



Co-digestion of Ensiled Napier Grass and Commercial Bakery Wastewater for Bioenergy Production

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Abstract Bioenergy derived from anaerobic digestion has gained attention for a decade because of its ability to convert waste into biogas. The advantages of this biotechnology could substitute conventional energy, lower greenhouse gas emissions, and produce less biodegradable waste. This study focused on methane production from ensiled Napier grass (Pak Chong 1) by co-digestion with anaerobic mixed cultures of an Expanded Granular Sludge Bed (EGSB) from a bakery factory. The ratio of co-digestion between ensiled Napier grass feedstock and inoculum (F/I ratio) was 1:1 and 1:3 based on volatile solids (VS). The potential of methane production was evaluated using a batch experiment for 30 days in the laboratory at room temperature ($32-35 \pm 5$ °C). To enhance biogas production, an alkaline pre-treatment of Napier grass was prepared by adding 1% NaOH. The results demonstrated that the methane production potential of the F/I ratio at 1:1 and 1:3 was 311.11 ± 29 and 255.56 ± 91 mL CH₄/g COD, respectively. The maximum energy production based on methane production was approximately 9.17 kJ/L. The range of pH was 6.96 ± 0.2 to 9.93 ± 0.2 and the percentage of SS, TS, and COD removal were 60.10, 24.69, and 48.63, respectively. The results of this study indicated that the feedstock and inoculum ratio (F/I) including pretreatment of feedstock and inoculum is necessary for biogas potential production. The potential biogas production of the CSTR reactor and its economic feasibility should be further considered.

Keywords anaerobic co-digestion, methane production, Napier grass, bakehouse wastewater

INTRODUCTION

Currently, biogas obtained from waste conversion has gained substantial attention due to its benefits. Biogas can be used as a substitute for fossil fuels, and it can reduce the CO₂ and greenhouse gas emissions of the waste treatment process. Anaerobic digestion is one of the traditional technologies for the biological treatment of organic waste (Ren et al., 2018). Previously, anaerobic digestion, in which organic compounds are degraded and converted to biogas in the absence of O₂, was generally used in many single biodegradable feedstocks including domestic or industrial waste, kitchen waste, and agricultural waste (Zarkaya et al., 2013; Pandey et al., 2019). Agriculture residues or crops are the second feedstocks that are utilized as sources of renewable energy. The complex substrates such

as cellulose and hemicellulose contain primarily glucose and xylose which could be fermented to produce bioenergy using the process of microorganisms. Lignocellulosic biomass could provide approximately 10% of the world's energy. (Zhang et al., 2007; Sawadeenarunat et al., 2016).

Napier grass (*Pennisetum purpureum*) has considerable attention as a promising crop for energy production, due to the fact that it is fast-growing, easy to harvest, has a high biomass, is highly lignocellulosic, and is environmentally friendly (Mehmood et al., 2017). Although it is native to Africa, it has been cultivated in many tropical regions of the world, including Thailand. Napier grass (Pakchong 1 strain) is not only one of the available biomass sources in Thailand (approximately 221,760,000 tonnes) but also has a high potential for bioenergy production from perennial grasses (Waramit and Chaugool, 2014; Sawasdee and Pisutpaisal, 2021). However, the anaerobic digestion of a single lignocellulosic feedstock has limitations including low biogas production efficiency, long duration, and acidification. Several studies have shown later that co-digestion results in higher biogas production compared to using a single feedstock. Successful research demonstrated that co-digestion of dairy manure with wheat straw and chicken manure produced more methane than using a single feedstock (Wang et al., 2012). Wall et al., (2014) reported that co-digestion of silage and dairy slurry yielded the highest specific methane yield of 349 L CH₄/kg VS. Typically, pretreatment is also considered an advanced option to enhance the biogas production of anaerobic digestion because it is necessary to break down lignin to hydrolyze cellulose and hemicellulose. Pretreatment methods are generally categorized as physical (grinding or milling), chemical (acid and alkaline), and biological methods (Vargas et al., 2015). Furthermore, grass silage is one of the appropriate options to minimize the feedstock preparation costs and provide adequate raw materials for biogas production operations. Grass ensiling is a simple preservation method that is implemented in biomass to extend the storage time (Pahlow et al., 2003). The bakery factory or bakehouse industry typically consumes an amount of water and energy while wastewater from the processes also causes environmental impacts. On the other hand, this kind of wastewater contains highly biodegradable and organic compounds including nutrients, carbohydrates, and lipids which are suitable for bioenergy production of anaerobic digestion (Ali et al., 2017; Pilarska, 2018).

