Influence of pH on biogas production in a batch anaerobic process of tofu wastewater

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Abstract. As a popular soybean-based food in Indonesia, tofu production might cause a potential environmental problem, especially to the water body around the tofu industries. Approximately 17-45 tonnes of tofu wastewater are generated for every tonne of soybean used. The wastewater has a high organic concentration at pH of 3.5-4.5. Anaerobic digestion not only could be an efficient method to treat wastewater with high organic content, but also it produces less sludge and generate biogas as a renewable energy source. Anaerobic microorganisms normally work well in neutral pH. By pH lower than 5 the metabolism of methanogenic community could be easily hampered. The objective of this research was to test the influence of pH on anaerobic digestion of tofu wastewater. The experiments were performed in a batch mode using tofu whey waste water indigenous at pH 3.7 and neutralized wastewater at pH 7 Concentrations were varied at 20, 50, and 100% wastewater. Our results showed that at low pH of tofu wastewater did not have a negative influence on anaerobic digestion of tofu wastewater in a batch mode. A further addition of wastewater into the reactor (batch-wise) also showed a similar trend. It suggests that the presence of adequate buffer capacity was more important than adjustment of wastewater pH.

1. Introduction

Tofu or soybean curd is a soybean-based food that is common and popular in South East Asia. It average consumption is of 7 kg/person/year [1]. In Indonesia, there are around 80,000 tofu factories—mostly are small and medium enterprises—with a production capacity of 2.6 million tonnes of soybean per year [2]. Tofu production requires 17-45 kg of water per kg of soybean [3,4], some of which are discharged as wastewater. Tofu wastewater contain 7.5-14 g-COD/l [4], which should be treated properly before discharged into water bodies.

Anaerobic digestion is an efficient method to treat wastewater with high organic content with more than 5 g-COD/l. The process requires low energy and nutrient input, and correspondingly low biomass or sludge production [5]. The high energy-efficiency and environmentally beneficial technology for bio-energy production are the advantages of anaerobic process [6]. Anaerobic digestion performance is influenced by several factors such as temperature, pH, ratio of carbon and nitrogen, organic loading rate, and hydraulic retention time [7].

Anaerobic digestion consists of several reactions such as hydrolysis, acidogenesis, and methanogenesis. These reactions are performed by different microbial consortia. Due to these microorganisms and their enzymatic activities, anaerobic digestion is highly dependent on pH [7]. Hydrolysis is reported to be optimum around neutral pH [8,9], although this is also subject to the type of substrate. The optimum pH of carbohydrate hydrolysis is between 6-7.5 [10,11]. Protein and lipid hydrolysis are more efficient at neutral or slightly alkaline pH [12–14]. The optimum pH of acidogenesis is 6 [15]. Methanogenesis takes place between pH 6.7-7.8; the optimum pH is 7 [14,16]. In a batch system or a single-step continuous reactor where these reactions may take place simultaneously, pH 6.8-7.4 has been suggested as optimum [7].

Tofu making process in Indonesia uses acidic solution to coagulate soymilk [3,4]. This process results in wastewater with a pH between 3-5 [4], which is lower than the optimum pH of anaerobic digestion. The objective of this research was to test the influence of the pH of the wastewater on anaerobic digestion of tofu wastewater. Experiments were performed in a batch mode using waste water indigenous at pH 3.7 and neutralized wastewater at pH 7. Biogas production was observed and compared.

2. Material and Methods

2.1. Materials

Tofu wastewater used in the experiments was obtained from a tofu factory located in Bandung, West Java, Indonesia. The wastewater used was the liquid (whey) that was discharged after coagulation of soymilk.

The inoculum used in this experiment was cow rumen obtained from a local slaughterhouse. Cow rumen was diluted with water (1 : 1), and molasse was added as nutrition source. The mixture was placed in a closed container at room temperature, and incubated for two months prior to experiments.

2.2. Experimental Setup

During the experiments, 2.5 L bottles were used as anaerobic reactors (Figure 1).

