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The cassava starch wastewater contains organic materials (as BOD, COD) in high concentrations so it has the potential to cause pollution in the aquatic environment. Several methods of cassava starch wastewater treatment have been used to reduce the concentration of organic matter (pollutants) in cassava starch wastewater, including Activated Sludge, Stabilization Pond, Anaerobic-Aerobic filter process. However, various studies continue to be carried out to get higher processing efficiency on the factors that influence it. Several factors influence the efficiency of wastewater treatment processes, including the type and origin of decomposing microorganisms, hydraulic residence time (HRT), organic load rate (OLR), process design, pH, and temperature. The research aimed to evaluate the performance of the AnF2B reactor in treating cassava starch wastewater, in which the reactor performance is shown by changes in organic matter removal (COD removal) and biogas production. The research is conducted using 3 types of AnF2B reactors wherein each AnF2B reactor contains a bee nest-shaped bio-filter as a growth medium for the consortium of indigenous bacteria. The AnF2B reactor operates in anaerobic conditions with a set temperature of 29-30 °C and a pH of 4.5-7. In each AnF2B reactor, cassava starch wastewater is fed with different OLR so that each reactor has an HRT of 5, 6, and 7 days. The concentration of COD at the influent and effluent of the reactor was measured and the biogas was produced using the APHA standard method. The results showed that the AnF2B reactor had a satisfactory performance in COD removal and biogas production, which at HRT: 6 days and OLR of 1.72 g/L day found that the maximum COD removal was 98 % and the volume of biogas of 4.8 L/L·day was produced on the 12<sup>th</sup> day

Keywords: biogas, bee nest, cassava starch, HRT, indigenous bacterial consortium, OLR -0 D

Received date 29.12.2020 Accepted date 01.02.2021 Published date 23.02.2021

### 1. Introduction

The cassava starch wastewater (CSWW) is wastewater from washing cassava and starch extraction in the cassava starch industry where wastewater has the potential to cause pollution of the receiving water bodies (rivers), for example, anaerobic conditions, odor, dead fish [1]. This is because CSWW contains a high concentration of organic matter, among others: BOD (3,000-6,000 mg/L), COD (7,000-30,000 mg/L), TSS (1,500-5,000 mg/L), and pH (4-7.5) [2]. However, CSWW has great potential as a raw material for biogas production [3].

Some studies on cassava starch wastewater treatment have been used both to reduce the concentration of contaminants and to produce biogas, among others: Upflow Anaerobic Sludge Blanket (UASB) [4], Anaerobic Baffled Reactor (ABR) [5], Fixed Bed Reactor (FBR) [6], Anaerobic \_ \_ \_ \_ \_ \_ \_ \_

### UDC 628.353

DOI: 10.15587/1729-4061.2021.225324

# AN ANALYSIS OF PERFORMANCE OF **AN ANAEROBIC FIXED FILM BIOFILTER** (AnF2B) REACTOR **IN TREATMENT OF CASSAVA** WASTEWATER

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Horizontal Tubular Reactor (AHTR) [7]. Likewise, the type and origin of the microorganisms used have also been studied [8]. However, most cassava starch industries use lagoon or activated sludge systems, where both systems have the disadvantage of producing gases such as CO<sub>2</sub>, CH<sub>4</sub>, and H<sub>2</sub>S that are released into the atmosphere so they have the potential impact on the global environment. This requires a more effective study of the cassava wastewater treatment system.

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The CSWW that contains high organic matter had been categorized in the high-rate system, where the bio-filter is the right choice for the high-rate system [9]. In high-rate anaerobic systems, it is necessary to keep biomass in the reactor, which is got by attachment on inert support or even by maintaining biomass in sludge beds.

Studies on the inert support and organic loading in anaerobic reactors are factors that significantly influence the reac-

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tor performance [10], especially in high strength wastewaters with high levels of solids such as effluents from starch and alcohol-producing industries. The support material promotes higher biomass retention in the reactor, higher substrate degradation, increases resistance to toxic substances, and shock loads [9]. The important aspects considered in selecting support material type are:

1) ability to biomass attachment;

2) high contact surface;

3) low weight does not require reactor structure increase; 4) easiness and low getting costs. Thus, several materials are used as an inert support in anaerobic reactors, such as polyurethane foam, low-density polyethene [11–13].

