre-treatment is necessary to enhance digestibility of the substrates (Rutz et al., 2008).

The methane yield of the substrate for anaerobic digestion is one of the most significant criteria when the substrate is chosen for anaerobic digestion process. Benchmarks for specific methane yields are shown in Figure 2.4. According to the Figure 2.4, the methane yield of animal manure is quite low. For this reason, animal manure is added to other co-substrates which have high methane yields. Oily remains from food or feed production processes, alcohol wastes, from brewery and sugar production processes, particularly cultivated energy crops are common co-substrates. In addition, flotation sludge, used cooking oil, and raw glycerin are the substrates which have the highest methane yields. Corn silage also has a high methane yield (Rutz et al., 2008).

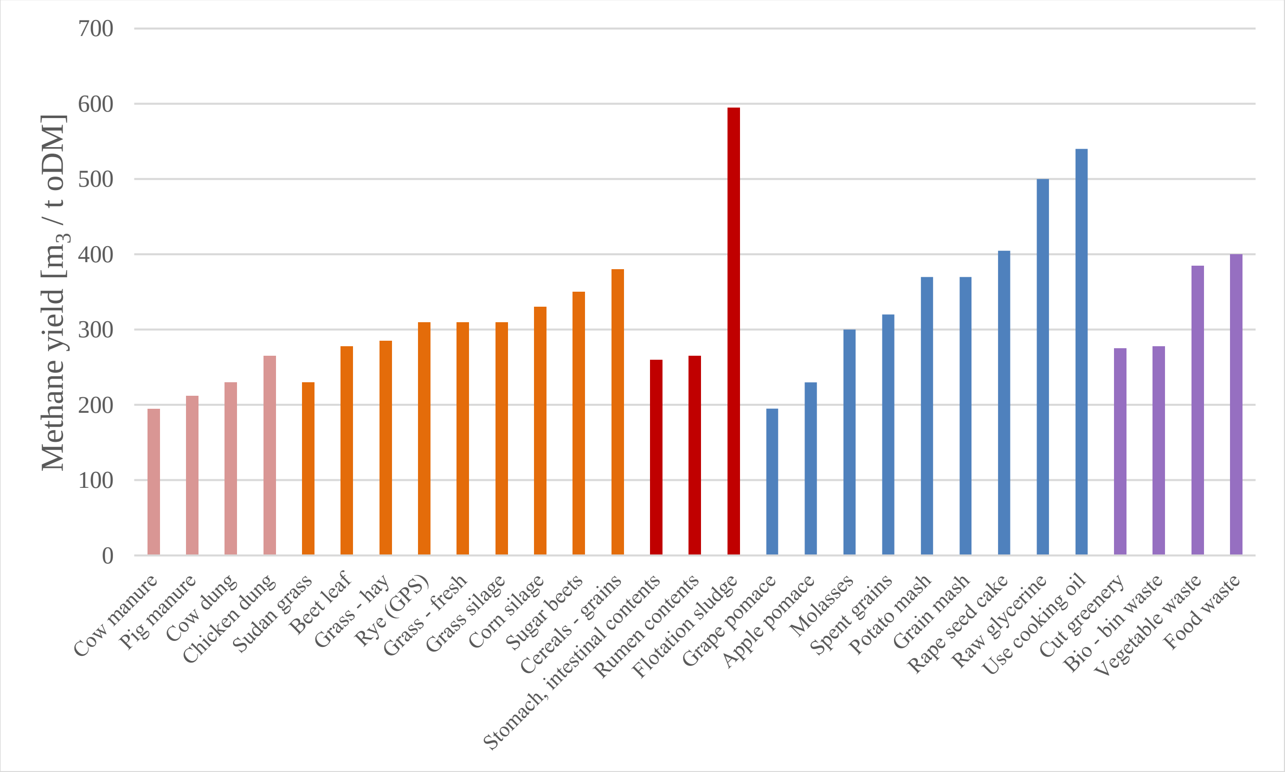

Figure 2.4. Benchmarks for specific methane yields (Rutz et al., 2008).

The safety of anaerobic digestion process is an important issue. The substrate for anaerobic digestion may include chemical, physical and biological pollutants, so the substrates should be controlled to provide a safe anaerobic digestion process. The possible contaminants for anaerobic digestion substrates are given in Table 2.5. According to the Table 2.5, if the wastes of animal origin are used for anaerobic digestion, it requires special attention (Rutz et al., 2008).

Table 2.5. The possible contaminants for anaerobic digestion substrates (Rutz et al., 2008).

		Risk			
		Safe	Hygienic risks	Contains problem materials	Risks of contaminants
Feedstock	Communal residue material	Greenery, grass cuttings		Biowaste, Roadside greenery	
	Industrial residue materials	Vegetable waste, mash, pomace, etc.	Expired foodstuff, foods with transport damage		Residue from vegetable oil production
	Agricultural residues	Fluid dung, solid dung			Cu and Zn
		Beet leaves, straw			
	Renewable raw materials	Corn silage, grass silage			
	Slaughter waste		Rumen stomach-intestinal contents, separated fats, blood flour, etc.		Separated-fats
	Miscellaneous		Industrial kitchen waste, household waste		

It is important to measure some parameters to carry out anaerobic digestion process smoothly. These measurements can be considered as temperature, pH and redox potential (ORP), dry matter (DM) content, water content, nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sulfur (S), heavy metals like lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), zinc (Zn), mercury (Hg), content of short - chain fatty acids, principally acetic acid, propionic acid, butyric acid, and C/N ratio (Deublein and Steinhauser, 2008).

Biogas has many socioeconomic and environmental advantages as given below (Rutz et al., 2008; Moestedt et al., 2016):

- Renewable energy
- Decreased greenhouse gas emissions and solving of global warming problem
- Decreased dependency on fossil fuels from abroad

- Promote to EU energy and environmental goals
- A good method to eliminate waste
- Provide employment opportunity
- Flexible and effective end use opportunities for produced biogas
- Production of bio-fertilizer

Biogas production also provides a lot of benefits for the farmers such as extra income, flexibility to use varied materials as substrate, and lower odors. In addition, the digestate, which is a nutrient-rich material, also can be utilized as excellent fertilizer (Rutz et al., 2008; Wei et al., 2014). However, biogas could not have a bigger role in energy sector because of some reasons such as the high cost of substrates, difficulty of obtaining substrates and the lack of innovations that help for reaching more economical biogas production technologies (Budzianowski, 2016).

2.1.1. Phases of Anaerobic Digestion Process

Anaerobic digestion, which is a very complicated process, consists of four stages, called hydrolysis, acidogenesis, acetogenesis and methanogenesis. Different types of microorganisms have a very important role on anaerobic digestion process. Biochemistry of the methane generation is given in Figure 2.5. (Deublein and Steinhauser, 2008).

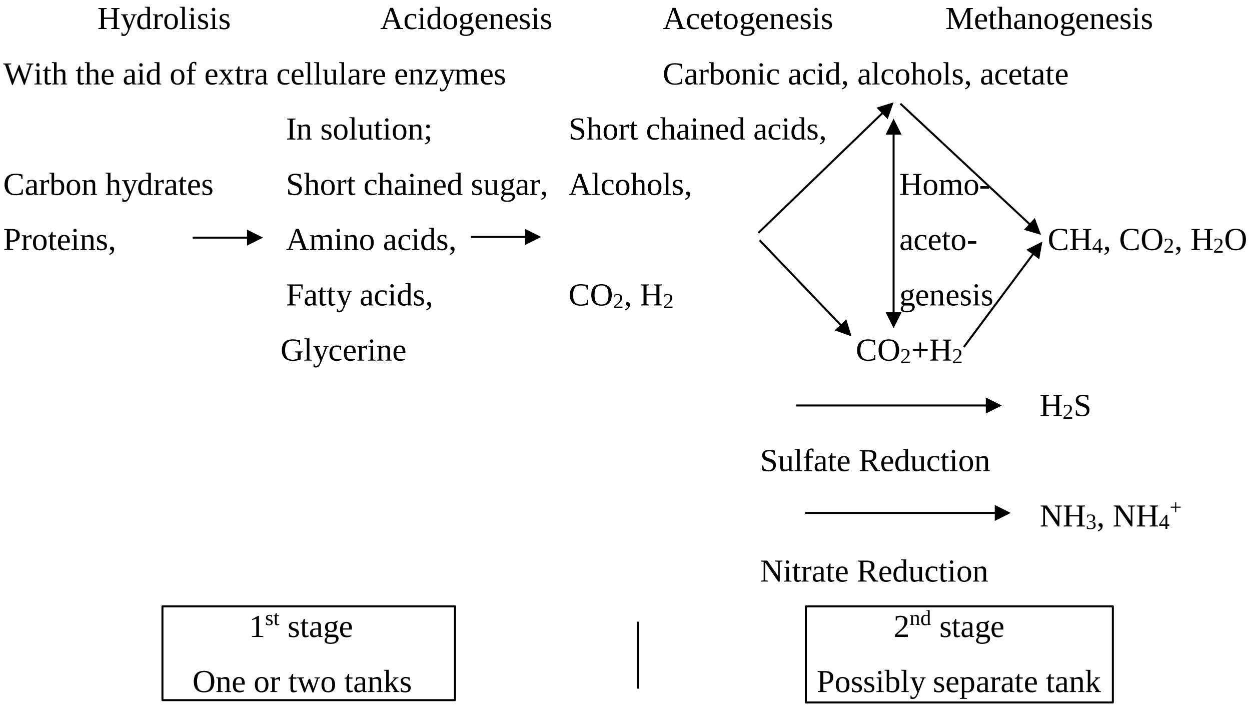

Figure 2.5. Biochemistry of the methane generation (Deublein and Steinhauser, 2008).

2.1.1.1. Hydrolysis. The first stage of the anaerobic digestion process is hydrolysis. In the hydrolysis phase, undissolved compounds like cellulose, lipids, proteins and fats are decomposed into water-soluble fragments (monomers) (Deublein and Steinhauser, 2008).

Hydrolytic enzymes are excreted by hydrolytic microorganisms to convert biopolymers into simpler and soluble compounds. Polymers like carbohydrates, lipids and proteins are converted into glucose, glycerol, purines and pyridines as shown below (Rutz et al., 2008).

$$\text{Lipids} \xrightarrow{\text{lipase}} \text{fatty acids, glycerol}$$

$$\text{Polysaccharide} \xrightarrow{\text{Cellulase, cellobiase, xylanase, amylase}} \text{monosaccharide}$$

$$\text{Proteins} \xrightarrow{\text{protease}} \text{amino acids}$$

For a couple hours, the hydrolysis of carbohydrates takes place, while the hydrolysis of proteins and lipids within a couple days. The lignocellulose and lignin degradation process is not fast and complete (Deublein and Steinhauser, 2008).

The dissolved oxygen in the water is taken by the facultative anaerobic microorganisms, and this causes the low redox potential essential for obligatorily anaerobic microorganisms (Deublein and Steinhauser, 2008).

It is important that the whole anaerobic digestion process can adversely be affected by a poor hydrolysis phase (Ezebuiro and Körner, 2017).

2.1.1.2. Acidogenesis. During acidogenesis phase, which is the second phase of anaerobic digestion process, the monomers are degraded to volatile fatty acids, ammonia, alcohols, hydrogen, and carbon dioxide by different facultative or obligatory anaerobic bacteria. The kind of products of fermentation depends on the concentration of the intermediately formed hydrogen ions. If the hydrogen partial pressure is lower than 60 Pa, the product is acetate. As the hydrogen partial pressure is higher than 60 Pa, butyrate is produced. If the hydrogen partial pressure is much higher, ethanol and lactate are the products (Deublein and Steinhauser, 2008).

2.1.1.3. Acetogenesis. During acetogenesis stage, the microorganisms use the products from the acidogenesis phase as substrate. During the acetogenesis phase, carbon compounds are converted into

CO_2, H_2, and acetic acid. Methane conversion by methanogens cannot occur directly from the outputs of acidogenesis stage. Thus, these substances (alcohols and propionic and butyric acids) are converted into acetate, hydrogen and carbon dioxide (Deublein and Steinhauser, 2008; Rutz et al., 2008; Choong et al., 2016).

Acetogenic bacteria are the H_2 producers. Very low partial pressure is necessary for the oxidation of long- chain fatty acids to form acetate. The required energy for the growth and survival of acetogenic bacteria can be obtained at very low H_2 concentration. However, the methanogenic microorganisms can live only with higher hydrogen partial pressure. Therefore; acetogenesis and methanogenesis occur parallel, as symbiosis of two groups of microorganisms (Deublein and Steinhauser, 2008; Rutz et al., 2008).

If the hydrogen partial pressure is low, the acetogenic bacteria produce H_2, CO_2, and acetate. Butyric, capronic, propionic, and valeric acids, and ethanol are formed if the hydrogen partial pressure is higher. H_2, CO_2, and acetate can only be processed by the methanogens. (Deublein and Steinhauser, 2008).

2.1.1.4. Methanogenesis. Methane and carbon dioxide are produced by the methanogens during methanogenesis. Methanogens converts acetic acid into methane and carbon dioxide. Also, small quantity of methane in the produced biogas is produced from the reaction of hydrogen and carbon dioxide (Rutz et al., 2008):

$$\text{Acetic acid} \xrightarrow{\text{methanogens}} \text{methane + carbon dioxide}$$

$$\text{Hydrogen + carbon dioxide} \xrightarrow{\text{methanogens}} \text{methane + water}$$

Methanogenesis is the slowest biochemical reaction of the anaerobic digestion process, and it is a very crucial phase in the whole anaerobic digestion process. Various operational conditions such as composition of feedstock, feeding rate, temperature and pH affect the methanogenesis process. Temperature changes, digester overloading or large amount of oxygen input may cause the termination of the anaerobic digestion process (Rutz et al., 2008).

2.2. Environmental and Operational Parameters Influencing Anaerobic Digestion Process

Some important parameters affect the effectiveness and stableness of anaerobic digestion process. Environmental parameters like temperature, pH and nutrients, and operational factors like organic loading rate (OLR), hydraulic retention time (HRT), and sludge retention time (SRT) influence anaerobic digestion process significantly (Rutz et al., 2008).

2.2.1. Environmental Factors

Environmental parameters like temperature, ORP, pH, mixing, availability of nutrients, alkalinity, inhibition and toxicity are very important parameters for biogas production.

2.2.1.1. Temperature. The anaerobic digestion process can occur at varied temperature ranges. These temperature ranges are psychrophilic, mesophilic and thermophilic ranges. Hydraulic retention time (HRT) is affected directly from the temperature of the digestion process. This relationship is given in Table 2.6 (Rutz et al., 2008; Neshat et al., 2017).

Table 2.6. Thermal stage and typical retention times (Rutz et al., 2008).

Thermal stage	Process temperatures	Minimum retention time
psychrophilic	< 20 ^{o}C	70 to 80 days
mesophilic	30 to 42 ^{o}C	30 to 40 days
thermophilic	43 to 55 ^{o}C	15 to 20 days

For an effective anaerobic digestion process, temperature should be stable. The stability and productivity of anaerobic digestion process are affected by temperature. Anaerobic digestion process is very sensitive to temperature changes. Especially; metabolic activity of microorganisms, settling characteristics and gas transfer rates are affected by temperature changes. Thermophilic bacteria are more sensitive to temperature fluctuation than mesophilic bacteria. If the temperature is not kept constant, the gas loses over 30% may occur. Floor or wall heating systems are used to obtain the necessary process temperature. The feedstock is an important parameter in terms of the selection of the operation temperature. Relative biogas yields, based on temperature and retention time are shown in Figure 2.6 (Deublein and Steinhauser, 2008; Rutz et al., 2008; Neshat et al., 2017).

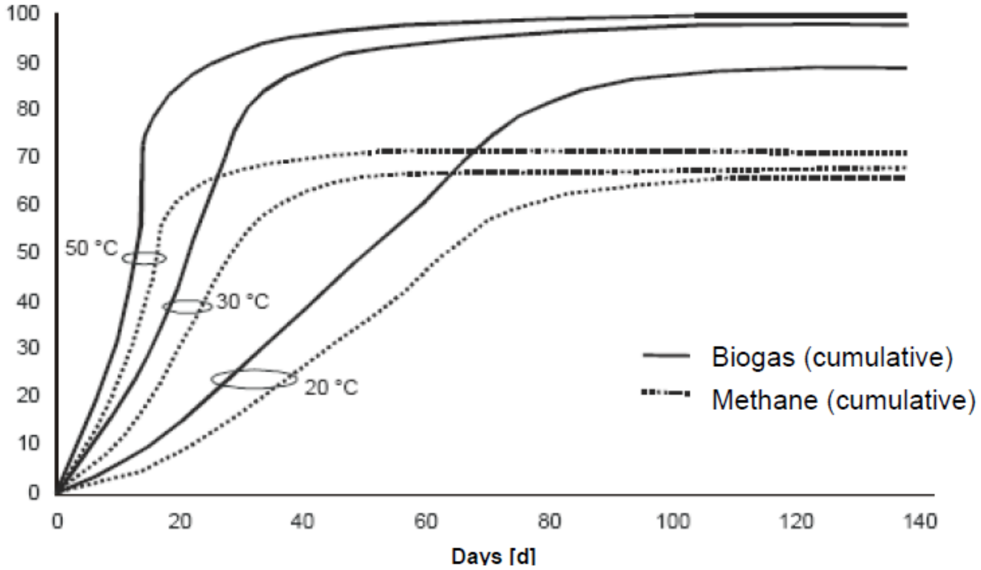

Figure 2.6. Relative biogas yields, based on temperature and retention time (Rutz et al., 2008).

Thermophilic process temperatures are generally chosen by lots of biogas plants. Thermophilic process has many advantages such as effective destruction of pathogens, higher grow rate of methanogenic bacteria at higher temperature, increasing chemical and biochemical reaction rates, decreased retention time, increasing the speed of the process and more efficient, higher solubility of organic compound, better degradation of solid substrates and better substrate utilization and, better opportunity for separating liquid and solid fractions. However, thermophilic process has a lot of disadvantages, too. These disadvantages can be considered as larger degree of imbalance, higher energy requirement due to high temperature, and higher inhibitory effect of ammonia and VFA. In addition, the solubility of many compounds such as NH_3, H_2, CH_4, H_2S, and VFA depends on temperature (Rutz et al., 2008; Neshat et al., 2017).

Acidogenic microorganisms are only active in a specific temperature range, and even any temperature changes have negative impact on the acidogenic bacteria. Also, methanogens cannot tolerate temperature changes above 1^0C/d (Neshat et al., 2017).

2.2.1.2. pH. The growth of methanogens is affected by the pH. The optimum range is 7.0- 8.0 for most methanogens. According to the studies conducted, when the pH value decreases below 6.8, the growth of methanogenic bacteria also decreases (Neshat et al., 2017). Lower pH values are usually appropriate for acidogenic microorganisms (Rutz et al., 2008).

Temperature is an important factor for the solubility of carbon dioxide in water. The solubility of carbon dioxide declines at increasing temperature, and the dissolved carbon dioxide reacts with water and forms carbonic acid. For this reason, the pH value in thermophilic digesters is higher than that of the mesophilic digesters. The optimum pH range is 6.5- 8.0 for mesophilic digesters (Rutz et al., 2008).

The bicarbonate buffer system is used to control the value of pH in anaerobic reactors. The pH value in the digesters depends on some factors such as the partial pressure of carbon dioxide and the concentration of alkaline and acid components in the liquid phase (Rutz et al., 2008).

2.2.1.3. Nutrients. The growth and existence of microorganisms are necessary for anaerobic digestion process. Microorganisms responsible for the anaerobic digestion process need trace elements like iron, nickel, cobalt, selenium, molybdenum or tungsten and macronutrients carbon, nitrogen, phosphor and; sulphur for survival and growth of the anaerobic microorganisms. The optimum ratio of macronutrients (C: N: P: S) is considered as 600: 15: 5: 1. However, inhibition and toxicity can occur because of insufficient amount of nutrients or too high digestibility of the substrate. If substrates with a too low C/N ratio are used, ammonia generation will increase, and inhibition of methane generation will occur. When substrates with a too high C/N ratio are used, it means lack of nitrogen, from which negative results for the formation of protein and thus the energy and structural material metabolism of the microorganisms result. Thus, an appropriate composition should be provided (Deublein and Steinhauser, 2008; Rutz et al., 2008).

2.2.1.4. Mixing. Mixing is also a significant parameter in terms of anaerobic digestion process. Blending the fresh substrate with digestate consisting of bacteria is the purpose of mixing in a digester. A homogeneous mixture of substrate and inoculum can be obtained due to a well-mixed digester. By this way, an optimum digestion conditions can be obtained by providing a homogeneous temperature, trace element and nutrition distribution in the digester. Moreover, the other advantages of mixing are preventing foam formation and avoiding temperature gradients in the reactor. In addition, slow mixing is preferred because excessive mixing may disrupt the microorganisms. The amount of mixing and kind of mixing materials can be chosen according to the type of reactor and the solids content in the digester. Careful as well as intensive mixing action should be chosen for an effective anaerobic digestion process (Deublein and Steinhauser, 2008; Verma, 2002; Kowalczyk et al., 2013).

