Shock-adaptation in a three-stage anaerobic reactor treating tofu whey wastewater

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Abstract. Anaerobic digestion is often used to treat wastewater with high organic content. The effectiveness of anaerobic digestion is determined by organic loading rate and hydraulic retention time, as well as influenced by temperature, pH, and wastewater composition. The multi-stage reactor system is stable towards sudden changes in the influent and fluctuations in hydraulic and organic loadings. The objective of the study was to investigate the adaptability and stability of the three-stage anaerobic reactor after a hydraulic shock loading. This study used tofu whey wastewater due to its enormous generated volume, varying COD concentration, and the low pH. A three-stage anaerobic packed-bed reactor system that had operated for 26 days at a total hydraulic retention time of 3.75 days or 1.25 day per reactor (0.33 l-wastewater per hour) was used in the experiment. The reactor was filled with wastewater at 4 l/h, twelve times the usual flow rate, for one hour. During this period, the changes in pH, COD concentration, and biogas production were monitored. Compared with the performance before the shock loading, COD and pH did not show changes after the 24-hour observation period, except for the pH in the second stage. In addition, biogas and methane production in the second stage was lower than production before the shock loading, even though the total production remained constant. This suggests that the second stage acted as the buffering reactor during the shock loading.

Keyword: tofu whey wastewater; biogas production; three-stage reactor; anaerobic digestion

1. Introduction

Anaerobic digestion is a biological process often used in wastewater treatment plants to treat wastewater with high organic content. The process is robust, requires low nutrient and energy inputs, and do not produce excessive sludge. Instead, it can produce energy in the form of biogas (methane) that can be used e.g. for heating and electricity generation [1,2]. The effectiveness of anaerobic digestion in treating wastewater is determined by organic loading rate and sludge retention time, as well as influenced by temperature, pH, and wastewater composition [3].

Anaerobic digestion occurs in a series of reactions grouped as hydrolysis, acidogenesis, and methanogenesis. Each of these reactions has different optimum conditions. The multi-stage reactor system is an alternative to provide different condition in each stage, therefore facilitates optimum condition for each reaction [4]. In addition, the multi-stage reactor system is also more stable towards fluctuations in hydraulic and organic loadings, pH, temperature, and sudden changes in the influent. Anaerobic digestion of food waste in a three-stage stirred-tank system and cassava wastewater in an up-flow anaerobic sludge blanket (UASB) system could increase biogas production and stabilise the
pH compared with the one-stage system [5,6]. At a higher organic loading rate, anaerobic digestion of sucrose-based wastewater in a two-stage UASB and fixed-bed reactor yielded more methane than the single-stage fixed-bed reactor [7].

Tofu whey wastewater is generated from the production of tofu (soybean curd). The annual consumption of soybean in Indonesia is 3 million tonnes, primarily for tofu and tempeh (fermented soybean cake) productions [8]. In traditional tofu productions in Indonesia, one tonne of processed soybean requires 22 m³ process water and generates 18 m³ wastewater [9]. Most of the traditional tofu production is done by small-scale factories that do not have wastewater treatment facilities and discharge the wastewater directly to the environment. Tofu whey wastewater contains a high organic content in the range of 5 to 37 g-COD/L while the wastewater pH is acidic in the range of 3.6 to 5.5 [10–12].

In this study, tofu whey wastewater was used due to its enormous volume, varying COD concentration, and the low pH. A three-stage anaerobic packed-bed reactor system was used in the experiment. Each reactor had the same effective volume. The cut-bamboo packed-bed provided attachment surface area for the anaerobic microorganisms, enabled the biomass to stay in the reactor for longer than the hydraulic retention time of the wastewater. The objective of the study was to investigate the adaptability of the three-stage anaerobic reactor system during a hydraulic shock loading. Before the shock loading experiment, hydraulic retention time (HRT) in each reactor was gradually decreased from 2.5 days to 1.1 days in six months, followed by two months pause without any input (Table 1). After two months of pause, it was started again by 1.25 days HRT for about a month. The shock loading was done by increasing the wastewater flowrate at 12 times the normal rate in an hour, and then back to the normal rate like before the shock. The performance of the system was observed after the shock period.

