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Anaerobic Digestion of Industrial Tempeh Wastewater with Sludge from Cow Manure Biogas Digester as Inoculum: Effect of F/M Ratio on the Methane Production

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Abstract— **Industrial tempeh wastewater contains high organic compounds that will negatively affect the water ecosystem's quality whenever discharged without proper treatments. Biological treatment, in particular, anaerobic digestion, is the potential to be an effective solution for eliminating that contaminant. Considering its advantages, especially to produce methane gas, a study to evaluate the anaerobic digestion process's performance to reduce the organic substances in industrial tempeh wastewater was conducted. Sludge from cow manure biogas digester was used as inoculum to foster an anaerobic decomposition process. Hence the particular focus was given to elaborate the effect of F/M ratio on methane production. With a practical volume of 4L each, controlled batch reactors were employed with several operational parameters (pH, COD, VFAs) to be monitored to understand the process. Results elucidated that methane production was closely related to the composition of the substrate to microorganism. The maximum methane production (8720 mL) followed by the highest organic reduction (67.7%) were found in F/M = 1.12. The measured operational parameters informed a sequence of the process involved in the complete anaerobic decomposition. Accumulation of VFAs, because of higher substrate loading, tended to hamper methanogens metabolism resulted in low methane production. In addition to that, inoculum from biogas digester of cow manure was proved to play a significant role in the anaerobic decomposition for industrial tempeh wastewater. This finding is essential for arranging an effective yet low-cost wastewater treatment for tempeh producers, which is a basis for further study.**

Keywords— **Anaerobic decomposition; F/M ratio; pH; COD; VFAs; methane.**

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I. INTRODUCTION

Tempeh is one of the famous local foods in Indonesia, especially on Java island. It is usually served either in fried form or mixed with vegetables and meats in soup and curry. Besides, tempeh is also converted to crispy form and is known as a popular snack. Tempeh is made from soybean through a controlled fermentation process by using *Rhizopus oligosporus* as a binding agent. It is noted that this food is a good source of protein, dietary fiber, and vitamins (especially vitamin B). Centre for Information and Documentation–National Standardization Agency of Indonesia mentioned that there were 81,000 tempeh companies all over Indonesia, mainly in Java island, putting the country as the largest Tempeh producer among others [1]. This number could be more since the unit production scale mostly in medium, small, or even micro (home industry) level.

Despite nutritional facts and positive economic stimulation, tempeh also produces wastewater as a by-product with harmful impacts on the ecosystem. During the production process, wastewater comes from washing, boiling, and soaking activities. Washing contributes the most considerable part of waste in terms of quantity, but boiling and soaking produce more concentrated wastewater. In practice, the amount of produced wastewater varies from one industry to another. However, the Ministry of Environment and Forestry, The Government of Indonesia, has set the maximum allowable quantity of wastewater as 10 m^3 per ton treated raw material (soybean) [2]. Considering the used raw materials and involved processes, the characteristic of industrial tempeh wastewater is highly organic. It was reported that the COD and BOD values of industrial tempeh wastewater could reach the value of 21,500 mg/L and 12,000 mg/L, respectively, with low pH conditions in the range of 3.7 to 5.0 [3]. Those values are far above the applied standard for the effluent industrial tempeh wastewater, which are 300 mg/L, 150 mg/L, and 6–9 for COD, BOD, and pH, respectively. Direct discharge of high loading organic wastewater to the river will seriously affect the ecosystem's balance by causing oxygen to sag. Rate of oxygen depletion in line with the amount of discharged organic substances, leading to the anaerobic condition. Furthermore, the impact will also link to the climate change issue because, with less or no oxygen content and the river's complexity condition, it will possibly result in greenhouse gasses production such as nitrous oxide (N_2O) and methane (CH_4).

Therefore, a reliable treatment method is needed to reduce the organic content to the lowest level to minimize industrial tempeh wastewater's negative impact. Among several options, biological treatment, particularly anaerobic digestion/fermentation, is considered the most effective solution for wastewater with high organic content. Anaerobic digestion requires less energy consumption than aerobic processes, especially from not using aeration system; it creates less environmental impact mainly due to low excessive sludge production and more straightforward operation [4]. Moreover, the anaerobic process will produce biogas (biohydrogen and methane) that substantially useful for alternative energy and, at the same time, will potentially reduce the treatment cost [5]. Despite the advantages, the removal efficiency of anaerobic treatment is known smaller than aerobic. For this reason, the application in the field scale is usually conducted in combination between anaerobic – anaerobic or anaerobic-aerobic to make sure the allowable effluent limit can be reached.