2.2.1.5. Alkalinity. Alkalinity is an important environmental parameter for anaerobic digestion process. The pH should be kept above 7.0 in the digester for maximum methane production. The alkalinity level in the anaerobic digestion reactor should be enough because the pH is decreased due to the acids that are produced in the first part of the anaerobic digestion process. Alkalinity buffers the effect of the acids in digester. Alkalinity measurements are necessary for efficient digester operations. If the level of acid in the digester increases and raises the ratio out of the normal operating range, the process may be damaged. The alkalinity should not be below 1000 mg/L, and the volatile acid to alkalinity ratio should be below 0.4 (Ragsdale, 2000).

During anaerobic digestion process, carbon dioxide is generated and, this causes the formation of carbonic acid and VFA. In case of the usage of substrates with high alkalinity, an optimum pH level which is necessary for methane production could be obtained (Neshat et al., 2017).

Some chemicals such as sodium bicarbonate and lime could be used to obtain the optimum pH level, and enhance the required alkalinity level. However, if these chemicals generate specific ions, it can cause an inhibition in the anaerobic digestion process (Neshat et al., 2017).

2.2.1.6. Inhibition and Toxicity. Another factor that affects the activity of anaerobic microorganisms is the presence of toxic and inhibitory compounds. There are basically two reasons of the presence of toxic compounds. Toxic compounds can be generated during the anaerobic digestion process or the feedstock can include the toxic compounds and they can be introduced to the anaerobic digestion system. It is difficult to control toxic compounds in the anaerobic digestion system due to the capacity of anaerobic microorganisms to adapt to environmental conditions. The other reason is that chemical processes bind toxic compounds, herewith to the presence of toxic compounds (Rutz et al., 2008).

There are lots of different parameters that cause to inhibition of the anaerobic digestion process like pH, temperature, substrate features and inoculum and ammonium concentration in the reactor (Yenigün and Demirel, 2013).

Ammonia affects the anaerobic digestion process for both negatively and positively. For example, it could help to obtain the optimum pH level and decrease the formation of VFAs. Also, ammonia concentration should be at an optimum level for bacterial growth. However, ammonia inhibition can be observed because of the high concentration of ammonia (Neshat et al., 2017; Yenigün and Demirel, 2013).

Especially, ammonia is considered as an inhibitor when complex materials like manure are used as substrate in the anaerobic digestion process (Yenigün and Demirel, 2013).

Many studies performed to investigate to not only the inhibitor impact of ammonia, but also the methods for reducing the ammonia inhibition in anaerobic digestion process. According to the studies, inhibitory impact of ammonia can be decreased by using pre-treated substrate in anaerobic digestion process. Also, in case of an inhibition by high concentration of free ammonia nitrogen, the conducted studies showed that a lot of methods can be used to enhance biogas and methane yields. For example, adjusting reactor pH and C:N ratio of the substrate, rarefaction of the substrate and reactor content and using some additives such as zeolite and activated carbon can be regarded as recovery methods for ammonia inhibition (Yenigün and Demirel, 2013).

In case of usage of animal manure, which could be contained antibiotics, in the anaerobic digestion system, the high level of antibiotics, which conclude to kill the microorganisms, can cause inhibitory impact on anaerobic digestion process (Neshat et al., 2017)

2.2.1.7. Oxidation Reduction Potential (ORP). The oxidation reduction potential of a digester is the measure of the oxidizability or reducibility of its content. The oxidation promoting substrates include oxygen, sulphate or nitrate groups. Such substrates can change the oxidation reduction potential and result in a shift in the pH. Oxidation reduction potential should be measured continuously to prevent shifting of the pH (Wiese and König, 2007).

Oxidation reduction potential should be low in the bioreactor. For instance; an oxidation reduction potential interval between -300 and -330 mV is optimum for monocultures of methanogenic bacteria. Additionally, the oxidation reduction potential in the fermenter can rise to 0 mVs. Some oxidizing agents such as sulfates, no oxygen, nitrates, or nitrites should be provided to keep a low oxidation reduction potential (Deublein and Steinhauser, 2008).

2.2.2. Operational Factors

The operational factors that affect the anaerobic digestion process can be considered as hydraulic retention time, organic loading rate, and sludge retention time.

2.2.2.1. Hydraulic Retention Time (HRT). The volume of the digester is determined according to the hydraulic retention time (HRT). The HRT can be defined as the average time that the substrate is kept in the digester. The digester volume and the volume of substrate fed per time unit are related to the HRT (Rutz et al., 2008).

$\mathrm{HRT} = V_R / Q$
HRT: Hydraulic retention time [days]
V_R: Digester volume [m^3]
Q: Volume of substrate fed per time unit (Flow Rate) [m^3/day]

According to the equation, if the organic load increases, it causes a reduction in the hydraulic retention time. The amount of microorganisms removed with the digestate (effluent) should not be higher than the amount of reproduced microorganisms. In order to ensure that the hydraulic retention time must be long enough. 10 days is generally accepted as the duplication rate of anaerobic microorganisms. If the hydraulic retention time is short, gas yield will be low, however substrate flow rate will be good. Hence, the hydraulic retention time should be determined extensively to supply required time for microorganisms for synthesizing the substrate and should be adapted to the specific decomposition rate of the utilized substrate. The necessary digester volume can be calculated by knowing the targeted hydraulic retention time, the daily feedstock input and the decomposition rate of the substrate (Rutz et al., 2008; Neshat et al., 2017).

2.2.2.2. Organic Loading Rate (OLR). Organic loading rate is the amount of dry organic solids that is added to the anaerobic digestion system per volume per time (Munk and Lebuhn, 2014). OLR is a measure of the biological conversion capacity of anaerobic digestion process. If the system is fed above its sustainable organic loading rate, some inhibiting substances like fatty acids may accumulate, and low biogas yields can be obtained. Organic loading rate must be reduced in such a case (Verma, 2002).

Hydrolytic and acidogenic microorganisms are more affected than methanogens in case of high organic loadings. According to the studies carried out, 4 kg DS/ m^3/day was determined as the maximum OLR which could be tolerated by microorganisms (Neshat et al., 2017).

2.2.2.3. Solid Retention Time (SRT). The solid retention time (SRT) is the most significant parameter in terms of controlling the anaerobic digestion process and maintaining digester stability. The volatile solids conversion to gas depends on solid retention time. If the solid retention time is low, bacterial growth is influenced negatively, and bacteria cannot replace the bacteria lost in the effluent. When the rate of bacterial lost is higher than the rate of bacterial growth, wash out occurs. “Critical SRT” is the SRT at which “wash out” begins to occur. SRT/HRT ratio is used to measure the achievement of biomass retention. In traditional digesters, the ratio is 1.0. Efficient retention systems will have SRT/HRT ratios exceeding 3.0 (Dennis and Burke, 2001).

2.3. Anaerobic Digestion of Energy Crops for Production of Renewable Energy

In addition to conventional AD feedstock types, dedicated energy crops for biogas generation were introduced in many regions (Rutz et al., 2008). According to the researches, energy-dense biomass can be produced only from dedicated plantations of high-yielding energy crops. In European countries such as Germany, France and Sweden, agricultural biomass is one of the most being preferred substrate for biogas generation as a resource of renewable energy (Bozym et al., 2015; Herrmann et al., 2015).

It was reported that, when energy crops were used as substrate, the main pathway of methane generation was determined as the hydrogenotrophic methanogenesis (Oleszkiewicz and Sharma, 1990).

The aims of the studies are to enhance performance and increase the type of energy crops and assest the potential of their biogas production potential. Agriculture of energy crops provides new farming opportunities and systems, where combined crop agriculture is also subject of many research (Rutz et al, 2008).

2.3.1. Anaerobic Digestion of Maize Silage

Maize silage regards as one of the most preferred substrates for biogas generation since maize silage is a complex lignocellulosic material and, it has high energy yield per hectare and methane yield based on volatile solids in the range of 268-366 L/kg and 251-349 L/kg, respectively. The picture of the maize silage is given in Figure 2.7. In Europe, many biogas plants use maize silage as sole substrate. For instance, the rate of maize usage for biogas production is 73% in Germany. In Figure 2.8, the methane yields of various crops are shown. As it is seen, the methane yield of maize silage is higher than that of the energy yields of biodiesel from canola and corn ethanol. However, a certain decrease in biogas production at some biogas plants has been observed after a period of time because of the inadequacy of trace elements. Trace elements are necessary for anaerobic digestion process. Methane formation and microbial growth are dependent on trace element concentration (Pobeheim et al., 2010; Bozym et al., 2015; Linke et al., 2015; Herrmann et al., 2015; Martin et al., 2017; Kalinichenko et al., 2016).

Figure 2.7. Maize silage (Rutz et al., 2008).

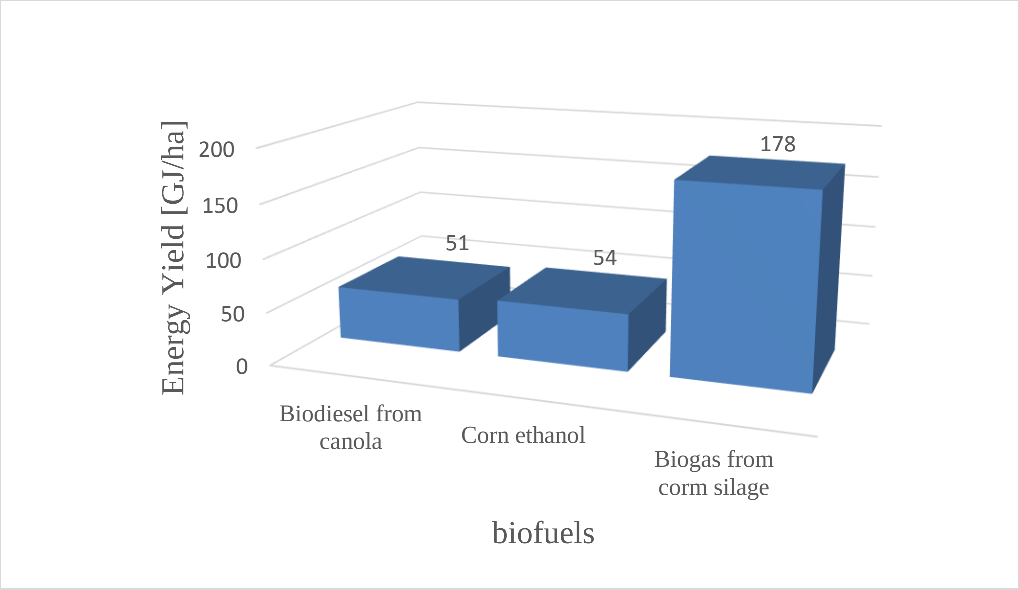

Figure 2.8. Methane yield of various crops (Kalinichenko et al., 2016).

Demirel and Scherer (2011) reported about the micro nutrient requirements on biogas generation from energy crops, crop remains, animal droppings and the organic fraction of municipal solid wastes (OFMSW). In this review, the studies in the literature about the impact of essential trace elements like Fe, Ni, Co and Mo was investigated. As a result of the literature studies, performance and stability of anaerobic digestion process is related to the availability of trace elements in the digester. if there is no any other clear reason, the lack of trace elements can be the main cause in case of any underperformance in anaerobic digestion process, especially when energy crops was utilized as substrate in mono-digestion process. For this reason, the determination and understanding of the most optimum amount of trace elements of anaerobic digestion is very important, especially for commercial biogas production applications (Demirel and Scherer, 2011).

The essential trace elements for anaerobic digestion process are manganese, nickel, selenium, molybdenum and cobalt. For example; manganese is an enzyme cofactor for the superoxide dismutase. Also, manganese is essential for Mn-dependent enzymes such as the lactate dehydrogenase. Xylose isomerase Manganicatalase, Manganisuperoxide dismutase and the NADH oxidase are affected from Mn concentration. Researches show that Mn has a very advantageous impact on cell growth rate (Hang et al., 2016).

Anaerobic digestion of maize silage, which is a preferable way of production of renewable energy, has been investigated for many years. Some of these researches are summarized below.

The methane potential of maize can be affected from the inoculum to substrate ratio. This effect was studied in 2006 (Raposo et al., 2006). In the experiment, completely batch reactors were used. 1, 1.5, 2 and 3 were selected as volatile solid ratios. The experiment was performed at 35°C. A reactor without substrate was also operated as a control reactor. Soluble chemical oxygen demand (CODs), total alkalinity, volatile fatty acids (VFA), pH and gas characteristics were measured to monitor of the reaction from solid substrate to biogas in case of the progress and stability. According to the result of the study, the average methane yield was 211 mL CH_4/g VS_{added} at standard temperature and pressure. The slight variation of methane yield coefficient at four different inoculum to substrate ratios (3, 2, 1.5 and 1) was only seen at the higher ratio. The maximum specific methane generation rate which ranged from 10 ml CH_4/g VSS day for an inoculum to substrate ratio $r_{I/S}$ of 3- 23 ml CH_4/g VSS day for a $r_{I/S}$ of 1was determined by analyzing the methane production curves. It was observed that the highest proportion of the CODs was in the VFA form. Also, an accumulation of longer chain acids was observed at the reactor which loaded with the highest VS rate (Raposo et al., 2006).

The effect of rumen microorganisms on anaerobic digestion from corn stover was studied (Hu and Yu, 2005). Corn stover was collected after the harvesting of the corn. Then, it was dried by sunlight for 5 days. Dried material was crumbled to 1.0 mm particle size. Batch and semi-continuous reactors are used of anaerobic digestion of corn stover by rumen microorganisms. The substrate put to obtain concentrations of 5.0, 10.0, and 15.0g volatile solids (VS)/L in the batch reactors. After 240 h incubation at 25-40°C, the volatile solids conversion efficiency was high as 65-70 % in the batch reactors. The volatile fatty acids yield is between 0.59 and 0.71 g/g VS added. The main aqueous products were acetate, propionate and butyrate. In addition to these products, small amount of i-butyrate and valerate were generated. Biogas consisted of carbon dioxide and methane. The semi continuous reactors were operated at 35-40^{o}C. The loading rates were 10, 20 and 30 g VS /Ld. In the semi-continuous reactor, the conversion efficiency of 65% was observed at loading rates of 10-30 g VS$^{/}$L d. The solids retention time and the hydraulic retention time were 96 h and 18 h, respectively. The volatile fatty acids yield is from 0.56 to 0.59 g /g VS added, and biogas consisted of hydrogen and carbon dioxide. However, there was not any methane in the biogas. According to the results of the study, the degradation of the volatile solids and the production of useful VFAs with high yields were achieved by the anaerobic digestion of corn stover by rumen microorganisms (Hu and Yu, 2005).

The effect of anaerobic digestion under the mesophilic and thermophilic conditions on biogas generation was studied by Vindis et al. (2009). The experiment was carried out to compare the impact of the different temperature conditions on biogas production. Some parameters were measured and compared to determine mesophilic (37^{o}C) and thermophilic (55 ^{o}C) conditions. Biogas production and composition were calculated with a gas analyzer. In the experiment, mini digesters were performed, and three different types of maize were used as substrate. Every day, the substrates are mixed for ten minutes. According to the study, anaerobic digestion in thermophilic conditions has many advantages. The biogas quality which generated under the thermophilic conditions is higher than the biogas quality generated under the mesophilic conditions. Anaerobic digestion in thermophilic temperature range is four times more intense, and has also higher VSS removal efficiency. In addition, the biogas yield is higher, and thermophilic stabilization is very economical. However, the thermophilic digestion process has a defect that more energy is needed to heat digesters. If a biogas plant has mesophilic digesters, establishing of additional mesophilic digesters is more expensive than upgrading of existing mesophilic digesters (35°C) to thermophilic digesters (55°C) (Vindis et al., 2009).

Variety, harvesting time and pretreatment are important factors that affect the anaerobic digestion process. The variety, harvest time and pretreatment of the anaerobic digestion of maize was studied to indicate the effect of these parameters on biogas production. Different maize varieties were selected as substrate. All parts of the maize plant were used. Six types of fresh maize harvested at three different times (after 116, 137, and 157 days after seeding) and of maize silage in two particle size were digested in batch reactors. Inoculum (Anaerobic digested effluent from a thermophilic biogas facility (55 ^{o}C) digesting cow manure) and substrate were added into reactors. No pH adjustment was made. Blank sample consisting of only inoculums and water also prepared. Some parameters such as total solids, volatile solids, ash content, ammonia and total Kjeldahl nitrogen of maize silage and fresh maize were analyzed. The methane production was measured with a gas chromatograph. According to the results, the biodegradability of the fresh maize was high. It was observed that at late harvest time, the energy yield from fresh maize whole plant was highest. Harvest time and maize variety had no remarkable effect on the methane yield. Mechanical pretreatment of maize silage (whole plant) enhanced the methane yield by 0.04 ± 0.01 m^3 CH_4/(kg VS). Both fresh maize and maize silage determined as appropriate substrates for anaerobic digestion. The degradation rate of the fresh maize and maize silage without pretreatments is very high (Bruni et al., 2010).

For biogas generation, animal manure and energy crops are very important substrates. Biogas generation from maize hybrids was analyzed. In this study, the determination of the maturity class of maize which has the highest biogas and biomethane production was aimed. In addition, the composition of gases was determined. Experiments were carried out using a laboratory digester for 35 days within four series of analyses. 15 corn hybrids (FAO 300 - FAO 400, FAO 400 - FAO 500 and FAO 500 - FAO 600) were analyzed. Until the relatively small amount of gas produced, the experiment was carried out. According to the result of the experiments, the biogas and biomethane yield of FAO 400 and FAO 500 (the higher maturity corn type) increased. The maize diversity showed a characteristic methane generation performance. In addition, methane production potential depended on their composition. The biogas generation also hinged on the crude protein content (Oslaj et al., 2010).

Pretreatment of substrates can be necessary to increase the biogas yields. Biological ensilage additive as pretreatment for maize to enhance the biogas generation was studied. Many biological ensilage additives were analyzed, and the effect of different types of biological ensilage additives on biogas generation and preservation of ODM content was determined. For instance; Bonsilage Mais (Lactosan) which contains Lactobacillus plantarum spp., Lactobacillus pentosaceus spp., and Lactobacillus buchneri spp., and Silasil Energy (Lactosan) are both homo- and hetero-fermentative bacterial mixtures. The particle size of maize was reduced to 2 cm by a garden chopper to homogenize. Some parameters like organic dry matter content, dry matter content, pH, total alkalinity were analyzed by following standard methods. A homogeneous subsample and filtered digestate from different farm reactors using cow manure and co-products mixed. The incubation occurred at 37°C, and the produced gas was collected. The amount of biogas production was measured. According to the study, biological additives for ensiling of maize affect the methane generation per ODM in subsequent anaerobic digestion by up to 22.5%. Successful ensiling was achieved by the addition of only homo-fermentative and hetero-fermentative LAB (Bonsilage Mais) with a high lactic acid generation capacity. However, restricted the biogas and biomethane yield compared with the addition of more complex additives that might enable the hydrolysis stage. For this reason, the inoculum for ensiling maize should be considered carefully (Vervearen et al., 2010).

Linke et al. (2015) performed a study to identify the performance of a novel biogas reactor. Maize silage was selected as substrate, and fed to the reactor semi-continuously. 35 L leach-bed reactor and 22 L anaerobic filter were used in the experiment. Six different feeding models were analyzed at average 4.5 g/L d of VS loading rate. The leach bed reactor was connected to the anaerobic filter. The anaerobic digestion process was conducted at mesophilic conditions. Maize silage and inoculum analyzed and, TS, VS, Total Ammonium Nitrogen, COD, pH, values were determined. Once a week, a sample from the digestate removed from the reactor for measurement. As a result of the experiment, methane production changed according to the VS loading rates. Different feeding methods did not have any impact on methane yield. Effective biogas generation was obtained (Linke et al., 2015).

Ammonia inhibition during biogas generation from maize silage and chicken manure was examined by Sun et al (2016). 5 L reactors were used as digesters. The purpose of this experiment was to investigate a multi-inhibited anaerobic digestion process. Maize silage and chicken manure were selected as substrate. During the study, no water was added to the digesters. Feeding of the digesters was conducted daily, and the digestate was analyzed every week in terms of alkalinity, TS, VS, TKN, pH and VFA. Biogas production was measured during the study. Also, element content was measured. TAN (Total Ammonia Nitrogen) was chosen as indicative parameter. Every 11 days, organic loading rate (OLR) was increased as 0.5 g VS/L day, and OLR were increased from 1 to 3 g VS/L day at the first 44 days. In order to obtain process stability, the chicken manure rate in the mixed substrate was adjusted according to the TAN concentration. According to the results of the experiment, at a TAN concentration of 9 g N./L, methanogenesis phase of the anaerobic digestion process was inhibited. Methane production was affected negatively by the low digestibility of the mixed substrate. TAN concentration of 7 g N/L was determined as the critical concentration in the reactor. Reactor performance was increased by decreasing the chicken manure concentration in the digester (Sun et al., 2016).

