



Treatment and Utilization of Industrial Tofu Waste in Indonesia

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Received: 25 May 2015;

Accepted: 11 July 2015;

Published online: 5 December 2015;

AJC-17639

Tofu is a popular food for the people because it contains good and healthy nutrition. Furthermore, it is low-cost for many people. The tofu processing industry, which usually is done by the small industries, can be found in almost every city in Indonesia. As a result of the large number of the small tofu industries, the waste gives a lot of impacts towards the environment. The great amount of water used for the tofu processing results in the great amount of the wastewater. The waste from the tofu processing usually has a high amount of organic substances; the BOD level is around 6000-8000 mg/L, the COD is around 7.500-14.000 mg/L. If all wastes are disposed without any treatments at all, this will surely result in polluting the surrounding environment. This article aims to discuss about the treatment process of the tofu waste in Indonesia and some other alternative treatment systems from previous researches. In general, small tofu industries do nothing about their wastes. It is because the price that they need to pay for the waste treatment is considerably high. On the other hand, some bigger tofu industries are usually treating the waste of their tofu processing with the anaerobic system with the treatment efficiency up to 50-70 %. Even though the waste has been treated, the organic substances in the waste (BOD and COD) released to the water still has a considerably high level of substances, above 500 mg/L. Therefore, further treatments are needed so that the organic content in the waste effluent meets the wastewater quality standard. Several researches shows that the liquid waste from the tofu industries can be treated by using the anaerobic fix domedigester, fixed bed anaerobic, anaerobic baffle reactor, thermophilic anaerobic stirred tank reactor, up flow anaerobic sludge blanket, up flow anaerobic filter process, anaerobic fluidized bed reactor and several others. All of those anaerobic processes produce methane gas which can be used as a source of energy. Some researchers have also successfully utilized the liquid waste of the tofu industries as the source of hydrogen gas, as well as the media lactic acid production. On the other hand, the solid waste (okara) can be used as snacks, fertilizers and feed for livestock. Okara can also be used as the basic ingredients of isoflavones.

Keywords: Tofu waste, Okara, Anaerobic digestion, Methane, Hydrogen.

INTRODUCTION

Tofu is one of the most commonly consumed side dishes in Indonesia. Tofu contains a lot of protein and vitamins needed by the body. According to the data from the Ministry of Agriculture in 2013, the tofu consumption in Indonesia is considerably great. Table-1 shows per capita consumption of some foodstuffs in Indonesia. Indonesian tofu consumption per capita reached 7,039 kg/person with a growth rate of 0.09 %. This number is considerably higher than per capita consumption of beef and chicken. Tofu can also serve as snacks and for some people tofu is also considered as the side dishes are eaten with the staple food.

Indonesia has the average of 84,000 tofu factories ranging from the household scale with the number of workers from five to eight people to the large scale with more than 100 workers. The tofu industries in Indonesia can spend about 2.56 million tons of soybeans every year for the tofu making

TABLE-1
PER CAPITA CONSUMPTION OF SOME FOODSTUFFS IN
INDONESIA (2012-2013) (SOURCE: DEPARTMENT OF
AGRICULTURE OF INDONESIA, 2013)

No	Type of food	2012 (%)	2013 (%)	Growth average (%)
1	Tofu	6.987	7.039	0.09
2	Ras chicken	3.494	3.650	4.60
3	Kampong chicken	0.521	0.469	-1.67
4	Chicken eggs	6.518	6.153	1.61
5	Duck eggs	2.190	1.825	-9.78
6	Tempe	7.091	7.091	0.23
7	Beef	0.365	0.261	-2.53

processes. Of this amount, each tofu industry produces an average of 20 million m³/year of liquid waste and about 1,024 million tons of solid waste [1]. The amount of the waste results in the average emission of 1 million tons of CO₂ equivalent.

One of environmental problems caused by tofu industries is the waste generated from the tofu production process. The

waste generated by tofu industries can be divided into 2 types, *i.e.* the solid waste and the liquid waste. The tofu industries generally produce solid waste for about 40 % of the total capacity of 100 kg soybean production. For every 100 kg soybean used for the production, it takes about 1.5-2 m³ of water [2].

The solid waste of tofu (okara) contains about 20.93 % protein, fiber 21.43 %, 10.31 % crude fat, 0.72 % calcium, 0.55 % phosphor and 36.69 % of other compounds [3]. The okara have a very little impact on the environment because most of the okara are resold by the tofu makers as fodder for goats or cows since the protein contents of the okara are still quite high.

The liquid waste was generated from the washing, pressing and molding process. Therefore, the liquid waste generated from the industries is quite high. The liquid waste from the tofu making contains very high organic substances, temperatures reached 40-46 °C, the level of biochemical oxygen demand (BOD₅) of 6000-8000 mg/L, the chemical oxygen demand (COD) of 7500-14000 mg/L and the low acidity at pH of 5-6 [4,5]. The liquid wastes from the tofu industries are generally discharged into the river and will surely disrupt the biotic lives of the river, decline the water quality caused by the high content of organic substances and can cause a bad odour and pollution of the surface and ground water [5-8].

