Determination of the suitable condition for producing biogas from the co-digestion of broiler manure and stillage

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การหาสภาวะที่เหมาะสมสำหรับการผลิตก๊าซชีวภาพจากการย่อยสลายร่วมของมูลไก่เนื้อและน้ำกากส่า

นางสาวชนิตา ตระกูลพานิชย์กิจ

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Thesis Title	Determination of the suitable condition for producing biogas from the co-digestion of broiler manure and stillage
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Field of Study	Hazardous Substance and Environmental Management
Thesis Advisor	Assistant Professor Patiroop Pholchan, Ph.D.

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ชนิตา ตระกูลพานิชย์กิจ : การหาสภาวะที่เหมาะสมสำหรับการผลิตก๊าซชีวภาพจากการย่อยสลายร่วมของมูลไก่เนื้อและน้ำกากส่า (Determination of the suitable condition for producing biogas from the co-digestion of broiler manure and stillage) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.ปฎิรูป ผลจันทร์, 107 หน้า.

พลังงานหมุนเวียนได้รับความสนใจมากขึ้นในช่วงสองทศวรรษที่ผ่านมาเนื่องจากการลดลงของเชื้อเพลิงฟอสซิลและปัญหาภาวะโลกร้อนจากการใช้เชื้อเพลิงฟอสซิล งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาวิธีการผลิตก๊าซชีวภาพจากมูลไก่เนื้อที่เป็นของเสียผสมกับวัสดุที่ใช้รองที่เกิดขึ้นในปริมาณมากในประเทศไทย วัตถุประสงค์แรกของงานวิจัยนี้คือเพื่อศึกษาหาสภาวะที่เหมาะสมในการปรับสภาพมูลไก่เนื้อโดยใช้กระบวนการปรับสภาพร่วมด้วยความร้อนและสารอัลคาไล ปูนขาวเป็นสารอัลคาไลที่ถูกเลือกใช้ เลือกใช้ Full Factorial Design (FFD) ในการออกแบบการทดลอง พร้อมด้วยปัจจัยสามอย่าง คือ อุณหภูมิ ความเข้มข้นของปูนขาว และระยะเวลาในการปรับสภาพ ผลการทดลองพบว่าสภาวะที่เหมาะสมในการปรับสภาพมูลไก่เนื้อ คือ ที่อุณหภูมิ 150 °C, ความเข้มข้นของปูนขาวที่ 3% Ca(OH)$_2$ w/v และระยะเวลาในการปรับสภาพหนึ่งชั่วโมง ได้ประสิทธิภาพการกำจัดลิกนินสูงสุดเท่ากับ 49.9%

อีกวัตถุประสงค์คือเพื่อศึกษาผลกระทบของอัตราส่วนของคาร์บอนต่อไนโตรเจน (C/N) ที่มีต่อประสิทธิภาพการผลิตก๊าซชีวภาพโดยการหมักร่วมระหว่างมูลไก่เนื้อกับน้ำกากส่า การทดลองเลือกทำที่อัตราส่วน C/N แตกต่างกันสามค่าคือ 30, 40 และ 50 ผลผลิตของมีเทนที่ได้สูงสุดคือ 247.73 ± 6.10 mlCH$_4$/gVS ได้จากการใช้น้ำกากส่าเป็นสารตั้งต้นอย่างเดียว ปริมาณของก๊าซมีเทนที่ได้ใกล้เคียงกันระหว่างมูลไก่เนื้อที่ไม่ได้รับการปรับสภาพ (164.39 ±6.05mlCH$_4$/gVS) กับมูลไก่เนื้อที่ได้รับการปรับสภาพ (160.70 ±0.93mlCH$_4$/gVS) เช่นเดียวกับการหมักร่วมที่ C/N 30 ปริมาณมีเทนที่เกิดขึ้นจากการย่อยมูลไก่เนื้อที่ได้รับการปรับสภาพกับน้ำกากส่า (141.37±6.99 mlCH$_4$/gVS) มีค่าแตกต่างอย่างไม่มีนัยสำคัญกับการย่อยมูลไก่เนื้อที่ไม่ได้รับการปรับสภาพกับน้ำกากส่า(154.53±5.79 mlCH$_4$/gVS) จากผลการทดลองสามารถสรุปได้ว่าการปรับสภาพมูลไก่เนื้อโดยใช้กระบวนการปรับสภาพร่วมด้วยความร้อนและสารอัลคาไลไม่ประสบผลสำเร็จในการช่วยเพิ่มการผลิตก๊าซชีวภาพ อีกทั้งยังเกิดผลที่เป็นปรปักษ์กัน ของการย่อยร่วมระหว่างมูลไก่เนื้อกับน้ำกากส่า ในส่วนของโมเดล โมเดล Gomperzt สามารถใช้ทำนายถึงปริมาณสุดท้ายของการเกิดก๊าซมีเทนในแต่ละการทดลองได้ดีกว่า First-order โมเดล โดยใช้ระยะเวลาที่น้อยกว่า และสามารถทำนายได้มากกว่า 95 เปอร์เซนของปริมาณของก๊าซมีเทนสุดท้ายที่เกิดขึ้นจริงจากการทดลอง

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ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

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KEYWORDS: BROILER MANURE / PRETREATMENT / CO-DIGESTION / BIOGAS PRODUCTION / BIOCHEMICAL METHANE PRODUCTION TEST / GOMPERZT MODEL

CHANITA TRAKUNPHANITKIT: Determination of the suitable condition for producing biogas from the co-digestion of broiler manure and stillage. ADVISOR: ASST. PROF. PATIROOP PHOLCHAN, Ph.D., 107 pp.

Renewable energy has gained more interest in the last two decades as the fossil fuel has been depleted and its use contributes to global warming problem. This research aimed to investigate the method to produce biogas from broiler manure (BM), a waste with bedding material that has been generated in large amounts in Thailand. In The first objective of this work was to examine the optimum condition for pretreating BM using the thermal-alkali pretreatment process. Lime was chosen as an alkali substance. Full factorial design (FFD) was used to design the experiments with three factors, i.e. temperature, lime concentration, and pretreatment duration. According to FFD results, the optimum condition for pretreating BM was at 150°C, 3% w/v Ca(OH)$_2$ solution, and 1 h pretreatment duration. The highest lignin removal efficiency from BM was 49.9%.

The second objective was to investigate the effects of C/N ratio on efficiency of biogas production by co-digesting BM with stillage. Three different C/N ratios were chosen, i.e. 30, 40, and 50. The highest methane yield (247.73±6.10 mlCH$_4$/gVS) was achieved from stillage as the sole substrate. The similar methane yields found from the non-pretreated BM (164.39±6.05 mlCH$_4$/gVS) and the pretreated BM (160.70±0.93 mlCH$_4$/gVS). Same for the co-digestion at C/N 30, the methane yield obtained from the pretreated BM co-digested with stillage (141.37±6.99 mlCH$_4$/gVS) was not significantly different from the non-pretreated BM co-digested with stillage (154.53±5.79 mlCH$_4$/gVS). From the results, it could conclude that the thermal-alkali pretreatment could not achieve to increase the biogas production and the co-digestion between BM and stillage had an antagonistic effect in biogas production. Gomperzt model was found to fit better to the BMP results than the first-order model for all the experiment results. Within the relatively shorter period of time (around 5 or 7 d), Gompertz model could predict more than 95% of the final methane production.

Field of Study: Hazardous Substance and Environmental Management
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Student's Signature ....
Advisor's Signature ....

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ABBREVIATION

λ	Lag phase
γ	The maximum volume accumulated at an infinite digestion time
α	The synergistic effect
AD	Anaerobic digestion
BM	Broiler manure
BMP	Biochemical methane potential
℃	Degree Celsius
CCD	Central Composite Design
CH_4	Methane
Co-d	Co-digestion
d	Day
exp	Experiment
FFD	Full Factorial Design
FO	The First-order model
g	gram
GM	The Gompertz model

h	Hour
k	The reaction rate coefficient
L	Liter
mL	Milliliter
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
P	The prediction of the methane production
Pret.BM	Pretreated Broiler manure
Rm	The maximum specific methane production rate
r^2	Coefficient of determination
TS	Total solids
Non Pret.BM	Non-pretreated Broiler manure
VS	Volatile solids

CHAPTER 1
INTRODUCTION

1.1 Background

Nowadays, renewable energy is essential for replacing fossil fuels in order to reduce carbon dioxide emissions that contribute to greenhouse effect. Recently, Thailand has renewable energy policy which is supported by the government's long-term renewable energy (RE) plans called the 10-Year Alternative Energy Development Plan (AEDP 2012-2021). The AEDP is planned by Department of Alternative Energy Development and Efficiency, the Ministry of Energy (MoE). This plan aims to extend renewable energy usage to 25 percent of the final energy consumption of the country (Department of Alternative Energy Development and Efficiency). Anaerobic digestion (AD) is a biological process that has gained high interest within the few decades due to increasing of renewable energy requirements. Biogas is one form of recovering energies produced by biological treatment of wastes and can be utilized as fuel to generate heat and energy. There are different kinds of biomass that can be utilized to produce biogas such as industrial waste, agriculture biomass, and livestock manure.

In Thailand, poultry industry is one of the important livestock production systems which have more than 1.7 million tons of boiler chicken for consumption and export (Office of agricultural economics, 2014). Therefore, high volume of poultry litter has been generated. Poultry litter consists of the poultry waste and bedding material which is mostly rice husk. This waste can cause serious damage to the environment if it is improperly managed. Poultry waste contains many components which are suitable for biogas production, though poultry litter is generally reused as fertilizers, soil amendment, animal feed and fuel source. In addition to poultry litter reused, another sustainable way for eliminating poultry waste is to be used as biomass for biogas production (Reza, 2016)

Some of the challenges for using poultry litter as the feedstock are high ammonia level and high lignocelluloses (Costa *et al.*, 2012; Sakar *et al.*, 2009). Rice husk is lignocellulosic biomass that contains high lignin content preventing accessibility of enzymatic hydrolysis. Hence, to expose the cellulose and hemicellulose which are embedded within lignin network and to increase bioconversion efficiency, the proper pretreatment must be selected for removing lignin content (Iiyama *et al.*, 1994).

There are several pretreatment methods to enhance the digestibility of ligno cellulosic biomass. For thermal pretreatment, the temperature used is around 150-180°C. Heat will affect and increase hemicellulose and lignin solubilization (Bobleter, 1994; Garrote *et al.*, 1999). Alkaline pretreatment causes solubilization, redistribution of lignin and modification in the crystalline of the cellulose (Gregg & Saddler, 1996). Combination of both pretreatment encourages the accessibility of enzymatic hydrolysis of lignocellulosic biomass. Gandi *et al.* (1997) used lime for thermal-alkaline pretreatment because it is relatively cheap and safe. The temperature required for the thermal-alkali pretreatment process is 100-150°C which is less than that when the thermal pretreatment is used alone (Chang *et al.*, 2001).

Nevertheless, another problem for using poultry litter as the feedstock for biogas production is low C/N ratio. Several studies show that to improve anaerobic digestion of poultry litter, combining with other wastes is necessary. Abouelenien *et al.* (2014) studied the co-digestion of poultry litter with mixture of agriculture waste; Gelegenis *et al.* (2007) studied poultry litter co-digest with whey; Sharma *et al.* (2013) studied the co-digestion of poultry litter with thin stillage. Co-digestion of manure with crop residues can provide better C/N ratio in the feedstock and improve on the biogas yield. There are several kinds of waste which has high organic content that can be used for co-digestion with poultry litter such as wastewater from cassava factory, palm oil factory, and distillery.

Thailand has around 82 distilleries, not including local distilleries (Department, 2014), which mostly use molasses for alcohol production. High amount of stillage is generated and generally, the waste stream is treated while some kinds of stillage (stillage containing high yeast concentration) will be reused as fertilizer thanks to its high N, P, and K components. Although stillage could contain high concentration of components which are necessary for plant's growth, the waste has very high organic content which is difficult to treat. Accordingly, stillage discharged to environment has caused many problems to nature, animals, and also human. Anaerobic digestion can be used with stillage to produce biogas. However, due to its high organic content, nitrogen content is not sufficient for anaerobic biodegradation. Therefore, mixing stillage with other wastes is required for high organic content dilution and C/N ratio adjustment.

To our knowledge, the study of anaerobic co-digestion for biogas production using poultry litter and stillage as the feedstock is still lacking. Hence, the aims of this study are to find the optimum condition for broiler manure pretreatment using thermo-alkali process and to investigate effects of C/N ratio on biogas production efficiency when the pretreated broiler manure is co-digested with stillage.

1.2 Objectives

1. To find the optimum temperature, lime concentration and pretreatment duration for pretreating broiler manure using the thermal-alkaline pretreatment process
2. To investigate effects of C/N ratios on efficiency of biogas production from the co-digestion of broiler manure and stillage.

1.3 Hypothesis

1. Pretreatment of broiler manure using thermal-alkali process can beneficially modify broiler manure characteristics for biogas production.

2. Enhancement of biogas production by co-digesting pretreated broiler manure with stillage can be done by manipulating the C/N ratio.

1.4 Scope of Study

1. Sources of broiler manure and stillage used in this study are shown in Table 1.

Table 1 Broiler manure and stillage sources

Types of feedstock	**Source**
Broiler manure	Pi-kul farm Meawang District, Chiang Mai, Thailand
Stillage	Tanapakdee Co., Ltd. (Thai whiskey factory) 315 Village No.4, Maefag Sub-District, Sansai District, Chiang Mai, Thailand

2. Thermal-alkali pretreatment for broiler manure

2.1 Alkali substance used in this study is calcium hydroxide ($CaOH_2$).

2.2 Temperature, lime concentration and duration are studied factors.

Full Factorial Design and Central Composite Design (CCD) theories are used to design the experiment. High and low values of each factor are selected and shown in Table 2

Table 2 High and low values of each studied factor

Factor	**Low value**	**High value**
Temperature	85°C	150 °C
Lime ($CaOH_2$) concentration	3% w/v $CaOH_2$	10% w/v $CaOH_2$
Time	1 hour	3 hours

2.3 The ratio of biomass to lime solution is 1:15 (w/v, 10 g broiler manure and 150 mL solution) (Suhardi *et al.*, 2013).

2.4 The optimum condition for pretreating broiler manure is selected by considering % lignin removal.

3. Effect of C/N ratios on efficiency of biogas production from the mixture of broiler manure and stillage are conducted using Biochemical Methane Potential (BMP) tests. The temperature in laboratory is controlled at 35 (±2) °C.

4. All experiments are conducted at the Department of Environmental Engineering, Faculty of Engineering, and Chiang Mai University.

The experiment framework of this research is showed in Figure 1;

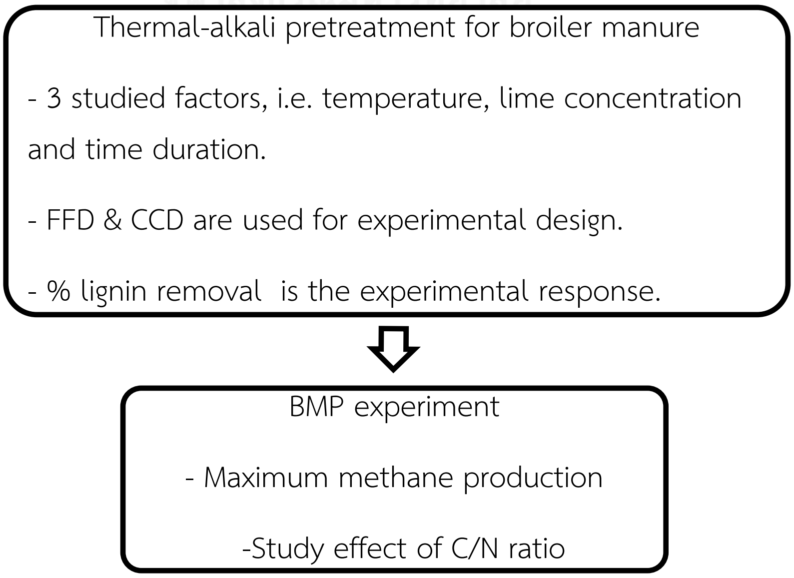


Figure 1 The experiment framework

CHAPTER 2

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Biogas

Biogas generally refers to a mixture of different gas converting from organic matter in the absence of oxygen. Biogas can be produced by anaerobic digestion with anaerobic bacteria. Biogas consists of methane (50–70%), carbon dioxide (30–50%), and trace levels of other gases such as hydrogen, carbon monoxide, nitrogen, oxygen, and hydrogen sulfide.Biogas is a renewable energy that can be used as a fuel for heating, electricity, and many other operations that use a reciprocating internal combustion engine.

2.1.1 Anaerobic digestion

Anaerobic treatment is the biological treatment without the use of air or elemental oxygen. In anaerobic treatment, organic pollutants are converted by anaerobic microorganisms to biogas which are methane (CH_4), carbon dioxide (CO_2) and other products as showed in Equation 2.1 (Metcalf&Eddy, 2004).

$$\text{CHONS} \longrightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S + \text{Other products} \qquad \text{(Eq. 2.1)}$$

(Organic substances)

The overall anaerobic conversion of biodegradable organic materials to final end products, methane and carbon dioxide, is occurred from the co - operation of two types of bacteria; acid forming or non – methanogenic bacteria and methanogenic bacteria. Anaerobic digestion comprises four steps (Figure 2) which occurred in order as followed;

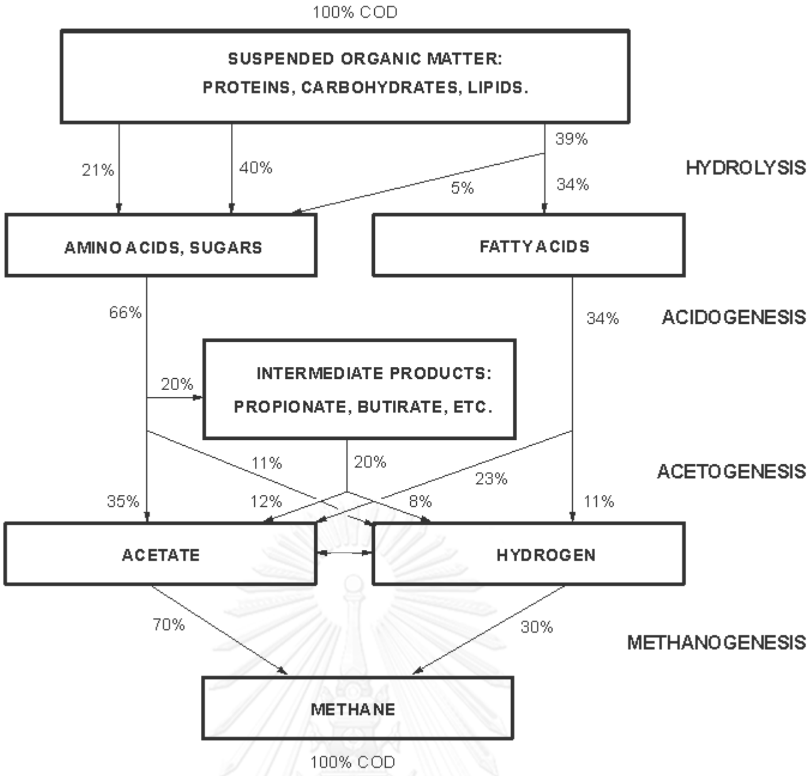


Figure 2 Anaerobic treatment processes

Step 1 Hydrolysis

Large organic matter molecules, i.e. carbohydrate, protein and fat, are hydrolyzed into their simple monomer compounds such as glucose, amino acid and some fatty acids. This process is mediated by extracellular enzymes produced by microorganisms.

Step 2 Acidogenesis

The simple monomer compounds from the hydrolysis step are degraded further to volatile fatty acid such as propionic, butyric, valeric and acetic acid.

Step 3 Acetogenesis

The volatile fatty acids from the acidogenesis step are transformed by acid forming bacteria and hydrogen forming bacteria to acetic acid, hydrogen gas (H_2) and carbon dioxide (Eq. 2.2 and 2.3).

$$CH_3CH_2COOH + H_2O \longrightarrow CH_3COOH + CO_2 + H_2 \qquad \text{(Eq. 2.2)}$$

(Propionic acid)

$$CH_3CH_2CH_2COOH + 2H_2O \longrightarrow 2CH_3COOH + 2H_2O \qquad \text{(Eq. 2.3)}$$

(Butyric acid)

These reactions are brought about by the facultative bacteria and the obligate bacteria both of which known as acid formers or non – methanogenic bacteria. During this step the pH of the system decreases because the production of acid by these bacteria.

Step 4 Methanogenesis

Finally, methane producing bacteria, known as methanogenic bacteria, convert acetic acid and hydrogen gas produced in the acetogenesis step to final products which are mainly CH_4 and CO_2. This step is called the methanogenic phase or methanogenesis. These reactions (Equation 2.4 and 2.5) are also known as methane formation.

