DEVELOPMENT OF METHANE ENHANCEMENT SYSTEM OF BIOGAS PRODUCTION FROM WATER PRIMROSE AND COW DUNG

NONG THI THU HUYEN

MASTER OF ENGINEERING IN RENEWABLE ENERGY ENGINEERING

MAEJO UNIVERSITY

2021

DEVELOPMENT OF METHANE ENHANCEMENT SYSTEM OF BIOGAS PRODUCTION FROM WATER PRIMROSE AND COW DUNG

NONG THI THU HUYEN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING

IN RENEWABLE ENERGY ENGINEERING

ACADEMIC ADMINISTRATION AND DEVELOPMENT MAEJO UNIVERSITY

2021

DEVELOPMENT OF METHANE ENHANCEMENT SYSTEM OF BIOGAS PRODUCTION FROM WATER PRIMROSE AND COW DUNG

NONG THI THU HUYEN

THIS THESIS HAS BEEN APPROVED IN PARTIAL FULFLLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING
IN RENEWABLE ENERGY ENGINEERING

APPROVED BY **Advisory Committee**

Chair ....

(Dr. Rameshprabu Ramaraj)

................../................../ ....

Committee ....

(Assistant Professor Dr. Nigran Homdoung)

................../................../ ....

Committee ....

(Assistant Professor Dr. Yuwalee Unpaprom)

................../................../ ....

Program Chair, Master of Engineering ....

in Renewable Energy Engineering (Assistant Professor Dr. Tanate Chaichana)

................../................../ ....

CERTIFIED BY ACADEMIC ....

ADMINISTRATION AND DEVELOPMENT

(Associate Professor Dr. Yanin Opatpatanakit)

Vice President for the Acting President of Maejo University

................../................../ ....

ชื่อเรื่อง	การพัฒนาระบบการปรับปรุงก๊าซมีเทนในการผลิตก๊าซชีวภาพจากต้นเทียนนาและมูลโค
ชื่อผู้เขียน	Miss Nong Thi Thu Huyen
ชื่อปริญญา	วิศวกรรมศาสตรมหาบัณฑิต สาขาวิชาวิศวกรรมพลังงานทดแทน
อาจารย์ที่ปรึกษาหลัก	Dr. Rameshprabu Ramaraj

บทคัดย่อ

ปัจจุบันแหล่งทรัพยากรด้านพลังงานและสิ่งแวดล้อมได้รับความสนใจเพิ่มมากขึ้น และการศึกษาในครั้งนี้ได้ศึกษาเกี่ยวกับแหล่งทางเลือกของพลังงาน การหมักแบบไร้ออกซิเจนหรือเทคโนโลยีการผลิตก๊าซชีวภาพเป็นพลังงานทางเลือกแบบหนึ่ง และกระบวนการทางชีวภาพนี้ได้ใช้ชีวมวลเป็นวัตถุดิบหลัก วิทยานิพนธ์นี้ได้ศึกษาศักยภาพของต้นเทียนนา (*Ludwigia hyssopifolia*) ซึ่งเป็นวัชพืชทางการเกษตรสำหรับการผลิตก๊าซชีวภาพ โดยงานวิจัยแรกนี้ได้ทำการทดลองแบบกะ (batch) และหมักเชิงเดี่ยว ดำเนินการทดลองเป็นเวลา 45 วัน ซึ่งแบ่งการทดลองออกเป็นวัตถุดิบแบบสดและแห้ง ทำการปรับสภาพต้นเทียนนาโดยใช้สารละลายโซเดียมไดออกไซด์ที่ความเข้มข้นแตกต่างกัน (1, 2, 3 และ 4 เปอร์เซ็นต์) และวัตถุดิบต้นเทียนนาในอัตราส่วนของแข็งทั้งหมด 10 เปอร์เซ็นต์ ผลการทดลองพบว่าที่ความเข้มข้นของสารละลายโซเดียมไดออกไซด์ 2 เปอร์เซ็นต์ ให้ผลการทดลองที่ดีที่สุด โดยให้ผลผลิตก๊าซชีวภาพทั้งหมดสูงที่สุด (8,072.00 มิลลิลิตร) ความเข้มข้นของก๊าซมีเทนสูงที่สุดเช่นกัน (64.72 เปอร์เซ็นต์) สำหรับการประเมินความเป็นไปได้ในการหมักแบบร่วมกับวัตถุดิบอื่น โดยทำการศึกษาระยะเวลาในการปรับสภาพของต้นเทียนนา (1, 2 และ 3 สัปดาห์) โดยใช้สารละลายโซเดียมไดออกไซด์ที่ความเข้มข้น 2 เปอร์เซ็นต์ จากนั้นผสมกับมูลโคที่อัตราส่วนต้นเทียนนาต่อมูลโค 1:1, 2:1 และ 1:2 (น้ำหนัก/น้ำหนัก) ในการหมักแบบร่วมที่อัตราส่วน 2:1 โดยปรับสภาพต้นเทียนนาเป็นระยะเวลา 2 สัปดาห์ ให้ผลการทดลองที่ดีที่สุด ผลการผลิตก๊าซชีวภาพทั้งหมด 8,610 มิลลิลิตร ความเข้มข้นก๊าซมีเทน 68.2 เปอร์เซ็นต์ ค่าประสิทธิภาพการย่อยสลายต่างๆ ได้แก่ ของแข็งทั้งหมด (TS) มีค่า 70.84 เปอร์เซ็นต์ ของแข็งที่ระเหยได้ (VS) มีค่า 64.76 เปอร์เซ็นต์ และ COD มีค่า 66.55 เปอร์เซ็นต์ เพื่อสนับสนุนผลการทดลองจากระดับห้องปฏิบัติการ จึงได้ดำเนินการทดลองระดับต้นแบบ (pilot-scale) ภายใต้สภาวะแวดล้อมที่แท้จริงและเพื่อสามารถปรับใช้ได้จริงในอนาคต โดยได้ผลของระยะเวลาการปรับสภาพด้วยโซเดียมไฮดรอกไซด์และอัตราส่วนมูลวัวต่อต้นเทียนนาที่ดีที่สุดมาใช้ในการหมักระดับต้นแบบ การทดลองระดับต้นแบบได้ใช้ถังหมักขนาด 1,000 ลิตร ซึ่งได้ผลการผลิตก๊าซชีวภาพทั้งหมด 1.7 มิลลิตร/กรัมของแข็งทั้งหมด/วัน ความเข้มข้นของก๊าซ

มีเทนสูงที่สุดเท่ากับ 68.6 เปอร์เซ็นต์ ซึ่งสามารถสรุปได้ว่า ผลการทดลองจากระบบห้องปฏิบัติการสามารถนำมาปรับใช้ในระบบต้นแบบได้จริง จากนั้นจึงดำเนินการทดลองเพื่อทำบริสุทธิ์ก๊าซ โดยการนำก๊าซที่ได้ไหลผ่านสารละลายโซเดียมไฮดรอกไซด์และแคลเซียมไฮดรอกไซด์ที่ความเข้มข้น 1, 2 และ 3 เปอร์เซ็นต์ (น้ำหนัก/ปริมาตร) นอกจากนี้ยังใช้ขี้เลื่อยเหล็กและฟองน้ำในการลดก๊าซซัลเฟอร์ไดออกไซด์และความชื้น ในการทำบริสุทธิ์ก๊าซชีวภาพนั้นมีวัตถุประสงค์เพื่อลดปริมาณคาร์บอนไดออกไซด์ ซึ่งสารละลายด่างอ่อนสามารถใช้ดูดซับคาร์บอนไดออกไซด์ไว้ได้ดี ประสิทธิภาพของวิธีการที่ใช้สารละลายแคลเซียมไฮดรอกไซด์ภายใต้สภาวะปกติได้ผลดีกว่าการใช้โซเดียมไฮดรอกไซด์ นอกจากนี้การดูดซับคาร์บอนไดออกไซด์ของแคลเซียมไฮดอกไซด์มีประสิทธิภาพถึง 60.33 และ 64.00 เปอร์เซ็นต์ เมื่อใช้ความเข้มข้น 1 และ 3 เปอร์เซ็นต์ ตามลำดับ ในขณะที่โซเดียมไฮดรอกไซด์มีประสิทธิภาพการดูดซับเท่ากับ 58.38 และ 62.91 เปอร์เซ็นต์ เมื่อใช้ความเข้มข้น 1 และ 3 เปอร์เซ็นต์ ตามลำดับ ก๊าซที่ผ่านขั้นตอนการเพิ่มความเข้มข้นของมีเทนด้วยแคลเซียมไฮดรอกไซด์ 3 เปอร์เซ็นต์แล้ว พบว่าค่าความร้อนของก๊าซชีวภาพเพิ่มขึ้นถึง 28.73 เปอร์เซ็นต์ จะเห็นได้ว่าก๊าซที่มีความเข้นข้นของไดไฮโดรเจนซัลไฟด์ คาร์บอนไดออกไซด์ต่ำ และการผลิตก๊าซชีวภาพจากระดับห้องปฏิบัติการ รูปแบบการผลิตก๊าซชีวภาพอย่างง่าย และการเพิ่มคุณภาพของก๊าซชีวภาพจากการศึกษาในครั้งนี้ มีความเป็นไปได้และสามารถเป็นทางเลือกที่ดีสำหรับพื้นที่ชนบทต่อไปได้

คำสำคัญ : การผลิตก๊าซชีวภาพ, การหมักร่วมแบบปราศจากออกซิเจน, ต้นเทียนนา, มูลโค, การทำให้บริสุทธิ์

Title	DEVELOPMENT OF METHANE ENHANCEMENT SYSTEM OF BIOGAS PRODUCTION FROM WATER PRIMROSE AND COW DUNG
Author	Miss Nong Thi Thu Huyen
Degree	Master of Engineering in Renewable Energy Engineering
Advisory Committee Chairperson	Dr. Rameshprabu Ramaraj

ABSTRACT

Currently, energy resources and the environment have increased interest and this study concerns alternative sources of energy. Anaerobic digestion or biogas technology is alternative energy and this biological process using biomass as the primary feedstock. This thesis investigates the potential of agricultural weed such as water primrose (*Ludwigia hyssopifolia*) for biogas production. The first research was carried out for 45 days of operation from anaerobic mono-digestion of water primrose in fresh and dry form by using a batch experiment. Pretreatment was applied for substrate using sodium hydroxide (NaOH) solution (w/v) at different concentrations (0, 1, 2, 3, and 4%) with 10% of total solids (TS) based on dry matter. The results showed that the treatment with 2% NaOH was the best condition for water primrose in dry form with the highest performance in biogas yield (8,072.00 mL) and methane content (64.72%). In order to examine the ability of co-digestion with other substrates, different pretreatment time (1, 2 and 3 weeks) of water primrose at 2% NaOH was mixed with cow dung at different ratios (1:1, 2:1 and 1:2 (w/w), water primrose to cow dung). Among the three mixing ratios of co-substrate tested, the best performance in this experiment was achieved at mixing ratio 2:1 for 2 weeks' pretreatment time on water primrose, including all measurements as biogas production (8,610 mL), methane concentration (68.2%), and percentage of total solids (70.84%), volatile solids (64.76%), and chemical oxygen demand (66.55%) removal efficiency. To further support the result from laboratory-scale experiments, pilot-scale work of anaerobic digestion was implemented under more realistic conditions and facilitated future practical application. The best performance of pretreatment time of sodium hydroxide and cow dung ratio to water primrose was chosen for the pilot-scale experiment. A pilot-scale experiment was performed in 1000 L of the digester, which gave an average biogas yield of 1.7 mL/ gTS/day with the highest methane content of 68.6%, thereby, proved that the results from the lab-scale experiment is reliable. The gas produced was collected for quality upgrading by aqueous solutions of sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$) at various concentrations of 1, 2 and 3% (w/v). Also, steel wool and sponge were employed to reduce H_2S and humidity in raw biogas. Biogas purification in this study aimed to achieve high CO_2 removal efficiency in biogas stream at minimal alkali consumption and that feasibility was confirmed. The method's efficiency using $Ca(OH)_2$ solution under normal conditions was competitive to the method using NaOH. Moreover, CO_2 absorption was more prospective for $Ca(OH)_2$ absorbent when 60.33 to 64.00% CO_2 removal efficiency was achieved compared to 58.38 to 62.91% for NaOH absorbent at 1% and 3% concentration, respectively. After enriched methane process, the purified biogas of 3% $Ca(OH)_2$ improved the highest value of calorific heating value reached to 28.73%. Consequently, with a low concentration of H_2S, CO_2, and small operation scale, a simple biogas production and biogas upgrading model implemented in this study is feasible and would be an appropriate choice for rural areas.

Keywords : Biogas Production, Anaerobic Co-digestion, Water Primrose, Cow Dung, Purification

ACKNOWLEDGEMENTS

First and foremost, I would like to express my special thanks of gratitude to my advisor Dr. Rameshprabu Ramaraj as well as my co-advisor Asst. Prof. Dr. Yuwalee Unpaprom who gave me the great opportunity for doing a Master's Degree. Their suggestions and providing invaluable guidance helped me all the time of research and writing of this thesis. Without their persistent, this Master's study could not be completed.

I would aslo like to thank to my committee members, Asst. Prof. Dr. Nigran Homdoung for your advise, valuable comments and encouragement countless times helped me to finish this assignment.

My sincere thanks also goes to Asst. Prof. Dr. Nattawud Dussadee for giving me the opportunity to do research. Your vision and sincerity deeply inspired me. Additionally, a thank you to Asst. Prof. Dr. Tanate Chaichana and Asst. Prof. Dr. Sei- ew Rotjapun for allowing me to use with their Lab equipments as part of my thesis. I also thanks to all the lectures in the School of Renewable Energy, Maejo University for their careness leading me to accomplish this study successfully.

I am truly grateful for the scholarship support of the School of Renewable Energy, Maejo University. This scholarship allows me to concentrate more of my time on studying.

Last but not least, I am depply thank you to Ms. Sawitree Tudtou for letting me take advantage of your lab to complete the experiment and always cheering me on. Finally, I would like to express my tremendous gratitude to everyone in Maejo University supporting me a lot during my research pursuit. I am always thankful for your support.

Nong Thi Thu Huyen

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays energy resources and the environment has increased interest, and this study concern regarding alternative sources of energy. Especially fossil fuels, which play a significant role in the development of various industries, transportations, agriculture sectors and to meet many other basic human needs in modern civilization (Owusu and Asumadu-Sarkodie, 2016). However, the more fossil fuels use, the more toxic gases produce on the environment, such as CO_2, SO_X, and NO_x, which is the primary source of greenhouse gases (Al Seadi, 2008). Therefore, it is necessary to find out renewable energy to replace energy sources derived from fossil fuels. In this context, the anaerobic digestion process could be a better option. Furthermore, this is one of the solutions to solve the biomass waste problems from the crops, agricultural waste, industrial waste, food waste, chicken waste or animal wastes (Al Seadi, 2008). Compared to other renewable energy (such as solar, wind, hydro energy) the anaerobic digestion of biomass was involved less capital investment. In addition, available biomass sources can easily be found in the rural areas. It is not depended on world price or the supply uncertainties as of imported and conventional fuels (Rao et al., 2010).

The production of biogas through anaerobic digestion is not only getting rid of unwanted wastes but also known to minimize the impact on the environment, energy-rich methane can be generated biofuel and energy for electricity and heat. Apart from biogas production, the bio-slurry also produces as a by-product from the anaerobic process, this is a mixture of digested matter and water with a high concentration of mineral substances and nutrients that suitable to be used as fertilizer. Therefore, by changing natural waste into vitality, biogas is using nature's abundant to reuse substances into valuable properties (Bonten et al., 2014).

Almost of microorganisms need oxygen to survive, but in specific environments, there is oxygen-free. Under such an environment, some microorganisms will grow and develop thanks to the amount of oxygen taken from the material and methane (Schnurer and Jarvis, 2010). Mostly, these microorganisms exist in swamps, landfills, covered lagoons, or enclosed tanks called anaerobic digestion. Anaerobic digestion refers to a process producing biogas by fermenting organic materials in absence of air or oxygen with the support of microorganisms to breakdown materials into intermediates to generate mainly methane (CH_4) and carbon dioxide (CO_2) and along with other trace gases. The biogas typically has 50-70% methane and 50% carbon dioxide (Schnurer and Jarvis, 2010).

Once anaerobic digestion is operated, there are some sensitive factors that should be considered. The imbalances could lead to inhibit or fail the process. Factors represent important parameters that can affect the efficiency of anaerobic digestion listed as pH, temperature, total solids, volatile solids, nitrogen ratio, retention time, etc. Moreover, in order to improve biogas yield, their quality or may reduce the retention time needed, pre-treatment methods are applied for substrates such as using fungal, chemical, mechanical, and thermal techniques (Kim et al., 2003). In short, comprehensive knowledge of anaerobic digestion is a key to ensure a stable operation and cost-effective final product. Biogas is a clean fuel and does not cause air pollution. It is considered as a better fuel than natural gas because it does not contain sulfur. Sulfur on burning gets converted into sulfur dioxide, which is responsible for many lung diseases. The efficient utilization of biogas technology has positive effects on the national economy and can readily be integrated with rural development as it provides: no smoking, cleaning fuel for cooking, lighting and running agro-machinery (Kim et al., 2003).

Water primrose is a semi-aquatic plant, rapid growth and spreading in the shallow areas of ponds, lakes, and streams, usually in standing water, rice paddies, that common belonging agricultural area land. *Ludwigia hyssopifolia (L. hyssopifolia)* is the scientific name of water primrose, commonly known as seedbox or 'Tianna' in Thai, belongs to Onagraceae family. Extensively in China, South and Southeast Asia, including Thailand and other tropical countries. Semiaquatic water primrose plants are growing with food crops. Eradicating weeds with herbicides has been adverse effects in food production because weeds compete with crops for water, nutrients, and soil. Also, weeds can harbor insect and disease pests, and noxious weeds and weed seeds can significantly affect crop quality. Recently, in Thailand agricultural processes focusing on organic agriculture. Therefore these weeds possible to remove and gradually reduces the population of weeds from the croplands. On the other hand, this large quantity of potential biomass can be utilized for biofuel applications directly. Up to now, the potential energetic of this biomass has not been investigated, meanwhile the biogas industry's strategy is the input material sources that does replace energy crops and does not a non-food competition for fuel (Zehnsdorf et al., 2018). There is no literature available on water primrose related to biogas production. Therefore, it is a new energy material for biogas production. In this study, the whole parts of water primrose such as flowers, leaves, stems, fruits, roots will be used as a material to produce biogas production. Typically, the material should undergo pre-treated before going the anaerobic process to release much more simple sugars that hold inside the cell wall of lignocellulosic material. Thus, chemical pre-treatment is applied for water primrose to increase biogas yield. Besides that, co-digestion of water primrose and cow dung through anaerobic conditions using different ratios also investigated. The final process in this study is enhancing methane production from raw biogas, which will be conducted for a big scale-up by biogas upgrading technologies using a water scrubber system.

1.2 Objectives of research

1. To investigate the potential of biogas production and energy analyzing from water primrose and cow dung.

2. To figure out the pre-treatment time and mixing ratio affecting biogas production under anaerobic conditions.

3. To design and enhance the quality of biogas production through the purification absorption process and optimize with the engineering model.

1.3 Scope of research

1. Determination of compositions and characteristics of the new energy weed plant from water primrose and cow dung.

2. Evaluation biogas production of pretreated water primrose with sodium hydroxide at different periods of time (one to three weeks).

3. Identify the proper mixing ratio of cow dung and water primrose at various fermentation ratios from 1:1 to 2:1.

4. Pilot-scale testing for biogas production with a capacity of 1000 L volume for 45 days operation.

5. Design biogas upgrading technologies on a lab scale system with chemical scrubbing process.

1.4 Benefit of research

1. Utilizing the available waste sources from agriculture and livestock to produce energy-rich gas.

2. It is controlling the rapid spread of weeds in the field without using toxic methods affecting the environment (pesticides).

3. Appling the research results in rural areas or large farms with high economic efficiency.

4. This study will be bringing high nutritional value to plants from waste generated in the anaerobic process.

CHAPTER 2
LITERATURE REVIEW

2.1 Overview of biogas production

Biogas is known as a mixture of gases where organic materials are decomposed in the absence of oxygen or air by the activity of fermentation bacteria. Biogas consists mainly of 55-70% methane (CH_4), 30-45% carbon dioxide (CO_2) and also contains mall amounts of hydrogen (H_2), hydrogen sulfide (H_2S), oxygen (O_2), Nitrogen (N_2) as presented in Table 2.1 (Jørgensen and Jacob, 2009). Biogas can be produced in a different environment, including at the bottom of ponds and marshes or the digestive of ruminants, in landfills, wastewater treatment plants, or anaerobic digestion (Zhao et al., 2010).

Table 2.1 Typical composition of biogas

Compound	Percentage
Methane, CH_4	55-70
Carbon dioxide, CO_2	30-45
Ammoniac, NH_3	
Hydrogen, H_2	1-2
Sulfide, H_2S	
Oxygen, O_2	
Nitrogen, N_2	Trace
Carbon monoxide (CO)	

Depending on the end-use, biogas will undergo various types of treatment technologies. If the applications require more energy content from gas, there must be implemented a process of improving the quality or usefulness of biogas, called up-gradation. Example for this applications such as fuel for vehicles, in order to produce heat and electricity with fuel cells, or for grid injection that mixed with natural gas (Abatzoglou and Boivin, 2009).

There is a largely diverse type of feedstock as organic matter or organic waste can be used as substrates for biogas production. It is necessary to utilize all fractions from biomass to different value products and generate a minimum amount of waste. Agriculture and forest products industries are two important sectors that contributed to the world economy, the common products provide such as food, feed, fiber shelter, packaging, clothing, and communications. Nevertheless, there are various biomasses and fractions, which are not tailored to these products. However, which could be converted to energy instead of waste through biological technologies (Chum and Overend, 2001). Usually, any biomass with the main component, such as carbohydrates, cellulose, and hemicelluloses, proteins, fats, can be converted to biogas. However, there are not all types of biomass are always given a high yield of biogas and methane, or being applied in AD, even given the right conditions. This may explain that the biomass is not suited for the biological process due to its inert (lignin), take a long time to digest or it is not degraded at all, or maybe the energy created from biomass rather low, not enough to meet the demand (Schnurer and Jarvis, 2010). Today, biogas is widely produced from different biomasses, energy efficiency is achieved when two or more biomasses are mixed, or the opposite can occur. The optimum is that the substrate component should provide a high gas output in a reasonable time, stable and powerful. A substrate with high gas-generating potential, may require an unreasonable long decomposition time or cause disturbance to the process. Therefore, selecting a substrate or a substrate mixture needs to consider not only the potential for gas production but also its composition. A material that works well in anaerobic digestion when it meets the nutritional requirements of microorganisms as well as creating favorable conditions for parameters such as the organic loading rate, retention time and temperature. It also depends on what pre-treatment of the substrate used (Schnurer and Jarvis, 2010).

Biogas is commonly made from the animal slurry, sludge settled from wastewater and landfills containing organic wastes. However, biogas, also is made from almost any organic waste, has the ability to produce to biogas: human excreta, slurry, animal slurry, fruit and vegetable waste, slaughterhouse waste, dairy factory waste. Many wastewaters contain an organic compound that may be converted to biogas, including municipal wastewater, food processing wastewater and much industrial wastewater. Raja and Wazir (2017) stated that solid and semi-solid materials that include plant or animal matter can be converted to biogas. The potential of methane yield from some typical materials given as Table 2.2:

Table 2.2 Percentage of methane from a typical substrate

Substrate	Methane %
Cattle manure	65
Poultry manure	60
Farmyard manure	55
Straw	59
Grass	70
Leaves	58
Kitchen waste	50
Algae	63
Water hyacinths	52

In addition, according to Sawyerr et al. (2019), the biomass sources are varied and can identify as bellow:

Biogas from wood and weeds

Without pre-treatment, the woody biomass is not suitable for biogas production. According to Milke et al. (2010) in anaerobic digestion, the best estimated for untreated wood degradation to be <20 percentage, or 10 percentage for average estimate value, the ability convert to methane was about 5 percentage of carbon. Besides that, the author reported at very tiny particle sizes could be got higher conversion efficiencies.

There is a considerable potential of biogas production from weeds because of several reasons (Gunaseelan, 1997): Weed thrives on soils without input and watering, less affected by pests and weather. The use of weed to produce biogas is an excellent idea to remove it from crops as well as control its growth.

Biogas from leaves and grass

The leaves biomass produced higher methane than terms. However, some toxic compound exists on leaves can inhibit the process and methane production can decrease. The co-digestion of leaves and animal manure achieved higher biogas production compared to the digestion of manure alone (Chynoweth et al., 1993). Regarding the grass, the age affects much on methane, the younger grass, the more methane obtained, and opposite to older grass. This may be due to younger grass contains less lignin (Shiralipour and Smith, 1984).

A study by Sidibe and Hashimoto (1990) was investigated that methane yield obtained from grass straw quite higher than dairy manure, the result was achieved 356 ± 8 ml/g volatile solids from fescue grass and 341± 5ml/g volatile solids from dry grass straw, the significant lower yield was found in dairy cattle manure (288 ±3 ml/g volatile solids). In this case, nitrogen is not a limiting nutrient in the fermentation of grass straw to methane. Depending on the nature of feedstock, their biogas yield potential will be different. De Renzo (1977) stated that biomass from aquatic plants, for instance, algae and moss, are decomposed better than terrestrial plants in anaerobic digestion due to its toughness. For a material easy to digest, that results in more biogas production.

Biogas from fruit, vegetable solid waste and organic municipal solid waste

The laboratory trials from fruit and vegetable solid waste are featured by high percentages of moisture (>80 %) and VS (>95 %) and have a very high biodegradability percentage, result in high methane yield (Sawyerr et al., 2019). The municipal solid waste originates from various material sources and contains different compositions. At a 35°C, the maximum of methane obtained from organic municipal waste, without paper and wood (Mata-Alvarez et al., 1990).

Water primrose

Water primrose is a non-woody plant, stands erect along with wet soil or float out across the water surface. The plant is easy to cut down or dig up but it is difficult to control their spreading because it will re-live from seeds or remaining roots. The plant systematics as below:

Common name: Water primrose

Scientific name: Ludwigia hyssopifolia

Taxonomic Tree

Kingdom: Plantae

Class: Dicotyledonae

Family: Onagraceae

Genus: Ludwigia

Researching in the Philippines, Pancho (1964) has shown that one plant of *Ludwigia hyssopifolia* growth in rice, resulting in 75,000 seeds and amount to 16,000 seeds per gram with the long in 0.5mm. The ability to strongly spread along the water surface may result in the formation of dense floating mats. It may become a nuisance and necessary control from the unwanted area once it grows too rampantly that affecting crops or aquatic life. Manual weeding is not the best choice to remove it completely, this way can be time-consuming and labor-intensive, so, farmers, mostly depend mainly on the use of herbicides. However, the plant is considered as a medicinal plant due to various compounds in leaves, fruits and roots that have medicinal properties such as saponins, tannins, polyphenols, alkaloids and flavonoids etc., which are used as astringents, anthelmintics, carminatives and diuretics. Moreover, the decoction from water primrose can be used to treat diarrhea, dysentery, leucorrhoea and spitting of blood (Pancho, 1964).

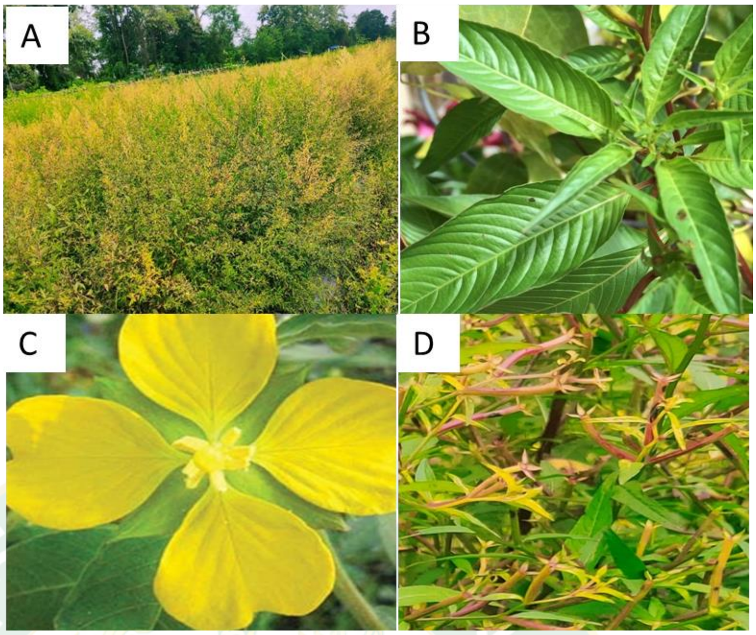


Figure 2.1 Water primrose

(A) Plants in nature, (B) Leaves, (C) Flowers, (D) Fruits

2.2 Anaerobic digestion

Anaerobic digestion (AD) sometimes it is also called biomethanation or in a simple word as biogas treatment. AD is a biochemical process that takes place through four successive biological and chemical stages in absence of oxygen, by various types of anaerobic microorganisms in order to produce biogas as an end product. The biogas potential depends on the type of complex substrates and the ability of biodegradable carbohydrates (cellulose, hemicellulose and lignin fractions), proteins and lipids (Mulat and Horn, 2018). It can be defined as mono-digestion or co-digestion depending on substrates that are fed into AD. Common to most biogas digestion today is co-digestion, which is a homogenous mixture of two or more substrate types (typically animal manure with waste from crops or industries) (Braun and Wellinger, 2003). Reactor design varies, but working based on general principle is to allow microorganisms to break down organic matter in a closed system without oxygen enter.

Even though anaerobic digestion happened partly, however, it can be divided in to four stages, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During the process, the large organic compounds are broken down into smaller molecules by hydrolyzing and fermenting microorganisms and produce mainly acetate, hydrogen and different amounts of volatile fatty acids, methane is produced from two groups of methanogenic bacteria, one is acetate and the other are hydrogen and carbon dioxide (Raja and Wazir, 2017). The degradation of organic matter into biogas was presented in Fig. 2.2.

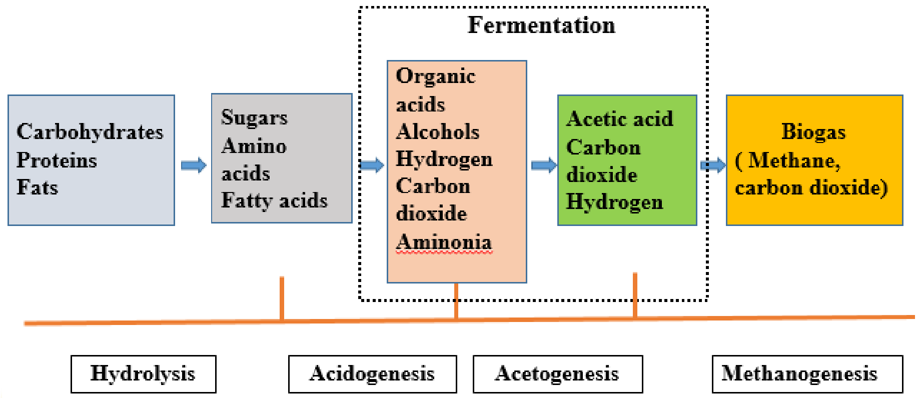


Figure 2.2 Four stages process of anaerobic digestion

2.3 Anaerobic digestion process

Hydrolysis

Biomass is made up of organic matter such as carbohydrates, proteins and fats. The cells of these materials are still intact, which constituent long-chain molecules (polymers), typically of carbon and hydrogen, the monomers are held within the cell walls and are not available to the microorganism to digest. In the hydrolysis stage, these organic matters are hydrolyzed by microorganisms into smaller molecules such as simple sugars, amino acids and fatty acids. Some of by-products were formed, including volatile fatty acids (acetate, butyrate, propionate, and lactate) and hydrogen being precursors for methanogens stage in later steps of the anaerobic digestion process. Hydrolysis is the slowest of the four degradation steps. Most of the microorganisms secrete a number of specific extracellular enzymes to break down complex organic material into tiny parts. And then microorganisms can easily access and directly absorb into the cells of microbial groups as well as use as a source of energy and nutrition. Each type of organic matter has different groups of extracellular enzymes, for examples, saccharolytic is an enzyme that microorganisms use to break down different sugars, while proteolytic is considered that break down proteins. Normally, cellulose and hemicellulose take more time to decompose than proteins and fats in the same process at the hydrolysis phase due to its solubility and character (Schnurer and Jarvis, 2010).

The reaction equation of the process can occur as shown below (Anukam et al., 2019):

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow (C_6H_{12}O_6)_n + nH_2 \qquad \text{..........Equation 1}$$

The molecules are still relatively large, and therefore in the following acidogenesis step, the microorganism continues further decompose the products resulted from hydrolysis in order to produce methane.

Acidogenesis

Acidogenesis is the next step of anaerobic digestion, this stage can be named as the fermentation process and occurs several reactions rather than hydrolysis. Many microorganisms used in the fermentation step are similar to the first stage but others are available active (Schnurer and Jarvis, 2010). The products from the hydrolysis step (sugars, amino acids, alcohols) need to further degradation for utilized directly by methanogenic and fermenting microorganisms that can be used as substrates. However, fatty acids were not degraded by fermentation microbial groups until the next stage of anaerobic digestion. During anaerobic digestion, the acidogenesis stage is known as the fastest step decomposed complex organic matter (Vavilin et al., 1996).

During acidogenesis, depending on the substrates, environmental conditions and type of microorganism present, compounds are formed through reactions. The products in the hydrolysis step mainly converted into volatile fatty acids (acetic acid, propionic acid, butyric acid, succinic acid, lactic acid etc. along with alcohols, ammonia and hydrogen sulfide (from amino acids), carbon dioxide and hydrogen (Schnurer and Jarvis, 2010). The organic matter is still quite a large volume and not suitable for methane production (Muzenda, 2014). In this stage, the reaction could be summarized as below (Anukam et al., 2019):

$$C_6H_{12}O_6 \rightleftharpoons 2CH_3CH_2OH + 2CO_2 \qquad \text{..........Equation 2}$$

$$C_6H_{12}O_6 + 2H_2 \rightleftharpoons 2\,CH_3CH_2COOH + 2\,H_2O \qquad \text{..........Equation 3}$$

$C_6H_{12}O_6 \longrightarrow 3CH_3COOH$ .... Equation 4

Acetogenesis

Under various anaerobic oxidation conditions, the products created through the acidogenesis are further digested by acetogenins to produce mostly acetic acid, CO_2 and H_2. Acetogens break down the substrates to the point that methanogens can create as much methane as possible (Raja and Wazir, 2017). The products form this stage were then used as the substrates for the last step in anaerobic digestion, named methanogenesis. Both processes of acetogenesis and methanogenesis usually work parallel as the symbiosis of two groups of organisms (Al Seadi, 2008).

The relative reactions are presented (Anukam et al., 2019):

$CH_3CH_2COO^- + 3H_2O \rightleftharpoons CH_3COO^- + H + HCO_3^- + 3H_2$ ....Equation 5

$C_6H_{12}O_6 + 2H_2O \rightleftharpoons 2CH_3COOH + 2CO_2 + 4H_2$ ....Equation 6

$CH_3CH_2OH + 2H_2O \rightleftharpoons CH_3COO^- + 3H_2 + H^+$ ....Equation 7

Methanogenesis

Mostly formation of methane and carbon dioxide are the final step of the biogas process, methanogenesis. In this stage, the intermediate products are consumed by methanogenic bacteria based on the substrates at the step of acetogenesis, namely hydrogen gas, carbon dioxide, and acetate. Under stable conditions, major methane production (70 %) was created from the degradation of acetic acid, while the other (30%) was created from carbon dioxide and hydrogen, the growth rate of the methanogens is one fifth compared to the acid-forming bacteria (Jørgensen and Jacob, 2009). It should be noted that methanogens are strongly influenced by several factors, such as pH changes and the presence of heavy metals and organic pollutants, the equation occurs as following (Anukam et al., 2019):

$CH_3COOH \longrightarrow CH_4 + CO_2$ ....Equation 8

$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$ ....Equation 9

$2CH_3CH_2OH + CO_2 \longrightarrow CH_4 + 2CH_3COOH$ ....Equation 10

2.4 Factors affecting biogas production

pH and volatile fatty acids, alkalinity

The pH parameter indicates the health of the anaerobic digestion. During the anaerobic digestion process, the value of pH corresponds to the mixture of the substrate. Generally, pH environment lies at narrow value interval of 5.5 to 8.0 and optimum operating with neutral pH between 6 to 7, either upper or lower than that value, the methanogen population will be affected leads to inhibit or stop producing biogas (Kumar, 2012). Therefore, the pH in AD should be as close to neutral as possible. At the acidogenesis stage of AD process, pH falls lower than optimum value due to acid transformation (fatty acids, acetic acids), while most methanogens growth a pH environment between 6.5 -7.5 (Al Seadi, 2008). The pH value can be increased by the substrate degradation of protein-producing ammonia or decreased by the accumulation of VFA inside the digester.

pH and VFA have quite closely related. The VFA is mainly presenting of acetic, propionic, butyric, and vary acid formed in the fermentation stage, these by-product will be transformed into final production in AD (methane and carbon dioxide). High VFA concentration decreases the pH-value causing a toxic environment and inhibition of the growth of methanogenic bacteria, therefore, leading to a decrease in gas productions. On the other side, the higher accumulative VFA obtains, the more biogas potential produces because it is a key to constituting the methanogenic stage later.
The drop of pH value not only expressed by the accumulative VFA but also the buffer capacity of the digester. The anaerobic digestion will be detrimental if the environment is too acidic or too alkaline condition. Alkalinity is a parameter showing the amount of alkaline (base) content in the substrate and represents in AD process as a buffering capacity, which controlling pH value when the acidity derived from the acidogenesis process (Chen et al., 2010; Meegoda et al., 2018).

Alkalinity involves the component of proteins and amino acids from the nitrogen-rich substrate which on degradation generates ammonia. Ammoniac react with CO_2 to generate bicarbonate, in the digester, the value of pH is mostly controlled by the bicarbonate buffer system (Al Seadi, 2008). According to Schnurer and Jarvis (2010), high alkalinity can ensure for increasing fatty acids without affected to decreasing pH, however, too high alkalinity concentration will inhibit methanogenic bacteria due to ammonia release; the acid production also influents on alkalinity, low concentration of alkalinity caused by the high acid production in the process. Alkalinity supplementation by added several chemicals into the digester in order to adjust pH such as NaOH, KOH, $Ca(OH)_2$ $CaCO_3$, Na_2CO_3 (Demirel and Scherer, 2008).

The ratio of VFA to alkalinity can be used to assess process stability, at the ratio lower than 0.3 then the process is stable; at the ratio higher than 1.0, the decreasing biogas produced along with foaming is high; at the value between 0.3-1.0, there is some instability in the process (Schnurer and Jarvis, 2010).

Temperature

The AD microorganisms are highly susceptible to the different elements; even small changes in the substrate can lead to collapse biogas system. The very first parameter affects to microorganism operating in AD is temperature as it influences the activity of enzyme, the waste quality and the gas production (Keskin et al., 2018), and it should keep a constant temperature as microorganisms take quite long time to get used to new temperature (Al Seadi, 2008). The higher yield of biogas can be achieved at high temperature. However this must be in a range of suitable temperatures due to the metabolic process will be decline if AD operates at too high temperature (Kumar, 2012). Anaerobic microbes and bacteria can divide into three temperature ranges are below 25oC for psychrophilic, mesophilic (25°C- 45°C) and thermophilic (45°C– 70°C) (Jørgensen and Jacob, 2009), the optimum temperature for microorganism grows at a faster rate thus can produce a lot of biogas is 10°C, 20-45°C and > 50°C for psychrophilic, mesophilic and thermophilic, respectively (Muzenda, 2014). Cooler digesters take more time to break down the biodegradable feedstock, while hotter ones may not break down the biodegradable feedstock due to bacteria remaining in the dormant stage.

Carbon/nitrogen: Nitrogen ratio

In order to make ensure that biogas is produced stably and continuously, it must provide sufficient raw materials for microorganisms to grow and develop. Two of the most important nutrients are carbon (C) and nitrogen (N). Mostly carbon contains in agriculture waste, green grass while nitrogen can be found in domestic sewage and animal and poultry wastes, these nutrients are necessary for gas production and organism's growth (Raja and Wazir, 2017).

Normally, microorganisms in digester consume carbon 20-30 times greater than nitrogen, it is necessary to keep the proper ratio of carbon and nitrogen in substrates. (Muzenda, 2014) recommended the optimum ratio of substrates would be 20-30:1. Otherwise microorganisms are restricted to grow. If C/N ratio is higher than that range, the microbial groups during methanogenesis stage will rapidly consume much nitrogen for meeting their protein demands and will not occur any reacts with the carbon remaining in the substrates therefore, it will reduce the biogas production. Materials with low C/N, will accumulate in the form of ammonia causing rising pH value that leads to a toxic environment for methanogenic bacteria during the digestion (Kumar, 2012).

Retention time

The gas produced in the digestion depends partly on composition of substrate, some materials are easily and quickly to digested such as sugar and starch, due to not undergo hydrolysis stage in AD process, thus, that type of materials require short retention time while fiber and cellulose plant matter requires hydrolysis then will take more or longer retention time for decomposition process. In the digestion tank, carbohydrates, proteins, fats contain in materials will be decomposed and converted into methane and carbon dioxide gas (Muzenda, 2014). The retention time is defined as the time need for organic material to digest completely by microorganisms, it is also used for pre-estimate size and cost of the digester.

Total solids and volatile solids

Total solids (TS) along with volatile solids (VS), reflect performances of the digestion process. Total solids are known as solids retrieved after evaporation and drying of an organic matter at 105^{o}C (Orhorhoro et al., 2017). Typically, the content of TS represents below 10% of the total volume (Nelson, 2010).

Yavini et al. (2014) reported that from agricultural waste, the optimum biogas yield was achieved at TS of 9%, any changes, even increasing (12%) or decreasing (2%, 5%, and 7%) the percentage TS concentration both affect to gas production. In this case, beyond the optimum TS value, the efficiency of biogas production has tended to decrease. This can be explained that when TS value increases, the water volume drops, therefore, reducing the level of microbial community activity and then affect to biogas yield.

Volatile solids are determined by the solids that remaining from total solids were furthermore burned at 600^{o}C (Orhorhoro et al., 2017). The VS content can be predicted the potential of methane produced from a substrate. Orhorhoro et al. (2017) found that 10.16% of TS was recommended for operating a biogas system, above that value, the biogas yield was reduced. In addition, when VS content increases, higher quantities of biogas production creates.

2.5 Mono-digestion and co-digestion

Biogas can be produced by a substrate or combine various substrates from the feedstock. From a study by Bouallagui et al. (2005), traditionally, a mono-digestion, which single substrate being applied in AD, such as fruit and vegetable wastes contain high content of solid around 8–18% total solids, volatile solid of 86–92%, and up to 75% easy biodegradable material (sugars and hemicellulose) that made up 95% methane. However, the significant limitation of AD from fruit and vegetable wastes is the imbalanced nutrients of carbon and nitrogen resulted in the rapid formation of volatile fatty acid production cause inhibits methane bacteria. In order to enhance biogas yield of solid waste, co-digestion is a great selection when more substrate applied at once time due to positive synergisms in the digestion environment and improve nutrient balance by the co-substrates (Mata-Alvarez et al., 2000). A study from De Vries et al. (2012) was investigated the environmental consequences of anaerobic mono- and co-digestion of pig manure to produce bio-energy. Six trials were evaluated: mono-digestion of manure, co-digestion with: maize silage, maize silage and glycerin, beet tails, wheat yeast concentrate, and roadside grass. The results indicated that mono digestion created a limited source for bio-energy but reduced most impacts. Co-digestion with animal feed increased bio-energy production but had an environmental impact. The study showed the best environmental performance from co-digestion with wastes or residues like roadside grass.

Aragaw and Gessesse (2013) examined a series of experiments of co-digestion from cattle manure with organic kitchen waste using rumen fluid at a different ratio. The results show the highest methane yield, increased 24-47% over the control, was obtained with ratio with 75% organic kitchen waste and 25% cattle manure, addition cattle manure cause inhibition process or significant methane yield produced.

