Tofu Liquid Waste Treatment Using Effective Volume of Anaerobic Sequencing Batch Reactor (ASBR)

Herawati Budiastuti1,2, Anissa Nurul Rachmawati1, Delliana Agustin1, Tifa Paramitha1, Rusdianasari2

1) Chemical Engineering Department, Politeknik Negeri Bandung, Bandung 40559, Indonesia
2) Chemical Engineering Department, Politeknik Negeri Sriwijaya, Palembang 30128, Indonesia

Abstract. The tofu industry produces liquid waste containing high organic compounds. Organic compounds in liquid waste can threaten aquatic ecosystems if discharged directly into water bodies. One of the most effective wastewater treatment systems is the treatment using Anaerobic Sequencing Batch Reactor (ASBR). Besides being able to reduce the content of organic compounds, ASBR can produce biogas, and its decomposing microorganisms do not flow into the effluent stream. The purpose of this study was to evaluate the performance of ASBR at an effective reactor volume according to the design. This is because the use of ASBR in previous studies had not reached the operational stage and did not use an effective volume reactor. The stages of waste treatment using ASBR include the seeding, acclimatization, and operation stages. Waste treatment took place at room temperature in the reactor with an effective volume of 6 L. The operating parameters tested were MLVSS, COD, BOD, pH, and cumulative biogas volume. The results obtained show that the seeding process took 56 days, acclimatization took 10 days, and the operation lasted for 17 days. The efficiency of reducing COD concentration was 60%, the efficiency of reducing BOD concentration was 35.65%, and the cumulative volume of biogas produced was 24,120 mL at the operating stage. The use of the ASBR system at an effective volume of 6 L resulted in the successful processing of tofu liquid waste.

Keywords: Anaerobic Sequencing Batch Reactor (ASBR), tofu liquid waste, effective volume of reactor


Kata kunci: Anaerobic Sequencing Batch Reactor (ASBR), limbah cair tahu, volume efektif reaktor

Received: 15 July 2023, Accepted: 29 April 2024
INTRODUCTION

The tofu industry is one of the industries producing organic waste. Tofu waste is produced in solid and liquid forms. Solid waste in the form of dregs can be used as animal feed, while liquid waste is generally discharged directly into the waters. The impact of tofu liquid waste causes a foul odor and pollutes the river. This is because tofu liquid waste contains organic matter in the form of carbohydrates, proteins, and fats which causes high COD and BOD content. High organic content causes oxygen levels in the water to decrease so that the water becomes dirty and turbid and threatens the survival of aquatic biota. The solution to reduce the organic content can be done by first treating the wastewater either aerobically or anaerobically (Budiastuti et al., 2021).

Anaerobic waste treatment involves microorganisms to reduce organic content by decomposition, namely the process of decomposing organic compounds in waste without the involvement of oxygen. This oxygen-tight condition must be maintained as an operating condition for anaerobic treatment, but this anaerobic waste treatment has the advantage of the presence of biogas products (Ghozali et al., 2018). The resulting biogas is used by the industry as an energy source.

One of the anaerobic industrial wastewater treatment technologies is sequential processing technology using the Anaerobic Sequencing Batch Reactor (ASBR). ASBR is an activated sludge process development under anaerobic conditions. The advantage of ASBR is that the sequential conditions in its stages can ensure a high conversion rate of organic substrates (Agua et al., 2015, Budiastuti et al., 2021). Other advantages of this sequential method are that it produces less biomass sludge and decomposing microorganisms do not flow in the treated wastewater (Budiastuti et al., 2023).

Research on tofu wastewater treatment using ASBR has been carried out by Rahayu et al. (2018). Based on their research, the efficiency of reducing COD was 87.51% with a biogas volume of 5.82 L. The inoculum used was sewage mud. Their research complements that of Agua et al. (2015) who used an inoculum in the form of anaerobic sludge from a brewery and city WWTP which resulted in a COD reduction efficiency of 64% with a methane gas yield of 0.27 L CH4/g COD. Gunawan and Nuraini (2022) also treated tofu liquid waste using inoculum in the form of cattle rumen extract, however this was only successful at the seeding and acclimatization stages. One of the reasons stated was the use of an ASBR volume of 5 L, while the reactor design was intended for an effective volume of 6 L (Muhammad and Salsabila, 2021). To be able to perfect Gunawan and Nuraini’s research (2022) and apply the reactor designed by Muhammad and Salsabila, (2021), this research was carried out with an effective volume of ASBR used of 6 L. Therefore, the stages carried out were not only seeding and acclimatization but up to operation of ASBR to process tofu liquid waste.