Anaerobic digestion has been reported to treat various types of industrial wastewater with high organic content such as automotive, petrochemical, sugar, cassava, palm oil, tofu, slaughterhouse/meat processing, and municipal wastewater [6]–[12]. Hence, based on the above considerations, the same process will be applied to treat wastewater from tempeh industry. In this study, anaerobic digestion experiments were done in batch mode, focusing on the effect of F/M (Food to Microorganism) ratio on methane (CH_4) production by using sludge from cow manure biogas digester.

II. Materials and Methods

A. Materials

The wastewater used in this work was obtained from a medium-sized tempeh company in Surabaya, Indonesia. The company has followed the standardization system (ISO) and good manufacturing practice (GMP) with relatively stable market demand. The daily production capacity was 1000 Kg resulting in wastewater at about 6 m^3 per day. In order to get characteristic information, wastewater was collected once a week during three months of observation. Each collected sample was analyzed for several parameters, and the mean value of the results is presented in Table 1. The inoculum was taken from a running biogas reactor of cow manure in the form of sludge. The inoculum's acclimation process was conducted to increase the affinity to the substrate by introducing the wastewater to the sludge. The mixture ratios (%v/v) were gradually adjusted by increasing the proportion of tempeh wastewater (75:25; 50:50; and 25:75). In every cycle, the pattern of microorganism growth was observed, and when it came to the stationary phase, the harvested culture was introduced to the new ratio condition. The total solids (TS) and volatile solids (VS) values of acclimated inoculum were 29.8 g/L and 24.7 g/L, respectively.

For analysis purposes, the entire chemicals in this work ($K_2Cr_2O_7$, potassium hydrogen phthalate, Hg_2SO_4, H_2SO_4, Ag_2SO_4, NaOH) were in analytical grade (Merck and Sigma Aldrich) and used directly without any modifications or pre-treatments.

B. Methods

1) Experimental Set-up: Controlled batch reactor equipped with a paddle mixer, gas tube, sampling port, and control channel was used to run the anaerobic digestion experiments (Fig. 1). The reactor had a capacity of 5L, and the working volume for each run was 4L. In the beginning, pure nitrogen gas was injected into the reactor through a sampling port (elongated pipe up to the bottom of the reactor was available) to remove the remaining air (via gas tube) and create anaerobic conditions. F/M (Food to Microorganism) ratio, expressed in gCOD/gVSS, was set by using the same value of wastewater COD concentration (2 times diluted, 50% of actual concentration of tempeh wastewater) and adjusted the volatile solids (VS) of inoculum to result in several F/M ratio values (0.56; 1.12 and 1.92). pH of the mixture was adjusted at 7.8 by NaOH 2M before loading into the reactor [13]. The headspace was flushed with pure nitrogen gas for 5 minutes to avoid free oxygen intervention. During the anaerobic process, a gentle mixing (50 rpm) was applied for creating effective contact between the organic substances with the microorganism. The produced gas was collected into the Tedlar bag (CEL Scientific) and analyzed for its methane content daily. As for the liquid phase, sampling on wastewater was done every day through the sampling port, and analyses were done for several parameters (COD, VFA). The value of pH was monitored continually with pH-probe (SI-Analytic) through the control channel. All experiments were conducted in mesophilic conditions (30 ± 1ºC) with three repetitions for each variation.

2) Analytical Method: Methane gas analysis was carried out by using Gas Chromatography (GC–Hewlett Packard 680 series) with FID detection and Helium gas as a carrier in HP-PLOT/Al_2O_3 column. The same instrument was used for VFA analysis, but in different conditions and detectors (Hydrogen as the carrier with ECD detector in HP-5, cross-linked 5% phenyl-methyl silicone column). The collected gas volume in the Tedlar bag was measured by vacuuming with a 60 mL syringe [14]. COD measurement was conducted following the APHA (American Public Health Association) standard with closed reflux and colorimetric method using UV-Spectrometer (Agilent) in 620 nm wavelength.