Lehtomäki et al. (2007) conducted laboratory batch tests on grass silage, sugar beet tops and oat straw with the cow in manure continuously stirred tank reactors. The highest methane yield was found in co-digestion of cow manure with grass, sugar beet tops, and straw, with up to 30% VS of crops in the feedstock. Further supply more volatile solid of feedstock up to 40%, the methane yield went down 4%-12%, meanwhile doubling the loading rate from 2-4 kg VS/ m^3 day, methane yield decrease from 16-26%, leaving a lot of untapped methane potential in the substrates.
Another researcher was documented that field grass that has high level of gas flammability could be improved biogas production in case combined with rabbit and cow dung, the fastest onset of gas flammability from the blend of grass-rabbit leading to produce 3 times gas production higher than that of field grass alone (Uzodinma and Ofoefule, 2009).

2.6 Pre-treatment biomass

Pre-treatment relate the disruption of recalcitrant material of biomass, including essentially physical and thermochemical process. Lignocellulosic biomass, is a major composition, content in many types of feedstock such as woody biomass, agricultural residues, energy crops, various types of cellulosic wastes (Kim et al., 2016). The convert of lignocellulose biomass to biogas start with a numerous bacteria breakdown of the large molecular into monomolecular, namely the simple sugars. In order for bacteria to enter the cell walls of biomass to release simple sugars, because of the tight binding of lignin, hemicellulose, and cellulose, making it difficult to separate, therefore need a further step that desires lower lignin content, pre-treatment (Patinvoh et al., 2017). There are many methods of pre-treatment treated to the substrate for the biogas process to enhance its available degradation, the methods produce maximum biogas production depends on the substrate's chemical composition and structure (Schnurer and Jarvis, 2010). The pre-treatment process can be started with a mechanical disruption method, using a mill, blender, or rotating knives that will surface area of the material increases making an easy way to bacteria attach to the material.

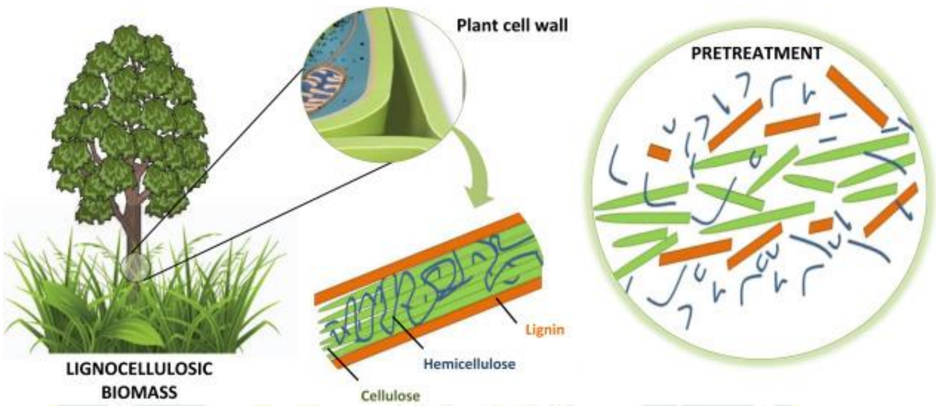


Figure 2.3 Pre-treatment process beak down the parts of the biomass (Mussatto and Dragone, 2016)

Physical pretreatment

The physical process means a method that does not use any chemical reagent or microorganism in the process. Some techniques can be used, including: Comminution, steam-explosion, liquid hot water pretreatment (Zheng et al., 2014).

Comminution

Comminution aims to reduce particle size and the degree of cellulose crystallinity, the degree of cellulose polymerization and increase the accessible surface area. Comminution can use the machine as milling and grinding, including some tools ball, vibrio, hammer, knife, two-roll, colloid, and attrition mills, or extruders. The suitable methods depend on the content of moisture of the feedstock (Zheng et al., 2014). With the moisture content from 10 to 15% (wet basis) the best method to use for feedstock is two-roll, hammer, attrition and knife mills. Higher moisture over 20%, suitable method are used: Colloid mills and extruders (Kratky and Jirout, 2011; Taherzadeh and Karimi, 2008).

Fernández-Cegrí et al. (2012) documented that the optimum methane yield achieved with the largest size (1.4-2.0 mm) of 213 mL/g volatile solids (VS) when compared with smaller particle sizes of 0.36- 0.55 mm and 0.71- 1.0 mm. These results proved that reducing oversize leading to overproduction of VFAs during the process, therefore, inhibiting methane production.

However, it also based on its chemical composition, further reducing particle size, could be achieve more methane yield, Kivaisi and Eliapenda (1994) reported that when reducing particle sizes of bagasse and coconut fibers from 5 mm to less than 0.85 mm, that make increasing over 40% degradation of total fiber and volatile fatty acids production. Thus, the methane yield was enhanced by an average of 30%.

Steam- explosion

Steam- explosion can be called autohydrolysis, are heated with high-pressure saturated steam, will heat biomass particles in a short time and the pressure is quickly reduced to stop the reactions, which causes the biomass to undergo an explosive decompression. In general, the pre-treatment pressure of 0.69- 4.83 MPa, temperature operates within the range of 160-260°C, time from seconds to a few minutes (Sun and Cheng, 2002).

Pre-treatment with steam- explosion makes biomass easy to degrade, the hydrolysis of hemicellulose based on the formation of acetic acids and other acids from acetyl or other functional groups. In addition, at high temperatures, water has certain acid properties, this will help to improve catalyzes hemicellulose hydrolysis. Thus, the degradation to form single sugars can occur during steam-explosion (Weil et al., 1997).

From the bulrush, Wang et al. (2010) found that using the steam-explosion method can improve methane yield compare to raw material. The optimum condition to achieve the highest methane yield at 205.3 ml per degradable VS (24% higher than that of untreated bulrush) with 11% moisture content, steam pressure of 1.72 MPa and residence time of 8.14 min. However, Teghammar et al. (2010) shown that the method has a negative on methane production from paper tube residuals; the methane reduced when increasing temperature from 10 to 30 minutes from 220°C. This phenomenon can be explained by steam-explosion had a weak ability to remove lignin. The addition of NaOH chemical combination has been shown to increase methane production.

Liquid hot water

In this method, no chemicals are added in the process. The pressure is used to maintain water in the liquid state at high temperatures, this pretreatment is highly effective for the expansion of the accessible and sensitive surface area of cellulose and improvement breaking down cellulose into bacteria and enzymes. During pretreatment, water can penetrate biomass cell structure, hydrate cellulose, dissolve hemicellulose and remove slightly lignin (Zheng et al., 2014).

A pretreatment by boiling pre-treatment with different retention time to boost methane yield was tested by In the 1:1 ratio of co-digestion mixture of buffalo grass and buffalo dung, the highest methane yield was obtained. Boiling pre-treatment was continuously kept at a temperature of 100^oC with changing different retention time at 0.5, 1, 1.5 and 2 hours for buffalo grass. The best optimal condition in this study was found at 100^oC with 2h retention time. In addition, at the same that optimal conditions, methane yield obtained in the co-digestion (grass and dung) were 5% higher than mono digestion (grass). The study indicated that the upgraded biogas through biological purification could achieve high methane yield up to 90.42%, the digestate contended high nutrient concentrations would be an efficient alternative fertilizer. Wheat straw can be used as a substrate for producing biogas, the biogas potential with pre-treatment as hydrothermal method given an increase of 9.2% in biogas production and 20% in methane production compared to that of the raw wheat straw substrate (Chandra et al., 2012). Fernández-Cegrí et al. (2012) reported effecting of pre-treatment with different temperatures from 25 to 200^oC for sunflower oil. At 100^oC, the overall highest methane yield was achieved, however, this value was only 6.5% higher than pretreatment at 25^oC.

Chemical pretreatment

Chemicals pretreatment involves the use of chemicals such as base (NaOH, $Ca(OH)_2$, CaO, KOH), acids (H_2SO_4, HCl, HNO_3, H_3PO_4, acetic acid, maleic acid) and ionic liquids (Zheng et al., 2014).

Alkaline pretreatment

Alkaline pretreatment uses the base to eliminate lignin for improving the biomass more degradable (Zheng et al., 2014). The purpose of alkaline pretreatment is to remove lignin-carbohydrate bonds and part of lignocellulose (Tarkow and Feist, 1969). By eliminating cross-linking, alkali pretreatment leads to increased porosity and inside the surface area, swollen structure, reduce the level of polymerization and crystals, disrupt lignin and structure (Fang et al., 1987).

Many researchers have been investigated the effect of alkaline pretreatment on the chemical composition and methane yield. Wheat straw was soaked in NaOH solution 1 and 10% g NaOH/g TS with a TS concentration of 160 g TS/L in closed bottles, at 40^{o}C for 24 h. The methane production improved from 14 to 31% for ensiled sorghum forage at 1 and 10% NaOH dosages. NaOH pre-treatment improved the hydrolysis of cellulose and hemicelluloses also be found in this experiment (Sambusiti et al., 2012).

Chen et al. (2010) performed 3 sets of experiments, using a straw with NaOH treatment (pretreatment), digested straw with NaOH treatment (post-treatment) and straw digested only (control). The cell wall was destroyed at the concentration of NaOH 5% for 48 h. the paper indicated that NaOH treatment on the digested rice straw saved 50% NaOH uses, therefore post-treatment was economical and feasible for biogas production. Zhu et al. (2010) conducted experiments using different NaOH loading in order to tested solid-state pretreatment of corn stover. The degradation of lignin increase following NaOH concentration from 9.1% to 46.2% at 1% to 7.5% (w/w), respectively. The highest methane yield was 372.4 L/kg VS with 5% pre-treatment, compared to untreated corn stover, it was 37.0% higher than that. With the loading 1% NaOH concentration, there was no improvement in biogas yield.

Ofoefule et al. (2009) was investigated the production of biogas from different pre-treatment methods from Water Hyacinth. Methods were applied in these experiments namely: Dried and crushed alone, dried and treated with alkaline (KOH), dried and blended with cow dung and freshwater Hyacinth used as control. The retention time was 32 days under a mesophilic temperature. The highest biogas production from the mixture of dried and cow dung with the composition of methane was 64% and 35.94% of carbon dioxide but the highest methane percentage was contained from dried and treated with alkaline (71.0% of methane, 28.94% of carbon dioxide). The authors indicated that biogas yield from water hyacinth could be significantly enhanced by drying and combining it with cow dung.

Acid pretreatment

The most common acid pretreatment used was sulfuric acid (H_2SO_4), the experiments can be conducted under high temperature (230°C) and dilute acids (0.1%) or low temperature (40°C) and high concentration of acids (30-70%). High concentrated acids given more effect on cellulose hydrolysis than dilute acids, because it is a toxic chemical, corrosive dangerous as well as requires expensive materials. Also, it must be recovered after biomass treatment for economic reasons, as it is energy-intensive and costly. Therefore, dilute acids are preferred over concentrated acids for lignocellulose biomass pretreatment. The aim of using acid pretreatment is to increase cellulose susceptibility to microbial degradation and enzyme hydrolysis (Zheng et al., 2014). Methane potential was investigated by Monlau et al. (2013) from sunflower oil cakes by dilute acid pretreatment. Methane yield without pretreatment was obtained 195 mL CH_4/g VS, the significant higher achieved (302 ± 10 mL CH_4/g VS) after acid pretreatment at 170°C. At the same temperature, further thermal treatment alone and low concentration of acid (<1%) had no effect on methane yield due to the formation of recalcitrant in the liquid phase. A pretreatment using 2% w/v of H_2SO_4 at ambient temperature and 121°C for 1h did not improve methane potential on all test feedstock (rapeseed and sunflower meal and straws) was proved by Antonopoulou et al. (2010). The reason made methane yield fall maybe from the toxic compound, which was released from the pretreatment process.

Biological pretreatment

Biological pretreatment for improvement of biogas production in anaerobic digestion generally focus on fungal pretreatment, pretreatment by microbial consortium, and enzymatic pretreatment. This method do not require chemical addition and lower energy input compared to physical and chemical pretreatment method (Zheng et al., 2014). One of the most benefits of biological pretreatment is that the process is green without using chemicals. Therefore, there is non- release any hazardous or toxic compounds to the environment (Sindhu et al., 2016; Ummalyma et al., 2019).

2.7 Design of experiments (DoE)

A design of experiment (DoE) or experimental design is a collection of tools used in many technical fields, especially in areas of science and industry (Aydar, 2018). DoE is a method based on the relationship between factors affecting a process and the output of that process. More clearly, a unit of the experiment is conducted based on the application of treatments, and then on a scientific method to measure one or more subsequent reactions (Aydar, 2018). This method has given researchers a big picture of their experiments through the control process inputs and optimizes the output.

DoE is a process including planning, implementing, analyzing a set of experiments, then evaluate the influents of variables on that system. According to Mäkelä (2017), an experiment aims to predict the outcome by a statistically valid model that contains information about one or several independent variables, known as input variables or predictor variables. Once one or more dependent variables are changed, that results in a change of one or more dependent variables, also known as an output variable or response variables. At this point, the statistically valid model will be used to predict future observations within the design principle. DoE, therefore, not only saving time, effort, and money but also being valid, reliable and scalable.

Response surface methodology (RSM)

One of the most common applications of experimental designs is the response surface methodology (RSM). RSM is used to examine the relationship between one or more response variables and a set of quantitative experimental variables or factors. This method is usually applied after having identified several important controllable factors and a desire to find the values of the factors for an optimal response. In other words, RSM is a group of mathematical and statistical techniques to develop, improve and optimize processes. RSM may be used to figure out the element values (conditionals) for best response or that satisfy the characteristics of the process, identify new implementation conditions that will improve product quality (response) compared to current conditions and demonstrate the relationship between quantitative factors and response (Bradley, 2007).

The surface response is a method based on surface position. Therefore, the main objective of the study is to understand the topography of the response surface including local maximum lines, local area, minimum and slopes and find areas that occur most appropriate response (Bradley, 2007). The linear function is the basic model that can be used in RSM. For its application, the respond response must be obtained following the following equation (Bezerra et al., 2008) :

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \varepsilon$$ ....Equation 11

Where:

k: The number of variables

β_0: The constant term

β_i : Represents the coefficients of the linear parameters

x_i : Represents the variables

ε: The residual associated to the experiments.

Central composite design

The central composite design was presented by Box and Wilson, which can be called A Box-Wilson Central Composite Design. This is one of the most popular standards of RSM design (Bezerra et al., 2008). According Chauhan et al. (2013), the central composite design has 3 groups: (1) two-level factorial or fractional factorial design points, (2) axial points (sometimes called 'star' or 'alpha' points), and (3) center points. CCD is used to estimate the coefficients of a quadratic model. All point descriptions are in terms of the coded values of the factors.

Factorial points

The two-level factorial part of the design consists of all possible combinations of the +1 and -1 levels of the factors, this is four design points: (-1, -1) (+1, -1) (-1, +1) (+1, +1)

Axial, star or alpha points

The star points have all of the factors set to 0, the midpoint, except one factor which has the value +/- α. For a two-factor problem, the star points are: (-α, 0) (+α, 0) (0, α) (0, + α)

Center points

Center point is the points with all levels set to coded level 0; and the midpoint of each factor range (0, 0). Center points are usually repeated 4-6 times to get a reasonable estimate of an experimental error (pure error). Figs.2.4 (a and b) illustrates the full central composite design for the optimization of two and three variables.

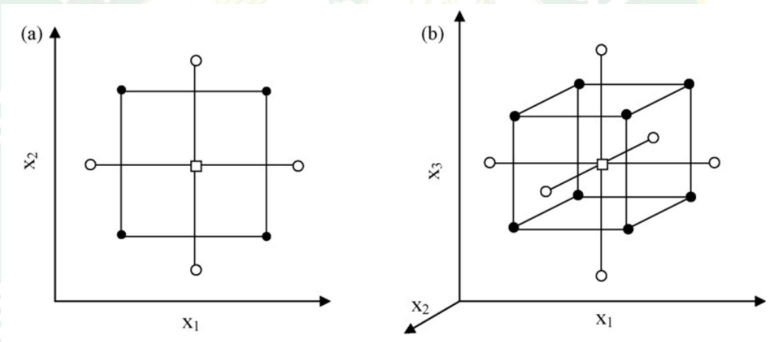


Figure 2.4 *Central composite designs for the optimization of: (a) two variables (α= 1.41) and (b) three variables (α= 1.68). (●)Points of factorial design, (O) axial points and (□) central point (Bezerra et al., 2008)*

Box-Behnken designs

The authors, Box and Behnken, suggested how to select points from the three-level factorial arrangement, which allows the efficient estimation of the first-and second-order coefficients of the mathematical model. In order to describe linear, quadratic and interaction effects, second-order polynomial has to be used in the modeling.

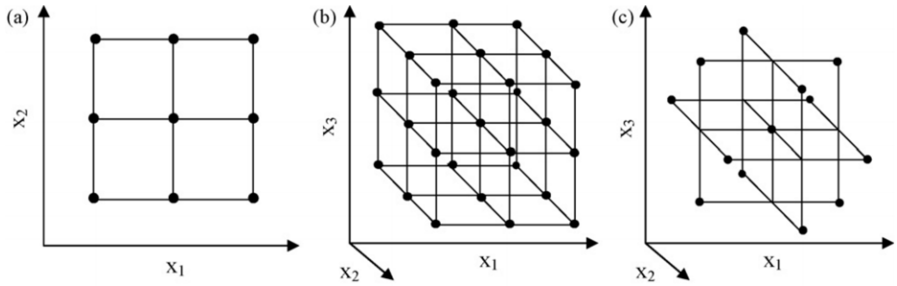


Figure 2.5 Experimental designs based on the study of all variables in three levels: a three-level factorial design for the optimization of (a) two variables and (b) three variables and (c) Box–Behnken design for the optimization of three variables

For 3 factors, the BBD offers some advantage in requiring a fewer number of runs. For 4 or more factors, this advantage no longer exists. Thus, the select of a suitable design when applying RSM depends on the number of factors to optimize the performance.

2.8 Purification

Although biogases consist mainly of methane and carbon dioxide and refer as fuel for many applications as cooking, lighting, cooling, drying. Nevertheless, biogases also contain significant quantities of unwanted compounds as hydrogen sulfide, ammonia, oxygen, moisture. Some problems can be caused by these compounds, firstly, these gases can be detrimental to any heat or biogas heat exchanger (for example, corrosion, erosion, and blockage), besides that, they also create harmful environmental. In order to remove sour gas or reduce machine and environmental harm as well as further utilization, biogas purification steps are necessary for its final use processes (Abatzoglou and Boivin, 2009).

Water scrubbing and organic solvent scrubbing

The removal of impurities by water scrubbing is applied to remove trace gases from biogas production because these gases are more soluble in water than methane, such as CO_2 and H_2S. The process of absorption is purely physical. Based on the solubility of different gases constituents in a liquid scrubbing solution, the effect to separate will be different. H_2S is considered to remove from mixture gases before removal CO_2 gas, due to dissolved H_2S is highly corrosive and unpleasant odor can cause operational problems. In general, the solubility of H_2S in water is higher than that of CO_2. Therefore, CO_2 will be removed at the same time with H_2S (Zhao et al., 2010).

Usually, the biogas is pressurized and fed to the bottom of a packed column while water is fed on the top and so the absorption process is operated counter-currently (Zhao et al., 2010). The water which is used in the column to absorbed CO_2 or H_2S can be regenerated in the desorption column with, either air or steam that releases the CO_2 from the water at a decreased pressure (Awe et al., 2017). However, according to Zhao et al. (2010) biogas with high levels of H_2S can make water quickly becomes contaminated due to elementary sulfur which causes operational problems. Methanol and dimethyl ethers of polyethylene glycol are an organic solvent which can be employed in CO_2 removal. Furthermore, in polyethylene glycol solvent can absorb CO_2, H_2S and H_2O at the same time because the solvent has a higher solubility than CH_4. The process of polyethylene glycol scrubbing based on the same underlying mechanism as water scrubbing (Zhao et al., 2010). The common solvents used in the process is called as trade name Selexol (dimethyl ethers of polyethylene glycol) and Genosorb, which exhibit higher affinity for CO_2 and H_2S than water by 5 times, especially, Selexol (Tock et al., 2010) which results in smaller absorbent volume is required with compact size and little pumping with the same quantity of biogas, thereby reducing the investment and operating cost (Awe et al., 2017). The water scrubbing and organic solvent scrubbing are illustrated in Fig. 2.6.

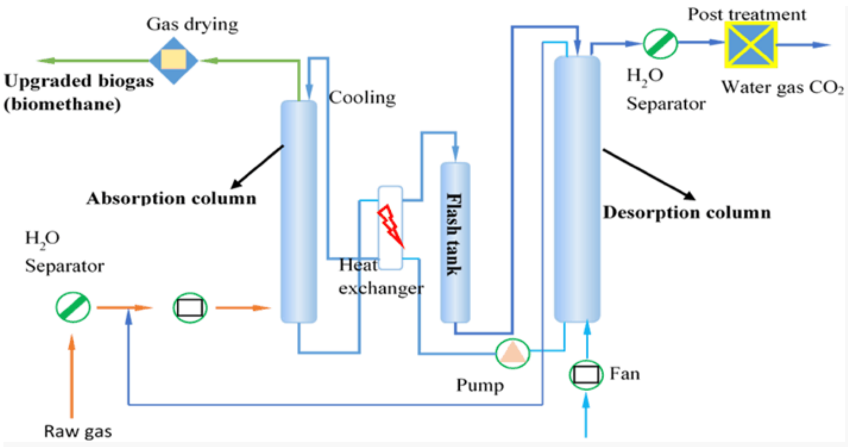


Figure 2.6 Biogas upgrading by water scrubbing and organic solvent scrubbing to remove CO_2 from biogas stream

Chemical absorption

Chemical absorption is the same way with water scrubbing and organic solvent scrubbing for biogas–liquid mass transfer principles, this process involves a chemical reaction between the solute and the solvent. Chemical solvents often use amine (mono ethanolamine or dimethyl ethanolamine, and alkali aqueous solutions such as KOH, K_2CO_3, NaOH, $Fe(OH)_3$, $FeCl_2$ (Zhao et al., 2010). Amin solution is the most used to absorber CO_2 with lowest losses of methane (0.1–1.2%) and over 99% methane can be recovered because the chemical solvent reacted selectively with CO_2 (Awe et al., 2017).

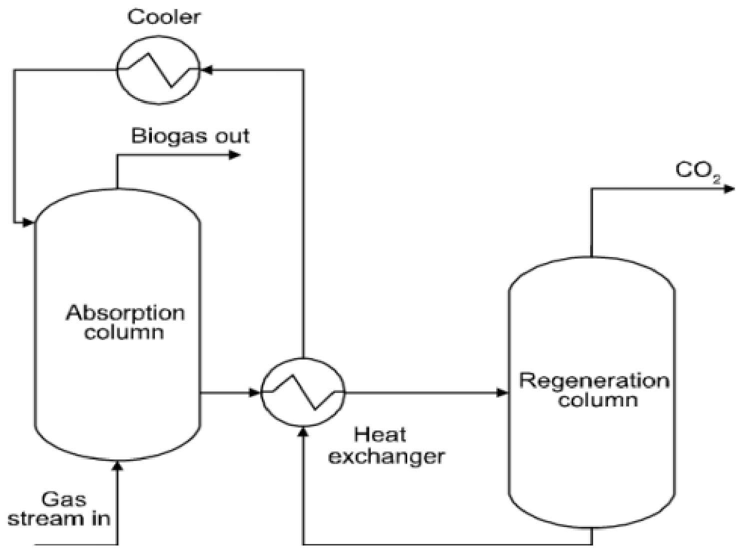


Figure 2.7 Flow chart of chemical absorption process (Zhao et al., 2010)

Tippayawong and Thanompongchart (2010) conducted a method using aqueous solutions such as sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) and mono-ethanolamine (MEA) to reduce CO_2 and H_2S content from biogas stream. The process was investigated in a packed column, liquid solvents were circulated through the column, contacting the biogas in countercurrent flow. The experiment results show that over 90% of CO_2 was removed and H_2S was removed below the detection limit. These results proved that the aqueous solutions used were effective in reacting with CO_2 in biogas.

Pressure swing adsorption

Pressure Swing Adsorption is a dry method relied on the pressure used to separate some gases unique from a mixture of gases according to the species' molecular characteristics and affinity for an adsorbent material (Bauer et al., 2013). Considering (PSA) on a macro level, the raw biogas is compressed at high pressure and then fed into an adsorption column which methane-rich (CH_4) gas passed through while carbon dioxide (CO_2) is adsorbed. Carbon dioxide is released when every time the column material is saturated with CO_2 and then CO_2 can be desorbed and led into an off-gas stream (Bauer et al., 2013). The most commonly used unique adsorptive materials are zeolite, activated carbon, activated charcoal, silica gel and synthetic resins (Zhao et al., 2010).

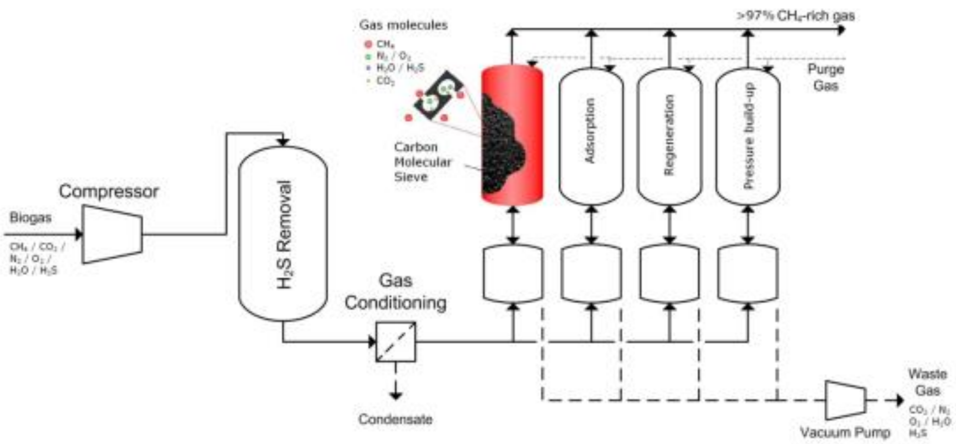


Figure 2.8 Process diagram for upgrading of raw biogas with PSA (Zhao et al., 2010)

Membrane separation

The principle of membrane separation is that based on the selective permeability property of the membranes, some components of the raw gas are transported through a thin membrane while others are retained. This process, which can be gas–gas separation (gas phase on both sides of the membranes) or gas-liquid separation (liquid absorbs the H_2S and CO_2 molecules diffusing through the membranes) (Awe et al., 2017; Zhao et al., 2010). This allows the permeability of H_2S while retaining the CH_4 on the other side of the membranes. In the process of gas-liquid separation, the liquid absorbs can be amine and the system is highly selectively compared to the solid membrane systems, and takes place at low pressure, approximately atmospheric pressure. The amine solution can be regenerated with heating to release the CO_2, which can be collected separately (Persson et al., 2006; Zhao et al., 2010). On the other hand, the gas-gas separation operates either at high pressure greater than 20 bar or at lower pressures of 8–10 bar. The separation is determined by the fact that different molecules of different sizes have different permeability through the membrane. Other important factors for the separation are a pressure difference between the two sides of the membrane and the temperature of the gas. This technology allows CO_2, H_2S, H_2O, O_2 to pass through the membrane to the permeate side while retaining CH_4 on the inlet side. Enhancing the purity of gas can be improved by increasing the size or number of the membrane modules, however, more of the methane will permeate through the membranes and be lost (Awe et al., 2017; Bauer et al., 2013; Zhao et al., 2010). The applications of technology on the European market today require a methane concentration of 97-98% and the upgrading process needs to have a methane recovery above 98% (Bauer et al., 2013).

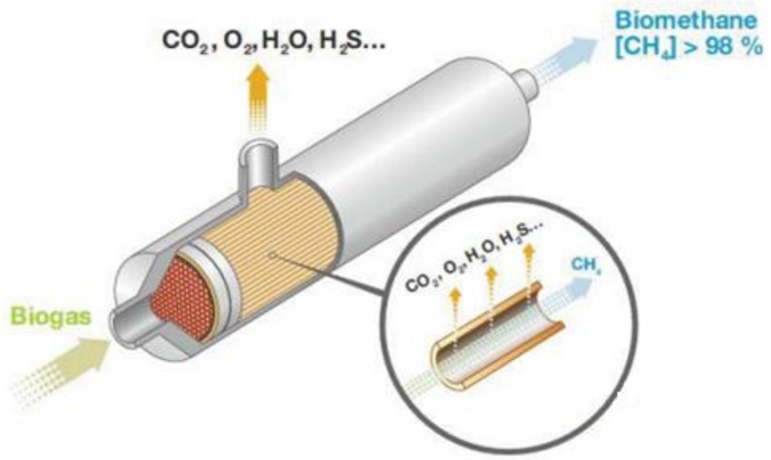


Figure 2.9 Illustration showing the separation of membrane biogas purification process (Bauer et al., 2013)

CHAPTER 3
MATERIALS AND METHODS

3.1 Conceptual framework

The study is carried out in this study to describe the potential methane generation. The detailed procedure is illustrated in Fig. 3.1. Before investigating a big scale test, lab-scale experiments is tested first. To calculate the energetic potential of the substrate, the preparation of basic physical and chemical parameters is examined. In the big scale-up experiment, the raw biogas production fed into an absorption column with water. This pure methane was tested for lighting and cooking applications.

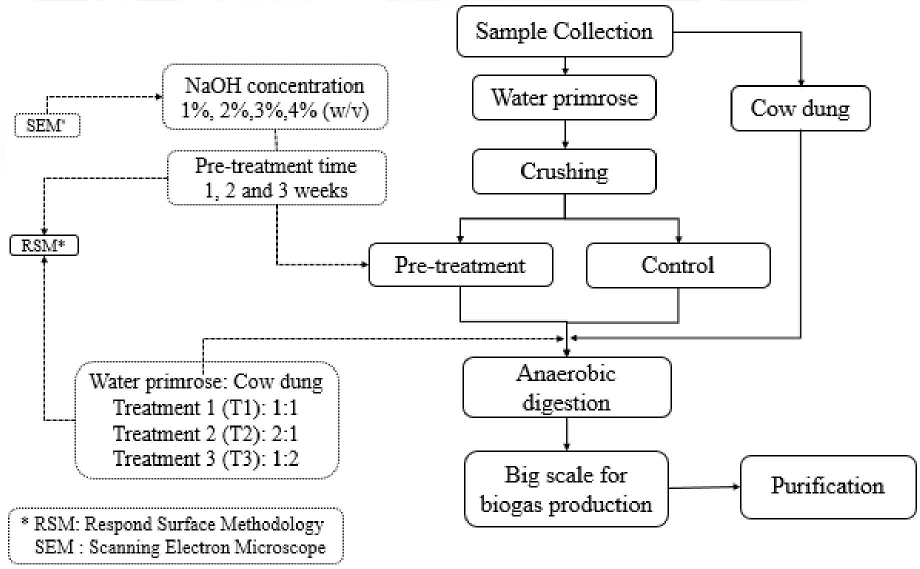


Figure 3.1 Process flow diagram of biogas production

3.2 Preparation of materials

Water primrose used in research experiments has been collected from a local field in Nong Han commune, San Sai district, Chiang Mai city, Thailand at coordinates 18°53'24.3"N- 99°02'11.5"E. This material was screened manually to remove visible impurities such as soil, strange plants. The whole parts of the plant (flowers, leaves, stems, fruits, roots) are then cutting into small particles by shredder model MJU-EB8 (physical pre-treatment, particle size reduction) into the small particle size range of 2-3 cm, then generally grounded into 5-10 mm with the help of a laboratory blender at high speed as a final size. Depends on the usage of fresh or dry material, part of the fresh material was immediately loaded in the digesters for the first test run, and another part was air-dried for two weeks.

The fermentative inoculum in the digester was taken from the faculty of animal science's farm at Maejo University, Thailand, at coordinates 18°55'04.5" N-99°01'26.9" E. Collecting materials is shown in Figs. 3.2 and 3.3. Those materials were stored in the freezer for further analysis to prevent biological decomposition.

Figure 3.2 Water primrose field and collection

Figure 3.3 Cow dung collection at Faculty of animal science and technology, Maejo University

3.3 Experiments in laboratory scale

To achieve the highest biogas efficiency from the big scale, it is necessary to conduct experiments at the lab scale first. The research on the substrates' biogas production efficiency is carried out in the laboratory of Energy Research Center, Maejo University.

Anaerobic mono-digestion

On the first batch, fresh and dry water primrose will be investigated on their biogas potential under anaerobic digestion. The best performance from this batch will be chosen for the next experiment, co-digestion with cow dung. Firstly, 10% of total solids concentration (100gTS/L) is calculated in this experiment based on dry matter. Accordingly, 70 g of fresh and dry water primrose are weighed against doing the pretreatment process before entering fermentation. An alkaline solution is prepared at different doses of NaOH as 0% (control), 1%, 2%, 3% and 4 % (w/v) then soaked with weighed samples following ratio 3:1 (v/w, ml NaOH: g water primrose) for fresh samples or 5:1 (v/w, ml NaOH: g water primrose) for dry samples in a plastic container with the capacity of 1.5 L. All containers are kept in room temperature, the duration of pretreatment time for fresh samples were 7 days and dry sample was 14 days. Hence, all samples were mixed manually, and the pH was recorded daily. In each of the treatments, three samples were conducted. Besides, cow dung was implemented in this batch as a control test to evaluate anaerobic mono-digestion from this study's substrates. In total, 33 digesters need to be prepared for the fermentation process.

Figure 3.4 Alkaline pretreatment of water primrose (fresh)

(A) Shredding material; (B) Sample preparation; (C) Alkaline solution preparation; (D) Pretreated sample

Figure 3.5 Alkaline pretreatment of water primrose (dry)
(A) Shredding material; (B) Sample preparation; (C) Alkaline solution preparation; (D) Pretreated sample

Anaerobic co-digestion

In this batch experiment, the best performance of NaOH concentration on the sample from the previous experiment will be selected to further investigate their biogas potential by co-digestion of water primrose with cow dung. For anaerobic co-digestion, two factors will be tested: the time for pretreated water primrose and the other factor is the mixing ratio of pretreated water primrose and cow dung. Each factor consists of 3 levels. The water primrose will be pretreated by NaOH solution at 1 week, 2 weeks, and 3 weeks, corresponding to the mixing ratio of 1: 1, 2: 1 and 1: 2 (water primrose to cow dung, w/w). The three experimental groups of the different mixing ratio of water primrose and cow dung (w/w) corresponding to the pretreatment time (1, 2, 3 weeks) were referred to as treatment 1 (T1, mixing ratio 1:1), treatment 2 (T2, mixing ratio 2:1), and treatment 3 (T3, mixing ratio 1:2). In the mixing ratio, 1:1 containing 3 pretreatments time, named as follows: T1- A (pretreatment time: 1 week), T1-B (pretreatment time: 2 weeks), and T1-C (pretreatment time: 3 weeks). Similarly, in the mixing ratio, 2:1 and 1:2 named T2-A, T2-B, T2-C and T3-A, T3-B, T3-C, respectively. The anaerobic co-digestion set-up in this experiment consisted of 27 digesters. The best performance from this experiment will be chosen out for the next batch, a big scale. The prepared samples during pretreatment time are illustrated in Fig. 3.6.

Figure 3.6 Co-digestion of water primrose and cow dung
(A) Sample preparation; (B) Alkaline solution preparation;
(C) Pretreated sample; (D) Sample mixing

Scanning Electron Microscope (SEM) and Light Microscope (LM) of water primrose

An observation of a water primrose stem cell cross-section was made by an Olympus CH30 light microscope (Fig. 3.7). The visualization of cells of water primrose was observed by magnifying their images. Also, the water primrose (raw material) and pretreated water primrose, including fresh and dry, were investigated under scanning electron microscopy (SEM) to examine the substrate's physical structure. SEM was carried out at the Institute of Product Quality and Standardization (IQS), Maejo University, Chiang Mai, Thailand.

Figure 3.7 Sample preparation for Light Microscope (LM) of water primrose (A) Sample preparation; (B) Size reduction; (C) Light microscope

All the samples were prepared by crushing manually into powder form using a pestle and mortar (Fig. 3.8). The pretreated sample was conducted by soaking 2% NaOH solution and kept at room temperature. The fresh sample was kept for 1 week and 2 weeks for a dry sample. Afterward, samples were dried in an oven for 24 hours. Before imaging, samples were sputtered with a fragile layer of gold to guarantee its electrical conductivity. The samples were then sputter-coated with and fixed with the brass stub for examination under the field emission scanning electron microscope (SEM) (Nova Nanosem 450, USA). The instrument used was JSM-5410LV and operated with a field emission gun, and observations of SEM images were performed at × 500 magnification with an accelerating voltage of 15 kV.

Figure 3.8 Sample preparation for SEM of water primrose (A) Sample preparation; (B) Dried sample; (C) Scanning electron microscope

The experimental digesters setup

In the laboratory-scale experiments, each biodigester was of 1000 mL of Duran bottles transparent laboratory bottles of diameter 94 mm and 222 mm length, each with 700 mL of working volume. Tap water is used to dilute substrate and makeup digester to desire working volume, which amounts needed more than about two times the original mass. The pH adjustment for all digesters before fermentation is 8.5-9 by using calcium oxide (CaO) powder; this pH is maintained throughout the process without any further adjustment.

After adding the substrate, glass bottles are then sealed airtight with a rubber cap and parafilm to ensure an anaerobic environment and prevent any possible gas leakage. Afterward, fitting the pipe inside the hole of the rubber cap. The produced biogas is transferred via the pipe to the gas holder, which is designed as an inverted cylinder immersed in water with 500 mL, placed inside the water bath. The water bath is transparent glass with a rectangular box (60cm x 30cm x 40cm). A biogas system with the gasholder can help offset temporary imbalances between gas supply and demand and establish a biogas reserve.

When the gas is consumed faster than production, stored gas can be pulled out of the holder to complement the production and meet the biogas consumers. In this way, the gasholder becomes a buffer for short-term process imbalance. The gas produced is measured equivalent to the volume of water decreased from gasholders. When the biogas produces constantly, the water is pulled down in a water tank due to the biogas pressure. The gas holder is prevented from tilting by a guide frame and connected to the pipeline between the digesters and the biogas consumers' vapor space.

The process was conducted at ambient temperature for 45 days. Mixing was implemented for all digesters two times a day by handshaking for one min to ensure full mix. The measurement of daily biogas production was taken every 24 hours. Biogas was collected in the gas bag every time the gas volume achieve 450 ml based on the downward displacement of water in the cylinder link to the volume of gas produced. Then, the daily volume gas was taken every 24 hours; the composition of gases such as methane, carbon dioxide, oxygen and hydrogen sulfide is quantified by gas analyzer Geotech GA5000 every 3 days.

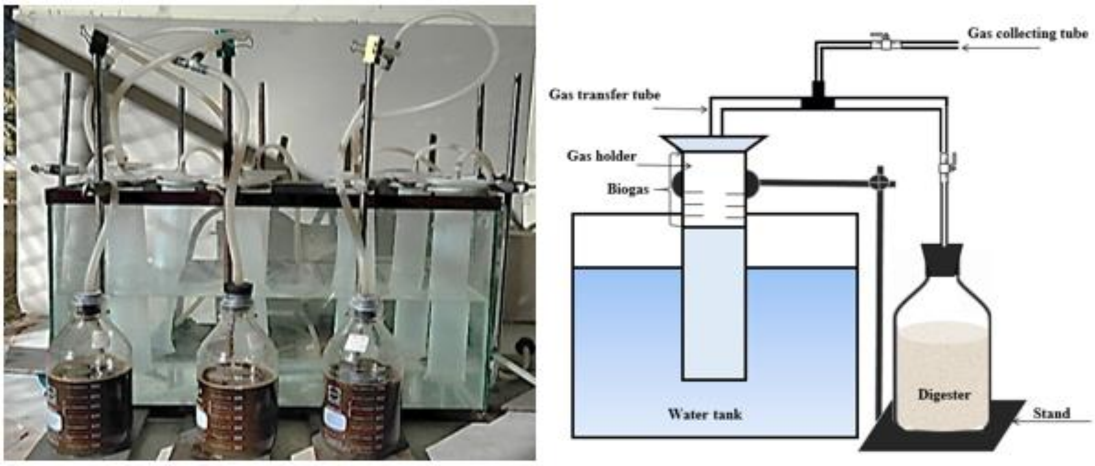


Figure 3.9 Anaerobic digestion system of laboratory scale

3.4 Pilot scale-up of biogas production

The big scale of anaerobic digestion is conducted based on the results obtained from laboratory-scale experiments. The best performance of pre-treatment time of sodium hydroxide and cow dung ratio to water primrose will be implemented for the big-scale experiment.

In this experiment, the amount of samples was calculated based on dry matter of water primrose which was 10% total solids (TS) content (100g TS/L). Accordingly, 34 kg of water primrose was prepared while 14 kg for cow dung as mixing ratio of 2:1 (w/w, water primrose to cow dung) which was chosen from lab-scale experiment. The substrate slurry was made by the mixture of pretreated water primrose, cow dung and tap water with the final slurry volume of 500 L.

The biogas production was measured based on water displacement method. The digester tank is made of stainless steel, with a semi-cylindrical shape from the bottom and from the top is a rectangle shape. The batch experiment was conducted using a 1,000-liter fermenter (1 m^3) for fermenting materials. For a safety margin, the fermenter with a working volume of 500 L (0.5 m^3) was chosen to prevent the high pressure of gas produced and avoid the gas overflowing from the lid. Furthermore, for observation of the substrate during the process, two longitudinal viewing glasses have installed on side walls and bottom with area of 0.15 m^2. The feeding and gas storage system is removable lids placed at the top of the fermenter. Dimension of feeding system is 0.35 m x 0.35 m. The top removable lid of the gas storage has a capacity of 0.4 m^3 and is equipped with connector pipes, valves and rubber seals. A 7 cm diameter valve inserted at the bottom allows the unloading of digested material. After the addition of the substrate, tap water was used to dilute the substrate and produced a liquid volume of 500 L. Calcium oxide (CaO) was then added to adjust the pH of the digester as a buffer. Finally, the digester was water-sealed for the duration of the process. The accumulative biogas was stored in a gas holder made of 120 L of a cylinder. The volume of gas produced was measured daily by the rising height of the floating cylinder; the gas record was done by reading the measurement attached along the length of gas holder. In addition, the digester was stirred twice a day for 10-15 min. The stirring system for achieving mixing substrate in the digester is also constructed from stainless steel and worked by an inverter through the control panel. The anaerobic digestion system is illustrated in Fig.3.10.

Figure 3.10 Anaerobic digestion system of big scale

3.5 Enhancement quality of biogas via purification process

In this study, the experimental biogas purification was performed as lab-scale experiment. The experiment was aimed to evaluate the amount of CO_2 removal after the process by using different concentrations of an alkaline chemical such as sodium hydroxide (NaOH) and calcium hydroxide ($CaOH)_2$) solution. Besides, hydrogen sulfide (H_2S) and moisture removal also investigated. The biogas purification process was done by using steel wool, alkaline solution and sponge as purification substrates (Fig. 11). The steel wool is to remove H_2S while alkaline solution is to react with the carbon dioxide and sponge aimed to remove moisture.

The experimental device is concluded of gas holder, vacuum pump for transferring gas flow and two scrubbing columns for biogas purification. The raw biogas was obtained from a big digester of 1,000 L capacity conducted in the previous experiment. For each purification test, 50 L of biogas was prepared in a gas holder and recorded its components by gas analyzer GA 5000 prior to the purified column.

Figure 3.11 Purification substrates

The biogas scrubbing system consisted of two columns, the hydrogen sulfide (H_2S) removing column and the other column was combined of carbon dioxide (CO_2) removal and moisture trap. Columns were designed and constructed from a Polyvinyl Chloride (PVC) cylinder with a diameter of 20 cm and a height of 70 cm. PVC cylinders have an actual of the volume of 20 L. These cylinders are sealed at the top and the bottom by plastic round lids for creating an airtight seal. At the top lid of cylinders were plug two holes with 2 valves as incoming and outgoing biogas. Furthermore, the incoming gas hole was attached by a small PVC tube of 63 cm in height and 2.7 cm in diameter. This design created a that allowed the biogas to flow from the bottom to the top to ensure that the biogas flow from the bottom to the top to ensure good gas distribution and increase gas reaction with the material.

