A review: biogas production from tofu liquid waste

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Abstract. The tofu consumption will increase in the future. As consequent, the tofu production increases significantly. In other side, the amount of tofu wastes also increases. The solid waste can be utilized as solid compost but the liquid waste is discharged directly to the waster bodies at the moment. The latter is potential to be used as biogas feedstock. Some important factors affecting biogas rate need to be considered such as power Hydrogen (pH), Chemical Oxygen Demand (COD) to Nitrogen ratio, Total solid (TS) content, biological agent addition, initial concentration and two-stage anaerobic digestion. Furthermore, purification of the biogas formed by digestion of the tofu liquid waste is needed to be conducted to increase the methane content and decrease carbon dioxide (CO₂) and hydrogen sulfide (H₂S) content. The H₂S has to be removed prior to CO₂ removal. The H₂S can be removed by biofiltration and CO₂ can be removed by biological absorption with help of microalgae. The effluent of anaerobic digester (digestate) may still contain high pollutant so it can be used as nutrient sources for microalgae. The zero waste system for tofu industries is successfully proposed in this paper. Future trend in treating tofu liquid waste in not only for biogas production but also for biohydrogen production.

1. Introduction
Tofu is a traditional food made from soybean [1]. It is very useful for human body as a protein source. In addition, its price is low. Hence, many Indonesian people consume it [2]. Tofu consumption in households in Indonesia per year is shown in Fig. 1. Development of the tofu consumption for the year 2002-2018 is fluctuation with average value of 7.41. Furthermore, the tofu consumption is predicted to be 8.38, 8.52, 8.67 in year of 2019, 2020, 2021 respectively [3]. In other word, the demand of tofu is predicted to become increasing for the future years. According to BPS [4], as protein source, tofu is more desirable than the others because consumption (kg/capita/week) for tofu is higher (0.158) than ras chicken meat (0.121) or beef (0.009). More than 84,000 tofu industry units are operated in Indonesia and total tofu production per year is more than 2.56 million tons [5].

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The shape of tofu in Indonesia is shown in Fig. 2. To produce the tofu traditionally, some process are needed [6-7].

a) The soybeans are washed and then are soaked in clean water for 8-12 hours.
b) The soaked soybeans are drained and then grinded to be a slurry.
c) The slurry is mixed with water (water:beans of 10:1) and then it is cooked at 100-110°C for 10 min.
d) It is filtered or centrifuged to separate the soymilk from solid waste (okara).
e) Next step is coagulation in which coagulants (usually calcium chloride or magnesium chloride) are added to allow for curdling.
f) Then, the supernatant is separated and the curds are pressed to form blocks of tofu. In this step, the liquid waste (whey) is generated.
g) Finally, it is cut and packaged.

The tofu production scheme is shown in Fig. 3.
2. Tofu wastewater characteristic and its environmental impacts

Based on Fig. 3, in tofu production, industries generate two kinds of wastes i.e. solid waste (okara) and liquid waste (whey). To produce tofu as much as 80 kg, the industries need soybeans of 60 kg and water of 2,700 kg. While, the solid and liquid wastes are generated as much as 70 kg and 2,610 kg respectively [9]. The tofu solid waste has a very little impact on the environment because it is mostly used as fodder for cattle animals or biofertilizer for crops [2]. Chemical content of tofu solid waste and solid compost is shown in Table 1. In the other side, usually, tofu liquid waste is discharged directly to the water bodies. However, this activity is forbidden because its characteristics are not below the quality standard. Comparison between chemical characteristics of fresh tofu liquid waste with quality standard of tofu liquid waste that can be discharged to the water bodies is presented in Table 2.
Table 1. Comparison of chemical content of tofu solid waste and solid compost [2]

| Parameters       | Tofu solid waste | Solid compost |
|------------------|------------------|---------------|
| N (%)            | 1.24             | 1.44          |
| P₂O₅ (mg/L)      | 5.54             | 2.37          |
| K₂O              | 1.34             | 3.03          |
| Protein (%)      | 7.72             | -             |

If the waste is discharged directly to the water bodies, some problems will occur. The high organic compound (high COD and BOD) will decrease the dissolved oxygen, so it will disturb the aquatic organisms such as fish. The high nitrogen content also results bad odor because of ammonia gas. Its murky color will decrease the water quality and decrease the sunlight penetration to the water plants in which it cannot do photosynthesis process easily. Furthermore, when the water is used for human daily activity, the human can get skin diseases, stomachache, etc [2,10-11].