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \qquad \text{(Eq. 2.4)}$$

$$CH_3COOH \longrightarrow CH_4 + 2H_2O \qquad \text{(Eq. 2.5)}$$

The accumulation of acetic acid and hydrogen gas from the previous step can affect methane formation, as the methanogenesis bacteria cannot survive in acidic conditions (Raja Priya *et al.*, 2009).

2.1.2 Advantages of anaerobic digestion

2.1.2.1 Less energy required

Anaerobic process is the net energy producer instead of energy user, as in the case of aerobic process. The anaerobic treatments need no air supply. In contrast with the aerobic process that requires energy in aeration step. On the other hand, the anaerobic process produces methane which is the source of energy. Aerobic treatment are energy-intensive process for the removal of organic matter, requiring 0.5-0.75 kWh of aeration energy for 1 kg of COD removed (Adrianus *et al.*, 1994).

2.1.2.2 Low production of biomass

Anaerobic treatment processes utilize more than 90% of the biological degradable organic matter (COD) for methane production, with only 10% or less converted to biomass. Because of the relatively lower growth rate of anaerobic microorganisms, the sludge was produced small amount. Aerobic treatment process generates considerable amounts of sludge. Biological oxidation of every kilogram of soluble BOD produces 0.5 kg of sludge. The costs of treatment and disposal of sludge account for 30-60% of the total operational costs in a conventional activated sludge process.

2.1.2.3 Smaller reactor volume required

The volumetric organic loading rates normally used for that anaerobic process are 5-10 times higher than for aerobic process (Speece, 1996), so smaller reactor volumes and less space may be required for treatment. The large volumetric organic loading rate can be applied. Moreover, the land requirements for the anaerobic treatment unit are reduced.

2.1.2.4 Low nutrient requirement

Owing to the lower biomass synthesis rate during the anaerobic process, the nutrient requirements are considerably lower, with the anaerobic process requiring just 20% of the nutrients required for the aerobic process. The cost for nutrient addition is much lesser in anaerobic process for anaerobic process because less biomass is produced.

2.1.2.5 Ability to reduce concentrations of refractory organics

With proper acclimation, many of the previously identified refractory organics such as carbon tetrachloride, chloroform, trichloroethylene, formaldehyde, and phenol have been successfully transformed to a lower toxic by anaerobic microorganisms (LaGrega *et al.*, 2006).

2.1.3 Disadvantages of anaerobic treatment process

2.1.3.1 Operation consideration

Anaerobic processes require long start-up time, their sensitivity to possible toxic compounds, operational stability, the potential for odor production, and corrosiveness of the digester gas are considered to be problematic. However, with proper wastewater characterization and process design these problems can be avoided and/or managed.

2.1.3.2 Need for alkalinity addition

Alkalinity in wastewater results from the presence of hydroxide (OH^-), carbonates (CO_3^{2-}) and bicarbonates (HCO_3^{2-}). The alkalinity in wastewater helps to resist changes in pH cause by the presence of acid. Alkalinity concentration of 2000 to 3000 mg/l as $CaCO_3$ may be needed in anaerobic process to maintain an acceptable pH with the high gas phase CO_2 concentration (Metcalf&Eddy, 2004).

2.2 Pretreatment methods for substrates

When considering particulate substrates like solid wastes or lignocellulosic waste, both accessibility of hydrolytic microorganisms to the solid matter and hydrolysis of the complex polymeric components constitute the rate-limiting step (Eastman & Ferguson, 1981). Therefore, one way of improving performance of digesters treating solid wastes is reduction in the size of the particles: thus, pretreatment of the substrate by mechanical disintegration should have positive effects on the anaerobic biodegradability of the substrate, through an increase of the available specific surface to the medium. The other way of improving performance is to promote hydrolysis of organic matter by a pretreatment of the substrate. Such pretreatments, breaking the polymer chains into soluble components, can be mainly biological, chemical, or physico-chemical.

Whatever the pretreatment may be, the objectives are to obtain an extension and an acceleration of the anaerobic process, an increased amount of biogas as well as reduction of the amount of anaerobic sludge and of the digestion time (Hartmann, 2003).

There are many types of the pretreatment methods in anaerobic digestion.

1. Physical pretreatments: mechanical: ultrasound, mechanical jet, and mechanical ball mill, thermal, and ultrasonic pretreatment

2. Chemical pretreatments: alkali, green solvents (ionic liquid), wet oxidation, and acid hydrolysis

3. Physicochemical pretreatments: steam-explosion, liquid hot water (LHW), ammonia fiber explosion (AFEX), ammonia recycles percolation (ARP), supercritical fluid (SCF)

4. Biological pretreatments: microorganisms, and enzymatic

5. Combination

According to Taherzadeh and Karimi (2008), an effective pretreatment should meet the following requirements:

- Avoiding formation of the possible inhibitors for hydrolytic enzymes and fermenting microorganisms
- Minimizing the energy demand
- Reducing the cost of size reduction for feedstock
- Reducing the cost of material for the construction of pretreatment reactors
- Producing less residues
- Consumption of little or no chemical and using a cheap chemical

When the common pretreatments do not have efficient results, combined pretreatment can be used.

Table 3 Effect of pretreatment on the chemical composition and structure of lignocellulose biomass

Pretreatment method	Increases accessible surface area	Decrystallization cellulose	Solubilization hemicellulose	Solubilization lignin	Alteration lignin structure	Inhibitor compound formation
Physical	+	+				
Acid	+		+	-	+	+
Alkaine	+		-	+/-	+	-
Oxidative	+	ND		+/-	+	-
Ionic liquids	+	+	-			
Stream-explosion	+		+	-	+	+
AFEX	+	+	-	+	+	-
ARP	+	+	-	+	+	
LHW	+	ND	+	-	-	-
SCF	+	+	+		-	
Biological	+	ND	+	+	+	
Thermal acid	+	ND	+			+
Thermal alkaline	+	ND	-	+/-	+	-

(Adapted from Brodeur *et al.* (2011); Hendriks and Zeeman (2009); Zheng *et al.* (2014)) + = major effect, - = minor effect, ND = not determined, Blank = no effect

Physical pretreatment involves breakdown of particle size and crystalline structure mostly by milling and grinding. The energy requirement depends on the final particle size and reduction in crystallinity of lignocelluloses biomass which in most case, energy consumption is higher than the energy content in biomass (Brodeur *et al.*, 2011).

Biological pretreatment involves using the microorganisms which focus on degrade lignin and hemicelluloses by lignin degrading enzyme in fungi. Although the biological pretreatment can work in mild conditions and has low cost, the drawbacks are low hydrolysis rate and long pretreatment period (Brodeur et al., 2011).

Alkali pretreatment refers to the application of alkaline solutions such as NaOH, Ca(OH)2 (lime) or ammonia that causes structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose, and partial solvation of hemicellulose (Brodeur et al., 2011). The alkali pretreatment can result in a sharp increase in saccharification, with manifold yields (Kassim & El-Shahed, 1986). Pretreatment can be performed at low temperatures but with a relatively long time and high concentration of the base. Sun *et al.* (1995) studied the effect of various alkali solutions to wheat straw by analyzing the delignification and the dissolution of hemicellulose. They found that using 1.5% sodium hydroxide at 20°C for 144 hours achieved the best results in 60% lignin removal and 80% release of hemicellulose. Silverstein *et al.* (2007) studied the effectiveness of sulfuric acid, sodium hydroxide, hydrogen peroxide, and ozone pretreatments for enzymatic conversion of cotton stalks. They found that sodium hydroxide pretreatment resulted in the highest level of delignification (65% with 2% NaOH in 90 min at 121°C) and cellulose conversion (60.8%). With including high temperature, the alkali pretreatment can perform in shorter time. Compared with acid or oxidative reagents, alkali treatment appears to be the most effective method in breaking the ester bonds between lignin, hemicellulose and cellulose, and avoiding fragmentation of the hemicellulose polymers (Gáspár *et al.*, 2007).For using lime pretreatment, A positive effect of lime is that it is relatively cheap and safe (Gandi et al., 1997).

Thermal-chemical pretreatment is a physical-chemical pretreatment. Thermal-chemical hydrolysis of particulate COD is commonly carried out with alkaline agents. Stuckey and McCarty (1978), working with waste-activated sludge (WAS), reported that under thermal-chemical pretreatment, WAS would react in the following ways: lipid are hydrolyzed under acid or alkaline conditions to glycerol and fatty acids; carbohydrates, and more particularly bacterial polysaccharides, are hydrolyzed to simpler polysaccharides or sugars; protein are hydrolyzed by acid solutions to amino acid monomers. Amino acids can be further degraded to ammonia and organic acids. Under alkaline conditions, proteins can also be hydrolyzed; however, the rate and extent are generally less than with acid. High temperature and extremes in pH increase the rate of polymerization. Many authors have compared the efficiency of thermal, chemical or thermal-chemical pretreatment (Prenaud, 1998; Stuckey & McCarty, 1978; Tanaka *et al.*, 1997) and observed that the best performances in terms of COD solubilization and anaerobic biodegradability were obtained when thermal-chemical pretreatment was used.

Hendriks and Zeeman (2009) reviewed the pretreatments to enhance the digestibility of lignocellulosic biomass. They reported that for thermal pretreatment, if the temperature increases above 150-180°C. Heat will affect and increase hemicellulose and lignin solubilization (Bobleter, 1994; Garrote et al., 1999). A systematic study of alkali pretreatment conditions suggested that for short pretreatment times (1-3 h), high temperatures (85-135°C) were required to achieve high sugar yields, whereas for long pretreatment times (e.g., 24 h), low temperatures (50-65°C) were effective (Chang *et al.*, 1998). Therefore, the combination of thermal pretreatment with alkali pretreatment was used. This pretreatment is usually carried out at temperature of 100-150°C (Chang et al., 2001).

Table 4 Advantages and disadventages of different pretreatment of lignocellulose biomass

Pretreatment process	**Advantages**	**Limitation and disadvantages**
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars: alters lignin structure	High cost; equipment corrosion; formation of toxic substances
	Increase in porosity/increased enzymatic hydrolysis	Generation of furfural/ hydroxymethyl furfural; need for recycling; costly
Alkaline hydrolysis	Removes hemicelluloses and lignin; increase accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass
		Formation of salts of calcium and magnesium
Organosolv	Hydrolyzes lignin and hemicellulose; pure lignin obtained and used as value added product	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost; solvents inhibit enzymatic hydrolysis
AFEX	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes; decrystallization of cellulose	Not efficient for biomass with high lignin content; costly

Pretreatment process	Advantages	Limitation and disadvantages
Ammonia treatment	Removal of lignin/ decrystallizing cellulose	Removal of ammonia; costly
Mechanical comminution	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Stream explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
CO_2 explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Pyrolysis	Produces gas and liquid products	High temperature; ash production
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
	Lignin is damaged; cellulose/ hemicellulose unaltered	
Biological	Degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low; a part of fermentable sugars are utilized as carbon source
Wet oxidation	Treatment of wastes	Costly
Microwave treatment	Cheap; generates less pollution	Degradation of cellulose/ hemicellulose

(Adapted from Chaturvedi and Verma (2013); Kumar *et al.* (2009))

Table 5 Alkaline pretreatment of lignocellulosic feedstock for biogas production

Feedstock	Pretreatment conditions	Results	References
- Agricultural residuals: Wheat straw, rice straw, corn stover, sugar beet leaves, maize, ensiled hay, sugarcane bagasse, rapeseed, sunflower stalks, grape pomace, and OPEFB - Forest residuals: fallen leaves - Hardwood: birch - Softwood: spruce and pine - Grass: switch grass, smooth cord grass and Jose tall wheatgrass - MSW: OFMSW and paper pulp/ sludge	- Chemicals: NaOH, Ca(OH)2, CaO, KOH, and NH3H2O - Chemical loading: 1-10% (g/g) - Temperature: 15-170°C - Time: 1 h to 10 days	- Positive effect in most cases with 3.2% to 2.3 folds increase of methane yield. - Negative effects also occurred in very few cases. - In general, it is more effective on biomass containing more lignin	(Azzam & Nasr, 1993; Chandra et al., 2012; Himmelsbach et al., 2010; Liew et al., 2011; Mirahmadi et al., 2010; Zhu et al., 2010)

2.3 Co-digestion

Co-digestion is defined as anaerobic treatment of a mixture of at least two different waste types. The mixing of several waste types has positive effects both on the anaerobic digestion process and on the treatment economy.

The profit of co-digestion in the anaerobic degradation process is mainly within the following areas:

- Increasing the methane yield
- Improving the process stability
- Achieving a better handling of the waste

Generally, the key for co-digestion lies in balancing several parameters in the co-substrate mixture. Some qualities of each co-substrate can be advantageous for use in the biogas process whereas other qualities can hinder the degradation solely of each waste type (Hartmann, 2003), these qualities are;

- Macro- and micronutrients
- C/N ratio
- pH
- Inhibitors/toxic compounds
- Biodegradable organic matter
- Dry matter

The balance of nutrients, an appropriate C/N ratio and a stable pH are prerequisites for a stable process performance with low C/N ratio. The optimal range of C/N ratio is 20:1-30:1 (Hawkes, 1980). Nutrient deficiency of a given waste can be adjusted by co-digestion together with a nutrient-rich waste type. The problem associated with ammonia toxicity can be corrected by dilution of the ammonia concentration in the liquid phase, or by adjusting the C/N ratio of the feedstock (Kayhanian & Tchobanoglous, 1992). The pH can be balanced by addition of waste with a high buffer capacity, which protects the process against failure due to pH drop when the VFA concentration increases. Referring to the effect on the degradation of toxic substances by co-digestion, it is not only the dilution by addition of other waste that serves as a benefit (Hamzawi *et al.*, 1998). Furthermore, detoxification of toxic compounds can be achieved in the co-substrate mixture by, for example, cometabolic mechanisms, where a compound is transformed along with the general metabolism of microbes using a primary substrate. For example, it has been shown that waste containing tetrachloroethene (PCE) in concentration up to 100 ppm can be degraded in co-digestion with manure (Ahring *et al.*, 1996).

In the treatment of organic waste with a high content of recalcitrant organic matter (i.e. lignocellulose), the co-digestion with waste rich in easily biodegradable organic matter will be advantageous for obtaining a higher biogas yield. Besides achieving a better economic feasibility of the treatment, the addition of easily degradable material has been shown to stabilize the anaerobic digestion process if added in a controlled fashion (Mathrani *et al.*, 1994). This effect could partly be due to a higher active biomass concentration in the reactor, which will be more resistant to inhibitory compounds. Furthermore, the inorganic parts of some organic wastes, such as clays and iron compounds, have been shown to counteract the inhibitory effect of ammonia and sulphide, respectively (Ahring *et al.*, 1992b). Finally, the dilution of waste with high TS such as the organic fraction of municipal solid wastes (OFMSW) by co-digestion with waste with a lower TS concentration such as manure resolves problems of pumping and mechanical treatment of solid waste (Angelidaki, 1997).

Abouelenien et al. (2014) studied the co-digestion of chicken manure (CM) with mixture of agriculture wastes (AWS). Two types of anaerobic digestion process were used, process 1 (P1) using fresh CM (FCM) and process 2 (P2) using treated CM (TCM), ammonia stirpped CM, were conducted. The results showed that methane production in P1 was increased by 93% and 50% compared to control (without AWS) with the maximum methane production of 502 and 506 ml/gVS obtained at 55°C and 35°C, respectively. Additionally, 42% increase in methane production was observed with maximum volume of 695 506 ml/gVS comparing P2 test with P2 control under 55°C.

Sharma et al. (2013) studied the Improving biogas production by the co-digestion of poultry litter and thin stillage. The experiment was conducted at 4 different poultry litter: thin stillage ratios: 80% poultry litter: 20% thin stillage, followed by successive ratios of 60:40, 40:60, and 20:80. The control of this experiment was 100% poultry litter. They found that after a period of adaptation to 20% and 40% stillage, digester performance showed increases in biogas, percent methane and COD removal. Peak performance occurred with 60% thin stillage. However, 80% thin stillage caused significant reduction of performance, including declines of methanogenic activity and COD removal. In conclusion, co-digestion of poultry litter with thin stillage improved biogas production, but thin stillage became inhibitory at high concentration.

2.4 Biochemical Methane Potential (BMP) Test

The BMP assay was developed as a standardized method to determine the ultimate biodegradability and associated methane yield during the anaerobic digestion of organic substrates (Chynoweth *et al.*, 1993).

In the past, the cause of anaerobic system failures has been difficult to assess because of the complex mixture being treated. Other difficulties include analysis for the great variety of potential inhibitors, and the lack of understanding of the interactions between inhibitors, other constituents in the digesting mixture, and the methanogenic bacteria. Bioassay techniques for measuring the presence or absence of inhibitory substances offer the most promise for resolving anaerobic treatment problems because they are relatively simple and inexpensive, and do not require knowledge of specific inhibitory substances. Also, bioassay techniques are essential for determining biodegradability since no chemical procedure is available which distinguishes between biodegradable and non-biodegradable organics (Owen *et al.*, 1979).

The BMP tests are conducted in batch conditions and in bench scale, measuring the maximum amount of biogas or bio-methane produced per gram of volatile solid (VS) contained in the organics used as substrates in the anaerobic digestion process. Also, the BMP are available to assist with site specific design criteria. Through stoichiometric conversion, CH_4 production is directly related to organic degradation; 395 mL CH_4 equals 1 g COD reduction at 35 °C (Speece, 1996).

Nielfa *et al.* (2015) studied methane production from the co-digestion of organic fraction municipal solid waste and biological sludge. Biochemical Methane Potential (BMP) test was used to determining the optimum co-digestion ratio. Several equations were used to study not only methane production, but also COD removal, element composition analysis, and biodegradability. Two different models first-order model (FO) and Gompertz model (GM) were applied to the experiment BMP results to determine the optimum equation that fit with these kinds of wastes and evaluate the parameter that had influence on the anaerobic digestion process. Predictions of maximum methane production by both models were compared to the final methane production achieved by the experiment. The results indicated that all the co-digestion mixtures increased the methane production from sole substrates. Co-digestion of 80% OFMSW and 20% biological sludge obtained the highest increase. Gompertz model was fit better than first-order model in these two substrates and could predict the final production in just 7 days after startup the experiment, saving time and costs for the experiment, with high reliability.

CHAPTER 3

METHODOLOGY

3.1 MATERIALS

3.1.1 Chemical reagents

1. Analytical reagent grade calcium hydroxide ($CaOH_2$)	RCI Labscan
2. Cetyltrimethylammonium bromide (CTAB)	MERCK
3. Sulfuric acid (H_2SO_4) 98%	RCI Labscan

3.1.2 Analytical Instruments

1. 29 L oil bath (Oil bath one 29, Memmert)
2. Foil
3. Palm oil
4. Deionized water
5. wire screen
6. sintered glass filter crucible 30 ml
7. Vacuum filtration apparatus
8. Vacuum pump
9. COD reflux condenser
10. Hot plate
11. Oven
12. Furnace
13. pH meter
14. Desiccator
15. Normal saline glass bottle 1000 ml
16. Septum
17. Hypodermic needle (27Gx1”, 23Gx1”, NIPRO)

18. Portable gas pressure meter
19. Portable biogas analyzer (BIOGAS 5000, Geotech)

3.1.3 Materials

1. Broiler manure

Broiler manure was collected from Pi-kul farm, Meawang District, Chiang Mai, Thailand. In this research, broiler manure comprised both manure and bedding materials which was rice husk. The collected broiler manure was manually disintegrated and completely mixed before using in the experiment.

2. Stillage

Stillage was collected from Tanapakdee Co., Ltd. (Thai whiskey factory) which is located in Sansai District, Chiang Mai, Thailand. This factory produces whiskey from fermented molasses. The collecting point was the effluent storage pond of Distillery section. The collected stillage was stored in 4°C room prior use. The characteristics of the collected stillage are shown in Table 6.

Table 6 Characteristics of stillage

Characteristic	Stillage
Flow rate (m^3/d)	420
pH	5.10
Temperature (°C)	54
Color	Very black and turbid
BOD	57,810
COD	194,850

3. The inoculums

The inoculums (seed) was collected from an Up flow Anaerobic Sludge Blanket Reactor of Tanapakdee Co., Ltd. (Thai whiskey factory). The collected seed was stored in 4°C room prior use.

The characteristic of broiler manure, stillage, and inoculums are represented in Table 7.

Table 7 Characteristics of broiler manure, stillage, and inoculums

Characteristic	Broiler manure	Pretreated broiler manure	Stillage	Inoculum
Moisture,%	9.18	3.74	86.28	91.04
TS,%	90.82	96.26	13.72	8.96
VS,%TS	78.16	73.33	74.19	52.10
VS, g/g	0.7098	0.7059	0.1018	0.0467
MLSS, mg/l				593312*
MLVSS, mg/l				24750*
Density, g/ml	0.73	0.69	1.06	1.02
C/N ratio	26.77		112.05	

***** Ratio of MLVSS to MLSS of the inoculum was rather low. This was the result of the UASB reactor used at Tanapakdee Co., Ltd. Was not fed regularly. Considering only its MLVSS concentration, this inoculum could still be used as the seed for the BMP test

4. Calcium hydroxide

Analytical reagent grade calcium hydroxide (RCI Labscan, Thailand) was used in the experiment. The properties of $CaOH_2$ were summarized in Table 8.