The Aerobic Fixed Film Bio-filter (AF2B) system using a bee nest shaped bio-filter and a consortium of indigenous bacteria can effectively reduce the concentration of pollutants in hospital wastewater and tofu industry wastewater [14]. The bee nest shaped bio-filter has a specification that is materials (plastic), density ( $0.125 \text{ g/cm}^3$ ), volume ( $2.160 \text{ cm}^3$ ), specific surface area ( $150-240 \text{ m}^2/\text{m}^3$ ), where HRT of 3 hours can reduce the concentration of BOD, COD, and phenol of 92 %, 86 %, and 63 %, respectively [15].

Therefore, the use of a wasp nest-shaped bio-filter and the use of a consortium of indigenous bacteria in an anaerobic reactor need to be studied in order to provide answers to optimal operating conditions in treating tapioca starch wastewater. Furthermore, the results of this study are expected to be used as a reference in implementation or practice in the field.

### 2. Literature review and problem statement

Biological wastewater treatment technology, especially anaerobic biological wastewater treatment, has developed very rapidly as shown by the increased efficiency of processing in reducing pollutants. Besides that, anaerobic biological wastewater treatment also contributes to producing a renewable energy source (biogas). Several anaerobic biological wastewater treatments that use suspended growth and attached growth processes include Upflow Anaerobic Sludge Blanket (UASB), Hybrid Upflow Anaerobic Sludge Blanket HUASB, Anaerobic Baffled Reactor (ABR), Rotating Biological Contactor (RBC), Multistage Anaerobic Reactor (UMAR), Anaerobic – Aerobic Sequencing Batch Reactors (SBR), Anaerobic Fluidized Bed Reactor (AFBR), and Anaerobic – Aerobic Fixed Film Bio-filter (A2F2B).

The paper [4] presents research on cassava starch wastewater treatment using the UASB reactor with a COD concentration in the feed of approximately 3,000 mg/L. The results showed that at OLR of  $10-16 \text{ kg COD/m}^3 \cdot \text{day}$ , flow velocity of 0.5 m/h with a recirculation ratio of 4:1, COD removal >95 % and gas productivity of  $5-8 \text{ m}^3/\text{m}^3$ ·day are obtained. While in the study [6] using the same UASB reactor with a volume of 4-24 L, OLR of 5-18 kg/m<sup>3</sup>·day, and a recirculation ratio of 1:1, optimum results were obtained at a COD load rate of 15 kg/m<sup>3</sup>·day, COD removal of 92.5 %, and  $H_2$  and  $CH_4$  production of 0.43 mL  $H_2/g$  COD applied, and 328 mL CH<sub>4</sub>/g COD applied, respectively. The two studies indicate that the UASB reactor has a high ability to take COD and produce methane gas. However, there are weaknesses, namely what if the process takes place horizontally and the process is top-down. The authors of the work [17] explained that OLR has the same effect on the performance of UASB and HUASB reactors in treating cassava starch wastewater. While the results of this research show that the COD removal (%) and OLR values are between 78.6 % to 58.9 % and 0.088 to 0.633 (kg COD/kg VSS·day) for the UASB reactor and between 83.1 % to 72.1 % and 0.076 to 0.629 (kg COD/kg VSS·day) for the HUASB reactor. While the biogas produced shows that the biogas yield gradually increases initially with an increase in COD removal (%), OLR, VLR, and HRT (i.e., from 0.25 to 0.29 m<sup>3</sup>/kg COD removal for UASB reactors and from 0.27 to 0.30 m<sup>3</sup>/kg. COD removal for the HUASB reactor and thereafter the yield decrease with a decrease in COD removal (%) (i.e., from 0.27 to 0.18 m<sup>3</sup>/kg. COD removal for the UASB reactor and from 0.28 m<sup>3</sup> to 0.24 m<sup>3</sup>/kg COD removal for the HUASB reactor).

