CHALMERS

Addition of Esters on Anaerobic Digestion: Inhibiting or Boosting Biogas Production?

Master of Science Thesis

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CHALMERS UNIVERSITY OF TECHNOLOGY
Göteborg, Sweden, 2012

MASTER'S THESIS

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ABSTRACT

The effects of addition of fruit flavour compounds such as esters on anaerobic digestion have been examined in this work. The aim was to evaluate effects of different ester concentrations and chain length in anaerobic digestion. The investigations of different ester concentrations were conducted using methyl butanoate, ethyl butanoate, ethyl hexanoate, and hexyl acetate. The experimental results showed that biogas production increases by addition of esters at concentration up to 10 g/L for methyl butanoate and 5 g/L for ethyl butanoate, ethyl hexanoate and hexyl acetate. Adding esters above these concentrations showed inhibitory effect on anaerobic digestion. The minimum inhibitory concentration for methyl- and ethyl butanoate, ethyl hexanoate, and hexyl acetate were in the range 10 to 20 g/L and 5 to 10 g/L respectively.

The investigation of the chain length of esters added were conducted using methy-, ethyl-, butyl-, and hexyl acetate, ethyl butanoate and ethyl hexanoate at concentration 5 g/L without any substrate added. The results obtained in this experiment showed that all esters added increased the biogas production, except hexyl acetate that showed strong inhibitory effect. The increase of biogas production varies with increasing length of the ester chains. Even though addition of esters at certain concentration on anaerobic digestion boosts biogas production, it also decreases the methane content ratio. Further work is required to better understand the mechanism that deals with the effect of addition of esters in anaerobic digestion.

Keywords: ester, fruit flavour, biogas, inhibition.

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NOMENCLATURES

MB-S : Methyl butanoate with substrate addition

MB : Methyl butanoate without substrate addition

EB-S : Ethyl butanoate with substrate addition

EB : Ethyl butanoate without substrate addition

EH-S : Ethyl hexanoate with substrate addition

EH : Ethyl hexanoate without substrate addition

HA-S : Hexyl acetate with substrate addition

HA : Hexyl acetate without substrate addition

MA : Methyl acetate with substrate addition

EA : Ethyl acetate with substrate addition

BA : Butyl acetate with substrate addition

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I. INTRODUCTION

1.1. Background

Fruit is one of the most important trading commodities on the global market. The fruit production in the world has been increasing every year since 2006 to 2010 approximately 2.42% [1]. The increase is due to population growth and improvement of living standard in many countries. The total global production in the world is approximately 609.2 million tons in 2010 [1]. The main part of the fruit are consumed as fresh and processed into juice.

The increase in production and consumption of fruit results in accumulation of fruit waste. Approximately, 30% of the fruit production becomes waste [2]. The high number of fruit waste is mostly caused by improper harvest and storage and/or fruit handling. Due to careless harvesting and improper storage, flavour, colour, size, and storability of fruits are affected. Improper harvest and storage can cause post-harvest losses where fruit wastes are created [2]. Traditionally, fruit waste is disposed in landfills. However, this process requires large area of land, which potentially cause water and soil pollution, and produces greenhouse gases. Therefore, a preferable method to treat fruit waste is needed to overcome the problems caused by accumulation of fruit waste.

One possible solution to handle the abundant fruit waste is anaerobic digestion. Anaerobic digestion has been applied to stabilize municipal organic solid waste since 3,000 years ago for the production of biogas and fertilizer [3]. Fruit waste still contains major amount of sugars, polysaccharides and organic acids, while minor constituent include pigments, and flavour substances. High amount of sugar content in fruit waste increases the possibility to convert fruit waste into biogas. According to Narayani and Priya, 1 g volatile solid of mixed fruit waste produced 363 ml (3.63×10^{-4} m^3) biogas [4].

The possibility of energy production based on fruit waste is interesting since it can solve problems of energy shortage and environmental issues. Today, the world is facing problems because of the depletion of fossil fuels and accumulation of greenhouse gas emission from the combustion of fossil fuels. These sustainability problems increase the demand for fuel produced from renewable recourses. Utilization of fruit waste for biogas production can provide comparably clean fuel that can reduce greenhouse gas

emissions and dependency on fossil fuel. It has been reported that 1 m^3 biogas (approx. 6 kWh/m^3) is equivalent to 0.5 kg (approx. 12 kWh/kg) of diesel oil. If biogas is used for vehicle fuels, it can reduce CO_2 emission by 75 – 200 % compared with the use of fossil fuels [5].

There are wide ranges of raw material that can be used in anaerobic digestion, like wastes from households, animals, agriculture, etc. Generally, biogas is produced through anaerobic digestion from manure. Manure is easy to degrade by microorganism and gives relatively high yield, however with a limited production rate. Therefore, sometimes manure is only used as co-substrate to enhance the biogas production. Utilization of biomass like fruit waste for the production of biogas offers some advantages. Beside as a waste management strategy, the cost for raw material is cheap, available in high quantities and the biogas yield is relatively the same as biogas produced from manure [6].

Preliminary works on biogas production from fruit waste results in much lower biogas production compared to theoretical biogas production. These results might be due to the presence of natural organic compound in fruit correlated with the defence system in plant. Plants have defence mechanism including physical and chemical barrier to protect them from microbial attack [7]. One of the defence systems in fruit is the presence of fruit flavour. Some of fruit flavours are considered as antimicrobial agents, for instance limonene as flavour compound in orange peel is reported to inhibit biogas production [8, 9]. Another study reported that hexenal, (E)-2-hexenal, and hexyl acetate have significant inhibitory effect against *E. Coli, S. Enteritidis,* and *L. Monocytogenes* and improved the safety of fresh-sliced apples [10]. Therefore, in order to increase biogas production or even prevent from failure of digestion process, it is necessary to investigate different types of fruit flavour that have a potential to inhibit bacteria activities.

1.2. Objective

Some of flavour compounds of fruit have been reported as antimicrobial agents. However, limited research on effect of flavour compounds on biogas production has been reported. Therefore, the aim of this study was to investigate the effect of addition of ester fruit flavours on biogas production and to determine the concentration range allowable of ester added.

II. LITERATURE STUDY

2.1. Fruit waste

2.1.1. How is fruit waste produced?

The main fruit waste stream originates from the following [11]:

- Agricultural production: The losses due to improper harvest operation, for example during fruit picking. Improper harvest can cause mechanical damage of fruit for instance skin breaks, bruises, or lesions. This damage increases the risk of microbial damage.
- Postharvest handling and storage: These losses can occur during handling, storage and transportation between farm and consumer. Harsh handling of fruits increases the potential of fruit spoilage. The fruit spoilage includes physical change in terms of colour and/or flavour.
- Processing: Processing of fruits produces loss of during industrial processing, e.g. juice production, canning, jam production, etc. The composition of the waste such as peels, seeds, waste pulp.
- Distribution: Involves losses during transportation from storage to market or retailers.
- Consumption: Includes waste from consumption of fresh fruit in the household.

Estimate of global fruit and vegetable losses are shown on Figure 2.1. The global fruit and vegetables losses are dominated by agriculture production losses and followed by consumption. At least 10% of fruit losses come from agriculture production, mostly due to improper handling during harvest or postharvest, and causes rejection by retailers.

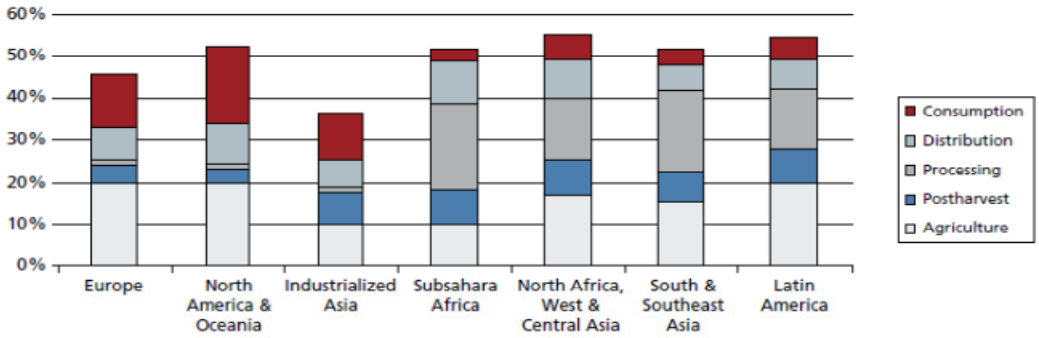


Figure 2. 1. Global fruit and vegetable losses [11]

2.1.2. Global fruit waste production

The global fruit waste production can be estimated to 30% from world fruit production [2]. Kantor *et.al*. reports that 20 – 40% of fruit production in developing countries becomes food losses due to pest and pathogens [12]. The abundant amount of fruit waste will create environment problems.

The fruit waste usually ends up in a landfill because it is the easiest and quickest way to handle this waste. However, this method does not really solve the environment problems. Since, it potentially causes contamination of groundwater, soil contamination, generation of green house gaseous, spreading of diseases vectors, etc. European commission reported that the environmental impacts of fruit and vegetable production in the EU27 are estimated 9.2E+10 (kg CO_2 eq./yr) and (kg PO_4 eq/yr) for GWP 100 and eutrophication, respectively [13].

2.1.3. Fruit waste composition

Fruit waste is organic compounds that contain carbohydrates, protein, etc. Therefore, fruit waste will finally decompose due to natural biomass circulation. However, if the amount of fruit waste is too high, the environment needs a long time to decompose it. The composition of fruit waste depends also on where the fruit waste is produced. The production of fruit waste in Gemah Ripah fruit market in Yogyakarta Province Indonesia is reported in the 2 to 11 tonnes/day with composition orange, mango and apple with percentage as 65%, 25%, and 5 % respectively [14]. The remaining 5% consists of pineapple, watermelon, melon, grape, zalacca, avocado, longan, starfruit, rambutan, papaya, the rose-apple, guava, and mangosteen [14].

2.2. Fruit Flavour

Fruits consist of hundreds of different types of fruit flavours comprising only 0.001 – 0.01% of the fruit's fresh weight [2]. Even though the fruit flavours are only present in low concentration, fruit flavour is one of the important factors for freshness of the fruits and it can be detected by human olfactory [2].

Fruit flavours are not formed during early fruit formation but produced during ripening and postharvest. The fruit flavours are classified based on biogenesis and chemical

structure. By biogenesis, the formation of fruit flavours through many metabolic pathways, depend on species, variety, climate, maturity, and pre and postharvest handling. As direct products of a metabolism or as result of interactions between pathways or end products, the fruit flavours can be classified by biogenesis: fatty acids (FA), amino acids (AA), glucosinolates, terpenoids, phenols, and related compounds as can be seen in Table 2.1 [2, 7, 16].

Table 2. 1. Precursors of some fruit flavours

Precursor	Fruit flavour
Carbohydrates - Glucose - Fructose - Sucrose	- Organic acids: pyruvic acid, acetic acid, propionic acid, acetoacetic acid, butyric acid, hexanoic acid, octanoid acid - Esters: pyruvates, acetates, propionates, butyrates, acetonacetates, hexanoates, octanoates - Alcohols: ethanol, propanol, butanol, hexanol, octanol - Aldehydes: acetaldehyde, propanal, butanal, hexanal, octanal - Terpenes: monoterpene, linalool, limonene, α-pinene, citronellal, citral, geranial
Amino acids - Alanine - Valine - Leucine - Isoleucine - Phenylalanine - Sirene theronine - Glycine - Cystine/cysteine sirene	- Pyruvic acid, acetaldehyde, ethanol - Isopropanal, isopropanol, α-keto-isobutyric acid - 3-methylebutanal, 3-methylbutanol, α-keto-isocaproic acid - 2-methylbutanal, 2-methylbutanol - Benzaldehyde, phynilacetaldehyde, cinnamaldehyde - Hydrocinnamaldehyde, p-hydroxybenzaldehyde, p-hydroxy phenylacetaldehyde, p-hydroxy cinnamaldehyde, p-hydroxy cinnamaldehyde - Pyruvic acid - Thiazoles - Glyoxal
Fatty acids - linoleic acid	Trans-2-trans-4-decadienal, hexenal, trans-2-octenal Trans-2-pentanal, trans-2-hexenol, hexanal Cis-3-hexenal, cis-3-hexenol Trans-2-trans-4-heptadienal, propanal
Vitamin - Carotene - β-carotene	β-ionone

The fruit flavours are divided by chemical structure into 6 classes which are esters, alcohols, aldehydes, ketones, lactones, and terpenoids [1, 5]. Table 2.2. shows major

flavour compounds divided in different classes. Fruit flavours in different types of fruit are summarized in Table 2.3.

Table 2. 2. Major flavour compounds [2]

Classes	Flavour compound
Esters	Butyl acetate, butyl butanoate, ethyl acetate, ethyl butanoate, ethyl hexanoate, methyl acetate, hexyl acetate, etc.
Alcohols	Benzyl alcohol, butan-1-ol, (E)-cinnamyl alcohol, 1-hexanol, (E)-2-hexenol, (Z)-3-hexenol, 1-octanol, (Z)-6-nonenol, hexan-1-ol, etc.
Aldehydes	Acethaldehyde, benzaldehyde, (E)-cinnamaldehyde, (E,E)-2,4-decadienal, hexanal, (E)-2-hexenal, nonanal, (Z)-6-nonenal, (E,Z)-2,6- nonadienal, (E)-2-nonenal, phenylacetaldehyde, etc.
Ketones	2,3-butanedione, β-damsenone eucalyptol, eugenol, 2-heptanone, 4-(*p*-hydroxyphenyl)-2-butaanone, 2-hydroxy-2-butanone, β-ionone, linalool, 6-methyl-5-heptene-2-one, nerolidol, 1-octen-2one, 2-pentanone (z)-1,5-octadien-3-one, terpenes, etc.
Lactones	γ-butyrolactone, γ-decalactone,δ-decalactone γ-dodecalactone, δ-dodecalactone, γ-octalactone, δ-octalactone
Terpenoids	Citral, β-damascenone, dihydroedulan, farnesyl acetate, geraniol, hotrienol, α-ionone, β-ionone, limonene, linalool,etc.