The gas was pumped throughout the system with the support of a vacuum pump. The valve of the columns' incoming gas was opened to let the gas flow through. The pump flow of raw biogas was then entering the first column. This column contained steel wood and was provided up to 50% of the column's height to desulphurize raw biogas. The remaining H_2S was then continuing reaction with the alkaline solution in the next column. The other hole of this column was connected with the pipe to a second scrubber column valve for further purification. In this adsorption column, the aqueous solution low-cost commercial chemicals were prepared at various doses of NaOH or $Ca(OH)_2$ as 1%, 2% and 3% concentration. The amount of solution in the column was 10 L, haft- space of the column used for motion of the gas flow and prevented the high pressure occurring during the process as well as increasing the reaction time. Hence, sponges were also provided in the column up to 50% height. The liquid solution absorbed carbon dioxide, hydrogen sulfide and moisture in raw biogas; thus, the concentration of these impurities gas was increased in the scrubbing column. In other words, the enrichment of the methane content in purified biogas was achieved. The biogas flow was continuing passed the outlet valve of the scrubbing column into the gasholder. The process was then repeating called a continuous process. The removal of impurities from gases was evaluated every 30 min for 2 hours (30 min, 60 min, 90 min and 120 min by taking a small quantity of gas at the exit valve of the second column for gas component checking using the Tedlar bag (500 mL). The experimental setup for biogas purification is shown in Figs 3.12 and 3.13.

Figure 3.12 Experimental set-up for biogas purification:
(a) before experiment; (b) after experiment

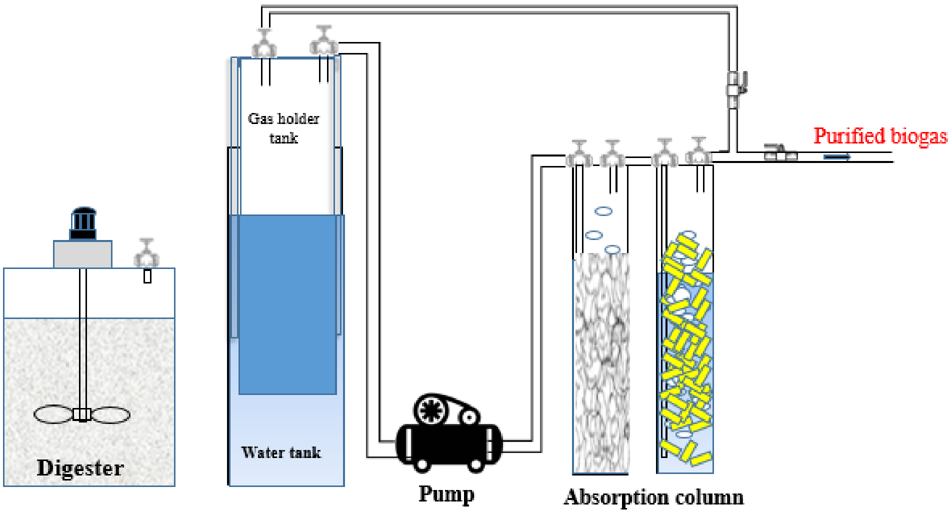


Figure 3.13 Schematic diagram of biogas purification

Theoretical modeling of CO_2 absorption

The absorption kinetics reflect the development of the absorption process versus time. In the present of aqueous solutions of sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$) of different strength, the CO_2 and H_2S in gas mixture was absorbed through direct from gas to liquid contact in scrubbing column.The relationship between the fractions of absorbed CO_2 (C) with fraction of CO_2 (C_0) in the inlet column reactor can be expressed as (Tippayawong and Thanompongchart, 2010):

$$A=1-(C/C_0) \qquad \text{..........Equation 12}$$

Assuming that the rate of declining CO_2 absorption is proportional to the fraction of absorbed CO_2 and the fraction that passes through. It can be denoted as (Lin and Shyu, 1999):

$$dA/dt=-kA(1-A) \qquad \text{..........Equation 13}$$

Integration of the above equation:

$$\ln\left(\frac{A(1-A_0)}{A_0(1-A)}\right) = k(\tau - t) \qquad \text{..........Equation 14}$$

The equation can be rearranged as:

$$t = \tau + \frac{1}{k}\ln\left(\frac{C}{C_0-C}\right) \qquad \text{..........Equation 15}$$

In which k is an absorption constant and τ is the characteristic absorption time when 50% concentration of CO_2 at the outlet occurs. From the above equation, it can be implied that the solutions in column should be saturated with CO_2 after a period of 2 τ .

3.6 Analysis of basic physicochemical parameters

Some of the essential parameters were examined based on the standard methods:

Table 3.1 Physicochemical parameters (APHA, 2015)

Parameters	Unit	Method	Analysis
pH	-	pH meter	Before and after
TS	mg/l	Gravimetric method	Before and after
VS	mg/l	Gravimetric method	Before and after
COD	mg/l	Gravimetric method	Before and after
Alkalinity	mg/l-$CaCO_3$	Titration method	Before and after

VFA	mg/g	Reflux equipment	Before and after
Cumulativebiogas production	ml	Gas sampling bags	Daily

Total solids

Total solids are the amount of solid remaining in the sample after evaporating water in it under higher temperatures. In other words, it is to indicate the quantity of the material residue left in the crucible after evaporation of the sample and its subsequent drying in a laboratory oven at 105°C for one hour. The TS is calculated as the following equation after cooling the sample in a desiccator:

$$TS(mg/L) = \frac{W_{Total} - W_{Crucible}}{V_{Sample}}$$ ....Equation 12

$$TS(\%) = \frac{W_{Total} - W_{Crucible}}{V_{Sample}} \times 100$$ ....Equation 13

Where:

W_{Total} : Weight of dried residue and crucible (mg)

$W_{Crucible}$: Weight of crucible (mg)

V_{Sample} : Volume of the sample (L)

Volatile solids

Volatile solids (VS) is the amount of solid remaining in a sample after evaporating water and heated at 550°C. The residue obtained from total solids is continuously heated at 550oC for 30 minutes to two hours using a muffle furnace. After cooling in a desiccator, VS is calculated to the following equation:

$$VS(mg/L) = \frac{W_{Total} - W_{Volatile}}{V_{Sample}}$$ ....Equation 14

$$VS(\%) = \frac{W_{Total} - W_{Volatile}}{V_{Sample}} \times 100$$ ....Equation 15

Where:

W_{Total} : Weight of dried residue and crucible (mg)

$W_{Volatile}$: Weight of residue and crucible after ignition 550°C (mg)

V_{Sample} : Volume of the sample (L)

Chemical oxygen demand (COD)

Chemical oxygen demand is defined as the amount of specified oxidant that reacts with the sample under controlled conditions. It is measured by the titration method (APHA, 2015). The method for measurement COD is the reflux tube method. Potassium dichromate crystals ($K_2Cr_2O_7$) is used to standardize the Ferrous Ammonium Sulfate (FAS) solution. Ferroin indicator is used as a solution. The concentration of H_2SO_4 is 0.01M and titrate against FAS solution using Ferrioin indicator. The titration is accomplished when the color change from blue-green to brown. The equation below used for COD calculation:

$$COD(mg/L) = \frac{8000 \times C_{FAS} \times (V_1 - V_2)}{V_0} \times 100$$ ....Equation 16

$$COD(mg/Kg) = \frac{8000 \times C_{FAS} \times (V_1 - V_2)}{m_0} \times 100$$ ....Equation 17

Where: C_{FAS} : The concentration (M)

V_0 : Volume of the sample before dilution (mg)

m_0 : Mass of the sample before dilution (mg)

V_1 : Volume of FAS used for blank (ml)

V_2 : Volume of FAS used for sample (ml)

Alkalinity and volatile fatty acid (VFA)

Alkalinity is measured by the titration method, using 0.01 M sulfuric acid (H_2SO_4) as an indicator reagent. Other reagents are also used as phenolphthalein and methyl orange. The sample will be diluted with distilled water using a centrifuge for 30 minutes, after finished, 20 ml supernatant liquid is collected, then 20 ml distilled water is added to the liquid. The contents are well mixed before added 3 drops of phenolphthalein and methyl orange are added respectively to the liquid. Titration by 0.01M H_2SO_4, the endpoint is the first pink coloration that persists on standing for a short time. The equation to calculate total alkalinity perform as below:

$$\text{TotalAlkalinity}(mg/L - CaCO_3) = \frac{V_{H_2SO_4} \times N \times 50{,}000}{V_{sample}} \quad \text{..........Equation 18}$$

Where:

$V_{H_2SO_4}$: Volume of sulfuric acid used in mL

N: Normality of acid used to titrate

V_{sample} : Volume of the sample used in ml

50,000: Mass equivalent of $CaCO_3$

The volatile fatty acid is measured by the combination of the reflux machine and titration. The volatile acid determination, in conjunction with pH measurements is valuable in controlling environmental conditions during the initiation of the methane digestion. The following equation is used to calculate volatile fatty acid:

$$VFA(mg/L) = \frac{V_{NaOH} \times 6{,}000}{V_{sample} \times 0.7} \quad \text{..........Equation 19}$$

Where: V_{NaOH}: Volume of sodium hydroxide used in mL

V_{sample} : Volume of the sample used in ml

0.7: It is assumed that only 70% of the total volatile acids are collected during distillation.

Energy analysis

The higher calorific values (HCV) and lower calorific value (LCV) of pure methane were 39.82 and 35.87 MJ/m^3, respectively. HCV and LCV of produced biogas were determined as below equation:

$$HCV_{biogas} = 0.3989 \times MC$$
$$LCV_{biogas} = 0.3593 \times MC$$
..........Equation 20

Where: MC is the methane content in biogas (%)

In addition, the calculation of energy and power potential is adopted by (Wellington et al., 2017) using the biogas collected and its flame to heat water as follows:

$$E = M_C\, C_C\, \Delta\theta + M_W C_W \Delta\theta$$
..........Equation 21

Where E is the amount of heat energy dissipated, Mc is the mass of calorimeter (g), Cc is the specific heat capacity of the calorimeter (390 J kg-1K-1), $\Delta\theta$ is the temperature change (^{o}C), M_w is the mass of water (g) and C_w is the specific heat capacity of water (4200 Jkg-1K-1).

Power = E/P ....Equation 22

Where E is the amount of heat energy calculated in Eq. 21 and t is the time taken for the energy to be dissipated (s).

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Characteristics of feedstock used for anaerobic digestion

Water primrose has bright yellow flowers and oval-shaped, typically having four petals. Flowers vary in size from 2 cm to 4 cm in diameter and bloom all season, except winter. The stems are long, trailing, branched. Stems of water primrose can be green to reddish appearance, can reroof from cutting, it fleshy and grow to the height up to 120 or even 210 cm. Leaves are long and slender in shape and arranged alternately on the stem, up to 10 cm long, 1-2 cm wide, shiny, dark green and lighter green central vein or in yellow color. The plants contain small seeds inside. Capsule pubescent, more-or-less cylindrical or swollen towards the apex, up to 30 mm long with many brown, oblong seeds about 0.5 mm long.

At the beginning, fresh material has 3.8 kg/m^2. After drying, the dry weight of the material remaining 0.9 kg/m^2. The plant constitutes 60% of the stem's dry weight, 15% of the flower and fruit, 15% of root and 10% of the leaf. Water primrose is an agricultural disturbance plant and spreads easily to become naturalized. It is well known as a troublesome aquatic noxious weed that invades water ecosystems and can clog waterways; also, it spreads croplands. Therefore, this nuisance plant biomass is a good source for bioenergy applications.

Cow dung is the second bio-solid waste used for biogas production. The digestate can be an important source of fertilizer due to its high nutrient contents. Co-digestion of water primrose and cow dung reduces water consumption, energy and cost consumption for waste collection and transportation. It also provides the opportunity for the recovery of valued nutrients and energy as biogas.

This work's novelty utilizes biomass from water primrose and cow dung that effectiveness related to biogas generation and improvement of methane production. Determining the material's characterization leads to a better estimate of the relationship between substrate and biogas potential. The substrate components as carbohydrates, fats, and proteins are converted into methane (biogas) production under anaerobic digestion (Bücker et al., 2020). Therefore, it is necessary to determine the organic content in substrates as well as other correlated parameters. The initial characteristics of water primrose and cow dung are related to the physical and chemical presented in Table 4.1. Furthermore, compositional analysis of water primrose, such as proximate and ultimate analysis also investigated.

TS's content was determined by igniting samples at high temperatures in a muffle furnace based on dry matter. In the water primrose samples, it is observed that the content of TS, VS was found 900,000 ± 4,165 mg/L and 836,667 ± 3,754 mg/L, respectively. Hence, the chemical oxygen demand of water primrose was 61,667 ± 4,812 mg/L. Those parameters indicate a high amount of organic compounds are available and the biodegradability of the substrate, which could contribute to the biogas conversion under anaerobic digestion.

The pH of the sample was 5.05 ± 0.02 shown that water primrose is acidic, which implied that alkaline pretreatment by NaOH solution could help increase alkaline buffer capacity and reduce the quantity of CaO powder used for adjusting pH in the digester. The proximate of water primrose also verified the percentage of moisture content (MC), volatile content (VC), fixed carbon (FC), and ash were 7.28, 63.1, 1.28 and 28.4, respectively.

In term of the ultimate analysis, the elemental composition of water primrose is detected in the elemental analyzer and contented carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S) elements. Based on the weight percentage on a dry basis, the major elemental water primrose composition was C with 40.2 wt %, followed by O (22.13 wt %) and H (5.03 wt %).

Other N and S elements were also found at a low percentage (1.8 wt% and 0.24 wt %). Nevertheless, not all elements (CHONS) are taken part in the anaerobic digestion process as nutrients. For example, the oxygen element does not contribute to the anaerobic process due to the process requires strictly free-oxygen conditions (Matheri et al., 2018). Thus, the essential organic matter contribution elements are referred to as CHNS content (Chan and Wang, 2016).

Table 4.1 Initial characteristics of water primrose and cow dung

	Measured values	
	WP	CD
TS (mg/L)	900,000 ± 4,165	196,666 ± 1,064
VS(mg/L)	836,667 ± 3,754	140,000 ± 984
pH	5.05 ± 0.02	8.15 ± 0.02
COD (mg/L)	61,667 ± 4,812	153,333 ± 5,695
VFAs (mg/L)	3,218 ± 182	4,376 ± 896
Alkaline (mg/L)	1,917 ± 312	3,4458 ± 295
Proximate analysis (wt%,d.b)		
MoisturecontenT (MC)	7.28	-
Volatile content (VC)	63.1	-
Fixed carbon (FC)	1.28	-
Ash	28.4	-
Ultimate analysis wt%,d.b)		
Carbon (C)	40.2	-
Hydrogen (H)	5.03	-
Oxygen (O)	22.13	-
Nitrogen (N)	1.8	-
Sulfur (S)	0.24	-

TS's content from cow dung was lower than water primrose, which was 196,666 ± 1,064 (mg/L) and 140,000 ± 984 (mg/L), respectively. Cow are fed grasses containing greater lignin complexes with cellulose, further digested into biogas in anaerobic digestion. According to the pH values in Table 4.1, the pH measurement of water primrose was in the acidic range (5.05); meanwhile, the pH of cow dung was used as co-substrate in the range of basic range (8.15). In the fermentation process, anaerobic co-digestion of water primrose and cow dung could prevent the risk of acidification and improve buffer capacity in digesters than conducted with one substrate in digesters. Also, manure is considered a nitrogen-bearing material in anaerobic digestion that during the fermentation process, the buffering system is more adjustable by releasing ammonia (Zamanzadeh et al., 2017). Overall, the initial values of feedstock showed a suitable condition for the anaerobic system.

4.2 Light and scanning electron microscopy of water primrose

An observation of a water primrose stem's cross-section was made by a light microscope (LM) presented in Fig. 4.1. A large calcium oxalate crystal was found in the cross-section of the stem of water primrose. Calcium oxalate crystal usually is located in all parts of the plant as roots, leaves, stems, seeds, and other parts (Franceschi, 2001). The crystals might contribute to the photosynthetic process and protect against insects and foragers (Franceschi, 2001; Konyar et al., 2014). However, accumulate oxalate can cause poisoning symptoms for ruminants in toxic concentrations (Konyar et al., 2014).

Still, imaging with scanning electron microscopy (SEM) was investigated before and after alkaline pretreatment. Scanning Electron Microscope observed the difference in the lignocellulosic structure of water primrose before and after pretreatment with 2% NaOH. Pictures were taken at 15 kV and magnifications × 500, which are shown in Fig. 4.2. As shown on the SEM picture, water primrose's surface morphology, the significant difference between untreated and treated surface was observed. The apparent structure of water primrose is closely regulated, and there are rough particle bulges. Also, it has an intact morphology form and the pores did not happen in a large amount, thus making it more recalcitrant challengingfor enzymes to access the plant cell wall. Generally, intact cells can be seen clearly in Fig. 4.2A. Tetard et al. (2010) explained that depending on the linkage of lignin and cellulose in biomass, different topography could have occurred, such as the holes and gaps layer appeared in the same region after the pretreatment process.

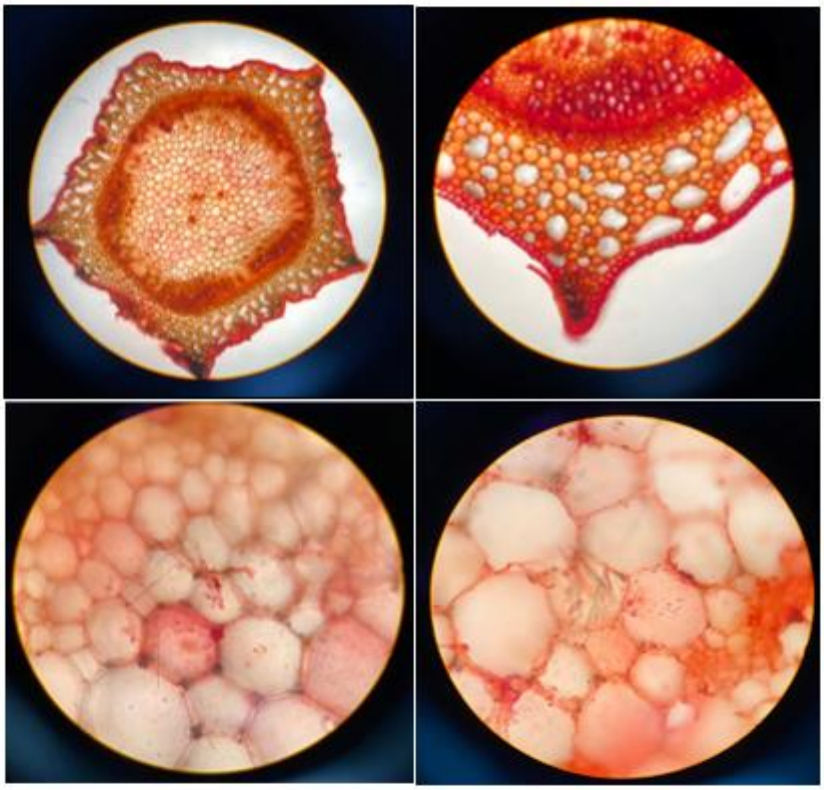


Figure 4.1 Cross-section plant stem under the microscope

Morphological changes by alkaline pretreatment are first noticeable after pretreatment at 2% of NaOH. In the fresh sample, the cell wall boundaries were clearly disrupted after the pretreatment (Fig. 4.2B and 4.2C) and the large-sized pores were visible that revealed the following layers within the cell walls in the dry sample (Fig. 4.2B). By 2% of NaOH, the apparent structure became looser, more holes appeared on the surface of solids and the size became larger, the surface area increased accordingly. Here, slight defibrillation was observed, consisting of the separation of individual fibers and an enlarging of the reactive area. The structure is more pronounced structural changes in the biomass were seen due to the solubilization of hemicellulose and cellulose increasing led to the changes of pronounced structural biomass and lignin re-localization changes. Still, a reduction in fiber length scales was significantly observed in the dry sample. This phenomenon may help enzymes attack the surface area easily during hydrolysis (Fang et al., 2015), enhance glucose production (Lei et al., 2013) thus, resulting in higher methane yield (Moset et al., 2018).

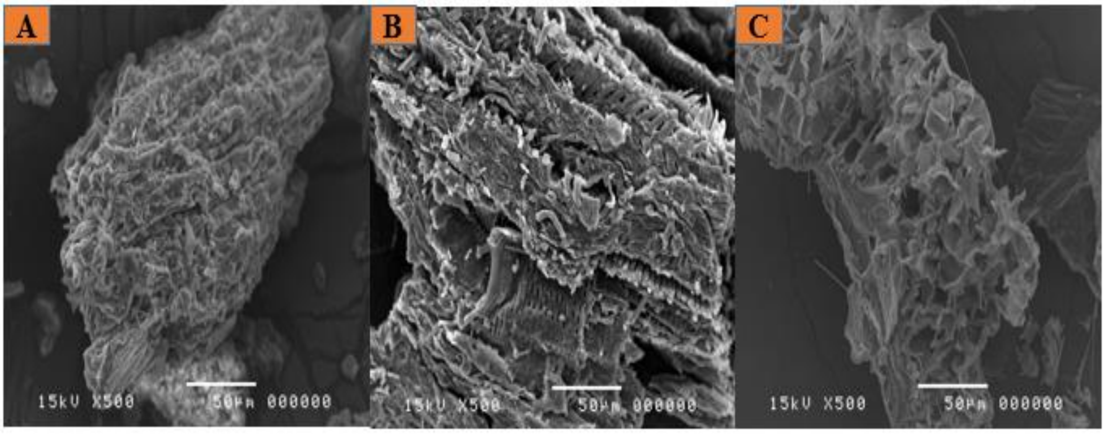


Figure 4.2 Scanning Electron Microscope image of water primrose: A) Raw water primrose, (B) Fresh pretreated water primrose and (C) Dry pretreated water primrose

4.3 Biogas production from anaerobic mono-digestion

Almost biomass undoubtedly can apply to anaerobic digestion as long as it contains a high amount of nutritious matters as carbohydrate, protein, fat (Saengsawang et al., 2020); meanwhile, they are not free from drawbacks. The complex structure of these lignocellulosic biomass causes microorganisms to hardly attack and degrade substrates because it bonds tightly together (Pang et al., 2008). Therefore, in order to enhance biogas yield in the final step of anaerobic digestion, there is a must to create a good condition for hydrolysis step in the first step of the process (Zheng et al., 2014), which means pretreatment of lignocelluloses biomass is an important step to reform its properties, make hemicellulose and cellulose more soluble, accessible to enzymatic hydrolysis (Amin et al., 2017). According to Du et al. (2019), the favorite pretreatment time for lignocellulosic biomass should not exceed thirty days. Beyond that ranges, the application of pretreatment technology is not conducive.

Generally, biogas can be produced from various kinds of organic waste materials, for example municipal, agricultural, and industrial wastes and energy crops, through an anaerobic digestion process (Bücker et al., 2020). Numerous researchers have been studying potential feedstock for biogas production from natural sources as rice straw (Mancini et al., 2018), Napier grass (Dussadee et al., 2014), teak leaves (Wannapokin et al., 2018), common reed (Van Tran et al., 2019) buffalo grass (Chuanchai and Ramaraj, 2018). In general, these biomass feedstocks are abundant in rural area and bring no benefit in economic and environmental aspects. Thus, the effectiveness of using biomass for anaerobic digestion is a better choice to consider to reduce the problems associated with organic waste disposal (Sonakya et al., 2001). Furthermore, the results obtained from these studies evidenced that the efficiency of applying pretreatment processes on biomass feedstock boosted biogas yield.
Mono digestion is the foundation for further development of co-digestion; investigated biogas production on a single substrate before mixing with one or more than one substrates would obviously evaluate each substrate's influence that involved in the fermentation process. One of the biggest factors in deciding operating anaerobic digestion is to investigate biogas potential on the feedstocks used. The more amount of dissolved carbon in the substrate, the more gas could be converted by bacterial. The second factors are the time given to the bacteria, called hydraulic retention time (HRT). In case the substrate has enough time for bacterial to digest, there may get full conversion of organics to the end products.

Typically, HRT works in the mesophilic temperature range of 20-35^{o}C for 15-40 days (Dareioti and Kornaros, 2014). Investigation of water primrose from biogas potential under the mesophilic condition shown that methane production decreased rapidly after 45 days of operation. Thus, the ideal time for the decomposition of this material was performed for 45 days.

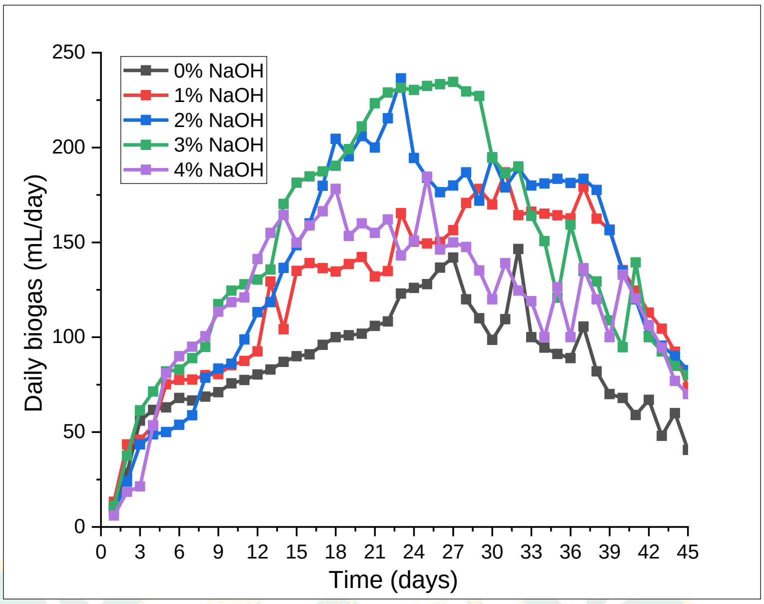
At a given time of 45-days operation, the substrate was decomposed through the microorganism's anaerobic process. It is important to determine that how much biogas produced and the time it reaches the greatest amount during a period of time. All fermenters were shaking twice a day manually to prevent scum formation and increase contact between microorganisms and substrate, leading to improving the fermentation process. Besides, high pressure could be built up via gas produced inside the fermenter. Therefore, 30% of the headspace of each fermenter was spent on gas space. The pH was adjusted before fermentation to the value of 8.5-9 by adding CaO to increase digester alkaline and prevent a rapid change of pH at the acidogenesis step. After that, the pH of the process was maintained by itself. The gas production was measured every 24 hours and the biogas components as methane, carbon dioxide, oxygen and hydrosulfide obtained from all treatments were measured every 3 days.

This experiment has investigated that the processing time for pretreatment samples was 7 days on the fresh sample and 14 days for dry samples by different NaOH concentrations (0%, 1%, 2%, 3% and 4%) at room temperature before entering the fermentation process. The performance of the biogas production observed in the present study was significantly influenced by the pretreatment process. The daily and cumulative biogas production from water primrose in the form of fresh and dry increases, and the obvious results are shown in Figs. 4.3-4.4.

Investigating on the fresh sample, control (0% NaOH) was the lowest yield of all the others (Fig.4.2). This indicated that even a mild concentration of NaOH (1, 2 % concentration) could improve biogas yield. In other words, NaOH pretreatment increased the amount of biodegradable material and its digestibility. In the case of an increasing concentration of NaOH from 1% to 3 %, the maximum biogas yield was found. Hence, although the maximum yield was obtained at 3% NaOH, the associated maximum biogas yield was not observed with a higher concentration (4% NaOH). The results were similar to a study by Wicaksono et al. (2017). The author has investigated the effect of NaOH solution on biogas production from rice straw. NaOH's concentration was chosen with 2%, 4%, and 6% then rice straw was soaked in the solution for 30 minutes before fermentation anaerobic digestion process. The highest volume of biogas produced was found in 4% NaOH solution with 21.1ml/gTS. The following was 20.4 ml/gTS from 2% NaOH solution; higher concentration (6% NaOH) showed the lower yield without pretreatment (17.2 ml/gTS). The possible reason can be explained that NaOH concentration could make lignocellulose (hemicellulose and cellulose) more dissolvable and increase the concentration of the substrate (Chandra et al., 2012; Du et al., 2019). Moreover, methanogenic bacteria have an optimal operation capacity at 4% NaOH in the anaerobic digestion process, higher than that concentration, and methanogenic bacteria is inefficient in biogas production. These results also stated that pretreatment using NaOH solution obtained more biogas volume than using acetic acid solution because the alkaline solution was more effective in reducing the substrate's lignin compound.

This experiment's maximum biogas yield was achieved at 3% NaOH with 6,692.55 mL; this was higher (39.4%) than control (4,054.13 mL). This result was similar to 2% NaOH with 6,272.37 mL volume of biogas. The minimum biogas yield was obtained at 1 and 4% NaOH with 5,581.13 mL and 5,407.7 mL, respectively. The results demonstrated that the biogas yield gradually increased with an increase in NaOH concentration. However, with more than 3% NaOH concentration, the total volume of biogas tended to decrease. The maximum (3%) attainment rate was not much different with 2% NaOH.

Regarding methane content from fresh material (Fig.4.3), the highest content was obtained from the highest biogas volume that was from 3% NaOH treatment with 61.23%, followed by treatment of 2%, 1%, 4% and lastly 0% of NaOH (with the methane percentage of 58.76%, 55.53%, 55.32% and 51.32%, respectively).



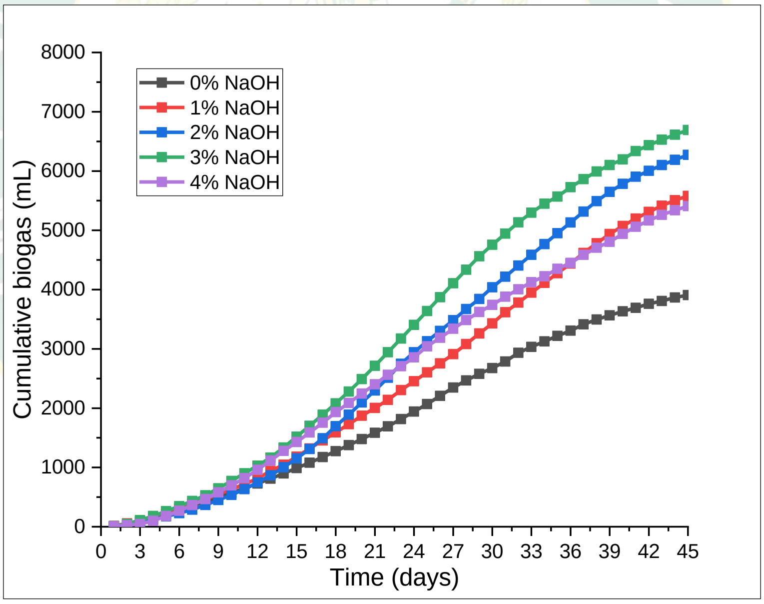


Figure 4.3 Daily and cumulative biogas production of mono-digestion from fresh samples

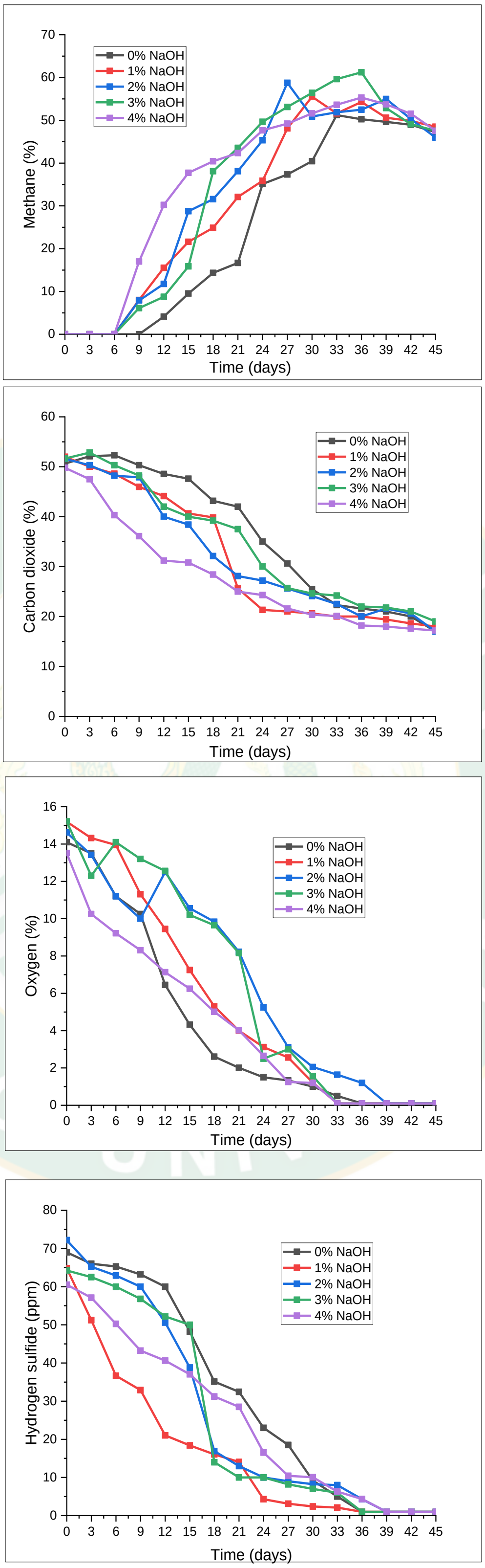


Figure 4.4 Biogas composition of mono-digestion from fresh samples

Investigating on dry samples, biogas started producing immediately on the first day from all treatments (control, 1, 2, 3, 4% NaOH). During the first two weeks, there was no significant different gas production among these treatments. From the third week onward, the biogas curves of 2 and 3% NaOH treatment were higher than the rest of the treatment and this curve was maintained till the end of the process.

The highest rates of biogas production per day reached 257.5 mL/ day using 2% NaOH. This peak value was obtained on day 35. The maximum biogas yield of control, 1%, 3% and 4%, gradually reached their peak value on day 33, 37, 27 and 45, respectively. In all treatments, the daily biogas volume curve was mostly flat and there was no clear peak in daily productivity. The digesters with 2% NaOH have a 71.6% higher peak volume than the controls (150 mL). Followed by 3% NaOH, which 40.6% higher than the controls but 18.1% lower than 2% NaOH. At 4 and 1% of NaOH, the peak volume was not much different from the controls, reaching 169.5 and 172 mL, respectively. Hence, the pretreatment samples reached the peak value of the controls (150 mL) then about 2 days and 18-20 days for 1%, 4% and 2%, 3% NaOH, respectively.

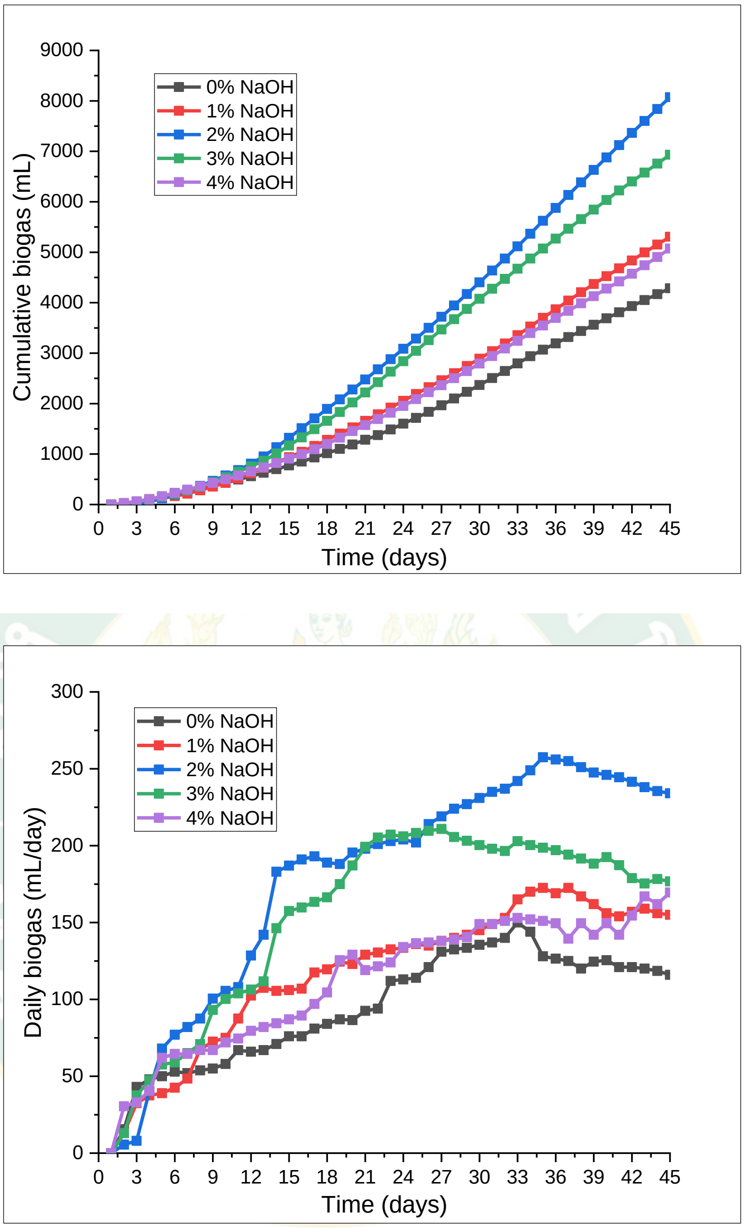


Figure 4.5 Biogas production of mono-digestion from dry samples (daily and cumulative volume)

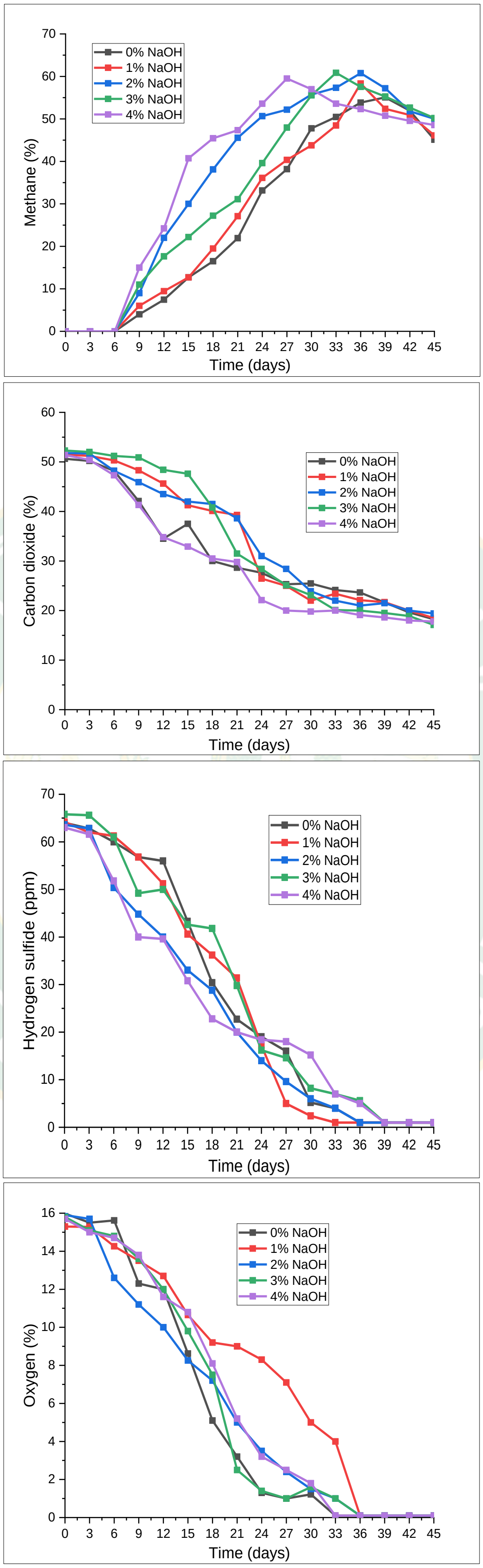


Figure 4.6 Biogas compositions of mono-digestion from dry samples

The accumulative biogas yield was calculated based on the gas production per day that varied from 4,285.80 to 8,072.00 mL. There was no significant different volume in the treatment between 1% and 4% NaOH and control. The highest biogas yield was obtained from 2% NaOH; it was by 16.4 %, 52.1% and 59.1% higher than yield of pretreatment samples of 3%, 4% and 1%, respectively. Moreover, it was much higher 88.4% for biogas yield of control. The biogas production started producing from day one, while the methane concentration was detected from day 6 onward for all treatments (Fig.4.5). Generally, the difference of methane formation in biogas varied from a minimum value of 55.07% (0% NaOH) to a maximum value of 64.72% (2% NaOH).

During the first six-day of fermentation, the methane was recorded at zero percent at all treatments. Thereafter, methane concentration steadily rose till the end of the process. At the beginning of the anaerobic digestion, the methane concentration at 4% NaOH treatment was slightly higher than the rest of the treatments. The 4% NaOH had the earliest peak value on day 27 with 59.51% of methane, indicating that at a high concentration of NaOH treatment on water primrose would positively improve the initial methane concentration. However, this experiment's highest methane formation was at 2% NaOH, with 64.72% obtained near the last day of the process (day 42). This is further supported that 2% NaOH of pretreatment on water primrose had a positive effect on the quantity of the total volume of gas produced and improves the quality of the gas.

In addition, an average of 1132.3 cm^3 biogas was used for calculation; the results of biogas power potential were 25.34 W or 22.3822 W/L or 22,382.19 W/m^3. The pure methane content could generate 37,258.9 J of energy or 37,258.9 W/m^3. As a result, 60.07% of methane content was estimated from 22,382.19 W/m^3 power. In addition, it is noted that the energy of 10 m^3 of gas liquefied petroleum (LPG) used for cooking is equal to 25 m^3 of biogas. In other words, the ratio of energy from LPG gas to energy from pure methane is 5:2. Consequently, biogas production from water primrose is a potential feedstock in anaerobic digestion with achievable energy efficiency.

Table 4.2 Biogas energy and power potential calculation

	The volume of gas used	Mass of water and calorimeter	Change in temperature	Energy gained by water & calorimeter	Time taken	Power dissipated
	V_{gas} (cm^3)	M (g)	$\Delta\theta$ (^0C)	E (J)	(s)	P (watts)
Calorimeter		47.3				
Test 1	1975	53	17	4,097.8	67	24.5
Test 2	874	47	14	3,021.8	14	26.5
Test 3	548	40	13	2,423.8	7	24.9
Average	1,132.3			3,181.1		25.3

Comparison of anaerobic mono-digestion from fresh and dry water primrose

At the beginning stage of anaerobic digestion, the anaerobic bacteria started acting on the organic matter of substrate for all of the digesters. The bacteria population increased and digested on more substrate leading to increase biogas production. After an adaptation period, the bacteria were active on the largest amount of readily biodegradable organic matter in the substrate, resulting in obvious influence on daily gas production that performed as the peak value. Thereafter, the carbon and nutrient in the substrate decreased. Biogas production started to drop and gradually stop producing.

The obvious results ere shown in general: (1) the accumulated biogas production increased with increased retention time with an increase in the retention time. (2) The pretreated samples with NaOH solutions achieved a higher volume of biogas compared to untreated samples. (3) As different doses of NaOH were used in pretreatment of water primrose, the organic matter degradation was observed through the volume of biogas production and methane content per all treatments. The complex structure of lignocellulosic biomass was significant affected by NaOH pretreatment; the experimental results demonstrated that the adding concentration of NaOH leads to an increased content of the biodigestibility of the substrate under anaerobic condition and make it easy to access to hydrolytic bacteria at the early stage of the digestion process.

The cumulative biogas from fresh and dry of water primrose under anaerobic mono-digestion were 4,054.13 mL and 4,285.80 mL, respectively, which showed no significant difference. However, the methane obtained was improved by dry samples, which was 55.07% higher than fresh samples (51.32%). Furthermore, when the samples were pretreated with NaOH, the quantity and quality of biogas production was increased. The optimal results for fresh samples were at 3% NaOH with a total biogas yield of 6,692.55 mL and 61.23% of CH_4. Meanwhile, dry samples were at 2% NaOH with a biogas yield of 8,072 mL and 64.72% of CH_4. Considering the efficiency and cost of the pretreatment process in anaerobic digestion, treatment of 2% NaOH concentration is the best option for pretreatment of the dry substrate, which quickly reaches the highest volume and achieves the highest cumulative biogas volume.

Overall, all treatments' peak methane value was found at the final stage of anaerobic digestion. This is because the acidogenic and methanogenic bacteria took a long time to adapt and balanced growth. Another reason is maybe the specific amount of organic inhibitors in the substrate was further degraded and converted to biogas generation, which contributed to methane concentration (Wang et al., 2020; Zheng et al., 2017).