The three studies show that both the UASB and HUASB reactors can process cassava wastewater into biogas and reduce COD, where biogas production and COD removal (%) are influenced by OLR, the recirculation ratio. But there are unsolved issues, namely the type and shape of microorganisms, horizontal flow direction, the shape of the support of media, and HRT used.

The paper [9] describes the results of observing the performance of the CSTR reactor, which operates on one phase in processing food waste through changes to process parameters such as HRT and OLR. The results show that HRT and OLR are fixed to have a more stable performance and higher yields than the varied HRT and OLR. However, this study has not shown the value of HRT and OLR to achieve stable performance. The paper [18] using the same type of reactor, namely one-phase horizontal reactors shows that the higher HRT, the greater the COD removal, where the HRT of 13 days COD removal is 99.2 %. This research shows that the horizontal reactor has a high ability to remove COD at 13 days HRT. However, lower HRT and types of microorganisms have not been observed. The authors of the work [19] investigated the wastewater treatment of tapioca flour using an up-flow multistage anaerobic reactor (UMAR), which showed that an HRT of 6 days and OLR at 10.2–40.00 kg COD/m<sup>3</sup>·day could reduce COD by a maximum of 97.9 %. The study shows that UMAR with lower HRT (6 days) has a high ability to remove COD but for horizontal reactors with top-down flow and the type of microorganism growth media (bio-filter) has not been observed.

In [5], research on evaluating the performance of an anaerobic baffled reactor (ABR) in the treatment of cassava wastewater is presented. The ABR reactor performance evaluation was made in terms of several parameters, including COD removal, pH, turbidity, alkalinity, acidity, and total suspended solids. The system showed buffering ability as acidity decreased along with compartments, while alkalinity and pH values were increased. There were particulate material retention and COD removal varied from 83 to 92 % for HRT of 3.5 days. This study shows that the presence of baffles can reduce HRT, which causes an increase in COD removal. However, the shape of the baffles and types of microorganisms has not been explained. Is the bio-filter bee nest-shaped? The paper [20] describes the effect of hydraulic retention time (HRT) and organic loading rate (OLR) on biological hydrogen production, which was assessed using an anaerobic fluidized bed reactor fed with cassava wastewater. The HRT of this reactor ranged from 1 to 8 h (28 to 161 kg COD/m<sup>3</sup>·day). The inoculum was obtained from a facultative pond sludge derived from wine wastewater treatment. The hydrogen yield production increased from 0.13 to 1.91 mol  $H_2$ /mol glucose as the HRT decreased from 2 to 8 h. The hydrogen production rate significantly increased from 0.20 to 2.04 L/L·h when

the HRT decreased from 1 to 8 h. Overall, we conclude that the best hydrogen yield production was got at an HRT of 2 h. This study shows that a fluidized medium for the growth of microorganisms can increase the production of hydrogen gas. However, for media with a wider surface such as bio-filters and types of microorganisms, it has not been observed.

The paper [14] explains hospital wastewater treatment using a consortium of indigenous bacteria in a batch reactor. It is shown that the substrate volume (microbial) and airflow rate influence the removal of pollutants, namely COD (86%), BOD (92%), NH3-N. (88%) and phenol (88%). The research was carried out in a batch reactor, but for continuous reactors it has not been done. Meanwhile, the researchers used the AF2B reactor, which contains a bee nest-shaped bio-filter and an indigenous bacteria consortium where the HRT of 3 hours can reduce pollutants such as BOD, fecal coli, and phenol by 92 %, 85 %, and 63 %, respectively [14, 22]. The research was carried out in a batch reactor and aerobic process, but for continuous reactors and anaerobic process it has not been done. Thus, the use of indigenous bacteria and bio-filters in wastewater treatment can minimize space and be more efficient at low HRT. However, there is an unsolved issue to improve the efficiency of the process in cassava starch wastewater treatment as the form of the support media (bio-filter), the form of bacteria (indigenous consortium).

For this study, we tried to investigate the performance of the AnF2B reactor to identify the effect of HRT and OLR on the COD removal and production of biogas, which AnF2B reactor containing a bee nest-shaped bio-filter and a consortium of indigenous bacteria.