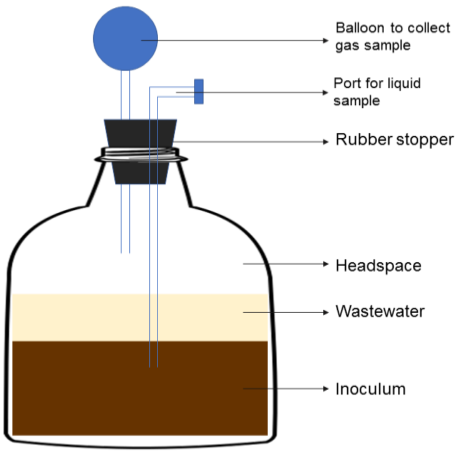

Figure 1. Experimental setup.

Table 1. Wastewater addition (ml).

	Concentration (%)					
	20		50		100	
Day	Wastewater	Water	Wastewater	Water	Wastewater	Water
0	100	400	250	250	500	0
13-16[a]	30	0	75	0	150	0

[a] Volume of wastewater addition per day

The experiments were performed using wastewater indigenous at pH 3.7 and pH 7. Solution of 5 N NaOH was used to increase the wastewater pH to 7. Waste water concentration was varied by 20, 50, and 100%.

Each of 2.5 L reactor contained 1 L of inoculum and 0.5 L mixture of wastewater and water (Table 1). An experiment with only 0.5 L water was used as control. The high amount of inoculum was to ensure that anaerobic digestion took place. Subsequently, the reactor was flushed with nitrogen gas to remove remaining oxygen, immediately sealed with rubber stopper, and incubated at room temperature. During the 13 incubation days, gas production from the balloon (Figure 1) was measured each day by using a syringe. After 13 days, wastewater with corresponding volume (Table 1) was added to the reactors. The incubation and gas monitoring were continued for 4 days. The experiments were performed in duplicates.

2.3. Analysis and calculations

Biogas produced during the experiment was collected in a balloon attached to the reactor (Figure 1). A syringe was used to collect and measure the volume of biogas in the balloon.

Liquid samples were drawn during day 13-16 for measurement of pH, bicarbonate alkalinity, and total volatile fatty acids (VFA). For alkalinity and VFA measurement, samples were filtered using paper filter. Using 0.1 N H_2SO_4, titration was performed on 20 ml of filtrate to pH 5 and pH 4.4. The volume of H_2SO_4 that was used for titration from the initial pH to pH 5 (A ml) and from pH 5 to pH 4.4 (B ml) were used in calculations as follows [17]:

$$\textit{Bicarbonate alkalinity} = A \times 250 \text{ mg/ml (as } CaCO_3\text{)}$$

$$\textit{Total VFA} = 500 \times (1{,}66\, B - 0{,}15) \text{ mg/l (as acetic acid)}$$

3. Results

3.1. Biogas production

Biogas formation during the first 13 days of the experiment is shown in Figure 2. Figure 2a and 2b show at 20 and 50% wastewater concentration and there was no significant difference between biogas formation from wastewater at pH 3.7 and 7.

At 100% wastewater concentration, between day-8 and day-13 more biogas was produced with wastewater pH 3.7 (Figure 2c). However, biogas formation with wastewater at pH 3.7 could not be measured, possibly due to leakage in the reactor. Therefore, the result for the first 13 days generated from a single experiment instead of duplicate. Between day 14 and 16 from duplicate results, it is apparent that biogas production from wastewater pH 3.7 was higher than pH 7.

3.2. Buffer capacity

After 13 days of experiment, wastewater was added to the reactor and pH was measured (Figure 3).

Figure 3a-c show that on day-13, the pHs for all experiment were in the range of 7.1-7.3. After wastewater addition, the lowest pH of 6.7 was observed on day-16 for 100% wastewater at pH 3.7 (Figure 3c). In this experiment, the control experiment pH also decreased to 6.4. For all experiments with wastewater pH of 7, the pH after wastewater addition ranged between 7.4-7.9.

Bicarbonate alkalinity is an indicator of alkaline buffer capacity toward changes of pH. Figure 4a shows the alkalinity of experiments with wastewater pH 3.7 decreased between day-13 and day-16. On the other hand, the alkalinity of experiments with wastewater pH 7 slightly increased between day-13 and day-16 (Figure 4b).