Based on information above, a treatment is needed to solve the problems. The one of potential methods is anaerobic digestion (AD) because (1) it is very good to treat wastes having high COD content, (2) it has low cost, (3) it converts a waste to biogas (renewable energy), (4) it is easy to be operated.

Table 2. Quality standard of tofu liquid waste and characteristics of fresh tofu liquid waste

| Parameters                        | Values                                    |
|-----------------------------------|-------------------------------------------|
|                                   | Quality standard for tofu liquid waste (Based on PP No. 82 year of 2001) | Fresh tofu waste [10,12] |
| pH                                | 5-9                                       | 4.82-5.5                  |
| Chemical oxygen demand (COD) (mg/L) | 500                                       | 5,000-8,640               |
| Biological oxygen demand (BOD) (mg/L) | 100                                       | 3,575.5-6,586             |
| Phosphate (mg/L)                  | 5                                         | 0.97-95.5                 |
| Nitrogen (mg/L)                   | 20                                        | 297.5                     |
| Nitrate (mg/L)                    | 20                                        | 25.355                    |
| TSS (mg/L)                        | 400                                       | 2,350                     |

3. Biogas production

3.1. Biogas production process

Anaerobic digestion is one of interesting methods to treat wastes. During digestion, the organic compound in the tofu liquid waste is converted to be biogas with help of anaerobic bacterial activity. Biogas contains methane (CH₄) as main product, carbon dioxide (CO₂) as main by products, and other gas in little amount (such as carbon monoxide (CO), hydrogen (H₂), ammonia (NH₃), hydrogen sulfide (H₂S), etc.) [13-14]. The percentage of the gasses in biogas depends on the biogas feedstocks. Some phases occur in biogas production i.e. hydrolysis, acidogenesis, acetogenesis, methanogenesis [1,15]. In hydrolysis phase, complex organic compounds (such as polysaccharides, lipids, protein) are degraded to become simple organic compounds (such as glucose, long-chain fatty acids, amino acids). In acidogenesis phase, the simple organic compounds are degraded to become volatile fatty acids (acetic acid, butyric acid, propionic acid, hydrogen, carbon dioxide, methanol, ethanol). In acetogenesis phase, propionic acid, butyric acid and ethanol are converted to become acetic acid. In methanogenesis, the methane is formed in which there are three mechanisms i.e. (1) hydrogenotrophic methanogenesis: reaction between carbon dioxide and hydrogen results methane and water (equation 1), (2) acetoclastic methanogenesis: acetic acid is converted to methane and carbon dioxide (equation 2), (3) methyltropic methanogenesis: methanol is converted to methane, carbon dioxide and water (equation 3). Scheme of biogas production is presented in Fig. 4.

\[ 2H_2 + CO_2 \rightarrow CH_4 + 2H_2O \] (1)

\[ CH_3COOH \rightarrow CH_4 + CO_2 \] (2)
\[ 4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \] (3)

Figure 4. Phases in anaerobic digestion [15].

3.2. Factor affecting biogas production

3.2.1. Power of hydrogen level. The fundamental factor for microbial growth is power of hydrogen (pH) level [16]. Syaichurrozi et al. [1] has studied the effect of initial pH on biogas production from tofu liquid waste with variation of initial pH of 5, 6, 7, 8, 9. The results are shown in Fig. 5. Based on Fig. 5, the best initial pH is 8. Lay et al. [17] also has a conclusion in which the optimum initial pH range for biogas from tofu wastewater is 7.5-8. The pH of tofu liquid waste is 4.82-5.5 (table 2), so the base compounds (such as NaOH) can be used to increase the pH level prior the waste is treated in anaerobic digestion.

3.2.2. Total solid content. Tofu liquid waste contains low total solid (TS) content (below 7%). According to Budiyono et al. [18], the optimum range of TS in AD is 7.4-9.2%. Hence, Rahmat et al. [11] have added some solid wastes (sheep dung, paddy straw, bamboo litter). The results showed that biogas increased drastically. If TS content more than the optimum range, organic materials is overload in digester. In other side, TS content less than the optimum range, fermentation process is unstable so biogas production rate is low [19].
3.2.3. COD to nitrogen ratio. Ratio between COD to nitrogen (COD/N) affects the success of biogas production. Anaerobic bacteria can grow in substrates containing COD/N ratio with range of 350/7 to 1000/7 [20]. If the ratio is higher than the optimum range, volatile fatty acids (VFAs) is produced in a large amount. The abundant of the VFAs decreases the substrate pH, so bacterial activity will be disturbed and biogas volume is low [21]. In other side, if the ratio is lower than the optimum range, ammonia and ammonium are easy to be formed. The high concentration of ammonia (more than 150 mg/L) and ammonium (more than 30,000 mg/L) is toxic for anaerobic bacteria. The ammonia is more toxic than ammonium [22]. According to Syaichurrozi et al. [21], tofu liquid waste contains COD/N ratio of 270/7. This value is lower than the optimum range. Biogas yield produced from AD of tofu liquid waste is 77.01 mL/g COD. To increase the COD/N ratio, carbon source such as glucose can be added to the tofu liquid waste. Besides that, a waste containing high carbon content also can be used as carbon source. This concept is called as co-digestion. Syaichurrozi et al. [21] utilized vinasse as co-partner for tofu wastewater because it contains high COD/N ratio (1436/7). The best mixture of vinasse and tofu wastewater to produce high biogas yield (153.7 mL/g COD) is 20:80 (volume basis) with COD/N of 1042/7.