### 3. The aim and objectives of the study

The aim of the study is to analyzes the performance of the AnF2B reactor in treating tapioca starch wastewater.

To achieve the aim, the following objectives are accomplished:

- to analyze the effect of HRT on COD removal;

– to analyze the effect of HRT on biogas production.



## 4. 1. Cassava starch wastewater (CSWW)

The CSWW was collected from a full-scale cassava starch factory in the southern region of Malang-East Java, Indonesia. The CSWW was generated during washing cassava roots and starch extraction. In the laboratory, the CSWW was homogenized and kept in a freezer (-20 °C) until use. Prepare CSWW as materials in the experiment, in which CSWW is diluted and then several parameters are measured, including temperature, pH, COD, and TSS.

### 4.2. Acclimation of the indigenous bacterial consortium

The indigenous bacterial consortium isolates were the mixed culture of several types of bacteria *Bacillus*, *Proteus*, *Lactobacillus*, *Acinetobacter*, *Pseudomonas*, *Saccharomyces*, *Candida*, *Aspergillus*, and *Mucor*, where the bacteria con-

sortium was got from the selection and isolation of potential bacteria in CSWW.

The acclimatization process of the indigenous bacteria consortium: in a container, 200 g of isolates culture of indigenous bacteria consortium were added to 2 liters of distilled water, nutrients of 1,000 ppm, stirring and checking the pH until pH=6–7. Further, the indigenous bacterial consortium solution is shaken continuously in a continuous shaker until growth increases, then the number of bacteria is checked using the hemocytometer method. Further, the indigenous bacteria consortium solution was made 5 %, 10 %, and 20 % by volume that adding the CSWW. The acclimatized indigenous bacteria consortium is known as a starter. The starter has gone under further acclimatization in the AnF2B reactor, which is filled with a starter as much as 5 % of the total volume and then nutrients were gradually added followed by the addition of CSWW (substrate) until the starter growth is maximum.

### 4. 3. Reactors, support materials, and experiment

The experimental apparatus system used was the AnF2B reactor designed as illustrated in Fig. 1. The volume of the AnF2B reactor is 50 L. The AnF2B is divided into 3 equal parts, where each part contains a bio-filter made of plastic with a bee nest shape. The material of the bio-filter is plastic with a density of  $0.125 \text{ g/cm}^3$ , a volume of  $40 \text{ cm}^3$ , and a specific surface area of  $150-240 \text{ m}^2/\text{m}^3$ . Bio-filter functions as a growth medium for indigenous bacteria consortium. A pH and flow controllers are fitted on the inlet of the AnF2B while the pump is fitted on the sedimentation tank to recycle a part of the sludge into the first part of the bio-filter of 10 % volume. The flow in the reactor is made circulating to have a large residence time. Nitrogen gas at the beginning of an operation to maintain anaerobic conditions.

The experiment was started by filling the starter (a consortium of indigenous bacteria) as much as 5 % of the total volume of the reactor and then flowing nitrogen gas from the nitrogen tank to maintain anaerobic conditions.



1. Feed Tank 2. Feed Pump 3. Flowmeter 4. Biofilter (bee nest shaped) 5. Recycle pump

Fig. 1. Sketch of experimental equipment (AnF2B reactor)

CSWW in the feed tank with a certain organic load (OLR) flows into the reactor using a feed pump at a flow rate that has been "set" so that the reactor has an HRT of 5 days, 6 days, 7 days. Wastewater flows in circulation and passes through the bio-filter, where on the surface of the bio-filter a bacterial consortium layer has been formed so that the biodegradation process of pollutants (organic matter) occurs by a consortium of indigenous bacteria. Biogas from biodegradation flows to the top of the reactor, which is

then collected in a gas storage tank to be measured using a gas analyzer. Meanwhile, the effluent is collected in a storage tank to measure the concentration of COD, TSS, and pH. To maintain the growth of the bacterial consortium in the reactor, some effluent is recycled into the reactor. By measuring the concentration of COD, TSS, pH, and flow rate on the influent and effluent, the pattern of percent COD taken, TSS taken and the volume of biogas produced can be obtained.