The tofu waste which seeps into the ground and mixed with ground water can cause many health problems for the people living around the place where the tofu industry is located. Some of the health problems are the skin diseases, stomach ache and several others. The tofu waste dumped into the river without any treatment might cause the death of river organism, causing damage to river ecosystems and harm the human who use the river for fishing, washing, *etc.* [9]. Liquid Waste Quality Standard for the industry (Decree of the Minister of the Environment of Indonesia No. 51, 1995) can be seen in Table-2.

Parameter	Concentration (mg/L)	Load capacity (kg/ton)
COD	300	6
BOD	150	3
TSS	200	4
pH		6-9
The highest quantity of waste water (m ³ /ton)		20

Tofu's waste treatments: The tofu's waste treatments are divided into 2 types, namely the solid waste treatment and liquid waste treatment. The treatment is done with the aim to reduce the direct or indirect impact of waste of tofu's industries.

Solid waste treatment: The solid wastes from tofu industries contain highly concentrated organic substances, so that there are some kinds of utilizations done to reduce the solid waste generated from tofu production activities.

Live stock feed: The solid wastes from the tofu industries (okara) contain a lot of organic substances and if it will cause a bad odour if being left for too long. To get the okara stored longer, the okara can initially be dried using the sunlight.

Another way to preserve the okara is by making the silage. First, the water containment of okara can be deducted up to 75 % and then is stored in a sealed plastic in order to make it air-tight. After that, it can be stored for 21 days and be used based on requirement. Okara is rich in dietary fibre, mainly as insoluble fibre, beside protein and fat [10].

Fertilizer: The content of okara similar to compost makes the okara have the potential to be used as fertilizer which can be used to fertilize crops. Table-3 shows a comparison of chemical content in green valley compost and okara. Research conducted by Asmoro and Sutoyo [11] concerning the utilization of the tofu waste to increase crop yields shows that the okara can be used as a fertilizer and is done by mixing natural soil and okara with a ratio of 5:1.

Parameter	Okara	Solid compost
N (%)	1.24	1.44
P ₂ O ₅ (ppm)	5.54	2.37
K ₂ O (%)	1.34	3.03
Protein (%)	7.72	–
Fat(%)	–	–
Carbohydrate(%)	–	–

(Source: [Ref. 11])

Various food products: The okara can be used as the various food products such as flour, soy sauce, crackers and several others. The high protein content in the okara allows the okara to be reprocessed into several other various food products.

Liquid waste treatment: Liquid waste treatment which contains high organic substances is usually done with the help of microorganisms. The waste treatment using bacteria can be done in 3 ways, which are aerobic, anaerobic and the combination of both. Liquid waste treatment usually uses anaerobic method [12]. There are several types of anaerobic treatments commonly used in Indonesia, among others:

Fix dome anaerobic digester: Reactors of this type (Fig. 1) are included into the kind of low-rate anaerobic sludge digester. The reactor has no stirring to mix the influent. In this reactor, the decomposition occurs due to contact between the influent with sediment containing the bacteria. The biogas as the result of the decomposition releases and brings most sediment particles containing the anaerobic bacteria. In the reactor, there is not more than 50 % of the reactor volume that is used in the process of decomposition. A large volume reactor is needed in order to produce maximum result [12].

Fixed bed anaerobic: Reactors of this type (Fig. 2) using a bed that becomes the growing spots of the bacteria. The bacteria moored on the bed decompose the liquid waste that is passed slowly. The average residence time that bacteria need in order to optimally process is above 20 days [12].

Anaerobic baffled reactor: Reactors of this type using the chambers that causes liquid waste to flow and contact with the sludge containing the bacteria, which is under the baffle and the upper surface of the reactor causing the liquid waste to experience the flow upward and downward (down flow and up flow) as in Fig. 3. The reactor of this type also has a large size to produce optimum results [13,14].