Figure 5a shows that after addition of wastewater (day 14-16), total VFA for experiment at wastewater pH 3.7 slightly increased. On the other hand, at wastewater pH 7 the increases of total VFA after wastewater addition were more apparent (Figure 5b).

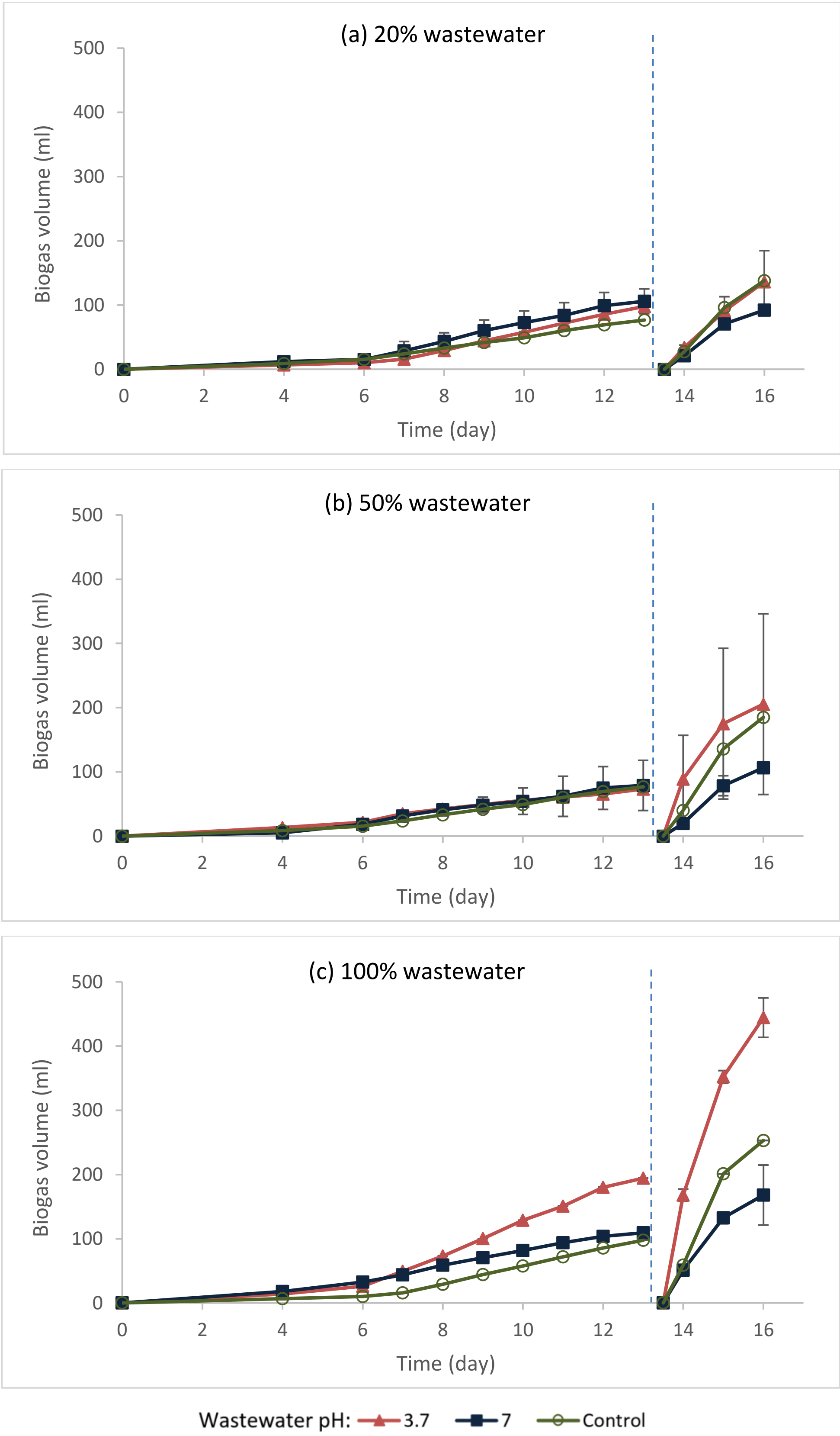

Figure 2. Cumulative biogas formation at wastewater concentration of (a) 20%, (b) 50%, and (c) 100%.