OBJECTIVE

The study aimed to investigate the methane production potential and the energy recovery from the co-digestion of ensiled Napier grass with commercial bakery wastewater in a batch experiment to achieve optimal proportions of substrates for further scale-up experiments.

METHODOLOGY

This study investigated the methane production potential of the co-digestion of ensiled Napier grass with commercial bakery wastewater on a laboratory scale as follows:

Inoculum and Feedstocks

In this study, anaerobic sludge obtained from an expanded granular sludge bed (EGSB) from a bakehouse wastewater treatment process (Bangkok, Thailand) was provided as an inoculum. It was preserved in the refrigerator at 4 °C. Before use, the anaerobic mixed culture was activated by adding 5% CH₃COOH under anaerobic conditions for a week. In this experiment, the 1:1 and 1:3 ratios of co-digestion between ensiled Napier grass feedstock and inoculum (F/I ratio) were investigated, while the initial pH of this was adjusted to a neutral level.

Preparation of the Ensiled Napier Grass

Napier grass (*Pennisetum purpureum*) Pakchong 1 strain was collected from the Lamtakhong demonstration field of the Thailand Institute of Scientific and Technological Research (Nakhon Ratchasima, Thailand). The grass was harvested at 70 days old and delivered to the laboratory. The

fresh grasses were chopped into small pieces (1-2 cm) by machine. Afterwards, 1L of the compressed-grass extraction was ensiled in the laboratory reactor and sealed airtight with a screw top for 60 days. Before the experiment, the ensiled Napier grass suspension was passed through a 47 mm filter to remove all the debris. The physiochemical characteristics of ensiled Napier grass were evaluated, including pH, TS, SS, TKN, TP, and VFA. In addition, alkaline pre-treatment of ensiled Napier grass was applied in this study by adding 1% NaOH (W/V) for 1 hour.

Methane Production and Analytical Method

Methane production was conducted in a batch experiment in the laboratory. The biological methane potential (BMP) was evaluated using the following 1:1 and 1:3 ratios of F/I with an initial pH of 6.8–7.2. The headspace of the BMP bottle was flushed with nitrogen gas for 5 minutes to displace the air, and the bottle was sealed with a rubber stopper. All BMP experiments were set up at a temperature of $32\text{--}35 \pm 5$ °C for 30 days. During the experiment, the daily methane gas was measured using the wetted gas syringe method (Owen et al., 1979). Methane production potential was calculated as the following equation (Eq. 1), while the energy production was calculated from the maximum methane converting to energy unit (kJ/L) (Sittijunda, 2015). All treatments were carried out in triplicate. The results are given as average values with standard deviations.

$$\text{Methane production potential} = \frac{\text{Total CH}_4 \text{ production (mL)}}{\text{g COD of substrate}} \quad (1)$$

pH was measured using a pH meter (Hach 1130, USA). Concentrations of COD, TS, and VS were analyzed according to the standard methods of water quality (APHA, 2015). The energy production in this study was estimated based on methane production and its heating value (Sittijunda, 2015).

RESULTS AND DISCUSSION

An ensiled Napier grass was applied to co-digestion with commercial bakery wastewater in this study. The ensiled Napier grass characteristics, including pH, TS, SS, COD, and VFA, were 4.08 ± 0.1 , 32.2 ± 0.38 g/L, 12.53 ± 0.38 g/L, 45.28 ± 0.44 g/L, and 3.52 ± 0.29 g/L, respectively. Due to the low pH of ensiled Napier grass, which could affect the hydrolysis reactions, 1% NaOH was provided to neutralize the pH level. The initial pH values were 6.8-7.2 and gradually increased to 8.1-9.3 at the end of the experiment. The increase in pH might relate to the concentration of NH_3 in the systems, which affected methanogen bacteria and the methane production potential. As in the previous study, Sompong (2019) reported that the high concentration of NH_3 influenced the methanogenic bacteria and tended to decrease methane production. Thus, pH values during anaerobic digestion processes including VFA should be considered for enhancing the methane production yields.

The daily methane production of both F/I ratios is shown in Figure 1. The maximum methane production of the 1:1 ratio was 230 ± 15 mL on the day 15th and 170 ± 20 mL of the 1:3 ratio at the day 14th. The COD removal percentages of 1:1 and 1:3 ratios were 48.63 and 43.11, while the percentages of SS and TS removal for the 1:1 ratio were 60.10 and 24.69, and 55.70 and 23.85 for the 1:3 ratio of F/I, respectively (Table 1). The energy production was determined based on the conversion of the maximum methane production, the density of methane, and its heating value (Sittijunda, 2015). The energy production of 1:1 and 1:3 F/I ratios were 9.17 and 6.81 kJ/L, respectively.

