EVALUATION OF BIOGAS PRODUCTION FROM PARA GRASS (*BRACHIARIA MUTICA*) WITH CO-DIGESTION OF BUFFALO DUNG

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MASTER OF ENGINEERING IN RENEWABLE ENERGY ENGINEERING

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ชื่อเรื่อง การประเมินผลการผลิตก๊าซชีวภาพจากหญ้าขนโดยกระบวนการหมักร่วมกับมูลกระบือ
ชื่อผู้เขียน นางสาวอัจฉราภา ชวนชัย
ชื่อปริญญา วิศวกรรมศาสตรมหาบัณฑิต สาขาวิชาวิศวกรรมพลังงานทดแทน
อาจารย์ที่ปรึกษาหลัก Dr. Rameshprabu Ramaraj

บทคัดย่อ

การผลิตก๊าซชีวภาพเป็นเทคโนโลยีที่สำคัญอย่างหนึ่งที่ช่วยในการขับเคลื่อนแผนพัฒนาพลังงานอย่างยั่งยืน ซึ่งมีวัตถุประสงค์เพื่อรองรับความต้องการในการใช้พลังงาน และเพื่อช่วยลดสภาวะการปลดปล่อยก๊าซเรือนกระจก การผลิตก๊าซชีวภาพสามารถทำได้โดยกระบวนการย่อยสลายทางชีวภาพแบบไม่ใช้ออกซิเจน (AD) กระบวนการย่อยสลายทางชีวภาพนี้ต้องอาศัยชีวมวลเป็นแหล่งพลังงาน ในการศึกษาครั้งนี้ได้ทำการศึกษาการประเมินการผลิตก๊าซชีวภาพจากการหมักร่วมระหว่างหญ้าขนและมูลกระบือ ซึ่งหญ้าขนถือว่าเป็นพืชน้ำที่เจริญได้ทั่วไปในพื้นที่ชุ่มน้ำและไม่มีภาวะแข่งขันกับพืชอาหาร ดังนั้นการศึกษานี้ได้มุ่งเน้นวิธีการปรับสภาพวัตถุดิบหญ้าขน และการหมักร่วมกับมูลกระบือโดยแบ่งการทดลองออกเป็น 3 ส่วน ในส่วนแรก ทำการศึกษาลักษณะของวัตถุดิบและปรับสภาพด้วยความร้อนโดยการต้มที่ 100 องศาเซลเซียสเป็นเวลา 2 ชั่วโมง และปรับสภาพด้วยสารเคมีโดยใช้โซเดียมไฮดรอกไซด์ เป็นเวลา 72 ชั่วโมง ทดสอบประสิทธิภาพของการปรับสภาพหญ้าขนโดยตรวจสอบด้วยภาพใต้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) การศึกษาส่วนที่ 2 ศึกษาประสิทธิภาพและอัตราส่วนระหว่างหญ้าขนและมูลกระบือต่อผลผลิตก๊าซชีวภาพระดับห้องทดลองที่อุณหภูมิห้อง ผลผลิตก๊าซชีวภาพสูงที่สุดได้จากชุดการทดลองที่ปรับสภาพด้วยโซเดียมไฮดรอกไซด์ 2% เป็นเวลา 72 ชั่วโมง โดยผลผลิตก๊าซชีวภาพ มีค่าเท่ากับ 12.11 ลิตร และความเข้มข้นของก๊าซมีเทน มีค่าเท่ากับ 69.30% สภาวะที่เหมาะสมที่สุดสำหรับการผลิตก๊าซชีวภาพ พบได้จากอัตราส่วนของการหมักร่วมระหว่างหญ้าขนและมูลกระบือในอัตราส่วน 2:1 ในส่วนที่ 3 การศึกษาการผลิตก๊าซชีวภาพในขนาดที่ใหญ่ขึ้นโดยใช้อัตราส่วนที่เหมาะสมที่ได้จากการทดลองที่ผ่านมา ชุดถังปฏิกรณ์การหมักมีขนาด 200 ลิตร ปริมาตรในการหมัก 150 ลิตร ผลการทดลองพบว่า ก๊าซชีวภาพที่ผลิตได้มีปริมาตร 1,620.65 ลิตร และความเข้มข้นของก๊าซมีเทน มีค่าเท่ากับ 69.70% ซึ่งวัตถุประสงค์อีกประการหนึ่งที่ต้องการคือ การศึกษาสภาวะที่เหมาะสมของกระบวนการและพัฒนารูปแบบทางวิศวกรรมหรือทางคณิตศาสตร์ ดังนั้นจึงมีการนำวิธีพื้นผิวตอบสนอง (RSM) วิเคราะห์ปัจจัยที่มีผลในการผลิตก๊าซชีวภาพเพื่อให้ได้ผลผลิตก๊าซชีวภาพสูงที่สุด ในกรณีนี้ปัจจัยที่มีผลต่อการผลิตก๊าซชีวภาพ

ที่นำมาใช้ในวิเคราะห์คือเวลาและอัตราส่วนของหญ้าขนและมูลกระบือ จากนั้นได้ทำการวัดค่าความร้อนของก๊าซชีวภาพที่ผลิตได้ ซึ่งค่าความร้อนที่ได้ มีค่าเท่ากับ 39.4 เมกะจูล/ลูกบาศก์เมตร ค่าความร้อนสูงสุด มีค่าเท่ากับ 27.80 เมกะจูล/ลูกบาศก์เมตร และค่าความร้อนต่ำสุด มีค่าเท่ากับ 25.04 เมกะจูล/ลูกบาศก์เมตร ในการเพิ่มขนาดของงานทดลองมีวัตถุประสงค์เพื่อยืนยันผลในการประยุกต์ใช้จริงและเพื่อวิเคราะห์กระบวนการทางเทคโนโลยีเชิงเศรษฐศาสตร์ นอกจากนี้ยังทำการศึกษาสมดุลมวลจากการทดลองเพื่อใช้ในการวิเคราะห์ระบบทางกายภาพ จากผลการทดลองโดยรวม สรุปได้ว่าหญ้าขนที่หมักร่วมกับมูลกระบือสามารถเป็นวัตถุดิบที่มีศักยภาพสูงในการผลิตก๊าซชีวภาพได้อย่างดี ข้อดีอีกประการหนึ่งจากระบบการผลิตก๊าซชีวภาพคือ ของเหลือจากกระบวนการย่อยสลายนั้นประกอบไปด้วยมหธาตุและจุลธาตุมากมาย ซึ่งเหมาะสำหรับการใช้ประโยชน์ในการทำปุ๋ยต่อไป

Title	EVALUATION OF BIOGAS PRODUCTION FROM PARA GRASS(*BRACHIARIA MUTICA*) WITH CO-DIGESTION OF BUFFALO DUNG
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Degree	Master of Engineering in Renewable Energy Engineering
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ABSTRACT

Biogas production is an important technology in the improvement of sustainable energy source schemes that aims to reduce consumption of conventional fuel, therefore reducing greenhouse gas emissions. Biogas production can be accomplished through anaerobic digestion (AD), a biological process which uses biomass as an energy source. In this study, wetland aquatic plant (para grass) and buffalo dung substrates were used for biogas production. These substrates are widely available and hence do not compete with food production. This study focused on the pretreatment methods on para grass and co-digestion with buffalo dung which was divided into 3 parts. The first part was to evaluate raw material pretreatments: thermal pretreatment (hot water 100°C with 2 h) and chemical pretreatment (2%NaOH with 72 hour). The effect of pretreatments on para grass was demonstrated by using scanning electron microscopy (SEM) images. In the second part, efficiency of pretreatment on para grass for biogas production and different ratios between para grass and buffalo dung was performed at a lab scale. The experiment was conducted at room temperature and the highest biogas yield was 12.11 L and the concentration of methane was at 69.30% by using 2% NaOH as pretreatment at 72 residence time. This optimal condition of biogas production was obtained from co-digestion with a 2:1 ratio between para grass and buffalo dung. In the third part, the best ratio was used in the final scale up experiment. Each reactor was made from a 200 L tank with working volume of 150 L. The biogas yield was

1,620.65 L with 69.70% methane. The other objective is to optimize the condition process and develop an engineering/mathematical model. Response Surface Methodology (RSM) can be employed to maximize biogas production. An experiment was used to optimize operational factors. In this case, variables like time, ratio of para grass and buffalo dung were used as the factors on the response of biogas yield. Moreover, heating value of biogas was measured. The heating value of biogas was 39.40 MJ/m^3. High heating value (HHV) was 27.80 MJ/m^3 and low heating value (LHV) was 25.04 MJ/m^3. The volume size was increased to ensure future large-scale applications; and techno-economic process was verified. The result of mass balance analysis suggested that this study apply conservation of mass to the analysis of physical systems. All in all, this study indicated that co-digestion of para grass and buffalo dung was a promising approach in improving biogas production. Also, digestate from the biogas systems containing many macro and micro nutritious can be used as an effective fertilizer.

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Ajcharapa Chuanchai

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ABBREVIATIONS

AD	Anaerobic digestion
Alk	Alkalinity
BMP	Biochemical methane potential
C/N	Carbon/Nitrogen
CCD	Central composite design
COD	Chemical Oxygen Demand
DM	Dry matter
e.g.	Exempli gratia
etc	The end of the sentence
h	Hour
HCV	Higher calorific values
IQS	Institute of product quality and standardization
LCV	Lower calorific values
MC	Methane content
NA	Not available
RSM	Response surface methodology
SD	Standard deviation
SEM	Scanning electron microscopy
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids

CHAPTER 1
INTRODUCTION

Background

The environmental and global warming consciousness has become an important policy in all countries around the world. Thai government has increasingly given an importance on how to solve this problem issues among the first priority in local development. Energy is primary importance in response to the basic need of the people and a fundamental factor of production in the business sector and industry. Therefore, the power supply it has to get enough energy supply, reasonable price and good quality according with customer required. Coal, oil and natural gas are the three kinds of fossil fuels that we have mostly depended on for our energy needs, from home heating and electricity to fuel for our automobiles and mass transportation. That energy is nonrenewable energy and will run out. Presently many agencies have focused on renewable energy such as solar energy, wind energy, hydro energy and geothermal energy. Renewable sources of energy and consumer products are required for sustainable development of modern society. Thailand is an agricultural area suitable for growing of many plants, especially annual crops that can be used as an energy crop or raw material of agricultural biogas plant (Perlack and Wright, 1995). Energy demand required to meet the economic growth of Thailand is high and growing every year. Accordingly, Thailand, as the country has the potential biogas as a country with a lot of agriculture; including raw materials from crops and livestock, it can be used to develop renewable energy in the form of biogas is methane gas caused by the decomposition of organic matter in the system (Dussadee et al., 2017).

Interest has recently been growing in using the anaerobic digestion of organic waste of farm origin, such as manure, crop residues and organic residues from food and agro-industries, to generate renewable energy (Gebrezgabher et al., 2010). Agricultural residues from the agricultural, agriculture industry and grassland biomass are usually used as feed materials in anaerobic digestion systems in Thailand are suitable in numerous ways for producing energy. This can be used as the raw materials for biogas production as environmentally friendly renewable energy (Dechrugsa et al., 2013). Using grassland biomass for producing energy especially biogas production currently is the most common. Plant biomass is the main source of renewable materials on earth and represents a potential source of renewable energy and bio based products. There are so many types of grasses that are popularly grown in Thailand (Ramaraj et al., 2015). Animal manures have been used as a resource of excellent material for anaerobic digestion with clear environmental benefit. Since Thailand economy depend mainly on agriculture activities. Therefore, utilization of natural resources for energy production is an extremely important issue. Biogas is a green renewable type of energy is generated from a digestion process under anaerobic conditions whose application is rapidly emerging as a viable means for providing continuous gaseous fuel and power generation. Biogas application includes ensuring energy security, decreasing carbon emission, improving economic activity and can be compressed, the same way as natural gas is compressed to CNG, and used to power motor vehicles. It can be produce by a single raw material such as pig manure, cow manure and buffalo manure. In present, the production of biogas has been evolving to enhance the efficiency like co-digestion of animal manure with grass. Co-digestion Para grass with buffalo dung in farm's around community existing digester become a valid approach to enhance biogas production. Para grass (*Brachiaria mutica*) is the tropical weed that no value and pervasive around the farm. It need to cut down and removed frequently for fire hazard and disease and vector controls (Sahoo et al., 2017). Addition of grass can help raise C:N of the feedstock to be suitable for metabolic activities in anaerobic digestion system (Xie et al., 2011). The physical structure and chemical composition of lignocelllulosic materials can be altered through various methods of pretreatment, breaking down the linkage between polysaccharides and lignin, thus making cellulose and hemicelluloses more accessible to hydrolytic enzymes (Hendriks and Zeeman, 2009). Therefore, pretreatments could accelerate the hydrolysis process and improve the final can get more methane production. Consequently, the main purpose of this research was to produce biogas yield from Para grass through anaerobic co-digestion with buffalo dung using different ratios. And study was to examine the effects of pre-treatment and after results the suitable method is selected for scale up study and future applications. Various technologies have been developed and available for produce biogas and biological processes are environmentally friendly and feasible.

Objectives

1. To study the suitable ratio of para grass and buffalo dung for biogas production.
2. To study optimization of biogas using co-digestion.

Scope of study

1. Experimental production of biogas from para grass co-digestion with buffalo dung with different ratio to produce in laboratory scale.
2. Identify the proper ratio for enriched bio-methane in the biogas production process.

Benefits

1. Abundantly available raw materials including Para grass to biogas produce and applicable for energy security.
2. Reduce the cost of using other gases if produce more enriched methane in the biogas production process enough to use.
3. Can be applies this study to the household.
4. Encourage the community to produce the energy from simple materials by themself that involve in save efficiency sustainable theory.
5. Alternative energy.

CHAPTER 2

LITERATURES REVIEW

Biogas

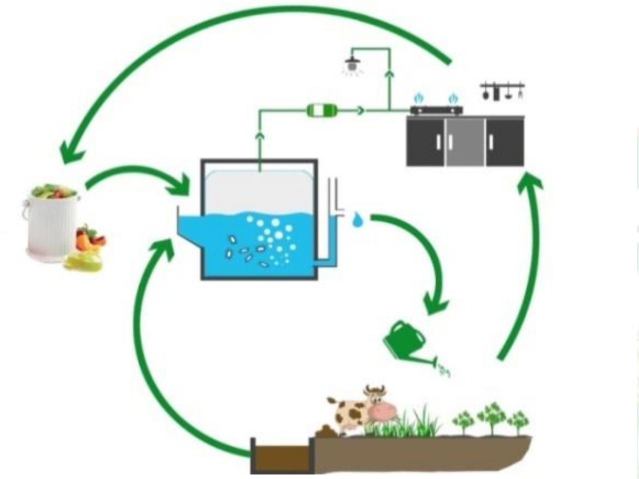


Figure 1 Biogas processes

Biogas is a green renewable type of energy. It can be produced from raw materials such as agricultural waste, manure, municipal waste, plant material, sewage, green waste or food waste (Figure 1). Application of biogas includes ensuring energy security, decreasing carbon emission and improving economic activity. Biogas is often used for cooking, heating, lighting or electricity generation. Larger plants can feed biogas into gas supply networks. Biogas contains 50–70% methane and 30–50% carbon dioxide, depending on the substrate (Ward et al., 2008). As well as small amounts of other gases including hydrogen sulphide (Table 1).

Table 1 Typical composition of biogas

Compound	Formula	%
Methane	CH_4	50-75
Carbon dioxide	CO_2	25-50
Nitrogen	N_2	0-10
Hydrogen	H_2	0-1
Hydrogen Sulfide	H_2S	0.1-0.5
Oxygen	O_2	0-0.5

Source: www.kolumbus.ft,2007

The composition of biogas varies from site to site, depending on the type of feedstock and also the applied digestion technology. In general, biogas has two major components, CH_4 and CO_2, and also contains impurities such as H_2S, N_2, and NH_3 (Table 2).

Table 2 Typical composition of biogas and natural gas, adapted from (Yang et al., 2014)

Character	Unit	AD biogas	Landfill biogas	Natural gas
CH_4	vol%	53-70	30-65	81-89
CO_2	vol%	30-50	25-47	0.67-1
N_2	vol%	2-6	<1-17	0.28-14
O_2	vol%	0-5	<1-3	0
H_2	vol%	NA	0-3	NA
Higher hydrocarbons	vol%	NA	NA	3.5-9.4
H_2S	ppm	0-2000	30-500	0-2.9
NH_3	ppm	<100	0-5	NA

The biogas yield and methane content depends upon the feedstock and its composition. Table 3 presents the composition for biogas substrates and the final methane yield and content. This table is based on theoretical assumptions but in practical the yields are much lower than theoretical yield.

Table 3 Biogas yield and methane content (Patterson et al., 2011)

Nutrients	Biogas yield (m^3/t VS)	Methane content by volume %
Fat	1000-1250	70-75
Protein	600-700	68-73
Carbohydrate	700-800	50-55

Methane

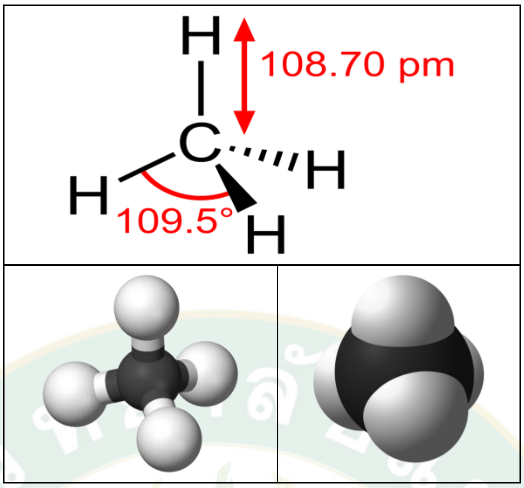


Figure 2 Chemical formula CH_4 (one atom of carbon and four atoms of hydrogen)

IUPAC name Methane other names Marsh gas, Natural gas, Carbon tetra hydride, Hydrogen carbide. Methane is a chemical compound with the chemical formula CH_4 (Figure 2). It is a group-14 hydride and the simplest alkane, and is the main constituent of natural gas. The relative abundance of methane on earth makes it an attractive fuel, though capturing and storing it poses challenges due to its gaseous state under normal conditions for temperature and pressure. The methane properties show on Table 4.

Table 4 Methane property

Properties	
Chemical formula	CH_4
Molar mass	16.04 $g.mol^{-1}$
Appearance	Colorless gass
Odor	Odorless
Density	0.657 $g.L^{-1}$ (gas, 25°C, 1 atm)
	0.717 $g.L^{-1}$ (gas, 0°C, 1 atm)
	422.62 $g.L^{-1}$ (liquid, -162°C)
Melting point	-182.5°C; -296.4 °F; 90.7 K

Boiling point	-164.00°C; -263.20°F; 109.15 K
Solubility in water	22.7 $mg.L^{-1}$
Solubility	Soluble in ethanol, diethyl ether, benzene, toluene, methanol, acetone
log *P*	1.09
Henry's law constant (k_H)	14 $nmol.pa^{-1}.kg^{-1}$
Magnetic susceptibility (X)	-12.2×10^{-6} $cm^3.mol^{-1}$
Thermochemistry	
Specific heat capacity (*C*)	35.69 $J \cdot (K \cdot mol)^{-1}$
Std molar entropy (S^{o}_{298})	186.25 $J \cdot (K \cdot mol)^{-1}$
Std enthalpy of formation ($\Delta_f H^{o}_{298}$)	−74.87 $kJ \cdot mol^{-1}$
Std enthalpy of combustion ($\Delta_c H^{o}298$)	−891.1 to −890.3 $kJ \cdot mol^{-1}$
Liquid; Heat capacity, *Cp*	52.93 J/(mol K)
Hazards	
GHS pictograms	
GHS signal word	DANGER
GHS precautionary statements	P210
NFPA 704	
Flash point	−188 °C (−306.4 °F; 85.1 K)
Autoignition temperature	537 °C (999 °F; 810 K)
Explosive limits	4.4–17%
Thermodynamic properties	
Triple point	90.67 K (−182.48 °C), 0.117 bar
Critical point	190.6 K (−82.6 °C), 46 bar
Std enthalpy of fusion, $\Delta_{fus}H^{o}$	1.1 kJ/mol
Std entropy change of vaporization, $\Delta_{vap}H^{o}$	8.17 kJ/mol

source: National Institute of Standards and technology. Retrieved 21 October 2013.

Methane is the component chiefly responsible for a typical calorific value of 21–24 MJ/m^3 or around 6 kWh/m^3. In anaerobic digestion, organic materials are degraded by bacteria, in the absence of oxygen, converting it into a methane and carbon dioxide mixture. The digestate or slurry from the digester is rich in ammonium and other nutrients used as an organic fertilizer (Ramaraj et al., 2016). The performance of the anaerobic digestion (AD) process is highly dependent on the characteristics of feedstock as well as on the activity of the microorganisms involved in different degradation steps (Carrère et al., 2009). The biochemical composition of different feedstock types varies is determinant for their theoretical methane yield, as seen in Table 5. (House, 1981) and the methane yield of the AD substrates depends on the content of proteins, fats, and carbohydrates, as shown in Table 6 (House, 1981).

Table 5 Methane yields of different feedstock material

Feedstock	Methane yield (%)	Biogas yield (m^3/tFF*)
Liquid cattle manure	60	25
Liquid pig manure	65	28
Distillers grains with soluble	61	40
Cattle manure	60	45
Pig manure	60	60
Poultry manure	60	80
Beet	53	88
Organic waste	**61**	**100**
Sweet sorghum	54	108
Forage beet	51	111
Grass silage	54	172
Corn silage	52	202

*FF=Fresh feedstock

Table 6 Biochemical composition of different feedstock types

Substrate	Liter Gas / kg TS	CH_4(%)	CO_2 (%)
Raw protein	700	70 to 71	29 to 30
Rae fat	1200 to 1250	67 to 68	32 to 33
Carbohydrates	790 to 800	50	50

Flexibility to use different feedstock

Various types of feedstock can be used for the production of biogas: animal manure and slurries, crop residues, organic wastes from dairy production, food industries and agroindustry, wastewater sludge, organic fraction of municipal solid wastes, organic wastes from households and from catering business as well as energy crops (Figure 3). Biogas can also be collected, with special installations, from landfill sites. One main advantage of biogas production is the ability to use “wet biomass” types as feedstock, all characterized by moisture content higher than 60–70% (e.g. sewage sludge, animal slurries, flotation sludge from food processing etc.). In recent years, a number of energy crops (grains, maize, rapeseed), have been largely used as feedstock for biogas production in countries like Austria or Germany. Besides energy crops, all kinds of agricultural residues, damaged crops, unsuitable for food or resulting from unfavorable growing and weather conditions, can be used to produce biogas and fertilizer. A number of animal by-products, not suitable for human consumption, can also be processed in biogas plants.

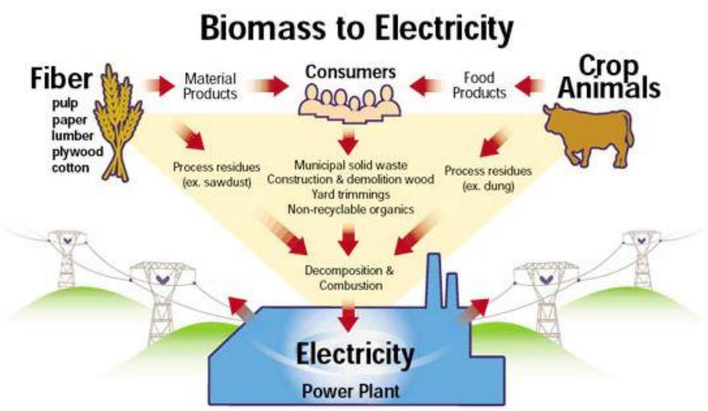


Figure 3 The sustainable cycle of biogas

Benefits and costs of a biogas plant

Biogas is a clean energy coming from anaerobic digestion of biomass, agriculture residue, animal manure, organic waste etc. A biogas plant supplies energy and fertilizer. Are improves life in the country and working conditions for the housewife. It reduces the dependency of people on inefficient and expensive fuel sources. The use of these traditional energy sources cause emission of harmful substances in case of incomplete combustion. Biogas can be used at household and industrial purposes and the energy contained in the biogas can be transformed into various forms of energy such as: heat, electricity, light, mechanical etc. And can helps to reduce the greenhouse gases emission thus contribution towards environmental protection also biogas helps to reduce the indoor pollution due to kitchen smoke and reduce the incidence of illness caused by smoke. We can build new facilities such as toilet facilities and link them to biogas digesters, which improves sanitation whilst allowing even human waste to become useful. The local communities have a double advantage through purchase of livestock such as cows, buffaloes. It supports continuous feed to the digester and helps increase in income through sell of milk, meat and other by-products. The digested slurry produced in the process of biogas formation can be used as excellent organic manure in the field, thus increasing the crop yield. 1 m^3 Biogas (Approximately 6 kWh/m^3) is equivalent to Table 7.

Table 7 Biogas is able to substitute almost the complete consumption of firewood in rural households

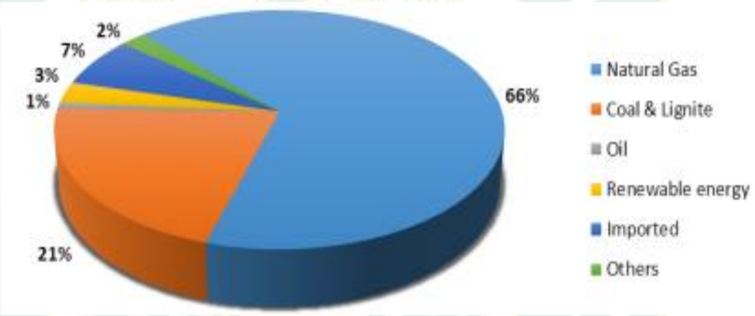
Diesel, Kerosene	12 kWh/kg	0.5 kg
Wood	4.5 kWh/kg	1.3 kg
Cow dung	5 kWh/kg dry matter	1.2 kg
Plant residues	4.5 kWh/kg d.m.	1.3 kg
Hard coal	8.5 kWh/kg	0.7 kg
City gas	5.3 kWh/m^3	1.1 m^3
Propane	25 kWh/m^3	0.24 m^3

Large units or communal units produce biogas in large quantities and can be used to power engines and generators for mechanical work or power generation.

