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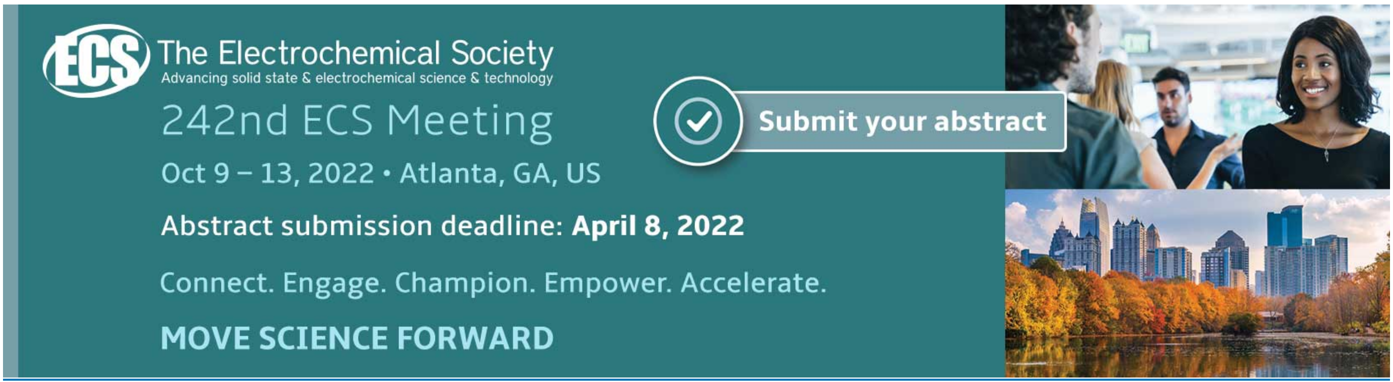
The role of C/N ratio on anaerobic decomposition of industrial tempeh wastewater for optimizing methane production

To cite this article: Y Fransiscus and T L Simangunsong 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **963** 012036



The role of C/N ratio on anaerobic decomposition of industrial tempeh wastewater for optimizing methane production

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Abstract. Considering its high organic contents, A biological process, in particular anaerobically decomposition is considered to be the most suitable treatment for industrial tempeh wastewater. Among the important parameters that determine the success level of the process, C/N ratio was mentioned to play a significant role. Thus, this study was carried out to investigate the effects of different C/N ratios (10; 20; 30 and 40) on the decomposition process to produce methane gas. The experiment was conducted using 1L batch reactors with sludge from active cow manure biogas digester as inoculum. Several parameters such as pH, COD, BOD, VFAs and methane gas were monitored during the process to understand the mechanism. The experimental results confirmed that the C/N ratio significantly affected the anaerobic decomposition of tempeh wastewater, giving the best achievement for C/N = 30, with organic removal as high as 89% (COD) and total methane produced as much as 1240 mL in 14 days of observation. The balanced composition between carbon and nitrogen is important for the anaerobic process, higher organic loading (C/N = 40) will potentially lead to VFAs accumulation, meanwhile higher nitrogen concentration (C/N = 10) will stimulate complex transition, mainly denitrification and free ammonia formation. Both conditions will block the methanogenesis stage, resulting in a nonoptimal decomposition process and minimum methane production.

1. Introduction

A comprehensive approach involving economic perspective is needed to tackle environmental problems. This proposed scheme would encourage industrial sectors since environmental preservation efforts will not only be considered as cost center, but also as a prospective profit post. Tempeh making process creates severe environmental impacts both from solid and liquid waste. Tempeh is a well-known dish in Indonesia, made from soybean through fermentation with *oligosporus* as the binding agent, and involving a significant amount of water during the making process. About 10 m^3 water per ton soybean is used in tempeh making process, mainly in the activity of washing, boiling and soaking, resulting in a wastewater with high amount of organic content. Several reports mentioned that the organic content in tempeh wastewater was in the range of 14,000 – 24,000 mg/L and 6000 - 12,000 mg/L expressed in COD and BOD respectively, with a very acid pH condition as low as 4, far from the National Standard of effluent wastewater [1,2]. Direct discharge of the untreated wastewater to the water body will pose negative impacts and raise complex environmental problems such as dissolved oxygen depletion, water ecosystem destruction, nuisance odors and clean water shortage. Thus, a decent treatment method is mandatory to fulfill the National Standard level and, most importantly, to preserve the environment.

However, considering that most tempeh producers in Indonesia are medium – small enterprise level, a high cost wastewater treatment system would not seem suitable.