MATERIALS AND METHODS

Materials

The materials used in this study include cow dung extract, tofu liquid waste, KNO₃, KH₂PO₄, glucose, KMnO₄, K₂Cr₂O₇, H₂SO₄, Ferro Ammonium Sulfate, ferroin indicator, CaCl₂, FeCl₃, MgSO₄, MnSO₄, NaOH, oxalic acid, sodium thiosulphate, starch, and whatman filter paper no.40. All chemicals were purchased from Kimia Mart.
**Equipment**

The equipment used (Figure 1) includes a series of Anaerobic Sequencing Batch Reactor (ASBR), vacuum pump (Gast DOA-P504-BN), biogas volume meter, oven (Binder B115 Incubator), COD centrifuge (Labogene-Scanspeed 460), furnace (Carbolite Gero ELF 1100), analytical balance (OHAUS CP 214), pH meter (OHAUS 3100), turbidimeter (micro TPW), and a set of laboratory glassware.

**Figure 1.** Equipment schematic of ASBR

**Procedure**

The overall research procedures are shown in Figure 2, with the following details:

**Preparation phase**

The equipment preparation stage involved checking the ASBR series including temperature indicators, pressure indicators, pumps, biogas storage tanks, and hoses. The anaerobic process was carried out in conditions without oxygen; therefore, water and gas leak tests were carried out by filling the reactor with compressed air and closing all inlet and outlet channels, then pouring foam liquid onto the walls and parts of the reactor that were checked for leaks.

**Figure 2.** Flow chart of research procedures

The material preparation stage involved testing the characteristics of the cow dung extract and tofu liquid waste. The cow manure used was extracted by filtering, then tested for COD and pH. The tofu liquid waste used was obtained from a tofu factory in the Bandung area which was collected in plastic containers which were stored in the refrigerator when not used immediately. Analysis of the tofu liquid waste includes COD, BOD, MLVSS, and pH.

**Seeding stage**

Seeding is a process of providing nutrition that is carried out so that microorganisms can grow and later be able to adapt to the liquid waste to be treated (Budiastuti et al., 2023). The seeding stage takes place with the provision of nutrition as much as 600 mL/day. The nutritional ratio given is BOD:N:P of 250:5:1 with glucose as a carbon source, KNO₃ as a nitrogen source, and KH₂PO₄ as a phosphorus source.

**Acclimatization stage**

Acclimatization is a process of adaptation or adjustment to the conditions of
microorganisms with the liquid waste to be treated (Budiastuti et al., 2023). The acclimatization stage takes place by providing feed as much as 600 mL/day but the feed is given with a certain ratio between tofu liquid waste and nutrients. The feed is given in stages with a ratio of tofu liquid waste and nutrients from 10:90, 20:80, 30:70, 50:50, 60:40, 70:30, 80:20, to 100:0.

**Operation stage of ASBR**

ASBR was operated by providing 600 mL/day of tofu liquid waste with a COD concentration of 24,000 mg/L. One cycle of the ASBR operating system lasts for 24 hours with details of the filling stage for 15 minutes, the reaction stage for 20 hours, the settlement stage for 3 hours, the decant stage for 30 minutes, and the stabilization stage (idle) for 15 minutes.

The concentration of COD is measured in the effluent sample so that the efficiency of reducing COD can be known. In addition, the MLVSS, pH, turbidity parameters, and volume of biogas were analyzed to determine the process conditions that occurred in this reactor.

**RESULTS AND DISCUSSION**

**Characteristics of Cow Dung Extract**

This study used cow dung extract as an inoculum for anaerobic microorganisms because it contains many methanogenic bacteria which are methane-forming bacteria (Irawan and Suwanto, 2016). Cow dung extract has the characteristics as shown in Table 1.