Biogas potential

The existing biomass resources on our plant can give us an idea of the global potential of biogas production. This potential was estimated by different experts and scientists, on the base of various scenarios and assumptions (Figure 4). Accordingly, results from these estimations and overall assumption showed that each part of process is very important to utilize and possible to increase the biogas production considerably.

Thailand's Power Generation by Energy Sources, 2014



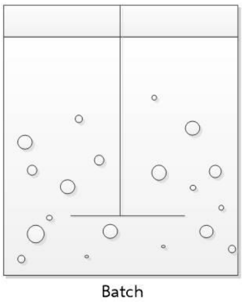
Source: Energy Policy and Planning Office, Ministry of energy

Figure 4 Thailand power generation by energy sources, 2014

Anaerobic digestion (AD)

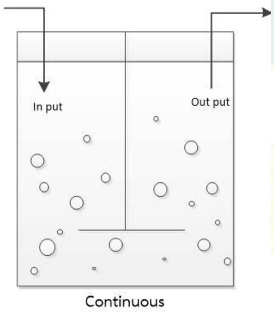
AD is a biochemical process during which complex organic matter is decomposed in absence of oxygen, by various types of anaerobic microorganisms. The process of AD is common to many natural environments such as the marine water sediments, the stomach of ruminants or the peat bogs. In a biogas installation, the result of the AD process is the biogas and the digestate. If the substrate for AD is a homogenous mixture of two or more feedstock types (e.g. animal slurries and organic wastes from food industries), the process is called "co–digestion".

Fermentation can be divided into 2 types of processes



Type 1 Batch fermentation: In a closed system with limited initial nutrient content, all necessary medium components and the inoculum are added at the beginning and not during period of fermentation. The products, be they internal or external, are harvested only at the end of the run (Figure 5).

Figure 5 Bath fermentation



Type 2 Continuous fermentation: Newly refilled foods and old meals are left out of the system at the same rate all the time. In a continuous operation, one or more feed streams containing the necessary nutrients are fed continuously (Figure 6).

Figure 6 Continuous fermentation

Substrates for anaerobic digestion

A wide range of biomass types can be used as substrates (feedstock) for the production of biogas from AD such as animal manure and slurry, agricultural residues and by-products, digestible organic wastes from food and agro industries (vegetable and animal origin), organic fraction of municipal waste and from catering (vegetable and animal origin), sewage sludge, dedicated energy crops (e.g. corn cob, rice straw, sorghum) Figures 7. The most common biomass categories used in Thailand biogas production are listed below and in Table 8.

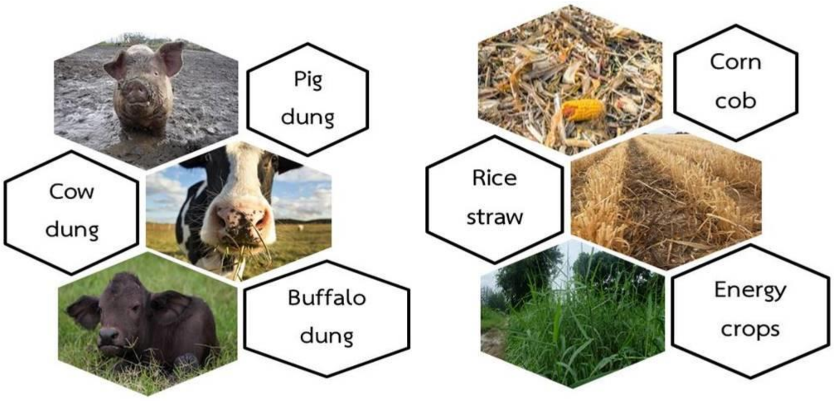


Figure 7 Animal manure and Agricultural residues

Table 8 The characteristics of some digestible feedstock types (Holm-Nielsen et al., 2009)

Type of feedstock	Organic content	C:N ratio	DM%	VS% of DM	Biogas yield $m^3*kg^{-1}VS$	Unwanted physical impurities	Other unwanted matters
Pig slurry	Carbohydrates, proteins, lipids	3-10	3-8	70-80	0.25-0.50	Wood shavings, bristles, water, sand, cords, straw	Antibiotics, disinfectants
Cattle slurry	Carbohydrates, proteins, lipids	6-20	5-12	80	0.20-0.30	Bristles, soil, water, straw, wood	Antibiotics, disinfectants, NH_4+
Straw	Carbohydrates, lipids	80-100	70-90	80-90	0.15-0.35	Sand, grit	-
Garden wastes	Carbohydrates, proteins, lipids	100-150	60-70	90	0.20-0.50	Soil, cellulosic, components	Pesticides
Grass	Carbohydrates, proteins, lipids	12-25	20-25	90	0.55	Grit	Pesticides
Grass silage	Carbohydrates, proteins, lipids	35	15-20	75	0.25-0.50	-	-

Substrates containing high amounts of lignin, cellulose and hemicelluloses can also be co-digested, but a pre-treatment is usually applied in this case, in order to enhance their digestibility. The potential methane yield is one of the important criteria of evaluation of different AD substrates (Figure 8). It is noticeable, that animal manure has a rather low methane yield. This is why, in praxis, animal manure is not digested alone, but mixed with other co-substrates, with high methane yield, in order to boost the biogas production. Common co-substrates, added for co-digestion with manure and slurries, are oily residues from food, fishing and feed industries, alcohol wastes, from brewery and sugar industries, or even specially cultivated energy crops.

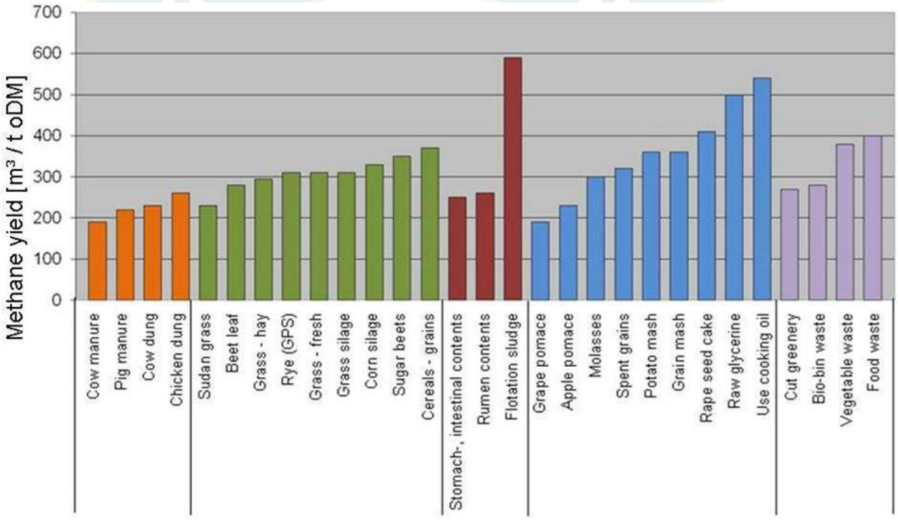


Figure 8 Benchmarks for specific methane yields (Peer et al., 1993)

The biochemical process of AD

Anaerobic digestion (AD) is a process in which microorganisms break down biodegradable material in the absence of oxygen. Anaerobic digestion can be used to treat various organic wastes and recover bio-energy in the form of biogas, which contains mainly CH_4 and CO_2 The reactions of this process require the cooperative action of several organisms. It occurs in each stage as the result of the activity of a variety of microorganisms. The degradation process can be divided into four phases: Hydrolysis, Acidogenesis, Acetogenesis, and Methanogenesis. The anaerobic digestion cycle represents an integrated system of a physiological process of microbial and energy metabolism, as well as the processing of raw materials under specific conditions (Figure 9). However, the microbial community is process can be possibly integrated with other conversion processes. It could be applicable to improve their sustainability and energy balance. On the other hand, biogas system is different from other biofuels like bio-hydrogen, bioethanol and biodiesel which uses only carbohydrates and lipids. Biogas is produced from all the convertible biomass macromolecules under anaerobic conditions.

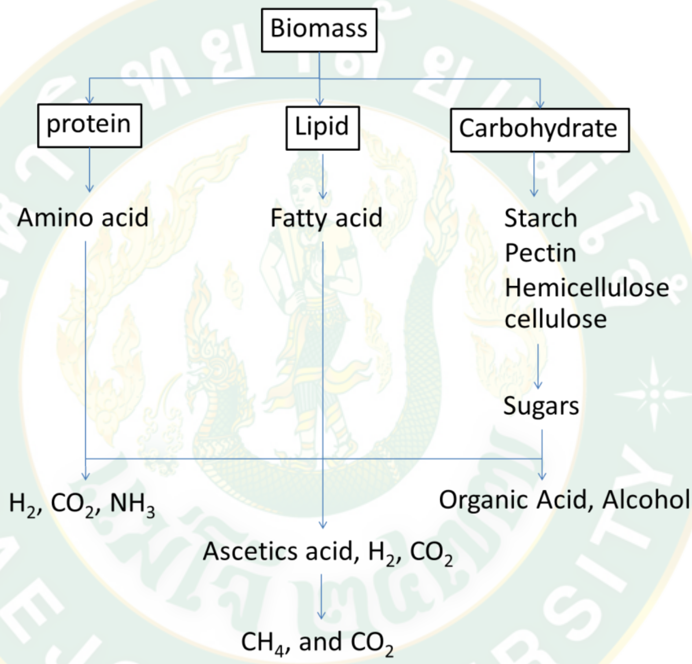


Figure 9 Process achieved

Hydrolysis:

Is first step of AD, during which the complex organic matter (polymers) is decomposed into smaller units (mono and oligomers). During hydrolysis, polymers like carbohydrates, lipids, nucleic acids and proteins are converted into glucose, glycerol, purines and pyridines. Hydrolytic microorganism excretes hydrolytic enzymes, converting biopolymers into simple and soluble compounds (House, 1981). The hydrolytic activity is of significant importance high biomass and may become rate limiting. Some operations methods overcome this limitation by the use of chemical reagents to enhance hydrolysis. The application of chemicals to enhance the first step has been found to result in a shorter digestion time and provide a higher methane yield (Ward et al., 2008).

Acidogenesis:

During acidogenesis, the products of Hydrolysis are converted by Acidogenic (fermentative) bacteria into methanogenic substrates. Simple sugars, amino acids and fatty acids are degraded into acetate, carbon dioxide and hydrogen (70%) as well as into volatile fatty acids (VFA) and alcohols (30%) (House, 1981), provide a higher methane yield.

Acetogenesis:

Products from acidogenesis, which cannot be directly converted to methane by methanogenic bacteria, are converted into methanogenic substrates during acetogenesis. VFA, with carbon chains longer than two units and alcohols, with carbon chains longer than one unit, are oxidized into acetate and hydrogen. The production of hydrogen increases the hydrogen partial pressure. This can be regarded as a waste product of acetogenesis and inhibits the metabolism of the acetogenic bacteria. During methanogenesis, hydrogen is converted into methane. Acetogenesis and methanogenesis usually run parallel, as symbiosis of two groups of organisms (House, 1981).

Methanogenesis:

The production of methane and carbon dioxide from intermediate products is carried out by methanogenic bacteria. 70% of the formed methane originates from acetate, while the remaining 30% is produced from conversion of hydrogen (H) and carbon dioxide (CO_2). Methanogenesis is a critical step in the entire anaerobic digestion process, as it is the slowest biochemical reaction of the process. Methanogenesis is severely influenced by operation conditions. Composition of feedstock, feeding rate, temperature, and pH are examples of factors influencing the methanogenesis process. Digester overloading, temperature changes or large entry of oxygen can result in termination of methane production (House, 1981).

Influence of factors on performance of anaerobic digestion

Many factors are very important on the performance of anaerobic digestion system. Manure quality, temperature, and storage time. The operating parameters of the digester must be controlled to enhance the microbial activity and increase the anaerobic degradation efficiency of the system. Some of these parameters are discussed in the following.

Temperature

Effect of the temperature on biogas production like to the other renewable energy sources (e.g. wind, solar) biogas generation is also affected by the weather. In cold climates, digesters require heat energy to maintain a constant biogas supply. In an anaerobic system, there are three optimal temperature ranges for methanogenesis: psychrophilic, mesophilic and thermophilic (Table 9). Anaerobic conversion has its highest efficiency is psycrophiles at 5-15°C, mesophiles 35-40°C and thermophiles 55°C (Sharma et al., 2013).

Table 9 Thermal stage and typical retention times

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

The temperature stability is decisive for anaerobic digestion. In practice, the operation temperature is chosen with consideration to the feedstock used and the necessary process temperature is usually provided by floor or wall heating systems, inside the digester (Figure 10).

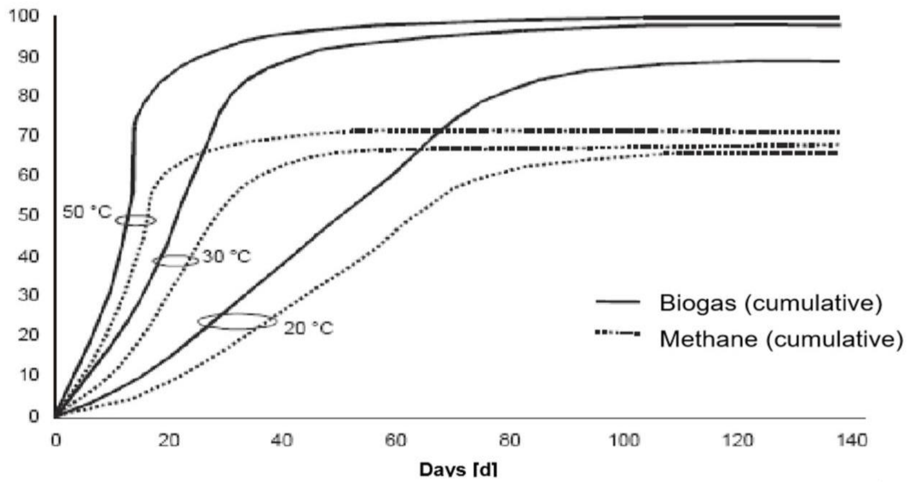


Figure 10 Relative biogas yields, depending on temperature and retention time (Weiland, 2010)

pH parameter

The pH-value is the measure of acidity/alkalinity of a solution and is expressed in part per million (ppm). The pH value of the AD substrate influences the growth of methanogenic microorganisms and affects the dissociation of some compounds of importance for the AD process (ammonia, sulphide, organic acids). In the anaerobic digestion process, pH is a very important parameter.

Experience from the biogas handbook shows that methane formation takes place within a relatively narrow pH interval, from about 5.5 to 8.5, with an optimum interval between 7.0 - 8.0 most methanogens. Acidogenic microorganisms usually have lower value of optimum pH Weiland (2010) stated that methane formation takes place within a relatively narrow pH interval, from about 6.5 to 8.5 with an optimum interval between 7.0 and 8.0. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5. The pH value increases by ammonia accumulation during degradation of proteins, while the accumulation of VFA decreases the pH value.

Volatile fatty acids (VFA)

VFA are important intermediate products and most of the CH_4 produced is derived from VFA. The VFA are intermediate compounds (acetate, propionate, butyrate, lactate), produced during acidogenesis, with a carbon chain of up to six atoms. In most cases, AD process instability will lead to accumulation of VFA inside the digester, which can lead furthermore to a drop of pH-value. Animal manure e.g. has a surplus of alkalinity, which means that the VFA accumulation should exceed a certain level, before this can be detected due to significant decrease of pH value. At such point, the VFA concentration in the digester would be so high, that the AD process will be already severely inhibited. When the process is inhibited by ammonia, an increase in the concentration of volatile fatty acids (VFA) will lead to a decrease in pH which will partly counteract the effect of ammonia. Peter Weiland., 2010, the process is severely inhibited if the pH decreases below 6.0 or rises above 8.5. The pH value increases by ammonia accumulation during degradation of proteins, while the accumulation of VFA decreases the pH value. The accumulation of VFA will often not always result in a pH drop, due to the buffer capacity of the substrate. The changes in VFA production can also be explained by the type of substrate (Demirel and Yenigün, 2006). The toxicity of VFAs is also pH dependent, since only the non-ionized forms are toxic to microorganisms. That mean excessive VFAs accumulation can inhibit methanogenesis. The concentration of acetic, propionic, and butyric acids are considered to be the best indicators of the metabolic state of the most sensitive microbial groups in the anaerobic system and are important in process monitoring (Gunaseelan, 1994).

Ammonia

Ammonia (NH_3) is an important compound, with a significant function for the AD process. NH_3 is an important nutrient, serving as a precursor to foodstuffs and fertilizers and is normally encountered as a gas, with the characteristic pungent smell. Proteins are the main source of ammonia for the AD process (House, 1981). Too high ammonia concentration inside the digester, especially free ammonia (the unionized form of ammonia), is considered to be responsible for process inhibition. This is common to AD of animal slurries, due to their high ammonia concentration, originating from urine.

Mixing

Mixing provides good contact between microbes and substrates, increasing the mass transfer, reduce the buildup of intermediates and stabilize environmental conditions. When mixing is inefficient, overall rate of process will be reduced by mass of material at different stage has a difference pH and temperature. Mixing can be accomplished through mechanical mixing, biogas recirculation or through slurry recirculation (Karim et al., 2005). It was found that mixing improved the performance of digesters treating waste with higher concentration while slurry recirculation showed better results compared to impeller and biogas recirculation mixing mode. Mixing also improved gas production as compared to unmixed digesters. Rapid mixing is not encouraged as methanogens can be less efficient in this mode of operation. Examples of systems with optimal flow include the continuously stirred tank reactor (CSTR) where incoming material is dispersed evenly throughout the vessel by perfect mixing and the plug flow reactor (PFR) where material moves through the vessel (Ward et al., 2008).

Wet digestion; the wet anaerobic digestion process works with a total solid concentration less than 15% (Bagge et al., 2005). The wet process for manure and energy crops can be operated in a single-stage or two-stage mode under mesophilic or thermophilic conditions depending on the waste input and the site conditions. Some reactors reinject biogas to the bottom of the reactor tank to create a loop in the digester and to obtain better homogenisation; other reactors use simple mechanical mixing (Lehtomäki et al., 2007).

C/N ratio

The carbon-nitrogen ratio of organic waste that can be used for biogas is from 8-30, but the optimal ratio for biogas production is about 23 (Wu et al., 2010). If the carbon-nitrogen ratio is high, nitrogen is used by methanogen to supplement the protein. And it will run out quickly. If the C / N Ratio are very low, it will cause a lot of nitrogen and stick together as ammonia. Ammonia is added to the pH. If the pH reaches 8.5, it begins to be toxic to bacteria, reducing the amount of methane. In addition, if the C / N ratios are outside of the range of 8-30, the proportion of other gaseous gases Like carbon dioxide higher. Animal dung, especially cow and buffalo, has the best carbon-nitrogen ratio. Secondly, they are the crockery, sprouts and food waste. While straw has a relatively high carbon-nitrogen ratio. However, high carbon-nitrogen ratios can be mixed with low carbon-nitrogen ratios. To obtains raw materials with a desired carbon-nitrogen ratio.

Biomass

Biomass has been defined as organic matter formed by photosynthetic capture of solar energy and stored as chemical energy (Gunaseelan, 1994), which includes agricultural crops and wastes, animal wastes, forest and mill residues, wood and wood wastes, livestock operation residues, aquatic plants, fast-growing trees and plants, and municipal and industrial wastes (Figure 11). The solar energy stored in biomass could be released as biogas, a mixture of methane (CH_4), carbon dioxide (CO_2), and some trace gases, through anaerobic digestion.

- Burning is only one way to release the energy in biomass. Biomass can be converted to other useable forms of energy such as methane gas or transportation fuels such as ethanol and biodiesel.
- Methane gas is a component of landfill gas or biogas that forms when garbage, agricultural waste, and human waste decompose in landfills or in special containers called digesters.
- Crops such as corn and sugar cane are fermented to produce fuel ethanol for use in vehicles. Biodiesel, another transportation fuel, is produced from vegetable oils and animal fats.

Figure 11 Types of biomass and converting biomass to other forms of energy

Advantages

- Biomass used as a fuel reduces need for fossil fuels for the production of heat, steam, and electricity for residential, industrial and agricultural use.
- Biomass is always available and can be produced as a renewable resource.
- Biomass fuel from agriculture wastes maybe a secondary product that adds value to agricultural crop.
- Growing biomass crops produce oxygen and use up carbon dioxide.
- The use of waste materials reduce landfill disposal and makes more space for everything else.
- Carbon dioxide which is released when biomass fuel is burned is taken in by plants.
- Less money spent on foreign oil.

Disadvantages

- Agricultural wastes will not be available if the basic crop is no longer grown.
- Additional work is needed in areas such as harvesting methods.

- Land used for energy crops maybe in demand for other purposes, such as faming, conservation, housing, resort or agricultural use.
- Some Biomass conversion projects are from animal wastes and are relatively small and therefore are limited.
- Research is needed to reduce the costs of production of Biomass based fuels.

Feedstock

Biogas can be generated from a wide range of feedback that is suitable for anaerobic digestion. It can be made from most biomass and waste material and over a large of moisture contents, with limited feedstock preparation. Therefore, feedback for biogas production may be solid, slurries, and both concentrated and dilute liquids. But the feedstock needs to be a liquid mixture with suitable moisture content. For example, mesophilic complete mix tank digesters typically operate best with a mixture of 4 to 8% solids in water (Callaghan et al., 2002). Beside this, feedstocks are energy crops including: sugarcane, sorghum, Napier grass, as well as, woody crops, corn, oilseed, switch grass. The best crops should have low fertility requirements, and low energy costs for planting and harvesting. Biogas production from different feedstock is difficult as performance data for specific types. It is under a wide variety of experimental condition are shown in Table 10 which in adopted from (Ward et al., 2008).

Table 10 Biogas yield from various types of crop residue

Type	Retention time (day)	Dry matter (%)	Gas yield (L/kg DM)	Gas composition (% V/V)	
				CH_4	CO_2
Rice straw	33	46	5.67	22.8	24.8
Para grass	36	30	5.05	4.3	23.2
Duck weed	41	22	5.46	11.3	32.2
Corn top	32	19	5.43	7.6	28.0
Water hyacinth	46	12	20.30	8.2	16.6

DM is dry matter; Source: (Nijaguna, 2002)

Para grass

Kingdom: Plantae

Order: Poales

Family: Poaceae

Genus: *Brachiaria*

Speccies: *B. mutica*

Figure 12 Para grass (*Brachiaria mutica*)

Para grass is a common name of *Brachiaria mutica*, also known as *Urochloa mutica* (Figure 12) which is perennial crop that can grow on wet and flooded soils in the higher rainfall areas. They are a tropical and invasive growing plant in rural area has only value to be feedstock for animal feeding. These exotic grass weeds are overgrown in abundantly available resources in the Northern region of Thailand. It needs to cut down and removed frequently for fire hazard and disease and vector controls. In which is found as aquatic weeds is the weed of no value and pervasive around area wetlands, along drainage channels, around lakes and dams, in roadside ditches and in other damp habitats, particularly in tropical climate. In areas where para grass in not grazed on by cattle, it has become a serious weed. It is a burden to the since it needs to be cut down and removed frequently for fire hazard, and disease and vector controls. Para grass and is estimated to contain about 42% of cellulose and about 20% hemicellulose, the hydrolysis of which can yield fermentable sugars and hence will serve as an excellent feedstock (Sahoo et al., 2017).

Description

Para grass is in the family Poaceae, along with other familiar grass such as Heterachne, Melica and many grass species. A perennial crop that can grow on wet soils area. It has steams and stolon which grow up to 5 m long and 1 m height. Leaves and leaf sheaths are generally hairy; leaves are 6-20 cm long and 1-2 cm wide

(Figure 13). Dry matter yield of 4-7 t/ha has been achieved in pastures with no N fertilizer, if use about 10-15 t/ha/year (Ramachandra et al., 2000). Absolutely it is found in space wetlands area. Para grass was cut down to keep it clean and good environmental. So, Para grass is the waste as well organic waste from unwanted locations.

Figure 13 Characteristics of para grass

Impacts

Para grass can form floating mats in drainage ditches or irrigation canals, resulting in cause's obstacles to the flow of water. The nature of para grass can create large monocultures through rapid growth and high productivity (Figure 14). Livestock on para grass seem to keep this invasive in check and is used extensively by many producers as forage (Bond and Templeton, 2011). However, education on the problems associated with para grass should be used to prevent unwanted infestations. If ungrazed in wetlands of northern Australia, Para grass may become a fuel for fires that occur during the dry season. It was reported to represent a much bigger fuel load than native grasses and is thus more likely to burn every dry season (Hannan-Jones et al., 2012).

Figure 14 Para grass floating in deep water (stems are rooted to the bank)

Invasiveness

As a long-lived, vegetative propagating pioneering species of disturbed areas, para grass has potential for invasiveness. It is reported to benefit from cultivation, browsing pressure, mutilation and fire (Rojas-Sandoval et al., 2014). It may have deleterious effects on native plant species such as wild rice (Oryza australiensis) whose seeds provide food for indigenous birds. In 1977, para grass was listed as a serious weed in Australia, Fiji and Thailand, as a weed in Sri Lanka, Colombia, Hawaii, Jamaica, Malaysia, Peru, the Philippines, Puerto Rico and Trinidad, and as a common weed in Borneo and Mauritius (Holm-Nielsen et al., 2009) the picture has shown that in Figure 15.

Figure 15 Pure stand of para grass on a highly disturbed urban floodplain

Uses/Applications

Planted for grazing in flat poorly drained or high rainfall environments. Also used as a cut-and-carry forage. Can be cut for hay but is generally slow to dry in the humid environments where it grows productively. Rested wetland areas can be used a dry season reserves of green feed. A similar system uses shallow water ponding on the edges of which Para grass continues to grow as the water recedes. Para will grow in water to 1.2 m deep in the tropics.

Structure of the composition in para grass

Para grass is a type of lignocellulosic material. In general, lignocellulosic materials consist of three main components: cellulose, hemicellulose, lignin, and other compounds (Figure 16).