Table 2. 3. Fruit flavours in different types of fruit [2]

Fruit name	Fruit flavours
Apple	Esters: 1-butyl acetate, butyl 2-methylbutanoate, ethyl butanoate, ethyl 2-methylbutanoate, 2–methylbutyl acetate, hexyl acetate, etc. Alcohols: 1-hexanol, hexen-1-ol Aldehydes: *n* - hexanal, *trans*-2- hexenal, and *trans* - 2 - hexen - 1 – ol Terpenoids: β – damascenone
Orange	Esters: ethyl acetate, ethyl butanoate, ethyl propanoate, ethyl - 2 - methyl butanoate, ethyl hexanoate, methyl butanoate, etc. Alcohols: ethanol, (*Z*)-3-hexen - 1 - ol, 3 – methyl butanol, 1 - octanol Aldehydes: acetaldehyde, (*E*,*E*)-2,4-decadienal, decanal, dodecanal, hexanal,(*E*) - 2 – hexenal, etc Ketone: 1 - penten - 3 - one, 1 - octen - 3 - one Terpenoids: damascenone, limonene, α - terpincol,terpinen - 4 - ol
Strawberry	Esters: butyl acetate, ethyl butanoate, ethyl hexanoate, hexyl acetate, methyl and ethyl acetates, methyl butanoate, etc. Aldehydes: hexanal, (*E*) - 2 - hexenal, methyl cinnamates Lactones: decalactone, γ - dodecalactone, Ketones: 2 - heptanone, linalool, 1 - octen - 3 - one Terpenoids: geraniol
Peach	Esters: *cis*-3-hexenyl acetate, ethyl acetate, ethyl butanoate, ethyl octanoate, (*Z*)-3-hexen-1–ethyl acetate, methyl octanoate Alcohols: benzyl alcohol, (*E*) - 2 - hexen - 1 - ol

	Aldehydes: benzaldehyde, (*E*,*E*)-2,4-decadienal, hexanal, etc. Ketones: γ - caprolactone Lactones: γ - decalactone, δ - decalactone, γ - dodecalactone, etc. Terpenoids: β - damascenone, linalool, terpinolene
Pear	Esters: butyl acetate, butyl butanoate, hexyl acetate, ethyl hexanoate, ethyl octanoate, ethyl (*E*) - 2 - octenoate, ethyl (*E* , *Z*) - 2,4 - decadienoate, methyl (*E* , *Z*) - 2,4 - decadienoate, and pentyl acetate
Mango	Esters: ethyl butenoate, ethyl dodecanoate, ethyl decanoate, ethyl octanoate Alcohols: butan-1-ol, *p*-cymene, *cis*-hex-3-en-1-ol, ethanol, etc. Aldehydes: hexanal Terpenoids: β-caryophyllene, limonene, α-phellandrene, etc. Other: β-carotene
Papaya	Esters: ethyl acetate, methyl butanoate, ethyl butanoate Terpenoids: linalool, α - terpineol Alcohols: 3 - methylbutanol, benzyl alcohol, butanol
Pineapples	Esters: methyl esters of β - hydroxybutyric, 2 - propenyl hexanoate Alcohols: *p* - allyl phenol Ketones: 4 - methoxy - 2,5 - dimethyl - 2(H) - furan - 3 - one, etc. Lactanones: butyrolactone, γ – octalactone
Plum	Esters: ethyl nonanoate Aldehyds: benzaldehyde, (*E* , *E*) - 2,4 - decadienal, (*Z*) - 3 - hexenal Lactanones: δ - decalactone, γ - decalactone, δ - octalactone Terpenoids: linalool

2.2.1. Ester

Esters are chemical compounds formed from alcohols and acids and the general chemical formula can be written as RCO_2R', where R and R' are the hydrocarbon parts of carboxylic acid and alcohol, respectively. The chemical structure of ester can be seen in Figure 2.3.

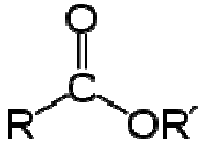


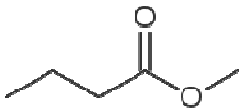
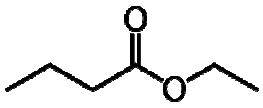
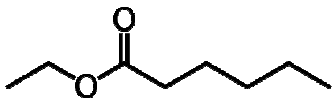
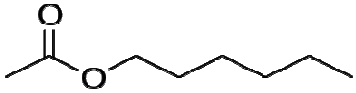
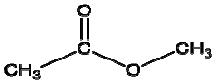
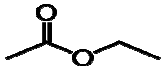
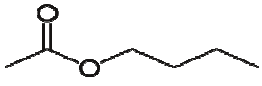
Figure 2. 2. Chemical structure of esters

Esters are the major volatile compounds that are found in fruit, for instance apple (*Malus domestica*, 78-92% of total volatile mass), pear (*Pyrus cummunis*), banana (*Musa sapientum*), strawberry (25 – 90% of the total volatile mass), raspberry (13% of the total volatile mass), etc. [2, 7, 17] as can be seen in Table 2.3. Esters are usually present in fruits in very low concentration, approximately between 1 and 100 ppm. Even

though it is only found in trace amount, ester is responsible for the character of the fruit [16]. Some esters that can be found in fruit are shown in Table 2.4.

The formation of esters during ripening of the fruits will influence the levels and type of esters. Esters are expected that are formed from unsaturated fatty acids by β-oxidation, lipoxygenase catalysis, oxidation of the radical to the carbonium ion and decarboxylation [2, 7, 17]. Other precursors for the esters are lipids and amino acids.

Table 2. 4. Some esters in different types of fruit [2, 7, 17]

Ester name	Chemical formula and structure	Molecular weight	Presence in fruit
Methyl butanoate or methyl butyrate	$C_5H_{10}O_2$ 	102	Strawberry, pineapple, orange, black currant, kiwi, papaya
Ethyl butanoate or ethyl butyrate	$C_6H_{12}O_2$ 	116	Citrus, apple, pear, passion fruit, strawberry, banana, orange
Ethyl hexanoate or ethyl caproate	$C_8H_{16}O_2$ 	144	Passion fruit, apple, strawberry, blackberry, black currant, pear, pineapple
Hexyl acetate or capryl acetat	$C_8H_{16}O_2$ 	144	apple, orange, pear, peach, nectarine, apricot, strawberry
Methyl acetate	$C_3H_6O_2$ 	74	Pineapple, apple, black currant, kiwi
Ethyl acetate	$C_4H_8O_2$ 	88	Apple, orange, apricot, black currant, blue barriers, grape, strawberry, peach, papaya, pear, banana
Butyl acetate	$C_6H_{12}O_2$ 	116	Apple, strawberry, pear, banana

2.2.2. Antimicrobial activity

Antimicrobial properties of a compound give positive as well as negative impacts on a process and/or the quality of a product. The positive impact if it can improve the properties of the component or process, for instance if it is used as food preservative, it can prolong the life shelf of the food. On the other hand, it can give negative impact if it reduces the quality of the component or process, for example inhibit the methanogenic bacteria on biogas production.

A natural preservative is used to reduce or eliminate the use of chemical synthesized additive in food, which has been increased in recent years. The preservative in food is used to prevent bacterial and fungal growth and for prolong the shelf life. The interest of natural preservative by consumers may due to the fact that it can be eaten raw or cooked [11, 15, 18]. Fruit flavours are one option that can be considered as natural preservative. Fruit flavours act as defence systems to protect fruit from microbial attack as well as to keep it fresh. The rank of antimicrobial property of fruit flavours has been proposed as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons [16].

As previously mentioned, esters are the major fruit flavours presented in fruit. It has been reported that hexyl acetate can be used to improve the safety of freshly sliced apples. The utilisation of hexanal, hexyl acetate, and (E)-2-hexenal at the level used 150, 150, and 20 ppm, respectively exhibited significant extension of lag phase of *E. Coli* and *S. Enteritidis* inoculated at levels of 10^4-10^5 CFU/g [10].

Another study involved fruit flavour as antimicrobial agent reported the inhibitory effect of d-limonene on biogas production from orange peel [8, 9]. By treating the orange peel in order to extract the d-limonene before it is used in digestion process increases the methane yield [8, 9].

2.3. Anaerobic digestion

Anaerobic digestion refers to process involving biological breakdowns of organic matter in the absence of oxygen to produce biogas. The composition of biogas depends on organic matter as raw material but it consists mainly of methane and carbon dioxide [6]. The biogas composition from agricultural biomass is shown in Table 2.5. The yield of anaerobic digestion depends also on the type of the substrate, see Table 2.6.

Table 2. 5. Composition of biogas from agricultural biomass [6]

Gas composition	Formula	Units	Gases
Methane	CH_4	% by vol.	45 – 75
Carbon dioxide	CO_2	% by vol.	25 - 55
Carbon monoxide	CO	% by vol.	< 0.2
Nitrogen	N_2	% by vol.	0.01 – 5.00
Oxygen	O_2	% by vol.	0.01 – 2.00
Hydrogen	H_2	% by vol.	0.5
Hydrogen sulfide	H_2S	mg/Nm^3	10 – 30.000
Mercaptan sulfur	S	mg/Nm^3	<0.1 – 30
Ammonium	NH3	mg/Nm^3	0.01 – 2.50

Table 2. 6. Maximum gas yields per kg dry matter of different substrates [6].

Substrate for biogas production	DM (%) or ODM in DM (%)	Biogas yield (m^3/kg OTS)
Spent fruits	25 – 45 90 – 95	0.4 – 0.7
Mash from fruits	2 – 3 95	0.3 – 0.7
Liquid manure from cattle	6 – 11 68 – 85	0.1 – 0.8
Excreta from cattle	25 – 30 80	0.6 – 0.8
Vegetables waste	5 – 20 76 - 90	0.4
Market wastes	8 – 20 75 – 90	0.4 – 0.6

OTS : organic total solid

DM : dry matter

ODM : organic dry matter

Anaerobic digestion is a technology that has many environmental benefits. Firstly, this process acts on the putrid solid waste and wastewater, to reduce organic content of the waste before being sent to landfill. Secondly, the wide range of raw material that can be used in this process, the utilization of household, animals, agricultural waste will contribute to waste management issues. The thirdly, biogas provides solution to reduce dependency on fossil fuels with limited availability and predicted only enough to fulfil energy demand for several next decades. The last, increasing world attention to this

process is the contribution to produce renewable energy, biogas, which is claimed to be an environmental friendly energy source, especially due to its low contribution to the greenhouse effect.

Related to raw material for biogas production, there is a wide range of raw materials that can be used such as organic residues from agriculture (crop residues), waste from animals (manure), municipal organic waste, industrial waste, sewage sludge, by-products from production of bioethanol and biodiesel, energy crops and algae etc. [18]. The production technology as well as energy efficiency varies substantially depending on type of feedstock used. Compared to aerobic digestion, anaerobic digestion is a cheaper process, since no need of oxygen supply and less sludge production that needs to be treated further.

Generally, biogas production is based on 3 main stages including hydrolysis, acid formation, and methane formation. Literatures describe four stages that consist of hydrolysis, acidogenesis, acetogenesis and methanogenesis [6, 19]. The anaerobic digestion pathway is shown in Figure 2.4.

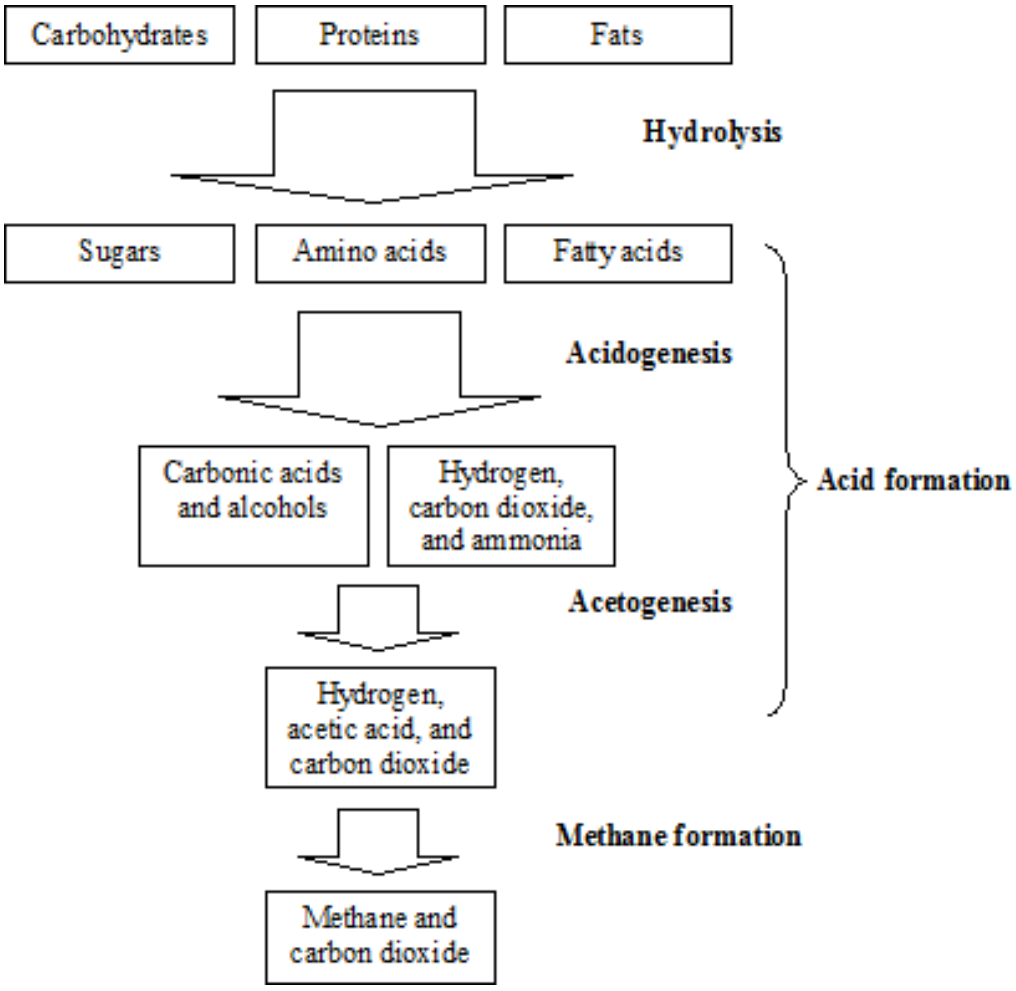


Figure 2. 3. Anaerobic Digestion Pathway [6, 19]

2.3.1. Hydrolysis

In the first stage, facultative and obligatory anaerobic bacteria convert biopolymer like cellulose, proteins, and fats into soluble compounds. The hydrolysis stage is also known as the polymer breakdown stage. The complex chain of carbohydrates, proteins and lipids are decomposed into shorter compounds. The hydrolysis of carbohydrates takes place within a few hours which obtain sugar monomers as products, while protein and lipid are hydrolyzed within few days. Hydrolysis of protein and lipid obtain fatty acids and amino acids as hydrolysis products, respectively [6]. The hydrolysis reaction of carbohydrates is shown below:

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2$$

2.3.2. Acidogenic

During acidogenesis, the monomers formed in the hydrolytic stage are taken up anaerobic bacteria and are degraded in the acidogenic stage. The goal in this stage is to degrade the products result in hydrolysis stage into shorter chain and convert it into

alcohols, hydrogen, ammonia, carbon dioxide and organic acids, such as butyric acid, propionic acid, acetic acid. Organic acids produced in this stage are called intermediate products. Major acids and alcohols produced through acidogenesis are shown in Table 2.7 [19].

