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Enhancement of Biogas Production from Bakery Waste by *Pseudomonas aeruginosa*

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***Abstract*—**Production of biogas from bakery waste was enhanced by additional bacterial cell. This study was divided into 2 steps. First step, grease waste from bakery industry's grease trap was initially degraded by *Pseudomonas aeruginosa*. The concentration of by-product, especially glycerol, was determined and found that glycerol concentration increased from 12.83% to 48.10%. Secondary step, 3 biodigesters were set up in 3 different substrates: non-degraded waste as substrate in first biodigester, degraded waste as substrate in secondary biodigester, and degraded waste mixed with swine manure in ratio 1:1 as substrate in third biodigester. The highest concentration of biogas was found in third biodigester that was 44.33% of methane and 63.71% of carbon dioxide. The lower concentration at 24.90% of methane and 18.98% of carbon dioxide was exhibited in secondary biodigester whereas the lowest was found in non-degraded waste biodigester. It was demonstrated that the biogas production was greatly increased with the initial grease waste degradation by *Pseudomonas aeruginosa*.

***Keywords*—**Biogas production, carbon dioxide, methane, *Pseudomonas aeruginosa*

I. Introduction

BIOGAS is one of the products of anaerobic degradation. Anaerobic degradation is the breakdown of organic substrates, which is one of the oldest processes used for the treatment of industrial wastes and stabilization of sludge [1]-[6]. The organic substrates, for examples; food processing waste: bakery waste or potato waste; restaurant kitchen waste: grease, oil or fats; animal manure: chicken, swine or cow manure, can be digested and produced useful energy for the world [7]. This energy can be used directly as cooking fuel, in combined heat and power gas engines [8] or upgraded to natural gas quality biomethane. The utilization of biogas as a fuel helps to replace fossil fuels, which the use of fossil fuels raise environmental concerns. Nowadays, the gas demand is market-driven. There is a need to improve and also increase the efficiency of biogas production. The several methods have been reported, for example; optimizing the various operational parameters, satisfying the nutritional requirements of microbes, using different biological and chemical additives, and manipulating the feed proportions [9]-[16].

The bacterial additive is one of the methods for enhancing biogas production. Some strains of bacteria and fungi have been found to increase biogas production in the range of 8.4-44% from cattle dung [17], [18]. In addition, lipid degradation prior to the biodigestion process can induce in lipid liquefaction, bioavailability for anaerobic microorganisms [19], [20] and also more complete biodegradation as bacterial cells are only able to uptake small molecules. *Pseudomonas aeruginosa* has been reported that it can degrade lipid to produce fatty acid and glycerol [21]. Glycerol generated during bacterial degradation process can be converted to biofuels and also biogas in anaerobic degradation process [2], [4], [22]. From these reason, glycerol from the bacterial additive in biodegradation process should be considered. The objectives of this research were to study the concentration of by-product, especially glycerol, from bacterial additive in biodegradation process by *Pseudomonas aeruginosa* and to study the biogas production by comparison in three different substrates.

II. Materials and Methods

A. Sources of Bakery Waste and Swine Manure

Bakery waste was collected from bakery industry's grease trap at a Bakery industry in Nakhon Ratchasima, Thailand. Swine manure was collected from organic garden at Suranaree University of Technology, Nakhon Ratchasima, Thailand. All samples were kept in the laboratory at 25°C for 24 hrs prior to experiment.

B. Source of Microorganism

Pseudomonas aeruginosa was taken from the Center for Scientific and Technological Equipment, Suranaree University of Technology, Nakhon Ratchasima in Thailand. It was purified by repeatedly transferring the cells to nutrient medium or nutrient broth (NB). A nutrient medium composed of beef extract 3 g and peptone 5 g in 1 l of deionized water and pH was adjusted to 7.0. The medium was autoclaved for 15 min at 15 psi and 121°C before use. Bacto Agar (18 g/l) was added when the nutrient agar was used. About 1 loop of this bacterium from stock solution were inoculated into 100 ml of this medium and incubated for 7 days at 30°C on a rotary shaker (150 rpm). In order to prepare cells for biodegradation, 2 ml of *P. aeruginosa* in nutrient broth flask were transferred into 500 ml Erlenmeyer flask containing 100

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ml of NB and 3 ml of bakery waste and incubated at 30°C on a rotary shaker (150 rpm). After 3 days, the purity of *P. aeruginosa* was checked by a streak plate technique on nutrient agar plates. The plates were incubated at 30°C for 3 days. After that the morphology of colonies were observed under a light microscope.

C. Biodegradation Experiment

This experiment was set in the first step of this study. Grease waste from bakery waste was initially degraded by *P. aeruginosa*. The biodegradation of grease was set at 2% (v/v) for 72 hrs. The experiment was performed in 50 l of polyethylene tank. 40 l of grease waste was added into this tank and then *P. aeruginosa* was transferred at 2% (v/v). Physical and chemical characteristics: glycerol concentration, pH and temperature were determined before and after this experiment.