The most important characteristic possessed by cow dung extract as a growth medium for microorganisms is pH. The pH value of the cow dung extract in this study was included in the optimum condition range, namely 6.4–7.4 so that microorganisms could grow and work properly (Dewi et al., 2018).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Cow Dung Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>mg/L</td>
<td>6,400</td>
</tr>
<tr>
<td>MLVSS</td>
<td>mg/L</td>
<td>32,565</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>7.08</td>
</tr>
<tr>
<td>Odor</td>
<td>-</td>
<td>Bad smell</td>
</tr>
</tbody>
</table>

**Characteristics of Tofu Liquid Waste**

Tofu liquid waste as the main material for waste treatment has characteristics as shown in Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Tofu liquid waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>mg/L</td>
<td>24,000</td>
</tr>
<tr>
<td>BOD</td>
<td>mg/L</td>
<td>14,725</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>3.69</td>
</tr>
<tr>
<td>Color</td>
<td>-</td>
<td>Yellowish white</td>
</tr>
<tr>
<td>Odor</td>
<td>-</td>
<td>Bad smell</td>
</tr>
<tr>
<td>Turbidity</td>
<td>NTU</td>
<td>79.47</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>26</td>
</tr>
</tbody>
</table>

The characteristics of the tofu liquid waste indicate that the waste contains high COD and BOD concentrations and is acidic in nature. When compared with existing literature, the concentration of tofu liquid waste in this study was higher than the tofu liquid waste tested by Gunawan and Nuraini (2022) which was only 6,000 mg/L and the tofu liquid waste tested by Budiastuti et al. (2021) of 10,268 mg/L. The COD of tofu liquid waste is 24,000 mg/L because the tofu liquid waste in this study comes from the clumping process. In this process, whey is added which is used repeatedly. Based on the characteristics of tofu liquid waste in Table 2, the waste needs to be processed first so that it can approach or reach the Tofu Liquid Waste Quality Standard according to Minister of Environment Regulation No. 05 of 2014. The permitted
thresholds for COD and BOD are 300 mg/L and 150 mg/L respectively.

**Seeding Stage**

The seeding stage lasts for 56 days with nutrient feeding and effluent sampling every day. The success parameter of the seeding process is the concentration of MLVSS which represents the concentration of microorganisms in the reactor which has increased.

![MLVSS and COD concentration against time during the seeding stage](image)

**Figure 3.** MLVSS and COD concentration against time during the seeding stage

Based on Figure 3, the concentration of MLVSS at the seeding stage fluctuated from day 1 to day 26. According to Arief et al. (2022), a decrease in the concentration of MLVSS shows that not all of the microorganisms in the sludge have adapted so that not all of the microorganisms are able to survive. On the 28th day, MLVSS tended to experience a steady increase with MLVSS concentrations above 30,000 mg/L. This shows that anaerobic microorganisms have adapted and grown well in the reactor.

In addition to the MLVSS concentration, the parameters for the success of the seeding process can also be seen from the value of COD, pH, and volume of biogas. Testing the COD concentration at the seeding stage aims to determine nutrients as organic substrates that can meet the nutritional needs for the growth of microorganisms (Ananda et al., 2018).

Based on Figure 3, COD concentrations fluctuated from increasing to decreasing on the last day of seeding. On days 1 to 40, COD concentrations tend to increase because the concentration of organic substrates in nutrients continues to increase. According to Paramarta (2016) an increase in nutrient concentrations that is too fast results in the accumulation of organic substrates in the reactor due to the slow growth of anaerobic microorganisms. However, the COD concentration on day 51 decreased because decomposing microorganisms had longer contact time to decompose organic substrates, which on day 42 to day 51 there was no addition of organic substrates to provide opportunities for microorganisms to degrade remaining organic compounds in the feed. According to Semarta et al. (2020) the longer the contact time between the waste and the inoculum media, the greater the removal efficiency. On the following day (52nd day) until the last day of the seeding stage (57th day), COD concentrations tended to be stable (Figure 3).

The pH parameter is an operational parameter that must be controlled in anaerobic waste treatment because the growth and performance of decomposing microorganisms is very susceptible to changes in pH. Figure 4 shows the curve of the pH value against time during the seeding process.
At the seeding stage, the pH initially decreased from 6.68 (day 2) to 4.8 (day 5) due to the formation of organic acids by acid-forming bacteria in the acidogenesis and acetogenesis stages (Budiastuti et al., 2016). The pH then rose again from 4.8 (day 5) to 5.21 (day 7). If we pay attention to the slightly acidic pH of the nutrients, the acid-forming bacteria work optimally to decompose organic substrates into organic acids which are then converted again into biogas. This is supported by data on an increase in the volume of biogas. However, the pH slowly continued to decrease to 4.14 (day 53) due to the high VFA accumulation so that not all organic substrates could be consumed by microorganisms degrading organic substrates in tofu liquid waste (Kahar, 2018).