A comparison of methane content in biogas from water primrose with another feedstock was gathered and is presented in Table 4.3. The results of methane value from different biomass sources compared to the result obtained from this study showed that the potential maximum methane content available in water primrose is highly competitive with other aquatic plants or terrestrial plants.

Table 4 3 Comparison of methane concentration from different feedstocks.

No.	Feedstock name	Methane (%)	References
1	Eichhornia crassipes	40.3	(Pereira et al., 2011)
2	Corn stover	51	(Wang et al., 2020)
3	Food waste	59.0	(Li et al., 2010)
4	Fruit/vegetablewaste	63.4	(Qiao et al., 2011)
5	Food waste	68.0	(Qiao et al., 2011)
16	WildMexicanSunflower	65	(Dahunsi et al., 2017)
7	Silage maize straw	67.83	(Li et al., 2017)
8	Rice straw	63	Li et al. (2017)
9	Tobacco straw	63.37	Li et al. (2017)
10	Dry maize straw	65.47	Li et al. (2017)
11	Soybean residues	57.14	(Onthong and Juntarachat, 2017)
12	Papaya peels	54.00	(Onthong and Juntarachat, 2017)
13	Sugarcane bagasses	49.12	(Onthong and Juntarachat, 2017)
14	Rice straws	56.25	(Onthong and Juntarachat, 2017)
15	Maize straw	42.05	(Wei et al., 2019)
16	Water primrose	64.72	This study

4.4 Biogas production from anaerobic co-digestion

The water primrose substrate has undergone pretreatment by alkaline (NaOH) with different doses. Study findings revealed that 2% NaOH concentration was the best condition for dry samples. Thus, this treatment was further continued to investigate its biogas potential in anaerobic co-digestion with cow dung. Anaerobic digesters of all treatments in this study were using 1-L bottles containing samples of T1 (T1-A, T2-B, T3-C), T2 (T2-A, T2-B, T2-C), and T3 (T3-A, T3-B, T3-C). The anaerobic digestion experiment was started immediately after samples pretreated by NaOH solution were done at 7-day, 14-day, and 21-day period. The cow dung was co-substrate with water primrose at ratios prepared as each treatment corresponds to 1:1, 1:2, and 2:1 on TS basis. The characteristics of pH, TS, VS, COD, VFAs, and alkaline of co-substrate before fermentation are given in Table 4.4.

The daily and total biogas production, methane concentration of co-digestion (water primrose and cow dung) at the three mixing ratios of 1:1, 2:1, and 1:2, are shown in Figs.4.7 and 4.8. The final total volume of biogas was calculated after 45 days of operation by the amount of gas obtained per day. The gas components were measured by gas analyzer GA5000. The trend was similar to the lowest total biogas obtained at 1 week and highest at 3 weeks'pretreatment time for each mixing ratio. Additionally, the increasing volume of biogas obtained, the higher the concentration methane achieved. Hence, the methane content in biogas indicates the amount of solubilization of the substrate was degraded by microorganisms in anaerobic digestion.
The maximum biogas volume in the digesters from treatment 1 to treatment 3 was 7200 mL, 8610 mL, and 8100 mL obtained in T1-C, T2-B, and T3-B, respectively. As a result, the methane concentration was distributed as T2-B (68.20%) > T3-B (66.05%) > T1-C (64.55%). In comparison with the biogas volume between each treatment, there was no significant different volume observed. However, these values were 67.9%, 88.9%, and 100.01% higher if compared with the control of water primrose in terms of biogas volume and 32.4%, 48.9%, and 51.9% if compared with the control of cow dung. Even though at the co-digestion of water primrose to cow dung in mixing ratio 1:2 carried more microorganisms than others, the efficiency of this treatment did not achieve the highest value of biogas production and methane concentration. The possible reason is that the substrate's limited organic matter was not enough supplied for anaerobic microorganisms to convert to methane product. The pretreatment of NaOH reflected the higher value of TS, VS, and COD than the controls made more biodegradability of the components in the feedstock, thus obtained higher biogas production.

Table 4.4 Parameters of co-digestion before the fermentation process

Treatment		Parameter					
		pH	TS (mg/L)	VS (mg/L)	COD(mg/L)	ALK (mg-$CaCO_3$/L)	VFA (mg/L)
T1	T1-A	8.8±0.11	47,500±1,500	36,000±1,322	50,667±2,542	4,708±212	1,216±395
	T1-B	8.8 ± 0.09	56,500 ± 2,500	45,000±1,412	52,000±2,832	6,000 ± 270	1,596 ± 211
	T1-C	8.8 ± 0.10	58,500±2,500	46,000±1,548	53,333±2,266	6,292±257	1,474 ±871
T2	T2-A	8.8±0.10	74,353±3,405	50,000±1,644	105,128±4,211	7,500±468	1,033 ± 415
	T2-B	8.9 ± 0.12	79,667±3,503	62,000 ± 1,459	133,667 ± 4,257	7,375±417	1,409±542
	T2-C	8.9 ± 0.08	77,500 ± 3,143	51,500 ± 1,355	132,167 ± 4,221	7,813±355	1,226±496
T3	T3-A	8.7 ± 0.13	64,000 ± 3,214	48,333 ±1,724	59,333±2,158	5,563 ± 313	1,052 ± 484
	T3-B	8.7 ± 0.13	65,000 ±3,256	48,361 ± 1,641	74,667±2,551	7,438 ± 629	1,332 ± 394
	T3-C	8.8 ± 0.10	69,667 ± 3,521	48,500 ± 1,270	90,667 ± 3,344	6,750 ± 375	1,439 ± 580

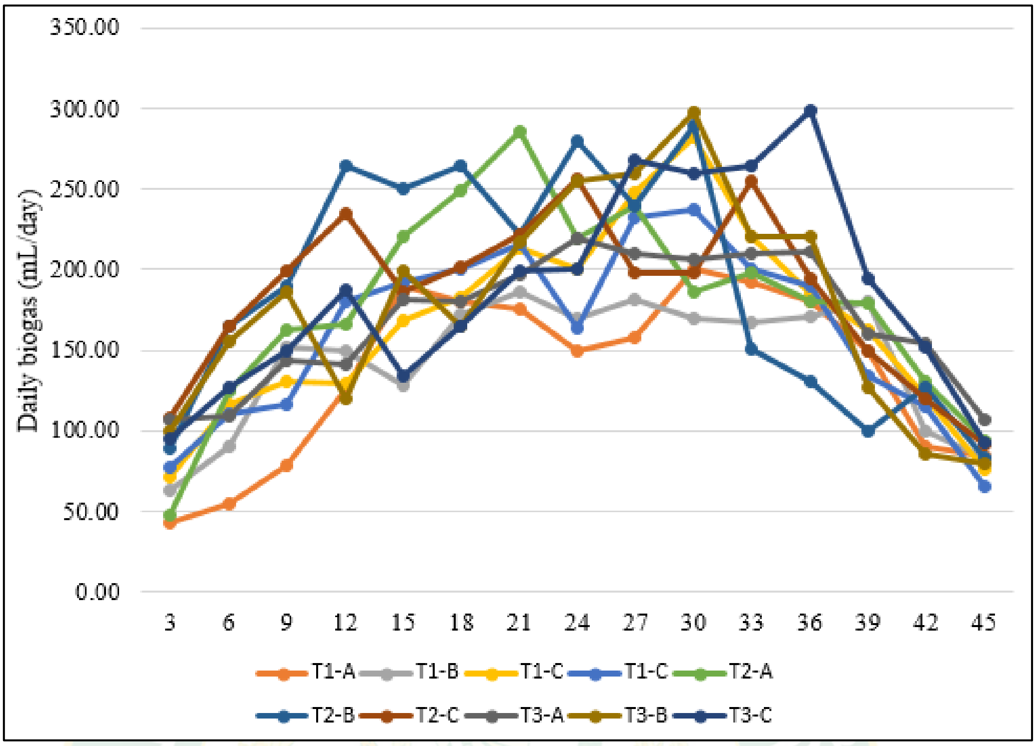


Figure 4.7 Daily biogas production of anaerobic co-digestion

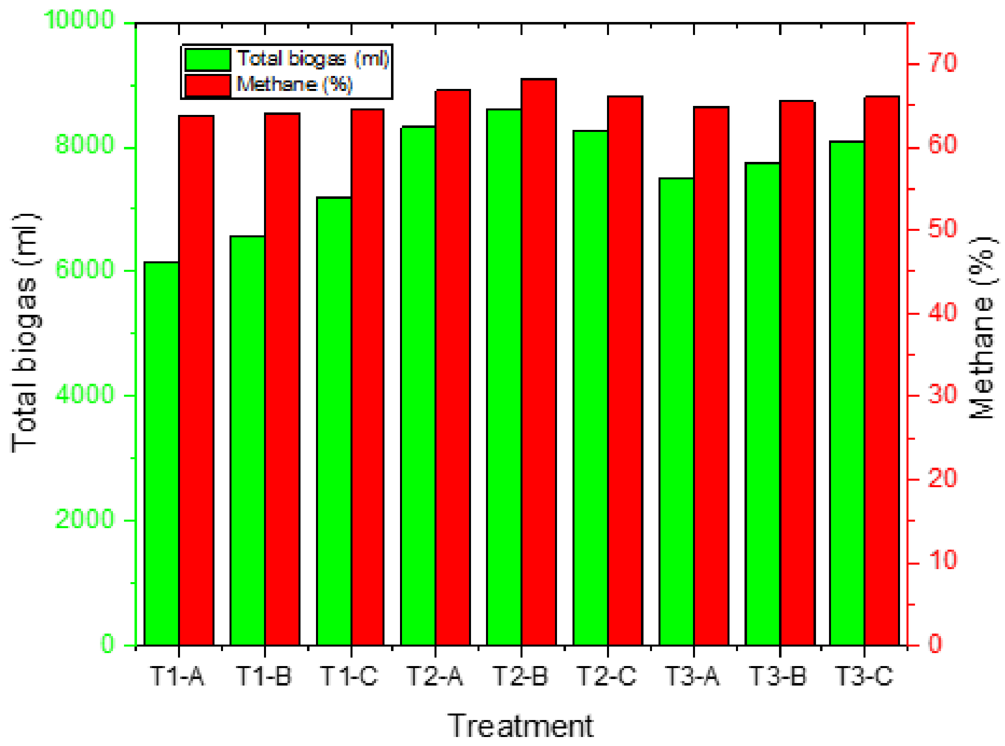


Figure 4.8 Biogas production and methane concentration of anaerobic co-digestion

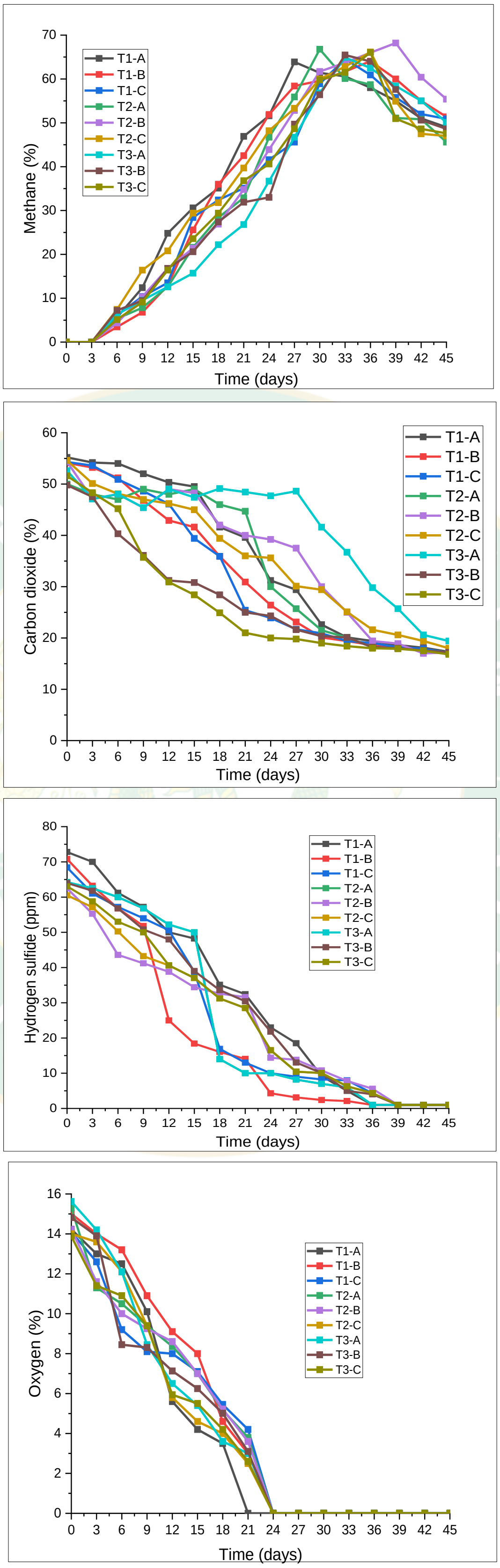


Figure 4.9 Biogas compositions of anaerobic co-digestion

Degradation efficiency of TS, VS, and COD

In the anaerobic digestion process, the biodegradable TS, VS, and COD represented by the amount of organic matter in the substrate was converted into the final product named methane and carbon hydroxide. In other words, the degradation of organic fractions in the substrate is proportional to the biogas produced (Feng et al., 2019). The efficiency of TS, VS, and COD reduction from all treatments was calculated by the initial and final value relationship and was expressed as a percentage, shown in Table 4.4. Besides the volume of biogas production, the measurement of TS, VS, and COD reduction could further evaluate the efficiency of biodegradability of feedstock. It can be seen that after 45 days of anaerobic digestion, the 2% NaOH pretreatment has a positive effect on degradability compared with the controls.

Moreover, the removal efficiency of organic material increased with increasing initial value performed by the higher percentage of TS, VS degradation rate, and COD removal rate. As mentioned earlier, the highest initial value of TS, VS, and COD was obtained from mixing ratio 2:1 at 2 weeks' pretreatment time. Thereby, the reported data has verified the results that the COD removal rate and VS degradation rate at T2-B treatment were achieved higher than the rest treatments.

As shown in Table 4.4, the percentage of TS and VS reductions for NaOH-treated water primrose was from 57.55– 58.95% to 50.22–53.31% in treatment 1, in the range of 61.65–70.84% and 54.36–64.76% in treatment 2, and lastly, in treatment 3 were 59.13–64.45% and 51.62–59.74% accordingly. Compared with the controls, these results were slightly higher. Pang et al. (2012) mentioned that after NaOH pretreatment, the organic material was increased, and more quantity soluble components were utilized by bacteria, which led to a higher biogas production related to increasing TS and VS reductions. The experimental results showed that the COD degradation was obtained at a range of 51.46– 66.55% removal efficiency for all mixing ratios in co-digestion, whereas the COD reduction control of water primrose and cow dung was 45.25 and 40.84%, respectively. Stabilization of the COD reduction after 21 days may be due to exhausting nitrogen and carbon content and the aging of the microbial cells (Kumar et al., 2020).

Table 4 5 Removal efficiency of the mixture in anaerobic digestion

Treatments		**Removal efficiency (%)**		
		TS	**VS**	**COD**
T1	T1-A	57.55	50.22	52.64
	T1-B	58.95	51.41	55.19
	T1-C	58.01	53.31	51.46
T2	T2-A	65.65	60.55	59.86
	T2-B	70.84	64.76	66.55
	T2-C	61.65	54.36	57.88
T3	T3-A	59.13	51.62	54.74
	T3-B	62.44	56.28	55.06
	T3-C	64.45	59.74	55.44

The significant removal of physico-chemical parameters of concentrated liquid, semiliquid, or solid biomass was used for biogas production. The results proved that co-digestion gave more biodegradability material for biogas production due to the nutrient in co-substrate, which helped the anaerobic microorganisms thrive more smoothly.

Response Surface Methodology (RSM) modeling for anaerobic co-digestion

In order to achieve the optimum biogas production, the factor of different pretreatment times of water primrose and mixing ratio with cow dung considered were optimized using response surface methodology (RSM). RSM was employed for investigating the influence of two different factors on experimental methane yield from pretreatment time, mixing ratio and the relationship between them. A three-level-two-factorial experiment was designed by central composite design (CCD) in order to optimize the parameters. The 20 runs CCD for biogas production are shown in Table 4.5 with both replicates of factorial points, and star points were 2 and center points were 4. The factors and levels were numbered as flowing:

Factor 1: NaOH (7 days), NaOH (14 days), and NaOH (21 days)

Factor 2: Ratio 1 (1:1), ratio 2 (1:2) and ratio (2:1)

The biogas production was selected as the dependent variable, namely responses to the test. In order to predict the optimal point and the peak value, the Design- Expert 11 software was used and the second-order polynomial formulation (Eq. 23) was employed to fit the independent variables and the responses.

$$\text{Biogas production} = +8436.07 + 271.67A + 605.00B - 62.50AB - 4.64A^2 - 1254.64B^2 \quad \text{..........Equation 23}$$

Where: A= NaOH (day)

B= Ratio

The equation in terms of coded factors can be used to make predictions about the response for each factor's given levels. 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.

From the above equation, the increase of two factors of pretreatment time by NaOH and ratio led to the negative of biogas production as the se response can be seen clearly in Fig. 4.10. The ANOVA for the response surface quadratic model for biogas yield was presented in Table 4.6.

Table 4 6 RSM design of experiments and obtained results

Run	Factor 1	Factor 2	Biogas production		Residual
	A:NaOH (day)	B:Ratio	Actual value	Predicted Value	
1	21	3	8100	7990.95	109.05
2	14	2	8610	8436.07	173.93
3	14	3	7750	7786.43	-36.43
4	7	2	8320	8159.76	160.24
5	21	2	8500	8703.10	-203.10
6	21	2	8500	8703.10	-203.10
7	14	1	6570	6576.43	-6.43
8	21	1	7000	6905.95	94.05
9	14	1	6570	6576.43	-6.43
10	21	3	8100	7990.95	109.05
11	7	1	6150	6237.62	-87.62
12	7	1	6150	6237.62	-87.62
13	14	2	8610	8436.07	173.93
14	14	3	7750	7786.43	-36.43
15	7	3	7500	7572.62	-72.62
16	21	1	7000	6905.95	94.05
17	14	2	8000	8436.07	-436.07
18	7	3	7500	7572.62	-72.62
19	7	2	8320	8159.76	160.24
20	14	2	8610	8436.07	173.93

The Model F-value of 74.35 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, A, B, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 2.70 implies a 9.66% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. The coefficient of determination (R^2) is the proportion of variation in the response due to the fitting model rather than to random error, and it is favorable that the R^2 value is above 80% (Joglekar and May, 1987). The R^2 of 0.9637 in Figure 4.9 is as close to the Adjusted R^2 of 0.9507. The Predicted R^2 of 0.9361 is in reasonable agreement with the Adjusted R^2 of 0.9507; i.e., the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 24.188 indicates an adequate signal. This model can be used to navigate the design space in Table 4.7.

Using Design Expert software, contour plots and 3D surface plots were generated to find the optimum operating conditions of the biogas production's anaerobic digestion process. A contour plot provides a two-dimensional view where all points with the same response are connected to produce contour lines of constant responses. A surface plot provides a threedimensional view that may provide a clearer picture of the response surface (Rao and Baral, 2011). The response surface plots and corresponding contour plots of biogas yield are shown in Figs. 4.10. These plots are drawn by keeping one variable at its central point level and varying the others within the experimental range.

As shown in Fig. 4.10, the interaction between NaOH pretreatment time and mixing ratio suggests that in order to obtain the maximum biogas production, the NaOH pretreatment time needed in an anaerobic digestion system is different under different conditions of time pretreated and mixing ratio. As can be seen in the plots, at the short time of the pretreatment process, the biogas production was considerably low and it increased first and then decreased with the increased pretreatment time of NaOH from 7 days to 21 days. The maximum biogas yield was achieved at the central point. Thus, it decrease when there is an increase or decreasing the ratio from the central point. The yield started decreasing when the ratio greater than 2.5. The optimum region for biogas production rate is in the time range of 14 days and the ratio is in the range of 2 (2 to 1), respectively.

Table 4.7 ANOVA for the response surface quadratic model for biogas production

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.287E+07	5	2.575E+06	74.35	< 0.0001	significant
A-NaOH	8.856E+05	1	8.856E+05	25.57	0.0002	
B-Ratio	4.392E+06	1	4.392E+06	126.82	< 0.0001	
AB	31250.00	1	31250.00	0.9023	0.3583	
A^2	100.60	1	100.60	0.0029	0.9578	
B^2	7.346E+06	1	7.346E+06	212.10	< 0.0001	
Residual	4.849E+05	4	34633.59			
Lack of Fit	2.058E+05	3	68598.41	2.70	0.0966	not significant
Pure Error	2.791E+05	1				
Cor Total	1.336E+07	9				

Table 4 8 Fit statistics of biogas production

Std. Dev.	186.10	**R^2**	0.9637
Mean	7680.50	**Adjusted R^2**	0.9507
C.V. %	2.42	**Predicted R^2**	0.9361
		Adeq Precision	24.1875

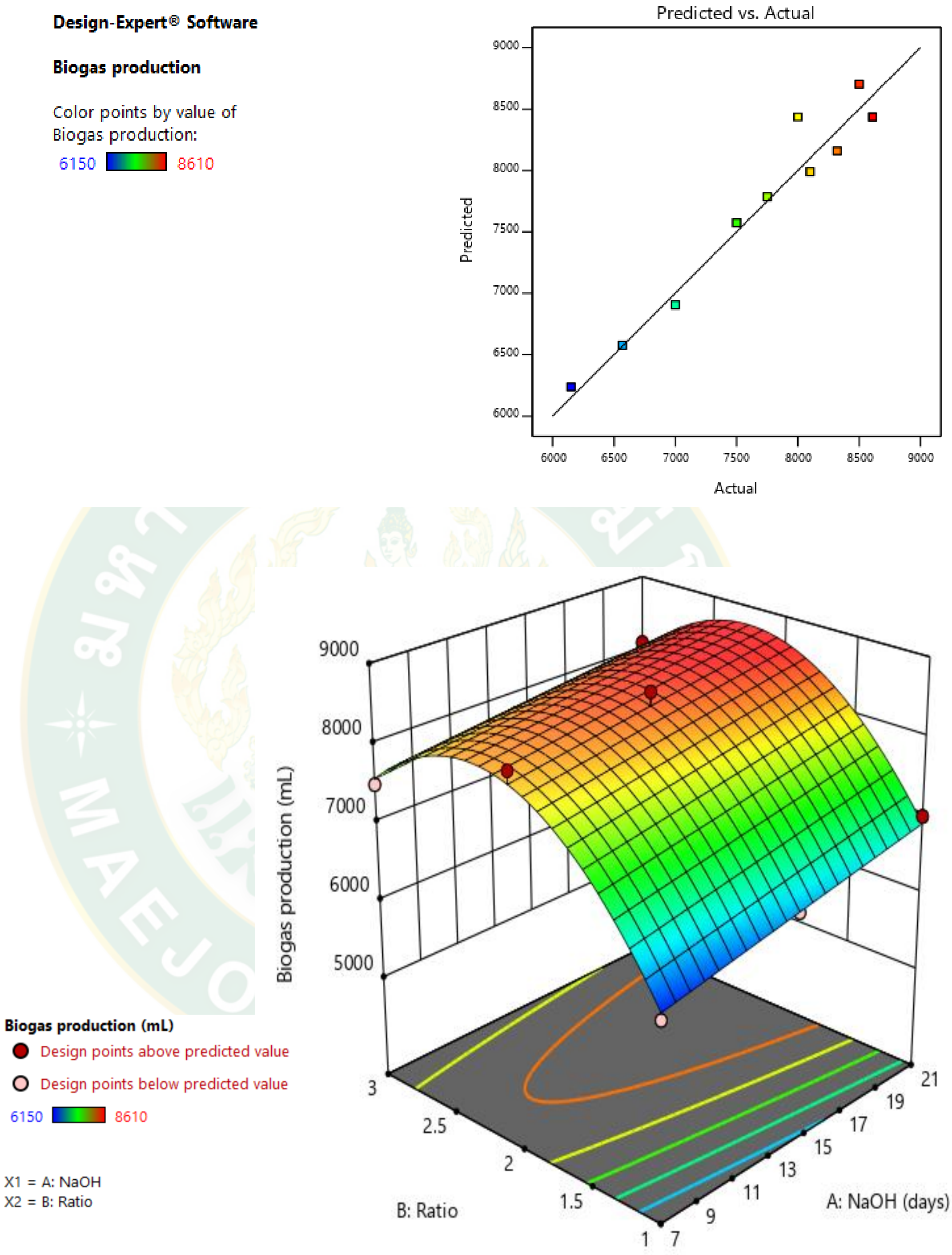


Figure 4 10 Comparision of the predicted and actual value of biogas production from RSM modelling

4.5 Pilot-scale for biogas production from anaerobic co-digestion

In order to support the result from laboratory-scale experiments, pilot- scale work of anaerobic digestion was implemented under more realistic conditions and also was to facilitate future practical application. The best performance of pre-treatment time of sodium hydroxide and cow dung ratio to water primrose was chosen for the pilot-scale experiment. The substrates converted the water primrose and cow dung into biogas under anaerobic digestion. Pretreated water primrose and cow dung using as co-substrates were calculated based on dry matter of water primrose with 10% TS per liter and added into the digester at the start of the process. The materials collected from the same agricultural field (water primrose) and cattle farm (cow dung) to ensure the most uniform feed characteristics possible.

The pH is an important indicator reflecting the growth of microorganism in anaerobic fermenter varied from 6.8 to 7.2 (Sreekrishnan et al., 2004) with the optimum value at 7.0-7.2 (Khalid et al., 2011). Similarly, the pilot-scale digester was operated at pH 7-7.5 in mesophilic temperature (25-37^{o}C) as shown in Fig. 4.11. In practical, the beginning pH of digester was 8.66 then dropped to 6.15 during the first week operation system due to the hydrolysis stage occurs of biodegradable material producing fatty acids (Khayum et al., 2018). The methanogens was quickly adapted to growth and develop in digester from week two onwards since the pH was more stable in digester ranged from 7.04- 7.67. This is demonstrated by gradually increasing methane content to reach 50% on day 23th and 68.6% on day 35th as the highest value in this study.

The retention time of conventional anaerobic process is in the range of 30-60 days (Khanto and Banjerdkij, 2016), whereby, the experiment was operated until 45 days to assess the stability of the process in this study. The performance of daily biogas and cumulative biogas production in anaerobic co-digestion is illustrated in Fig. 12 Results showed that, the digestion started producing gas on the first day of process and gradually increased with no fluctuation of biogas production. However, it took about 3-4 days from beginning process to detect methane gas in biogas (Fig 4.12) as this time the hydrolysis process occurs with pH drop from 8.66 to 6.15 and the acidification inhibited methanogens activity. In contrast to CH_4, carbon dioxide was produced at high concentration from the beginning of the experiment, which was due to the reaction of dissolved organic matter converted into CO_2 at the acidogenesis stage (Zemiński and Frąc, 2012).

From week two till week five, biogas produced was more stable at high volume obtained and fluctuated in ranged of 100-135 L/day. In addition, it can be observed from Fig. 6 that the first peak volume reached on 16th day with 100 L/day, and quickly reached second peak volume on 35th day with 135 L/day. Then daily biogas decreased after 36 day of digestion time as less organic matter remaining in the material for bacterial survive and growth (Khayum et al., 2018). It indicates that substrate decomposition taking place over a period of 12-15 days of retention time to achieve optimal biogas yield. In another word, the VFAs accumulation was consumed by methanogen activity and thus buffer recovered at pH of 7.15-7.23 (Chandra et al.Vijay et al., 2012). Accordingly, the initial methane in biogas generated on 4th day with 2.6% and in ranged of 50.9%- 68.6 % from 24th day onwards. In this study, the maximum daily biogas production associated highest methane content which was obtained on day 35. The biogas production reached 1,026 L on day 17^{th} and quickly reached 2,077 L after 10 days, the total gas was 4,160 L at the end of digestion process.Generally, anaerobic digestion of lignocellulosic material may not feasible in biogas generation due to the lack of the active microbial community, whereas, cow dung mixed with lignocellulosic substances would enhance the biogasifiability as cow dung has essential bacterial nutrients to support digested biomass as well as maintain the nutrient values in slurry (Kumar et al., 2020). Earlier studies was done by investigating anaerobic co-digestion of biomass and cow dung via different mixing ratios (Kumar et al., 2020; Latinwo and Agarry, 2015; Mel et al., 2015; Muthukumar et al., 2018).These studies confirmed that the mixing ratio of cow dung and biomass boosted material degradation, and at the same time, resulting in high biogasifiability and methane potential. In comparison, the current 1000 L pilot-scale produced biogas yield of 1.7 mL/ gTS/day which was lower than 1 L of lab-scale.

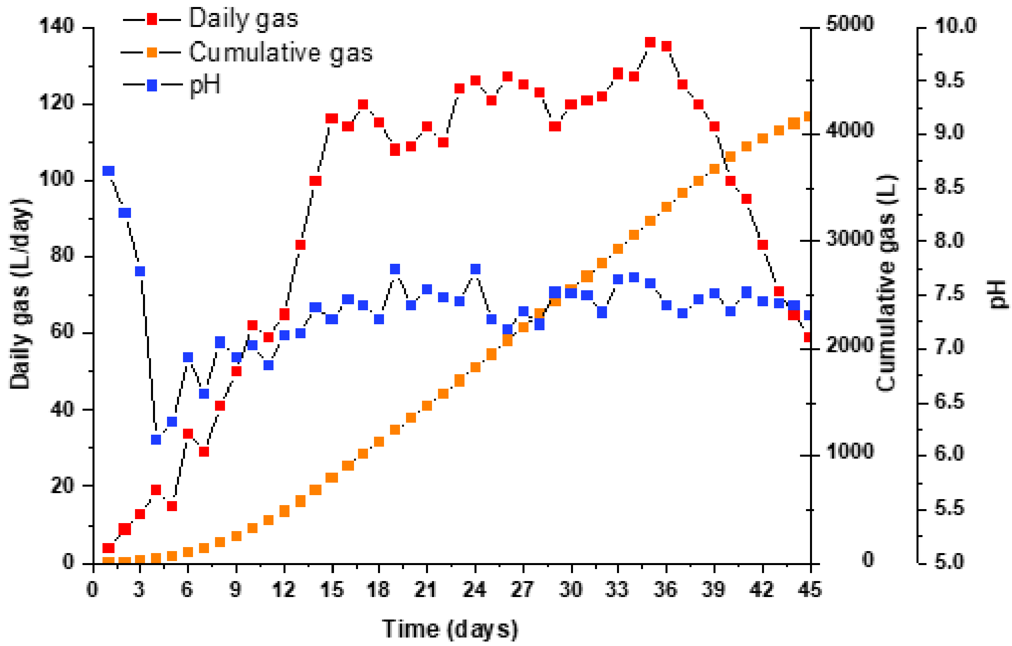


Figure 4.11 Daily, cumulative gas produced and pH recorded in pilot-scale digester

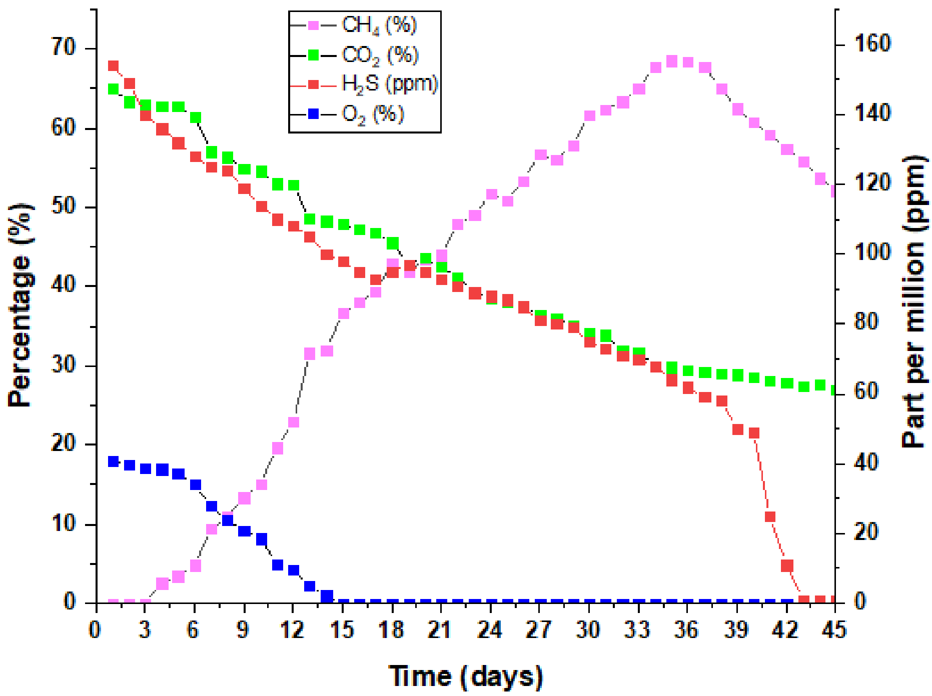


Figure 4 12 Biogas composition in pilot-scale digester

This may due to unreasonable control conditions such as uneven temperature and heterogeneous mixing because a larger dead mass in the digester (Zhang et al., 2018).

4.6 Biogas upgrading using chemical absorption

Absorption is a reactive absorption process of transferring a component of its gas into a chemical solvent (gases being absorbed by a liquid) and involves chemical reaction of CO_2 and aqueous solutions to form a weak binding intermediate compound (Lawal et al., 2010).

In this experimental study, raw biogas was passed through two purification columns, each column provides different purification substrates. The first column was filled with adsorbent substrate of iron oxide, hereby, when the biogas enters through the purification device, the corrosive gasses will react with iron oxide to form insoluble iron sulfide. Iron oxide presents in different shapes and types, in this experiment, the steel wool (iron sponge) is made from iron fiber that exists in everyday life was used. The reaction can be expressed as (Sarperi et al., 2014):

$$FeO + H_2S \rightarrow FeS + H_2O$$ ....Equation 23

$$Fe_2O_3 + 3H_2S \rightarrow Fe_2S_3 + 3H_2O$$ ....Equation 24

During scrubbing process, several reaction of iron oxide with H_2S do occur as following:

$$Fe_3O_4 + 4H_2S \rightarrow 3FeS + 4H_2O + S$$ ....Equation 25

$$Fe_3O_4 + 6H_2S \rightarrow 3FeS_2 + 4H_2O + 2H_2$$ ....Equation 26

$$FeS + S \rightarrow FeS_2$$ ....Equation 27

The reduction of H_2S in gas stream was effectively reduces by using iron sponge resulting in the insoluble salt (FeS) as the product of the reaction ($Fe^{2+} + S^{2-} \rightarrow FeS$). The resultant FeS was easily taken from the system along with discharged solids. It will be oxidized in the atmosphere to the formation of dissolved salts and used as plant nutrients (Angelidaki et al., 2018).

In the second purification column, investigation was focus on two gases removal of CO_2 of alkaline solution and raw biogas called chemical absorption process. The solubility of CO_2 was examined at different doses of NaOH/ $Ca(OH)_2$ (1%, 2% and 3%). Calcium hydroxide or sodium hydroxide was prepared by the reaction of calcium oxide/sodium hydroxide pellets with water. These columns were vigorously shaken to mix well to produce an aqueous solution. The precipitate was then settled and removed from the solution after letting it stand for 15-20 min. Firstly, when sodium hydroxide and calcium hydroxide are dissolved in water, due to it is strong alkaline, negative hydroxide ions (anions, OH^-) and positive sodium/calcium ions (cations, Na^+/ Ca^{2+}) are almost fully ionized in water (Yoo et al., 2013). This part of the reaction can be shown as (Rajagukguk and Satria, 2019):

$$Ca(OH)_2 \rightarrow Ca^{2+} + 2OH^- \qquad \text{..........Equation 28}$$

$$NaOH \rightarrow Na^+ + OH^- \qquad \text{..........Equation 29}$$

Secondly, the dissolved carbon dioxide and either water or hydroxide ions to form H^+ ions and bicarbonate by reversible reactions (Da Silva et al., 2007). It occurs in very fast rate and in high level of pH (pH>10) (Yincheng et al., 2011):

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad \text{..........Equation 30}$$

$$CO_2 + OH^- \rightleftharpoons H^+ + HCO^{3-} \qquad \text{..........Equation 31}$$

Bicarbonate ions are immediately react with hydroxyl ion (OH^-) to form carbonate ions:

$$HCO^{3-} + OH^- \rightleftharpoons CO_3^{2-} + H_2O \qquad \text{..........Equation 32}$$

A decreasing of OH- anions occurs when CO_2 continuously is absorbed by an alkaline solution, the overall reaction may in general be expressed (Rajagukguk and Satria, 2019):

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O \qquad \text{..........Equation 33}$$

$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$ ....Equation 34

After reaching the saturated condition of CO_3^{2-}, further CO_2 is fed in aqueous solution can be occurred according to the reaction:

$Na_2CO_3 + CO_2 + H_2O \rightarrow NaHCO_3$ ....Equation 35

$CaCO_3 + CO_2 + H_2O \rightarrow Ca(HCO_3)_2$ ....Equation 36

In addition, the H_2S content in the outlet of the first column was further dissolved in alkali chemisorption process:

$H_2S + OH \rightarrow HS^- + H_2O$ ....Equation 37

$H_2S + NaOH \rightarrow NaHS + H_2O$ ....Equation 38

$H_2S + 2Ca(OH)_2 \rightarrow Ca(HS)_2 + 2H_2O$ ....Equation 39

The ability to absorb CO_2 and H_2S by using chemical solvents as NaOH, $Ca(OH)_2$ and steel wool is presented in Table 4.9. The difference of gas composition in inlet was the result of gas produced in different days from the pilot digester, however, it was not affect the comparability of the experimental results. Carbon dioxide and hydrogen sulfide were simultaneously removed after purification process, generated methane enriched biogas. The absorption of CO_2 and H_2S was simultaneously absorbed by reacting rapidly with purification substrate resulting in remaining gas outlet was less than 13% of CO_2 and 1.5 ppm of H_2S for all trials after purification process with the initial value of CO_2 in ranged 30.2-31.2% and H_2S in ranged 40.8- 46.1 ppm. The results from Table 4.9 are compared the removal efficiency of CO_2 and H_2S and also CH_4 enrichment in biogas from different concentration of NaOH and $Ca(OH)_2$.

It is observed that the concentration of absorbents has a great influence on the CO_2 removal efficiency in which the removal efficiency increased about proportionally with increasing doses of the absorption liquids. The maximum value in the measured CO_2 removal efficiency was found in 3% NaOH with 62.91% and lower removal efficiency in 1% and 2% NaOH which was 58.33% and 59.68%, respectively. Similar result was recorded by using $Ca(OH)_2$ with the highest concentration reached highest CO_2 removal which was 64.74% at 3% $Ca(OH)_2$, followed by 2% and 1% which was 61.97% and 60.33%, respectively. This can be explained that increasing concentration produces a greater quantity of OH^- concentration and react with CO_2 which leading to increase CO_3^{2-} concentration compared to HCO_3^- and therefore, enhance absorption rate as well as higher CO_2 removal efficiency. Notably, the forward of reaction 13 was dominated during initial time of the absorption process due to the presence of a very high alkalinity in absorbent.

Table 4.9 Comparison biogas composition and its efficiency

Absorbent	Gas input			Gas output			Efficiency (%)		
	CH_4 (%)	CO_2 (%)	H_2S (ppm)	CH_4 (%)	CO_2 (%)	H_2S (ppm)	CH_4 enrichment	CO_2 removal	H_2S removal
1% NaOH	67.2	31.2	46.1	81.8	13.0	1.2	21.73	58.33	97.4
2% NaOH	67.9	31	40.8	83.8	12.5	1.4	23.42	59.68	96.57
3% NaOH	68.5	30.2	39.1	85.2	11.2	0.8	24.38	62.91	97.95
1% $Ca(OH)_2$	68.1	30.5	40.8	84.5	12.1	1.5	24.08	60.33	96.32
2% $Ca(OH)_2$	68.5	30.5	42.5	86.1	11.6	1.1	25.69	61.97	97.41
3% $Ca(OH)_2$	68.5	31.2	41.3	88.2	11.0	0.3	28.76	64.74	99.27

However, it also can be found that CO_2 removal efficiency was reduced with reaction time within each measurement series when the reduction of the outlet stream every 30 min was not significant after one hour. In another word, the absorbent tends to be more saturated when the time increases. Hence, high CO_3^{2-} accumulation forced the backward reaction (32) and leading to the forward reaction (31) resulting in decreasing HCO_3^- concentration and lower CO_2 removal efficiency. When CO_2 was continuous fed after the exhaustion of OH^-, the carbonate was converted to bicarbonate as reaction (35) and (36). This chance occurs at low rate about 4-5% (Kordylewski et al., 2013).

Regarding to H_2S removal efficiency, solid desulfurizing substances such as steel wool was utilized in this study. The selection was given low-cost investigation and ability to regeneration (Kulkarni and Ghanegaonkar, 2019). In overall, a nearly constant H_2S level was obtained after the gas flow passed through two consecutive columns for all experiment series which were maintained below 1.5 ppm at differing H_2S concentration input varied from 46.1 ppm to 40.8 ppm. The efficiency achieved at high level of greater than 96% for all experiments. That implies the input H_2S concentration does not much effect to the removal efficiency in this substrate investigation. The H_2S was readily reacted with iron oxide to form iron sulfide and the accumulation of elemental sulfur covered on steel wool after purification process is shown in Fig. 8. Due to the H_2S removal decreases considerably as less than 1.5 g/l for each experiment, therefore, it is possible to use steel wool in order to support desulfurization process. On the other hand, when absorption of steel wool is saturated, once regeneration, its activity will reduce 1/3 compared to its original, therefore, it has to be renewed after once or twice used (Abatzoglou et al., 2009). In fact, with low concentration of H_2S (<1%) and small scale of experiment, using steel wool is an effective method for removal of H_2S.

Figure 4.13 The accumulation of elemental sulfur after purification process

On the concern of methane enrichment, Table 4.10 shows the comparison of CH_4 increased from raw to purified biogas. It is found that the CH_4 enrichment is proportional to the increase in the doses of absorbent. In the same condition and quantity of chemical reagents, the $Ca(OH)_2$ was more effective than NaOH when it achieved CH_4 concentration higher after purified. The CH_4 concentration was 81.8%, 83.8% and 85.2% after purified by using 1%, 2% and 3% NaOH, respectively, which enriched CH_4 concentration up to 21.73%, 23.42% and 24.38% compared to its original concentration. While in using 1%, 2% and 3% $Ca(OH)_2$, the methane concentration increased to 84.5%, 86.1% and 88.2%, respectively, also methane enrichment was 24.08%, 25.69% and 28.67%, respectively. Therefore, the efficiency of CH_4 enrichment is possible to reach above 80%, even at mild concentration of alkaline solution or nearly 90% could able to obtain at higher concentration (3% $Ca(OH)_2$).

Besides, the sponge used in this study are non-reactive and tasteless, odorless, non-toxic and non-corrosive. Hence, the structural characteristics of sponge which contains empty space between fibers in the sponge making it to swell when soaking up with solution. In that way, the liquid solution is kept in within the sponge and can easily accumulate moisture from raw biogas.

The aim of biogas purification in this study was to achieve high CO_2 removal efficiency in biogas stream at minimal alkali consumption and that feasibility was confirmed. The efficiency of method using NaOH solution under normal conditions was competitive to the method using $Ca(OH)_2$. Nevertheless, CO_2 absorption was more prospective for $Ca(OH)_2$ absorbent when 60.33% to 64% CO_2 removal efficiency achieved compared to 58.38% to 62.91% for NaOH absorbent at 1% to 3% concentration, respectively.

The kinetic of CO_2 absorption

As the absorption proceed continuously within 2 hours, the carbon dioxide in the mixture gas was absorbed and accumulated into the aqueous solution. In scrubbing column, at an early stage in scrubbing column, due to a high concentration presence, CO_2 was quickly started reacting according to the stoichiometry of the reaction to form carbamate and bicarbonate until the solvent was completely saturated or neutralized at pH 7-8 (Tippayawong et al., 2010). At a certain time, the concentration of CO_2 inlet was equal to CO_2 outlet of gas stream, this demonstration is shown in Fig. 14, the plots showed that NaOH and $Ca(OH)_2$ solution could able to absorb 50% CO_2 inlet gas at the first 30 minutes of the process. More clearly, the first measurement of the rate C/C_0 of CO_2 absorption experiments were above 50% and gradually increased to above 90% at the end of the absorption process. The 3% $Ca(OH)_2$ absorbent was achieved almost saturated with 96.75% meanwhile the 3% NaOH absorbent reached 93.79% after 120 min absorption time. Alternatively, other concentrations of the aqueous solution were also highly effective with the rate of declining CO_2 absorption of 1% and 2% NaOH was 91.54% and 92.71% while 91.61% and 91.78% for 1% and 2% $Ca(OH)_2$, respectively.