$$\begin{split} & \text{COD}_{\text{removal}} (\%) = \{(\text{COD}_{\text{inf}} - \text{COD}_{\text{eff}}) / \text{COD}_{\text{inf}}\} \times 100 \% \\ & \text{TSS}_{\text{removal}} (\%) = \{\text{TSS}_{\text{inf}} - \text{TSS}_{\text{eff}}) / \text{TSS}_{\text{inf}}\} \times 100 \%. \end{split}$$

## 5. Research results of the performance of the AnF2B reactor in treating tapioca starch wastewater

### 5.1. COD removal

One of the indicators to measure a reactor's performance is to see the ability of a reactor to remove organic materials (COD) in wastewater. While the influent COD concentration varied, so the organic load of the feed varied as shown by the amount of OLR. Where OLR is the concentration of COD in influent per unit time. Based on the analysis results of COD influent – effluent, and feed flow rate for various HRTs, experimental data are obtained as shown in Fig. 2.

Fig. 2 shows the reactor with HRT of 5 days and initial COD of 7 g/L, then on the 5th day the sampling data are obtained, namely COD effluent of 4 g/L and then sampling the influent – effluent reactor daily for 20 days, and then the COD influent and effluent data are obtained every day, which is then obtained COD data were taken, and OLR.

Likewise, the experiments on the reactor with HRT of 6 days (Fig. 2, *b*) or the reactor with the HRT of 7 days (Fig. 2, *c*). Fig. 2, *b* shows that COD effluent was only obtained on day 6, while in the reactor with HRT of 7 days (Fig. 2, *c*) COD effluent data were only obtained on the 7<sup>th</sup> day.

Fig. 2 shows that the longer the process of biodegradation of organic matter (COD) in the reactor, the greater the COD removal, where on the 12<sup>th</sup> day the maximum COD removal was 87 % with an OLR of 1.5 g/L·day. COD removal was relatively stable until the 20<sup>th</sup> day at 83 %. For the reactor with an HRT of 5 days, the OLR value is influenced by the COD influent, where the influent COD feed is 7–7.6 g/L, so the OLR is obtained ranging from 1.4 to 1.54 g/L·day. Furthermore, through a mathematical process, it is shown that the COD removal pattern for the reactor with HRT of 5 days has a mathematical equation  $Y=-3E-05X^{6}+0.0013X^{5}-0.0047X^{4}-0.05211X^{3}+8.1407X^{2}-29.546X+25.139$  with  $R^{2}$ :0.9764.

Fig. 2, *b* shows that the maximum COD removal on the 13<sup>th</sup> day is 98 % with an OLR of 1.72 g/L·day, and then COD removal is relatively stable until the 20<sup>th</sup> day of 92 %. For the reactor with an HRT of 6 days, with an influent COD of 10–10.3 g/L, the OLR is 1.67–1.73 g/L·day and the COD removal pattern has the following mathematical equation:  $Y=-0.0001X^{6}+0.0087X^{5}-0.1911X^{4}+1.6544X^{3}-3.334X^{2}-6.5127X+11.955$  with  $R^{2}:0.9724$ . While Fig. 2, *c* shows that on the 12<sup>th</sup> day, there was a maximum COD removal of 89 % with an OLR of 3.03 g/L·day, then COD removal was relatively stable until the 20<sup>th</sup> day, namely 84 %.

For the reactor with an HRT of 7 days, with the characteristics of the feed containing COD of 21-22 g/L, the OLR obtained is 3-3.16 g/L·day. The pattern of COD removal has a mathematical equation:  $Y=-0.0003X^{6}+0.0171X^{5}-0.427X^{4}+4.8526X^{3}-24.135X^{2}+47.946X-28.729$  with  $R^{2}$ :0.9707.



Fig. 2. Performance of the AnF2B reactor in COD removal in various HRT: a - HRT: 5 days; b - HRT: 6 days; c - HRT: 7 days

Based on the experimental data for various HRTs as shown in Fig. 2, it is shown that COD removal has the same trendline where the percentage of COD removal has increased from time to time along with the amount of COD influent, HRT, and OLR, where the maximum COD removal occurs on the  $12^{\text{th}}$  to the  $13^{\text{th}}$  day, and thereafter is stable until the  $20^{\text{th}}$  day.