2.3.2. Effect of Trace Elements on Anaerobic Digestion of Maize

Micro elements are vital for anaerobic digestion process. Macro and micro elements are needed for the growing of anaerobic bacteria. Inhibition of anaerobic digestion process occurs via the absence of macro and micro nutrients. One of the most significant parameters for the anaerobic digestion process is enzyme. Enzymes include trace elements like tungsten, selenium, nickel, chromium, molybdenum, iron and cobalt. As a result, the absence of these elements influences the activity of enzymes, and if any of these elements is limited, the whole anaerobic degradation process may be damaged. In addition, methanogens convert organic acids and hydrogen to biogas and, if trace elements are not adequate for methanogenic bacteria, the total anaerobic degradation rate can be adversely affected. The comparison between nutrient demand of anaerobic bacteria and their content in varied mono substrates is given in Table 2.7. According to the Table 2.8., important trace elements content of maize silage such as iron, nickel, cobalt and molybdenum are not sufficient. Consequently,this may result in the destabilization of the anaerobic degradation process and the reduction of biogas production. (Hinken et al., 2008; Schattauer et al., 2011).

Table 2.7. The comparison between nutrient requirement of anaerobic bacteria and their content in varied mono substrates (Hinken et al, 2008).

		Nutrient content of average data from substrate		
	Calculated nutrient requirement	Maize silage	Rye crops	Wheat crops
	[mg/kg COD_{in}]	[mg/kg COD]	[mg/kg COD]	[mg/kg COD]
Nitrogen	7,410	14,737	26,365	18,845
Phosphorus	7,710	2,411	3,925	4,164
Potassium	1,140	12,823	2,770	4,505
Calcium	456	2,082	346	352
Magnesium	342	1,534	1,270	932
Iron	205	111	28	56
Zinc	7	38	39	36
Manganese	2	32	28	35
Copper	1	4	5	4
Nickel	11	<1	-	-
Cobalt	9	<1	-	-
Molybdenum	7	<1	-	-

By trace element addition, digester stability can be obtained, methane and biogas yields can be increased, and fatty acids concentration can be decreased. Also, the addition of micro- and macro-nutrients provides high methane yields at very short hydraulic retention times (30-40 days) (Romero-Güiza et al., 2016; Choong et al., 2016).

Micro elements have a brutal role in hydrolysis step of anaerobic digestion process. Hydrolysis of the substrate can be a limiting stage for biogas generation via anaerobic digestion process when complex substrates are digested (Ezebuiro and Körner, 2017).

Apart from the trace element requirement for growth and enzyme activity in the anaerobic digestion process, if the optimum dose could not be determined, it could be toxic impact on anaerobic digestion process. Also, in such a case, digestate could not be used as fertilizer because of the over-dosing heavy metal concentration. So, trace element concentrations should be determined before the anaerobic digestion process begins (Bozym et al., 2015).

According to the studies, lead concentration should be maximum 340 mg/dm^3, and optimum concentration is reported as 0.02-200 mg/dm^3 for anaerobic digestion. Toxic level for cadmium is 180 mg/kg (Bozym et al., 2015).

Methane production could be inhibited by copper level at 40 mg/kg, and also biogas production could be inhibited by the copper concentration is higher than 400 mg/dm^3 (Bozym et al., 2015).

Zinc has an improving impact on methane production. However, when the zinc concentration exceeds 100 mg/kg DM or 0.400 mg/dm^3, it has a toxic effect (Bozym et al., 2015).

In anaerobic digestion process, process stability can be obtained and methane yield could be improved by sufficient nickel concentration. According to the studies, while 0.11-0.25 mg/kg Ni concentration improve biogas production, 1.2 mg Ni/dm^3 has inhibiting effects on biogas generation (Bozym, et al., 2015; Thanh et al., 2016; Ortner et al., 2014).

Maize is a crop which contains low trace concentration because of the inadequate uptake from the soil. So, the trace elements concentration of the maize silage needs to be determined extensively before the digestion process (Hang et al., 2016).

Cobalt, molybdenum, nickel, zinc and iron can be regarded as examples of essential trace elements. A lot of researches about the influences of micro elements on anaerobic digestion of maize have been carried out (Hinken et al., 2008).

In 2008, a study about the assessment of malnutrition of the anaerobic digestion of maize silage was carried out. In the study, batch reactors were used. The effect of three trace elements (iron, nickel and cobalt) on anaerobic digestion of maize was investigated at varied sludge loading rates, and maize and acetate were used as substrate. First of all, the content of different biomasses from lots of anaerobic facilities was determined by using ICP-MS (Inductively Coupled Plasma Mass Spectrometry). The connection of facility operation, substrate features, and micro element concentration were determined. Ten biogas plants (one municipal sludge digester, one co-digestion facility, five mono-digestion facilities using energy crops as substrate, and three agricultural co-digestion facilities using energy and manure as substrate) were selected for the experiment. The determination of the kinetic parameters such as biomass activity and degradability of substrate is made with automatic anaerobic batch tests (AABT). The automatic anaerobic batch tests are also used to determine the impact of inhibiting compounds and the effect of nutrient or micro-nutrient inadequacies. The methanogenic microorganisms need iron, cobalt and cobalt. In the study, different amount of elements were added to the biomass according to the sludge loading rates. According to the experiments, trace element concentration is very important for anaerobic digestion process. Especially, an inadequate amount of trace element can cause a reduction in biogas production. However, too high concentrations of micro nutrients may have a negative influence and result in inhibition. For biomass of a municipal sludge digestion, the trace element addition did not affect the biogas production. However, for biomass of a mono-digestion facility, the trace elements addition enhanced the biogas production up to 35% for maize silage used as substrate, and up to 70% for acetate used as substrate (Hinken et al., 2008).

Weiland et al. (2011) studied the abundance of micro elements in demonstration biogas plants. Ten biogas facilities in Europe (Austria, Czech Republic, Denmark, Germany, Italy, Poland and UK) were investigated in this experiment. The concentrations of micro elements in the digestates of these facilities were determined. Two of these plants were operated at thermophilic temperature (51^{o}C). The other biogas facilities were operated at mesophilic temperature ($35-42^{o}$C). Two- stage anaerobic digestion process was used at two plants. According to the results, iron concentration ranged from 48-1421 mg/kg. Magnesium concentration ranged from 65-890 mg/kg. Sulphur concentrations were between 45-230 mg/kg. The study showed that calcium was the most abundant micro element, and calcium concentrations ranged from 500-3600 mg/kg. In many feedstocks, iron, calcium, sulphur and magnesium were considered as micronutrients with considerable abundance in many feedstocks. The other elements which measured were manganese, molybdenum, zinc, copper, cobalt, nickel, selenium, chromium, and boron. In the study, a considerable uniformity was found out for molybdenum. In conclusion, a large variation in feedstock may enhance the abundance of micro elements. Manure or slurry, pig or cattle had effect on all the biogas facilities analyzed. The highest concentrations of nutrients were examined at biogas facilities feed by bleaching earth. Biogas facilities operated with wastes such as blood and food waste also revealed higher concentrations of trace elements. In addition, all of the biogas plants investigated had sufficient quantities of trace elements (Schattauer et al., 2011).

Features of the population of anaerobic microorganisms digesting a model substrate for maize with the addition of micro elements was investigated (Pobeheim et al., 2010a). The effect of an identified micro element solution on anaerobic digestion of maize was determined. The trace element solution consists of (μM) Fe^{2+} 7.5 ($FeCl_2.4H_2O$), Zn^{2+} 0.5 ($ZnCl_2$), Mn^{2+} 0.5 ($MnCl_2.4H_2O$), B^{3+} 0.1 (H_3BO_3), Co^{2+} 0.8 ($CoCl_2.6H_2O$), Cu^{2+} 0.01 ($CuCl_2.2H_2O$), Ni^{2+} 0.1 ($NiCl_2.6H_2O$), Se^{6+} 1.0 ($Na_2SeO_3.5H_2O$), Mo^{6+} 0.15 ($Na_2MoO_4.2H_2O$), W^{6+} 0.1 ($Na_2WO_42H_2O$). Nickel ($NiCl_2.6H_2O$) was also added in different concentrations as an extra. A synthetic model substrate consisting of microcrystalline cellulose, starch and urea as carbon and nitrogen source in a ratio simulating the basic combination of maize silage was used. 50% microcrystalline cellulose and 46% starch were utilized as carbon source. 4% urea was also used as a nitrogen source. Two batch reactors were carried out around 21days. 2L glass reactors were used, and the pH value was selected as 7.8± 0.2 for anaerobic digestion process. A reactor including only the identified model substrate and inoculum sludge was used as control reactor. According to the results of the study, an increase in nickel concentrations augmented methane generation by up to %20 (Pobeheim et al., 2010a).

Pobeheim et al. (2010b) studied the effect of micro elements on methane production from a synthetic model substrate for maize silage in batch reactor experiments. The impact of an identified micro element solution, and the elements named nickel, cobalt and molybdenum on anaerobic digestion of a synthetic model substrate for maize silage was analyzed. The synthetic model substrate was comprised of 50% xylan and 46% starch as carbon sources, 4% urea as nitrogen sources, and phosphorous requirement was provided with a 0.1 M potassium phosphate buffer. Macro elements and micro element concentrations in inoculum sludge and in maize silage were measured with an ICP-OES (inductively coupled plasma- optical emission spectrometer). The reactors were performed for 30 days at 35°C. As a consequence of the experiment, the addition of micro element solution causes an increase of methane production of up to 30%. 25th day of the operation, a yield of 407 L/kg

ODM was obtained, and an improved methane generation was seen due to addition of nickel at 10.6 µM. A decrease in methane formation and process stability was observed through total elimination of nickel from the micro element solution. Cobalt in a concentration range of 0.4 up to 2.0 µM enhanced the methane generation by up to 10%. However, methane production was not influenced by addition of molybdenum (Pobeheim et al., 2010b).

Nickel and cobalt can be regarded as important trace elements for anaerobic digestion process. Effect of nickel and cobalt on biogas generation and process stability during semi-continuous anaerobic digestion process before and after trace nutrient deficiency was determined by Pobeheim et al. (2010c). Maize silage was utilized as the model substrate. A well-designed model substrate for maize silage was designed for the experiment. Five semi-continuous reactors were run for 250 days at 35°C. A micro element solution was put to all of the digesters. Cellulose, starch, and hemicellulose were used to design a model substrate for maize silage. Carbon, nitrogen, and phosphorus (the basic nutrients) were mixed in a C:N:P ratio of 125:5:1. Starch and cellulose were added as carbon sources. Also, urea was added as nitrogen source, and. Phosphorous was provided from 0.1M potassium phosphate buffer. The composition of added micro element solution is shown in Table 2.8. (Pobeheim et al., 2010c).

Table2.8. Composition of used micro element solution (Pobeheim et al, 2010c).

Element	**Compound**	**Concentration (µM)**
Fe^{2+}	$FeCl_2.4H_2O$	7.5
Zn^{2+}	$ZnCl_2$	0.5
Mn^{2+}	$MnCl_2. 4H_2O$	0.5
B^{3+}	H_3BO_3	0.1
Cu^{2+}	$CuCl_2. 2H_2O$	0.01
Se^{6+}	$Na_2SeO_3. 5H_2O$	1.0
Mo^{6+}	$Na_2MoO_4. 2H_2O$	0.15
W^{6+}	$Na_2WO_4. 2H_2O$	0.1

The result of the study showed that limitation of cobalt and nickel affected the biogas production and stability of anaerobic digestion process negatively. In addition, organic loading rates could be enhanced with a proper addition of a chosen micro element solution, nickel and cobalt, respectively (Pobeheim et al., 2010c).

The long term process stability and necessities of biogas generation by anaerobic digestion of maize silage was investigated (Lebuhn et al., 2008). Six continuously stirred reactors were used. Fermenters were daily fed, and chemical and microbiological tests were performed continuously. The parameters which were analyzed were gas composition (CH_4, CO_2, H_2S, H_2, and O_2), TS, VS, pH, VFA, TVA/TAC (total volatile acids/total alkalinic carbonate), NH_4-N, macroelements (total C, N, P, S, K, Ca, Mg), micro nutrients (Fe, Cu, Ni, Co, Se, Al, Mo, Mn, Zn, B, Cd), ambient temperature and air pressure. The experiments were carried out under mesophilic conditions. Biogas generation was determined by Milligascounters. After 8 hours of operation, the reactors acidified at low organic loading rate of 2 g VS/L d. The most reliable parameter to determine process instabilities leading to acidification was the TVA/TAC ratio. A TVA/TAC threshold of 0.5 should not be exceeded. A micro element mixture was added to the reactors. The trace element cocktail consisted of $FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $MnCl_2.4H_2O$, $NaMoO_4.2H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, H_3BO_3, Na_2SeO_4, and $NiCl_2.6H_2O$. After the addition of the micro element mixture, the stability of the acidified reactors improved. The acidified reactors also recovered better, and allowed a higher organic loading rate. Hydrolysis was not process- limiting stage; however, methanogens were influenced. According to the result of the study, the most limiting element was cobalt in long term mono-digestion of maize silage. In addition, molybdenum and selenium should be provided. The amount of trace elements added should be enough to meet the actual needs. The trace element supplementation is the key issue for biogas production in both mono and co-digestion process (Lebuhn et al., 2008).

Lübken et al. (2015) determined the long-term process stability of a two-stage agricultural biogas production process under trace element limitation. For this reason, The Anaerobic Digestion Model No 1, which was designed in recent years to model the anaerobic digestion processes, was modified. At the pilot plant, the reactors were run for 494 days. Maize silage was used as mono substrate. A horizontal digester, which had a volume of 240 L, and a second vertical reactor (had a volume of 750L), were used. The study was conducted in two periods. At the first period, reactors were run for 150 days with organic loading rates (OLR) between 1.65 - 3.33 g VS/L/day. From the vertical fermenter, a homogenous mixture of the fermenter was recycled. At the second period, reactors were operated for 344 days. Any recycling process was not applied. At the laboratory experiment system, reactors were operated separately in terms of xylan, starch, cellulose and protein. The substrate was analyzed in terms of TS, VS, COD, TKN, NH_3-N and C, H, O, N, S concentrations. Raw lipid, neutral detergent fiber, raw protein, raw fiber, N-free extract, and acid detergent lignin concentrations were also determined, and expressed as percentage of TS (%TS). Volatile fatty acids concentrations like propionic acid, lactic acid and acetic acid were measured. Reactors were operated at 38 ± 1^0C. As a result of the experiment, different hydrolysis rates for starch, xylan, cellulose and protein were determined as 1.20/day, 0.70/day, 0.18/day, and 0.30/day, respectively. Also, at organic loading rate higher than 2.5 g $_{VS}$/L day, micro elements should be put to the reactors for anaerobic digestion process. At this rate of OLR, a stable anaerobic digestion process was obtained by addition of Fe(III)Cl_3 (Lübken et al., 2015).

Munk and Lebuhn (2014) investigated the stability of anaerobic digestion process under trace element depleted conditions. Maize silage was used as substrate. Cobalt, selenium and sodium were selected as depleted trace elements. Digesters were run semi-continuously at mesophilic temperature. Selenium was added to the one of the reactors, while cobalt was added to the second reactor. The third reactor was control reactor, which no trace element was added. Produced biogas quantity was measured continuously. Also, CH_4 and CO_2 concentrations were analyzed. As a result of the experiment, firstly, acidification was observed in the reactor without trace element addition at an OLR of 4.0 kg VS m^3/day. Selenium and cobalt supplementation had a positive effect on process stability. Reactor with cobalt acidified less than that of the selenium supplemented reactor. So, cobalt was determined as the most limiting element in this experiment (Munk and Lebuhn, 2014).

Studies conducted to analyze the impact of micro elements on biogas generation showed that trace elements have different impacts on anaerobic digestion according to the type of substrate and digestion conditions (Wei et al., 2014). The purpose of this experiment is to analyze the impacts of trace element (Co, Ni and Mo) addition on biogas generation from maize silage.

3. MATERIAL AND METHODS

3.1. Anaerobic Batch Reactors

Anaerobic digestion analyses were carried out in the laboratory. Batch reactors used for anaerobic digestion of maize silage are shown in Figure 3.1. The content of the anaerobic batch reactors is also presented in Table 3.1. In total, 6 sets of trials were performed in this experimental work. During the experiments, the impacts of the selected trace metals, namely Ni, Co and Mo, solely and in combination, on biogas production from maize silage was investigated.

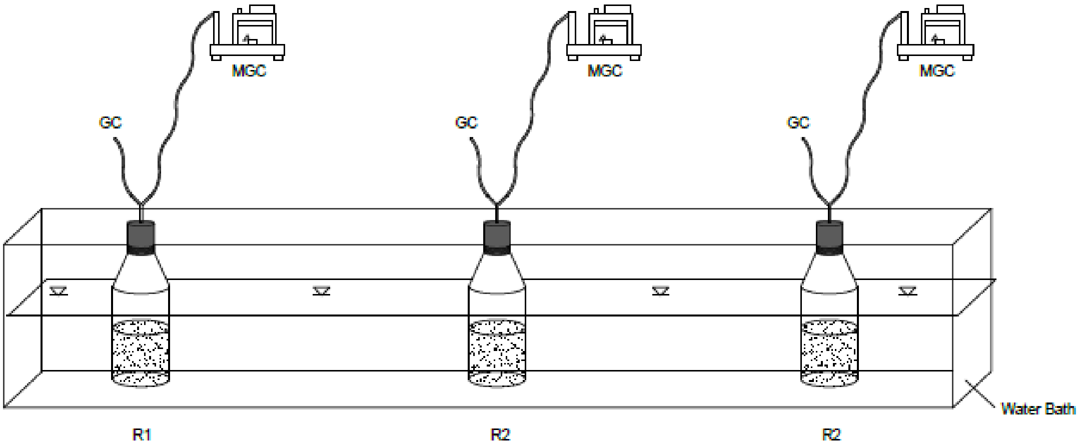

Figure 3.1. Configuration of the system for anaerobic digestion.

Table 3.1. Content of anaerobic batch reactors.

Set	**Reactor**	**Maize Silage + Water**	**I/S**	**Anaerobic Seed Sludge**	**Buffer Solution (1N $NaHCO_3$)**	**Ni**	**Co**	**Mo**
1	R1 (Control Reactor)	250mL	1:3	250mL	100mL	-	-	-
	R2 (3 parallel)	250mL	1:3	250mL	100mL	0.1mg/L	-	-
2	R1 (Control Reactor)	250mL	1:3	250mL	100mL	-	-	-
	R2 (3 parallel)	250mL	1:3	250mL	100mL	-	0.1mg/L	-
3	R1 (Control Reactor)	250mL	1:3	250mL	100mL	-	-	-
	R2 (3 parallel)	250mL	1:3	250mL	100mL	0.1mg/L	0.1mg/L	-
4	R1 (Control Reactor)	250mL	1:3	250mL	100mL	-	-	-
	R2 (3 parallel)	250mL	1:3	250mL	100mL	-	-	0.05mg/L
5	R1 (Control Reactor)	250mL	1:3	250mL	100mL	-	-	-
	R2 (3 parallel)	250mL	1:3	250mL	100mL	0.1mg/L	0.1mg/L	0.05mg/L
6	R1 (Control Reactor)	250mL	1:3	250mL	100mL	-	-	-
	R2 (3 parallel)	250mL	1:3	250mL	100mL	0.5mg/L	0.5mg/L	0.25mg/L

Borosilicate glass bottles were used as reactors for anaerobic digestion. The total volume, effective volume and headspace of each bottle were 1000 mL, 600 mL and 400 mL, respectively.

A water bath (Nüve, NB 20) was used to keep the reactors at a fixed at 37^oC by using a heat controller, and the bottles were put in the water bath. A picture of the water bath is illustrated in

Figure 3.2. A thermometer was utilized to ensure that the mesophilic condition was sustained in the water bath that would improve the anaerobic digestion period. In addition, the level of water bath was checked every day and completed with water as it evaporated. The reactors were also mixed every day to ensure the complete contact between the microorganisms and the substrate.

Figure 3.2. The water bath (Nüve, NB 20).

The digesters were seeded with the anaerobically digested sludge to start and improve anaerobic degradation rate of the methane generation. The seed sludge utilized in this experiment was taken from a chips factory named Fritolay in İzmit. Total solids (TS) concentration and the volatile solids (VS) concentration of anaerobic sludge were 3 and 68%, respectively.

The reactors were filled with maize silage as the solo substrate and anaerobically digested sludge as inoculum. Maize silage was provided from a farm in İzmit. Maize silage and anaerobic granular sludge were kept in the cold room at 4°C prior to use. Before the addition to the reactors, maize silage and sludge were left at the room temperature and, their total solid (TS) and volatile solid (VS) values were measured. The results of these analyzes are shown in Results and Discussion part. The measured

TS values were used to determine the amount of maize silage and anaerobic sludge, which would be added to the digesters.

A specific concentration of maize silage and anaerobic seed sludge mixture (5% TS) was prepared for batch studies. An experimental trial picture is given in the Figure 3.3.