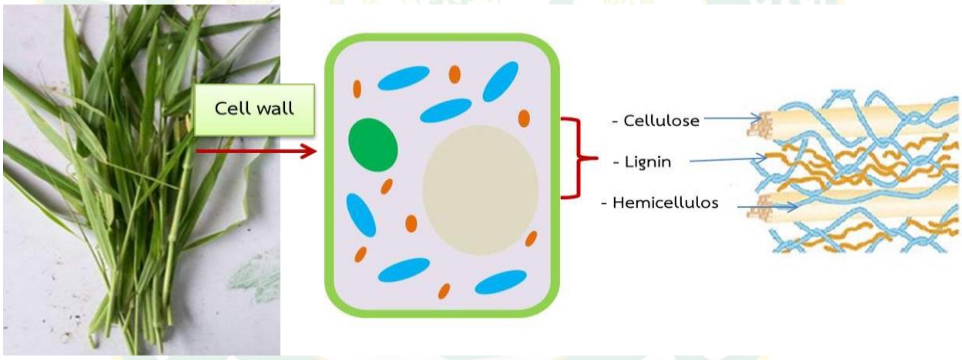


Figure 16 Structure of the composition in para grass

Nutritional attributes of Para grass

Para grass has a variable nutritional value, with protein content in the 7-10% DM range. Dry and old forage can contain as little as 3-4% protein but protein content higher than 20% DM have been recorded (Table 11).

Table 11 Para grass (*Brachiaria mutica*), aerial part and fresh

Main analysis	Unit	Avg	SD	Min	Max	
Dry matter	% as fed	27.7	8.9	11.1	56.8	
Crude protein	% DM	8.4	3.8	3.5	21.4	
Crude fiber	% DM	35.5	3.4	25.7	43.5	
Ether extract	% DM	1.7	0.7	0.5	4.5	
Ash	% DM	9.7	2.3	4.9	17	
Insoluble ash	% DM	4.1	1.8	0.6	14.6	
Neutral detergent fiber	% DM	72.3	5.6	56.8	86.2	*
Acid detergent fiber	% DM	41.7	5.9	30.5	58.1	*
Lignin	% DM	5.9	1.4	3	10	*
Gross energy	MJ/kg DM	18	0.4	17.3	19	*

The asterisk * indicates that the average value was obtained by an equation.

Pre-treatment

Pre-treatments for lignocellulosic materials include mechanical comminution, alkali swelling, acid hydrolysis, steam and other fiber explosion techniques, and exposure to supercritical fluids. These processes act by a variety of mechanisms to render the carbohydrate components of lignocellulosic materials more susceptible to enzymatic hydrolysis and microbial conversion. A variety of methods are effective on representative biomass feedstocks such as agricultural residues, herbaceous crops, and hardwoods. This chapter reviews pre-treatment techniques, focussing on the importance of biomass structure and composition in determining pre-treatment efficacy and the mechanisms by which different pre-treatments act. The chapter concludes by recommending approaches for achieving further improvements in pre-treatment technologies. Physical pre-treatment refers to those methods that do not use external compounds such as chemicals, water or microorganisms during the pre-treatment process. In this thesis physical pre-treatment methods studied are classified as: mechanical (Figure 17).

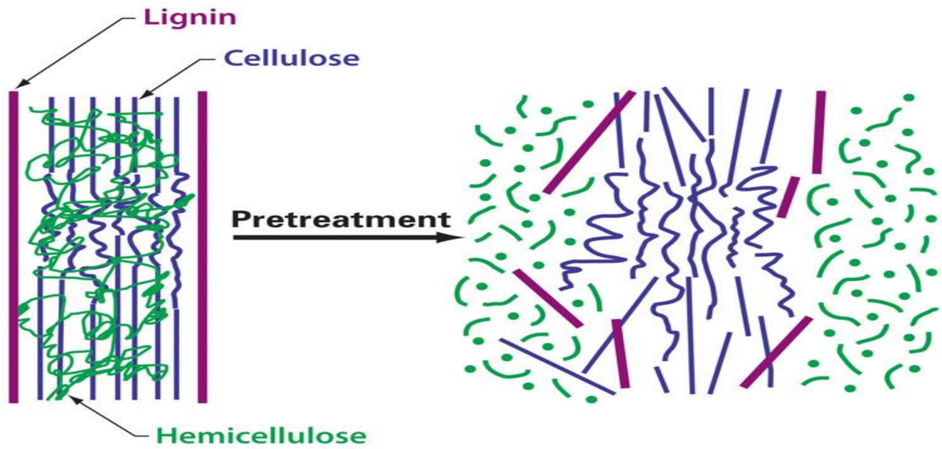


Figure 17 Pre-treatments for lignocellulosic materials

Mechanical

The breaking is a mechanical pre-treatment that is popularly used for waste materials such as agricultural residues from straw, cone, grass etc. The objective of a mechanical pre-treatment is a reduction of particle size. The reduction in particle size leads to an increase of available specific surface and a reduction of the degree of polymerization (Amin et al., 2017). The type of grass used is another factor that affects the biogas production, depending on the grass specie, its composition vary, therefore the substrates available for anaerobic digestion are different for each grass type (Rodríguez-López et al., 2012). Cutting is a mechanical pre-treatment that is widely used for big waste materials such as agricultural residues from straw, corn stove or any other crops and forestry residues as wood chips (Lima et al., 2013). The objective of chipping is to reduce heat and mass transfer limitations caused by large size particles (Behera et al., 2014). After chipping the final particle size of materials is usually 10-30 mm (Alvira et al., 2010).

Thermal pre-treatment method

Thermal pre-treatment is effective in the degradation of lignin and hemicellulose, heat break up the hydrogen bonds in crystalline complexes of cellulose and lignocellulose, causing the biomass to swell, thus increasing the accessible surface area. Thermal pre-treatment is carried out in most cases in autoclaves, pressure cookers or jacketed reactors (laboratory scale). It was shown that thermal pre-treatment reduces the crystal density and viscosity when it is used as feedstock for anaerobic digestion (Carrère et al., 2009).

Chemical pre-treatment method

The most commonly alkalis used (sodium, ammonium, calcium and potassium hydroxides) have been used for the pre-treatment of lignocellulosic materials, the alkali pre-treatment is effectiveness depends on the lignin content of the biomass. This pretreatment produces the saponification and breakage of lignin-carbohydrate linkages, increases the porosity and internal surface area of biomass, and decreases the degree of polymerization and crystallinity of feedstock. Chemical pre-treatment technology is generally considered attractive economically, used in lignin rich biomass that otherwise could not be digested. The residual alkali remaining in alkali pre-treatment biomass could help to prevent a drop in pH during the acidogenesis step (Rodríguez-López et al., 2012).

Co-digestions

Co-digestion has been defined as the anaerobic treatment of a mixture of at least two different substrates with the aim of improving the efficiency of the anaerobic digestion process. At present, there are an increasing number of full-scale co-digestion plants treating manure and industrial organic wastes. Co-digestion of mixed substrates offers many advantages, including ecological, technological, and economic benefits, compared to digesting a single substrate. However, combining two or more different types of feed stocks requires careful selection to improve the efficiency of anaerobic digestion. The main reason for co-digestion of feedstock is the adjustment of the carbon to nitrogen (C/N) ratio. Microorganisms generally utilize carbon and nitrogen in the ratio of 25-30:1 (Ward et al., 2008). The main resource is represented by animal manure and slurries from cattle and pig production units as well as from poultry, fish, etc. And agricultural substrate suitable for anaerobic digestion is represented by energy crops, of which most common are grain crops, grass crops, and maize. Grass crops are among the most promising energy crops for biogas production. Biogas from co-digestion of animal manure and suitable organic wastes is also a very attractive solution from a socio-economic point of view, when biogas externalities, including environmental, human and animal health benefits are quantified and integrated in the overall economic benefits. For the socio-economic point of view, admixture of organic waste to animal manure digestion brings about important benefits concerning increased production of biogas and energy sales, savings related to organic waste treatment, improved fertilizer value of digestate and reduction of GHG emissions from manure and organic wastes (Holm-Nielsen et al., 2009).

Dung is a west from animal. Since Thailand economy depend mainly on agricultural activities and little bit on livestock therefore, utilization of natural resources for energy production is an extremely important issue. However, large availability of buffalo dung in Thailand forms sound base for use of biogas as a prominent renewable energy source. Anaerobic digestion (AD) of animal manure is considered to improve their fertilizer value.

- Manure from different animals (cattle, pig, poultry etc.) are mixed and co-digested, providing a more balanced content of nutrients.
- AD breaks down complex organic material such as organic nitrogen compounds, increasing the amount of plant-available nutrients.
- Co-digestion of manure with other substrates adds various amounts of nutrients to the feedstock mixture.

Theoretical biochemical methane potential (BMP)

The characteristics of the feedstock are important in the design, economy and management of the AD process. Methane potential is the most important characteristics and commonly analyzed by the BMP test (F Owen et al., 1979). These methods are applied considering that all the organic material is degraded; therefore, a proper adjustment of this value is necessary, using the biodegradability obtained from the experimental BMP tests. The stoichiometric equation based on the atomic composition of the waste material is also used to calculate the theoretical methane composition by taking into account the elements C, O, H and N from the elemental composition of plants can be calculated the amount of methane and carbon dioxide (Pavlostathis and Giraldo-Gomez, 1991).

Statistical experiment design

Statistics is a process for converting information into knowledge and making knowledge useful for the advancement of science. Many scientists use statistical methods to analyze their data in order to better understand a given research problem at hand and to help discover the unknown, and they regard statistical analysis to be an integral part of their research. The fundamental element of statistical analysis is the variable, the characteristic or outcome, which is measured or counted. The values reported in the present study were the mean of three replicates. And data are reported as mean ± SE from triplicate observations. All Statistical analyses of data were performed using the program SPSS 20.0 (SPSS Inc., Chicago, IL, USA). A significant difference was considered at the level of $p < 0.05$. Statistics deals with all aspects of data including the planning of data collection in terms of the design of surveys and experiments.

Design of experiment

Design of experiments (DOE) is an elementary statistical tool for engineering field. DOE is a systematic method to determine the relationship between factors affecting a process and the output of that process. This improves the process by considering only most significant factor, and also to reducing operation costs and saving time (Percival Zhang, 2008). Several DOE methods have been applied for experiment for optimization such as full or fractional factorial design, the central composite design (CCD), Box-Behnken design (BBD), Plackett-Burman design (PB) are shown in Figure 18 which in adopted from (Witek-Krowiak et al., 2014).

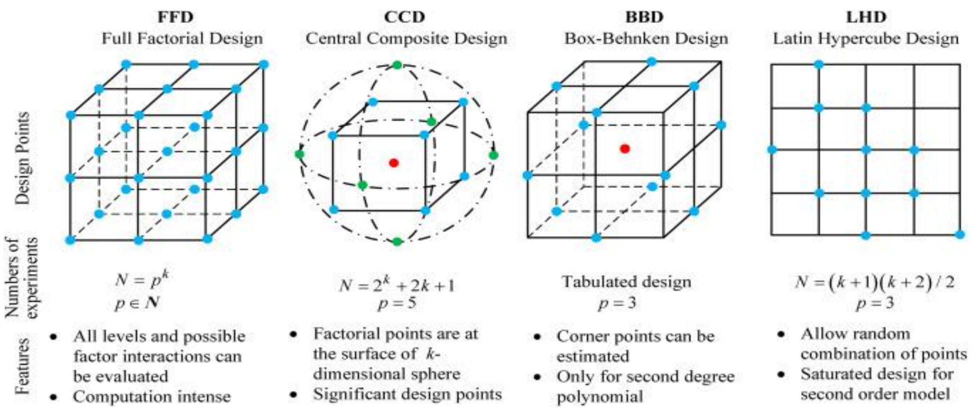


Figure 18 Basic design of experimental (Lian et al., 2017)

Optimization design of experiment

The second aim of this research was to predict and optimize the AD process after beating treatment for maize silage and waste of potato through applying Response Surface Methodology (RSM) via Design Expert software to develop mathematical models that relate the process input parameters to the output features as responses. The two main input of AD process considered are ratio and time. The output features investigated are production of biogas compositions. For each material, mathematical models were developed to predict the required responses. Moreover, the main effects of the process parameters on the responses were discussed and presented graphically. Furthermore, the developed models were optimized by determining the best combinations of input process parameters in order to reach an excellent output.

RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. The most extensive applications of RSM are in the particular situations where several input variables potentially influence some performance measure or quality characteristic of the process. Thus performance measure or quality characteristic is called the response. The input variables are sometimes called independent variables, and they are subject to the control of the scientist or engineer (Aghaie et al., 2009). RSM is usually applied for modeling and analyzing a process to study the relation among several independent factors and one or more response and the optimization of a process. The types of RSM were discussed below.

Full factorial design (FFD)

A common experimental design is the full factorial design, where all input parameters are set at two levels. FFD includes all possible combinations of variables with multiple levels. The full factorial design allows determining the main and low-order interaction effects with great flexibility and efficiency.

Central composite design (CCD)

CCD is an experimental design, useful in response surface methodology, for building a second order (quadratic) model for the response variable without needing to use a complete three-level factorial experiment. The central composite design yields as much information as the 3n full factorial design; however this methodology requires a smaller number of experimental runs than FFD. Additionally CCD provides high quality predictions of linear and quadratic interaction effects of parameters affecting the process. The obtained model could be used to predict and optimize the value of significant factors without doing more experiments.

Box-Behnken design (BBD)

Box and Behnken (1960) developed a 3-level incomplete factorial design as an alternative to the labor extensive full factorial design. To accurately describe linear, quadratic and interaction effects, second order polynomial has to be used in the modeling. Box and Behnken created this design to minimize the number of experiments, specifically in quadratic model fitting. Experiment matrices are built by means of two level factorial designs (+1, −1) with incomplete block designs. The final matrix is completed with several replications of the central point, what improves precision. There are no experimental points in this design, where all factors have extreme values. This feature might be beneficial in experiments where undesired phenomena might occur in extreme conditions. The BB is slightly more labor efficient than the CCD and much more labor efficient than the FFD. The BBD has only two significant restrictions: the number of experimental factors has to be equal or higher than three and the BBD should not be used for fitting other equations than second order polynomial (Witek-Krowiak et al., 2014).

Economic analysis

Energy demand is continuously rising because of increase in population and industrial development. Currently there is huge difference in consumption and availability of energy resources. Energy shortage in developing countries is one of the major challenges for sustainable development. Such challenges can be met and managed via indigenous, clean and reliable alternate energy sources like biogas and bioenergy especially at household levels. Biogas is a methane rich gas that is being generated by anaerobic fermentation of organic material and a biogas plant can effectively utilize various feedstock sources including animal manure, vegetable-fruit waste, sugar, poultry waste and molasses etc. Research (Yasar et al., 2017) the results shown that 1 kW of energy can be generated from 0.65 m3 of biogas by such household biogas units; furthermore it was evident that fixed dome type biogas plants were more economical with shortest payback period of about four months. Additionally effluent slurry being generated by such biogas plant can be a profitable provision in-terms of bio-fertilizer for agricultural.

Total cost

Total cost is the total economic cost of production and is made up of variable costs, which vary according to the quantity of a good produced and include inputs such as labor and raw materials, plus fixed costs, which are independent of the quantity of a good produced and include inputs (capital) that cannot be varied in the short term, such as buildings and machinery. Total cost in economics includes the total opportunity cost of each factor of production as part of its fixed or variable costs.

Average fixed cost

In economics, average fixed cost (AFC) is the fixed costs of production (FC) divided by the quantity (Q) of output produced. Fixed costs are those costs that must be incurred in fixed quantity regardless of the level of output produced (Eq. 1).

$$AFC = \frac{FC}{Q} \qquad \text{Eq. 1}$$

Average variable cost

The average variable cost (AVC) is the total variable cost per unit of output. This is found by dividing total variable cost (TVC) by total output (Q). Total variable cost (TVC) is all the costs that vary with output, such as materials and labor. The easiest way to determine if a cost is variable is if the output changes, the cost changes as well (Eq. 2).

$$AVC = \frac{TVC}{Q} \qquad \text{Eq. 2}$$

Average cost

Average cost and/or unit cost is equal to total cost divided by the number of goods produced (the output quantity, Q). It is also equal to the sum of variable costs (total variable costs divided by Q) plus average fixed costs (total fixed costs divided by Q). Average costs may be dependent on the time period considered (increasing production may be expensive or impossible in the short term, for example). Average costs affect the supply curve and are a fundamental component of supply and demand (Eq. 3).

$$AC = \frac{TC}{Q} \qquad \text{Eq. 3}$$

Data from lab scale-up studies to obtain mass balance which are incorporated into the model. Energy balance can also be pursued at this stage. Applying mass yield along with measured calorific values from biomass to fuels and chemicals provides measured energy yields from the pathway. These energy yields along with energy consumption from each of the processes allow us to establish energy balance.

The economic and employment dimension of dung

It is difficult to estimate dung employment at the national level, as there is no official data available on this specific area of employment. Information on the structure and size of farm is also limited. Farmers who only keep a small number of animals in scattered rural areas do not usually employ additional labor and have, therefore, a limited impact on downstream activities. However, small numbers of animals in scattered rural areas do not usually employ addition labor and have, therefore, a limited impact on downstream activities. However, small farm could create employment in the construction sector if investments were made in small biogas in stations (The economics of biogas; Marek Harsdorff)

Economic effects

There are several economic benefits resulting from the biogas plant (Taleghani and Shabani Kia, 2005).

• Treatment of solid waste without long-term follow-up costs usually due to soil and water pollution.

• Reduction of foreign exchange needs: through production of compost to reduce fertilizer, chemical herbicides and pesticides demand through direct utilization of energy produced (biogas/electricity/heat) in the treatment process to reduce fossil energy demand.

• Generation of income through compost and energy sales (biogas/electricity/heat) to the public/public grid.

• Improved soil/agricultural productivity through long-term effects on soil structure and fertility through compost utilization.

CHAPTER 3
MATERIALS AND METHODS

This study firstly conducts with lab scale experiments to test and select optimal pretreatment condition and then scale up. The sample is collected from fields and undergoes pretreatment and fermentation processes to produce biogas (Figure 19).

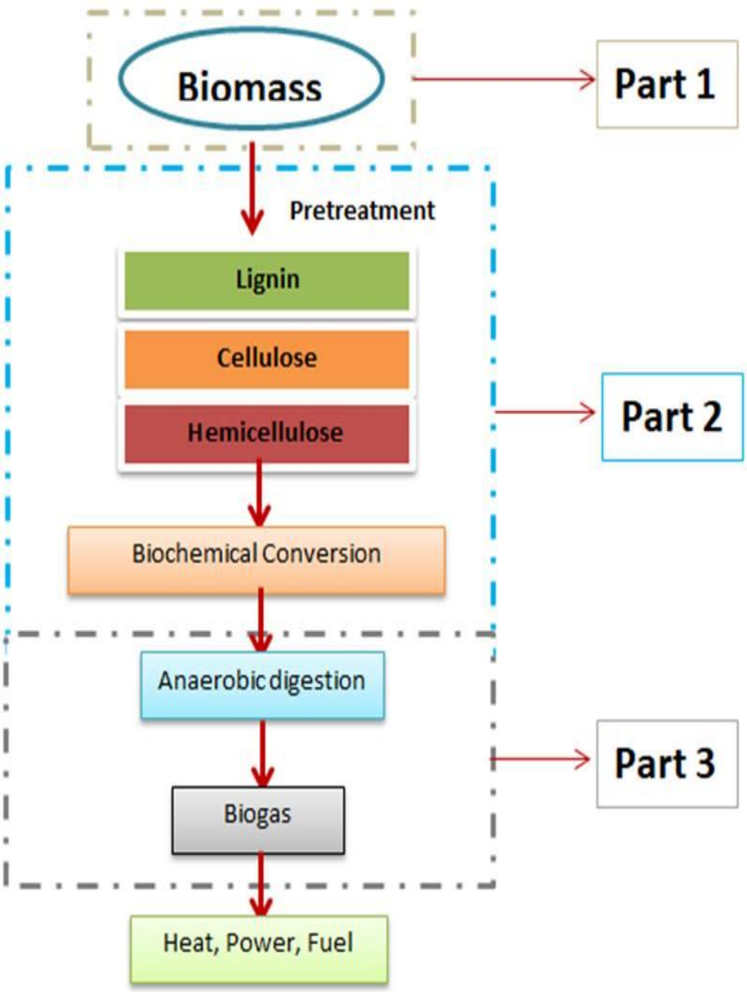


Figure 19 The process of biogas production

This research contain 3 experiments

Experiment 1: Primary evaluation of raw material and pre-treatment. (Mono digestion substrate)

Experiment 2: Efficiency of pre-treatment on Para grass for biogas production and different ratios between Para grass and buffalo dung. (Co-digestion with pretreatment + ratio)

Experiment 3: Evaluation of biogas production from Para grass co-digestion with buffalo dung. (Scale up)

Experiment 1: Primary evaluation of raw material and pre-treatment. (Mono digestion substrate)

Material collection and preparation

Para grass was obtained at Sansai (18° 53’ 37’ N; 99° 01’ 08’ E), Chiang Mai, Thailand. The fresh material was crushed into small particles by grinding machine and stored in the freezer at 4°C for further using (Figure 20).

Figure 20 Para grass collection (A and B); Para grass preparation (C and D)

Buffalo dung (BD) was collected from Learning Center of Agriculture Maejo University, Chiang Mai, Thailand (Figure 21). The collected samples were transferred to the lab of Energy Center Research, Maejo University.

Figure 21 Buffalo dung soure (A); Buffalo dung collection (B)

Biogas estimation by Biochemical methane potential (BMP)

The first step of the present study was the characterization of the considered leaf biomass in order to obtain their composition. In fact, the maximum theoretical biogas production and the amount of methane fraction may be foreseen on the grounds of the organic matter elemental composition. The theoretical methane potential was calculated based on the elemental analysis which is derived by stoichiometric conversion of the compound to CH_4, CO_2 and NH_3 results according to the Bushwell and Boruff, 1932 formula Eq. 4.

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3^- \quad \text{Eq. 4}$$

Theoretical yield of biogas, calculated from the chemical composition of para grass ($C_aH_bO_cN_d$). When the C, H, O and N composition of a substrate is known (Table 14) can be used to estimate theoretical gas composition on a percentage molar basic. However, it must be kept in mind that this theoretical approach does not take into account needs for cell maintenance and anabolism.

Experiment 2: Efficiency of pre-treatment on Para grass for biogas production and different ratios between Para grass and buffalo dung. (Co-digestion with pretreatment + ratio)

Pre-treatment

In this study, para grass was treated with two different pretreatment methods: thermal (boiling) and chemical (NaOH) methods. The experiments were divided into 6 treatments including control, physical and chemical treatments with different treated para grass/ buffalo dung ratios (Figure 22). The effects of different pre-treatment were compared the efficiency of biogas production from para grass. Thermal and alkaline pre-treatment is effective in the degradation of lignin and hemicellulose is carried out in boiled.

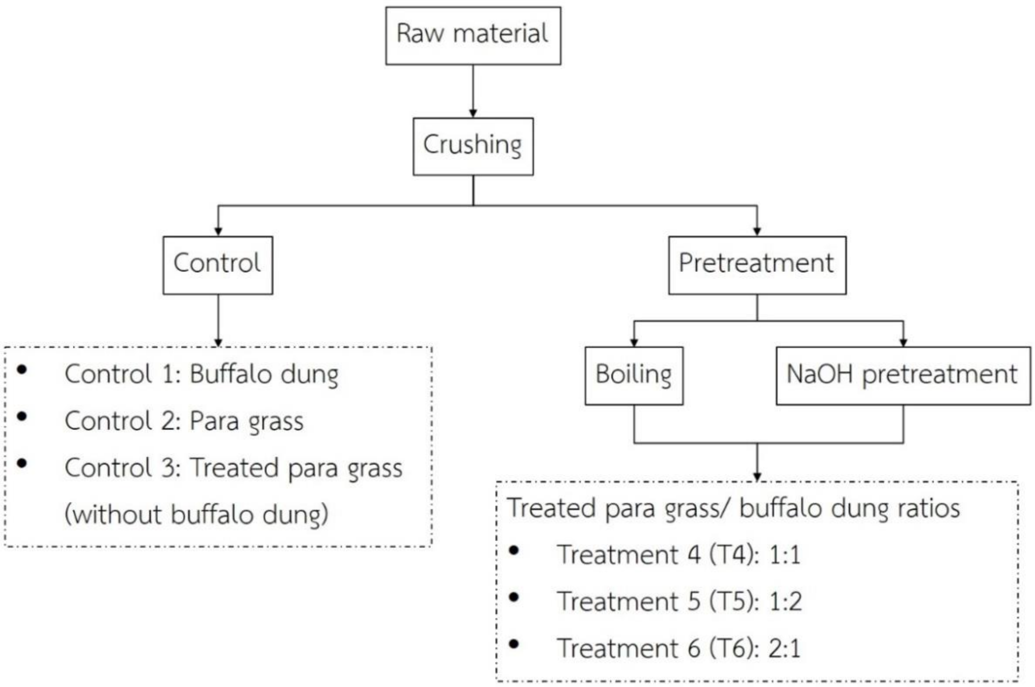


Figure 22 Schematic of different pretreatment methods applying on para grass for biogas production

Thermal pre-treatment

The purpose of thermal pre-treatment is to breaking down the linkage between polysaccharides and lignin, thus making cellulose and hemicelluloses more accessible to hydrolytic enzymes (Hendriks and Zeeman, 2009). Para grass was boiled in hot water for 2 hours (Figure 23).

Figure 23 Thermal pre-treatment of para grass

Chemical pre-treatment

For alkali pre-treatment, crushed para grass was soaked in 2% sodium hydroxide (NaOH) for 3 days (Figure 24). The residual alkali remaining in alkali-pretreated biomass could help to prevent a drop in pH during the acidogenesis step.