Table 8 Chemical properties of Calcium hydroxide used in this study

Properties	Calcium hydroxide
Molecular formula	$Ca(OH)_2$
Molecular weight	74.09 g/mol
Purity	>95%
Density	2.24 g/cm^3
Melting point	550°C

(RCI Labscan, Thailand)

3.2 METHODS

In order to determine the optimum condition for broiler manure pretreatment using the thermo-alkali process and investigate effect of C/N ratios on biogas production potential from the co-digestion of pretreated broiler manure and stillage, the methodology of this research can be explained as following;

3.2.1 Broiler manure pretreatment using thermal-alkali process

3.2.1.1 Details of experiments

Thermo-alkaline process was used for pretreating broiler manure contaminating by considerable amounts of rice husk. $Ca(OH)_2$ was chosen as it has been found to be effective for pretreating the lignocellulosic biomass (Table 3 and 4) and had low cost compared to other alkalis.

1. **The experimental designs**

To obtain the pretreatment optimum condition, experiments were designed using the 2-level Full Factorial and Central Composite Design of experiment. For the Full Factorial Design, numbers of experiment would equal to2^k. k is a number of factors in the experiments and the levels of each factor are defined as high and low. MINITAB17 program calculated all states by using considered factors in the experiment which were temperature, alkaline concentration, and pretreatment duration. In total, 2^3or 8 experiments were conducted. High and low levels of each studied factor are shown in Table 9. Each experiment was replicated 2 times.

Table 9 Levels of each studied factor

Factor	Level		
	High	Center	Low
Temperature, °C	**150**	**117.5**	**85**
$Ca(OH)_2$ concentration, %w/v	**10**	**6.5**	**3**
Duration, h	**3**	**2**	**1**

Level of each studied factor was set using the appropriate range reported in previous studies (Hendriks & Zeeman, 2009; Silverstein et al., 2007; Sun et al., 1995a; and table 5).

In addition, to analyze the possible curvature of the experiment response, 3 experiments were also done at the center point value of each studied factor. The response for result analysis was lignin removal efficiency. Results from the full factorial experiment showed the curvature relationship between the studied factors and lignin removal efficiency. Therefore additional experiments designed based on the Central Composite Design of experiments were also conducted. These additional six experiments were conducted at the axial point and also replicated. Details of each experiment are shown in Table 10.

2. Experiment method

All 31 experiments shown in Table 10 were carried out in 250 ml flasks. Each flask contained 10 g of broiler manure mixed with 150 ml $CaOH_2$ solution (Suhardi et al., 2013). Temperature of the flask content was controlled by submerged in the 29 L oil bath (Oil bath one 29, Memmert) filled with palm oil. After completion of experiment, the mixture was filtered through a wire screen to separate the solid residue. The residue was washed with distilled water until neutral pH was achieved. The sample was air dried and used for lignin measurement (Appendix A).

Table 10 Studied conditions of experiments for determining pretreatment optimum condition

No.	Coded value			Actual value			Experiment portion
	Temp. (°C)	$Ca(OH)_2$ conc. (%)	Time (h)	Temp. (°C)	$Ca(OH)_2$ conc. (%)	Time (h)	
F1	-1	-1	-1	85	3	1	Factorial Portion
F2	1	-1	-1	150	3	1	
F3	-1	1	-1	85	10	1	
F4	1	1	-1	150	10	1	
F5	-1	-1	1	85	3	3	
F6	1	-1	1	150	3	3	
F7	-1	1	1	85	10	3	
F8	1	1	1	150	10	3	
F9*	-1	-1	-1	85	3	1	Replicated Factorial Portion
F10*	1	-1	-1	150	3	1	
F11*	-1	1	-1	85	10	1	
F12*	1	1	-1	150	10	1	
F13*	-1	-1	1	85	3	3	
F14*	1	-1	1	150	3	3	
F15*	-1	1	1	85	10	3	
F16*	1	1	1	150	10	3	

No.	Coded value			Actual value			Experiment portion
	Temp. (°C)	$Ca(OH)_2$ conc. (%)	Time (h)	Temp. (°C)	$Ca(OH)_2$ conc. (%)	Time (h)	
C1	0	0	0	117.5	6.5	2	Center points
C2	0	0	0	117.5	6.5	2	
C3	0	0	0	117.5	6.5	2	
A1	-1.633	0	0	64.4	6.5	2	Axial points
A2	1.633	0	0	170.6	6.5	2	
A3	0	-1.633	0	117.5	0.8	2	
A4	0	1.633	0	117.5	12.2	2	
A5	0	0	-1.633	117.5	6.5	0.37	
A6	0	0	1.633	117.5	6.5	3.63	
A7*	-1.633	0	0	64.4	6.5	2	Replicated Axial points
A8*	1.633	0	0	170.6	6.5	2	
A9*	0	-1.633	0	117.5	0.8	2	
A10*	0	1.633	0	117.5	12.2	2	
A11*	0	0	-1.633	117.5	6.5	0.37	
A12*	0	0	1.633	117.5	6.5	3.63	

* Replicated experiment

3.2.2 Effects of C/N ratios on biogas production potential from the co-digestion of pretreated broiler manure and stillage

3.2.2.1 Details of experiments

1. Experiment method

The Biochemical Methane Potential (BMP) test was performed in this part of the study in 1000 ml glass bottles. Firstly, seed was added into the bottle.

Then substrate or mixture of substrates was added. The substrate/Inoculum ratio (S/I ratio) was kept constant at 1:2gVS/gVS. S/I ratio can be represented as the amount of VS in the substrate per the amount of VS originating from inoculum. Chynoweth et al. (1993) reported that maximal methane yields were obtained with S/I ratios of 0.5 to 1.0. If the volume of seed and substrate was less than working volume of each bottle (500 ml), deionized water was added. The bottle was purged with N_2 gas for 3 minutes to get rid of O_2 and then sealed with a septum. Volume of biogas produced daily was calculated from the measured pressure inside the bottle. The bottle was shaken completely daily before pressure measurement. When the pressure of biogas reached 400 mbar, biogas composition was measured using the portable biogas analyzer (BIOGAS5000, Geotech) (Appendix A). The reaction was completed when the daily biogas production of less than 1% of the whole production was detected for at least 3 days (Nielfa et al., 2015). Details of substrate or mixture of substrate used in each experiment and BMP test design are shown in Table 11 and 12. Each experiment was replicated 3 times.

Table 11 Substrates for BMP and ratio of mixture

Experiment	**Substrate**
Blank	Only inoculum
nonPret.BM	Non-pretreated broiler manure
Pret.BM	Pretreated broiler manure
Stillage	Stillage
Co-digestion 1	Non-pretreated broiler manure + stillage at C/N ratio = 30
Co-digestion 2	Pretreated broiler manure+ stillage, C/N ratio = 30
Co-digestion 3	Pretreated broiler manure+ stillage, C/N ratio = 40
Co-digestion 4	Pretreated broiler manure+ stillage, C/N ratio = 50

Table 12 BMP test design

Experiment	Substrate		
	Inoculum, ml	Broiler manure, g	Stillage, ml
Blank	209.93	-	-
nonPret.BM	209.93	7.04	-
Pret.BM	209.93	7.04	-
Stillage	209.93	-	46.34
Co-digestion 1	209.93	6.17	5.77
Co-digestion 2	209.93	6.17	5.77
Co-digestion 3	209.93	4.28	18.21
Co-digestion 4	209.93	3.06	26.23

(Appendix B)

2. BMP mathematical models

Anaerobic digestion process could be described by different kinetics depending on various parameters e.g. the experiment condition and types of organic matter. Biodegradable kinetics and methane production could be used to predict methane potential of specific substrates. In this work, some kinetic parameters were derived by fitting the results from the BMP tests to two mathematical models using Excel program analyzed for the specific methane yield (mlCH4/gVS).

- **First-order model (FO)**

Generally, anaerobic digestion is described as a first-order reaction which is the simplified model. The hydrolysis rate of particular organic matter and the cumulative methane yield are kinetics that can be derived from this equation (Eq. 3.1).

$$P = \gamma \times \left(1 - exp(-kt)\right) \qquad \text{(Eq. 3.1)}$$

Where P is the specific methane yield at given time (mlCH4/gVS): γ is the maximum methane potential at an infinite time (t) (mlCH4/gVSadded): k is the hydrolysis rate constant (d-1).

- **Modified Gomperzt model (GM)**

For this model, biogas production is assumed to be proportional to microbial activity. This model is originally used for describing the growth of bacteria in batch mode and is used to predict the methane production (Nielfa et al., 2015) (Eq. 3.2)

$$P = \gamma exp\left(-exp\left(\frac{K(\lambda - t)e^{1}}{\gamma} + 1\right)\right) \qquad \text{(Eq 3.2)}$$

Three parameters are needed for the prediction of methane production (P):

- The maximum volume accumulated at an infinite digestion time (t); γ(mlCH$_4$/gVS$_{added}$)
- The specific rate constant; Rm(mlCH$_4$/gVS/d)
- The lag phase time constant; λ (d)

However, as the model could not completely predict the experimental results, a relative error should also be calculated according to (Eq. 3.3).

$$error\ (\%) = \left(\frac{\gamma_{exp} - \gamma_{th}}{\gamma_{exp}}\right) \times 100 \qquad \text{(Eq. 3.3)}$$

Where γ_{exp} is the maximum methane yield from the experiment (mlCH4/gVS): $\gamma_{th}$is the maximum methane yield from the mathematical model (mlCH4/gVS).

3. Synergistic effects

Synergistic effects are from the inner reactions created by the co-digestion of substrates that influence biogas production. According to the proportions of each substrate in the mixture and the individual methane potential, the synergistic effect could calculate according to this equation (Eq. 3.4)

$$\gamma_{th} = (\gamma_{solesubstrate} \times X_1) + (\gamma_{solesubstrate} \times X_2) \qquad \text{(Eq. 3.4)}$$

Where γ_{th} is the maximum methane yield from the co-digestion (mlCH4/gVS): $\gamma_{solesubstrate}$ is the maximum methane yield from the substrate alone (mlCH4/gVS): X1 and X2 are the percentages of each sole substrate in the co-digestion mixture.

3.3 Physical and chemical analysis

Samples were taken for measurement during each experiment. Details of parameter, sampling, frequency, and analytical method used are tabulated in Table 13.

Table 13 Details of parameter and analysis

Parameter	Frequency		Analytical Method
	Pretreatment	BMP	
pH	-	Startup - final	pH meter
TS	-	Startup - final	Gravimetric Method
VS	-	Startup - final	Gravimetric Method
% lignin	Startup - final	-	Acid detergent fiber and lignin
Biogas pressure	-	Everyday	Portable gas pressure meter
Biogas component	-	When pressure reach 400 psi	Portable biogas analyzer

(APHA, 2006)

CHAPTER 4

RESULTS AND DISCUSSION

In order to determine the optimum condition for broiler manure pretreatment using the thermo-alkali process, the experiments were designed by Full Factorial design (FFD). Results obtained from all experiments can be presented as following

4.1 Results of thermal-alkali pretreatment

4.1.1 Full Factorial Design (FFD)

4.1.1.1 Factorial analysis

Values of 3 studied factors, i.e. temperature, lime concentration, and pretreatment duration, at each condition (F1-F16) along with three experiments conducted at center point (C1-C3) are shown in Table 9. Response of each experiment used for result analysis was % lignin removal. Results of the experiments are shown in Table 14.

Table 14 Lignin removal efficiency obtained from experiments

No.	Temperature (°C)	$Ca(OH)_2$conc.(%)	Time (hr)	% lignin removal
F1	85	3	1	26.6
F2	150	3	1	54.0
F3	85	10	1	2.9
F4	150	10	1	17.0
F5	85	3	3	4.9
F6	150	3	3	33.5
F7	85	10	3	19.2
F8	150	10	3	51.2

No.	Temperature (°C)	$Ca(OH)_2$conc.(%)	Time (hr)	% lignin removal
F9*	85	3	1	11.0
F10*	150	3	1	43.7
F11*	85	10	1	-3.7
F12*	150	10	1	52.4
F13*	85	3	3	-2.1
F14*	150	3	3	49.9
F15*	85	10	3	21.4
F16*	150	10	3	35.3
C1	117.5	6.5	2	43.4
C2	117.5	6.5	2	38.0
C3	117.5	6.5	2	38.2

* Replicated experiments

From factorial analysis of experiments obtained from MINITAB 17, results of analysis of variance (ANOVA) and linear regression analysis to determine the effects of studied factors on lignin removal are shown in Table 15 and Table 16, respectively.

Table 15 ANOVA of lignin removal

Source	DF	Adj SS	Adj MS	F	P
Model	5	5378.34	1075.67	10.80	0.000
Linear	3	4172.42	1390.81	13.96	0.000
Temperature	1	4124.85	4124.85	41.42	**0.000**
Lime Concentration	1	41.93	41.93	0.42	0.528
Duration	1	5.64	5.64	0.06	0.816
2-Way Interactions	1	724.96	724.96	7.28	0.018

Source	DF	Adj SS	Adj MS	F	P
Conc.*Duration	1	724.96	724.96	7.28	**0.018**
Curvature	1	480.97	480.97	4.83	**0.047**
Error	13	1294.77	99.60		
Lack of fit	3	164.47	54.82	0.49	0.700
Pure Error	10	1130.30	113.03		
Total	18	6673.11			

Table 16 Linear regression analysis

Term	Effect	Coef.	SE Coef.	T	P
Constant		26.07	2.49	10.45	0.000
Temperature	**32.11**	16.06	2.49	6.44	**0.000**
Lime Concentration	-3.24	-1.62	2.49	-0.65	0.528
Duration	1.19	0.59	2.49	0.24	0.816
Conc.*Duration	**13.46**	6.73	2.49	2.70	**0.018**
Ct Pt		13.80	6.28	2.20	**0.047**
S=9.97986	R^2 = 80.60%		R^2 adjusted = 73.13%		

Results showed significant interaction effect (P=0.018) between lime concentration and pretreatment duration (Table 14). However, when the main effect was considered, only temperature affected lignin removal efficiency significantly (P=0.000). These results were reflected by levels of effect shown in Table 16, in which effect level of temperature was the highest at 32.11 while interaction effect level between lime concentration and pretreatment duration was the second highest at 13.46. The plus or minus impact value indicates a positive or negative effect of each studied factor on % lignin removal. For instance, high plus effect (32.11) of temperature on lignin removal percentage meant that increasing temperature resulted in higher lignin removal percentage being obtained. Though samples of broiler manure were highly nonhomogeneous and experimental errors were expected to be high, accuracy of the model obtained from linear regression analysis (Eq. 4.1) was still considerably high (adjusted coefficient of determination = 73.13%). Equation 4.1 was constructed using all factors having significant effects on % lignin removal. Terms of duration and lime concentration, though their main effects were found to be not significant, were needed to be included in the equation as significant interaction effects of both factors were found.

$$\text{Lignin Removal Efficiency} = 26.07 + (16.06 \times \text{Temperature}) - (1.62 \times \text{Lime Conc.}) + (0.59 \times \text{Duration}) + (6.73 \times (\text{Conc.} * \text{Duration})) \quad \text{(Eq. 4.1)}$$

This equation can be used to predict lignin removal efficiency from broiler manure by thermal-alkali pretreatment. This equation was most eligible for temperature 85-150°C, 3-10% w/v $Ca(OH)_2$ concentration and 1-3 h treatment duration, respectively.

4.1.1.2 Interaction effect on lignin removal efficiency

Interaction effect is the effect of one factor which is influenced by levels of the other factor. Thus, interaction effect is essential for estimating the effect of a particular factor on the interested response. Figure 3 shows the interaction effect of three studied factors on lignin removal efficiency.

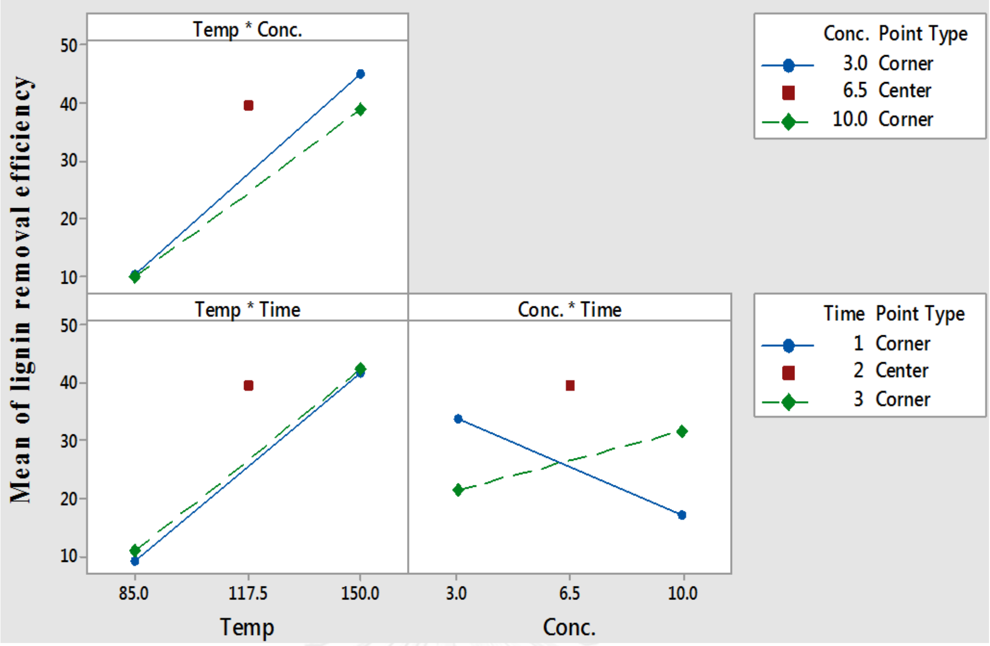


Figure 3 Interaction effects of three studied factors

Significant interaction effect was found between lime concentration and pretreatment duration at the confidence level of 95% (P=0.018; Fig 3). Higher lignin removal was achieved at 10% $Ca(OH)_2$ when the treatment duration is 3 h, while at 1 h treatment duration the higher lignin removal was gained at lower lime concentration (3% $Ca(OH)_2$). These results might be partly contributed by the evaporation of water of lime solution in the flask. At higher lime concentration, the volume of water in the flask was lesser compared to that at lower lime concentration. When the temperature higher than 100°C was utilized, the solution was boiled and evaporated. Lower volume of water used for higher lime concentration resulted in the content of experimented flask being dried quicker. As dry lime had less pH adjusting effect compared to that in the solution form, experiments with higher lime concentration required longer pretreatment duration to have similar effect compared to those conducted at lower concentration. This assumption is supported by Song *et al.* (2013) who found that below water boiling point temperature (25±2°C), the highest lime concentration (12% $Ca(OH)_2$) was found to render the highest lignin removal efficiency when used to pretreat rice straw regardless of the pretreatment duration (3, 7 and 11 day).

4.1.13 Main effect on lignin removal efficiency

Main effect is the effect of one factor on the response when level of that factor has changed. Figure 4 showed the main effect of three studied factors to lignin removal efficiency.