### 5.2. Biogas production

By using the same reactor, namely the AnF2B reactor, the performance of the AnF2B reactor can be analyzed based on its ability to produce biogas.

While the results of measurements of biogas volume, pH, and TSS on various HRTs obtained experimental data as shown in Fig. 3.

For biogas production in each reactor, the HRT treatment of 5, 6, and 7 days is shown in Fig. 3.

Fig. 3 shows that in the reactor with the HRT of 5 days, the biogas product is obtained on the  $6^{\rm th}$  day, and the biogas production has increased maximally on the  $12^{\rm th}$  day with a total biogas volume of 1.7 L/L·day with TSS removal of 73.1%. Biogas production has decreased on the  $17^{\rm th}$  day then stabilized until the  $20^{\rm th}$  day. And the biogas production

pattern has formed a mathematical equation:  $Y=-6E-07X^{6}+$ +7 $E-05X^{5}-0.0024X^{4}+0.036X^{3}-0.2258X^{2}+0.5638X-0.4149$  with  $R^{2}$ :0.9632.

The volume of biogas produced is in line with the percentage of TSS removal, and the degradation of organic materials into biogas is shown by changes in TSS removal and pH of the effluent.

Fig. 3, *b* shows that in the reactor with HRT of 6 days, the biogas product is produced on the 6<sup>th</sup> day, and biogas production increased to a maximum on the 12<sup>th</sup> day with a total biogas volume of 4.8 L/L·day with TSS removal of 94.6 %. Biogas production has decreased on the 18<sup>th</sup> day and then stabilized until the 20<sup>th</sup> day. And the biogas production pattern has formed a mathematical equation:  $Y=-7E-06X^{6}+0.0005X^{5} -0.0135X^4+0.1575X^3-0.7685X^2+1.4591X-0.8331$  with  $R^2$ : 0.9887. Whereas Fig. 3, c shows the biogas production for the reactor with HRT of 7 days, where the maximum biogas production was reached on the 13<sup>th</sup> day with a total biogas volume of 3.3 L/L·day with TSS removal of 98.1 %. Biogas production is relatively stable until the 20<sup>th</sup> day. And the biogas production pattern has formed a mathematical equation:  $Y = -4E - 06X^{6} + 0.0003X^{5} - 0.0072X^{4} - 0.0796X^{3} - 0.0072X^{4} - 0.0796X^{3} - 0.0072X^{4} - 0.0072X^{4}$  $0.3554X^2 + 0.5676X - 0.2435$  with  $R^2: 0.9623$ .



Time (day)

pH effluent
Biogas production (L/L·day)
TSS removal (%)

с

Fig. 3. Performance of the AnF2B reactor in the production of biogas at various HRT: a - HRT: 5 days; b - HRT: 6 days; c - HRT: 7 days

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This shows that biogas production has the same trendline for various HRT values, where biogas production increases with increasing HRT value. Biogas production is also influenced by TSS removal, where the maximum biogas production is achieved at the TSS removal of 98.1 % in the reactor with HRT: 7 days. Biogas production is also followed by changes in the pH of the effluent, where the greater the volume of biogas produced, the higher the pH of the effluent.

### 6. Discussion of experimental results

### 6. 1. Effect of HRT on COD removal

The performance of a reactor is its ability to remove organic matter (COD) to become other materials that are not a pollutant where the amount of COD removal is influenced by the OLR and HRT. The performance of the AnF2B reactor can be shown from the changes and removal of COD at various HRTs as shown in Fig. 2. The concentration of COD in the influent from time to time varies, expressed as OLR, where OLR also shows the content of organic matter in feed per time.