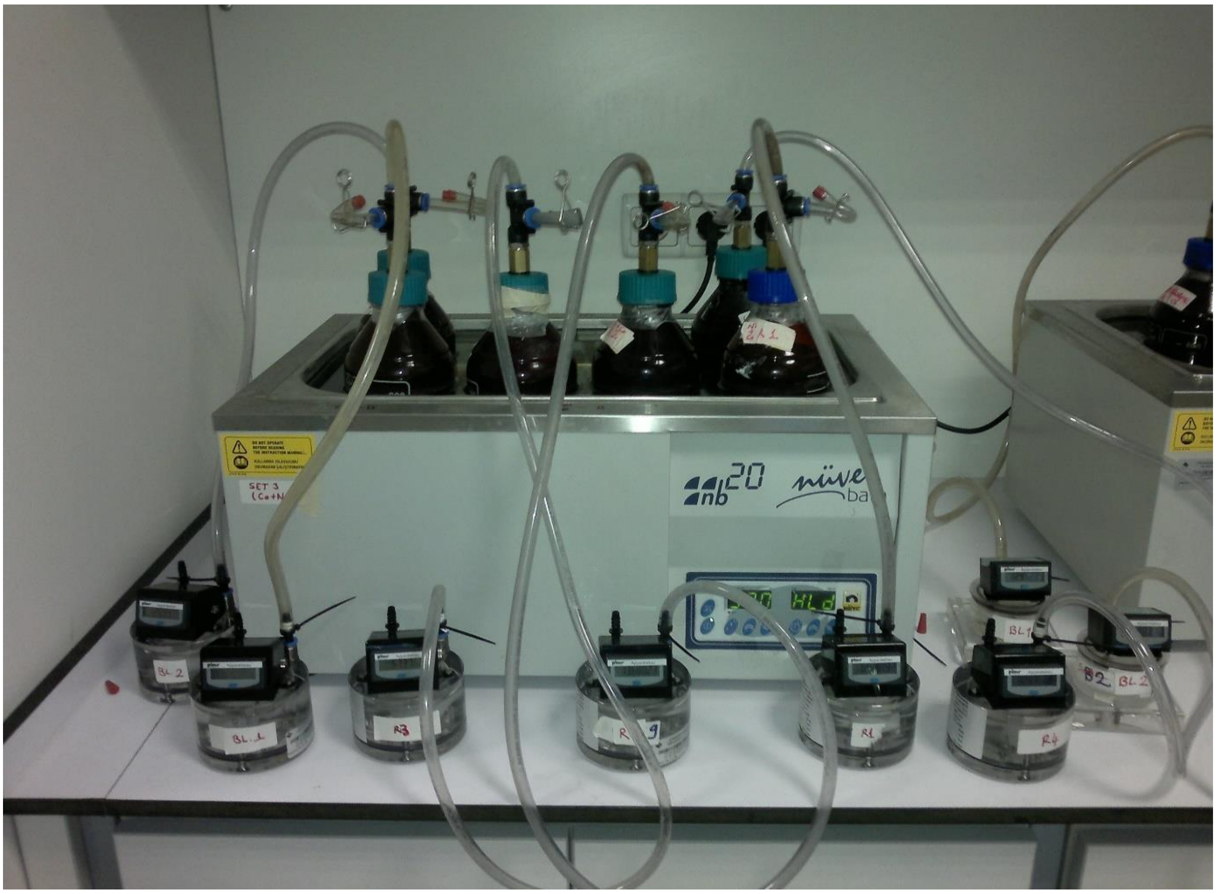

Figure 3.3. Experimental trial layout.

To analyze the impact of various micro elements on biogas generation from maize silage, 6 different trial were performed at different times for this experiment. The batch reactors were run in triplicate together with a control reactor (R1). Control reactors included only maize silage and anaerobic sludge, and trace elements were not added to control reactors. Throughout the anaerobic digestion process, the results of the control reactors were compared with the results of the reactors with trace elements, and the effect of trace elements addition was determined.

Maize silage and anaerobic sludge were analyzed, and trace element contents of silage and sludge were specified. According to the initial results obtained, Ni, Co and Mo were absent (or they were below detection limits) in the maize silage, so these elements were selected for the experiments. For the first set-up 0.6 mL Ni solution was added to the reactor to provide 0.1 mg/L Ni concentration, and the impact of Ni on biogas production was analyzed. For the second set-up 0.6 mL Co solution was added to the reactor to provide 0.1 mg/L Co concentration to determine the influence of Co. For the third set-up both 0.6 mL Ni and 0.6 mL Co solutions were added to the reactor to provide 0.1 mg/L Ni and 0.1 mg/L Co concentrations. 0.3 mL Mo solution was added to the reactor to provide 0.05 mg/L Mo concentration for the fourth set-up. For the fifth set-up, the effect of all of the trace elements was investigated. 0.6 mL Ni, 0.6 mL Co and 0.3 mL Mo were added to the reactor to provide 0.1 mg/L Ni, 0.1 mg/L Co and 0.05 mg/L Mo concentrations. For the sixth set-up the amount of trace elements added was increased by five times, and 3.0 ml Ni, 3.0 mL Co and 1.5 mL Mo were added to the reactor to provide 0.5 mg/L Ni, 0.5 mg/L Co and 0.25 mg/L Mo concentrations.

According to the preliminary studies, preparation of maize silage such as grinding provides large surface area and enhance the contact between microorganisms and substrates (Deublein, and Steinhauser, 2008). Hence, maize silage was grinded using a shredder. In addition, anaerobic sludge was mixed and homogenized. Subsequently, homogenized sludge and silage were added to the reactors. Then buffer solution (sodium bicarbonate) was added to the reactors. The mixture was diluted to 600 mL with deionized (DI) water. Micro element solution was prepared and added to the reactors. Stock solutions for Ni, Co and Mo were prepared utilizing reagent-grade metal solutions of 1000 mg/L $Co(NO_3)_2$ in HNO_3 (2-3%), 1000 mg/L $(NH_4)_6Mo_7O_{24}$ in H_2O and 1000 mg/L $Ni(NO_3)_2$ in HNO_3 (2-3%). Same procedure was followed and equal amounts of crop and sludge was used for the preparation of samples.

Preliminary studies demonstrated that the generation of methane could be observed within the pH range of 7.0–8.5 (McKendry, 2002). So, the pH of the digesters was initially adapted to 7.0-7.2. 6 N NaOH and 6N H_2SO_4 were used to obtain appropriate situations for the growth of methanogens.

Thereafter, plastic caps were used to close the reactors, and all connections and joints were controlled to be sure that there was no gas in and out. For 5 minutes, nitrogen (N_2) gas was fed to the bottles to maintain the anaerobic conditions in the bottles by displacing oxygen (O_2). The generated biogas in the bottles was collected and measured to determine the amount and content of the biogas. Milligascounters ® (MGC-1, Ritter, Bochum, Germany) were used to measure the generated daily biogas volume. The reactors were equipped with a gas gathering port which was fixed to the Milligascounter with a PVC hose placed at the top of the bottles. The other outlet was closed by a rubber tap to be used to take gas samples for GC analyses. To avoid flow of the siliconic fluid present in the MGC’s to the digesters, MGC’s were placed at a lower level than the bottles (Evranos and Demirel, 2015).

Figure 3.4. Milligascounter® (MGC-1) utilized for the biogas volume measuring.

3.2. Analytical Methods

First of all; maize silage, anaerobic sludge and their mixture were analyzed to determine total solid (TS), volatile solid (VS), pH, alkalinity, Total Kjeldahl Nitrogen (TKN), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), orthophosphate (PO_4^{3-}), Chemical Oxygen Demand (COD), C/N ratio, total organic carbon (TOC) and heavy metals content. The samples were measured in triplicate. The biogas generation volume was monitored day to day, and the content of biogas was determined approximately three times a week during the experiment. From parallel digesters, double samples were utilized for biogas analyses. The content of produced biogas (CH_4 and CO_2 composition) was determined using a HP 6850 gas chromatograph (Carboxen 1010 plot column 30 m x 0.53 mm). The chromatograph was also fitted with a thermal conductivity detector (TCD). For the carrying gas (2 mL min^{1-}), Helium gas was utilized. Calibration was performed by utilizing 99.99 % Supelco methane and carbon dioxide standards and 5% gas mix. The operation temperature for injection port and detector were selected as at 150 °C and 160 °C, respectively. The temperature of the oven was adjusted to begin at 70 °C and was raised 5 °C per minute step by step till the final temperature was

150 °C. In order to correct gas chromatograph and achieve correction curves, methane, gas mixture and pure carbon dioxide standards were utilized.

All of the measurements were carried out by following the Standard Methods for the Examination of Water and Wastewaters (APHA, 1998). Analytical protocol performed during the studies is given in the Table 3.2.

Table 3.2. Analytical protocol of the study.

Parameter	Maize Silage	Inoculum (Sludge)	Substrate + Inoculum before digestion	Substrate + Inoculum after digestion	Biogas
TS, %	+	+	+	+	
VS, % of TS	+	+	+	+	
TSS, g/kg	+				
TKN, g/kg	+				
NH_4^+, g/kg	+				
TP, mg/L	+				
PO_4^{3-}, g/kg	+				
C, %	+				
N, %	+				
H, %	+				
pH	+		+	+	
COD, mg/L	+				
NH_3-N, mg/L	+				
NH_3^+, mg/L	+				
Trace Elements (Cr, Mn, Fe, Ni, Cu, Zn, Al, Cd, Pb, Si, Co, Mo), µg/g	+	+			
Biogas Volume					+
Biogas Content (CH_4, CO_2), %					+

3.2.1. Total Solid (TS)

Standard Methods were used to identify Total Solid (TS) content of substrate and inoculum separately and as a mixture. Examples were made homogeneous. Then, the examples were placed in tared ceramic dishes, and weights of the examples were measured. On the steam bath (Julabo Ecotemp TW 12), the examples were evaporated. Then, the examples were held at 105^0C in the drying oven (Nüve-FN 500). Afterwards, the examples in the dishes were cooled in the desiccator and weighed.

3.2.2. Volatile Solid (VS)

Volatile solids (VS) are rough approximation of organic matter present in the solid part of the sample. After the identification of total solid composition, incineration process was applied to the dried examples to constant weight at 550 ± 50 °C in the oven (Nüve – MF 120). Solids remaining after ignition are fixed solids since the weight of lost on ignition represents the volatile solids.

3.2.3. pH

The pH values of the digester content were determined because of its indicator feature. For this reason, pH values were measured at the beginning and at the end of the trials. A pH probe connected with a WTW Inolab pH 7110 pH meter and Hanna Instruments H1221 were used after the calibration with pH values of 4, 7 and 10.

3.2.4. Total Kjeldahl Nitrogen (TKN)

Nessler Method was used to measure Total Kjeldahl Nitrogen (TKN). When the organic nitrogen and ammonia nitrogen are summed, Total Kjeldahl Nitrogen value can be determined. The procedure in HACH/DR 2010 Spectrophotometer Handbook was implemented. Sample was digested with concentrated sulphuric acid at 440 °C in Digesdahl Digestion Apparatus and Hydrogen Peroxide was added. One drop of TKN indicator and 8N KOH solution were added to the sample until the first permanent blue color was observed. The specimen volume was completed to 25 mL and then mineral stabilizer, polyvinyl alcohol dispersing agent were added. Same procedure was followed by using deionized water as the control. The TKN of the sample was read as mg/L at 460 nm by using HACH DR / 2010 Spectrophotometer.

3.2.5. Total Phosphorus (TP)

Digesdahl Digestion Device was used to digest the samples at 440 °C. Then, Phosver 3 Method was used to measure Total Phosphorus content of the sample. 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.

3.2.6. Phosphate (PO_4^{3-})

Ascorbic Acid Methods was used to identify the Phosphate (PO_4^{3-}) content of the examples which was defined above. However, the examples were used without digested for Phosphate analysis.

3.2.7. Ammonium Nitrogen (NH_4^+-N)

Ammonium nitrogen (NH_4^+-N) content of the digestate samples was monitored by using Nessler Method. As a first step, 1 g dried sample was poured in 25 mL mixing graduated cylinder. Then, three drops of mineral stabilizer and polyvinyl alcohol dispersing agent were added and 1 mL of Nessler Reagent was poured into each cylinder. Ammonium concentration as mg/L $NH4^+$-N read at 425 nm by using HACH DR / 2010 Spectrophotometer.

3.2.8. Chemical Oxygen Demand (COD)

COD is widely utilized to characterize organic compounds in liquid mixtures. It is the predominant parameter for most of the wastewater treatment processes. Although organic matter is predominantly described in terms of VS for digestion of maize silage, soluble s(COD) concentration is also determined in order to have more information on the characteristics of substrate and sludge mixture. This analysis was made by closed reflux colorimetric method. Firstly, 2.5 mL samples were placed into HACH vials. Afterwards, 1.5 mL potassium dichromate and 3.5 mL of acid digestion mixture were put 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 determined colorimetrically at

600 nm by utilizing HACH DR / 2010 Spectrophotometer. Potassium hydrogen phthalate (KHP) solutions were used for preparing calibration curves (0-800 ppm).

3.2.9. C, H and N Ratios

C, H and N ratios of the maize silage were identified using a COSTEC ECS 4010 CHNSO elemental analyzer.

3.2.10. Heavy Metal Concentrations

Heavy metal content of maize silage was determined. Measurement of the pollutants becomes possible when all the desired metals are extracted from the soil media to aqueous phase. For this reason, to determine the heavy metal concentration of samples they were digested by strong acids in order to set metals free into a certain amount of liquid solution. During the digestion, organic part of the soil is completely destroyed, releasing the metal ions bound to it (Güney, 2006). Total metal concentrations in soils were determined on filtered liquids extracts with an Inductively Coupled Plasma Instrument Perkin Elmer OES Optima 2100 DV ICP-OES after microwave digestion.

4. RESULTS AND DISCUSSION

4.1. Characteristics of Substrate and Seed Sludge

4.1.1. Maize Silage Analysis

Maize silage was utilized as the mono substrate in this experiment. Before the experiments, maize silage was measured for pH, total solids (TS %), volatile solids (VS %), total suspended solids (TSS), COD, C, N, H, TKN, TP, ortophosphate, ammonium-N and heavy metals. The amount of maize silage (substrate), which should be added to the anaerobic batch reactors, was calculated according to the TS content of maize silage. Two parallel samples were obtained for each sample. The characterization of maize silage is given in Table 4.1. The metal content of maize silage is given in Table 4.2.

Table 4.1. Characterization of maize silage.

PARAMETER	**VALUE**
TS, %	38
VS, % of TS	96
TSS, g/kg	0.7
TKN, g/kg	22.1
NH_4^+, g/kg	0.9
TP, mg/L	1.4
PO_4^{3-}, g/kg	1.1
C, %	44.6
N, %	2.4
H, %	6.3
pH	5.8
COD, mg O_2/L	5981.7
NH_3^+, mg/L	133

Table 4.2. Metal content of maize silage.

TRACE ELEMENT	**CONTENT (µg/g)**
Cr	1
Mn	25.3
Fe	318.3
Ni	ND
Cu	5.6
Zn	29.7
Al	405.9
Cd	ND
Pb	1.5
Si	2587.6
Co	ND
Mo	0.5

ND: None detected.

4.1.2. Anaerobic Seed Sludge Analysis

Anaerobic seed sludge (inoculum) was analyzed for TS, VS, and heavy metals before the experiments. TS and VS content of inoculum are given in Table 4.3, and metal content of anaerobic sludge are given in Table 4.4.

Table 4.3. TS and VS content of anaerobic sludge.

PARAMATER	**VALUE**
TS, %	3
VS, % of TS	68

Table 4.4. Metal content of anaerobic sludge.

TRACE ELEMENT	**CONTENT (µg/g)**
Cr	27
Mn	145
Fe	9261.1
Ni	4.8
Cu	47.2
Zn	290.5
Al	9701.8
Cd	0.5
Pb	15.4
Si	2431.1
Co	4.3
Mo	2.9

4.2. Substrate Analysis Before and After the Digestion

Before and after the experiments, the substrate was analyzed for TS, VS and, pH values. The results are given in Table 4.5.

Table 4.5. The condition of the reactors at the beginning and at the end of the experiments.

Set	Reactor	Initial TS (%)	Initial VS (% of TS)	Final VS (% of TS)
1	R1 (Control)	5.0	75.8	48.3
1	R2 (0.1 mg/L Ni)	5,0	75.2	47.2
2	R1 (Control)	5.7	85.6	49.1
2	R2 (0.1 mg/L Co)	5.6	85.4	48.2
3	R1 (Control)	5.2	85.7	50.0
3	R2 (0.1 mg/L Ni + 0.1 mg/L Co)	5.3	83.7	47.7
4	R1 (Control)	5.5	82.7	44.6
4	R2 (0.05 mg/L Mo)	5.3	81.6	47.1
5	R1 (Control)	5.6	82.8	46.6
5	R2 (0.1 mg/L Ni + 0.1 mg/L Co + 0.05 mg/L Mo)	5.0	81.1	46.7
6	R1 (Control)	5.3	81.6	49.2
6	R2 (0.5 mg/L Ni + 0.5 mg/L Co + 0.25 mg/L Mo)	5.6	83.1	49.0

Volatile solids content of each reactor was measured to identify the organic matter content of each reactor before and after the anaerobic digestion tests and the consumption rate of organic matter as a consequence of the degradation. As a result of biological degradation, the VS content of all of the reactors decreased at the end of the experiments. The highest VS degradation rate was seen for the control reactor of set 4 including maize silage and inoculum without trace element addition. However, in order to identify the performance of anaerobic digestion process, VS degradation should be considered together with biogas and methane yields.

Table 4.6. The pH values at the beginning and at the end of the experiments.

Set	Reactor	Initial pH	Final pH
1	R1 (Control)	7.14	7.73
1	R2 (0.1 mg/L Ni)	7.16	7.74
2	R1 (Control)	7.06	7.71
2	R2 (0.1 mg/L Co)	7.06	7.71
3	R1 (Control)	7.07	7.68
3	R2 (0.1 mg/L Ni + 0.1 mg/L Co)	7.08	7.72
4	R1 (Control)	7.07	7.71
4	R2 (0.05 mg/L Mo)	7.09	7.71
5	R1 (Control)	7.05	7.73
5	R2 (0.1 mg/L Ni + 0.1 mg/L Co + 0.05 mg/L Mo)	7.06	7.76
6	R1 (Control)	7.02	7.70
6	R2 (0.5 mg/L Ni + 0.5 mg/L Co + 0.25 mg/L Mo)	7.02	7.70

The pH is also one of the most major factors which has a very important effect on anaerobic digestion process. Even a slight change in pH of the reactor could cause reduction of biogas production. During anaerobic digestion process, the quantity of volatile fatty acids and carbon dioxide affects the pH level of the reactor. The alkalinity level of the anaerobic sludge was quite high, so the pH of the mixture in the reactors prepared above the desired level. In the output of the batch reactors, a pH range of between 7.70-7.76 was observed after the digestion. These pH values showed that the anaerobic digestion process were well-balanced in the reactors.

4.3. Gas Analysis

Biogas volume (daily gas volume and cumulative gas volume), biogas quality (considerably the methane and carbon dioxide content of biogas), biogas yield and specific methane yield were analyzed as gas analysis. Biogas volume can be considered as the main indicative parameter of a succeeded anaerobic digestion process. Methane and carbon dioxide are the main output of anaerobic conversion of biomass, so the biogas quality was also determined. In addition to these parameters, biogas yield and specific methane yield were also analyzed since these two parameters are utilized to identify the economic viability of the biogas generation system. The results of daily gas production, cumulative gas production, gas composition, biogas yield and specific methane yield are given in the following sections.

4.3.1. Daily Biogas Production

The quantity of biogas produced in the anaerobic batch reactors was measured and recorded every day. The daily biogas volume are given in Figure 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6, respectively.

In Set 1, the maximum daily biogas volume was observed with a value of 1105 mL in the reactor 1 (Control reactor without Ni addition) at the 2nd day. Since the fifth day of the experiment, the daily biogas volume in reactor 2, containing 0.1 mg/L of Ni, was higher than that of the control reactor. The daily biogas volume was always higher than that of the control reactor since the 5th day to the last day of the test. In Set 1, daily biogas volume started to decrease after the 11th day, and at 30th day, it reached the minimum value.

In Set 2, reactor 2 with 0.1 mg/L Co supplementation showed the highest biogas volume with a value 1820 mL at second day of the set up. The experiment was conducted for 30 days. During the set-up, the reactor 2 always showed higher daily biogas production than those of the control reactor. After the 15th day, the biogas production started to decrease.

In Set 3, at 3rd day of the experiment, the maximum daily biogas production was observed with a value of 1784 mL in the reactor 2 with 0.1 mg/L Ni and 0.1 mg/L Co supplementation. After the 16th day of the experiment, the biogas production started to decrease. During the set-up, reactor 2 with Ni and Co addition produced more biogas than that of the control reactor without trace element supplementation.

Throughout the methane fermentation process, enzymes are needed and trace elements play like cofactors in enzymes, so obtaining the optimum concentration of micro elements in the reactor are very brutal for an effective biogas generation process. Especially, in case of mono digestion of energy crops, the optimum micro element content should be obtained because, if the trace element content is inadequate and any manure is not added, it might cause a decrease in biogas generation. If the trace element content is too high, it also causes lots of difficulty in anaerobic digestion process. Before the experiment, the trace element content of maize silage was measured and it was determined that maize silage did not have any Ni and Co (Pobeheim et al, 2010b; Hinken et al, 2008; Zandvoort et al, 2006; Bartacek et al, 2008; Nordberg et al., 2007).

In Set 4, Mo addition increased the anaerobic digestion period. The experiment was conducted for 45 days. Also, it prompted to increase biogas generation. The control reactor showed the maximum daily biogas volume with a value 1912 mL, while the biogas production of the reactor 2 (0.05 mg/L of Mo addition) was 1900 mL. After the 15th day of the experiment, the daily biogas production started to decrease. After the 26th day of the experiment, the biogas volume produced daily in reactor 2 with Mo supplementation was always higher than that of the control reactor.