D. Biodigester Set-up

Schematic of the biodigester system is shown in Fig. 1. The biodigester was made from 50 l of polyethylene tank and the working volume of the biodigester was maintained at 40 l. The experiments composed of 3 biodigesters were set-up in 3 different substrates. The first biodigester was composed of 40 l of non-degraded waste as substrate. In order to enhance biogas production, the secondary and third biodigester were performed with grease waste degraded by *Pseudomonas aeruginosa*. Thus, the secondary biodigester was composed of 40 l of degraded waste and the third biodigester was composed of 40 l of mixture between degraded waste and swine manure in ratio 1:1 as substrate. Biogas volume and concentration were determined during day 0, 7, 14, 17, 20, 23, 26, and 30 of experiment.

E. Analytical Methods

The duplicate analysis was done for all. During the biodegradation process, pH and temperature were measured by multi parameter analyzer (CONSORT C532, Belgium). In addition, glycerol concentration was determined according to the methodology proposed by Thai Industrial Standards Institute, Ministry of Industry [23]. Biogas volume was measured with water displacement. Biogas concentration in form of methane and carbon dioxide was assayed with gas chromatograph (SHIMADZU GC-14: Active carbon packed column, 80°C) equipped with a thermal conductivity detector (TCD) (100°C for detector, 70°C for oven, 80°C for inlet, helium gas as carrier gas, 1 ml for injection volume).

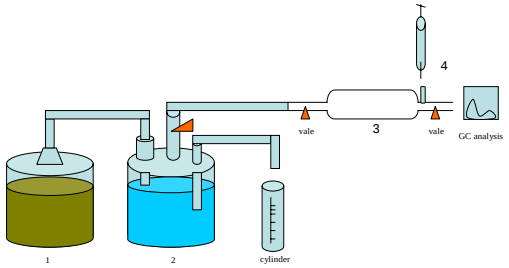

Fig. 1 Schematic diagram of the biodigester system: 1, biodigester tank; 2, water displacement tank; 3, gas sampling bulk; 4, biogas outlet point

III. Results and Discussion

A. Biodegradation by Pseudomonas aeruginosa

Grease waste taken from bakery industry's grease trap was initially degraded by *Pseudomonas aeruginosa*. The physical characteristic of grease waste was observed before and after experiment (Table I). Before experiment, grease mass contained very large particles, large fat scum layers, and small water content, which did not easy for anaerobic digestion in biogas production [2]. Therefore, *Pseudomonas aeruginosa* was added for increasing lipid liquefaction and bioavailability. After this additive process, grease was degraded and converted into small particles, little fibrous structure, and more water content. This product is a suitable substrate for biodigestion in biogas production because bacterial cells are only able to uptake small molecules for their growth [4], [24]. In addition, temperature was decreased from 30°C to 29°C whereas pH was increased from 5.3 to 6.0. These characteristics are suitable for growth of *P. aeruginosa* [25]. The most important by-product in this degradation was glycerol. Glycerol concentration was increased from 12.83% to 48.10% as shown in Table I. This glycerol can be used as a carbon source for the synthesis of cell mass and produce biogas as a product [22]. In this experiment, *P. aeruginosa* can degrade waste and produce glycerol. As similar result to the study of Prasad *et al*. [26], they reported that *P. aeruginosa* exhibited very good lipid degradation in palm oil effluent, soap effluent, and domestic wastewater. This bacterium has an important enzyme that is lipase [26]. Lipase can catalyze the hydrolysis of lipid to fatty acid and also glycerol [27]. This enzyme showed to be a very encouraging alternative for degrading rich-lipids wastewaters generated by dairy and slaughterhouses industries [28].

TABLE I
CHEMICAL AND PHYSICAL CHARACTERISTICS OF GREASE WASTE IN BIODEGRADATION PROCESS

Grease degradation	Physical characteristic			Chemical characteristic
	Grease waste	Temperature	pH	Glycerol concentration (%)
Before process	Grease mass contained very large particles, large fat scum layers, and small water content	30°C	5.3	12.83
After process	Grease mass contained small particles, little fibrous structure, and more water content	29°C	6.0	48.10

B. Biogas Production

After biodegradation process, 3 biodigester systems were set up and recorded for biogas volume and concentration. The volume of biogas occurred very low amount in first and secondary biodigester tank which did not detected. It may be due to the first and secondary biodigester had only bakery waste in non-degraded or degraded waste as a substrate, which it did not much for biogas production [11]. However, the first biodigester produced low concentration of biogas that was 2.15%, 0.07%, 0.12%, and 0.58% of methane on day 17, 23, 26, and 30, respectively, and it also produced 0.70%, 0.08%, 0.13%, and 0.86% of carbon dioxide on day 17, 20, 26, and 30, respectively (Fig. 2 and 3). The secondary biodigester also exhibited the biogas, which showed higher concentration of methane and carbon dioxide than the first. This biodigester produced 3.55%, 0.22%, 0.30%, 0.38%, and 24.9% of methane and 12.75%, 0.19%, 0.47%, 18.98%, and 18.98% of carbon dioxide on day 17, 20, 23, 26, and 30, respectively (Fig. 2 and 3). It may be due to suitability of substrate in secondary biodigester, which it composed of initially degraded waste from biodegradation process. Therefore, the biodegradation by *Pseudomonas aeruginosa* presented high efficiency for biogas production.

