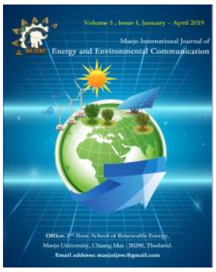
Maejo International Journal of
Energy and Environmental Communication



ARTICLE

Green biomass to biogas – A study on anaerobic monodigestion of para grass

Ajcharapa Chuanchai[1*], Sawitree Tipnee[2], Yuwalee Unpaprom[2], Keng-Tung Wu[1,*]

[1]Department of Foreatry, National Chung Hsing University, Taichung 402, Taiwan.
[2]Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, 50290, Thailand

*Corresponding author, E-mail address: d107033001@mail.nchu.edu.tw , wukt@nchu.edu.tw

ARTICLE INFORMATION

Received 12th September 2019
Accepted 28th November 2019

Keywords:
Green biomass
Para grass
Pretreatments
Fermentation

ABSTRACT

Recently, biogas production through anaerobic digestion technology has advanced massively. At the moment, caused by high energy demand and environmental concerns as the world's population increases, the drive for anaerobic digestion processes is achievement drive within research and the industry for sustainable energy generation. The study evaluated biogas production from anaerobic mono-digestion of para grass in laboratory scale studies. In addition, improvement of the biogas yield from the grass via chemical pretreatment and leaching bed reactors was studied. Methane content of biogas was 54.36 % by mono-substrate. The results revealed that para grass can be treated anaerobically and are a good source of biogas.

1. Introduction

Energy is of primary importance in response to the basic need of the people and a fundamental factor of production in the business sector and industry (Unpaprom et al., 2015, 2016, 2017). Therefore, the power supply has to get enough energy supply, reasonable price and good quality according to customer required (Ramaraj et al., 2016a,b,c). Coal, oil and natural gas are the three kinds of fossil fuels that we have mostly depended on for our energy needs, from home heating and electricity to fuel for our automobiles and mass transportation. That energy is nonrenewable and will run out. Presently many agencies have focused on renewable energy such as solar energy, wind energy, hydro energy and geothermal energy. Renewable sources of energy and consumer products are required for the sustainable development of modern society. Thailand is an agricultural area suitable for growing of many plants, especially annual crops that can be used as an energy crop or raw material of agricultural biogas plant (Dussadee et al. 2014 b). The energy demand required to meet the economic growth of Thailand is high and growing every year. Accordingly, Thailand, as the country has the potential biogas as a country with a lot of agriculture; including raw materials from crops and livestock, it can be used to develop renewable energy in the form of biogas is methane gas caused by the decomposition of organic matter in the system (Dussadee et al. 2014 a).

Interest has recently been growing in using the anaerobic digestion of organic waste of farm origin, such as manure, crop residues and organic residues from food and agro-industries, to generate renewable energy. (Gebrezgabher et al., 2010). Agricultural residues from the agricultural, agriculture industry and grassland biomass are usually used as feed materials in anaerobic digestion systems in Thailand are suitable in numerous ways for producing energy (Manmai et al., 2017a; Manmai et al., 2017b; Manmai et al., 2017c; Manmai et al., 2018a; Manmai et al., 2018b). This can be used as the raw materials for biogas production as environmentally friendly renewable energy (Dussadee et al. 2014a,b; Chuanchai and Ramaraj, 2018). Using grassland biomass for producing energy especially biogas production currently, is the most common. Plant biomass is the primary source of renewable materials on earth and represents a potential source of renewable energy and bio-based products. There are so many types of grasses that are popularly grown in Thailand (Dussadee et al. 2014b). Animal manures have been used as a resource of excellent material for anaerobic digestion with clear environmental benefit. Since Thailand economy depend mainly on agriculture activities. Therefore, utilization of natural resources for energy production is an extremely important issue (Manmai et al., 2019a; Manmai et al., 2019b; Manmai et al., 2019c; Manmai et al., 2020).