Table 2. 7. Major acids and alcohols produced during acidogenesis

Name	Formula
Acids	
- Acetate	CH_3COOH
- Butyric acid	$CH_3(CH_2)_2COOH$
- Caproic acid	$CH_3(CH_2)_4COOH$
- Formate	$HCOOH$
- Lactate	$CH_3CHOHCOOH$
- Propionate	CH_3CH_2COOH
Alcohols	
- Butanol	$CH_3(CH_2)_2CH_2OH$
- Ethanol	CH_3CH_2OH
- Methanol	CH_3OH
- Propanol	$CH_3CH_2CH_2OH$

Some products (hydrogen, carbon dioxide and acetic acid) from this stage will skip the third stage, and be utilized directly in the final stage by methanogenic bacteria [6, 20]. The general reactions in the acidegonic stage are:

$$C_6H_{12}O_6 \leftrightarrow 2CH_3CH_2OH + 2CO_2$$

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2H_2O$$

2.3.3. Acetogenic

The products on the acidogenic stage are transformed by acetogenic bacteria into hydrogen, carbon dioxide and acetic acid. Hydrogen plays an important intermediate product in this stage. It is necessary for hydrogen to have a low partial pressure in order to allow the conversion of all the acids [18]. The reactions are as follow [6, 20]:

Propionic acid : $CH_3(CH_2)COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$

Butyric acid : $CH_3(CH_2)_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$

Capronic acid : $CH_3(CH_2)_4COOH + 4H_2O \rightarrow 3CH3COOH + 5H_2$

Carbondioxid/hydrogen : $2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$

Ethanol : $CH_3(CH_2)OH + H_2O \rightarrow CH_3COOH + 2H_2$

2.3.4. Methanogenic

In the final stage, the reaction takes place under strictly anaerobic condition. The methanogenic reaction converts the hydrogen, carbon dioxide and acetic acid into methane gas. The reactions can be summarized based on substrate types as follow [6, 21]:

CO_2 : $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$

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

$4HCOO^- + H_2O + H^+ \rightarrow CH_4 + 3HCO_3^-$

Acetate : $CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3$

Methyl : $4CH_3OH \rightarrow 3CH_4 + HCO_3^- + H^+ + H_2O$

$CH_3OH + H_2 \rightarrow CH_4 + H_2O$

There are different types of bacteria that contribute in each anaerobic digestion stage. Some different types of bacteria are described in the Table 2.8.

Table 2. 8. Some bacteria that contribute in the anaerobic digestion stages [6]

Stages	**Microorganism**
Hydrolysis	*Bacteroides* (*B.uniformis*, *B. acidifaciens*) *Lactobacillus* (*L. pentosus*, *L. plantarum*) *Propionibacterium* (*P. microaerophilum, P. granulosum*), *Sphingomonas* (*S. aromaticivorans*, *S. subterranea*), *Sporobacterium olearium*, *Megasphora elsdenii,* *Clostridium (C. celerecrescens, C. butyricum, C. aerotolerans)*
Acidogenesis	*Clostridium* (*C. tyrobutyricum*, *C. methylpentosum*, *C. clostridiformis*, *C. spiroforme*) *Ruminococcus* (*R. albus*, *R. bromii*, *R. gnavus*) *Paenibacillus*
Acetogenesis	*Desulfovibrio* (*D. desulfuricans, D. termitidis*), *Aminobacterium colombiens*, *Acidaminococcus*
Methanogenesis	*Methanobacterium formicium* *Mb. Thermoantotrophicum* *Methanococcus frisius* *Methanococcus mazei* *Methanosarcina bakerii*

2.4. Anaerobic digestion factors

There are some factor that can influence the anaerobic digestion process, such as substrate, temperature, pH, ratio C/N, volatile fatty acids and alkalinity, and inhibitory substances. The factors are described below.

2.4.1. Substrate

Substrate is material and energy source for the microorganism. Substrate will be consumed by microorganism and converted to methane as well as the use for growth. Types of substrate determine the rate of the digestion process, and lack of substrate ends the metabolism of the microorganism. It also determines the time of digestion, since more complex substrate will take longer time for degradation by microorganism. The general equation for methane formation from biomass is as follows [6]:

$$C_cH_hO_oN_nS_s + yH_2O \rightarrow xCH_4 + nNH_3 + sH_2S + (c - x)CO_2$$

During the digestion process, microorganism produces intermediate products. Intermediate products usually are short lived and do not accumulate in the reactor. However, the production rate of intermediate products depends on the composition of the substrate and can lead to the accumulation of intermediate products. The change of operational conditions likes pH or temperature can also induce the accumulation of intermediate products. The accumulation of intermediate products can inhibit digestion process. For instance substrate containing high fats can give high production of the fatty acids and induce to decrease pH, which will inhibit the microorganism activity further [6, 19].

2.4.2. Temperature

Temperature is an important factor that influences microbial activity. Anaerobic digestion can be carried out at three different temperatures range, which are psychrophilic (below 25^{o}C), mesophilic (30 – 42^{o}C) and thermophilic (43 – 55^{o}) [6]. There is correlation between temperature and retention time, as shown in Table 2.9. and Figure 2.5.

Anaerobic digestion is usually carried out under mesophilic or thermophilic condition. Most of methanogenic microorganisms work under mesophilic condition, while only few of them are thermophilic. Anaerobic digestion is sensitive to temperature change, especially thermophilic methanogen. Small changes in temperature can cause significant

decrease in activity of microbial and gas production up to 30%. Therefore, the temperature should be kept exactly in the range of +/-2^{o}C [6].

Table 2. 9. Thermal stage and typical retention times [6]

Thermal stage	Temperature (^{o}C)	Minimum retention time (day)
Psychrophilic	< 20	70 – 80
Mesophilic	30 – 42	30 – 40
Thermophilic	43 – 55	15 - 20

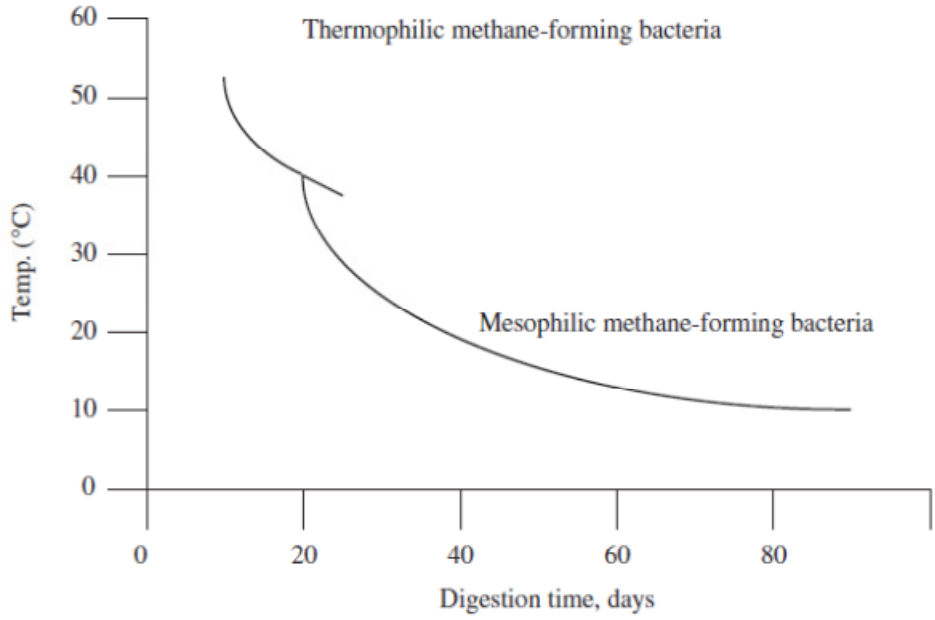


Figure 2. 4. Correlation between time and digestion time on anaerobic digestion [19]

There are some advantages and disadvantages for both mesophilic and thermophilic conditions. The inhibition of ammonium is low under mesophilic operation condition since ammonia toxicity increases with increasing temperature. The energy balance in the mesophilic condition is also better than in the thermophilic condition. Moreover, thermophilic condition requires larger energy due to high temperature [6, 19].

On the other hand, thermophilic condition gives higher growth rate of methanogenic bacteria and leads to higher biogas yield compared to mesophilic condition. Special hygienic procedures are not necessary since the epidemic and phytopathogenic germs are inactivated by higher temperature process. The high temperature condition also gives low oxygen solubility, so that the process can reach optimal condition more quickly. Moreover, thermophilic condition can operate in high loading rate with shorter retention time and gives better degradation of solid substrates. The summary of

advantages and disadvantages for both conditions is shown in Table 2.10. One can apply different temperature in anaerobic digestion, which are mesophilic in the hydrolysis stage while thermophilic condition in the methanogenesis stage [6, 22].

Table 2. 10. Comparison between mesophilic and thermophilic [6, 19, 22]

	Mesophilic	**Thermophilic**
Loading rate	Low	High
Retention time	Long	Short
Destruction of pathogens	Low	High
Sensitivity to toxicants	Low	High
Temperature control	Less difficult	More difficult
Methane yield	Low	High
Energy consumption	Low	High
Operational cost	Low	High

2.4.3. pH

pH is an importance factor that affects microbial activity and control the anaerobic digestion process. The pH of the substrate influences the growth of methanogenic bacteria. The optimum pH for anaerobic digestion is between 6.7 – 7.5 [9, 20]. Anaerobic digestion that operated in a pH below 6.5 decreases the organic acid production by hydrolytic bacteria, as well as decreases the methane production. In the digester, the pH value is kept in a neutral range. This condition is ensured by two buffering systems, carbon dioxide/hydrogen carbonate buffer system for strong acidification and ammonia-ammonium buffer system for weak acidification [6, 19]. The buffer system reaction occurs as follow:

$$CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + 2CO_3^{2-}$$

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

$$NH_3 + H^- \leftrightarrow NH_4^-$$

2.4.4. Nutrients (C/N-ratio)

The range of C/N-ratio in the substrate is 16:1 – 25:1. Too low value of the C/N ratio in the substrate causes an increase of ammonia production that leads to inhibition of the methane production. On the other hand, too high value of the C/N ratio gives negative effect in protein formation [6]. The microorganisms also need minimum concentration

of trace elements, such as Fe, Co, Ni, Se, W, and Mg [6, 21]. The amount depends on the type of microorganisms.

2.4.5. Volatile fatty acids and alkalinity

Volatile fatty acids (VFA) such as acetate, propionate, butyrate, lactate, etc. are may produced in the acidogenic and acetogenic stages. VFA are substrate for methanogenic bacteria. However, accumulation of volatile fatty acids leads to decrease pH as well as failure of the anaerobic digestion process. Accumulation of volatile fatty acids has direct relationship to alkalinity. Alkalinity is used as a buffer to keep the pH in the allowable range. Alkalinity is usually used as a buffer in the form of bicarbonate. The ratio of recommended volatile fatty acids and alkalinity for a good digestion process is 0.1 and 0.35, respectively for a proper digestion process [21].

2.4.6. Inhibition

There are some inhibitory substances that can affect the anaerobic digestion process. The inhibitor can be organic and inorganic material. Organic acids may be presented in the substrate, such as fatty acid and amino acid. Whereas, the inorganic material that can be inhibited the digestion are heavy metal cations, hydrogen sulphide, salts and ammonia [6, 21]. The degree of inhibition is affected by many factors, such as [19]:

1. Antagonism is when the existence of several substances gives a lower degree of inhibition compared to effect of inhibition for each of substance.
2. Synergism is when the existence of several substances gives a higher degree of inhibition compared to effect of inhibition for each of substance.
3. Adaptation or acclimatization is when the microorganisms are capable to adapt to the toxic environment and start to grow. In this type of inhibition, there are several mechanisms of adaptation of microorganisms to toxic environment, such as the microorganisms lowering the concentration of the inhibitory substance by degraded the inhibitory substance into nontoxic compounds, or the microorganisms build a defence system in their cell to make it more resistant to inhibitory substance.

In term of biogas from fruit waste, a suggested compound that may have antimicrobial activity is fruit flavour that is present in the fruit waste. Fruit flavour acts as defence system to protect plant from microbial attack [7]. Unfortunately, the present of fruit flavours on the anaerobic digestion give negative impact on biogas production. Since

they have antimicrobial properties that potentially can inhibit microbial activities, they give low yield on biogas production.

Several findings related to antimicrobial activity of flavour compound of fruit have been reported [21]. For example, 10 major flavour compounds in cashew *Anacardium occidentale* (Anacardiaceae) apple which are car-3-ene, limonene, (E)-2-hexenal, furfural, hexanal, benzaldehyde, nonanal, 2-methylpentan-1-ol, α-terpinene, and β-caryopyllene shows activity against one or more of 14 microorganisms with concentration in range 6.25 to 800 µg/mL. Most noticeably, (E)-2-hexenal showed gram-negative bacteria with concentration of either 200 or 400 µg/mL for four bacteria, which are *P. Aeruginosa, E. Aerogenes, E. Coli,* and *P. Vulgaris*. However, no research has been considerably reported the effect of the 10 major compounds in cashew apple on biogas digestion [23].

Beside in cashew apple, limonene is also found as a major flavour compound in orange peel. The effect of d-limonene on biogas production using orange peel as feedstock has been tested [8, 9]. The study showed that methane production increased by treating orange peel in order to remove d-limonene content before the digestion process [8, 9]. By reduction of d-limonene approximately 94% leads to increase of the methane production up to 426% compare with the untreated feedstock [9].

