# Biogas Production from Bakery Wastewater in Two-Stage Anaerobic Digestion System

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#### **Abstract**

Biogas production from bakery wastewater was studied using a semi-continuous, two-stage anaerobic digestion system, consisting of 2 l-first-stage digester and 5 l-second-stage digester under temperature of 35°C. Substrate feed rates were examined in a batch experiment by varying in the range of 50 to 200 ml/l/d. Characteristics of substrate and effluent of the digester (i.e. pH, SS, TS, VS, VFAs, COD, sCOD, TN, TP, TSO<sub>4</sub><sup>2-</sup>), biogas yields and compositions were investigated. The experimental results showed that substrate feed rate of 100 ml/l/d produced maximum yield of biogas. The biogas yield was directly proportional to concentration of VFAs in the second-stage digester. The approximate pH values in the first stage and the second stage digesters were 6.13 and 7.25, respectively. The average biogas yield of 0.481 l/ g VS removed and 0.609 l/ g sCOD removed was observed at a hydraulic retention time (HRT) of 10 days. Biogas contained 46.4%-60.8% methane. Removal efficiencies of SS, VS, COD and sCOD in this system were 85.58%, 93.35%, 87.91% and 75%, respectively. The amounts of total nitrogen and total phosphorus after digestion increased whereas that of sulfate decreased.

Keywords: biogas, bakery wastewater, two-stage anaerobic digestion

# 1. Introduction

Anaerobic digestion is a degradation process of organic substances by microorganisms in the absence of oxygen. It is considered to be one of the most environmentally friendly, cost-effective and commercially viable technologies for alternating fossil fuel [1]. Under anaerobic conditions, the organic substrates are converted into biogas and digestate, which can be used as a renewable energy and soil conditioning, respectively [2]. There are four steps in anaerobic digestion of organic matters. Firstly, complex organic compounds (i.e. carbohydrates, proteins, fats) are degraded into simple soluble substances (i.e. sugars, short chain peptides, amino acids, long chain fatty acids) by hydrolytic bacteria. Secondly, acidogenic bacteria transform these soluble substances into organic acids (i.e. formate, acetate, propionate, butyrate) including alcohols, ketones, aldehydes, carbon dioxide (CO<sub>2</sub>), hydrogen (H<sub>2</sub>), ammonia (NH<sub>3</sub>), hydrogen sulfide (H<sub>2</sub>S) and water (H<sub>2</sub>O). Thirdly, acetogenic bacteria convert these soluble organic matters into volatile fatty acids (VFAs) comprising mainly acetic acid (CH<sub>3</sub>COOH) as well as CO<sub>2</sub>, H<sub>2</sub> and so on [3]. Finally, about 75% of methane (CH<sub>4</sub>) is produced from decarboxylation of acetate by acetate-consuming methanogens and the remaining is generated from the reduction of CO<sub>2</sub> and H<sub>2</sub> by

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H<sub>2</sub>/CO<sub>2</sub>-consuming methanogens [3, 4]. Biogas is generally composed of a mixture of CH<sub>4</sub> (55-70%) and CO<sub>2</sub> (30-45%), and trace amounts of other gases (i.e. H<sub>2</sub>S, NH<sub>3</sub>, H<sub>2</sub>O) [1]. There are several important factors affecting biogas production rate such as pH, temperature, substrate type, microorganism species, hydraulic retention time (HRT), etc. [1, 3, 5, 6]. A thermophilic process has higher specific growth rates and higher biogas production however it is more sensitive to environmental changes than the mesophilic process [3]. Waste materials containing high organic fraction, i.e. food waste, agricultural residues, industrial waste, municipal waste, sewage sludge can potentially be used as substrate for biogas production [1, 2, 6, 7]. Therefore, suitable anaerobic digestion system is required to achieve high production rate.