Anaerobic decomposition is a solid treatment to tackle a high organic content waste and wastewater and transform it into methane gas [3]. This method is considered as a low-cost treatment since there is no excessive energy needed for the operation, and a great potential of energy cost reduction exist because the produced methane can be used as an alternative energy source. Adding to that, application of an anaerobic system in tropical areas such as Indonesia is favorable as the system is more suitable for operational temperature above 20 – 25°C [4]. Based on those advantages, anaerobic decomposition is technically and economically the best option to be applied for industrial tempeh wastewater.

However, the setting of an ideal anaerobic decomposition process is tricky since there are several factors that play a significant role. One of the crucial factors in determining the success of anaerobic decomposition to produce methane gas is C/N ratio. The presence of carbon source is essential since this is the main substrate for anaerobic microbial, while nitrogen is needed as a key element to support the growth of microorganisms because of its role in protein synthesis [5]. There were many studies to describe the role of C/N ratio in anaerobic decomposition and the suggested ratio was largely various. This condition is highly possible since the characteristic of substrate and inoculum may differ from one to another. Based on understanding the importance of C/N ratio in determining methane gas production and limited available information of the application in tempeh wastewater, this study was conducted.

2. Materials and method

2.1. Materials

Tempeh wastewater was collected from a medium scale of tempeh industry in Surabaya, East Java, Indonesia. This industry treated 1 – 2 ton soybean per day with daily wastewater generation as much as 6 – 12 m^3. The collected wastewater was then analyzed to get characteristic informations as provided in Table 1. Filtration with Whatman filter paper was conducted to reduce suspended solids and other physical impurities prior to the decomposition process. Inoculum to boost the anaerobic decomposition was obtained from an active biogas digester of cow manure. This inoculum was acclimatized progressively to achieve better affinity with tempeh wastewater. The final total solids (TS) and volatile solids (VS) concentration of acclimated inoculum were 33.37 g/L and 27.71 g/L respectively.

All chemicals used for analysis purposes were in analytical grade (Merck and Sigma-Aldrich) and are in original condition without pre-treatment.

2.2. Method

Anaerobic decomposition experiments in batch condition were performed by using 1 L bottle (Duran), which had four holes; three of which were used for gas collection (figure 1), pH monitoring and liquid sampling port. Effective volume in each run was 800 mL, with initial COD concentration was maintained at 8000 mg/L and $NaNO_3$ was added to obtain targeted C/N ratio (10; 20; 30 and 40 - represented actual wastewater condition). The initial pH of wastewater was adjusted to 7.8 with NaOH 2M before loading it into the reactor. Pure nitrogen gas was injected into the headspace of the bottle in order to avoid oxygen intervention. Lastly, the anaerobic reactor was placed on a magnetic stirrer for gentle mixing during the process.

During the experiment, daily sampling was taken and analyzed for several parameters (COD, VFAs, NO_3-N, NO_2-N) while the pH was continually monitored with a pH probe (SI-Analytic). Free ammonia (NH_3-N) was measured at the end of the run for each variation. The generated gas was collected inside tedlar bag (CEL Scientific) and measured for its methane content and volume on a daily basis. All runs were conducted at room temperature (29 ± 1°C) with three repetitions for each variation.

2.3. Analytical methods

All parameters in this study were measured in accordance to standard methods (APHA). A gas chromatography (Hewlett Packard 680 series) equipped FID and ECD detector was used to determine the methane content and VFAs concentration. The volume of evolved gas in tedlar bag was measured by vacuuming with 60mL and 100µL syringe [6].

Table 1. Characteristic of tempeh wastewater.

Parameter	**Unit**	**Average Concentration**
pH	-	4.4
COD	mg/L	16,000
BOD	mg/L	8800
TSS	mg/L	11,500
TA	mg/L ($CaCO_3$)	1200
TN	mg/L	380
SO_4	mg/L	90