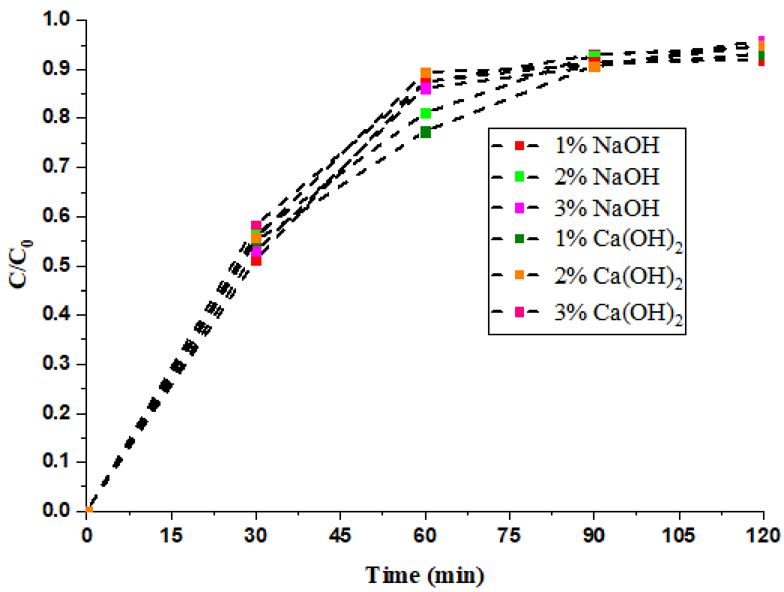


Figure 4.14 Plot of absorption time (min) vs C/C_0 for CO_2 absorption

In addition, the model parameters of the theoretical predictions is shown in Table 4.10. As τ dedicated the absorption time when the CO_2 inlet reaches 50% of CO_2 outlet at which $\ln[C/(C-C_0)]$ is zero. Accordingly, τ reach its value at very short time of the absorption process which was no longer than 40 min reaction. In case of using NaOH solution, value of τ was 30.6 min, 33.7 min and 31 min for 1%, 2% and 3% concentration respectively meanwhile $Ca(OH)_2$ reached its value at slightly longer time of 34.88 min and 37.03 min for 1% and 2% concentration compared to NaOH solution. Still, at 3% Ca $(OH)_2$, the absorption time had tendency to be shorter with 26.98 min, this demonstrated that $Ca(OH)_2$ was very reactive and contributed to more efficient CO_2 absorption. This evidence is clearly observed in Fig.4.13. A similar results were reported by Tippayawong et al., the study employed different aqueous solutions of sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$) and mono-ethanolamine (MEA) to remove CO_2 from biogas in a packed column reactor. These solutions were achieved saturation with CO_2 after saturation with CO_2 after 50 min for $Ca(OH)_2$, and 100 min for NaOH and MEA corresponding to value of $Ca(OH)_2$ was 29 min, NaOH and MEA were 42 min and 36 min, respectively.

Table 4.10 Kinetic parameters for CO_2 absorption

Parameters	Absorbent					
	1%NaOH	2%NaOH	3%NaOH	1% $Ca(OH)_2$	2% $Ca(OH)_2$	3% $Ca(OH)_2$
k (min)	0.90	0.80	0.80	0.85	0.75	0.88
τ (min)	30.61	33.7	31.04	34.88	37.03	26.98
R^2	0.9493	0.89865	0.86813	0.98316	0.96125	0.88579

Moreover, the correlation coefficient R^2 reflected the relationship of prediction of model and obtained data from experiments which given a goodness of fit with R^2 values of greater than 0.89 for all the experimental series (0.89-0.99).

4.7 Energy analysis

The primary energy inputs to mineral-based resources are deliberated. It refers to the energy that has not undertaken any conversion or transformation process. Many parameters may define the efficiency of biogas production, so the multi-criteria analysis is needed to evaluate the performance of a biogas plant. The process investigation for agricultural and any waste biomass (i.e., weeds) streams and food industry residues revealed the energy inputs for feedstock collection, transport, and pretreatment. The analyses for energy crops considered the entire supply chain from planting and cultivation, through harvesting and transport processes. However, utilizing weeds (for example, water primrose) provides more benefits if it could reduce the cultivation cost. Also, it was on arable land with no conflict with food and fodder production. Results from this study show that there could be significant and energy efficiency for biogas plants arising from feedstock resource and process adopted a single feedstock, conversion technology applied, and digestate management technique.

Biogas, in a raw form, has limited applications within only heating purposes since the large percent of CO_2 has considered as the incombustible gas and it alleviates heat value also interfering with further compression and transport (Yousef et al., 2018). The caloric content of the biogas was determined with results reported as high heating value (HHV) and low heating value (LHV). One of the crucial reasons for the determination of the energy content of feeds is the calorific value. The difference between HHV and LHV represents caloric content lost to the generation of water vapor in the combustion process. The HHV represents the heat released if the test conditions are returned to 25°C and energy from condensing the water vapor is recovered. In contrast, the LHV reports the heat released if the water produced in combustion remains a vapor. Generally, researchers have reported the calorific value concerning LHV and HHV (Li et al., 2014). Komilis et al. (2014) suggested that the LHV has a practical application in energy estimation and utilization of the biogas released from the burner.

In this study, HCV and LCV were 35.18 MJ/m^3 and 31.69 MJ/m^3, respectively. HCV and LCV are considerably greater than biogas production from traditional anaerobic digestion (LCV of 18.0–23.4 MJ/m^3 and HCV of 20.0–25.9 MJ/m^3. Notably, further investigation on concentration and condition of experiment in this study could able to achieve the calorific value of natural gas (36.5 MJ/m^3). Nevertheless, Hosseini and Wahid (2014) stated that the lower heating value of biogas at standard condition can be around 13.720-27.440 MJ/m^3.

CHAPTER 5
SUMMARY

The study demonstrates that the massive weed (water primrose) availability in the agricultural field takes more effort to collect, transportation, storage, or even treat material before getting its bio-methane production as renewable energy. On the other hand, this weed causes many problems in a variety of ways for agricultural land. Above all, implementing a project to produce biogas would mean that renewable energy producing biogas energy for generating electricity and heat. This would considerably reduce agricultural weed, and evaluating its energy potential is necessary for environmental evaluation.

The result of this study has clearly demonstrated that water primrose is a potential feedstock for anaerobic digestion. The application of alkaline pretreatment on lignocellulosic biomass achieved a significant outcome for biogas production and methane concentration. Also, pretreatment of water primrose in co-digestion with other substrate (cow dung) enhanced biogas volume, methane content. In order to support the result from laboratory-scale experiments, pilot-scale work of anaerobic digestion was implemented under more realistic conditions and also was to facilitate future practical application. The study stated with low concentration of H_2S, CO_2 and small operation scale, a simple model of biogas production and biogas upgrading implemented in this study are feasible and would be an appropriate choice for rural areas. The purpose of this thesis work is to report the research findings as follows:

- Alkaline pretreatment (2% NaOH) of water primrose has achieved the highest performance in biogas yield and methane content.
- At a mixing ratio 2:1 of water primrose to cow dung, not only biogas production but also methane percentage gave the highest value compared with other treatments in this study.
- The purified biogas of using 3% $Ca(OH)_2$ gave maximum CO_2 and H_2S removal efficiency and high-calorific value.

Besides, there are some recommendations from this study findings that would benefit for further research:

- Research could explore the efficiency of using CaO solution for pretreatment methods instead of NaOH because CaO is not only has the same effect on lignocellulosic biomass but also it is the most economically favorable alkaline reagent.
- Research to develop initial pH approaches and carry out above 10 for all digesters of the anaerobic digestion process and the mixing should conduct one time per day in the digester.
- Extended decomposition time is needed for biogas production that the HRT should be operated above 50 days until the measured methane is below 50%.
- It would be helpful to further quantify the value research by economic analysis.

REFERENCES

REFERENCES

Abatzoglou, N. & Boivin, S. 2009. A review of biogas purification processes. **Biofuels, Bioproducts and Biorefining**, 3(1), 42-71.

Al Seadi, T. 2008. Biogas handbook.

Amin, F. R., Khalid, H., Zhang, H., u Rahman, S., Zhang, R., Liu, G. & Chen, C. 2017. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. **Amb Express**, 7(1), 72.

Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H. & Kougias, P. G. 2018. Biogas upgrading and utilization: Current status and perspectives. **Biotechnology advances**, 36(2), 452-466.

Antonopoulou, G., Stamatelatou, K. & Lyberatos, G. 2010. **Exploitation of rapeseed and sunflower residues for methane generation through anaerobic digestion: the effect of pretreatment**.

Anukam, A., Mohammadi, A., Naqvi, M. & Granström, K. 2019. A Review of the Chemistry of Anaerobic Digestion: Methods of Accelerating and Optimizing Process Efficiency. **Processes**, 7(8), 504.

Aragaw, T. & Gessesse, A. 2013. Co-digestion of cattle manure with organic kitchen waste to increase biogas production using rumen fluid as inoculums. **International Journal of Physical Sciences**, 8(11), 443-450.

Awe, O. W., Zhao, Y., Nzihou, A., Minh, D. P. & Lyczko, N. 2017. A review of biogas utilisation, purification and upgrading technologies. **Waste and Biomass Valorization**, 8(2), 267-283.

Aydar, A. Y. 2018. Utilization of response surface methodology in optimization of extraction of plant materials. **Statistical Approaches With Emphasis on Design of Experiments Applied to Chemical Processes. InTech**, 157-169.

Bauer, F., Hulteberg, C. & Persson Tobias, D. T. 2013. Biogas upgrading-REVIEW of commercial technologies (biogasuppgradering-Granskning av kommersiella tekniker. **SGC rapport**, 270(83).

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S. & Escaleira, L. A. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. **Talanta**, 76(5), 965-977.

Bonten, L. T. C., Zwart, K. B., Rietra, R. P. J. J., Postma, R., De Haas, M. & Nysingh, S. 2014. **Bio-slurry as fertilizer: is bio-slurry from household digesters a better fertilizer than manure: a literature review** 1566-7197

Bouallagui, H., Touhami, Y., Cheikh, R. B. & Hamdi, M. 2005. Bioreactor performance in anaerobic digestion of fruit and vegetable wastes. **Process biochemistry**, 40(3-4), 989-995.

Bradley, N. 2007. **The response surface methodology.**Indiana University South Bend.

Braun, R. & Wellinger, A. 2003. **Potential of co-digestion**.

Bücker, F., Marder, M., Peiter, M. R., Lehn, D. N., Esquerdo, V. M., de Almeida Pinto, L. A. & Konrad, O.2020. Fish waste: An efficient alternative to biogas and methane production in an anaerobic mono-digestion system. **Renewable Energy**, 147(798-805.

Chandra, R., Takeuchi, H., Hasegawa, T. & Kumar, R. 2012. Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. **Energy**, 43(1), 273-282.

Chandra, R., Vijay, V., Subbarao, P. & Khura, T. 2012. Production of methane from anaerobic digestion of jatropha and pongamia oil cakes. **Applied Energy**, 93(148-159.

Chen, G., Zheng, Z., Luo, Y., Zou, X. & Fang, C. 2010. Effect of alkaline treatment on anaerobic digestion of rice straw. **Huan jing ke xue= Huanjing kexue**, 31(9), 2208-2213.

Chuanchai, A. & Ramaraj, R. 2018. Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and bio-fertilizer. **3 Biotech**, 8(3), 151.

Chum, H. L. & Overend, R. P. 2001. Biomass and renewable fuels. **Fuel processing technology**, 71(1-3), 187-195.

Chynoweth, D., Turick, C., Owens, J., Jerger, D. E. & Peck, M. 1993. Biochemical methane potential of biomass and waste feedstocks. **Biomass and bioenergy**, 5(1), 95-111.

Dahunsi, O., Oranusi, S. & Efeovbokhan, E. 2017. Anaerobic mono-digestion of Tithonia diversifolia (Wild Mexican sunflower). **Energy Conversion and Management**, 148,128-145.

Dareioti, M. A. & Kornaros, M. 2014. Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system. **Bioresource technology**, 167,407-415.

De Renzo, D. J. 1977. Energy from bioconversion of waste materials. **Park Ridge, NJ, Noyes Data Corp.(Energy Technology Review, No. 11; Pollution Technology Review, 33, 236**

De Vries, J., Vinken, T., Hamelin, L. & De Boer, I. 2012. Comparing environmental consequences of anaerobic mono-and co-digestion of pig manure to produce bio-energy–a life cycle perspective. **Bioresource technology**, 125,239-248.

Demirel, B. & Scherer, P. 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. **Reviews in Environmental Science and Bio/Technology**, 7(2), 173-190.

Du, J., Qian, Y.-t., Xi, Y.-l., Jin, H.-m., Kong, X.-p., Zhu, N., Lv, X.-w., Zhang, Y.-p. & Ye, X.-m. 2019. The Feasibility of Shortening the Pretreatment Time for Improvement of the Biogas Production Rate from Rice Straw with Three Chemical Agents. **BioResources**, 14(2), 3808-3822.

Du, J., Qian, Y., Xi, Y. & Lü, X. 2019. Hydrothermal and alkaline thermal pretreatment at mild temperature in solid state for physicochemical properties and biogas production from anaerobic digestion of rice straw. **Renewable Energy**, 139,261-267.

Dussadee, N., Reansuwan, K. & Ramaraj, R. 2014. Potential development of compressed bio-methane gas production from pig farms and elephant grass silage for transportation in Thailand. **Bioresource Technology**, 155,438-441.

Fang, L., Gharpuray, M. & Lee, Y.1987.**Cellulose hydrolysis biotechnology monographs**: Springer, Berlin.

Fang, W., Weisheng, N., Andong, Z. & Weiming, Y. 2015. Enhanced anaerobic digestion of corn stover by thermo-chemical pretreatment. **International Journal of Agricultural and Biological Engineering**, 8(1), 84-90.

Feng, S., Hong, X., Wang, T., Huang, X., Tong, Y. & Yang, H. 2019. Reutilization of high COD leachate via recirculation strategy for methane production in anaerobic digestion of municipal solid waste: Performance and dynamic of methanogen community. **Bioresource technology**, 288(121509.

Fernández-Cegrí, V., De la Rubia, M. Á., Raposo, F. & Borja, R. 2012. Effect of hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing on fibre composition. **Bioresource technology**, 123,424-429.

Franceschi, V. 2001. Calcium oxalate in plants. **Trends in Plant Science**, 6(7), 331.

Gunaseelan, V. N. 1997. Anaerobic digestion of biomass for methane production: a review. **Biomass and bioenergy**, 13(1-2), 83-114.

Hosseini, S. E. & Wahid, M. A. 2014. Development of biogas combustion in combined heat and power generation. **Renewable and Sustainable Energy Reviews**, 40, 868-875.

Joglekar, A. & May, A. 1987. Product excellence through design of experiments. **Cereal foods world**, 32(12), 857.

Jørgensen & Jacob, P. 2009. Biogas-green energy. **Faculty of Agricultural Sciences, Aarhus University**.

Keskin, T., Ersoy, Y. & Azbar, N. 2018. Evaluation of the effect of initial solid matter concentration and season on anaerobic biodegradation of municipal solid wastes. **Sakarya University Journal of Science**, 22(5), 1409-1417.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T. & Dawson, L. 2011. The anaerobic digestion of solid organic waste. **Waste management**, 31(8), 1737-1744.

Khanto, A. & Banjerdkij, P. 2016. Biogas Production from Batch Anaerobic Co-Digestion of Night Soil with Food Waste. **EnvironmentAsia**, 9(1).

Khayum, N., Anbarasu, S. & Murugan, S. 2018. Biogas potential from spent tea waste: A laboratory scale investigation of co-digestion with cow manure.**Energy**, 165(760-768.

Kim, J., Park, C., Kim, T.-H., Lee, M., Kim, S., Kim, S.-W. & Lee, J. 2003. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. **Journal of bioscience and bioengineering**, 95(3), 271-275.

Kim, J. S., Lee, Y. & Kim, T. H. 2016. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. **Bioresource technology**, 199(42-48.

Kivaisi, A. K. & Eliapenda, S. 1994. Pretreatment of bagasse and coconut fibres for enhanced anaerobic degradation by rumen microorganisms.**Renewable Energy**, 5(5-8), 791-795.

Komilis, D., Kissas, K. & Symeonidis, A. 2014. Effect of organic matter and moisture on the calorific value of solid wastes: An update of the Tanner diagram. **Waste management**, 34(2), 249-255.

Konyar, S. T., Öztürk, N. & Dane, F. 2014. Occurrence, types and distribution of calcium oxalate crystals in leaves and stems of some species of poisonous plants. **Botanical studies**, 55(1), 32.

Kordylewski, W., Sawicka, D. & Falkowski, T. 2013. Laboratory tests on the efficiency of carbon dioxide capture from gases in NaOH solutions. **Journal of ecological engineering**, 14(2), 54-62.

Kratky, L. & Jirout, T. 2011. Biomass size reduction machines for enhancing biogas production. **Chemical Engineering & Technology**, 34(3), 391-399.

Kulkarni, M. & Ghanegaonkar, P. 2019. Hydrogen sulfide removal from biogas using chemical absorption technique in packed column reactors. **Global Journal of Environmental Science and Management**, 5(2), 155-166.

Kumar, S. 2012. **Biogas**. BoD–Books on Demand.

Kumar, V., Kumar, P., Kumar, P. & Singh, J. 2020. Anaerobic digestion of Azolla pinnata biomass grown in integrated industrial effluent for enhanced biogas production and COD reduction: Optimization and kinetics studies. **Environmental Technology & Innovation**, 17, 100627.

Latinwo, G. K. & Agarry, S. E. 2015. Modelling the kinetics of biogas production from mesophilic anaerobic co-digestion of cow dung with plantain peels. **International Journal of Renewable Energy Development**, 4(1), 55.

Lawal, A., Wang, M., Stephenson, P., Koumpouras, G. & Yeung, H. 2010. Dynamic modelling and analysis of post-combustion CO2 chemical absorption process for coal-fired power plants. **Fuel**, 89(10), 2791-2801.

Lehtomäki, A., Huttunen, S. & Rintala, J. 2007. Laboratory investigations on co-digestion of energy crops and crop residues with cow manure for methane production: effect of crop to manure ratio. **Resources, Conservation and Recycling**, 51(3), 591-609.

Lei, H., Cybulska, I. & Julson, J. 2013. Hydrothermal pretreatment of lignocellulosic biomass and kinetics. **Journal of Sustainable Bioenergy Systems**, 3(04), 250.

Li, R., Chen, S. & Li, X. 2010. Biogas production from anaerobic co-digestion of food waste with dairy manure in a two-phase digestion system. **Applied biochemistry and biotechnology***,* 160(2)*,* 643-654.

Li, X., Liu, Y.-H., Zhang, X., Ge, C.-M., Piao, R.-Z., Wang, W.-D., Cui, Z.-J. & Zhao, H.-Y. 2017. Evaluation of biogas production performance and dynamics of the microbial community in different straws. **J Microbiol Biotechnol***,* 27(3)*,* 524-534.

Li, Y., Zhang, R., He, Y., Zhang, C., Liu, X., Chen, C. & Liu, G. 2014. Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). **Bioresource technology***,* 156, 342-347.

Lin, S. H. & Shyu, C. T. 1999. Performance characteristics and modeling of carbon dioxide absorption by amines in a packed column. **Waste Management***,* 19(4)*,* 255-262.

Mäkelä, M. 2017. Experimental design and response surface methodology in energy applications: a tutorial review. **Energy Conversion and Management***,* 151(630-640.

Mancini, G., Papirio, S., Riccardelli, G., Lens, P. N. & Esposito, G. 2018. Trace elements dosing and alkaline pretreatment in the anaerobic digestion of rice straw. **Bioresource technology***,* 247, 897-903.

Mata-Alvarez, J., Cecchi, F., Pavan, P. & Llabres, P. 1990. The performances of digesters treating the organic fraction of municipal solid wastes differently sorted. **Biological wastes***,* 33(3)*,* 181-199.

Mata-Alvarez, J., Mace, S. & Llabres, P. 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. **Bioresource technology***,* 74(1)*,* 3-16.

Meegoda, J. N., Li, B., Patel, K. & Wang, L. B. 2018. A review of the processes, parameters, and optimization of anaerobic digestion. **International journal of environmental research and public health***,* 15(10)*,* 2224.

Mel, M., Ihsan, S. I. & Setyobudi, R. H. 2015. Process improvement of biogas production from anaerobic co-digestion of cow dung and corn husk. **Procedia Chemistry**, 14, 91-100.

Milke, M., Fang, Y. & John, S. 2010. Anaerobic biodegradability of wood: a preliminary review.

Monlau, F., Latrille, E., Da Costa, A. C., Steyer, J.-P. & Carrère, H. 2013. Enhancement of methane production from sunflower oil cakes by dilute acid pretreatment. **Applied energy**, 102, 1105-1113.

Moset, V., Xavier, C. d. A. N., Feng, L., Wahid, R. & Møller, H. B. 2018. Combined low thermal alkali addition and mechanical pre-treatment to improve biogas yield from wheat straw. **Journal of Cleaner Production**, 172, 1391-1398.

Mulat, D. G. & Horn, S. J. 2018. Biogas Production from Lignin via Anaerobic Digestion. **Energy and Environment Series**, 391-412.

Mussatto, S. I. & Dragone, G. (2016). Biomass pretreatment, biorefineries, and potential products for a bioeconomy development. In **Biomass fractionation technologies for a lignocellulosic feedstock based biorefinery** (pp. 1-22): Elsevier.

Muthukumar, V., Makendar, V., Pasupathy, S., Kishore, R. N. & Raja, T. 2018. Optimization of anaerobic bio-digester process parameters for biogas production using taguchi method. **Advanced Science, Engineering and Medicine**, 10(3-4), 518-521.

Muzenda, E. 2014. Bio-methane generation from organic waste: a review.

Nelson, R. 2010. Methane generation from anaerobic digesters: considering different substrates. **Environmental Biotechnology, Lowa State University: Ames, IA, USA**.

Ofoefule, A., Uzodinma, E. & Onukwuli, O. 2009. Comparative Study of The Effect of Different Pretreatment Methods on Biogas Yield From Water Hyacinth (Eichhornia Crassipes.

Onthong, U. & Juntarachat, N. 2017. Evaluation of biogas production potential from raw and processed agricultural wastes. **Energy Procedia**, 138, 205-210.

Orhorhoro, E. K., Ebunilo, P. O. & Sadjere, E. 2017. Experimental Determination of Effect of Total Solid (TS) and Volatile Solid (VS) on Biogas Yield. **American Journal of Modern Energy**, 3(6), 131-135.

Owusu, P. A. & Asumadu-Sarkodie, S. 2016. A review of renewable energy sources, sustainability issues and climate change mitigation. **Cogent Engineering**, 3(1), 1167990.

Pancho, J. 1964. Seed sizes and production capacities in common weed species of the rice fields of the Philippines. **Philipp. Agric**, 48, 307-316.

Pang, C., Xie, T., Lin, L., Zhuang, J., Liu, Y., Shi, J. & Yang, Q. 2012. Changes of the surface structure of corn stalk in the cooking process with active oxygen and MgO-based solid alkali as a pretreatment of its biomass conversion. **Bioresource technology**, 103(1), 432-439.

Pang, Y., Liu, Y., Li, X., Wang, K. & Yuan, H. 2008. Improving biodegradability and biogas production of corn stover through sodium hydroxide solid state pretreatment. **Energy & Fuels**, 22(4), 2761-2766.

Patinvoh, R. J., Osadolor, O. A., Chandolias, K., Horváth, I. S. & Taherzadeh, M. J. 2017. Innovative pretreatment strategies for biogas production.**Bioresource technology**, 224, 13-24.

Pereira, R. G., Romeiro, G. A., Damasceno, R. N., Junior, L. A. F., d'Agua, J. A. C. B. & de Andrade, E. T. 2011. Characterization of char and oil from low temperature conversion of biomass from Eichhornia crassipes. **International Journal of Chemistry**, 3(4), 121.

Persson, M., Jönsson, O. & Wellinger, A. 2006. **Biogas upgrading to vehicle fuel standards and grid injection**.

Qiao, W., Yan, X., Ye, J., Sun, Y., Wang, W. & Zhang, Z. 2011. Evaluation of biogas production from different biomass wastes with/without hydrothermal pretreatment. **Renewable energy***,* 36(12)*,* 3313-3318.

Raja, I. A. & Wazir, S. 2017. Biogas Production: The Fundamental Processes.

Rajagukguk, K. & Satria, A. W. 2019. Design of biogas purification to reduce carbon dioxide (co2) and hydrogen sulfide (h2s). **JURNAL TEKNIK MESIN***,* 7(1).

Rao, P. V. & Baral, S. S. 2011. Experimental design of mixture for the anaerobic co-digestion of sewage sludge. **Chemical Engineering Journal***,* 172(2-3)*,* 977-986.

Rao, P. V., Baral, S. S., Dey, R. & Mutnuri, S. 2010. Biogas generation potential by anaerobic digestion for sustainable energy development in India. **Renewable and sustainable energy reviews***,* 14(7)*,* 2086-2094.

Saengsawang, B., Bhuyar, P., Manmai, N., Ponnusamy, V. K., Ramaraj, R. & Unpaprom, Y. 2020. The optimization of oil extraction from macroalgae, Rhizoclonium sp. by chemical methods for efficient conversion into biodiesel. **Fuel***,* 274, 117841.

Sambusiti, C., Ficara, E., Rollini, M., Manzoni, M. & Malpei, F. 2012. Sodium hydroxide pretreatment of ensiled sorghum forage and wheat straw to increase methane production. **Water Science and Technology***,* 66(11)*,* 2447-2452.

Sarperi, L., Surbrenat, A., Kerihuel, A. & Chazarenc, F. 2014. The use of an industrial by-product as a sorbent to remove CO2 and H2S from biogas. **Journal of Environmental Chemical Engineering***,* 2(2)*,* 1207-1213.

Sawyerr, N., Trois, C., Workneh, T. & Okudoh, V. 2019. An Overview of Biogas Production: Fundamentals, Applications and Future Research. **International Journal of Energy Economics and Policy***,* 9(2)*,* 105-116.

Schnurer, A. & Jarvis, A. 2010. Microbiological handbook for biogas plants. **Swedish Waste Management U**, 1-74.

Shiralipour, A. & Smith, P. H. 1984. Conversion of biomass into methane gas. **Biomass**, 6(1-2), 85-92.

Sidibe, A. & Hashimoto, A. 1990. **Conversion of grass straw to methane**. American Society of Agricultural Engineers.

Sindhu, R., Binod, P. & Pandey, A. 2016. Biological pretreatment of lignocellulosic biomass–An overview. **Bioresource technology**, 199,76-82.

Sonakya, V., Raizada, N. & Kalia, V. C. 2001. Microbial and enzymatic improvement of anaerobic digestion of waste biomass. **Biotechnology letters**, 23(18),1463-1466.

Sun, Y. & Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. **Bioresource technology**, 83(1), 1-11.

Taherzadeh, M. J. & Karimi, K. 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. **International journal of molecular sciences**, 9(9), 1621-1651.

Tarkow, H. & Feist, W. 1969. A mechanism for improving digestibility of lignocellulosic materials with dilute alkali and liquid ammonia. **Advances in Chemistry Series**, 95), 197.

Teghammar, A., Yngvesson, J., Lundin, M., Taherzadeh, M. J. & Horváth, I. S. 2010. Pretreatment of paper tube residuals for improved biogas production. **Bioresource technology**, 101(4), 1206-1212.

Tetard, L., Passian, A., Farahi, R., Kalluri, U., Davison, B. & Thundat, T. 2010. Spectroscopy and atomic force microscopy of biomass. **Ultramicroscopy**, 110(6), 701-707.

Tippayawong, N. & Thanompongchart, P.2010. Biogas quality upgrade by simultaneous removal of CO2 and H2S in a packed column reactor. **Energy**, 35(12), 4531-4535.

Tock, L., Gassner, M. & Marechal, F. 2010. Thermochemical production of liquid fuels from biomass: Thermo-economic modeling, process design and process integration analysis. **Biomass and Bioenergy**, 34(12), 1838-1854.

Ummalyma, S. B., Supriya, R. D., Sindhu, R., Binod, P., Nair, R. B., Pandey, A. & Gnansounou, E. (2019). Biological pretreatment of lignocellulosic biomass—Current trends and future perspectives. In **Second and Third Generation of Feedstocks** (pp. 197-212): Elsevier.

Uzodinma, E. & Ofoefule, A. 2009. Biogas production from blends of field grass (Panicum maximum) with some animal wastes. **Int. J. Phys. Sci**, 4(2), 091-095.

Van Tran, G., Unpaprom, Y. & Ramaraj, R. 2019. Methane productivity evaluation of an invasive wetland plant, common reed. **Biomass Conversion and Biorefinery**, 1-7.

Vavilin, V., Rytov, S. & Lokshina, L. Y. 1996. A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. **Bioresource technology**, 56(2-3), 229-237.

Wang, F., Yi, W., Zhang, D., Liu, Y., Shen, X. & Li, Y. 2020. Anaerobic co-digestion of corn stover and wastewater from hydrothermal carbonation. **Bioresource Technology**, 123788.

Wang, J., Yue, Z.-B., Chen, T.-H., Peng, S.-C., Yu, H.-Q. & Chen, H.-Z. 2010. Anaerobic digestibility and fiber composition of bulrush in response to steam explosion. **Bioresource technology**, 101(17), 6610-6614.

Wannapokin, A., Ramaraj, R., Whangchai, K. & Unpaprom, Y. 2018. Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. **3 Biotech**, 8(2), 123.

Wei, L., Qin, K., Ding, J., Xue, M., Yang, C., Jiang, J. & Zhao, Q. 2019. Optimization of the co-digestion of sewage sludge, maize straw and cow manure: microbial responses and effect of fractional organic characteristics. **Scientific reports**, 9(1), 1-10.

Weil, J., Sarikaya, A., Rau, S.-L., Goetz, J., Ladisch, C. M., Brewer, M., Hendrickson, R. & Ladisch, M. R. 1997. Pretreatment of yellow poplar sawdust by pressure cooking in water. **Applied Biochemistry and Biotechnology***,* 68(1-2)*,* 21-40.

Wellington, A., Baraza, L. D., Mageto, M. & Orori, K. F. 2017. Energy Evaluation and Qualitative Analysis of Biogas Produced from Co-Digesting Kitchen Waste and Cow Dung. **Physical Science International Journal***,* 1-13.

Wicaksono, A., Rahmawan, A., Matin, H. H. A., Wardani, L. G. K., Kusworo, T. D. & Sumardiono, S. 2017. **The effect of pretreatment using sodium hydroxide and acetic acid to biogas production from rice straw waste**.

Yavini, T. D., Chia, A. I. & John, A. 2014. Evaluation of the effect of total solids concentration on biogas yields of agricultural wastes. **Int Res J Environ Sci***,* 3(2)*,* 70-75.

Yincheng, G., Zhenqi, N. & Wenyi, L. 2011. Comparison of removal efficiencies of carbon dioxide between aqueous ammonia and NaOH solution in a fine spray column. **Energy Procedia***,* 4, 512-518.

Yousef, A. M., El-Maghlany, W. M., Eldrainy, Y. A. & Attia, A. 2018. New approach for biogas purification using cryogenic separation and distillation process for CO2 capture. **Energy***,* 156, 328-351.

Zamanzadeh, M., Hagen, L. H., Svensson, K., Linjordet, R. & Horn, S. J. 2017. Biogas production from food waste via co-digestion and digestion-effects on performance and microbial ecology. **Scientific reports***,* 7(1)*,* 1-12.

Zehnsdorf, A., Moeller, L., Stabenau, N., Bauer, A., Wedwitschka, H., Gallegos, D., Stinner, W. & Herbes, C. 2018. Biomass potential analysis of aquatic biomass and challenges for its use as a nonconventional substrate in anaerobic digestion plants. **Engineering in Life Sciences***,* 18(7)*,* 492-497.

Zhang, L., Zhang, J. & Loh, K.-C. 2018. Activated carbon enhanced anaerobic digestion of food waste–Laboratory-scale and Pilot-scale operation.**Waste management***,* 75, 270-279.

Zhao, Q., Leonhardt, E., MacConnell, C., Frear, C. & Chen, S. 2010. Purification technologies for biogas generated by anaerobic digestion. **Compressed Biomethane, CSANR, Ed**.

Zheng, M., Schideman, L. C., Tommaso, G., Chen, W.-T., Zhou, Y., Nair, K., Qian, W., Zhang, Y. & Wang, K. 2017. Anaerobic digestion of wastewater generated from the hydrothermal liquefaction of Spirulina: Toxicity assessment and minimization. **Energy conversion and management**, 141, 420-428.

Zheng, Y., Zhao, J., Xu, F. & Li, Y. 2014. Pretreatment of lignocellulosic biomass for enhanced biogas production. **Progress in energy and combustion science**, 42, 35-53.

Zhu, J., Wan, C. & Li, Y. 2010. Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. **Bioresource technology**, 101(19), 7523-7528.

Ziemiński, K. & Frąc, M. 2012. Methane fermentation process as anaerobic digestion of biomass: Transformations, stages and microorganisms. **African Journal of Biotechnology**, 11(18), 4127-4139.

Zwietering, M., Jongenburger, I., Rombouts, F. & Van't Riet, K. 1990. Modeling of the bacterial growth curve. **Applied and environmental microbiology**, 56(6), 1875-1881.

APPENDIX

APPENDIX A

PUBLICATIONS

Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-01065-6

ORIGINAL ARTICLE

Sustainable valorization of water primrose with cow dung for enhanced biogas production

Huyen Thu Thi Nong [1,2] · Yuwalee Unpaprom [2,3] · Kanda Whangchai [4] · Rameshprabu Ramaraj [1,2]

Received: 28 August 2020 / Revised: 25 September 2020 / Accepted: 2 October 2020

Abstract

In this study, the effects of alkaline pretreatment (2% NaOH) of water primrose on its biogas production efficiency and anaerobic co-digestion of cow dung were investigated. A scanning electron microscope (SEM) was used to analyze the changes in main compositions and physico-chemical structure of water primrose after pretreatment and untreated biomass. Furthermore, the experiments evaluate the efficiency and optimization mixing ratio of co-digestion using water primrose and cow dung. The pretreatment of water primrose at different periods for the examination of the biodegradability matters in the substrate and biogas production. Among the three mixing ratios of co-substrate tested, the best performance in this study was achieved at mixing ratio 2:1 (water primrose to cow dung, w/w) for 2 weeks' pretreatment time on grass, including all measurements as biogas production (8610 mL), methane concentration (68.2%), and percentage of total solids (70.84%), volatile solids (64.76%), and chemical oxygen demand (66.55%) removal efficiency.

Keywords Water primrose · Cow dung · Pretreatment · Biogas production · Energy analysis

1 Introduction

Currently, energy resources and the environment have increased interest and this study concerns regarding alternative sources of energy. Fossil fuels play a significant role in the development of various industries, transportations, agriculture sectors, and to meet many other basic human needs in modern civilization [1, 2]. However, the more fossil fuels use, the more toxic gases produce on the environment, such as CO_2, SO_X, and NOx, which is the primary source of greenhouse gases [3, 4]. Therefore, it is necessary to find out renewable energy to replace energy sources derived from fossil fuels. In this context, the anaerobic digestion process could be a better option.

Furthermore, this is one of the solutions to solve the biomass waste problems from crops, agricultural waste, industrial waste, food waste, chicken waste, or animal wastes [5, 6]. Compared with other renewable energy (such as solar, wind, hydro energy), the anaerobic digestion of biomass was involved less capital investment. In addition, available biomass sources can easily be found in rural areas. It is not dependent on world prices or the supply uncertainties as of imported and conventional fuels [6, 7].

The production of biogas through anaerobic digestion is not only getting rid of unwanted wastes but also known to minimize the impact on the environment; energy-rich methane can be generated biofuel and energy for electricity and heat [8, 9]. Apart from biogas production, the bio-slurry also produces as a by-product of the anaerobic process; this is a mixture of digested matter and water with a high concentration of mineral substances and nutrients that suitable to be used as fertilizer. Therefore, by changing natural waste into vitality, biogas is using nature's abundant to reuse substances into valuable properties [10].

Almost of microorganisms need oxygen to survive, but in specific environments, there is oxygen-free such an environment, some microorganisms will grow and develop thanks to

✉ Rameshprabu Ramaraj
rrameshprabu@gmail.com; rameshprabu@mju.ac.th

1 School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand

2 Sustainable Resources and Sustainable Engineering Research Lab, Maejo University, Chiang Mai 50290, Thailand

3 Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand

4 Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

Published online: 08 October 2020

the amount of oxygen taken from the material and methane [11, 12]. These microorganisms exist in swamps, landfills, covered lagoons, or enclosed tanks called anaerobic digestion. Anaerobic digestion refers to producing biogas by fermenting organic materials in the absence of air or oxygen with the support of microorganisms to breakdown materials into intermediates to generate mainly methane (CH_4) and carbon dioxide (CO_2) and along with other trace gases. The biogas typically has 50–70% methane and 50% carbon dioxide [13].

Once anaerobic digestion is operated, some sensitive factors should be considered. The imbalances could lead to inhibit or fail the process. Factors represent important parameters that can affect the efficiency of anaerobic digestion listed as pH, temperature, total solids, volatile solids, nitrogen ratio, retention time, etc. Moreover, in order to improve biogas yield, their quality, or may reduce the retention time needed, pretreatment methods are applied for substrates such as using fungal, chemical, mechanical, and thermal techniques [14]. In short, comprehensive knowledge of anaerobic digestion is vital to ensure a stable operation and cost-effective final product. Biogas is a clean fuel and does not cause air pollution. It is considered as a better fuel than natural gas because it does not contain sulfur. Sulfur on burning gets converted into sulfur dioxide, which is responsible for many lung diseases. The efficient utilization of biogas technology has positive effects on the national economy and can readily be integrated with rural development as it provides no smoking, cleaning fuel for cooking, lighting, and running agro-machinery [15, 16].

Water primrose is a semi-aquatic plant, rapid growth, and spreading in the shallow areas of ponds, lakes, and streams, usually in standing water, rice paddies, that common belonging agricultural area land. *Ludwigia hyssopifolia* (*L. hyssopifolia*) is the scientific name of water primrose, commonly known as seedbox or "Tianna" in Thai, belongs to the Onagraceae family. Extensively in China, South, and Southeast Asia, including Thailand and other tropical countries. Semiaquatic water primrose plants are growing with food crops. Eradicating weeds with herbicides has been adverse effects in food production because weeds compete with crops for water, nutrients, and soil. Also, weeds can harbor insect and disease pests, and noxious weeds and weed seeds can significantly affect crop quality. Recently, in Thailand, agricultural processes were focusing on organic agriculture. Therefore, these weeds were possible to remove and gradually reduce the population of weeds from the croplands [17].

On the other hand, this large quantity of potential biomass can be utilized for biofuel applications directly. There is no literature available on water primrose related to biogas production. Therefore, it is a new energy material for biogas production. In this study, the whole parts of water primrose, such as flowers, leaves, stems, fruits, roots, will be used as a material to produce biogas production. Typically, the material should undergo pretreated before going the anaerobic process to release much more simple sugars that hold inside the cell wall of lignocellulosic material [17–19]. Thus, chemical pretreatment is applied for water primrose to increase biogas yield. Besides that, co-digestion of water primrose and cow dung through anaerobic conditions using different ratios was also investigated.

2 Materials and methods

2.1 Feedstock preparation

Water primrose was collected from the agricultural field (Fig. 1) nearby Maejo University, San Sai, Chiang Mai, Thailand (coordinates 18° 53′ 24.3″ N–99° 02′ 11.5″ E). The collected grass underwent air-dried for 2 weeks. A shredder crushed air-dried samples into 1–2 cm pieces. Then used a blender to reduce the size into 5–10 mm pieces. Also, the samples were kept in airtight plastic bags for further use. The co-substrate of cow dung was obtained from the cow farm at the Faculty of Animal Science and Technology, Maejo University, Thailand. The cow dung was transferred to the laboratory and put in the fermenter within a day.

2.2 Alkaline pretreatment on feedstock

Water primrose biomass (10% TS of dried) as treated with 2% NaOH and applied all the experiments. Subsequently, the mixing ratio of water primrose with cow dung and pretreatment time processes were investigated. Water primrose was allowed 1, 2, and 3 weeks' period of pretreatment time. The water primrose (WP) and cow dung (CD) were used as a control (without pretreatment). The three experimental groups of the different mixing ratio of water primrose and cow dung (w/w) corresponding to the pretreatment time (1, 2, 3 weeks) were referred to as treatment 1 (T1, mixing ratio 1:1), treatment 2 (T2, mixing ratio 1:2), and treatment 3 (T3, mixing ratio 2:1). In the mixing ratio, 1:1 containing 3 pretreatments time. It was applied to water primrose, named as follows: T1-A (pretreatment time: 1 week), T1-B (pretreatment time: 2 weeks), and T1-C (pretreatment time: 3 weeks). Furthermore, similarly, in the mixing ratio, 1:2 and 2:1 named as T2-A, T2-B, T2-C and T3-A, T3-B, T3-C, respectively.

2.3 Scanning electron microscopy analysis

The dried water primrose (raw material) and pretreated water primrose were investigated under scanning electron microscopy (SEM) to examine the changes in the physical structure of the substrate. Before imagining, the samples were crushed manually into powder form used a pestle and mortar then coated by a carbon layer to improve the electrical conductivity of the samples, as well as fixed sample positions during SEM

Fig. 1 Water primrose: Growing field (**a**) Whole plant (**b**) Fruits (**c**) and Flowers (**d**)

that may ruin the image captured. The JEOL JSM-5410LV scanning electron microscopy (SEM) was used. Observations of SEM images were performed at × 500 magnification with an accelerating voltage of 15 kV.

2.4 The experimental setup

All the samples (treated with NaOH and untreated) were fermented in batch anaerobic digesters of 1 L with a working volume of 700 mL (Fig. 2). Each treatment was conducted in triplicates with a solid loading of 10% TS based on dry matter content of water primrose. Pretreated water primrose and cow dung were used as co-substrates with the different mixing ratios of 1:1, 1:2, and 2:1 (water primrose: cow dung, w/w). The control was prepared as mono-substrates for anaerobic digestion and only contained grass and cow dung (without pretreatment). The pH adjustment for all treatments before fermentation was 8.5–9 by using calcium oxide (CaO) powder. In total, the experiment consisted of 33 digesters, including control and pretreated samples. During the fermentation

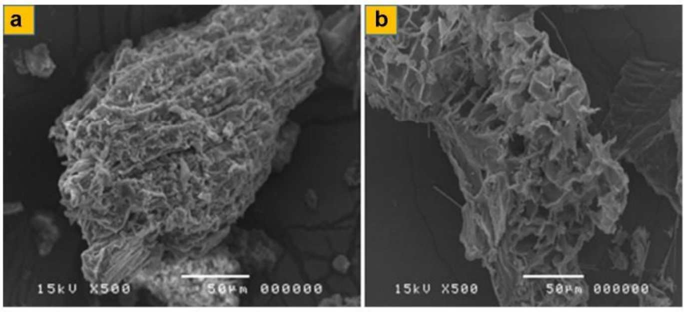


Fig. 2 SEM image of water primrose untreated material (**a**) and pretreated material (**b**)

process, all digesters were manually shaken and mixed twice a day (morning and afternoon) for 30 s to 1 min before the gas record. The experiments were run for 45 days under mesophilic conditions.

2.5 Analytical methods

The pH value of the substrate was measured using the pH meter (Oakton PCSTestr 35 Waterproof pH). Total solids (TS) measuring, samples were ignited in a muffle furnace at a temperature of 105 °C for 24 h, and TS was determined by the quantity of residue materials that left after igniting. The volatile solids (VS) were the remaining solids that were lost on ignition of the residue materials from TS at 550 °C for a period time of 30 min to 1 h.