In a previous study, methane generation was not influenced by supplementation of Mo when maize silage used as a substrate in anaerobic digestion process (Pobeheim et al., 2010b). However, in this work, a stimulative effect of Mo addition could be observed for the concentration range studied.

In Set 5, the experiment was conducted for 45 days. At the 3rd day, the reactor 2 with Ni, Co and Mo addition with concentrations of 0.1 mg/L, 0.1 mg/L and 0.05 mg/L, respectively produced the maximum daily biogas volume with a value 2340 mL at 3rd day. The same day, the daily produced biogas was 1223 mL. After the 13th day of the start, the biogas production started to decrease.

In Set 6, the experiment was conducted for 40 days. The maximum daily produced biogas volume was observed at control reactor (R1) with a value 2401 mL at 4th day of the trial. Reactor 2 with 0.5 mg/L of Ni, 0.5 mg/L of Co and 0.25 mg/L of Mo supplementation produced approximately three times more biogas than the control reactor. At that day, the biogas production value of the reactor 2 was 1896 mL while this value of the control reactor was 591 mL. After the 4th day, the biogas production at the reactor 2 was always higher than that of the control reactor. The daily biogas generation started to reduce after the 14th day of the experiment.

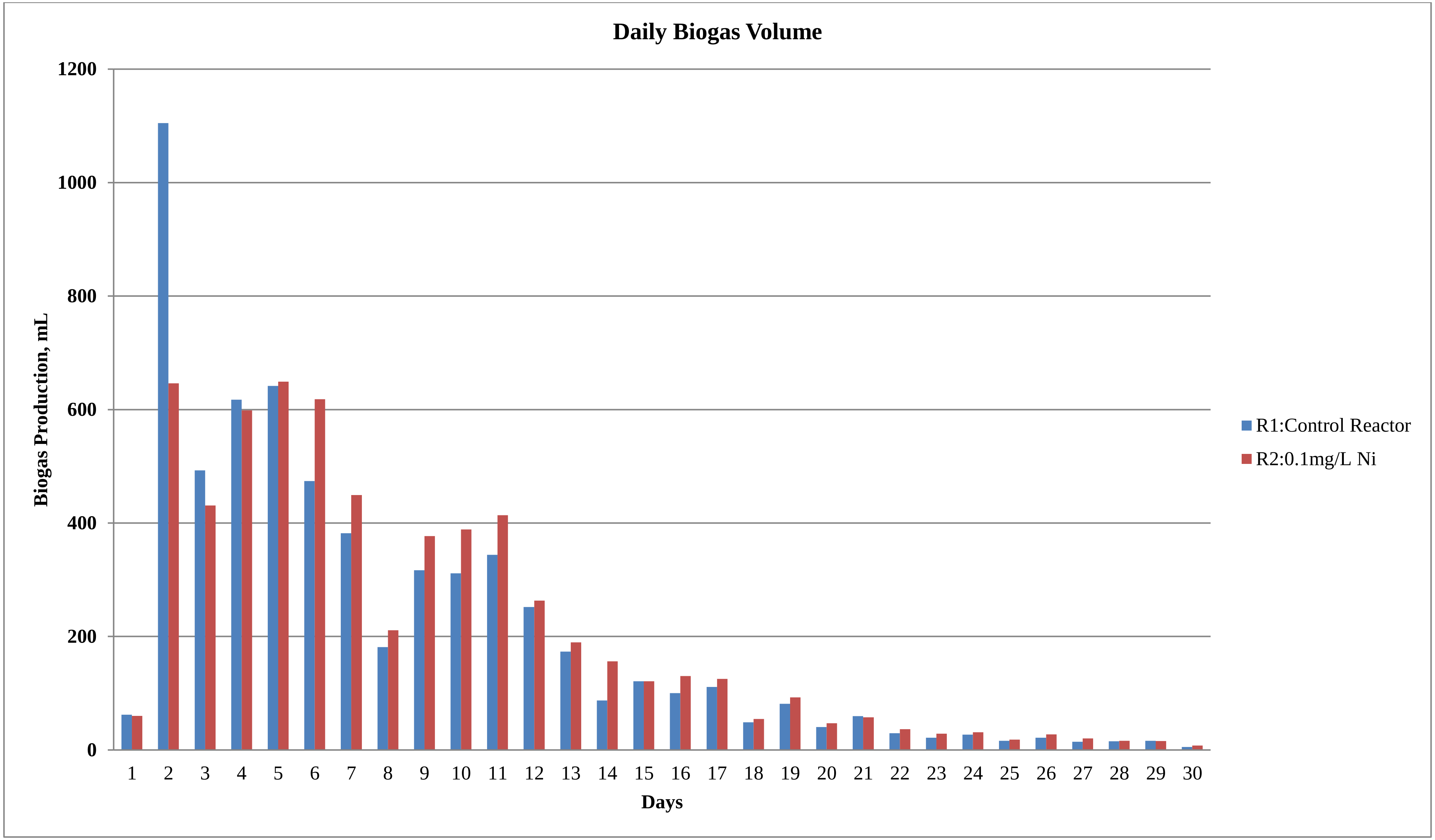

Figure 4.1. Daily biogas production in the reactors for the 1st set-up.

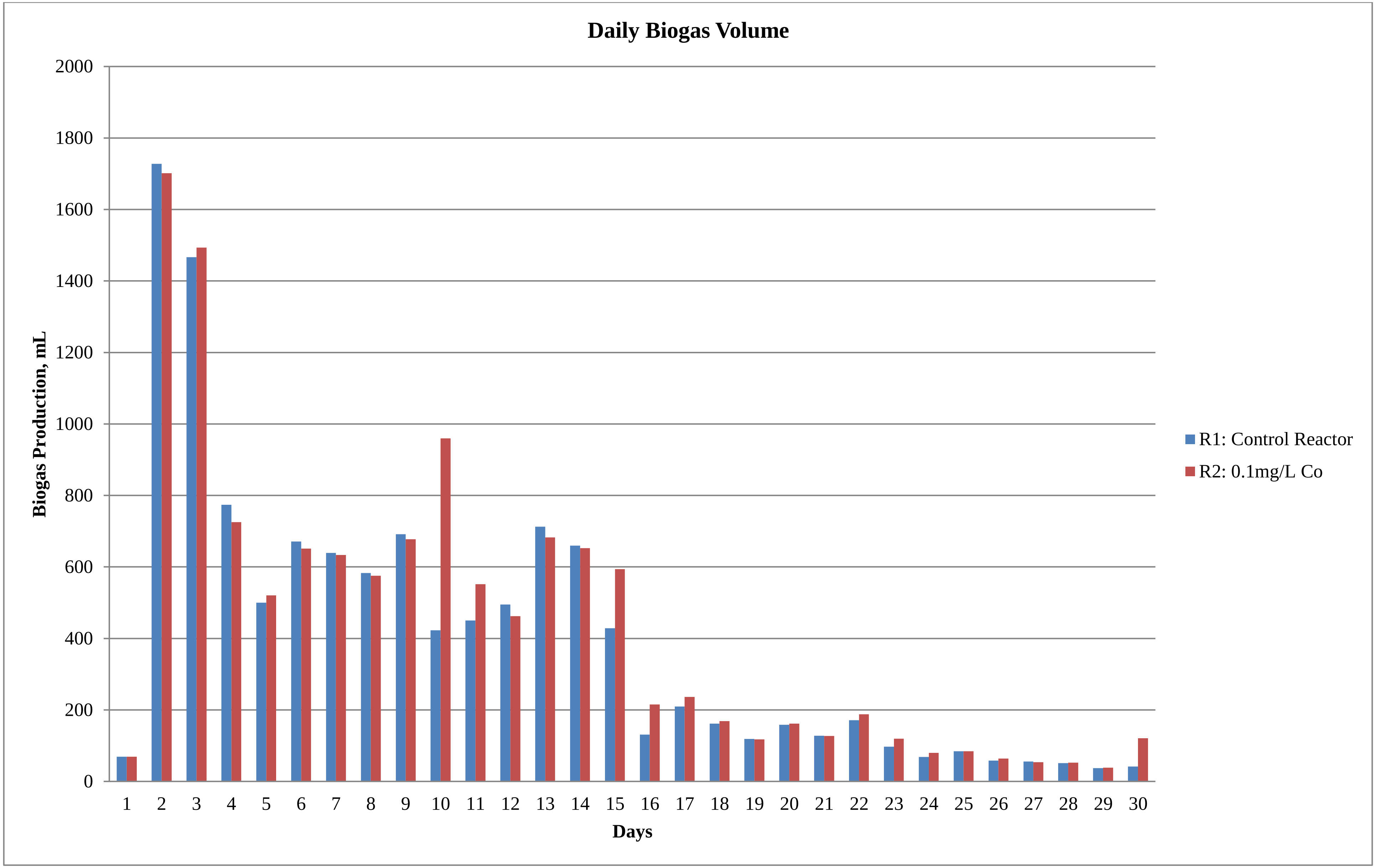

Figure 4.2. Daily biogas production in the reactors for the 2nd set-up.

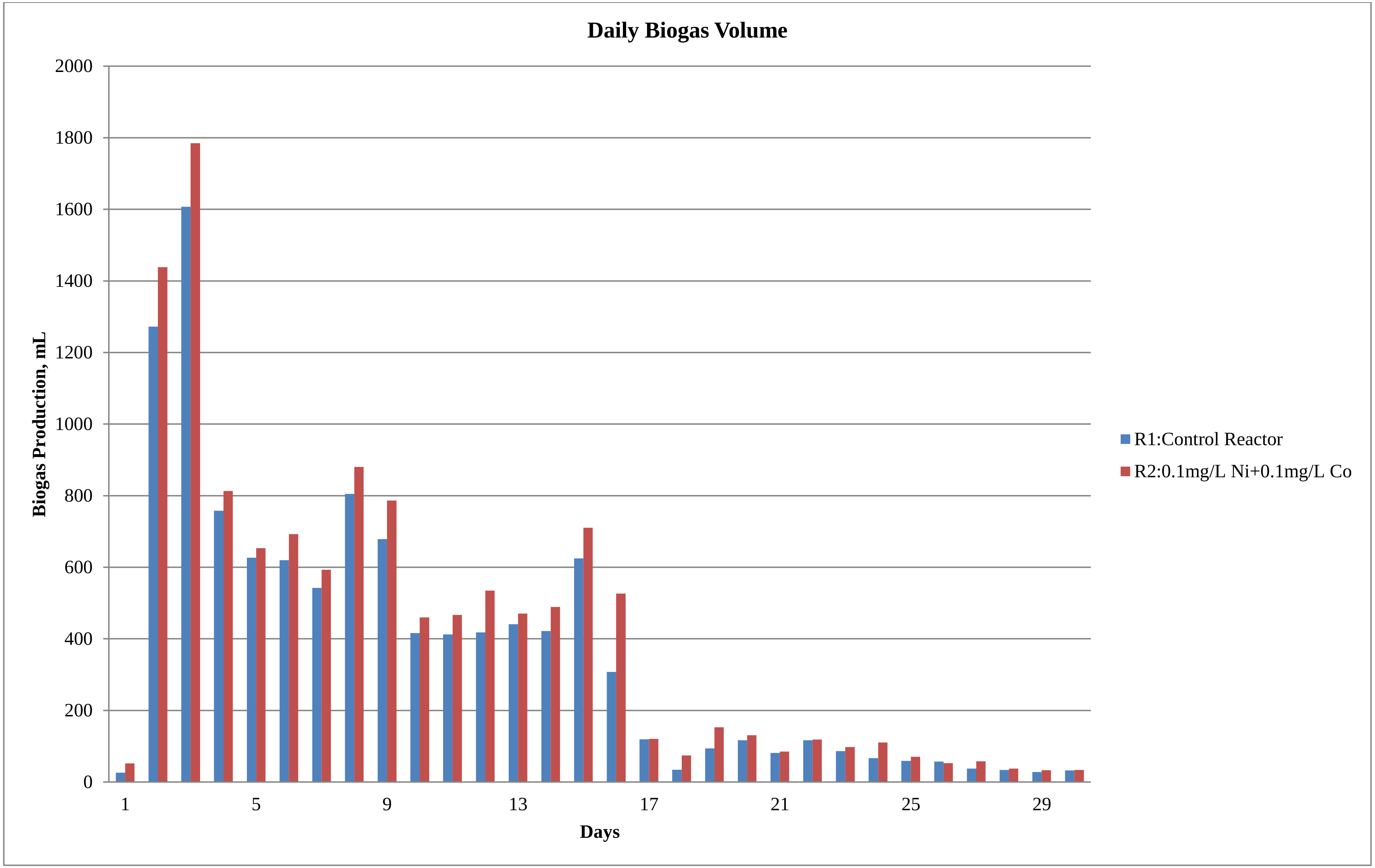

Figure 4.3. Daily biogas production in the reactors for the 3rd set-up.

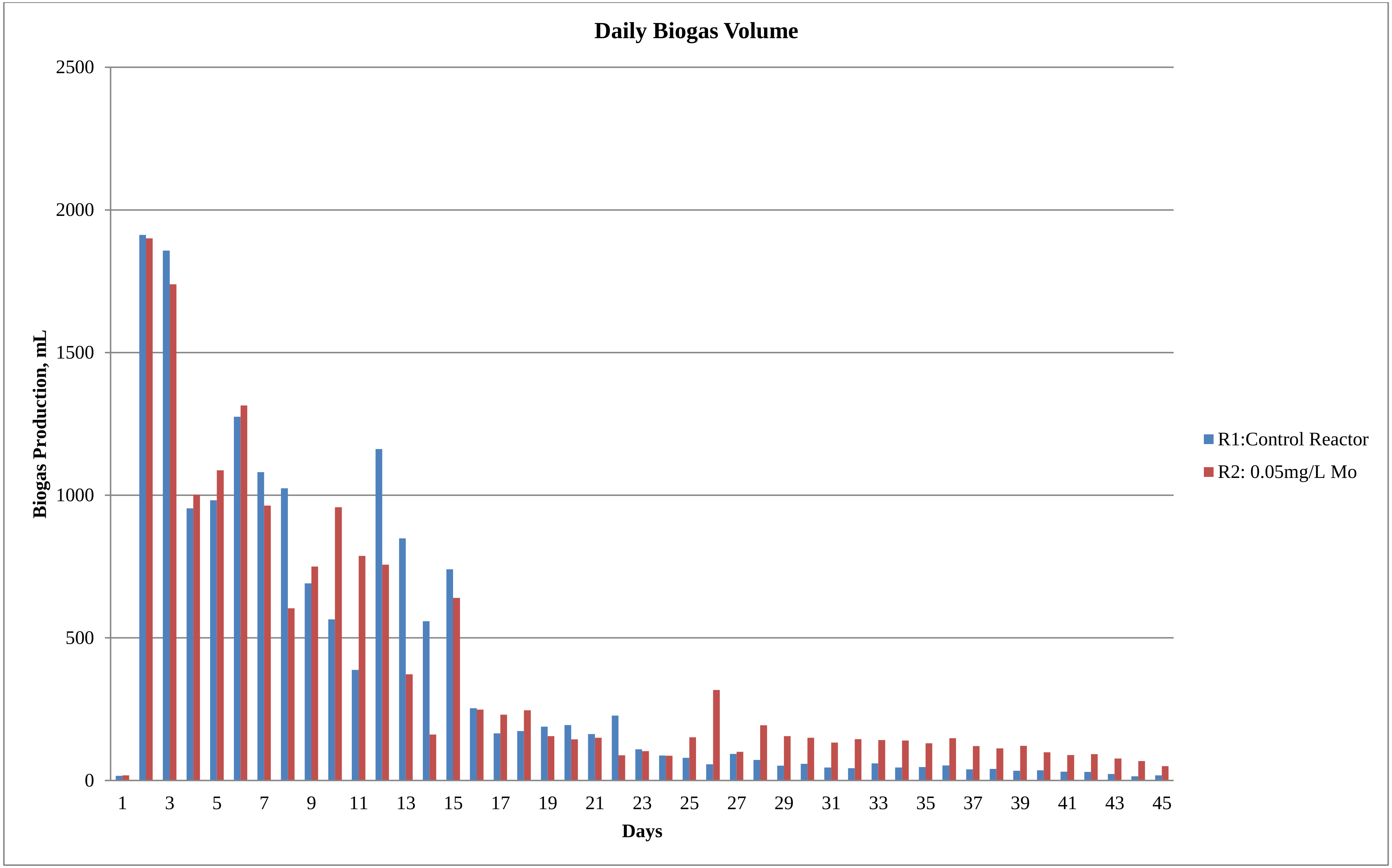

Figure 4.4. Daily biogas production in the reactors for the 4th set-up.

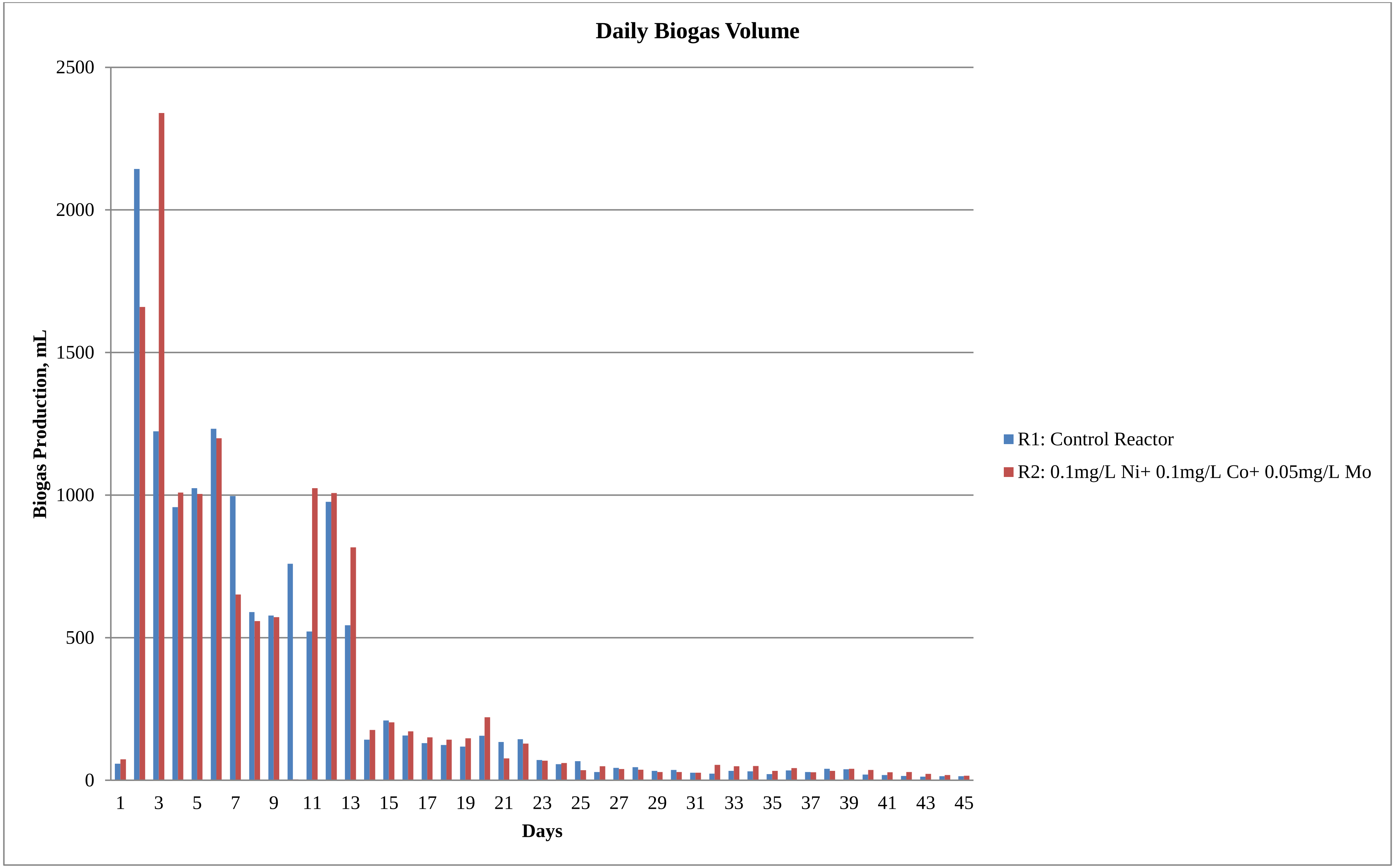

Figure 4.5. Daily biogas production in the reactors for the 5th set-up.