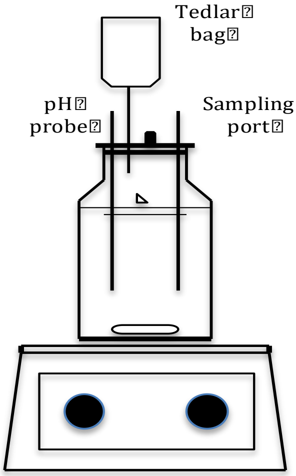


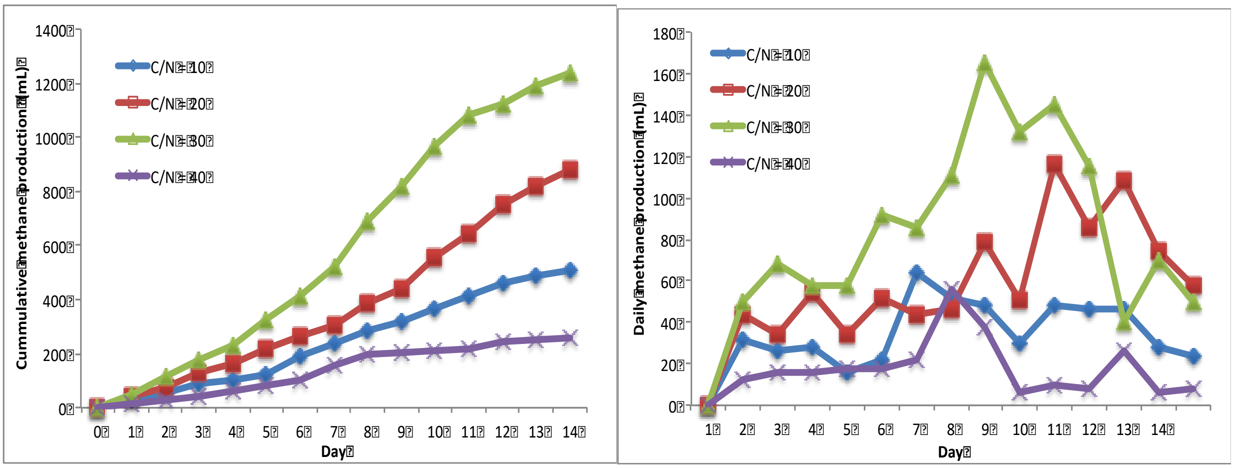
Figure 1. Batch reactor for anaerobic decomposition.

3. Result and discussion

3.1. Methane production

Anaerobic decomposition process for tempeh wastewater occurred in all C/N ratio variations, as indicated by the production of methane gas. As presented in figure 2(a), the total volume of gas produced in 14 days of experiment differed from one to other variations. Experiment with C/N = 30 produced the highest volume of methane gas as much as 1240 mL, followed by C/N = 20 and C/N = 10 as the second and third largest producers with total volume of 880 mL and 510 mL respectively. Meanwhile, run with C/N = 40 created the least amount of methane which is only 21% from the highest production or 260 mL on the same duration. In order to get a better understanding on the system's response to different C/N ratios, figure 2(b) describes the daily methane production. Daily production on C/N = 30 showed an increasing trend achieving a maximum rate of 165 mL/day at day 9 prior to the declining rate. In a lower rate, a similar trend to that was actually applied for C/N = 20. However, the daily methane production for this variation was relatively flat in the first 8 days preceding the peak at day 11, with production rate as high as 116 mL/day. As for C/N = 10 and C/N = 40, their production rates were considered even, with no significant peak in the entire measurement time. But in general, C/N = 10 had two times higher production rate than C/N = 40.

These results spotted that different compositions of carbon as substrate (food) for microorganisms and nitrogen, which was expected to be the nutritional element for the growth, drove the anaerobic mechanism to produce methane. There were several reports proposing various recommended C/N ratio for an optimum anaerobic decomposition process in solid and liquid conditions. In a solid-state fermentation, several values were reported such as 29.6 for rice straw [7]; 20 – 25 for algal sludge decomposition [8] and 15 for onion juice [9]. Meanwhile, more diverse ratios were reported in liquid condition. Chen and Lin (1993) mentioned that by using methanol as the carbon source, methanogenesis will only start whenever COD/N ratio was as low as 7.8; Her and Huang (1995) explained that by rising the C/N ratio to 25, an optimum decomposition with acetate or glucose as the carbon source will occur; while Akuna et al., 1992 reported that at COD/N-NO_3 ratio of 53, a maximum methane production was achieved by using glucose as the carbon source [10,11,12]. The diversity of optimum C/N ratio values may be attributed to several factors that play a significant role in anaerobic decomposition such as type of carbon sources (wastewater characteristics), organic loading rate, type/source of inoculum (microbial populations), the availability of inhibitors, pH, alkalinity and temperature. The finding of maximum methane production at C/N ratio of 30 with tempeh wastewater as a substrate is elaborated in the following section by presenting carbon and nitrogen transformation profile.



(a) (b)

Figure 2. (a) Cumulative methane production from different C/N ratio. (b) Daily methane production from different C/N ratio.