Before measurements of alkaline, volatile fatty acids (VFAs), and chemical oxygen demand (COD), a sufficient amount of the samples was first centrifuged at 1500 rpm for 15 min and the supernatant liquid was used for analysis. The estimation of alkaline was verified by a combination of 2 indicators (phenolphthalein and methyl orange) and titrated with 0.01 M H_2SO_4 to the endpoint of red color in solution. VFAs were measured by adjusting the pH of samples to 3.3–3.5 then boiling for 3 min. The samples were subsequent titration from pH 4 to 7 with 0.1 M NaOH solution. COD was determined by titrating samples with 0.1 N ferrous ammonium sulfate to the first red-brown endpoint in closed reflux. These parameters, including TS, VS, alkaline, VFAs, COD, were performed according to standard methods [20].

Proximate and ultimate analyses of the water primrose were carried out by using X-ray fluorescence (XRF) spectrometers and an elemental analyzer. Proximate analysis of samples was performed by moisture content (MC), volatile content (VC), fixed carbon (FC), and ash, and for ultimate analysis included elemental chemical: carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S).

2.6 Biogas determination, energy analysis, and bio-fertilizer

The measurement of daily biogas was regularly read on the scale displayed on the gasholder every 24 h. The main biogas compositions, including methane and carbon dioxide, trace gases of hydrogen sulfide, and oxygen, were determined by a portable gas analyzer (Geotech GA5000). Energy analysis of high heating value (HHV) and low heating value (LHV), also bio-fertilizer estimation methods, was adopted from our previous studies [7, 21].

2.7 Statistical analysis

The results from anaerobic digestion experiments data were reported as mean ± SE from triplicate observations. Significant differences between means were analyzed. All statistical analyses were performed using SPSS Version 20.0. A correlation was assumed significant when $P < 0.05$.

3 Results and discussion

3.1 Characteristics of feedstock used for anaerobic digestion

In order to estimate the biogas potential from the substrates for anaerobic digestion, the general characteristics are measured by parameters related to the physical and chemical of water primrose and cow dung and are presented in Table 1. In the water primrose samples, it is observed that a high concentration of TS and VS was found 900,000 mg/L and 836,667 mg/L, respectively. Furthermore, in terms of proximate analysis, volatile content was 63.07% indicated the high amount of organic fractions are available and easily biodegradable in the substrate, which is highly beneficial for biomethane generation [21, 22].

According to the ultimate analysis, the elemental contents of water primrose consisted of CHONS (based on the weight percentage on a dry basis). The major element in the grass was C content with 40.2 wt%, followed by O and H with 22.13 and 5.03 wt%, respectively. N and S contents were also detected in the grass with a percentage of 1.8 and 0.24. Nevertheless, the content of oxygen was not taken into account as a part of

Table 1 General characteristics of water primrose and cow dung

Parameter	Measured values	
	WP	CD
TS (mg/L)	900,000 ± 4165	196,666 ± 1064
VS (mg/L)	836,667 ± 3754	140,000 ± 984
pH	5.05 ± 0.02	8.15 ± 0.02
COD (mg/L)	61,667 ± 4812	153,333 ± 5695
VFAs (mg/L)	3218 ± 182	4376 ± 896
Alkaline (mg/L)	1917 ± 312	34,458 ± 295
Proximate analysis (wt%)		
MC	7.28	-
VC	63.1	-
FC	1.28	-
Ash	28.4	-
Ultimate analysis (wt%)		
C	40.2	-
H	5.03	-
O	22.13	-
N	1.8	-
S	0.24	-

nutrients due to the operation of digesters requires strictly anaerobic condition [21]. Therefore, CHNS content is considered as the basic element in the contribution of organic matter [23]. In addition, the pH measurement of water primrose was in the acidic range (5.05); meanwhile, the pH of cow dung was used as co-substrate in the range of basic range (8.15). In the process of fermentation, in anaerobic co-digestion of water primrose and cow dung could lead to preventing the pH drops in digesters.

3.2 The effect of pretreatment on co-digestion in fermentation

Anaerobic digesters of all treatments (untreated and treated) in this study were using 1-L bottles containing samples of T1 (T1-A, T2-B, T3-C), T2 (T2-A, T2-B, T2-C), and T3 (T3-A, T3-B, T3-C). The anaerobic digestion experiment was started immediately after samples pretreated by NaOH solution were done at 7-day, 14-day, and 21-day period. The cow dung was used as co-substrate with water primrose at ratios prepared as each treatment corresponding to 1:1, 1:2, and 2:1 on TS basis. The working volume of digesters was 700 mL containing substrates and tap water. The adjustment for the initial pH of all digesters was in the range of 8.5–9. The experiments were performed under mesophilic anaerobic digestion for 45-day operation. In order to evaluate the effect of co-digestion on the biogas potential, before the fermentation process, a sufficient samples in each treatment were taken out for parameter analysis. The characteristics of pH, TS, VS, COD, VFAs, and alkaline of co-substrate before fermentation are given in Table 2.

The value of TS, VS, and COD is good parameters to indicate the ability of biodegradation of the substrate; the higher the initial VS and COD, the more organic matter in it [24]. From the results, as the treatment duration increased, the value of some parameters was increasing in each treatment, including TS, VS, and COD. While the parameters showed a gradually increasing trend with increasing pretreatment time, however, in mixing ratio of 2:1 at 2 weeks pretreatment, the highest values of TS, VS, and COD were found. Overall, co-digestion of WP:CD in 2:1 mixing ratio provided the highest initial value of COD, TS, and VS which reached to 133,667 ± 4257 (mg/L), 79,667 ± 3503 (mg/L), and 62,000 ± 1459 (mg/L), respectively. Whereas in case of 1:1 mixing ratio, the lowest amount of value in COD, TS, and VS was found (50,667 ± 3542, 47,500 ± 1500, 36,000 ± 1322). Interestingly, the value of TS, VS, and COD became higher when increasing the amount of water primrose in co-digestion. This value indicated that NaOH solution had a positive effect on water primrose during pretreatment duration, which made lignin content broken down in solution and thus, the carbohydrate of the substrate was easily soluble in the liquid phase [25]. Thereby, the bacteria was more

Table 2 Parameters of co-digestion before fermentation process

Parameter	Treatments								
	T1			T2			T3		
	T1-A	T1-B	T1-C	T2-A	T2-B	T2-C	T3-A	T3-B	T3-C
pH	8.8 ± 0.11^{a}	8.8 ± 0.09^{a}	8.8 ± 0.10^{a}	8.7 ± 0.13^{a}	8.7 ± 0.13^{a}	8.8 ± 0.10^{a}	8.8 ± 0.10^{a}	8.9 ± 0.12^{a}	8.9 ± 0.08^{a}
TS (mg/L)	47,500 ± 1500^{d}	56,500 ± 2500^{c}	58,500 ± 2500^{c}	64,000 ± 3214^{bc}	65,000 ± 3256^{bc}	69,667 ± 3521^{b}	74,353 ± 3405^{ab}	79,667 ± 3503^{a}	77,500 ± 3143^{a}
VS (mg/L)	36,000 ± 1322^{d}	45,000 ± 1412^{cd}	46,000 ± 1548^{cd}	48,333 ± 1724^{c}	48,361 ± 1641 ^{c}	48,500 ± 1270^{c}	50,000 ± 1644^{bc}	62,000 ± 1459^{a}	51,500 ± 1355^{bc}
COD (mg/L)	50,667 ± 2542^{e}	52,000 ± 2832^{e}	53,333 ± 2266de	59,333 ± 2158^{d}	74,667 ± 2551^{c}	90,667 ± 3344^{bc}	105,128 ± 4211^{b}	133,667 ± 4257^{a}	132,167 ± 4221^{a}
ALK (mg-$CaCO_3$/L)	4708 ± 212^{d}	6000 ± 270^{bc}	6292 ± 257^{b}	5563 ± 313^{c}	7438 ± 629^{ab}	6750 ± 375^{b}	7500 ± 468^{a}	7375 ± 417^{ab}	7813 ± 355^{a}
VFA (mg/L)	1216 ± 395^{b}	1596 ± 211^{a}	1474 ± 871^{a}	1052 ± 484^{c}	1332 ± 394^{ab}	1439 ± 580^{a}	1033 ± 415^{c}	1409 ± 542^{ab}	1226 ± 496^{b}

All statistical analyses were performed using SPSS Version 20.0. Means with the same letter (superscript) are not significantly different from each other. A correlation was assumed significant when $P < 0.05$

accessible to the substrate; the hydrolysis degree was enhanced, resulting in high solubility organic matter converted into biogas production under anaerobic conditions [26, 27].

The stability of the anaerobic process was evaluated by indicators of VFAs and alkaline in digesters. The presence of volatile fatty acids and alkaline is conducive factors of digesters related to the environment of biomethane generation. The concentration of VFAs throughout treatment groups was quite constant, with the values ranged from 1033 ± 68 to 1596 ± 111 mg/L. The higher concentration of VFAs causes decrease pH in digesters, led to the inhibited microorganism in reactors [28]. However, higher alkalinity could help prevent the drop of pH by the accumulation of VFAs in the digesters, which ranged from 4708 ± 212 to 7813 ± 313 (mg $CaCO_3$/L) in this study.

3.3 Influence of alkaline pretreatment on the physical structure of water primrose

The main purposes of alkaline pretreatment are to increase the surface structure by swelling and to decrease or break down the lignin content in lignocellulosic biomass, which facilitated the entry of enzymes to hemicellulose and cellulose [29, 30], thus, improve the reaction time of chemical solution and substrate as well as shortening hydrolysis [31]. The physical structure changes of water primrose before and after pretreatment were performed by SEM images taken at × 500 magnification which are shown in Fig. 3. As these images show the surface morphology of water primrose, the significant differentiation between untreated and treated surface was observed. With the untreated samples, the surface was too rigid and in intact morphology form, which made it challenging to enzymes to access the plant cell wall. Tetard et al. [32] explained that depending on the different linkage of lignin and cellulose in biomass could be led to different topography, such as the holes and gaps layer appeared in the same region after the pretreatment process. This phenomenon evident is exposed in Fig. 3b; the cell walls were disrupted and the surface became loose with a large number of holes which are visible in the length scales and revealed the following layers within the cell walls. This may help enzymes attack the surface area easily during hydrolysis [33], resulting in higher methane yield [34].

3.4 Specific biogas production and methane potential

The total biogas production and methane concentration of the controls, co-digestion (water primrose and cow dung) at the three mixing ratios of 1:1, 1:2, and 2:1, are shown in Table 3. The final total volume of biogas was calculated after 45 days of operation by the sum of the amount gas obtained per day. The gas components were measured by gas analyzer GA 5000. For each mixing ratio, the trend was similar to the lowest total biogas obtained at 1 week and highest at 3 weeks' pretreatment time. Additionally, the increasing volume of biogas obtained, the higher the concentration methane achieved. Hence, the methane content in biogas indicates the amount of solubilization of the substrate was degraded by microorganisms in anaerobic digestion [35].

The maximum biogas volume in the digesters from treatment 1 to treatment 3 was 7200 mL, 8100 mL, and 8610 mL obtained in T1-C, T2-C, and T3-B, respectively. As a result, the methane concentration was distributed as T3-B (68.20%) > T1-C (64.55%) > T2-C (66.05%). In comparison with the biogas volume between each treatment, there was no significant different volume observed. However, these values were 67.9%, 88.9%, and 100.01% higher if compared with the control of water primrose in terms of biogas volume and 32.4%, 48.9%, and 51.9% if compared with the control of cow dung. Even though at the co-digestion of water primrose to cow

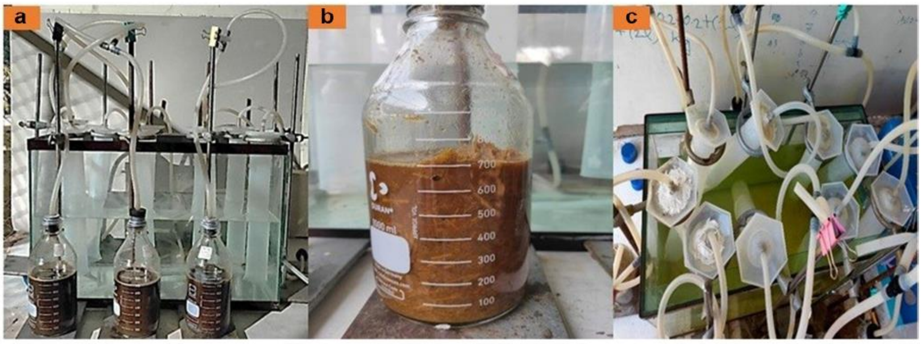


Fig. 3 Lab-scale anaerobic digestion for biogas production. **a** Experimental setup. **b** Working volume of digester. **c** Gas collection and storage in gas holder

Table 3 Biogas production and methane concentration

Treatments		Total biogas (mL)	Methane (%)
WP (control 1)		4285^{d}	55.07^{b}
CD (control 2)		5436^{d}	53.00^{b}
T1	T1-A	6150^{cd}	63.88^{ab}
	T1-B	6570^{cd}	64.05^{ab}
	T1-C	7200^{c}	64.55^{ab}
T2	T2-A	7500^{c}	64.91^{ab}
	T2-B	7750^{c}	65.47^{ab}
	T2-C	8100^{b}	66.05^{a}
T3	T3-A	8320^{ab}	66.78^{a}
	T3-B	8610^{a}	68.20^{a}
	T3-C	8260^{ab}	66.15^{a}

All statistical analyses were performed using SPSS Version 20.0. Means with the same letter (superscript) are not significantly different from each other. A correlation was assumed significant when $P < 0.05$

Table 4 Removal efficiency of mixture in anaerobic digestion

Treatments		Removal efficiency (%)		
		TS	VS	COD
WP (control 1)		51.29c	49.15^{d}	45.25^{cd}
CD (control 2)		48.56^{c}	45.63^{d}	40.84^{d}
T1	T1-A	57.55^{bc}	50.22^{cd}	52.64^{cd}
	T1-B	58.95^{bc}	51.41^{c}	55.19^{bc}
	T1-C	58.01^{bc}	53.31^{bc}	51.46^{cd}
T2	T2-A	59.13^{bc}	51.62^{c}	54.74^{bc}
	T2-B	62.44^{b}	56.28^{bc}	55.06^{bc}
	T2-C	64.45^{ab}	59.74^{b}	55.44^{bc}
T3	T3-A	65.65^{ab}	60.55^{b}	59.86^{b}
	T3-B	70.84^{a}	64.76^{a}	66.55^{a}
	T3-C	61.65^{b}	54.36^{bc}	57.88^{bc}

All statistical analyses were performed using SPSS Version 20.0. Means with the same letter (superscript) are not significantly different from each other. A correlation was assumed significant when $P < 0.05$

dung in mixing ratio 1:2 carried more microorganisms than others, but the efficiency of this treatment did not achieve the highest value of biogas production as well as methane concentration. The possible reason is that the limited organic matter in the substrate was not enough supplied for anaerobic microorganisms to convert to methane product. The pretreatment of NaOH reflected the higher value of TS, VS, and COD than the controls made more biodegradability of the components in the feedstock, thus obtained higher biogas production.

3.5 Degradation efficiency of TS, VS, and COD

In the anaerobic digestion process, the biodegradable of TS, VS, and COD represented by the amount of organic matter in substrate was converted into the final product named as methane and carbon hydroxide. In other words, the degradation of organic fractions in the substrate is proportional to the biogas produced [36]. The efficiency of TS, VS, and COD reduction from all treatments was calculated by the relationship of the initial and final value and was expressed as a percentage, which is shown in Table 4. Besides the volume of biogas production, the measurement of TS, VS, and COD reduction could further evaluate the efficiency of biodegradability of feedstock. It can be seen that after 45 days of anaerobic digestion, the 2% NaOH pretreatment has a positive effect on degradability compared with the controls.

Moreover, the removal efficiency of organic material increased with increasing initial value which was performed by the higher percentage of TS, VS degradation rate, and COD removal rate. As mentioned earlier, the highest initial value of TS, VS, and COD was obtained from mixing ratio 2:1 at 2 weeks' pretreatment time. Thereby, the reported data has verified the results that the COD removal rate and VS degradation rate at T3-B treatment were achieved higher than the rest treatments.

As shown in Table 3, the percentage of TS and VS reductions for NaOH-treated water primrose was from 57.55–58.95% to 50.22–53.31% in treatment 1, in the ranged of 59.13–64.45% and 51.62–59.74% in treatment 2, and lastly, in treatment 3 were 61.65–70.84% and 54.36–64.76% accordingly. Compared with the controls, these results were slightly higher. Pang et al. [37] mentioned that after NaOH pretreatment, the organic of the material was increased and more quantity soluble components were utilized by bacteria, which led to a higher biogas production related to increasing TS and VS reductions.

The experimental results showed that the COD degradation was obtained at a range of 51.46–66.55% removal efficiency for all mixing ratios in co-digestion, whereas the COD reduction control of water primrose and cow dung was 45.25 and 40.84%, respectively. Stabilization of the COD reduction after 21 days may be due to exhausting nitrogen and carbon content along with the aging of the microbial cells [38]. Also, the significant removal of physico-chemical parameters of concentrated liquid, semiliquid, or solid biomass was used for biogas production. The results proved that co-digestion gave more biodegradability material for biogas production due to the nutrient in co-substrate which helped the anaerobic microorganisms thrive more smoothly.

3.6 Energy analysis and bio-fertilizer

The caloric content of the biogas was determined with results reported as high heating value (HHV) and low heating value (LHV). One of the crucial reasons for the determination of the energy content of feeds is the calorific value. The difference between HHV and LHV represents caloric content lost to the generation of water vapor in the combustion process. The HHV represents the heat released if the test conditions are returned to 25 °C and energy from condensing the water vapor is recovered. In contrast, the LHV reports the heat released if the water produced in combustion remains a vapor. Generally, researchers have reported the calorific value concerning LHV and HHV. Komilis et al. [39] suggested that the LHV has a practical application in energy estimation and utilization of the biogas released from the burner. In this study, HCV and LCV were 27.21 MJ/m^3 and 24.50 MJ/m^3, respectively. HCV and LCV are considerably greater than biogas production from traditional anaerobic digestion (LCV of 18.0–23.4 MJ/m^3 and HCV of 20.0–25.9 MJ/m^3) [40]; consequently, the study results confirmed that high-calorific biogas was obtained in this study system of the sustainable valorization of water primrose with cow dung.

The digestate called biogas slurry (i.e., bio-fertilizer) refers to the liquid part of digestates produced from the anaerobic digestion process; it contains huge content of nitrogen, phosphorus, and other macronutrients and micronutrients. Nitrogen and phosphorus are the two main nutrients in the digestates used for agricultural purposes [2, 7, 21]. The nutrients in the digestate examined in the current study were gathered efficiently. Biogas digestate physicochemical characteristics which include nutrients, total nitrogen, total phosphorus, fluoride, chloride, sulfate, sodium, potassium, calcium, and magnesium were 15.54, 4.71, 0.61, 1.2, 0.75, 2.41, 0.50, and 0.28, respectively. This digestate offered high contents of nutrients that are important for use as fertilizer, generating agronomic benefits.

4 Conclusion

The result of this study has clearly demonstrated that water primrose is a potential feedstock for anaerobic digestion. Furthermore, the application of alkaline pretreatment on grass achieved a significant outcome for biogas production and methane concentration. Especially, after NaOH pretreatment of 2 weeks on water primrose, at a mixing ratio 2:1 (water primrose to cow dung), not only biogas production but also methane percentage gave the highest value compared with other treatments in this study (8610 mL and 68.20%, respectively). Hence, in order to evaluate the production efficiency of the co-digestion of the fermentation process, the removal efficiency of TS, VS, and COD was calculated. The results indicated that the increased percentage of TS, VS degradation rate, and COD removal rate represented the improved biodegradability of feedstock and thus the high biogas production gained.

References

1. Sophanodorn K, Unpaprom Y, Whangchai K, Homdoung N, Dussadee N, Ramaraj R (2020) Environmental management and valorization of cultivated tobacco stalks by combined pretreatment for potential bioethanol production. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-00992-8
2. Nguyen TV, Unpaprom Y, Manmai N, Whangchai K, Ramaraj R (2020) Impact and significance of pretreatment on the fermentable sugar production from low-grade longan fruit wastes for bioethanol production. Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00977-7
3. Ramaraj R, Unpaprom Y (2019) Optimization of pretreatment condition for ethanol production from *Cyperus difformis* by response surface methodology. 3 Biotech 9(6):218
4. Ramaraj R, Kawaree R, Unpaprom Y (2016) A newly isolated green alga, *Pediastrum duplex* Meyen, from Thailand with efficient hydrogen production. IJSGE 4:7–12
5. Unpaprom Y, Pimpimol T, Whangchai K, Ramaraj R (2020) Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer. Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00850-72
6. Wannapokin A, Ramaraj R, Unpaprom Y (2017) An investigation of biogas production potential from fallen teak leaves (*Tectona grandis*). Emergent Life Sci Res 1:38–45
7. Wannapokin A, Ramaraj R, Whangchai K, Unpaprom Y (2018) Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. 3. Biotech 8:123
8. Dussadee N, Unpaprom Y, Ramaraj R (2016) Grass silage for biogas production. Advances in Silage Production and Utilization. 16:153
9. Dussadee N, Reansuwan K, Ramaraj R (2014) Potential development of compressed bio-methane gas production from pig farms and elephant grass silage for transportation in Thailand. Bioresource Technology 155:438–441
10. Pantawong R, Chuanchai A, Thipbunrat P, Unpaprom Y, Ramaraj R (2015) Experimental investigation of biogas production from water lettuce, *Pistia stratiotes* L. Emergent Life Sci Res 1:41–46
11. Unpaprom Y, Intasaen O, Yongphet P, Ramaraj R (2015) Cultivation of microalga Botryococcus braunii using red Nile tilapia effluent medium for biogas production. J Ecol Environ Sci 3:58–65
12. Ramaraj R, Dussadee N, Whangchai N, Unpaprom Y (2016) Microalgae biomass as an alternative substrate in biogas production. IJSGE 4:13–19
13. Ramaraj R, Unpaprom Y, Whangchai N, Dussadee N (2015) Culture of macroalgae *Spirogyra ellipsospora* for long-term experiments, stock maintenance and biogas production. Emergent Life Sci Res 1:38–45
14. Ramaraj R, Unpaprom Y, Dussadee N (2016) Potential evaluation of biogas production and upgrading through algae. IJNTR 2:128–133
15. Ramaraj R, Dussadee N (2015) Biological purification processes for biogas using algae cultures: a review. IJSGE 4(1 – 1):20–32
16. Dussadee N, Ramaraj R, Cheunbarn T (2017) Biotechnological application of sustainable biogas production through dry anaerobic digestion of Napier grass. 3. Biotech 7(1):47
17. Ramaraj R, Unpaprom Y, Dussadee N (2016) Cultivation of green microalga, Chlorella vulgaris, for biogas purification. IJNTR 2:117–122
18. Manmai N, Unpaprom Y, Ramaraj R (2020) Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00602-7

19. Zhang Y, Banks CJ, Heaven S (2012) Co-digestion of source segregated domestic food waste to improve process stability. Bioresource technology 114:168–178
20. APHA (2005) Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington, DC
21. Van Tran G, Unpaprom Y, Ramaraj R (2019) Methane productivity evaluation of an invasive wetland plant, common reed. Biomass Conv Bioref. 10:689–695. https://doi.org/10.1007/s13399-019-00451-z
22. Chuanchai A, Ramaraj R (2018) Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and bio-fertilizer. 3 Biotech 8(3):151
23. Van Tran G, Unpaprom Y, Ramaraj R (2019) Effects of co-substrate concentrations on the anaerobic co-digestion of common reed and cow dung. AJARCDE| Asian Journal of Applied Research for Community Development and Empowerment 3(1):28–32
24. Matheri AN, Ntuli F, Ngila JC, Seodigeng T, Zvinowanda C, Njenga CK (2018) Quantitative characterization of carbonaceous and lignocellulosic biomass for anaerobic digestion. Renewable and Sustainable Energy Reviews 92:9–16
25. Chan WP, Wang JW (2016) Comprehensive characterisation of sewage sludge for thermochemical conversion processes–based on Singapore survey. Waste management 54:131–142
26. Du J, Qian Y, Xi Y, Lü X (2019) Hydrothermal and alkaline thermal pretreatment at mild temperature in solid state for physico-chemical properties and biogas production from anaerobic digestion of rice straw. Renewable Energy 139:261–267
27. Devlin DC, Esteves SRR, Dinsdale RM, Guwy AJ (2011) The effect of acid pretreatment on the anaerobic digestion and dewatering of waste activated sludge. Bioresource Technology 102(5):4076–4082
28. Rabii A, Aldin S, Dahman Y, Elbeshbishy E (2019) A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. Energies 12(6): 1106
29. Zheng Y, Zhao J, Xu F, Li Y (2014) Pretreatment of lignocellulosic biomass for enhanced biogas production. Progress in energy and combustion science 42:35–53
30. Amin FR, Khalid H, Zhang H, Rahman U, Zhang SR, Liu G, Chen C (2017) Pretreatment methods of lignocellulosic biomass for anaerobic digestion. Amb Express 7(1):72
31. Deepanraj B, Sivasubramanian V, Jayaraj S (2017) Effect of substrate pretreatment on biogas production through anaerobic digestion of food waste. International Journal of Hydrogen Energy 42(42):26522–26528
32. Tetard L, Passian A, Farahi RH, Kalluri UC, Davison BH, Thundat T (2010) Spectroscopy and atomic force microscopy of biomass. Ultramicroscopy 110(6):701–707
33. Fang W, Weisheng N, Andong Z, Weiming Y (2015) Enhanced anaerobic digestion of corn stover by thermo-chemical pretreatment. International Journal of Agricultural and Biological Engineering 8(1):84–90
34. Moset V, Xavier CDAN, Feng L, Wahid R, Møller HB (2018) Combined low thermal alkali addition and mechanical pre-treatment to improve biogas yield from wheat straw. Journal of Cleaner Production 172:1391–1398
35. Mao C, Zhang T, Wang X, Feng Y, Ren G, Yang G (2017) Process performance and methane production optimizing of anaerobic co-digestion of swine manure and corn straw. Scientific reports 7(1):1–9
36. Feng S, Hong X, Wang T, Huang X, Tong Y, Yang H (2019) Reutilization of high COD leachate via recirculation strategy for methane production in anaerobic digestion of municipal solid waste: Performance and dynamic of methanogen community. Bioresource technology 288:121509
37. Pang C, Xie T, Lin L, Zhuang J, Liu Y, Shi J, Yang Q (2012) Changes of the surface structure of corn stalk in the cooking process with active oxygen and MgO-based solid alkali as a pretreatment of its biomass conversion. Bioresour Technol 103:432–439
38. Kumar V, Kumar P, Kumar P, Singh J (2020) Anaerobic digestion of Azolla pinnata biomass grown in integrated industrial effluent for enhanced biogas production and COD reduction: Optimization and kinetics studies. Environmental Technology & Innovation 17: 100627
39. Komilis D, Kissas K, Symeonidis A (2014) Effect of organic matter and moisture on the calorific value of solid wastes: an update of the Tanner diagram. Waste Manage 34:249–255
40. Li Y, Zhang R, He Y, Zhang C, Liu X, Chen C, Liu G (2014) Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). Bioresour Technol 156:342–347

Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-01083-4

ORIGINAL ARTICLE

Development of sustainable approaches for converting the agro-weeds *Ludwigia hyssopifolia* to biogas production

Huyen Thu Thi Nong [1,2] · Kanda Whangchai [3] · Yuwalee Unpaprom [2,4] · Churat Thararux [1] · Rameshprabu Ramaraj [1,2]

Received: 9 September 2020 / Revised: 28 September 2020 / Accepted: 9 October 2020

Abstract

This study aimed to evaluate potential biogas production from *Ludwigia hyssopifolia* (water primrose) and examine the effect of alkaline pretreatment on samples through biogas production efficiencies. The research was carried out for 45 days of operation from anaerobic mono-digestion of water primrose by using a batch experiment. Pretreatment was applied for substrate using sodium hydroxide (NaOH) solution (w/v) at different concentrations (0, 1, 2, 3, and 4%) with 10% of total solids (TS) based on dry matter. The scanning electron microscopy (SEM) images were captured to investigate the characteristics of the raw material and pretreated biomass. The results showed that the treatment with 2% NaOH has the highest performance in biogas yield (8072.00 mL) and methane content (64.72%). Notably, the increase (3, 4%) or decreasing (0, 1%) NaOH concentration in treating water primrose did not achieve a significant improvement. Further investigation in the power potential of produced biogas was calculated, and the result was 22,382.19 W/m^3 power. Consequently, the feasibility of the alkaline pretreatment method for biogas production and achievable potential for energy efficiency indicates that water primrose is appropriate agro-weed biomass for bioenergy applications.

Keywords Water primrose · Pretreatment · Mono-digestion · Biogas production

1 Introduction

The energy consumption in the world is overexploitation due to population development and economic growth has led to several problems that need to be tackled, such as climate change, the loss of natural resources, or the environmental population [1, 2]. Also, the energy demand has risen significantly, which may face the fossil fuels crisis as today more than 88% of the main energy used still relies on fossil fuels [3] and once combusted, a large amount of carbon dioxide (CO_2) emission to be released [4].

✉ Rameshprabu Ramaraj
rameshprabu@mju.ac.th; rrameshprabu@gmail.com

1 School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand

2 Sustainable Resources & Sustainable Engineering Research Lab, Maejo University, Chiang Mai 50290, Thailand

3 Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

4 Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand

This matter has given a growing concern in greenhouse gas emission as well as energy security that is leading to an increase in the proliferation of various research on alternative energy [5, 6]. The concept of alternative energy is often referred to as renewable energy; it includes the energy supplied from natural sources such as biomass, solar energy, geothermal, hydropower, wind energy, and ocean energy [7, 8]. Among these energy generation sources, converting biomass sources to biogas generation has been widely attracted huge attention through anaerobic digestion [9].

Anaerobic digestion or biogas technology is alternative energy and this biological process using biomass as the primary feedstock, mostly from agricultural and agro-industrial wastes such as asparagus, wheat, barley, rice straw and maize stalks; sorghum forage, wheat straw, and corn stover [10–12]. The process takes place in the oxygen-free condition for degradation organic matter while producing biogas, which contains principally of methane (CH_4, 50–75%), carbon dioxide (CO_2, 25–50%), and other trace gas approximating hydrogen sulfide (H_2S), hydrogen (H_2), and nitrogen (N_2) [11, 12].

Biomass principally comprises cellulose, hemicellulose, and lignin, which is called lignocellulosic biomass [13]. Cellulose and hemicellulose can degrade after the hydrolysis

Published online: 13 October 2020

step in anaerobic digestion, making lignocellulosic biomass a potential candidate for biofuel generation [14]. On the contrary, lignin plays a function in the formation of the cell wall (impermeability) [15] and does not allow microbes to attack or degrade at the anaerobic system [16] and, more importantly, high lignin content in the substrate could lead to process failure [17]. The pretreatment step is typically required to break down the complex structure and chemical properties of lignocellulosic biomass, making it resistant to degradation by enzymes and bacteria during anaerobic digestion [15–17].

Generally, pretreatment methods have been accomplished by the physical method (comminution, steam-explosion, liquid hot water pretreatment), chemical method (alkaline, acid), and biological method (use of microorganisms and enzymes) [14, 17, 18]. The alkaline pretreatment is considered to be the most effective that is typically applied for lignocellulosic materials. This method of pretreatment causes the structure to swell, increases the area of the interval surface, and breaks down structural linkages between lignin and cellulose/hemicellulose [19]. Furthermore, this method involves lower temperature and pressure, thereby offers lower production costs and easy operation compared to other pretreatment methods [20].

Water primrose (*Ludwigia hyssopifolia*) is an invasive aquatic weed, growing equally well in or adjacent to water. This plant is growing with food crops. Eradicating weeds with herbicides has been adverse effects in food production because weeds compete with crops for water, nutrients, and soil. Also, weeds can harbor insect and disease pests, and noxious weeds and weed seeds can significantly affect crop quality [21]. So far, there is no literature available on water primrose related to biogas production. Therefore, this is a novel energy material for biogas production. In this study, the whole parts of water primrose, such as flowers, leaves, stems, fruits, roots, will be used as a mono-substrate to produce biogas production. The highlight of this investigation is the effect of alkaline pretreatment on the substrate. Sodium hydroxide (NaOH) is selected as the chemical reagent to determine the optimum conditions for dry water primrose pretreatment.

2 Materials and methods

2.1 Sample collection and preparations

Water primrose was collected from a field near Maejo University, Chiang Mai city, Thailand. Before use, the whole part of the plant was air-dried for 1 to 2 weeks, shredded by machine into small pieces, and milled by a blender. The final size of blended water primrose was 5–10 mm and then it was stored in plastic bags at room temperature. Based on the dry matter of water primrose, 10% of total solids concentration was calculated for each treatment in the current study. Consequently, 70 g of water primrose samples was prepared separately for pretreatment and control (untreated samples). Each treatment was done in triplicate samples.

2.2 Pretreatment of water primrose

The pretreatment of water primrose was conducted in a closed container with a capacity of 1.5 L. Solutions were prepared at different doses of NaOH as 1, 2, 3, and 4% (w/v). Afterward, in each container, the sample with a total solids (TS) content of 10% (100 g TS/L) was soaked with NaOH following ratio 1:5 (w/v) and kept for 2 weeks of pretreatment time. In each treatment, three samples were conducted. The collecting water primrose and sample preparation are shown in Fig. 1.

2.3 Imaging with scanning electron microscopy

In order to document the disruptive effect of alkaline on the pretreated samples, scanning electron microscopy (SEM) was carried out at the Institute of Product Quality and Standardization (IQS), Maejo University, Chiang Mai, Thailand. First, dried water primrose was sputtered with a very thin layer of gold to guarantee its electrical conductivity. The samples were then sputter-coated with and fixed with the brass stub for examination under the field emission scanning electron microscope (FESEM) (Nova Nanosem 450, USA). The instrument used was JSM-5410LV and operated with a field emission gun, and observations were performed at a total magnification of × 500.

2.4 Anaerobic mono-digestion design

The capacity of the digester was 1 L with an operating volume of 700 mL used in current experiments that fed with 10% TS per liter and retention time of 45 days. All experiments were prepared based on the dry matter content of the sample in triplicates. In total, 70 g of samples was added to each digester. After the addition of the substrate, the digesters were recapped by rubber stoppers and gas outlet released from the top of digesters via a gas transfer tube into a gas holder which placed inside a water bath (Fig. 2). The gas produced was measured equivalent to the volume of water decreased from gasholders. Then, the daily volume gas was taken every 24 h; the composition of gases such as methane (CH_4), carbon dioxide (CO_2), oxygen (O_2), and hydrogen sulfide (H_2S) was quantified by gas analyzer Geotech GA5000 every 3 days. In all experiments, the pH of samples was adjusted up to 8.5–9 by added calcium oxide (CaO). This pH was maintained throughout the process without any further adjustment.

Fig. 1 Sample preparation of water primrose. **a** Source of material. **b** Collecting material. **c** Air-dry material. **d** Shredding material. **e** Sample. **f** Pretreatment sample

2.5 Analytical methods

The physical and chemical analysis of water primrose was conducted in the Laboratory of Faculty of Science. The solids content, including total solids (TS) and volatile solids (VS), was characterized using a 10 g of sample drying at 105 °C for 24 h and 550 °C for 30 min to 1 h, respectively [21]. The pH was measured by pH meter (Oakton PCSTestr 35 Waterproof). A three-point calibration of the pH probe was checked before analysis.

2.6 Biogas energy and power potential calculation

The calculation of energy and power potential is adopted by [22] using the biogas collected and its flame to heat water as follows:

$$E = M_c C_c \Delta\theta + M_w C_w \Delta\theta \quad (1)$$

where E is the amount of heat energy dissipated, M_c is the mass of calorimeter (g), C_c is the specific heat capacity of the calorimeter (390 J kg^{-1} K^{-1}), $\Delta\theta$ is the change in temperature (°C), M_w is the mass of water (g), and C_w is the specific heat capacity of water (4200 Jkg^{-1} K^{-1}).

$$\text{Power} = \frac{(E)}{(t)} \quad (2)$$

where E is the amount of heat energy calculated in Eq. 1 and t is the time taken for the energy to be dissipated (s).

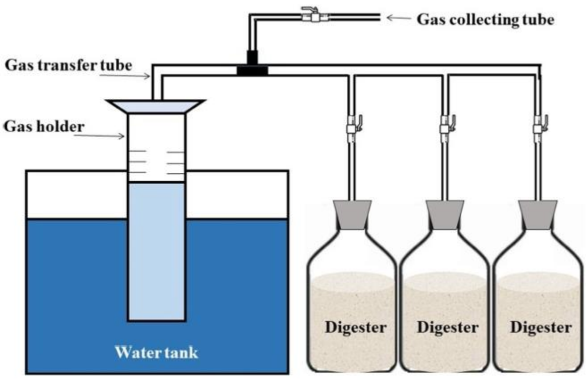


Fig. 2 Schematic diagram of the gas collecting and measurement setup

3 Results and discussion

3.1 Substrate characterization

Determining the characterization of material used in the experiment leads to a better estimate of the relationship between substrate and biogas potential. The initial characteristics of water primrose for TS and VS were 900,000 mg/L and 836,000 mg/L, respectively, based on dry matter. High TS and VS contents show that the organic compounds in the water primrose could contribute high organic conversion to the biogas generation. Since the pH of the substrate was 5.05, therefore, applying NaOH pretreatment on water primrose results in reduced amounts of CaO that was used to adjust pH in the fermenter before fermentation and increases alkaline buffer capacity as acids were produced causing the acidic environment in the digester. This is the advantage of using NaOH pretreatment for the substrate.

Water primrose is a semi-aquatic annual herb widely distributed worldwide as Asia, Africa, and Australia. This plant stands erect along with wet soil or floats out across the water surface with bright yellow flowers, can grow up to 2-m or even 3-m high. Leaves are long and slender in shape and arranged alternately on the stem, up to 10-cm long, 1–2-cm wide, shiny, dark green and lighter green central vein or in yellow color [23]. Water primrose has been growing in unwanted places, their growth does not meet the purposes for either food or feed, and thus their value for a specific purpose as biogas energy in the anaerobic digestion is in a manner conforming with the sustainability principles.

3.2 Effect of NaOH on the substrate

According to Du et al. [24], the pretreatment time should be in the ranges of 3 to 30 days; beyond that ranges, the application of pretreatment technology is not conducive. Biogas production from lignocelluloses biomass in general and water primrose in particular is well known that the rate-limiting step in the anaerobic digestion process is the hydrolysis step and possible inhibition in the overall process due to the complicated polymer of lignin [20, 24, 25]. Thus, pretreatment substrates show not only profound concerning increase the anaerobic biodegradability, but improving biogas production of biomass [14, 17, 24].

In the current research, the pretreatment test was prepared following by adding a sample with NaOH solution ratio 1:5. The effect of alkaline pretreatment on water primrose was evaluated through biogas and methane efficiency shown in Table 1. The results show that adding concentration of NaOH leads to an increased content of the biodigestibility of the substrate under anaerobic conditions. The complex structure of lignocellulosic biomass was significantly effected by NaOH pretreatment. As different doses of NaOH were used in pretreatment of water primrose, the organic matter degradation was observed through the volume of biogas production and CH_4 content per all treatments differently. The cumulative biogas production was highest for 2% NaOH (8072.00 mL), followed by 3% (6933.03 mL), 1% (5307.00 mL), 4% (5073.50 mL), and lastly, control (4285.80 mL). In terms of methane content in biogas, after 45 days of fermentation, the percentage of methane was similar in all treatments (55–65%). However, the highest methane obtained at 2% NaOH treatment (64.72%) was 9.65% more than the control (55.07%).

Table 1 Total biogas and methane content of different alkaline treatments on substrate

Treatment	Total biogas (mL)	Methane (%)
Control (no treatment)	4285.80	55.07
1% NaOH	5307.00	58.33
2% NaOH	8072.00	64.72
3% NaOH	6933.03	60.84
4% NaOH	5073.50	59.51

Generally speaking, biodegradability improvement of the alkaline-pretreated water primrose enhanced not only by the total biogas volume but also by the methane concentration. After alkaline pretreatment, the complex structure of lignocellulosic biomass would change due to the reaction of alkaline. These changes resulted in the high solubilization rate of lignin and hemicellulose, making substrate easily for anaerobic microorganisms to metabolize and produce gases [13, 14, 17].

Furthermore, there was no significant biogas yield improvement by applying 1 and 4% NaOH treatment compared to the control. This indicated that at the mild concentration, alkaline did not become strong enough to increase the degradation of organic matter. On the contrary, high chemical concentration loading results in the disadvantage or even toxic to microbes [13, 20].

Similar biogas yield results were also observed by Chandra et al. [25]; the author used 4% NaOH (g/g TS) pretreated substrate for a wheat straw at mesophilic temperature. The total biogas obtained from the untreated substrate was 3349 L. Meanwhile, the value of biogas yield using 4% NaOH was observed higher than that of the untreated substrate, with the total gas was 6279 L. The results evidenced that the efficiency of 4% NaOH treatment on wheat straw had increased biogas production by 87.5% compared to the untreated wheat straw substrate. Effective comparison of using three alkali chemicals by Yang et al. [26], including lime ($Ca(OH)_2$), sodium hydroxide (NaOH), and potassium hydroxide (KOH) on rice straw. Alkaline loading at vary concentration of 2%, 6%, and 10% levels. The study reported that among these chemicals evaluated, the biogas production was achieved 50% higher than the untreated sample in case using either KOH or NaOH pretreatment. However, NaOH treatment was the most effective at 20 °C and 10% chemical loading for 24 h achieved the highest lignin removal of 35% for rice straw. Besides, the study implied that in the future, the pretreatment on rice straw might concentrate on different doses of NaOH to optimize the pretreatment conditions further.

3.3 Scanning electron microscopy of a raw and pretreated water primrose

SEM analyses of native and 2% NaOH-pretreated samples were carried out to assess changes in morphology. Figure 3(a) shows the SEM micrograph of native water primrose, the surface of which shows to have a stable and compact structure. As can be seen, the apparent structure of water primrose is closely regulated, and there are rough particle bulges. Morphological changes induced by alkaline pretreatment are first noticeable after pretreatment at 2% of NaOH, as shown in Fig. 3(b); the apparent structure became looser, more holes appeared on the surface of solids and the size became larger, the surface area increased accordingly. Here, a slight defibrillation was observed, consisting of the separation of individual fibers and an enlarging of the reactive area. By 2% of NaOH, more pronounced structural changes in the biomass were seen due to solubilization and break down of the hemicellulose and cellulose. As hemicellulose operates as cementing material, its solubilization causes a significant defibrillation effect on the biomass. Also, a reduction in fiber length and the formation of entangled clusters can be seen. After pretreatment at 2% NaOH concentration, the fiber structure is almost entirely disintegrated due to the higher solubilization of hemicellulose and lignin re-localization.

3.4 Evaluate biogas production and methane content from the substrate

Biogas can be produced from various organic substrates such as municipal, agricultural, and industrial wastes and energy crops through anaerobic digestion process [9, 15, 27]. Among those sources, the most popular using for biogas production is grasses as it contains high energy content and easy to harvest [28, 29]. Mono-digestion is the foundation for further development of co-digestion; investigated biogas production on a single substrate before mixing with one or more than one substrates would obviously evaluate the influence of each substrate that is involved in the fermentation process. One of the most significant factors in deciding operating anaerobic digestion is to investigate biogas potential on the feedstocks used. The more amount of dissolved carbon in the substrate, the more gas could be converted by bacterial. The second factors are the time given to the bacteria called hydraulic retention time (HRT). In case the substrate has enough time for bacterial to digest, there may get a full conversion of organics to the end products.

Typically, HRT works in the mesophilic temperature range of 20–35 °C for 15–40 days [30]. The investigation from dry water primrose shows that methane production decreased rapidly after 45 days of operation. Therefore, the ideal time for the decomposition of this material was performed for 45 days. The daily and accumulative biogas production from all treatment is presented in Fig. 4 and Fig. 5. Biogas started producing immediately on the first day from all treatments (control, 1, 2, 3, 4% NaOH). During the first 2 weeks, there was no significant different gases production among these treatments. From the third week onward, the biogas curves of 2 and 3% NaOH treatment were higher than the rest of the treatment and this curve maintained until the end of the process.