TABLE I
CHARACTERISTICS OF INDUSTRIAL TEMPEH WASTEWATER

Parameter	Unit	Value/Concentration
pH	-	4.62 ± 0.1
COD	mg/L	13,850 ± 618
BOD	mg/L	9200 ± 166
TS	mg/L	13,635 ± 280
TA	mg/L ($CaCO_3$)	2000 ± 86
TN	mg/L	116 ± 4
N-NH_3	mg/L	81.5 ± 5
SO_4	mg/L	208 ± 12

TS = Total Solids, TA = Total Alkalinity, TN = Total Nitrogen

Fig. 1 Anaerobic batch reactors to perform anaerobic digestion for industrial tempeh wastewater.

III. RESULTS AND DISCUSSION

A. Methane Gas Production

The accumulation and daily production of methane gas from three different pre-treatments are presented in Fig. 2 and Fig. 3. The highest amount of methane gas for 21 days digestion period was produced from F/M ratio = 1.12 with a total volume 8720 mL. The second and third producers were F/M = 1.92 and 0.56 with total methane production of 6840 mL and 2460 mL, respectively. Meanwhile, as expected, in the control treatment (reactor with no additional inoculum/microorganism) insignificant volume of biogas was produced. As depicted in Fig.2, lag phase for F/M = 0.56 and 1.12 are similar, considered shorter than for F/M = 1.92. In the first 5 days, the total volume of methane from F/M = 0.56 and 1.12 are comparable but higher than F/M = 1.92. The production in F/M = 1.12 tends to be more stable, giving a constant increase throughout the experiment period. Although exhibiting slow progress initially, total biogas production from F/M = 1.92 starts to outweigh F/M = 0.56 at day 11 and enormously elevated, putting F/M = 1.92 second-largest methane producer at the end of a run.

System's response to the different F/M ratio is also expressed from the daily rate of methane production. Fig.3 delineates a rapid response of F/M = 0.56. At day 3 the system could produce methane gas as much as 340 mL/day that is quite superior compare to the others. However, F/M = 0.56 indicated a non-stable performance after that, as the daily production fluctuated. F/M = 1.12 has more stable progress; this composition's increasing rate continues until day 15 prior to the declining rate. Meanwhile, F/M = 1.92 exhibits a slower production rate, just reach the 200 mL/day at day 8, but then progressively elevate to the maximum rate of 740 mL/day at day 16. Control treatment also produced methane gas, but the production rate was negligible if compared to the systems with inoculum addition.

Different methane production levels from anaerobic digestion are closely related to the substrate's ratio (food) to inoculum (microorganism). Lower biogas production in F/M ratio (0.56) indicates a limited substrate that is completely biodegraded. The incomplete decomposition could be related to the low metabolic activity of microorganisms [13]. Meanwhile, the slow increase in methane production in F/M = 1.92 could be attributed to the volatile fatty acids (VFA) accumulation, hindering a complete methanogenesis process. In higher F/M ratio less anaerobic inoculum was involved, meaning that the digesting ability was lower. Thus, organic biodegradability and substrate utilization rate decreased [15]. To better understand the process mechanism of methane production from industrial tempeh wastewater in different F/M ratios. Explanation about the dynamics of several operational parameters is given in the following sections.