Fig. 2 shows that the AnF2B reactor at the initial effluent with HRD: 6 days can remove more COD (61%) than the reactor with HRD: 5 days (45%) and HRD: 7 days (55%). It is shown that the AnF2B reactor that contains a bee nest-shaped bio-filter with a consortium of indigenous bacteria has a high ability to degrade organic materials (COD). There is a non-linear relationship between HRD and OLR with COD removal that HRD: 5–6 days and OLR: 1.48–1.72 g/L·day, there was an increase in COD removal. Meanwhile, HRD>7 days and OLR: 3 g/L·day, there was a decrease in COD removal.

Fig. 2 shows that the steady-state and maximum COD removal occurred at 12 days: HRT: 5 days (87%), HRT: 6 days (97%), and HRT: 7 days (89%), respectively.

Thus, the reactor with the HRT of 6 days has a greater ability than the reactor with the HRT of 5 days or HRT of 7 days. This shows that the reactor with an HRT of 6 days has a balance between the growth rate of the bacterial consortium and the organic load rate (OLR) of the feed, while on the HRT of 5 days, the OLR is greater than the bacterial growth rate so that bacteria have less ability to remove organic matter (COD). With the increasing time of the degradation process of organic matter, there was a decrease in COD removal along with the decrease in the ability of bacteria to digest organic matter. However, with a continuous recycling process of 10 %, the growth of bacteria has increased and is stable on days 12 to 13. And this is a limitation in this study, where the varying recycling rate can produce different COD removal patterns.

However, in a continuous flow reactor, the characteristics of the bio-filter and the growth rate and deathly rate of bacteria affect the rate of degradation of organic matter, where the steady-state can be an indicator of an equilibrium deathly rate – microorganism growth rate and feed rate [22].

[23] mentions that biological treatment of cassava wastewater by aerobic and combined anaerobic/aerobic reactors can provide removal of organic matter of 89 to 93 %, the cyanide was 95 to 99 %, and the food to microorganisms ratio was found to be between 0.166 to 0.242 per day, with an HRT of 1.4 to 4.2 days. While continuous stirred tank reactor (CSTR) confirmed that the co-digestion ratio of WAS:SS (1:1) can enhance biogas yield and methane yield at 301 and 142 L/kg TVS, respectively, at a retention time of 11 days. There is a non-linear relationship between the organic loading and the rate of removal of COD [24]. This is because the higher the HRD and OLR, the smaller the feed flow rate and the greater the organic material entering the reactor per unit time, so the higher the degradation process of organic matter (COD) by microorganisms in the bio-filter to be decomposed into other products (gas, new cells, H<sub>2</sub>O) [25].

[5] mentions that buffering ability as acidity decreased along with compartments while alkalinity and pH values were increased. There were particulate material retention and COD removal varied from 83 to 92% for HRT of 3.5 days. [6] using two fixed bed reactors filled with different support materials, bamboo rings and flexible PVC rings with OLR ( $15 \text{ g/L} \cdot \text{day}$ ) and low HRT (0.8 day), show that COD removal increased with the OLR increase resulting in COD removal values of up to 99 %. Total solids removal efficiency was 86.2 and 85.5 %, respectively. Thus, the AnF2B reactor containing bee-nest shaped bio-filter and indigenous bacterial consortium has a better performance in COD removal so it can be considered in increasing the efficiency of cassava starch wastewater treatment. However, this study has several limitations, including: the reactor volume is 50 L, the HRT range: 5–7 days, the recycle flow rate is 10 % (volume), and the initial starter is 5 % (volume). Furthermore, to get more comprehensive research results on the effect of HRT on COD removal, it is necessary to develop it for further research, including more enlarged HRT range (HRT: 1-10 days), varied recycle flow rate, and enlarged starter volume. Besides that, several things can be developed from this research, including mathematical models for COD removal and biogas production, COD removal kinetics, and more varied HRT.

### 6. 2. Effect of HRT on biogas production

The performance of the AnF2B reactor in the production of biogas for various HRT and pH can be seen in Fig. 3, where there is a significant increase in the volume of biogas produced with increasing HRT. Besides, there is a relationship between the volume of biogas produced and the pH and TSS, where the pH is correlated with the stages of the process of degradation of organic matter and TSS to biogas. While TSS removal is correlated with organic matter converted to biogas.

