ALGAE BIOGAS PRODUCTION AND PURIFICATION THROUGH BIOLOGICAL PROCESS

RAMESHPRABU RAMARAJ

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING

IN RENEWABLE ENERGY

GRADUATE SCHOOL, MAEJO UNIVERSITY

JULY, 2016

ALGAE BIOGAS PRODUCTION AND PURIFICATION THROUGH BIOLOGICAL PROCESS

RAMESHPRABU RAMARAJ

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

APPROVED BY Advisory Committee

Chair ..... N. Dussadee .....
(Assistant Professor Dr. Natthawud Dussadee)
28 / 07 / 2016

Committee ..... Niwooti Whangchai .....
(Associate Professor Dr. Niwooti Whangchai)
28 / 07 / 2016

Committee ..... Yuwalee Unpaprom .....
(Dr. Yuwalee Unpaprom)
28 / 07 / 2016

Program Chair, Master of Engineering in Renewable Energy ..... Sarawut Polvongsri .....
(Dr. Sarawut Polvongsri)
28 / 07 / 2016

CERTIFIED BY GRADUATE SCHOOL ..... Jaturapatr .....
(Assistant Professor Dr. Jaturapatr Varith)
Dean of Graduate School
29 / 07 / 2016

ชื่อเรื่อง การผลิตก๊าซชีวภาพโดยสาหร่ายและการทำให้บริสุทธิ์ด้วยกระบวนการทางชีวภาพ

ชื่อผู้เขียน นายราเมศปราบู รามาราช

ชื่อปริญญา วิศวกรรมศาสตรมหาบัณฑิต สาขาวิชาพลังงานทดแทน

อาจารย์ที่ปรึกษาหลัก ผู้ช่วยศาสตราจารย์ ดร.ณัฐวุฒิ ดุษฎี

บทคัดย่อ

การใช้ประโยชน์จากพลังงานทดแทนเพิ่มขึ้นอย่างรวดเร็ว ดังนั้นจึงควรคำนึงถึงความปลอดภัยด้านพลังงาน ความคุ้มทุนทางเศรษฐกิจและสิ่งแวดล้อม ในปัจจุบันมีพืชพลังงานที่ไม่ใช่พืชอาหารและเป็นแหล่งสำคัญในการผลิตพลังงานชีวภาพหลายชนิด สาหร่ายเป็นแหล่งพลังงานชีวภาพอีกแหล่งหนึ่งที่มีข้อดีหลายอย่าง เช่น มีอัตราการเจริญเติบโตอย่างรวดเร็ว และมีผลผลิตชีวมวลสูง การผลิตก๊าซชีวภาพจากการย่อยสลายชีวมวลสาหร่ายแบบไม่ใช้ออกซิเจนจึงเป็นแหล่งพลังงานทดแทนที่สำคัญ ก๊าซชีวภาพเป็นเชื้อเพลิงชนิดหนึ่งที่มีข้อดีหลายประการเมื่อเปรียบเทียบกับเชื้อเพลิงชีวภาพชนิดทั้งในด้านสิ่งแวดล้อมและทรัพยากรที่มีประสิทธิภาพ องค์ประกอบหลักของก๊าซชีวภาพได้แก่ ก๊าซมีเทน (CH_4) และคาร์บอนไดออกไซด์ (CO_2) นอกจากนี้ยังประกอบด้วย ไฮโดรเจนซัลไฟด์ (H_2S) และสารประกอบกำมะถันอื่น น้ำ และก๊าซอื่นๆ ที่ปนเปื้อนมาด้วย การทำก๊าซให้บริสุทธิ์และการเพิ่มคุณภาพของก๊าซชีวภาพจึงเป็นสิ่งที่จำเป็นยิ่ง ซึ่งการลดคาร์บอนไดออกไซด์และไฮโดรเจนซัลไฟด์เป็นหลักสำคัญในการปรับปรุงคุณภาพก๊าซชีวภาพ การศึกษาครั้งนี้มีจุดประสงค์เพื่อประเมินระบบการผลิตก๊าซชีวภาพจากสาหร่ายขนาดใหญ่ และการทำก๊าซให้บริสุทธิ์โดยใช้จุลสาหร่าย ซึ่งอาศัยหลักการความสามารถในการสังเคราะห์แสงของสาหร่ายในการกำจัดสิ่งเจือปนในก๊าซชีวภาพ ทำการทดลองโดยใช้น้ำจากแหล่งน้ำธรรมชาติที่ปราศจากการเติมอาหารสำหรับเลี้ยงสาหร่าย เพื่อศึกษาศักยภาพที่แท้จริงของสาหร่ายในขั้นตอนการผลิตก๊าซชีวภาพ ทำการรวบรวมและจำแนกสาหร่ายโดยใช้ลักษณะสัณฐานวิทยาและกายวิภาค พบว่าเป็นสาหร่ายเทา (*Spirogyra ellipsospora*) ซึ่งเป็นสาหร่ายน้ำจืดที่มีลักษณะเป็นเส้นสาย ในการเลี้ยงสาหร่ายได้ใช้แนวคิดเพื่อลดต้นทุนการผลิต จึงออกแบบการเลี้ยงโดยใช้ถังปฏิกิริยาแสง และเลี้ยงภายนอกอาคาร ผลการศึกษาพบว่า ถังปฏิกิริยานี้สามารถใช้เลี้ยงสาหร่ายเทาได้ดีตลอดระยะเวลา 6 สัปดาห์ จากนั้นทำการวิเคราะห์คลอโรฟิลล์ ชีวมวล การผลิตก๊าซชีวภาพและการหมักแบบไม่ใช้ออกซิเจน พบว่า สาหร่ายมีศักยภาพสูงและสามารถนำมาใช้เป็นวัตถุดิบที่ดีสำหรับการผลิตก๊าซชีวภาพได้ เนื่องจากสาหร่ายเทาเป็นแหล่งที่อุดมด้วยโปรตีน คาร์โบไฮเดรตและไขมัน นอกจากนี้ยังได้ศึกษาองค์ประกอบทางเคมีของสาหร่ายเทา โดยการอบแห้งด้วยพลังงานแสงอาทิตย์ บดให้เป็นผง และวิเคราะห์ต่อไป ผลการศึกษาพบว่าสาหร่ายเทาเหมาะสมในการเป็นสารตั้งต้นแบบเดี่ยว ซึ่งสามารถนำใช้ผลิตมีเทนได้สูงที่สุดร้อยละ 64.64 คาร์บอนไดออกไซด์ร้อยละ 31.5 และไฮโดรเจนซัลไฟล์ 578 ppm โดยไม่มีกระบวนการปรับสภาพใด ๆ การ ศึกษาครั้งนี้แสดงให้เห็นว่า มีความเป็นไปได้สูงในการใช้สาหร่ายเทาเป็นสารตั้งต้นในการผลิตก๊าซชีวภาพในระดับอุตสาหกรรมหรือขนาดใหญ่ ปัจจุบันความสนใจในการผลิต

พลังงานชีวภาพจากสาหร่ายได้เพิ่ม มากขึ้นเพราะสาหร่ายเจริญเติบโตได้อย่างรวดเร็ว และสามารถเปลี่ยนพลังงานแสงอาทิตย์เป็นพลังงานเคมีโดยกระบวนการตรึงคาร์บอนไดออกไซด์ จึงได้ศึกษาบทบาทของจุลสาหร่ายในการทำบริสุทธิ์ก๊าซชีวภาพ ในการศึกษานี้ได้ใช้สาหร่ายคลอเรลลา *(Chlorella vulgaris)* ซึ่งเลี้ยงในบ่อซีเมนต์แบบระบบเปิด โดยมีวัตถุ ประสงค์เพื่อประเมินการเจริญเติบโตของสาหร่ายคลอเรลล่าและเลี้ยงในต้นทุนต่ำ โดยใช้สูตรอาหาร Rameshprabu ซึ่งประกอบด้วยปุ๋ยนาข้าว รำข้าว ปลาป่น ปูนขาว และปุ๋ยยูเรีย ซึ่งสูตรอาหารนี้ทำให้ได้ผลผลิตชีวมวล คาร์โบไฮเดรต โปรตีน และไขมันสูง สำหรับการกำจัดการปนเปื้อนในก๊าซชีวภาพด้วยกระบวนการทางชีวภาพโดยอาศัยความสามารถในการสังเคราะห์แสงของสาหร่ายซึ่งเป็นวิธีที่เป็นมิตรกับสิ่งแวดล้อมนั้น ได้ทำการทดลองเป็นระยะเวลา 8 ชั่วโมง จากนั้นทำการวัดปริมาณก๊าซต่างๆ พบว่ามีเทนมีปริมาณเพิ่มมากขึ้นจากร้อยละ 64.64 เป็นร้อยละ 81.35 นอกจากนี้ ปริมาณคาร์บอนไดออกไซด์มีปริมาณลดลงจากร้อยละ 31.5 เหลือร้อยละ 16.08 และปริมาณไฮโดรเจนซัลไฟด์ลดลงเหลือน้อยกว่า 0.01 ppm จากผลการลดปริมาณก๊าซคาร์บอนไดออกไซด์และไฮโดรเจนซัลไฟด์ได้อย่างมีนัยสำคัญ ดังนั้นวิธีนี้สามารถเพิ่มคุณภาพของก๊าซชีวภาพได้เป็นอย่างดี นอกจากนี้กากที่เหลือจากการย่อยสลายจากการผลิตก๊าซชีวภาพสามารถนำมาใช้เป็นปุ๋ยที่เป็นมิตรกับสิ่งแวดล้อม เรียกว่า “ปุ๋ยอินทรีย์" สรุปได้ว่า มีความเป็นไปได้สูงในการผลิตก๊าซชีวภาพจากชีวมวลของสาหร่ายขนาดใหญ่ที่เจริญตามธรรมชาติ และการทำให้บริสุทธิ์ทางชีวภาพโดยผ่านระบบการเจริญเติบโตของจุลสาหร่าย ถือว่า มีประโยชน์ทั้งในทางสิ่งแวดล้อมและเศรษฐกิจ

Title	Algae Biogas Production and Purification Through Biological Process
Author	Mr. Rameshprabu Ramaraj
Degree of	Master of Engineering (Renewable Energy)
Advisory Committee Chairperson	Assistant Professor Dr. Natthawud Dussadee

ABSTRACT

The utilization of renewable energy is significantly increasing, together with energy security concerns, environmental and economical benefits. Recently, energy crops have most important as a source for the production of bioenergy because they do not compete with food crops. Algae have numerous advantages such as fast growth rates and biomass yield. Biogas from anaerobic digestion of algal biomass is a renewable energy resource. From an environmental and resource-efficiency perspective biogas has several advantages in comparison to other biofuels. The main components of biogas are methane (CH_4) and carbon dioxide (CO_2), but usually biogas also contains hydrogen sulphide (H_2S) and other sulphur compounds, water, other trace gas compounds and other impurities. Purification and upgrading of the gas is necessary. Reducing CO_2 and H_2S content will significantly improve the quality of biogas. In this study aim was to systematically evaluate the macroalgal biomass as new substrates for biogas production, and purification using microalgal involved the use of algae's photosynthetic ability in the removal of the impurities present in biogas. The utilization of natural water medium for algal production without any extra added nutrition, lead to a better understanding of the potential to these use of algae based on its role in this study. Freshwater alga *Spirogyra ellipsospora*, filamentous charophytic characteristics was classified based on morpho-anatomical characters. The algae were cultivated using the natural water resource to develop the algae growth system by ecological engineering concept to reduce the production expenses. Based on the trial use of outdoor photo-reactor to grow macroalgae in natural water as medium, results showed that the reactor could well be used to grow algae within 6 weeks. After harvesting, the algae were analyses for chlorophyll, biomass, biogas production and anaerobic fermentation good material. It was found that algae could be used as good raw material for production of biogas. Macroalgae are suitable as a source of rich protein, carbohydrate and lipids. In this study, *S. ellipsospora* biomass was dried with solar dryer, and the materials were pulverized for chemical composition analysis. Results showed that macroalgae biomass was suitable as a monosubstrate for with highest CH_4 yield at 64.64 % and CO_2 along with H_2S are 31.5 % and 578 ppm, respectively,

without any pretreatment process. This study suggested that it is possible to achieve established operation using *S. ellipsospora*, as a substrate for biogas production in pilot or large scale biogas plant. Currently, renewed interest in producing bioenergy from microalgae has occurred because they can grow rapidly and convert solar energy into chemical energy via CO_2 fixation. Subsequently, microalgae are playing an important role in the biological purification of biogas. In this study, green microalgae, *Chlorella vulgaris* which was cultivated under open type cement pond system to produce biomass. The objective of this study was to evaluate the growth of green microalga *C. vulgaris* on low cost artificial medium consisting with rice fertilizer, rice bran, fish meal, lime and urea. This medium was named as, Rameshprabu medium. The best biomass in terms of high total carbohydrates, protein and lipid production was obtained through the use of Rameshprabu medium. For biogas impurity removal, biological processes considered environmentally friendly and feasible were employed using the photosynthetic ability of the algae. Photoautotrophic purification process was continued 8 hours. After purification, the CH_4 content has improved gigantically, from 64.64 % upgraded to 81.35%. Also CO_2 and H_2S amounts were reduce a lot. The CO_2 content was 31.5 % reduced to 16.08 %. Enhanced biogas confirms that less than 0.01 ppm of H_2S was noticed. Reducing CO_2 and H_2S content will significantly improve the quality of biogas. In addition production of biogas, the digestate could be used as an environmentally friendly fertilizer, so called "organic fertilizer". In conclusion, it is possible to produce biogas from naturally grown macroalgal biomass, also biological purification through microalgal growth system and this is beneficial from both an environmental and economic perspective.

ACKNOWLEDGEMENT

I express my sincere thanks to my supervisor, Assistant Professor Dr. Natthawud Dussadee, School of Renewable Energy, Maejo University, Chiang Mai, for his prudent suggestions, incessant support, inspiration and constructive critics throughout my project work. I also gratefully acknowledge to Co-advisors: Dr. Niwooti Whangchai, Associate Professor, Faculty of Fisheries Technology and Aquatic Resources and Dr. Yuwalee Unpaprom, Lecturer, Program in Maejo University, Chiang Mai for their kindness and encouragement to carry out my project work. I want to thank all our faculty members, office staff of School of Renewable Energy, Maejo University, Chiang Mai, for their co-operation.

I extend my gratefulness to external examiner Dr. Sirinuch Chindaruksa, Assistant Professor, Department of Physics, Faculty of Science, Naresuan University, Phitsanulok, Thailand, for her valuable comments and suggestions. I wish to extend my sincere thanks to Dr. Natthawud Dussadee, Director of Energy Research Center, Maejo University all for his help. I wish to thank all my lab mates, and Energy Research Center (ERC) staffs, research scholarship from School of Renewable Energy Maejo University for their help. I would like send my warmest thanks to Associate Professor Dr. Thep Phongparnich, former MJU President and "Pises Powder" Parachute Brand.

I express my abysmal adoration and heartfelt devotion to my beloved grandparents (late K. Balagurusamy, Thayammal and Valliammal), aunties (Pasuvathi and Ramalaxmi), parents, brothers and their family as well as sister and her family members for their love, countless blessings, affection and incessant inspiration that always has been the fighting power for me against the odds and shape my life and career till today.

Rameshprabu Ramaraj

July 2016

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

°C	Degree Celsius
% (v/v)	Present volume by volume
% (w/v)	Present weight by volume
μA	Microampere
μg	Microgram
μL	Microliter
BOD	Biological oxygen demand
CH_4	Methane
cm	Centimeter
CO_2	Carbon-dioxide
COD	Chemical oxygen demand
FS	Fixed solids
FSS	Fixed suspended solids
H_2S	Hydrogen sulfide
O_2	Oxygen
OD	Optical density
TN	Total nitrogen
TP	Total phosphorous
TS	Total solids
TSS	Total suspended solids
VS	Volatile solids
VSS	Volatile suspended solids

CHAPTER 1
INTRODUCTION

Principles, Theory, Rationale and/or Hypotheses

Due to the energy crisis, renewable energy becomes a popular issue in this world today and there are several alternatives such as bioenergy, solar, wind, tide, geothermal, etc. Bioenergy is a type of renewable energy made from biological sources including algae, trees, or waste from agriculture, wood processing, food materials, and municipalities. For bioenergy, algae are the third generation biofuel (Tsai, 2012). It provides an excellent biomass as a renewable energy source, so called "bioenergy", and turn algae as the most efficient bio-component (Ramaraj, 2013).

Macroalgae have recently received considerable attentions as a substrate for biofuels production, since they have higher growth rates compared to the plants. Generally, macroalgae (red, brown, and green) are obtained from natural and cultivated resources. *Spirogyra* sp. (Tao) is freshwater macroalgae, available in the north and northeast of Thailand. It contain high amount of chemical components including carbohydrates, fat, proteins and mineral substances (Peeraporn pisal, 2008). Regarding biofuel production, algae can provide different types of biofuels, including: biodiesel (from algal fatty acids); ethanol (produced by fermentation of starch); hydrogen (produced biologically); and methane (produced by anaerobic digestion of algal biomass). From an environmental and resource-efficiency perspective biogas has several advantages in comparison to other biofuels. The production of biogas via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product (Park et al., 2012; Frigon et al., 2013).

Biogas is composed of 40-70% methane (CH_4), 60-30% carbon dioxide (CO_2), 100-3000 ppmv hydrogen sulfide (H_2S) and water, other trace gas compounds and other impurities. Since the main components of biogas are CH_4, CO_2 and H_2S. Purification and upgrading of the gas is necessary because purified biogas provides reductions in green house gas emissions as well as several other environmental benefits when used as a

vehicle fuel. Reducing CO_2 and H_2S content will significantly improve the quality of biogas.

Various technologies have been developed and available for biogas impurity removal; and biological processes are environmentally friendly and feasible. Furthermore, microalgae are abundant and omnipresent. Biogas purification using microalgae involved photosynthetic ability in the removal of the impurities present in biogas. Therefore, in this study I will be testing the development in AD techniques for macroalgae as biogas producer and using microalgae as biogas purification.

Objective

1. To utilize the natural recourses and evaluation of algae growth environment.
2. To investigate the biogas potential from freshwater macroalgae (*Spirogyra* sp.).
3. To produce the biogas potential of *Spirogyra* sp.
4. To apply the biogas purification through microalgal (*Chlorella* sp.) biological purification method.

Scope

1. Applied sustainable resource, sustainable engineering and valuable bioenergy approach of *Spirogyra* sp. Utilization.

2. New innovative approach of utilizing macroalgae for biogas production and biogas impurity removal used by microalgae for biological purification.

Benefits

In Thailand, there is plenty of freshwater natural resource available. In this study, I will be utilize the natural recourses and obtain algal biomass from the resource directly for biogas production. It could be reduce the cultivation and production cost. In the same time this approach could be helpful to decrease algae degradation and pollution in the water body. In addition, it could reduce the algal bloom. However, biogas is containing with impurities. Various technologies have been developed and available for biogas impurity

removal; and biological processes are environmentally friendly and feasible. This is one of main benefit we can get it from this research because I will use microalgae for impurities removal from the biogas. It could be save environment effects such as green house gases and same time it could reduce the chemical cost and other related purification material cost.

CHAPTER 2

LITERATURE REVIEW

Fundamental of algae

Algae are the most important primary producer in aquatic ecosystem (Ramaraj et al., 2014). Many species of algae are present such as; green, red and brown algae which belong to the group of Chlorophyta, Rhodophyta and Phaeophyta, respectively. Algal growth is found in a wide range of habitats, like fresh water, marine water, in deep oceans, in rocky shores, the plank-tonic and benthic algae can become important constituents of soil flora and can exist even in such extreme conditions as in snow, sands/desert or in hot springs, open and closed ponds, photo bioreactors, sewage and wastewater, desert as well as CO_2 emitting industries etc (Ramaraj, 2013). Generally they are found in damp places or water bodies and are common in terrestrial as well as aquatic environments. Algae, a broad category encompassing eukaryotic microalgae, cyanobacteria and macroalgae, can be cultivated to produce biomass for a wide range of applications (Ramaraj et al., 2010).

Applied of algal biotechnology and bioengineering aspects

Algae are a very diverse group of predominantly aquatic photosynthetic organisms of tremendous ecological importance, because they were the beginning of the food chain for other animals. Algae played an important role in self-purification of contaminated natural waters and offered an alternative for advance nutrition removal in water or wastewater (Ramaraj et al., 2013; Ramaraj et al., 2014). The idea to incorporate microalgae as an agent of bioremediation was firstly proposed by Oswald and Gotaas in 1957 (Vílchez et al., 1997); the biomass recovered was converted to methane, which was a major source of energy (Oswald, 2003). Hence, algae provided the basis of the aquatic food chain and they were fundamental to keep CO_2 of carbon cycle via photosynthesis as a substantial role in biogeochemical cycles (Ramaraj et al., 2014b). Most algae were photoautotrophic, converting solar energy into chemical forms through photosynthesis.

The mechanisms of algal photosynthesis were very similar to photosynthesis in higher plants and their products are molecularly equivalent to conventional agricultural crops (Graham and Wilcox, 2000). The main advantages of culturing algae as a source of biomass were as follows: (1) high photosynthetic yields (up to a maximum of 5-6% conversion of light c.f. 1-2% for the majority of terrestrial plants); (2) the ability to grow in fresh, salt and wastewater; (3) high oil content; (4) the ability to produce non-toxic and biodegradable biofuels; (5) many species of algae can be induced to produce particularly high concentrations of chosen compounds–proteins, carbohydrates, lipids and pigments - that are of commercial value; (6) the ability to be used in conjunction with wastewater treatment (Vilchez et al., 1997; Graham, and Wilcox, 2000; Oswald, 2003; Tsai, 2012; Ramaraj, 2013).

In addition, algae application is widely accepted in practice as one of the best strategies in bioengineering. There are several reasons for this approach: (1) the best growth rate among the plants, (2) low impacts on world's food supply, (3) specificity for CO_2 sequestration without gas separation to save over 70% of total cost, (4) excellent treatment for combustion gas exhausted with NOx and SOx, (5) high value of algae biomass including of feed, food, nutrition, pharmaceutical chemicals, fertilizer, aquaculture, biofuel, etc (Tsai, 2012; Ramaraj, 2013). Algae an important application for the cultivation of algae is the production of biomass for energy purposes.

Applied of algal bioenergy and renewable energy aspects

Recently, macroalgae is one such source of aquatic biomass and potentially represents a significant source of renewable energy. The average photosynthetic efficiency of aquatic biomass is 6–8%, which is much higher than that of terrestrial biomass (1.8–2.2%). Macroalgae are fast growing marine and freshwater plants that can grow to considerable size (up to 60 m in length). Annual primary production rates (grams cm^{-2} yr^{-1}) are higher for the major marine macroalgae than for most terrestrial biomass (Gellenbeck and Chapman, 1983). Macroalgae can be subdivided into the blue algae (Cyanophyta), green algae (Chlorophyta), brown algae (Phaeophyta) and the red algae (Rhodophyta). Either Freshwater macroalgae or marine macroalgae (kelp or seaweed) could be used for solar energy conversion and biofuel production (Gellenbeck and Chapman, 1983). Macroalgae received a large amount of attention

as a biofuel feedstock due to its prolific growth in natural habitat of freshwater system, eutrophic coastal water fouling beaches and coastal waterways.

Freshwater macroalgae, Tao (*Spirogyra* sp.) available northern part of Thailand, it contains high amount of nutritional compositions, including basic nutrients, which are carbohydrates, fat, proteins, multivitamins, and mineral substances (Peeraporn pisal, 2008). *Spirogyra* sp. is a genus of filamentous freshwater green algae of the Division Chlorophyta order Zygnematales Family Zygnemataceae., named for the helical or spiral arrangement of the chloroplasts that is diagnostic. It is commonly found in freshwater areas and there are more than 400 species of *Spirogyra* in the world (Naik et al., 2012). They grow in the standing water of clean to moderate quality, clear water with the turbidity not exceeding 10 NTU, temperature 15-27°C and pH 6-7.8.

For bioenergy, algae are the third generation biofuel (Tsai, 2012). For the reasons of the best energy conversion efficiency of sunlight (Ramaraj et al., 2013) and the highest growth rate (Tsai, 2012; Ramaraj, 2013; Ramaraj et al., 2013), algae have the best potential among all the energy crops. Since algae were a key primary producer global-wide, algae biomass was essential biological natural resources. Algae produce biomass, which can be converted into energy or an energy carrier through a number of energy conversion processes. They include thermochemical conversion (gasification, direct combustion and pyrolysis), biochemical conversion (anaerobic fermentation, anaerobic digestion and photobiological hydrogen production) and esterification of fatty acids to produce biodiesel (Oswald, 2003; Tsai, 2012; Ramaraj, 2013).

Bioenergy should play an essential part in reaching targets to replace petroleum-based transportation fuels with a viable alternative, and in reducing long-term CO_2 emissions, if environmental and economic sustainability are considered carefully. The world continues to increase its energy use, brought about by an expanding population and a desire for a greater standard of living. This energy use coupled with the realization of the impact of CO_2 on the climate, has led us to reanalyze the potential of plant-based biofuels (Jones and Mayfield, 2012). The term biofuel is referred to as liquid or gaseous fuels for the transport sector that are predominantly produced from biomass. A variety of fuels can be produced from biomass resources including liquid fuels, such as ethanol, methanol, biodiesel, Fischer-Tropsch diesel, and gaseous fuels, such as biohydrogen and biogas.

Algal biogas

The process of biogas production from algal biomass is an alternative technology that has larger potential energy output compared to green diesel, biodiesel, bioethanol, and hydrogen production processes. Moreover, anaerobic digestion can be integrated into other conversion processes. The organic fraction of almost any form of biomass (from plants, algae and other microorganisms), including sewage sludge, animal wastes and industrial effluents, can be broken down through anaerobic digestion (AD) into CH_4 and CO_2 mixture called as ''biogas". The first methane digester plant was built at Bombay, India in 1859 (Meynell, 1976; Demirbas, 2008). AD approaches steadily growing role in the renewable energy mix in many countries. AD is the process by which organic materials are biologically treated in the absence of oxygen by naturally occurring bacteria to produce 'biogas' which is a mixture of CH_4 (40-70%) and CO_2 (30-60%) with traces of other gases such as hydrogen, hydrogen sulphide and ammonia (Lastella et al., 2002; Dussadee et al., 2013); the biogas process also produces potentially useful by-products in the form of a liquid or solid 'digestate' (Lastella et al., 2002).

Normally, biogas is comprised of CH_4, CO_2, and other trace gas compounds gases such as water vapour, H_2S, halogenated hydrocarbons, siloxanes, ammonia, nitrogen, and oxygen (Dussadee et al., 2013). Biogas is a valuable fuel which is produced in digesters filled with the feedstock like dung or sewage. All types of biomass can be used as substrates for biogas production as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. The composition of biogas and the methane yield depends on the feedstock type, the digestion system, and the retention time. In general, the use of plant biomass for energy generation today is problematic because of the competition with food or feed production. This is because most of the plants used for energy generation today (crop plants, sugar cane, sugar beets, canola, etc.) have to be grown on arable land. Low demand alternatives like switchgrass are only beginning to emerge. Algae have got a number of potential advantages compared to higher plants because of faster growth rates and the possibility of cultivation on non-arable land areas or in lakes or the ocean, therefore attenuating food and feed competition (Rittmann, 2008; Stephens et al., 2010). Of the potential sources of biogas the most efficient producers of biomass are the photosynthetic algae (micro and macroalgae).

Photosynthetic pigments, including chlorophyll, have an important role since it provides the oxygen and the source of energy for all living things. Plant and algae growth is affected by the photosynthesis speed which depends on the availability of CO_2. Biological CO_2 fixation by algae is another such form; i.e. sunlight being used to reduce CO_2 to carbon. Capturing CO_2 from flue gases is the precautionary principle which needs preventive action, at both national and international levels to minimize this potential action (Mussgnug et al., 2010). A promising approach therefore seems to be the use of fast-growing algae species for anaerobic fermentation to produce biogas, which then can substitute natural gas resources.

Macroalgae can be converted to biogas by process of AD to biogas (~ 60% CH_4) (Ross et al., 2008). Research conducted in the 1980's on macroalgae (giant brown kelp i.e. *Macrocystis*) (Chynoweth et al., 1981) still provides a bench mark for biogas yields for a number of macroalgal species, but since this time there have been developments in AD technology and an enormous increase in its use. In comparison to terrestrial biomass crops, macroalgae contain little cellulose and no lignin and therefore undergo a more complete hydrolysis. AD has been used to dispose and process this material for the production of biogas; the AD of macroalgae biomass could meet two currently important needs, the mitigation of the eutrophication effects and the production of renewable energy. Because of the abundance of seaweed/ freshwater macroalgae biomass its conversion can be highly desirable and convenient, mostly for countries with long coastlines or eutrophic environments (Hughes et al., 2012). Investigations on the use of macroalgae of the brown algae division in processes of methane fermentation were conducted by Vergara-Fernàndez (2008). He was examining the possibility of applying to this end the biomass of *Macrocystis pyrifera* and *Durvillea antarctica* macroalgae and a substrate based on the mixture of these species. His study proved that for all substrates tested the yield of biogas production was comparable and reached 180.4±1.5 dm3/kg d.m.d. Singh and Gu (2010) and Parmar et al. (2011) were also analyzing the yield of biogas production with the use of microphytobenthos plants as an organic substrate. They achieved the highest technological effects during fermentation of Laminaria digitata brown algae belonging to the order Laminariales. In that case, methane production was high and reached 500 dm^3 CH_4/kg o.d.m. The use of *Macrocystis* sp. enabled achieving 390–410 dm^3 CH_4/kg o.d.m., whereas upon the use of *Gracilaria* sp. and *Laminaria* sp. methane production accounted for 280–400 dm^3 CH_4/kg o.d.m. and 260–280 dm^3 CH_4/kg o.d.m., respectively (Debowsk, et al., 2013).