Two-stage anaerobic digestion system comprises of two separate reactors. The hydrolysis, acidogenesis and acetogenesis occur in the first stage reactor whilst the methanogenesis is conducted in the second stage reactor according to their different growing environmental conditions of microorganisms. Methanogens can grow under strictly defined pH range (pH 6.5-8.5), absence of oxygen and relative constant temperature conditions whereas hydrolytic bacteria can grow in the pH range of 4.7 to 7.0 and a wider range of environmental conditions than methanogens [8, 9]. Organic acids produced in the acidification process lead to decrease in pH, which likely inhibits the growth of methanogens [8, 10]. The advantages of two-stage anaerobic digestion over one-stage anaerobic digestion are to easily degrade high solid waste, enhance biogas production and decrease retention time of the digestion [10-12]. Several studies have reported that the two-stage anaerobic digestion system produced higher biogas yields than the single stage one [9-14]. However, there are only few studies investigated biogas production from bakery wastewater using two-stage anaerobic digestion system.

Wastewater from bakery factories is composed of high fraction of biodegradable organic materials such as flour dust, sugar, vegetable oil, which are promising substrates for biogas production [15]. Typically, bakery wastewater has biological oxygen demand (BOD<sub>5</sub>) varying from 906 to 24,000 mg/l, chemical oxygen demand (COD) varying from 2,910 to 50,400 mg/l, total solids (TS) varying from 848 to 36,700 mg/l, fat, oil and grease (FOG) varying from 429 to 10,000 mg/l [15]. In this study, the production of biogas from bakery wastewater was investigated using a semi-continuous, two-stage anaerobic digestion system under mesophilic condition (35 °C). Characteristics including pH, suspended solids (SS), total solids (TS), volatile solids (VS), chemical oxygen demand (COD), soluble chemical oxygen demand (sCOD), total nitrogen (TN), total sulfate (TSO<sub>4</sub><sup>2-</sup>), total phosphorus (TP) of substrate and effluent were analyzed. Compositions of biogas were also analyzed using Gas Data meter.

# 2. Material and Methods

# 2.1 Inoculum and substrate

In this study, anaerobic digester sludge and wastewater collected from a wastewater treatment plant of bakery factory in Bangkok, Thailand were used as inoculum and substrate, respectively. Characteristics of substrate including pH, SS, TS, VS, COD, sCOD, TN, TSO<sub>4</sub><sup>2-</sup>, TP were analyzed using Standard Methods for the Examination of Water and Wastewater [16] and ASTM [17].

#### 2.2 Set up of semi-continuous, two-stage anaerobic digestion system

A semi-continuous, two-stage anaerobic digestion system consisted of a two-liter Duran bottle including hydrolysis, acidogenic and acetogenic reactions (the first-stage digester) connected to a five-liter Duran bottle including methanogenic reaction (the second-stage digester). The second-stage digester was composed of a feed vent which connected via a Tygon tube to the first-stage digester and an exhaust gas vent which connected to a 600 ml-glass column containing 2% NaCl (w/v) for measuring the produced biogas by liquid displacement. The reactor was equipped with

an agitator operating at approximately 100 rpm. The first-stage digester comprised of feed vent which connected to a one-liter Duran bottle of feed tank and an effluent vent which connected to the second-stage digester. A schematic diagram of semi-continuous, two-stage anaerobic digestion system is illustrated in Figure 1.

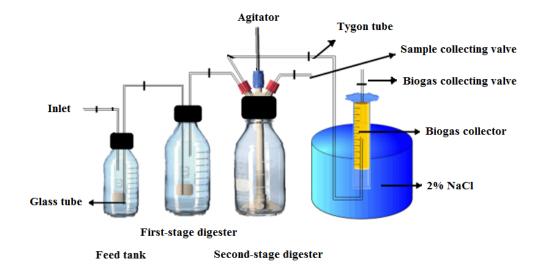


Figure 1. A schematic diagram of semi-continuous, two-stage anaerobic digestion system

#### 2.3 Experimental procedure

#### 2.3.1 Study on the biogas production in a batch reactor

A batch experiment was conducted in a five-liter Duran bottle with working volume 4.5 l. Ten percent (v/v) of sludge was inoculated into the digester. The digester was purged with nitrogen gas (99.99% purity) (Praxair (Thailand) Co., Ltd.) for 5 minutes to remove air, then sealed with rubber stopper and placed in a water-bath at a controlled temperature of 35°C. The volume of produced biogas was measured every 30 minutes by reading from scale of the liquid displacement column at ambient pressure and temperature until remained stable.