Biogas is a green renewable type of energy is generated from a digestion process under anaerobic conditions whose application is rapidly emerging as a viable means for providing continuous gaseous fuel and power generation. Biogas application includes ensuring energy security, decreasing the carbon emission, improving economic activity and can be compressed, the same way as natural gas is compressed to CNG, and used to power motor vehicles. Para grass (*Brachiaria mutica*) is the tropical weed that no value and pervasive around the farm. It needs to cut down and removed frequently for fire hazards and disease and vector controls. Addition of grass can help raise C: N of the feedstock to be suitable for metabolic activities in anaerobic digestion (Chuanchai and Ramaraj, 2018). The physical structure and chemical composition of lignocellulosic materials can be altered through various methods of pretreatment, breaking down the linkage between polysaccharides and lignin, thus making cellulose and hemicelluloses more accessible to hydrolytic enzymes (Ramachandra et al., 2000; Zhang et al., 2008). Therefore, pretreatments could accelerate the hydrolysis process and improve the final can get more methane production. Consequently, the main purpose of this research was to produce biogas yield from para grass through anaerobic mono-digestion. Furthermore, the study was to examine the effects of pre-treatment and after results the suitable method is selected for scale up study and future applications. Various technologies have been developed and available for produce biogas and biological processes are environmentally friendly and feasible.

2. Methodology

Para grass was obtained at Sansai (18° 53' 37' N; 99° 01' 08' E), Chiang Mai, Thailand. The fresh material was crushed into small particles by grinding machine and stored in the freezer at 4°C for further using; and inoculum (pig manure) was collected from Learning Center for Agriculture and swine farm at the Faculty of Animal Science and Technology Maejo University, Chiang Mai, Thailand, respectively. The collected samples were transferred to the lab of Energy Center Research, Maejo University.

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

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

Following parameters dry matter, crude protein, crude fiber, ether extract, ash, insoluble ash, neutral detergent fiber, acid detergent fiber, lignin, gross energy total solids, volatile solids, chemical oxidation demand, and volatile fatty acids concentrations were analyzed by using stand methods and procedures from our previous published papers (Dussadee et al. 2014a,b; APHA, 2005; Chuanchai and Ramaraj, 2018).

Nomenclature and Abbreviation

FTIR	Fourier Transform Infrared Spectroscopy
HDPE	High Density Polyethylene
LAF	Laminar AirflowAD Anaerobic digestion
Alk	Alkalinity
C/N	Carbon/Nitrogen
COD	Chemical Oxygen Demand
DM	Dry matter
etc	The end of the sentence
MC	Methane content
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids

3. Results and Discussion

3.1 Para grass (or bufflo grass)

Para grass is a common name of *Brachiaria mutica*, also known as *Urochloa mutica* which is perennial crop that can grow on wet and flooded soils in the higher rainfall areas (Figure 1, 2 and 3). They are a tropical and invasive growing plant in rural area has only value to be feedstock for animal feeding. These exotic grass weeds are overgrown in abundantly available resources in the Northern region of Thailand. It needs to cut down and removed frequently for fire hazard and disease and vector controls. In which is found as aquatic weeds is the weed of no value and pervasive around area wetlands, along drainage channels, around lakes and dams, in roadside ditches and in other damp habitats, particularly in tropical climate. In areas where para grass in not grazed on by cattle, it has become a serious weed. It is a burden to the since it needs to be cut down and removed frequently for fire hazard, and disease and vector controls. Para grass and is estimated to contain about 42% of cellulose and about 20% hemicellulose, the hydrolysis of which can yield fermentable sugars and hence will serve as an excellent feedstock (Ramachandra et al., 2000; Chuanchai and Ramaraj, 2018).

Figure 1. Para grass

3.2 Description

Para grass is in the family Poaceae, along with other familiar grass such as Heterachne, Melica and many grass species. A perennial crop that can grow on wet soils area. It has steams and stolon which grow up to 5 m long and 1 m height. Leaves and leaf sheaths are generally hairy; leaves are 6-20 cm long and 1-2 cm wide. Dry matter yield of 4-7 t/ha has been achieved in pastures with no N fertilizer, if use about 10-15 t/ha/year. Absolutely it is found in space wetlands area. Para grass was cut down to keep it clean and good environmental. So, Para grass is the waste as well organic waste from unwanted locations.