Pretreatment methods

Algae anaerobic biodegradability is limited by their complex cell wall structure. Thus, pretreatment techniques are being investigated to improve algal methane yield. Various pretreatment technologies have been developed in recent years. These pretreatment technologies aim to make AD faster, potentially increase biogas yield, and make use of new and/or locally available substrates, and prevent processing problems such as high electricity requirements for mixing or the formation of floating layers. Pretreatment methods can be divided into four categories: thermal, mechanical, chemical and biological processes (Figure 1).

Pretreatment methods have been studied in order to disintegrate microalgae cells, solubilise the organic content, and increase the anaerobic digestion rate and extent. Thermal pretreatments have been the most widely investigated already in continuous reactors and leading to net energy production (Markou et al., 2013; Schwede et al., 2013).

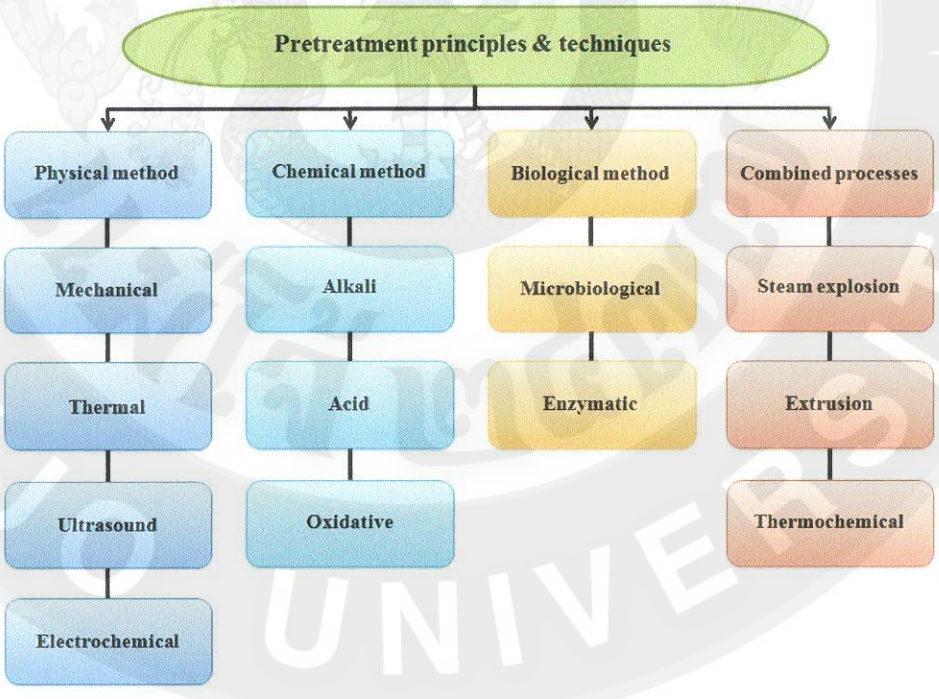

Figure 1 Pretreatments for improving algae biogas production

Mechanical pretreatments have mostly been investigated in batch assays using algae cultures (Cho et al., 2013). Thermal pretreatments have been the most widely studied already in continuous reactors and leading to net energy production (Passos et al., 2013). Mechanical pretreatments were less dependent on algae species, but required a higher energy input if compared with chemical, thermal and biological methods (Cho et al., 2013). Chemical pretreatments have been proved successful, particularly when combined with heat (Passos et al., 2013). Enzymatic pretreatment seem to improve microalgae hydrolysis (Mahdy et al., 2014), which is promising due to its low energy input.

Pretreatment of the algae is thus needed to aid both mechanical transport (pumping) as well as microbiological AD. Biogas can be derived via anaerobic fermentation of any organic matter, including the cellulose and hemicellulose within macroalgae, although the biomass must be subjected to pretreatment processes in order to liberate the sugars needed for fermentation. The effect of the pretreatment technologies, thermal treatment, thermochemical treatment, mechanical treatment, wet oxidation, hydrothermal pretreatment, steam explosion, plasma-assisted pretreatment and ball milling. One option is mechanical pretreatment of the algae; however a method which can handle the long fibrous material in macroalgae species is needed. Another method, which is relatively untested but promising, is enzymatic pretreatment which during recent years has been tested on many substrates to investigate effect on biogas potential (Zieminski et al., 2012).

The mechanical pretreatment effectively broke up the structure of all macroalgae into homogenous slurry. Mechanical pretreatment could increase the soluble COD-concentration of the tested algae by 1.5 to 3 times compared to raw algae. Enzymatic treatment increased it by 1.3 to 1.7 times. The best results were achieved by combining mechanical and enzymatic treatment where the concentration could was increased 3.5 times compared to raw algae (Kjerstadius et al., 2013). A mechanical pretreatment phase is usually the first step not only for methane (Tedesco et al., 2013; Jard et al., 2013). Nielsen and Heiske (2011) was discussed the effect on methane yield of U. lactuca by various pretreatments including mechanical maceration and autoclavation. Sodium hydroxide soaking at room temperature prior to AD led to a 18% increase in methane potential in macroalgae as (Palmaria palmata), possess a high methane potential (308 ± 9 mL gVS^{-1}) (Nielsen and Heiske, 2011). Nielsen and Heiske (2011) studied four macroalgae species-harvested in Denmark-for their suitability of bioconversion to methane. In

batch experiments (53°C) methane yields varied from 132 ml g volatile solids(-1) (VS) for *Gracillaria vermiculophylla*, 152 ml gVS-1 for *Ulva lactuca*, 166 ml g VS-1 for Chaetomorpha linum and 340 ml g VS^{-1} for *Saccharina latissima* following 34 days of incubation. With an organic content of 21.1% (1.5-2.8 times higher than the other algae) *S. latissima* seems very suitable for anaerobic digestion. However, the methane yields of *U. lactuca*, *G. vermiculophylla* and *C. linum* could be increased with 68%, 11% and 17%, respectively, by pretreatment with maceration.

Biogas purification methods and processes

To utilize biogas as a transport fuel, CO_2 and H_2S must be removed from the concentration to leave biomethane. Biogas purification is the process where any impurities are removed such as sulphides and ammonia. Biogas upgrading on the other hand is the process which removes CO_2 and the end product is bio-methane. The bio-methane which has been upgraded is suitable for injection into the national gas grid or vehicle fuel (Dussadee et al., 2013). Biogas needs cleaning for two main reasons; the first is to improve the calorific value of the product gas and the second is to reduce the chance of damaging downstream equipment which is due to the formation of harmful compounds (Ryckebosch et al., 2011). Thus, biogas has a wide availability and renewable nature due to the organic materials and microorganisms required for biogas synthesis. Biogas purification methods can be divided into two generic categories:

Those involving physicochemical phenomena (reactive or non-reactive absorption; reactive or non-reactive adsorption) and biological processes (contaminant consumption by living organisms and conversion to less harmful forms).

Biological processes are widely employed for CO_2 and H_2S removal, especially in biogas applications. For CO_2 capture from biogas, physical and chemical absorption methods are generally applied with fewer complications; however, these methods are needed to post treat the waste materials for regeneration of cycling utilization. The biological methods of CO_2 capture from biogas are potentially useful (Kao et al., 2012a). Biological processes are widely employed for H_2S removal, especially in biogas applications (Abatzoglou et al., 2009).

Microalgae are a group of unicellular or simple multicellular photosynthetic microorganisms that can fix CO_2 efficiently from different sources (Ramaraj et al., 2010; Ramaraj, 2013; Ramaraj et al., 2013; Ramaraj et al., 2014a; Ramaraj et al., 2014b), including the atmosphere, industrial exhaust

gases, and soluble carbonate salts. Furthermore, combination of CO_2 fixation, biofuel production, and wastewater treatment may provide a very promising alternative to current CO_2 mitigation strategies. Presence of chlorophyll and other pigments help in carrying out photosynthesis. The true roots, stems or leaves are absent. Mostly they are photoautotrophic and carry on photosynthesis, some of these are chemo heterotrophic and obtain energy from chemical reactions as well as nutrients from preformed organic matter. Beside the plants, since algae had high potential CO_2 fixation in the current knowledge.

Microalgae can fix CO_2 using solar energy with efficiency ten times greater than terrestrial plants (Ramaraj, 2013; Ramaraj et al., 2014b). The issue of greenhouse gas attracts an enormous attention worldwide recently. When atmospheric CO_2 concentration increased, it would gradually disturb the balance of global climate to cause unusual and astounding phenomena on earth. Therefore, we require the rapid development of bio-carbon-fixation technology to eliminate the adverse effects of CO_2, to transfer atmospheric CO_2 through the carbon cycle and to promote carbon balancing ecologically. At present, algae application of CO_2 sequestration has developed as a popular topic and the current interests are including: species, power plant flue gas utilization, reactor design, growth condition, growth kinetics and modeling. The most studies in the literature concerned the maximum CO_2 uptake rate by the artificial photo-bioreactors (Tsai, 2012; Ramaraj, 2013; Ramaraj et al., 2014a). Among those techs, bio-eco-technology is the most natural and ecological way to accomplish the designed targets by the utilization of "self-designed" bio-functions of nature (Ramaraj et al., 2010; Ramaraj, 2013; Ramaraj et al., 2013; Ramaraj et al., 2014a; Ramaraj et al., 2014b). Accordingly, algae production has a great potential for CO_2 bio-fixation process and deserves a close look.

Biogas purification/scrubbing using algae involved the use of algae's photosynthetic ability in the removal of the impurities (mainly CO_2 and H_2S) present in biogas, leaving a purified biogas containing almost pure methane, which could be used for energy generation. Biological purification technology is worth examining because has double impact. The method about removing CO_2 from biogas by microalgal culturing using the biogas effluent as nutrient medium and effectively upgrade biogas also simultaneously reduce the biogas effluent nutrient (Yan and Zheng, 2013). Using biogas as a source of carbon dioxide has two main advantages: the biomass production costs are reduced and the produced biomass does not contain harmful compounds, which can occur in flue gases. Hendroko et al. (2011) verified exhibit that microalgae (*Scenedesmus*

sp.) in laboratory experiments using biogas slurry as growing medium and biogas are given periodically generate 21% of CO_2 compared with 24% of controls. They summarized: digestion slurry with seed cake JatroMas cultivar as raw material is able to increase growth of microalgae Scenedesmus sp. higher than standard media; microalgae *Scenedesmus* sp. is able to capture CO_2 gas in bio-methane; with integration of slurry and bio-methane intake, there is tendency *Scenedesmus* sp. growth is more increasing; Mutualism symbiosis among slurry, bio-methane and microalgae *Scenedesmus* sp. will give impact to increasing of CH_4 content in bio-methane. In other word, microalgae can be work as purification biologic from bio-methane (Hendroko et al., 2011).

There are several authors (Rittmann, 2008; Hendroko et al., 2011; Yan and Zheng, 2013) reported that *Arthrospira* sp, *Chlorella vulgaris* SAG 211-11b, *Chlorella* sp. MM-2, *Chlorella* sp. MB-9, *Chlorella vulgaris* ARC1, Chlamydomonas sp. dan Scenedesmus sp. was a positive synergy with biogas. The productivity of the system with Zarrouk media and biogas almost 5 times higher than that for the same media without biogas when piggery waste was used, the utilization of biogas brings a productivity gain of about 2–5 times higher (Hendroko et al., 2011).

Kao et al. (2012b) demonstrates that the microalga *Chlorella* sp. MB-9 was a potential strain which was able to utilize CO_2 for growth when aerated with desulfurized biogas (H_2S<50ppm) produced from the anaerobic digestion of swine wastewater. The demonstrated system can be continuously used to upgrade biogas by utilizing a double set of photobioreactor systems and a gas cycle-switching operation. Furthermore, they demonstrated that the efficiency of CO_2 capture from biogas could be maintained at 50% on average, and the CH_4 concentration in the effluent load could be maintained at 80% on average, i.e., upgrading was accomplished by increasing the CH_4 concentration in the biogas produced from the anaerobic digestion of swine wastewater by 10%.

Some literatures mentioned about the cultivation microalgae using biogas as CO_2 provider. Kao et al. (2012a) used biogas that contained 20±2% CO_2 for *Chlorella* sp. culture with variation of light intensity which was at cloudy and at sunny day. Kao et al. (2012a) used biogas that contained 20±1% CO_2 for *Chlorella* sp. culture with variation flow rate of biogas which was 0.05; 0.1; 0.2; 0.3 vvm. Douškova et al. (2010) investigated the potential of biogas as CO_2 provider for *Chlorella vulgaris*; and optimization of biogas production from distillery stillage is described. The growth kinetics of microalgae *Chlorella* sp. consuming biogas or mixture of air and CO_2 in the

concentration range of 2–20% (v/v) (simulating a flue gas from biogas incineration) in laboratory-scale photo-bioreactors. It was proven that the raw biogas (even without the removal of H_2S) could be used as a source of CO_2 for growth of microalgae. The growth rate of microalgae consuming biogas was the same as the growth rate of the culture grown on a mixture of air and food-grade CO_2. Several species of algae can metabolize H_2S (Biebl and Pfennig, 1977). Using a biological system to remove H_2S has similar benefits to using one to remove CO_2: lower upkeep costs, more environmentally sustainable and non-hazardous waste.

Furthermore, Tongprawhan et al. (2014) used oleaginous microalgae to capture CO_2 from biogas for improving methane content and simultaneously producing lipid. They screened several microalgae for identify their ability to grow and produce lipid using CO_2 in biogas. Finally, they reported a marine *Chlorella* sp. was the most suitable strain for capturing CO_2 and producing lipid using biogas (50% v/v CO_2 in methane) as well as using 50% v/v CO_2 in air. Sumardiono et al. (2014) established to evaluate the design of the photobioreactor system for purifying biogas through the culturing of microalgae. This system represented a simple promising way for the current forthcoming technologies of biogas purification. It helps to decrease the concentration of CO_2 in biogas concomitantly producing microalgae biomass. The microalgae Nannochloropsis is able to use CO_2 from biogas produced from the anaerobic digestion of tannery sludge. The results show that cultivation of microalgae under the biogas to scrub out CO_2 and promote enrichment of methane in the biogas in this work and obtained scrubbing of 27% from 30%.

The biocapture of CO_2 by microalgae can be applied to improve the quality of biogas by reducing the CO_2 content as this would lead to an increase in the methane content (Mann et al., 2009). The microalgae *Chlorella* sp. was analyzed in terms of conditioning biogas. As a result the biogas components CO_2 and H_2S could be reduced up to 97.07% and 100%, respectively. Also an increase of microalgae cell count could be documented, which provides interesting alternatives for the production of algae ingredients. Consequently, the algae biological purification is an alternative to other biogas purification methods.

CHAPTER 3

MATERIALS AND METHODS

The potential of freshwater green macro and micro- algae as substrates for biogas production and biogas purification was investigated. This research methodology was classified as two categories. First category focused on macroalgae biogas production including study site selection, species identification, and lab scale macroalgae production, natural grown algae utilization, harvesting, along with applied for biogas production. For the macroalgae cultivation, natural water medium (Ramaraj et al., 2015) was used from slow running fresh water stream water.

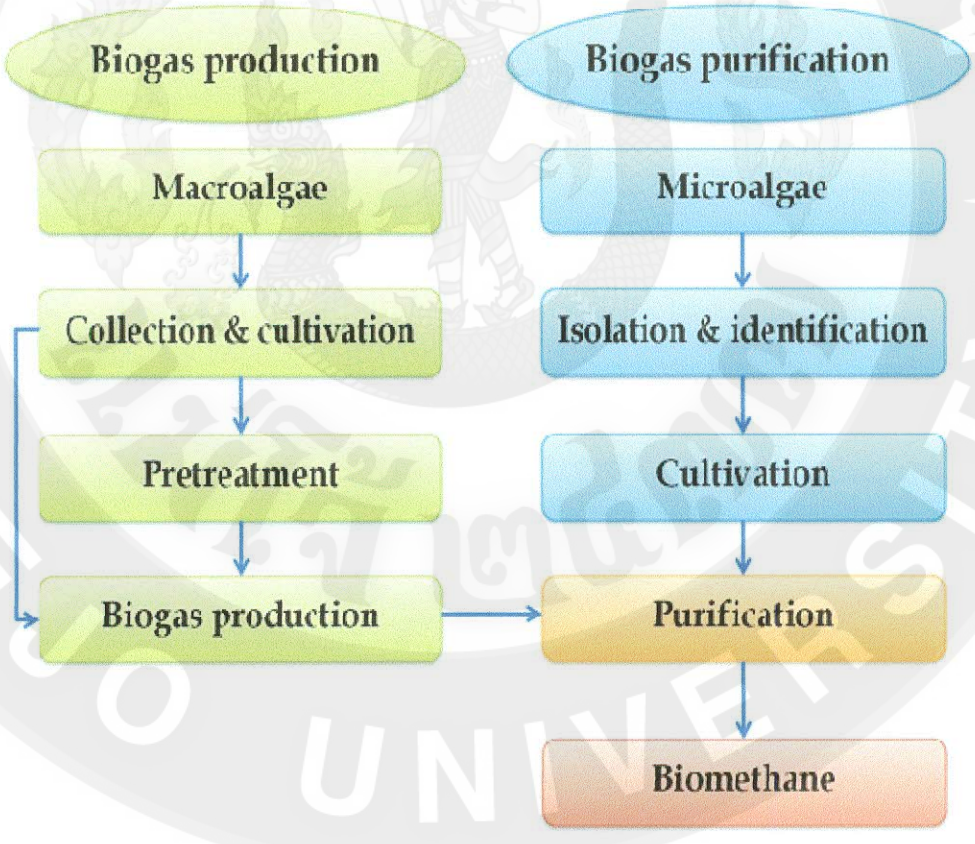

Figure 2 Flow chart of methodology

The nearby stream water was screened by 1x1 mm sieve (mesh No. 18) then the water was filtrated by 0.45 µm filter paper as feed macroalgae. Subsequently macroalgae was used biogas production. Biogas production processes were goes through by biochemical methane potential (BMP) test, pretreatment, and lab scale up biogas production subsequently the gas was stored further analysis. Second category was involved biogas purification through microalgae. The processes were together with microalgae isolation, identification, cultivation and then cultivated algae for used biogas purification. The study methodology is illustrated in Figure 3.1.

Experiment 1: Study on fresh water macroalgae *Spirogyra* sp. natural growth system analysis and biomass harvest

Spirogyra sp. were collected from the slow running fresh water stream at Sobpeng, MaeTeang, Chiang Mai Province, Thailand and transported to the School of Renewable Energy and Energy Research Center, Maejo University, Sansai, Chiang Mai-50290, within 2 h for identification and analysis. *Spirogyra* sp. was harvest by using the local traditional methods from the natural water body. Various physicochemical parameters were monitored in situ including temperature, dissolved oxygen, pH, conductivity, turbidity and water flow rate. Algae biomass was estimated gravimetrically by total suspended solids (TSS). TSS was measured by Method 209C with Whatman GF/C filter paper, chemical oxygen demand and dissolved nutrients were measured in the laboratory; all physico-chemical analyses were carried out whole growth period according to the standard method (APHA, 2005).

Algal sample collection, cultivation and experiment setup and Identification of alga

The freshwater macroalgae, *Spirogyra* was collected from the slow running fresh water stream at Tumbon Pang Yang (19° 18'42.41" N; 98° 48'44.11" and elevation 722 m), Mae Taeng district, Chiang Mai province, Thailand and transport to the Energy Research Laboratory at Maejo University, Chiang Mai, Thailand. The methodology was illustrated in (Figure 2). This investigation is to simulate the ecosystem in natural water body with macroalgae growth ecological engineering concepts. For the macroalgae cultivation, the nearby water was screened by 1x1 mm sieve

(mesh No. 18) to remove macro particles. According to Ramaraj et al. (2015a, 2015b) the stream water was used as medium. Water collected from the same sampling zone afore mentioned and the water was filtrated by 0.45 µm filter paper as feed. *Spirogyra* Sp. were grown in autotrophic conditions of 10 L open type outdoor jar. The jar containing 5L working volume and 5 L base filled with sterilized white sand and growth system was demonstrated in Figure 2.

Identification of alga

The algal samples were observed under light microscope and were then visualized with a Nikon Eclipse 80i microscope and photographs were taken with attached digital camera. Relevant publication of Prescott (1951) was referred for the identification of algal taxa and taxonomically determined with the help of authentic literature (Randhawa 1959, Transeau 1951, Vidyavati 1995, Kargupta and Jh 2004, Taft 2009). For the taxonomic description of taxa, dimensions were given in micrometer (µm). The measuring scales given for algae photographs were equal to 20 µm. The morphological characters including length, width, number of spiral chloroplasts, and number of granules were recorded for species confirmation. Plant materials (macroalgae)

Spirogyra ellipsospora biomass was collected from the slow running fresh water stream at Tumbon Pang Yang (19° 18'42.41" N; 98° 48'44.11" and elevation 722 m), Mae Taeng district, Thailand and transported to the Energy Research Center, Maejo University, Sansai, Chiang Mai-50290,Thaialnd. Species identification and morphological details were presented in our previous study (Pantawong et al., 2015; Ramaraj et al., 2015). Figure 3 demonstrated that study site, material collection, harvesting and drying through solar dryer for biochemical analysis. Samples of macroalgae biomass were collected by hand (traditional method) directly from the stream. Directly after acquisition macroalgae biomass was rinsed with tap water to remove sand and other pollutants.

Figure 3 Study site and plant material collection: (A) slow running fresh water stream, (B) traditional algae collection, (C), harvested algae, (D) solar dryer for biomass drying.

Experiment 2: Biogas production from fresh water macroalgae *Spirogyra ellipsospora*

Anaerobic biodegradability batch assays were performed according to the directives defined in Angelidaki et al. (2009). I was constructed with a small-scale anaerobic digester and maintain a constant flow rate of biogas using a regulator at room temperature. The digester was be fed with inoculum and designed to produce biogas similar in composition to that produced from landfills (45-75% methane, 25-55% CO_2, and 7-100 ppm H_2S). The digester was be kept in closed system, sealed with an airtight lid, and outfitted ports. Raw biogas was flow out of the port in the lid while a peristaltic pump was connected the body ports to mix digester contents. Intermittent stirring could increase interactions between microbes and the feed slurry (Wilkie et al., 2003). Periodically adding new feedstock ensures that the microbes have a fresh batch of organic materials to process. Concurrently, a portion of the digested materials were removed to

prevent overflow. The same volume of added feed could come out through the waste port. Biogas was accumulating above the waste port.

Lab study aims to optimize a wide range of factors including the flow rate, type of feed, feeding frequency, composition of biogas, and other logistical problems (including pretreatments). The flow meter was used to find the maximum biogas production rate and the time it takes to reach that rate. The pH determination procedure was adopted from Weiland (2010). Biogas composition in laboratory test (CH_4, CO_2, H_2, H_2S, and O_2) was measured using an automated gas analyzer according to Brettschneider et al. (2014). To test the entire system, the anaerobic digester and allow it to run until the flow rate approaches the maximum.

Anaerobic digestion batch tests

The anaerobic assays were conducted in 500 mL bottles (triplicate reactor) containing 40 mL of inoculum and 200 g of fresh *S. ellipsospora* and remaining make up with double distilled water. The total working volume is 400 mL. The biochemical methane potential (BMP) assay was used to determine the methane productivity of *S. ellipsospora*. The bottles were closed with a septum and flushed with N2 to remove oxygen. Triplicate, 500 mL fermenters were incubated in the room temperature. Production of biogas was monitored by measuring the overpressure in the bottle headspace at time intervals depending on the production of biogas.

Experimental procedure

The bioreactor system consists of flasks of 5000 ml in capacity. The equipment is constituted of: valves, quick release tubing connectors, plastic pipes and gas collector, shown in figure 4. To preserve anaerobic conditions, nitrogen has been flushed for 2 min into the reactors to clear up any residual trace of oxygen from within the flasks and pipes. Water-baths were used to keep the reactors at a mesophilic temperature in the laboratory. A biogas analyzer was used to verify anaerobic conditions were created correctly when preparing the reactors and to analyze the biogas biochemical composition. The experimental set up and methodologies are followed our previous studies (Ramaraj et al., 2005, 2016). The purpose of the first experiment is to identify whether a benefit in room temperature system for biogas production. The anaerobic assays were conducted in 5000 mL duran bottles (triplicate reactor) containing 400 mL of inoculum and 1000 g of fresh *S. ellipsospora* and remaining make up with double distilled water. The total working

volume is 4000 mL. After inoculation, all batch reactors were purged with nitrogen gas to create an anaerobic condition. Triplicate, 5000 mL fermenters were incubated in the room temperature (assumed as mesophilic conditions). Production of biogas was monitored by measuring the overpressure in the bottle headspace at time intervals depending on the production of biogas. Three digesters have been prepared with the exact amount of inoculums used. The anaerobic inoculum was obtained from a working anaerobic digester at Energy Research Center, Maejo University. Five liter batch fermenters were incubated at room temperature conditions for 70 days. The digesters were shaken two or three times everyday to prevent the formation of surface crust which may prevent contact between microorganisms and the substrate.

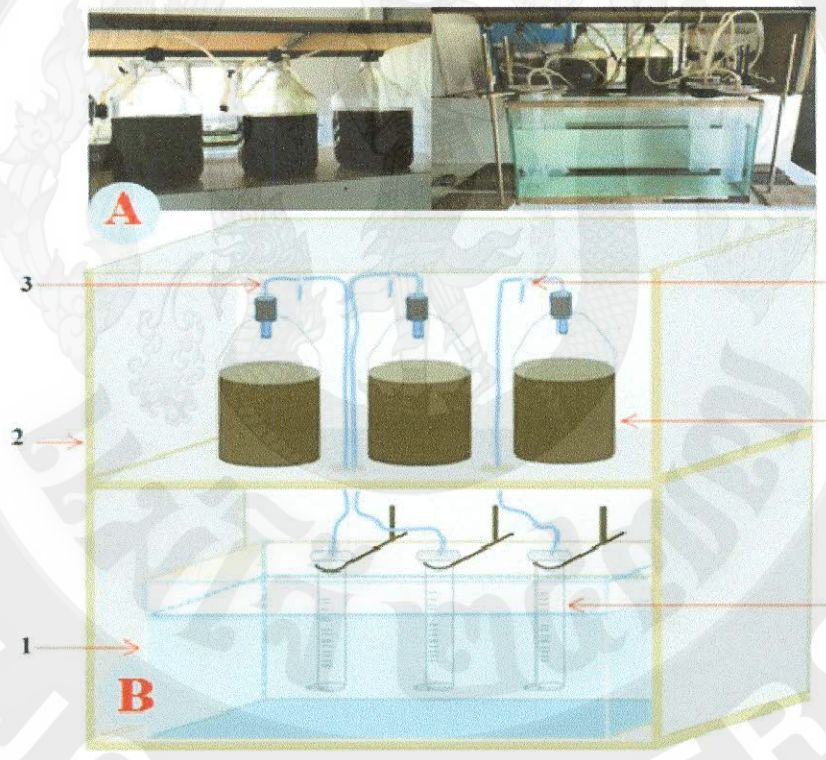

Figure 4 A) Batch system of macroalgae biogas production B) Schematic view of the experimental set up during anaerobic digestion 1) water tank 2) protection wooden box 3) gas transfer tube 4) gas sampling port 5) digester (5000 ml) 6) gas measuring cylinder

Experiment 3: microalgae cultivation for biogas purification and methane enrichment.

The growth of microalgae consuming biogas or mixture of air and CO_2 in the concentration range of 2–20% (v/v) (simulating a flue gas from biogas incineration) in laboratory-scale photo-bioreactors. It was proven that the raw biogas (even without the removal of H_2S) could be used as a source of CO_2 for growth of microalgae. The growth rate of microalgae consuming biogas was the same as the growth rate of the culture grown on a mixture of air and food-grade CO_2. Several species of algae can metabolize H_2S (Biebl and Pfennig, 1977). Using a biological system to remove H_2S has similar benefits to using one to remove CO_2: lower upkeep costs, more environmentally sustainable and non-hazardous waste. In this study I will use the different growth kinetics processes of microalgae consuming biogas. That is including different temperature, light intensities, pH level and different type of biogas aeration. These methods will be helpful for find out the optimized condition to impurity removal from the biogas and to enhance the biogas quality. In addition, the biogas calorific value, flash points, heating value and other related parameters were tested by standard methods. All treatments were conducted in three replicated treatments.