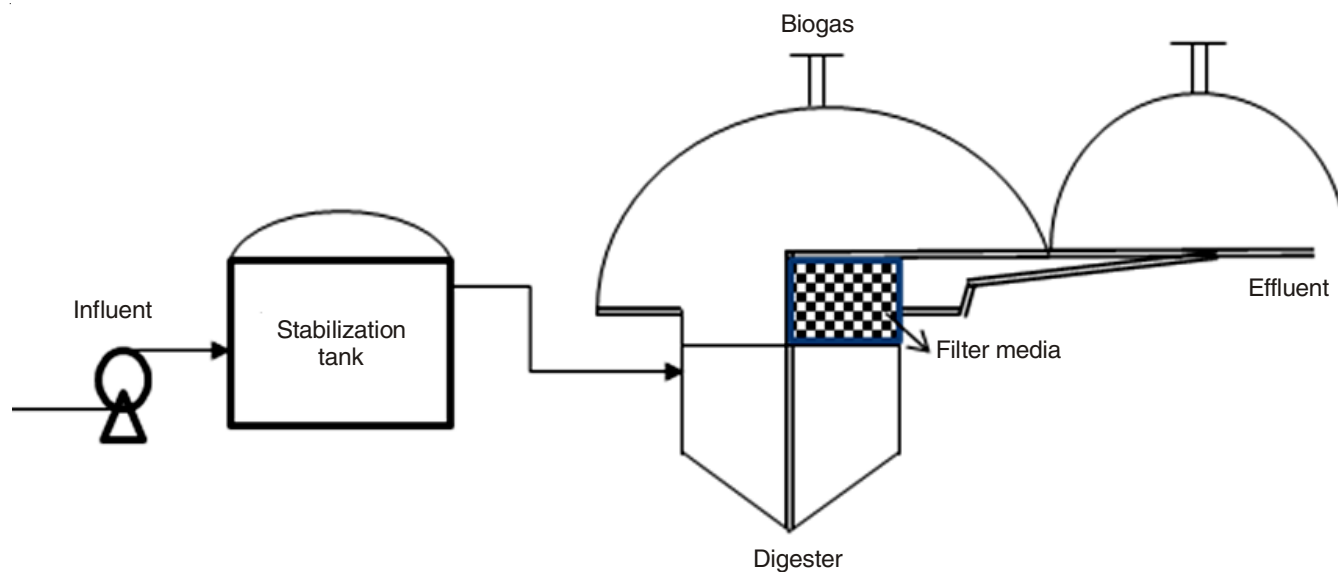


Fig. 1. Schematic diagram for fixed dome reactor [Ref. 12]

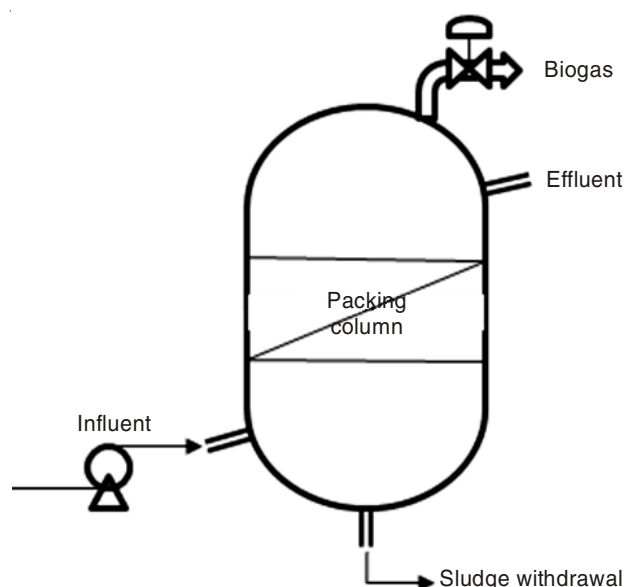


Fig. 2. Schematic diagram for fixed bed anaerobic reactor [Ref. 12]

Thermophilic anaerobic stirred tank reactor: Reactors of this type are included in anaerobic stirred tank reactors. Thermophilic bacteria are grown inside the reactor and will decompose organic substances in the liquid waste. Reactor of this type covers the lack of other reactor types that require large volumes. It is because inside this reactor, the mixing of the waste with decomposed bacteria occurs perfectly that the residence time required is far less. The schematic diagrams for thermophilic anaerobic stirred tank reactor can be seen in Fig. 4. This method has not yet been applied in Indonesia.

Another method of tofu waste treatment that can be used to treat the tofu waste is the up flow anaerobic sludge blanket, up flow anaerobic filter process, anaerobic fluidized bed reactor and with a combination of biological processes of anaerobic-aerobic.

Tofu's industrial waste treatments in Indonesia: The number of tofu industries in Indonesia that have been using the waste treatment plant (WTP) is still very small, but the public awareness of the importance to protect the environment