2.5. Biogas from fruit waste and sustainability

To meet the ambitious EU 20-20-20 Renewable Energy Directive goals, one promising bioenergy is biogas from fruit waste. It is expected become sustainable energy, since the production and utilisation biogas from fruit waste can reduce green house gaseous and it produces from bioresource. According to Swedish Energy Agency, to ensure bioenergy is sustainable, it has to fulfilling sustainability criteria which are greenhouse gas emission during the entire production to end use shall be at least 35% lower than its fossil comparator and the raw material used must not be harvested from land with high biodiversity or resulting in exploitation of land. According to those criteria, biogas can be categorized as sustainable energy. Because it is produced from is produced from biomass waste and if it is used as vehicle fuel it can reduce CO_2 emission by 75 – 200 % compared with fossil fuels [5]. On the other hand, the utilization fruit waste to produce biogas provides another advantage for environment which is as waste management strategy.

III. METHODOLOGY

3.1. Material

3.1.1. Medium

The medium used was synthetic medium prepared from nutrient broth, D-glucose, and yeast extract. 10 g of nutrient broth, 10 g of yeast extract and 10 g of D-glucose were dissolved in distilled water and diluted to 100 mL. The solution was then sterilized by filtration through a 0.2 μm membrane filter. The medium was prepared for concentration of 20 g/L according to what has been describes elsewhere [24].

3.1.2. Inoculums

Active inoculums were prepared from the municipal waste plant Sobacken (Borås, Sweden). The inoculums were stored in incubator at temperature 55^{o}C for 2-3 days. The purpose of storage is to acclimate the bacteria with the desired condition (55^{o}C).

3.1.3. Chemical

- Flavour compounds as inhibitor

Flavours compound were used as inhibitor that were added to the anaerobic digestion and the effect on biogas production was analyzed.

- Methyl butanoate
- Ethyl butanoate
- Ethyl hexanoate
- Hexyl acetate
- Methyl acetate
- Ethyl acetate
- Butyl acetate

- Preparation sample:

- Nutrient broth
- D-Glucose
- Extract yeast
- Gas mixture containing 80% N_2 and 20% CO_2

- Gas chromatography measurement:

- CH_4 standard
- CO_2 standard
- N_2

3.2. Equipment

The equipment used was:

- 120 ml-clear glass serum bottle (USA)
- 20mm/aluminium crimp cap with inserted gray PTFE/butyl rubber septa (USA)
- Hand crimper for 20 mm seal (USA)
- 0.25 ml-pressure lock syringe (VICI, USA)
- Microfilter 0.2 µm
- Thermostat at 55^{o}C (Incucell)
- Gas chromatograph (Varian 450 GC) with a capillary column, equipped with TCD detector and Software Galaxie Chromatography Data System v. 1.9 single instrument.

  Working condition:

  Injector : 75^{o}C

  Column flow : Nitrogen 2.0 ml/min

  Oven : 100^{o}C

  Detector (TCD) : 120^{o}C

3.3. Method

3.3.1. Anaerobic digestion

Two different investigations of anaerobic digestion were performed:

a) Investigation of effect of concentration of different types of ester added on biogas production
b) Investigation of the chemical structure of different type of esters addition on biogas production.

The method used for anaerobic digestion was adapted from the method described by Hansen *et al*. [24], and OECD [25]. The experiments were carried out in a 120 ml glass

bottle containing 50 ml of sludge, 1 ml of medium, and 2.5 ml of inhibitor solution or distilled water (control). In order to measure gas production from the inoculums, the sludge was incubated without addition of medium in glass bottles containing 50 ml of sludge and 3.5 ml of distilled water (blank). The glass bottles were closed tightly with butyl-rubber seals and aluminium caps. The bottles were then flushed with 80% N_2 and 20% CO_2 for 2 minutes to remove oxygen (anaerobic condition). The bottles were incubated at 55^{o}C in incubator for 28 days incubation. The bottles were shaken twice a day using water bath shaker (55^{o}C) at 15 rpm. During incubation, gas sample was taken from the headspace of the bottles through the septum using a syringe with pressure lock on certain days and analyzed using GC. All samples were carried out in triplicate batch experiment and the result was presented in average. All data are presented subtracted with methane production of inoculums (blank).

3.3.2. Investigation the effect of concentration of ester addition on anaerobic digestion

The purpose of this experiment was to analyze the effect of concentration of ester added as inhibitor on biogas production.

The first experiment series used methyl butanoate, ethyl butanoate, ethyl hexanoate, and hexyl acetate. Three different concentrations for each ester were varied for this experiment, (5 g/L, 0.5 g/L, and 0.05 g/L) [23]. The anaerobic digestion was executed for 28 days. The gas sample was taken from the glass bottles and analyzed using Gas Chromatography.

The second experiment series used the same ester as the first experimental series with concentration was increased into 10 and 20 g/L. The experiment was also performed at concentration ester of 5 g/L without substrate addition to see if the ester was consumed by microorganisms or not. The anaerobic digestion was run for 28 days. The gas sample was taken from the glass bottles and analyzed using Gas Chromatography.

3.3.3. Investigation of the effect of alkyl or acid groups of the ester addition on biogas production

The purpose of this experiment was to find out whether the carboxylic or the acetate groups give stronger effect on the anaerobic digestion process. Seven different esters were used methyl acetate, ethyl acetate, butyl acetate, hexyl acetate, methyl butanoate, ethyl butanoate, and ethyl hexanoate. The added ester concentration was 5 g/L with

variation with and without substrate addition. The anaerobic digestion was run for 28 days. The gas sample was taken from the glass bottles and analyzed using Gas Chromatography.

3.3.4. Method of analysis

Methane and carbon dioxide produced from the samples were measured using Varian 450-GC Gas Chromatography and Galaxie Chromatography Data System v 1.9 Single Instrument as the software. Gas sample was taken from the glass bottles using a 0.25 ml syringe with pressure lock. The methane content in the reactor headspace could be calculated from the fixed volume sample and the measured mass of methane in the sample, without measuring the actual pressure in the bottle [23]. It was necessary to release the gas in order to avoid overpressure due to the accumulation of gas production on the bottle and to maintain the pressure below 2 bars. The overpressure condition could lead to leakage of gas. Each sample was analyzed twice, before and after release. The measurements were then transferred into accumulated CH_4 as a function of incubation time. The methane production of the samples was subtracted with the methane production of inoculums. Thus, the result only represents the methane production from samples. Flow diagram of batch anaerobic digestion and illustration of batch reactor and sampling were shown on Figure 3.1. and 3.2, respectively.

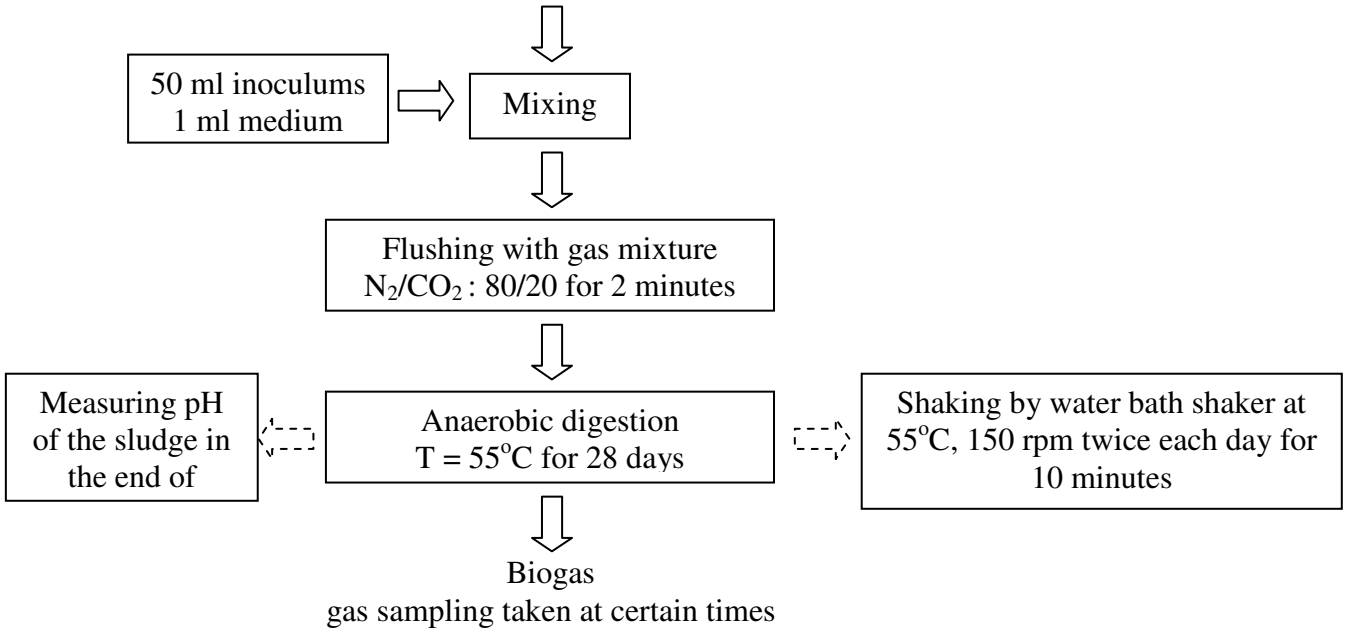


Figure 3. 1. Flow diagram of batch anaerobic digestion

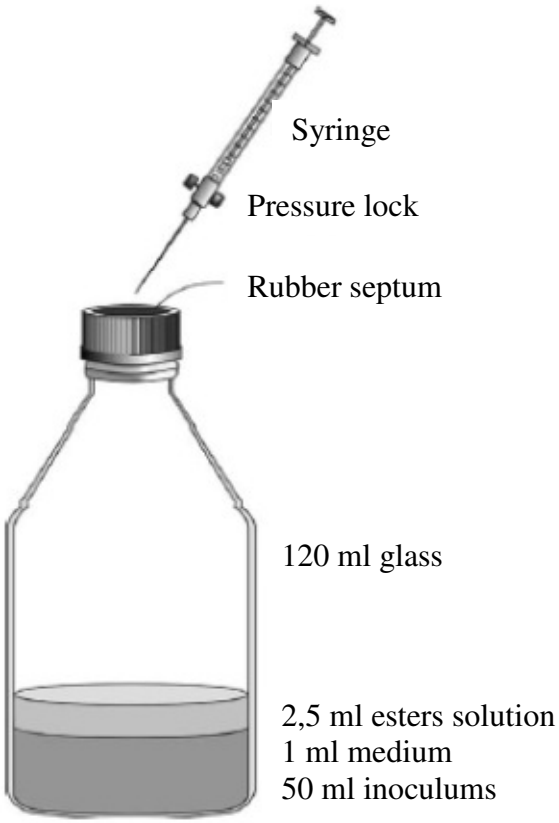


Figure 3. 2. Illustration of batch reactor and gas sampling [23]

IV. RESULT AND DISCUSSION

4.1.1. Esters concentration effects on biogas production

- The first experiment series

In the first experiment series the effect of different concentration of esters added was studied. The cumulative methane production during 28 days of digestion is shown on Figure 4.1. In general, all variations of different esters concentration added gave higher methane production compared to that of control, except ester added at concentration of 0.05 g/L. The higher concentration of esters added resulted in higher methane production.

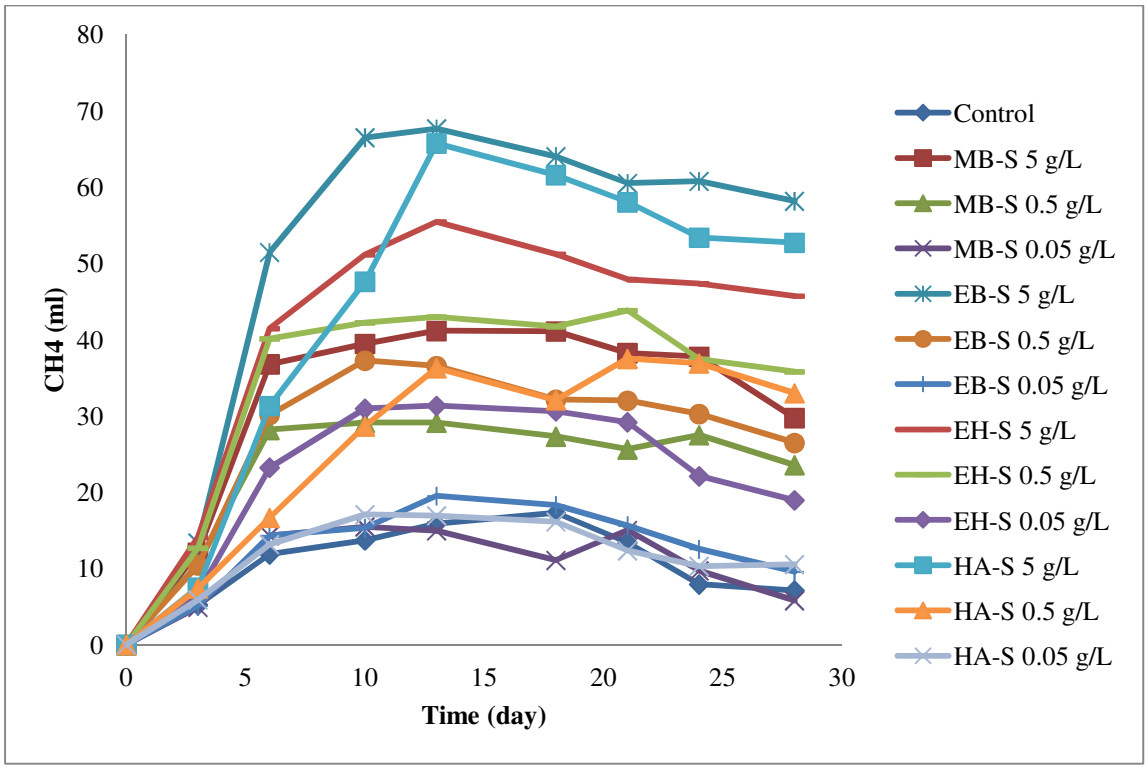


Figure 4. 1. The cumulative methane production of different concentration of esters added on biogas production

In the beginning of digestion, the methane production for all sample variations was relatively the same. But after 6 days of digestion, the methane production increased drastically compared to that of control, except addition the ester at concentration of 0.05 g/L which was nearly the same with methane production from control. After 13 days of digestion, the rate of methane production for all variations was relatively constant.