Isolation and Identification of Microalgae

The methodology of microalgae collection, isolation and identification process were adopted our previous published papers (Unpaprom et al., 2015a, b; Ramaraj et al., 2015a, b). The sample was collected by plankton net (20-µm pore size) from freshwater fish pond (18° 55′ 4.2″N; 99° 0′ 41.1 ″E) at a location near Maejo University, Sansai, Thailand. The collected samples were samples of about 5 ml were inoculated into 5-ml autoclaved Bold Basal Medium (BBM) in 20-ml test tubes and cultured at room temperature (30±1°C) under 50 µmol-1 m^2 sec-1 intensity with 16:8 h photoperiod for 10 days. After incubation, individual colonies were picked and transferred to the same media for purification in 250 mL conical flask.

The culture broth was shaken manually for five to six times a day. The pre-cultured samples were streaked on BBM medium-enriched agar plates and cultured for another 10 days with cool white fluorescent light using the same light intensity. The single colonies on agar were

picked up and cultured in liquid BBM medium, and the streaking and inoculation procedure was repeated until pure cultures were obtained. The purity of the culture was monitored by regular observation under microscope. The isolated microalgae were identified microscopically using light microscope with standard manual for algae (Bligh and Dyer, 1959; APHA, 2005).

Maintenance of microalgae cultures

Isolated and purified microalgae were inoculated in 250 ml Erlenmeyer flasks containing 125 ml BBM medium. Flasks were placed on a reciprocating shaker at 120 rpm for 7 d at room temperature of 30±1°C. Light was provided by cool white fluorescent lamps at an intensity of 50 µmol-1 m2 sec-1. The algae culture was then transferred to 500-ml Erlenmeyer flasks containing 450 ml. Algae growth were monitored by measuring the optical density of the algal medium with spectrophotometer (Spectronic Genesys 20, Thermo Fisher Scientific) at a wavelength of 665 nm. Measurements were taken daily and three replicates were measured.

Production of microalgae

Figure 5 showed the open type algal cement pond and detailed descriptions through schematic diagram. Two litters' cultured microalgae were transferred to open type cement pond (total volume 200L) for large amount of biomass production through artificial medium. The medium was prepared with rice fertilizer (100g), rice bran (400g), fish meal (100g), lime (50g) and urea (200g). This medium was named as, Rameshprabu medium. Algal cement pond height was 40 cm and length was 80 cm. Furthermore, pond was filled 150 liters water and medium. It was reached 25 cm height in the pond. All ingredients were filled with 10L water subsequently mixed by stir then transfer to the pond; it associated with air pump. Pond was left for one night to release ammonia and medium dissolve in the water properly. Next day stock algae were transfer to the triplicate cement ponds. Algal growth was measured and stirred the everyday to prevent algae precipitation.

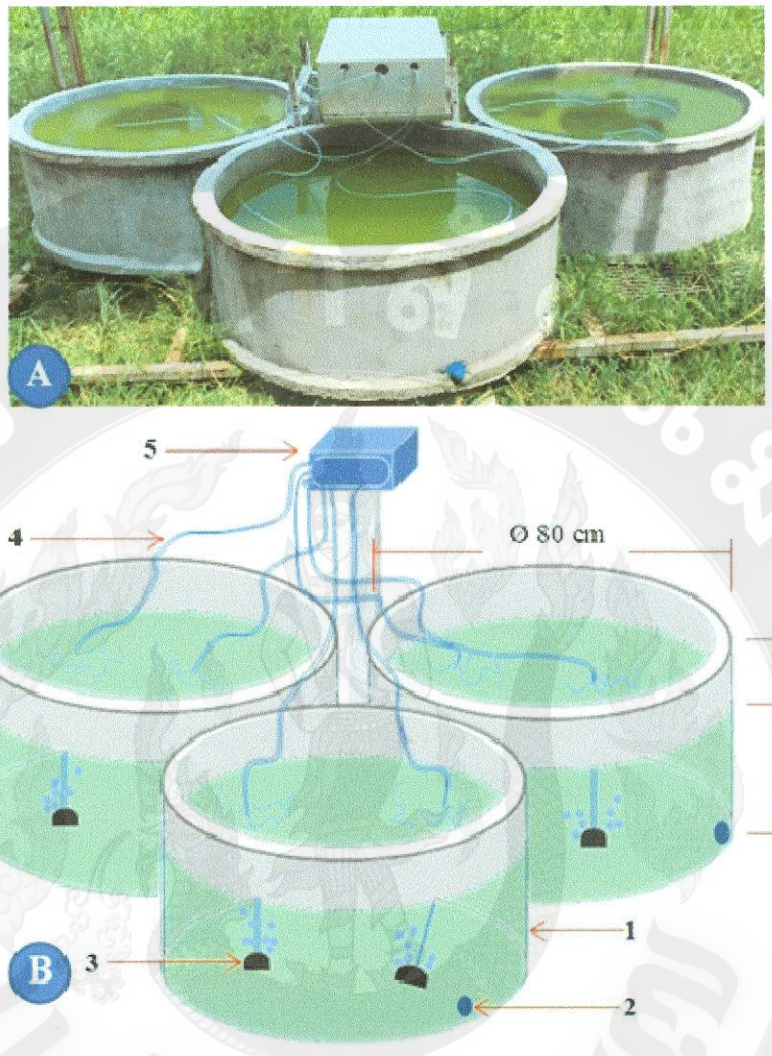

Figure 5 (A) Open type algal cement pond; (B) Schematic diagram of algal cement pond: 1. algae growth, 2. outlet of pond, 3. aeration bubble, 4. tube, 5. air pump.

The cultured microalgae, C. vulgaris were obtained from Energy Research Center, Maejo University, Sansai, Chiang Mai 50290, Thailand. The algae were cultivated using open type cement pond and low cost artificial medium which is named as Rameshprabu medium.

Analytical methods

Chlorophylls estimation Ten ml of sample was taken and centrifuged at 6000 rpm for 15 minutes. Supernatants were discarded and re-suspended in a known volume of methanol, while pellets extracted with 5 ml of 96% methanol extraction. The tubes were wraped with aluminum foil and kept in dark. The samples were centrifuged again and the supernatant were used for measuring the optical density at 663 nm and 645 nm against 96% methanol as a blank by spectrophotometer (Spectronic Genesys 20, Thermo Fisher Scientific). After extraction chlorophyll concentration was determined spectrophotometrically and calculated Chlorophyll content (Chlorophyll a, chlorophyll b and total chlorophyll) were computed using the following equations:

$$\text{Chlorophyll-a } (\mu g\ g/ml) = \{(15.65 \times A_{666} - 7.340 \times A_{653}) \times V/\ 50 \times W\} \times \text{dilution}$$

$$\text{Chlorophyll-b } (\mu g/ml) = \{(27.05 \times A_{653} - 11.21 \times A_{666}) \times V/\ 50 \times W\} \times \text{dilution}$$

$$\text{Total chlorophyll} = \text{chlorophyll-a} + \text{chlorophyll-b}$$

The solids contents, including total solids (TS) and volatile solids (VS), chemical oxygen demand (COD), total nitrogen (TN), total phosphorous (TP), further all the indices including cell count, optical density (OD), pH, alkalinity, biological oxygen demand (BOD), total nitrogen (TN), total phosphorous (TP), total suspended solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) were continuously monitored throughout the study, following the standard protocols were characterized using the Standard Methods for the Examination of Water and Wastewater (method # 2540) (APHA-AWWA-WEF, 2005). Metrohm 774 pH-meter was used in all pH measurements. Fatty acid content was performed by GC-MS analysis. Protein, carbohydrates, lipids determination procedures were adopted from Tipnee et al. (2015). Moisture content of raw materials was determined following the procedure given in ASTM Standard D 4442-07. The residual sample in the crucible was heated without lid in a muffle furnace at 700 ± 50°C for one half hour. The crucible was then taken out, cooled first in air, then in desiccators and weighed. Heating, cooling and weighing was repeated, till a constant weight obtained.

Fatty acid content was performed by GC-MS analysis. Protein, carbohydrates, lipids determination procedures were adopted from Tipnee et al. (2015). Biogas estimation method was adopted from literature (Pavlostathis and Giraldo-Gomez 1991, von Sperling and Chernicharo 2009).

Total fat, ash, moisture, fiber contents and volatile fatty acids (VFA) were determined using AOAC official method [15]. The entire experiments were done in triplicate. Elemental composition (C, H, N, O, S) was analyzed using the element analyzer (Perkin–Elmer 2004). Biogas estimation method was adopted from literature (Pavlostathis and Giraldo-Gomez 1991, von Sperling and Chernicharo 2009). The composition of biogas (CH_4, CO_2, H_2S, H_2 and O_2) was measured using a biogas analyzer (GFM 416 series, UK).

The inoculums characteristics including TS, VS, COD were 296.1 ± 0.05 mg/L, 158.5 ± 1.15 mg/L and 1241.6 ± 2.01 mg/L, respectively; along with alkalinity of 136.4 ± 0.04 mg/L as $CaCO_3$, VFA of 136.4 ± 0.25 mgCH_3COOH/L and pH was 6.66 ± 0.03.

Statistical analyses

All experiments were determined in biological triplicate to ensure the reproducibility. Experimental results were obtained as the mean value ± SD. Statistical analyses were performed using Microsoft Excel and SPSS statistical package (SPSS Inc., Chicago, IL, USA). The statistical significances were achieved when $p<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

Algae Cultivation

Macroalgae Cultivation

Morphological study of Spirogyra Spirogyra is a genus of filamentous green algae in the order Zygnematales. The name indicates the helical or spiral arrangement of the chloroplasts, which is the main diagnostic characteristic of the genus. The *Spirogyra* species typically develops unbranched filaments and is one cell thick, which grows longer through normal cell division. There are more than 400 species of *Spirogyra* in the world. Vegetative growth of *Spirogyra* can be recognized by three characteristics: (1) type of cross walls (plane, replicate, semireplicate or colligate), (2) cell length and width and (3) chloroplast numbers.

There are classical and standard morphological methods that were used in the identification of the *Spirogyra* specimens with help of specific literatures (Randhawa 1959, Transeau 1951, Vidyavati, 1995, Kargupta and Jh 2004, Taft 2009). The morphological characteristics of each sample were recorded via cell dimensions, along with the number and arrangement of chloroplast spirals/pyrenoids. The morphological characteristics of biological parameters were also studied and presented in Table 2. The classical morphologically based methods are used for the identification of *Spirogyra* specimens. The structure of species from this study demonstrated definitive identity matches in the range of 99% for the agreement of *S. ellipsospora*. Light microscopic picture of macroalgae *S. ellipsospora* was presented in Figure 6.

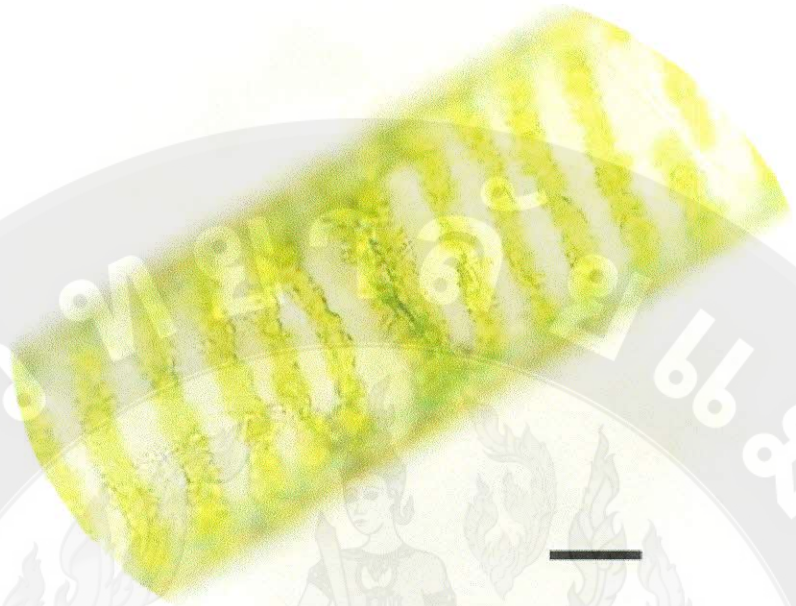

Figure 6 Light microscopic pictures of macroalgae *Spirogyra ellipsospora*

Table 1 Morphological characteristic of *S. ellipsospora*.

Type of parameters	Parameters	Equipments and methods	Characteristics
Biological	Width of cell	Light microscope	120–150
	Length of cell		90–280
	Chloroplasts per cell		5–8
	Vegetative cell width (μm)		35–85
	Vegetative cell Length (μm)		80–190
	L/W ratio vegetative cell		2.0–3.4
	Number of chloroplasts		2–5
	Shape of zoospore		Ellipsoid
	Zoospore width		60–73
	Zoospore length		75–95

S. ellipsospora growth and biomass measurement

Biomass was a critical measurement in the algal harvesting process for applications. A number of methods had been developed to estimate and quantify, which were useful in different cases (Ramaraj 2013, Ramaraj et al. 2015c). Different methods were available such as dry weight: Total suspended solids, volatile suspended solids and fixed suspended solids; wet weight method; chlorophyll (Chl) method: Chl-a, Chl-b and Chl-a+b), epifluorescence microscopy, bioluminescence, photometric, turbidity, packed cell volume and cell count etc (Unpaprom et al. 2015). According to Ramaraj et al. (2013), algal biomass measurement and roughly we could classify into two groups, (1) direct index such as dry weight and (2) indirect index such as chlorophyll, so-called proxy index.

Chlorophyll is the most widely used proxy measurement of algae or phytoplankton and their determination is relatively simple and straightforward. In this study, we used chlorophylls measurement to analysis biomass. Chl-a as an algal biomass measurements in natural systems was very popular. Chl-b is used to calculate pigment concentrations. The total Chl-(a + b) is used to measure algal growth (Ramaraj et al. 2010). Growth system was setup outdoor conditions. Algae biomass measured by Chl-a, Chl-b and total chlorophyll results were average as 9.36 µgmL-1, 3.88 µgmL-1 and 13.24 µgmL-1, respectively. Accordingly, this study presents results to produce algae biomass using the natural water and result was encouraging.

The potential of natural water medium for *S. ellipsospora* long-term experiments and stock maintenance

The utilization of natural water medium which came from water body directly without any extra nutrition addition, demonstrated the potential to adopt the algal function for natural growth and long time surveillance. The study confirmed that macroalgae can get essential nutrition from natural water body (natural water medium). Utilizing this growth uptake function we could apply the natural medium in controlled environments such as lab (outdoor lab scale) or field scale growth units or even further applied in natural environment, but nowadays most of researchers and algae manufactures are using artificial medium which is expensive to produce algal biomass. Our study could take advantage of nutrients available in natural water to reduce the total cost, long-term experiments and stock maintenance.

Figure 7 Macroalgae growth system

The productivity of macroalgae cultured for 6 cycles of 6 days using with outdoor lab environment to imitate the natural system. The culture that is continuously provided natural water medium each of cycle ends. Macroalgae were placed in 10 L cylindrical tanks in an outdoor system to be cultured for 36 days (Figure 7). Biomass was initially stocked at 2 g/L fresh weight (fw) for *S. ellipsospora*. The algae were cultivated in a batch culture system, described in detail previously (in methodology part). Biomass was harvested every 6 days (6 cycles of 6 days each in total) using a net , spun to a constant fresh weight, weighed and subsequently re-stocked at initial stocking densities for a new cycle. Stock maintenance, long time experiment and growth of *S. ellipsospora* are an essential for its subsequent use in biotechnology. For this purpose, we tested the suitability of the stock maintenance and growth of algal species is essential for their use in biotechnology. Therefore, natural water medium is the most suitable culture media and ease of laboratory culture is relevant topics. This environmental friendly process offers a substantial potential source of algae biomass to provide bioenergy and to reduce the greenhouse gas, carbon dioxide.

Microalgae Cultivation

Species Identification

For many years, strains of *Chlorella* (Chlorophyceae, Chlorococcales) have served as model organisms in plant physiology and biochemical research. Currently available information was positively identifying an environmental strain of the *Chlorella* genus of freshwater unicellular green algae. The genus *Chlorella* encompasses spherical or ellipsoidal non-motile green cells that produce autospores, and inhabit freshwater, soil and marine habitats. Its commercial potential has been considered since 1960, being the first microalga to be mass cultured for food, feed and as a source of nutraceuticals. More recently, it has also been suggested that they are good candidates for fuel production and biogas purification (Castorena-Gonzalez et al., 2013; Ramaraj and Dussadee, 2015).

Since the characteristics traditionally used for taxonomy are sufficient and morphological criteria for the identification of *Chlorella* species. According to taxonomical grouping based on morphology and physiological properties, it belongs to genus *Chlorella*, family Oocystaceae, order Chlorococcales, class Chlorophyceae, division Chlorophyta of the kingdom Plantae. *C. vulgaris* is a spherical microscopic cell with 2-10 µm diameter and has many structural elements similar to plants (Blair et al., 2014). The individual cells of the colonies were in the range of 10 µm. Cells are green color, unicellular, spherical in shape; figure 8 shows the morphology of *C. vulgaris* observed under a light microscope. The cell wall contains hemicelluloses, which accounts for the stability and rigidity of the cells. It has an asexual reproductive cycle, with the production of autospores from the mature large cell, by dividing the cell into smaller units. One mature cell divides into four new ones every 16-20 hours. The algal cells utilize sunlight for photosynthesis. The photosynthetic rate exceeds the respiration rate of *Chlorella* cells by 10–100 times.

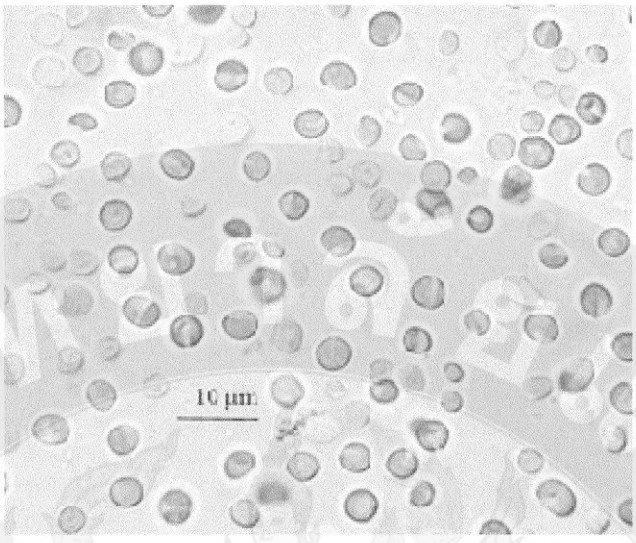

Figure 8 *Chlorella vulgaris.*

Determination of algae growth and biomass production

Algae growth in this study would be presented by optical density (OD 685) condition and cell densities were determined through cell counts under optical microscope using an improved bright lined haemocytometer. Optical density and cell count clearly indicated that the best growth of *C. vulgaris* was through Rameshprabu medium using open type cement ponds shown in figure 9 and 10. Their growth increased rapidly in the first six days, then slowed down in the next six days, and again from thirteen days growing up, which could be due to the gradual consumption of certain nutrient elements like nitrogen and phosphorus in the medium. C. vulgaris have been widely used in numerous field applications for their strong survival abilities and efficient utilization of nitrogen and phosphorus (Pantawong et al., 2015; Ramaraj et al., 2015; Unpaprom et al., 2015; Ramaraj and Unpaprom, 2016).

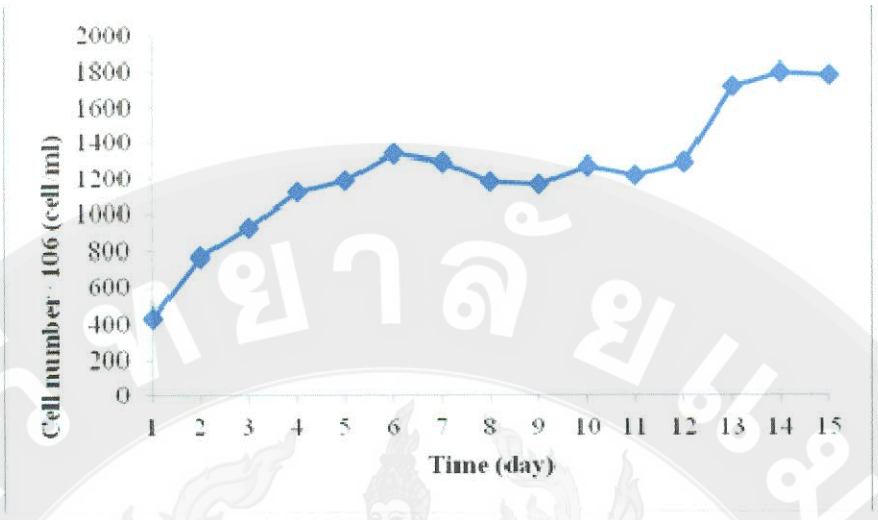

Figure 9 *C. vulgaris* growth determined through cell count.

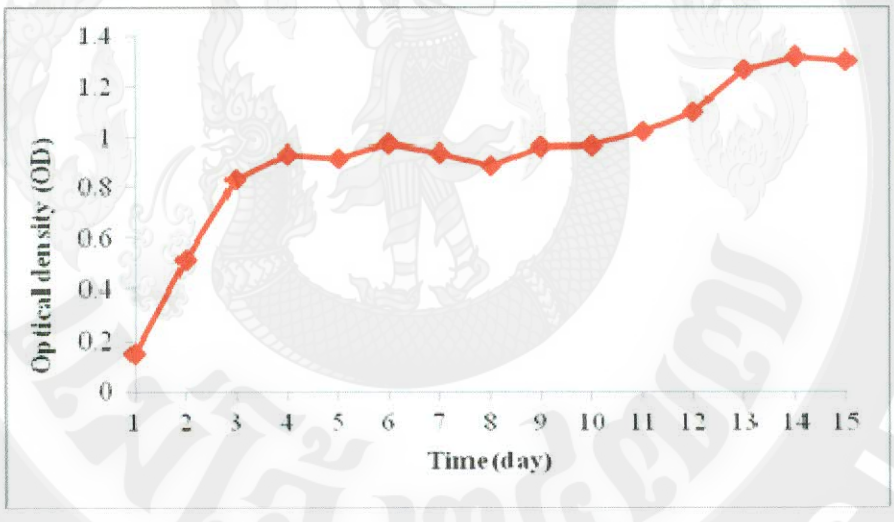

Figure 10 *C. vulgaris* growth estimated through cell densities.

While CO_2 is available abundantly in the atmosphere as well as from anthropogenic sources, the availability of light is very important for the algae growth (Blair et al., 2014). It is known that environmental factors such as light intensity, temperature and nutrients can significantly affect the composition of microalgae (Guschina et al., 2015). Furthermore, Ramaraj et al. (2015) demonstrated that algae's ability to uptake CO_2 from the atmosphere and much greater biomass productivity compared to land plants. This study also proved that the microalga C. vulgaris could grow well in open type cement pond utilization atmospheric CO_2 and might be

a good candidate microalga for the biogas purification if could apply in the closed system utilizing biogas as a carbon source. Algal biomass by direct measurement of total suspended solids and volatile suspended solids resulted as 474.12±0.15 mg/L and 396.25±0.03 mg/L, respectively.

Chlorella vulgaris cultural conditions

The values of measured physicochemical and biological parameters in the microalgal growth conditions of this study are summarized in table 1 and figure 5. The most important parameters are nutrient quantity/quality, light, temperature and pH (Guschina and Harwood, 2004; Ramaraj et al., 2015a). Growth system was setup in the open pond system for utilize solar energy directly. This study was aimed to find a strategy to reduce the costs and environmental impacts of *Chlorella* biomass production under autotrophic conditions used open type cement ponds. Ramaraj et al. (2015b) confirmed that photoautotrophic microalgae require several things to grow. Because they are photosynthetic, they need a light source, CO_2 as energy and carbon sources, water, and inorganic salts (Ramaraj, 2013).

The biomass produced absorption of atmospheric CO_2, possibly driven by a decline in the CO_2 partial pressure resulted from photosynthesis. Carbon, phosphorus and nitrogen are considered to be the most important nutrients for algae growth; the results are shown in Table 1. COD measured the nutrition carbon on natural water medium and reactor effluent; the medium was 998±1.16 mg/L, while the reactor was 802.5±0.71 mg/L. After algae growth, the concentration of COD increased significantly. In the medium, nitrogen was 105.5±0.22 mg/L, while in it ponds were 50.59±70.71 mg/L. For alkalinity, the content was reduced medium to ponds. Alkalinity was an important buffering to maintain a fairly optimal growth range in the water body and the changes were consumed by algae growth from its role as one of possible carbon sources. Chlorophyll a production by phytoplankton cells is known to vary with growth conditions; in our experiments, the maximum production of chlorophyll a content of *C. vulgaris* was 28.89±1.33 (mg/g).

Table 2 Physiochemical parameter

Parameters	Rameshprabu medium		Alga cement pond	
	Mean (± S.D.)	Range	Mean (± S.D.)	Range
pH	7.53 (±0.15)	7.4-8.2	8.38 (±0.22)	8.2-8.6
Temperature (°C)	-	-	32.56 (±0.78)	31.4-33.7
Light intensity ($\mu mol^{-1}m^{-2}$)	-	-	32.56 (±0.11)	31.4-33.7
DO (mg/L)	8.5 (±1.25)	7-10	13.5 (±0.05)	10-17
TN (mg/L)	105.5 (±0.22)	94-117	50.59 (±70.71)	24.6-84.6
TP (mg/L)	42 (±0.43)	36-48	31.9 (±0.35)	20.7-42.5
Alkalinity ($mgCaCO_3/L$)	29.03 (±1.52)	25-33	9.51 (±2.5)	1.5-20.7
COD (mg/L)	998 (±1.16)	1006-1013	802.5 (±0.71)	560-987
BOD (mg/L)	600 (±2.01)	615-630	427 (±0.66)	190-640
TSS (mg/L)	-	-	474.12 (±0.15)	319-737
VSS (mg/L)	-	-	396.25 (±0.03)	266-537
FSS (mg/L)	-	-	77.63 (±0.02)	60-199

Chlorella vulgaris chemical compositions

Photoautotrophic microalgae can effectively transform the inorganic nutrients, CO_2, H_2O and other substances into organic compounds such as protein, carbohydrate, lipid and other ingredients through photosynthesis. Microalgal biomass, containing lipids, starch, cellulose, proteins, and so on, is considered a promising feedstock for producing a variety of renewable fuels, such as biodiesel, bioethanol, biohydrogen and biogas (Ramaraj et al., 2015; Unpaprom et al., 2015; Ramaraj and Unpaprom, 2016). The proximate and ultimate analysis of *C. vulgaris* illustrated in table 2. The major fatty acid composition was determined using GC- MS system (table 3). Green algae have the bulk of their fatty acids as saturated and unsaturated C18 s, a composition similar to that of vegetable oils (Benemann and Oswald, 1996; Ramaraj, 2013). In this study, *C. vulgaris*, palmitic acid (C16:0), physetoleic acid (C16:1), and oleic acid (C18:1) were commonly dominant. In particular, lipids with high content of unsaturated fatty acids had been reasonable balance of fuel properties.

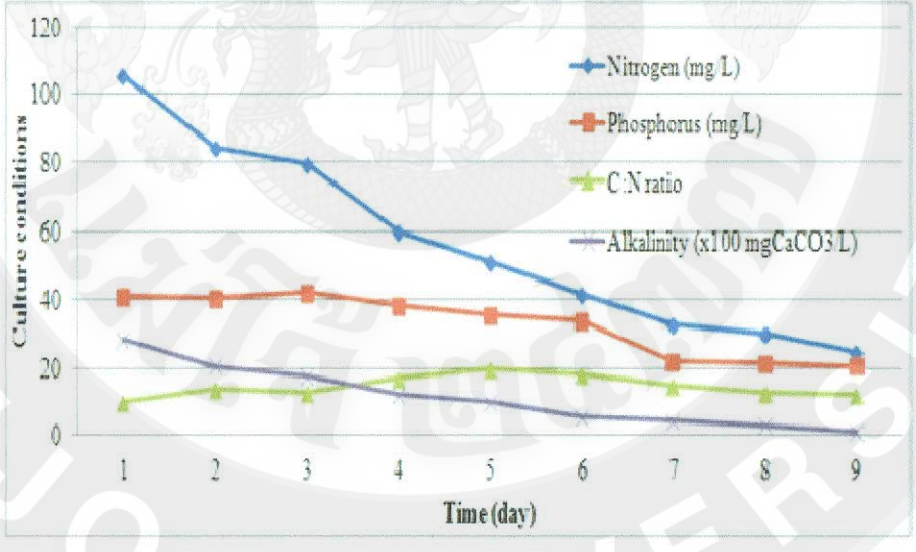

Figure 11 Culture conditions of *Chlorella vulgaris*.

Table 3 Fatty acids analysis of *C. vulgaris*

Parameters		*C. vulgaris*
Proximate Analysis (%)	Moisture	9.87
	Ash	14.87
	Carbohydrate	29.85
	Protein	48.88
	Lipid	13.60
	Fiber	17.06
Ultimate Analysis (%)	Carbon	48.56
	Hydrogen	6.40
	Oxygen	33.71
	Nitrogen	6.26
	Sulphur	0.79

Table 4 Fatty acids analysis of *C. vulgaris*

Fatty acids	Value
C14:0	3.0± 0.2
C16:0	36.2± 1.5
C16:1	1.8± 0.1
C16:2	1.1± 0.2
C16:3	1.3± 0.1
C18:0	5.46± 0.3
C18:1	18.33± 1.2
C18:2	16.7± 0.6
C18:3	19.8± 0.7
MUFA [a]	20.1± 1.2
PUFA [b]	36.4± 2.2
UFA [c]	57.2± 2.7
DUS [d]	1.16 ± 0.03

Note: a percentage of total fatty acids (%); b MUFA= monounsaturated fatty acids, c PUFA= polyunsaturated fatty acids, d UFA= unsaturated fatty acid, e DUS = degree of fatty acid unsaturation.