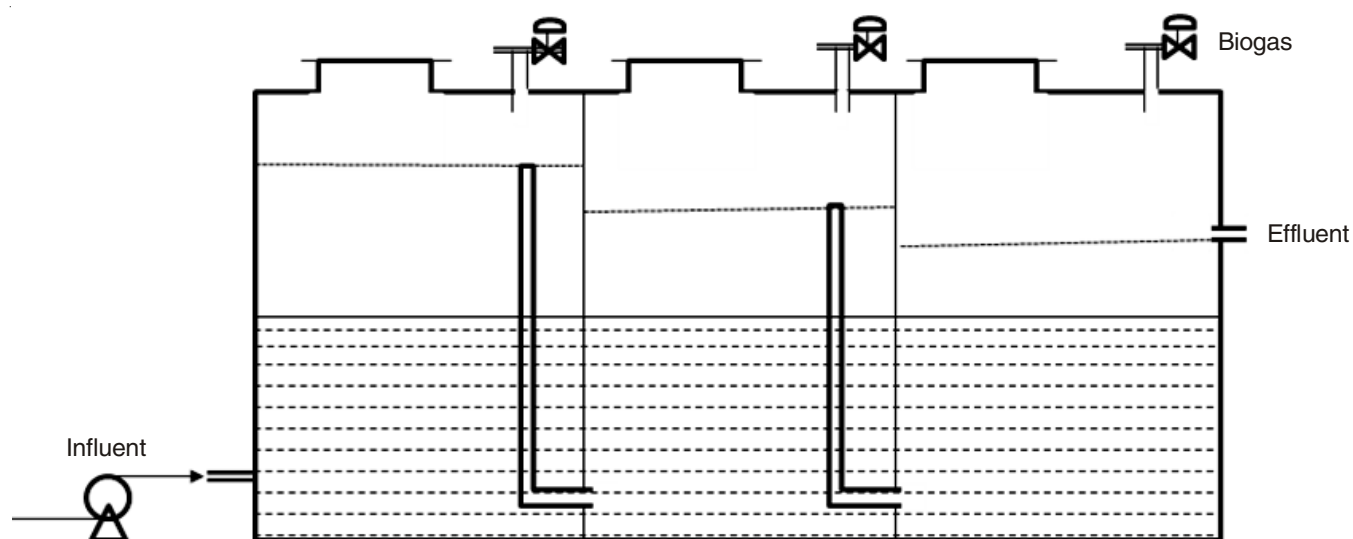


Fig. 3. Schematic diagram for anaerobic baffled reactor [Ref. 12]

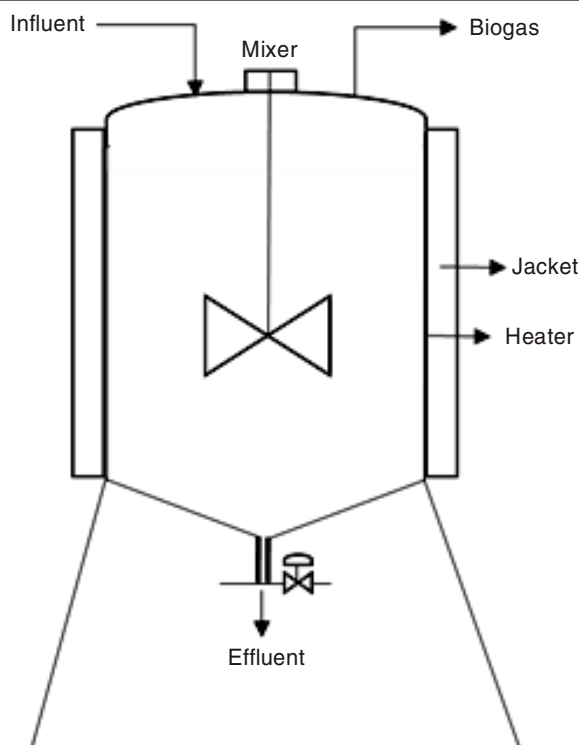


Fig. 4. Schematic diagram for thermophilic anaerobic stirred tank reactor

causing the number of the tofu industries using the waste treatment plant is increasing. The tofu waste treatment, especially the liquid waste is closely connected with economic problems. The treatments usually require a large amount of cost. Therefore, many tofu industries in Indonesia do not treat the waste, causing the water pollution. The condition is also due to the great number of the tofu makers that do not yet understand the cleanliness of the environment. As an addition to that, the low economy level becomes another factor, so that the waste treatment would be a considerable burden to them. On the other hand, the solid waste of okara on small industries sold directly to the people to be used as livestock feed. However, this okara waste can also be renewed into the nutritious food or the basic material to make the isoflavones if the okara is properly treated [15].

Treatments of solid waste: The okara still contains 27 g of protein and 41.3 g carbohydrates that it is possible to be renewed into soy sauce, taoco (soybean paste), flour that can be used in the process of making the various foods (pastries, cake, side dishes, crackers, *etc.*). In making cakes and snacks, the use of the tofu flour can be the substitute material of the grain flour. The use of tofu flour as the wheat substitute material has the advantage such as to produce a product that still has nutritional and economic value as well as the cleaner environment [16]. The okara are mostly used as the basic ingredients of Tempe Gembus (a side dish made of soy bean) by many people. This is done because the process of making Tempe Gembus is easy and the cost is quite cheap. Besides Tempe Gembus, the okara are also processed to be used as livestock feed [16].

Treatment of liquid waste: Currently, the industrial tofu wastewater management is usually done by making the wastewater tank in order to make the anaerobic process occurs. The substances of the organic pollutants in the wastewater can be reduced through the biological anaerobic process. However, the efficiency treatment of this process is only ranging from 50 to 70 %. Thus, if the BOD concentration in the wastewater is 6000 ppm, then the BOD level that comes out is still considerably high, about 1800 ppm. Therefore, this treatment is still considered as the source of the environmental pollutant.