2. Materials and methods

2.1. Material
Tofu whey wastewater, obtained from a tofu factory (Bandung City, West Java, Indonesia) was used as a substrate in the experiment. The wastewater had 15.9 g/L total COD, 13.5 g/L soluble COD, and a pH of 3.5.

2.2. Three-stage packed-bed reactor system
The experiment was performed in a three-stage anaerobic packed-bed reactor system as previously described [13].

Table 1. Anaerobic system operation before the experiment.

| Parameter          | Unit    | 0-28 | 28-71 | 71-93 | 93-157 | 157-184 [13] |
|--------------------|---------|------|-------|-------|--------|-------------|
| Flowrate           | L/h     | 0.17 | 0.25  | 0.38  | -      | 0.33        |
| HRT system         | day     | 7.5  | 5.0   | 3.3   | 3.75   |             |
| HRT per reactor    | day     | 2.5  | 1.7   | 1.1   | -      | 1.25        |
| Effluent pH        | -       | 7.3  | 7.5   | 7.1   | -      | 7.3         |
| Effluent COD       | g/L     | 4.6  | 2.2   | 5.1   | -      | 7.4         |
| Biogas production | L/day   | 2.9  | 5.0   | 14.0  | -      | 19.5        |
Before the experiment, the system had been operated for six months (Table 1), with an eight-week break period without substrate influent. After the break, the reactor was able to maintain an average of 51% total COD removal within 26 days of operation [13]. The steady-state flow rate was 0.33 l-wastewater/h, corresponded to HRT of 1.25 day per reactor or 3.75 days for the entire system.

### 2.3. Shock loading experiment

The setup of the shock loading experiment is shown in Figure 1. The system consisted of three reactors in series with an effective volume of 10 litres per reactor; the first, second, and third reactors were assigned as R1, R2, and R3, respectively. The wastewater was fed using a peristaltic pump (IKA, Germany) to R1 and continuously flowed by gravity to R2 and R3. The gas generated in each reactor was combined and passed through a wet gas meter (Ritter, Germany), to be collected in a gas bag.

![Figure 1. Experimental setup in a three-stage anaerobic packed-bed reactor system.](image)

The experiment was performed at day-183 of the system operation and already stable reaching a flow rate of 8 L/day or 0.33 L/h for a month. At the start of the shock loading experiment, tofu wastewater flowed into the reactor at the flow rate of 4 L/h for an hour. Afterwards, the flow rate was returned to 0.33 L/h.

Liquid samples were collected every one hour from reactor effluent (R3 outlet) and every two hours from R1 and R2 outlets in the first eight hours after shock loading. Afterwards, R3 outlet was sampled at t = 10, 12, 14, 20, and 22 hours. At the end of the experiment (24 hours), liquid samples were collected from R1, R2, and R3 outlets. The gas bag was collected and replaced at t = 0, 2, 4, 6, 8, and 24 hours.

### 2.4. Analysis

Liquid samples were analysed for total and soluble chemical oxygen demand (COD), bicarbonate alkalinity, and total volatile fatty acids (VFA). COD was analysed using the potassium dichromate method using acetic acetate standard; the absorbent at 615 nm was measured with a UV-VIS Spectrophotometer (Shimadzu GC14-A, Japan). Total COD is measured from the total homogenised sample, and soluble COD is measured from the supernatant of the centrifuged sample. Bicarbonate alkalinity and total VFA were analysed using the titrimetric method [14]. The collected gas was analysed for composition using a biogas analyser (Gasboard 3200 plus, China).
3. Results and Discussions

3.1. COD concentration

Between day-183 and day-197, the average total COD concentration was $14.7 \pm 0.9 \text{ g/l}$ in R1, $12.5 \pm 1.1 \text{ g/L}$ in R2, and $8.2 \pm 1.6$ in R3 (Figure 2a). There were no significant differences between total COD concentration before [13], during (Figure 2b), and after the shock loading experiment. The soluble COD concentration (Figure 3) also showed a similar trend with the total COD concentration.