Biogas Production form Macroalgae

Biomethane Potential Analysis

The well known use of the microbiological process of anaerobic digestion (AD) to generate biogas (mixture of methane and carbon dioxide) is now widely implemented for the production of renewable energy worldwide; bio-methane potential (BMP) tests are commonly used in studies concerning the AD of organic solids (Ramaraj et al., 2015c). Macroalgae can be converted to biofuels by various processes including thermal processes and fermentation. The most direct route to obtaining biofuel from macroalgae is via AD to biogas (Hughes et al., 2012, Montingelli et al., 2015). Algal biomass contains considerable amount of biodegradable components such as carbohydrates, lipids and proteins. This makes it a favorable substrate for anaerobic microbial flora and can be converted into methane rich biogas (Sialve et al., 2009). In spite of the fact that macroalgae have high potential for biogas production, there are some studies on anaerobic digestion of macroalgal biomass utilizing Chaetomorpha linum, Saccharina latissima, Gracillaria vermiculophylla and Ulva lactuca biomass.

Apparently, the BMP of algae depends mainly on its composition, which itself depends on the growth conditions and and is specific species. The concentration of substrate in the BMP assay also impacts on the final biodegradability and methane productivity (Sialve et al. 2009). When the C, H, O and N composition of a wastewater or substrate is known, the stoichiometric relationship reported by Buswell and Boruff (1932) and Angelidaki and Sanders (2004), and can be used to estimate the theoretical gas composition on a percentage molar basis. In this equation, the organic matter is stoichiometrically converted to methane, carbon dioxide and ammonia. The specific methane yield expressed in liters of CH_4 per gram of volatile solids (VS) can thus be calculated as:

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

Eq. (1) is a theoretical approach that allows estimation of the maximum potential yields. Using Eq. (1), it is possible to compute a theoretical specific methane yield. Composition of methane and biogas production from *S. ellipsospora* details were presented in Table 3 (by dry weight basis). The biogas composition of carbon dioxide (44%), methane (48%) (less than 1~) and hydrogen sulphide of was estimated from the biogas. The carbon, hydrogen, nitrogen, oxygen and hydrogen sulphide content tested in this study. The algae showed distinct differences in their chemical composition. The carbon, hydrogen, nitrogen, oxygen and sulphur contents of were in *S. ellipsospora*, 41.87%, 6%, 35.77%, 4.27% and 0.43%, respectively. Consequently *S. ellipsospora* has plenty of nutrients for biogas production process; it is suitable to be used as energy crops for biogas production.

Gross composition of several algae species were presented by Becker (2007). As expected, the species that can reach higher lipid content have a higher methane yield. COD is commonly used in the water and wastewater industry to measure the organic strength of liquid effluents. It is a chemical procedure using strong acid oxidation. The strength is expressed in 'oxygen equivalents' i.e. the mg O2 required to oxidize the C to CO_2. However, the COD concept could be estimate the methane yield (Pavlostathis and Giraldogomez 1991, von Sperling and Oliveira 2009, Than et al. 2014). One mole of methane requires 2 moles of oxygen to oxidise it to CO_2 and water, so each gram of methane produced corresponds to the removal of 4 grams of COD.

$$\underset{16}{CH_4} + \underset{64}{2O_2} \rightarrow CO_2 + H_2O \quad \text{..... Eq. (2)}$$

or

1 kg COD is equivalent to 250 g of methane.

1 kg COD $\Rightarrow$ 250g of CH_4

250 g of CH_4 is equivalent to 250/16 moles of gas = 15.62 moles

1 mole of gas at

standard temperature and pressure (STP) = 22.4 liters

Therefore 15.62 x 22.4 = 349.8 liters = 0.35 m^3.

In our study, the sample content of total solids (TS) and volatile solids (VS) was measured; the results were average as 16622 mg/kg and 13959 mg/kg, respectively. The average pH was 7.4 and average COD 14236 mg/L. Methane formation takes place within a relatively narrow pH interval, from about 6.5 to 8.5 with an optimum interval between 7.0 and 8.0. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5. The pH value increases by ammonia accumulation during degradation of proteins, while the accumulation of VFA decreases the pH value. The accumulation of VFA will often not always result in a pH drop, due to the buffer capacity of the substrate (Mösche and Jördening 1999, Wang et al. 1999, Weiland 2010). According to the COD estimation, our study shows the mixed culture microalgal biomass is a potentially valuable fermentation substrate, and produce 1.9930 L (0. 002 m^3) of methane gas.

In conclusions, production of biofuels is undoubtedly one of the best solutions for declining the crude oil reserves and global warming due to excessive greenhouse gasses emissions. As fossil fuel prices increase and environmental concerns gain prominence, the development of alternative fuels from biomass has become more important. Biogas is considered a renewable energy carrier. As demonstrated here, macroalgal biogas is technically feasible. Macroalgae have several advantages over terrestrial plants such as higher photosynthetic efficiencies, lower need for cultivation area, higher growth rates, more continuous biomass production, no direct competition with food production, and possibility to use artificial medium, natural water medium (freshwater/marine water) and wastewater for biomass production. The algae biomass thus produced constitute an additional source of organic substrate in the installation for biogas production. The biogas production was 910.10 L/kg. Therefore, fast growing, high-yielding and rich in organic matter of *S. ellipsospora* was promising energy crops for biogas production. This suggested that it is possible to achieve stable operation using *S. ellipsospora*, as a substrate for biogas production in pilot or large scale biogas plant in the future.

Table 5 Composition of methane and biogas production from *S. ellipsospora*

Elements	Percent	Mol[a]	CH_4[b]	CO_2	NH_3	Biogas (L/kg)
C	41.87	3.49				
H	6.00	6.00				
O	35.77	2.24	48.00 (%)	43.96 (%)	8.04 (%)	910.10
N	4.27	0.31				
S	<1					

[a] Mol=g/Mw ; [b] Volume content of CH_4 calculated from

Laboratory Scale Macroalgae Biogas Production

Macroalgae, *Spirogyra ellipsospora* to biogas: anaerobic digestion Macroalgae can be converted to biofuels by various processes including thermal treatment (Zhou et al., 2010) and fermentation (Adams et al., 2009; Goh and Lee, 2010) but the most direct route to obtaining biofuel from macroalgae is via its anaerobic digestion to biogas (~60% methane). Methane can be used to produce heat and electricity or compressed for use as a transport fuel. Since this time there have been developments in AD technology and an enormous increase in its use Hughes et al. (2009). The study results of biogas and methane production from macroalgae, S. *ellipsospora* presented in figure 3 and 4. Without any pretreatment methane content was achieved 64.67 %. Our studies agreed with Hughes et al. (2009) and also confirmed better results; on 70th day day CO_2 level was 31.5 % and H_2S was 578 ppm (i.e. 0.0578%). For the comparison of freshwater macroalgae biogas production and methane content from *S. ellipsospora* was much higher than *Spirogyra* neglecta, Chara fragilis and Cladophora glomerata, which was reported by Baltrenas and Misevičius (2012).

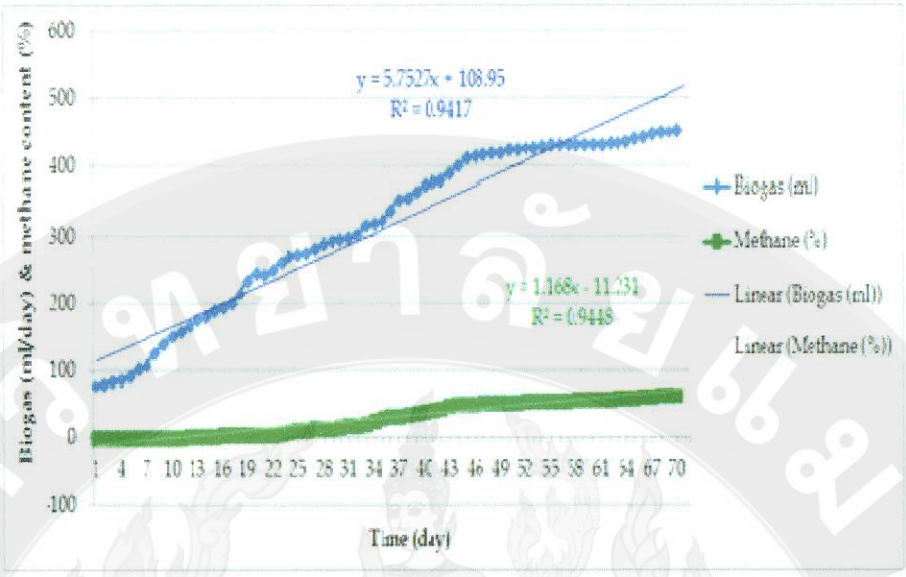

Figure 12 biogas and methane production from macroalgae, *S. ellipsospora*.

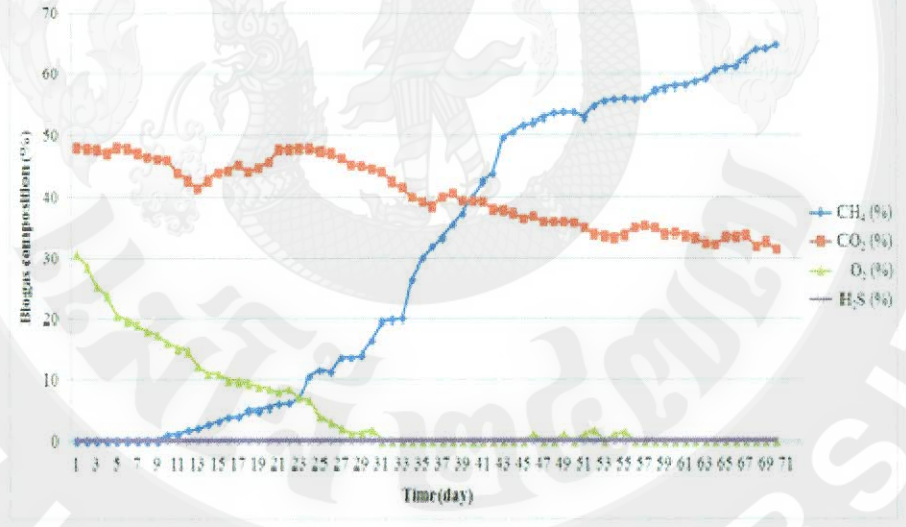

Figure 13 biogas composition of macroalgae, *S. ellipsospora* during anaerobic digestions.

Effect of pre-treatment on solubilization and biogas yield

Beside pure mechanical comminuting by chopping, milling or shredding various other forms of physical and chemical or biological pre-treatment are known (Hendriks and Zeeman, 2009), leading not only to particle size reduction, but to as the rate-limiting step in anaerobic

digestion of solid materials such as energy crops and crop residues is hydrolysis of complex polymeric substances (Mata-Alvarez et al., 2000). Since, cellulose, hemi-cellulose and lignin comprise up to 75% of dry matter of biomass (van der Weijde er al., 2013). Macroalage was lignocellulose rich materials and lignocellulose is in most cases extremely resistant to anaerobic digestion (de Araújo et al., 2013). Macroalgae contains lignin, cellulose and hemicelluloses (de Araújo et al., 2013). Hence, pretreatment can enhance the bio-digestibility of the wastes for biogas production and increase accessibility of the enzymes to the materials. It results in enrichment of the difficult biodegradable materials, and improves the yield of biogas from the biomass. Therefore, pretreatment process was needed to be able to get a high biogas yield. The study results showed in figure 3 and 4.

Two different pretreatment methods for macroalage were investigated. In order to determine how each method affects on the composition of the grass, the digestibility of the grass in biogas production. In addition pretreatment methods also compared with untreated grass (i.e. control); the effect of pre-treatment characteristics and biogas yield. The pre-treatments were performed in batch mode with TS, VS, COD and pH, and all pre-treatment conditions. The biogas yield was calculated from VS and composition of biogas was measured using a biogas analyzer (GFM 416 series, UK).

The study results were clearly exhibited that NaOH pretreated sample produced high yield of biogas than untreated (raw) and hot water pretreated samples. Furthermore, the results show that alkali pre-treatment resulted in higher at 1% of NaOH reacted with 48 h biogas result was 490 mg/l VS, moreover 1% of NaOH and 48 h reaction time could be better for cost and time saving. The VS/TS ratio is very important for biogas production. The range of VS/TS was 0.6-0.8 was produced higher amount methane (Lübken, et al., 2010; Deublein and Steinhauser, 2008). This study results also demonstrated similar range with literature. Also, alkali pretreatment opened the bonds between cellulose, hemicelluloses and lignin. Pretreatment most likely dissolved a portion of the lignin and hemicelluloses components, producing a soluble substance and allowing more access for the hydrolysis process. The substrate porosity for pretreated material increase after alkali pretreatment, which could improve contact between microorganism, and thus facilitate the hydrolysis which is the first phase of biogas fermentation.

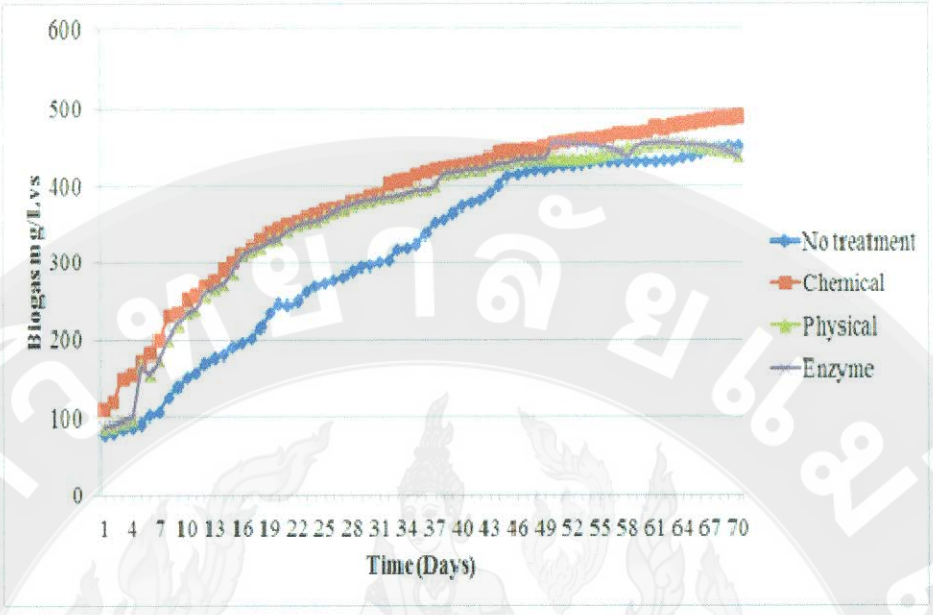

Figure 14 biogas productions with different pretreatments.

Ward et al. (2008) declared that a higher concentration of alkali might result in a decline in biogas yield. Extra induction of sodium (Na+) would inhibit the activity of anaerobic microbes and thus cause a decline in biogas yield. Na+ could be increased with increasing of NaOH concentration; that reason may be 2% and 3% of NaOH inhibited the microbial function in the system and 1% of NaOH concentration on pretreatment process was suitable for biogas production of macroalgae as monosubstrate. Consequently, this study was selected 1% of NaOH with 48 h reaction time for further scale up study.

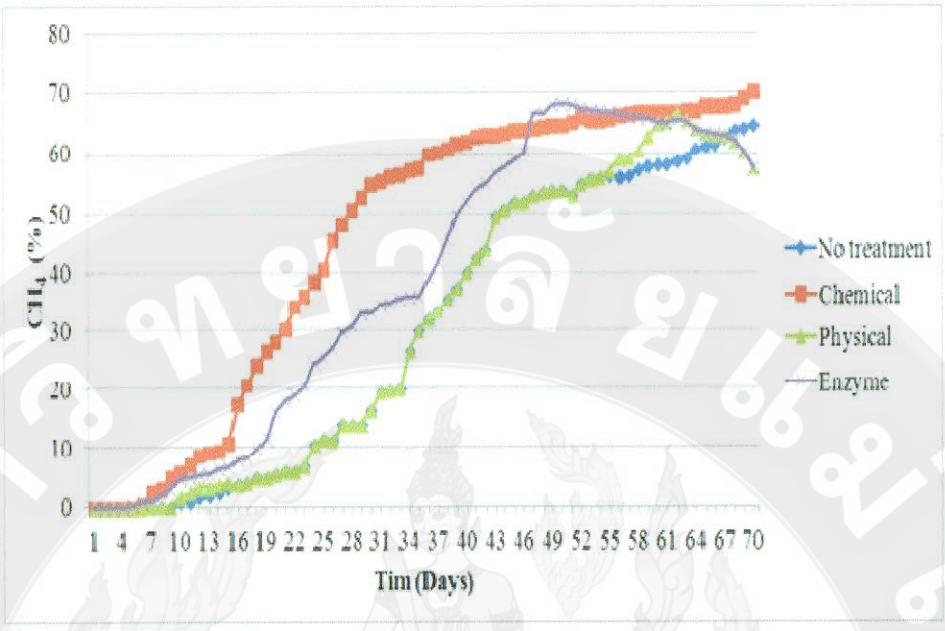

Figure 15 methane contents with different pretreatments

Biogas Purification

Biogas Purification Through Biological Process

The main components of biogas are CH_4 and CO_2, but usually biogas also contains H_2S other sulphur compounds, water, other trace gas compounds and other impurities. Purification and upgrading of the gas is necessary because purified biogas provides reductions in green house gas emissions as well as several other environmental benefits when used as a vehicle fuel [7]. Reducing CO_2 and H_2S content will significantly improve the quality of biogas. For biogas impurity removal, biological processes are environmentally friendly and feasible. Biogas purification using algae involved the use of algae's photosynthetic ability in the removal of the impurities present in biogas.

Ramaraj and Dussadee (2015) stated that algae application of CO_2 sequestration has developed as a popular topic and the current interests are including: species, power plant flue gas utilization, reactor design, growth condition, growth kinetics and modeling. The most studies in the literature concerned the maximum CO_2 uptake rate by the artificial photo-bioreactors (Ramaraj et al., 2014a). Among those techs, bio-eco- technology is the most natural and ecological way to accomplish the designed targets by the utilization of "self-designed" bio-functions of nature (Frigon

et al., 2003; Tsai et al., 2015, 2016). Further our establishment study, *C. vulgaris* applicable for biogas purification.

CO_2 Microalgae can fix CO_2 using solar energy with efficiency ten times greater than terrestrial plants (Ramaraj, 2013; Ramaraj et al., 2013). Therefore, we require the rapid development of bio-carbon-fixation technology to eliminate the adverse effects of CO_2, to transfer atmospheric CO_2 through the carbon cycle and to promote carbon balancing ecologically. The most studies in the literature concerned the maximum CO_2 uptake rate by the artificial photo-bioreactors (Ramaraj et al., 2014a; b). Among those techs, bio-eco-technology is the most natural and ecological way to accomplish the designed targets by the utilization of "self-designed" bio-functions of nature (Tsai et al., 2015, 2016). Accordingly, algae production has a great potential for CO_2 bio-fixation process and deserves a close look.

Biogas purification/scrubbing using algae involved the use of algae's photosynthetic ability in the removal of the impurities (mainly CO_2 and H_2S) present in biogas, leaving a purified biogas containing almost pure methane, which could be used for energy generation. Biological purification technology is worth examining because has double impact. The method about removing CO_2 from biogas by microalgal culturing using the biogas effluent as nutrient medium and effectively upgrade biogas also simultaneously reduce the biogas effluent nutrient (Yan and Zheng, 2013). Using biogas as a source of carbon dioxide has two main advantages: the biomass production costs are reduced and the produced biomass does not contain harmful compounds, which can occur in flue gases. Hendroko et al. (2011) verified exhibit that microalgae (*Scenedesmus* sp.) in laboratory experiments using biogas slurry as growing medium and biogas are given periodically generate 21% of CO_2 compared with 24% of controls. There are several authors (Rittmann (2008), Hendroko et al. (2011) and Yan and Zheng (2013) reported that *Arthrospira* sp, Chololera vulgaris SAG 211-11b, *Chlorella* sp. MM-2, *Chlorella* sp. MB-9, *Chlorella vulgaris* ARC1, *Chlamydomonas* sp. and *Scenedesmus* sp. was a positive synergy with biogas. The productivity of the system with Zarrouk media and biogas almost 5 times higher than that for the same media without biogas when piggery waste was used, the utilization of biogas brings a productivity gain of about 2–5 times higher (Hendroko et al., 2011).

Kao et al. (2012) demonstrates that the microalga *Chlorella* sp. MB-9 was a potential strain which was able to utilize CO_2 for growth when aerated with desulfurized biogas (H_2S<50ppm) produced from the anaerobic digestion of swine wastewater. The demonstrated system can be continuously used to upgrade biogas by utilizing a double set of photobioreactor systems and a

gas cycle-switching operation. Furthermore, they demonstrated that the efficiency of CO_2 capture from biogas could be maintained at 50% on average, and the CH_4 concentration in the effluent load could be maintained at 80% on average, i.e., upgrading was accomplished by increasing the CH_4 concentration in the biogas produced from the anaerobic digestion of swine wastewater by 10%.

Some literatures mentioned about the cultivation microalgae using biogas as CO_2 provider. Kao et al. (2012a) used biogas that contained 20±2% CO_2 for *Chlorella* sp. culture with variation of light intensity which was at cloudy and at sunny day. Kao et al. (2012) used biogas that contained 20±1% CO_2 for *Chlorella* sp. culture with variation flow rate of biogas which was 0.05; 0.1; 0.2; 0.3 vvm. Douškova et al. (2010) investigated the potential of biogas as CO_2 provider for *Chlorella vulgaris*; and optimization of biogas production from distillery stillage is described. The growth kinetics of microalgae *Chlorella* sp. consuming biogas or mixture of air and CO_2 in the concentration range of 2–20% (v/v) (simulating a flue gas from biogas incineration) in laboratory-scale photo-bioreactors. It was proven that the raw biogas (even without the removal of H_2S) could be used as a source of CO_2 for growth of microalgae. The growth rate of microalgae consuming biogas was the same as the growth rate of the culture grown on a mixture of air and food-grade CO_2. Several species of algae can metabolize H_2S. Using a biological system to remove H_2S has similar benefits to using one to remove CO_2: lower upkeep costs, more environmentally sustainable and non-hazardous waste.

Furthermore, Tongprawhan et al. (2014) used oleaginous microalgae to capture CO_2 from biogas for improving methane content and simultaneously producing lipid. They screened several microalgae for identify their ability to grow and produce lipid using CO_2 in biogas. Finally, they reported a marine *Chlorella* sp. was the most suitable strain for capturing CO_2 and producing lipid using biogas (50% v/v CO_2 in methane) as well as using 50% v/v CO_2 in air. Sumardiono et al. (2014) established to evaluate the design of the photobioreactor system for purifying biogas through the culturing of microalgae. This system represented a simple promising way for the current forthcoming technologies of biogas purification. It helps to decrease the concentration of CO_2 in biogas concomitantly producing microalgae biomass. The microalgae Nannochloropsis is able to use CO_2 from biogas produced from the anaerobic digestion of tannery sludge. The results show that cultivation of microalgae under the biogas to scrub out CO_2 and promote enrichment of methane in the biogas in this work and obtained scrubbing of 27% from 30%.

The biocapture of CO_2 by microalgae can be applied to improve the quality of biogas by reducing the CO_2 content as this would lead to an increase in the methane content [33]. The microalgae *Chlorella* sp. was analyzed in terms of conditioning biogas. As a result the biogas components CO_2 and H_2S could be reduced up to 97.07% and100%, respectively. Also an increase of microalgae cell count could be documented, which provides interesting alternatives for the production of algae ingredients. Consequently, the algae biological purification is an alternative to other biogas purification methods.

Biogas Purification Through Biological Process Microalgae

The entire unit of biogas purification through microalgae *C. vulgaris* system and schematic view of the experimental set up during biogas upgrading processes were shown in figure 5. The microalgal culture obtained directly from the open pond system which was grown near this experimental zone. Ten liter of *C. vulgaris* cultures was used through 10 L duran bottles. The biogas obtained from macroalgae fermenter which is involved in the study. The macroalgae, *S. ellipsospora* biogas production processes are above mentioned.

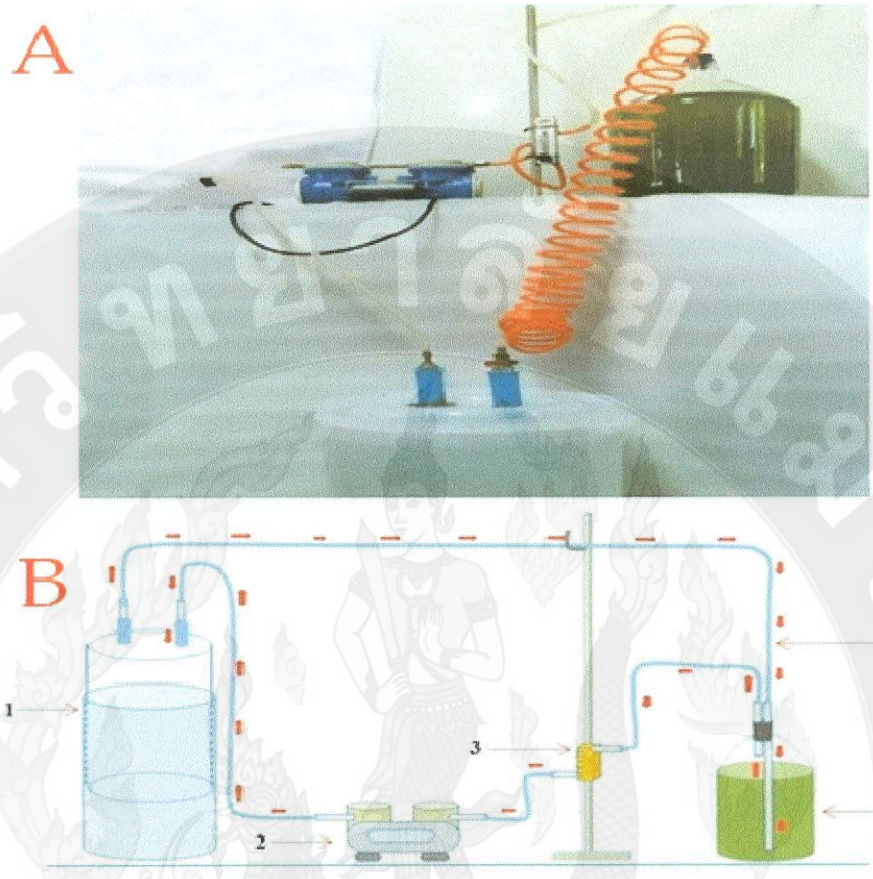

Figure 16 A) biogas purification system through microalgae, *C. vulgaris* B) schematic diagram of set up during biogas upgrading 1) biogas storage container 2) air pump 3) air flow meter 4) biogas pumping direction and 5) microalgae unit.

Table 6 Test and evaluation of the system performance through biological upgrading.

Component	Biogas production analysis	
	Before the system improve	After the system improvement
CH_4	64.67%	81.35%
CO_2	31.5 %	16.08%
O_2	0%	1.11%
H_2S	578 ppm	Less than 0.01 ppm

Table 7 algae cells concentration, biogas composition performance through biological upgrading.

Time (hour)	Algae cell (cells/ml)*	CH_4 (%)	CO_2 (%)	O_2 (%)	H_2S (ppm)
0	1167	64.67	33.5	0	578
1	1200	66.04	32.36	0.08	511
2	1287	66.86	32.02	0.17	463
3	1313	68.02	29.83	0.45	377
4	1398	70.43	27.29	0.62	302
5	1432	73	24.85	0.79	224
6	1505	76	21.73	0.94	145
7	1586	79.33	18.38	1.01	82
8	1654	81.35	16.08	1.11	0.01

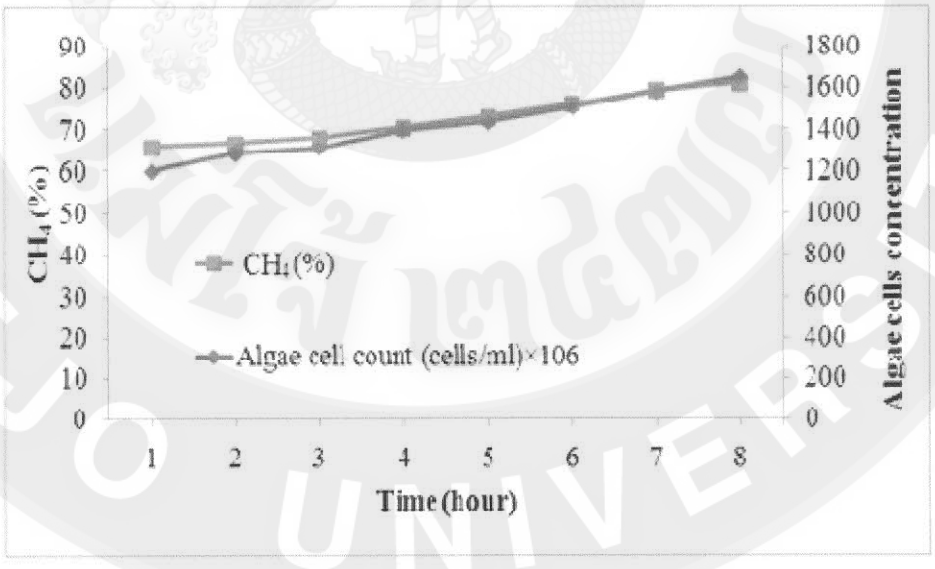

Figure 17 methane enrichment and algal cells concentrations

Photoautotrophic purification process was continued 8 hours. The results were presented in table 1. After purification, the CH_4 content has improved gigantically. Also CO_2 and H_2S amounts were reduce a lot. Due to algal photosynthesis process O_2 was slightly increased. Reducing CO_2 and H_2S content will significantly improve the quality of biogas. However this study suggested that long time purification process needed. For biogas impurity removal, biological processes are environmentally friendly and feasible. Consequently biogas purification using algae involved the use of algae's photosynthetic ability in the removal of the impurities present in biogas. Further our establishment study, *C. vulgaris* applicable for biogas purification.