This paper presents some examples of tofu factories in Indonesia using the waste treatment plant. Some of them are the Gagak Sipat Boyolali Tofu industry and Sederhana Kendal Tofu industry, located in Java island. Their great efficiencies in reducing the environmental pollution will be discussed and served as the model for the tofu industries that use the waste treatment plant in their industry.

Waste treatments in Boyolali Tofu industry: This industry is using the waste treatment plant with the anaerobic processes of the fixed dome anaerobic reactor. Fig. 5 shows the schematic of waste treatments in Boyolali tofu industry. Before being poured into the reactor, the wastewater is pumped into the tank with the size of 0,768 m³ with a residence time of 3.6 h. After that, the wastewater flowed into the reactor (volume of 30 m³) with a residence time of 6 days. Before being discharged

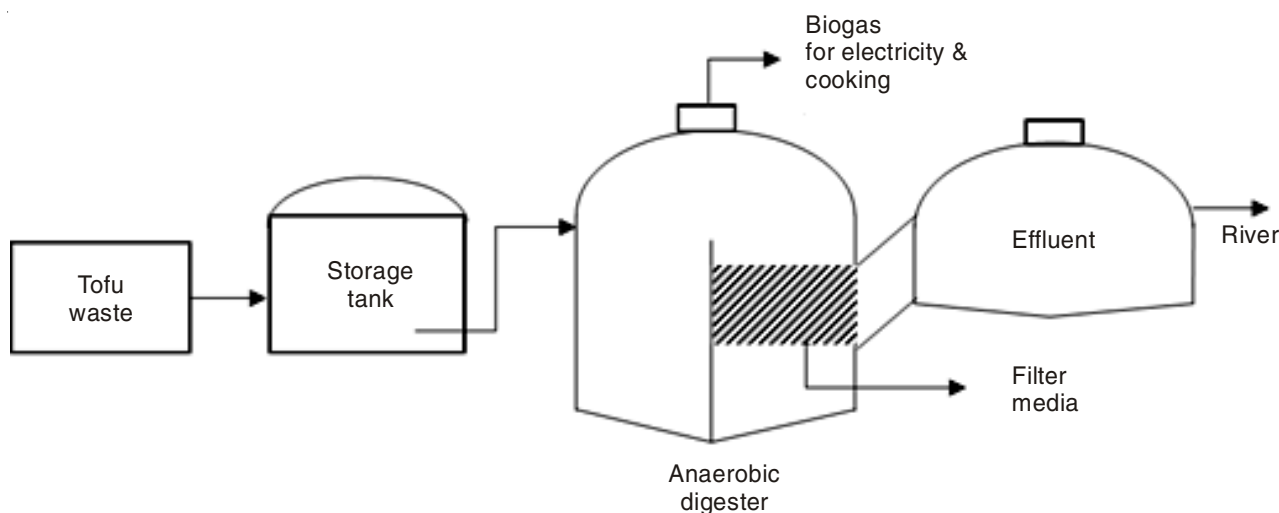


Fig. 5. Schematic diagram for waste treatment process at Boyolali tofu industry

into the rivers, the wastewater out of the reactor was firstly flowed into a tub-sized of 2.4 m³ with a residence time for 11.5 h. Waste treatment plant owned by this tofu industry operates at a flow waste rate of 5 m³ [16]. The performance of these reactors can be seen in Table-4. From Table-4 it can be seen that the COD and BOD in the treatments of Boyolali Tofu industry have not meet the standardization of the wastewater quality standard, on the other hand the pH standard has been met. Therefore, further treatments are needed for this industry.

TABLE-4
PERFORMANCE OF WASTEWATER TREATMENT
FACILITY OF BOYOLALITOFU INDUSTRY

Parameter	Influent	Effluent	Efficiency
BOD	3283 mg/L	337.9 mg/L	0.89 %
COD	6486 mg/L	759.8 mg/L	0.88 %
TSS	575 mg/L	116 mg/L	0.79 %
pH	4.53	7.32	—

(Source: [Ref.16])

Waste treatments in Sederhana Kendal tofu industry:

This tofu factory is using waste treatment plant with two types of treatments, which are the anaerobic and aerobic. The reactor used in this waste treatment plant is type of anaerobic baffled reactor (ABR). Before the waste being poured into the reactor, it is firstly accommodated into the tank (with the volume of 63 m³). Upon pouring the reactor with the residence time of 1.5 days, the wastewater is put into 300 m³ sized reactors with the residence time of 7.5 days. The liquid waste coming out of the reactor is streamed into the aeration pond (with the volume of 6 m³) with a residence time for 3.6 h. The waste treatment plant used by this tofu industry is able to accommodate the wastewater with a flow rate of 35 m³. The performance of this waste treatment plant can be seen in Table-5. The results of waste treatments done in Sederhana Kendal Tofu industry have already met the permitted quality standards [16].