Figure 24 Chemical pretreatment using NaOH of para grass

Characterization of sample by scanning electron microscopy (SEM)

To observe the changes happen in the structure of the biomass before and after the pre-treatment process, SEM was carried out at the Institute of product quality and standardization (IQS). First, samples were sputtered with a very thin layer of gold to guarantee their electrical conductivity. Scan coat SEM sputter coater (Edwards, UK) was used for coating; the scanning electron microscope (JSM-5410LV, USA) operates with a field emission gun and is additionally equipped with an energy-dispersive X-ray spectrometer (EDS) EDAX Apollo X-SDD (Edax, USA). Observations were performed at a total magnification of 200X and 1000X (Figure 25).

Figure 25 The procedure of scanning electron microscopy analysis (SEM)

Experiment design

The experiments were conducted with a working volume of 5 L. the percentage of para grass, buffalo dung and inoculum inside fermenter were 10% total solid (TS) of grass biomass, 10% TS of buffalo dung and 5% inoculum. Figure 26 presented perform of the design of fermenters and experimental setting up. The fermenters was sealed and closed with brass valve to ensure the anaerobic condition and collect biogas. The accumulated biogas was stored and measured using plastic cylinders (500 ml). The fermenters were carried out in room temperature at 30–34 °C for 36 days. Fermenters were manually mixed three times a day during fermentation time. The concentration of biogas including methane (CH_4), carbon dioxide (CO_2), hydrogen sulfide (H_2S) and oxygen (O_2) were all determined by a portable gas analyzer (Biogas 5000, UK). Some of the important parameters were determined during biogas production process (Table 12).

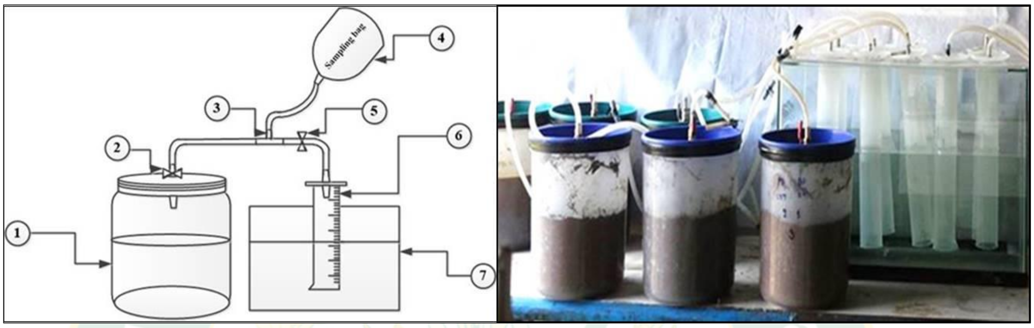


Figure 26 Schematic view of the experiment set up for anaerobic digestion of para grass. (1) Batch fermenter, (2&4) Valve, (3) Gas sampling port, (5) Gas measuring cylinder

Table 12 Physicochemical parameters

Parameters	Method	Reference
pH	meter	pH meter
TS	Gravimetric method	
VS	Gravimetric method	
COD	Closed reflux method	
VFA	Titration method	APHA- AWWA- WEF, 2005
Alkalinity	Titration method	
Cumulative biogas productions	Gas collection	
Composition of biogas	Gas checks	

Statistical analysis

Data of the chemical and elemental composition were expressed as the mean ± standard deviation (SD) of three replicates. To examine the statistical analyses of data were performed using the program SPSS 20.0 (SPSS Inc., Chicago, IL, USA). The level for accepted statistical significance was $p < 0.05$.

Response surface methodology (RSM)

The RSM approach was used to conduct the optimization of all experimental work in this study. The two main factors of AD process considered are time and temperature. The output features investigated are biogas yield. This study was should a central composite design (CCD) for determining the effects and statistical significance. In order to test effect and the interaction of ratio (para grass: buffalo dung) and retention time. The Design-Expert software (Stat-Ease, USA), version 11.0.3.0 was used to build and analyze the experimental design. The software displayed totally 11 base runs with 2 runs at middle points (Table 13).

Table 13 The low and high level of the factors by CCD

Factor	Unit	Symbol	Coded level		
			-1	0	1
Ratio	-	A	0	1	2
Time	day	B	0	18	36

The variables that significantly affected the response were determined using a confidence level above 95% which p-value less than 0.05 and also the statistical significant of model was estimated using analysis of variance (ANOVA) with p-value less than 0.05.

Experiment 3: Evaluation of biogas production from Para grass co-digestion with buffalo dung. (Scale up)

Scale up study

After lab scale experiment, a scale of 200 L was conducted for. The design of scale-up experiment is showed as simulation diagram in Figure 27 and real study of scale-up fermenter shown in Figure 28. The percentage of para grass, buffalo dung, and inoculum were 10% TS of grass biomass, 10% TS of buffalo dung and 5% inoculum. Water was added into fermenter to enhance the viscosity of the mixture. The materials inside fermenter were mixed for 10 minutes automatically by propellers every 2 hours. The fermentation time of this system lasts to 45 days. Experimental results were obtained by means of water displacement. Record volume for the accumulated biogas was stored carefully until check gas components by a gas analyzer operating manual (Biogas 5000, UK).

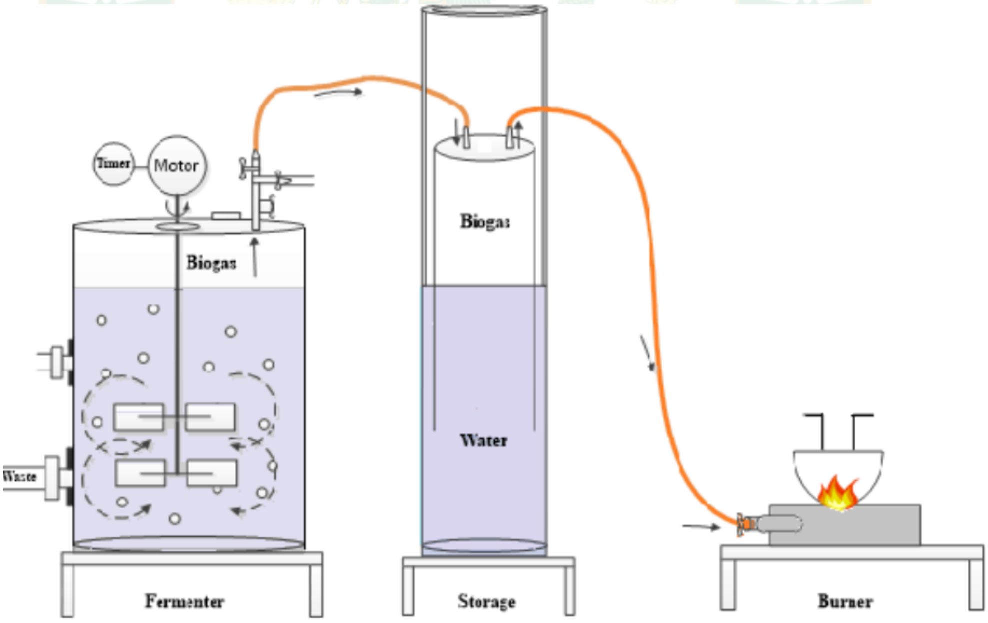


Figure 27 Simulation diagram

Figure 28 Scale-up fermenter

Analytical methods

The samples were analyzed for total solids (TS), volatile solids (VS), chemical oxygen demand (COD) (Federation and Association, 2005) and pH by standard methods. Elemental composition (C, H, N, O, S) was analyzed using the element analyzer Perkin–Elmer.

Moisture content (%) was determined by drying at 105°C for 4 hours (Singh et al., 2017). The moisture content of sample was estimated by percentage of mass loss at 105°C. Moisture and total solids (TS) were calculated as weight percentage using Eq 5, 6.

$$\%Moisture = \frac{Weight_{oven\,sample\,and\,crucible} - Weight_{crucible}}{Weight_{initial\,sample}} \times 100 \qquad \text{Eq. 5}$$

$$\%Total\,solid(TS) = 100 - \%Moisture \qquad \text{Eq. 6}$$

Ash content (%) was estimated using muffle furnace at 575 °C for 4 hours (NREL, 2008). The crucible was then taken out, cooled first in air, then in desiccators and weighed. Heating, cooling and weighing was repeated, till a constant weight obtained. The residue was reported as ash on percentage basis (Eq. 7).

$$\%Ash = \frac{Weight_{ash}}{Weight_{initial\,sample}} \times 100 \qquad \text{Eq. 7}$$

For estimation of volatile solid (VS), the crucibles and sample were kept in a muffle furnace at 925 °C for 7 min (Singh et al., 2017). The percentage of volatile solid was the difference in weight loss at 925°C (Eq. 8).

$$\%VS = \%TS - \left(\frac{Weight_{initial\ sample} - Weight_{oven\ sample}}{Weight_{initial\ sample}} \times 100 \right) \qquad \text{Eq. 8}$$

Economic analysis

In this work, a scale-up (200 L) biogas production from para grass co-digestion with buffalo dung was used for evaluation. All of the value of currency used in this test is on the year of 2018. Biogas and bioenergy technologies have been proven the environmentally safer with fewer or lowest health impacts, economically effective and helpful in energy conservation. This study was calculate by use average cost or unit cost is equal to total cost divided by the number of goods produced (the output quantity, Q). Eq. 3

Energy content analysis

Calorific values were estimated according to Li et al. (2014).The higher calorific values (HCV) and lower calorific values (LCV) of pure methane was 39.82 and 35.87 MJ/m^3, respectively. HCV and LCV of produced biogas were determined according to the following formula Eq. 9, 10.

$$HCV_{biogas} = 0.3989 \times MC = 0.0213 \ (R2 = 1) \qquad \text{Eq. 9}$$

$$LCV_{biogas} = 0.3593 \times MC = 0.0192 \ (R2 = 1) \qquad \text{Eq. 10}$$

Where; MC is the methane content in biogas (%).

Digestate fertilizer analysis

The samples were analyzed for organic carbon, nitrogen (alkaline $KMnO_4$ method), 0.5 M $NaHCO_3$ (pH 8.5) extractable P and 1 N NH_4OAc- extractable K and other trace elements (Page et al., 1982). In addition, Emission, atomic absorption, volumetric, colorimetric, and photometric methods were used to determine physicochemical digestate properties measurements were adopted from Kinyua et al. (2016).

CHAPTER 4
RESULTS AND DISCUSSIONS

Feedstock characterization

Table 14 presents the characteristics of para grass biomass and buffalo dung; the study parameters including proximate analysis (moisture and ash, wt. %), ultimate analysis (carbon, hydrogen, nitrogen, oxygen and Sulphur, wt. %) and biochemical analysis (TS and VS %, COD mg/l, Alk mg/l- $CaCO_3$, VFA mg/g, pH, C:N ratio, cellulose, hemi cellulose and lignin). In our study, the composition of para grass and buffalo dung used clearly indicates that they are containing high nutritious matters. The proximate measurement in the Para grass biomass and buffalo dung was verified; the results moisture and ash were average as 77.3, 2.79 and 83.01, 5.79 %, respectively. Ultimate analysis both materials have plenty of nutrients for biogas containing for para grass 41.5% of carbon, 5.3% of hydrogen, 1.3% of nitrogen, 27.3% of oxygen and 0.3% of sulfide and buffalo dung 37.2% of carbon, 6.6% of hydrogen, 1.4% of nitrogen, 54.1% of oxygen and 0.15% of sulfide. Meanwhile, the pH was adjusted between the ranges of 7.40 to 7.70 for suitable anaerobic digestion. Methane formations take place within a relatively narrow pH interval. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5 when the C, H, O, and N composition of a wastewater or substrate is known, the stoichiometric relationship reported by Rodriguez et al. (2017).

The fiber content of para grass comprised mostly of hemi cellulose and cellulose, in order. Higher lignin composition makes it more difficult to degrade in anaerobic group. Para grass had higher total solid (TS %) and Volatile solid (VS %) than buffalo dung as shown in Table 14. The carbon in Para grass was higher than buffalo dung whereas the nitrogen content was lower. C/N ratios were 32.2 and 24 in para grass and buffalo dung, respectively and had high moisture suitable for anaerobic digestion.

Table 14 Characteristics of para grass biomass and buffalo dung

Parameters	Para Grass	Buffalo dung
Proximate analysis (wt. %)		
Moisture	77.3	83.01
Ash	2.79	5.79
Ultimate analysis (wt. %)		
Carbon	41.5	37.2
Hydrogen	5.3	6.6
Nitrogen	1.3	1.4
Oxygen	27.3	54.1
Sulphur	0.3	0.15
Biochemical analysis		
TS (%)	26.29	16.98
VS (%)	23.25	10.90
COD (mg/l)	26,600	61,300
Alk (mg/l- $CaCO_3$)	1,740	1,460
VFA (mg/g)	3,000	3,365
pH	8.26	8.02
C:N Ratio	32.2	24
Cellulose	42	
Hemi cellulose	20	
Lignin	19	

Theoretical analysis of para grass biogas and biochemical methane production

The elemental composition of plants can be used to calculate the amount of methane and carbon dioxide; calculation process is shown in Eqs. 4. Calculated from para grass is composed of methane had high percentage which means that organic matter in the grass. It was decomposed and converted into methane by 54.36% TS, respectively, as shown in Table 15 clearly demonstrated the huge potential of biogas production capacity from para grass. Which was consistent with available literature; biomass is composed of higher methane than carbon dioxide.

Table 15 Biogas composition, total biogas production and theoretical biogas yield of biomass

Biomass	Gas composition (%)			Total gas production (m^3)			Total theoretical amount of gas	
	CH_4	CO_2	NH_3	CH_4	CO_2	NH_3	m^3/Kg	L/kg
Napier grass	48.45	47.82	3.73	0.43	0.42	0.03	0.89	886.90
Duck weed	50.34	48.81	0.85	0.47	0.45	0.01	0.93	928.94
Para grass	54.36	43.03	2.61	0.53	0.45	0.02	0.99	996.78

At; 100% of gas composition

Mechanism and theoretical estimation of biogas from para grass

The anaerobic fermentation process has achieved growing importance in practice in recent years. Anaerobic fermentation is especially valuable because its end product is methane, a renewable energy source. In order to produce biogas, any organic substrate that is microbiologically accessible can be used. Anaerobic digestion is a synergistic process of a consortium of microbes which can be classified along with a series of metabolic pathways (Pavlostathis and Giraldo-Gomez, 1991). Anaerobic degradation of organic matter is a complex series of metabolic interactions among different anaerobic microorganisms and is classified into four main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

The first step involves the enzyme-mediated transformation of insoluble organic material and higher molecular mass compounds such as lipids, polysaccharides, proteins, fats, nucleic acids, etc. into soluble organic materials. This step is called the hydrolysis and is carried out by strict anaerobes such as Bactericides, Clostridia and facultative bacteria such as Streptococci, etc. In the second step, acidogenesis, another group of microorganisms ferments the break-down products to acetic acid, hydrogen, carbon dioxide and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted to acetic acid. In the third step, these acetic acid, hydrogen and carbon dioxide are converted into a mixture of methane and carbon dioxide by the methanogenic bacteria. The final stage is called as methanogenesis. Acetate is converted into methane; also carbon dioxide converts organic matter into methane.

Effect of pre-treatments on Para grass by imaging with scanning electron microscopy (SEM)

Generally, changes in chemical structure, chemical composition and physical characteristics of crop residues containing large molecules of lignocellulosic biomasses are expected to occur during various pretreatment processes. As a result, lignin network destruction from its complex structures and swelling of carbohydrate fibers could occur due to both physical and chemical interactions between the biomass and boiling/alkali in the pretreatment process (Park et al., 2010). Accordingly, morphological changes in the treated and untreated para grass during the hydrothermal pretreatments were observed using scanning electron microscope. SEM analyses was carried out to assess changes in morphology of the native and pretreated samples boiled at 100 °C with 2h retention time and NaOH chemical pretreatment. Rodriguez et al. (2017) stated that thermal pretreatment is effective in the degradation of lignin and hemicellulose, heat break up the hydrogen bonds is crystalline complexes of cellulose and lignocellulose, causing the biomass to swell, thus increasing the accessible surface area. Hot water pretreatment is physical pretreatment by thermal heat treatment for modification of raw materials to destruction the cellulose tissue. The most often used temperatures at 95-100 °C.

Jiang et al. (2016) that said stated the performance of giant reed by hot water pretreatment 170 °C 5 min can extract cellulose and lignin content with 40.20% and 4.4%, respectively.

Although cellulose has a crystalline structure and great resistance to acids and alkalis the NaOH pretreatment are chemical pretreatment by alkali treatment. The methods of pretreatment by alkali treatment can be Improve quality of general plant fiber to the effect on lignocellulosic materials. The effect of alkali is based on the amount of lignin contained in fiber. The principles of alkali pretreatment are used for to increase swelling within the molecule of hemicellulose and increasing surface area for enzyme from bacteria and can be breaking down the linkage between polysaccharides and lignin (Percival Zhang et al., 2006).

Figure 29 shows the SEM micrograph of native Para grass stem (Figure 29 a, d, g), leaves upper epidermis boiling pre-treatment (Figure 29 b, e, h), and leave lower epidermis NaOH pre-treatment (Figure 29 c, f, i) structure. Morphological changes induced by pre-treatment are first noticeable after a pretreatment on para grass. The result of SEM shown that pre-treatment by NaOH is the best in this study when compere with pre-treatment by boiling pre-treatment and non-pretreated samples. A slight defibrillation was observed the separation of individual fibers, enlargement of the reactive area and more pronounced structural changes in the biomass were seen due to a possible solubilization of the hemicellulose. As hemicellulose operates as a cementing material, its solubilization causes a significant defibrillation effect on the biomass. In addition, a reduction in fiber length and the formation of entangled clusters can be seen in Figure 29 c, f and i. The fiber structure was almost entirely disintegrated due to the higher solubilization of hemicellulose and lignin re-localization. It was found that the fibers were greatly affected by NaOH with 72 h soak retention time. In addition, the swelling of fibers is also observed in alkaline pretreated biomass. This result was also supported by the structural changes observed from the SEM images of the stem, upper and lower leaf epidermis of the para grass samples.

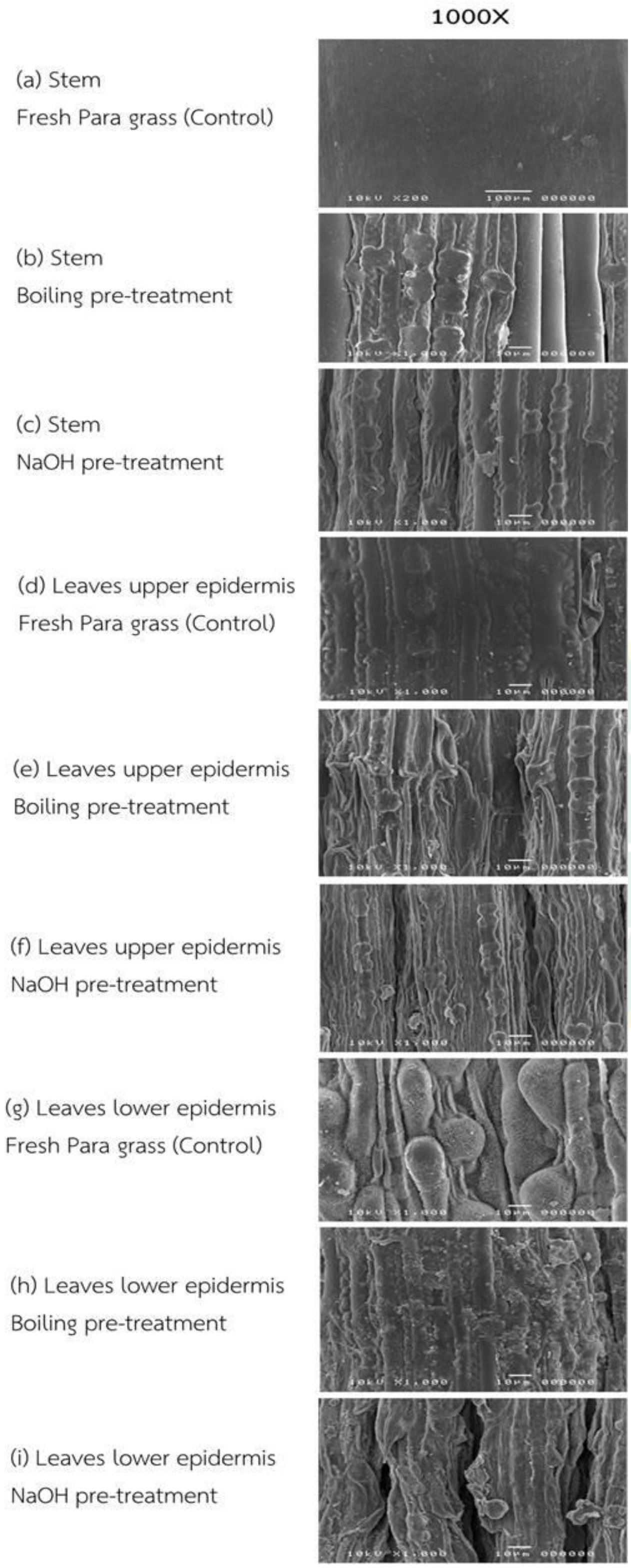


Figure 29 Scanning electron micrographs of morphological characteristics of non-pretreated and pretreated of Para grass samples

Experiment 1: mono-digestion

Control 1 Buffalo dung = no pretreatment, produce biogas

Control 2 Para grass) = no pretreatment, produce biogas

All types of biomass can be used as substrates for biogas production as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. The composition of biogas and the methane yield depends on the feedstock type, the digestion system, and the retention time (Braun 2007). The result of raw material TS, VS, COD, Alk, VFA and pH are reported in the Table 16 Found control 1 can remove well than control 2. Alkali after fermentation them increase all. In fact, the increase of alkalinity was normally due to the activity of the methanogen bacteria, which could produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate (Turovskiy et al., 2006). VFA and COD all decreases after fermentation. All types of biomass can be used as substrates for biogas production as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. The results showed that the initial total solids, volatile solids, chemical oxidation demand, and volatile fatty acids concentrations were significantly reduced after 36 days with mono-digestion biogas production process.

Table 16 Parameter of mono-digestion substrate

Parameter	Mono-digestion		
	Treatment	Start	End
TS (ml/l)	Control 1	141,813	74,277
	Control 2	122,810	65,260
VS (ml/l)	Control 1	95,253	65,413
	Control 2	81,567	35,650
COD (ml/l)	Control 1	73,000	42,000
	Control 2	15,605	8,530
Alk (ml/l)	Control 1	2,400	3,833
	Control 2	2,740	3,133
VFA (mg/l)	Control 1	3,259	3,544
	Control 2	4,033	3,835
pH	Control 1	7.68	6.31
	Control 2	7.23	6.45

TS and VS removals were 47.62%, 31.33% at control 1 and 46.86%, 56.29% at control 2 (Table 17). Found in control 1 can remove well than control 2. Furthermore, grass, due to its high digestible organic matter content, is also an excellent feedstock for anaerobic digestion. Para grass is one of the most promising grasses available for large production in tropics and subtropics. Biogas component was present control 1 get high CH_4 52.27% total biogas productions 8,982 ml and control 2 CH_4 50.35% total biogas production 7,184 ml in Table 17.

Table 17 Degradation efficiency of mono-digestion substrate

Treatment	Degradation efficiency (%)		Total biogas productions (ml)	CH_4 (%)
	TS	VS		
Control 1	47.62	31.33	8,982	52.27
Control 2	46.86	56.29	7,184	50.35

This study investigated the potential of para grass biomass as a feedstock for biogas production. Para grass is a fast-growing and highly nutritious especially. So it is suitable for use as energy crop for biogas production. These results indicated that para grass contains rich organic substances and these substances are suitable to use in the anaerobic fermentation process to be used to sustain microbial life and transform nutrients into biogas. Anaerobic digestion is a biological method used to convert organic substances into a stable product for land application without adverse environmental effects. Methane content of 50.35% was found in total biogas from anaerobic fermentation in 36 day hydraulic detention time. This suggested that it is possible to achieve stable operation using para grass as a substrate or increase performance by co-digestion process for biogas production in pilot or large-scale biogas plant in the future.

Experiment 2: Co-digestion with pretreatment + ratio

In this study, para grass was investigated for biodegradability improvement under different pre-treatment methods. Biogas is generated from the biological conversion of substrates. The TS, VS and COD reductions in the grass using the two different pretreatment methods the results are shown in the Table 18.

Para grass was investigated in order to determine how each method affects the composition of the grass and the digestibility of the grass in biogas production. In addition, pretreatment methods also compared with untreated grass (i.e. control); the effect of pretreatment characteristics, the optimum grass concentration in the each ratio and biogas yield. The effects of pretreatment of each ratio and biogas yield were performed in bath mode with TS, VS, COD and pH is presented in the Table 18. The biogas yield was measured using a biogas analyzer (BIO5000, UK).

Table 18 The changes of important parameters before and after boiling and chemical pretreatment

Parameter	Treatment	Boiling		NaOH	
		Start	End	Start	End
TS (ml/l)	Control 3	133,049	80,884	84,256	55,217
	T4 (1:1)	347,514	98,520	214,986	124,740
	T5 (1:2)	126,730	78,267	247,189	142,830
	T6 (2:1)	232,742	81,770	229,196	111,560
VS (ml/l)	Control 3	98,274	50,538	75,512	48,297
	T4 (1:1)	122,746	67,667	180,766	105,010
	T5 (1:2)	124,221	56,173	185,239	101,680
	T6 (2:1)	136,638	65,668	213,700	45,020
COD (ml/l)	Control 3	15,667	8,734	93,333	6,666
	T4 (1:1)	44,667	8,267	58,666	4,666
	T5 (1:2)	64,333	15,000	104,000	6,666
	T6 (2:1)	55,667	15,667	128,000	7,333

Alk (ml/l)	Control 3	1,700	5,133	3,166	100
	T4 (1:1)	2,533	4,800	4,000	166
	T5 (1:2)	2,767	8,233	100	333
	T6 (2:1)	1,866	4,600	300	100
VFA (mg/l)	Control 3	6,079	2,129	1,688	798
	T4 (1:1)	3,766	1,311	1,246	607
	T5 (1:2)	3,558	1,649	1,218	584
	T6 (2:1)	6,394	3,246	1,688	731
pH	Control 3	7.68	6.42	7.5	6.64
	T4 (1:1)	7.68	6.68	7.6	6.55
	T5 (1:2)	7.56	6.56	7.6	6.58
	T6 (2:1)	7.65	6.65	7.6	6.64

Para grass/ buffalo dung ratio (T4, T5, T6)

The study results clearly exhibited that NaOH pretreatment at the T6 (2:1) ratio sample produced high yield of biogas than untreated (raw) and hot water pretreated sample. The TS and VS removal efficiencies of 2% NaOH at 72 hour pretreated substrate were observed to be 64.86% and 51.94% respectively. Biogas result was 12,113 ml/L CH_4 69.3% (Table 19). Also, alkali pretreatment most likely dissolved a portion of the lignocellusic biomass, is producing a soluble substance and allowing more access for pretreated material increases after alkali pretreatment (Dussadee et al., 2017). Alkali treatment can be particularly advantageous when using plant material in anaerobic digestion. Gunaseelan (1994); compared the anaerobic digestion of parthenium, an invasive weed with high lignin content, with and without alkali pre-treatment and found that methane production and cellulose reduction were significantly enhanced in the presence of alkali. The degradation rate of paper waste was also found to increase by adding NaOH at 10% (Clarkson and Xiao, 2000).