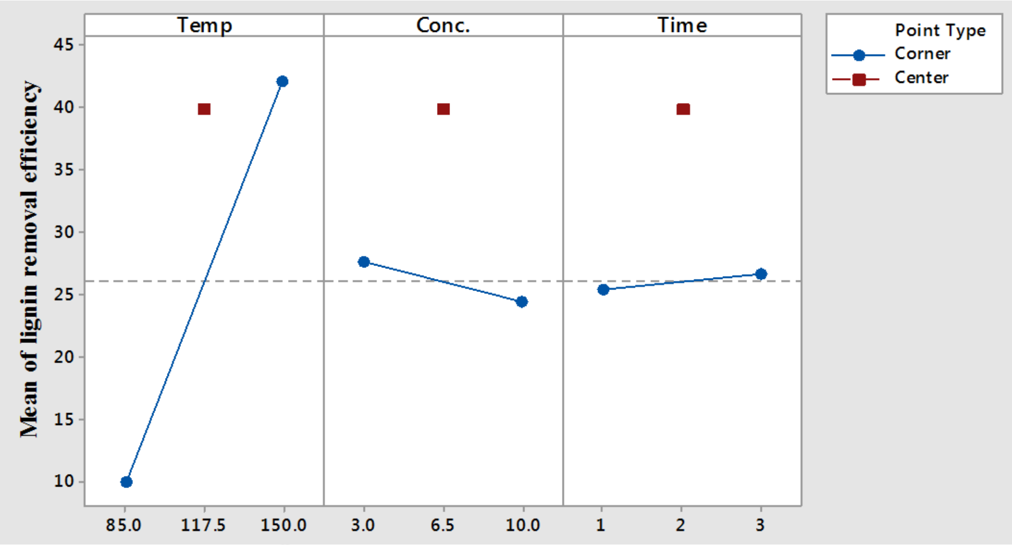


Figure 4 Main effects of three studied factors

From Fig.4, the result showed clearly that only temperature affected lignin removal significantly at confidence level of 95% (P=0.000). According to Li et al. (2014) for lignocellulosic biomass, usually, hemicellulose was thermal decomposed at temperature of 200-320°C, cellulose was decomposed at temperature of 280-380°C, and lignin was found to be decomposed at 200-600°C. This implies that elimination of lignin composition is more effective at higher temperature. Surprisingly, lime concentration and treatment duration were found not to significantly affect % lignin removal. Chang *et al.* (1997); (1998) found that 0.1 g $Ca(OH)_2$/g raw biomass was critical value of lime loading. As lime solubility in the water is low, the excess amount of lime beyond the saturation point in solution did not bring about any more effect. Also, high lime concentration resulted in more amount of water for rinsing the pretreated products being increased (Xu *et al.*, 2010), which was not practically desirable. In addition, the results from the same study by Xu et al. (2010) showed that at a wide range of temperatures (21°C-121°C), the significant lime loading for maximizing total reducing sugar yield was 0.1 g $Ca(OH_2)$/g raw biomass (Switchgrass) regardless of the temperature used. For the duration of pretreatment, they found that at lower temperature, the pretreatment process required longer duration to achieve the similar value of the maximum total reducing sugar yield (At 121°C, 0.5h; 50°C, 24h; 21°C, 96h). At the same temperature and duration, they found that increasing the amount of lime concentration lowered the level of lignin reduction.

4.1.1.4 Cube plot for lignin removal efficiency

Figure 5 shows all the results of experiments conducted at each condition to determine effects of temperature, lime concentration and treatment duration on % lignin removal.

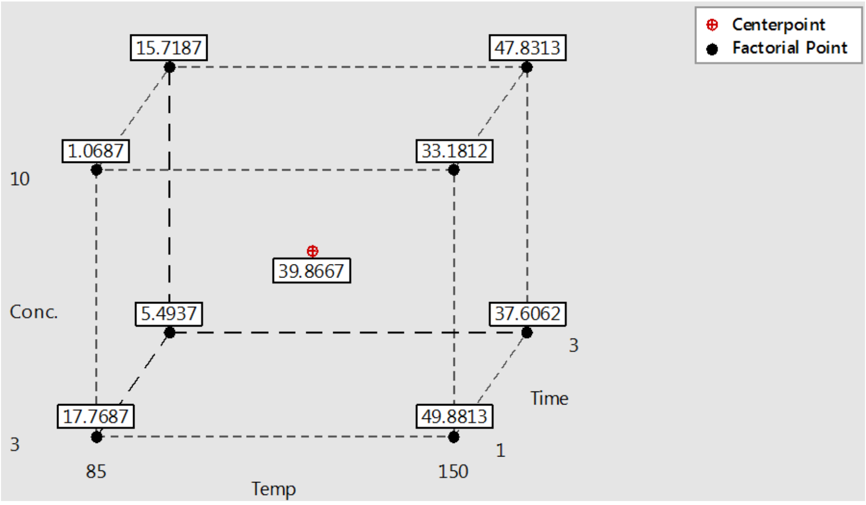


Figure 5 Cube plot

From Fig.5, the highest efficiency (49.9%) for lignin removal from broiler manure was detected at temperature 150°C, 3% w/v $Ca(OH)_2$ solution, and 1 h pretreatment duration. As temperature was found to be the only significant factor affecting lignin removal, higher lignin removal was obtained at higher temperature regardless of the levels of lime concentration and pretreatment duration. Nevertheless, the result from factorial analysis also revealed the significant curvature (P=0.047) between studied factors and lignin removal (Table 14), indicating that the Central Composite Design of Experiment (CCD) was needed in order to find the optimum pretreatment condition in the next step.

4.1.2 The Central Composite Design (CCD)

From Factorial analysis, the significant curvature was found (P=0.047) and % lignin removal at center point in Table 13 showed higher values than most values gained from the FFD experiments. Thus, to find the optimum pretreatment condition for the highest lignin elimination, CCD experiments (A1-A12 in Table 9) were carried out. Table 17 shows % lignin removal obtained from those experiments.

Table 17 % Lignin removal obtained from experiments conducted at axial points

No.	Temperature (°C)	$Ca(OH)_2$conc.(%)	Time (hr)	% lignin removal
A1	64.4	6.5	2	61.0
A2	170.6	6.5	2	31.9
A3	117.5	0.8	2	46.7
A4	117.5	12.2	2	61.0
A5	117.5	6.5	0.37	66.4
A6*	117.5	6.5	3.63	32.6
A7*	64.4	6.5	2	42.1

No.	Temperature (°C)	Ca(OH)$_2$conc.(%)	Time (hr)	% lignin removal
A8*	170.6	6.5	2	44.5
A9*	117.5	0.8	2	43.4
A10*	117.5	12.2	2	12.0
A11*	117.5	6.5	0.37	30.2
A12*	117.5	6.5	3.63	6.7

* Replicated experiments

From response surface analysis, results of analysis of variance (ANOVA) and linear regression analysis to determine the effects of studied factors on lignin removal are shown in Table 18 and Table 19, respectively.

Table 18 ANOVA of lignin removal obtained from experiments conducted at axial points

Source	DF	Adj SS	Adj MS	F	P
Model	5	3806.1	761.2	2.79	0.035
Blocks	3	1001.3	1001.3	3.67	0.065
Linear	1	2079.8	693.3	2.54	0.076
Temperature	1	1706.1	1706.1	6.26	**0.018**
Lime Concentration	1	108.6	108.6	0.40	0.533
Duration	1	265.0	265.0	0.97	0.332
2-Way Interactions	1	725.0	725.0	2.66	0.114
Conc.*Duration	1	725.0	725.0	2.66	0.114
Error	29	7908.4	272.7		
Lack of fit	10	4299.6	430.0	2.66	0.060
Pure Error	19	3608.6	189.9		
Total	34	11714.4			

Table 19 Linear regression analysis

Term	**Effect**	**Coef.**	**SE Coef.**	**T**	**P**
Constant		34.49	2.82	12.23	0.000
Blocks		-5.40	2.82	-1.92	0.065
Temperature	**16.00**	8.00	3.20	2.50	**0.018**
Lime Concentration	-4.04	-2.02	3.20	-0.63	0.533
Duration	-6.31	-3.15	3.20	-0.99	0.332
Conc.*Duration	13.46	6.73	4.13	1.63	0.114
S=16.5137	R^2 = 32.49%		R^2 adjusted = 20.85%		

From Table 18, only temperature affected lignin removal efficiency significantly (P=0.018). These results were supported by levels of effect shown in Table 18, in which effect level of temperature was the highest at 16.00. The interaction effect level between lime concentration and pretreatment duration was the second highest at 13.46 but this effect was not significant (P=0.114). However, the adjusted coefficient of determination (R^2 adjusted) of the linear regression analysis was found to be relatively low (20.85%). Highly nonhomogeneous characteristic of broiler manure was assumed to be the main reason of this low R^2 adjusted level. Several additional experiments had been conducted in order to increase R^2 adjusted level, however the results obtained did not significantly improve the reliability of the model.

Owing to the less reliability of the CCD model (R^2 adjusted=20.85%), the optimum condition for broiler manure pretreatment was selected from the FFD model. From Fig.5 which shows all the results from pretreatment experiments, at 150°C, 1h with 3% w/v $Ca(OH)_2$, the highest efficiency of lignin removal (49.9%) was obtained.

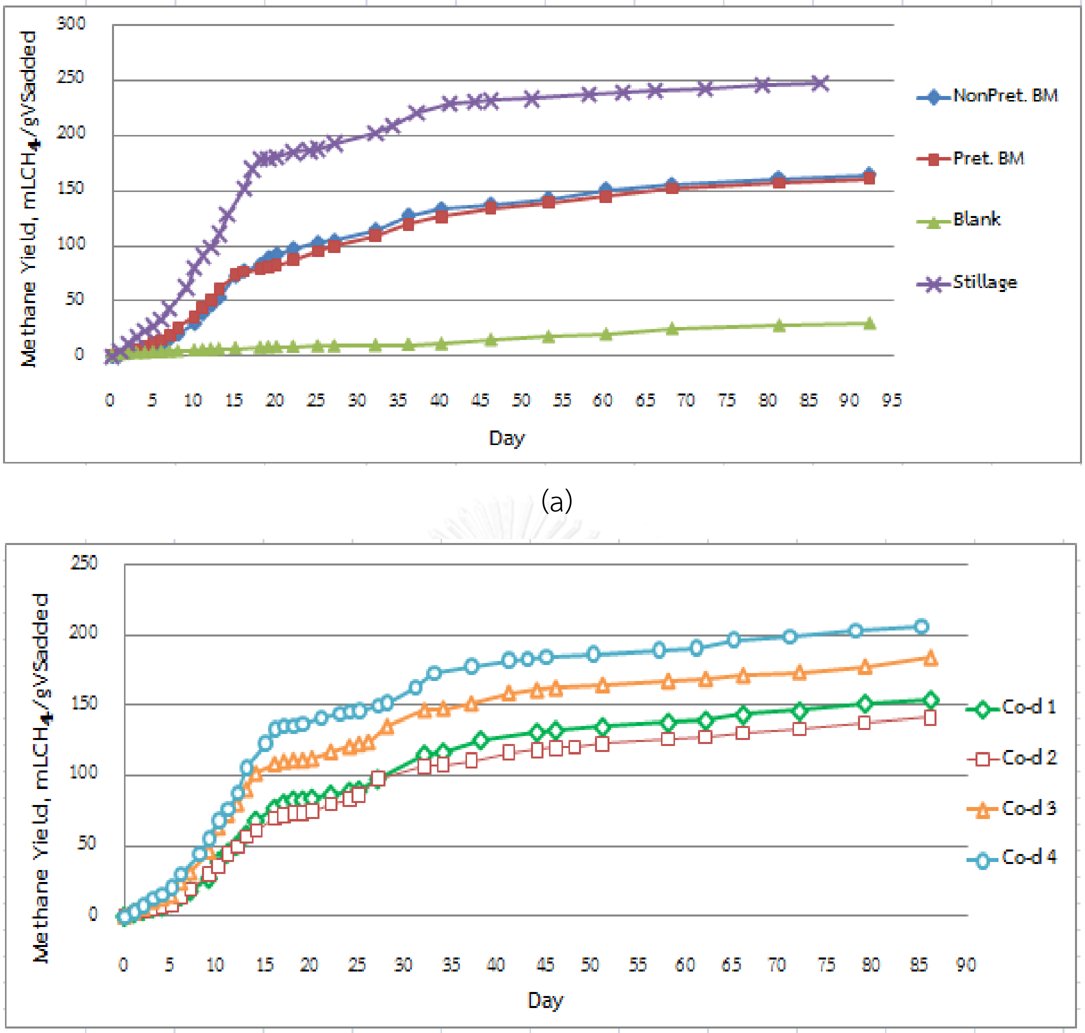
Accordingly, the pretreated broiler manure used in the BMP test to investigate efficiency of the co-digestion between broiler manure and stillage was pretreated at the aforementioned conditions.

4.2 Biochemical Methane Potential (BMP) test

BMP tests were conducted in order to investigate the efficiency of co-digestion between the pretreated BM and stillage and to determine effects of C/N ratios on efficiency of biogas production from the mixture of both substrates. The non-pretreated BM (nonPret.BM), pretreated BM (Pret.BM) and stillage were used as the sole substrate. To investigate effects of C/N ratios, BMP tests were done at different C/N ratios, i.e. 30 (Co-digestion 2), 40 (Co-digestion 3) and 50 (Co-digestion 4), of the mixture of pretreated BM and stillage. For comparison, mixture of non-pretreated BM and stillage at the C/N ratio of 30 (Co-digestion 1) was also tested. Methane potential of seed was determined as the control (Blank). Details of all conditions used in this study are tabulated in Table 12.

4.2.1 Results from the BMP tests

The final cumulative methane yields were obtained after 86-92 d of experiment when the dairy methane production of less than 1 % of the whole production was detected at least 3 d consecutively.



(a)

(b)

Figure 6 BMP results from all experiments

(a) the inoculum (blank) and sole substrates (Non pretreated broiler manure, pretreated broiler manure and stillage)

(b) the co-digestions (Co-d 1 = NonPret.BM + stillage at C/N ratio 30, Co-d 2 = Pret.BM + stillage at C/N ratio 30, Co-d 3 = Pret.BM + stillage at C/N ratio 40, Co-d 4 = Pret.BM + stillage at C/N ratio 50)

The cumulative methane yields achieved from all experiments are shown in Fig. 6. For the inoculum and the sole substrates (BM and stillage) (Fig. 6a), only

29.53±1.49mlCH_4/gVS of methane yield occurred from the inoculum itself. The highest methane yield (247.73±6.10 mlCH_4/gVS) was gained when stillage was used as the sole substrate. This was possibly due to its high organic matter. Similar methane yields were found from the non-pretreated BM (nonPret.BM) and the pretreated BM (Pret.BM) which was 164.39±6.05 mlCH_4/gVS and 160.70±0.93 mlCH_4/gVS, respectively. It had been expected that the methane yield of the pretreated BM would be higher than that of the non-pretreated BM as the pretreatment process could transform nearly half of the lignin content in the original BM. This unexpected finding was the result of the solid residue separation method used after pretreatment process. As the solid residue was separated from the alkaline solution by filtering through a wire screen, some amounts of cellulose or hemicellulose extracted from the BM during the pretreatment process and existed in the solution forms could be lost with the separated liquid. Solubilization, redistribution and condensation of lignin and modifications in the crystalline state of the cellulose from the pretreatment of the lignocellulosic biomass were reported by Gregg and Saddler (1996). Another assumption of this low methane yield for the pretreated BM could be that lime was still attached on solid residue. During the solid residue separation method, hot water was used to wash off lime from BM. it was possible that some amounts of lime was still left on the residue and was the one that restrained the accessibility of enzymes. However, considering that considerable amounts of easily degraded organic substances could be lost with the separated liquid, similar methane yields obtained from the pretreated BM compared to that of the non-pretreated BM, in which all organic substances was still intact, tentatively suggested that the pretreatment method facilitated and improved the accessibility of enzyme used for biodegradation..

Co-digestion of pretreated BM with stillage at C/N ratio of 50 rendered the highest methane yield (206±4.56mlCH_4/gVS) followed by co-digestion of both substrates at the C/N ratio of 40 (183.76±4.30mlCH_4/gVS). The lowest methane yield (141.37±6.99mlCH_4/gVS) was obtained when the pretreated BM was co-digested with stillage at the C/N ratio of 30 which was not significantly different from the value obtained (154.53±5.79mlCH_4/gVS) when the non-pretreated BM was co-digested with stillage at the same C/N ratio. Results achieved from different BMP tests suggested that methane yield had the positive relationship with amount of stillage added into the flask. It also indicated that methane yield was mainly influenced by stillage. From the results of measured gas components, only biogas generated from stillage, used as the sole substrate, contained methane more than 60% after 17 d of BMP test, while less than 60% methane was detected in the biogas for the whole experimental period of other tests (Appendix C). Mixing stillage with BM in order to manipulate the C/N ratios did not improve the biodegradation but, instead, it was detrimental to the methane yield. Even though the C/N ratio of stillage (112.05) was found to be very high, this ratio could be much lesser if only the biodegradable carbon content of stillage was used in the calculation instead of the total carbon. This assumption is supported by a report that only some amounts of carbon contained in any substances are suitable for biodegradation, while almost all nitrogen will be available (Kayhanian & Tchobanoglous, 1992). The fact that stillage contains considerable amounts of the protein-rich yeast cell (Krzywonos *et al.*, 2009) means that nitrogen concentration of stillage could be quite high. Results from the BMP test of this current study and the aforementioned assumptions implied that stillage (though, having high C/N ratio) might not as much be nutrient-deficient substrate as previously expected. This could be the main reason why co-digesting stillage with the low C/N ratio BM did not bring about the improved effect. It also tentatively revealed that the co-metabolism effect could not be enhanced by mixing these substrates together. Suitability of co-digestion can be verified by analyzing the synergistic effects as described in the next topic. Table 20 summarized results obtained from the BMP test.

Table 20 Methane yields obtained from the BMP tests

Experiment	Methane yield ($mlCH_4/gVS_{added}$)*
Blank	29.53±1.49
Non Pret.BM	164.39±6.05
Pret.BM	160.70±0.93
Stillage	247.73±6.10
Co-digestion 1	154.53±5.79
Co-digestion 2	141.37±6.99
Co-digestion 3	183.76±4.30
Co-digestion 4	206±4.56

* Milliliter methane occur/gram of volatile solid of substance added

4.2.2 Synergistic effects

Generally, the synergistic or antagonistic effects are produced during the co-digestion of substrates. The synergistic impact would be noticed as an enhancing of methane production by co-digestion over the weight average of the sole substrates. On the other hand, lower productivity compared to the theoretical production of the sole substrates would call the antagonistic effect. The synergistic effect of BM and Stillage was analyzed and showed in Table 21

As explained in the previous topic, all co-digestion experiments indicated an antagonism between BM and stillage which meant the mixture had a competitive effect in the final production. Interestingly, the highest synergistic effect was found from the co-digestion 4-sample (non-pretreated manure and stillage). This finding suggested that the thermal-alkaline pretreatment process used might not be as effective as expected. Pettersen (1984) found that change of the cellulose structure to a form that was denser and thermodynamically more stable than the native cellulose could be caused by alkaline pretreatment. As rice husk presenting in the broiler manure was a high-lignin containing biomass, thermal-alkaline pretreatment might not be sufficient to increase the digestibility compared to the low-lignin containing biomass as reported by Kaar and Holtzapple (2000)

Table 21 Results of the synergistic or antagonistic effects produced by the co-digestion

Sample	Experimental production	Theoretical production	α
Blank	29.53±1.49	29.53	-
NonPret.BM	164.39±6.05	164.39	-
Pret.BM	160.70±0.93	160.70	-
Stillage	247.73±6.10	247.73	-
Co-digestion 1	154.53±5.79	204.25	0.76
Co-digestion 2	141.37±6.99	202.23	0.70
Co-digestion 3	183.76±4.30	231.24	0.79
Co-digestion 4	206±4.56	238.76	0.86

4.2.3 BMP mathematical models

To determine the optimum model that could simulate patterns of methane production from the studied substrates and evaluate some kinetic coefficients influencing the anaerobic digestion process, two different models, i.e. first-order model (FO) and Gompertz model (GM), were applied to the BMP experimental results.

1. Suitability of the model
2. Comparison of model predictability for BMP experimental results

The maximum methane productions (P) were predicted in various days of the experiment (5, 7, 18, 32, 51, 53, 86, and 92 d) and then compared to the final methane production of BMP results (Table 21).

Generally, the Gomperzt model was found to fit better than the first-order model for all the experiment results (in conditions that % error < 5% and R-square > 0.95; Table 21). Even, the Blank experiment that both models could not predict in each day, as there was less food for the microorganism to consume, GM model could still predict the maximum methane yield by using the kinetic parameters from the last day of the experiment. These models can explain 95% of the BMP results.

From the comparison between nonPret.BM and Pret.BM, GM could predict the methane yield for Pret.BM in 7 d with a relative error < 5%and the r^2 > 0.95(relative error =4.09%and r^2 = 0.98) while it took until 32 d for nonPret.BM (relative error = 0.38%and r^2 = 0.95). Possible reason to describe the difference in prediction day could be that nonPret.BM had lesser accessibility for enzyme to attack the organic matter than the Pret.BM led to unstable digestibility. Moreover, as GM has the assumption that biogas production is assumed to be proportional to microbial activity, the nonPret.BM would require longer contact time for microorganisms to access and digest the degradable portions.

For the stillage and the co-digestion 3 and 4, GM was able to predict the maximum methane production within 5 d with a relative error < 5% and the r^2 > 0.99. Also the kinetic parameters k and R_m were higher for stillage when used as the sole substrate compared to other experiments. This suggested that organic substances of stillage were more easily biodegradable. In addition, the lag phase (λ) parameter for stillage was relatively lesser compared to those of other substrates referring to rapid biodegradability or short biodegradability periods. This result could, to some extent, explain the negative effect of co-digesting the mixture of stillage and the broiler manure. Even though, broiler manure contained high nitrogen contents, the biodegradability of its carbon contents was significantly lower than those of the stillage. Mixing these wastes together, though the C/N ratio was improved, created the more recalcitrant matrix of organic carbons. Even when the pretreated broiler manure was used, the biodegradability of its remained organic contents might not be considerably improved as parts of the easily biodegradable cellulose and hemicelluloses were dissolved and separated from the pretreated solids. This more complex organic content could overpower the positive effect of improved C/N ratio of the mixture.