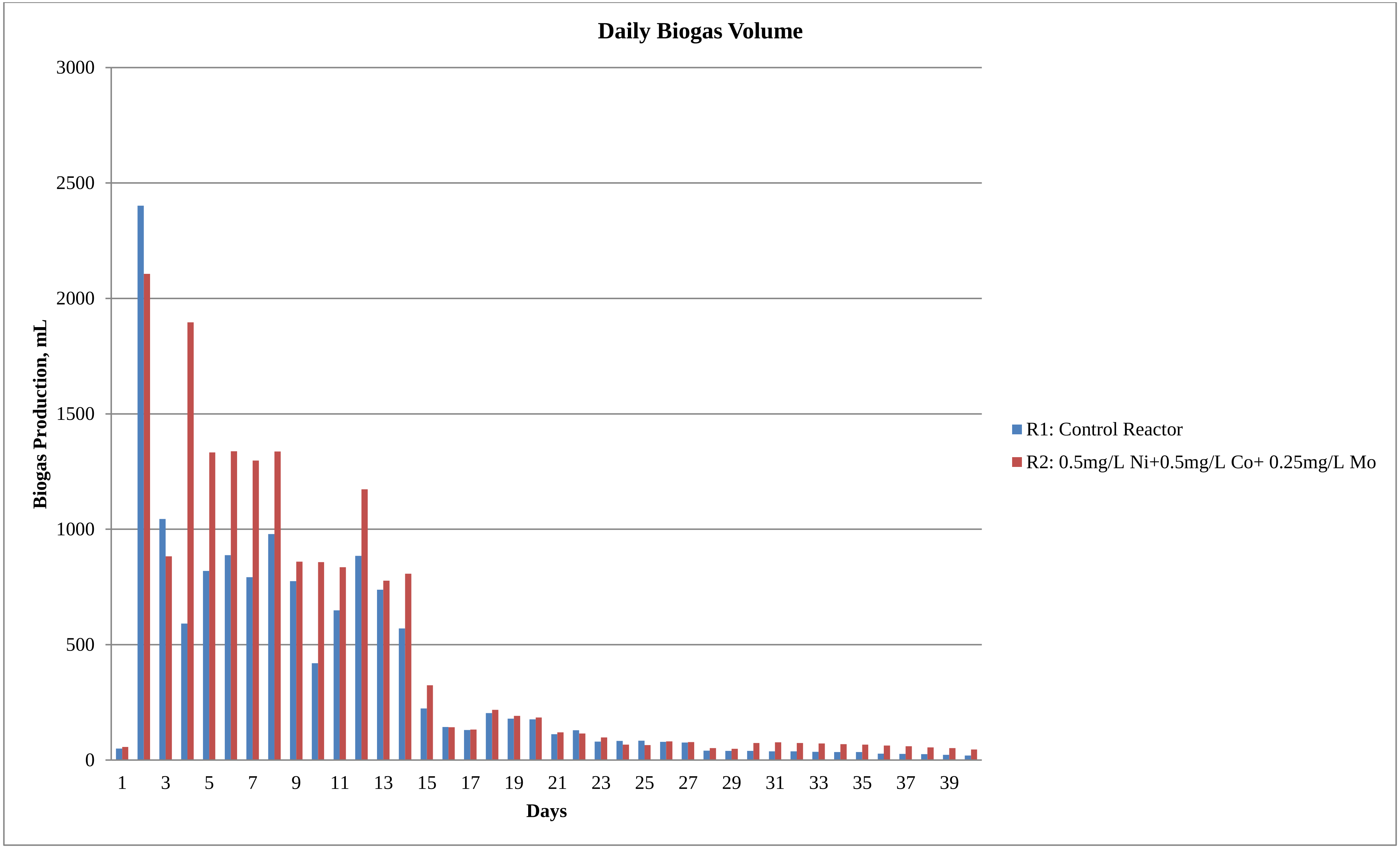

Figure 4.6. Daily biogas production in the reactors for the 6th set-up.

4.3.2. Cumulative Biogas Production

In addition to the daily biogas generation, cumulative biogas generation was also plotted and given in Figures 4.7, 4.8, 4.9, 4.10, 4.11, and 4.12, respectively. The batch anaerobic digestion tests were performed for a period of time between 30-45 days, depending on the period of time when the cumulative biogas generation curve reached a plateau in each experiment.

In Set 1, Ni supplementation did not have a significant impact on cumulative biogas generation. According to the results given in Figure 4.7, after the 16th day, the cumulative biogas production of reactor 2 (0.1 mg/L Ni supplementation) started to be higher than that of the control reactor.

In Set 2, Co addition increased the cumulative biogas generation. Since the beginning of the experiment, the cumulative biogas production of the reactor 2, with 0.1 mg/L of Co addition, was higher than that of the control reactor. Especially, after the 15th day of the trial, the difference between the reactor 1 (Control reactor) and reactor 2 (Co supplementation) started to increase. In Set 3, the cumulative biogas generation was also higher than that of the control reactor.

A study about long- term anaerobic digestion process of maize silage determined that when Co concentration was higher than 0,03 mg/L, it caused an inhibitory impact (Munk et al., 2010).

In Set 3, the difference between the cumulative biogas production of Control Reactor and Reactor 2 (0,1 mg/L Ni + 0,1 mg/L Co supplementation) was also higher than that of the set-up 1 and 2.

In Set 4, Mo supplementation did not have a big effect on cumulative biogas production. At the beginning of the trial, the cumulative biogas production of the control reactor and rector 2 (with Mo addition) was not different from each other. In a recent study, Mo had an additive effect on anaerobic digestion process when it was added together with Ni and Co (Schönheit et al., 1979).

In Set 5, biogas production lasted about 45 days, but Ni + Co + Mo supplementation together did not increase the cumulative biogas generation. The cumulative biogas generation in Reactor 2 (with Ni, Co and Mo addition) was higher than that of the control reactor, however, the difference was quite low.

In Set 6, the higher rates of Ni + Co + Mo supplementation together raised the cumulative biogas generation significantly in reactors with respect to control reactor without trace metal supplementation.

In this study, the C/N ratio was at the optimum value. In addition, via the nutrient supplementation, a stable anaerobic digestion process and the natural pH were achieved (Masse et al, 2013; Mohaibes and Heinonen-Tanski, 2012).

Also, it should not be forgotten that the operational conditions such as obtaining adequate mixing, optimum temperature, and pH values are as important as obtaining optimum nutrient concentration in the biogas plant (Masse et al, 2013; Mohaibes and Heinonen-Tanski, 2012).

In a recent study, the most appropriate concentration of Co in the reactor was determined as 5.9 mg/m^3 - 120 mg/m^3 This value for Mo was determined as 48 mg/m^3 (Takashima and Speece, 1990a).

Also, in another study, 100 mg/m^3d and 200 mg/m^3 d^3 was determined as the minimum concentrations of Co and Ni, respectively for methane generation (Takashima and Speece, 1989b).

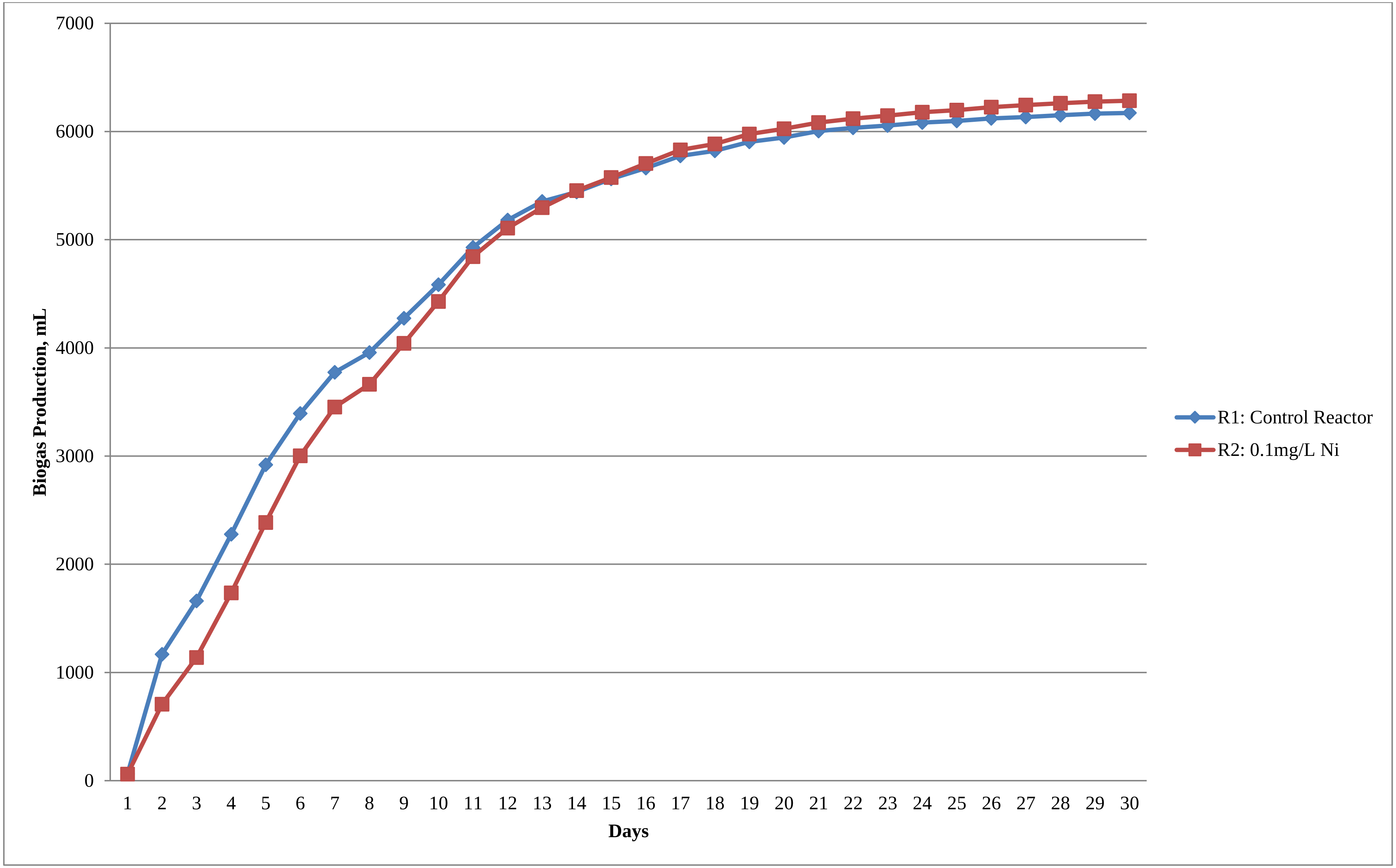

Figure 4.7. Cumulative biogas production in the reactors for the 1st set-up.

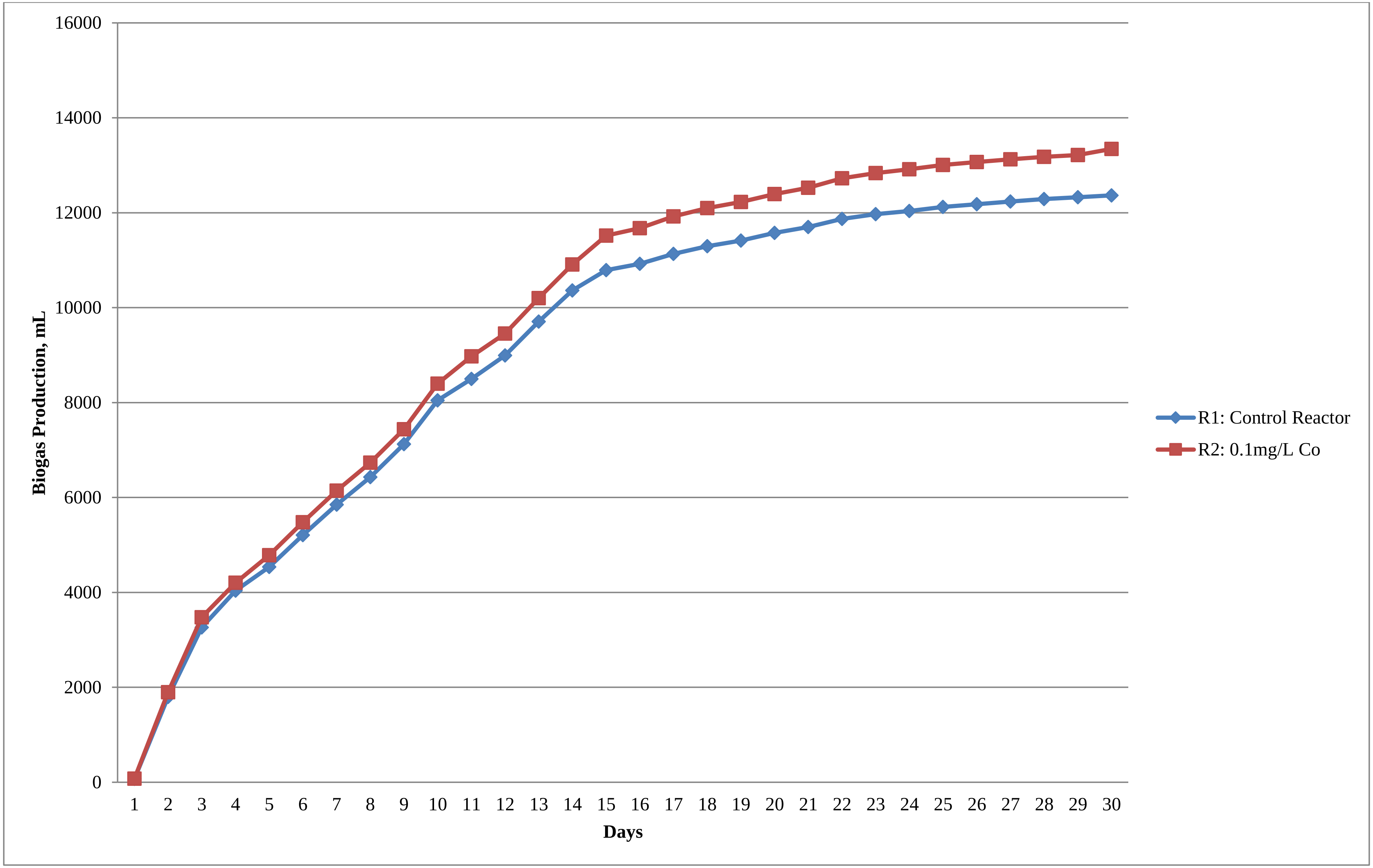

Figure 4.8. Cumulative biogas production in the reactors for the 2^{nd} set-up.

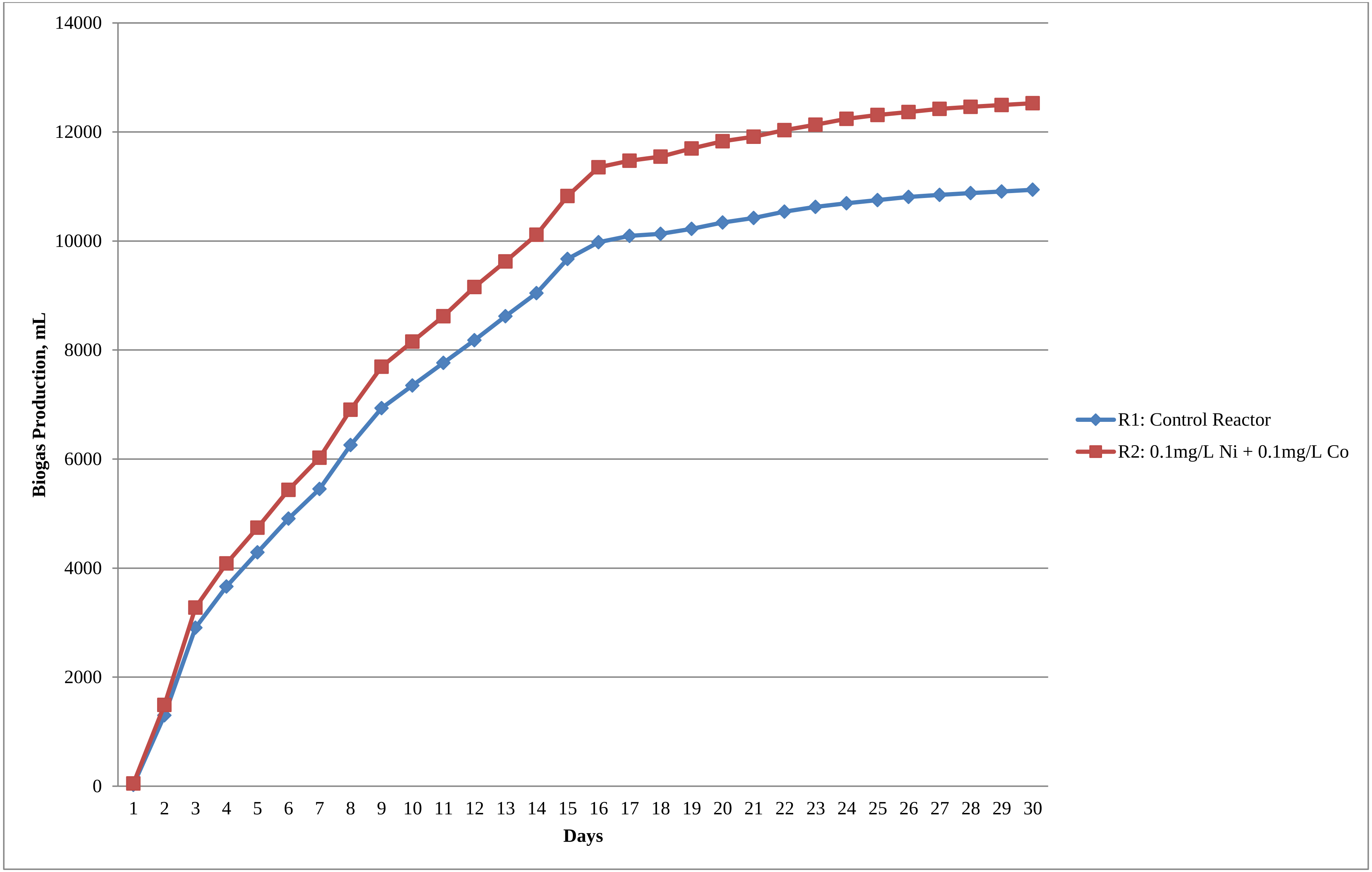

Figure 4.9. Cumulative biogas production in the reactors for the 3rd set-up.

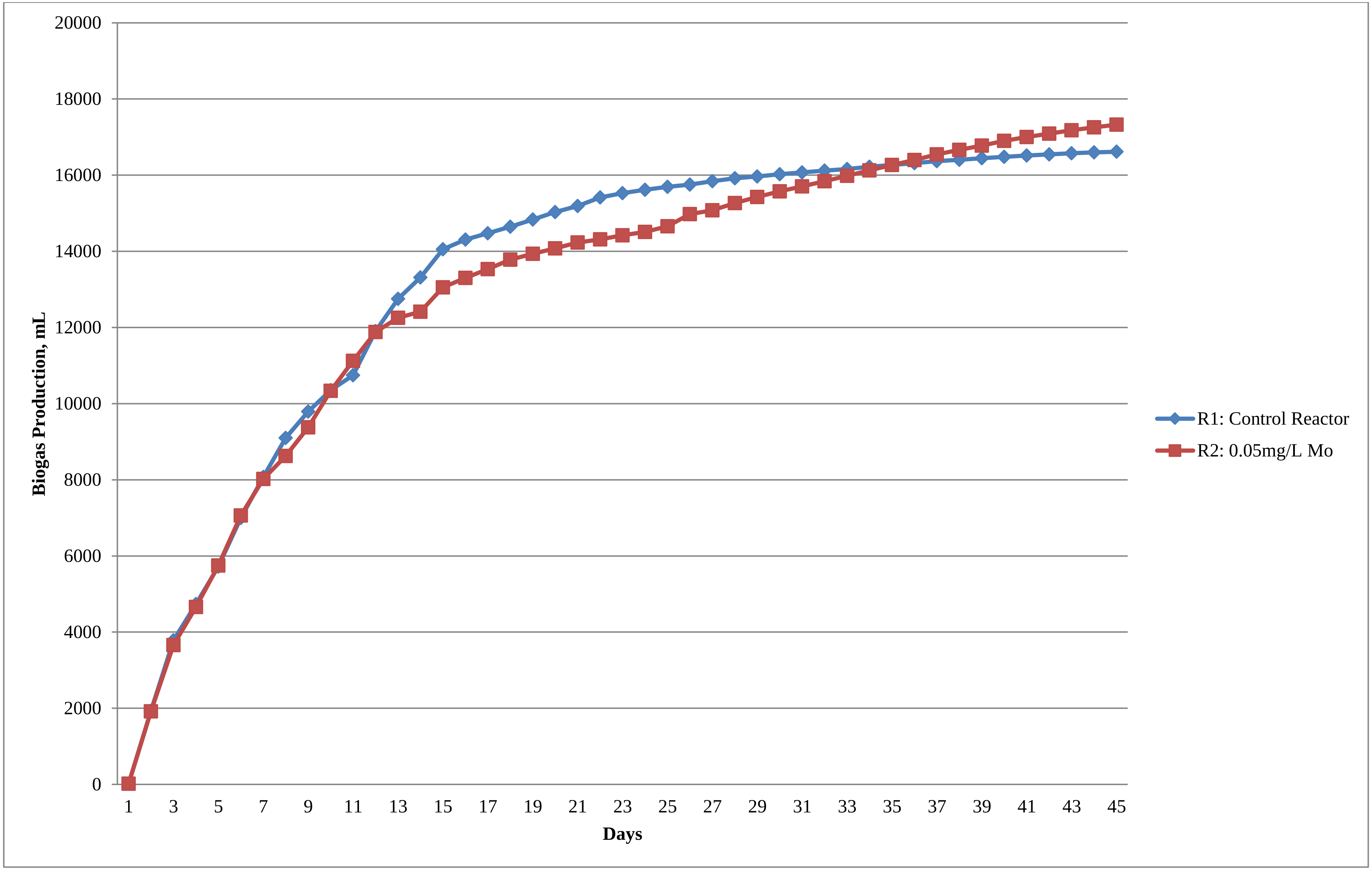

Figure 4.10. Cumulative biogas production in the reactors for the 4th set-up.