CHAPTER 5

SUMMARY

In conclusions, production of biofuels is undoubtedly one of the best solutions for declining the crude oil reserves and global warming due to excessive greenhouse gasses emissions. As fossil fuel prices increase and environmental concerns gain prominence, the development of alternative fuels from biomass has become more important. Biogas is considered a renewable energy carrier. As demonstrated here, macroalgal biogas is technically feasible. Macroalgae have several advantages over terrestrial plants such as higher photosynthetic efficiencies, lower need for cultivation area, higher growth rates, more continuous biomass production, no direct competition with food production, and possibility to use artificial medium, natural water medium (freshwater/marine water) and wastewater for biomass production. The algae biomass thus produced constitute an additional source of organic substrate in the installation for biogas production. The biogas production was 910.10 L/kg. Therefore, fast growing, high-yielding and rich in organic matter of *S. ellipsospora* was promising energy crops for biogas production. This suggested that it is possible to achieve stable operation using *S. ellipsospora*, as a substrate for biogas production in pilot or large scale biogas plant in the future.

The cultivation of the microalga, *C. vulgaris* utilized low cost artificial medium which named as Rameshprabu medium. The medium was prepared through rice fertilizer, rice bran, fish meal, lime and urea. This medium was named as, Rameshprabu medium. The aim of the current work was to evaluate the potential of the green alga *C. vulgaris* as a cheap renewable energy source in term of biofuels. The algae grew faster, providing higher productivities of biomass, lipids, carbohydrates and proteins. Furthermore, *C. vulgaris* biomass production and carbohydrate consumption were enhanced by supplementing the inorganic culture. Photoautotrophic conditions cultivation of *C. vulgaris* can be considered as a feasible strategy to reduce the costs of microalgal biomass production, while also contributing to solve the environmental problem. The cultivation of the microalga, *C. vulgaris* utilized low cost artificial medium which named as Rameshprabu medium. The medium was prepared through rice fertilizer, rice bran, fish meal, lime and urea. This medium was named as, Rameshprabu medium. The aim of the current work was to evaluate the potential of the green alga *C. vulgaris* as a cheap renewable energy source in term of biofuels. The

algae grew faster, providing higher productivities of biomass, lipids, carbohydrates and proteins. Furthermore, *C. vulgaris* biomass production and carbohydrate consumption were enhanced by supplementing the inorganic culture. Photoautotrophic conditions cultivation of *C. vulgaris* can be considered as a feasible strategy to reduce the costs of microalgal biomass production, while also contributing to solve the environmental problem. Furthermore, biogases upgraded through biological processes considered environmentally friendly and feasible were employed using the photosynthetic ability of the algae; the purification process was continued 8 hours. Before entering the improve system biogas containing CH_4, CO_2, O_2 are 64.64 %, 31.5 % and 0% with H_2S 578 ppm. After the enhancement process the biogas having CH_4, CO_2, O_2 are 81.35%, 16.08%, 1.11 and less than 0.01 ppm with H_2S. Consequently, H_2S was removed to below the detection limit and methane content was reached about 81%. The results were demonstrated the amount of CO_2, H_2S gas was reduced along with CH_4 was improved efficiently. Biogas will lead to reduced use of fuel wood and diesel generators hence an innovative technology to the reduction of greenhouse gas emissions. Beside of energy production, other valuable products, such as high quality bio-fertilizer are obtained from the anaerobic digestion of macroalgae and this will minimize the use of expensive mineral fertilizer. The option of biogas production as a way of energy exploration using macroalgae could be improve environmental sustainability by improving the social, economic and physical well being of the environment.

BIBLIOGRAPHY

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

Adams, J. M., Gallagher, J. A. and Donnison, I. S. 2009. Fermentation study on Saccharina latissima for bioethanol production considering variable pre-treatments. **Journal of Applied Phycology** 21(5): 569-574.

APHA, AWWA and WPCF. 2005. **Standards Methods for the Examination of Water and Wastewater**. 21st ed. APHA-AWWA-WPCF, Washington, DC.

Angelidaki, I. and Sanders, W. 2004. Assessment of the anaerobic biodegradability of macropollutants. **Reviews in Environmental Science and Biotechnology** 3(2): 117-129.

Angelidaki., I. Alves., M. Bolzonella., D. Borzacconi., L. Campors., J. Guwy., A. Kalyuzhnyi., S. Jenicek., P. and Van Lier., J. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. **Water Science Technology** 59(5): 927-934.

Becker, E. W. (2007). Micro-algae as a source of protein. **Biotechnology Advances** 25(2): 207-210.

Benemann, J. R. and Oswald, W. J. 1996. **Systems and economic analysis of microalgae ponds for conversion of carbon dioxide to biomass.** Final Report: Grant No.DEFG2293PC93204.

Biebl, H. and Pfennig, N. 1977. Growth of sulfate-reducing bacteria with sulfur as electron acceptor. **Archives of Microbiology** 112(1): 115-117.

Blair., M. Kokabian., B. and Gude., G. 2014. Light and growth medium effect on *Chlorella vulgaris* biomass production. **Journal of Environmental Chemical Engineering** 2(1): 665-674.

Bligh, E. G. and Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology** 37(8): 911-917.

Brettschneider., O. Thiele., R. Faber., R. Thielert, H. and Wonzny, G. 2004. Experimental investigation and simulation of the chemical absorption in a packed column for the system NH_3 –CO_2 –H_2S–NaOH–H_2O. **Separation and Purification Technology** 39(3): 139-159.

Buswell, A. M. and Boruff, C. S. 1932. The relationship between chemical composition of organic matter and the quality and quantity of gas produced during sludge digestion. **Sewage Works Journal** 4(3): 454-460.

Castorena-Gonzalez., J. A. Foote., C. MacVittie., K. Halámek., J. Halámková., L. Martinez-Lemus, L. A. and Katz. E. 2013. Biofuel Cell Operating in Vivo in Rat. **Electroanalysis** 25(7): 1579-1584.

Champenois ., J. Marfaing, H. and Pierre, R. 2014. Review of the taxonomic revision of *Chlorella* and consequences for its food uses in Europe. **Journal of Applied Phycology** 27(5): 1845-1851.

Cho., S. Park., S. Seon., J. Yu, J. and Lee, T. 2013. Evaluation of thermal, ultrasonic and alkali pretreatments on mixed-microalgal biomass to enhance anaerobic methane production. **Bioresource Technology** 143: 330-336.

Chynoweth., D. P. Klass, D. L. and Ghosh, S. 1981. **Anaerobic digestion of kelp**. In Biomass conversion processes for energy and fuels, Edited by Sofer SS, Zaborsky OR. New York: Plenum Press, 315-318.

Demirbas, A. 2008. Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. **Energy Conversion and Management** 49(8): 2106-2116.

Debowski., M. Zielinski., M. Grala, A. and Dudek, M. 2013. Algae biomass as an alternative substrate in biogas production technologies Review. **Renewable and Sustainable Energy Reviews** 27(11): 596-604.

Douškova., F. Kaštanek., F. Maleterova., Y. Kaštanek., P. Doucha, J. and Zachleder, V. 2010. Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: Biogas-cogeneration-microalgae-products. **Energy Conversion and Management** 51(3): 606-611.

Dussadee., N. Reansuwan, K. and 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.

Frigon., J.-C. Matteau-Lebrun., F. Hamani Abdou., R. McGinn., P. J. O'Leary, S. J. B. and Guiot, S. R. 2013. Screening microalgae strains for their productivity in methane following anaerobic digestion. **Applied Energy** 108: 100-107.

Gellenbeck, K. W. and Chapman, D. J. 1983. Seaweed uses: the outlook for mariculture. **Endeavour** 7(1): 31-37.

Goh, C. S. and Lee, K. T. 2010. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. **Renewable Sustainable Energy Review** 14(2): 842-848.

Graham, L. E. and Wilcox, L. W. 2000. **Algae**. Prentice Hall Inc. Upper Saddle River, New Jersey.

Guschina, I. A. and Harwood, J. L. 2006. Lipids and lipid metabolism in eukaryotic algae. **Progress in Lipid Research** 45(2): 160-86.

Hendroko., R. Kawaroe., M. Salafudin., Saefurahman., G. Fitrianto., N. E. Sari, D. W. and Sakri, Y. 2011. **Biorefinery preliminary studies: integration of slurry and CO_2 as biomethane digester waste for microalgae *Scenedesmus* sp. Growth.** International seminar on chemical engineering Soehadi Reksowardojo, Bandung, October 5-7.

Hughes., D. Kelly., M. S. Black, K. D. and Stanley, M. S. 2012. Biogas from Macroalgae: is it time to revisit the idea? **Biotechnology for Biofuels** 5: 86.

Jard., G. Dumas., C. Delgenes., J. P. Marfaing., H. Sialve., B. Steyer, J. P. and Carrère, H. 2013. Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalga *Palmaria palmate*. **Biochemical Engineering Journal** 79: 253-258.

Jones, C. S. and Mayfield, S. P. 2012. Algae biofuels: versatility for the future of bioenergy. **Current Opinion in Biotechnology** 23(3): 346-351.

Kao., Y. Chiu., S. Y. Huang., T. T. Dai., L. Wang., G. H. Tseng., C. P. Chen, C. H. and Lin, C. S. 2012a. A mutant strain of microalga *Chlorella* sp. for the carbon dioxide capture from biogas. **Biomass and Bioenergy** 36: 132-140.

Kao., Y. Chiu., S. Y. Huang., T. T. Dai., L. Hsu, L. K. and Lin, C. S. 2012b. Ability of a mutant strain of the microalga *Chlorella* sp. to capture carbon dioxide for biogas upgrading. **Applied Energy** 93: 176-183.

Kargupta, A. N. and Jha, R. N. 2004. **Algal flora of Bihar (Zygnemataceae)**. Bishen Singh Mahendra Pal Singh, Dehra Dun, India.

Krupek., R. A. Empinotti., A. Santos, R. K. and Araujo, E. A. T. 2014. Influence of physical characteristics of environment (light and current velocity) on the substrate occupation by *Spirogyra* sp. in stream ecosystems. **Brazilian Journal of Botany** 37(4): 453-459.

Kumar., J. P. Dhar., A. B. Tayade., D. Gupta., O. P. Chaurasia, Upreti., D. K. Toppo., K. Arora., R. Suseela, M. R. and Srivastava. R. B. 2015. Chemical composition and biological activities of Trans-Himalayan alga Spirogyra *porticalis* (Muell.) Cleve. **PLoS ONE** 10: e0118255.

Lastella., G. Testa., C. Cornacchia., G. Notornicola., M. Voltasio, F. and Sharma, V. K. 2002. Anaerbic digestion of semi-solid organic waste: biogas production and its purification. **Energy Conversion and Management** 43(1): 63-75.

Lawton., R. J., de Nys, R. and Paul, N. A. 2013. Selecting reliable and robust freshwater macroalgae for biomass applications. **PLoS ONE** 8: e64168.

Li., H. Kjerstadius., H. Tjernström, E. and Davidsson, Å. 2013. **Evaluation of pretreatment methods for increased biogas production from macro algae** (Utvärdering av förbehandlingsmetoder för ökad biogasproduktion från makroalger). SGC Rapport, 278: 136 [http://www.sgc.se/ckfinder/userfiles/files/SGC278.pdf]

Mahdy., L. Mendez., S. Blanco., S. Ballesteros, M. and González-Fernández, C. 2014. Protease cell wall degradation of *Chlorella vulgaris*: effect on methane production. **Bioresource Technology** 171: 421-427.

Mann., G. Schlegel., M. Schumann, R. and Sakalauskas, A. 2009. Biogas-conditioning with microalgae. **Agronomy Research** 7: 33-38.

Markou., G. Angelidaki., I. Nerantzis, E. and Georgakakis, D. 2013. Bioethanol Production by Carbohydrate-Enriched Biomass of *Arthrospira* (Spirulina) *platensis*. **Energies** 6(8): 3937-3950.

Meynell, P. J. 1976. **Methane: planning a digester**. Schocken Books, New York.

Mösche, M. and Jördening, H. J. **(1999).** Comparison of different models of substrate and product inhibition in anaerobic digestion. **Water Research** 33(11): 2545-2554.

Mussgnug., J. H. Klassen., V. Schlüter, A. and Kruse, O. 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. **Journal of Biotechnology** 150(1): 51-56.

Naik., A. Hemavani, C. and Thippeswamy, B. 2012. Evaluation of antimicrobial property of *Spirogyra* species. **International Multidisciplinary Research Journal** 2(2): 13-15.

Nielsen, H. B. and Heiske S. 2011. Anaerobic digestion of macroalgae: methane potentials, pre-treatment, inhibition and co-digestion. **Water Science Technology** 64(8): 1723-1729.

Official Methods of Analysis of AOAC International. 19th Ed., AOAC International, 420 Gaithersburg, MD, USA. 2012.

Oswald, W. J. 2003. My sixty years in applied algology. **Journal of Applied Phycology** 15: 99-106.

Pantawong., R. Chuanchai., A. Thipbunrat., P. Unpaprom. Y. and Ramaraj, R. 2015. Experimental investigation of biogas production from water lettuce, *Pistia stratiotes* L. **Emergent Life Sciences Research** 1(2): 41-46.

Parmar., N. Singh., K. Pandey., A. Gnansounou, E. and Madamwar, D. 2011. Cyanobacteria and microalgae: a positive prospect for biofuels. **Bioresource Technology** 102: 10163-10172.

Passos., F. Solé, M. Garcia, J. and Ferrer, I. 2013. Biogas production from microalgae grown in wastewater: effect of microwave pre-treatment. **Applied Energy** 108: 168-175.

Pavlostathis, S. G. and Giraldogomez, E. 1991. Kinetics of anaerobic treatment. **Water Science and Technology** 24(5-6): 35-59.

Peerapornpisal, Y. 2008. Edible freshwater macroalgae in Northern Thailand. **Journal of Fisheries Technology Research** 1: 178-189.

Prescott, G. W. 1951. **Algae of the western great lakes area**. Cranbrook Institute of Science, Bloomfield Hills, Michigan, USA.

Randhawa, M. S. 1959. **Zygnemaceae**. ICAR, New Delhi, India.

Ramaraj., R. Tsai, D. D-W. and Chen, P. H. 2010. Algae Growth in Natural Water Resources. **Journal of Soil and Water Conservation** 42: 439-450.

Ramaraj, R. 2013. **Freshwater microalgae growth and Carbon dioxide Sequestration**, PhD thesis. National Chung Hsing University, Taichung, Taiwan.

Ramaraj, R. Tsai, D. D-W. and Chen, P. H. 2013. Chlorophyll is not accurate measurement for algal biomass. **Chiang Mai Journal of Science** 40(4): 547–555.

Ramaraj., R. Tsai, D. D-W. and Chen, P. H. 2014a. Freshwater microalgae niche of air carbon dioxide mitigation. **Ecological Engineering** 68: 47-52.

Ramaraj., R. Tsai, D. D-W. and Chen, P. H. 2014b. An exploration of the relationships between microalgae biomass growth and related environmental variables. **Journal of Photochemistry and Photobiology B: Biology** 135: 44-47.

Ramaraj., R. Tsai, D. D-W. and Chen, P. H. Biomass of algae growth on natural water medium. **Journal of Photochemistry and Photobiology B: Biology** 2015; 142: 124-128.

Ramaraj., R. Dussadee., N. Whangchai, N. and Unpaprom, Y. 2015. Microalgae biomass as an alternative substrate in biogas production. **International Journal of Sustainable and Green Energy** 4(1-1): 13-19.

Ramaraj, R. and Dussadee, N. 2015. Biological purification processes for biogas using algae cultures: a review, **International Journal of Sustainable and Green Energy** 4(1-1): 20-32.

Ramaraj., R. Kawaree, R. and Unpaprom Y. 2015. A newly isolated green alga, *Pediastrum duplex* Meyen, from Thailand with efficient hydrogen production. **International Journal of Sustainable and Green Energy** 4(1-1): 7-12.

Ramaraj, R. and Unpaprom, Y. 2016. Effect of temperature on the performance of biogas production from Duckweed. **Chemistry Research Journal** 1(1): 58-66.

Rittmann, B. E. 2008. Opportunities for renewable bioenergy using microorganisms. **Biotechnology and Bioengineering** 100(2): 203-212.

Ross., B. Jones, J. M. and Kubacki, M. L. 2008. Since macroalgae are again receiving attention as a substrate for anaerobic digestion. **Bioresource Technology** 99: 6494-6504.

Ryckebosch., E. Drouillon, M. and Vervaeren, H. 2011. Techniques for Transformation of Biogas to Biomethane. **Journal of Biomass and Bioenergy** 35(5): 1633-1645.

Safi., C. Zebib., B. Merah., O. Pontalier, P. and Vaca-Garcia, C. 2014. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: a review. **Renewable and Sustainable Energy Reviews** 35: 265-278.

Schwede., S. Rehman., Z.-U. Gerber., M. Theiss, C. and Span, R. 2013. Effects of thermal pretreatment on anaerobic digestion of *Nannocloropsis salina* biomass. **Bioresource Technology** 143: 505-511.

Sialve., B. Bernet, N. and Bernard O. 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. **Biotechnology Advances** 27: 409-416.

Singh, J. and Gu, S. 2010. Commercialization potential of microalgae for biofuels production. **Renewable and Sustainable Energy Reviews** 14: 2596-2610.

Stephens., E. Ross., I. L. King., Z. Mussgnug., J. H. Kruse., O. Posten., C. Borowitzka, M. A. and Hankamer, B. 2010. An economic and technical evaluation of microalgal biofuels. **Nature Biotechnology** 28(2): 126-128.

Sumardiono., S. Budiyono, I. S. and Sasongko, S. B. 2014. Utilization of Biogas as Carbon Dioxide Provider for *Spirulina platensis* Culture. **Current Research Journal of Biological Sciences** 6(1): 53-59.

Taft, C. E. 2009. Some Oedogoniacea and Zygnemataceae from Texas and Louisiane. **Transactions of the American Microscopical Society** 65: 18-26.

Tedesco, S. Barroso, T. M. and Olabi, A. G. 2013. Optimization of mechanical pre-treatment of *Laminariaceae* spp. biomass-derived biogas. **Renewable and Sustainable Energy Reviews** 27: 596-604.

Than, N. V., Cheng, S-S. and Tien, Y-M. 2014. Biochemical methane production potential of soluble leachate from biological solid state fermentation with batch jar tests. **Arabian Journal for Science and Engineering** 4(6): 169-175.

Tipnee., S. Ramaraj, R. and Unpaprom., Y. 2015. Nutritional evaluation of edible freshwater green Macroalga *Spirogyra varians*. **Emergent Life Sciences Research** 1(2): 1-7.

Tongprawhan., W. Srinuanpan, S. and Cheirsilp, B. 2014. Biocapture of CO_2 from biogas by oleaginous microalgae for improving methane content and simultaneously producing lipid. **Bioresource Technology** 170: 90-99.

Transeau, E. N. 1951. **The Zygnemataceae**. Ohio. State Univ. Press, Columbus.

Tsai, D. D-W. 2012. **Watershed Reactor Analysis, CO_2 Eco-function and Threshold Management Study**. PhD thesis, National Chung Hsing University, Taichung, Taiwan.

Tsai., D. D-W. Chen., P. H. Chou M-j. Hsu., C-F. Ramaraj, R. and Chen, P. H. 2015. Carbon sequestration by alga ecosystems. **Ecological Engineering** 84: 386-389.

Tsai., D. D-W. Ramaraj, R. and Chen, P. H. 2016. Carbon dioxide bio-fixation by algae of high rate pond on natural water medium. **Ecological Engineering** 92: 106-110.

Unpaprom., Y. Tipnee, S. and Ramaraj, R. 2015. Biodiesel from green alga *Scenedesmus acuminatus*. **International Journal of Sustainable and Green Energy** 4(1-1): 1-6.

Unpaprom., Y. Intasaen., O. Yongphet, P. and Ramaraj, R. 2015. Cultivation of microalga *Botryococcus braunii* using red Nile tilapia effluent medium for biogas production. **Journal of Ecology and Environmental Sciences** 3 58-65.

Vergara-Fernàndez, A., Vargas, G., Alarcon, N. and Antonio, A. 2008. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. **Biomass and Bioenergy** 32(4): 338-344.

Vílchez., C., Garbayo., I. Lobato, M. V. and Vega J. M. 1997. Microalgae-mediated chemicals production and waste removal. **Enzyme and Microbial Technology** 20(8): 562-572.

Vidyavati and Noor, M. N. 1995. **Biology of conjugales.** Pritwell. Jaipur.India.

von Sperling, M. and Oliveira, S. C. 2009. Comparative performance evaluation of full-scale anaerobic and aerobic wastewater treatment processes in Brazil. **Water Science and Technology** 59(1): 15-22.

Yan, Z. and Zheng, Z. 2013. Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae *Chlorella* sp. **Bioresource Technology** 139: 292-299.

Wang., Q. H. Kuninobu., M. Ogawa, H. and Kato, Y. (1999). Degradation of volatile fatty in highly efficient anaerobic digestion. **Biomass and Bioenergy** 16(6): 407-416.

Weiland, P. 2010. Biogas production: current state and perspectives. **Applied Microbiology and Biotechnology** 85(4): 849-860.

Wilkie., A. C. Smith, P. H. and Bordreaux, F. M. 2003. An economical bioreactor for evaluating biogas potential of particular biomass. **Bioresource Technology** 92: 103-109.

Zieminski., K. Romanowska, I. and Kowalska, M. 2012. Enzymatic pretreatment of lignocellulosic wastes to improve biogas production. **Waste Management** 32(6): 1131-1137.

Zhou., D, Zhang., L. A. Zhang., S. C. Fu., H. B. and Chen, J. M. 2010. Hydrothermal liquefaction of macroalgae Enteromorpha prolifera to Bio-oil. **Energy Fuel.** 24: 4054-4061.

APPENDIX

APPENDIX A

PUBLICATIONS

Emer Life Sci Res (2015) 1(1): 38-45

E-ISSN: 2395-6658
P-ISSN: 2395-664X

Research Article

Culture of Macroalgae Spirogyra ellipsospora for Long-Term Experiments, Stock Maintenance and Biogas Production

Rameshprabu Ramaraj, Yuwalee Unpaprom, Niwooti Whangchai, Natthawud Dussadee

Received: 20 March 2015, Accepted: 3 April 2015

Abstract

The freshwater alga Spirogyra ellipsospora, a filamentous charophyte, collected from the stream, was identified on the basis of morpho-anatomical characters. In this study, we tried to utilize the natural water resource to develop the algae growth system by ecological engineering concept to develop a low cost medium for macroalgae growth. The outdoor photo-reactor was used to grow macroalgae through using natural water as medium. The results showed that the reactor had good performance on algae growth. Culture media for growth of this study species have not yet been tested for long-term experiments, stock maintenance and biogas production. Here we tested the S. ellipsospora growth with natural water medium in a 6-weeks laboratory experiment. Consequently, the study consists of laboratory tests showing S. ellipsospora growth, harvesting, chlorophyll extraction, biomass analysis and anaerobic fermentation for biogas production.

Keywords Bio-methane, Biogas, Macroalgae, Stream, Spirogyra culture

Introduction

Algae are the dominant primary producers in aquatic ecosystems. Since algae are highly varied group organisms, which have important functions in ecosystem; they are widely distributed around the world and closely connected with human life (Ramaraj et al. 2015a; 2015b). Furthermore algae biomass is an essential biological resource. Algal biomass has been recently investigated as a possible and complementary alternative to lignocellulosic substrates to produce biofuels/biotechnological products, due to several advantages, such as (1) a higher productivity yields, (2) they do not require arable lands for growth and therefore do not outcompete food resources, (3) they can grow in a variety of environments including fresh water, salt water and municipal wastewaters, (4) many species of algae can be induced to produce particularly high concentrations of chosen compounds – proteins, carbohydrates, lipids and pigments - that are of commercial value, and (5) the ability to produce non-toxic and biodegradable biofuels (Ramaraj 2013; Ramaraj et al. 2014a; 2014b).

Recently, macroalgae are receiving a considerable attention due to their ability to synthesize valuable compounds, accumulate high energy compounds and sequester carbon (Lawton et al. 2013). They are therefore considered as a third generation feedstock for biofuel production and have a great potential as renewable feedstock (Hughes et al. 2012). The genus Spirogyra has recently drawn attention to researchers due to its various biotechnological and industrial applications. Spirogyra, one of the commonest green filamentous freshwater macroalgae, is named because of the helical or spiral arrangement of the chloroplasts (Krupek et al. 2014). There are more than 400 species of Spirogyra in the world. This genus is

R. Ramaraj, N. Dussadee✉
School of Renewable Energy, Maejo University, Sansai, Chiang Mai 50290, Thailand
Y. Unpaprom,
Biotechnology Program, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand
N. Whangchai
Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Sansai, Chiang Mai 50290, Thailand
✉ natthawu@yahoo.com, natthawu@mju.ac.th

photosynthetic, with long bright grass-green filaments having spiral-shaped chloroplasts. It is bright green in the spring, when it is most abundant, but deteriorates to yellow. In nature, Spirogyra grows in running streams of cool fresh water, and secretes a coating of mucous that makes it feel slippery. This freshwater alga is found in shallow ponds, ditches amongst vegetation at the edges of large lakes, small stagnant water bodies, rivers, and streams. Under favorable conditions, Spirogyra forms dense mats that float on or just beneath the surface of the water. Blooms cause a grassy odour and clog filters, especially at water treatment facilities.

Spirogyra sp. contains about 11–21% of lipids and a high content of sugar, about 33–64% (Becker 2007). Spirogyra sp. contains Chlorophyll-a and Chlorophyll-b which are responsible for its green color. However, in some culture/stress conditions the macroalga appears yellow or orange due to the presence of secondary pigments (carotenoids). Spirogyra sp. are promising source of novel biochemically active compounds like fatty acids, steroids, carotenoids, polysaccharides, lectins, vitamins and phyco-proteins, amino acids, dietary minerals, halogenated compounds, polyketides, diverse antioxidants, antibiotic, antiviral, anti-inflammatory and other positive biological activities (Kumar et al. 2015). The high productivity of the macroalga Spirogyra and its capacity to accumulate high amounts of sugar, make this biomass also attractive as substrate for bioenergy production. Biofuel is a renewable energy, which may be instead of the fossil fuel resources in the future with decreasing of the fossil fuel on a daily basis (Unpaprom et al. 2015). The application of anaerobic digestion (AD) technology is growing worldwide because of its economic and environmental benefits (Dussadee et al. 2014). As a consequence, a number of studies and research activities dealing with the determination of the biogas potential of solid organic substrates have been carrying out in the recent years.

The biogas production by this macroalga is still in development and so far, there are no references in the literature related to biomethane gas production from Spirogyra using natural water medium. Consequently, this study was to examine the Spirogyra ellipsospora growth conditions, biomass and biogas potential through natural water medium also long-term experiments, stock maintenance.

Methodology

Algal sample collection, cultivation and experiment setup

The freshwater macroalgae, Spirogyra was collected from the slow running fresh water stream at Tumbon Pang Yang (19° 18'42.41" N; 98° 48'44.11" and elevation 722 m), Mae Taeng district, Chiang Mai province, Thailand and transport to the Energy Research Laboratory at Maejo University, Chiang Mai, Thailand. The methodology was illustrated in (Figure 1). This investigation is to simulate the ecosystem in natural water body with macroalgae growth ecological engineering concepts. For the macroalgae cultivation, the nearby water was screened by 1x1 mm sieve (mesh No. 18) to remove macro particles.

Figure 1. A flow chart of study methodology

According to Ramaraj et al. (2015a; 2015b) the stream water was used as medium. Water collected from the same sampling zone afore mentioned and the water was filtrated by 0.45 µm filter paper as feed. Spirogyra Sp. were grown in autotrophic conditions of 10 L open type outdoor jar. The jar containing 5 L working volume and 5 L base filled with sterilized white sand and growth system was demonstrated in Figure 2.

Identification of alga

The algal samples were observed under light microscope and were then visualized with a Nikon Eclipse 80i microscope and photographs were taken with attached digital camera.