**Figure 2.** Total COD concentration during (a) the observation period and (b) shock loading experiment.

**Figure 3.** Soluble COD concentration during (a) the observation period and (b) shock loading experiment.
Within the 24 hours of the experiment, fluctuation mainly occurred in R3. The fluctuation indicated that the reactors tried to overcome both the hydraulic and organic shock loadings. An increase of flow inside the reactor reduced the contact time between wastewater and biofilm, and in severe cases might result in biomass washout [2]. The latter did not seem to occur in our experiment, however, R1 showed the behaviour of a completely-mixed reactor. An additional organic loading and changes in contact time might result in the fluctuation of COD concentration.

3.2. Biogas production
Typical biogas produced from anaerobic digestion consists of CH$_4$ and CO$_2$. Other gases such as H$_2$, CO, and H$_2$S are often present at 1 to 2 % at most [15], therefore their presence is sometimes neglected.

Before the shock loading experiment, the average (total) biogas and CH$_4$ productions were 20 L/day and 10 L/day, respectively [13]. Figure 4 shows that at the start of the experiment, biogas production increased to 35 L/day as a direct response to the flow rate increase. Peak biogas production occurred between $t = 4$ and 6 hours at 56 L/day (57 % CH$_4$), despite the reactors could not convert all the added COD as indicated by the increased concentration in Figure 2b and 3b. Afterwards, biogas production decreased to 37 L/day but CH$_4$ production was almost similar, producing biogas with 75 % CH$_4$ concentration between 8 and 24 hours.

After the experiment (Figure 5a), the average biogas production was $21 \pm 7$ L/day, similar to before the experiment. The average CH$_4$ production and concentration was $13 \pm 7$ L/day and $58 \pm 13$ %, respectively. The average CH$_4$ production after the experiment was higher than before, however, the difference was not significant.

Figure 5b and 5c show that biogas and methane production in R2 were lower than R1. Biogas and methane production in R2 were also lower than production before the shock loading experiment (7 and 4 L/day, respectively [13]). Biogas and methane productions in R3 (Figure 5c) were similar to before the shock loading experiment.

3.3. System stability
Alkalinity/VFA ratio is a measure of the stability of an anaerobic digestion system [14]. At the second start-up (day-157 until 163) and during the shock loading experiment, alkalinity/VFA ratio in the R3 was higher than 3 (Figure 6), which indicate a high organic input to the reactor. The ratio was stabilised between day-164 until 183 and after the shock loading experiment, showing that the reactor was able to digest the organic load.

![Figure 4. Biogas and methane production during the shock loading experiment.](image-url)
Figure 5. Biogas and methane production during the observation period: (a) Total, (b) R1, (c) R2, (d) R3.

Figure 6. Alkalinity/VFA ratio in R3
Figure 7. (a) pH during the observation period (b) and shock loading experiment

Before the experiment, the pH increased from 4.9 in R1 to 5.2 in R2 and 7.0 in R3 [13]. Figure 7, however, shows that the pH did not change from R1 to R2 during and after the shock loading experiment. This suggests that R2 acted as the buffering reactor during and after the shock loading.

The pH in R3 was higher than R1 and R2 since most VFAs were converted to methane in this stage. A drop of pH in R3 would indicate VFA accumulation. The pH in R3 after the shock loading was stable at 7.4 ± 0.3, which showed no VFA accumulation and in addition was the ideal pH for methanogenesis. This also concurred with biogas production and alkalinity/VFA ratio.