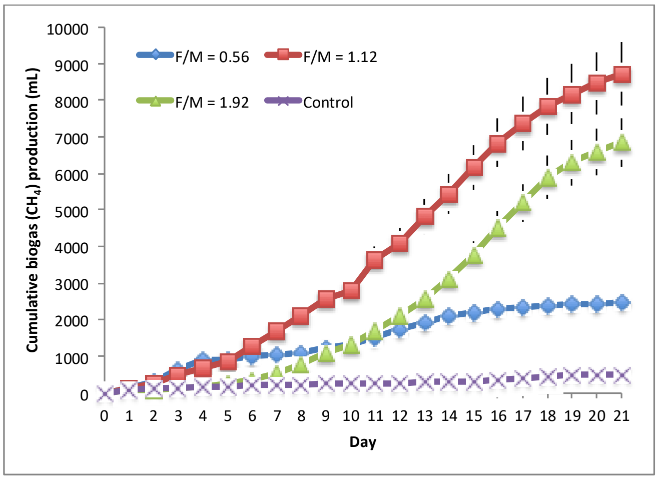

Fig. 2 Cumulative methane production from different F/M ratio

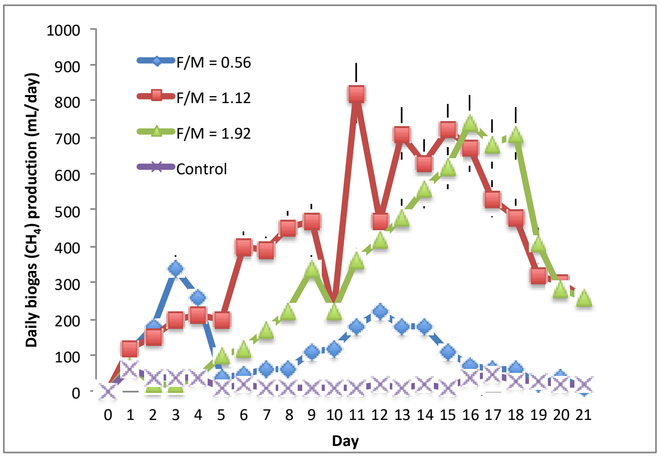

Fig. 3 Daily production of methane from different F/M ratio

B. The Dynamics of Process Parameters

Anaerobic digestion, a biological decomposition with no oxygen present, to convert organic substances into methane gas comprises four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [16]. At the first stage, complex organic compounds (carbohydrates, lipids, proteins) are decomposed into soluble monomers (monosaccharides, fatty acids, amino acids) by involving several groups of hydrolytic microorganisms. In the acidogenesis process, hydrolysis products are converted into volatile fatty acids (VFAs) in the form of acetic acid, butyric acid and propionic acid, ammonia (NH_3) and gases (hydrogen/H_2, carbon dioxide/CO_2, and hydrogen sulfide/H_2S). VFAs are the main component for making methane gas, but the compounds shall be changed into acetate. In the third stage, acetogenesis, that conversion has occurred with carbon dioxide/CO_2 and hydrogen/H_2 as additional products. Lastly, in methanogenesis stage, methane gas can be produced from two possible pathways: acetotrophic by transforming acetate into methane/CH_4 and carbon dioxide/CO_2 and/or hydrogenotrophic by using carbon dioxide/CO_2 and hydrogen/H_2 gasses as the reactants [17]. Some parameters have been monitored in order to trace the methane production process in regard to F/M ratio.

1) pH: pH is one of the critical parameters to monitor the normality of the anaerobic process. Fig. 4 describes the transition of pH value from different F/M variation.