3.2. *Volatile Fatty Acids (VFAs)*

Conversion of organic substrate into methane gas is not a direct process. Instead, it involves four sequence major stages, which are hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the beginning, suspended carbohydrates, proteins and fats will be converted into amino acids, sugars and fatty acids. Those products from the initial stage will then transform into hydrogen, carbon dioxide, acetates and VFAs in the acidogenesis stage. Following that, VFAs (acetic, propionic and butyric acid) will shift into acetate, hydrogen gas and carbon dioxide. This third stage is required because VFAs cannot be transformed directly into methane by the methanogens. Lastly, the final stage of methanogenesis will convert the intermediate products into methane gas. This stage consists of two groups, namely acetotrophic that converts the acetate from acetogenesis stage into methane and carbon dioxide and hydrogenotrophic that changes hydrogen and carbon dioxide into methane [13]. Considering that VFAs are crucial intermediate products to produce methane gas, measurement of these materials during the experiments was conducted. Figure 3 displays the VFAs product from different C/N ratio experiments, which are consistently dominated by acetic acid. This is favorable since acetic acid was reported to be the main precursor for methane production. Around 65% - 95% methane is directly produced from this component [14]. However, the transition of VFAs concentration during the experiment period indicated a different mechanism. It was noticed that the higher C/N ratio is, the depletion of acetic acid is getting slower. At C/N ratio = 10, the acetic acid produced sharply declined, and only during 6 days the concentration decreased to below 100 mg/L. A similar pattern in different levels was observed for C/N = 20 and C/N = 30, where acetic acid in both treatments declined slower than the one in C/N = 10. As for C/N = 40, the acetic acid concentration reached peak concentration of 2596 mg/L at day 1 and then lessened and remained stable at around 1200 mg/L until the end of the run. Phenomena in C/N = 40 could be triggered by a different rate of VFAs production and consumption which led to VFAs accumulation. This hypothesis was affirmed by the pH transition during the experiment, as seen in figure 4(a), where pH for C/N = 40 dropped drastically to 4.4, reflecting the progress of acidogenesis and acetogenesis stages to produce VFAs as the intermediate compounds. Although the pH turned back, the achieved highest value until day 14 was only 5.8 – 5.9, still far from the ideal condition to foster methanogenesis stage. Mao *et al*., 2015 reported that the optimum pH range for an anaerobic single stage or batch process should be 6.8 – 7.4 [15]. Similar findings on the obstruction of methane production because of VFAs accumulation and low pH were reported by Cuff *et al*., 2018 and Zhang *et al*., 2019 [16,17]. C/N = 20 and C/N = 30 had comparable patterns of pH transition, and the recovered pH was similar in the range of 6.6 – 7.1, suggesting the methanogenesis could smoothly occur. However, interestingly the amount of methane produced in C/N = 20 was 30% less than in C/N = 30. A plausible explanation for this result could be related to the presence of nitrogen substances in the system. Nitrogen concentration in C/N = 20 was higher than one in C/N = 30, indicating that nitrogen took some portion of substrates for its evolution. The lower amount of methane produced in C/N = 10 asserted that nitrogen plays a significant role in anaerobic decomposition process.