Table 19 Degradation efficiency of TS and VS, biogas production, and percentage of methane from different pretreatment and experiments.

Parameters	Pretreatment	Treatments			
		Control 3	T4 (1:1)	T5 (1:2)	T6 (2:1)
Degradation efficiency of TS (%)	Boiling	39.20	71.65	38.24	64.86
	NaOH	34.46	41.97	42.21	51.32
Degradation efficiency of VS (%)	Boiling	48.57	44.87	54.77	51.94
	NaOH	36.04	41.90	45.10	78.93
Total biogas productions (ml)	Boiling	6,899	10,481	8,935	7,368
	NaOH	7,840	7,818	10,044	12,113
CH_4 (%)	Boiling	54.10	68.57	63.78	66.10
	NaOH	65	58	65.3	69.3

The highest degradation efficiency of TS is 71.65% at the ratio 1:1 when underwent boiling condition. This explains for the highest obtained biogas (10,481ml) and methane percentage (68.57%) when sample was boiled as pretreatment. In contrast, under NaOH pretreatment condition, the highest degradation efficiency of TS reached 51.32% with treatment 6 (ratio of 2:1). The highest biogas (12,113 ml) and methane yield (69.3%).

Biogas composition

In general, it can be seen that the percentage of methane increases along with fermentation time. In contrast, the other components such as CO_2, O_2, and H_2S decrease and reach nearly zero value at the end of fermentation. With the help of chemical pretreatment, methane was produced from co-digestion of para grass and buffalo dung faster than the sample treated by boiling (Figure 30 A1, A2). Sodium hydroxide was proved to be an effective reagent to disturb the recalcitrant structure of lignocellulosic biomass, especially non-woody biomass (Ramachandra et al., 2000). In addition, the results from the control experiment of both pretreatment yielded lower methane when; compared to other treatments with the presence of buffalo dung. This indicates that buffalo dung enhances the digestion process of para grass. The quality of obtained biogas also depends on the presence of others components, CO_2, O_2, and H_2S. The percentages of other gases produced from control fermenter are quite high, especially H_2S shown in Figure 30 B1, C1, D1. On the other hand, there is more oxygen produced in control fermenter of chemical pretreatment.

In conclusion, chemical pretreatment using NaOH has better effects on methane yield. Thus, this method was chose to optimize the condition using response surface methodology.

Boiling NaOH

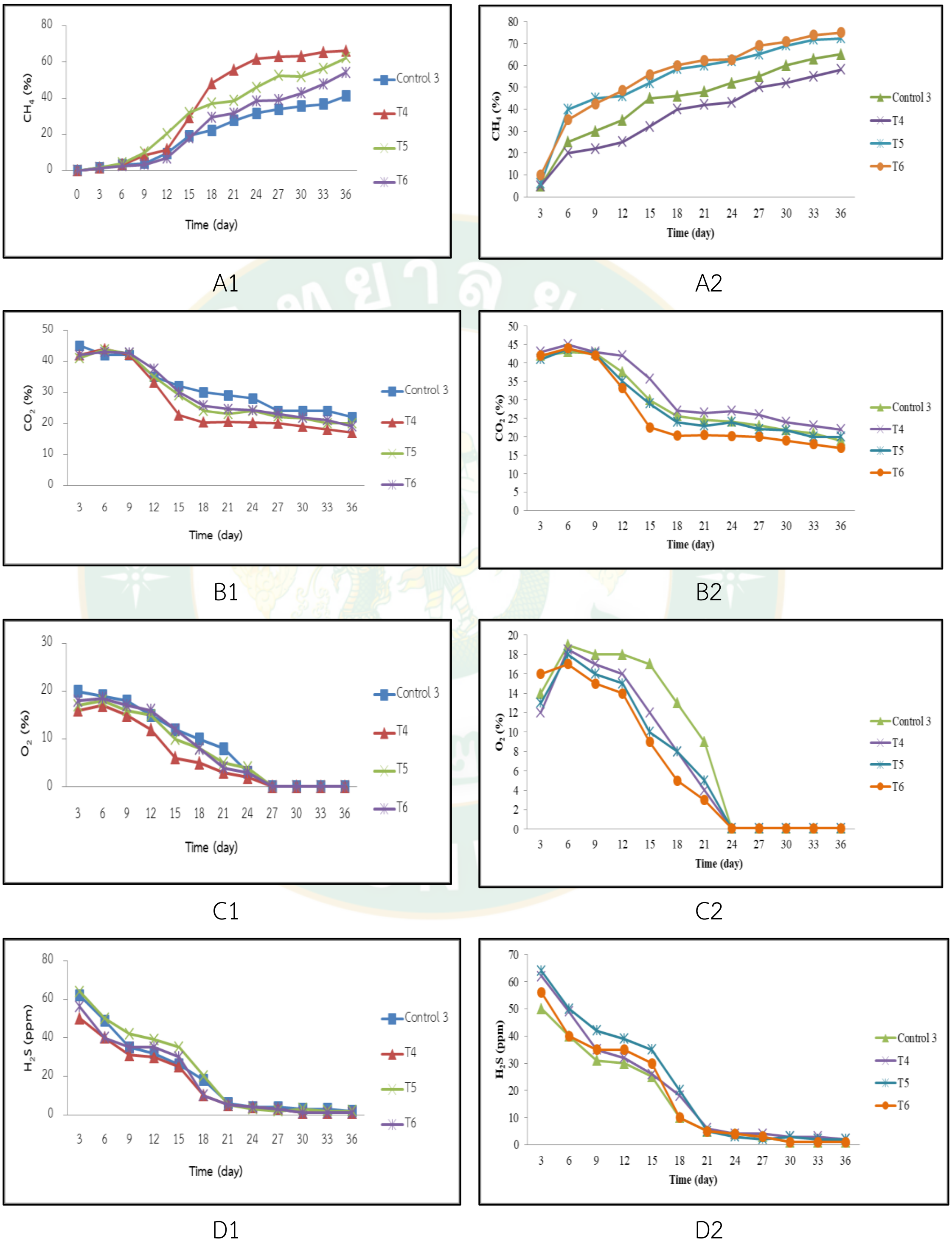


Figure 30 The compositions of produced biogas from boiling and chemical pretreatment of para grass during fermentation time (36 days)

Compare mono substrate with co-digestion

The main advantage of mono-digestion of energy crops is the increased volumetric methane yields (Banks and Humphreys, 1998). However, experience with these plants showed that mono-digestion of energy crops is more sensitive to process imbalance than co-digestion with manure (Lehtomäki et al., 2007). Energy crops and crop residues can be digested either alone or in co-digestion with other materials, employing either wet or dry processes (Dussadee et al., 2017). It can be seen that the comparison of mono-digestion and co-digestion result from other researches (Table 20) points out that co-digestion have high methane yield more than mono-digestion seen that the co-digestion systems were stable in operation in terms of pH, VFA/alkalinity ratios and concentrations of ammonium/free ammonia (Wannapokin et al., 2017). Improvements in biodegradation of grass through thermal and chemical technologies are further examined, and the results demonstrate that grass can be an excellent feedstock for subsequent biogas production. It is practical for animal farms to co-digest grass with animal manure at existing on-site biogas. Based on the lab scale experiments, was put on the RSM for fine the optimum conditions by CCD.

Table 20 Summaries of comparisons with other studies

Substrates	Fermenter	Methane yield (L/kg_{VS})	Reference
Grass silage	Mono-digestion	0.26	Koch et al., 2009
Grass wast	Mono-digestion	0.17	Yu et al., 2002
PM:PP	Co-digestion	0.33	Kaparaju and Rintala, 2005
PM:DGS	Co-digestion	0.27	Xie eu al., 2012
PM:Maize	Co-digestion	2.1	Bulkowska et al., 2012

PM: Pig manure, PP: potato peel, DGS: dried grass silage

Optimization study of mono and co-digestion by CCD

The RSM approach was used to conduct the optimization of all experimental work in this study. The contribution of RSM in this study to ratio and time improvement was introduced as a new pretreatment technique for para grass and dung as a way of accelerating the hydrolysis process, biogas yield during anaerobic digestion. This also verifies the success of RSM as a method of predicting and optimizing anaerobic digestion of grass biomass after optimized treatment.

RSM is a widely used modeling technique functioned to develop, improve and optimize the response variable in the statistical design of experiments. And it is applicable when a response of interest is influenced by several parameters or variables and the objective is to optimize this response. Consequently, in this study RSM was specified the relationships among one or more measured responses and the essential controllable input factors. This is very useful for further scale up design proved biochemical engineering aspects of biogas production from buffalo grass and dung. The mono-digestion and co-digestion, were compare performance as a promising feedstock for biogas production. CCD was applied to optimaize the biogas yeild with two selected independent variables. The study results were presented in Table 21 to 28 and Figure 31 to 36. The effects of each of the parameters on responses were identified, and this enabled the determination of parameters settings that would lead to optimal outcomes.

The equation in terms of coded factor can be used to make predictions about the response for given of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Mono-digestion of alkali pretreatment

$$Y_1 = 4372.67 + 0.0000A + 8249.83B + 894.50AB - 837.67A^2 - 509.50B^2 - 5224.33A^2B + 894.50AB^2 \quad \text{Eq. 11}$$

Where Y_1 are biogas yield (ml) from mono-digestion; A and B are respectively, the ratio and relation time.

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Table 21 ANOVA analysis of model for optimization for mono-digestion of alkaline pretreatment

Source	Sum of Squares	df	Mean Square	F-value	p-value	Conclusion
Model	1.30E+08	7	1.85E+07	954.36	< 0.0001	significant
A-Ratio	0	1	0	0	1	
B-Time	3.32E+07	1	3.32E+07	1711.34	< 0.0001	
AB	3.66E+06	1	3.66E+06	188.74	0.0008	
A^2	8.42E+05	1	8.42E+05	43.45	0.0071	
B^2	3.61E+05	1	3.61E+05	18.64	0.0229	
A^2B	1.17E+07	1	1.17E+07	603.57	0.0001	
AB^2	1.11E+06	1	1.11E+06	57.44	0.0048	
Pure Error	58140.67	3	19380.22			
Cor Total	1.30E+08	10				
Std. Dev.	139.21	R^2	0.9996			
Mean	4036.64	Adjusted R^2	0.9985			
C.V. %	3.45					

Table 22 The experiment designed runs with actual and predicted values

Run	A:Ratio	B:Time	Biogas yield (ml)		Residual
	-	day	Predicted	Actual	
1	2	0	0	0	0
2	1	36	12,113.00	12,113.00	0
3	2	0	0	0	0
4	0	36	4,262.00	4,262.00	0
5	2	36	7,840.00	7,840.00	0
6	1	18	4,372.67	4,373.00	0.33
7	1	18	4,372.67	4,543.00	170.33
8	0	0	0	0	0
9	0	18	3,535.00	3,535.00	0
10	2	18	3,535.00	3,535.00	0
11	1	18	4,372.67	4,202.00	- 170.67

Co-digestion of alkaline pretreatment

Y_2 =2900.11 + 332.50A + 2186.50B + 517.50AB + 2025.24A^2 + 171.24B^2 + 3352.50A^2B + 185.00AB^2 Eq. 12

Where Y_2 are biogas yield (ml) from mono-digestion; A and B are respectively, the ratio and relation time.

Table 23 ANOVA analysis of model for optimization for co-digestion of alkaline pretreatment

Source	Sum of Squares	df	Mean Square	F-value	p-value	Conclusion
Model	1.46E+08	7	2.09E+07	12.49	0.0312	significant
A-Ratio	2.21E+05	1	2.21E+05	0.132	0.7404	
B-Time	9.56E+06	1	9.56E+06	5.71	0.0968	
AB	1.07E+06	1	1.07E+06	0.6397	0.4823	
A^2	1.04E+07	1	1.04E+07	6.21	0.0884	
B^2	74282.54	1	74282.54	0.0444	0.8467	
A^2B	1.50E+07	1	1.50E+07	8.95	0.0581	
AB^2	45633.33	1	45633.33	0.0273	0.8794	
Residual	5.02E+06	3	1.67E+06			
Lack of Fit	4.96E+06	1	4.96E+06	153.04	0.0065	significant
Pure Error	64800	2	32400			
Cor Total	1.51E+08	10				
Std. Dev.	1294.02	R^2	0.9668			
Mean	4098.18	Adjusted R^2	0.8894			
C.V. %	31.58					

Table 24 The experiment designed runs with actual and predicted values

Run	A:Ratio	B:Time	Biogas yield (ml)		Residual
		ml	Predicted	Actual	
1	5	18	2900.11	3490	589.89
2	5	0	884.84	0	-884.84
3	7.5	0	-442.42	0	442.42
4	2.5	0	-442.42	0	442.42
5	5	18	2900.11	3310	409.89
6	2.5	36	9600.58	10043	442.42
7	7.5	36	11670.58	12113	442.42
8	7.5	18	5257.84	4373	-884.84
9	5	18	2900.11	3670	769.89
10	5	36	5257.84	4373	-884.84
11	2.5	18	4592.84	3708	-884.84

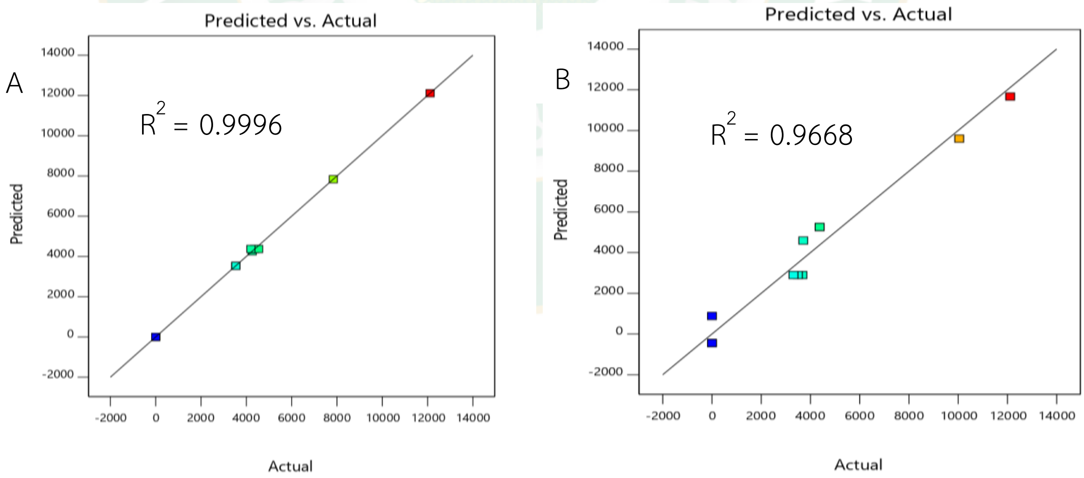


Figure 31 Eperimentel data plotted against RSM model predicted data of A mono-digestion and B Co-digestion of alkaline pretreatment

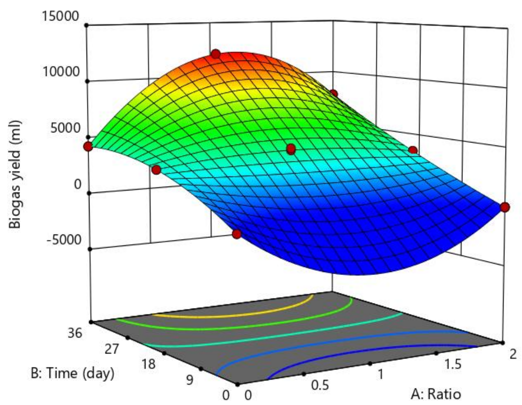


Figure 32 The effect of ratio and time on biogas yield of mono-digestion for alkaline pretreatment

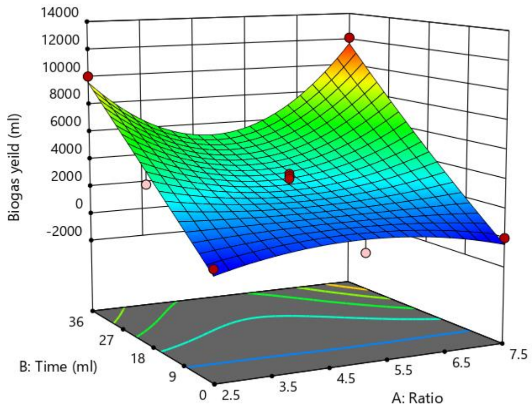


Figure 33 The effect of ratio and time on biogas yield of co-digestion for alkaline pretreatment

Mono-digestion of boiled pretreatment

$$Y_3 = 4905.42 + 441.67A + 5240.33B + 659.08AB - 2025.22A^2 + 51.78B^2 - 2449.92A^2B + 217.42AB^2 \quad \text{Eq. 13}$$

Where Y_3 are biogas yield (ml) from mono-digestion; A and B are respectively, the ratio and relation time.

Table 25 ANOVA analysis of model for optimization for co-digestion of alkaline pretreatment

Source	Sum of Squares	df	Mean Square	F-value	p-value	Conclusion
Model	1.01E+08	7	1.44E+07	77.86	0.0022	significant
A-Ratio	3.90E+05	1	3.90E+05	2.11	0.2426	
B-Time	5.49E+07	1	5.49E+07	296.45	0.0004	
AB	1.74E+06	1	1.74E+06	9.38	0.0549	
A^2	1.04E+07	1	1.04E+07	56.08	0.0049	
B^2	6792.48	1	6792.48	0.0367	0.8604	
A^2B	8.00E+06	1	8.00E+06	43.2	0.0072	
AB^2	63026.68	1	63026.68	0.3402	0.6007	
Residual	5.56E+05	3	1.85E+05			

Lack of Fit	5.08E+05	1	5.08E+05	21.11	0.0442	significant
Pure Error	48092.67	2	24046.33			
Cor Total	1.02E+08	10				
Std. Dev.	430.42		R^2	0.9945		
Mean	3829		Adjusted R^2	0.9818		
C.V. %	11.24					

Table 26 The experiment designed runs with actual and predicted values

Run	A:Ratio	B:Time day	Biogas yield (ml) Predicted	Biogas yield (ml) Actual	Residual
1	2	0	141.57	0	-141.57
2	2	36	7040.57	6899	-141.57
3	1	36	10197.54	10480.7	283.13
4	1	18	4905.42	4559	-346.42
5	2	18	3321.87	3605	283.13
6	1	18	4905.42	4722	-183.42
7	1	18	4905.42	4869	-36.42
8	0	36	4404.23	4262.67	-141.57
9	1	0	-283.13	0	283.13
10	0	18	2438.54	2721.67	283.13
11	0	0	141.57	0	-141.57

Co-digestion of boiled pretreatment

Y_4 = 4846.23 - 445.50A + 5240.33B - 391.75AB - 873.07A² + 199.76B² - 1164.58A²B + 53.75AB² Eq. 14

Where Y_4 are biogas yield (ml) from mono-digestion; A and B are respectively, the ratio and relation time.

Table 27 ANOVA analysis of model for optimization for co-digestion of boiled pretreatment

Source	Sum of Squares	df	Mean Square	F-value	p-value	Conclusion
Model	1.25E+08	7	1.79E+07	186.36	0.0006	significant
A-Ritio	3.97E+05	1	3.97E+05	4.14	0.1346	
B-Time	5.49E+07	1	5.49E+07	573.51	0.0002	
AB	6.14E+05	1	6.14E+05	6.41	0.0853	
A^2	1.93E+06	1	1.93E+06	20.16	0.0206	
B^2	1.01E+05	1	1.01E+05	1.06	0.3798	
A^2B	1.81E+06	1	1.81E+06	18.88	0.0225	
AB^2	3852.08	1	3852.08	0.0402	0.8539	
Residual	2.87E+05	3	95765.14			
Lack of Fit	2.39E+05	1	2.39E+05	9.95	0.0875	not significant
Pure Error	48092.67	2	24046.33			
Cor Total	1.25E+08	10				
Std. Dev.	309.46		R^2	0.9977		
Mean	4478.97		Adjusted R^2	0.9924		
C.V. %	6.91					

Table 28 The experiment designed runs with actual and predicted values

Run	A:Ratio	B:Time day	Biogas yield (ml) Predicted	Biogas yield (ml) Actual	Residual
1	2.5	36	9032.17	8935	-97.17
2	5	18	4846.23	4869	22.77
3	5	36	10286.32	10480.7	194.34
4	5	18	4846.23	4559	-287.23
5	5	18	4846.23	4722	-124.23
6	2.5	0	97.17	0	-97.17
7	2.5	18	4418.66	4613	194.34
8	7.5	36	7465.17	7368	-97.17
9	7.5	0	97.17	0	-97.17
10	5	0	-194.34	0	194.34
11	7.5	18	3527.66	3722	194.34

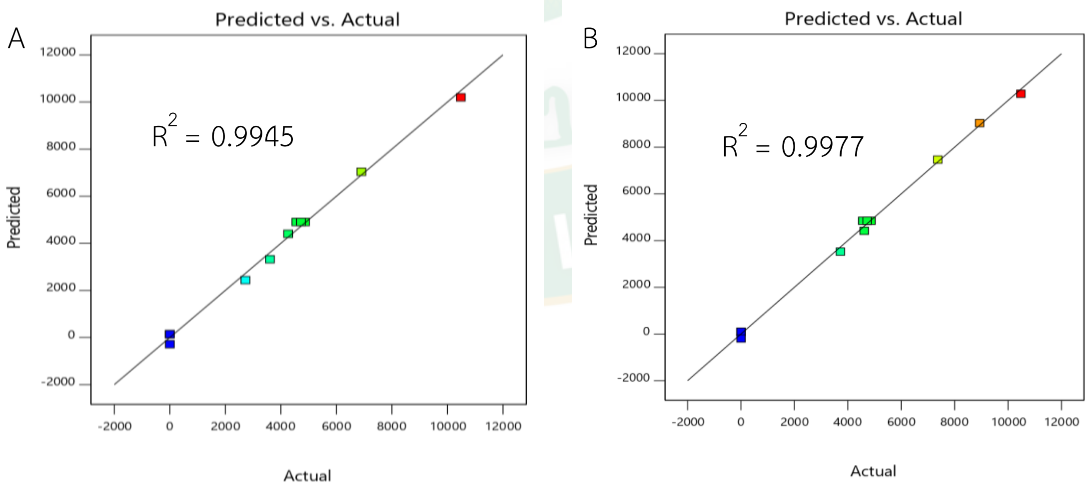
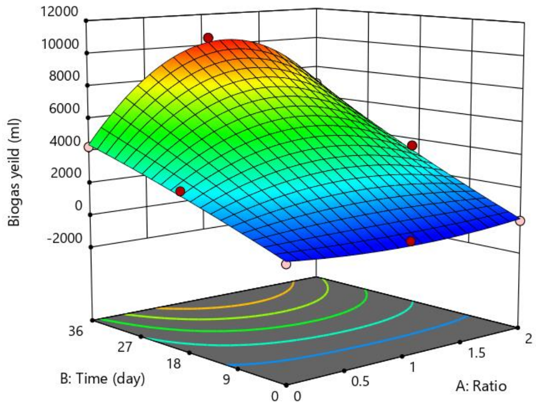


Figure 34 Eperimentel data plotted against RSM model predicted data of A mono-digestion and B Co-digestion of boiled pretreatment



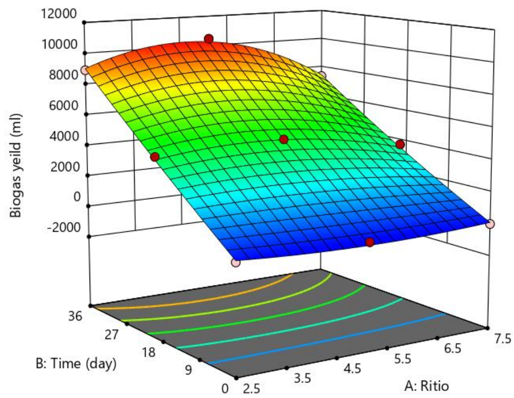


Figure 35 The effect of ratio and time on biogas yield of mono-digestion for boiled pretreatment

Figure 36 The effect of ratio and time on biogas yield of co-digestion for boiled pretreatment

RSM is a widely used modelling technique functioned to develop, improve and optimize the response variable in the statistical design of experiments. And it is applicable when a response of interest is influenced by several parameters or variables and the objective is to optimize this response. Consequently, in this study RSM was specified the relationships among one or more measured responses and the essential controllable input factors. This is very usfel for further scle up degin via biochemical engineering aspects of biogas production from buffalo grass and dung. From RSM analy was use for the scale up in experiment 3.

Experiment 3: scale up

After optimization, the scale up study was performed. In the scale-up study, a digester (200 L) with a working volume of 150 L was used. It consisted of a sampling outlet, a gas sampling port, and a feed inlet. It was sealed using a faucet that could be used as a valve in which there was a pipe to extract biogas. The digester was connected to a gas-collection system consisting of a displacement container and a storage container. The total fermentation period was 45 days was examined sample weekly once also gas composition was measured daily basis and the digester was mixed thrice a day. Physiochemical conditions of fermenter were presented in Table 29 and Figure 37. Solid contents were gradually decreased during fermentation period.