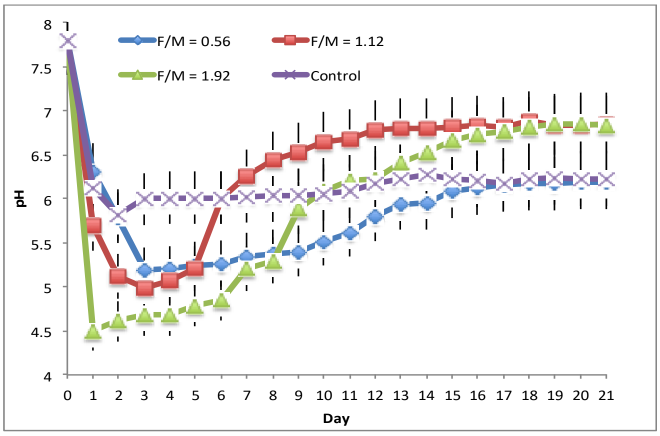

Fig. 4 pH transition during anaerobic digestion process from different F/M ratio

All three experiments had a similar pattern on the pH transition, decreased from the initial pH value (7.8) in the beginning phase before went up in the following days. F/M = 1.92 had the sharpest decline, reaching the lowest pH value of 4.5 and then progressively increased until 6.8 – 6.85 at day 18 to 21. F/M = 0.56 and 1.12 had similar pH value movement at the early days (5.0 – 5.25) but different increasing pattern and value at the end of the experiment, which was 6.19 and 6.85 respectively. The control experiment indicated a stagnant progression of pH after the small drop, keeping the value in the range of 6.01 – 6.23 from day 4 until the end of a run. The decrease of pH at initial stage connects to the VFAs production process, thus in some extent, the level of pH drop reflects the magnitude of produced VFAs [18]. The turn-back pattern designates that the acidogenesis process will be gradually completed and followed by acetogenesis or methanogenesis. This pH turn-back is important since methanogenesis is reported to have optimum performance at the pH of 6.8–7.2 [17], [19]. The pH increase's ability may be determined by the alkalinity of the substrate/inoculum involved (adjusted) and the breakdown result of protein compounds. It was suggested to have alkalinity concentration in the range of 2500 – 5000 mg/L as $CaCO_3$ to provide strong buffering capacity for smoothing the methanogenesis process [13,14]. This study shows that the alkalinity of the system increased at the end of the experiment. Fig. 5 portrays the different alkalinity at the initial condition (before running) and at the end of the experiment (day 21). The variation of alkalinity concentration on the initial condition was attributed to the addition of NaOH and inoculum.

Meanwhile, the increase of alkalinity during the anaerobic progress is firmly due to the role of nitrogen. Besides various amino acid compounds, nitrogen, mostly in the form of ammonia (NH_3), is another product of protein decomposition in the hydrolysis phase. The presence of ammonia (NH_3) triggers two reactions that affect the alkalinity of the solution. The first reaction, reported by Zhang et al. [20], explains that ammonia will neutralize VFA via ionization as given in the following equation:

$$CxHyCOOH + NH_3.H_2O \longleftrightarrow CxHyCOO^- + NH_4^+ + H_2O \quad (1)$$

The second transformation of ammonia (NH_3) is following this stoichiometry equation:

$$NH_3 + H_2O + CO_2 \longrightarrow NH_4^+ + HCO_3^- \quad (2)$$

Both reactions will boost the buffering capacity enabling the pH level to move up along the substrate decomposition. The indication of ammonia (NH_3) transformation was corroborated by the different concentrations between day 1 (when the hydrolysis phase occurred) and day 21, as shown in Fig. 6.

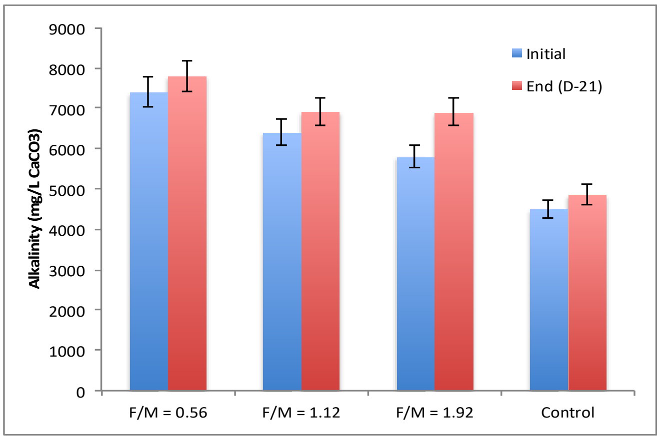

Fig. 5 The difference of alkalinity (as $CaCO_3$) concentration at the initial and end of the experiment

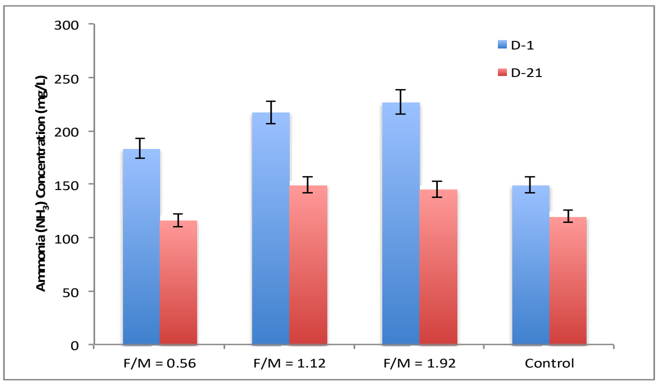

Fig. 6 The reduction of ammonia (NH_3) concentration during the anaerobic decomposition