The first-order model could predict the BMP experiment results mostly on day 32 (a relative error <3% and the $r^2 > 0.95$) except for the co-digestion 2 and 3 that FO could predict the productivity just from day 18 which both had a relative error less than 3% and the r^2 more than 0.95.

Nielfa et al. (2015) studied the BMP experiments using biological sludge and organic fractions of municipal solid waste (OFMSW). They also applied GM model and FO model to their experiment BMP results to determine the optimum equation that fit with these kinds of wastes. They found that Gompertz model could fit better than first-order model for these two substrates and could predict the final production in just 7 days after startup the experiment. The prediction from model could explain around 99% of the methane production achieved by the experiment. The kinetics from model also showed that biological sludge was easily biodegradable and had less biodegradability periods than OFMSW. The increase in proportion of biological sludge in the co-digestion resulted in a growth of k and R_m (rate of anaerobic digestion process). In contrast, the decrease of lag phase occurred with the increase of biological sludge.

The result found from this current study showed that GM model was more suitable for predicting the BMP results for broiler manure and stillage compared to the FO model. Within the relatively shorter period of time (around 5 or 7 d), Gompertz model could predict more than 95% of the final methane production from the kinetic parameters.

Table 22 Results of the prediction models at different days. (The numbers in the bold indicate both % error less than 5% and r² more than 0.95)

Times (d)	γ ($mlCH_4/gVSadded$)		Error (%)		r^2		Kinetic parameters		
	FO	GM	FO	GM	FO	GM	FO	GM	
							k (d^{-1})	R_m($mlCH_4/gVS/d$)	λ (d)
Blank	BMP experimental: 29.41 $mlCH_4/gVS_{added}$								
5	25.83	29.02	12.16	1.34	0.91	0.90	0.02	0.63	0.91
7	24.00	28.20	18.40	4.13	0.90	0.89	0.02	0.50	1.63
18	22.15	26.14	24.70	11.11	0.98	0.96	0.02	0.38	3.14
32	19.75	22.24	32.85	24.38	0.96	0.94	0.01	0.29	6.44
53	20.60	20.98	29.96	28.66	0.94	0.95	0.01	0.27	7.52
92	26.35	30.21	10.40	**-2.72**	0.81	**0.98**	0.02	0.35	2.98
nonPret.BM	BMP experimental: 164.39 $mlCH_4/gVSadded$								
5	105.18	136.85	36.02	16.75	1.00	1.00	0.01	1.77	0.07
7	116.47	149.73	29.15	10.74	0.95	0.96	0.01	2.10	0.36
18	160.43	164.11	2.41	0.17	0.91	0.94	0.04	4.98	2.25
32	161.19	163.76	**1.95**	**0.38**	**0.97**	**0.95**	0.04	4.38	1.42
53	161.00	160.87	**2.06**	2.14	**0.99**	0.91	0.04	3.24	1.80
92	161.94	151.14	**1.49**	8.06	**0.99**	1.00	0.05	5.43	3.75

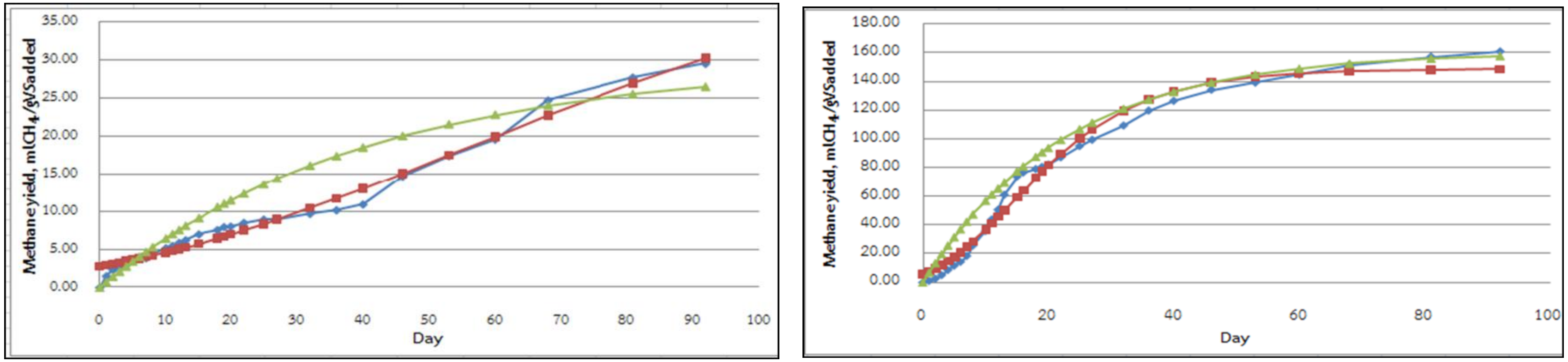
Times (d)	**γ** ($mlCH_4/gVSadded$)		Error (%)		r^2		Kinetic parameters		
	FO	GM	FO	GM	FO	GM	FO	GM	
							k (d^{-1})	Rm($mlCH_4/gVS/d$)	**λ** (d)
Pret.BM	BMP experimental: 160.70 $mlCH_4/gVSadded$								
5	121.73	150.22	24.25	6.52	0.96	0.96	0.02	2.39	0.46
7	129.45	154.12	19.44	**4.09**	0.97	**0.98**	0.02	2.70	0.62
18	157.33	160.49	2.10	**0.13**	0.94	**0.97**	0.04	5.03	1.87
32	156.38	159.83	**2.69**	**0.54**	**0.98**	**0.95**	0.04	4.02	0.54
53	156.65	156.26	**2.52**	2.76	**0.99**	0.92	0.04	3.03	2.72
92	157.82	148.53	**1.79**	7.57	**0.99**	0.99	0.04	4.63	2.14
Stillage	BMP experimental: 247.73 $mlCH_4/gVSadded$								
5	216.28	244.34	12.69	**1.37**	1.00	**1.00**	0.02	5.60	0.02
7	221.93	245.26	10.42	**1.00**	0.98	**0.99**	0.03	5.95	0.14
18	246.95	247.68	0.31	**0.02**	0.91	**0.98**	0.07	10.16	1.58
32	246.70	247.32	**0.42**	0.16	**0.95**	0.93	0.06	7.88	0.24
51	246.51	240.65	**0.49**	2.86	**0.98**	0.85	0.06	5.18	6.09
86	246.03	247.73	**0.69**	**0.00**	**0.99**	**1.00**	0.06	12.86	4.08

Times (d)	**γ** (mlCH$_4$/gVSadded)		Error (%)		r²		Kinetic parameters		
	FO	GM	FO	GM	FO	GM	FO	GM	
							k (d^{-1})	Rm(mlCH$_4$/gVS/d)	**λ** (d)
Co-digestion 1	BMP experimental: 154.53 mlCH$_4$/gVSadded								
5	99.94	129.76	35.32	16.03	0.98	0.98	0.01	1.82	0.16
7	117.78	144.13	13.48	6.73	0.94	0.95	0.02	2.43	0.34
18	151.60	154.32	1.92	**0.14**	0.94	**0.97**	0.05	5.13	1.92
32	150.70	153.58	**2.48**	**0.61**	**0.97**	**0.95**	0.04	4.05	0.48
51	151.11	149.58	**2.22**	3.21	**0.99**	0.91	0.04	3.03	3.09
86	151.19	142.85	**2.16**	7.56	**0.98**	0.98	0.04	4.78	2.26
Co-digestion 2	BMP experimental: 141.37 mlCH$_4$/gVSadded								
5	89.68	116.88	36.56	17.33	0.98	0.98	0.01	1.61	0.18
7	113.30	135.09	19.85	4.44	0.90	0.91	0.02	2.49	0.52
18	138.52	141.17	**2.02**	**0.14**	**0.96**	**0.97**	0.06	4.66	1.73
32	138.32	140.58	**2.16**	**0.56**	**0.98**	**0.96**	0.04	3.76	0.42
51	137.84	135.50	**2.50**	4.15	**0.99**	0.91	0.04	2.64	4.11
86	137.55	129.04	**2.70**	8.72	**0.99**	1.00	0.04	4.39	2.04

γ (mlCH$_4$/gVSadded) Error (%) r² Kinetic parameters

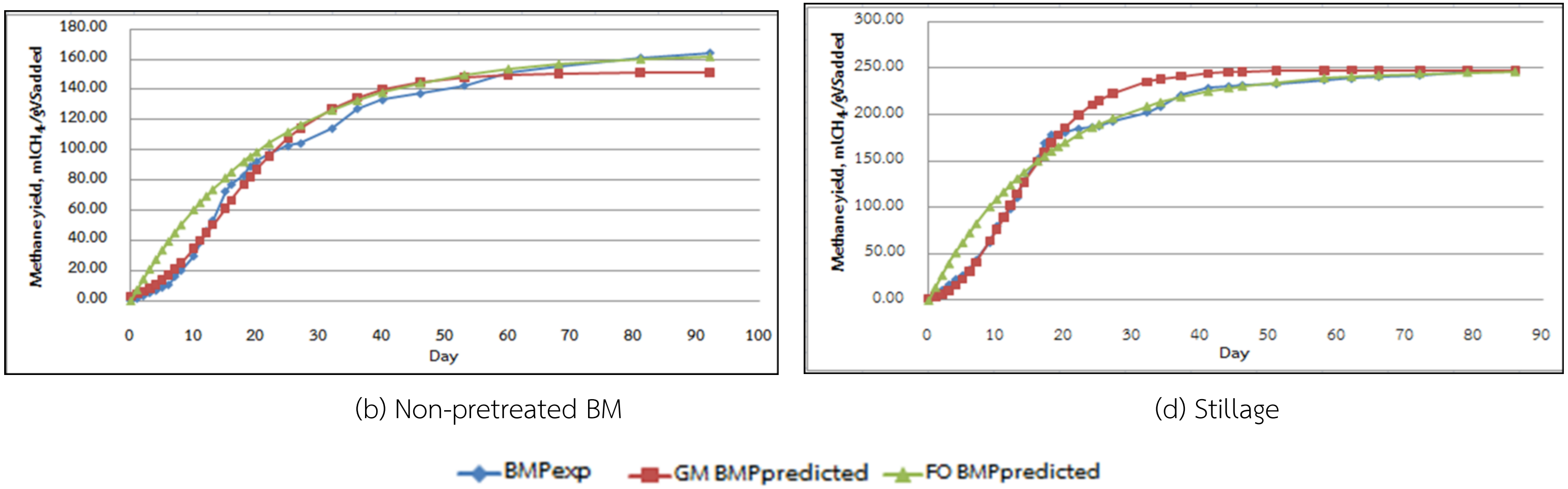
Times

Times (d)	**γ** (mlCH$_4$/gVSadded)		Error (%)		r²		Kinetic parameters		
	FO	GM	FO	GM	FO	GM	FO	GM	
							k (d^{-1})	Rm(mlCH$_4$/gVS/d)	**λ** (d)
Co-digestion 3	BMP experimental: 183.76 mlCH$_4$/gVSadded								
5	144.00	174.59	21.64	**4.99**	0.99	**0.99**	0.02	3.12	0.05
7	162.17	181.31	11.75	**1.33**	0.94	**0.95**	0.02	4.20	0.43
18	182.62	183.70	**0.62**	**0.03**	**0.96**	**0.97**	0.06	7.19	1.46
32	181.39	182.96	**1.29**	0.44	**0.97**	0.92	0.05	5.11	0.90
51	180.82	176.55	**1.60**	3.92	**0.99**	0.87	0.05	3.57	6.18
86	179.05	167.15	**2.56**	9.04	**0.98**	0.98	0.04	6.68	1.48
Co-digestion 4	BMP experimental: 206.00 mlCH$_4$/gVSadded								
5	172.59	200.94	16.22	**2.46**	1.00	**1.00**	0.02	4.19	0.09
7	188.78	204.64	8.36	**0.66**	0.96	**0.97**	0.03	5.40	0.51
18	205.25	205.95	0.36	**0.02**	0.94	**0.98**	0.07	8.53	1.54
32	204.13	205.30	**0.91**	0.34	**0.96**	0.92	0.06	6.02	0.92
51	203.58	198.75	**1.17**	3.52	**0.98**	0.85	0.05	4.17	6.39
86	202.96	188.95	**1.48**	8.28	**0.98**	0.98	0.05	8.50	2.07



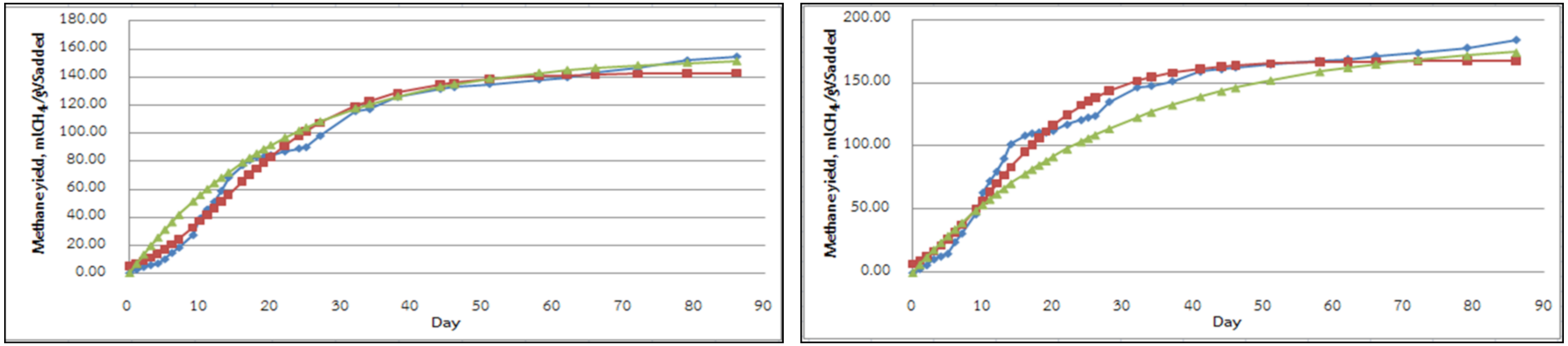
(a) Blank

(c) Pretreated BM



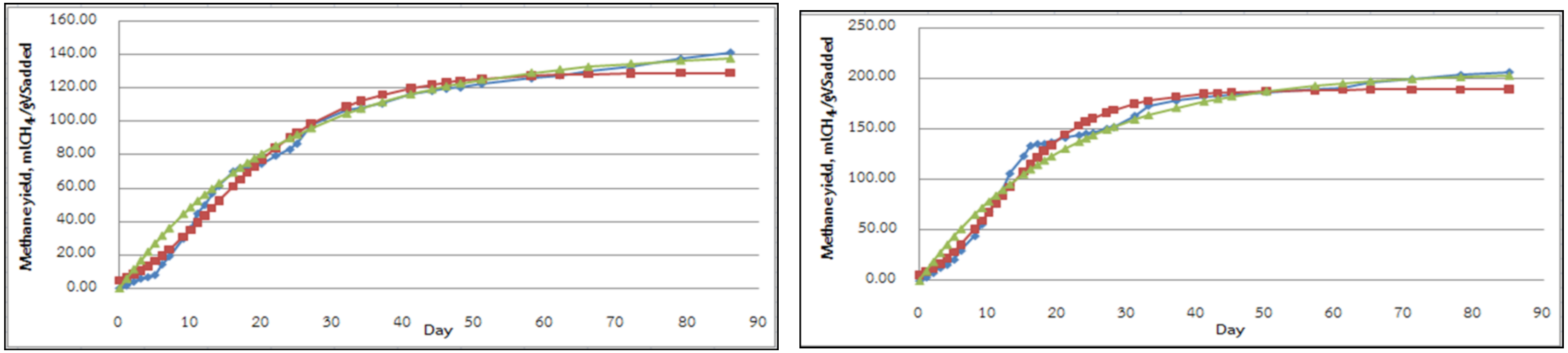
(b) Non-pretreated BM

(d) Stillage



(e) Co-digestion 1

(g) Co-digestion 3



(f) Co-digestion 2

(h) Co-digestion 4

Figure 7 Comparison of the maximum methane yield between the experiment, Gomperzt model (GM), and First-order model (FO) of each experiment

As seen in Fig. 7, it is obvious that Gomperzt model fitted the BMP results better and could predict the result at the shorter time duration onwards until the completion of the experiment. On the other hand, the first-order model failed to simulate the results especially at the early stage of the experiment (e.g. as can be seen in Fig 7 (b) to (e)). Superiority of GM over FO model could be explained by the model assumptions. Related the biodegradable efficiency to the microbial activity, GM was more reliable when the mixture contained stillage (Fig 7 (d) to (h)). In contrary, as FO model assumes that anaerobic digestion efficiency is governed by the hydrolysis rate, it could not simulate well when the waste mixture contained the more readily biodegradable stillage. When broiler manure was the main ingredient (as shown in Fig 6 (b) and (c)), FO was found to fit to the BMP results better as hydrolysis became an important rate limiting step.

CHAPTER 5
CONCLUSION

5.1 Conclusion

For thermal-alkali pretreatment process, the experiments were designed by Full Factorial design (FFD) to determine the optimum condition for broiler manure pretreatment using the thermal-alkali pretreatment method. From the experiments, only temperature was found to significantly affect lignin removal efficiency ($P=0.000$). However, significant interaction effect was detected between lime concentration and pretreatment duration ($P=0.018$). By the Full Factorial Analysis, an equation that can be used to predict the extent of lignin removal is;

$$\textbf{\textit{Lignin Removal Efficiency}} = 26.07 + (16.06 \times \textit{Temperature}) - (1.62 \times \textit{Lime Conc.}) + (0.59 \times \textit{Duration}) + (6.73 \times (\textit{Conc.*Duration}))$$

However, The ranges of conditions that the equation can predict are at temperature 85-150°C, 3-10% w/v $Ca(OH)_2$ concentration and 1-3 h treatment duration. The optimum conditions for pretreating broiler manure using the thermal-alkaline pretreatment process were at temperature 150°C, 3% w/v $Ca(OH)_2$ solution, and 1 h pretreatment duration, which achieved the highest lignin removal efficiency from broiler manure at 49.9%. Accordingly, the pretreated broiler manure used in the BMP test was pretreated at these conditions.

Stillage rendered the highest methane yield ($247.73 \pm 6.10\ \mathrm{mlCH_4/gVS}$) when used as the sole substrate while similar methane yields were found from the non-pretreated broiler manure ($164.39 \pm 6.05\mathrm{mlCH_4/gVS}$) and the pretreated broiler manure ($160.70 \pm 0.93\mathrm{mlCH_4/gVS}$). Co-digestion of pretreated BM with stillage at C/N ratio of 50 rendered the highest methane yield ($206 \pm 4.56\mathrm{mlCH_4/gVS}$) followed by co-digestion of both substrates at the C/N ratio of 40 (183.76±4.30mlCH_4/gVS). The lowest methane yield (141.37±6.99mlCH_4/gVS) was obtained when the pretreated BM was co-digested with stillage at the C/N ratio of 30 which was not significantly different from the value obtained (154.53±5.79mlCH_4/gVS) when the non-pretreated BM was co-digested with stillage at the same C/N ratio. Therefore, in this research, the results show that the thermal-alkali pretreatment could not achieve to increase the biogas production.

The synergistic effect revealed an antagonism between BM and stillage which meant the mixture had a competitive effect in the final production. Gomperzt model was found to fit better to the BMP results than the first-order model for all the experiment results (at conditions that % error < 5% and R-square > 0.95). In addition, Gomperzt model was more suitable for predicting the BMP results for broiler manure and stillage compared to the first-order model. Within the relatively shorter period of time (around 5 or 7 d), Gompertz model could predict more than 95% of the final methane production from the kinetic parameters.

5.2 Recommendations

1. To investigate the potential of the pretreated BM, continuous experiment using the appropriate anaerobic reactor, e.g. CSTR, should be conducted.
2. Some other pretreatment methods, e.g. enzyme pretreatment, ultrasonic, can be tested to find the best method for preparing BM as the feedstock for biogas production.
3. Economic analysis should be conducted to evaluate the feasibility of pretreatment method.

4. As it was presumed that significant amounts of easily biodegradable products might be dissolved in the solution, liquid portion of pretreatment BM should be used in the BMP experiments.
5. Liquid portion of pretreatment BM should be reused in the pretreatment process or calcium could be recovered by neutralizing it with carbon dioxide (CO_2) and using lime kiln technology.
6. Other nutrient deficient co-substrates, e.g. starch producing wastewater, could be used in the co-digestion process with the pretreated BM to investigate the advantages of the co-digestion process.

REFERENCES

Abouelenien, F., Namba, Y., Kosseva, M. R., Nishio, N., & Nakashimada, Y. (2014). Enhancement of methane production from co-digestion of chicken manure with agricultural wastes. *Bioresource Technology, 159*, 80-87. doi:http://dx.doi.org/10.1016/j.biortech.2014.02.050

Adrianus, Van Haandel, & Lettinga, G. (1994). *Anaerobic Sewage Treatment*. Baffins Lane, Chichester, Wast Sussex PO19 1UP, England.: John Wiley & sons.