Figure 2. Macroalgae growth system

Relevant publication of Prescott (1951) was referred for the identification of algal taxa and taxonomically determined with the help of authentic literature (Randhawa 1959; Transeau 1951; Vidyavati 1995; Kargupta and Jh 2004, Taft 2009). For the taxonomic description of taxa, dimensions were given in micrometer (µm). The measuring scales given for algae photographs were equal to 20 µm. The morphological characters including length, width, number of spiral chloroplasts, and number of granules were recorded for species confirmation.

Chlorophylls estimation

Ten ml of sample was taken and centrifuged at 6000 rpm for 15 minutes. Supernatants were discarded and re-suspended in a known volume of methanol, while pellets extracted with 5 ml of 96% methanol extraction. The tubes were wraped with aluminum foil and kept in dark. The samples were centrifuged again and the supernatants were used for measuring the optical density at 663 nm and 645 nm against 96% methanol as a blank by spectrophotometer (Spectronic Genesys 20, Thermo Fisher Scientific). After extraction chlorophyll concentration was determined spectrophotometrically and calculated Chlorophyll content (Chlorophyll a, chlorophyll b and total chlorophyll) were computed using the following equations:

Chlorophyll-a (µg/ml) = {(15.65xA_{666} – 7.340xA_{653}) x V/ 50 x W} x dilution

Chlorophyll-b (µg/ml) ={(27.05xA_{653} – 11.21xA_{666}) x V/ 50 x W} x dilution

Total chlorophyll = chlorophyll-a + chlorophyll-b

Inoculum

Anaerobic sludge was obtained from a leachate recirculation digester (Napier grass biogas fermenter), located in the Energy Research Center at Maejo University, Chiang Mai, Thailand, was used as inoculum in all biodegradability assays.

Anaerobic digestion batch tests

The anaerobic assays were conducted in 500 mL bottles (triplicate reactor) containing 40 mL of inoculum and 200 g of fresh S. ellipsospora and remaining make up with double distilled water. The total working volume is 400 mL. The biochemical methane potential (BMP) assay was used to determine the methane productivity of S. ellipsospora. The bottles were closed with a septum and flushed with N_2 to remove oxygen. Triplicate, 500 mL fermenters were incubated in the room temperature. Production of biogas was monitored by measuring the overpressure in the bottle headspace at time intervals depending on the production of biogas.

Analytical methods

Table 1 Methods employed for determination of physico-chemical parameters. The solids contents, including total solids (TS) and volatile solids (VS), chemical oxygen demand (COD) were characterized using the Standard Methods for the Examination of Water and Wastewater (method # 2540) (APHA-AWWA-WEF, 2005). Metrohm 774 pH-meter was used in all pH measurements. The entire experiments were done in triplicate. Biogas estimation method was adopted from literature (Pavlostathis and Giraldo-Gomez 1991; von Sperling and Chernicharo 2009).

Statistical analysis

All analytical results were conducted at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation. The standard deviations were analyzed by using Microsoft Excel 2003 for Windows.

Results and Discussion

Morphological study of Spirogyra

Spirogyra is a genus of filamentous green algae in the order Zygnematales. The name indicates the helical or spiral arrangement of the chloroplasts, which is the main diagnostic characteristic of the genus. The Spirogyra species typically develops unbranched filaments and is one cell thick, which grows longer through normal cell division. There are more than 400 species of Spirogyra in the world. Vegetative growth of Spirogyra can be recognized by three characteristics: (1) type of cross walls (plane, replicate, semi-replicate or colligate), (2) cell length and width and (3) chloroplast numbers.

Table 1. Physicochemical parameters

Parameter	Method	Reference
TS	Method 2540 C	APHA-AWWA-WEF, 2005
VS	Method 2540 E	
COD	Method 5220	
Chlorophylls	spectrophotometric method	-
pH	Metrohm 774 pH-meter	-
Biomethane estimation	via COD	Pavlostathis and Giraldo-Gomez, 1991; Ramaraj et al. 2014
Percentage of CH_4, CO_2 and H_2S	BMP analysis	

There are classical and standard morphological methods that were used in the identification of the Spirogyra specimens with help of specific literatures (Randhawa 1959; Transeau 1951; Vidyavati 1995; Kargupta and Jh 2004; Taft 2009). The morphological characteristics of each sample were recorded via cell dimensions, along with the number and arrangement of chloroplast spirals/pyrenoids. The morphological characteristics of biological parameters were also studied and presented in Table 2. The classical morphologically based methods are used for the identification of Spirogyra specimens. The structure of species from this study demonstrated definitive identity matches in the range of 99% for the agreement of S. ellipsospora. Light microscopic pictures of Spirogyra ellipsospora is presented in Figure 3.

S. ellipsospora growth and biomass measurement

Biomass was a critical measurement in the algal harvesting process for applications. A number of methods had been developed to estimate and quantify, which were useful in different cases (Ramaraj 2013; Ramaraj et al. 2015c).

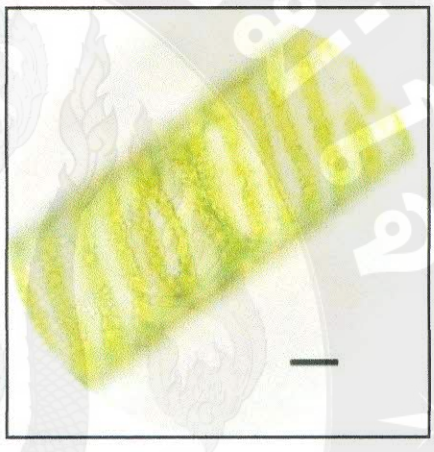

Figure 3. Light microscopic pictures of macroalgae Spirogyra ellipsospora

Different methods were available such as dry weight: Total suspended solids, volatile suspended solids and fixed suspended solids; wet weight method; chlorophyll (Chl) method: Chl-a, Chl-b and Chl-a+b), epifluorescence microscopy, bioluminescence, photometric, turbidity, packed cell volume and cell count etc (Unpaprom et al. 2015). According to Ramaraj et al. (2013), algal biomass measurement and roughly we could classify into two groups, (1) direct index such as dry weight and (2) indirect index such as chlorophyll, so-called proxy index.

Chlorophyll is the most widely used proxy measurement of algae or phytoplankton and their determination is relatively simple and straightforward. In this study, we used chlorophylls measurement to analysis biomass. Chl-a as an algal biomass measurements in natural systems was very popular. Chl-b is used to calculate pigment concentrations. The total Chl-(a + b) is used to measure algal growth (Ramaraj et al. 2010). Growth system was setup outdoor conditions. Algae

biomass measured by Chl-a, Chl-b and total chlorophyll results were average as 9.36 μgmL^{-1}, 3.88 μgmL^{-1} and 13.24 μgmL^{-1}, respectively. Accordingly, this study presents results to produce algae biomass using the natural water and result was encouraging.

Table 2. Morphological characteristic of S. ellipsospora

Type of parameters	Parameters	Equipments and methods	Charac teristics
Biological	Width of cell	Light microscope	120–150
	Length of cell		90–280
	Chloroplasts per cell		5–8
	Vegetative cell width (µm)		35–85
	Vegetative cell length(µm)		80–190
	L/W ratio vegetative cell		2.0–3.4
	Number of chloroplasts		2–5
	Shape of zoospore		Ellipsoid
	Zoospore width		60–73
	Zoospore length		75–95

The potential of natural water medium for S. ellipsospora long-term experiments and stock maintenance

The utilization of natural water medium which came from water body directly without any extra nutrition addition, demonstrated the potential to adopt the algal function for natural growth and long time surveillance. The study confirmed that macroalgae can get essential nutrition from natural water body (natural water medium). Utilizing this growth uptake function we could apply the natural medium in controlled environments such as lab (outdoor lab scale) or field scale growth units or even further applied in natural environment, but nowadays most of researchers and algae manufactures are using artificial medium which is expensive to produce algal biomass. Our study could take advantage of nutrients available in natural water to reduce the total cost, long-term experiments and stock maintenance.

The productivity of macroalgae cultured for 6 cycles of 6 days using with outdoor lab environment to imitate the natural system. The culture that is continuously provided natural water medium each of cycle ends. Macroalgae were placed in 10 L cylindrical tanks in an outdoor system to be cultured for 36 days. Biomass was initially stocked at 2 g/L fresh weight (fw) for S. ellipsospora. The algae were cultivated in a batch culture system, described in detail previously (in methodology part). Biomass was harvested every 6 days (6 cycles of 6 days each in total) using a net, spun to a constant fresh weight, weighed and subsequently re-stocked at initial stocking densities for a new cycle. Stock maintenance, long time experiment and growth of S. ellipsospora are an essential for its subsequent use in biotechnology. For this purpose, we tested the suitability of the stock maintenance and growth of algal species is essential for their use in biotechnology. Therefore, natural water medium is the most suitable culture media and ease of laboratory culture is relevant topics. This environmental friendly process offers a substantial potential source of algae biomass to provide bioenergy and to reduce the greenhouse gas, carbon dioxide.

Theoretical analysis of S. ellipsospora biogas production and biochemical methane potential

The well known use of the microbiological process of anaerobic digestion (AD) to generate biogas (mixture of methane and carbon dioxide) is now widely implemented for the production of renewable energy worldwide; bio-methane potential (BMP) tests are commonly used in studies concerning the AD of organic solids (Ramaraj et al. 2015c). Macroalgae can be converted to biofuels by various processes including thermal processes and fermentation. The most direct route to obtaining biofuel from macroalgae is via AD to biogas (Hughes et al. 2012; Montingelli et al. 2015). Algal biomass contains considerable amount of biodegradable components such as carbohydrates, lipids and proteins. This makes it a favorable substrate for anaerobic microbial flora and can be converted into methane rich biogas (Sialve et al. 2009). In spite of the fact that macroalgae have high potential for biogas production, there are some studies on anaerobic digestion of macroalgal biomass utilizing Chaetomorpha linum, Saccharina latissima, Gracillaria vermiculophylla and Ulva lactuca biomass.

Apparently, the BMP of algae depends mainly on its composition, which itself depends on

the growth conditions and and is specific species. The concentration of substrate in the BMP assay also impacts on the final biodegradability and methane productivity (Sialve et al. 2009). When the C, H, O and N composition of a wastewater or substrate is known, the stoichiometric relationship repo rted by Buswell and Boruff (1932) and Angelidaki and Sanders (2004), and can be used to estimate the theoretical gas composition on a percentage molar basis.

Elements	Percent	Mol[a]	CH_4[b]	CO_2	NH_3	Biogas (L/kg)
C	41.87	3.49				
H	6.00	6.00				
O	35.77	2.24	48.00 (%)	43.96 (%)	8.04 (%)	910.10
N	4.27	0.31				
S	0.43					

Table 3. Composition of methane and biogas production from S. ellipsospora

In this equation, the organic matter is stoichiometrically converted to methane, carbon dioxide and ammonia. The specific methane yield expressed in liters of CH_4 per gram of volatile solids (VS) can thus be calculated as:

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

Eq. (1) is a theoretical approach that allows estimation of the maximum potential yields. Using Eq. (1), it is possible to compute a theoretical specific methane yield. Composition of methane and biogas production from S. ellipsospora details were presented in Table 3 (by dry weight basis). The biogas composition of carbon dioxide (44%) and methane (48%) of was estimated from the biogas. The carbon, hydrogen, nitrogen, oxygen and sulphur contents of were in S. ellipsospora, 41.87%, 6%, 35.77%, 4.27% and 0.43 respectively. Consequently S. ellipsospora has plenty of nutrients for biogas production process; it is suitable to be used as energy crops for biogas production.

Laboratory analysis of S. ellipsospora biogas production and biochemical methane potential

Gross composition of several algae species were presented by Becker (2007). As expected, the species that can reach higher lipid content have a higher methane yield. COD is commonly used in the water and wastewater industry to measure the organic strength of liquid effluents. It is a chemical procedure using strong acid oxidation. The strength is expressed in 'oxygen equivalents' i.e. the mg O_2 required to oxidise the C to CO_2. However, the COD concept could be estimate the methane yield (Pavlostathis and Giraldogomez 1991; von Sperling and Oliveira 2009; Than et al. 2014). One mole of methane requires 2 moles of oxygen to oxidise it to CO_2 and water, so each gram of methane produced corresponds to the removal of 4 grams of COD.

$$\underset{16}{CH_4} + \underset{64}{2O_2} \rightarrow CO_2 + 2H_2O \quad \text{..... Eq. 2}$$

Or

1kg COD is equivalent to 250g of methane.

1kg COD $\Rightarrow$ 250g of CH_4

250g of CH_4 is equivalent to 250/16 moles of gas = 15.62 moles

1 mole of gas at STP = 22.4 liters

Therefore 15.62 x 22.4 = 349.8 liters = 0.35 m^3.

In our study, the sample content of total solids (TS) and volatile solids (VS) was measured; the results were average as 16622 mg/kg and 13959 mg/kg, respectively. The average pH was 7.4 and average COD 14236 mg/L. Methane formation takes place within a relatively narrow pH interval, from about 6.5 to 8.5 with an optimum interval between 7.0 and 8.0. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5. The pH value increases by ammonia accumulation during degradation of proteins, while the accumulation of VFA decreases the pH value. The accumulation of VFA will often not always result in a pH drop, due to the buffer capacity of the substrate (Mösche and Jördening 1999; Wang et al. 1999; Weiland 2010). According to the COD estimation, our study shows the mixed culture microalgal biomass is a potentially valuable fermentation substrate, and produce 1.9930 L (0.002 m^3) of methane gas.

In conclusions, production of biofuels is undoubtedly one of the best solutions for declining the crude oil reserves and global warming due to excessive greenhouse gasses emissions. As fossil fuel prices increase and environmental concerns gain prominence, the development of alternative

fuels from biomass has become more important. Biogas is considered a renewable energy carrier. As demonstrated here, macroalgal biogas is technically feasible. Macroalgae have several advantages over terrestrial plants such as higher photosynthetic efficiencies, lower need for cultivation area, higher growth rates, more continuous biomass production, no direct competition with food production, and possibility to use artificial medium, natural water medium (freshwater/marine water) and wastewater for biomass production. The algae biomass thus produced constitute an additional source of organic substrate in the installation for biogas production. The biogas production was 910.10 L/kg. Therefore, fast growing, high-yielding and rich in organic matter of S. ellipsospora was promising energy crops for biogas production. This suggested that it is possible to achieve stable operation using S. ellipsospora, as a substrate for biogas production in pilot or large scale biogas plant in the future.

References

APHA-AWWA-WPCF **(2005)**. Standards Methods for the Examination of Water and Wastewater. 21st ed. APHA-AWWA-WPCF, Washington, DC.

A. D. Hughes, M. S. Kelly, K. D. Black and M. S. Stanley **(2012)**. Biogas from macroalgae: is it time to revisit the idea? Biotechnol Biofuels., **5:** 1-7.

A. M. Buswell and C. S. Boruff **(1932)**. The relationship between chemical composition of organic matter and the quality and quantity of gas produced during sludge digestion. Sewage Works J., **4:** 454-460.

A. N. Kargupta and R. N. Jha **(2004)**. Algal flora of Bihar (Zygnemataceae). Bishen Singh Mahendra Pal Singh, Dehra Dun, India.

B. Sialve, N. Bernet and O. Bernard **(2009)**. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol. Adv., **27:** 409-416.

C. E. Taft **(2009)**. Some Oedogoniacea and Zygnemataceae from Texas and Louisiane. Trans Am Microsc Soc., **65:** 18-26.

E. N. Transeau **(1951)**. The Zygnemataceae. Ohio. State Univ. Press, Columbus.

E. W. Becker **(2007)**. Micro-algae as a source of protein. Biotechnol Adv., **25:** 207-210.

G. W. Prescott **(1951)**. Algae of the western great lakes area. Cranbrook Institute of Science, Bloomfield Hills, Michigan, USA.

I. Angelidaki and W. Sanders **(2004)**. Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Biotechnol., **3:** 117-129.

J. Kumar, P. Dhar, A. B. Tayade, D. Gupta, O. P. Chaurasia, D. K. Upreti and K. Toppok et al. **(2015)**. Chemical composition and biological activities of Trans-Himalayan alga Spirogyra porticalis (Muell.) Cleve. PLoS ONE **10:** e0118255.

M. Mösche, H. J. Jördening **(1999)**. Comparison of different models of substrate and product inhibition in anaerobic digestion. Water Res., **33:** 2545-2554.

M. S. Randhawa **(1959)**. Zygnemaceae. ICAR, New Delhi, India.

M. von Sperling and S. C. Oliveira **(2009)**. Comparative performance evaluation of full-scale anaerobic and aerobic wastewater treatment processes in Brazil. Water Sci. Technol., **59:** 15-22.

N. V. Than, S-S. Cheng and Y-M. Tien **(2014)**. Biochemical methane production potential of soluble leachate from biological solid state fermentation with batch jar tests. AJESE., 4: 169-175.

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

P. Weiland **(2010)**. Biogas production: current state and perspectives. Appl. Microbiol. Biotechnol., **85:** 849-860.

Q. H. Wang, M. Kuninobu, H. Ogawa and Y. Kato **(1999)**. Degradation of volatile fatty in highly efficient anaerobic digestion. Biomass Bioenergy., **16:** 407-416.

R. Ramaraj, D. D. W. Tsai and P. H. Chen **(2015a)**. Carbon dioxide fixation of freshwater microalgae growth on natural water medium. Ecol. Eng., **75:** 86-92.

R. Ramaraj, D. D-W. Tsai and P. H. Chen **(2015b)**. Biomass of algae growth on natural water medium. J. Photochem. Photobiol. B., **142:** 124-128.

R. Ramaraj, N. Dussadee, N. Whangchai, Y. Unpaprom **(2015c)**. Microalgae biomass as an

alternative substrate in biogas production. International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. **4:** 13-19.

R. Ramaraj, D. D-W. Tsai and P. H. Chen **(2014a).** An exploration of the relationships between microalgae biomass growth and related environmental variables. J. Photochem. Photobiol. B., **135:** 44-47.

R. Ramaraj, D. D-W. Tsai and P. H. Chen **(2014b).** Freshwater microalgae niche of air carbon dioxide mitigation. Ecol. Eng., **68:** 47-52.

R. Ramaraj **(2013).** Freshwater microalgae growth and carbon dioxide sequestration. PhD thesis, National Chung Hsing University, Taichung, Taiwan.

R. Ramaraj, D. D-W. Tsai and P. H. Chen **(2013).** Chlorophyll is not accurate measurement for algal biomass. Chiang Mai J. Sci., **40:** 547-555.

R. Ramaraj, D. D-W. Tsai and P. H. Chen **(2010).** Algae growth in natural water resources. J. of Soil and Water Conser., **42:** 439-450.

R. A. Krupek, A. Empinotti, R. K. Santos and E. A. T. Araujo **(2014).** Influence of physical characteristics of environment (light and current velocity) on the substrate occupation by Spirogyra sp. in stream ecosystems. Braz. J. Bot., **37:** 453-459.

R. J. Lawton, R. de Nys and N. A. Paul **(2013).** Selecting reliable and robust freshwater macroalgae for biomass applications. PLoS ONE **8:** e64168.

S. G. Pavlostathis and E. Giraldogomez **(1991).** Kinetics of anaerobic treatment. Water Sci. Technol., **24:** 35-59.

Y. Unpaprom, S. Tipnee and R. Ramaraj **(2015).** Biodiesel from green Alga Scenedesmus acuminatus. International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. **4:** 1-6.

Vidyavati and M. N. Noor **(1995).** Biology of conjugales. Pritwell. Jaipur.India.

International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-3, March 2016 Pages 117-122

Cultivation of Green Microalga, Chlorella vulgaris for Biogas Purification

Rameshprabu Ramaraj, Yuwalee Unpaprom ,Natthawud Dussadee*

Abstract— Algal biomass can be utilized for renewable energy sources, such as hydrogen, biodiesel and biogas. Currently, renewed interest in producing bioenergy from microalgae has arisen because they can grow rapidly and convert solar energy into chemical energy via CO_2 fixation and, thus, are now considered one of the most promising energy sources. Subsequently, microalgae are playing important role in the biological purification of biogas. In this study, samples were collected from fish pond water and samples were enriched and isolated. The strain was identified as species of green microalgae Chlorella vulgaris. It was cultivated under open type cement pond systems to produce biomass. The objective of this study was to evaluate the growth of green microalga C. vulgaris on low cost artificial medium. The medium was prepared with rice fertilizer, rice bran, fish meal, lime and urea. This medium was named as, Rameshprabu medium. In this research, we investigated the growth, biomass production and biochemical composition of C. vulgaris using batch culture. The best biomass in terms of high total carbohydrates, protein and lipid production was obtained through using Rameshprabu medium. Furthermore, algal growth removed nitrogen, phosphorus, and chemical oxygen demand (COD) from the medium.

Index Terms— Microalgae, Chlorella vulgaris, Cultivation, Biomass production.

I. INTRODUCTION

The global demand for biomass for food, feed, biofuels, and chemical production is expected to increase in the coming decades. Microalgae are a promising new source of biomass that may complement agricultural crops [1] – [4]. Biofuels productions from microalgae received wide attention recently and have high potential to replace fossil fuels. Although there is much excitement about the potential of algae biofuels such as bioethanol, biodiesel and biogas, much work is still required in the field [5]. The production of biogas via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product [6]. From an environmental and resource-efficiency perspective biogas has several advantages in comparison to other biofuels.

Biogas is composed of 40-70% methane (CH_4), 20-30% carbon dioxide (CO_2), 100-3000ppmv hydrogen sulfide (H_2S) and water, other trace gas compounds and other impurities. Since the main components of biogas are CH_4, CO_2 and H_2S. To utilize biogas as a transport fuel, CO_2 and H_2S must be removed from the concentration to leave biomethane. Biogas purification is the process where any impurities are removed such as sulphides and ammonia. Biological processes are widely employed for CO_2 and H_2S removal, especially in biogas applications [7]. According to Ramaraj et al. [8] confirmed that microalgae is the best candidate to uptake CO_2 efficiently. In addition, biological methods of CO_2 capture from biogas are potentially useful [9].

Since algae are easy to grow and cultivate anywhere with less energy requirements and using very few of the nutrients. It can be cultivated all year round under autotrophic, mixotrophic or heterotrophic conditions. Mixotrophic and heterotrophic cultures have a place as alternative modes of producing algae biomass. The ideal growth conditions for microalgal cultures are strain specific and the biomass productivity depends upon many factors [5]. These include abiotic factors for example temperature, minerals, CO_2, pH, water quality, light cycle and intensity; biotic factors include cell fragility and cell density. Mechanical factors include continuous mixing, gas bubble size and distribution and mass transfer, all these are of particular concern in photo-bioreactors [5], [6]. Light and temperature are the two most important factors that affect algae biomass productivity. The energy for growing algae is provided by light via photosynthesis. Sufficient light energy must be effectively utilized to achieve higher biomass productivity.

Algae cultivation also depends on pH levels and optimum pH influences the carbon availability, metabolism and biochemical composition of cells [7], [8]. For efficient use of algae as a source of bioenergy, it is very important to focus on the native algal species and to select that algal species which not only has a high growth rate but has greater lipid content. Identification of local algal species, optimization of conditions for native algal species was preferable for further studies. Hence, present study is a significant step forward in utilization and cultivation technique applied as an algae as a source of renewable energy and biological process of biogas purification.

The coccoid green microalgae genus Chlorella is one of the most important commercial microalgae [10]. Chlorella (Chlorophyta, Trebouxiophyceae), is commercially cultivated by many countries in the world. The annual production of Chlorella biomass exceeds 2,000 tonnes, mostly used for dietary supplements and nutraceuticals, with a minor share destined to the cosmetic market and aquaculture. C. vulgaris is a robust and fast growing microalgae species commonly cultivated and interesting regarding the production of secondary metabolites with health beneficial properties. The main purpose in this topic is

Rameshprabu Ramaraj, School of Renewable Energy, Maejo University, Sansai, Chiang Mai 50290, Thailand;
Yuwalee Unpaprom, Program in Biotechnology, Faculty of Science, Maejo University, Sansai, Chiang Mai-50290, Thailand;
Natthawud Dussadee, School of Renewable Energy, Maejo University, Sansai, Chiang Mai 50290, Thailand;

to minimize the production costs as low possible. That is main reason in this study open type cement ponds were employed. In this paper, we aim to cultivate the isolated and an identified strain C. vulgaris for further establishment of biogas purification.

II. Materials and Methods

A. Isolation and Identification of Microalgae

The methodology of microalgae collection, isolation and identification process were adopted our previous published papers [2], [3], [5], [6]. The sample was collected by plankton net (20-µm pore size) from freshwater fish pond (18° 55′ 4.2″N: 99° 0′ 41.1″E) at a location near Maejo University, Sansai, Thailand. The collected samples were samples of about 5 ml were inoculated into 5-ml autoclaved Bold Basal Medium (BBM) in 20-ml test tubes and cultured at room temperature (30±1°C) under 50 μmol^{-1} m^2 sec^{-1} intensity with 16:8 h photoperiod for 10 days. After incubation, individual colonies were picked and transferred to the same media for purification in 250 mL conical flask.

The culture broth was shaken manually for five to six times a day. The pre-cultured samples were streaked on BBM medium-enriched agar plates and cultured for another 10 days with cool white fluorescent light using the same light intensity. The single colonies on agar were picked up and cultured in liquid BBM medium, and the streaking and inoculation procedure was repeated until pure cultures were obtained. The purity of the culture was monitored by regular observation under microscope. The isolated microalgae were identified microscopically using light microscope with standard manual for algae [11], [12].

B. Maintenance of microalgae cultures

Isolated and purified microalgae were inoculated in 250-ml Erlenmeyer flasks containing 125 ml BBM medium. Flasks were placed on a reciprocating shaker at 120 rpm for 7 d at room temperature of 30±1°C. Light was provided by cool white fluorescent lamps at an intensity of 50 μmol^{-1} m^2 sec^{-1}. The algae culture was then transferred to 500-ml Erlenmeyer flasks containing 450 ml. Algae growth were monitored by measuring the optical density of the algal medium with spectrophotometer (Spectronic Genesys 20, Thermo Fisher Scientific) at a wavelength of 665 nm. Measurements were taken daily and three replicates were measured. .

C. Production of microalgae

Figure 1 showed the open type algal cement pond and detailed descriptions through schematic diagram. Two litters' cultured microalgae were transferred to open type cement pond (total volume 200L) for large amount of biomass production through artificial medium. The medium was prepared with rice fertilizer (100g), rice bran (400g), fish meal (100g), lime (50g) and urea (200g). This medium was named as, Rameshprabu medium. Algal cement pond height was 40 cm and length was 80 cm. Furthermore, pond was filled 150 liters water and medium. It was reached 25 cm height in the pond. All ingredients were filled with 10L water subsequently mixed by stir then transfer to the pond; it associated with air pump. Pond was left for one night to release ammonia and medium dissolve in the water properly. Next day stock algae were transfer to the triplicate cement ponds. Algal growth was measured and stirred the everyday to prevent algae precipitation.

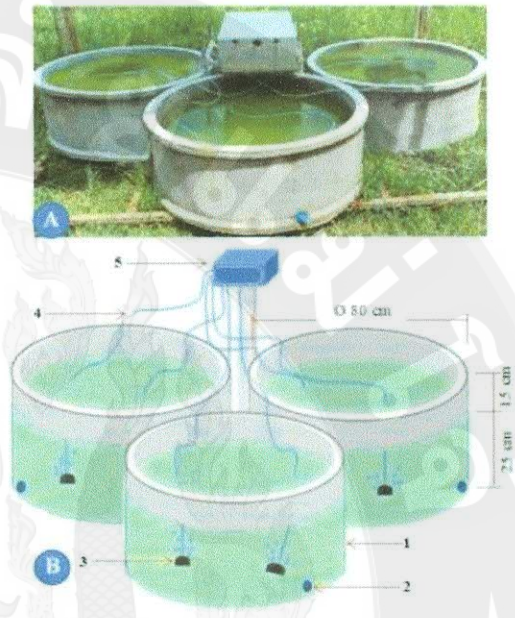

Figure 1 – (A) Open type algal cement pond; (B) - Schematic diagram of algal cement pond: 1. algae growth, 2. outlet of pond, 3. aeration bubble, 4. tube, 5. air pump.

D. Analytical Methods

All the indices including cell count, optical density (OD), pH, alkalinity, chemical oxygen demand (COD), biological oxygen demand (BOD), total nitrogen (TN), total phosphorous (TP), Total suspended solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) were continuously monitored throughout the study, following the standard protocols of APHA [11]. Fatty acid content was performed by GC-MS analysis. Chlorophylls, protein, carbohydrates, lipids determination procedures were adopted from Tipnee et al. [13]. Elemental composition (C, H, N, O, S) was analyzed using the element analyzer (Perkin–Elmer 2004). Moisture content of raw materials was determined following the procedure given in ASTM Standard D 4442-07. The residual sample in the crucible was heated without lid in a muffle furnace at 700 ± 50 °C for one half hour. The crucible was then taken out, cooled first in air, then in desiccators and weighed. Heating, cooling and weighing was repeated, till a constant weight obtained.

E. Statistical analyses

All experiments were determined in biological triplicate to ensure the reproducibility. Experimental results were obtained as the mean value ± SD. Statistical analyses were performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). The statistical significances were achieved when $p<0.05$.