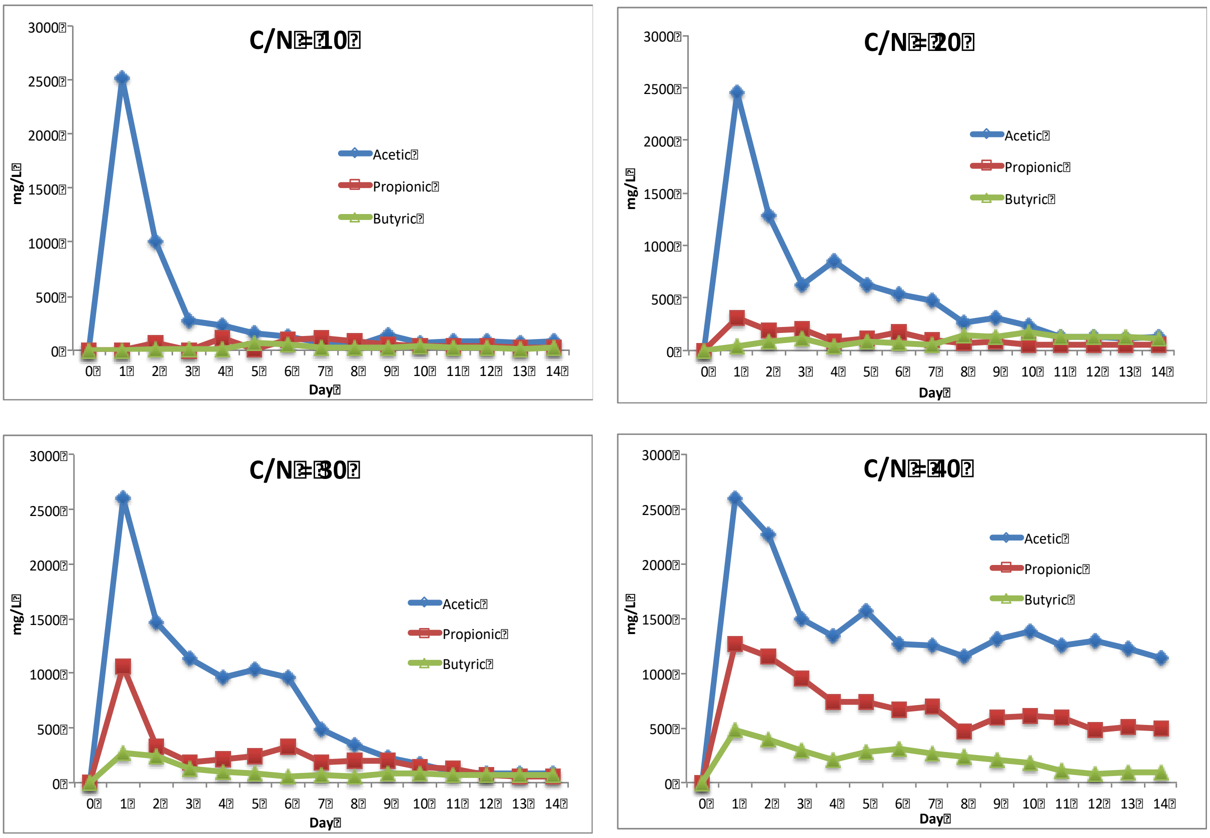
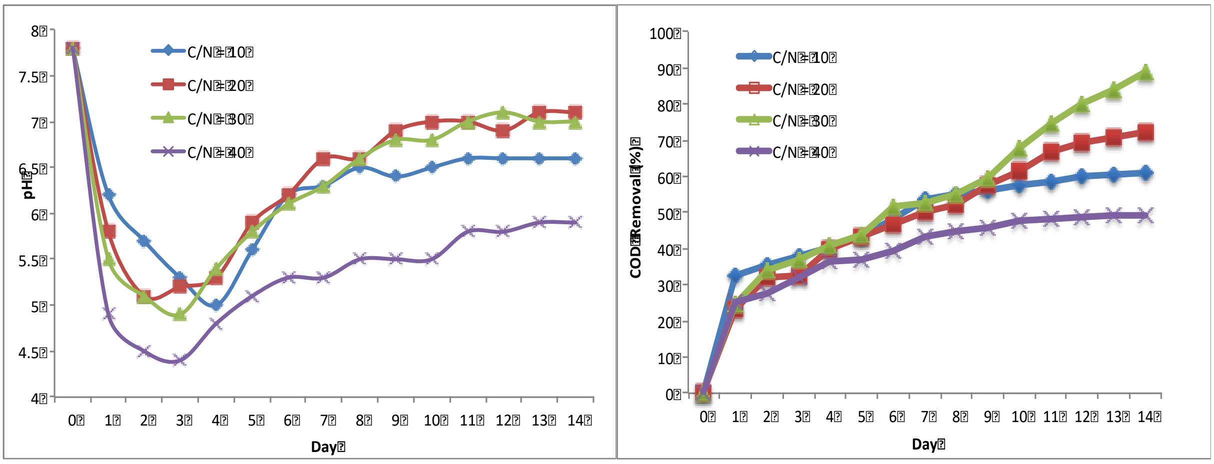


Figure 3. Volatile fatty acids (VFAs) transition in different C/N ratio.