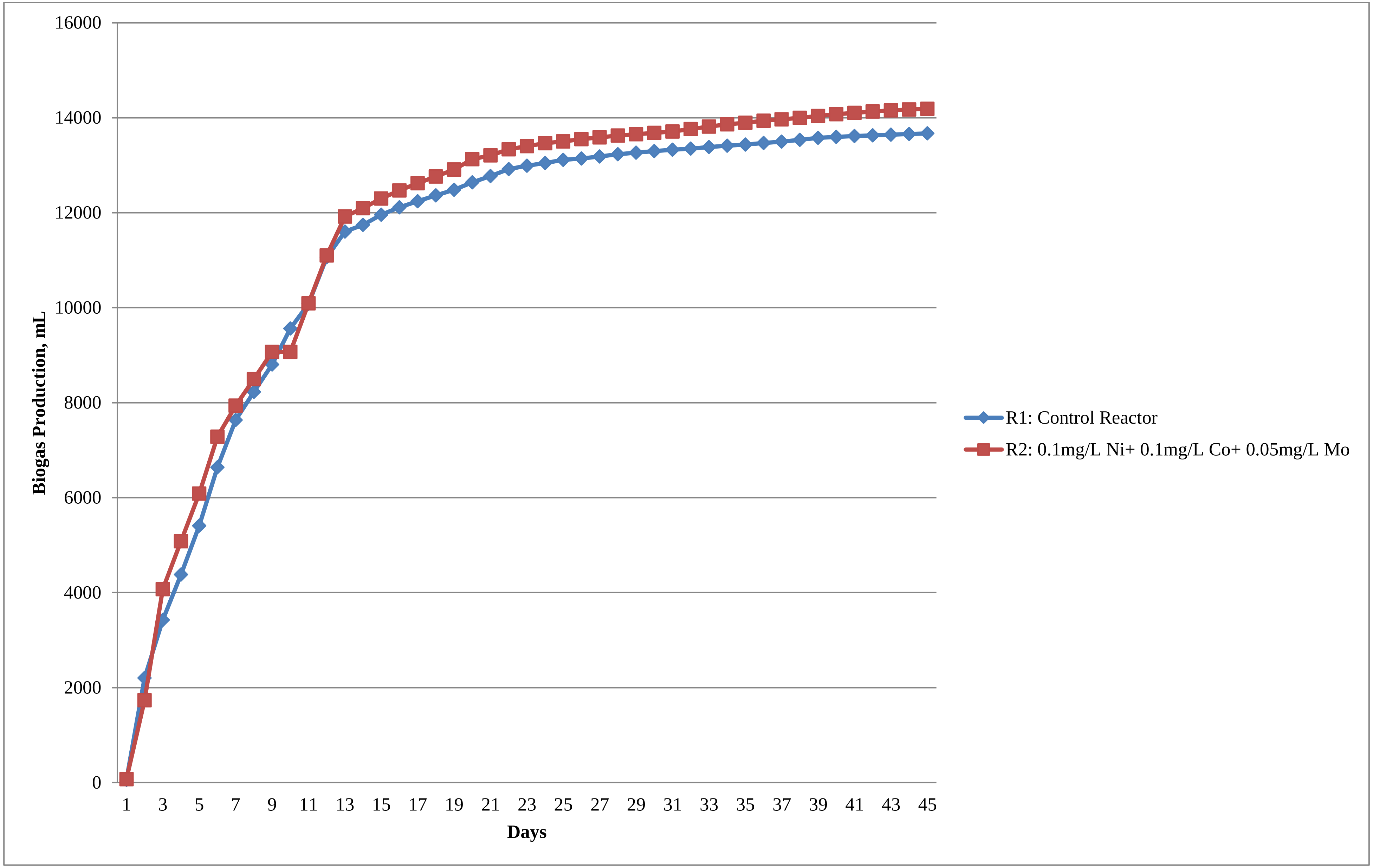

Figure 4.11. Cumulative biogas production in the reactors for the 5th set-up.

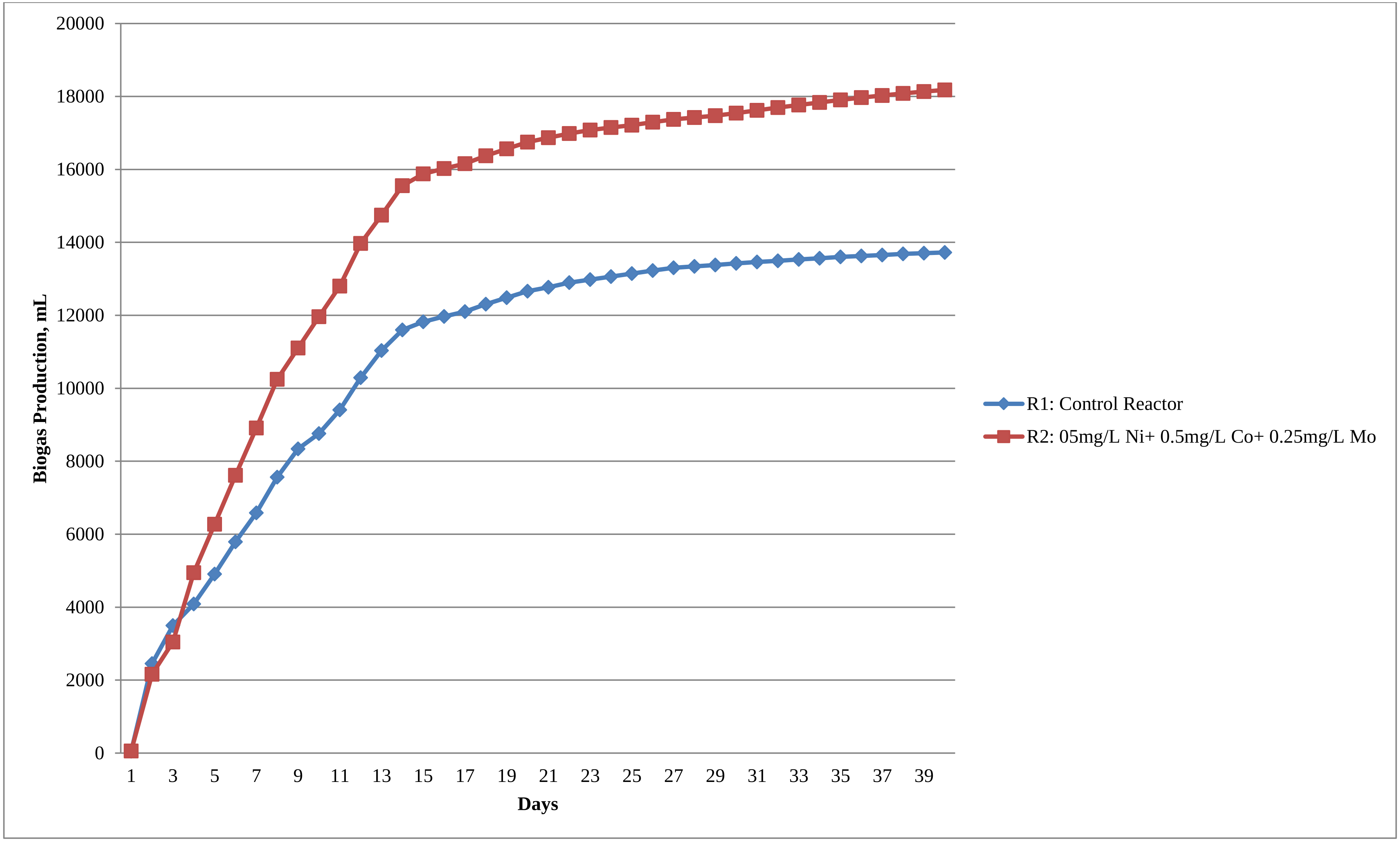

Figure 4.12. Cumulative biogas production in the reactors for the 6th set-up.

4.3.3. Biogas Composition

The biological activity and organic substance conversion in the digesters are represented by the composition of the biogas with regards to methane and carbon dioxide composition. Therefore, while analyzing the achievement of a biogas production process, composition of the biogas is one of the most important parameters that should be paid attention. Composition of biogas (CH_4, CO_2) in anaerobic batch reactors was measured in every few days (8-12 times) during the 30-45 days biogas generation process. The methane content of the biogas produced in the anaerobic batch reactors is given in Figure 4.13, 4.14, 4.15, 4.16, 4.17, and 4.18, respectively.

In a study, the effect of micro elements on biogas generation from maize silage in CSTR was investigated. It was found out that the most restrictive trace element was Co, Se and Mo (Lebuhn et al, 2008). In Set 1, the methane content of R2 (0.1 mg/L Ni) was about 60% and it was higher than that of the control reactor at all times during the anaerobic digestion process.

In Set 2, Co supplementation has a drastic effect on methane content of biogas. In Set 3, Co and Ni supplementation did not differ much from the control reactor.

In a study about the anaerobic digestion of maize silage, it was shown that process stability was affected by the limitation of Ni and Co negatively. The experiment was conducted in semi- continuous reactors under mesophilic conditions. Also, biogas generation did not increase when Ni and Co content was higher than a threshold value. An unstable anaerobic digestion process was also observed (Pobeheim et al., 2010a).

In Set 4, addition of Mo, higher methane production rates reached at the end of the experiment. In Set 5, methane content was also between 50-60%, and the methane content of the reactor was higher than that of the control reactor. In a recent study, Mo had an additive effect on anaerobic digestion process when it was added together with Ni and Co (Murray and Van Den Berg, 1981).

Trace element requirement of agricultural anaerobic digestion system should be supplied to improve the performance of reactors. In set 6, the methane content of produced biogas was increased by 0.5 mg/L Co + 0.5 mg/L Ni + 0.05 mg/L Mo supplementation.

The bioavailability of trace elements, which is necessary for bacteria for an efficient anaerobic digestion process, is an important parameter. The bioavailability of the trace elements does not hinge on the total trace element content of the reactor. Because, usually, there is actually in small quantities of trace element in solution. The bioavailability of trace elements could also have been influenced by some substances present in the inoculum. Chloride and sulphate can be given as examples of these substances, but the concentration of these elements in the inoculum was not determined in this study (Oleszkiewicz and Sharma, 1990).

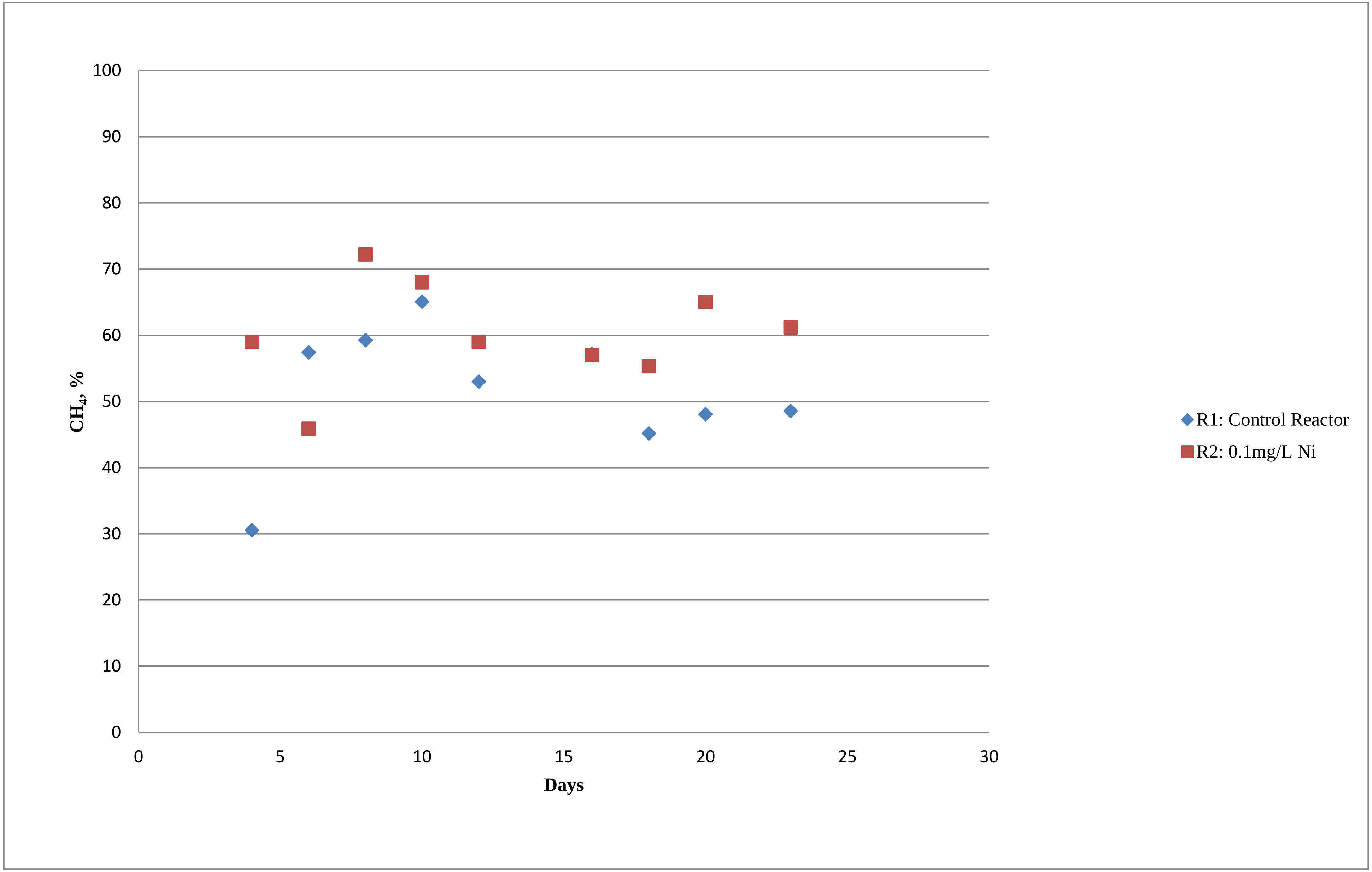

Figure 4.13. The methane (CH_4) content of the biogas produced in the reactors for the 1st set-up.

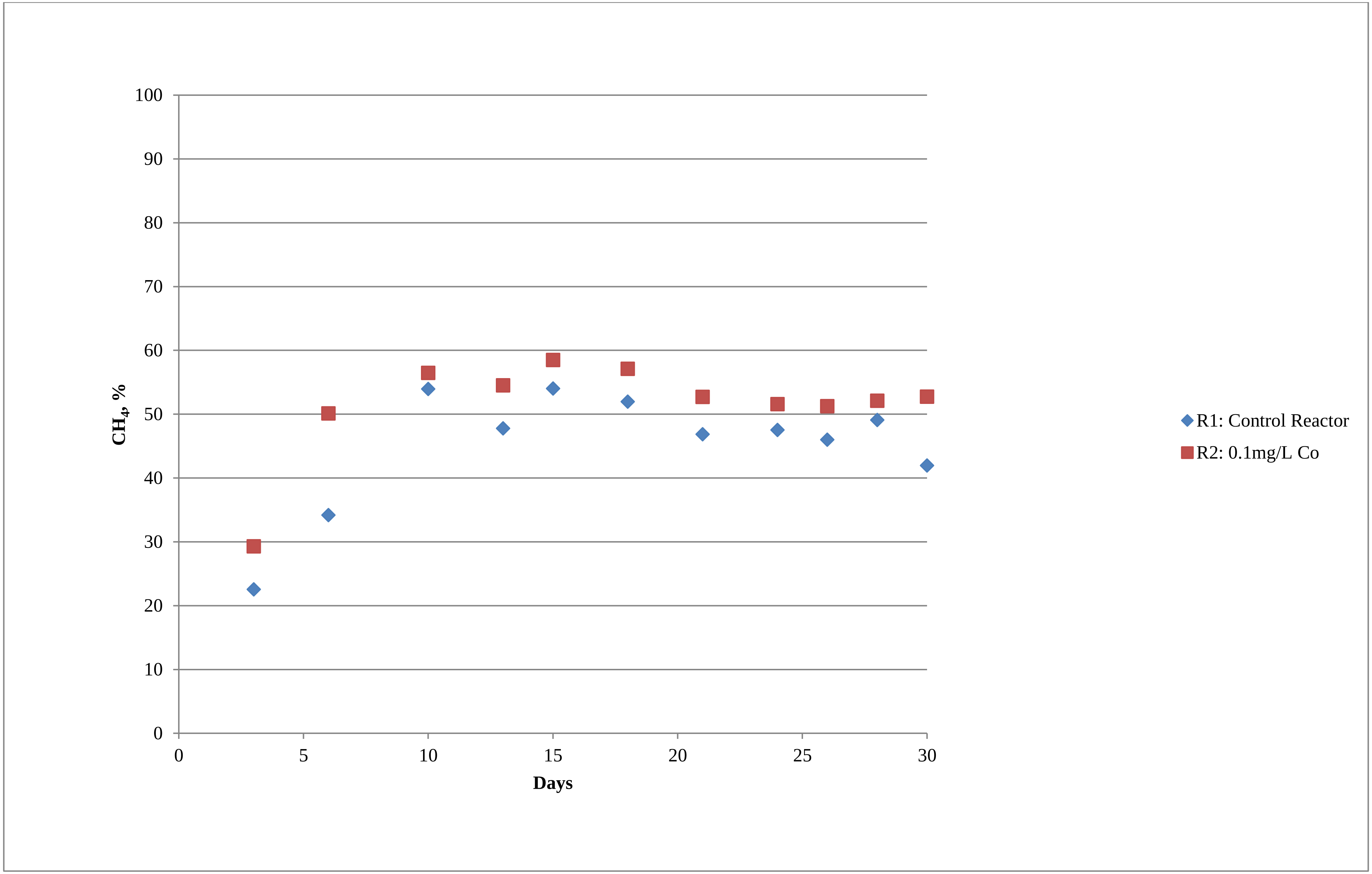

Figure 4.14. The methane (CH_4) content of the biogas produced in the reactors for the 2nd set-up.

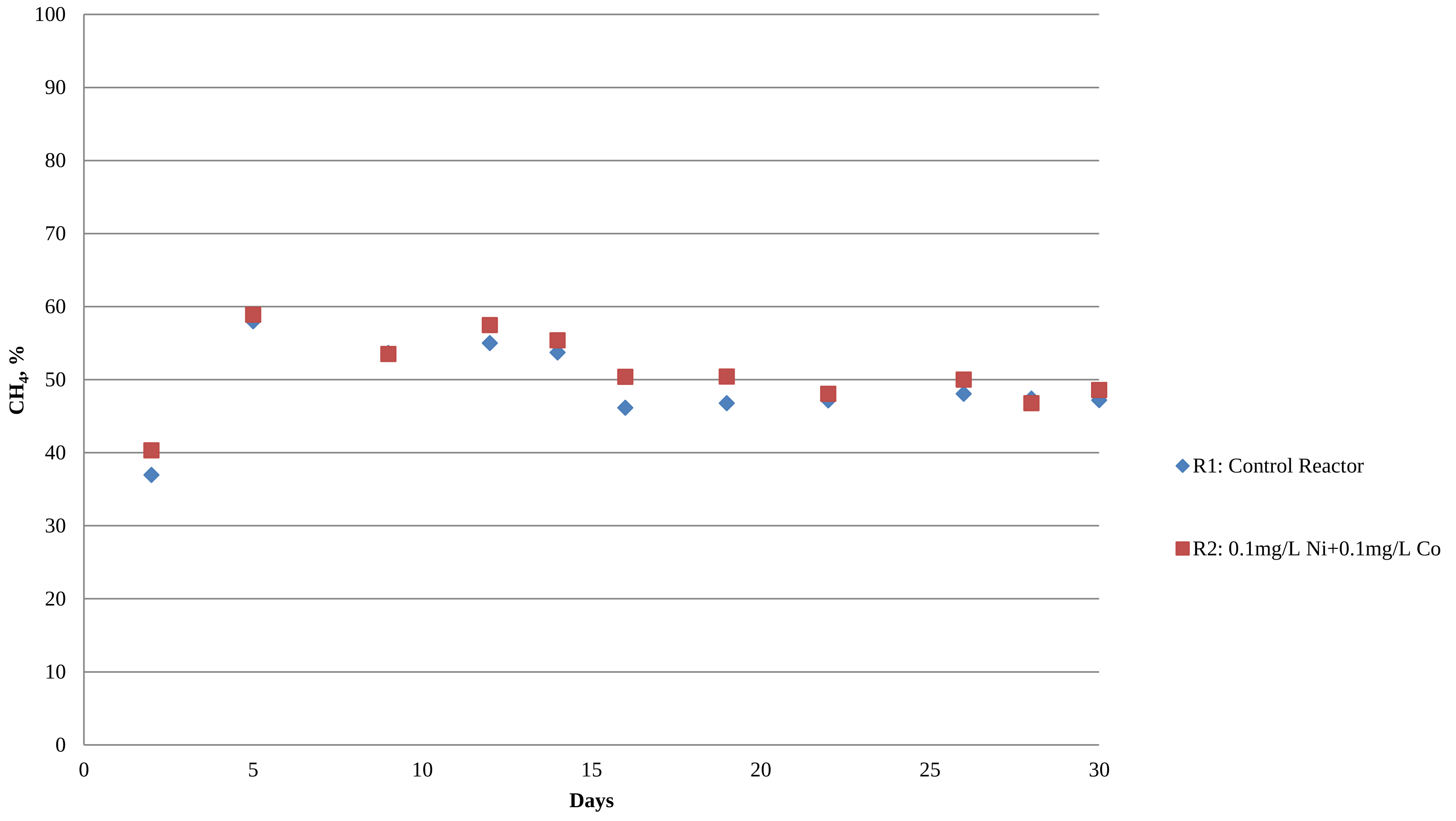

Figure 4.15. The methane (CH_4) content of the biogas produced in the reactors for the 3rd set-up.