International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-3, March 2016 Pages 117-122

III. Results and Discussion

Species Identification

For many years, strains of Chlorella (Chlorophyceae, Chlorococcales) have served as model organisms in plant physiology and biochemical research. Currently available information was positively identifying an environmental strain of the Chlorella genus of freshwater unicellular green algae. The genus Chlorella encompasses spherical or ellipsoidal non-motile green cells that produce autospores, and inhabit freshwater, soil and marine habitats. Its commercial potential has been considered since 1960, being the first microalga to be mass cultured for food, feed and as a source of nutraceuticals. More recently, it has also been suggested that they are good candidates for fuel production and biogas purification [7] , [14].

Since the characteristics traditionally used for taxonomy are sufficient and morphological criteria for the identification of Chlorella species. According to taxonomical grouping based on morphology and physiological properties, it belongs to genus Chlorella, family Oocystaceae, order Chlorococcales, class Chlorophyceae, division Chlorophyta of the kingdom Plantae. C. vulgaris is a spherical microscopic cell with 2-10 µm diameter and has many structural elements similar to plants [15]. The individual cells of the colonies were in the range of 10 µm. Cells are green color, unicellular, spherical in shape; figure 2 shows the morphology of C. vulgaris observed under a light microscope. The cell wall contains hemicelluloses, which accounts for the stability and rigidity of the cells. It has an asexual reproductive cycle, with the production of autospores from the mature large cell, by dividing the cell into smaller units. One mature cell divides into four new ones every 16-20 hours. The algal cells utilize sunlight for photosynthesis. The photosynthetic rate exceeds the respiration rate of Chlorella cells by 10–100 times.

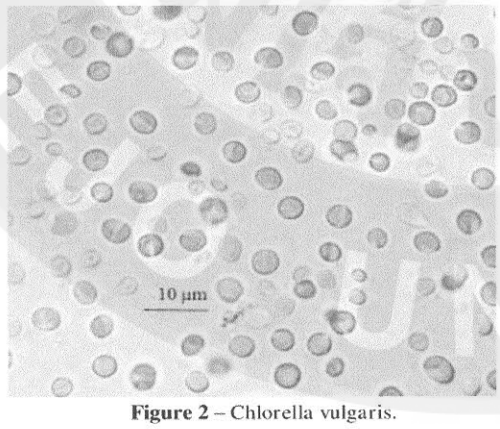

Figure 2 – Chlorella vulgaris.

Determination of algae growth and biomass production

Algae growth in this study would be presented by optical density (OD 685) condition and cell densities were determined through cell counts under optical microscope using an improved bright lined haemocytometer. Optical density and cell count clearly indicated that the best growth of C. vulgaris was through Rameshprabu medium using open type cement ponds shown in figure 3 and 4. Their growth increased rapidly in the first six days, then slowed down in the next six days, and again from thirteen days growing up, which could be due to the gradual consumption of certain nutrient elements like nitrogen and phosphorus in the medium. C. vulgaris have been widely used in numerous field applications for their strong survival abilities and efficient utilization of nitrogen and phosphorus [1]–[4].

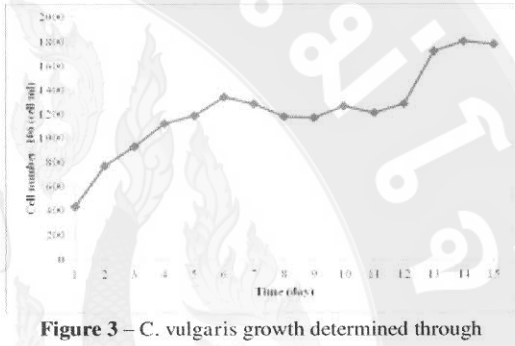

Figure 3 – C. vulgaris growth determined through cell count.

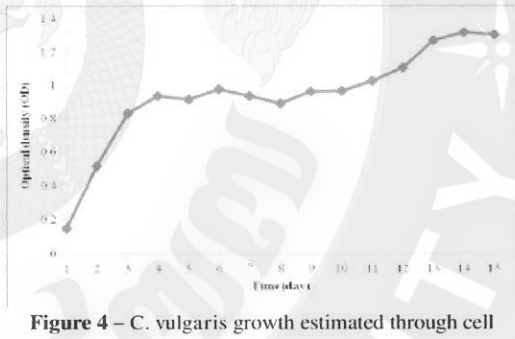

Figure 4 – C. vulgaris growth estimated through cell densities.

While CO_2 is available abundantly in the atmosphere as well as from anthropogenic sources, the availability of light is very important for the algae growth [16]. It is known that environmental factors such as light intensity, temperature and nutrients can significantly affect the composition of microalgae [17]. Furthermore, Ramaraj et al. [18] demonstrated that algae's ability to uptake CO_2 from the atmosphere and much greater biomass productivity compared to land plants. This study also proved that the microalga C. vulgaris could grow well in open type cement pond utilization atmospheric CO_2 and might be a good candidate microalga for the biogas purification if could apply in the closed system utilizing biogas as a carbon source. Algal biomass by direct measurement of total suspended solids and volatile suspended solids resulted as 474.12±0.15 mg/L and 396.25±0.03 mg/L respectively.

Chlorella vulgaris cultural conditions

The values of measured physicochemical and biological

parameters in the microalgal growth conditions of this study are summarized in table 1 and figure 5. The most important parameters are nutrient quantity/quality, light, temperature and pH [8], [18]. Growth system was setup in the open pond system for utilize solar energy directly. This study was aimed to find a strategy to reduce the costs and environmental impacts of Chlorella biomass production under autotrophic conditions used open type cement ponds. Ramaraj et al. [19] confirmed that photoautotrophic microalgae require several things to grow. Because they are photosynthetic, they need a light source, CO_2 as energy and carbon sources, water, and inorganic salts [19].

The biomass produced absorption of atmospheric CO_2, possibly driven by a decline in the CO_2 partial pressure resulted from photosynthesis. Carbon, phosphorus and nitrogen are considered to be the most important nutrients for algae growth; the results are shown in Table 1. COD measured the nutrition carbon on natural water medium and reactor effluent; the medium was 998±1.16 mg/L, while the reactor was 802.5±0.71 mg/L. After algae growth, the concentration of COD increased significantly. In the medium, nitrogen was 105.5±0.22 mg/L, while in it ponds were 50.59±70.71 mg/L. For alkalinity, the content was reduced medium to ponds. Alkalinity was an important buffering to maintain a fairly optimal growth range in the water body and the changes were consumed by algae growth from its role as one of possible carbon sources. Chlorophyll a production by phytoplankton cells is known to vary with growth conditions; in our experiments, the maximum production of chlorophyll a content of C. vulgaris was 28.89 ± 1.33 (mg/g).

Table 1– Physiochemical parameter

Parameters	Rameshprabu medium		Alga cement pond	
	Mean (± SD)	Range	Mean (± SD)	Range
pH	7.53 (±0.15)	7.4-8.2	8.38 (±0.22)	8.2-8.6
Temperature (°C)	-	-	32.56 (±0.78)	31.4-33.7
Light intensity ($\mu mol^{-1}m^{-2}$)	-	-	32.56 (±0.11)	31.4-33.7
DO (mg/L)	8.5 (±1.25)	7-10	13.5 (±0.05)	10-17
TN (mg/L)	105.5 (±0.22)	94-117	50.59 (±70.71)	24.6-84.6
TP (mg/L)	42 (±0.43)	36-48	31.9 (±0.35)	20.7-42.5
Alkalinity ($mgCaCO_3/L$)	29.03 (±1.52)	25-33	9.51 (±2.5)	1.5-20.7
COD (mg/L)	998 (±1.16)	1006-1013	802.5 (±0.71)	560-987
BOD (mg/L)	600 (±2.01)	615-630	427 (±0.66)	190-640
TSS (mg/L)	-	-	474.12 (±0.15)	319-737
VSS (mg/L)	-	-	396.25 (±0.03)	266-537
FSS (mg/L)	-	-	77.63 (±0.02)	60-199

Chlorella vulgaris chemical compositions

Photoautotrophic microalgae can effectively transform the inorganic nutrients, CO_2, H_2O and other substances into organic compounds such as protein, carbohydrate, lipid and other ingredients through photosynthesis. Microalgal biomass, containing lipids, starch, cellulose, proteins, and so on, is considered a promising feedstock for producing a variety of renewable fuels, such as biodiesel, bioethanol, biohydrogen and biogas [1]–[7]. The proximate and ultimate analysis of C. vulgaris illustrated in table 2. The major fatty acid composition was determined using GC- MS system (table 3). Green algae have the bulk of their fatty acids as saturated and unsaturated C18 s, a composition similar to that of vegetable oils [19], [20]. In this study, C. vulgaris, palmitic acid (C16:0), physetoleic acid (C16:1), and oleic acid (C18:1) were commonly dominant. In particular, lipids with high content of unsaturated fatty acids had been reasonable balance of fuel properties.

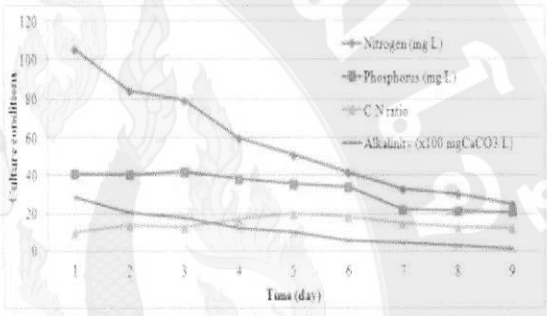

Figure 5 – Culture conditions of Chlorella vulgaris.

Table 2 – The proximate and ultimate analysis of C. vulgaris

Parameter		C. vulgaris
Proximate Analysis (%)	Moisture	9.87
	Ash	14. 87
	Carbohydrate	29.85
	Protein	48.88
	Lipid	13.60
	Fiber	17.06
Ultimate Analysis (%)	Carbon	48.56
	Hydrogen	6.40
	Oxygen	33.71
	Nitrogen	6.26
	Sulphur	0.79

Table 3 – Fatty acids analysis of C. vulgaris

Fatty acids	Value
C14:0	3.0± 0.2
C16:0	36.2± 1.5
C16:1	1.8± 0.1
C16:2	1.1± 0.2
C16:3	1.3± 0.1
C18:0	5.46± 0.3
C18:1	18.33± 1.2
C18:2	16.7± 0.6
C18:3	19.8± 0.7
MUFA [a]	20.1± 1.2
PUFA [b]	36.4± 2.2
UFA [c]	57.2± 2.7
DUS [d]	1.16 ± 0.03

Note: [a] percentage of total fatty acids (%); [b] MUFA= monounsaturated fatty acids, [c] PUFA= polyunsaturated fatty

International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-3, March 2016 Pages 117-122

acids, [d] UFA= unsaturated fatty acid, [e] DUS = degree of fatty acid unsaturation.

Biological purification processes for biogas

The main components of biogas are CH_4 and CO_2, but usually biogas also contains H_2S other sulphur compounds, water, other trace gas compounds and other impurities. Purification and upgrading of the gas is necessary because purified biogas provides reductions in green house gas emissions as well as several other environmental benefits when used as a vehicle fuel [7]. Reducing CO_2 and H_2S content will significantly improve the quality of biogas. For biogas impurity removal, biological processes are environmentally friendly and feasible. Biogas purification using algae involved the use of algae's photosynthetic ability in the removal of the impurities present in biogas.

Ramaraj and Dussadee [7] stated that algae application of CO_2 sequestration has developed as a popular topic and the current interests are including: species, power plant flue gas utilization, reactor design, growth condition, growth kinetics and modeling. The most studies in the literature concerned the maximum CO_2 uptake rate by the artificial photo-bioreactors [20]. Among those techs, bio-eco-technology is the most natural and ecological way to accomplish the designed targets by the utilization of "self-designed" bio-functions of nature [8], [18], [19]. Further our establishment study, C. vulgaris applicable for biogas purification.

IV. CONCLUSIONS

The cultivation of the microalga, C. vulgaris utilized low cost artificial medium which named as Rameshprabu medium. The medium was prepared through rice fertilizer, rice bran, fish meal, lime and urea. This medium was named as, Rameshprabu medium. The aim of the current work was to evaluate the potential of the green alga C. vulgaris as a cheap renewable energy source in term of biofuels. The algae grew faster, providing higher productivities of biomass, lipids, carbohydrates and proteins. Furthermore, C. vulgaris biomass production and carbohydrate consumption were enhanced by supplementing the inorganic culture. Photoautotrophic conditions cultivation of C. vulgaris can be considered as a feasible strategy to reduce the costs of microalgal biomass production, while also contributing to solve the environmental problem.

REFERENCES

[1] Ramaraj R, Unpaprom Y. Effect of temperature on the performance of biogas production from Duckweed. Chemistry Research Journal. 2016; 1: 58–66.
[2] Unpaprom Y, Tipnee S, Ramaraj R. Biodiesel from green alga Scenedesmus acuminatus. International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. 2015; 4: 1–6.
[3] Ramaraj R, Kawaree R, Unpaprom Y. A newly isolated green alga, Pediastrum duplex Meyen, from Thailand with efficient hydrogen production. International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. 2015; 4: 7–12.
[4] Pantawong R, Chuanchai A, Thipbunrat P, Unpaprom Y, Ramaraj R. Experimental investigation of biogas production from water lettuce, Pistia stratiotes L. Emergent Life Sciences Research. 2015; 1: 41–46.
[5] Ramaraj R, Dussadee N, Whangchai N, Unpaprom Y. Microalgae biomass as an alternative substrate in biogas production. International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. 2015; 4: 13–19.
[6] Unpaprom Y, Intasaen O, Yongphet P, Ramaraj R. Cultivation of microalga Botryococcus braunii using red Nile tilapia effluent medium for biogas production. Journal of Ecology and Environmental Sciences 3 (2015) 58–65.
[7] Ramaraj R, Dussadee N. Biological purification processes for biogas using algae cultures: a review. International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. 2015; 4: 20–32.
[8] Ramaraj R, Tsai D D-W, Chen, PH. Carbon dioxide fixation of freshwater microalgae growth on natural water medium. Ecological Engineering. 2015; 75: 86–92.
[9] Kao CY, Chiu SY, Huang TT, Dai L, Wang GH, Tseng CP, Chen CH, Lin C S. A mutant strain of microalga Chlorella sp. for the carbon dioxide capture from biogas. Biomass and Bioenergy. 2012; 36: 132–140.
[10] Champenois J, Marfaing H, Pierre R. Review of the taxonomic revision of Chlorella and consequences for its food uses in Europe. Journal of Applied Phycology. 2014; 27: 1845–1851.
[11] APHA, AWWA, WPCF. Standards methods for the examination of water and wastewater. 21st ed. APHA-AWWA-WPCF, Washington, DC, 2005.
[12] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 1959; 37: 911–917.
[13] Tipnee S, Ramaraj R, Unpaprom Y. Nutritional evaluation of edible freshwater green Macroalga Spirogyra varians. Emergent Life Sciences Research. 2015; 1: 1–7.
[14] Castorena-Gonzalez JA, Foote C, MacVittie K, Halámek J, Halámková L, Martinez-Lemus LA, Katz E. Biofuel Cell Operating in Vivo in Rat. Electroanalysis. 2013; 25: 1579–1584.
[15] Safi C, Zebib B, Merah O, Pontalier P, Vaca-Garcia C. Morphology, composition, production, processing and applications of Chlorella vulgaris: areview. Renewable and Sustainable Energy Reviews. 2014; 35: 265–278.
[16] Blair M, Kokabian B, Gude G. Light and growth medium effect on Chlorella vulgaris biomass production. Journal of Environmental Chemical Engineering. 2014; 2, 665–674.
[17] Guschina IA, Harwood JL. Lipids and lipid metabolism in eukaryotic algae. Progress in Lipid Research. 2006; 45: 160–86.
[18] Ramaraj R, Tsai DD-W, Chen, PH. Biomass of algae growth on natural water medium. Journal of Photochemistry and Photobiology B: Biology. 2015; 142: 124–128.
[19] Ramaraj R. Freshwater microalgae growth and carbon dioxide sequestration. Taichung, Taiwan, National Chung Hsing University, PhD thesis, 2013.
[20] Benemann JR, Oswald WJ. Systems and economic analysis of microalgae ponds for conversion of carbon dioxide to biomass. Final Report: Grant No.DEFG2293PC93204; 1996.

Dr. Rameshprabu Ramaraj, M.Sc., M.Phil., Ph.D., Lecturer, School of Renewable Energy, Maejo University, Sansai, Chiang Mai 50290, Thailand and Energy Research Center, Maejo University, Sansai, Chiang Mai-50290, Thailand; he also pursuing M.Eng, master of engineering (renewable energy) in the same institution. **Professional in scientific and engineering field:** biology (animal, plant & microbes), aquatic insects, medical entomology, ecology and environmental science, algae cultivation, isolation, identification and biomass analysis, algae growth fermenters and reactor analysis, biochemical analysis and water quality analysis, sustainable resource engineering, environment and ecological engineering, bio-statistical analysis and related software applications, biofuels (biodiesel, hydrogen, ethanol & biogas) and solid fuels.
List of publications:
https://www.researchgate.net/profile/Rameshprabu_Ramaraj/publications

Dr. Yuwalee Unpaprom, M.Sc., Ph.D., Lecturer, Program in Biotechnology, Faculty of Science, Maejo University, Sansai, Chiang Mai 50290, Thailand; **Professional in scientific and engineering field:** plant biotechnology, plant microbial therapeutic products and natural products, plant physiology and biochemistry, plant hormone, nutrient analysis, plant anatomy, tissue culture and cell culture, bacterial, algal, fungal isolation and cultivation, Ornamental plants (expert in native Thai orchids) and alternate fuels/bioenergy.
List of publications:
https://www.researchgate.net/profile/Yuwalee_Unpaprom/publications

Dr. Natthawud Dussadee, M.Eng., M.Sc., Ph.D., Assistant Professor & Director, School of Renewable Energy, Maejo University, Sansai, Chiang Mai-50290, Thailand. **Professional:** 1. Agricultural Processes (Drying of Foods and Cereal Grains), 2. Energy Conservation (Energy Conservation in building and Industry), 3. Solar energy and Thermal Process (Solar Heating, Heat exchanger), 4. Computer Application (Computer Simulation, Computer Programming) and 5. Renewable Energy (Wind, Hydro, Biomass, Biofuel).
List of publications:
https://www.researchgate.net/profile/Natthawud_Dussadee/publications

International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-3, March 2016 Pages 128-133

Potential Evaluation of Biogas Production and Upgrading Through Algae

Rameshprabu Ramaraj, Yuwalee Unpaprom ,Natthawud Dussadee*

Abstract— Algae are known to be a potential feedstock in the production of biogas. Many studies has been focused on microalgal species, macroalgae are also suitable as a source of rich protein, carbohydrate and lipids. In this study, a locally abundant (Chiang Mai Province, Thailand) and naturally grown filamentous algae, Spirogyra ellipsospora has been harvested from a slow running freshwater stream; subsequently biomass was dried with solar dryer, and the materials were pulverized for chemical composition analysis. The feasibility of utilizing macroalgae biomass as a monosubstrate for the biogas production was investigated. Results showed that the highest methane yield was reached 65 % without any pretreatment process. This study suggested that it is possible to achieve stable operation using S. ellipsospora, as a substrate for biogas production in pilot or large scale biogas plant. Therefore, S. ellipsospora as energy crop can be an alternative energy resource.

Index Terms— Algae, Freshwater stream, Spirogyra ellipsospora, Chlorella vulgaris, Biogas.

I. Introduction

Due to the energy crisis, renewable energy becomes a popular issue in this world today and there are several alternatives such as bioenergy, solar, wind, tide, geothermal, etc. Bioenergy is a type of renewable energy made from biological sources including algae, trees, or waste from agriculture, wood processing, food materials, and municipalities. For bioenergy, algae are the third generation biofuel [1]. It provides an excellent biomass as a renewable energy source, so called "bioenergy", and turn algae as the most efficient bio-component [2]. Recently, macroalgae have recently received considerable attentions as a substrate for biofuels production, since they have higher growth rates compared to the plants. The average photosynthetic efficiency of aquatic biomass is 6–8%, which is much higher than that of terrestrial biomass (1.8–2.2%).

Macroalgae are fast growing marine and freshwater plants that can grow to considerable size (up to 60 m in length). Annual primary production rates (grams cm-2 yr-1) are higher for the major marine macroalgae than for most terrestrial biomass [3]. Macroalgae can be subdivided into the blue algae (Cyanophyta), green algae (Chlorophyta), brown algae (Phaeophyta) and the red algae (Rhodophyta). Either Freshwater macroalgae or marine macroalgae (kelp or seaweed) could be used for solar energy conversion and biofuel production [3]. Macroalgae received a large amount of attention as a biofuel feedstock due to its prolific growth in natural habitat of freshwater system, eutrophic coastal water fouling beaches and coastal waterways. Generally, macroalgae (red, brown, and green) are obtained from natural and cultivated resources.

Spirogyra sp. (Tao) is freshwater macroalgae, available in the north and northeast of Thailand. It contain high amount of chemical components including carbohydrates, fat, proteins and mineral substances [4]. It is a genus of filamentous freshwater green algae of the Division Chlorophyta order Zygnematales Family Zygnemataceae., named for the helical or spiral arrangement of the chloroplasts that is diagnostic. It is commonly found in freshwater areas and there are more than 400 species of Spirogyra in the world [5]. They grow in the standing water of clean to moderate quality, clear water with the turbidity not exceeding 10 NTU, temperature 15-27°C and pH 6-7.8. Regarding biofuel production, algae can provide different types of biofuels, including: biodiesel (from algal fatty acids); ethanol (produced by fermentation of starch); hydrogen (produced biologically); and methane (produced by anaerobic digestion of algal biomass). From an environmental and resource-efficiency perspective biogas has several advantages in comparison to other biofuels. Hughes et al. [6] suggested that energy conversion via anaerobic digestion was successful as the biochemical composition of macroalgae makes it an ideal feedstock. The production of biogas via anaerobic digestion (AD) is the most feasible and cost-effective route to an energy product [7], [8].

Biogas is composed of 40-70% methane (CH_4), 60-30% carbon dioxide (CO_2), 100-3000 ppmv hydrogen sulfide (H_2S) and water, other trace gas compounds and other impurities. Since the main components of biogas are CH_4, CO_2 and H_2S. Purification and upgrading of the gas is necessary because purified biogas provides reductions in green house gas emissions as well as several other environmental benefits when used as a vehicle fuel. Reducing CO_2 and H_2S content will significantly improve the quality of biogas. Various technologies have been developed and available for biogas impurity removal; and biological processes are environmentally friendly and feasible. Furthermore, microalgae are abundant and omnipresent. Biogas purification using microalgae involved photosynthetic ability in the removal of the impurities present in biogas. In Thailand, there is plenty of freshwater natural resource available. In this study, we utilized the natural recourses and obtain algal biomass from the resource directly for biogas production. It could be reduce the cultivation and production cost. In the same time, this approach could be helpful to

Rameshprabu Ramaraj, School of Renewable Energy, Maejo University, Sansai, Chiang Mai 50290, Thailand
Yuwalee Unpaprom, Program in Biotechnology, Faculty of Science, Maejo University, Sansai, Chiang Mai-50290, Thailand
Natthawud Dussadee, School of Renewable Energy, Maejo University, Sansai, Chiang Mai 50290, Thailand

decrease algae degradation and pollution in the water body. In addition, it could reduce the algal bloom. However, biogas is containing with impurities. Biogas impurity removals through microalgal system are beneficial for environment and economical aspects [9]. It could be save environment effects such as green house gases and same time it could reduce the chemical cost and other related purification material cost. Therefore, this study focused on the development in AD techniques for macroalgae as biogas producer and using microalgae as biogas purification. The main objectives are (1) to utilize the natural recourses and evaluation of algae growth environment, (2) to investigate the biogas production potential from freshwater macroalgae (Spirogyra ellipsospora) and (3) to apply the biogas purification through microalgal (Chlorella vulgaris) biological purification process.

II. Materials and Methods

A. Plant materials (macroalgae)

Spirogyra ellipsospora biomass was collected from the slow running fresh water stream at Tumbon Pang Yang (19° 18'42.41" N; 98° 48'44.11" and elevation 722 m), Mae Taeng district, Thailand and transported to the Energy Research Center, Maejo University, Sansai, Chiang Mai-50290,Thaialnd. Species identification and morphological details were presented in our previous study [10], [11]. Figure 1 demonstrated that study site, material collection, harvesting and drying through solar dryer for biochemical analysis. Samples of macroalgae biomass were collected by hand (traditional method) directly from the stream. Directly after acquisition macroalgae biomass was rinsed with tap water to remove sand and other pollutants.

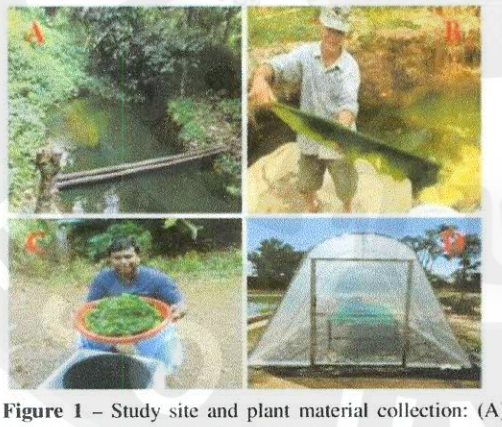

Figure 1 – Study site and plant material collection: (A) slow running fresh water stream, (B) traditional algae collection, (C), harvested algae, (D) solar dryer for biomass drying.

B. Microalgae collection for biogas purification

The cultured microalgae, C. vulgaris were obtained from Energy Research Center, Maejo University, Sansai, Chiang Mai-50290, Thailand. The algae were cultivated using open type cement pond and low cost artificial medium which is named as Rameshprabu medium.

C. Experimental procedure

The bioreactor system consists of flasks of 5000 ml in capacity. The equipment is constituted of: valves, quick release tubing connectors, plastic pipes and gas collector, shown in figure 2. To preserve anaerobic conditions, nitrogen has been flushed for 2 min into the reactors to clear up any residual trace of oxygen from within the flasks and pipes. Water-baths were used to keep the reactors at a mesophilic temperature in the laboratory. A biogas analyzer was used to verify anaerobic conditions were created correctly when preparing the reactors and to analyze the biogas biochemical composition. The experimental set up and methodologies are followed our previous studies [10], [11]. The purpose of the first experiment is to identify whether a benefit in room temperature system for biogas production. The anaerobic assays were conducted in 5000 mL duran bottles (triplicate reactor) containing 400 mL of inoculum and 1000 g of fresh S. ellipsospora and remaining make up with double distilled water. The total working volume is 4000 mL. After inoculation, all batch reactors were purged with nitrogen gas to create an anaerobic condition. Triplicate, 5000 mL fermenters were incubated in the room temperature (assumed as mesophilic conditions). Production of biogas was monitored by measuring the overpressure in the bottle headspace at time intervals depending on the production of biogas. Three digesters have been prepared with the exact amount of inoculums used. The anaerobic inoculum was obtained from a working anaerobic digester at Energy Research Center, Maejo University.

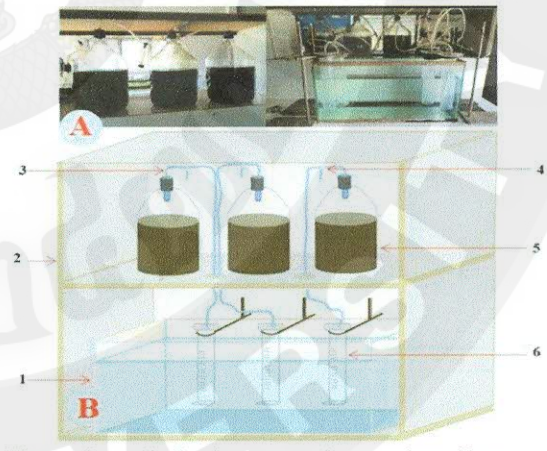

Figure 2 – A) Batch system of macroalgae biogas production B) Schematic view of the experimental set up during anaerobic digestion (1) water tank 2) protection wooden box 3) gas transfer tube 4) gas sampling port 5) digester (5000ml) 6) gas measuring cylinder

The inoculums characteristics including TS, VS, COD were 296.1 ± 0.05 mg/L, 158.5 ± 1.15 mg/L and 1241.6 ± 2.01 mg/L, respectively; along with alkalinity of 136.4 ± 0.04 mg/L as $CaCO_3$, VFA of 136.4 ± 0.25 mgCH_3COOH/L and pH was 6.66 ± 0.03 [12]. Five liter batch fermenters were

International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-3, March 2016 Pages 128-133

incubated at room temperature conditions for 70 days. The digesters were shaken two or three times everyday to prevent the formation of surface crust which may prevent contact between microorganisms and the substrate.