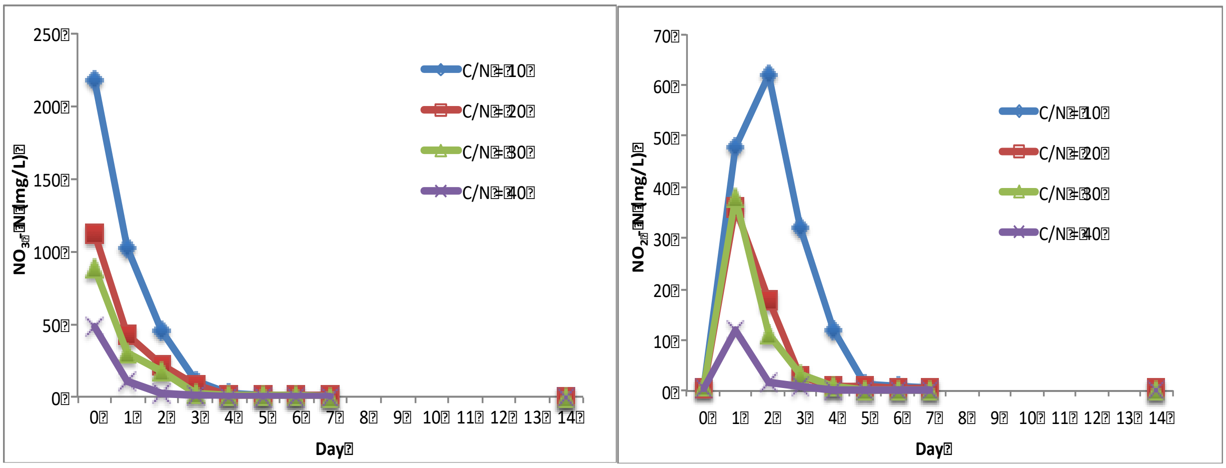


(a) (b)

Figure 4. (a) pH transition during the anaerobic decomposition in different C/N ratio. (b) Substrates (COD) removal efficiency in different C/N ratio.

3.3. *Nitrogen transformation*

Nitrogen is required in an anaerobic process to support microbial development and reproduction [18], besides that, the presence of nitrogen in the form of ammonia will neutralize VFAs through ionization in the liquid condition. The neutralization process is beneficial to prevent pH drop; thus the methanogenesis stage will perhaps take place smoothly [19]. However, since $NaNO_3$ was used to adjust nitrogen concentration in the system, the nitrogen formation during the experiment was rather complex because it may be converted to organic nitrogen, reduced to the form of ammonia or denitrified. Measurement of nitrate and nitrite concentrations indicated that denitrification process took place during the anaerobic decomposition experiments. Figures 5 (a) and (b) reveal the dynamic transition of nitrate and nitrite in the system. Nitrate concentration in all C/N variations progressively decreased down to negligible level whilst nitrite concentration increased up to a certain time prior to the depletion. Different level of nitrate concentrations in the beginning was referred to the different adjusted total nitrogen concentration in each reactor.



(a) (b)

Figure 5. (a) Nitrate transition during the anaerobic decomposition in different C/N ratios. (b) Nitrite transition during the anaerobic decomposition in different C/N ratio.

Figure 5 depicts that C/N = 10 had the highest concentration of nitrate and nitrite compared to others, indicating that the denitrification level was also higher. In anaerobic condition, heterotrophs microbial will consume organic matters to proceed reduction of nitrate to nitrite, in this case VFAs. Product from the first two stages of anaerobic process can be used directly as the carbon source. Thus, methanogenesis theoretically will start only after denitrification is complete and whenever there is still carbon available. Considering that C/N = 10 had the highest concentration of nitrogen, it can be concluded that organic reduction, as displayed in figure 4(b), were mostly due to denitrification process, leaving smaller portion for the production of methane gas. Several reports mentioned that methanogenesis stage was getting slower or completely hampered during the denitrification, and recovered after all nitrate oxides have been converted into nitrogen gas [20,21,22]. Unfortunately, nitrogen gas analysis was not conducted during this study.

Profile of nitrate – nitrite transformation for C/N = 20 and C/N = 30 described in figure 5 were similar, indicating that there were enough substrates for reduction. However, the amount of methane gas produced was different (figure 2). This phenomenon may be elucidated from the different free ammonia concentration in both reactors, which were 180 mg NH_3-N/L and 76 mg NH_3-N/L for C/N = 20 and C/N = 30 respectively at day 14. At higher pH, the presence of ammonia is more dominant than ammonium [23]. Therefore, the ammonia existence was possible in both reactors as the achieved turned back pH to the same range, 6.6 – 7.1. Since the initial concentration of nitrogen is higher in C/N = 20, more nitrogen was converted into ammonia form. This would be the case if we considered the report of Shin et al., 2002 [24], which mentioned that acidogenesis pushed nitrate reduction to ammonia at alkalinity below 2000 mg $CaCO_3$/L, while the alkaline concentration for the used tempeh wastewater in this study was 1200 mg $CaCO_3$/L. Total ammonia (ammonium and ammonia) was reported to be the main inhibitor for methanogenic microbial. Various total ammonia nitrogen (TAN) thresholds have been reported by researchers for smooth methane production, such as 3900 – 5600 mg-N/L by Liu et al., 2020; 1500 – 7000 mg-N/L by Yuan and Zhu (2016) and 1500 mg-N/L by Xia et al., 2016 [25,26, 27]. Free ammonia has been identified to be much more dangerous compared to ammonium ions because it can passively diffuse into the cell membrane and lead to imbalance and/or potassium deficiency [28,29]. In order to maintain the optimum methanogenesis stage, Yuan and Zhu (2016) and Duarte et al., 1982 advised to keep the free ammonia level as much as 80 mg/L and 53 mg/L respectively [26,30].