The higher methane production of samples compared to control indicates no inhibitory effect, of esters addition at concentration up to 5 g/L, on biogas production. In fact, the esters probably acted as second carbon source for the microorganisms. Esters are formed from alcohols and acids which the general chemical formula written as RCO_2R', where R and R' are the hydrocarbon parts of carboxylic acid and alcohol, respectively. The esters were probably hydrolyzed by microorganisms into carboxylic acid and alcohol. Carboxylic acids and alcohols are known as intermediate products on the acidogenesis stage [6, 19]. Therefore, the carboxylic acid and alcohol could have become substrate addition for the microorganisms beside the substrate that was added. The carboxylic acid and alcohols further will degrade by microorganisms through acetogenesis stage into acetic acid and carbon dioxide. Methanogenic bacteria then convert acetic acid into methane. Liu and Suflita [26], reported the ability of *Acetobacterium woodii* and *Eubacterium limosum* to degrade methyl esters under anaerobic biodegradation. Both bacteria hydrolyzed methyl butanoate into carboxylate and methanol. The alcohols further oxidized to produce formate. The reaction of microbial biodegradation of methyl esters was proposed as the following [26]:

$$RCOOCH_3 + OH^- \rightarrow RCOO^- + CH_3OH \quad (1)$$

Based on reaction 1, the hydrolysis reactions for the other three esters probably follow the general reaction below:

$$RCO_2R' + H_2O \rightarrow RCO_2H + R'OH \quad (2)$$

$$\text{Ethyl butanoate} \rightarrow \text{butyric acid} + \text{ethanol} \quad (3)$$

$$\text{Ethyl hexanoate} \rightarrow \text{caproic acid} + \text{ethanol} \quad (4)$$

$$\text{Hexyl acetate} \rightarrow \text{acetic acid} + \text{hexanol} \quad (5)$$

The highest methane production was obtained by addition of ethyl butanoate at concentration 5 g/L and followed by hexyl acetate, ethyl hexanoate, and methyl butanoate at the same concentration. Ethyl butanoate with 6 carbons produced more methane compared to methyl butanoate with 5 carbons. Even though ethyl hexanoate and hexyl acetate have 8 carbons, the methane produced was lower than that of ethyl butanoate. It might due to caproic acid that has a longer carbon chain and could be more difficult to be degraded compared to butyric acid. For hexyl acetate, the acetic acid can be directly converted by methanogenic bacteria into methane. However, hexyl seems more difficult to degrade into methane [26].

At concentration 0.05 g/L, the methane production for methyl butanoate, ethyl butanoate, and hexyl acetate were mostly the same as the production of control. While with addition of ethyl hexanoate, methane production was higher than that of control. The level was even similar when adding methyl butanoate at concentration of 0.5 g/L.

Methane content ratio (by assuming only methane and carbon dioxide produced) of biogas mixture during 28 days of digestion is shown on Figure 4.2. It was shown that control gave higher methane content than all types of added esters. It seems the addition of ester enhance the carbon dioxide production. The percentage of carbon dioxide increases by increasing concentration of added ester.

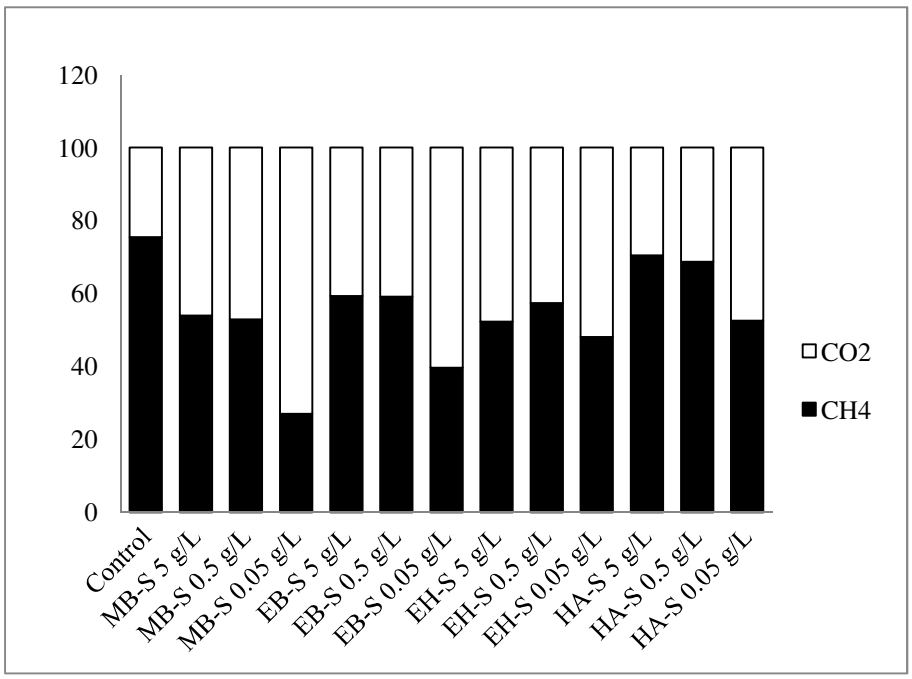


Figure 4. 2. Methane content ratio of different concentration of esters added on biogas production

The theoritical methane production can be calculated based on the general reaction of methane formation as follows [6]:

$$C_cH_hO_oN_nS_s + yH_2O \rightarrow xCH_4 + nNH_3 + sH_2S + (c - x)CO_2 \quad (6)$$

Mole of methane produced (x) = 1/8(4c + h – 2o - 3n – 2s)

The calculation results for the esters consumed during digestion is shown in Table 4.1. The methane produced is shown in the table originating only the production of methane from esters by bacteria. The value was obtained by substracting the methane production each variations with methane production of control. The highest percentage of esters consumed was obtained by additon esters at concentration 0.05 g/L. By increasing

concentration of added esters, the percentage of esters consumed decreased. It was probably due to high concentration of ester added which caused accumulation of volatile acids produced [26]. Table 4.1. shows that the addition of esters with concentration higher than 5 g/L probably will inhibit the digestion process due to accumulation of volatile acids.

Table 4. 1. Percentage of esters consumed by microorganism

Ester	Concentration added (g/L)	Methane production (ml)	Theoretical gas production (ml)	Ester consumed (%)
Methyl butyrate	5	25,28	190,92	13,24
	0,5	13,25	19,09	69,41
	0,05	1,63	1,91	85,22
Ethyl butyrate	5	51,78	206,62	25,06
	0,5	20,68	20,66	100
	0,05	3,65	2,07	100
Ethyl hexanoate	5	39,56	228,86	17,28
	0,5	27,11	22,89	100
	0,05	15,47	2,29	100
Hexyl acetate	5	49,86	228,86	21,79
	0,5	20,39	22,89	89,09
	0,05	1,07	2,29	46,73

- The second experiment series

In the second experiment series, the concentration of esters added was increased to 10 and 20 g/L to examine if the microbial still are capable to consume it or not. The experiments were also carried out with addition of esters of 5 g/L without addition of substrate. The cumulative methane production is shown in Figure 4.3. After 2 days of digestion, the methane production for all sample variations was lower than that of control. For addition of hexyl acetate, the methane production was even lower than the blank which without addition of substrate for all variations until 28 days of digestion. It seems hexyl acetate gives strong inhibitory effect for the microorganisms. Hexyl acetate was hydrolyzed by hydrolytic bacteria into hexanol and acetic acetate. Acetic acetate probably can be consumed by microorganisms which were not the case for hexanol, an alcohol with 6 carbons that seem toxic for microorganisms. The alcohol with 6 carbons

1-hexanol at 0,28 mg/L was reported significantly inhibiting proliferation of *E. Coli* and completely inhibited at 3,8 mg/L [27].

For sample with concentration of esters 5 g/L without substrate addition, the methane production was lower than control due to lack of substrate for the microorganisms. While for concentration 10 and 20 g/L, it seems the concentration was too high for the microorganisms. However, after 6 days of digestion, the methane production at 5 g/L ester added without substrate increased much even higher than control did. The action might be due to synthesis of enzyme involving in ester degradation since there was no substrate addition on the samples. The esters acted as substrate for the microorganisms. The production of methane for this sample increased further until 28 days of digestion.

At concentration of ester added at 10 g/L esters, the methane production increased significantly after 6 days of digestion. It gave higher methane than control. It seems the microorganisms started to adapt with high concentration of ester added. The microorganisms have ability to adapt to inhibitors after a certain times [26]. But the increased methane production was only occurred for methyl butanoate. While for ethyl butanoate and ethyl hexanoate, the methane production was relatively constant after 6 days of digestion. It occurs probably due to the longer chain of the esters compared to methyl butanoate.

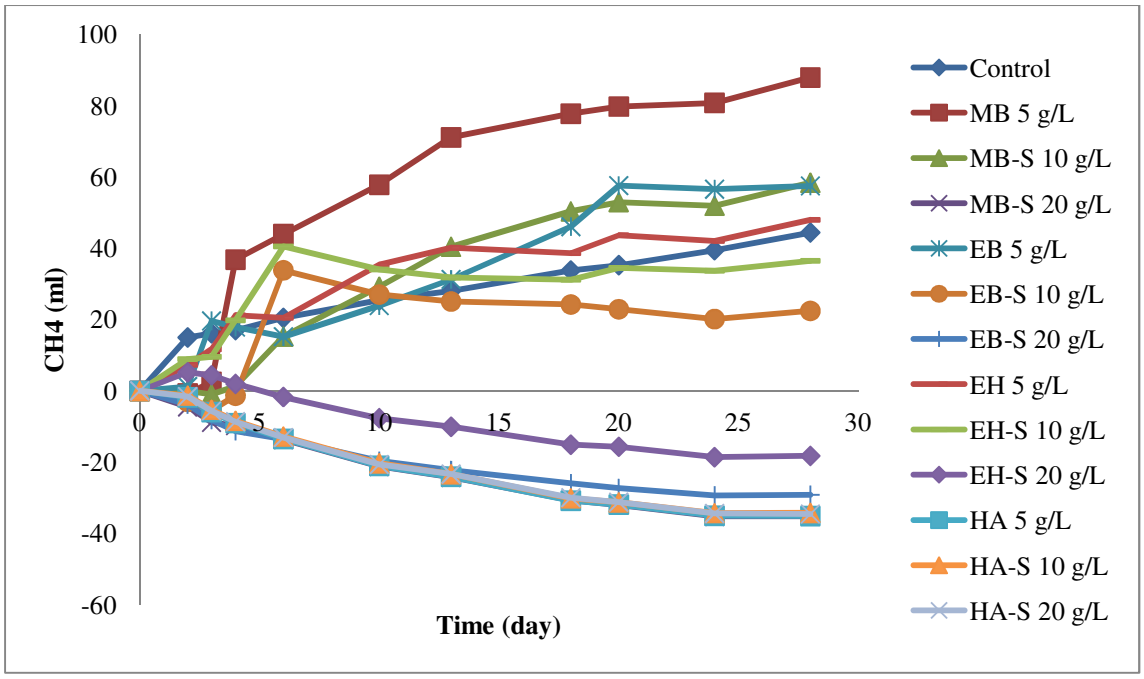


Figure 4. 3. The cumulative methane production of different concentration of esters added on biogas production

By addition of 20 g/L of esters added, methane production for all esters gave decreased methane production compared to control. At this concentration, the microbial activity was strongly inhibited by the esters.

The methane content ratio of the second experiment series is shown in Figure 4.4. The addition of esters decreased the methane content ratio. For all variations of concentration of added hexyl acetate and the other esters at level concentration of 20 g/L, gave zero methane production. It means the bacteria only produced carbon dioxide during digestion.

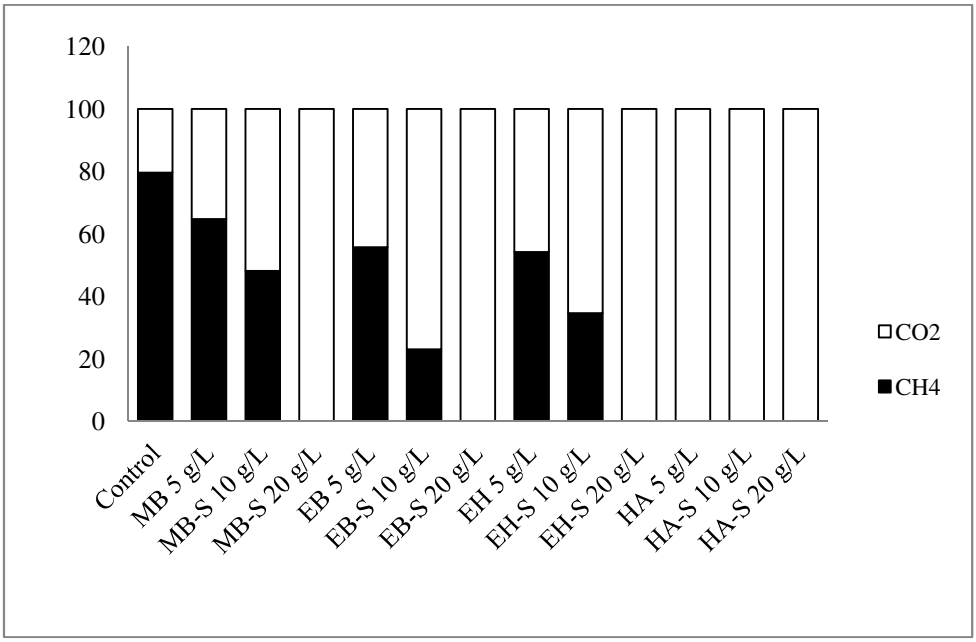


Figure 4. 4. Methane content ratio of different concentration of esters added on biogas production

The theoritical gas produced is shown in Table 4.2. Additon of esters at concentration of 20 g/L inhibited biogas production by factor of about 144%. The minimum inhibitor concentration (MIC) of methyl butanoate was between 10 and 20 g/L, while for ethyl butanoate, ethyl hexanoate, and hexyl acetate the MIC was between 5 to 10 g/L. Moreover, hexyl acetate at concentration of 5 g/L inhibited the microorganism activity if no substrate was present on the medium. While for the three other ester, addition of 5 g/L esters was used for consumption and acted as substrate for the digestion.

