Production of biogas using AnF2B reactor from cassava starch wastewater with consortium bacteria as biocatalyst

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Abstract. Cassava starch wastewater contains organic biodegradable (BOD, COD) with high concentrations so that it can be used as raw material for biogas through an anaerobic decomposition process. The purpose of this study was to evaluate the behavior of the continuous flow AnF2B reactor, related to the production of biogas in a variety of organic loads, from cassava starch wastewater. The AnF2B reactor has three parts of space with a total volume of 50 L, where each space contains biofilter (wasp nest-shaped) which is useful for the growth of bacterial consortium. The experiments were carried out by flowing wastewater into the AnF2B reactor continuously with organic loading of 2 g, 3 g, 4 g, and 5 g. The decomposition time was 1-10 days. The temperature in the reactor was maintained at 37 - 39 °C and pH: 6.5 - 8. The results showed that biogas production was influenced by the organic load of wastewater, where the maximum biogas production of 6.926 L was obtained at an organic load of 5 g of COD and a biodegradation time of 3 days. The biogas produced contains CH\(_4\) (85%), CO\(_2\) (14.5%), and CO (0.5%).

1. Introduction
The effluent from cassava starch industrial contains organic biodegradable materials which causes pollution to the receiving water body (rivers). Some of the issues that could be caused by cassava starch industrial effluent are death of fish, unpleasant smell, disturbance of aquatic ecosystems [1-3]. Cassava starch wastewater has characteristics, among others: BOD (3,000 - 6,000 mg·L\(^{-1}\)), COD (7,000 - 30,000 mg·L\(^{-1}\)), TSS (1,500 - 5,000 mg·L\(^{-1}\)), dan pH (6.5 - 8) [2,4-6]. Thus, cassava starch wastewater has potential as a raw material in biogas production, where through anaerobic decomposition the organic material will decompose into CH\(_4\), CO\(_2\), CO and a small amount of NH\(_3\), N\(_2\), H\(_2\)S, O\(_2\) and H\(_2\) [2,5,11]. The factors that affect biogas production, among others: Organic Loading Rate (OLR), Hydraulic Retention Time (HRT), kind of bacterial, pH, temperature, and inhibitors [3,6,10,11]. While several wastewater treatment processes are used to produce biogas, including: Anaerobic Digestion, UASB, Fixed Bed Reactor (FBR), Horizontal Reactor, Anaerobic Baffled Reactor (ABR) [12-16].

Methane production using anaerobic digesters shows that digesters with HRT and fixed load have better performance and larger biogas products than digesters with HRT and varying loading [12]. Biogas production using a UASB reactor at an optimum rate of COD load of 15 kg·m\(^{-3}\)·d\(^{-1}\) can produce H\(_2\) and CH\(_4\) of 0.43 ml H\(_2\)·g\(^{-1}\) COD and 328 ml CH\(_4\)·g\(^{-1}\) COD applied, respectively [13]. Whereas in the FBR reactor, the highest biogas production was obtained at the highest influent concentration with OLR of 5.6 g·L\(^{-1}\)·d\(^{-1}\), and HRT of 2.7 d [14].
The maximum biogas production was 0.65 L at the HRT of 8.3 days, with CH$_4$ content of 59%. While the maximum COD removals and biogas production were obtained at the HRT of 8.3 days [17]. Furthermore, Kusworo [18] stated that the mixture of cassava starch wastewater, yeast, ruminant bacteria and urea can increase biogas production namely 726.43 ml·g$^{-1}$. The AnF2B reactor has a good performance in COD removal and biogas production, where the maximum COD removal of 98% and biogas of 4.8 L·L$^{-1}·d^{-1}$ are obtained at HRT of 6 days and OLR of 1.72 g·L$^{-1}·d^{-1}$[19]. So, the objective of the research was to analyze the biogas production in an AnF2B reactor from various organic loading using consortium bacteria as biocatalyst.