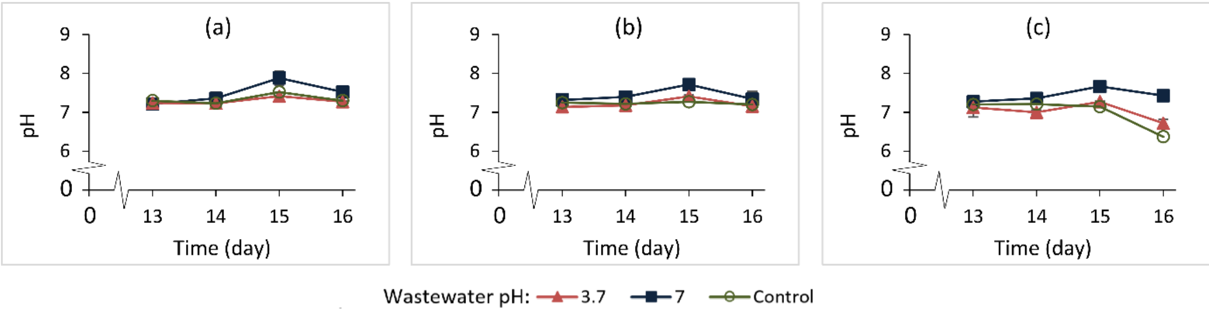

Figure 3. pH of the experiments at wastewater concentration of (a) 20%, (b) 50%, and (c) 100%.

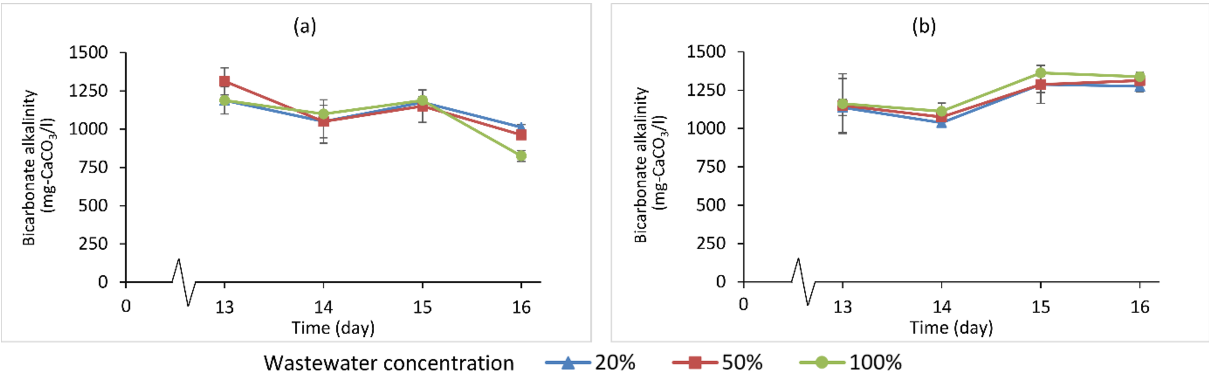

Figure 4. Bicarbonate alkalinity of the experiments at wastewater pH (a) 3.7 and (b) 7.