Table 4. 2. Percentage of ester consumed and inhibition effect

Ester	Concentration added (g/L)	Methane production (ml)	Theoretical gas production (ml)	Ester consumed (%)	Inhibition (%)
Methyl butyrate	5	87,90	190,92	46,04	-
	10	13,98	381,84	3,66	-
	20	-79,56	763,69	-	190,83
Ethyl butyrate	0	57,49	206,62	27,83	-
	10	-21,99	413,24	-	28,27
	20	-73,56	826,48	-	176,78
Ethyl hexanoate	5	48,01	228,86	20,98	-
	10	-7,90	457,72	-	7,67
	20	-62,66	915,44	-	144,63
Hexyl acetate	5	-35,09	228,86	-	191,14
	10	-78,65	457,72	-	189,05
	20	-78,96	915,44	-	188,61

Figure 4.5. shows the pH of all samples at 10, 20, and 30 days of digestion. The pH for all samples at the beginning of digestion was between 7.5 to 8. The pH of sampels after 10 days of digestion for esters added of 10 and 20 g/L decreased. For 20 g/L of esters added the pH around 5. This condition gave inhibitory effect for the digestion. The optimum condition of digestion process is at pH 6.7 – 7.5 [9, 20]. The decrease of pH is probably due to the accumulation of acids and the buffering system can not handle it [6, 19]. But for hexyl acetate addition, the pH only slightly decrease after 20 and 30 days of digestion. The pH was around 7 which is still in the optimum condition for digestion process. However, the methane production was minus (see Figure 4.3). The inhibition mechanisms of hexanol was not due to the accumulation of volatile acids, but it was probably due to change of the permeability of cell membranes bacteria, thus causing leakage of cellular component and infuencing the methabolism of bacteria. It can be also due to the conversion of hexanol to more toxic compounds [27].

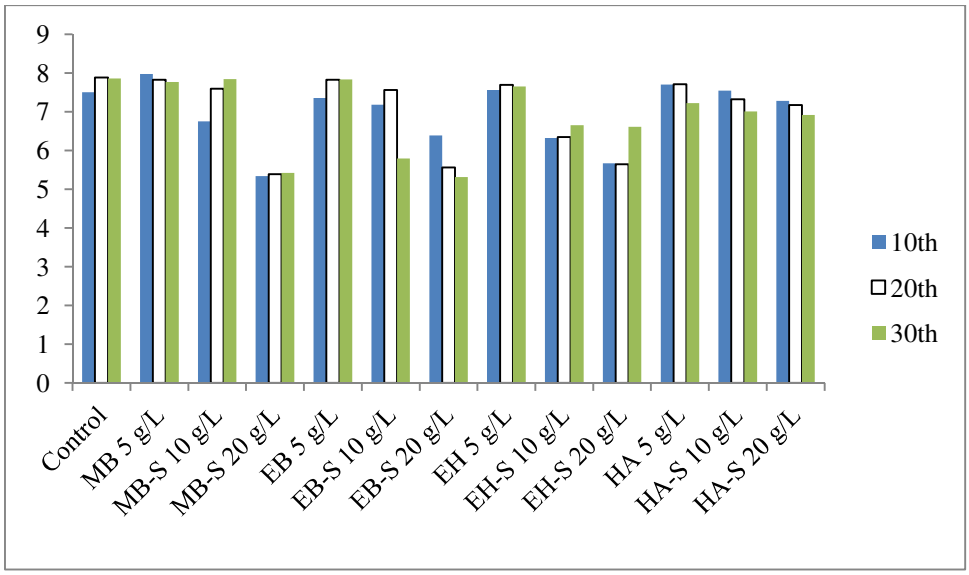


Figure 4. 5. pH of different type of esters addition after 10, 20 and 30 days incubation

4.1.2. Acetate ester effect on biogas production

In this experiment, four different esters with acetate groups were used which are methyl, ethyl, butyl, and hexyl acetate. The purpose is to investigate effect of the length of the chain of the ester bond that gave inhibitory effect on biogas production. The experiment was carried out by addition of esters at concentration of 5 g/L without substrate added. The cumulative methane production during 28 days of digestion for this experiment is shown in Figure 4.6.

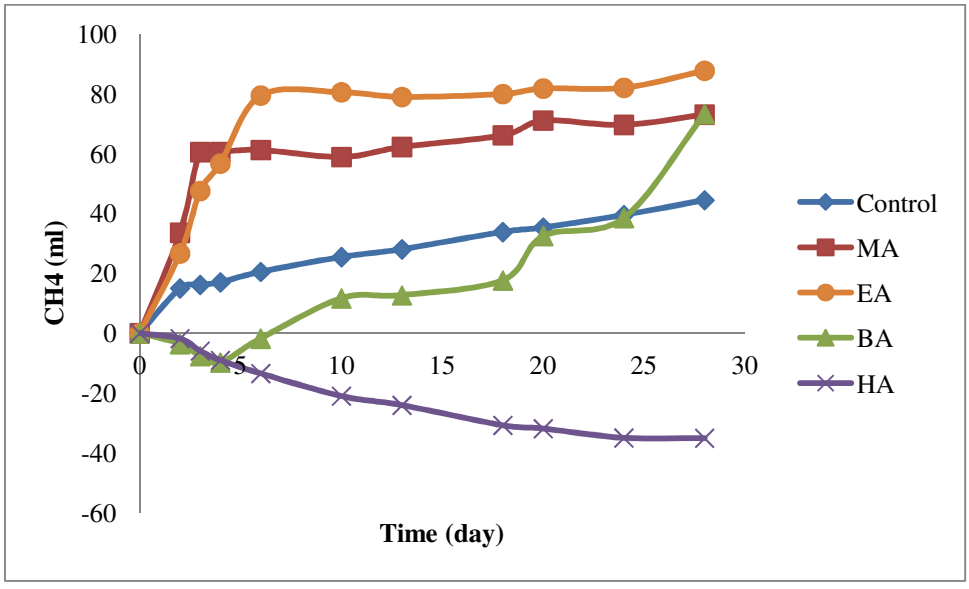


Figure 4. 6. Cumulative methane production of esters added at concentration 5 g/L without substrate added

By addition of methyl or ethyl acetate, the methane production gives always higher methane production compared to control since the beginning of digestion. Even though the experiment was carried out without substrate addition, the methane production was still higher than that of control (with substrate addition). This occured because methyl and ethyl acetate was consumed by microorganisms easily. Ethyl acetate, butyl acetate and hexyl acetate was reported to increase conidial germination of *B.* Cinerea that was injected on apple at low concentration, but it inhibited the conidial germination at higher concentration [28]. The hydrolization of methyl and ethyl acetate is probably as follows

$$\text{Methyl acetate} + H_2O \rightarrow \text{Methanol} + \text{acetic acid} \quad (7)$$

$$\text{Ethyl acetate} + H_2O \rightarrow \text{Ethanol} + \text{acetic acid} \quad (8)$$

Acetic acid will be directly converted into methane by methanogenic bacteria. While methanol and ethanol will be converted by acetogenic bacteria into formate and acetic acid (HCOOH and CH_3COOH) before it is used by methanogen bacteria to produce methane [6, 19, 21].

Different phenomenon occurs for butyl and hexyl acetate. At the beginning and until 4 days of digestion, butyl acetate inhibited the digestion process. It was probably due to the fact that no substrate added in this experiment. The bacteria were in the condition lacking nutrition for growing and producing methane. Butyl acetate was probably degraded by hydrolytic bacteria into butanol and acetate acid. The bacteria could consume acetic acid as substrate. While the butanol with longer chain carbon than methanol and ethanol was probably toxic to the bacteria. But after 4 days of digestion, the production of methane started to increase even higher than production by control after 24 days of digestion. After 4 days of digestion, the bacteria started to adapt to their environment and probably it was already able to consume the butanol. It has been reported that *C. Acetobutylicum* can grow in media supplemented with butanol concentration up to 1.8% and *E. Coli* and yeast host cells can adapt with butanol toxicity and exhibit limited growth in 2% butanol [29]. Addition of hexyl acetate at concentration of 5 g/L without substrate strongly inhibited the digestion process. Since the beginning of digestion until 28 days of digestion, the methane production was negative.

From this experiment, it seems the methane production was more affected by increasing number of carbon of the alkyl compared to the acid group. Methane production increases with decreased number of carbon of the alkyl.

Figure 4.7 shows the methane content ratio for esters addition with acetate groups. The methane content ratio for esters addition was lower than methane content ratio of the control. Even though the cumulative methane production by esters addition, except hexyl acetate, was higher than control production but the rate production of carbon dioxide was also high. In the end, it gave lower methane content ratio than control.

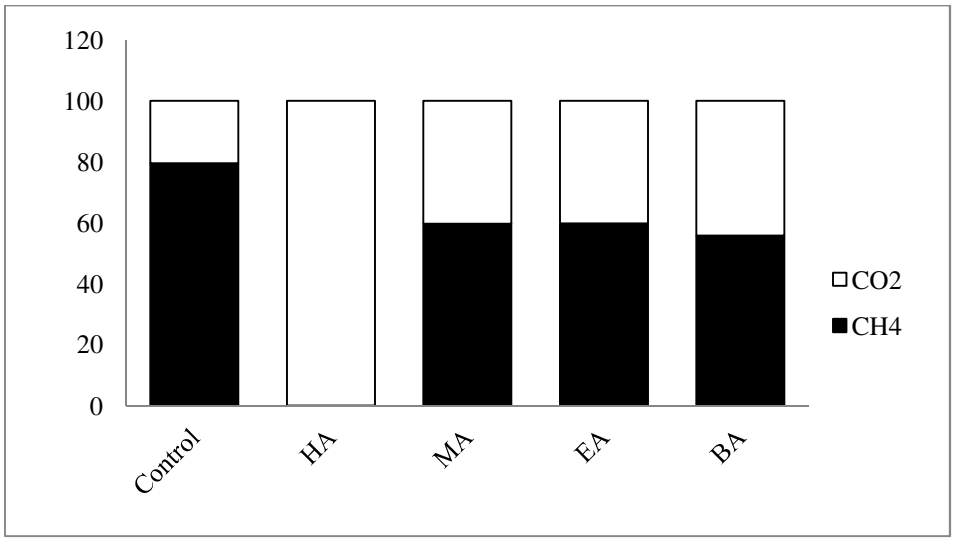


Figure 4. 7. Methane content ratio during 28 days of digestion

4.1.3. Ethyl esters effect on biogas production

In this experiment, there were ethyl esters added on anaerobic digestion. The experiment was carried out without substrate addition. Figure 4.8. shows the cumulative methane production for ethyl esters addition on anerobic digestion.

Ethyl acetate addition gave higher methane production compared to control since in the beginning of digestion followed by ethyl butanoate and ethyl hexanoate. The suggested reactions of ester degradation are:

$$\text{Ethyl acetate} + H_2O \rightarrow \text{ethanol} + \text{acetic acid} \quad (9)$$

$$\text{Ethyl butanoate} + H_2O \rightarrow \text{ethanol} + \text{butanoic acid} \quad (10)$$

$$\text{Ethyl hexanoate} + H_2O \rightarrow \text{ethanol} + \text{hexanoic acid} \quad (11)$$

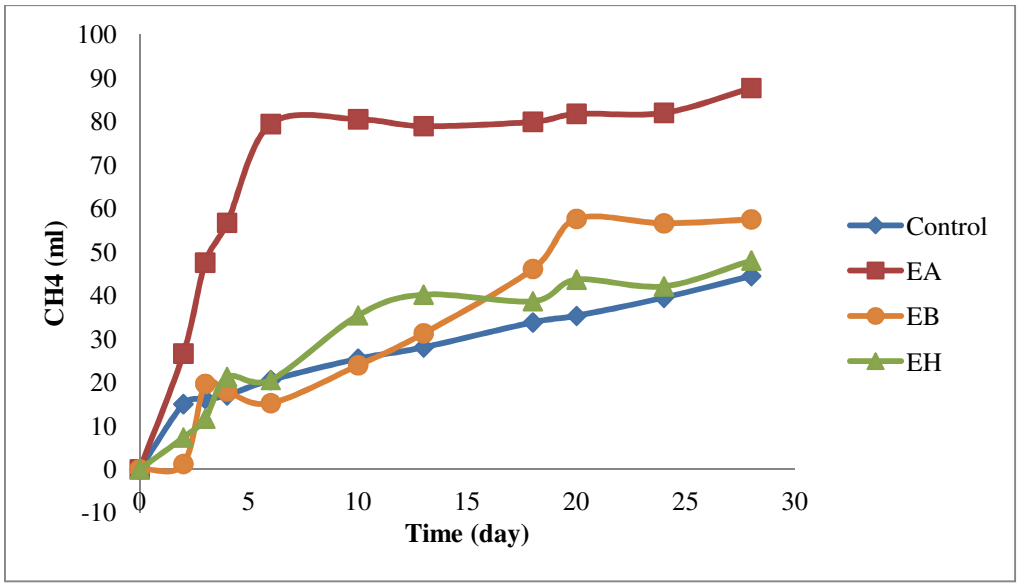


Figure 4. 8. Cumulative methane production of ethyl esters added at concentration 5 g/L without substrate added

Ethanol and acids groups produced during acidogenesis will be converted by acetogenesis bacteria into acetic acid that further will be degraded by methanogenic bacteria into methane. Short chain fatty acids C2 – C5 at concentration up to 2.5 M exhibited minimal activity of microorganisms by killing 80% of microorganisms. [30]. From this experiment, the acid groups on the esters affected methane production. Methane production increased with decreasing number of carbon on acid groups.

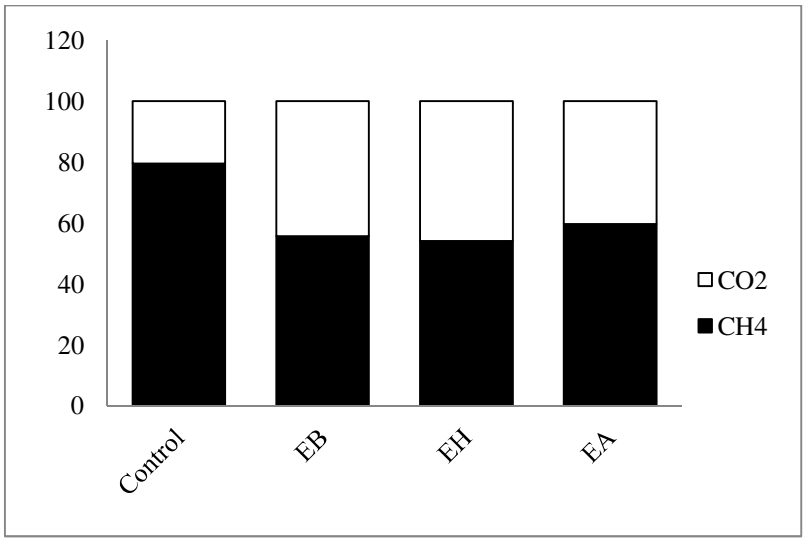


Figure 4. 9. Methane content ratio of anaerobic digestion with addition ethyl esters at 5 g/L without substrate added

The methane content ratio for experiment with addition ethyl ester is shown on Figure 4.9. The methane content ratio for three variations of ethyl ester addition was lower than control (50%).