2. Methods and Materials

2.1 Starter (decomposer) preparation
The isolate of the bacterial consortium was obtained from cassava starch effluent (indigenous process). 1 L of wastewater is taken and put in Erlenmeyer and added with nutrient agar (NA) as much as 5% by volume, then closed Erlenmeyer, then put it into the incubator shaker. NA was added every day. After incubation in a shaker, part of the wastewater in Erlenmeyer was taken to be carried out by identification, characterization and bacteria isolation. The results of identification, characterization and isolation, then selected 5 types of potential bacteria as bacterial isolates. A total of 5 types of bacterial isolates were poured into Erlenmeyer as a bacterial consortium isolate or known as a starter of bacterial consortium. Furthermore, the starter is acclimatized by maximizing growth in a growth medium in a reactor with a growth scale of 1 - 200 times. In order to obtain a starter volume of 2 L. This starter is a decomposer (biocatalyst).

2.2 Studied wastewater
This study uses cassava starch wastewater from the cassava starch industry in East Java, Indonesia. The wastewater contained BOD (3,500 - 6,000 mg·L$^{-1}$), COD (9,000 - 20,000 mg·L$^{-1}$), and TSS (500 - 2,500 mg·L$^{-1}$). Furthermore, wastewater is collected in a tank at 2,000 L for each running experiment. The pH and COD concentration of wastewater as feed are maintained by adding acid or base and dilution.

2.3 AnF2B operation
The reactor of AF2B is made of acrylic material in the form of a horizontal tank with a volume capacity of 50 L. The AnF2B reactor has 3 chambers where each chamber contains a biofilter wasp nest-shaped for bacterial consortium (as biocatalyst). The reactor of AnF2B operated at 29 - 31 C and pH at 6 - 7.5 by adjusting using a pH adjuster. The reactor operated continuously without recycle flow, and to maintain the anaerobic conditions of the reactor that is flowed nitrogen gas at the start of the operation. The starter produced from the acclimatization process of the bacterial consortium isolate is then poured into the AnF2B reactor as much as 2 L, and cassava starch effluent is added gradually until it reaches a volume of 10 L, which is furthermore known as acclimatized wastewater. The acclimatization process of cassava starch effluent in the AnF2B reactor takes place until maximum growth is achieved. If the growth of the bacterial consortium (biocatalyst) has been maximized, fresh cassava starch effluent will flow into the reactor with several variations of COD load.

The COD loading were used of 2 g, 3 g, 4 g, and 5 g of COD. In this research, a COD loading of 2 g mean that 10 L of acclimated cassava starch wastewater is taken from the AnF2B reactor and 40 L of fresh cassava starch wastewater (COD: 10,000 mg·L$^{-1}$) was flowed into the reactor with flowrate of 8 L·h$^{-1}$, so the waste volume in the reactor was kept constant at 50 L (Hydraulic Residence Time, HRT: 5 h). Liquid samples were withdrawn from the effluent valve and then COD concentration measurements using open reflux method which is in accordance with APHA standards [20], while the CH$_4$ gas that effluent of the top of the reactor is collected and measured using Gas Analyzer type AS8900.
3. Result and Discussion

3.1 Effect of Organic Loading on Biogas Production Rate

In general, AnF2B reactor operate properly to decomposing organic compounds in cassava starch wastewater into biogas, where the analysis results show that the maximum biogas produced on the 3rd day contains CH$_4$ (85%), CO$_2$ (14.5%), and CO (0.5%) with a total volume of biogas of 5.889 L. Figure 2, shows that the organic load affects the volume of CH$_4$ produced where the greater the value of organic load in wastewater, the greater the CH$_4$ gas produced. This is because the greater the organic load, the greater the organic content in tapioca flour wastewater so that more organic material is decomposed into biogas or CH$_4$ gas. The organic materials in cassava starch wastewater would be converted by anaerobic bacteria into biogas through hydrolysis, acidogenesis, acetogenesis, and methanogenesis process [5,10,21]. At the initial process, anaerobic bacteria require around 5-6 day to reach the methanogenesis process. However, this experiment using HRT for 5 hours has produced methane gas at the 12th hour and reached maximum production on the 3rd day (48 hours). This depends on HRT, the form of the media and the type of anaerobic bacteria used, where in this experiment using an HRT of 5 hours, the type of wasp nest-shaped biofilter as media and a consortium of indigenous bacteria have a faster ability to decompose organic matter into gas methane [13,22–26].