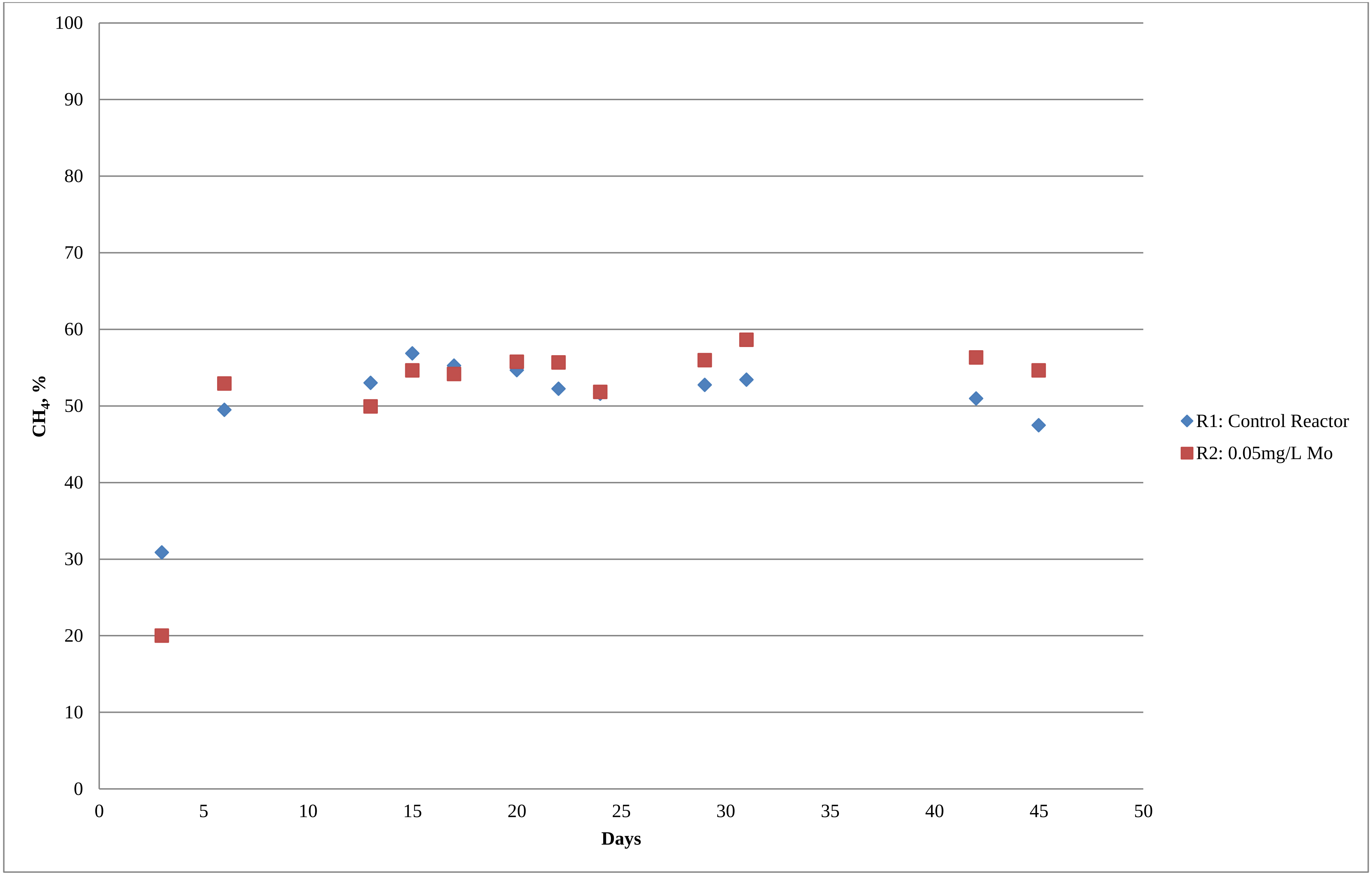

Figure 4.16. The methane (CH_4) content of the biogas produced in the reactors for the 4th set-up.

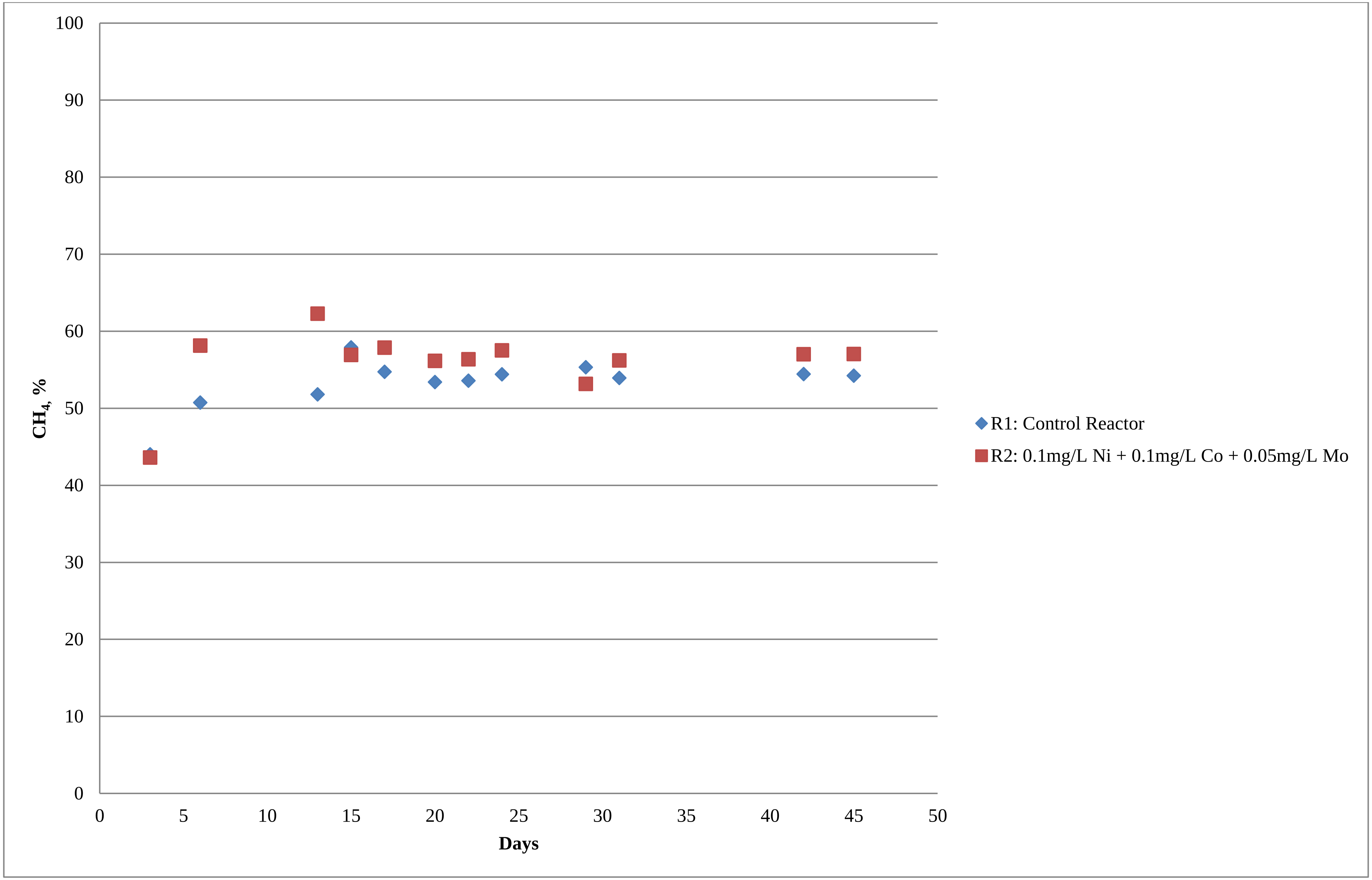

Figure 4.17. The methane (CH_4) content of the biogas produced in the reactors for the 5th set-up.

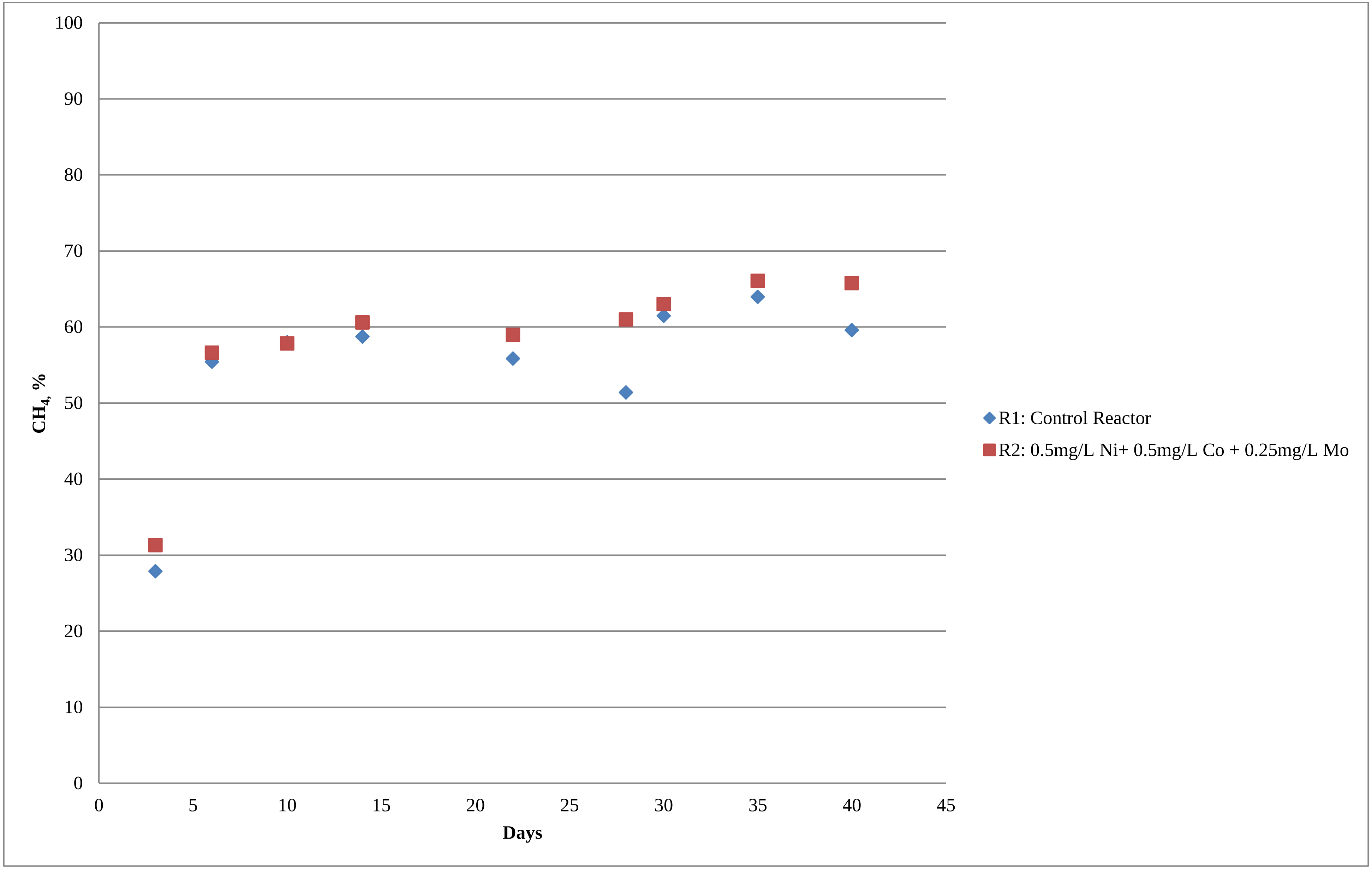

Figure 4.18. The methane (CH_4) content of the biogas produced in the reactors for the 6th set-up.

4.3.4. Biogas and Methane Yield

Biogas and methane yields are also important parameters to identify the effects of micro elements supplementation on biogas generation from maize silage. According to the research work conducted about the effects of Co, Ni and Mo on the methane yield and process stability of the semi-continuous anaerobic digestion of maize silage, Co and Ni were found to be more efficient on increasing the methane yield and process stability than Mo (Wei et al., 2014).

In a previous study, it was determined that when energy crops, especially maize silage, were used as substrate in anaerobic digestion process, addition of nutrients seemed to be very essential to reach high methane and biogas yields at short HRT. In addition, low VFA generation would also be achieved as well (Demirel et al, 2008; Nges et al, 2012).

Within this scope, biogas and methane yields obtained anaerobic digestion of maize silage were also evaluated. The biogas and methane yields are given in Table 4.7. In addition, all the results are summarized in Table 4.8.

According to the experimental findings obtained, trace element addition by Ni, Co and Mo, singly or in combination at varied doses, biogas and methane yields increment was observed in each experiment. According to the results of the experiments, Reactor R2 from Set 4 (0.05 mg/L Mo) has the highest biogas yield with a value of 0.669 L Biogas/g $VS_{degraded}$. However, drastic improvements were determined for Set 3 (0.1 mg/L Ni + 0.1 mg/L Co), 5 (0.1 mg/L Ni + 0.1 mg/L Co + 0.05 mg/L Mo) and 6 (0.5 mg/L Ni + 0.5 mg/L Co + 0.25 mg/L Mo) after supplementation by trace metals.

0,429 L CH_4/g $VS_{degraded}$ was monitored as the highest methane yield at the reactor including 0,5 mg/L Ni, 0,5 mg/L Co and 0,25 mg/L Mo supplementation during 6th set-up. In Set 6, methane yield was increased by approximately 36%.

In previous works, Ni, Co and Mo supplementation, in combination, enhanced the methane yield by 30%. In addition, Ni and Co supplementation, alone, enhanced the methane yield by 30 and 15%, respectively. However, it was determined that Mo supplementation has no impact on methane yield. Co supplementation had a stimulatory impact. When Ni was not supplemented to the reactors, the stability of the process and methane generation seemed to be reduced (Pobeheim et al., 2010b; Pobeheim et al., 2010c). In this experiment, Mo addition raised the methane yield by 20% in Set 4.

Table 4.7. The biogas yield and methane yield provided from anaerobic digestion of maize silage.

Set	**Reactor**	**Biogas Yield (L Biogas/g $VS_{degraded}$)**	**Methane Yield (L CH_4/g $VS_{degraded}$)**
1	R1 (Control)	0.274	0.131
1	R2 (0.1 mg/L Ni)	0.279	0.167
2	R1 (Control)	0.422	0.177
2	R2 (0.1 mg/L Co)	0.465	0.245
3	R1 (Control)	0.387	0.183
3	R2 (0.1 mg/L Ni + 0.1 mg/L Co)	0.472	0.229
4	R1 (Control)	0.609	0.289
4	R2 (0.05 mg/L Mo)	0.669	0.365
5	R1 (Control)	0.492	0.267
5	R2 (0.1 mg/L Ni + 0.1 mg/L Co + 0.05 mg/L Mo)	0.583	0.333
6	R1 (Control)	0.530	0.316
6	R2 (0.5 mg/L Ni + 0.5 mg/L Co + 0.25 mg/L Mo)	0.652	0.429

Table 4.8. The results of the experiments.

Set	**Reactor**	**Initial pH**	**Final pH**	**Initial TS (%)**	**Initial VS (% of TS)**	**Final VS (% of TS)**	**Methane Yield (L CH_4/g $VS_{degraded}$)**
1	R1 (Control)	7.14	7.73	5.0	75.8	48.3	0.131
	R2 (0.1 mg/L Ni)	7.16	7.74	5,0	75.2	47.2	0.167
2	R1 (Control)	7.06	7.71	5.7	85.6	49.1	0.177
	R2 (0.1 mg/L Co)	7.06	7.71	5.6	85.4	48.2	0.245
3	R1 (Control)	7.07	7.68	5.2	85.7	50.0	0.183
	R2 (0.1 mg/L Ni + 0.1 mg/L Co)	7.08	7.72	5.3	83.7	47.7	0.229
4	R1 (Control)	7.07	7.71	5.5	82.7	44.6	0.289
	R2 (0.05 mg/L Mo)	7.09	7.71	5.3	81.6	47.1	0.365
5	R1 (Control)	7.05	7.73	5.6	82.8	46.6	0.267
	R2 (0.1 mg/L Ni + 0.1 mg/L Co + 0.05 mg/L Mo)	7.06	7.76	5.0	81.1	46.7	0.333
6	R1 (Control)	7.02	7.70	5.3	81.6	49.2	0.316
	R2 (0.5 mg/L Ni + 0.5 mg/L Co + 0.25 mg/L Mo)	7.02	7.70	5.6	83.1	49.0	0.429

5. CONCLUSION

This lab-scale experimental work was carried out in order to determine the impact of trace elements on biogas production. Maize silage was utilized as the mono substrate for anaerobic digestion process. Trace elements selected were Nickel (Ni), Cobalt (Co), and Molybdenum (Mo). These trace elements are among the essential micro-nutrients required by diverse group of microorganisms in anaerobic digestion process.

Therefore, Ni, Co and Mo were added to the mesophilic anaerobic batch reactors, alone or in combination, at pre-determined concentration ranges that were determined based on literature data available. The Ni and Co content of the maize silage used in the experiment was below detection range. In addition, Ni and Co were available in the inoculum added to the reactors to initiate biological activity.

According to the results obtained from the experiments, the methane content of generated biogas was not influenced by 0,1 mg/L of Ni and Co addition, solely or together. In addition, Ni, Co and Mo addition increased the biogas and methane yields from anaerobic digestion of maize silage. However, impressive improvements were observed for Sets 3, 5 and 6. In set 3, 5 and 6, the biogas yields increased by 22%, 18% and 23%, respectively.

According to the results, cumulative biogas production was not affected by Mo addition. However, the experiment was conducted 2 more weeks longer than the previous experimental set-ups with Ni and Co. In set 4, a plateau for biogas curve was obtained in 45 days. This period was 30 days in previous experimental set-ups with Ni and Co.

In set 4, methane content was between 50-60 %. Mo supplementation did not have any significant effect on methane content of the produced biogas for the first 30 days of the trial. However, the period between 30 and 45 days, the methane production increased.

In set 5, cumulative biogas production was not affected by addition of 0,1 mg/L Ni, 0,1 mg/L Co and 0,05 mg/L Mo together.

In set 6, the trace element supplementation was five times higher than that of the previous trial (Set 5). This increase contributed to higher biogas production in set 6. In this set-up, methane content was also between 50-60%. Reactor R2, including 0,5 mg/L Ni, 0,5 mg/L Co and 0,25 mg/L Mo, showed the highest methane yield with a value of 0,429 L CH_4/g $VS_{degraded}$ in Set 6.

In case of daily biogas production and cumulative biogas production, it was determined that there was no difference between control reactors and rectors with trace element supplementation at the beginning of the study. The daily and cumulative biogas production increased in the reactors with trace element addition, and the difference between the gas production amounts of the control reactor and trace element supplemented reactors were higher after a couple of days since the first day of the experiments.

Indeed, the results showed that it was difficult to determine the required trace element quantity for an agricultural biogas digester. Furthermore, in addition to the initial trace element content of the substrate and the characteristics of inoculum, the operational and environmental conditions should also be considered for each particular substrate type for biogas production in agricultural biogas plants. In summary, it can be concluded that supplementation by Ni, Co and Mo provided higher biogas and methane yields during mesophilic batch anaerobic digestion of maize silage as mono-substrate, indicating that addition of trace metals as micro-nutrients played an important role during mono digestion of agricultural biomass, since energy crops often lack macro and micro nutrients for anaerobic digestion.

6. RECOMMENDATION

In order to determine the most effective trace elements concentrations for anaerobic digestion of maize silage, further studies should be carried out. It is recommended to investigate the effect of Co, Ni and Mo individually or in combination at different doses on anaerobic digestion process. The effect of Mo addition to agricultural biogas reactors operated under mono-digestion process should also be investigated more thoroughly. The operational conditions during trace elements supplementation to the energy crops should also be studied to determine the most appropriate operational conditions such as temperature, organic loading rate and feeding regime for anaerobic digestion to obtain the most suitable biogas rates. Also, further studies about trace element supplementation should be done with the other types of substrates and different reactor types such as semi-continuous or continuously stirred reactors. Finally, in addition to Ni, Co and Mo, the effect of other important trace element such as W, Se, Cu and Zn should also be studied as well.

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