In order to enhance the biogas production, the mixture between the degraded waste and swine manure was set as substrate in the third biodigester. The highest volume of 11,600 ml of biogas occurred in this biodigester on day 20 of experiment (Fig. 4). Furthermore, it produced higher concentration of biogas than the two previous biodigesters. The concentration was 11.86%, 10.50%, 39.69%, 6.35%, 44.33%, and 35.44% of methane and 63.71%, 38.03%, 21.04%, 2.23%, 13.78%, and 13.78% of carbon dioxide on day 14, 17, 20, 23, 26, and 30, respectively (Fig. 2 and 3). In addition, the highest concentration of biogas exhibited 44.33% of methane and 63.71% of carbon dioxide on day 26 and day 14, respectively. From these results, the third biodigester showed very high efficiency for biogas production, which it was the mixture of degraded waste and swine manure in ratio 1:1 similar to the study of Adelekan and Bamgboye [29] who reported that slurry containing cassava peels-piggery waste in ratio 1:1 produced more biogas. It has been found that the appropriate amount of substrate and the biological additive may induce the biogas production [30], [31]. In addition, the mixture between pure bacterium or mixed consortia and cattle manure have been found to improve biogas production [17], [18], [32] Thus, the mixture in the third biodigester presented suitable substrate for anaerobic digestion in the biogas production process.

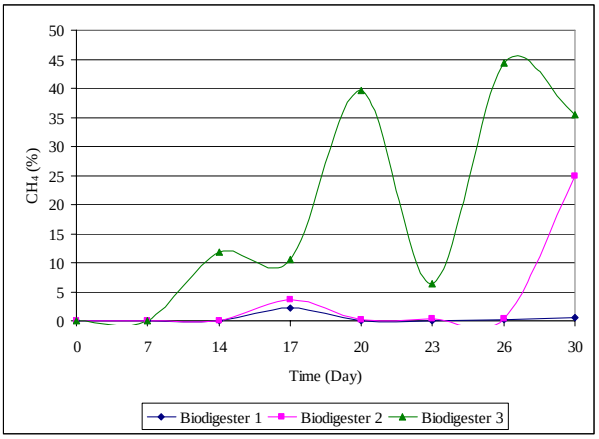

Fig. 2 Methane concentration in each biodigester

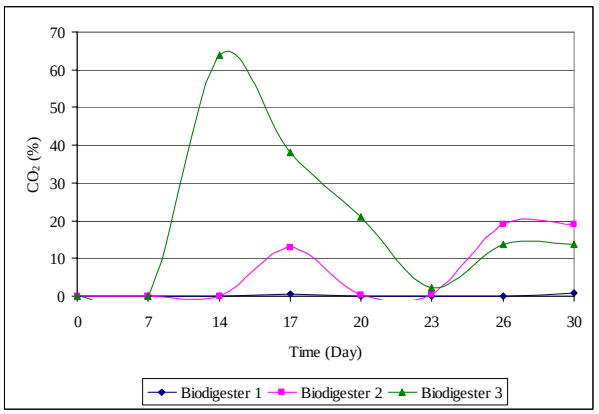

Fig. 3 Carbon dioxide concentration in each biodigester

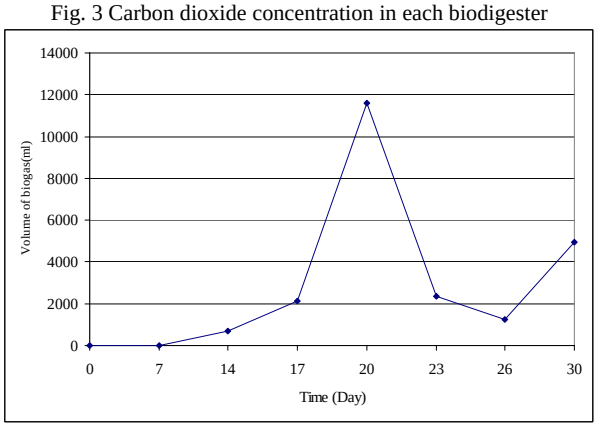

Fig. 4 The volume of biogas in third biodigester during experiment

IV. Conclusion

Pseudomonas aeruginosa was added into the biodegradation process for biogas production enhancement. The most important by-product in this degradation was glycerol. Glycerol concentration was increased from 12.83% to 48.10%. This glycerol is very suitable for growth of anaerobic microorganisms. After that this degraded waste was used as a substrate for biodigester. Methane and carbon dioxide generated from the biodigester was found to be the highest concentration when the degraded waste and swine manure were used as substrate. It was 44.33% of methane and 63.71% of carbon dioxide. Furthermore, the biogas production of biodigester of degraded waste and swine manure was enhanced by 12-20 times of methane and 22-70 times of carbon dioxide, which showed higher than that of degraded waste or non-degraded waste alone. Therefore, the initial addition of *Pseudomonas aeruginosa* increased the biogas production in form of methane and carbon dioxide.

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