Another parameter of anaerobic wastewater treatment is the formation of biogas. Biogas is produced from the decomposition of organic substrates by methanogenic bacteria. Figure 5 shows the cumulative biogas volume curve during the seeding process. It can be seen that the formation of biogas occurs at the seeding stage up to a cumulative volume of biogas of 15,310 mL.

This indicates that organic substrates undergo decomposition into biogas by microorganisms degrading organic compounds in wastewater (Dewi et al., 2018).

If we attention to the MLVSS curve, starting on the 30th day the MLVSS begins to stabilize and tends to increase at around 30,000 mg/L and it is supported by a decrease in COD concentration (Figure 3) as well as the volume of biogas formed 15,310 mL (Figure 5) then the seeding stage can be terminated and continued to the next stage, the acclimatization stage.

The COD concentration is a value that represents the concentration of organic substrates in the waste. The decrease in COD concentration indicates that anaerobic microorganisms have adapted and can act to decompose organic substrates in tofu wastewater.

Based on Figure 6, the COD concentration at the acclimatization stage decreased from 88,000 mg/L to 20,000 mg/L. According to Ananda et al. (2018) that a decrease in COD concentration indicates that decomposing

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**Figure 4.** pH curve against time during the seeding process

**Figure 5.** Cumulative biogas volume curve during seeding process

**Acclimatization Stage**

The acclimatization stage begins after the seeding process is complete and lasts for 10 days. The acclimatization process is carried out by gradually changing the nutritional feed with tofu liquid waste. The success parameter of the acclimatization stage is COD concentration.

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microorganisms have adapted and are active optimally. The decrease in COD concentration is supported by MLVSS data which represent the concentration of decomposing microorganisms. The concentration of COD as an organic substrate becomes nutrients for decomposing microorganisms. Microorganisms decompose organic substrates which are measured as COD parameters in the form of COD concentrations into biogas. Therefore, the higher the MLVSS concentration, the higher the COD concentration will decrease.

The COD concentration and MLVSS concentration are affected by the operating conditions of the waste treatment, one of which is the degree of acidity (pH). Optimum pH conditions will facilitate the performance of microorganisms to increase the MLVSS concentration so that the COD concentration will decrease.

Based on Figure 7, the pH at the acclimatization stage decreased from 4.14 to 3.99 due to the addition of tofu liquid waste which is acidic. The pH then increased to 4.42 due to the addition of NaOH solution into the tofu liquid waste to obtain or approach the optimum pH conditions of 6 to 9 (Ghozali et al., 2018).

The activity of decomposing microorganisms can be seen from the biogas products formed (Figure 8). The presence of biogas indicates that microorganisms have performed their role in decomposing organic substrates from tofu liquid waste.

According to Figure 6, the MLVSS concentration fluctuated but tended to increase from 30,450 mg/L to 41,992.5 mg/L. The increase in MLVSS concentration indicated an increase in the growth rate of the decomposing microorganism. Microorganisms have grown and reproduced because they have adapted to tofu liquid waste.

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volume of biogas is 5,250 mL. The increase in biogas volume is in line with the increase in substrate concentration. Tofu liquid waste contains organic substrates needed by anaerobic microorganisms to produce biogas.

Based on the decrease in COD concentration and increase in MLVSS concentration in Figure 6, it can be said that the acclimatization stage can be ended. According to the Herald in Ananda et al. (2018) the acclimatization process can be stopped when the COD removal efficiency is constant with fluctuations of not more than 10%. This decision is supported by the formation of stable biogas at around 600 mL/day (Figure 8). This indicates that the microorganisms have adapted and are able to decompose organic substrates or COD in tofu liquid waste into biogas.

**Operation Stage of ASBR**

The ASBR operation phase takes place after an acclimatization process of 17 days. ASBR is operated by feeding tofu liquid waste with a load of 600 mL/day. The success parameters of the operation stage as the last stage of tofu wastewater treatment are COD and BOD concentrations. The concentration of COD and BOD is a standard requirement for the quality of tofu liquid waste so that it is not dangerous to be discharged into the environment.