3.2.4. Initial concentration. Widyarani et al. [23] stated that initial concentration of substrate affects biogas production from tofu liquid waste. The waste concentration is varied to be 20, 50, 100%. The concentration of 100% (without dilution) results the higher biogas volume than the two others. It shows that the organic materials (total solid) in tofu wastewater is needed to be increased not to be decreased. Thus, addition of the other waste is better to get suitable total solid content (correlation with point 3.2.2).

3.2.5. Biological agent addition. Addition of a biological agent also can increase biogas production. Addition of yeast of Saccharomyces cerevisiae successfully increases total biogas volume of tofu wastewater in which the total biogas increases 22.91-81.97% compared to without yeast addition [1]. In fermentation process, the yeast helps anaerobic bacteria to convert glucose to become acetic acid, butyric acid and ethanol. The composition of these compounds depends on pH condition [24]. Thus, optimum pH level is needed to be obtained to get optimum condition for growth of both the yeast and the anaerobic bacteria in order to biogas is produced maximally. The lower the pH level, the higher the ethanol concentration presents in system so it disturbs the anaerobic bacteria activity. In other side, the higher the pH level, the lower the ethanol concentration is generated but too high pH level is also not good for...
anaerobic bacteria. The best initial pH for collaboration of the yeast and anaerobic bacteria is 8-9 [1]. A previous study predicts the scheme of biogas production when the yeast is added and rumen fluid is used as inoculum (Fig. 6). The rumen fluid contains bacteria of *Clostridium* *sp.*, *Clostridium sporogenes*, *Clostridium butyricum* and methanogenic bacteria [1].

3.2.6. Two-stage anaerobic digestion. This concept is proposed to separate the hydrolysis-acid formations (acidogenesis and acetogenesis) and methane formations (methanogenesis). Commonly, biogas production process occurs in one-stage anaerobic digestion where all phases occurs in one system. However, this technique is not optimal because acidogenic and acetogenic bacteria requires a acid acidic condition and methanogenic bacteria needs a neutral condition. The scheme of the two-stage anaerobic digestion is shown in Fig. 7. Hence, the two-stage anaerobic digestion is proposed to treat tofu liquid waste (Iriani et al., 2018). The pH is adjusted to be 5.3-5.7 in first reactor and 7 in second reactor [25]. The hydrogen (H₂) and carbon dioxide (CO₂) is generated in first digester and then the methane (CH₄) and carbon dioxide (CO₂) is generated in second digester [26]. Purification to decrease the CO₂ can be applied to get H₂ and CH₄ gas in high purity. The CO₂ removal in biogas will be discussed in section of 4.1.
4. Biogas Purification

Based on Harihastuti et al. [27], biogas generated from AD of tofu liquid waste contains 45.89% of CH$_4$, 29.69% of CO$_2$, 0.33% of H$_2$S, 0.0006% of NH$_3$, and 24.10% of H$_2$O. Hence, it is needed to be purified to increase the biogas quality.

4.1. Removal of CO$_2$

The CO$_2$ is a main by product in biogas. The presence of CO$_2$ can decrease caloric value. Hence, purification methods are needed to remove the CO$_2$ in biogas.

4.1.1. Physical absorption.

The principle of this method is based on different solubility of gas components in liquid absorbent. Commonly, waster is used as the absorbent. The solubility of CO$_2$ is 26 times higher than that of CH$_4$. However, the solubility of H$_2$S is higher than CO$_2$. The H$_2$S will be dissolved and disturb the operation because it has corrosive characteristic. Thus, it is better to remove H$_2$S in biogas prior the CO$_2$ removal process is conducted [28]. The advantages of this method are (1) high efficiency (more than 97% CH$_4$), (2) adjustable capacity by changing temperature or pressure, (3) low CH$_4$ loss (less than 2%). The disadvantages of it are (1) high cost in investment and operation, (2) clogging by bacterial growth, (3) foaming [28].