#### 2.3.2 Study on effect of substrate feed rate

A batch experiment was carried out in a two-liter Duran bottle with working volume 1.8 l. Ten percent (v/v) of sludge was inoculated into the digester. Feed rates of substrate were examined by varying in 50, 100, 150 and 200 ml/l/d. The volume of produced biogas was measured every 30 minutes by reading from scale of the liquid displacement column as described above. The experiments were run in triplicate. Hydraulic retention time (HRT) was determined by dividing volume of the medium in the reactor by feed rate of substrate.

#### 2.3.3 Study on biogas production in a semi-continuous, two-stage anaerobic digestion system

The experiment was carried out in a semi-continuous, two-stage anaerobic digestion system at temperature of 35°C. The system was set up as described in section 2.2 using a two-liter Duran bottle, obtained from section 2.3.2, as the first-stage digester and a five-liter Duran bottle, obtained from section 2.3.1, as the second-stage digester. Biogas production was investigated with the optimum feed rate of substrate obtained from the experimental results. The reactor was fed once a day with wastewater after removal of the same volume of effluent. The liquid organic fraction in the second-stage digester was agitated at 100 rpm for 5 minutes before removal meanwhile the

same volume of hydrolysate from the first-stage digester was transferred into the second-stage digester. At the same time, wastewater from feed tank was added into the first-stage digester using a peristaltic pump (Model 777201-60, Cole-Parmer Instrument Company, USA). Sample from the second-stage digester was taken every day in order to measure pH, SS, TSO<sub>4</sub><sup>2-</sup>, VS, VFAs, sCOD using analytical procedures in Standard Methods [16, 17].

The volume of produced biogas was measured every 30 minutes by reading from scale of the liquid displacement column as mentioned above. Biogas compositions were daily analyzed by a Gas Data Meter (GFM 416 model, Coventry Co., Ltd., UK). After reaching the level of 500 ml, the biogas was drawn from the biogas collector then passed through the Gas Data meter. The data of gas compositions were read from the gauge. After the end of the experiment, characteristics of hydrolysate in the first-stage digester (including pH, SS, TS, VS, COD, sCOD) and liquid organic fraction from the second-stage digester (i.e. pH, SS, TS, VS, VFAs, COD, sCOD, TS, TN, TP) were analyzed. All chemicals used in this study were analytical grade. All measurements were run in triplicate. The result data were analyzed statistically by one-way analysis of variance and multiple comparison at 5% level.

# 3. Results and Discussion

# 3.1 Biogas production in a batch reactor

The experimental results revealed that bakery wastewater used in this study had COD 5,360±792 mg/l, TS 4,230±65.6 mg/l, VS 2,887±113.7 mg/l and SS 756±61.9 mg/l as illustrated in Table 1. The cumulative biogas production generated from the bakery wastewater in batch fermentation is shown in Figure 2. Biogas yield increased with increase in time until remained stable (approx. 7 days of digestion). The average production rate of biogas was 1.043 l/d. This result indicated that the bakery wastewater can potentially be used as substrate for biogas production.

**Table 1.** Characteristics of substrate in feed tank, hydrolysate in the first-stage digester and effluent from the second-stage digester

Parameters	Substrate in Feed tank	Hydrolysate in the first-stage digester	Effluent from the second-stage digester	% Removal
pН	6.14	6.13	7.25	-
Temperature (°C)	30	35	35	-
SS (mg/l)	756 ±61.9	481±6.9	109±26.7	85.58
TS (mg/l)	$4,230 \pm 65.6$	3,560±324.5	$4,713 \pm 170.4$	-11.42
VS (mg/l)	$2,887 \pm 113.7$	$2,193 \pm 202.6$	$192 \pm 14.4$	93.35
COD (mg/l)	$5,360 \pm 792$	2,088	648	87.91
sCOD (mg/l)	1,728	1,008	432	75
TN (mg/l)	121	-	264.99	-119
$TSO_4^{2-}$ (mg/l)	62.40	-	6.13	90.18
TP (mg/l)	73.44	-	91.17	-24.14