The unfavorable effect of limited nitrogen concentration on the methanogenesis was delineated in the experiment with C/N = 40. From the nitrate – nitrite formation and depletion as seen in figure 5 and the remaining free ammonia concentration (27 mg NH_3-N/L), it seems that the majority of nitrogen has been converted. A ssufficient amount of organic nitrogen is needed, especially in the system with high organic loading, because higher concentration of ammonia can neutralize the decrease of pH due to massive VFAs production through ionization [19]. It was clearly noted that the pH of C/N = 40 was not able to turn back to the ideal condition for methanogenesis. This could be related to the minimum remaining nitrogen concentration.

4. Conclusion

The effect of C/N ratio on anaerobic decomposition for tempeh wastewater to produce methane gas was investigated. The optimum methane production was found in C/N = 30 followed by C/N = 20 with the total amount of 1240 mL and 880 mL respectively. The limited methane production in C/N = 10 was attributed to denitrification, in which some portion of the intermediate substrates were utilized by denitrifiers for reduction. Meanwhile, the least produced methane in C/N = 40 was ascribed to VFAs accumulation resulting in a nonideal pH condition for methanogenesis. This result is valuable for the purpose of wastewater treatment setting, and to maximize the generation of bio-energy at the same time. However, further study is especially needed in elaborating the complex nitrogen transition and its effect on methanogenesis for tempeh wastewater.

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Acknowledgments

The authors would like to thank the Institute of Research and Community Service, University of Surabaya, for the internal scheme of research funding no.003/SP-Lit/LPPM-01/Int/PSL/III/2019. The staffs in Laboratory Bioprocess Technology and Environmental Process are highly appreciated for their excellent technical support.

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Preface – International Bioprocessing Association Subject Conference (IBASC 2021)

To cite this article: 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **963** 011001

Preface – International Bioprocessing Association Subject Conference (IBASC 2021)

The International Bioprocessing Association Subject Conference (IBASC 2021) was organized jointly by the Chemical Engineering Department, Faculty of Engineering, Universitas Gadjah Mada (UGM) and International Bioprocessing Association (IBA). The conference was held virtually on August 3rd - 5th, 2021 in conjunction with the Workshop on High Impact Publication by the Editor in Chief of Bioresource Technology (Elsevier), Mini Class: Anaerobic Digestion Research, and Indonesian Society for Smart Biomaterial and Tissue Engineering Meeting.

Due to the invaluable role of bioresources for the survival and growth of humankind, the emerging need for their exploration, conservation, and sustainable utilization is undeniable. The conference with the theme “Sustainable technologies for bioresource utilization: Bio-based products, bioenergy, and environmental protection” brought together speakers and experts in various branches of bioprocess engineering from universities, industries, research institutions, and students to foster a fruitful collaboration. The conference was a precious stage for participants to share the knowledge, experience, and state of the art in the research and technology for bioprocessing applications. Furthermore, the forum could provide opportunities to enlarge collaboration among the participants.

A total of 83 papers from seven countries (Indonesia, India, Taiwan, Malaysia, Iraq, Germany, and Sweden) were presented at the conference as an oral presentation. Finally, 59 papers were decided to be published in the IOP Conference Series: Earth and Environmental Science, which cover a broad scope, ranging from biomaterial engineering, environmental biotechnology, upstream/downstream bioprocess engineering, bioenergy/biofuel, food technology/engineering, and waste (water) to resources.

Due to the recent global pandemic, it is unfortunate that IBASC 2021 cannot be held in person. However, the committee is very grateful for all parties effort to contribute and be together virtually in this event. We express sincere gratitude to our peer-review team, who allocated their time to review papers – their contribution is indispensable. We would also like to express deep gratitude to the many experts and all supporting parties whom we cannot mention one by one for making this event possible.