The highest rates of biogas production per day reached 257.5 mL/day using 2% NaOH; this peak value was obtained on day 35. The maximum biogas yield of control, 1%, 3%, and 4% gradually reached their peak value on days 33, 37, 27, and 45, respectively. In all treatments, the daily biogas volume curve was mostly flat and there was no clear peak in daily productivity. The digesters with 2% NaOH have a 71.6% higher peak volume than the controls (150 mL), followed by

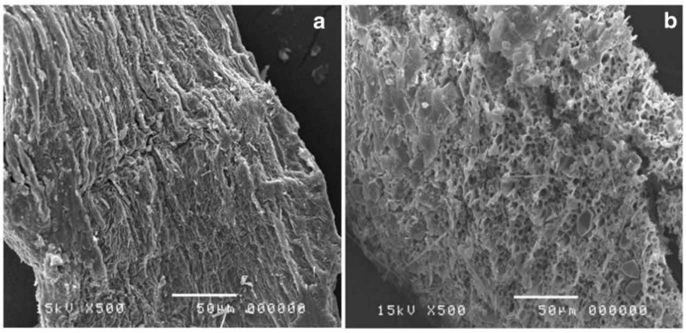


Fig. 3 SEM images of the untreated (**a**) and 2% NaOH-treated water primrose sample (**b**) under magnification of × 500

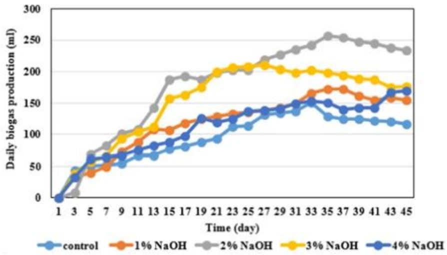


Fig. 4 Daily biogas production from dry water primrose

3% NaOH, which is 40.6% higher than the controls but 18.1% lower than 2% NaOH. At 4 and 1% of NaOH, the peak volume was not much different compared to the controls, which reached 169.5 and 172 mL, respectively. Hence, the pretreatment samples reached the peak value of the controls (150 mL) than about 2 days and 18–20 days for 1%, 4%, and 2%, 3% NaOH, respectively.

For all of the digesters, at the beginning stage of anaerobic digestion, the anaerobic bacteria started acting on the organic matter of the substrate [10, 11]. The bacteria population increased and digested on more substrate leading to increase biogas production. After an adaptation period, the bacteria were active on the most considerable amount of readily biodegradable organic matter in the substrate, resulting in an apparent influence on daily gas production that performed as the peak value [17]. After that, the carbon and nutrient in the substrate decrease, biogas production started to drop and gradually to stop producing.

The accumulative biogas yield calculated based on the gas-producing per day that varied from 4,285.80 to 8072.00 mL that shows in Fig. 5. There was no significant different volume in the treatment between 1, 4% NaOH, and control. The highest biogas yield was obtained from 2% NaOH; it was by 16.4%, 52.1%, and 59.1% higher than the yield of pretreatment samples of 3%, 4%, and 1%, respectively. Moreover, it was much higher 88.4% for biogas yield of control. Therefore, the treatment of 2% NaOH concentration is the best option for pretreatment substrate, which not only quickly reaches the highest volume but also achieves the highest cumulative biogas volume. The rapid culmination of volume obtained from treated samples due to the bacteria initially is quickly digesting easily biodegradable materials. This result indicates that the pretreated water primrose is easy to access hydrolytic bacteria at the early stage of the digestion process.

During the forty-fifth day of anaerobic mono-digestion, the methane concentration obtained from all treatments is shown in Fig. 6. The methane content was measured every 3 days of the fermentation process. The biogas production started producing from day one while the methane concentration was detected from day 6 onward for all treatments. Generally, the difference of methane formation in biogas varied from a minimum value of 55.07% (control) to a maximum value of 64.72% (2% NaOH). All through the first 6 days of fermentation, the methane was recorded at 0% at all treatments. After that, methane concentration steadily rose until the end of the process. It is observed that at the beginning of the anaerobic digestion, the methane concentration at 4% NaOH treatment was slightly higher than the rest of the treatments. The 4% NaOH had the earliest peak value on day 27 with 59.51% of methane, which indicated that at a high concentration of NaOH treatment on water primrose would positively improve the initial methane concentration. However, the highest methane formation from this study was at 2% NaOH, with 64.72% obtained near the last day of the process (day 42). This is further supported that 2% NaOH of pretreatment on water primrose not only had a positive effect on the quantity of the total volume of gas produced but also improves the quality of the gas. The biogas compositions, including CH_4, CO_2, H_2S, and O_2 were 64.72, 35.09, 0.09, and 0.1%, respectively, observed in the digesters applied 2% NaOH pretreated substrate.

Overall, the peak methane value of all treatment was found at the final stage of anaerobic digestion; this is because the acidogenic and methanogenic bacteria took a long time to adapt and balanced growth. Another reason is maybe the certain amount of organic inhibitors in the

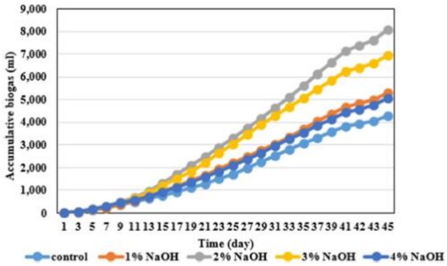


Fig. 5 Cumulative biogas from dry water primrose

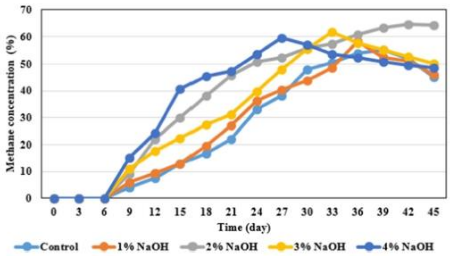


Fig. 6 Methane concentration from dry water primrose

substrate was further degraded and converted to biogas generation, which contributed to methane concentration [31]. The comparison of methane content in biogas from water primrose with other feedstocks is presented in Table 2. Water primrose provided higher methane content compared to water hyacinth, corn stover, rice straw, tobacco straw, soybean residues, papaya peels, sugarcane bagasse, rice straws, maize straw, food waste, and fruit/vegetable waste [32–38], moreover, almost similar methane concentration compared to wild Mexican sunflower and dry maize straw [36, 39]. Therefore, the methane value from different biomass sources compared to the result obtained from this study showed that the potential maximum methane content available in water primrose is highly competitive with other sources of biomass feedstocks.

3.5 Energy and power analysis

The primary energy inputs to mineral-based resources are deliberated. It refers to the energy that has not undertaken any conversion or transformation process. Many parameters may define the efficiency of biogas production, so the multi-criteria analysis is needed to evaluate the performance of a biogas plant. The process investigation for agricultural and any waste biomass (i.e., weeds) streams and food industry residues revealed the energy inputs for feedstock collection, transport, and pretreatment. Pöschl et al. [40] stated that the analyses for energy crops considered the entire supply chain from planting and cultivation, through harvesting and transport processes. However, utilizing weeds (for example, water primrose) provides more benefits if it could reduce the cultivation cost. Also, it was on arable land with no conflict with food and fodder production. Results from this study show that there could be significant and energy efficiency for biogas plants arising from feedstock resource and process adopted a single feedstock, conversion technology applied, and digestate management technique. From Eqs. (1) and (2), the energy and power of the gas burned were computed, which shows in Table 3. An average of 1132.3 cm^3 biogas was used for calculation; the results of biogas power potential were 25.34 W or 22.3822 W/L or 22,382.19 W/m^3. The pure methane content could generate 37,258.9 J of energy or 37,258.9 W/m^3 [41]. As a result, 60.07% of methane content was estimated from 22,382.19 W/m^3 power. In addition, it is noted that the energy of 10 m^3 of gas liquefied petroleum (LPG) used for cooking is equal to 25 m^3 of biogas. In other words, the ratio of energy from LPG gas to energy from pure methane is 5:2 [41]. Consequently, biogas production from water primrose is a potential feedstock in anaerobic digestion with achievable energy efficiency.

4 Conclusions

This study indicates that the utilization of water primrose is effective for biogas production through anaerobic mono-digestion. The NaOH pretreatment has significant positive effects in enhancing biogas yield as well as methane concentration. The best optimal treatment for water primrose was at 2% NaOH with the cumulative biogas yield of 8072 mL, which is 88.4% higher than that of control, 16.4% higher than 3% NaOH, and also 52.1% and 59.1% higher than 4% and 1% NaOH, respectively. Furthermore, the highest methane content (64.72%) obtained from 2% NaOH was also higher than the rest of the treatments. The energy assessment by testing its

Table 2 Comparison of methane concentration from different feedstocks

No.	Feedstock name	Methane (%)	References
1	Water hyacinth	40.3	Pereira et al. [32]
2	Corn stover	51	Li et al. [33]
3	Food waste	59.0	Li et al. [34]
4	Fruit/vegetable waste	63.4	Qiao et al. [35]
5	Rice straw	63	Li et al. [36]
6	Tobacco straw	63.37	Li et al. [36]
7	Dry maize straw	65.47	Li et al. [36]
8	Soybean residues	57.14	Onthong and Juntarachat [37]
9	Papaya peels	54.00	Onthong and Juntarachat [37]
10	Sugarcane bagasses	49.12	Onthong and Juntarachat [37]
11	Rice straws	56.25	Onthong and Juntarachat [37]
12	Maize straw	42.05	Wei et al. [38]
13	Wild Mexican sunflower	65	Dahunsi et al. [39]
14	Water primrose	64.72	This study

Table 3 Biogas energy and power potential calculation

	The volume of gas used V_{gas} (cm^3)	Mass of water and calorimeter M (g)	Change in temperature $\Delta\theta$ (°C)	Energy gained by water and calorimeter E (J)	Time taken t (s)	Power dissipated P (Watts)
Calorimeter		47.3				
Test 1	1975	53	17	4097.8	167	24.5
Test 2	874	47	14	3021.8	114	26.5
Test 3	548	40	13	2423.8	97	24.9
Average	1132.3			3181.1		25.3

flame also given an energy efficiency potential of 22,382.19 W/m^3 power. Consequently, the results of the research paper would contribute to the increasing estimation of energy efficiency and the influence of NaOH treatment for biogas production on a new biomass material.

Acknowledgments The authors gratefully acknowledged the School of Renewable Energy, Program in Biotechnology, and Energy Research Center, Maejo University, Chiang Mai, and Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand, for the research facilities to accomplish this experimental study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Nomenclature E, amount of heat energy dissipated (J); M_c, mass of calorimeter (g); C_c, specific heat capacity of the calorimeter (390 J kg^{-1} K^{-1}); $\Delta\theta$, change in temperature (°C); M_w, mass of water (g); C_w, specific heat capacity of water (4200 J kg^{-1} K^{-1}); t, time taken for the energy to be dissipated (s); NaOH, sodium hydroxide; KOH, potassium hydroxide; $Ca(OH)_2$, calcium hydroxide; CH_4, methane; CO_2, carbon dioxide; H_2, hydrogen; N_2, nitrogen; H_2S, hydrogen sulfide; HRT, hydraulic retention time; LPG, liquefied petroleum; TS, total solids; VS, volatile solids

References

1. Manmai N, Unpaprom Y, Ramaraj R (2020) Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00602-7
2. Vu PT, Unpaprom Y, Ramaraj R (2018) Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production. Bioresour Technol 247:125–130
3. Sophanodorn K, Unpaprom Y, Whangchai K, Homdoung N, Dussadee N, Ramaraj R (2020) Environmental management and valorization of cultivated tobacco stalks by combined pretreatment for potential bioethanol production. Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00992-8
4. Vu PT, Unpaprom Y, Ramaraj R (2017) Evaluation of bioethanol production from rice field weed biomass. Emergent Life Sci Res 3(2):42–49
5. Saengsawang B, Bhuyar P, Manmai N, Ponnusamy VK, Ramaraj R, Unpaprom Y (2020) The optimization of oil extraction from macroalgae, *Rhizoclonium* sp. by chemical methods for efficient conversion into biodiesel. Fuel 274:117841
6. Ramaraj R, Unpaprom Y (2019) Optimization of pretreatment condition for ethanol production from *Cyperus difformis* by response surface methodology. 3 Biotech 9(6):218
7. Bhuyar P, Sundararaju S, Rahim MH, Ramaraj R, Maniam GP, Govindan N (2019) Microalgae cultivation using palm oil mill effluent as growth medium for lipid production with the effect of CO2 supply and light intensity. Biomass Conv Bioref 26:1–9
8. Bhuyar P, Rahim MH, Yusoff MM, Gaanty Pragas Maniam NG (2019) A selective microalgae strain for biodiesel production in relation to higher lipid profile. Maejo Int J Energy Environ Commun 1:8–14
9. Pantawong R, Chuanchai A, Thipbunrat P, Unpaprom Y, Ramaraj R (2015) Experimental investigation of biogas production from water lettuce, *Pistia stratiotes* L. Emergent Life Sci Res 1:41–46
10. Ramaraj R, Dussadee N, Whangchai N, Unpaprom Y (2016) Microalgae biomass as an alternative substrate in biogas production. IJSGE 4:13–19
11. Ramaraj R, Dussadee N (2015) Biological purification processes for biogas using algae cultures: a review. IJSGE 4:20–32
12. Ramaraj R, Dussadee N, Whangchai N, Unpaprom Y (2015) Microalgae biomass as an alternative substrate in biogas production. IJSGE 4:13–19
13. Unpaprom Y, Pimpimol T, Whangchai K, Ramaraj R (2020) Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer. Biomass Conv Bioref. https://doi.org/10.1007/s13399-020-00850-72
14. Wannapokin A, Ramaraj R, Whangchai K, Unpaprom Y (2018) Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. 3 Biotech 8:123
15. Van Tran G, Unpaprom Y, Ramaraj R (2019) Methane productivity evaluation of an invasive wetland plant, common reed. Biomass Conv Bioref 10:689–695. https://doi.org/10.1007/s13399-019-00451-z
16. Ramaraj R, Unpaprom Y, Dussadee N (2016) Cultivation of green microalga, *Chlorella vulgaris*, for biogas purification. IJNTR 2: 117–122
17. Chuanchai A, Ramaraj R (2018) Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and bio-fertilizer. 3 Biotech 8(3):151
18. Atelge MR, Atabani AE, Banu JR, Krisa D, Kaya M, Eskicioglu C, Kumar G, Lee C, Yildiz YŞ, Unalan SE, Mohanasundaram R (2020) A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. Fuel 270:117494

19. Tsapekos P, Kougias PG, Angelidaki I (2015) Anaerobic mono-and co-digestion of mechanically pretreated meadow grass for biogas production. Energy Fuel 29(7):4005–4010
20. Pang YZ, Liu YP, Li XJ, Wang KS, Yuan HR (2008) Improving biodegradability and biogas production of corn stover through sodium hydroxide solid state pretreatment. Energy Fuel 22(4):2761–2766
21. APHA (2005) Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington, DC
22. Ummalyma SB, Supriya RD, Sindhu R, Binod P, Nair RB, Pandey A, Gnansounou E (2019) Biological pretreatment of lignocellulosic biomass—Current trends and future perspectives. Second and Third Generation of Feedstocks:197–212
23. Chauhan B, Pame AP, Johnson D (2011) Compensatory growth of ludwigia (*Ludwigia hyssopifolia*) in response to interference of direct-seeded rice. Weed Sci 59(2):177–181
24. Du J, Qian YT, Xi XL, Jin HM, Kong XP, Zhu N, Ye X (2019) The feasibility of shortening the pretreatment time for improvement of the biogas production rate from rice straw with three chemical agents. BioResources 14(2):3808–3822
25. Chandra R, Takeuchi H, Hasegawa T, Kumar R (2012) Improving biodegradability and biogas production of wheat straw substrates using sodium hydroxide and hydrothermal pretreatments. Energy 43(1):273–282
26. Yang D, Zheng Y, Zhang R (2009) Alkali pretreatment of rice straw for increasing the biodegradability. American Society of Agricultural and Biological Engineers 095685
27. Dussadee N, Ramaraj R, Cheunbarn T (2017) Biotechnological application of sustainable biogas production through dry anaerobic digestion of Napier grass. 3 Biotech 7(1):47
28. Van Tran G, Unpaprom Y, Ramaraj R (2019) Effects of co-substrate concentrations on the anaerobic co-digestion of common reed and cow dung. AJARCDE 3(1):28–32
29. Dussadee N, Unpaprom Y, Ramaraj R (2016) Grass silage for biogas production. Advances in Silage Production and Utilization 16: 153
30. Koch K, Wichern M, Lübken M, Horn H (2009) Mono fermentation of grass silage by means of loop reactors. Bioresour Technol 100(23):5934–5940
31. Dareioti MA, Kornaros M (2014) Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system. Bioresour Technol 167:407–415
32. Pereira RG, de Jesus V (2011) Production and characterization of biogas obtained from biomass of aquatic plants. Renewable Energy Power Qual J:9
33. Li Y, Zhang R, Liu X, Chen C, Xiao X, Feng L, He Y, Liu G (2013) Evaluating methane production from anaerobic mono-and co-digestion of kitchen waste, corn stover, and chicken manure. Energy Fuel 27(4):2085–2091
34. Li R, Chen S, Li X (2010) Biogas production from anaerobic co-digestion of food waste with dairy manure in a two-phase digestion system. Appl Biochem 160(2):643–654
35. Qiao W, Yan X, Ye J, Sun Y, Wang W, Zhang Z (2011) Evaluation of biogas production from different biomass wastes with/without hydrothermal pretreatment. Renew Energy 36(12):3313–3318
36. Li X, Liu YH, Zhang X, Ge CM, Piao RZ, Wang WD, Cui ZJ, Zhao HY (2017) Evaluation of biogas production performance and dynamics of the microbial community in different straws. J Microbiol Biotechnol 27(3):524–534
37. Onthong U (2017) Juntarachat N (2017) Evaluation of biogas production potential from raw and processed agricultural wastes. Energy Procedia 138:205–210
38. Wei L, Qin K, Ding J, Xue M, Yang C, Jiang J, Zhao Q (2019) Optimization of the co-digestion of sewage sludge, maize straw and cow manure: microbial responses and effect of fractional organic characteristics. Sci Rep 9(1):1–10
39. Dahunsi OS, Oranusi S, Efeovbokhan EV (2017) Anaerobic mono-digestion of *Tithonia diversifolia* (Wild Mexican sunflower). Energy Convers Manag 148:128–145
40. Pöschl M, Ward S, Owende P (2010) Evaluation of energy efficiency of various biogas production and utilization pathways. Appl Energy 87(11):3305–3321
41. Balat M, Balat H (2009) Biogas as a renewable energy source—a review. Energy Sources 31(14):1280–1293

Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-01115-z

ORIGINAL ARTICLE

Assessment of the effects of anaerobic co-digestion of water primrose and cow dung with swine manure on biogas yield and biodegradability

Huyen Thu Thi Nong [1,2] · Yuwalee Unpaprom [2,3] · Kanda Whangchai [4] · Sermsuk Buochareon [1] · Rameshprabu Ramaraj [1,2]

Received: 2 October 2020 / Revised: 19 October 2020 / Accepted: 23 October 2020

Abstract
This study aims to investigate the potential of water primrose for biogas production in batch anaerobic digestion. To examine the ability of co-digestion with other substrates, cow dung and swine manure was chosen to mix with pretreated water primrose in a ratio of 1:1 (w/w, based on dry matter of water primrose). The pretreatment of water primrose was conducted by using sodium hydroxide at 2% concentration for one week. A modified Gompertz equation was employed to estimate parameters, including estimated biogas yield potential (Y_m), maximum biogas production rate (R_m), and duration of lag phase (λ). The equation showed a good approximation of cumulative biogas production with a coefficient of determination (R^2) over 0.997. The overall results indicate that all treatments had successfully produced biogas production in the range of 4285 to 6150 mL with methane (CH_4) content above 50%. The maximum biogas yield of 6150 mL was obtained at co-digestion with cow dung and high methane content of 63.88%. This value was given 25.50 MJ/m^3 for high calorific value (HCV) and 22.97 MJ/m^3 of low calorific value (LCV).

Keywords Water Primrose · Cow dung · Swine manure · Biogas production · Energy analysis · Kinetic model

1 Introduction

In the 21st century, the widespread assumption of fossil fuels reflects the ease of its application and product diversity compared to other energy sources, mainly because petroleum and natural gas are considered the largest sources from it [1, 2]. As a result, fossil fuel production is becoming increasingly expensive, yet, it is difficult to access due to this source is non-renewable [3, 4] and when burned, the large quantities regard to the emission of carbon dioxide (CO_2) produced which contributes to climate change and global warming [5]. For those reasons, fossil fuels are not attributed as sustainable energy in correlation with ecology and environment. According to Kang et al. [6], to counteract the decline of fossil fuel, it is necessary to accelerate finding alternative renewable energy sources, minimizing dependence. In this regard, bioenergy, such as biogas, biodiesel, and bioethanol, appears as clean. Renewable energy could be the best option for an alternative path of sustainability which is a suitable solution to ecological-environmental problems against the degradation time of fossil fuels and the reduction of CO_2 emission [7].

For useful energy generation in biorefinery, natural sources such as biomass material, including lignocellulosic biomass, animal waste, or municipal waste, have been widely investigated through anaerobic digestion process by numerous studies [8, 9] because of its wide distribution, less expensive and renewable. Different types of biomass waste are used as a feedstock under an anaerobic digestion system and decomposed with microorganisms' help to form biogas as the final product that could convert to various forms of energy, such as heating, electricity, transportation, and hydrogen through biorefinery technologies [10, 11].

✉ Rameshprabu Ramaraj
rameshprabu@mju.ac.th; rrameshprabu@gmail.com

[1] School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand

[2] Sustainable Resources & Sustainable Engineering Research Lab, Maejo University, Chiang Mai 50290, Thailand

[3] Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand

[4] Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

Published online: 28 October 2020

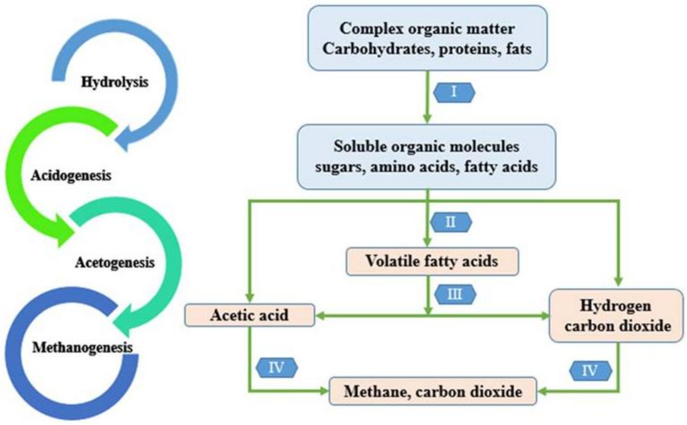
The anaerobic mono-digestion is commonly used animal manure as feedstock to produce biogas production. However, many studies indicated that instead of using one substrate indigestion, the combination of two or more substrates, called co-digestion, would make the overall process economically profitable because of offering high biogas yield, more biodegradable carbon matter, increase nutrient, reduce toxicity, advance the efficient use of the equipment, and cost-sharing [12, 13]. Thereby, it was evident that the anaerobic digestion process proves its advantages in biogas energy and utilizing digestion residue as a plant fertilizer, resulting in the reduction of waste disposal and energy cost [14, 15].

Even though anaerobic digestion happened partly, it can be divided into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During the process, the large organic compounds are broken down into smaller molecules by hydrolyzing and fermenting microorganisms and produce mainly acetate, hydrogen, and different amounts of volatile fatty acids; methane is produced from two groups of methanogenic bacteria, one is acetate and the other is hydrogen and carbon dioxide [16, 17]. The degradation of organic matter into biogas process was presented in Fig. 1.

A mostly diverse feedstock type as organic matter or organic waste can be used as substrates for biogas production. It is necessary to utilize all fractions from biomass to different value products and generate a minimum amount of waste. Also, it depends on plant materials, lignin, cellulose, and hemicellulose in lignocellulosic biomass. Noteworthy is that the lignin fraction structure inhibits the biogas produced because it is not fermented under an anaerobic environment, whereas cellulose and hemicellulose take a long time to hydrolysis [18]. Cellulose is covered by lignin. Therefore, the cellulolytic enzymes are difficult to access and convert the carbohydrate fractions (cellulose and hemicellulose) into sugars [19]. To overcome this obstacle, the pretreatment process of lignocellulosic biomass is necessary for enzymes and microbes easily to enter soluble organics; this step performs before the digestion process to achieve more efficient production of biogas. Chemical pretreatment, especially alkaline, is found as a cost-beneficial method; moreover, increasing alkaline leads to an increase of pH buffer in the anaerobic digestion, which is highly favorable for growing microorganisms [20]. Notably, the substrate's pretreatment process can be applied by combined treatments of physical and chemical methods to improve the effectiveness of a single pretreatment method [21].

Water primrose is one kind of agricultural weed available in the wetland and crop field in Thailand considered an aquatic invasive plant. Up to now, the potential energy of this biomass has not been investigated; meanwhile, the biogas industry's strategy is the input material sources that do replace energy crops and do not a non-food competition for fuel [22]. Thus, this study aims to focus on the novel material for biogas potential from water primrose and the combination of water primrose with cow dung or swine manure in anaerobic co-digestion was also investigated. The best performance in methane content in this study was then evaluated for high calorific value (HCV) and low calorific value (LCV). Besides, to boost biogas yield in anaerobic co-digestion, the physicochemical pretreatment method was applied on water primrose at 2% sodium hydroxide (NaOH) for 1 week.

Fig. 1 Stage of anaerobic digestion



2 Material and methods

2.1 Material collection

The water primrose was collected at the agricultural field in the fresh form nearby Maejo University, Chiang Mai, Thailand. The collected raw material was transferred to the laboratory at Energy Research Center, then air-dry for 2 weeks. The shredding machine and a blender were employed to cut the material into small particles of 5–10 mm as a final size (Fig. 2). Fresh cow dung and swine manure were collected at cow's farm and pig's farm at the Faculty of Animal Science and Technology, Maejo University, then weighed for the experiment (Fig. 3).

2.2 Alkaline pretreatment

The dry sample of water primrose with a size of 5–10 mm was used to investigate the effect of chemical pretreatment on water primrose. The alkaline reagent was prepared at 2% of NaOH and mixed with sample following ratio 5:1 (mL NaOH:g water primrose) in a plastic container with 1.5-L capacity. The amount of sample was calculated based on the desire set treatment of experiments. All containers were then sealed with the plastic cover and stored in the laboratory at room temperature for 1 week. Additionally, during pretreatment time, all containers were mixed manually every 24 h.

2.3 Biodigester design

The experiment consisted of 15 digesters was made of 1000 mL of Duran bottles transparent laboratory bottles of diameter 94 mm and 222 mm length, each with 700 mL of working volume. The substrate was digested anaerobically in the digesters at mesophilic temperature. The lab-scale anaerobic experiments were divided into three sets. Set I conducted 3 control samples such as water primrose, cow dung, and swine manure, while sets II and III performed co-substrates with a mixing ratio of 1:1 (w/w) of pretreated water primrose and cow dung or swine manure, respectively. In sets II and III, the batch anaerobic digestion was carried out with 2% NaOH pretreatment on water primrose for one week, each set run in triplicates.

The quantity of substrates (water primrose, cow dung, and swine manure) was computed based on total water primrose (10% TS). Accordingly, 70 g of water primrose, cow dung, and swine manure was determined in set I, while 35 g of each substrate was added and mixed in sets II and III as following the mixing ratio 1:1. Before sealing the digester with rubber corks, the tap water was used to dilute the substrates up to the desired volume, which amount needed more than about two times the original mass of the substrate, and then, calcium oxide (CaO) powder was used to adjust the initial pH to 8.5–9 for each set of experiment. The accumulative biogas was stored in a gas holder made of 500 mL of a graduated cylinder placed in a water tank (Fig. 4). All digesters were

Fig. 2 Water primrose preparation: **a** source of the material, **b** material collection, **c** air-dry material, **d** shredded material, **e** final particle size, **f** stored material

Fig. 3 Cow dung (**a**) and swine manure (**b**) collection

allowed for 45 days and during that period, digesters stirred twice a day for 1–2 min. The gas produced was measured every 24 h and methane concentration was determined by gas analyzer GA5000.

2.4 Analytical methods

The percentage of total solids (TS) and volatile solids (VS) was measured according to the standard methods of the American Public Health Association [23]. The samples were ignited in a muffle furnace at 105 °C for 24 h, and then the samples were cool down to room temperature in a desiccator for 15–30 min. TS is the residue remaining after ignition. The content of VS was the loss of sample mass after ignited total solids at 550 °C for 30 min. Before analyzing other parameters, all samples were diluted and centrifuged for 15 min at 1500 rpm. The permanent of homogeneous samples were taken out for analysis. The pH value was determined by an electronic pH meter. Titration methods analyzed chemical oxygen demand (COD), volatile fatty acids (VFAs), and alkaline. All parameters, including pH, TS, VS, ash, moisture content, COD, VFAs, and alkaline, were determined according to the standard process. The biogas produced was continuously recorded every day, then released into a gas sampling bag (500 mL) every 3 days. The methane content was regularly measured from the gas sampling bag using a gas analyzer GA 5000.

2.5 Data analysis

In determining the efficiency of anaerobic digestion, the decomposition of organic matter during the process was

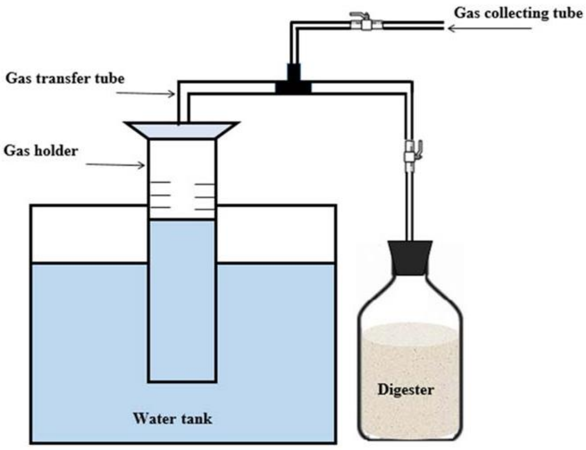


Fig. 4 Schematic diagram of anaerobic digestion

predicted by the curve fitting of the modified Gompertz equation [24] and the observed biogas cumulative production:

$$Y = Y_m \ \exp\left\{-\exp\left[\frac{R_m e}{Y_m}(\lambda - t) + 1\right]\right\} \quad (1)$$

where Y (mL/gTS) is the cumulative biogas production at a given time t (day), Y_m is biogas potential of the substrate (mL/gTS), R_m is the maximum biogas production rate (mL/gTS/day), "e" is exp (1) = 2.718, λ is the lag phase time (day), and R^2 is a measure of how well the model fits the biogas production curve.

2.6 Energy analysis

The calorific values were calculated, according to Li et al. [25]. Based on methane concentration obtain from the feedstock, higher calorific values (HCV) and lower calorific values (LCV) were determined as follows:

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

$$LCV_{biogas} = 0.3593 \times MC + 0.0192 \left(R^2 = 1\right) \quad (3)$$

where MC is the methane content in biogas (%). The HCV and LCV of pure methane were 39.82 and 35.87 MJ/m^3, respectively.

2.7 Statistical analysis

The data and graphs were processed using Originlab 2020 (Origin Lab, USA). The mean value and standard deviation of the data were analyzed using analysis of variance (ANOVA).

3 Results and discussion

3.1 Feedstock characterization

Initial characterization of water primrose, cow dung, and swine manure used in this experiment is presented in Table 1. The high content of TS and VS were found in water primrose, which reached 900,000 ± 4165 and 836,667 ± 3754 mg/L, respectively. These values indicated a high organic matter in the material that could convert to biogas production in the anaerobic digestion process. According to the data of the pH values in Table 1, the combination of pretreated water primrose with cow dung or swine manure were preferred for anaerobic digestion that may prevent the risk of acidification and improve buffer capacity in digesters than conducted one substrate in digesters. Also, manure is considered as a nitrogen-bearing material in anaerobic digestion that during the fermentation process, the buffering system is more adjustable by releasing ammonia [26]. Overall, the initial values of feedstock showed a suitable condition for the anaerobic system.

Table 1 Characteristics of water primrose, cow dung, and swine manure

Parameter	Measured values		
	Water primrose	Cow dung	Swine manure
TS (mg/L)	900,000 ± 4165	196,666 ± 1064	98,760 ± 846
VS (mg/L)	836,667 ± 3754	140,000 ± 984	69,000 ± 548
pH	5.05 ± 0.02	8.15 ± 0.02	6.14 ± 0.20
COD (mg/L)	61,667 ± 4812	153,333 ± 5695	105,645 ± 652
VFAs (mg/L)	3218 ± 182	4376 ± 896	2792.79 ± 268.12
Alkaline (mg/L)	1917 ± 312	34,458 ± 295	2041 ± 155.9

Water primrose is a non-woody plant, stands erect along with wet soil or float out across the water surface with bright yellow flowers and oval-shaped, typically having four petals. Flowers vary in size from 2 to 4 cm in diameter and bloom all season, except winter. The stems are long, trailing, branched. Stems of water primrose can be of green to reddish appearance, can reroof from cutting, can be fleshy, and can grow to the height up to 120 or even 210 cm. Leaves are long and slender in shape and arranged alternately on the stem, up to 10 cm long, 1–2 cm wide, shiny, dark green, and lighter green central vein or in yellow color. The plants contain small seeds inside. It shows the capsule pubescent, more-or-less cylindrical or swollen towards the apex, up to 30 mm long with many brown, oblong seeds about 0.5 mm long.

An observation of a cross-section of water primrose stem was made by a microscope presented in Fig. 5. A large calcium oxalate crystal was found in the cross-section of the stem of water primrose (Fig. 5 a, b, c, and d). Calcium oxalate crystal is normally located in all parts of the plant as roots, leaves, stems, seeds, and other parts [27]. The crystals might contribute to the photosynthetic process and protect against insects and foragers [27, 28]. However, accumulate oxalate can cause symptoms of poisoning for ruminants in toxic concentrations [28]. Also, imaging with scanning electron microscopy (SEM) was investigated before and after alkaline pretreatment (Fig. 5e, f). The surface morphology of water primrose was observed × 500 magnification. The untreated sample showed a crystallinity of solid composition as lignin, hemicellulose, and cellulose content were rigid and high ordered fibrils. After alkaline pretreatment, some particles were broken down and separated from the original topology due to the lignin and cellulose removal. Thus, the structure of water primrose was deformed, and the external surface area increased, which led to more sugar exposure, resulting in higher biogas generation.

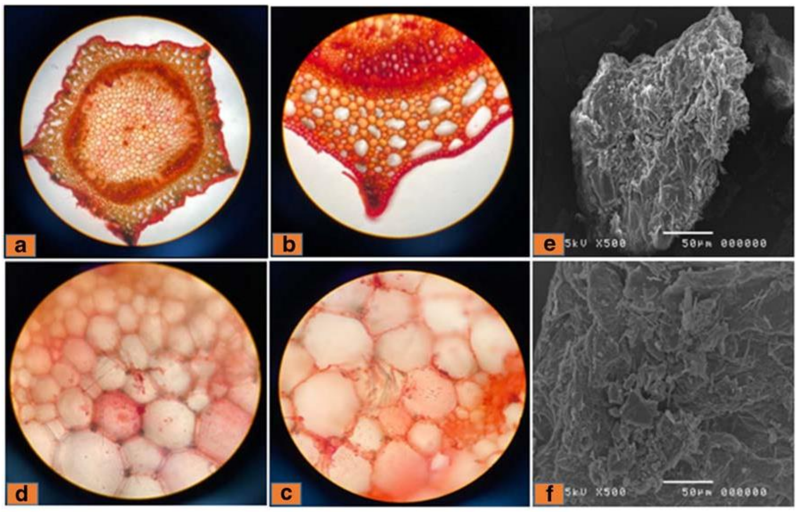


Fig. 5 Cross-section plant stem under the microscope (**a**–**d**) and SEM images of the untreated (**e**), and 2% NaOH-treated (**f**) water primrose

3.2 Potential biogas production and methane concentration

Many studies regarding alkaline pretreatment for lignocellulosic biomass are effective in improving biogas yield. Liang et al. [29] have investigated solid-state NaOH treatment on rose stalk resulting in anaerobic digestion with the highest biogas production, which was 144% higher than untreated samples. Also, by way of increasing NaOH concentration, the lignocelluloses are more likely to be disrupted, which is shown in the changes of the TS and VS reductions. The observed enhancement in biogas yield of NaOH-pretreated biomass such as corn stover was reported by Zhu et al. [30]; the investigation was found the highest biogas yield of 372.4 L/kg VS at 5% NaOH-pretreated corn stover. This result was 37.0% higher than the untreated corn stover.

The effect of alkaline on water primrose in this study was performed via biogas production and methane content. The potential of water primrose for biogas production was investigated for 45 days of anaerobic conditions. The comparison of co-digestion of water primrose with cow dung and swine manure was made through biogas performance. The cumulative biogas production was calculated based on the volume of daily biogas produced. The final volume biogas of 3 sets of lab-scale anaerobic experiments is shown in Fig. 6. All these sets had successfully produced biogas production, which in the range of 4285–6150 mL throughout the experiment as water primrose was treated with alkaline pretreatment, the simple soluble organic fraction presented in this biomass to be digested faster and easier in the fermentation process, thereby enhancing biogas yield, which was indicated in sets II and III.

The cow dung produced more biogas production (5436 mL) than the others (water primrose: 4285 mL, swine manure: 4847 mL) in set I but less than the biogas obtained from co-digestion in set II (6150 mL) and set III (5887 mL). This result agreed with Lu et al. [31]; the authors highlighted that anaerobic co-degradation could prevent rapid acidification in the system rather than individually digest the substrate.

It could be observed that the high content of TS, VS, and COD demonstrated the quality of cow dung than swine manure (Table 2). Moreover, the final volume biogas indicated it was well-operated in anaerobic digestion. Therefore, it could be expected that cow dung has positively affected the biogas production in co-substrate with water primrose. This finding was evidence by the total biogas production in set II obtained higher than in set I and set III. Akintokun et al. [32] also found that rice husk co-substrate with cow dung improved biogas production than mono-substrate. Specifically, the biogas yield obtained from rice husk was 150 cm^3 and 4327.65 cm^3 from cow dung, while the combination of these substrates in anaerobic digestion was achieved 4730.55 cm^3 with 11.4% methane improvement. The higher biogas production by combining two or more substrates could be explained by the mixture's nutrient balance and favorable pH in the digester [33].

The percentage of methane in biogas was sampled and measured every 3 days; the highest methane concentration obtained from all experimental sets is shown in Fig. 6. The

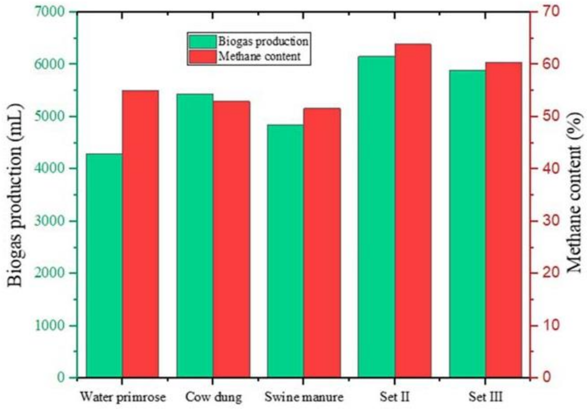


Fig. 6 Total biogas production (mL) and methane concentration (%) of 3-set experiment

methane content typically ranges from 50 to 70% [34]; the higher methane content in the anaerobic process reflects the digestive efficiency. The potential of methane content in biogas produced from water primrose was 55.07%. Compared to other biomass material, the methane obtained from this study was higher than rice straw, common reed, or sewage sludge [13, 35, 36].

Overall, in sets I, II, and III, experiments produced an excellent biogas volume with methane content above 50%. In set I, CH_4 content was 55.07% (water primrose), 53.0% (cow dung), and 51.62% (swine manure); set II obtained higher methane content than in set I and set III, which was 63.88% methane and whereas in set III was 60.49%. Consequently, the addition of cow dung or swine manure increased not only biogas production but also methane quality in anaerobic digestion.

In evaluating anaerobic digestion performance, further investigation on TS, VS, and COD removal efficiency was made in this study, shown in Fig. 7. In fact, biogas production is the result of the degradability efficiency of organic fraction contained in substrate representing as TS, VS, and COD content. Overall removal percentages ranged between 46.8 and 57.55% for TS, 44.25 and 50.22% for VS, and 40.84 and 52.64% for COD. In set II, the highest percentage of all parameters could be observed from Fig. 7, followed by set III and lastly, in set I. The TS, VS, and COD reduction was no significant difference in set I; the higher value of water primrose than cow dung and swine manure was observed due to the high organic component available in the substrate. Similar TS, VS, and COD removal were observed for cow dung and swine manure (48.56%, 45.63%, 40.84% and 46.8%, 44.25%, 41.56%, respectively). Higher removal rates of TS, VS, and COD reported for anaerobic co-digestion of pretreated water primrose and swine manure (set III) than set I, which was 52.63%, 48.97%, and 46.33%, respectively. A similar result was found by Cordoba et al. [37]; the VS and COD removal from mono-digestion of swine manure was 32.0% and 18.4%, respectively, whereas the increasing efficiency could be achieved at 43% for VS and 53% for COD. The difference was not very large, but the removal efficiency of TS, VS, and COD value in set II was achieved the highest in this study (57.55%, 50.22%, and 52.64%, respectively). The TS removal represents the relationship of biogas yield, as Li et al. [38] reported the efficiency of TS reduction in co-digestion of corn stover and manure was 52.5%. Hence, the average value of TS reduction in anaerobic digestion of dairy manure with crop residues lies from 38 to 45%. Therefore, the efficiency of pretreated water primrose and cow dung with swine manure on biodegradability was achieved in this study.

Table 2 Total biogas production (mL) and methane concentration (%) of 3-set experiment

Treatments		Total biogas (mL)	Methane (%)
Set I	Water primrose	4285	55.07
	Cow dung	5436	53.00
	Swine manure	4847	51.62
Set II	Pretreated water primrose and cow dung	6150	63.88
Set III	Pretreated water primrose and swine manure	5488	60.49

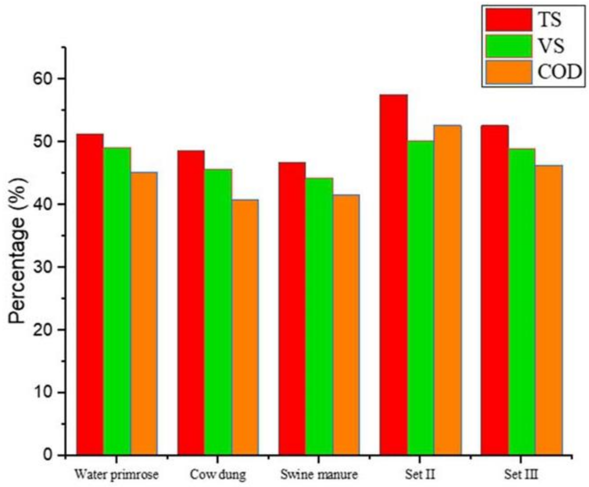


Fig. 7 Percentage removal efficiency of TS, VS, and COD

3.3 Gompertz model for biogas production

The study of biogas production from water primrose, cow dung, swine manure, and their mixture was investigated for 45 days. The biogas produced was measured and recorded every day until the process end. The modified Gompertz equation was plotted and evaluated based on the curve fitting of the predicted cumulative biogas values from the model and the cumulative biogas production data from the experiment shown in Fig. 8. The summary of estimated parameters, including estimated biogas yield potential (Y_m), maximum biogas production rate (R_m), and duration of lag phase (λ), from the model are given in Table 2.

The biogas production potential of water primrose was estimated at 110.05 mL/gTS, higher than cow dung or the value in set 3 treatment. Simultaneously, the co-digestion of pretreated water primrose with cow dung caused biogas to increase from 110.05 mL/gTS to 122.09 mL/gTS. However, the treatment in set III, which combined pretreated water primrose and swine manure gave lower biogas (101.54 mL/gTS) compared to the water primrose substrate. Still, this substrate's lag phase indicated that the biomass might not produce gas immediately after the process started. It required a full adaption period from week 2 onward. However, combining water primrose with cow dung or swine manure reduced the minimum haft time to produce biogas (λ) from 11.70 days (water primrose) to 6.17 days (set II) and 5.74 days (set III).