D. Analytical Methods

All the indices including chemical oxygen demand (COD), total nitrogen (TN), total phosphorous (TP), Total solids (TS) and volatile solids were continuously monitored throughout the study, following the standard protocols of APHA [13]. Metrohm 774 pH-meter was used in all pH measurements. Fatty acid content was performed by GC-MS analysis. Protein, carbohydrates, lipids determination procedures were adopted from Tipnee et al. [14]. Elemental composition (C, H, N, O, S) was analyzed using the element analyzer (Perkin–Elmer 2004). Moisture content of raw materials was determined following the procedure given in ASTM Standard D 4442-07. The residual sample in the crucible was heated without lid in a muffle furnace at 700 ± 50°C for one half hour. The crucible was then taken out, cooled first in air, then in desiccators and weighed. Heating, cooling and weighing was repeated, till a constant weight obtained. Total fat, ash, moisture, fiber contents and volatile fatty acids (VFA) were determined using AOAC official method [15]. The composition of biogas (CH_4, CO_2, H_2S, H_2 and O_2) was measured using a biogas analyzer (GFM 416 series, UK). All the values or readings are the result of mean of three replicates. Data was reported as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel. The statistical significances were achieved when $p<0.05$.

III. Results and Discussion

Feedstock characteristics

Results of physical and chemical composition of the S. ellipsospora after harvested and biomass showed distinct differences in their chemical composition. The fixed carbon, volatile matter, moisture, ash, pH, carbon, hydrogen, oxygen and nitrogen contents were in S. ellipsospora, 13.38%, 66.16%, 8.27%, 15.02%, 7.4, 41.87%, 6.00%, 35.77% and 0.43 %, respectively. The macromolecules of carbohydrate, protein, lipid and fiber contents were 56.43%, 21.60%, 9.81%, and 6.50%, respectively. In this study, the sample content of total solids (TS) and volatile solids (VS) was measured; the results were average as 16622 mg/kg and 13959 mg/kg, respectively. The average was COD 14236 mg/L. Consequently S. ellipsospora has plenty of nutrients for biogas production process; this macroalgae is suitable to be used as energy crops for biogas production. .

Macroalgae, Spirogyra ellipsospora to biogas: anaerobic digestion

Macroalgae can be converted to biofuels by various processes including thermal treatment [16] and fermentation [17], [18] but the most direct route to obtaining biofuel from macroalgae is via its anaerobic digestion to biogas (~ 60% methane). Methane can be used to produce heat and electricity or compressed for use as a transport fuel. Since this time there have been developments in AD technology and an enormous increase in its use Hughes et al. [18]. The study results of biogas and methane production from macroalgae, S. ellipsospora presented in figure 3 and 4. Without any pretreatment methane content was achieved 64.67 %. Our studies agreed with Hughes et al. [18] and also confirmed better results; on 70th day day CO_2 level was 31.5 % and H_2S was 578 ppm (i.e. 0.0578%). For the comparison of freshwater macroalgae biogas production and methane content from S. ellipsospora was much higher than Spirogyra neglecta, Chara fragilis and Cladophora glomerata, which was reported by Baltrėnas and Misevičius [19].

Figure 3 – biogas and methane production from macroalgae, S. ellipsospora.

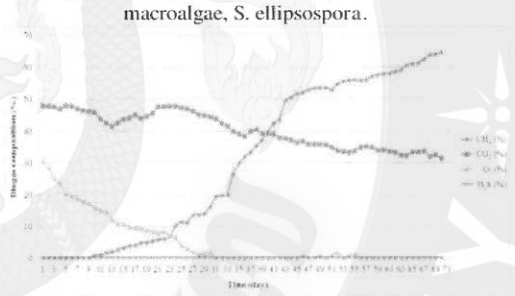

Figure 4 – biogas composition of macroalgae, S. ellipsospora during anaerobic digestions.

Biogas purification through biological process

CO_2 Microalgae can fix CO_2 using solar energy with efficiency ten times greater than terrestrial plants [20], [21]. Therefore, we require the rapid development of bio-carbon-fixation technology to eliminate the adverse effects of CO_2, to transfer atmospheric CO_2 through the carbon cycle and to promote carbon balancing ecologically. The most studies in the literature concerned the maximum CO_2 uptake rate by the artificial photo-bioreactors [22],[23]. Among those techs, bio-eco-technology is the most natural and ecological way to accomplish the designed targets by the utilization of "self-designed" bio-functions of nature [24],[25]. Accordingly, algae production has a great potential for CO_2 bio-fixation process and deserves a close look.

Biogas purification/scrubbing using algae involved the use of algae's photosynthetic ability in the removal of the impurities (mainly CO_2 and H_2S) present in biogas, leaving a purified biogas containing almost pure methane, which could be used for energy generation. Biological purification technology is worth examining because has double impact.

The method about removing CO_2 from biogas by microalgal culturing using the biogas effluent as nutrient medium and effectively upgrade biogas also simultaneously reduce the biogas effluent nutrient [26]. Using biogas as a source of carbon dioxide has two main advantages: the biomass production costs are reduced and the produced biomass does not contain harmful compounds, which can occur in flue gases. Hendroko et al. [27] verified exhibit that microalgae (Scenedesmus sp.) in laboratory experiments using biogas slurry as growing medium and biogas are given periodically generate 21% of CO_2 compared with 24% of controls. There are several authors [26], [27], [28] reported that Arthrospira sp, Chololera vulgaris SAG 211-11b, Chlorella sp. MM-2, Chlorella sp. MB-9, Chlorella vulgaris ARC1, Chlamydomonas sp. dan Scenedesmus sp. was a positive synergy with biogas. The productivity of the system with Zarrouk media and biogas almost 5 times higher than that for the same media without biogas when piggery waste was used, the utilization of biogas brings a productivity gain of about 2–5 times higher [27].

Kao et al. [29] demonstrates that the microalga Chlorella sp. MB-9 was a potential strain which was able to utilize CO_2 for growth when aerated with desulfurized biogas (H_2S<50ppm) produced from the anaerobic digestion of swine wastewater. The demonstrated system can be continuously used to upgrade biogas by utilizing a double set of photobioreactor systems and a gas cycle-switching operation. Furthermore, they demonstrated that the efficiency of CO_2 capture from biogas could be maintained at 50% on average, and the CH_4 concentration in the effluent load could be maintained at 80% on average, i.e., upgrading was accomplished by increasing the CH_4 concentration in the biogas produced from the anaerobic digestion of swine wastewater by 10%.

Some literatures mentioned about the cultivation microalgae using biogas as CO_2 provider. Kao et al. (2012a) used biogas that contained 20±2% CO_2 for Chlorella sp. culture with variation of light intensity which was at cloudy and at sunny day. Kao et al. [30] used biogas that contained 20±1% CO_2 for Chlorella sp. culture with variation flow rate of biogas which was 0.05; 0.1; 0.2; 0.3 vvm. Douškova et al. (2010) investigated the potential of biogas as CO_2 provider for Chlorella vulgaris; and optimization of biogas production from distillery stillage is described. The growth kinetics of microalgae Chlorella sp. consuming biogas or mixture of air and CO2 in the concentration range of 2–20% (v/v) (simulating a flue gas from biogas incineration) in laboratory-scale photo-bioreactors. It was proven that the raw biogas (even without the removal of H_2S) could be used as a source of CO_2 for growth of microalgae. The growth rate of microalgae consuming biogas was the same as the growth rate of the culture grown on a mixture of air and food-grade CO_2. Several species of algae can metabolize H_2S. Using a biological system to remove H_2S has similar benefits to using one to remove CO_2: lower upkeep costs, more environmentally sustainable and non-hazardous waste.

Furthermore, Tongprawhan et al. [31] used oleaginous microalgae to capture CO_2 from biogas for improving methane content and simultaneously producing lipid. They screened several microalgae for identify their ability to grow and produce lipid using CO_2 in biogas. Finally, they reported a marine Chlorella sp. was the most suitable strain for capturing CO_2 and producing lipid using biogas (50% v/v CO_2 in methane) as well as using 50% v/v CO_2 in air. Sumardiono et al. [32] established to evaluate the design of the photobioreactor system for purifying biogas through the culturing of microalgae. This system represented a simple promising way for the current forthcoming technologies of biogas purification. It helps to decrease the concentration of CO_2 in biogas concomitantly producing microalgae biomass. The microalgae Nannochloropsis is able to use CO_2 from biogas produced from the anaerobic digestion of tannery sludge. The results show that cultivation of microalgae under the biogas to scrub out CO_2 and promote enrichment of methane in the biogas in this work and obtained scrubbing of 27% from 30%.

The biocapture of CO_2 by microalgae can be applied to improve the quality of biogas by reducing the CO_2 content as this would lead to an increase in the methane content [33]. The microalgae Chlorella sp. was analyzed in terms of conditioning biogas. As a result the biogas components CO_2 and H_2S could be reduced up to 97.07% and100%, respectively. Also an increase of microalgae cell count could be documented, which provides interesting alternatives for the production of algae ingredients. Consequently, the algae biological purification is an alternative to other biogas purification methods.

Biogas purification through microalgae Chlorella vulgaris

The entire unit of biogas purification through microalgae C. vulgaris system and schematic view of the experimental set up during biogas upgrading processes were shown in figure 5. The microalgal culture obtained directly from the open pond system which was grown near this experimental zone. Ten liter of C. vulgaris cultures was used through 10 L duran bottles. The biogas obtained from macroalgae fermenter which is involved in the study. The macroalgae, S. ellipsospora biogas production processes are above mentioned.

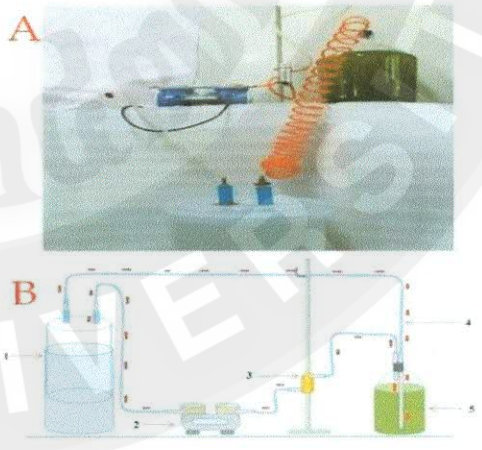

Figure 5– A) biogas purification system through microalgae, C. vulgaris B) schematic diagram of set up during biogas upgrading 1) biogas storage container 2) air pump 3) air flow meter 4) biogas pumping direction and 5) microalgae unit.

International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-2, Issue-3, March 2016 Pages 128-133

Table 1– Test and evaluation of the system performance through biological upgrading.

Component	Biogas production analysis	
	Before the system improve	After the system improvement
CH_4	64.67%	82.05%
CO_2	31.5 %	17.08%
O_2	0%	1.11%
H_2S	578 ppm	Less than 0.01 ppm

Photoautotrophic The purification process was continued 8 hours. The results were presented in table 1. After purification, the CH4 content has improved gigantically. Also CO_2 and H_2S amounts were reduce a lot. Due to algal photosynthesis process O_2 was slightly increased. Reducing CO_2 and H_2S content will significantly improve the quality of biogas. However this study suggested that long time purification process needed. For biogas impurity removal, biological processes are environmentally friendly and feasible. Consequently biogas purification using algae involved the use of algae's photosynthetic ability in the removal of the impurities present in biogas. Further our establishment study, C. vulgaris applicable for biogas purification.

IV. CONCLUSIONS

The cultivation of the microalga, C. vulgaris utilized low cost artificial medium which named as Rameshprabu medium. The medium was prepared through rice fertilizer, rice bran, fish meal, lime and urea. This medium was named as, Rameshprabu medium. The aim of the current work was to evaluate the potential of the green alga C. vulgaris as a cheap renewable energy source in term of biofuels. The algae grew faster, providing higher productivities of biomass, lipids, carbohydrates and proteins. Furthermore, C. vulgaris biomass production and carbohydrate consumption were enhanced by supplementing the inorganic culture. Photoautotrophic conditions cultivation of C. vulgaris can be considered as a feasible strategy to reduce the costs of microalgal biomass production, while also contributing to solve the environmental problem.

REFERENCES

[1] Tsai D D-W. Watershed Reactor Analysis, CO_2 Eco-function and Threshold Management Study. PhD thesis, National Chung Hsing University, Taichung, Taiwan. 2012.
[2] Ramaraj R. Freshwater microalgae growth and Carbon dioxide Sequestration. PhD thesis. National Chung Hsing University, Taichung, Taiwan. 2013.
[3] Gellenbeck KW, Chapman DJ. Seaweed uses: the outlook for mariculture. Endeavour. 1983; 7: 31–37.
[4] Peerapornpisal Y. Edible freshwater macroalgae in Northern Thailand. Journal of Fisheries Technology Research. 2008; 1: 178-189.
[5] Naik A, Hemavani C, Thippeswamy B. Evaluation of antimicrobial property of Spirogyra species. International Multidisciplinary Research Journal. 2012; 2:13–15.
[6] Hughes AD, Kelly MS, Black KD, Stanley MS. Biogas from Macroalgae: is it time to revisit the idea? Biotechnology for Biofuels. 2012; 5:86.
[7] Park K C, Whitney C, McNichol JC, Dickinson KE, MacQuarrie S, Skrupski BP, Zou J, Wilson KE, O'Leary SJB, McGinn PJ. Mixotrophic and photoautotrophic cultivation of 14 microalgae isolates from Saskatchewan, Canada: potential applications for wastewater remediation for biofuel production. Journal of Applied Phycology. 2012; 24: 339–348.
[8] Frigon J-C, Matteau-Lebrun F, Hamani Abdou R, McGinn PJ, O'Leary SJB, Guiot SR. Screening microalgae strains for their productivity in methane following anaerobic digestion. Applied Energy. 2013; 108: 100–107.
[9] Ramaraj R, Dussadee N. Biological purification processes for biogas using algae cultures: a review . International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. 2015; 4: 20–32.
[10] Pantawong R, Chuanchai A, Thipbunrat P, Unpaprom Y, Ramaraj R. Experimental investigation of biogas production from water lettuce, Pistia stratiotes L. Emergent Life Sciences Research. 2015; 1: 41–46.
[11] Ramaraj R, Dussadee N, Whangchai N, Unpaprom Y. Microalgae biomass as an alternative substrate in biogas production. International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology. 2015; 4: 13–19.
[12] Ramaraj R, Unpaprom Y. Effect of temperature on the performance of biogas production from Duckweed. Chemistry Research Journal. 2016; 1: 58–66.
[13] APHA, AWWA and WPCF. Standards Methods for the Examination of Water and Wastewater. 21st ed. APHA-AWWA-WPCF, Washington, DC. 2005.
[14] Tipnee S, Ramaraj R, Unpaprom Y. Nutritional evaluation of edible freshwater green Macroalga Spirogyra varians. Emergent Life Sciences Research. 2015; 1: 1–7.
[15] Official Methods of Analysis of AOAC International. 19th Ed., AOAC International, 420 Gaithersburg, MD, USA. 2012.
[16] Zhou D, Zhang LA, Zhang SC, Fu HB, Chen JM. Hydrothermal liquefaction of macroalgae Enteromorpha prolifera to Bio-oil. Energy Fuel. 2010; 24:4054–4061.
[17] Goh CS, Lee KT. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. Renewable Sustainable Energy Review. 2010; 14:842–848.
[18] Adams JM, Gallagher JA, Donnison IS. Fermentation study on Saccharina latissima for bioethanol production considering variable pre-treatments. Journal of Applied Phycology. 2009; 21:569–574.
[19] Hughes D, Kelly MS, Black KD, Stanley MS. Biogas from Macroalgae: is it time to revisit the idea? Biotechnology for Biofuels. 2012; 5: 86.
[20] Ramaraj R. Freshwater microalgae growth and Carbon dioxide Sequestration, PhD thesis. National Chung Hsing University, Taichung, Taiwan. 2013.
[21] Ramaraj R. Tsai DD-W, Chen PH., Chlorophyll is not accurate measurement for algal biomass. Chiang Mai Journal of Science. 2013; 40: 547–555.
[22] Ramaraj R. Tsai DD-W, Chen PH. Freshwater microalgae niche of air carbon dioxide mitigation. Ecological Engineering. 2014; 68: 47–52.
[23] Ramaraj R, Tsai D. D-W, and Chen P. H. An exploration of the relationships between microalgae biomass growth and related environmental variables. Journal of Photochemistry and Photobiology B: Biology. 2014; 135: 44–47.
[24] Tsai D D-W, Chen PH, Chou M-j, Hsu C-F, Ramaraj R, Chen PH. Carbon sequestration by alga ecosystems. Ecological Engineering. 2015; 84: 386–389.
[25] Tsai DD-W, Ramaraj R, Chen PHCarbon dioxide bio-fixation by algae of high rate pond on natural water medium. Ecological Engineering. 2016; 92: 106–110.
[26] Yan Z, Zheng Z. Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae Chlorella sp. Bioresource Technology 139: 292–299.
[27] Hendroko R, Kawaroe M, Salafudin, Saefurahman G,. Fitrianto NE, Sari DW, Sakri Y. Biorefinery preliminary studies: integration of slurry and CO2 as biomethane digester waste for microalgae Scenedesmus sp. Growth. International seminar on chemical engineering Soehadi Reksowardojo, Bandung, October 5–7, 2011.

[28] Rittmann BE. Opportunities for renewable bioenergy using microorganisms. Biotechnology and Bioengineering. 2008; 100: 203–212.
[29] Kao Y, Chiu SY, Huang TT, Dai L, Wang GH, Tseng CP, Chen CH, Lin CS. A mutant strain of microalga Chlorella sp. for the carbon dioxide capture from biogas. Biomass and Bioenergy. 2012; 36: 132–140.
[30] Kao Y, Chiu SY, Huang TT, Dai L, Hsu LK, Lin CS.. Ability of a mutant strain of the microalga Chlorella sp. to capture carbon dioxide for biogas upgrading. Applied Energy. 2012; 93: 176–183.
[31] Tongprawhan W, Srinuanpan S, Cheirsilp B. Biocapture of CO_2 from biogas by oleaginous microalgae for improving methane content and simultaneously producing lipid. Bioresource Technology. 2014; 170: 90–99.
[32] Sumardiono S, Budiyono IS, Sasongko SB. Utilization of biogas as carbon dioxide provider for Spirulina platensis culture. Current Research Journal of Biological Sciences. 2014; 6: 53–59.
[33] Mann G, Schlegel M, Schumann R, Sakalauskas A. Biogas-conditioning with microalgae. Agronomy Research. 2009, 7: 33–38.

Dr. Rameshprabu Ramaraj, M.Sc., M.Phil., Ph.D., Lecturer, School of Renewable Energy, Maejo University, Sansai, Chiang Mai 50290, Thailand and Energy Research Center, Maejo University, Sansai, Chiang Mai-50290, Thailand; he also pursuing M.Eng, master of engineering (renewable energy) in the same institution. **Professional in scientific and engineering field:** biology (animal, plant & microbes), aquatic insects, medical entomology, ecology and environmental science, algae cultivation, isolation, identification and biomass analysis, algae growth fermenters and reactor analysis, biochemical analysis and water quality analysis, sustainable resource engineering, environment and ecological engineering, bio-statistical analysis and related software applications, biofuels (biodiesel, hydrogen, ethanol & biogas) and solid fuels.
List of publications:
https://www.researchgate.net/profile/Rameshprabu_Ramaraj/publications

Dr. Yuwalee Unpaprom, M.Sc., Ph.D., Lecturer, Program in Biotechnology, Faculty of Science, Maejo University, Sansai, Chiang Mai 50290, Thailand; **Professional in scientific and engineering field:** plant biotechnology, plant microbial therapeutic products and natural products, plant physiology and biochemistry, plant hormone, nutrient analysis, plant anatomy, tissue culture and cell culture, bacterial, algal, fungal isolation and cultivation, Ornamental plants (expert in native Thai orchids) and alternate fuels/bioenergy.
List of publications:
https://www.researchgate.net/profile/Yuwalee_Unpaprom/publications

Dr. Natthawud Dussadee, M.Eng., M.Sc., Ph.D., Assistant Professor & Director, School of Renewable Energy, Maejo University, Sansai, Chiang Mai-50290, Thailand. **Professional:** 1. Agricultural Processes (Drying of Foods and Cereal Grains), 2. Energy Conservation (Energy Conservation in building and Industry), 3. Solar energy and Thermal Process (Solar Heating, Heat exchanger), 4. Computer Application (Computer Simulation, Computer Programming) and 5. Renewable Energy (Wind, Hydro, Biomass, Biofuel).
List of publications:
https://www.researchgate.net/profile/Natthawud_Dussadee/publications

APPENDIX B

CURRICULUM VITAE

CURRICULUM VITAE

NAME	Rameshprabu Ramaraj
DATE OF BIRTH	21 October 1981
EDUCATION	Jul 2013 Doctor of Philosophy in Soil & Water Conservation
	Jun 2006 Master of Philosophy in Zoology (Medical entomology)
	Jun 2004 Master of Science in Zoology
	Jun 2002 Bachelor of Science in Zoology with ancillary of Chemistry & Botany)
WORK EXPERIENCE	Aug 2015–Present Lecturer, School of Renewable Energy, Majeo University, Thailand.
	Aug 2013–Jul 2015 Foreign expert, School of Renewable Energy, Maejo University, Thailand.
	Aug 2014–Jul 2015 Project leader of algae biogas co-digestion of energy crops (Funded by Ministry of Energy, Thailand).
RESEARCH PROJECTS	Aug 2013–Jul 2014 Project Research associate & consultant, Project: Inland Aquaculture and Adaptation to Climate Change in Northern Thailand (Funded by International Development Research Centre, Ottawa, Canada, as a contribution to the AQUADAPT project).
AWARDS AND HONORS	Invited "Special lecturer" at Graduate School of Engineering, Nagasaki University, Japan, 2016
	Best research paper award, 2015 by Emergent Life Science Research

Best research student awarded by KUO HSI-LIU FOUNDATION, 2011

National Chung Hsing University Scholarship, 2009-2013

Outstanding student awarded with Taiwan Government Scholarship, 2006-2009

Best Behaved Student Award received during 2004.

PUBLICATIONS

David D-W. Tsai, **Rameshprabu Ramaraj** and Paris Honglay, Carbon dioxide bio-fixation by algae of high rate pond on natural Water medium, Ecological Engineering, (2016), 92: 106-110

Rameshprabu Ramaraj, Yuwalee Unpaprom, Natthawud Dussadee Cultivation of Green Microalga, *Chlorella vulgaris* for Biogas Purification, International Journal of New Technology and Research, 2016, (3): 117-122

Rameshprabu Ramaraj, Yuwalee Unpaprom, Natthawud Dussadee Potential Evaluation of Biogas Production and Upgrading Through Algae, International Journal of New Technology and Research, 2016, (3): 128-133

Piyaphong Yongphet, **Rameshprabu Ramaraj**, Natthawud, Effect of Greenhouse Cages Integrated with

using Solar Energy on the Growth Performance on Freshwater Fish , International Journal of New Technology and Research, 2016, (3): 100-107

Rameshprabu Ramaraj, Yuwalee Unpaprom, Effect of temperature on the performance of biogas production from Duckweed, Chemistry Research Journal, 2016, 1(1): 58-66

David D-W. Tsai, Paris Honglay Chen, Caroline Mengjung Chou, Cheng-Feng Hsu and **Rameshprabu Ramaraj**, Carbon sequestration by alga ecosystems, Ecological Engineering 84 (2015): 386–389.

Rungtawan Pantawong, Ajcharapa Chuanchai, Parichat Thipbunrat, Yuwalee Unpaprom, **Rameshprabu Ramaraj**, Experimental Investigation of Biogas Production from Water Lettuce, Pistia stratiotes L, Emergent Life Sciences Research (2015) 1(2): 41–46.

Rameshprabu Ramaraj, David Dah-Wei Tsai and Paris Honglay Chen, Detention time study of algal biomass production with natural water medium, Chiang Mai Journal of Science 42 (2015): 549–559.

Yuwalee Unpaprom, Orapin Intasaen, Piyaphong Yongphet and **Ramaraj Rameshprabu**, Cultivation of

microalga *Botryococcus braunii* using red Nile tilapia effluent medium for biogas production, Journal of Ecology and Environmental Sciences 3 (2015): 58–65.

Ramaraj Rameshprabu, Tsai David D-W and Chen, Paris Honglay, Carbon dioxide fixation of freshwater microalgae growth on natural water medium, Ecological Engineering 75 (2015): 86–92.

Rameshprabu Ramaraj, David Dah-Wei Tsai and Paris Honglay Chen,
Biomass of algae growth on natural water medium, Journal of Photochemistry and Photobiology B: Biology 142 (2015) : 124–128.

Yuwalee Unpaprom, **Rameshprabu Ramaraj**, and Murugan Kadarkarai, Mosquito larvicidal properties of *Anisomeles Malabarica* (L.) extracts against the Malarial vector, *Anopheles Stephensi* (Liston), Chiang Mai Journal of Science 42 (2015) : 148–155.

Rameshprabu Ramaraj, Yuwalee Unpaprom, Niwooti Whangchai, Natthawud Dussadee, Culture of macroalgae *Spirogyra ellipsospora* for long-term experiments, stock maintenance and biogas production, Emergent Life Sciences Research (2015) 1(1): 38–45.

Sawitree Tipneea, **Rameshprabu Ramaraj**, Yuwalee UnpapromNutritional evaluation of edible freshwater green macroalga *Spirogyra varians*, Emergent Life Sciences Research (2015) 1(2): 1–7.

Pairote Wongputtisin, **Rameshprabu Ramaraj**, Yuwalee Unpaprom, Rungthip Kawaree and Nongkran Pongtrakul, Raffinose family oligosaccharides in seed of Glycine max cv. Chiang Mai60 and potential source of prebiotic substances, International Journal of Food Science & Technology (2015) doi: 10.1111/ijfs.12842

Yuwalee Unpaprom, Sawitree Tipnee, **Ramaraj Rameshprabu,** Biodiesel from Green Alga *Scenedesmus acuminatus*, International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology 4 (2015): 1–6.

Ramaraj Rameshprabu, Rungthip Kawaree, Yuwalee Unpaprom A Newly Isolated Green Alga, *Pediastrum Duplex* Meyen, from Thailand with Efficient Hydrogen Production, International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology 4 (2015): 7–12.

Rameshprabu Ramaraj, Natthawud Dussadee, Niwooti Whangchai, Yuwalee Unpaprom, Microalgae Biomass as an Alternative Substrate in Biogas Production, International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology 4 (2015): 13–19.

Rameshprabu Ramaraj, Natthawud Dussadee, Biological Purification Processes for Biogas Using Algae Cultures: A Review, International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology 4 (2015): 20–32.

Rameshprabu Ramaraj, Natthawud Dussadee, Renewable Energy Application for Organic Agriculture: A Review, International Journal of Sustainable and Green Energy. Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology 4 (2015): 33–38.

Natthawut Dutsadee, Nigran Homdoung, **Rameshprabu Ramaraj**, Khamatanh Santisouk, Shangphuerk Inthavideth, Performance Analysis of Power Generation by Producer Gas from Refuse Derived Fuel-5 (RDF-5)

Rameshprabu Ramaraj, Natthawud Dussadee, Renewable Energy Application for Organic Agriculture: A Review, Special Issue: Renewable Energy Applications in the Agricultural Field and Natural Resource Technology 4 (2015): 33–38.

Rameshprabu Ramaraj, David D-W. Tsai and Paris Honglay Chen An exploration of the relationships between microalgae biomass growth and related environmental variables. Journal of Photochemistry and Photobiology B: Biology 135 (2014): 44–47.

Ramaraj Rameshprabu, Tsai David D-W and Chen, Paris Honglay, Freshwater microalgae niche of air carbon dioxide mitigation, Ecological Engineering 68 (2014): 47–52.

Nattawud Dussadee, Kamoldara Reansuwan, **Rameshprabu Ramaraj,** Potential development of compressed bio-methane gas production from pig farms and elephant grass silage for transportation in Thailand, Bioresource Technology 155 (2014): 438–441.

David D-W. Tsai, Caroline M. Chou, **Rameshprabu Ramaraj**, Wen-Cheng Liu and Paris Honglay Chen, Watershed safety and quality control by safety

threshold method, Geophysical Research Abstracts 6 (2014) EGU 2014–4880.

Rameshprabu Ramaraj, and Yuwalee Unpaprom, Medicinally Potential Plant of *Anisomeles malabarica* (L.) R.Br., Journal of Agricultural Research and Extension 3Suppl (2013): 29–39.

Ramaraj Rameshprabu, Tsai David DW and Chen, Paris Honglay, Chlorophyll is not accurate measurement for algal biomass, Chiang Mai Journal of Science 40 (2013): 547–555.

David Dah-Wei Tsai, **Rameshprabu Ramaraj**, Paris Honglay Chen, Growth condition study of algae function in ecosystem for CO_2 bio-fixation, Journal of Photochemistry and Photobiology B: Biology 107 (2012): 27–34.

David Dah-Wei Tsai, **Rameshprabu Ramaraj**, Paris Honglay Chen,A Method of Short-Circuiting Comparison, Water Resources Management 26, (2012): 2689–2702.

David Dah-Wei Tsai, **Rameshprabu Ramaraj** and Paris Honglay Chen, A method of short-circuiting comparison with mixing indexes, Journal of Water Supply: Research and Technology AQUA 60 (2011): 502–510.

Rameshprabu Ramaraj, David Dah-Wei Tsai and Paris Honglay Chen, Algae Growth in Natural Water Resources, Journal of Soil and Water Conservation 42 (2010): 439–450.

Paris Honglay Chen, David Dah-Wei Tsai and **Rameshprabu Ramaraj**, The Study of Bioenergy from Natural Water Resource, Journal of Soil and Water Conservation 41 (2009): 455–466.