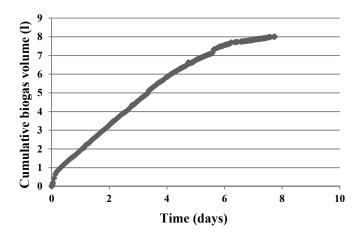
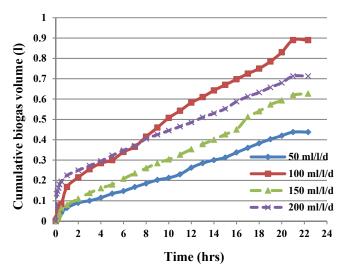


Figure 2. Cumulative biogas production in a batch, one-stage anaerobic reactor at 35 °C

# 3.2 Effect of substrate feed rate on biogas production

Figure 3 shows the cumulative biogas production as a function of time under different feed rates of substrate in a batch fermentation process. The results showed that the substrate feed rate of 100 ml/l/d (1.072 kg COD/l/d) produced the maximum yield of biogas with the average production rate of 0.768 l/d. There were statistically significant differences between feed rates as determined by one-way ANOVA at an alpha level of 0.05 (F(3,8) = 14.51, p = 0.007) as described in Figure 4. Tukey's comparison result indicated that feed rate of 100 ml/l/d was not statistically significant with feed rate of 200 ml/l/d. The higher feed rate may result in accumulation of volatile fatty acids (VFAs) in the system because acidogenic bacteria grow faster than methanogenic bacteria and converted organic substances to VFAs which inhibit the growth of methanogens [8, 9] whilst the lower feed rate may cause insufficient nutrients for growing bacteria in the reactor. The hydraulic retention time (HRT) of the substrate feed rate at 100 ml/l/d was 10 days.



**Figure 3.** Cumulative biogas production rate with various substrate feed rates in a batch, one-stage anaerobic reactor at 35°C

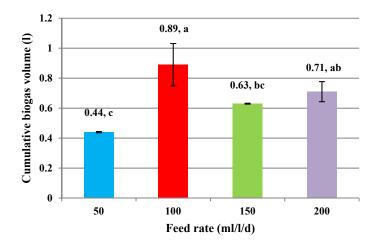
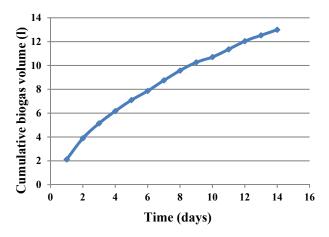


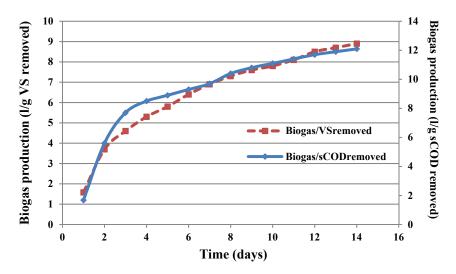
Figure 4. The statistical analysis of the relation of biogas production and substrate feed rates

# 3.3 Biogas production in a semi-continuous, two-stage anaerobic digestion system

The cumulative biogas production in a semi-continuous, two-stage anaerobic digestion system with substrate feed rate of 100 ml/l/d is illustrated in Figure 5. The digestion system was conducted with a hydraulic retention time (HRT) of 4 days in the first stage and 10 days in the second stage. This result was similar to other studies reporting that HRT of two-stage anaerobic reactor varying from 12 to 21 days depending on type of substrate, which is lower than one-stage anaerobic reactor [12]. The semi-continuous, two-stage anaerobic digestion system would maintain the average biogas production rate of 0.793 l/d, which is similar to the results of the batch experiment (approx. 0.768 l/d). Figure 6 shows that average biogas yield was remained relatively stable with the rate of 0.481 l/ g VS removed, which is slightly lower than that from codigestion of sugar beet pulp silage and vinasse (0.598 l/ g VS removed) [18] and 0.609 l/ g sCOD removed throughout the experimental period.