Also, from Table 2 and Fig. 8, it can be observed that in set I, the swine manure gave the highest kinetic parameters of the biogas production potential of 150.10 mL/gTS at a maximum biogas production rate 2.29 mL/gTS/day with a lag phase (λ) of 13.81 days. While set II and set III, which comprised an equal amount of water primrose with cow dung and swine manure, they have had lower biogas production potential (122.09 mL/gTS and 101.54 mL/gTS, respectively). However, the maximum biogas production and the lag phase were improved: 2.43 mL/gTS/day and 6.17 days in set II or in set III. The values were 2.47 mL/gTS/day and 5.74 days, respectively. Moreover, the modified Gompertz equation was observed a better coefficient of determination (R^2), which represented biogas production with a goodness of fit. The model fits the data for cumulative biogas production of all the set experiments (0.9976–0.9997).

This study implies that the water primrose content is more complicated than other substrates, which took a long time to degrade in the system. The cow dung and swine manure positively affected water primrose in anaerobic co-digestion indicated in enhanced maximum biogas production and shortened the lag phase duration. The reasonable explanation may be that the higher quantity and quality of anaerobic bacteria contain manure allowed to digest more kinds of substrate content than converted to biogas in the anaerobic digestion process [39].

3.4 Energy value

The study demonstrates that the massive weed (water primrose) availability in the agricultural field takes more effort to collect, transportation, storage, or even treat material before getting its bio-methane production as renewable energy.

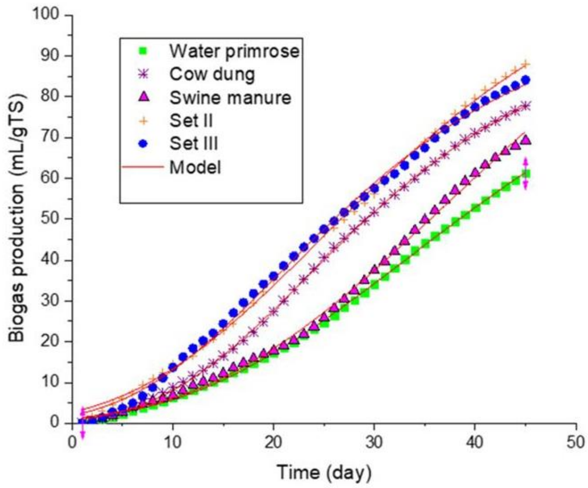


Fig. 8 Comparison of biogas production from experimental data and modified Gompertz model

Above all, implementing a project to produce biogas would mean that renewable energy produced must be larger and better affect the environmental evaluation [40]. On the other hand, this weed causes many problems in a variety of ways for agricultural land. Therefore, producing biogas energy for generating electricity and heat from water primrose in anaerobic digestion would considerably reduce agricultural weed and evaluating its energy potential is necessary.

The energy potential from biomasses has been investigated by many studies, which is expressed as heating value. An energetic measurement from rice straw was made by Nguyen et al. [41] and indicated that the heating value obtained from results was 14.2 and 15.3 MJ kg^{-1} for high heating value and the low heating value was 13 and 13.7 MJ kg^{-1}. On the concern in calorific value from 27 different special biomass materials, Özyuğuran et al. [42] have predicted the energy potential based on proximate analysis from herbaceous and woody biomasses, nutshells, fruit stones, stem and husks, pulps, and agricultural residues. The authors found that the lowest heating value was from tobacco waste with 14.51 MJ kg^{-1}, and the highest heating value was 21.23 MJ kg^{-1} for damson plum stone. Another study that investigated food waste from a canteen in China at different pressure levels showed that at high pressure of biogas, the possible HCV and LCV that could be obtained from the substrate was 36.2 MJ/m^3 and 32.6 MJ/m^3, respectively [43].

This study's highest methane content was taken to compute the heating value; thereby, 63.88% of the methane from co-digestion of pretreated water primrose with cow dung was calculated for HCV and LCV based on Eqs. (1) and (2). The results were obtained as 25.50 MJ/m^3 for HCV and 22.97 MJ/m^3 for LCV. Compared with biogas production obtained traditional AD (HCV of 20.0–25.9 MJ/m^3 and LCV of 18.0–23.4 MJ/m^3), the methane gained proved high-calorific biogas in this study [24]. Bastidas-Oyanedel et al. [44] stated that with the methane content of 65% in biogas production in anaerobic digestion, the HCV typically obtains about 7.2 kWh/Nm^3 and LCV about 6.5 kWh/Nm^3. Similarly, with 26 MJ m^{-3} from biogas, the calorific value could equal to 0.77 m^3 of natural gas of 33.5 MJ calorific value, or replace 1.1 kg of hard coal with 23.4 MJ calorific value or 2 kg of firewood with 13.3 MJ calorific value [45].

4 Conclusion

This study has demonstrated that water primrose is a suitable feedstock for biogas energy. Among the three substrates tested, the highest accumulative biogas and methane content of 6150 mL and 63.88%, respectively, were produced by the co-digestion of pretreated water primrose and cow dung. This methane content is given HCV with 25.50 MJ/m^3 and LCV with 22.97 MJ/m^3. The modified Gompertz equation gave a good fit with the coefficient of determination (R^2) for all treatment over 0.997. Based on results, the study suggests that pretreatment of water primrose in co-digestion with other substrates could enhance biogas volume, methane content, and high-calorific biogas.

Acknowledgments The authors gratefully acknowledged the School of Renewable Energy, Program in Biotechnology, and Energy Research Center, Maejo University, Chiang Mai, and Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand, for the research facilities to accomplish this experimental study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Nomenclature and abbreviation *Y,* cumulative biogas production is the at the given time; *t,* time; Y_m, biogas potential of the substrate; R_m, maximum biogas production rate; *E,* exp (1) = 2.718; λ, lag phase time; R^2, coefficient of determination; *HCV,* high calorific value; *LCV,* low calorific value; *MC,* methane content in biogas; CH_4, methane; CO_2, carbon dioxide; *CaO,* calcium oxide; *COD,* chemical oxygen demand; *TS* , total solids; *VS,* volatile solids; *VFAs,* volatile fatty acids

References

1. Bhuyar P, Sundararaju S, Rahim MH, Ramaraj R, Maniam GP, Govindan N (2019) Microalgae cultivation using palm oil mill effluent as growth medium for lipid production with the effect of CO_2 supply and light intensity. Biomass Convers Biorefin 26:1–9
2. Nguyen TV, Unpaprom Y, Manmai N, Whangchai K, Ramaraj R (2020) Impact and significance of pretreatment on the fermentable sugar production from low-grade longan fruit wastes for bioethanol production. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-00977-7
3. Khammee P, Ramaraj R, Whangchai N, Bhuyar P, Unpaprom Y (2020) The immobilization of yeast for fermentation of macroalgae *Rhizoclonium* sp. for efficient conversion into bioethanol. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-00786-y
4. Manmai N, Unpaprom Y, Ramaraj R (2020) Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-00602-7
5. Saengsawang B, Bhuyar P, Manmai N, Ponnusamy VK, Ramaraj R, Unpaprom Y (2020) The optimization of oil extraction from macroalgae, *Rhizoclonium* sp. by chemical methods for efficient conversion into biodiesel. Fuel 274:117841
6. Kanget Zhao C, Qiao X, Cao Y, Shao Q (2017) Application of hydrogen peroxide presoaking prior to ammonia fiber expansion pretreatment of energy crops. Fuel 205:184–191
7. Sophanodorn K, Unpaprom Y, Whangchai K, Homdoung N, Dussadee N, Ramaraj R (2020) Environmental management and valorization of cultivated tobacco stalks by combined pretreatment for potential bioethanol production. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-00992-8
8. Ramaraj R, Unpaprom Y (2019) Optimization of pretreatment condition for ethanol production from *Cyperus difformis* by response surface methodology. 3 Biotech 9(6):218
9. Pantawong R, Chuanchai A, Thipbunrat P, Unpaprom Y, Ramaraj R (2015) Experimental investigation of biogas production from water lettuce, *Pistia stratiotes* L. Emergent Life Sci Res 1:41–46
10. Ramaraj R, Kawaree R, Unpaprom Y (2016) A newly isolated green alga, *Pediastrum duplex* Meyen, from Thailand with efficient hydrogen production. IJSGE 4:7–12
11. Unpaprom Y, Intasaen O, Yongphet P, Ramaraj R (2015) Cultivation of microalga *Botryococcus braunii* using red Nile tilapia effluent medium for biogas production. J Ecol Environ Sci 3:58–65
12. Van Tran G, Unpaprom Y, Ramaraj R (2019) Methane productivity evaluation of an invasive wetland plant, common reed. Biomass Convers Biorefin 10:689–695. https://doi.org/10.1007/s13399-019-00451-z
13. Van Tran G, Unpaprom Y, Ramaraj R (2019) Effects of co-substrate concentrations on the anaerobic co-digestion of common reed and cow dung. AJARCDE 3(1):28–32
14. Chuanchai A, Ramaraj R (2018) Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and bio-fertilizer. 3 Biotech 8(3):151
15. Dussadee N, Unpaprom Y, Ramaraj R (2016) Grass silage for biogas production. Advances in Silage Production and Utilization 16:153
16. Ramaraj R, Dussadee N, Whangchai N, Unpaprom Y (2015) Microalgae biomass as an alternative substrate in biogas production. IJSGE 4:13–19
17. Ramaraj R, Unpaprom Y, Whangchai N, Dussadee N (2015) Culture of macroalgae *Spirogyra ellipsospora* for long-term experiments, stock maintenance and biogas production. Emergent Life Sci Res 1:38–45
18. Unpaprom Y, Pimpimol T, Whangchai K, Ramaraj R (2020) Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer. Biomass Convers Biorefin. https://doi.org/10.1007/s13399-020-00850-72
19. Vu PT, Unpaprom Y, Ramaraj R (2018) Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production. Bioresour Technol 247:125–130
20. Wannapokin A, Ramaraj R, Unpaprom Y (2017) An investigation of biogas production potential from fallen teak leaves (*Tectona grandis*). Emergent Life Sci Res 1:38–45
21. Wannapokin A, Ramaraj R, Whangchai K, Unpaprom Y (2018) Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. 3 Biotech 8:123
22. Zehnsdorf A, Moeller L, Stabenau N, Bauer A, Wedwitschka H, Gallegos D, Herbes C (2018) Biomass potential analysis of aquatic biomass and challenges for its use as a nonconventional substrate in anaerobic digestion plants. Eng Life Sci 18(7):492–497
23. APHA (2005) Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington, DC
24. Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet KJAEM (1990) Modeling of the bacterial growth curve. Appl Environ Microbiol 56(6):1875–1881
25. Li Y, Zhang R, He Y, Zhang C, Liu XC, Liu G (2014) Anaerobic co-digestion of chicken manure and corn stover in batch and continuously stirred tank reactor (CSTR). Bioresour Technol 156:342–347
26. Zamanzadeh M, Hagen LH, Svensson K, Linjordet R, Horn SJ (2017) Biogas production from food waste via co-digestion and digestion-effects on performance and microbial ecology. Sci Rep 7(1):1–12
27. Franceschi V (2001) Calcium oxalate in plants. Trends Plant Sci 6(7):331
28. Konyar ST, Öztürk N, Dane F (2014) Occurrence, types and distribution of calcium oxalate crystals in leaves and stems of some species of poisonous plants. Bot Stud 55(1):32
29. Liang YG, Cheng B, Si YB, Cao DJ, Li DL, Chen JF (2016) Effect of solid-state NaOH pretreatment on methane production from thermophilic semi-dry anaerobic digestion of rose stalk. Water Sci Technol 73(12):2913–2920

30. Zhu J, Wan C, Li Y (2010) Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. Bioresour Technol 101(19):7523–7528
31. Lu X, Jin W, Xue S, Wang X (2017) Effects of waste sources on performance of anaerobic co-digestion of complex organic wastes: taking food waste as an example. Sci Rep 7(1):1–9
32. Akintokun AK, Abibu WA, Oyatogun MO (2017) Microbial dynamics and biogas production during single and co-digestion of cow Dung and rice Husk. Appl Ecol Environ Res 39(2):67–76
33. Aragaw T, Gessesse A (2013) Co-digestion of cattle manure with organic kitchen waste to increase biogas production using rumen fluid as inoculums. Int J Phys Sci 8(11):443–450
34. Xiao Y, Yang H, Yang H, Wang H, Zheng D, Liu Y, Deng L (2019) Improved biogas production of dry anaerobic digestion of swine manure. Bioresour Technol 29:122188
35. Du J, Qian YT, Xi YL, Jin HM, Kong XP, Zhu N, Ye XM (2019) The feasibility of shortening the pretreatment time for improvement of the biogas production rate from rice straw with three chemical agents. BioResources 14(2):3808–3822
36. Wei L, Qin K, Ding J, Xue M, Yang C, Jiang J, Zhao Q (2019) Optimization of the co-digestion of sewage sludge, maize straw and cow manure: microbial responses and effect of fractional organic characteristics. Sci Rep 9(1):1–10
37. Córdoba V, Fernández M, Santalla E (2016) The effect of different inoculums on anaerobic digestion of swine wastewater. J Environ Chem Eng 4(1):115–122
38. Li X, Li L, Zheng M, Fu G, Lar JS (2009) Anaerobic co-digestion of cattle manure with corn stover pretreated by sodium hydroxide for efficient biogas production. Energy Fuel 23(9):4635–4639
39. Widiasa IN, Johari S (2010) The kinetic of biogas production rate from cattle manure in batch mode. Int J Chem Mol Eng 4(1):75–80
40. Croce S, Wei Q, D'Imporzano G, Dong R, Adani F (2016) Anaerobic digestion of straw and corn stover: the effect of biological process optimization and pre-treatment on total bio-methane yield and energy performance. Biotechnol Adv 34(8):1289–1304
41. Nguyen VH, Topno S, Balingbing C, Nguyen VCN, Röder M, Quilty J, Gummert M (2016) Generating a positive energy balance from using rice straw for anaerobic digestion. Energy Rep 2:117–122
42. Özyuğuran A, Yaman S (2017) Prediction of calorific value of biomass from proximate analysis. Energy Procedia 107:130–136
43. Li Y, Liu H, Yan F, Su D, Wang Y, Zhou H (2017) High-calorific biogas production from anaerobic digestion of food waste using a two-phase pressurized biofilm (TPPB) system. Bioresour Technol 224:56–62
44. Bastidas-Oyanedel JR, Schmidt JE (2019) Biorefinery: integrated sustainable processes for biomass conversion to biomaterials, biofuels, and fertilizers. Springer, Berlin/Heidelberg, Germany
45. Arbon IM (2002) Worldwide use of biomass in power generation and combined heat and power schemes. Proceedings of the Institution of Mechanical Engineers, Part A 216(1):41–57

knowvel
TAKE YOUR RESEARCH TO NEXT LEVEL

GLOBAL JOURNAL OF SCIENCE & ENGINEERING
Vol. 05 (2020) 1-5
www.knowvel.com

Original Research Article

BIOMETHANE POTENTIAL OF INVASIVE AQUATIC WEED WATER PRIMROSE

HUYEN THU THI NONG [1,2], YUWALEE UNPAPROM [2], CHUDAPAK CHAICHOMPOO [3], RAMESHPRABU RAMARAJ [1,2]

ABSTRACT

This study aims to examine the perspective of feedstock for producing biomethane from invasive aquatic weed water primroses (*Ludwigia Hyssopifolia*). The methane yield and methane content of biogas were analyzed and studied. The calculating methods of theoretical methane yield based on the elemental application or the theoretical chemical oxygen demand (COD) number were showed. The percentage of element chemicals, carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S) of raw materials were analyzed, and results were 40.2%, 5.03%, 22.13%, 1.8% and 0.24%, respectively. The plant biomass was contained moisture content, volatile carbon, fixed carbon and ash were 7.28%, 63.07%, 1.28% and 28.37%, respectively. From the water primroses calculated yield of methane (CH_4), carbon dioxide (CO_2) and ammonia (NH_3) results were 54.90%, 41.40% and 3.70%, respectively. Therefore, the aquatic weed water primroses biomass are suitable feedstock for biogas production as well as future scale-up studies.

Keywords: Invasive aquatic weed, water primroses, methane, biogas.

AUTHOR AFFILIATION

[1]*School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand*
[2]*Sustainable Resources and Sustainable Engineering Research Lab, Maejo University, Chiang Mai 50290, Thailand.*
[3]*Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand*
[4]*Faculty of Liberal Arts, Maejo University, Chiang Mai 50290, Thailand*

CORRESPONDENCE

Rameshprabu Ramaraj, School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand
Email: rrameshprabu@gmail.com
rameshprabu@mju.ac.th

PUBLICATION HISTORY

Received: June 30, 2020
Accepted: July 28, 2020

ARTICLE ID: GJSE-CP-25

1. INTRODUCTION

Energy is an essential tool for national and international development. Many countries face added dilemmas regarding environmental protection due to their heavy dependency on biomass and fossil fuel [1-3]. They opined that the Asian region is endowed with vast renewable energy resources and less amount of conventional energy resources [4]. The global quest for environmentally friendly and ecologically balanced and sustainable energy has increased over the last few decades. In addition to this has forced the world to search for other alternate sources of energy [5]. However, the new alternative energy sources demand massive economic investment and technical power to operate, making it a little difficult for developing and under-developing countries. Currently, biogas is a reliable [6-8], accessible, and economically feasible alternative and renewable energy source, which can be generated using agricultural, domestic and industrial materials employing simple technology.

The technology can be utilized to provide energy for households, rural communities, farms, and industries. One of the aims of organic farming is to reduce the use of non-renewable resources to a minimum. So far, however, only very little progress has been made to introduce renewable energy in organic farming [9, 10]. Biomass is a crucial energy carrier with good potential for on-farm development. Apart from utilizing farm manure and crop residues for biogas production [11,12], the production of nutrient efficient short rotation coppice is an option in organic farming: the habitat and the aquatic and semiaquatic weeds. The weeds "control" meaning is protection of these plants at same level and they can't economic damage. And the aquatic weeds can be taken under control to manageable limits by various methods.

These efforts come when plants' growth has become an expensive problematic, as machinists of canals look costs for de-weeding and disposing of water based biomass, much of it from the waterweeds. The biomass from these aquatic macrophytes (i.e., plants large enough to be seen by the naked eye) has swollen in volume [13, 14]. Their excessive growth upsets the local eco-balance and impairs rivers and lakes for sports and recreation. It is barely astonishing, then, to find that local lake owners and municipalities, feel compelled to have the waterways cleared and the biomass taken to a service company such as a composting plant for disposal, both of which incur high costs.

Similarly, semiaquatic weeds are a massive problem in agricultural land. These weeds compete for resources with crops, and weed infestation usually results in yield and quality reduction [15, 16]. Weed control has always been a significant concern in agricultural production because of the extensive labor required with traditional culture for weeding or modern agriculture, the wide use of herbicides. Agriculture is still the occupation of most Thai people, despite the rising share of industry and services. In terms of agricultural lands, Thailand is also one of the world's largest countries, especially in Asia [17, 18]. Presently, the market demand for organic food is increasing mainly due to consumer perceptions of these products' quality and safety. The major aim of organic agriculture is to optimize the health benefits and productivity of interdependent communities of soil life, plants, animals, and people.

Organic agriculture is estimated that this necessity will continue to grow shortly. Therefore, reducing herbicides and eradicate the weeds of essential in the organic farming system. Weed control is considering the major obstacle for the growers in organic farming. Lower plant productivity in organic farming is mainly related to poor weed control [9]. Oerke [10] stated that there is no reliable study of worldwide damage due to weeds. However, it is broadly known that losses produced by weeds have exceeded the losses from any category of agricultural pests. The potential crop yield loss without weed control was estimated by 43%, on a global scale. Invasive plants characterize a severe threat to the native biodiversity of inland aquatic ecosystems throughout the world. The damages caused by these species are mostly linked to their high biomass, and their presence modifies water quality, hydrology [9, 10], or the composition and structure of native communities. No chemical weed control measures were used on the energy crops. Accordingly, many semi-aquatic weeds can produce biofuels, and, at the same time, weed plants were removed then utilized for bioenergy feedstocks.

Vu et al. [19] study suggested that rice field weeds are a good source for bioethanol production. Non-indigenous aquatic plants currently invade aquatic ecosystems. Also, a significant challenge for biological invasion research is to develop the ability to predict the spread of species. Throughout the world, *Ludwigia peploides* subsp. *Montevidensis*, Ludwigia grandiflora subsp. *hexapetala* and *Ludwigia hyssopifolia* are now considered to be two of the most invasive aquatic plants. *Ludwigia hyssopifolia* (water primrose) usually grows in wet places, and it can be a severe rice weed in lowland rice fields via its allelopathic effects. It currently poses a threat to agricultural systems as a stubborn weed and has constituted a significant concern as no particular control measure has been sought for it before this research. However, the feedstock of water primrose for biogas production potential and theoretical values are address in the establishment of sole biomass as a viable biogas resource, which the current study addressed. Scope and objectives of this study to investigate the potential of biogas production and energy analysis from water primrose, to estimate the methane production potential of water primrose through the theoretical biochemical methane potential (TBMP) via elemental chemical compositions, and to the prediction from the calculation in this study can be applied for another biomass material.

2. Materials & Methods

Ludwigia hyssopifolia (water primrose) was obtained from the crop field which is located near to the nearby Maejo University, Chiang Mai Province, Thailand (coordinates 18° 53′ 24.3″ N–99° 02′ 11.5″ E). Classification of the plant, growing locations, flower blooms with seeds and mature plants nature was described in Figure 1. The methodology adopted in this study is illustrated in Figure 2. In these tests, one square meter was completely harvested in three repetitions for biomass quantity analysis.

The biochemical methane potential (BMP) of the substrates was performed according to [20, 21]. To apply the model to a specific feedstock, we need to know the feedstock's chemical components. The model considers only carbon, hydrogen, oxygen, nitrogen and sulfur as input elements, and the relative ratios of these elements can be taken from published values for ultimate analyses of water primrose biomass. Total solid (TS, %), volatile solid (VS, %), pH, ash and moisture content (MC, %) contents were analyzed using standard methods [22]. Proximate and ultimate analyses of the water primrose were carried out by using X-ray fluorescence spectrometers and an elemental analyzer (Elementar Analysensysteme Gmbh, Germany, Model: vario EL cube), and by for ultimate analysis included elemental chemical: carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S).

Theoretical biochemical methane potential (TBMP) is computed by the constants of chemical elements given by ratios of C:H:O:N from the stoichiometric formula. The principle chemical equation of methane production (adopted from Pavlostathis and Giraldogomez [23]):

$$C_aH_bO_cN_d + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8}\right)CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8}\right)CO_2 + dNH_3 \quad (1)$$

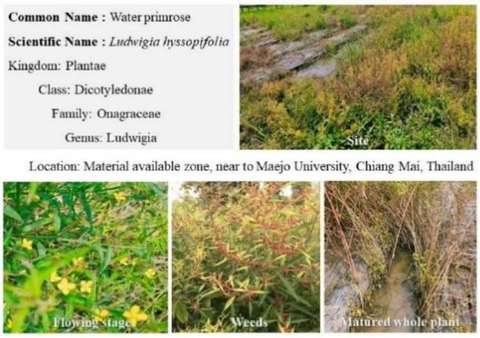


Figure 1. Classification of the plant, growing locations, flower blooms with seeds

Figure 2. Study outline and methodology

TBMP is assumed under STP (standard temperature and pressure) conditions:

$$\text{TBMP} = \frac{22.4 \times \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8}\right)}{12.017a + 1.00794b + 15.999c + 14.0067d} \quad (2)$$

Energy content analysis: The higher calorific values (HCV) and lower calorific values (LCV) of produced biogas were determined according to the following [20, 25, 25]:

$$\text{HCV biogas}(MJ/m^3) = 0.3989 \times MC \quad (3)$$

$$\text{LCV biogas}(MJ/m^3) = 0.3593 \times MC \quad (4)$$

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

Statistical analysis: the values reported in the present study were the mean of three replicates. Moreover, data are reported as mean ± SE from triplicate observations. All Statistical analyses of data were performed using the program SPSS 20.0.

3. Results & Discussion

Water primrose typical characterization was presented in Table 1. Theatrical Biochemical Methane Potential (TBMP) tests are a useful tool for determining the best substrate configurations; however, some methodologies are destined to save costs and time from this process by using the theoretical final methane potential of a substrate from its organic composition. TBMP tests are applicable when used to expose which types of substrates have the highest biochemical potential from various possibilities [21]. The biogas yield of the individual substrates varies significantly depending on their origin, organic substance content, and substrate composition. Moreover, biomass resources' chemical constituents include carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. Feedstocks differ markedly in their chemical composition. As such, the amount and the composition of biogas vary from one substrate to another.

Table 1. Water primrose typical characterization

Parameters	Compounds
Total Solid (TS, %)	90.0
Volatile Solid (VS, %)	83.67
pH	4.76
Ash Content (%)	28.37
Moisture (%)	7.28
Carbon (C, wt%)	40.2
Hydrogen (H, wt%)	5.03
Oxygen (O, wt%)	22.13
Nitrogen (N, wt%)	1.8

Water primrose element (CHONS) compositions as shown in Table 2. The theoretical methodologies' ability to accurately estimate methane yields of complex substrates was evaluated by comparing the experimental productivity from the TBMP tests with the theoretical productivity obtained from the different methodologies [22]. Although the theoretical results obtained for the elemental composition equation method follow behavior similar to the previous method and the experimental results, the values are lower, but it gets agreements higher than 90%.

Table 2. Biogas composition and elements

Biogas	CH_4 (%)	CO_2 (%)	NH_3 (%)		
composition	54.90054	41.40336	3.696099		
Elements	C (%)	H (%)	O (%)	N (%)	S (%)
	40.2	5.03	22.13	1.8	0.24

Table 3. Total biogas production from the water primrose

Biogas composition	The gas produced with 1 lb of water primrose		The gas produced with 1 Kg of water primrose		
CH_4	9.8625	ft³	CH_4	0.6162	m³
CO_2	7.4288	ft³	CO_2	0.4641	m³
NH_3	0.6661	ft³	NH_3	0.0416	m³
	17.9575	ft³/lb		1.1219	m³/Kg
	Total theoretical amount of gas			1,121.88	L/kg

TBMP is a simple but reliable procedure for determining maximum methane volume produced per gram of the substrate's volatile solid and indicates the rate and extent of converting biodegradable organics to methane in an anaerobic digestion set-up. Although the TBMP of water primrose has not been studied, other grasses, vegetables and food wastes have been studied using Buswell's and modified Dulong's equations with the elemental (CHNSO) compositions of substrates. The predicted biogas production results, as well as the determined parameters, are shown in Tables 3 and 4. Biomethane is produced by the breakdown of biodegradable materials with microorganisms' help in the absence of oxygen. Through anaerobic digestion, biomethane, carbon dioxide, and ammonia are coproduced, and the overall reaction is shown in equation 2.

Table 4. Biogas composition, total biogas production and theoretical amount of biogas

Gas composition (%)			Total gas production (m³)			The total theoretical amount of gas	
CH_4	CO_2	NH_3	CH_4	CO_2	NH_3	m³/Kg	L/kg
54.90054	41.40336	3.696099	0.616235	0.464068	0.041578	1.121881	1121.881

Table 5. Results of the theoretical biochemical methane potential method from different materials

Materials	Gas composition (%)			Total biogas (m³)			Total theoretical amount of gas (L/kg)	References
	CH_4	CO_2	NH_3	CH_4	CO_2	NH_3		
Musa sapientum	53.64	44.83	1.52	0.50	0.42	0.01	935.85	Mata-Alvarez et al. [26]
E. polybractea	52.74	45.09	2.17	0.53	0.45	0.02	997.83	Burton and Wu [27]
Eucalyptus	49.98	47.79	2.23	0.45	0.43	0.02	1023.63	Mishra et al. [28]
F. simplex	49.45	48.54	2.01	0.50	0.49	0.02	1000.73	Xiao et al. [29]
Teak leaves	55.47	43.57	0.96	0.60	0.47	0.01	1073.99	Wannapokin et al. [30]
Bambusoideae	45.81	52.74	1.46	0.35	0.40	0.01	764.65	Huang et al. [31]
Water primrose	54.90	41.40	3.69	0.61	0.46	0.04	1,121.88	This study

The overall biogas yields depend on the chemical composition of the water primrose. The target strain should be highly digestible. The volatile solids/ash-free dry weight of biomass plays a significant role in predicting theoretical biogas production potential, critical in determining biogas productivities. The biogas composition of carbon dioxide (43.57%) and methane (54.9%) were estimated. Total biogas yield was 1.1219 m³/kg achieved through the theoretical estimation, and total methane yield reached 0.6162 m³. Results of the theoretical biochemical methane potential method from different materials are shown in Table 5.

The biogas containing HCV was 21.83 MJ/m³ and LCV was 19.66 MJ/m³. It was much higher than biogas production from traditional AD (LCV of 18.0–23.4 MJ/m³ and HCV of 20.0–25.9 MJ/m³) [25]; accordingly, these study results verified that high-calorific biogas was obtained in this study system after methane was enriched through biological biogas purification.

4. Conclusion

A simplistic theoretical study of anaerobic digestion to predict the biogas amount of weeds waste biomass is proposed. This paper aims to calculate the amount of energy or chemicals produced using anaerobic digestion. This work provides a simplified model that predicts the biogas amount produced and could be applied for weeds energy feasibility studies dimensioning bioreactors digesting feedstock materials and expanding anaerobic digestion systems as a clean energy source. Therefore, the water primrose is a potential feedstock for biogas production. The model provides basic predictions that can aid agricultural farmer decisions. The theoretical study can give overall details and substrate information substrate for biogas production in pilot or large scale biogas plant in the future.

Conflict of Interests

The authors declare that there is no conflict of interest related to the publication of this article.

References

[1] R. Ramaraj, R. Kawaree, and Y. Unpaprom, "Direct Transesterification of Microalga *Botryococcus braunii* Biomass for Biodiesel Production," Emergent Life Sci. Res., vol. 2, no. 2, pp. 1–7, 2016.

[2] K. Khunchit, S. Nitayavardhana, R. Ramaraj, V. K. Ponnusamy, and Y. Unpaprom, "Liquid hot water extraction as a chemical-free pretreatment approach for biobutanol production from *Cassia fistula pods*," Fuel, vol. 279, 2020.

[3] M. Manmai, K. Bautista, Y. Unpaprom, and R. Ramaraj. "Optimization of combined pre-treatments on sugarcane leaves for bioethanol production," Maejo Int J Energ Environ Comm., vol. 1, no. 1, pp. 30–39, 2019.

[4] R. Ramaraj, Y. Unpaprom, and N. Dussadee, "Cultivation of Green Microalga, *Chlorella vulgaris* for Biogas Purification," Int. J. New Technol. Res., vol. 2, no. 3, p. 263569, 2016.

[5] P. Khammee, Y. Unpaprom, S. Buochareon, and R. Ramaraj, "Potential of bioethanol production from marigold temple waste flowers," Proceeding of The 1st Thailand Biorefinery Conference, The Future of Biorefinery for Thailand 4.0, Suranaree University of Technology, Nakhon Ratchasima, Thailand, pp. 25–26, 2019.

[6] P. Khammee, R. Ramaraj, N. Whangchai, P. Bhuyar, and Y. Unpaprom, "The immobilization of yeast for fermentation of macroalgae *Rhizoclonium* sp. for efficient conversion into bioethanol," Biomass Convers. Biorefinery, 2020.

[7] R. Ramaraj and Y. Unpaprom, "Optimization of pretreatment condition for ethanol production from *Cyperus difformis* by response surface methodology," 3 Biotech, vol. 9, no. 6, 2019.

[8] N. Manmai, Y. Unpaprom, V. K. Ponnusamy, and R. Ramaraj, "Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation," Fuel, vol. 278, 2020.

[9] H. F. Abouziena, and W. M. Haggag, "Weed control in clean agriculture: a review1," Planta daninha, vol. 34, no. 2, pp. 377–392, 2016.

[10] E. C. Oerke, "Crop losses to pests," J. Agric. Sci., vol. 144, pp. 31–43, 2006.

[11] J. Kaewdiew, R. Ramaraj, S. Koonaphapdeelert and N. Dussadee, "Assessment of the biogas potential from agricultural waste in northern Thailand," Maejo Int J Energ Environ Comm., vol. 1, no. 1, pp. 40–47, 2019.

[12] R. Ramaraj, N. Dussadee, N. Whangchai, and Y. Unpaprom, "Microalgae Biomass as an Alternative Substrate in Biogas Production," Int. J. Sustain. Green Energy. Spec. Issue Renew. Energy Appl. Agric. F. Nat. Resour. Technol., vol. 4, no. 1, pp. 13–19, 2015.

[13] R. Ramaraj, Y. Unpaprom, N.Whangchai, and N. Dussadee, "Culture of Macroalgae *Spirogyra ellipsospora* for Long-Term Experiments, Stock Maintenance and Biogas Production," Emer Life Sci. Res., vol. 1, no. 1, pp. 38–45, 2015.

[14] R. Rameshprabu, R. Kawaree, and Y. Unpaprom, "A Newly Isolated Green Alga, *Pediastrum duplex* Meyen, From Thailand With Efficient Hydrogen Production," Int. J. Sustain. Green Energy Int. J. Sustain. Green Energy. Spec. Issue Renew. Energy Appl. Agric. F. Nat. Resour. Technol., vol. 4, no. 1, pp. 7–12, 2015.

[15] R. Ramaraj, and N. Dussadee, "Renewable Energy Application for Organic Agriculture: A Review, IJSGE vol. 4, no. 1–1, pp. 33–38, 2015.

[16] P. T. Vu, Y. Unpaprom, and R. Ramaraj, "Evaluation of Bioethanol Production from Rice Field Weed Biomass," Emergent Life Sci. Res., vol. 3, pp. 42–49, 2017.

[17] N. Dussadee, K. Reansuwan, and R. Ramaraj, "Potential Development of Compressed Bio-Methane Gas Production from Pig Farms and Elephant Grass Silage for Transportation in Thailand," Bioresour. Technol., vol. 155, pp. 438–441, 2014.

[18] N. Dussadee, R. Ramaraj, and T. Cheunbarn, "Biotechnological Application of Sustainable Biogas Production Through Dry Anaerobic Digestion of Napier Grass," 3 Biotech, vol. 7, no. 1, p. 47, 2017.

[19] P. T. Vu, Y. Unpaprom, and R. Ramaraj, "Impact and Significance of Alkaline-Oxidant Pretreatment On the Enzymatic Digestibility of *Sphenoclea zeylanica* for Bioethanol Production," Bioresour. Technol., vol. 247, pp. 125–130, 2018.

[20] A. Chuanchai, and R. Ramaraj, "Sustainability Assessment of Biogas Production from Buffalo Grass and Dung: Biogas Purification and Biofertilizer," 3 Biotech, vol. 8, no. 3, 2018.

[21] G. Van Tran, Y. Unpaprom, and R. Ramaraj, "Methane Productivity Evaluation of an Invasive Wetland Plant, Common Reed," Biomass Convers. Biorefinery, 2019.

[22] APHA, Standard Methods for the Examination of Water and Waste Water, American Public Health Association, Washington, DC, USA, 18th edition, 1998.

[23] S. G. Pavlostathis, and E. Giraldogomez, "Kinetics of Anaerobic Treatment," Water Sci. Technol., vol. 24, no. 8, pp. 35–59, 1991.

[24] A. Chuanchai, S. Tipnee, Y. Unpaprom, and K. T. Wu, "Green Biomass to Biogas – A Study On Anaerobic Monodigestion of Para Grass," Maejo Int J Energ Environ Comm., vol 1, pp. 32–38, 2019.

[25] Y. Li, R. Zhang, Y. He, C. Zhang, X. Liu, C. Chen and G. Liu, "Anaerobic Co-Digestion of Chicken Manure and Corn Stover in Batch and Continuously Stirred Tank Reactor (CSTR)," Bioresour Technol., vol. 156, pp. 342–347, 2014.

[26] J. D. Mata-Alvarez, M. S. Romero-Güiza, X. Fonoll, M. Peces and S. Astals, "A critical review on anaerobic co-digestion achievements between 2010 and 2013," Renew. Sust. Energ. Rev., vol. 36, pp. 412-427, 2014.

[27] A. Burton, and H. Wu, "Bed agglomeration during the drying of mallee leaf in fluidized bed," Ind. Eng. Chem. Res., vol. 55, no. 6, pp. 1796–1800, 2016.

[28] V. Mishra, C. Balomajumder, and V. K. Agarwal, "Zn (II) ion biosorption onto surface of eucalyptus leaf biomass: isotherm, kinetic, and mechanistic modeling," Clean–Soil, Air, Water, vol. 38, no. 11, pp. 1062–1073, 2010.

[29] R. Xiao, X. Chen, F. Wang, and G. Yu, "The physicochemical properties of different biomass ashes at different ashing temperature," Renew. Energy, vol. 36, no. 1, pp. 244–249, 2011.

[30] A. Wannapokin, R. Ramaraj, and Y. Unpaprom, "An investigation of biogas production potential from fallen teak

leaves (Tectona grandis)," Emergent Life Sci. Res., vol. 3, pp. 1–10, 2017.
[31] Y. F. Huang, W. H. Kuan, P. T. Chiueh and S. L. Lo, "Pyrolysis of biomass by thermal analysis–mass spectrometry (TA–MS)," Bioresour. Technol., vol. 102, no. 3, pp. 3527–3534, 2011.

APPENDIX B

CERTIFICATES

CERTIFICATE OF ATTENDANCE, PARTICIPATION, & PRESENTATION

THIS IS PROUDLY GIVEN TO

HUYEN THU THI NONG

for attending, participating and conducting an oral presentation at

THE INTERNATIONAL ONLINE CONFERENCE ON INNOVATIVE SCIENCE, ENGINEERING, AND TECHNOLOGY (IOCISET-2020) (JULY 03, 2020 - JULY 05, 2020)

as per the details below:

Submission Type: Full Paper
Title: Biomethane Potential of Invasive Aquatic Weed Water Primroses

KNOWVEL
TAKE YOUR RESEARCH TO NEXT LEVEL

KNOWVEL JOURNALS
WWW.KNOWVEL.COM
INFO@KNOWVEL.COM

KNOWVEL JOURNALS TAKE YOUR RESEARCH TO NEXT LEVEL
DATE: 08/07/2020

CERTIFICATE OF ATTENDANCE, PARTICIPATION, & PRESENTATION

THIS IS PROUDLY GIVEN TO

HUYEN THU THI NONG

for attending, participating and conducting an oral presentation at

THE INTERNATIONAL ONLINE CONFERENCE ON INNOVATIVE SCIENCE, ENGINEERING, AND TECHNOLOGY (IOCISET-2020) (JULY 03, 2020 - JULY 05, 2020)

as per the details below:

Submission Type: Abstract
Title: Biomethane Potential of Invasive Aquatic Weed Water Primroses

KNOWVEL
TAKE YOUR RESEARCH TO NEXT LEVEL

KNOWVEL JOURNALS
WWW.KNOWVEL.COM
INFO@KNOWVEL.COM

KNOWVEL JOURNALS TAKE YOUR RESEARCH TO NEXT LEVEL
DATE: 08/07/2020

ICBB 2020

INTERNATIONAL CONFERENCE OF BIOMASS AND BIOENERGY 2020

“Advanced Technology and Digital Innovations in Biomass, Bioenergy and Agriculture”

Certificate

This is to certify that

Huyen Thu Thi Nong

Has participated as a

Presenter

10-11 August 2020

Chairman of International Conference of Biomass and Bioenergy

Dr. Dwi Setyaningsih, S.TP, M.Si

econference

BIEC 2020 Business Innovation and Engineering Conference

2nd ICRMIA 2020

Certificate

The New Dynamics of Business Innovation & Engineering:
Creating and Protecting Value in The Era of Industry 4.0 & Society 5.0

This certificate is awarded to

Huyen Thu Thi Nong

as a **Presenter** of a paper entitled:

Biogas production from fresh ludwigia hyssopifolia: Effect of pretreatment using different sodium hydroxide conditions

Bogor, 28 July 2020

Prof. Dr. Ir. Noer Azam Achsani, MS
Dean of School of Business - IPB University

Dikky Indrawan, PhD
Chair of BIEC & ICRMIA 2020

IPB University | SB-IPB | IRMAPA | PUTRA Business School | 広島大学 | Sponsored by CRMS


Certificate
International Virtual Conference
On
"Sustainable Development in Current Scenario:
Social Science, Commerce, Management, Education, Medical, Pharmacy, Agriculture, IT &
Engineering and Humanities in Global Environment" (SDCS-2021)
Organized By
AAFT University Raipur(CG), Christian Eminent Academy of Professional Studies-Indore, Research Foundation of India & JHERF
Association with
World Virtual Conference Forum, St. Paul Institute of Professional Studies, Indore, BM Group of Institutions Indore,
College of Professional Studies-ATC- Indore, SKITM Indore & SKIPER Indore, Bagula Mukhi Group Of Institutions Bhopal,
Smt. K G Mittal Commerce College, Mumbai
Sponsored by:
Academic Research Guide Association, International Research Guide Federation (IRGF), World Federation of Science and Technology (WFST) & Edu Academic
ICSDCS/2021/155
Date: 21 February, 2021
It is to be Certified that Prof./Dr./Mr./Ms./Mrs.
Huyen Thu Thi Nong
School of Renewable Energy, Maejo University, Chiang Mai, Thailand
Participated in The International Virtual Conference on ""Sustainable Development in Current Scenario:
Social Science, Commerce, Management, Education, Medical, Pharmacy, Agriculture, IT & Engineering and Humanities in Global Environment" (SDCS-2021)
Organized by AAFT University Raipur(CG), Christian Eminent Academy of Professional Studies-Indore, Research Foundation of India & JHERF
on 21 February 2021 as Participant / Author and Presented a Paper Titled
Enhancement of Biogas Production from Fresh Ludwigia Hyssopifolia by Chemical Pretreatment
in Technical Session I
Dr. Shikha Verma Kashyap
Associate Director,
AAFT University, Raipur, CG
Dr. Rajesh Vyas
Principal,
Christian Eminent College Indore
Prof. Ajay Jain
President, Central India Board,
Research Foundation of India
Dr. Ashok Kumar Gupta
Chief Managing Director
Research Foundation of India
Dr. Sourabh Jain
Chairman
Research Foundation of India
www.researchfoundationofindia.com

Certificate of MJU-TEP Results

This is to certify that

Miss Nong Thi Thu Huyen

took the Maejo University Test of English Proficiency (MJU-TEP) on

June 26th, 2020

The test results are as follows (see reverse side for more details):

1. Listening Comprehension Skill	19/30 marks
2. Semi-speaking Skill	18/25 marks
3. Semi-writing Skill	21/30 marks
4. Reading Comprehension Skill	20/35 marks
Total Score	**78/120 marks**

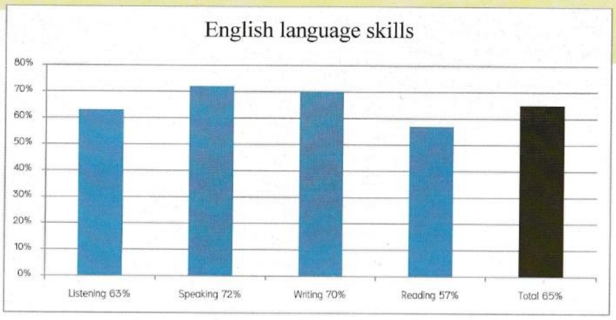
Given on July 2nd, 2020

(Dr. Sutkhet Sakunthong)

Acting Director, Maejo University Language Center

MJU-TEP Scores

*of **Miss Nong Thi Thu Huyen***



Meanings

1. Beginning users	0-20%	5. Competent users	61-70%
2. Very limited users	21-30%	6. Good users	71-80%
3. Limited users	31-40%	7. Very good users	81-90%
4. Modest users	41-60%	8. Expert users	91-100%

CURRICULUM VITAE

NAME	Nong Thi Thu Huyen
DATE OF BIRTH	05 Feb 1995
EDUCATION	2013-2018 Bachelor of Environmental Science, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam. 2019-Now Master of Renewable Energy Engineering, School of Renewable Energy, Maejo University, Chiang Mai 52090, Thailand.
WORK EXPERIENCE	2018-2019 Environmental Technology Centre (ENTEC), Ho Chi Minh, Viet Nam.