4.1.2. Chemical absorption.

In this method, a solvent is used as liquid absorbent. The common solvents applied are mono ethanol amine (MEA) and di-methyl ethanol amine (DMEA). The H$_2$S is removed prior to chemical absorption because it causes an amine poisoning [28]. The advantages of this method are (1) high efficiency (more than 97% CH$_4$), (2) low operating cost, (3) low CH$_4$ loss (less than 0.1%), (4) power cost of 0.05-0.25 kWh/m$^3$ of gas. The disadvantages of it are (1) Expensive investment, (2) heating for regeneration, (3) poisoning of the amine [28].

4.1.3. Biological absorption.

Microalgae can be applied to remove CO$_2$ in biogas. The microalgae will uptake it as direct carbon source to do photosynthesis. This method is very interesting because biogas purification and biomass production occur in same time. The advantages of this method are (1) high efficiency, (2) low operating cost, (3) microalgal biomass production, (4) decreasing cultivation cost.
The disadvantages of it are (1) drop in pH due to too high CO$_2$ content in biogas or too high the flow rate, (2) light energy is needed to do photosynthesis\cite{29}. Some microalgae can be applied in this method but the most interesting is *Spirulina* because (1) it has big biomass size so the biomass is easy to be harvested and (2) it has fast growth rate.

4.1.4. Cryogenic separation. This method utilizes the different boiling point of CO$_2$ (-78°C) and CH$_4$ (-160°C). The high pressure is applied (200 bar) \cite{28}. The advantages of this method are (1) high efficiency, (2) low extra energy cost to reach liquid biomethane. The disadvantages of it are (1) expensive investment and operation, (2) high energy for cooling, (3) high pressure \cite{28}.

4.2. Removal of H$_2$O

The high H$_2$O content in biogas gives some disadvantages because (1) it causes a corrosion in metal burner equipments or compressors and (2) it decrease the calorific value. The H$_2$O can react with H$_2$S, NH$_3$, CO$_2$ to form acids \cite{28}.

4.2.1. Physical separation (condensation). This method includes demisters, cyclone separators, moisture traps and water traps. However, the ways are low efficient \cite{28}. In previous study, the H$_2$O in biogas generated from tofu liquid waste can be removed by condensation \cite{27}.

4.2.2. Chemical separation. The principle of this method is absorption of water in glycol (a drying agent). The glycol has a binding component decreasing the dew point from −5 until −15 °C. Furthermore, the silica gel, magnesium oxide, activated carbon and alumina also can be used because they have a binding component decreasing the dew point until −40 °C at pressure of 6–10 bar \cite{28}.

4.3. Removal of H$_2$S

Some methods to remove H$_2$S in biogas are absorption by water, chemical absorption, membrane, adsorption with activated carbon \cite{28}. However, they need high investment and operating cost. In other side, biofiltration needs cheap investment and operating costs. Furthermore, it can be operated at low pressure and does not produce further waste \cite{30}. Biofiltration has a multi-phase system in which H$_2$S gas is dissolved and adsorbed in the biofilm, and then degraded by microorganisms that are immobilized on a packing material forming a thin layer (biofilm). The packing material can be made from natural and synthetic materials. The packing material must have (1) high porosity as well as high water retention capacity, (2) nutrient availability for microorganisms, (3) high surface area, high permeability and high adsorbance, (4) low price. Some materials such as soil, compost, wood chips, leaves, sawdust, bagasse ans salak seed can be used. There are several microorganisms that could be utilized in sulfur removal. Bacteria of Thiobacillus thioxidans, Thiobacillus ferrooxidans, Chromatiaceae (Chlorobiaceae) can be chosen in biofiltration method \cite{30}.

5. Treatment of AD effluent (digestate)

In AD, effluent of digester still contains organic compound. Hence, the effluent cannot be discharged directly since it has high COD content. Some methods have to be applied to utilize the effluent.