Ahring, B. K., Angelidaki, I., & Johansen, K. (1992b). Co-digestion of organic solid waste manure and organic industrial waste. In K. J. Thomé-Kozmiensky (Ed.), *Waste Management International* (pp. 661-666). Berlin: EF-VERLAG für Energie und Umwelttech nik Gmbh.

Ahring, B. K., Garcia, H., Mathrani, I., & Angelidaki, I. (1996). Codigestion of manure with organic toxic waste in biogas reactor In J. A. Hansen (Ed.), *Management of Urban Biodegradable Waste* London: James & James Science Publishers Ltd.

Angelidaki, I. a. A., B.K. . (1997). Codigestion of olive oil mill wastewaters with manure, house hold waste or sewage sluge. *Biodegradation 8*(4), 221-226.

APHA. (2006). Standard Methods for the Examination of Water and Wastewater. . Washington, D.C.: American Public Health Association

Azzam, A. M., & Nasr, M. I. (1993). Physicothermochemical Pretreatments of Food Processing Waste for Enhancing Anaerobic Digestion and Biogas Generation. *Journal of Environmental Science and Health . Part A: Environmental Science and Engineering and Toxicology, 28*(8), 1629-1649. doi:10.1080/10934529309375968

Bobleter, O. (1994). Hydrothermal degradation of polymers derived from plants. *Progress in Polymer Science, 19*(5), 797-841. doi:http://dx.doi.org/10.1016/0079-6700(94)90033-7

Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K. B., & Ramakrishnan, S. (2011). Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. *Enzyme Research, 2011*, 17. doi:10.4061/2011/787532

Chandra, R., Takeuchi, H., Hasegawa, T., & Kumar, R. (2012). Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. *Energy, 43*(1), 273-282. doi:http://dx.doi.org/10.1016/j.energy.2012.04.029

Chang, V. S., Burr, B., & Holtzapple, M. T. (1997). Lime pretreatment of switchgrass. *Applied Biochemistry and Biotechnology, 63*(1), 3. doi:10.1007/bf02920408

Chang, V. S., Kaar, W. E., Burr, B., & Holtzapple, M. T. (2001). Simultaneous saccharification and fermentation of lime-treated biomass. *Biotechnology Letters, 23*(16), 1327-1333. doi:10.1023/a:1010594027988

Chang, V. S., Nagwani, M., & Holtzapple, M. T. (1998). Lime pretreatment of crop residues bagasse and wheat straw. *Applied Biochemistry and Biotechnology, 74*(3), 135-159. doi:10.1007/bf02825962

Chaturvedi, V., & Verma, P. (2013). An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. *3 Biotech, 3*(5), 415-431. doi:10.1007/s13205-013-0167-8

Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E., & Peck, M. W. (1993). Biochemical methane potential of biomass and waste feedstocks. *Biomass and Bioenergy, 5*(1), 95-111. doi:http://dx.doi.org/10.1016/0961-9534(93)90010-2

Costa, J. C., Barbosa, S. G., Alves, M. M., & Sousa, D. Z. (2012). Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter. *Bioresource Technology, 111*, 141-147. doi:http://dx.doi.org/10.1016/j.biortech.2012.02.047

Department of Alternative Energy Development and Efficiency, M. o. E. The 10-Year Alternative Energy Development Plan. Retrieved from http://weben.dede.go.th/webmax/content/10-year-alternative-energy-development-plan.

Department, T. E. (2014). Retrieved from http://www.excise.go.th/cs/groups/public/documents/document/mjaw/mdc1/~edisp/webportal16200075520.pdf

Eastman, J. A., & Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J. - Water Pollut. Control Fed.; (United States)*, Medium: X; Size: Pages: 352-366.

Gandi, J., Holtzapple, M. T., Ferrer, A., Byers, F. M., Turner, N. D., Nagwani, M., & Chang, S. (1997). Lime treatment of agricultural residues to improve rumen digestibility. *Animal Feed Science and Technology, 68*(3), 195-211. doi:10.1016/S0377-8401(97)00050-3

Garrote, G., Domínguez, H., & Parajó, J. C. (1999). Hydrothermal processing of lignocellulosic materials. *Holz als Roh- und Werkstoff, 57*(3), 191-202. doi:10.1007/s001070050039

Gáspár, M., Kálmán, G., & Réczey, K. (2007). Corn fiber as a raw material for hemicellulose and ethanol production. *Process Biochemistry, 42*(7), 1135-1139. doi:http://dx.doi.org/10.1016/j.procbio.2007.04.003

Gelegenis, J., Georgakakis, D., Angelidaki, I., & Mavris, V. (2007). Optimization of biogas production by co-digesting whey with diluted poultry manure. *Renewable Energy, 32*(13), 2147-2160. doi:http://dx.doi.org/10.1016/j.renene.2006.11.015

Gregg, D., & Saddler, J. N. (1996). A techno-economic assessment of the pretreatment and fractionation steps of a biomass-to-ethanol process. *Applied Biochemistry and Biotechnology, 57*(1), 711-727. doi:10.1007/bf02941753

Hamzawi, N., Kennedy, K. J., & McLean, D. D. (1998). Technical Feasibility of Anaerobic Co-Digestion of Sewage Sludge and Municipal Solid Waste. *Environmental Technology, 19*(10), 993-1003. doi:10.1080/09593331908616757

Hartmann, H., Angelidaki, I., and Ahring, B.K. (2003). Co-digestion of organic fraction of municipal waste with orther waste types. Fundamentals of the anaerobic digestion process. In J. Mata-Alvarez (Ed.). *Biomethanization of organic fraction of municipal solid waste*, 181-197.

Hawkes, D. L. (1980). *Factors affecting net energy production from mesophilic anaerobic digestion.* Paper presented at the the first International Symposium on Anaerobic Digestion University College, Cardiff, Wales, September 1979.

Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology, 100*(1), 10-18. doi:http://dx.doi.org/10.1016/j.biortech.2008.05.027

Himmelsbach, J. N., Raman, D. R., Anex, R. P., Burns, R. T., & Faulhaber, C. R. (2010). Effect of ammonia soaking pretreatment and enzyme addition on biochemical methane potential of switchgrass. *Transactions of the ASABE, 53*(6), 1921-1927. doi:10.13031/2013.35791

Iiyama, K., Lam, T. B. T., & Stone, B. A. (1994). Covalent Cross-Links in the Cell Wall. *Plant Physiology, 104*(2), 315-320.

Kaar, W. E., & Holtzapple, M. T. (2000). Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover. *Biomass and Bioenergy, 18*(3), 189-199. doi:http://dx.doi.org/10.1016/S0961-9534(99)00091-4

Kassim, E. A., & El-Shahed, A. S. (1986). Enzymatic and Chemical hydrolysis of certain cellulosic materials. *Agricultural Wastes, 17*(3), 229-233. doi:http://dx.doi.org/10.1016/0141-4607(86)90097-1

Kayhanian, M., & Tchobanoglous, G. (1992). Computation of C/N ratios for various organic fractions *Biocycle*.

Krzywonos, M., Cibis, E., MiŚkiewicz, T., & Ryznar-Luty, A. (2009). *Utilization and biodegradation of starch stillage (distillery wastewater)*.

Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research, 48*(8), 3713-3729. doi:10.1021/ie801542g

LaGrega, D., M., & al., e. (2006). *Hazardous Waste Management* (USA, Trans. 2nd ed.): McGraw-Hill.

Liew, L. N., Shi, J., & Li, Y. (2011). Enhancing the solid-state anaerobic digestion of fallen leaves through simultaneous alkaline treatment. *Bioresource*

Technology, 102(19), 8828-8834. doi:http://dx.doi.org/10.1016/j.biortech.2011.07.005

Mathrani, I. M., Johansen, K., & Ahring, B. K. (1994). Experiences with Thermophilic Anaerobic Digestion of Manure, Organic Industrial and Household Waste of the Large Scale Biogas Plant in Vegger, Denmark (pp. 365-374): IAWQ Southern African National Committee.

Metcalf&Eddy. (2004). *Wastewater Engineering Treatment Disposal Reuse*. Mcgraw-Hill ,Singapore.

Mirahmadi, K. a., Kabir, M. M. a., Jeihanipour, A., Karimi, K., & Taherzadeh, M. J. (2010). Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production. *BioResources, 5*(2), 928-938.

Nielfa, A., Cano, R., & Fdz-Polanco, M. (2015). Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports, 5*, 14-21. doi:http://dx.doi.org/10.1016/j.btre.2014.10.005

Office of agricultural economics, c. f. (2014). Retrieved from http://www.oae.go.th/download/document_tendency/journalofecon2558.pdf

Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y., & McCarty, P. L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research, 13*(6), 485-492. doi:http://dx.doi.org/10.1016/0043-1354(79)90043-5

Pettersen, R. C. (1984). the chemical composition of wood (chapter 2). In R. M. E. Rowell (Ed.), *The chemistry of solid wood* (pp. 207). Washington, DC. : American Chemical Society

Prenaud, V., Delgenes, J.P. and Moletta, R. . (1998). Influence of thermo-chemical pretreatment conditions on solubilization and anaerobic biodegradability of a microbial biomass *Environmental Technology*.

Raja Priya, K., Sandhya, S., & Swaminathan, K. (2009). Kinetic analysis of treatment of formaldehyde containing wastewater in UAFB reactor. *Chemical Engineering Journal, 148*, 212-216.

Reza, S. (2016). Poultry litter – a great resource to utilize. Retrieved from https://permaculturenews.org/2016/03/11/poultry-litter-a-great-resource-to-utilize/

Sakar, S., Yetilmezsoy, K., & Kocak, E. (2009). Anaerobic digestion technology in poultry and livestock waste treatment — a literature review. *Waste Management & Research, 27*(1), 3-18. doi:doi:10.1177/0734242X07079060

Sharma, D., Espinosa-Solares, T., & Huber, D. H. (2013). Thermophilic anaerobic co-digestion of poultry litter and thin stillage. *Bioresource Technology, 136*, 251-256. doi:http://dx.doi.org/10.1016/j.biortech.2013.03.005

Silverstein, R. A., Chen, Y., Sharma-Shivappa, R. R., Boyette, M. D., & Osborne, J. (2007). A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresource Technology, 98*(16), 3000-3011. doi:http://dx.doi.org/10.1016/j.biortech.2006.10.022

Song, Z., Yang, G., Han, X., Feng, Y., & Ren, G. (2013). Optimization of the Alkaline Pretreatment of Rice Straw for Enhanced Methane Yield. *BioMed Research International, 2013*, 968692. doi:10.1155/2013/968692

Speece, R. E. (1996). *Anaerobic biotechnology for industrial wastewaters*. Nashville, Tennessee: Archae Press 5840 R.E. Lee Dr.

Stuckey, D. C., & McCarty, P. L. (1978). Thermochemical pretreatment of nitrogenous materials to increase methane yield. *Biotechnology and Bioengineering Symposium, 8*, 219-233.

Suhardi, V. S. H., Prasai, B., Samaha, D., & Boopathy, R. (2013). Combined biological and chemical pretreatment method for lignocellulosic ethanol production from energy cane. *Renewable Bioresources, 1*(1). doi:10.7243/2052-6237-1-1

Sun, R., Lawther, J. M., & Banks, W. B. (1995). Influence of alkaline pre-treatments on the cell wall components of wheat straw. *Industrial Crops and Products, 4*(2), 127-145. doi:http://dx.doi.org/10.1016/0926-6690(95)00025-8

Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *International Journal of Molecular Sciences, 9*(9), 1621-1651. doi:10.3390/ijms9091621

Tanaka, S., Kobayashi, T., Kamiyama, K., Lolita, M., & Signeybildan, N. (1997). Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. *Water Science and Technology, 35*(8), 209-215. doi:10.1016/s0273-1223(97)00169-8

Xu, J., Cheng, J. J., Sharma-Shivappa, R. R., & Burns, J. C. (2010). Lime pretreatment of switchgrass at mild temperatures for ethanol production. *Bioresource Technology, 101*(8), 2900-2903. doi:http://dx.doi.org/10.1016/j.biortech.2009.12.015

Zhu, J., Wan, C., & Li, Y. (2010). Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. *Bioresource Technology, 101*(19), 7523-7528. doi:http://dx.doi.org/10.1016/j.biortech.2010.04.060

APPENDIX

APPENDIX A

THERMAL-ALKALI PRETREATMENT METHOD

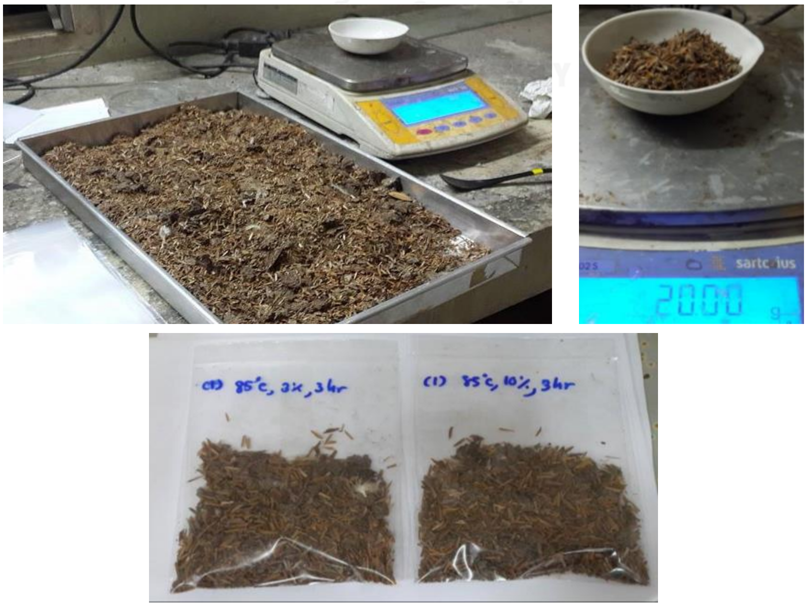
ACID DETERGENT FIBER AND LIGNIN ANALYSIS

THE BIOCHEMICAL METHANE POTENTIAL (BMP) TEST

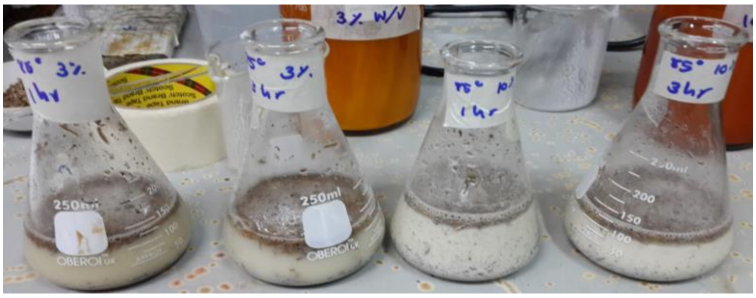
1. Pretreatment of broiler manure with thermal-alkali pretreatment

1.1 The collected broiler manure was manually disintegrated and completely mixed before using in the experiment

1.2 Separating broiler manure into 31 experiments, each experiment had 20g of manure (10g for pretreatment: 10g for control)



1.3 Pretreating in oil bath at each condition (w/v, 10 g broiler manure and 150 mL $Ca(OH)_2$ solution)



1.4 After pretreated, manure and lime solution were separated using a wire screen for lignin measurement.

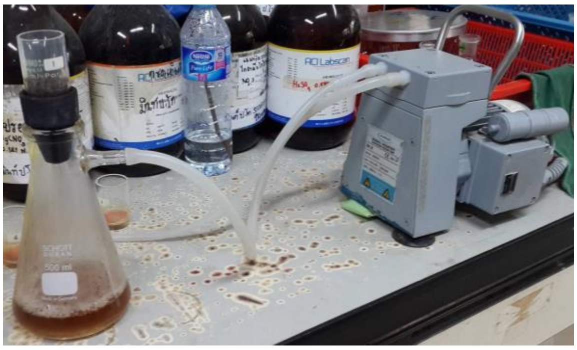
2. Acid detergent fiber and lignin analysis

2.1 Elimination moisture from manure by putting in oven 100 °C for 24h.

2.2 Dry 30 ml sintered glass filter crucible at 100 °C for 24h, cool in desicator and weight (W1)

2.3 Boiling 1 g manure (W2) together with 100 ml acid detergent solution (sulfuric acid 27 ml with CTAB 20 g in volumetic flask 1000 ml) for 60 min.

2.4 Filtering sample through a weighted glass filter crucible, using vacuum filtration apparatus, and rinsed it with some amount of hot water

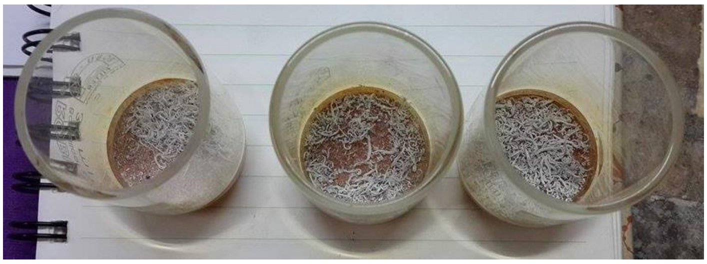


2.5 Dry the crucible at 100 °C for 24h, cool in desicator and weight (W3)

2.6 Sulfuric acid 72% (cool to 15°C) was added to the crucible every 1h until 3h and then sucked the acid out with vacuum filtration apparatus, rinsed with hot water about 3 -5 times

2.7 Dry the crucible at 100 °C for 24h, cool in desicator and weight (W4)

2.8 Heating the crucible in furnace at 550°C for 3h, cool in desicator and weight (W5)



%ADF = ((W3-W1)/W2)×100

%lignin = ((W4-W5)/W2)×100

%cellulose = %ADF - %lignin

W1 = Crucible weight, g

W2 = Broiler manure weight (determined as 1 g)

W3 = Crucible and fiber weight, g

W4 = Crucible and lignin weight, g

W5 = Crucible and ash weight, g

%ADF is determined gravimetrically as the residue remaining after extracting cell solubles, hemicellulose, and soluble minerals. The residues remaining are cellulose, lignin, heat damaged protein, a portion of cell wall protein, and mineral (ash)

3. **The Biochemical Methane Potential (BMP) test**

3.1 Adding seed and broiler manure and stillage into the 1 L glass bottle (The ratio of VSseed : Vssubstrate is kept constant at 2:1). The working volume of the bottle was 500 ml (deionized water was added to complete the working volume)

3.2 the bottle was purged with N_2 gas for 3 min and then sealed with the septum. Measuring the pressure of biogas occurred in the bottle daily (volume of biogas was calculated from the pressure inside the bottle)

3.3 when the pressure of biogas reached 400 mbar, biogas composition was measured using the portable gas analyzer (BIOGAS 5000, Geoteach)

APPENDIX B

INOCULUMS, BROILER MANURE, PRETREATED BROILER MANURE AND STILLAGE CHARACTERISTICS

CALCULATION OF BMP TEST DESIGN

1. Characteristics of broiler manure, stillage, and Inoculums

Characteristic	Broiler manure	Pretreated broiler manure	Stillage	Inoculum
Moisture,%	9.18	3.74	86.28	91.04
TS,%	90.82	96.26	13.72	8.96
VS,%TS	78.16	73.33	74.19	52.10
VS, g/g	0.7098	0.7059	0.1018	0.0467
MLSS, mg/l				593312*
MLVSS, mg/l				24750*
Density, g/ml	0.73	0.69	1.06	1.02
C/N ratio	26.77		112.05	
C, %	28.38		112.05	
N, %	1.06 (%dry weight)		4.482	

$$VS\ g_{VS}/g_{sample} = g\_sample \times ((\%TS)/100)\times((\%VS)/100)$$

The fermentation batch should contain 1.5-2% by weight of organic mass from the seeding sludge in order to ensure a comparable biomass concentration. For example, a fermentation batch of 500 ml requires 7.5-10 g_{VS} from the seeding sludge. (VDI 4630, Fermentation of Organic Materials (Characterization of the Substrates, Sampling, Collection of Material Data, Fermentation Tests. VDI-Handbuch Energietechnik (2006)).

2. Calculation of BMP test design

Experiment	Substrate		
	Inoculum, ml	Broiler manure, g	Stillage, ml
Blank	209.93	-	-
nonPret.BM	209.93	7.04	-
Pret.BM	209.93	7.04	-
Stillage	209.93	-	46.34
Co-digestion 1	209.93	6.17	5.77
Co-digestion 2	209.93	6.17	5.77
Co-digestion 3	209.93	4.28	18.21
Co-digestion 4	209.93	3.06	26.23

10 g_{VS} of the seeding sludge (g_{VSseed}) was chosen for 500 ml working volume of the BMP test.