4. Conclusions
An experiment with a three-stage anaerobic packed-bed reactor shows that the system could handle an increase in hydraulic loading up to 12 times the usual flow rate. The system was effective in maintaining the total biogas production and COD and pH at the effluent. Observation on individual stage shows that the first stage tended to act like a completely-mixed reactor, especially during the first eight hours after the shock loading, and could return to the initial performance eight hours after the shock. The pH and biogas productions in the second stage were lower than before the shock loading, which suggests that this stage was affected by the shock during the first eight hours, but then acted as the buffering reactor to keep the stability in the third stage.

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Author Contributions
Widyarani and N Sintawardani contributed equally as the main contributor of this work. All authors read and approved the final paper.

References
[1] Fehrenbach H, Giegrich J, Reinhardt G, Sayer U, Gretz M, Lanje K and Schmitz J 2008 Kriterien einer nachhaltigen Bioenergienutzung im globalen Maßstab UBA-Forschungsbericht 206 41–112
[2] Leitão R C, van Haandel A C, Zeeman G and Lettinga G 2006 The effects of operational and environmental variations on anaerobic wastewater treatment systems: A review Bioresour. Technol. 97 1105–18
[3] Mao C, Feng Y, Wang X and Ren G 2015 Review on research achievements of biogas from anaerobic digestion Renew. Sustain. Energy Rev. 45 540–55

[4] Mota V T, Santos F S and Amaral M C S 2013 Two-stage anaerobic membrane bioreactor for the treatment of sugarcane vinasse: Assessment on biological activity and filtration performance Bioresour. Technol. 146 494–503

[5] Jiraprasertwong A, Maitriwong K and Chavadej S 2019 Production of biogas from cassava wastewater using a three-stage upflow anaerobic sludge blanket (UASB) reactor Renew. Energy 130 191–205

[6] Zhang J, Loh K-C, Li W, Lim J W, Dai Y and Tong Y W 2017 Three-stage anaerobic digester for food waste Appl. Energy 194 287–95

[7] Mota V T and Zaiat M 2018 Two- vs. single-stage anaerobic reactors: evaluation of effluent quality and energy production potential using sucrose-based wastewater Water Sci. Technol. 78 1966–79

[8] Rittgers C, McDonald G and Rahmanulloh A 2019 Indonesia Oilseeds and Products Annual Indonesia Oilseeds and Products Annual 2019 (USDA)

[9] Faisal M, Machdar I, Mulana F and Daimon H 2014 Potential renewable energy from tofu processing waste in Banda Aceh City, Indonesia Asian J. Chem. 26 6601–4

[10] Anggarini S, Hidayat N, Sunyoto N M S and Wulandari P S 2015 Optimization of Hydraulic Retention Time (HRT) and Inoculums Addition in Wastewater Treatment Using Anaerobic Digestion System Agric. Agric. Sci. Procedia 3 95–101

[11] Chen Y, Zhang F, Wang T, Shen N, Yu Z-W and Zeng R J 2016 Hydraulic retention time affects stable acetate production from tofu processing wastewater in extreme-thermophilic (70 °C) mixed culture fermentation Bioresour. Technol. 216 722–8

[12] Faisal M, Mulana F, Gani A and Daimon H 2015 Physical and chemical properties of wastewater discharged from tofu industries in Banda Aceh city, Indonesia. Res. J. Pharm. Biol. Chem. Sci. 6 1053–8

[13] Sriwuryandari L, Widyarani, Priantroro E A, Muchlis, Hamidah U, Sembiring T and Sintawardani N 2019 Performance of the three-stages anaerobic tofu wastewater treatment during the second start-up process IOP Conf. Ser. Earth Environ. Sci. 277 012010

[14] Lossie U and Pütz P 2008 Targeted control of biogas plants with the help of FOS/TAC Pract. Rep. Hach-Lange

[15] Chen X Y, Vinh-Thang H, Ramirez A A, Rodrigue D and Kaliaguine S 2015 Membrane gas separation technologies for biogas upgrading RSC Adv. 5 24399–448