3.3 Impacts

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

3.4 Invasiveness

As a long-lived, vegetative propagating pioneering species of disturbed areas, Para grass has potential for invasiveness. It is reported to benefit from cultivation, browsing pressure, mutilation and fire (Rojas-Sandoval et al., 2014). It may have deleterious effects on native plant species such as wild rice (*Oryza australiensis*) whose seeds provide food for indigenous birds.

Figure 2. Para grass floating in deep water (stems are rooted to the bank)

In 1977, Para grass was listed as a serious weed in Australia, Fiji and Thailand, as a weed in Sri Lanka, Colombia, Hawaii, Jamaica, Malaysia, Peru, the Philippines, Puerto Rico and Trinidad, and as a common weed in Borneo and Mauritius (Holm-Nielsen et al., 2009).

3.5 Applications

Planted for grazing in flat poorly drained or high rainfall environments. Also used as a cut-and-carry forage. Can be cut for hay but is generally slow to dry in the humid environments where it grows productively.

Figure 3. Pure stand of para grass on a highly disturbed urban floodplain

Rested wetland areas can be used a dry season reserves of green feed. A similar system uses shallow water ponding on the edges of which Para grass continues to grow as the water recedes. Para will grow in water to 1.2 m deep in the tropics.

3.6 Structure of the composition in para grass

Para grass is a type of lignocellulosic material. In general, lignocellulosic materials consist of three main components: cellulose, hemicellulose, lignin, and other compounds, and structure of the composition in para grass shown in Figure 4.

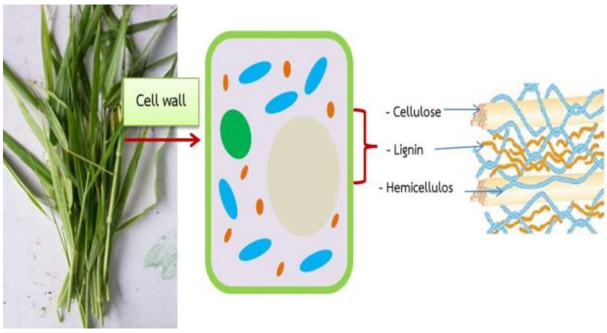


Figure 4. Structure of the composition in para grass

3.7 Nutritional attributes of Para grass

Para grass has a variable nutritional value, with protein content in the 7-10% DM range. Dry and old forage can contain as little as 3-4% protein but protein content higher than 20% DM have been recorded (Table 1).

Table 1 Para grass aerial part and fresh

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

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

3.8 Feedstock characterization

The characteristics of para grass biomass study parameters including proximate analysis (moisture and ash, wt. %), ultimate analysis (carbon, hydrogen, nitrogen, oxygen and Sulphur, wt. %) and biochemical analysis (TS and VS %, COD mg/l, Alk mg/l- $CaCO_3$, VFA mg/g, pH, C:N ratio, cellulose, hemi cellulose and lignin). In our study, the composition of para grass used clearly indicates that they are containing high nutritious matters. The proximate measurement in the Para grass biomass was verified; the results moisture and ash were average as 77.3, 2.79 and 83.01, 5.79 %, respectively. Ultimate analysis of materials has plenty of nutrients for biogas containing for para grass 41.5% of carbon, 5.3% of hydrogen, 1.3% of nitrogen, 27.3% of oxygen and 0.3% of sulfide. Meanwhile, the pH was adjusted between the ranges of 7.40 to 7.70 for suitable anaerobic digestion. Methane formations take place within a relatively narrow pH interval. The process is severely inhibited if the pH decreases below 6.0 or rises above 8.5 when the C, H, O, and N composition of a wastewater or substrate is known, the stoichiometric relationship reported by Rodríguez-López et al. (2012).