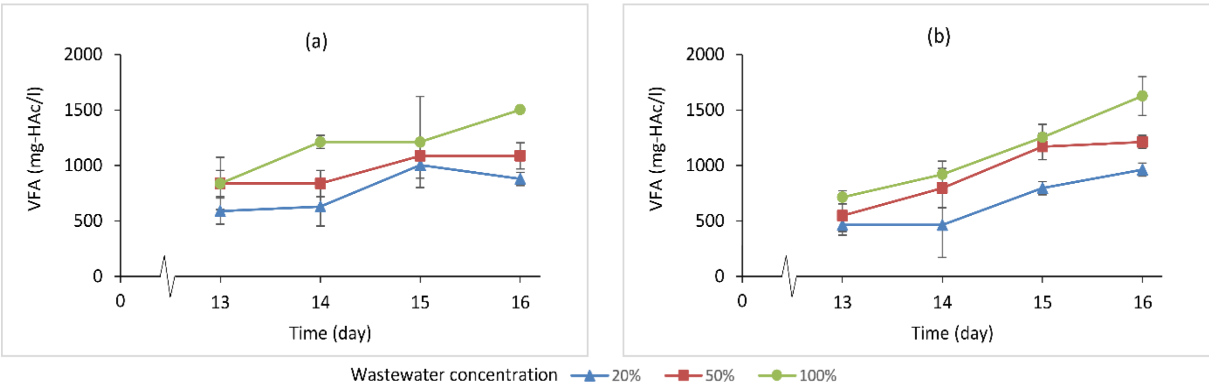

Figure 5. Total volatile fatty acids (VFA) at wastewater pH (a) 3.7 and (b) 7.

4. Discussion

Low pH is not expected in anaerobic digestion, since hydrolysis, acidogenesis, and methanogenesis processes are more optimum in pH around 7 [7]. However, Figure 2a-c show that the lower pH of the wastewater did not hamper anaerobic digestion. This might be due to the pHs of the mixture in all experiments were 7 or higher (Figure 3a-c), regardless the pH of the wastewater. This also shows that the system had sufficient buffer capacity to keep the pH stable.

Not only the low wastewater pH did not hamper anaerobic digestion, but also at 100% wastewater concentration there was indication that biogas production with wastewater pH 3.7 was higher than with wastewater pH 7. This might be due to the use of NaOH solution to adjust the pH. Sodium ions (Na^+) might have toxic effect that inhibit anaerobic processes, particularly on methanogenic microorganisms [18]. In our experiment, however, Na^+ concentration was probably too low to cause toxicity.

The other possible mechanisms is due to the accumulation of inhibiting compounds as the result of hydrolysis. NaOH aids carbohydrate hydrolysis, resulting in some sugar monomers and might also in some phenolic compounds from lignin degradation [19]. Some phenolic compounds might have inhibiting effects on anaerobic digestion [20].

Tofu wastewater in our experiment had a protein content of 1.6 g/l (unpublished result). The amount of NaOH might correlate with increased protein solubilisation and hydrolysis [21]. During anaerobic digestion of protein-rich substrate, ammonia might accumulate as a product of protein hydrolysis and acidogenesis [22]. The increase of ammonia with wastewater pH 7 is indicated by the higher pH (Figure 3). Since total ammonia (NH_3 and NH_4^+) inhibited anaerobic processes, ammonia accumulation might result in lower biogas production [22,23]. On the other hand, the presence of both Na^+ and ammonia was suggested to show less inhibition compared with when only one of the two inhibitors was present [23]. Furthermore, ammonia contributes to process stability by creating buffer at pH 6.4-9.3 [24].

In either case where the excess hydrolysis products from lignin and/or protein might cause inhibition of anaerobic digestion, the low biogas production might be temporary. After an adaptation period, the excess hydrolysis become available substrates for acidogenesis and methanogenesis processes, and increased biogas production.

Another indicator of process stability is the ratio of total VFA and alkaline buffer capacity. During the experiments, the alkalinity also changed as a result of the depletion of organic acid and volatile fatty acids. Lactic acid, valeric acid, butyric acid, propionic acid, and acetic acid in the medium were consumed by bacteria in their metabolism [14]. The value of stable operation is empirical and system-specific; however, ratio of 0.4-0.6 is often used as an indicator of stable continuous operation [17]. In this experiment, VFA/buffer capacity ratios were between 0.5 and 1.8. These values might indicate that the reactors were too low on substrate, as also indicated by the increase of biogas formation after wastewater addition, particularly at 100% wastewater concentration (Figure 2c).

5. Conclusions

Our results show that the low pH of tofu wastewater did not have a negative influence on anaerobic digestion of tofu wastewater in a batch mode. Contrary to expectation, pH adjustment of wastewater with high protein content might have negative influence, possibly due the presence of sodium ion. Our results suggest that the presence of adequate buffer capacity was more important than the adjustment of wastewater pH.

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