Thus, 10 g_{VSseed} had to use the inoculums = 10/0.0467 = 214.13 g, Volume = Mass/Density = 214.13/1.02 = **209.93 ml**

Chynoweth et al. (1993) reported that maximum methane yields were obtained with S/I ratios of 0.5 to 1.0. In this experiment, the S/I ratio was chosen at 0.5.

Thus, $g_{VSsubstrate}/10g_{VSseed} = 0.5$, $g_{VSsubstrate} = 10*0.5 = 5$ g

- For stillage, 5 $g_{VSstillage}$ had to use stillage 5/0.1018 = 49.12 g, V = M/D = 49.12g/1.06 = **46.34 ml**
- For broiler manure, 5 $g_{VSmanure}$ had to use broiler manure = 5/0.7098 = **7.04 g**

For C/N ratio,

1. Substrate 1 g, **Water** = 1 g*(%moisture/100) , g

   Dry matter = 1-Water , g

   N = Dry matter*(N, %dry weight/100) , g

   C = (C/N ratio)*N , g

2. Substrate 1 g, **N** = 1 g*(N, %wet weight/100) , g

   C = (C/N ratio)*N , g

Therefore, Broiler manure 1 g had N = 0.0097 g and C = 0.2584 g. Stillage 1 g had N = 0.0004 g and C = 0.04482 g

C/N = 30,

$$30 = \frac{\mathrm{Cstillage}+(\mathrm{Y})\mathrm{Cbroiler}}{\mathrm{Nstillage}+(\mathrm{Y})\mathrm{Nbroiler}}$$

$$30 = \frac{0.04482+(Y)0.2584}{0.0004+(Y)0.0097}$$

Y = 1.007 g broiler/ g stillage

C/N = 40, Y = 0.222 g broiler/ g stillage

C/N = 50, Y = 0.11 g broiler/ g stillage

$$\text{VS of the mixture} = \frac{Y(VSbroiler)+VSstillage}{Y+1}$$

- For Co-digestion 1 and 2 (C/N = 30),

  VS of mixture were 0.407 g/g, 5 $g_{VSCo\text{-}d1}$ had to use the mixture = 5/0.407 = 12.29 g.

  g of the mixture 2.007 g had broiler manure 1.007 g. Thus, the mixture had Broiler manure = 12.29*1.007/2.007 **= 6.17 g** and had stillage = 12.29 – 6.17 = 6.12 g = **5.77 ml**

- For Co-digestion 3 (C/N = 40),

  VS of mixture were 0.212 g/g, 5 $g_{VSCo\text{-}d1}$ had to use the mixture = 5/0.212 = 23.58 g.

g of the mixture 1.222 g had broiler manure 0.222 g. Thus, the mixture had Broiler manure = 23.58*0.222/1.222 = **4.28 g** and had stillage = 23.58 – 4.28 = 19.30 g = **18.21 ml**

- For Co-digestion 4 (C/N = 50),

  VS of mixture were 0.162 g/g, 5 $g_{VSCo\text{-}d1}$ had to use the mixture = 5/0.162 = 30.86 g.

  g of the mixture 1.11 g had broiler manure 0.11 g. Thus, the mixture had Broiler manure = 30.86*0.11/1.11 = **3.06 g** and had stillage = 30.86 - 3.06 = 27.80 g, = **26.23 ml**

APPENDIX C

PRETREATMENT RESULTS

BMP EXPERIMENTAL RESULTS

1. Pretreatment results

Sample	Temp. °, C	CaOH$_2$ Conc., %	Time, hr	W2	W1	W3	W4	W5	% ADF	% Lignin	% cellulose	%lignin removal
F1	85.0	3	1	1.004	30.0840	30.8425	30.3434	30.1587	75.55	18.40	57.15	26.6
Control				1.005	29.9276	30.6765	30.3207	30.0686	74.50	25.08	49.42	
F2	85.0	10	1	1.002	30.3093	31.0513	30.6322	30.3888	74.07	24.30	49.78	2.9
Control				1.007	29.9762	30.6765	30.3207	30.0686	69.52	25.03	44.50	
F3	85.0	3	3	1.009	29.9740	30.7674	30.3269	30.0527	78.63	27.18	51.46	4.9
Control				1.001	30.2665	30.9698	30.6193	30.3333	70.25	28.57	41.68	
F4	85.0	10	3	1.008	29.7880	30.6092	30.0438	29.8763	81.48	16.62	64.86	19.2
Control				1.005	30.2348	30.916	30.5126	30.3059	67.81	20.58	47.24	
F5	150.0	3	1	1.008	30.0774	30.9032	30.3232	30.1632	81.96	15.88	66.08	54.0
Control				1.007	29.9275	30.6113	30.3504	30.0028	67.88	34.51	33.38	
F6	150.0	10	1	1.008	30.2993	31.1224	30.5403	30.3866	81.62	15.24	66.38	17.0
Control				1.005	29.9719	30.6605	30.2293	30.0447	68.49	18.36	50.13	
F7	150.0	3	3	1.008	29.9675	30.7331	30.1709	30.0470	75.94	12.29	63.65	33.5
Control				1.009	30.2652	30.9705	30.5536	30.3670	69.87	18.49	51.39	
F8	150.0	10	3	1.010	29.7852	30.6026	30.0305	29.8600	80.94	16.88	64.06	51.2
Control				1.008	30.2328	30.9196	30.6574	30.3089	68.11	34.56	33.55	
R1*	85.0	3	1	1.004	30.0822	30.8019	30.4558	30.1759	71.72	27.89	43.83	11.0
Control				1.006	29.9310	30.6124	30.3194	30.0042	67.74	31.34	36.41	
R2*	85.0	10	1	1.003	30.2981	31.0161	30.6479	30.3839	71.58	26.32	45.26	-3.7

Sample	Temp. °, C	CaOH2 Conc., %	Time, hr	W2	W1	W3	W4	W5	% ADF	% Lignin	% cellulose	%lignin removal
Control				1.005	29.9745	30.6547	30.2976	30.0424	67.66	25.39	42.28	
R3*	85.0	3	3	1.008	29.9712	30.7222	30.2906	30.0653	74.52	22.36	52.16	-2.1
Control				1.004	30.2686	30.9726	30.5919	30.3721	70.11	21.89	48.22	
R4*	85.0	10	3	1.004	29.7882	30.5372	30.0967	29.8909	74.58	20.49	54.09	21.4
Control				1.005	30.2356	30.9116	30.5681	30.3061	67.27	26.07	41.20	
R5*	150.0	3	1	1.008	30.0789	30.8630	30.2778	30.1541	77.77	12.27	65.50	43.7
Control				1.006	29.9293	30.6234	30.2175	29.9981	68.97	21.80	47.17	
R6*	150.0	10	1	1.007	30.2971	31.1090	30.5189	30.3760	80.66	14.20	66.46	52.4
Control				1.007	29.9734	30.6852	30.3518	30.0513	70.67	29.84	40.84	
R7*	150.0	3	3	1.008	29.9696	30.7531	30.1706	30.0479	77.70	12.17	65.53	49.9
Control				1.009	30.2663	30.9601	30.5866	30.3416	68.76	24.28	44.48	
R8*	150.0	10	3	1.009	29.7869	30.5591	29.9775	29.8377	76.50	13.85	62.65	35.3
Control				1.008	30.2334	30.939	30.5322	30.3164	70.02	21.42	48.61	
C1	117.5	6.5	2	1.008	30.0757	30.8953	30.3302	30.1583	81.34	17.06	64.28	43.4
Control				1.004	29.9729	30.7092	30.3552	30.0523	73.31	30.16	43.15	
C2	117.5	6.5	2	1.006	29.9668	30.7706	30.2083	30.0409	79.94	16.65	63.29	38.0
Control				1.005	30.2308	30.9621	30.5790	30.3092	72.76	26.84	45.92	
C3	117.5	6.5	2	1.006	29.7847	30.5946	30.1388	29.8807	80.47	25.65	54.83	38.2
Control				1.007	29.9449	30.6755	30.4298	30.0121	72.56	41.48	31.08	

Sample	Temp. °, C	CaOH2 Conc., %	Time, hr	W2	W1	W3	W4	W5	% ADF	% Lignin	% cellulose	%lignin removal
A1	64.4	6.5	2	1.0043	30.0776	30.7849	30.3830	30.1650	70.43	21.71	48.72	61.0
Control				1.0034	29.7894	30.4976	30.4271	29.8684	70.58	55.69	14.90	
A2	170.6	6.5	2	1.0020	30.0779	30.8547	30.4253	30.1777	77.53	24.71	52.81	31.9
Control				1.0041	29.9279	30.6506	30.3747	30.0102	71.98	36.30	35.67	
A3	117.5	0.78	2	1.0043	30.2961	31.0360	30.7098	30.3828	73.67	32.56	41.11	-11.1
Control				1.0088	29.9745	30.6739	30.3450	30.0493	69.33	29.31	40.02	
A4	117.5	12.22	2	1.0073	29.9678	30.7563	30.2617	30.0535	78.28	20.67	57.61	61.0
Control				1.0090	30.2656	30.9814	30.8888	30.3534	70.94	53.07	17.88	
A5	117.5	6.5	0.37	1.0076	29.7881	30.5367	30.2523	29.8893	74.30	36.03	38.27	-7.6
Control				1.0088	30.2332	30.9037	30.7204	30.3826	66.47	33.49	32.98	
A6	117.5	6.5	3.63	1.0086	30.0756	30.8837	30.3866	30.1830	80.12	20.19	59.94	32.6
Control				1.0046	29.9387	30.6269	30.4117	30.1108	68.51	29.95	38.55	
A7*	64.4	6.5	2	1.0044	29.9667	30.6659	30.3087	30.0599	69.61	24.77	44.84	42.1
Control				1.0071	30.2640	31.0305	30.8252	30.3944	76.11	42.78	33.33	
A8*	170.6	6.5	2	1.0021	30.2963	31.0649	30.7481	30.3939	76.70	35.35	41.35	-4.2
Control				1.0007	30.2314	30.9350	30.6577	30.3184	70.31	33.91	36.40	
A9*	117.5	0.78	2	1.0035	29.7874	30.5284	30.1888	29.8905	73.84	29.73	44.12	12.0
Control				1.0035	29.9368	30.6578	30.366	30.0269	71.8485	33.79	38.06	
A10*	117.5	12.22	2	1.0051	29.9270	30.7131	30.3034	30.0342	78.21	26.78	51.43	-20.8

Sample	Temp. °,C	CaOH2 Conc., %	Time, hr	W2	W1	W3	W4	W5	% ADF	% Lignin	% cellulose	%lignin removal
Control				1.0053	30.0761	30.8070	30.3859	30.1630	72.71	22.17	50.53	
A11*	117.5	6.5	0.37	1.0043	29.9722	30.6900	30.2938	30.0690	71.47	22.38	49.09	30.2
Control				1.0031	30.2928	30.9772	30.6962	30.3744	68.23	32.08	36.15	
A12*	117.5	6.5	3.63	1.0052	30.2649	31.1127	30.6698	30.3970	84.34	27.14	57.20	6.7
Control				1.0045	29.9645	30.6603	30.3383	30.0462	69.27	29.08	40.19	
A13*	170.6	6.5	2	1.0043	29.7855	30.6124	30.2018	29.8807	82.34	31.97	50.36	44.5
Control				1.0078	30.2328	30.9516	30.9130	30.3324	71.32	57.61	13.71	
A14*	117.5	0.78	2	1.0035	30.2940	31.0191	30.6671	30.3944	72.26	27.18	45.08	46.7
Control				1.0009	29.9731	30.6571	30.5729	30.063	68.34	50.94	17.39	
A16*	117.5	12.22	2	1.0036	29.9653	30.7148	30.4109	30.1217	74.68	28.82	45.87	43.4
Control				1.0078	30.2646	30.9944	30.8994	30.3865	72.42	50.89	21.52	
A17*	117.5	6.5	0.37	1.0037	30.0769	30.7831	30.3670	30.1802	70.37	18.61	51.75	66.4
Control				1.0020	29.9277	30.6137	30.5701	30.0149	68.46	55.41	13.05	
C4*	117.5	6.5	2	1.0016	29.929	30.6928	30.2233	30.0242	76.25	19.88	56.38	44.1
Control				1.0072	30.0755	30.7858	30.5092	30.1511	70.52	35.55	34.97	
C5*	117.5	6.5	2	1.0041	29.9733	30.7442	30.387	30.0722	76.78	31.35	45.42	39.1
Control				1.0089	30.2921	31.0187	30.9029	30.3833	72.02	51.50	20.52	
C6*	117.5	6.5	2	1.0038	30.2654	31.0416	30.5588	30.3676	77.33	19.05	58.28	38.6
Control				1.0063	29.9645	30.6892	30.3663	30.0541	72.02	31.02	40.99	

*Replicate

$\%ADF = ((W3-W1)/W2)\times 100$

$\%lignin = ((W4-W5)/W2)\times 100$

$\%cellulose = \%ADF - \%lignin$

$$\%lignin\ removal = \frac{(\%lignin,control - \%lignin,pretreated\ manure)}{\%lignin,control}\times 100$$

W1 = Crucible weight, g

W2 = Broiler manure weight (determined as 1 g)

W3 = Crucible and fiber weight, g

W4 = Crucible and lignin weight, g

W5 = Crucible and ash weight, g

2. BMP experimental results

2.1 Blank

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
9-Jun	0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
10-Jun	1	57.9	20.3	9.5	0.2	70.0	36.48	7.42	7.42	1.48
11-Jun	2	31.7	20.3	9.5	0.2	70.0	19.97	4.06	11.48	2.30
12-Jun	3	16.2	20.3	9.5	0.2	70.0	10.18	2.07	13.55	2.71
13-Jun	4	9.2	20.3	9.5	0.2	70.0	5.82	1.18	14.74	2.95
14-Jun	5	19.7	20.3	9.5	0.2	70.0	12.41	2.52	17.26	3.45
15-Jun	6	11.1	20.3	9.5	0.2	70.0	7.01	1.43	18.69	3.74
16-Jun	7	4.3	20.3	9.5	0.2	70.0	2.71	0.55	19.24	3.85
17-Jun	8	17.9	20.3	9.5	0.2	70.0	11.28	2.29	21.53	4.31
19-Jun	10	33.6	20.3	9.5	0.2	70.0	21.17	4.31	25.84	5.17
20-Jun	11	11.0	20.3	9.5	0.2	70.0	6.95	1.41	27.25	5.45
21-Jun	12	16.3	20.3	9.5	0.2	70.0	10.29	2.09	29.34	5.87
22-Jun	13	13.0	20.3	9.5	0.2	70.0	8.19	1.67	31.01	6.20
24-Jun	15	30.0	20.3	9.5	0.2	70.0	18.90	3.84	34.85	6.97

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
27-Jun	18	22.7	20.3	9.5	0.2	70.0	14.28	2.90	37.76	7.55
28-Jun	19	15.0	20.3	9.5	0.2	70.0	9.45	1.92	39.68	7.94
29-Jun	20	3.0	20.3	9.5	0.2	70.0	1.89	0.38	40.06	8.01
1-Jul	22	18.7	20.3	9.5	0.2	70.0	11.76	2.39	42.45	8.49
4-Jul	25	16.3	20.3	9.5	0.2	70.0	10.29	2.09	44.54	8.91
6-Jul	27	0.0	20.3	9.5	0.2	70.0	0.00	0.00	44.54	8.91
11-Jul	32	30.0	20.3	9.5	0.2	70.0	18.90	3.84	48.39	9.68
15-Jul	36	18.7	20.3	9.5	0.2	70.0	11.76	2.39	50.78	10.16
19-Jul	40	29.3	20.3	9.5	0.2	70.0	18.48	3.76	54.54	10.91
25-Jul	46	104.4	27.9	13.1	0.4	58.6	65.79	18.90	73.44	14.69
1-Aug	53	76.6	27.9	13.1	0.4	58.6	48.28	13.64	87.08	17.42
8-Aug	60	63.4	27.9	13.1	0.4	58.6	39.92	10.82	97.90	19.58
16-Aug	68	111.2	36.6	16.4	0.4	46.7	70.06	25.70	123.59	24.72
29-Aug	81	65.3	36.6	16.4	0.4	46.7	41.16	15.05	138.64	27.73
9-Sep	92	39.0	36.6	16.4	0.4	46.7	24.59	9.00	147.64	29.53

2.2 Non Pretreated broiler manure

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
9-Jun	0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
10-Jun	1	84.2	15.1	19.1	0.0	65.9	53.02	8.00	8.00	1.60
11-Jun	2	91.4	15.1	19.1	0.0	65.9	57.56	8.68	16.67	3.33
12-Jun	3	101.1	15.1	19.1	0.0	65.9	63.71	9.60	26.27	5.25
13-Jun	4	83.0	15.1	19.1	0.0	65.9	52.29	7.88	34.15	6.83
14-Jun	5	108.7	15.1	19.1	0.0	65.9	68.46	10.32	44.47	8.89
15-Jun	6	90.3	15.1	19.1	0.0	65.9	56.91	8.58	53.05	10.61
16-Jun	7	151.2	28.8	25.8	0.1	45.2	95.25	27.45	80.50	16.10
17-Jun	8	111.3	28.8	25.8	0.1	45.2	70.14	20.21	100.71	20.14
19-Jun	10	262.0	28.8	25.8	0.1	45.2	165.06	47.56	148.27	29.65
20-Jun	11	186.3	39.0	27.6	0.1	33.3	117.35	45.78	194.06	38.81
21-Jun	12	151.0	39.0	27.6	0.1	33.3	95.13	37.11	231.17	46.23
22-Jun	13	141.7	39.0	27.6	0.1	33.3	89.25	34.83	266.00	53.20
24-Jun	15	327.1	46.8	26.9	0.1	26.2	206.07	96.58	362.58	72.52
25-Jun	16	77.3	46.8	26.9	0.1	26.2	48.72	22.83	385.42	77.08

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
27-Jun	18	99.7	46.8	26.9	0.1	26.2	62.79	29.38	414.80	82.96
28-Jun	19	95.1	49.7	30.1	0.2	20.0	59.93	29.78	444.58	88.92
29-Jun	20	51.8	49.7	30.1	0.2	20.0	32.63	16.21	460.79	92.16
1-July	22	82.3	49.7	30.1	0.2	20.0	51.87	25.76	486.55	97.31
4-July	25	84.0	49.7	30.1	0.2	20.0	52.92	26.30	512.85	102.57
6-July	27	27.3	49.7	30.1	0.2	20.0	17.22	8.56	521.41	104.28
11-July	32	158.0	49.7	30.1	0.2	20.0	99.54	49.64	571.05	114.21
15-July	36	202.2	51.4	31.1	0.1	17.4	127.36	65.50	636.55	127.31
19-July	40	89.6	51.4	31.1	0.1	17.4	56.45	28.98	665.53	133.11
25-July	46	65.3	51.4	31.1	0.1	17.4	41.16	21.16	686.68	137.34
1-Aug	53	83.0	51.4	31.1	0.1	17.4	52.29	26.89	713.57	142.71
8-Aug	60	129.9	51.2	32.3	0.2	16.3	81.81	41.91	755.48	151.10
16-Aug	68	65.1	51.2	32.3	0.2	16.3	41.03	21.02	776.51	155.30
29-Aug	81	90.1	51.2	32.3	0.2	16.3	56.74	29.07	805.58	161.12
9-Sep	92	50.7	51.2	32.3	0.2	16.3	31.92	16.35	821.93	164.39

2.3 Pretreated broiler manure

Date	Day	Daily pressure production mbar,	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
9-Jun	0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
10-Jun	1	74.0	12.0	16.1	0.0	71.8	46.60	5.46	5.46	1.09
11-Jun	2	103.2	12.0	16.1	0.0	71.8	65.04	7.98	13.44	2.69
12-Jun	3	170.5	12.0	16.1	0.0	71.8	107.39	12.27	25.71	5.14
13-Jun	4	135.1	20.9	21.9	0.1	57.1	85.09	17.90	43.61	8.72
14-Jun	5	110.1	20.9	21.9	0.1	57.1	69.34	14.59	58.21	11.64
15-Jun	6	79.6	25.5	23.8	0.1	50.6	51.43	13.95	72.16	14.43
16-Jun	7	129.5	25.5	23.8	0.1	50.6	81.58	20.53	92.69	18.54
17-Jun	8	180.3	32.3	24.9	0.0	42.8	113.57	36.04	128.73	25.75
19-Jun	10	227.8	35.7	25.2	0.0	39.1	143.51	50.54	179.28	35.86
20-Jun	11	152.5	41.7	25.0	0.0	33.3	96.05	40.15	219.43	43.89
21-Jun	12	127.3	41.7	25.0	0.0	33.3	80.22	33.48	252.91	50.58
22-Jun	13	179.1	44.8	25.0	0.0	30.2	112.85	52.25	305.16	61.03
24-Jun	15	197.3	50.0	25.0	0.1	24.8	124.30	62.64	367.80	73.56
25-Jun	16	46.3	50.0	25.0	0.1	24.8	29.19	14.58	382.38	76.48