Table 29 Physiochemical conditions of fermenter

Week	Parameters					
	TS (%)	VS (%)	COD (mg/l)	VFA (mg/l)	ALK	pH
1	121,188	111,583	53,333	5,500	2,800	7.16
2	116,922	106,708	44,000	10,065	1,666	6.28
3	106,290	92,796	26,667	13,169	1,833	6.22
4	122,563	106,278	21,333	13,584	2,733	6.38
5	62,359	51,643	32,000	15,643	3,433	6.26
6	31,828	19,633	10,267	16,422	3,233	6.12
7	30,321	20,643	12,267	18,201	3,333	6.13

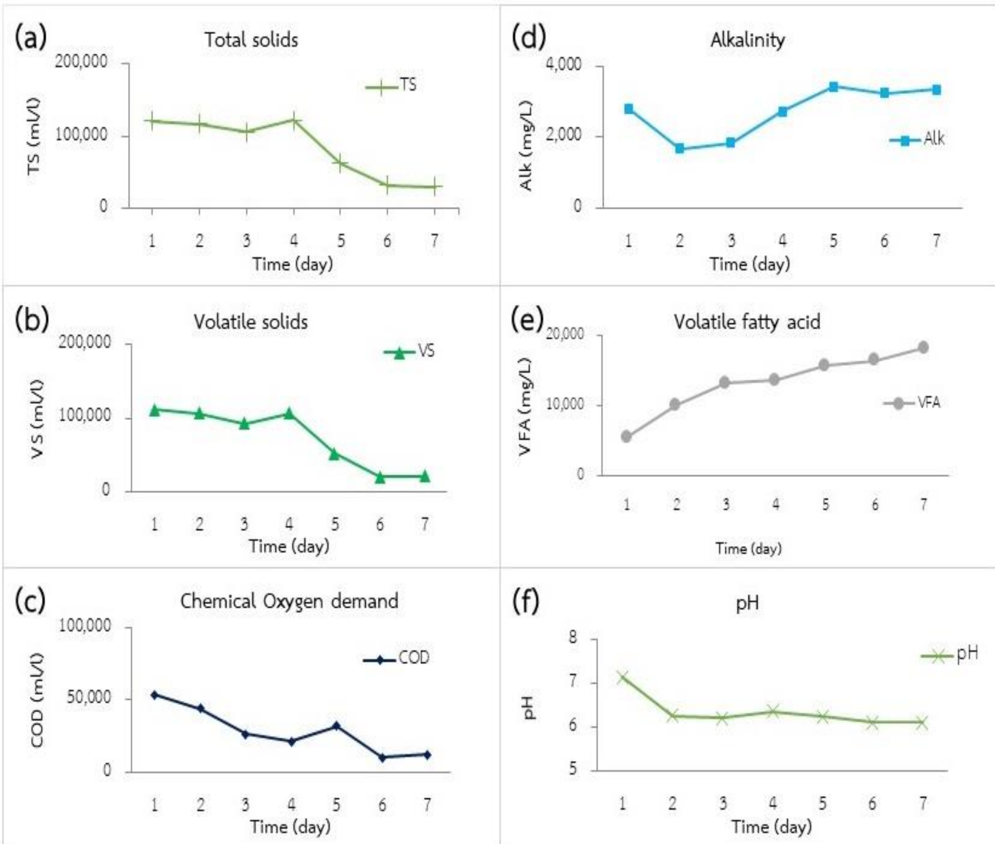


Figure 37 Physiochemical conditions on during fermentation

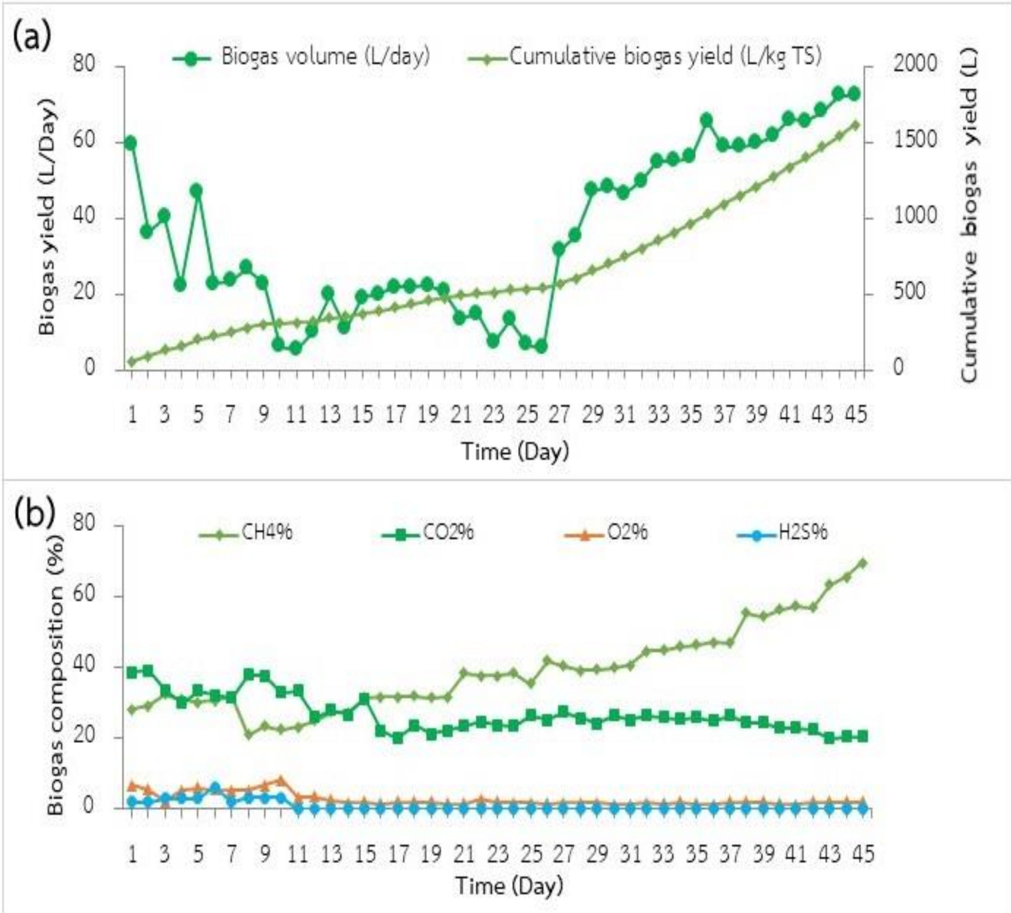


Figure 38 Biogas yield and cumulative biogas (a), and biogas composition of scale up study (b)

Cumulative gas production, biogas yield and composition were shown in Figure 38 a and b. Due to the crushing of leaves, the particle size decreased many times and the surface area was increased. As a result, daily gas production was increased gradually. It was observed that the lag phase prevailed for up to 5–6 days during the digestion period. After the lag period, the cumulative volume of gas increased up to 45 days of fermentation, and daily biogas was increased. All of the reactors were taken for further study of pH effect, VS destruction, COD reduction, and so on.

The methanogenic phase, which occurs after the acid phase in the biodigestion process, is characterized by a methane concentration at a level of 50 to 60%, with a decrease in the concentration of carboxylic acids and consequent increase in the pH of the environment (Barlaz et al., 1989). From Figure 54b it can be seen that, as the methanogenic phase advances, the methane concentration increases, while the carbon dioxide decreases, basically in the same proportion. It is also observed that, in my study reached 79.5% in the methane concentration. The steady state of anaerobic digesters in this investigation occurs after 15 days of the start-up process. In the steady state, the degradability efficiency of the average TS, VS, and COD are reported in Table 25. These values are comparable with the VS reductions reported in the literature for various substrates (Rouf et al., 2010; Thangamani et al., 2009). The concentration of VS in the slurry decreased with increasing digestion period. Chemical pretreatments have been used far less than thermal and mechanical ones. Among the chemical methods, mostly alkali pretreatments have been applied. Alkali reagents are commonly used to solubilize polymers, favoring the availability of organic compounds for enzymatic attacks (Bohutskyi and Bouwer, 2013). The small amount of residual alkali remaining in pretreated biomass may be helpful to prevent pH reduction during the subsequent acidogenesis step. Therefore, this co-digestion approach is feasible for application to farm-scale digesters, as it would improve methane production. Also these results indicate that para grass with buffalo dung can be successfully converted using AD. Consequently, the results of this study suggest that it is possible to achieve stable operation using para grass with buffalo dung as a substrate and co-substrate for biogas production in commercial scale biogas plants in the future.

Compere performance of small scale with up scale

After a small experiment to select the best ratio with good conditioning was going to scale up for ensure that can be work the result shown that Figure 39 A, B. At lab scale 5 L working volume can get cumulative biogas 12.11 L when scale to 200 L the cumulative is 1,620.65 L. So these results indicated that when scale up of the fermenter means that inside the fermenter was have more nutrients for anaerobic bacteria digestion and para grass contains rich organic substances and these substances are suitable to use in the anaerobic fermentation process to be used to sustain microbial life and transform nutrients into biogas.

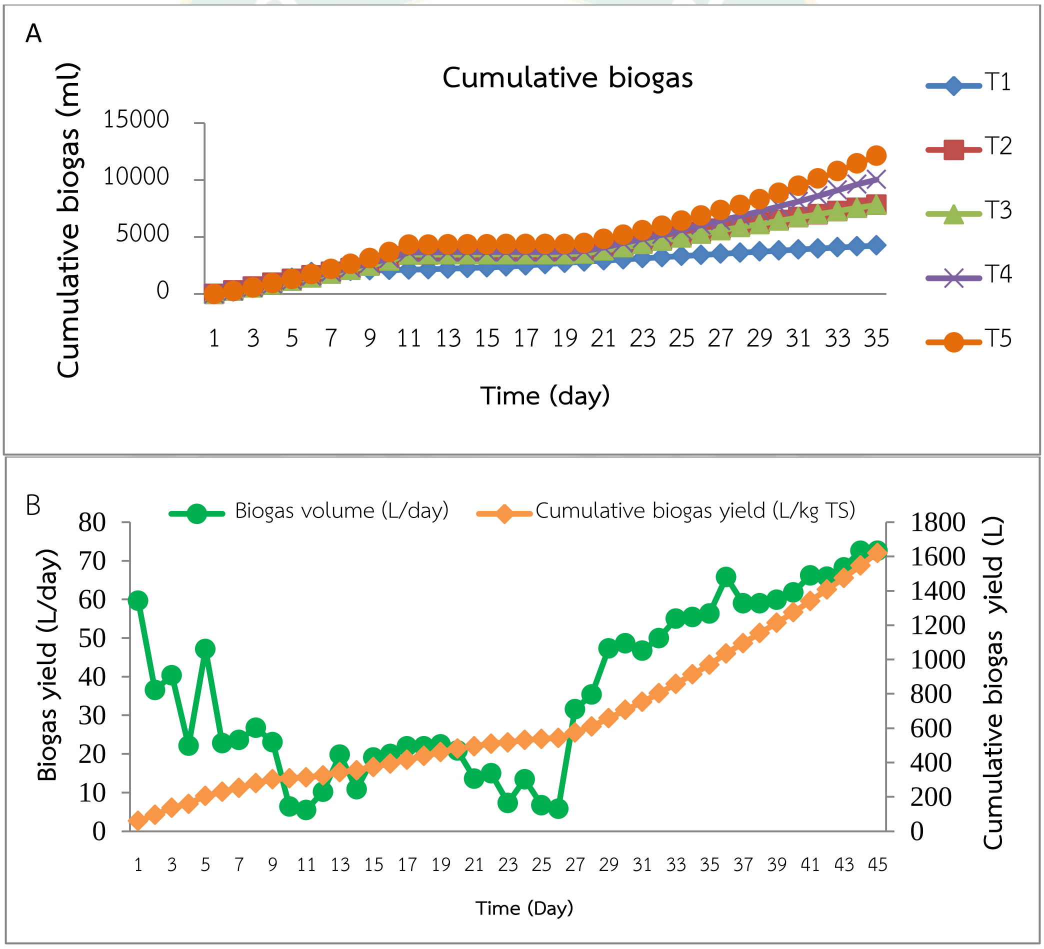


Figure 39 The comparison of cumulative biogas from A lab scale and B scale up

Digestate fertilizer

The study digestate and literature data were presented in Table 30. Digestate can be defined as liquid from anaerobic decomposition of animal and plant waste. It contains considerable amounts of mineral elements including nitrogen, phosphorus, potassium and others. In terms of rapidity of action, it resembles mineral fertilizers since N, P and K elements are easily available for plants. Govasmark, (2011) and Heviánková, (2013) proved that the possibility of occurrence of pathogenic bacteria and heavy metals in digestate.

Table 30 Chemical compositions of digestate from the different anaerobic digesters

Raw materials	TOC, g L^{-1}	N	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	Reference
		g kg^{-1} in the form of dry matter										
Poultry manure	452	67	24	24	5.3	92	6	1.8	0.66	0.58	0.11	Kirchmann and Witter (1992)
Biodegradable household waste	ND	152	16	78	7	50	10	ND	<0.001	0.08	0.01	Haraldsen et al. (2011)
Pig manure + sludge from wastewater treatment plant + biodiesel wastewater	247	200	6	52	ND	26	10	1	0.16	1.16	0.21	Alburquerque et al. (2012)
Maize silage	ND	41	34.8	5.9	ND	3.7	36.2	ND	ND	0.08	0.08	Pokój et al. (2015)
codigestion of buffalo grass + buffalo dung	389.17	77.53	13.39	37.86	5.73	35.76	12.11	2.14	0.36	0.75	0.19	This study

*ND = not determined.

This is why it is important that digestate is safe for use as a fertilizer, also highlighted the use of digestate as a fertilizer, in place of mineral fertilizers (Vázquez-Rowe et al., 2015). Na concentration is an important factor to assess the suitability of effluent irrigation. Phosphorus is essential for microorganism growth. Based on the results obtained in this research, an alternative to mitigate those problems is using biogas digestate, which could supply the chemical fertilizer demands.

Biogas enhancement through biological process and calorific values

There are a number of purification methods that have been applied in some countries, namely: absorption of liquids into the physics/chemical; adsorption on the surface of a solid adsorbent, membranes separation, cryogenic separation, and chemical change. However, these technologies showed that there is a high cost to purify methane, which is three times higher than that of the biogas production cost. An alternative technique to upgrade biogas is to use photosynthetic CO_2 uptake by microalgae. Microalgae have high carbon fixation ability and rapid growth rate, and can be adapted to various environmental conditions (Ramaraj et al., 2016). When microalgae are utilized for biogas upgrading, the photosynthesis can efficiently convert CO_2 in raw biogas into its biomass (Tang et al., 2011). This allows the valorization of biogas CO_2 in the form of a valuable microalgae biomass, which can be used as feedstock to produce biofuels or even high value-added by-product. This study biogas purification and methane enhancement through biological process presented Table 31.

Table 31 Biogas purification and methane enhancement through biological process

Parameters	Performance	Biogas composition (%)					
Biogas composition	Biogas Flow rate	CH_4 (%)	CO_2 (%)	O_2 (%)	H_2S (%)	Other trace gases (%)	Reference
Before purification	-	68.8	29.7	0	0.077	-	Dussadee et al., 2014
After purification	-	89.35	10.05	0.02	0.001	-	
Before purification	-	64.67	31.5	0	0.058	-	Ramaraj e al., 2016
After purification	-	82.05	17.08	1.11	0.001	-	
Before purification	-	69.70	28	0	0.013	0.99	This study
After purification	0.9 lpm	91	8.56	1.49	0	0.11	
	1.8 lpm	83	15	1.31	0	0.65	

Gupta, (2014) revealed that H_2S might lead the inhibitory effect on photosynthesis in the bioreactor system. In this is case, the study biogas doesn't have H_2S. Accordingly, there are no inhibitory effects due to lack of H_2S may be related to: (1) transport of CO_2 in photosynthesis and (2) interference on electron carrier protein of PSII (Photosystem II) for PSI (Photosystem I). Basically, SO_3^{2-} is known to inhibit photosynthetic CO_2 fixation in plants due to SO_3^{2-} outcompeting CO_2 in rubisco and inhibit mitochondrial ATP production and this study system doesn't meet this situation due to the lacked of H_2S. Also, H_2S concentrations present in raw biogas up to 3000 ppm did not exert notable inhibitory effects on microalgae growth (Yan et al., 2016).

Since the metabolism and photosynthesis of microalgae depend on microalgae growth, the law of nutrient and CO_2 removal efficiency changed as well as the variation tendency of microalgae growth. Furthermore, the study results indicated that biogas flow rate in the purification system very important, by 0.9 lpm methane content was increased to 91% and by 1.8 lpm methane content was reached to 83%, and other biogas components were demonstrated in Table 26. In addition, biogas flow rate (1.8 lpm) exposed the better performance compared to previous studies (Dussadee et al., 2014; Ramaraj et al., 2016). Zhu, (2015) was confirmed that CO_2 in biogas can be used as an important carbon source for microalgae cells growth. Also it is not difficult to conclude that N and P are more insufficient than carbon sources during the growth of microalgae according to the nutrient removal efficiency results. For the same reason, the CO_2 in the biogas was consumed during the photosynthesis of microalgae, so the biogas purification enhanced biogas (from co-digestion of buffalo grass and buffalo dung) HCV was 36.30 MJ/m^3 and LCV was 32.70 MJ/m^3. It was much higher than biogas production from traditional AD (LCV of 18.0–23.4 MJ/m^3 and HCV of 20.0–25.9 MJ/m^3) (Li et al., 2014), accordingly, this study results verified that high-calorific biogas was obtained in this study system after methane was enriched through biological biogas purification.

Economic analysis

Grass is one of the most abundant renewable energy sources in worldwide. Grass bio-methane has been shown to be a sustainable gaseous biofuel. It has an excellent energy balance; it is also shown to allow economic viability both to the producer and the consumer. Although both economic and financial analyses aim at appraising profitability of an investment project, the concept of benefit in economic analysis differs significantly from the financial analysis (Chakrabarty et al., 2013). Since this study was aimed for apply in rural area which can reduce the transportation and raw materials cost. This study results of economic analysis of biogas production from co-digestion of buffalo grass and buffalo dung presented in Table 32.

Table 32 Economic analysis of biogas scale-up

No.	Item	Economic Analysis	Units
1.	Electrical		
	system (0.164 kW/day) 45 day	39.36	Baht
	Blender (0.108 kW/time)	0.432	Baht
2.	Media and chemicals		
	NaOH 15 baht/kg (200g/time x 5 time)	15.00	Baht
	Water 9.50 baht/m^3 (0.17m^3/time)	1.62	Baht
	Total fixed costs	56.41	Baht
3.	Biogas production	1.62	m^3
4.	$AC = \frac{TC}{Q}$ Eq.3	34.82	Baht/m^3

Domestic biogas programs are frequently justified on the basis of financial cost-benefit analysis in terms of providing a superior cooking fuel (displaces dirtier and less efficient cooking fuels viz., firewood, kerosene etc.). Individual households judge profitability of biogas plants primarily from monetary surplus gained from utilizing biogas and bio-fertilizer in relation to the cost of the plants. The financial analysis is concerned with owner's private cost-benefit of the project without considering environmental or social externalities; however, an economic analysis considers externalities due to project execution (Campbell and Brown, 2003).

In this respect, economic analysis has a much broader scope than the financial analysis for policy consideration. Economic cost-benefit analysis is the most efficient and widely used tools for measuring whether any investment would be beneficial or not along with their environmental and social concern. The following effects, to be documented and provided with a monetary value, should be listed as benefits: expenditure saved by the substitution of other energy sources with biogas, income from the sale of biogas, replacing cost of using chemical fertilizer by slurry, income from the sale of slurry, time saved for collecting and preparing previously used fuel materials (Gebrezgabher et al., 2010). Time saved for cooking after utilizing biogas. Consequently, my study results explored that household type fermenter possible to replace the LPG and other fossil fuel usage; in addition it could bring the extra income to farmers.

Mass Balance for biomass and biogas production

Figure 40 present the mass balance for the scale up of fermentation system. The mass balance for this system has input is a para grass 42.8 kg/TS, buffalo dung 22.22 kg/TS, pig manure 7.5 kg/TS and water for adjust level mixed together at the fermenter after that was get biogas 60.3 L/day concentration of methane 69.70% and sludge become to fertilizer 4.5 kg.

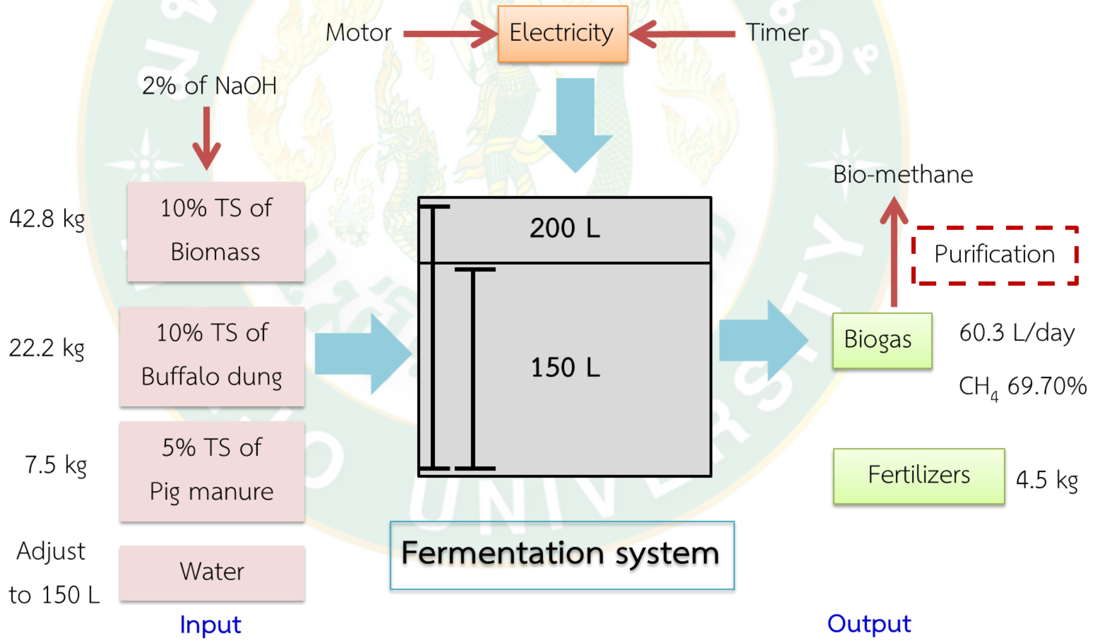


Figure 40 Block diagram for fermentation system

CHAPTER 5
SUMMERY

In conclusion, para grass is a good substrate for anaerobic digestion and used together with buffalo dung. The results showed that the initial total solids, volatile solids, chemical oxidation demand, and volatile fatty acids concentrations were significantly reduced after 36 days with biogas production process. The enhancement of the biogas yield was attributed to the improvement of biodegradability through pretreatment. In most cases, the use of co-substrate improves the biogas yields due to positive synergisms established in the digestion medium and the supply of missing nutrients by the para grass co-digestion with buffalo dung. The data obtained from this study would be used for designing large scale anaerobic digesters for treatment of para grass. Our future work is focused on pilot scale anaerobic digestion of para grass co-digestion with buffalo dung.

In the final part, the best ratio at 2:1 was used to last experiment operated between grass and dung. Each reactor was made from a 200 L tank with working volume of 150 L. The biogas yield shown that 1,620.65 L/day and the concentration of methane were 69.70%. The other objective is to optimize the condition of the process and develop an engineering/mathematical model of this process. Technique, Response Surface Methodology (RSM) has been used to optimize the mean factors of the process (temperature, NaOH concentrations and pH). From the biogas production, heat value of biogas was 6 kWh/m^3 and high heating value (HHV) was 8,937 BTU/ft3. The volume size increased to make sure for future large-scale applications and also techno-economic process was verified. The results suggested that co-digestion of para grass and buffalo dung was a promising approach for improving biogas production. Furthermore, the digestate has high nutrient concentrations that can potentially use as fertilizer.

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APPENDIX

APPENDIX A

PUBLICATION

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ORIGINAL ARTICLE

Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and bio-fertilizer

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Abstract

Biomass from wetland aquatic grass and buffalo grass can be exploited for biogas production, because this substrate is plenteous and does not compete with food production. In this study, the grass substrate was physically pretreated by boiling with different retention time to increase its biodegradability and was examined in batch mode. Boiling pretreatment suggested that 100 °C with 2 h retention time was the best condition. The results showed that the optimum grass concentration in the 1:1 ratio of co-digestion mixture with manure produced the highest methane yield. The results suggested that co-digestion of buffalo grass and buffalo dung was a promising approach for improving biogas production. This study was achieved the upgraded biogas through biological purification contained 90.42% CH_4 8.04% CO_2 1.43% O_2 and 0.11% other trace gases—a remarkable performance based on an efficiency criteria. Furthermore, the digestate has high nutrient concentrations that can potentially use as fertilizer.

Keywords Buffalo grass · Buffalo dung · Biogas production · Methane enhancement

Introduction

The environmental and global warming consciousness has become an important policy in all countries around the world. Furthermore, the fossil fuel use has been related to some alarming environmental problems such as global warming and climate change (Tsai et al. 2016; Vu et al. 2017). These increasing demands for energy, together with the weakening and limited source of fossil fuels, together with the harmful impacts in the environment, are the reasons industries and governments worldwide are pursuing renewable alternatives. Bioenergy, a renewable energy sources, draws responsiveness due to its accessibility and low carbon dioxide emission (Ramaraj et al. 2016a, b ,c). Thai government has increasingly given an importance on how to solve this problem issues among the first priority in local development.

At present, many agencies have focused on renewable energy such as solar energy, wind energy, hydroenergy and geothermal energy. Renewable sources of energy and consumer products are required for sustainable development of modern society (Unpaprom et al. 2017; Vu et al. 2017). Energy demand required to meet the economic growth of Thailand is growing higher in every year (Dussadee et al. 2017). Accordingly, Thailand has a huge potential to develop renewable energy from biomass as the country has an abundant agriculture sources such as raw materials from crops and livestock that can be used to produce biogas, specifically methane gas, through the decomposition of organic matter in the system (Dussadee et al. 2014; Vu et al. 2018).