Based on Figure 9, the concentration of COD at the operating stage decreased from 19,200 mg/L to 9,600 mg/L. This shows that wastewater treatment using ASBR can reduce the COD concentration of tofu liquid waste with a COD reduction efficiency of 60% from the initial COD of 24,000 mg/L. On the 11th day, the COD concentration increased and then decreased again on the 16th day. This is caused by the pH conditions which decreased and then rose again in at this range of time.

![Figure 9. COD concentration versus time during operation of ASBR](image)

When compared to Gunawan and Nuraini's research (2022) which used the same ASBR reactor, the COD concentration in the liquid waste has decreased by up to 60%. This is influenced by the difference in the effective volume of the reactor and therefore the process has been carried out successfully up to the operating stage. In this study, the effective reactor volume used was 6L, above the 5L volume of the research by Gunawan and Nuraini (2022), so that the liquid waste treatment process could take place more effectively. In addition, the operation process which is carried out after the seeding and acclimatization stages results in more optimal waste treatment. Therefore, the use of a reactor with an effective volume of 6L results in successful processing with a processing efficiency of 60%.

In addition to COD concentration, BOD concentration is a more significant parameter of successful tofu wastewater treatment. BOD testing was carried out on samples of the last day of the operating phase. The BOD concentration obtained was 9,475 mg/L. This value indicates a decrease in the concentration of BOD from the initial BOD concentration of
Budiastuti et al. tofu liquid waste with a value of 14,725 mg/L so that the efficiency of reducing the concentration of BOD is 35.65%. According to Wardhani et al. (2015) a decrease in the concentration of BOD indicates that the organic substrates in the waste have been decomposed by microorganisms.

The decrease in COD and BOD concentrations is supported by data on increasing MLVSS concentrations. Based on Figure 9, the MLVSS concentration fluctuated but tended to increase until it stabilized up to 39,542.5 mg/L. This shows that there are many decomposing microorganisms in the reactor so that the activity of decomposing organic matter in the waste occurs properly.

Parameters for the successful processing of tofu liquid waste at the ASBR operating stage which tends to be stable are influenced by stable operating conditions. The pH value as an operating condition tends to be stable so that the activity of microorganisms in the reactor is also stable in decomposing organic substrates (Figure 10).

\[ \text{Figure 10. pH against time during operation of ASBR} \]

During the ASBR operation stage with 100% tofu liquid waste feed, the pH fluctuated from 3.75 to 3.88. This condition certainly inhibits the performance of anaerobic bacteria, especially methanogenic bacteria. However, the process can still run with the decomposition of waste organic substrates into VFA which is carried out by acidifying bacteria which can work in acidic conditions (Kahar, 2018, Budiastuti et al., 2016).

The product of the tofu liquid waste treatment process is biogas. The success of anaerobic waste treatment is seen from the presence of biogas as a product.

Biogas produced at the operating stage with normal load has increased until the highest biogas production is obtained on the 10th day of 700 mL/day. Feeding with a normal load every day causes an increase in the volume of biogas, although it fluctuates, but more than 600 mL/day is obtained.

\[ \text{Figure 11. Biogas volume against time during operation of ASBR} \]

The main component in biogas as an alternative fuel is methane gas (CH4). To determine the presence of methane gas in biogas, a flame test was carried out. The result obtained is that no blue flame is produced. The sample tested is the accumulation of biogas in a day so the sample volume to qualitatively check methane gas is still insufficient.

According to Sanjaya and Haryanto (2015) the anaerobic process requires a lot of time to convert organic substrates into methane gas. In addition, a progressive decrease in pH causes accumulation of organic acids, thereby disrupting the decomposition process, especially for methanogenic bacteria which are susceptible to pH (Kahar, 2018, Budiastuti et al., 2016).
CONCLUSION

Research on tofu liquid waste treatment using ASBR on the effective volume of the reactor has been successfully carried out. The COD concentration decreased by 60% while the BOD concentration decreased by 35.65%. In addition, the biogas produced during the tofu liquid waste treatment process using ASBR accounted for of 24,120 mL at the operating stage. It is recommended that the active volume of the reactor is a determining factor for the success of liquid waste processing that needs to be considered.

ACKNOWLEDGMENT

We would like to convey our gratitude to Politeknik Negeri Bandung for providing laboratory facilities and research funding through the Applied Research Scheme Number B/66.5/PL1.R7/PG.00.03/2022.

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