After reaching optimal conditions, the biogas production process slowly decreases along with decreasing ratio of decomposer-substrate (organic material) in the biofilter, where with a continuous decomposition time there is a portion of the decomposer carried by effluent as Figure 1.
Figure 2 shows that the greater the COD load, the greater the volume of CH$_4$ gas produced. This means that the decomposition process of organic matter into CH$_4$ gas is in line with the concentration of organic matter in the wastewater [26-27].

The continuous stirred tank reactor (CSTR) confirmed that the co-digestion ratio of WAS:SS (1:1) can enhance biogas yield and CH$_4$ yield at 301 and 142 L·kg$^{-1}$ TVS, respectively at retention time of 11 days [28]. The Hybrid Upflow Sludge Blanket (HUASB) with an organic load of 0.155 kg COD·kg$^{-1}$ VSS·day can produce biogas of 54 - 56 % [27]. Whereas in this study, the AnF2B reactor contained a wasp nest-shaped biofilter and used a consortium of indigenous bacteria, at a COD loading rate of 5 g and decomposition time of 3 days which it could produce methane gas of 5.889 L (85.6% CH$_4$). Thus, the reactor AnF2B using wasp nest-shaped biofilter is more effective than CSTR reactors and HUASB.

3.2 COD performance in biogas production

The value of COD of the cassava starch wastewater from the cassava starch factory before it being fed into the AnF2B reactor from 9,000 - 20,000 mg·L$^{-1}$ and fluctuate from time to time, and it could decrease to around 860 mg·L$^{-1}$ (Figure 3). The maximum value of COD removal of 85.6% occurs on the 3$^{rd}$ day when CH$_4$ gas production is maximum and decreases fluctuatively according to the concentration of COD influent. The value of COD decreased because some of the organic materials was into CH$_4$ and other compounds [25-26,29-31].

The decreasing COD was influenced by the stages of metabolism and biocatalyst growth. On the first day, the hydrolysis and acidogenesis processes occurred so the value of COD decreased drastically. The next stage was methanogenesis process, which decomposed the organic substance in wastewater into CH$_4$. In this process, the biocatalyst grows well and then forms a layer on the surface of the biofilter so that it is able to convert organic materials (COD) into CH$_4$ gas. On the 3$^{rd}$ day, the bacterial consortium was able to decompose organic matter by 85.6% [7,8,11,32]. Furthermore, after the 3$^{rd}$ day, the bacterial growth rate was smaller than the bacterial death rate so that the high COD influent was unable to decompose optimally. This is shown by the higher concentration of Volatile Liquor Suspended Solid (VLSS) and COD effluent. Besides that, in this study there is no recycle flow from the effluent so that the concentration and growth of bacteria in the reactor cannot be maintained optimally.
Fig 4. The concentration of COD in influent-effluent during the decomposition process

Meanwhile, tapioca starch wastewater treatment using a batch biofilm reactor have COD removal of 88% [33]. The UASB reactor with COD load of 42 kg COD·m⁻³·d⁻¹ and HRT 8.3 - 8.4 hours had COD removal from 82.5 - 90.7% [34]. Thus, the continuous flow non-recycled AnF2B reactor has less capability than UASB or batch biofilm reactors.

4. Conclusions
The results showed that CH4 gas production and biogas composition were influenced by organic load, where biogas production increases with increasing amount of organic load. The maximum of COD loading was 5 g and decomposition time of 3 days can produce a biogas of 6.928 L. The biogas produced contains CH₄ (85%), CO₂ (14.5%), and CO (0.5%).

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