Date	Day	Daily pressure production mbar,	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
27-Jun	18	38.3	50.0	25.0	0.1	24.8	24.15	12.10	394.48	78.90
28-Jun	19	24.3	50.0	25.0	0.1	24.8	15.33	7.68	402.17	80.43
29-Jun	20	25.3	50.0	25.0	0.1	24.8	15.96	7.91	410.07	82.01
1-July	22	78.0	51.0	25.8	0.1	23.1	49.14	25.25	435.32	87.06
4-July	25	115.8	53.1	27.0	0.1	19.8	72.93	38.97	474.29	94.86
6-July	27	67.2	53.1	27.0	0.1	19.8	42.34	22.12	496.42	99.28
11-July	32	148.1	53.1	27.0	0.1	19.8	93.28	49.45	545.86	109.17
15-July	36	156.4	52.6	27.7	0.1	19.5	98.53	52.10	597.97	119.59
19-July	40	104.4	51.2	29.3	0.1	19.4	65.77	33.74	631.70	126.34
25-July	46	120.0	51.2	29.3	0.1	19.4	75.62	38.80	670.50	134.10
1-Aug	53	76.5	51.2	29.3	0.1	19.4	48.17	24.70	695.20	139.04
8-Aug	60	84.6	51.5	29.8	0.1	18.7	53.32	27.43	722.63	144.53
16-Aug	68	109.0	51.7	30.4	0.0	17.9	68.69	35.55	758.17	151.63
29-Aug	81	83.7	51.7	30.4	0.0	17.9	52.71	27.30	785.48	157.10
9-Sep	92	55.3	51.7	30.4	0.0	17.9	34.86	18.02	803.49	**160.70**

2.4 Stillage

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH₄/gVS
14-May	0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
15-May	1	451.7	8.4	23.4	0.1	68.1	284.55	23.82	23.82	4.76
16-May	2	367.3	14.0	33.7	0.1	52.2	231.42	32.32	56.14	11.23
17-May	3	261.5	18.1	39.1	0.2	42.6	164.76	29.77	85.91	17.18
18-May	4	152.1	30.4	38.6	0.2	30.8	95.80	29.16	115.07	23.01
19-May	5	106.0	30.4	38.6	0.2	30.8	66.78	20.33	135.39	27.08
20-May	6	155.7	30.4	38.6	0.2	30.8	98.07	29.85	165.24	33.05
21-May	7	184.8	44.8	31.1	0.2	24.0	116.40	52.55	217.79	43.56
23-May	9	331.7	44.8	31.1	0.2	24.0	208.99	93.69	311.48	62.30
24-May	10	261.8	53.0	28.2	0.1	20.2	164.93	88.38	399.86	79.97
25-May	11	156.5	55.5	26.9	0.1	18.9	98.59	54.79	454.65	90.93
26-May	12	109.0	55.5	26.9	0.1	18.9	68.67	38.11	492.76	98.55
27-May	13	172.0	55.5	26.9	0.1	18.9	108.36	60.14	552.90	110.58
28-May	14	245.3	58.6	24.0	0.0	17.4	154.54	90.49	643.40	128.68
30-May	16	317.3	58.6	24.0	0.0	17.4	199.92	117.08	760.47	152.09

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
31-May	17	222.1	62.2	24.1	0.1	13.5	139.94	87.03	847.50	169.50
1-Jun	18	114.3	62.2	24.1	0.1	13.5	72.01	44.80	892.30	178.46
2-Jun	19	0.0	62.2	24.1	0.1	13.5	0.00	0.00	892.30	178.46
3-Jun	20	28.8	62.2	24.1	0.1	13.5	18.12	11.33	903.63	180.73
5-Jun	22	51.7	62.2	24.1	0.1	13.5	32.55	20.25	923.89	184.78
7-Jun	24	22.3	62.2	24.1	0.1	13.5	14.07	8.73	932.62	186.52
8-Jun	25	16.3	62.2	24.1	0.1	13.5	10.29	6.40	939.02	187.80
10-Jun	27	67.2	59.4	26.3	0.0	14.3	42.34	25.15	964.17	192.83
15-Jun	32	128.9	59.4	26.3	0.0	14.3	81.18	48.22	1012.39	202.48
17-Jun	34	82.6	59.4	26.3	0.0	14.3	52.06	30.92	1043.31	208.66
20-Jun	37	164.3	59.4	26.3	0.0	14.3	103.53	61.51	1104.82	220.96
24-Jun	41	108.0	56.0	28.5	0.0	15.5	68.02	38.11	1142.93	228.59
27-Jun.	44	27.3	56.0	28.5	0.0	15.5	17.18	9.63	1152.56	230.51
29-Jun	46	17.9	56.0	28.5	0.0	15.5	11.26	6.31	1158.87	231.77
4-Jul	51	20.2	56.0	28.5	0.0	15.5	12.70	7.12	1165.99	233.20

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
11-Jul	58	59.2	56.0	28.5	0.0	15.5	37.30	20.89	1186.88	237.38
15-Jul	62	20.0	56.0	28.5	0.0	15.5	12.60	7.06	1193.95	238.79
19-Jul	66	28.7	56.0	28.5	0.0	15.5	18.06	10.12	1204.07	240.81
25-Jul	72	23.0	56.0	28.5	0.0	15.5	14.49	8.12	1212.18	242.44
1-Aug	79	44.7	56.0	28.5	0.0	15.5	28.14	15.77	1227.95	245.59
8-Aug	86	30.3	56.0	28.5	0.0	15.5	19.11	10.71	1238.66	247.73

2.5 Co-digestion 1

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
14-May	0	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
15-May	1	150.5	13.1	18.2	0.2	68.6	94.81	12.43	12.43	2.49
16-May	2	105.0	13.1	18.2	0.2	68.6	66.15	8.67	21.10	4.22
17-May	3	88.0	13.1	18.2	0.2	68.6	55.44	7.24	28.35	5.67
18-May	4	69.5	13.1	18.2	0.2	68.6	43.78	5.77	34.11	6.82

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
19-May	5	81.4	29.4	22.8	0.2	47.8	51.28	15.05	49.17	9.83
20-May	6	123.3	29.4	22.8	0.2	47.8	77.65	22.79	71.95	14.39
21-May	7	100.5	29.4	22.8	0.2	47.8	63.31	18.59	90.54	18.11
23-May	9	243.5	29.4	22.8	0.2	47.8	153.40	45.01	135.55	27.11
24-May	10	218.4	43.2	22.6	0.0	34.2	137.56	59.45	195.00	39.00
25-May	11	118.2	43.2	22.6	0.0	34.2	74.47	32.17	227.17	45.43
26-May	12	99.5	43.2	22.6	0.0	34.2	62.68	27.08	254.25	50.85
27-May	13	143.0	43.2	22.6	0.0	34.2	90.09	38.91	293.16	58.63
28-May	14	161.6	47.0	28.2	0.0	24.9	101.78	47.79	340.95	68.19
30-May	16	149.7	47.0	28.2	0.0	24.9	94.31	44.30	385.25	77.05
31-May	17	60.0	47.0	28.2	0.0	24.9	37.80	17.75	403.00	80.60
1-Jun	18	40.0	47.0	28.2	0.0	24.9	25.20	11.84	414.84	82.97
2-Jun	19	0.0	47.0	28.2	0.0	24.9	0.00	0.00	414.84	82.97
3-Jun	20	20.0	47.0	28.2	0.0	24.9	12.60	5.91	420.75	84.15
5-Jun	22	37.0	47.0	28.2	0.0	24.9	28.35	13.30	434.05	86.81

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
7-Jun	24	13.5	47.0	28.2	0.0	24.9	22.36	10.49	444.53	88.91
8-Jun	25	13.0	47.0	28.2	0.0	24.9	9.76	4.58	449.11	89.82
10-Jun	27	130.3	49.5	29.4	0.0	21.1	82.06	40.62	489.74	97.95
15-Jun	32	281.2	49.5	29.4	0.0	21.1	177.15	87.70	577.44	115.49
17-Jun	34	24.0	49.5	29.4	0.0	21.1	15.12	7.49	584.92	116.98
21-Jun	38	140.6	49.7	31.4	0.1	18.8	88.55	43.97	628.90	125.78
27-Jun	44	89.5	49.7	31.4	0.1	18.8	56.35	27.98	656.88	131.38
29-Jun	46	21.0	49.7	31.4	0.1	18.8	13.23	6.57	663.45	132.69
4-Jul	51	37.0	49.7	31.4	0.1	18.8	23.31	11.57	675.03	135.01
11-Jul	58	50.5	49.7	31.4	0.1	18.8	31.81	15.81	690.83	138.17
15-Jul	62	25.0	49.7	31.4	0.1	18.8	15.75	7.82	698.65	139.73
19-Jul	66	64.0	49.7	32.3	0.0	18.1	40.29	19.96	718.61	143.72
25-Jul	72	42.8	49.7	32.3	0.0	18.1	26.93	13.37	731.98	146.40
1-Aug	79	88.2	49.1	33.4	0.0	17.6	55.53	27.23	759.21	151.84
8-Aug	86	43.5	49.1	33.4	0.0	17.6	27.37	13.44	772.65	**154.53**

2.6 Co-digestion 2

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
14-May	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
15-May	1	82.4	16.1	12.9	0.0	71.0	51.9	8.4	8.4	1.67
16-May	2	111.3	16.1	12.9	0.0	71.0	70.1	11.3	19.7	3.93
17-May	3	91.0	16.1	12.9	0.0	71.0	57.3	9.2	28.9	5.78
18-May	4	42.3	16.1	12.9	0.0	71.0	26.7	4.3	33.2	6.64
19-May	5	63.0	16.1	12.9	0.0	71.0	39.7	6.4	39.7	7.93
20-May	6	151.8	33.2	20.8	0.1	45.9	95.6	31.7	71.4	14.27
21-May	7	113.7	33.2	20.8	0.1	45.9	71.7	23.8	95.2	19.04
23-May	9	254.6	33.2	20.8	0.1	45.9	160.4	53.4	148.6	29.71
24-May	10	138.7	33.2	20.8	0.1	45.9	87.4	29.1	177.6	35.52
25-May	11	167.7	43.7	21.0	0.0	35.3	105.6	46.2	223.9	44.77
26-May	12	91.8	43.7	21.0	0.0	35.3	57.8	25.3	249.2	49.83
27-May	13	126.0	43.7	21.0	0.0	35.3	79.4	34.7	283.9	56.78
28-May	14	83.3	43.7	21.0	0.0	35.3	52.5	23.0	306.9	61.38

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
30-May	16	142.9	47.9	24.3	0.0	27.8	90.0	43.1	350.0	70.00
31-May	17	34.5	47.9	24.3	0.0	27.8	21.7	10.4	360.4	72.09
1-Jun	18	18.5	47.9	24.3	0.0	27.8	11.7	5.6	366.0	73.21
2-Jun	19	0.0	47.9	24.3	0.0	27.8	0.0	0.0	366.0	73.21
3-Jun	20	22.8	47.9	24.3	0.0	27.8	14.4	6.9	372.9	74.59
5-Jun	22	81.9	47.9	24.3	0.0	27.8	51.6	24.7	397.7	79.53
7-Jun	24	60.7	47.9	24.3	0.0	27.8	38.2	18.3	416.0	83.20
8-Jun	25	57.3	47.9	24.3	0.0	27.8	36.1	17.3	433.4	86.67
10-Jun.	27	171.8	51.1	26.3	0.0	22.6	108.3	55.3	488.6	97.73
15-Jun	32	138.0	51.1	26.3	0.0	22.6	86.9	44.5	533.2	106.63
17-Jun	34	17.7	51.1	26.3	0.0	22.6	11.1	5.7	538.9	107.77
20-jun	37	45.3	51.1	26.3	0.0	22.6	28.6	14.6	553.4	110.69
24-Jun.	41	90.5	51.3	27.5	0.0	21.3	57.0	29.3	582.8	116.55
27-Jun	44	27.4	51.3	27.5	0.0	21.3	17.2	8.9	591.6	118.33
29-Jun	46	19.8	51.3	27.5	0.0	21.3	12.5	6.4	598.1	119.61

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
1-Jul	48	11.6	51.3	27.5	0.0	21.3	7.3	3.8	601.8	120.37
4-Jul	51	34.1	51.2	28.1	0.1	20.6	21.5	10.9	612.7	122.55
11-Jul	58	50.8	51.2	28.1	0.1	20.6	32.0	16.4	629.1	125.82
15-Jul	62	28.4	51.2	28.1	0.1	20.6	17.9	9.2	638.3	127.66
19-Jul	66	39.1	51.2	28.1	0.1	20.6	24.6	12.6	650.9	130.18
25-Jul	72	42.1	51.2	28.1	0.1	20.6	26.5	13.6	664.5	132.90
1-Aug	79	74.5	50.8	28.9	0.1	20.3	46.9	23.8	688.3	137.67
8-Aug	86	58.1	50.6	29.6	0.1	19.7	36.6	18.5	706.9	**141.37**

2.7 Co-digestion 3

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
14-May	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
15-May	1	210.1	11.7	16.6	0.1	71.6	132.4	15.5	15.5	3.10
16-May	2	195.2	11.7	16.6	0.1	71.6	123.0	14.4	29.9	5.97

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
17-May	3	170.7	22.0	24.3	0.0	53.7	107.6	23.7	53.6	10.71
18-May	4	78.7	22.0	24.3	0.0	53.7	49.6	10.9	64.5	12.89
19-May	5	78.0	22.0	24.3	0.0	53.7	49.1	10.8	75.2	15.05
20-May	6	194.6	37.5	26.5	0.1	35.9	122.6	45.9	121.2	24.23
21-May	7	144.6	37.5	26.5	0.1	35.9	91.1	34.1	155.3	31.06
23-May	9	320.7	37.5	26.5	0.1	35.9	202.0	75.7	231.0	46.21
24-May	10	266.3	50.9	23.3	0.0	25.8	167.7	85.4	316.4	63.28
25-May	11	142.7	50.9	23.3	0.0	25.8	89.9	45.8	362.1	72.43
26-May	12	116.0	50.9	23.3	0.0	25.8	73.1	37.2	399.3	79.87
27-May	13	160.0	50.9	23.3	0.0	25.8	100.8	51.3	450.6	90.12
28-May	14	168.5	53.6	26.7	0.1	19.6	106.2	56.9	507.6	101.51
30-May	16	100.9	53.6	26.7	0.1	19.6	63.6	34.1	541.6	108.33
31-May	17	24.3	53.6	26.7	0.1	19.6	15.3	8.2	549.8	109.97
1-Jun	18	12.0	53.6	26.7	0.1	19.6	7.6	4.0	553.9	110.77
2-Jun.	19	0.0	53.6	26.7	0.1	19.6	0.0	0.0	553.9	110.77

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
3-Jun	20	19.0	53.6	26.7	0.1	19.6	12.0	6.4	560.3	112.05
5-Jun	22	71.7	53.6	26.7	0.1	19.6	45.1	24.2	584.4	116.89
7-Jun	24	53.3	53.6	26.7	0.1	19.6	33.6	18.0	602.4	120.49
8-Jun	25	29.7	53.6	26.7	0.1	19.6	18.7	10.0	612.4	122.49
9-Jun	26	19.3	53.6	26.7	0.1	19.6	12.2	6.5	618.9	123.79
11-Jun.	28	161.1	54.7	28.3	0.1	16.8	101.5	55.5	674.4	134.89
15-Jun	32	165.9	54.7	28.3	0.1	16.8	104.5	57.3	731.7	146.34
17-Jun	34	17.3	54.7	28.3	0.1	16.8	10.9	6.0	737.7	147.54
20-Jun	37	52.7	54.7	28.3	0.1	16.8	33.2	18.2	755.9	151.17
24-Jun	41	112.7	53.2	29.6	0.0	17.2	71.0	37.7	793.6	158.72
27-Jun	44	29.2	53.2	29.6	0.0	17.2	18.4	9.8	803.4	160.67
29-Jun	46	21.2	53.2	29.6	0.0	17.2	13.4	7.1	810.5	162.10
4--Jul	51	33.7	53.2	29.6	0.0	17.2	21.2	11.3	821.8	164.35
11-Jul	58	42.5	53.2	29.6	0.0	17.2	26.8	14.2	836.0	167.20
15-Jul	62	21.3	53.2	29.6	0.0	17.2	13.4	7.1	843.2	168.63

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
19-Jul.	66	36.3	53.2	29.6	0.0	17.2	22.9	12.2	855.3	171.06
25-Jul.	72	36.0	53.2	29.6	0.0	17.2	22.7	12.1	867.4	173.48
1-Aug	79	60.3	53.2	29.6	0.0	17.2	38.0	20.2	887.6	177.52
8-Aug	86	95.2	52.1	31.4	0.0	16.5	60.0	31.2	918.8	183.76

2.8 Co-digestion 4

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
15-May	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
16-May	1	215.7	12.5	20.9	0.1	66.5	135.9	17.0	17.0	3.40
17-May	2	279.0	12.5	20.9	0.1	66.5	175.8	22.0	39.0	7.80
18-May	3	151.8	26.1	29.4	0.1	44.4	95.6	25.1	64.1	12.82
19-May	4	90.4	26.1	29.4	0.1	44.4	57.0	14.6	78.7	15.74
20-May	5	158.2	26.1	29.4	0.1	44.4	99.7	25.8	104.6	20.91
21-May	6	183.2	37.7	28.1	0.1	34.2	115.4	44.2	148.8	29.76

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, mlCH_4/gVS
23-May	8	302.7	37.7	28.1	0.1	34.2	190.7	72.2	221.0	44.21
24-May	9	208.3	42.7	26.9	0.1	30.4	131.2	57.1	278.2	55.63
25-May	10	204.3	49.9	24.5	0.0	25.6	128.7	64.3	342.5	68.50
26-May	11	118.3	49.9	24.5	0.0	25.6	74.5	37.3	379.8	75.96
27-May	12	192.0	49.9	24.5	0.0	25.6	121.0	60.3	440.1	88.03
28-May	13	253.9	55.7	24.0	0.0	20.3	159.9	89.1	529.2	105.85
30-May	15	245.3	55.7	24.0	0.0	20.3	154.6	86.1	615.4	123.07
31-May	16	141.5	57.4	25.9	0.1	16.6	89.1	51.1	666.4	133.29
1-Jun	17	26.1	57.4	25.9	0.1	16.6	16.4	9.5	676.0	135.19
2-Jun	18	0.0	57.4	25.9	0.1	16.6	0.0	0.0	676.0	135.19
3-Jun	19	21.5	57.4	25.9	0.1	16.6	13.5	7.8	683.8	136.75
5-Jun	21	62.5	57.4	25.9	0.1	16.6	39.4	22.6	706.3	141.27
7-Jun	23	34.3	57.4	25.9	0.1	16.6	21.6	12.4	718.7	143.75
8-Jun	24	21.3	57.4	25.9	0.1	16.6	13.4	7.7	726.5	145.29
9-Jun	25	10.0	57.4	25.9	0.1	16.6	6.3	3.6	730.1	146.02

Date	Day	Daily pressure production, mbar	CH_4, %	CO_2, %	O_2, %	BALANCE, %	Biogas Vol., ml	CH_4 Vol., ml	Cumulative CH_4 vol., ml	Yield gas, $mlCH_4/gVS$
11-Jun	27	56.0	57.4	25.9	0.1	16.6	35.3	20.3	750.4	150.07
12-Jun	28	22.3	57.4	25.9	0.1	16.6	14.1	8.1	758.4	151.68
15-Jun	31	155.3	57.4	25.9	0.1	16.6	97.9	56.2	814.6	162.93
17-Jun	33	141.6	55.4	28.2	0.1	16.3	89.2	49.5	864.1	172.82
21-Jun	37	69.1	55.4	28.2	0.1	16.3	43.6	24.1	888.2	177.65
25-Jun	41	62.8	55.4	28.2	0.1	16.3	39.6	21.9	910.2	182.04
27-Jun	43	10.0	55.4	28.2	0.1	16.3	6.3	3.5	913.7	182.73
29-Jun	45	20.0	55.4	28.2	0.1	16.3	12.6	7.0	920.7	184.13
4-Jul	50	31.3	55.4	28.2	0.1	16.3	19.7	10.9	931.6	186.32
11-Jul	57	43.7	55.4	28.2	0.1	16.3	27.5	15.3	946.9	189.37
15-Jul	61	21.3	55.4	28.2	0.1	16.3	13.4	7.5	954.3	190.86
19-Jul	65	82.7	53.3	31.3	0.0	15.3	52.1	27.7	982.1	196.41
25-Jul	71	41.4	53.3	31.3	0.0	15.3	26.1	13.9	996.0	199.20
1-Aug	78	59.4	53.3	31.3	0.0	15.3	37.4	19.9	1015.9	203.19
8-Aug	85	42.0	53.3	31.3	0.0	15.3	26.4	14.1	1030.0	**206.00**

VITA

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