TABLE-5
PERFORMANCE OF WASTEWATER TREATMENT
FACILITY OF SEDERHANA KENDAL TOFU INDUSTRY

Parameter	Influent	Effluent	Efficiency
BOD	2726 mg/L	57.60 mg/L	97.80 %
COD	4972 mg/L	203.2 mg/L	95.90 %
TSS	388 mg/L	62 mg/L	84.02 %
pH	5.51	7.06	—

(Source: [Ref. 16])

Another tofu industry that also has a wastewater treatment unit is the small tofu industry in Semanan, West Jakarta, which uses anaerobic lagoon system (Fig. 6). Lagoon systems used in the industry is able to lower the levels of organic substances down (BOD) of about 50 %. In Banyumas, there are also two waste treatment plants with a capacity of 21 m³ (equivalent to 1200 kg of soybeans per day for 20 owners) and 5 m³ (300 kg soybeans/day for 5 employers) [17]. The number and capacity of the unit is certainly not comparable to the number of tofu industry owners in the district. In the city of Banda Aceh, Aceh province, the tofu industry owners do not treat the waste because of the large cost for the treatments [5]. Some tofu industries



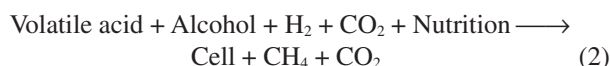
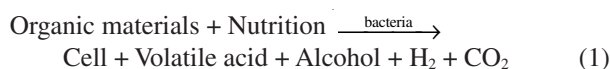
Fig. 6. Wastewater treatment of small tofu industry at Semanan, Jakarta Barat

were supported by National Group Organization (NGO) to treat the waste, but due to the lack of technical knowledge of the treatments, the system only lasts for 3 to 6 months, then the system is never be used again due to the damage caused (blockages, odour, no gas and so on).

Integrated concept in tofu waste treatments: With a high containment of the organic substances inside the tofu waste, the waste can be processed into a more useful material after going through several stages of processing. The integrated processing will be able to take advantage of all the potential that exists in the wastewater into useful materials or compounds. Fig. 7 describes one integrated concept in the process of tofu waste treatment. The tofu's waste consists of solid and liquid wastes. Every 1000 kg of basic ingredients (soybean) requires approximately 2200 kg of water. That amount of material will produce the tofu (product) as much as 1080 kg, the solid waste (okara) 1400 kg and about 1800 kg of liquid waste. By using the integrated concept, all of the waste will be utilized. The tofu's solid waste (okara) can be used as livestock feed. With further processing okara can be used as snacks. Okara can also be easily processed into organic fertilizer. Besides, through several stages of the process, the component inside okara can produce more useful isoflavones compounds.

The tofu waste will go through the process of anaerobic digestion treatment in the retention time for 8-24 h. The effluent product results can be used as liquid fertilizer or solid fertilizer that can be used directly. The main objective of using the anaerobic processes is to produce methane gas or biohydrogen. The mixing between the results of the final tofu's waste effluent and okara with a certain ratio can also produce organic fertilizer, which is quite good. The effluent as the result of this anaerobic reactor contains microbes that could accelerate the process of decomposition in composting.

The process of formation of methane (CH₄) can occur through the following reaction stages:



Propionic acid, acetic acid and other components can be used for the formation of methane:

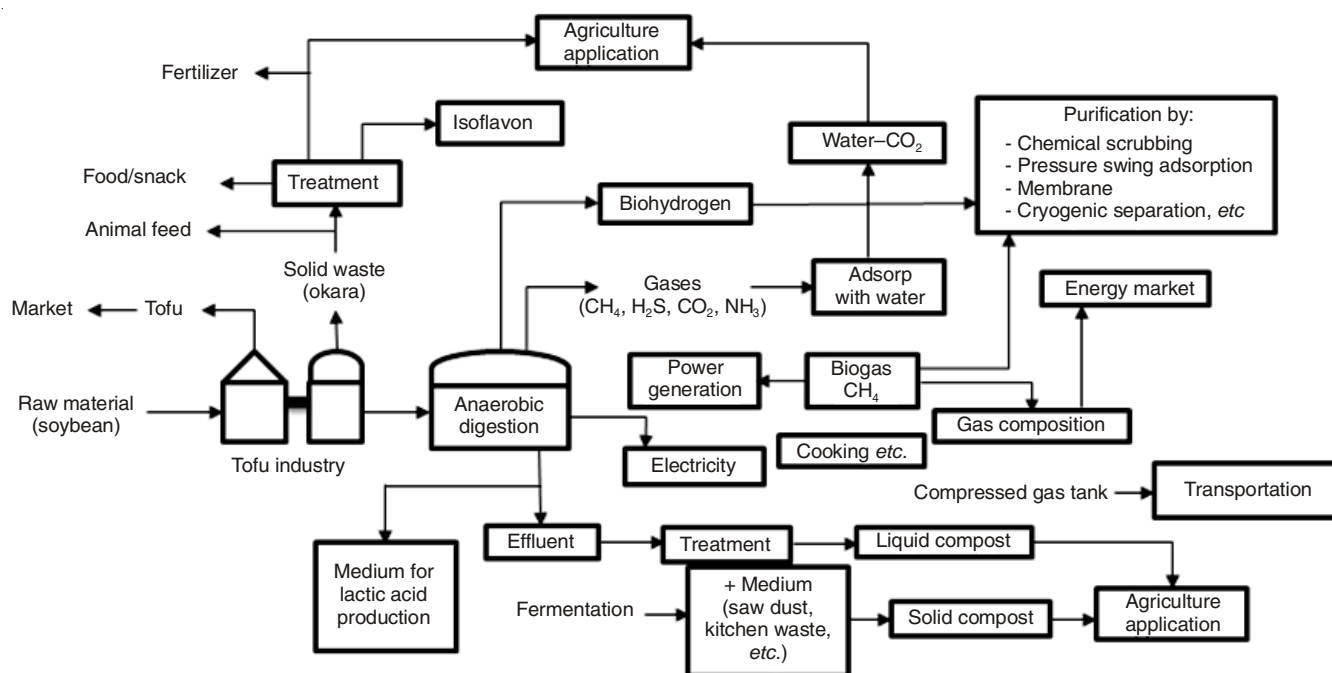
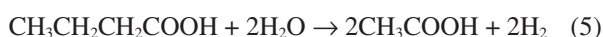


Fig. 7. Integrated concept for waste treatment at tofu industry



Lay *et al.* [18] have studied the formation of bioenergy from tofu's waste with anaerobic processes and a high amount of biogas can be produced. Theoretically, 1 Kg COD will generate 0.35 m³ CH₄, which can be used for the various applications. The methane gas production will increase with the increase of substrate loading rate until it reaches the maximum and then decreases if the loading rate is increased.

The purification process is needed because the gas that comes out of the processing system still contains CO₂, H₂S and NH₃. There are several methods that can be used to separate CO₂ from the fermentation reactor, such as the high pressure water scrubbing (HPWS), chemical scrubbing (AS) which uses an amine based solvent, organic physical scrubbing (OPS) which uses a commercial blend of polyethylene glycol, pressure swing adsorption (PSA), selective membrane (MS) separation and Cryogenic separation [19,20]. Although the separation technology can be used, but generally the industry is still using common methods which they do not store the CO₂ as the result of the process but by releasing it back to the atmosphere or using it for the industrial purposes, if it is sufficient in terms of both quality and quantity [21,22]. Basically, although the gas that comes out of the processing system still contains a lot of impurities, the gas can be directly used for cooking. However, the purification process is done, if the purer biogas is wanted, without any impurities, therefore further processing must be done. The pure biogas can be used as the main energy source of electricity, the use on the field transportations and so forth.

Furthermore, biohydrogen (H₂) can also be produced by using the tofu waste as raw material through photosynthesis or fermentation process. Fermentation is more preferable as it is technically simpler than that of the photosynthesis [23-25]. Fermentation produces hydrogen from the carbohydrate materials contained in the wastethrough the following reaction:



Bacteria such as *Clostridium perfringens*, cellulolytic bacteria M18, FS3, *Clostridium sartagoforme* FZ11 and *Clostridium acetobutylicum* have been use by many researchers to produce hydrogen from carbohydrate containing organic wastes [25-32]. The developed methods seem to be promising tool for hydrogen production.

Conclusion

Tofu industry produces the waste that contains a lot of organic substances. If the waste is discharged into the environment without any treatment process, it will cause the environmental pollution. In Indonesia, tofu industries (the small-scale industry) mostly do not treat their waste due to the problem of the high cost treatment process. For the cases of the big tofu industries, the treatment is done with the anaerobic method. This method can produce the methane gas and hydrogen as its main product. Although the initial treatment is already done, the level organic substances content in the tofu waste is still high and it cannot be discharged freely to the environment or the water. Further processing is needed so that the waste disposed has the COD and BOD level that will fit the standard allowed by the Liquid Waste Quality Standard. The integrated tofu waste management will be able to exploit all the potential that exists in the tofu waste into more useful materials. This integrated treatment concept requires expensive investment, so that the support of sponsors or government is totally needed. Through this concept, there will be biogas (CH₄

and H₂), snacks, fertilizers, fodder and other useful compounds that are produced.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support by Directorate General Higher Education of Indonesia for Research Grant of International Research Collaboration and International Publication (Grant No. 035/SP2H/PL/Dit.Litabmas/II/2015).