Plant biomass is the main source of renewable materials on Earth and represents a potential source of renewable energy and bio-based products (Guo et al. 2015; Wannapokin et al. 2017). Animal manures have been used as a resource of excellent material for anaerobic digestion (AD) with clear environmental benefit, especially for buffalo dung. Since Thailand economy depends mainly on agriculture activities, therefore, utilization of natural resources for energy production is an extremely important issue. Agricultural residues from the agricultural sector, agriculture industry and grassland biomass are usually used as feed materials in anaerobic digestion systems in Thailand which

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are suitable in numerous ways for producing energy. There are so many types of grasses that are popularly grown in Thailand (Dussadee et al. 2017). Deb et al. (2016) stated that the buffalo grass, traditionally raised in a mixed crop livestock system, has played an important role over the centuries, especially in Asia, for the lives of millions of people, by ensuring work power and food at the end of their career as work animals.

Buffalo grass a tropical and invasive growing plant in rural area has only value to be feedstock for animal feeding. These exotic grass weeds are overgrown in abundantly available resources in the Northern region of Thailand. It needs to cut down and removed frequently for fire hazard, and disease and vector controls (Sahoo et al. 2017). The present study investigates the possibility of buffalo grass as a feedstock for biogas production using certain pretreatment. Rösch et al. (2013) stated that grass is converted to silage that can be used as feedstock for anaerobic digestion. This can be utilized as raw materials for an environmentally friendly renewable energy, more specifically for biogas production. Additionally, the use of grassland biomass for the biogas production is currently the common practice. Biogas application includes ensuring energy security, decreasing carbon emission and improving economic activity. It can be produced by a single raw material such as pig manure, cow manure and buffalo manure. Furthermore, Thailand is being in top 11 in the countries of Asia for buffalo population.

In present, the production of biogas has been evolving to enhance the efficiency like co-digestion of buffalo dung with grass. Co-digestion of buffalo grass (para grass) with buffalo dung in farm's around community existing digester becomes a valid approach to enhance biogas production Also, the addition of grass can help raise C:N of the feedstock to be suitable for metabolic activities in anaerobic digestion system. The physical structure and chemical composition of lignocellulosic materials can be altered through various methods of pretreatment, breaking down the linkage between polysaccharides and lignin, thus making cellulose and hemicelluloses more accessible to hydrolytic enzymes (Wannapokin et al. 2018). Therefore, pretreatments could accelerate the hydrolysis process and improve the methane content in the biogas.

Strevett et al. (1995) stated that water vapor in biogas is problematic for compressibility and should be removed prior to storage. And biogas typically contains a high percentage of carbon dioxide (CO_2), which decreases its caloric value. Finally, hydrogen sulfide (H_2S), which is also present in biogas, is toxic and exhibits corrosive effects on process equipment if not removed prior to compression and storage. Physicochemical methods such as physical adsorption, physical absorption or chemical absorption are commonly used to treat biogas. However, these biogas purification methods require costly investment and maintenance which are not suitable for industrial scale and reduce the profit. Therefore, biological purification that takes advantages of photosynthesis process of plant such as microalgae to eliminate CO_2 from biogas can be applied to reduce the capital and operations cost as enhance the biogas quality (Ramaraj et al. 2016a, b ,c). Therefore, this study main aim is to assess different pre-treatment and fermentation techniques through experimentation and evaluate each process and improvement of biogas yield. Finally, biogas production from buffalo grass (*Brachiaria mutica*) co-digestion with buffalo dung) through anaerobic enhanced methane content achieved by microalgae pass biological purification. Additionally, this study aimed to use non-food plant source as a feedstock for biogas production, a renewable energy fuel.

Materials and methods

Collection and preparation of substrates

The study methodology is illustrated in Fig. 1. This experimental study was carried out at an Energy Research

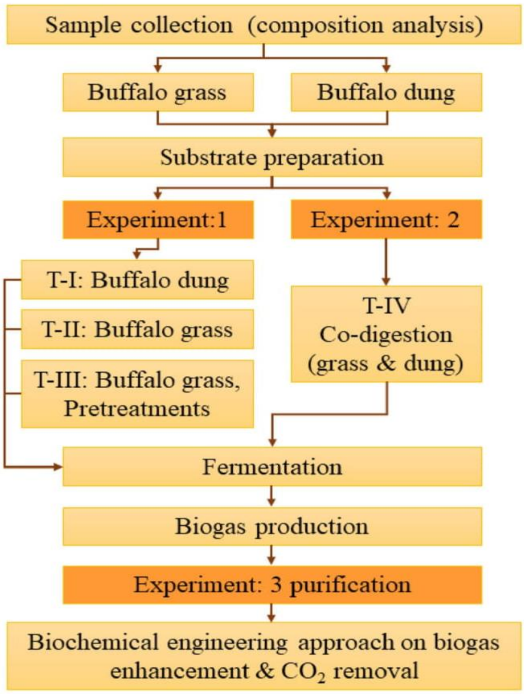


Fig. 1 The flowchart of study methodology

Center (ERC), Maejo University, Chiang Mai, Thailand (18°53′35″N; 99°01′10″E); additionally, the buffalo grass and buffalo dung were collected near to the experimental zone. The grass sample was crushed by a machine into small particles. Stored grass was pulverized into small particles (1.0 mm) before use. The inoculum was utilized from the Maejo pig farms located at the University campus. For biogas purification, the microalgae were obtained from ERC and the culturing details were described by Ramaraj et al. (2016a, b ,c).

Experimental setup

The buffalo grass was pretreated with boiling water at 100 °C with different reaction time ranging from 0.5 to 2 h. The experiments were carried out in batch type laboratory scale reactors and were categorized based on the different treatments applied: T-I (no treatment, buffalo dung), T-II (no TREATMENT, buffalo grass), T-III-A (buffalo grass, boiled 100 °C 0.5 h), T-III-B (buffalo grass boiled 100 °C 1 h), T-III-C (buffalo grass, boiled for 1.5 h at 100 °C), T-III-D (buffalo grass, boiled for 2 h at 100 °C) and T-IV (co-digestion of buffalo dung and buffalo grass, boiled for 2 h at 100 °C,). Experiment T-IV was operated with 1:1 ratio of grass and dung. Each reactor was made from a 7 L plastic container placed in a water bath. All reactors with 5 L working volume were run simultaneously for 35 days. The schematic configuration of the anaerobic biogas reactor system is given in Fig. 2. The accumulated biogas was stored carefully until the sufficient volume for purification experiments was reached.

Analytical methods

Parameters such as total solid (TS), volatile solids (VS), fixed solids (FS), chemical oxygen demand (COD), ash and moisture contents were measured according to the standard methods (APHA 2005). The compositions of sample (cellulose, hemicellulose, and lignin) were determined by Van Soest method (Van Soest et al. 1991). Metrohm 774 pH meter was used in all pH measurements. The pH was adjusted ranging from 7.40 to 7.70 for all experiments. Direct titration method for the determination of total volatile fatty acids and alkalinity was used (Ennouri et al. 2016). Samples were titrated with 0.1 N HCl (pH = 3), boiled over 3 min to remove CO_2, then back-titrated using 0.1 N NaOH until the pH reached 6.5. The biogas volume produced from the batch digester was determined using a water displacement unit. The pH of the substrate and digestate was determined using pH meter. The concentration of methane (CH_4) and other gases including carbon dioxide (CO_2), hydrogen sulfide (H_2S), and oxygen (O_2) in biogas produced were all determined by a portable gas analyzer (BIO5000, UK). The volume of biogas produced was measured at daily basis and biogas compositional analysis was performed every 3 days. The samples were analyzed for organic carbon, nitrogen (alkaline $KMnO_4$ method), 0.5 M $NaHCO_3$ (pH 8.5) extractable P and 1 (N) NH4OAc—extractable K and other trace elements (Page et al. 1982). In addition, emission, atomic absorption, volumetric, colorimetric, and photometric methods were used to determine physicochemical digestate properties and measurements adopted from Kinyua et al. (2016).

Calorific values were estimated according to Li et al. (2014).The higher calorific values (HCV) and lower calorific values (LCV) of pure methane were 39.82 and 35.87 MJ/m^3, respectively. HCV and LCV of produced biogas were determined according to the following formula:

$$HCV_{biogas} = 0.3989 \times MC = 0.0213(R^2 = 1) \quad (1)$$

$$LCV_{biogas} = 0.3593 \times MC = 0.0192(R^2 = 1) \quad (2)$$

where MC is the methane content in biogas (%).

Characterization of pretreated and untreated biomass

The biomass was characterized using scanning electron microscope, in order to observe the changes on the structure before and after applying pretreatment, characterization of biomass was done analysis using scanning electron microscopy analysis (JSM–5410LV, USA). The observation was performed at a total magnification of 100 ×.

Biogas through biological purification

Biogas enhancement was performed through photoautotrophic microalgae (*Chlorella vulgaris*). The experiment was continued for 8 h. Two types of biogas flow rate (0.9 and 1.8 lpm) in the algae growth unit were applied. The biological biogas purification process is described in Fig. 3.

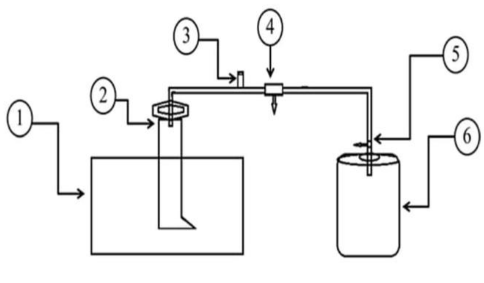


Fig. 2 The digester (1) water bath, (2) gas holder, (3) gas release valve, (4) gas line connecter, (5) gas line tube and (6) fermenter

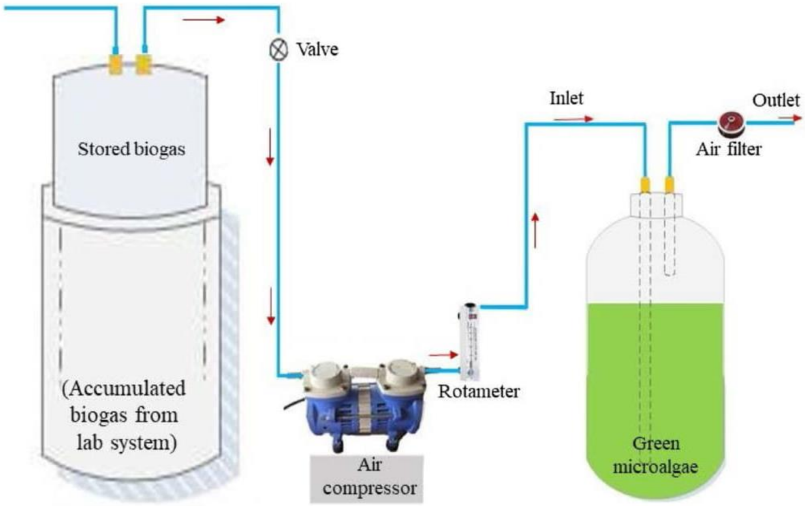


Fig. 3 Biogas enhancement through biological purification system

Statistical analysis

The values reported in the present study were the mean of three replicates. Statistical analyses of data were performed using the program SPSS 20.0 (SPSS Inc., Chicago, IL, USA). A significant difference was considered at the level of $p < 0.05$.

Results and discussion

Substrate characteristics

Feedstock characteristic is an important factor influencing digester's performance and stability. Buffalo grass (*Brachiaria mutica*) commonly known as Para grass is a member of the Poaceae family which is found as aquatic weeds throughout northern part of Thailand. Buffalo grass is estimated to contain about 40–44% of cellulose, about 18–22% hemicellulose and 18–21% of lignin. The initial pH, ash and moisture were 8.26, 2.79 and 77.3%, respectively. TS, VS, COD, alkalinity, volatile fatty acid were 349,813 mg/l, 128,275 mg/l, 62,333 mg/l, 2733 mg/l–$CaCO_3$, 4013 mg/l, respectively. The characteristics of buffalo dung TS, VS, COD, alkalinity, volatile fatty acid, pH, ash and moisture were 246,397 mg/l, 195,253 mg/l, 30,333 mg/l, 2400 mg/l–$CaCO_3$, 1260 mg/l, 8.02, 2.9 and 83.0%, respectively.

Imaging with scanning electron microscopy (SEM)

Morphological changes in the treated and untreated Buffalo grass during the hydrothermal pretreatments were observed using scanning electron microscope. SEM analyses was carried out to assess changes in morphology of the native and pretreated samples boiled at 100 °C with 2 h retention time. Figure 4a shows the SEM micrograph of native buffalo grass stem, the surface of which shows to have a regular and compact structure. Morphological changes induced by boiling are first noticeable after a pretreatment on buffalo grass stem, as shown in Fig. 4b.

A slight defibrillation was observed (shown in Fig. 4c, d); the separation of individual fibers, enlargement of the reactive area and more pronounced structural changes in the biomass were seen due to a possible solubilization of the hemicellulose. As hemicellulose operates as a cementing material, its solubilization causes a significant defibrillation effect on the biomass. In addition, a reduction in fiber length and the formation of entangled clusters can be seen in Fig. 4e, f; the fiber structure was almost entirely disintegrated due to the higher solubilization of hemicellulose and lignin re-localization. It was found that the fibers were greatly affected by boiling with 2 h retention time. In addition, the swelling of fibers is also observed in boiling pretreated biomass. This result was also supported by the structural changes observed from the SEM images of the stem, upper and lower leaf epidermis of the buffalo grass samples.

Pretreatment and biogas production

Hydrothermal pretreatment in lignocellulosic feedstock involves the usage of water only and has been widely accepted as a green technology without potential chemical consumption and potential pollution (Saha et al. 2013). Typically, it can remove most of hemicellulose and part of lignin in biomass by degrading them into soluble fractions and loosening the recalcitrant structure as well

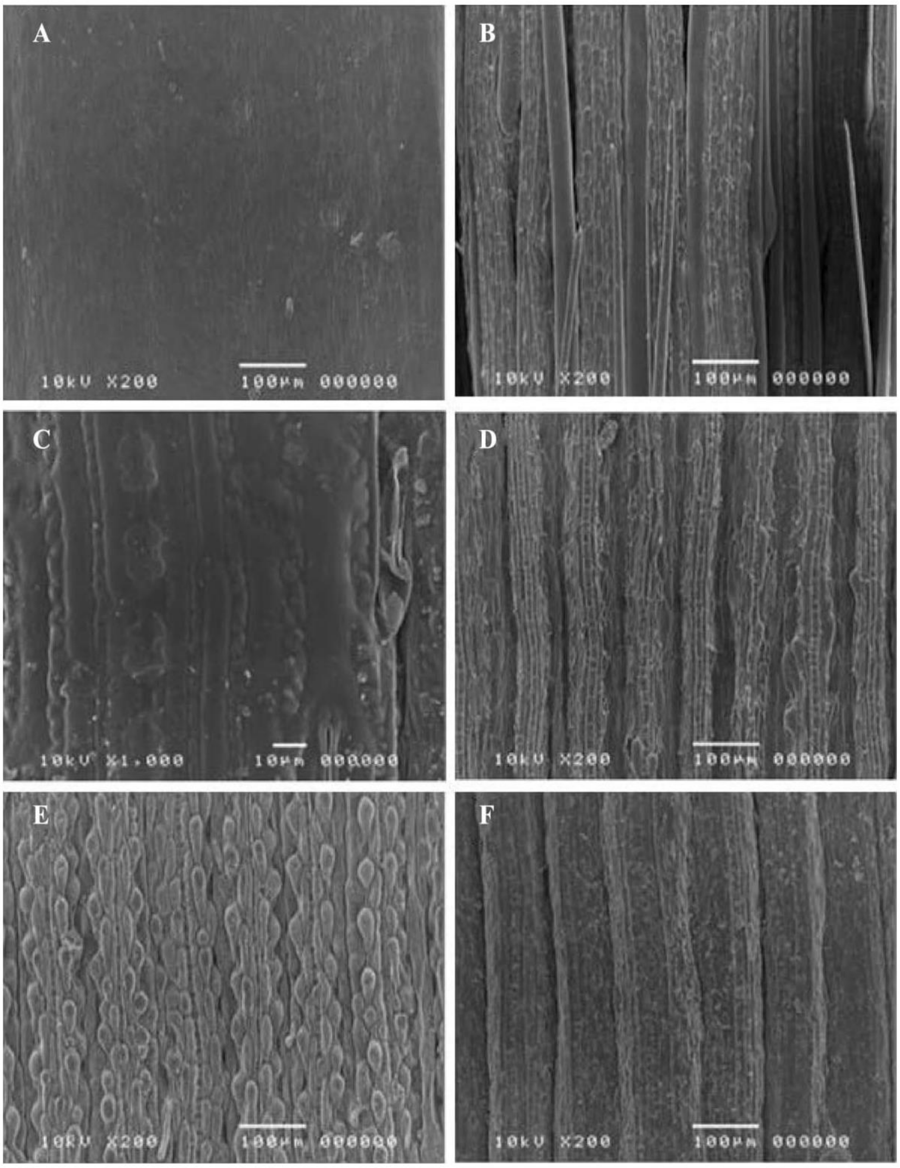


Fig. 4 Scanning electron micrographs of morphological characteristics of non-pretreated and pretreated of buffalo grass samples: **a** stem (not pretreated), **b** stem pretreated by boiling, **c** upper leaf epidermis (not pretreated), **d** pretreated upper leaf epidermis, **e** lower leaf epidermis (not pretreated) and **f** pretreated lower leaf epidermis

(Li et al. 2017). Therefore, hydrothermal pretreatment has been widely applied for facilitating biofuels production (Cybulska et al. 2014). They have long been used for enhancing particulate organic matter disintegration at temperatures from 50 to 270 °C. This study was applied with boiling pretreatment. Batch anaerobic fermentation was conducted to study the biogas potential of boiling preferment with mono and digestion of buffalo grass with buffalo dung. These experimental results are presented in Table 1. With 100 °C boiling water, the buffalo grass produces higher biogas yield and methane content by retention time (i.e., T-III-A < T-III-B < T-III-C < T-III-D = 58.13% CH_4 < 62.17% CH_4 < 63.78 CH_4% < 66.10 CH_4%. Furthermore, accumulated biogas yield was increased along with retention time. As a study

Table 1 The effect of pretreatment and biogas yield

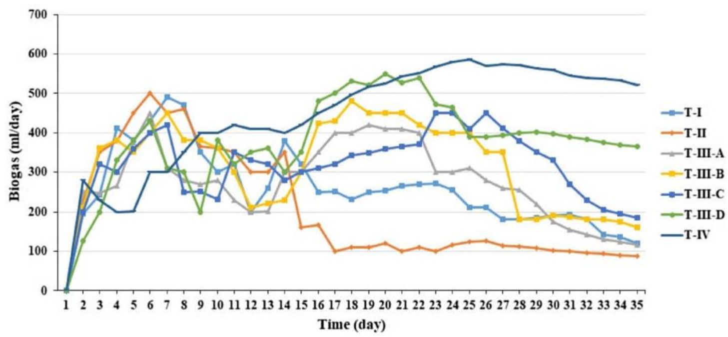
Items		Parameters				
		CH_4 (%)	CO_2 (%)	O_2 (%)	H_2S (ppm)	Accumulated biogas yield (ml)
No treatment, dung	T-I	52.27	42.8	0.1	454 (0.0454%)	8982
No treatment, grass	T-II	50.34	44.5	0.1	403 (0.0403%)	7184
Boiled 100 °C 0.5 h (grass)	T-III-A	58.13	39	0.1	384 (0.0384%)	9522
Boiled 100 °C 1 h (grass)	T-III-B	62.17	37	0.1	331 (0.0331%)	10,975
Boiled 100 °C 1.5 h (grass)	T-III-C	63.78	35.4	0.1	234 (0.0234%)	11,047
Boiled 100 °C 2 h (grass)	T-III-D	66.10	33	0	217 (0.0217%)	13,185
Co-digestion of grass (boiled 100 °C 2 h) and dung	T-IV	71.00	28	0	132 (0.0132%)	15,521

result, the main functions of hydrothermal pretreatment on converting the insoluble components into soluble fractions, breaking physical structure, and homogenizing feedstock sizes may improve anaerobic digestion.

The methane production rate reflects the biodegradability and amount of degradable matter. The daily biogas and gas composition including methane, carbon dioxide, hydrogen sulfide and oxygen production characteristics is shown in Figs. 5 and 6. Codigestion is defined as the digestion of mixtures of at least two waste materials for improving AD efficiency. Many successful codigestions of substrates have increased methane potential substantially compared to the mono digestion of the substrates (González–Fernández et al. 2011; Teghammar et al. 2013). These study results clearly demonstrated and agreed with González–Fernández et al. (2011) and Teghammar et al. (2013). Co-digestion of buffalo grass and buffalo dung produced higher accumulated biogas (15,521 ml) and rich methane content (71%) compared to mono digestion.

The total solids, volatile solids, chemical oxidation demand, alkalinity, volatile fatty acid and pH performance on before and after fermentation process was presented in Table 2 and Fig. 7. VFA formed during the acid phase of the anaerobic digestion tends to reduce the system pH, making the methanogenic bacteria, which are sensitive to low pH values, reduce their activity (Zhang et al. 2008). Thus, a balance between the production and consumption of acid during the refuse biodigestion is essential for the stability of the anaerobic process. The pH is one of the key factors in AD and the growth of methanogens can be significantly influenced by the pH level. VFA can maintain an efficient AD performance by influencing pH levels and alkalinity. The determination of volatile solids is a good parameter to follow the biodegradable organic matter degradation and its analysis is commonly applied to the biological stability measurement in sludge from liquid effluents (Metcalf and Eddy 1991). The anaerobic stabilization process starts when the volatile suspended solids of the system are hydrolyzed, resulting in soluble COD. The soluble COD represents the soluble organic matter of the system, which in turn is substrate for the methanogenesis, being converted into CH_4 and CO_2 (Zhang et al. 2008). Carbon is among the main nutrients for the microorganisms, as it is a source of energy for the microbial population; nitrogen is crucial for the microbial population growth (Igoni et al. 2008). Despite the volatile solid values

Fig. 5 Daily biogas production



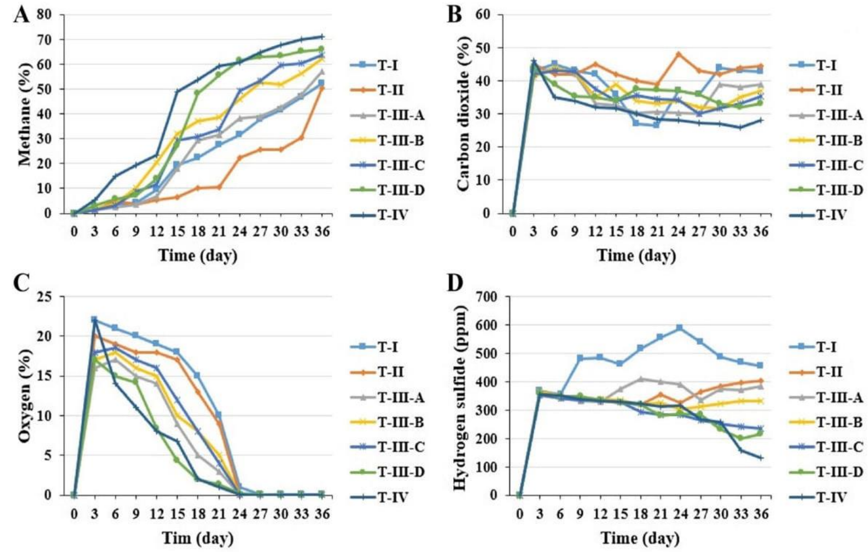


Fig. 6 Biogas composition: **a** methane, **b** carbon dioxide, **c** oxygen and **d** hydrogen sulfide

Table 2 Alkalinity, volatile fatty acid and pH performance on before and after fermentation

Treatments	Alkalinity (mg/l–$CaCO_3$)		Volatile fatty acid (mg/l)		pH	
	Before fermentation	After fermentation	Before fermentation	After fermentation	Before fermentation	After fermentation
T-I	2400	3833	3960	3844	7.55	7.06
T-II	2733	3133	4013	3820	7.55	6.53
T-III-A	2533	3800	4166	3912	7.55	6.55
T-III-B	2767	3233	4058	3949	7.55	6.54
T-III-C	2935	3324	4195	42,477	7.55	6.51
T-III-D	2787	3143	4004	3990	7.55	6.54
T-IV	2948	3072	4123	4246	7.55	6.52

being still relatively high at the end of the process, the final carbon values reveal that the biogas production develops to the end in the biodigesters; the TS, VS, and COD degradation efficiency were 79.48, 79.72 and 79.80%, respectively, which were consumed within the 35 days of the biodigestion.

Biogas enhancement through biological process

There are a number of purification methods that have been applied in some countries, namely absorption of liquids into the physics/chemical; adsorption on the surface of a solid adsorbent, membranes separation, cryogenic separation, and chemical change. However, these technologies showed that there is a high cost to purify biomethane, which is three times higher than that of the biogas production cost. An alternative technique to upgrade biogas is to use photosynthetic CO_2 uptake by microalgae. Microalgae have high carbon fixation ability and rapid growth rate, and can be adapted to various environmental conditions (Ramaraj et al. 2016a, b ,c). When microalgae are utilized for biogas upgrading, the photosynthesis can efficiently convert CO_2 in raw biogas into its biomass (Tang et al. 2011). This allows the valorization of biogas CO_2 in the form of a valuable microalgae biomass, which can be used as feedstock to produce biofuels or even high value-added by-product. In this study, biogas purification and methane enhancement through biological process are presented in Table 3.