V. CONCLUSION

The need of renewable sources of energy is increasing all over the world. This work is trying to investigate the effects of ester addition on anaerobic digestion: Inhibition or boosting biogas production? The conclusions from this work can be summarized as:

- The addition of esters inhibits and boosts methane production depending on the concentration of esters added.
- Addition of methyl butanoate, ethyl butanoate, ethyl hexanoate, and hexyl acetate at concentration of 5 g/L increase methane production with increasing number of carbon on esters.
- Minimum inhibitory concentration for addition methyl butanoate is between 10 and 20 g/L, while for ethyl butanoate, ethyl hexanoate, and hexyl acetate between 5 and 10 g/L.
- Higher than MIC without substrate added, the inhibition of esters increases with increasing number of carbon on esters.
- Addition of hexyl acetate at 5 g/L without substrate added strongly inhibits biogas production. While addition of methyl, ethyl, and butyl acetate do not show inhibitory effect.
- Addition of esters on anaerobic digestion decreases methane content ratio.

FUTURE WORK

Result from this work indicates the need for more work on following issues:

- Analyzing the concentration of esters added as function of time to see the change of concentration of esters on the reaction and in order to know if the esters are really consumed or convert to other compounds.
- Investigating the inhibition mechanism of ester addition on anaerobic digestion.
- Narrowing the range of minimum inhibitory inhibition ester added in order to get the limiting concentration of ester added that gives inhibitory effect on anaerobic digestion.
- Analyzing the composition of esters on fruit waste and investigating the effects on anaerobic digestion using fruit waste as a feedstock.

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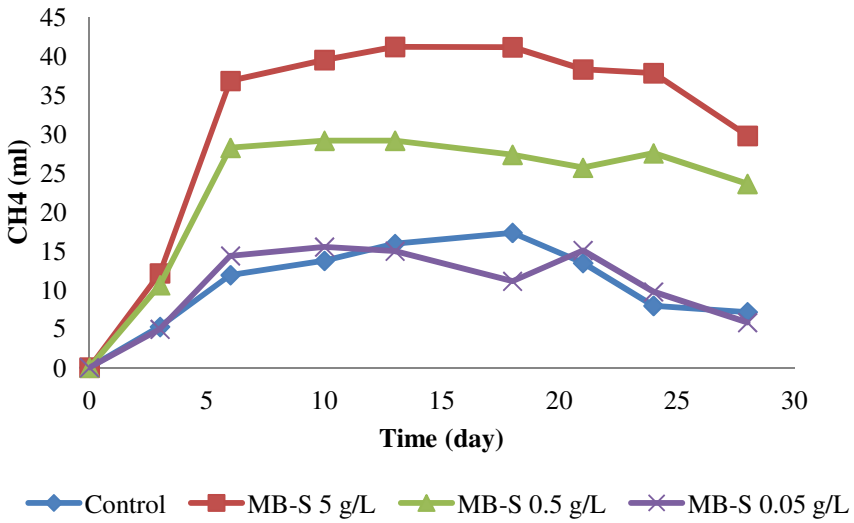
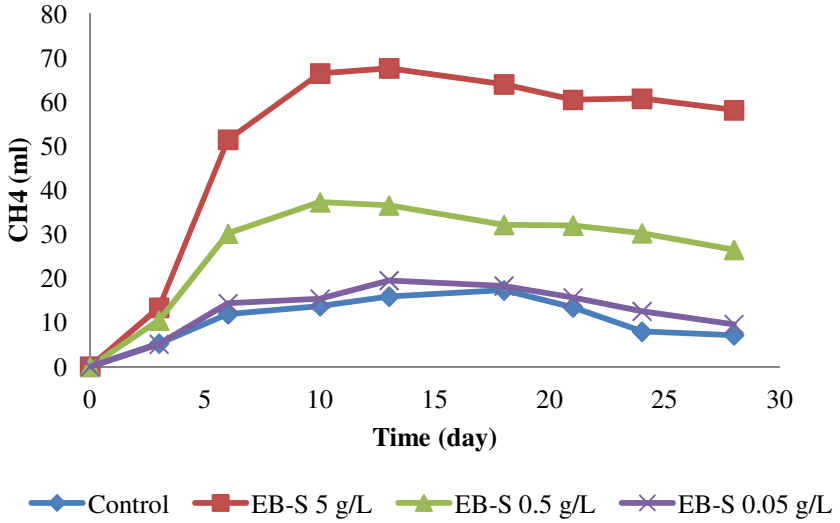
APPENDICES

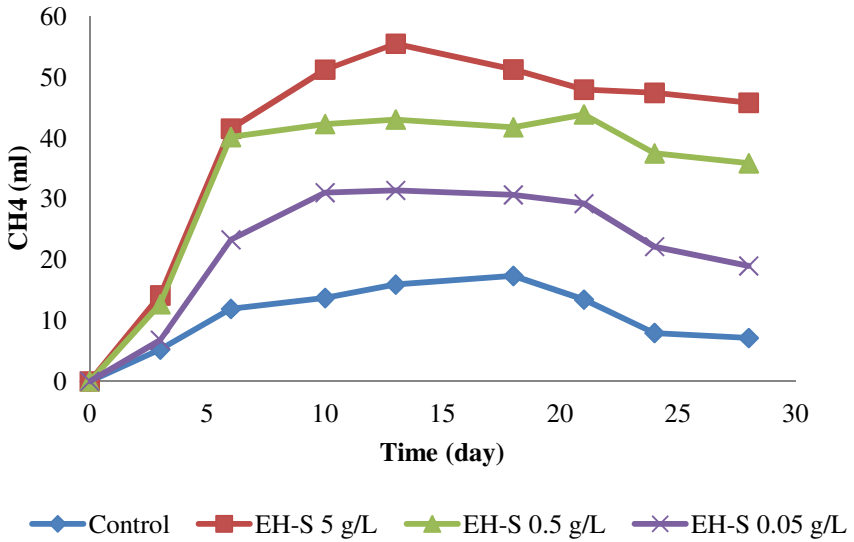
Appendix 1. Data of methane production of the first experimental series.

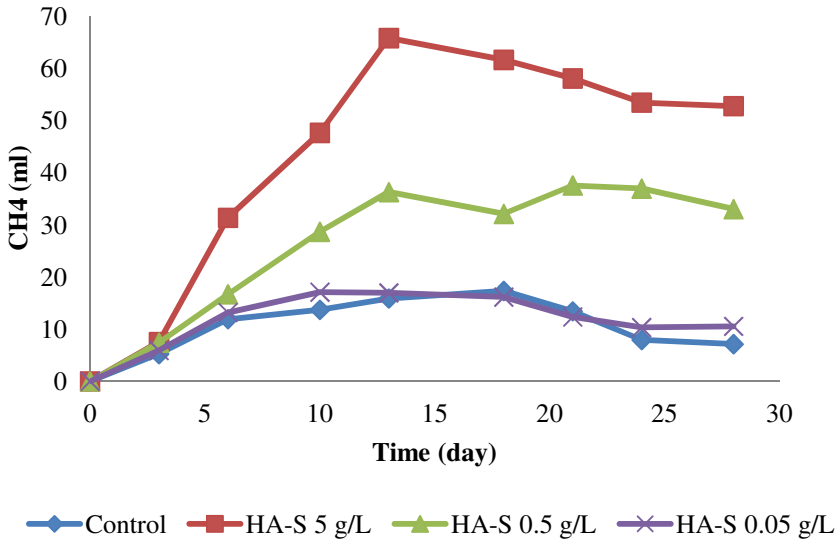
Sample code	Methane production for 28 days of digestion (ml)								
	0	3	6	10	13	18	21	24	28
Control	0	5,236	11,880	13,698	15,874	17,298	13,413	7,935	7,120
MB 5 g/L	0	12,102	36,780	39,458	41,156	41,096	38,257	37,782	29,734
MB 0.5 g/L	0	10,588	28,192	29,125	29,125	27,316	25,642	27,495	23,573
MB 0.05 g/L	0	4,927	14,335	15,476	14,949	11,082	15,040	9,715	5,794
EB 5 g/L	0	13,366	51,454	66,493	67,654	64,015	60,503	60,768	58,159
EB 0.5 g/L	0	10,429	30,149	37,261	36,552	32,147	32,012	30,266	26,472
EB 0.05 g/L	0	5,077	14,324	15,341	19,525	18,307	15,640	12,545	9,592
EH 5 g/L	0	14,107	41,477	51,171	55,432	51,193	47,876	47,352	45,727
EH 0.5 g/L	0	12,671	40,124	42,241	42,984	41,720	43,819	37,446	35,812
EH 0.05 g/L	0	6,755	23,231	30,972	31,346	30,602	29,183	22,113	18,953
HA 5 g/L	0	7,525	31,322	47,610	65,736	61,575	58,029	53,390	52,714
HA 0.5 g/L	0	7,418	16,668	28,659	36,264	32,069	37,513	36,909	32,988
HA 0.05 g/L	0	5,895	13,164	17,078	16,943	16,133	12,340	10,315	10,534

Appendix 2. Graphic data of methane production for the first experimental series









Appendix 3. Data of methane content ratio for the first experimental series

Sample code	Methane content ratio for 28 days of digestion (%)							
	3	6	10	13	18	21	24	28
Control	72,42	60,37	63,72	71,38	72,78	69,45	72,93	75,49
MB 5 g/L	51,47	61,29	62,94	59,68	55,44	56,61	59,05	53,98
MB 0.5 g/L	70,53	61,39	62,16	54,78	51,01	52,76	55,69	52,87
MB 0.05 g/L	62,97	48,83	50,75	45,53	38,26	47,37	37,03	27,00
EB 5 g/L	58,08	57,73	63,84	64,22	60,88	58,80	59,85	59,30
EB 0.5 g/L	72,14	60,33	65,28	64,98	60,74	60,93	61,23	59,12
EB 0.05 g/L	77,36	50,06	51,77	51,60	48,23	48,97	44,86	39,60
EH 5 g/L	46,89	48,70	53,95	56,82	53,86	53,59	52,63	52,26
EH 0.5 g/L	69,16	61,16	62,37	59,12	57,31	58,77	57,69	57,37
EH 0.05 g/L	73,10	51,82	58,91	56,34	55,05	54,13	50,91	48,09
HA 5 g/L	65,86	54,51	64,56	69,88	68,39	69,80	69,89	70,42
HA 0.5 g/L	57,86	54,70	67,49	69,27	63,46	73,75	69,90	68,69
HA 0.05 g/L	77,55	57,71	63,90	55,43	53,62	59,74	50,50	52,54

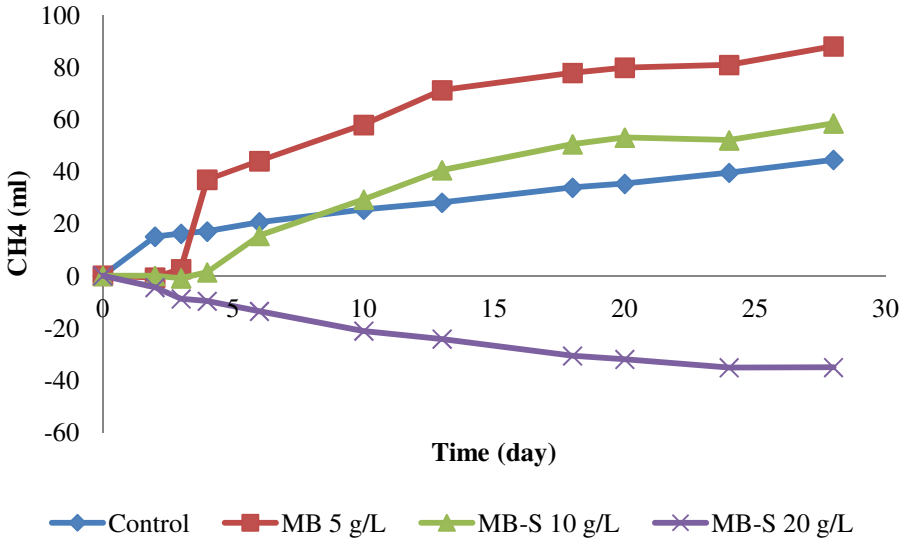
Appendix 4. Data of standard deviation for the first experimental series

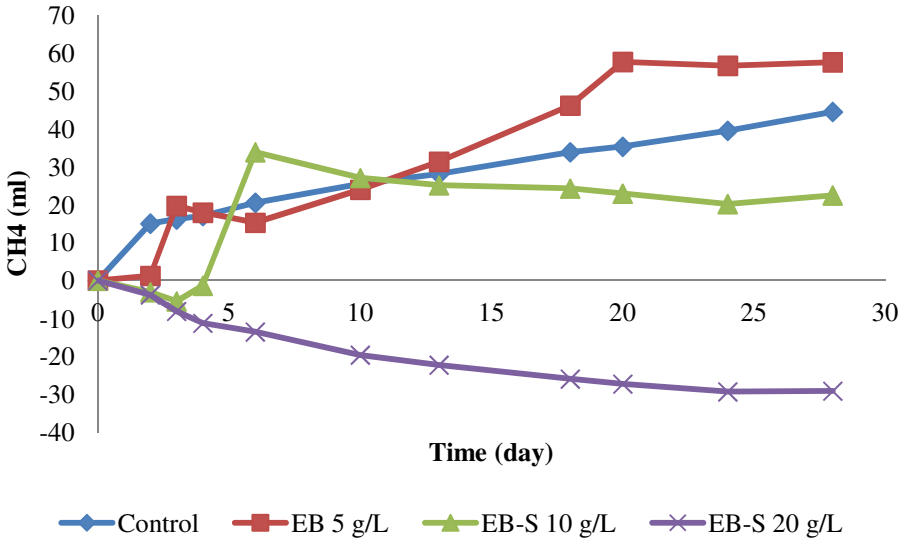
Sample code	Standard deviation for 28 days of digestion							
	3	6	10	13	18	21	24	28
Control	0,22	0,95	1,90	3,31	3,81	4,45	9,17	7,47
MB 5 g/L	0,63	1,99	4,83	4,84	3,64	4,51	3,20	0,68
MB 0.5 g/L	0,13	1,91	3,40	3,47	0,79	2,12	3,68	3,68
MB 0.05 g/L	1,14	1,96	1,20	1,25	3,68	3,32	2,41	2,41
EB 5 g/L	0,19	2,09	1,81	3,65	6,77	8,94	4,33	6,56
EB 0.5 g/L	0,95	0,79	0,74	0,60	2,82	5,71	2,70	2,60
EB 0.05 g/L	0,21	0,44	1,98	3,75	2,37	5,27	10,22	8,98
EH 5 g/L	0,50	2,80	2,24	2,02	2,52	5,93	5,26	5,07
EH 0.5 g/L	0,86	2,94	4,42	4,74	6,06	5,71	4,68	5,58
EH 0.05 g/L	0,05	1,42	0,07	1,67	2,45	3,68	1,69	2,77
HA 5 g/L	0,03	0,60	1,17	0,71	1,33	1,23	2,02	3,79
HA 0.5 g/L	0,88	3,80	4,39	0,69	2,49	0,77	0,48	0,48
HA 0.05 g/L	1,10	5,32	2,25	3,70	5,02	3,61	6,18	11,54