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

3.9 Theoretical analysis of para grass biogas and biochemical methane production

The elemental composition of plants can be used to calculate the amount of methane and carbon dioxide; calculation process is shown in Eqs. 4. Calculated from para grass is composed of methane had high percentage which means that organic matter in the grass. It was decomposed and converted into methane by 54.36% TS, respectively, as shown in Table 2 clearly demonstrated the huge potential of biogas production capacity from para grass. Which was consistent with available literature; biomass is composed of higher methane than carbon dioxide.

Table 2 Biogas composition, total biogas production and theoretical biogas yield of biomass

Biomass	Gas composition (%)			Total gas production (m^3)		
	CH_4	CO_2	NH_3	CH_4	CO_2	NH_3
Napier grass	48.45	47.82	3.73	0.43	0.42	0.03
Duck weed	50.34	48.81	0.85	0.47	0.45	0.01
Para grass	54.36	43.03	2.61	0.53	0.45	0.02

At; 100% of gas composition

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

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

3.10 Effect of pre-treatments on Para grass

Generally, changes in chemical structure, chemical composition and physical characteristics of crop residues containing large molecules of lignocellulosic biomasses are expected to occur during various pretreatment processes. As a result, lignin network destruction from its complex structures and swelling of carbohydrate fibers could occur due to both physical and chemical interactions between the biomass and boiling/alkali in the pretreatment process (Park et al., 2010). Accordingly, morphological changes in the treated and untreated para grass during the hydrothermal pretreatments were observed. Rodríguez-López et al. (2012) stated that thermal pretreatment is effective in the degradation of lignin and hemicellulose, heat break up the hydrogen bonds is crystalline complexes of cellulose and lignocellulose, causing the biomass to swell, thus increasing the accessible surface area. Hot water pretreatment is physical pretreatment by thermal heat treatment for modification of raw materials to destruction the cellulose tissue. The most often used temperatures at 95-100 °C. The performance of giant reed by hot water pretreatment 170 °C 5 min can extract cellulose and lignin content with 40.20% and 4.4%, respectively.

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

Results suggested that pre-treatment by NaOH is the best in this study when compere with pre-treatment by boiling pre-treatment and non-pretreated samples. A slight defibrillation was observed the separation of individual fibers, enlargement of the reactive area and more pronounced structural changes in the biomass were seen due to a possible solubilization of the hemicellulose. As hemicellulose operates as a cementing material, its solubilization causes a significant defibrillation effect on the biomass. In addition, a reduction in fiber length and the formation of entangled clusters. The fiber structure was almost entirely disintegrated due to the higher solubilization of hemicellulose and lignin re-localization. It was found that the fibers were greatly affected by NaOH with 72 h soak retention time. In addition, the swelling of fibers is also observed in alkaline pretreated biomass.

3.11 Fermentation

All types of biomass can be used as substrates for biogas production as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. The composition of biogas and the methane yield depends on the feedstock type, the digestion system, and the retention time (Park et al., 2010). The result of raw material TS, VS, COD, Alk, VFA and pH are reported in the Table 3 Found control 1 can remove well than control 2. Alkali after fermentation them increase all. In fact, the increase of alkalinity was normally due to the activity of the methanogenic bacteria, which could produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate.

VFA and COD all decreases after fermentation. All types of biomass can be used as substrates for biogas production as long as they contain carbohydrates, proteins, fats, cellulose, and hemicelluloses as main components. The composition of biogas and the methane yield depends on the feedstock type, the digestion system, and the retention time. The results showed that the initial total solids, volatile solids, chemical oxidation demand, and volatile fatty acids concentrations were significantly reduced after 36 days with mono-digestion biogas production process.