However, apart from its beneficial role, the presence of free ammonia in certain amount is reported to inhibit methanogenic microorganisms. Up to the maximum concentration of 200 mg/L as total ammonia nitrogen (TAN) is still positive for the anaerobic decomposition, but whenever the concentration is more than 1500 mg/L it will lead to serious suppression [21]. More specifically, it was advised to keep the free ammonia (NH_3) at below 200 mg/L to avoid obstruction [17]. On day 1, the ammonia concentration in F/M = 1.12 and 19.2 were slightly above the recommended level, 217 and 226 mg/L, respectively. Nevertheless, methane gas was still produced noting that the decrease of ammonia concentration during the progress smoothing the methanogenesis stage. Initial pH condition (7.8) in all F/M variations supported by alkalinity from the ammonia transformation was favorable for industrial tempeh wastewater's anaerobic digestion. In particular, since the pH for F/M = 1.12 and 1.92 were closed to the ideal condition, more methane was produced.

2) Volatile Fatty Acids (VFAs): VFAs, vital intermediate products for complete anaerobic digestion, are produced during the acidogenesis stage. The number of products is influenced by the substrate availability (organic loading) as well as biomass load and types. VFAs consist of several forms of acid such as acetic, butyric, propionic, n-butyric, iso-butyric, iso-valeric, and n-valeric can be used as a carbon source for the making of methane gas [22]. Based on that, VFAs production in anaerobic decomposition need to be monitored as an indicator for the potential of biogas production. Tempeh wastewater with sludge from cow manure digester tank as inoculum produced VFAs in all treatment conditions. The VFAs formation was dominated by acetic acid, while butyric and propionic were observed in smaller amount. The dominance of acetic acid was beneficial as it was reported that acetic acid (and butyric) could be used directly by methanogens; meanwhile, propionic acid was recognized as direct inhibitor for the methanogens, especially whenever the concentration reaches 1g/L [17], [23], [24].