REFERENCES

1. N. Sintawardani, Socio-Economic Problems on Reducing the Waste-water Pollution from Tofu Processing in the Cibuntu Area, Indonesia, Research Center for Physics Indonesian Institute of Science (2011).
2. P. Nurhassan and N. Pramudyanto, Utilization of Tofu Wastewater, Yayasan Bina Karya Lestari (1991).
3. N. Marlina and S. Askar, Chemical Composition of some Agricultural Wastes, Animal Research Center, Bogor (2004).
4. A. Herlambang, Waste Treatment Technology for Tofu Industrial Waste, BPPT, Indonesia (2002).
5. M. Faisal, I. Machdar, F. Mulana and H. Daimon, *Asian J. Chem.*, **26**, 6601 (2014).
6. F. Belén, J. Sánchez, E. Hernández, J.M. Auleda and M. Raventós, *J. Food Eng.*, **110**, 364 (2012).
7. A.S. Pablo and R.W. Jorge, *Información Tecnológica*, **20**, 65 (2009).
8. Y. Sudiyani, A. Syarifah, A. Yulia and A. Indri Badria, International Conference on Chemical Sciences (ICCS-2007), ANL Rep., p. 47 (2007).
9. A. Darmayanti, J. Hermana and A. Masduqi, *Purifikasi*, **5**, 151 (2004).
10. I. Mateos-Aparicio, C. Mateos-Peinado and P. Ruperes, *Innov. Food Sci. Emerg. Technol.*, **11**, 445 (2010).
11. Y.A. Suranto and D. Sutoyo, *J. Bioteknol.*, **5**, 51 (2008).
12. C.A.L. Chemicaro, Biological Wastewater Treatment Series: Anaerobic Reactors, Federal University of Minas Gerais, Brazil (2007).
13. M. Pirsaeheb, M. Rostamifar, A.M. Mansouri, A.A.L. Zinatizadeh and K. Sharafi, *J. Taiwan Inst. Chem. Eng.*, **47**, 137 (2014).
14. A. Ahamed, C.-L. Chen, R. Rajagopal, D. Wu, Y. Mao, I.J.R. Ho, J.W. Lim and J.-Y. Wang, *Bioresour. Technol.*, **182**, 239 (2015).
15. L. Jankowiak, O. Trifunovic, R.M. Boom and A.J. van der Goot, *J. Food Eng.*, **124**, 166 (2014).
16. K. Fibria, Ph.D. Thesis, Technical Study of Utilization of Solid and Liquid Wastes of Tofu Industries: Study Cases at Tofu Industry of Tandang Semarang, Sederhana Kendal and Gagak Sipat Boyolali, Diponegoro University, Indonesia (2007).
17. S.N. Idaman, I. Haryanto, R. Nugro and A. Herlambang, Treatment of Tofu Waste by Using Biofilter Anaerob and Aerob, BPPT, Indonesia (2010).
18. C.H. Lay, B. Sen, S.C. Huang, C.C. Chen and C.Y. Lin, *Renew. Energy*, **58**, 60 (2013).
19. A. Petersson and A. Wellinger, *IEA Bioenergy*, 12 (2009).
20. R. Lems and E. Dirkse, Making Pressurized Water Scrubbing the Ultimate Biogas Upgrading Technology with the DMT TS-PWS System, DMT Environmental Technology, Joure, Netherlands (2009).
21. R. Baciocchi, A. Corti, G. Costa, L. Lombardi and D. Zingaretti, *Energy Procedia*, **4**, 4985 (2011).
22. K. Starr, X. Gabarrell, G. Villalba, L. Talens Peiro and L. Lombardi, *Biomass Bioenergy*, **62**, 8 (2014).
23. S. Sung, D.A. Bazylinski and L. Raskin, US DOE Hydrogen, Fuel Cells & Infrastructure Technologies Program-2003 Annual Merit Review Meeting, May (2003).
24. S.K. Han and H.S. Shin, *Int. J. Hydrogen Energy*, **29**, 569 (2004).
25. I.K. Kapdan and F. Kargi, *Enzyme Microb. Technol.*, **38**, 569 (2006).
26. K.W. Jung, D.-H. Kim and H.-S. Shin, *Bioresour. Technol.*, **102**, 2745 (2011).
27. Y.T. Fan, G.S. Zhang, X.Y. Guo, Y. Xing and M.H. Fan, *Biomass Bioenergy*, **30**, 493 (2006).
28. J.J. Lay, *Biotechnol. Bioeng.*, **68**, 269 (2000).
29. O. Mizuno and T. Ohara, *Water Sci. Technol.*, **42**, 345 (2000).
30. D. An, Q. Li, X.Q. Wang, H. Yang and L. Guo, *Int. J. Hydrogen Energy*, **39**, 19928 (2014).
31. A. Avci, N.K. Kilic, G. Donmez and S. Donmez, *Environ. Technol.*, **35**, 278 (2014).
32. G.L. Cao, X.F. Xia, L. Zhao, Z.Y. Wang, X. Li and Q. Yang, *Int. J. Hydrogen Energy*, **38**, 15653 (2013).