Table 3. Parameter of mono-digestion substrate

Parameter	Mono-digestion of paragrass	
	Start	End
TS (ml/l)	122,810	65,260
VS (ml/l)	81,567	35,650
COD (ml/l)	15,605	8,530
Alk (ml/l)	2,740	3,133
VFA (mg/l)	4,033	3,835
pH	7.23	6.45

TS and VS removals were 47.62%, 31.33% control 1 and 46.86%, 56.29% control 2 (Table 5). Found in control 1 can remove well than control 2. Furthermore, grass, due to its high digestible organic matter content, is also an excellent feedstock for anaerobic digestion. Para grass is one of the most promising grasses available for large production in tropics and subtropics. Biogas component was present control 1 get high CH_4 54.36% total biogas productions 8,982 ml and control 2 CH_4 50.35% total biogas production 7,184 ml in Table 17.

Table 4. Degradation efficiency of mono-digestion substrate

Degradation efficiency (%)		Total biogas productions (ml)	CH_4 (%)
TS	VS		
46.86	56.29	7,184	54.36

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

4. Conclusion

In conclusion, para grass is a good substrate for anaerobic mono-digestion. The results showed that the initial total solids, volatile solids, chemical oxidation demand, and volatile fatty acids concentrations were significantly reduced after 36 days with biogas production process. The enhancement of the biogas yield was attributed to the improvement of biodegradability through pretreatment. The data obtained from this study would be useful for designing co-digestion / large scale anaerobic digesters for treatment of para grass. Moreover, abundantly available raw materials including Para grass to biogas produce and applicable for energy security; and reduce the cost of using other gases if produce more enriched methane in the biogas production process enough to use.

References

APHA, 2005. Standard methods for the examination of water and wastewater, American Public Health Association, American Water Works Association, Water Environmental Federation, 21 edn, Washington.

Casabar J.T., Unpaprom Y., Ramaraj R., 2019. Fermentation of pineapple fruit peel wastes for bioethanol production. Biomass Conversion and Biorefinery 9: 761.

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

Cybulska I., Chaturvedi T., Brudecki G.P., Kadar Z., Meyer A.S., Baldwin R.M., Thomsen M.H., 2014. Chemical characterization and hydrothermal pretreatment of *Salicornia bigelovii* straw for enhanced enzymatic hydrolysis and bioethanol potential. Bioresource Technology 153: 165-172.

Dussadee N., Reansuwan R., 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.

Dussadee N., Ramaraj R., Cheunbarn T., 2017. Biotechnological application of sustainable biogas production through dry anaerobic digestion of Napier grass. 3 Biotech 7: 47.

Gebrezgabher S.A., Meuwissen M.P.M., Prins B.A.M., Lansink A.G.J.M.O., 2010. Economic analysis of anaerobic digestion—A case of Green power biogas plant in The Netherlands. NJAS - Wageningen Journal of Life Sciences 57(2): 109-115.

Holm-Nielsen J.B., Al Seadi T., Oleskowicz-Popiel P., 2009. The future of anaerobic digestion and biogas utilization. Bioresource Technology 100(22): 5478-5484.

Manmai N., Siriboon T., Tipnee S., Unpaprom Y., Ramaraj R., 2017a. Bioconversion of lignocellulosic biomass, sorghum bicolor L. into bioethanol. Proceedings of Academics World 73 rd International Conference, Taipei, Taiwan, 41-45.

Manmai N., Siriboon T., Homdoung N., Unpaprom Y., Ramaraj R., 2017b. Comparison of sugarcane leaves biomass pretreatments for bioethanol production. Proceeding of 1st National graduate research conference and creative innovation competition, Chiang Mai, Thailand, 325-332.

Manmai N., Siriboon T., Unpaprom Y., Ramaraj R., 2017c. Bioethanol from lignocellulosic biomass: a comparison between sunflower stalk and sorghum stalk. Proceedings of the 24th Tri-University International Joint Seminar and Symposium, Mie University, Japan, 1-4.

Manmai N., Bautista K., Unpaprom Y., Ramaraj R., Jayabalan R., 2018a. Bioethanol production from pretreated-corn stalk waste by separate hydrolysis and fermentation process. Proceeding of The 9th

International Graduate Research Conference, Chiang Mai, Thailand, 383-391.