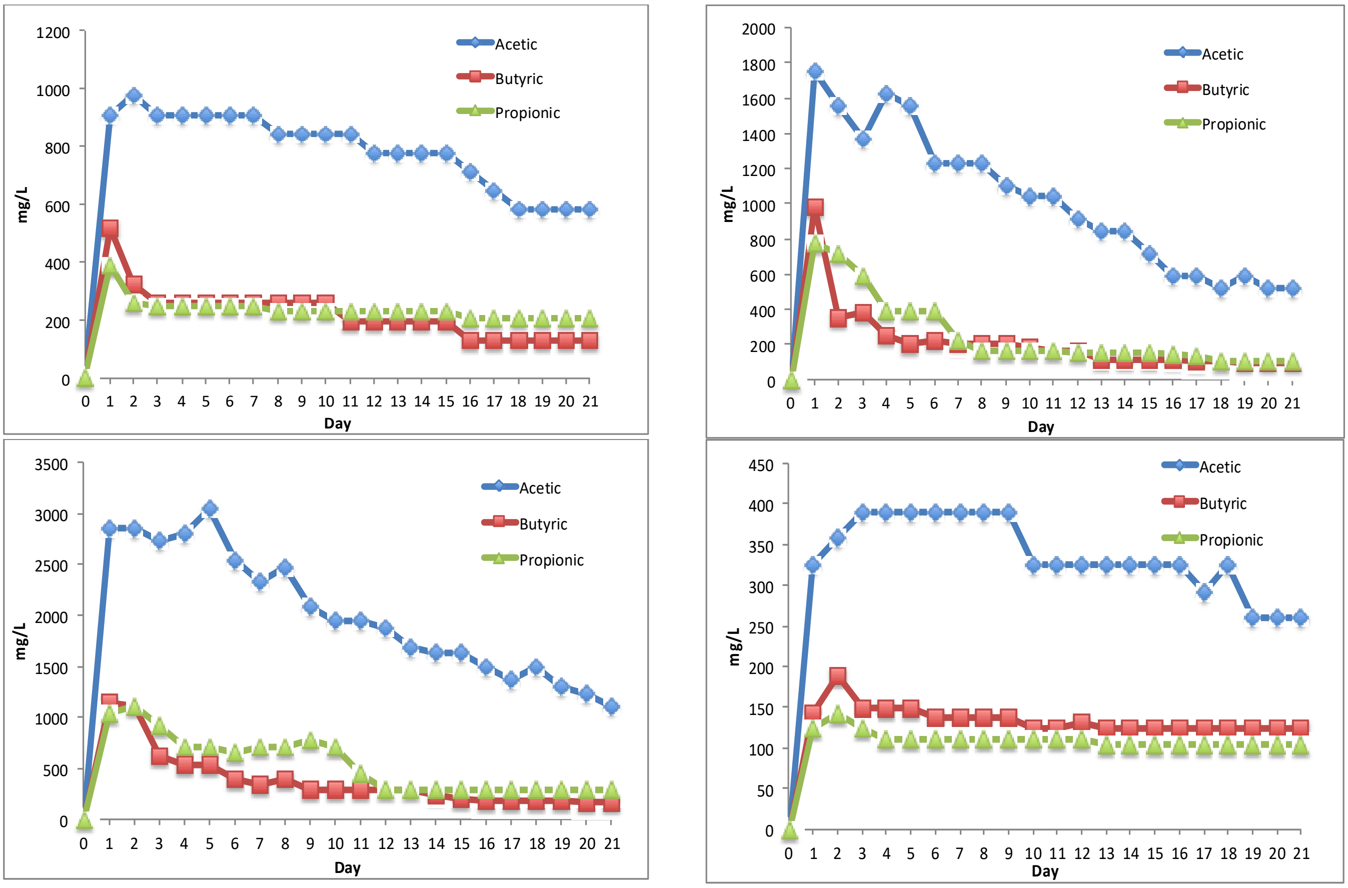

Fig. 7 Volatile fatty acids (VFAs) in three different F/M ratios and control treatment

The metabolism rate of methanogens is different from the production rate of VFAs. The activity of methanogens is susceptible to ammonia toxicity and a low level of pH [25]. The high organic loading to the system triggers level of ammonia toxicity and pH drop. More VFAs will be produced in this condition, resulting in pH drop, and subsequently affecting the composition of ionized ammonia (NH_4^+) and free ammonia (NH_3). In this case, free ammonia (NH_3) is the direct inhibitor to the methanogens because of its high permeability to the cell membranes [19]. In this study, it was indicated that ammonia (NH_3) did not severely affect biogas production, considering that the concentration still at an acceptable level (Fig. 6). Therefore, a plausible explanation for the different production gas in different F/M ratios could be attributed to the VFAs production. As can be seen in Fig. 7, this compound was fabricated at the early stage of the process, with the highest concentration of acetic acid found in F/M = 1.92, as much as 3000 mg/L, and subsequently followed by F/M = 1.12 (1800 mg/L) and F/M = 0.56 (900 mg/L). Thereafter, the acetic acid concentration tends to decline for the rest of the observation periods; a similar trend applied for butyric and propionic acids. The decrease of VFAs is related to the formation of methane gas; as the pH went up to the amicable level (as shown in Fig. 4), methanogens will start to convert acetate into the gas. A steeper decline is noticed in F/M = 1.12. This pattern corresponds to the shorter time needed by this variation to achieve higher pH value, indicating a better balance condition of the amount of produced VFAs and methanogens metabolism. Thus, process conversion of VFAs into methane was faster in F/M =1.12, compared to those in F/M = 0.56 and 1.92, giving the highest total volume of methane. Similar VFAs decrease and pH increase are also perceived for F/M = 0.56 and 1.92 reflecting the methanogenesis rate. In a high F/M ratio (1.92), meaning more substrate available, the produced VFAs was almost double than in F/M = 1.12 and triple than 0.56. The huge production also validated by the lowest drop of pH among others, as illustrated in Fig. 4. This condition is lowering the rate of methanogens metabolism resulting in lower methane gas production. However, as the VFAs were no more produced (the total accumulation reduced), methanogens converted it into biogas more optimally, supported by the achieved ideal pH condition. Hindrance to methanogens activity because of VFAs accumulation and pH drop in high substrate loading was also reported by Zhang *et al.* [26] and Cuff *et al*. [27]. As for the F/M = 0.56, it was expected that the rate of methane production could be the fastest, as the number of biomass was about double the available organic substrate, but the data revealed a divergent pattern. Indeed, at the beginning, F/M = 0.56 has higher daily production compared to others. However, the pattern changed unexpectedly (Fig. 3), indicating instability. The declining pattern of VFAs, especially for acetic acid, is relatively flat (Fig. 7), designating low bioactivity. Raposo *et al.* [13] pointed out that even though it will avoid inhibition effect at low substrate concentration, microorganisms tend to perform slow metabolism. It is reported that at a low F/M ratio, enzyme reactions for primary mineralization would be impeded [28], [29]. After the production stage, TVAs contents in control treatment remained constant. Without any inoculum, it was notable that the hydrolysis and acidogenesis process were very limited and methanogenesis did not significantly occur. By comparing to three others, it can be affirmed that inoculum from the biogas reactor of cow manure contained sufficient *acetogenins* and *methanogens*.