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PUBLICATION TYPE	ISSN	COVERAGE	INFORMATION
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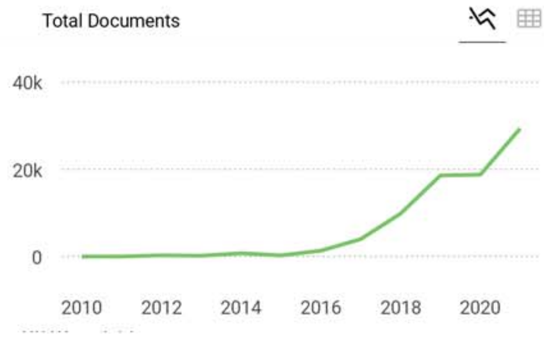
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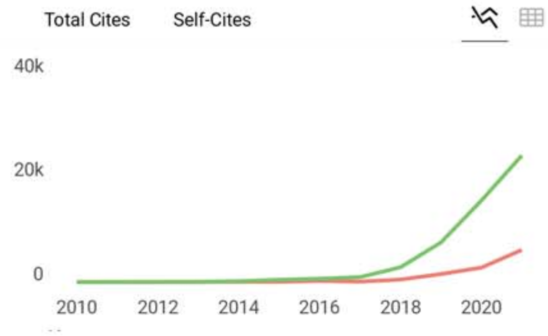
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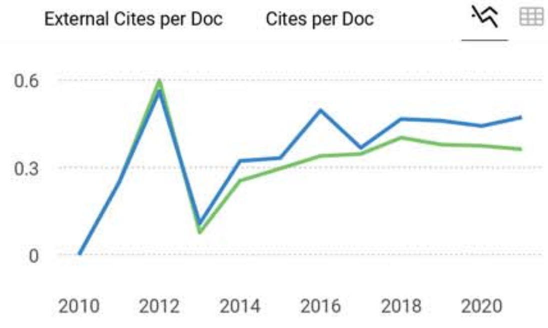
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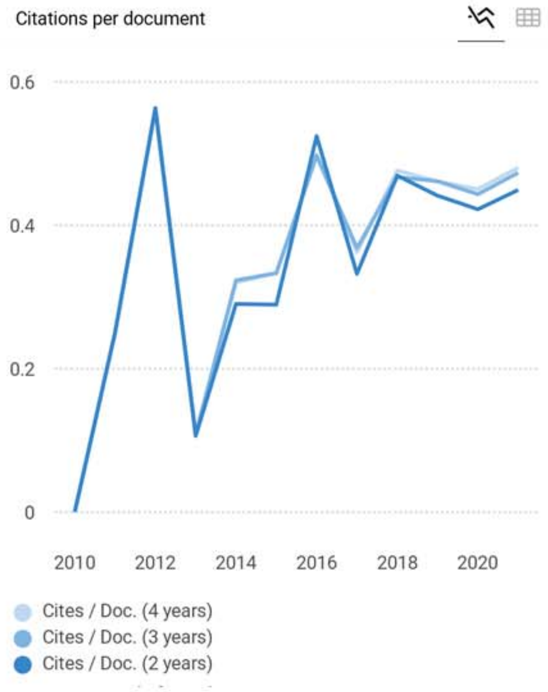
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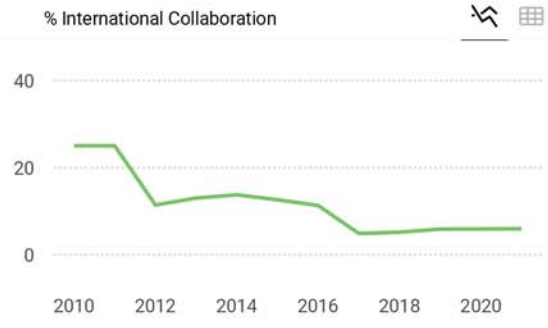
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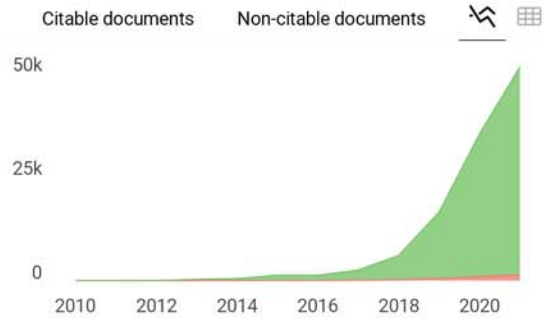
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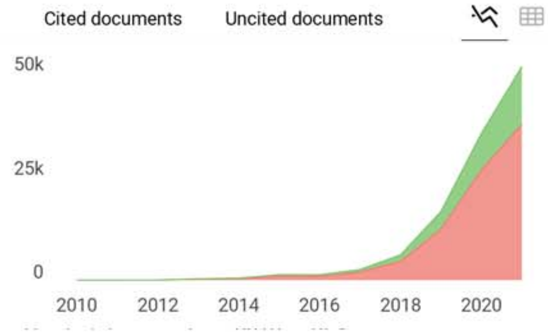
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