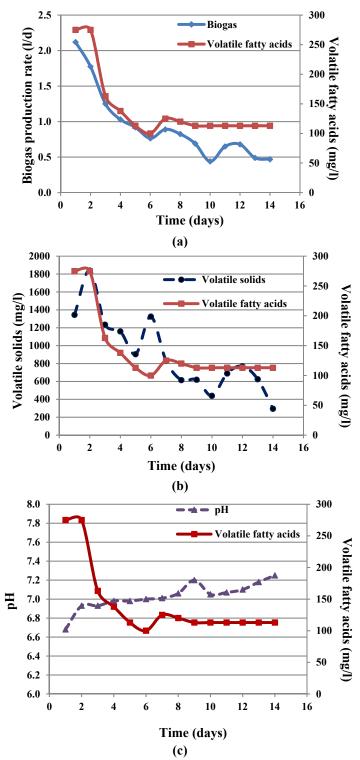


**Figure 5.** Cumulative biogas production in a semi-continuous, two-stage anaerobic digestion system at 35°C



**Figure 6.** Relation of biogas production and volatile solids removed and sCOD removed in effluent from the second-stage anaerobic digestion system at 35°C

The relation of volatile fatty acid contents and biogas production was observed during the fermentation in a semi-continuous, two-stage anaerobic digestion system as shown in Figure 7a. The results showed that biogas production was directly proportional to concentrations of volatile fatty acids (VFAs) because VFAs produced from acetogenesis were converted to biogas by methanogens [3]. Figure 7b shows the relation between concentrations of volatile solids and volatile fatty acids. In the two-stage anaerobic digester, volatile solids (VS) were transformed to VFAs in the first-stage then the VFAs were consecutively converted to biogas in the second-stage [3]. Therefore, the concentrations of VFAs tended to be directly proportional to those of VS in the second-stage anaerobic digester. The concentrations of VS and VFAs in the second-stage anaerobic reactor tended to decrease in the beginning of 4-days fermentation period. This may cause by the first-stage digester contained high VS and VFAs from the batch experiment. After 4 days, the volatile fatty acid contents remained relatively stable between 100-125 mg/l, which is related to the neutral pH results (Figure 7c). These results indicated that the pH condition in the second-stage digester was suitable for the growth of methanogens [9]. After digestion, the removal efficiencies of SS and VS were 85.58% and 93.35%, respectively whereas those of COD and sCOD were 87.91% and 75%, respectively (Table 1). Organic substances were reduced because they were hydrolyzed and degraded to VFAs by hydrolytic bacteria and acid forming bacteria, respectively then converted to biogas by methanogens [3]. This process gained not only biogas production but also waste reduction. However, total solid, total nitrogen and total phosphorus contents tended to increase because the effluent from the second-stage digester could be contaminated with bacterial cells. Thus, the effluent should be filtered before analysis in future work. About ninety percent of total sulfate was reduced. It may be transformed to hydrogen sulfide by sulfate reducing bacteria [3]. Methane contents ranged from 46.4% to 60.8% with average methane content of 55.8±6.4% of total biogas produced.



**Figure 7.** Relation of volatile fatty acids and biogas production rate (a) and volatile solids (b) and pH (c) in effluent from the second-stage anaerobic digestion system at 35°C

#### 4. Conclusions

Wastewater from treatment plant of bakery factory could be used as substrate of biogas production. The biogas production rate was affected by the substrate feed rate. Average biogas yield was 0.481 l/ g VS removed and 0.609 l/ g sCOD removed at substrate feed rate of 100 ml/l/d with HRT 10 days. The biogas production rate was directly proportional to concentrations of volatile fatty acids in the second stage. The approximate pH values in the second stage digesters was 7.25, which implied that there was no effect of VFAs accumulation in the system. After digestion, the removal efficiencies of SS, VS, COD and sCOD were 85.58%, 93.35%, 87.91% and 75%, respectively. Total solid, total nitrogen and total phosphorus contents tended to increase while about ninety percent of total sulfate was reduced. This anaerobic digestion system could produce biogas as renewable energy and reduce the organic matters in wastewater as waste reduction.

# 5. Acknowledgements

The authors would like to express their gratitude to President Bakery Public Company Limited, Ladkrabang Industrial Estate, Bangkok, Thailand for the supply of wastewater samples used in this study. We are also grateful to Dr. Pramote Sirirote, Department of Biology, Faculty of Science, KMITL for his help and advice.

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