5.1. Coagulation

This method utilize coagulants of iron hydroxide to adsorb the COD and then settled as sludge. The sludge is easy to be separated through sedimentation or filtration. There are two kinds of coagulation i.e. chemical coagulation and electrocoagulation. In chemical coagulation (CC), chemical compounds such as FeCl$_2$, FeCl$_3$, Fe(SO$_4$)$_2$, Fe$_2$(SO$_4$)$_3$ is added to the AD effluent. Furthermore, the compounds react with water to form coagulants (Fe(OH)$_2$ or Fe(OH)$_3$). Meanwhile, in electrocoagulation (EC), the Fe$^{2+}$ or Fe$^{3+}$ ion is generated from electrolysis. In EC, iron electrodes (anode and cathode) are immersed to the AD effluent and then the current is flowing, so the anode is oxidized to be Fe$^{2+}$ or Fe$^{3+}$ ion. In other side, the water is reduced to be OH$^{-}$ ion and H$_2$ gas in cathode. The Fe$^{2+}$ or Fe$^{3+}$ ion will react with OH$^{-}$.
to form coagulants. The CC is high cost because it needs chemical compounds. The EC needs no chemical compounds but it needs electrical sources. Application of CC and EC on effluent of AD (substrate of AD is not tofu liquid waste) is successfully conducted by Rodrigues et al. [31] and Liu et al. [32] respectively. Based on our literature study, application of CC and EC on anaerobically treated tofu liquid waste has not reported yet.

5.2. Utilization as biofertilizer
The AD effluent also can be used directly as biofertilizer since its characteristic is suitable with quality standard of compost. Tofu liquid waste contains high nitrogen sources so the AD effluent may contain high ammonia or ammonium. Hence, some further treatments are needed to decrease the ammonia or ammonium level prior the effluent is used as biofertilizer. Koszela and Lorencowicz [33] reports that digestate from agricultural biogas plants can be used as a fertilizer directly.

![Figure 8. Photosynthetic oxygenation principle [34].](image)

5.3. Utilization as organic nutrient for microalgae
The AD effluent can be used as nutrient source for microalgae because it still contains organic carbon and nitrogen sources. This concept is very interesting because some advantages can be reached at same time i.e. wastetreatment and biomass production. The AD effluent may contain oxidation bacteria. When it is applied as nutrient sources for microalge, the oxidation bacteria and microalgae present in the system. According to Oswald and Gotaas [34], during cultivation, there are collaboration between oxidation bacteria and microalgae that may occur (Fig. 8). At the first time of cultivation, the oxidation bacteria consumes organic compounds and produces energy and CO$_2$ via respiration and then the CO$_2$ reacts with water to form carbonate, so pH substrate to be low. After that, the carbonate is consumed by microalgae via photosynthetic process and released OH$^-$. Photosynthetic activity needs CO$_2$ as primary carbon source and light as primary energy source. Accumulation of OH$^-$ increases substrate pH. The microalgae can also consume organic carbon as source of carbon and generate energy and CO$_2$ via respiration. This growth is called as a heterotrophic growth. The CO$_2$ is applied as carbon source in photosynthesis. This growth is called as photoautotrophic growth. The microalgae that can grow through either photoautotrophic or heterotrophic processes is called as mixotrophic bacteria [35].
6. Scheme of zero waste system

A scheme that can be applied by tofu industries with zero waste can be seen in Fig. 9. Tofu industries produce tofu as a main product which is consumed by human as protein source. The solid waste is utilized as biofertilizer (as compost). The compost is applied in fields to increase soybean production. In other side, the liquid waste is brought to anaerobic digesters. In anaerobic digesters, the organic compounds in the liquid waste are converted to biogas by help of anaerobic bacteria. The biogas still contains impurities (H$_2$S and CO$_2$), so purification methods are applied to decrease these gas.
components. The H₂S and CO₂ gases are removed by biofiltration and biological absorption (microalgae) respectively so the biomethane is obtained. The H₂S in low concentration also can be removed by microalgae but it in high concentration is not suggested because it is toxic. Hence, biofiltration is better to be conducted prior the biogas is purified through microalgae. The biomethane can be used as energy source in tofu production. The other product of anaerobic digestion is effluent. The effluent is separated to obtain the solid part and liquid part. The solid part is used as solid compost and the liquid part is interesting to be applied as organic nutrient sources in microalgal cultivation. After cultivation, the biomass of microalgae is separated by filtration (harvesting). The biomass can be applied as a source of food, energy, and supplements.

7. Conclusion
Tofu liquid waste can be treated by anaerobic digestion and produce biogas as top product and effluent as bottom product. Some factor affecting biogas production are important to be considered i.e. pH, COD to N ratio, TS content, biological agent, initial concentration and two-stage anaerobic digestion. The biogas formed still contains high CO₂ and H₂S. Hence purification methods are applied to decrease these gas components. The H₂S is removed by biofiltration. The CO₂ can be removed by biological absorption with help of microalgae. In other side, the effluent can be used as organic nutrient sources for microalgae. The zero waste system is successfully proposed in this paper.

8. References
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