Fig. 3 shows that the initial pH of the effluent is acid (4-4.5), and along with the anaerobic processes that occur in the AnF2B reactor, namely, the hydrolysis, acetogenesis, and methanogenesis processes so that the pH of the effluent fluctuates. On 5 to 12 days, the degradation (bioconversion) process of organic matter produces more glycerol and acid (e.g., acetic acid, butyric acid) than biogas, CO<sub>2</sub>.

Thus, until the 12<sup>th</sup> day, the degradation process of organic substances was dominated by hydrolysis and acetogenesis processes rather than methanogenesis processes, thus increasing the alkalinity of the effluent. After the 12<sup>th</sup> day, more advanced processes dominated the methanogenesis process to produce methane gas (biogas). This is shown by a decrease in TSS, COD, and increasing rate of biogas production. However, factors other than the stages in the anaerobic process are also affected by HRT and OLR [24, 25].

This suggests that the degradation of organic matter by indigenous bacteria consortium is effective. This is due to the greater the HRT, the lower the feed flow rate so that the greater the degradation process of organic matter into products (glycerol, acid compounds, and biogas) and the increasing growth rate of new cells (bacterial consortium). However, greater HRT or lower feed flow rate can cause to a decrease in the degradation process of organic matter of products if the death rate is greater than the bacterial growth rate [25].

[4] mentions that the reactor with OLR of 2.25 g/L·day produces biogas of 787 mL/g·day. Reactor with fixed HRT showed better performance with higher biogas yield and better stability indicators. The up-flow anaerobic sludge blanket reactor (UASB) for cassava wastewater treatment using acidogenic reactor that the COD removal efficiency and biogas production were favored by OLR increased from 2 to 8 g/L·day. However, the OLR increase to 10 g/L·day leads to biogas reduction from 25 to 8 L/day, whereas the COD removal efficiency decreased discretely (66 to 62 %). The up-flow anaerobic fixed bed reactor for cassava starch wastewater treatment and observed that the biogas production increase (0.8 to 7.56 L/day) was proportional to OLR increase (2.5 to 10 g/L·day). However, the methane proportion in biogas decreased from 76.8 to 66 %, respectively [17].

Fig. 3 shows that the steady-state and maximum biogas produced occurred at 12 days, respectively: HRT: 5 days (1.7 L/L·day), HRT: 6 days (4.8 L/L·day), and HRT: 7 days (2.8 L/L·day). Thus, the AnF2B reactor which contains a bee nest-shaped bio-filter and indigenous bacteria consortium has a high ability to produce biogas of 4.8 L/L·day at HRT: 6 days and OLR: 1.72 g/L·day. [26] mentioned that the increase in organic and hydraulic loads can cause disturbances in the microbial community structure of the reactor. The reduction in biogas volume and methane contents are usually related to a decrease in the quantity and activity of the syntrophic bacteria and methanogenic archaea. [16] observed in a three-stage UASB reactor at mesophilic temperature (37 °C) at COD loading rates from 5 to 18 kg/m<sup>3</sup>·day (based at an optimum COD loading rate of 15 kg/m<sup>3</sup>·day) the system provided the highest COD removal level (92.5 %) and the highest H<sub>2</sub> and CH<sub>4</sub> yields of 0.43 mL H<sub>2</sub>/g COD applied, and 328 mL CH<sub>4</sub>/g COD, respectively. Thus, the AnF2B reactor has a better performance in the production of biogas. However, this study has several limitations, including biogas volume measurement methods, biogas content analysis, and several temperature and pH control equipment that have not been computerized. Furthermore, to obtain more valid research results, it is necessary to carry out experiments using more systemized equipment in a computerized system and use experimental variables that are more comprehensive.

### 6. Conclusions

1. The HRT affects COD removal, where with HRT: 6 days on day 12, the maximum COD removal is 96 %.

2. The HRT affects biogas production, where with HRT: 6 days on day 12, the maximum biogas production is 4.8 L/L·day.

### Acknowledgments

The authors gratefully acknowledge the financial support from the State Polytechnic of Malang, who has provided financial support through the DIPA research fund. Besides that, the researcher thanked the staff of the wastewater treatment laboratory that supported this research.

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