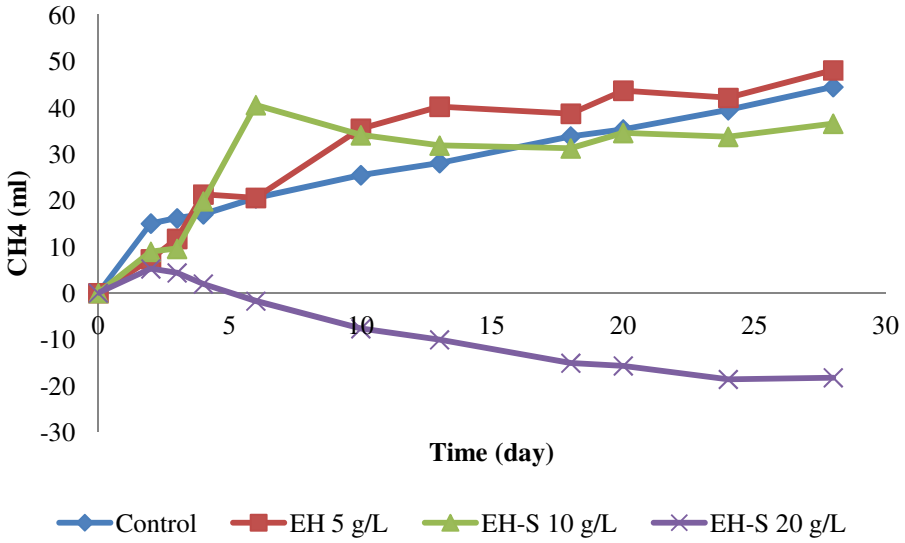
Appendix 5. Data of methane production for the second of experimental series

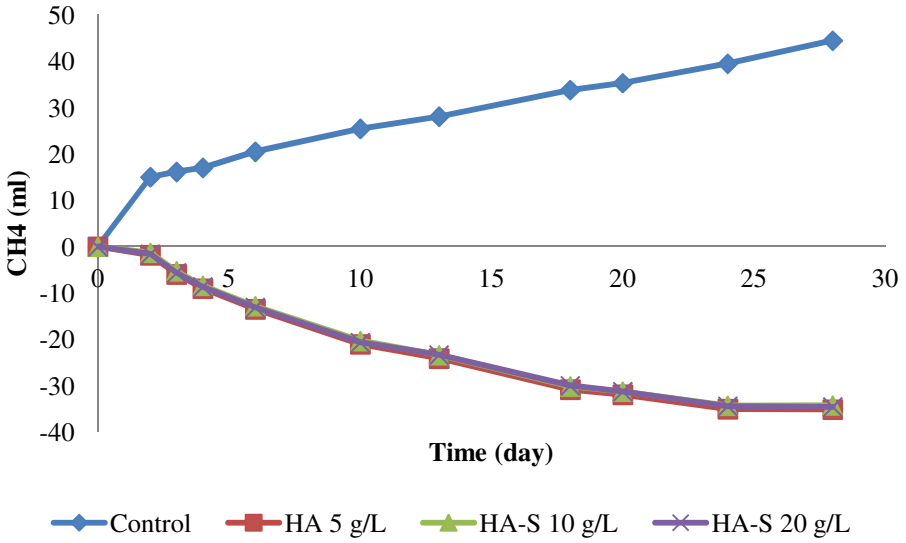
Sample code	Methane production for 28 days of digestion (ml)										
	0	2	3	4	6	10	13	18	20	24	28
Control	0	14,993	16,142	17,000	20,487	25,418	28,043	33,793	35,276	39,459	44,430
MB-S 5 g/L	0	-0,737	2,562	36,831	43,980	57,828	71,104	77,759	79,758	80,782	87,895
MB 10 g/L	0	-0,034	-0,963	1,335	15,280	29,228	40,463	50,390	52,961	51,940	58,408
MB 20 g/L	0	-4,467	-8,813	-9,730	-13,623	-21,178	-24,239	-30,696	-32,024	-35,189	-35,132
EB-S 5 g/L	0	1,224	19,652	17,902	15,212	23,932	31,281	46,085	57,602	56,581	57,494
EB 10 g/L	0	-3,058	-5,475	-1,347	33,833	27,029	25,078	24,240	22,911	20,161	22,443
EB 20 g/L	0	-3,785	-8,132	-11,258	-13,547	-19,639	-22,271	-25,945	-27,274	-29,309	-29,126
EH-S 5 g/L	0	7,321	11,697	21,266	20,543	35,416	40,179	38,663	43,647	42,098	48,013
EH 10 g/L	0	8,955	9,582	19,804	40,535	34,076	31,812	31,200	34,552	33,702	36,526
EH 20 g/L	0	5,235	4,363	1,985	-1,722	-7,665	-10,057	-15,083	-15,681	-18,588	-18,228
HA-S 5 g/L	0	-1,806	-5,936	-9,037	-13,471	-21,025	-24,086	-30,799	-31,894	-35,012	-35,094
HA 10 g/L	0	-1,298	-5,276	-8,401	-12,787	-20,292	-23,353	-30,093	-31,233	-34,266	-34,217
HA 20 g/L	0	-1,588	-5,622	-8,639	-13,076	-20,630	-23,325	-29,943	-31,215	-34,378	-34,528

Appendix 6. Graphic data methane production for the second experimental series









Appendix 7. Data of methane content ratio for the second experimental series

Sample code	Methane content ratio for 28 days of digestion (%)										
	0	2	3	4	6	10	13	18	20	24	28
Control	0	14,993	16,142	17,000	20,487	25,418	28,043	33,793	35,276	39,459	44,430
MB-S 5 g/L	0	-0,737	2,562	36,831	43,980	57,828	71,104	77,759	79,758	80,782	87,895
MB 10 g/L	0	-0,034	-0,963	1,335	15,280	29,228	40,463	50,390	52,961	51,940	58,408
MB 20 g/L	0	-4,467	-8,813	-9,730	-13,623	-21,178	-24,239	-30,696	-32,024	-35,189	-35,132
EB-S 5 g/L	0	1,224	19,652	17,902	15,212	23,932	31,281	46,085	57,602	56,581	57,494
EB 10 g/L	0	-3,058	-5,475	-1,347	33,833	27,029	25,078	24,240	22,911	20,161	22,443
EB 20 g/L	0	-3,785	-8,132	-11,258	-13,547	-19,639	-22,271	-25,945	-27,274	-29,309	-29,126
EH-S 5 g/L	0	7,321	11,697	21,266	20,543	35,416	40,179	38,663	43,647	42,098	48,013
EH 10 g/L	0	8,955	9,582	19,804	40,535	34,076	31,812	31,200	34,552	33,702	36,526
EH 20 g/L	0	5,235	4,363	1,985	-1,722	-7,665	-10,057	-15,083	-15,681	-18,588	-18,228
HA-S 5 g/L	0	-1,806	-5,936	-9,037	-13,471	-21,025	-24,086	-30,799	-31,894	-35,012	-35,094
HA 10 g/L	0	-1,298	-5,276	-8,401	-12,787	-20,292	-23,353	-30,093	-31,233	-34,266	-34,217
HA 20 g/L	0	-1,588	-5,622	-8,639	-13,076	-20,630	-23,325	-29,943	-31,215	-34,378	-34,528

Appendix 8. Data of standard deviation for the second experimental series

Sample code	Standard deviation for 28 days of digestion									
	2	3	4	6	10	13	18	20	24	28
Control	1,27	1,56	2,11	3,01	2,97	3,19	4,77	8,75	6,49	12,68
MB-S 5 g/L	0,94	3,34	9,51	9,47	14,07	16,21	11,02	10,44	14,30	10,18
MB 10 g/L	0,39	0,76	1,84	2,73	3,16	1,69	1,38	3,59	5,67	0,94
MB 20 g/L	0,00	0,00	0,03	0,25	0,25	0,25	0,24	0,24	0,24	0,61
EB-S 5 g/L	0,01	0,66	0,18	1,11	0,42	1,91	1,47	5,96	6,82	8,33
EB 10 g/L	1,04	1,53	5,59	1,10	0,04	1,53	1,58	1,58	0,99	2,45
EB 20 g/L	0,39	0,39	0,39	1,64	2,48	2,73	4,50	4,50	5,15	5,35
EH-S 5 g/L	1,38	3,56	5,52	1,25	5,42	8,72	9,99	11,08	10,44	14,46
EH 10 g/L	0,87	0,99	2,05	1,57	2,73	2,55	2,55	4,49	6,78	6,76
EH 20 g/L	1,97	2,80	2,24	2,27	2,30	2,08	2,47	2,45	2,85	3,33
HA-S 5 g/L	0,16	0,08	0,07	0,07	0,07	0,07	0,11	0,13	0,05	0,16
HA 10 g/L	0,53	0,62	0,62	0,55	0,63	0,63	0,63	0,74	0,55	0,65
HA 20 g/L	0,85	0,98	1,12	1,12	1,12	1,46	1,42	1,49	1,49	1,49

Appendix 9. Data of pH for 2nd experimental design for 28 days of digestion

Sample code	pH for 30 days of digestion		
	10th	20th	30th
Inoculum	7	7,96	7,89
Control	7,5	7,88	7,86
MB-S 5 g/L	7,97	7,82	7,77
MB 10 g/L	6,75	7,59	7,84
MB 20 g/L	5,34	5,39	5,42
EB-S 5 g/L	7,35	7,82	7,83
EB 10 g/L	7,18	7,56	5,80
EB 20 g/L	6,39	5,56	5,31
EH-S 5 g/L	7,56	7,69	7,65
EH 10 g/L	6,32	6,35	6,65
EH 20 g/L	5,67	5,64	6,61
HA-S 5 g/L	7,7	7,71	7,22
HA 10 g/L	7,54	7,32	7,00
HA 20 g/L	7,28	7,17	6,91

Appendix 10. Data of methane production for investigation of acetate ester on anaerobic digestion

Sample code	Methane production for 28 days of digestion										
	0	2	3	4	6	10	13	18	20	24	28
Control	0	14,993	16,142	17,000	20,487	25,418	28,043	33,793	35,276	39,459	44,430
MA 5 g/L	0	33,606	60,533	60,603	61,210	58,869	62,225	66,137	71,066	69,607	73,061
EA 5 g/L	0	26,628	47,530	56,708	79,426	80,523	78,944	79,927	81,770	81,993	87,696
BA 5 g/L	0	-3,615	-7,671	-9,815	-1,839	11,622	12,657	17,652	32,457	38,511	73,260
HA 5 g/L	0	-1,806	-5,936	-9,037	-13,471	-21,025	-24,086	-30,799	-31,894	-35,012	-35,094

Appendix 11. Data of methane content ratio for investigation of acetate ester on anaerobic digestion

	2	3	4	6	10	13	18	21	24	28
Control	62,57	100,00	86,05	79,06	86,17	82,06	75,40	75,66	85,87	79,56
MA 5 g/L	45,95	60,44	60,99	58,95	59,13	57,38	58,38	57,62	59,49	59,61
EA 5 g/L	44,23	49,92	54,77	63,32	64,29	60,64	59,27	58,50	60,17	59,77
BA 5 g/L	0,00	0,00	0,00	0,00	20,10	20,27	26,94	36,55	42,78	55,73
HA 5 g/L	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Appendix 12. Data of standard deviation for investigation acetate ester on anaerobic digestion

Sample code	Standard deviation for 28 days of digestion									
	2	3	4	6	10	13	18	20	24	28
Control	1,27	1,56	2,11	3,01	2,97	3,19	4,77	8,75	6,49	12,68
MA 5 g/L	4,20	3,76	2,90	5,41	4,71	8,69	7,11	4,97	6,30	9,80
EA 5 g/L	1,75	2,20	4,40	4,51	5,86	4,36	3,12	4,75	4,23	6,04
BA 5 g/L	0,11	0,53	1,17	18,35	8,44	13,20	16,40	13,43	4,26	15,66
HA 5 g/L	0,16	0,08	0,07	0,07	0,07	0,07	0,11	0,13	0,05	0,16

Appendix 13. Data of methane production for investigation of ethyl ester on anaerobic digestion

Sample code	Methane production for 28 days of digestion										
	0	2	3	4	6	10	13	18	20	24	28
Control	0	14,993	16,142	17,000	20,487	25,418	28,043	33,793	35,276	39,459	44,430
EA 5 g/L	0	26,628	47,530	56,708	79,426	80,523	78,944	79,927	81,770	81,993	87,696
EB 5 g/L	0	1,224	19,652	17,902	15,212	23,932	31,281	46,085	57,602	56,581	57,494
EH 5 g/L	0	7,321	11,697	21,266	20,543	35,416	40,179	38,663	43,647	42,098	48,013

Appendix 14. Data of methane content ratio for investigation of acetate ester on anaerobic digestion

	2	3	4	6	10	13	18	21	24	28
Control	62,57	100,00	86,05	79,06	86,17	82,06	75,40	75,66	85,87	79,56
EA 5 g/L	45,95	60,44	60,99	58,95	59,13	57,38	58,38	57,62	59,49	59,61
EB 5 g/L	0	1,224	19,652	17,902	15,212	23,932	31,281	46,085	57,602	56,581
EH 5 g/L	57,494	0	7,321	11,697	21,266	20,543	35,416	40,179	38,663	43,647

Appendix 15. Data of standard deviation for investigation acetate ester on anaerobic digestion

Sample code	Standard deviation for 28 days of digestion									
	2	3	4	6	10	13	18	20	24	28
Control	1,27	1,56	2,11	3,01	2,97	3,19	4,77	8,75	6,49	12,68
EA 5 g/L	4,20	3,76	2,90	5,41	4,71	8,69	7,11	4,97	6,30	9,80
EB 5 g/L	0,01	0,66	0,18	1,11	0,42	1,91	1,47	5,96	6,82	8,33
EH 5 g/L	1,38	3,56	5,52	1,25	5,42	8,72	9,99	11,08	10,44	14,46