The results suggested that the co-digestion of feedstock and inoculum showed better methane production potential than a single substrate. According to a study by Sirirote et al. (2014), the highest cumulative biogas production of 26.25 L was obtained using a ratio of Napier grass and inoculum of 1:2. Comparing the previous study, the methane production potential of this co-digestion study (255.56 and 311.11 mL CH_4 /g COD) showed higher methane production than single Napier grass digestion (122.4 mL CH_4 /g TVS) (Sawasdee and Pisutpaisal, 2021). Many studies reported the

methane production yields of different feedstocks and inoculums, for instance, the co-digestion of USAB granular and hydrolysate Napier grass (1:1) produced 40.9 percentage of methane yield (Sittijunda, 2015), and the sludge digested with ice cream wastewater was provided 320-340 mL CH₄/g COD (Ince, 1998). This indicated that the presence of organic materials is a crucial factor in the process of biogas production through anaerobic digestion. In a previous study, Sittijunda (2015) noticed that the amount of methane produced is affected by variables such as the organic loading rate, the particular substrate utilized, and the temperature at which the process is carried out. Furthermore, several pre-treatment methods of feedstocks are selected to enhance the biogas production yields. Grass ensiling is a method selected to make feedstock preparation more cost-effective, promote biomass degradation, and increase methane production (McEniry et al., 2014). The results suggested that ensiled Napier grass digested with commercial bakery wastewater can produce biogas and reduce greenhouse gases. However, it is necessary to conduct further research on scaling up the systems to obtain the required energy, using double-batch or CSTR reactors and investigating economic feasibility.

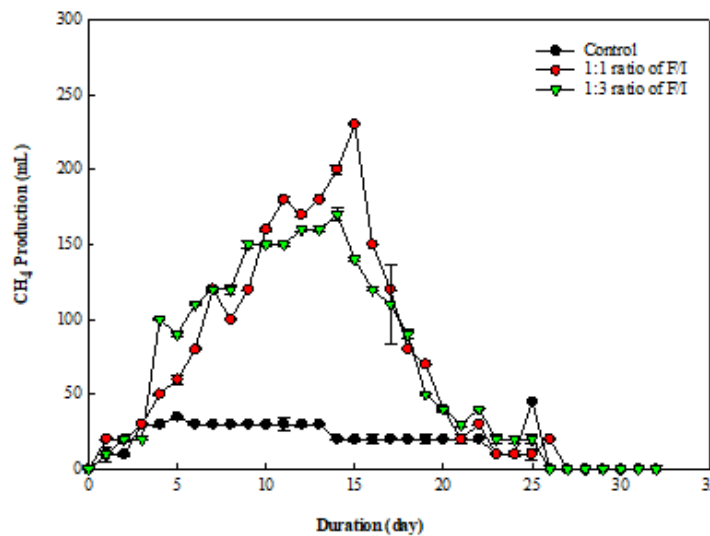


Fig. 1 The methane production of 1:1 and 1:3 ratios of F/I

Table 1 The removal percentage of COD, SS, TS, methane production potential, and energy production of the co-digestion of ensiled Napier grass and commercial bakery wastewater

F/I ratio	% COD removal	% SS removal	% TS removal	CH ₄ production potential (mL CH ₄ /g COD)	Maximum CH ₄ production (mL)	Energy production (kJ/L)
1:1	48.63	60.10	24.69	311.11 ± 29	230 ± 15	9.17
1:3	43.11	55.70	23.85	255.56 ± 91	170 ± 20	6.81

CONCLUSION

The results demonstrated that the co-digestion of ensiled Napier grass and commercial bakery wastewater can produce methane and energy. The study determined that the optimal feedstock and inoculum ratio was 1:1, resulting in a methane production potential of 311.11 ± 29 mL CH₄/g COD and energy production of 9.17 kJ/L. Additionally, it was found to remove 43.11 to 48.63% of COD, 55.70 to 60.10% of SS, and 23.85 to 24.69% of TS. Therefore, this study indicated that co-digesting of ensiled Napier grass with commercial bakery wastewater can generate biogas and reduce

greenhouse gas emissions. To enhance the methane production yield, pH, VFA, and methanogen bacteria dynamics could be necessarily considered for further research.

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