Manmaie N., Unpaprom Y. and Ramaraj R., 2018b. Effect of chemical pretreatment on enzymatic hydrolysis and fermentation of corn stalks for ethanol production. Proceedings of the 6th ASCON-IEEChE 2018, Sun Moon Lake, Taiwan, 375–382.

Manmai M., Bautista K., Unpaprom Y., Ramaraj R., 2019a. Optimization of combined pre-treatments on sugarcane leaves for bioethanol production. Maejo International Journal of Energy and Environmental Communication 1(1): 30-39.

Manmai M., Mariano A. P., Unpaprom Y., Ramaraj R., 2019b. Bioethanol production from sunflower stalk: comparison between the impact of optimal chemical and biological pretreatments. Proceeding of the 1st Thailand Biorefinery Conference "The Future of Biorefinery for Thailand 4.0", Suranaree University of Technology, Nakhon Ratchasima, Thailand, 24-31.

Manmai M., Saetang S., Unpaprom Y., Wu K. T., 2019c. Influential degree of polymerization of sugar extraction on alkali pretreatment from sunflower stalk wastes by applied statistical modelling 1(2): 37-43.

Manmaie N., Unpaprom Y. and Ramaraj R., 2020. Bioethanol production from sunflower stalk: application of chemicaland biological pretreatments by response surfacemethodology (RSM). Biomass Conversion & Biorefinery 1-15.

Park S., Baker J.O., Himmel M.E., Parilla P.A., Johnson D. K., 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. Biotechnology for Biofuels 3(1): 10.

Percival Zhang Y.H., Himmel M.E., Mielenz J.R., 2006. Outlook for cellulase improvement: Screening and selection strategies. Biotechnology Advances 24(5): 452-481.

Ramaraj R., Unpaprom Y., Dussadee N., 2016a. Cultivation of green microalga, *Chlorella vulgaris* for biogas purification. International Journal of New Technology and Research 3: 117-122.

Ramaraj R., Unpaprom Y., Dussadee N., 2016b. Potential evaluation of biogas production and upgrading through algae. International Journal of New Technology and Research 2: 128-133.

Ramaraj R., Kawaree R., Unpaprom Y., 2016c. Direct transesterification of microalga *Botryococcus braunii* biomass for biodiesel production. Emergent Life Sciences Research 2: 1-7.

Ramachandra T.V., Joshi N.V., Subramanian D.K., 2000. Present and prospective role of bioenergy in regional energy system. Renewable and Sustainable Energy Reviews 4(4): 375-430.

Rodríguez-López J., Romaní A., González-Muñoz María J., Garrote G., Parajó J.C., 2012. Extracting value-added products before pulping: Hemicellulosic ethanol from Eucalyptus globulus wood. Holzforschung, 66.

Tran G.V., Unpaprom Y., Ramaraj R., 2019. Biomass Conversion & Biorefinery. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-019-00451-z

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

Unpaprom Y., Intasaen O., Yongphet P., Ramaraj R., 2015. Cultivation of microalga Botryococcus braunii using red Nile tilapia effluent medium for biogas production. Journal of Ecology and Environmental Sciences 3(2): 58-65

Unpaprom Y., Ramaraj R., Whangchai K., 2017. A newly isolated green alga, *Scenedesmus acuminatus* from Thailand with efficient hydrogen production. Chiang Mai Journal of Science 44: 1270-1278.

Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74: 3583-3597.

Vu P.T., Unpaprom Y., Ramaraj R., 2018. Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production. Bioresource Technology 247: 125-130.

Wannapokin A., Ramaraj R., Unpaprom Y., 2017. An investigation of biogas production potential from fallen teak leaves (*Tectona grandis*). Emergent Life Sciences Research 3: 1-10.

Zhang P., Zeng G., Zhang G., Li Y., Zhang B., Fan M., 2008. Anaerobic co-digestion of biosolids and organic fraction of municipal solid waste by sequencing batch process. Fuel Process Technology 89: 485-489.