3) Organic Removal (COD): As the primary target substrate for microorganisms, organic substances in the wastewater will reduce as microbial activities occurred. If complete anaerobic digestion passes off through the final phase, methanogenesis, these compounds will be converted into gases, which are methane (CH_4) and carbon dioxide (CO_2) [30]. It means the presence of organic materials will be no longer traceable in the aqueous phase. Therefore, organic substances' transition could be one of the clear indicators to monitor anaerobic digestion's smooth running. Fig 8, denotes the passage of COD removal during the run for all F/M variations.

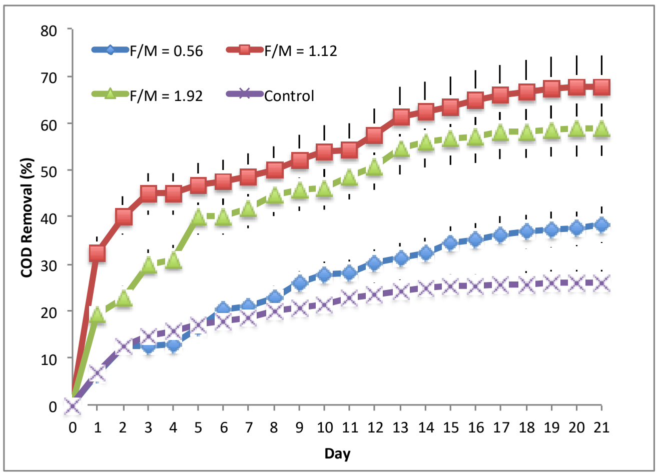

Fig. 8 COD removal in three different F/M ratios and control treatment

For the same period of the experiment, F/M = 1.12 has the biggest COD removal as high as 67.7% following by F/M = 1.92 and 0.56 which were 58.9% and 38.4% respectively. In correspond to Fig.7, total COD removal may be accounted for acetic, butyric, and propionic abatement. Total COD removal of 67.7% in F/M = 1.12 relevant to the reduction of 71.1% of acetic, 90.1% butyric and 86.7% propionic acid. For F/M = 1.92, 58.9% COD removal fit to the decrease of 63.2% acetic, 84.6% butyric and 74.1% propionic acid. Meanwhile 38.4% COD removal in F/M = 0.56, reflects the decline of 35.7% acetic, 75.0% butyric and 46.7% propionic acid. As a comparison, control treatment has total COD removal of 26.2% connected to the less 33.3% acetic, 34.5% butyric and 27.3% propionic acid.

IV. Conclusions

The use of sludge from cow manure biogas digester as inoculum successfully promoted an anaerobic digestion process for industrial tempeh wastewater. The composition of food to microorganism (F/M ratio) played a significant role in determining the total volume of produced methane and COD removal. In this study, F/M = 1.12 produced the biggest volume of methane with a total of 8720 mL and gave the highest COD removal as much as 67.7% for 21 days. The measured operational parameters divulge the number of available substrates connect to the extent of VFAs production, which induces the rate of methane production. This finding is very useful as basic information for arranging suitable and beneficial (in terms of bio-energy collection) wastewater treatment for tempeh producers, especially when considering that these industries are mostly in small-medium level.

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