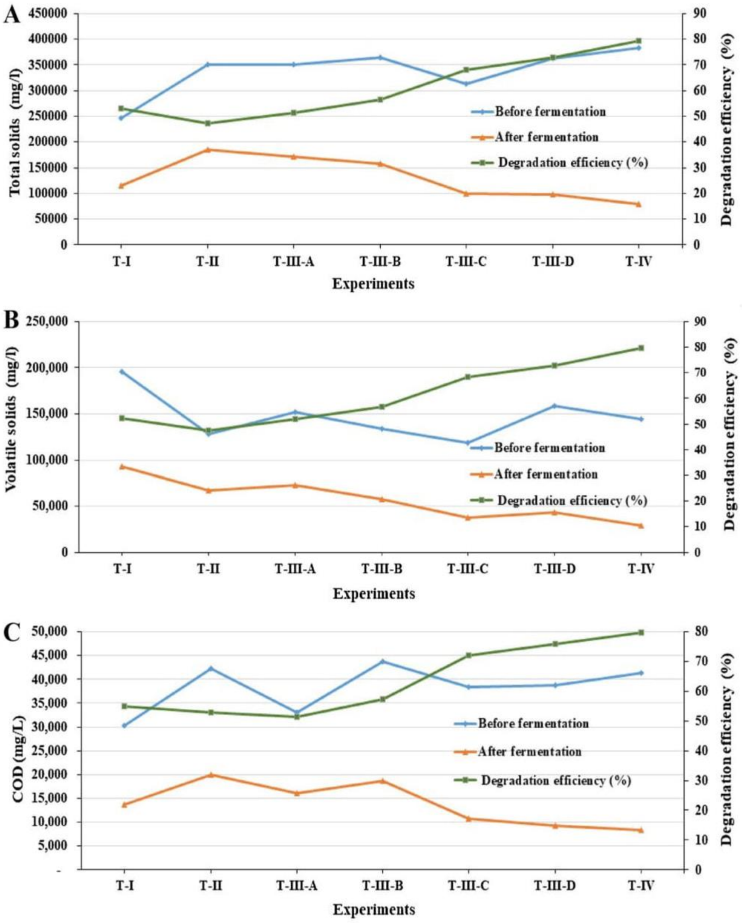


Fig. 7 Total solids, volatile solids and chemical oxidation demand of before and after fermentation

Table 3 biogas purification and methane enhancement through biological process

Parameters	Performance	Biogas composition (%)					
Biogas composition	Biogas flow rate	CH_4 (%)	CO_2 (%)	O_2 (%)	H_2S (%)	Other trace gases (%)	References
Before purification	–	68.8	29.7	0	0.077	–	Dussadee et al. (2014)
After purification	–	89.35	10.05	0.02	0.001	–	
Before purification	–	64.67	31.5	0	0.058	–	Ramaraj et al. (2016a, b ,c)
After purification	–	82.05	17.08	1.11	0.001	–	
Before purification	–	71	28	0	0.013	0.99	This study
After purification	0.9 lpm	91	8.56	1.49	0	0.11	
	1.8 lpm	83	15	1.31	0	0.65	

Gupta et al. 2014 revealed that H_2S might lead to the inhibitory effect on photosynthesis in the bioreactor system. In this is case, the study biogas does not have H_2S. Therefore, the inhibitory impact of H_2S on photosynthesis process that is relevant to biological purification using microalgae was ignored. Basically, SO_3^{2-} is known to inhibit photosynthetic CO_2 fixation in plants due to SO_3^{2-} outcompeting CO_2 in rubisco and inhibit mitochondrial ATP production and this study system does not meet this situation due to the lack of H_2S. Also, H_2S concentrations present in raw biogas up to 3000 ppmv did not exert notable inhibitory effects on microalgae growth (Yan et al. 2016).

Since the metabolism and photosynthesis of microalgae depend on microalgae growth, the law of nutrient and CO_2 removal efficiency changed as well as the variation tendency of microalgal growth. Furthermore, this study results revealed that flow rate as a vital factor for biogas purification. Different flowrates (0.9–1.8lpm) were achieved methane content of 83%–91%, and other biogas components were demonstrated in Table 3. In addition, biogas flow rate (1.8 lpm) exposed the better performance compared to the previous studies (Dussadee et al. 2014; Ramaraj et al. 2016a, b ,c). Zhu (2015) was confirmed that CO_2 in biogas can be used as an important carbon source for microalgae cells growth. Also it is not difficult to conclude that N and P are more insufficient than carbon sources during the growth of microalgae according to the nutrient removal efficiency results. For the same reason, the CO_2 in the biogas was consumed during the photosynthesis of microalgae, so the biogas purification capacity was also improved.

Enhanced biogas calorific value and digestate fertilizer

Enhanced biogas (from co-digestion of buffalo grass and buffalo dung) HCV was 36.30 MJ/m^3 and LCV was 32.70 MJ/m^3. It was much higher than biogas production from traditional AD (LCV of 18.0–23.4 MJ/m^3 and HCV of 20.0–25.9 MJ/m^3) (Li et al. 2014); accordingly, these study results verified that high-calorific biogas was obtained in this study system after methane was enriched through biological biogas purification. Finally, the digestate from codigestion of buffalo grass and buffalo dung was analyzed. The study digestate and the literature data are presented in Table 4. Digestate can be defined as liquid from anaerobic decomposition of animal and plant waste. It contains considerable amounts of mineral elements including nitrogen, phosphorus, potassium and others. In terms of rapidity of action, it resembles mineral fertilizers since N, P and K elements are easily available for plants. Govasmark et al. (2011) and Heviánková et al. (2013) proved the possibility of occurrence of pathogenic bacteria and heavy metals in digestate. This is why it is important that digestate is safe for use as a fertilizer and also highlighted the use of digestate as a fertilizer in place of mineral fertilizers (Vázquez–Rowe et al. 2015). Na concentration is an important factor to assess the suitability of effluent irrigation. Phosphorus is essential for microorganism growth. Based on the results obtained in this research, an alternative to mitigate those problems is using

Table 4 Chemical compositions of digestate from the different anaerobic digesters

Raw materials	TOC, g L^{-1}	N	P	K	S	Ca	Mg	Fe	Mn	Zn	Cu	References
		g kg^{-1} in the form of dry matter										
Poultry manure	452	67	24	24	5.3	92	6	1.8	0.66	0.58	0.11	Kirchmann and Witter (1992)
Biodegradable household waste	ND	152	16	78	7	50	10	ND	< 0.001	0.08	0.01	Haraldsen et al. (2011)
Pig manure + sludge from wastewater treatment plant + biodiesel wastewater	247	200	6	52	ND	26	10	1	0.16	1.16	0.21	Alburquerque et al. (2012)
Maize silage	ND	41	34.8	5.9	ND	3.7	36.2	ND	ND	0.08	0.08	Pokój et al. (2015)
Co-digestion of buffalo grass + buffalo dung	389.17	77.53	13.39	37.86	5.73	35.76	12.11	2.14	0.36	0.75	0.19	This study

ND not determined

biogas digestate, which could supply the chemical fertilizer demands.

Conclusions

In the present study, buffalo grass has been established as an efficient cosubstrate for buffalo dung to enhanced biogas production. While buffalo grass is a menacing aquatic biomass, it could also serve as an effective aquatic energy crop with controlled growth and proper maintenance in constructed wetlands and thus reduce the dependency of terrestrial energy crops for bioenergy generation in the near future. More specifically, the methane concentration from the co-digestion mixture was found to be the key parameters for an improved biomethanation process. The microalga biological purification of biogas enrichment was achieved successfully. Furthermore, the digestate from biogas fermenter was confirmed to be an efficient alternative fertilizer with high nutrients and environmentally-friendly comparing to chemical fertilizer.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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การประชุมวิชาการและประกวดนวัตกรรมบัณฑิตศึกษาแห่งชาติ ครั้งที่ 1
"เทิดพระเกียรติวันแม่แห่งชาติ สู่ความมั่นคง มั่งคั่ง ยั่งยืน"
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การประเมินผลการหมักร่วมระหว่างหญ้าขนและมูลกระบือแบบไม่ใช้ออกซิเจนเพื่อการเพิ่มประสิทธิภาพในการผลิตก๊าซชีวภาพ

Evaluation of anaerobic co-digestion of para grass (*Brachiaria mutica*) with buffalo dung for enhancing biogas production

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บทคัดย่อ

การผลิตก๊าซชีวภาพจากกระบวนการหมักแบบไร้ออกซิเจน เป็นเทคโนโลยีการผลิตพลังงานทดแทนอย่างหนึ่ง ซึ่งก๊าซชีวภาพที่ผลิตได้จากวัสดุเหลือทิ้งทางการเกษตรหรือพืชในพื้นที่ชุ่มน้ำเพื่อเป็นแหล่งพลังงานทางเลือกนั้นเป็นที่น่าสนใจในปัจจุบัน โดยเฉพาะอย่างยิ่งเทคโนโลยีการหมักร่วม ในการทดลองนี้ได้ทำการศึกษาความเป็นไปได้ในการหมักร่วมระหว่างหญ้าขนและมูลกระบือ ซึ่งหญ้าขนถือว่าเป็นวัชพืชอย่างหนึ่งในพืชที่ชุ่มน้ำ โดยทำการเก็บหญ้าขนจากบริเวณศูนย์การเรียนรู้ทางการเกษตร มหาวิทยาลัยแม่โจ้ อำเภอสันทราย จังหวัดเชียงใหม่ เปรียบเทียบการผลิตก๊าซโดยเปรียบเทียบอัตราส่วนการหมักร่วมกับมูลกระบือ ทำการหมักในถังพลาสติกขนาด 7 ลิตร เป็นระยะเวลา 35 วัน ซึ่งได้วิเคราะห์ค่าของแข็งทั้งหมด (TS) ของแข็งระเหย (VS) และซีโอดี (COD) ก่อนและหลังการหมัก ผลการทดลอง พบว่า หญ้าขนที่ปรับสภาพด้วยการต้มหมักร่วมกับมูลกระบือ ในอัตราส่วน 1:1 ให้ผลผลิตก๊าซชีวภาพสูงที่สุด คือ 1,187 มิลลิลิตร/ลิตร และความเข้มข้นของก๊าซมีเทนเท่ากับ 68.57% ค่าประสิทธิภาพการย่อยสลายของแข็งทั้งหมด (TS) ประสิทธิภาพการย่อยสลายของแข็งระเหย (VS) และประสิทธิภาพการย่อยสลายซีโอดี (COD) เท่ากับ 74.28%, 78.89% และ 87.60% ตามลำดับ ดังนั้นจึงสรุปได้ว่า หญ้าขนเป็นวัตถุดิบที่มีศักยภาพในการผลิตก๊าซชีวภาพ นอกจากนั้น การปรับสภาพด้วยการต้ม ถือว่าเป็นวิธีที่สามารถเพิ่มประสิทธิภาพการผลิตก๊าซชีวภาพได้มากยิ่งขึ้น

Abstract

Biogas production through anaerobic digestion (AD) has emerged as one of the renewable energy production technology. At the present, biogas production from agricultural waste or wetland plants as alternative energy source was interesting, particularly co-digestion technology. This experiment was focused on possibility of para grass *(Brachiaria mutica)* as a waste material co-digestion with buffalo dung, para grass is the weed of no value and pervasive around wetland areas. The grass was collected from wetlands located at the Learning Center for Agriculture, Maejo University, Chiang Mai, Thailand. Optimized fermentation by co-digestion with buffalo dung was compared together with para grass. The fermentation process was done in a 7 L plastic container of digester for 35 days. Total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) were determined at the start to end of the fermentation process. Co-digestion of para grass and buffalo dung presented highest biogas yield at ratio 1:1 with 1,187 ml/L with the concentration of methane at 68.57%. Degradation efficiency of TS, VS and COD were 74.28%, 78.89% and 87.60%, respectively. Therefore, para grass can be considered as a potential material for biogas production. In addition, boiling pretreatment is an effective method for increasing efficiency of biogas production.

Keywords: Biogas, anaerobic digestion, co-digestion, para grass, alternative energy

Introduction

The biogas application is used as renewable energy efficiency and suitable for improving energy security, decreasing environmental disruption caused by carbon emissions (Ramaraj et al., 2015a; 2015b). Biogas production through anaerobic digestion (AD) has emerged as one of the renewable energy production technology of choice because through AD biogas as a renewable fuel (Ramaraj et al., 2016; Sakar et al., 2009). Generally, the production of this gas involves a complex biochemical reaction that take place under AD condition in sensitive microbiological catalysts that are mainly bacteria.

Brachiaria mutica commonly known as para grass is a member of the Poaceae family which is found as aquatic weeds of wetland areas, drainage channels, lakes and dams, roadside ditches and in other damp habitats, particularly in tropical climate (Sahoo et al., 2017). It is a burden since it needs to be cut down and removed frequently for fire hazard, and for potential disease spread and vector controls (Xie et al., 2011). Reproduction and dispersal of this species can quickly cover large areas. Seeds and stem segments can be spread by floods and animals, and most long-distance dispersal occurs through its use as a pasture grass. Para grass consists of a mixture of lignin, hemicelluloses and cellulose (Vila et al., 2012; Soccol et al., 2010; Singh et al., 2017). Both the cellulose and hemicelluloses are polymers of sugars, and are thereby a potential source of sugars (Xie et al., 2012). Hemicellulose component of the lignocellulosic biomass is considered as an attractive raw material for the production of biogas. The relatively high content of hemicellulose in the para grass indicates that it could be a good source of hemicellulose for bioconversion. One of the possible solutions to lessen Para grass on the environment is to use it as substrate for the production of organic fertilizers via composting or vermicomposting, and a source for the production of biofuel (Ganesh et al., 2005).

New efficient and cost-effective small-scale renewable energy generation options are commercially available today. Alternative energy technologies are being disseminated in many countries with an objective to reduce the uses of traditional and commercial energy sources (Wannapokin et al., 2017; Marchetti et al., 2016). Realization of the non-renewable nature of fossil fuels has led to a search for effective alternative renewable sources to meet future energy demands. Conversion of organic matter to biogas is a well-established small and medium scale technology. This study aims to investigate the potential of para grass in co-digestion with buffalo dung for biogas production.

Materials and Methods

Sample preparation

Fresh para grass (*B. mutica*) were harvested from wetlands at Learning Center for Agriculture Maejo University Chiang Mai, Thailand. It was then crushed by a machine into small particles. The sample had undergone pretreatment by boiling as shown in figure 1. The buffalo dung was harvested from Learning Center for Agriculture Maejo, University Chiang Mai, Thailand.

Para grass pretreatment assay

The thermal pretreatments were performed in batch mode for 2 hours at 100°C. The pretreatment experiment was carried out in a 7 L anaerobic digester plastic bottle. Each pretreatment experiment was conducted with 10% total solid (TS) of grass biomass, 10%TS of buffalo dung and 5% inoculum.

Figure 1 Para grass biomass preparations for anaerobic digestion (A-D).

Determination of the biogas potential

The inoculum was collected from a swine farm at Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand. The inoculum was kept in air-tight buckets at 4°C in a walk-in cooler. Prior to use, the inoculum was acclimated and degassed at 35°C for 3 weeks to minimize the effect of methane production from inoculum. The anaerobic batch digestions were carried out at room temperature in 7 L digester plastic container, closed with brass valve in which there was a pipe to extract biogas. The anaerobic assays contained 5% of inoculums (the inoculum was obtained from pig manure), 10% TS of crushed fresh Para grass and 10% TS of buffalo dung, and the remaining is made up with doubled distilled water. The experiments were performed in triplicate.

The daily biogas yield and cumulative biogas yield for co-digestion of para grass with buffalo dung at mixing ratio of 0:2, 2:0, 1:1, 1:2, 2:1 were indicated by treatment (T1, T2, T3, T4 and T5). The total gas production was measured by water displacement method at each 24 hour interval. The contents of the bottle were mixed manually and regularly after gas measurement.

Experimental design

The tests were conducted in triplicate with a 7 L capacity of digester plastic container with working volume of 5 L. It was sealed using a brass valve in which there was a pipe to extract biogas. The digester was connected to a gas collection system which consists of a displacement container and storage containers shown in figure 2. Schematic view of the experiment set up for anaerobic digestion of para grass. Thereafter, the digesters were placed at room temperature (30–34°C) for 35 days. Each digester was manually mixed twice a day.

Figure 2 Batch digesters.

Analytical Methods

Samples were analyzed for total solids (TS), volatile solids (VS), chemical oxygen demand (COD) and alkalinity using standard methods (APHA-AWWA-WPCF., 1981; Federation and Association, 2005). Metrohm774 pH-meter was used in all pH measurements. Ash, moisture, volatile fatty acids (VFA) and carbohydrates were determined using AOAC official method (Helrick., 1990). Elemental composition analysis was carried out using a Perkin–Elmer 2004 element analyzer, to determine the carbon (C), hydrogen (H), nitrogen (N), sulfur (S) contents of the sample. The oxygen (O) content was subsequently calculated as the difference. The composition of biogas (CH_4, CO_2, H_2S and O_2) was measured using a biogas analyzer (BIO5000, UK).

Results and Discussion

The composition of para grass used clearly indicates that they are nutritionally rich than compared to buffalo dung (Table 1). The grass showed distinct differences in their chemical composition. Meanwhile, the pH was adjusted between the ranges of 7.40 to 7.70 for anaerobic digestion. Consequently, both materials have plenty of nutrients for biogas containing 41.5% of carbon, 5.3% of hydrogen, 27.3% of oxygen and 1.3% of nitrogen for para grass and buffalo dung with 37.2% of carbon, 6.6% of hydrogen, 54.1% of oxygen and 1.4% of nitrogen.

Table 1 Characteristics of para grass biomass and buffalo dung.

Parameters	Para Grass	Buffalo dung
pH	8.26	8.02
Proximate analysis (wt. %)		
Moisture	77.3	83.01
Ash	2.79	5.79
Ultimate analysis (wt. %)		
Carbon (C)	41.5	37.2
Hydrogen (H)	5.3	6.6
Nitrogen (N)	1.3	1.4
Oxygen (O)	27.3	54.1
Sulphur (S)	0.3	0.15
C:N Ratio	32.2	24
Biochemical analysis		

Total solid (TS %)	26.29	16.98
Volatile Solids (VS%)	23.25	10.90
Chemical oxygen demand COD (mg/l)	26,600	61,300
Alkalinity (Alk) (mg/l-$CaCO_3$)	1,740	1,460
VFA (mg/g)	3,000	3,365

In the all treatment pH fell immediately after of the experiment (Table 2). The pH biggest decrease was T2 and T5 similar with articles from Weiand et al. (2010). Methane formation takes place within a relatively narrow pH interval, from about 6.5 to 8.5 with an optimum interval between 7.0 and 8.0. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5. And the pH decrease from 7.2 to 5.3 because the substrate was rapidly degraded to VFA (Bouallagui et al., 2003).

Table 2 pH of the different ratios.

Treatments	**pH**	
	Before	**After**
T1 (BG)	8.02	7.06
T2 (Pretreated PG)	6.82	4.53
T3 (Mixed ratio 1:1)	6.83	6.35
T4 (Mixed ratio 1:2)	6.80	6.24
T5 (Mixed ratio 2:1)	6.82	4.40

PG: para grass, BD: buffalo dung

Determination of TS, VS, COD, and CH_4

TS and VS of all the treatments in this study were ranged 8.27%-14.98%, 6.57%-9.53%, respectively (Table 3). The fractional increase of methane yield in co-digestion with buffalo dung compared with control sample. The reason was that the bioreactor was unstable at the beginning of the anaerobic digestion which was the environmental adaptation stage for methane bacteria. The system was stable after day five, more methane yield was attained with T3 (1:1) fermenter because of the many soluble organic material contains. View the results shown in the table 3 all of the parameter after fermentation decreases and in T3 have degradation efficiency 74.28% higher than all experiment show in Table 4. The higher production rates and methane composition corresponding substrate show that the disappearance of solids results in higher production due to in the particle, these solids are converted to process of gas production and have reasonable ratio is 1:1

between para grass and buffalo dung. Co-digesting animal manure that has a low C/N ratio along with feedstock containing low levels of nitrogen (high C/N ratio) gives more stable operation performance and a higher methane yield than digesting manure only (Callaghan et al., 2002). Accordingly, the pretreatment of boiled 100 °C 2 hour was done to increase the efficiency of biogas production by pretreatment breaks down the complex organic structure into simpler molecules which are then susceptible to microbial degradation. Consequently, pretreatment to remove the lignin and enhance the hydrolysis of cellulose is essential. To overcome this problem and to optimize biogas production, the plants must undergo some kind of pre-treatment (Pantawong et al., 2015) and test the feasibility on the large scale set up. Hence, pretreatment can enhance the bio-digestibility of the wastes for biogas production and increase accessibility of the enzymes to the materials. It results in enrichment of the difficult biodegradable materials and improves the yield of biogas from the biomass. Therefore, pretreatment process was needed to be able to get a high biogas yield (Dussadee et al., 2017).

Table 3 Parameter of the experiment and biogas yield.

Treatments	Parameter						
	TS (%)		VS (%)		COD (mg/L)		CH_4 (%)
	Before	After	Before	After	Before	After	
T_1 (BD)	14.98	7.82	9.53	6.60	61,333	29,333	54.10
T_2(Pretreated PG)	12.34	8.08	7.84	7.05	26,667	13,333	56.23
T_3(Mixed ratio 1:1)	11.61	9.66	8.32	7.12	37,333	24,000	68.57
T_4(Mixed ratio 1:2)	9.66	9.52	7.02	6.74	56,000	45,333	63.48
T_5(Mixed ratio 2:1)	8.27	7.86	6.57	6.25	40,000	24,000	58.50

PG: para grass, BD: buffalo dung

Table 4 Degradation efficiency.

Treatments	Degradation efficiency (%)		
	TS	VS	COD
T_1 (BD)	52.46	55.07	55.93
T_2 (Pretreated PG)	60.83	53.70	66.66
T_3 (Mixed ratio 1:1)	74.28	78.89	87.60
T_4 (Mixed ratio 1:2)	68.13	61.15	62.54
T_5 (Mixed ratio 2:1)	67.48	64.38	66.66

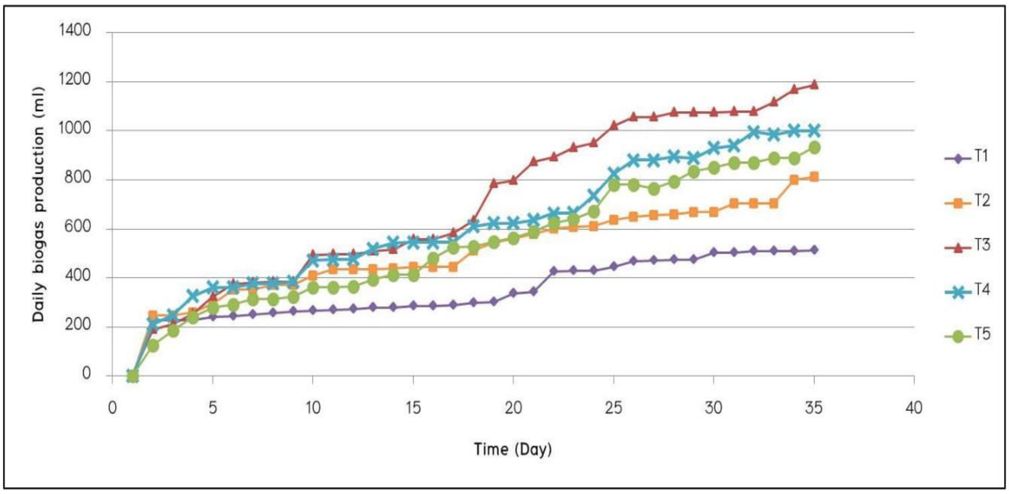

Figure 3 Biogas productions from para grass with different ratios.

In treatments T5, T2 and T1 (VS%: 6.57, 7.84 and 9.53, respectively) initial methane production was low during the period from day 1-35. After fifteen days, methane production of T3 and T4 increased sharply, and with a 6.35, 6.24 pH of indicating the enrichment of methanogens in the reactor. At the end of the experiment, methane production declined due to the lack of soluble biodegradable organic substances. Among the treatments, T_3 has the highest methane yield on day 35 Co-digestion of para grass with buffalo dung can increase the biogas yield maintain an optimal pH for methanogens decreasing ammonia inhibition, which may occur in AD of manure: providing a better carbon/nitrogen ratio (C/N) in the feedstock (Xie et al., 2011).

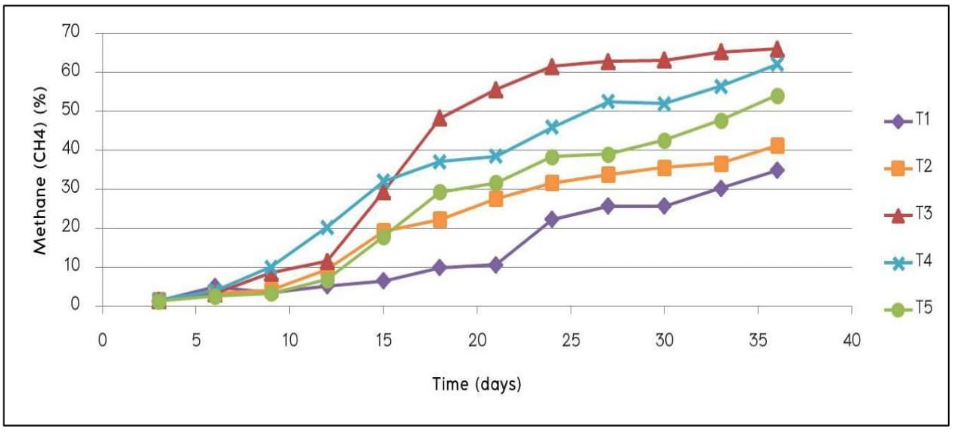

Figure 4 Potential production of biogas from para grass with different ratios.

In conclusion, para grass is a good substrate for anaerobic digestion and used together with buffalo dung. The results showed that the initial total solids, volatile solids, chemical oxidation demand, and volatile fatty acids concentrations were significantly reduced after 35 days with biogas production process. The enhancement of the biogas yield was attributed to the improvement of biodegradability through boiling. In most cases, the use of co-substrate improves the biogas yields due to positive synergisms established in the digestion medium and the supply of missing nutrients by the para grass co-digestion with buffalo dung. The data obtained from this study would be used for designing large scale anaerobic digesters for treatment of para grass. Our future work is focused on pilot scale anaerobic digestion of para grass co-digestion with buffalo dung. Production of biogas will enhance clean environment, during anaerobic digestion and thus producing fertilizer. Biogas finds application in cooking, lighting, electricity generation amongst other uses.

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