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Steady State of Anaerobic Hybrid Bioreactor with Acidogenesis Phase for Biohydrogen Production from Palm Oil Mill Wastewater

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ABSTRACT

The utilization of industrial waste as an alternative energy source, such as Palm Oil Mill Effluent (POME), represents a promising approach, as it has a high potential to be converted into energy while simultaneously reducing environmental pollution. This research aims to achieve a steady state condition, so that it can be operated continuously using a pilot scale acidogenesis phase anaerobic hybrid bioreactor with a capacity of 12.5 L. This study employed a 12.5 L laboratory reactor operated at a controlled 24-hour Hydraulic Retention Time (HRT) to realistically represent the interactions and operating conditions commonly encountered in practical environmental systems. The results of the study showed that the seeding and acclimatization process lasted for 16 days and the start-up stage lasted for 71 days until the steady state of anaerobic hybrid bioreactor was achieved with pH conditions of 7. The Chemical Oxygen Demand (COD) loading rate was 2,240 mg/L-hour, COD concentration of 3,750 mg/L, alkalinity concentration of 1,920 mg/L, the Volatile Fatty Acids (VFA) concentration of 589 mg/L. The Total Solids (TS) concentration was 30,800mg/L and Total Suspended Solids (TSS) concentration of 9,400mg/L. The Total Volatile Solids (TVS) concentration was 24,100mg/L, the Volatile Suspended Solids (VSS) concentration of 3,100mg/L and biogas production of 1.8 L/hour. The steady state of the acidogenesis phase anaerobic hybrid bioreactor with a hydraulic retention time of 1 day can obtain a COD removal efficiency of 93.3% with a VFA/Alkalinity ratio of 0.3.

KEYWORDS: *Fuel, Biohydrogen, Anaerobic hybrid bioreactor, POME, Energy transition.*

1. INTRODUCTION

The Government of the Republic of Indonesia is currently developing a Grand National Energy Strategy to ensure the availability of sufficient energy, with good quality, affordable prices and environmentally friendly. The implementation of the Grand National Energy Strategy also takes into account the current state of national energy development, by paying attention to available new and renewable energy sources (EBT), and adapting to the economic trends of EBT. On the other hand, the utilization of renewable energy in Indonesia has not been utilized optimally. Government Regulation of the Republic of Indonesia Number 79 of 2014 emphasizes minimizing the use of petroleum and maximizing the use of EBT by considering the economic level, including the utilization of biohydrogen-based energy, both for transportation and industrial activities. Biohydrogen energy is a non-carbon energy that has long-term potential as an alternative fuel to replace conventional fossil fuels [1]. Biohydrogen production can be obtained by an anaerobic process through the acidogenesis phase using raw materials containing carbohydrates, proteins and fatty oils. One of the abundant raw materials in Indonesia is palm oil mill wastewater (Palm Oil Mill Effluent, POME) because Indonesia is the largest contributor of crude palm oil in the world, namely 40% [2].

Biohydrogen from palm oil biomass offers various advantages, namely no carbon emissions, while the use of biohydrogen as a fuel to produce heat and pure water as a by-product [3]. In addition, biohydrogen is considered a future energy carrier that can be consumed for various purposes in the form of fuel. The development of biohydrogen production through anaerobic fermentation using bacterial communities derived from animal waste to produce new and renewable energy that is sustainable in the future by reviewing various aspects in order to produce process improvements in biohydrogen production by anaerobic bacteria in the acidogenesis phase [4]. Biohydrogen has an important role in future energy because it is environmentally friendly, renewable, sustainable and the energy used is also low [5]. In addition, fossil energy sources are a significant contributor to greenhouse gases.

Biohydrogen production involves several process stages, namely the hydrolysis process and the acidogenesis process. The performance of the acidogenesis phase depends on the hydrolysis process to break down polymeric organic compounds into simple organic compounds, then the simple organic compounds are converted into acetic acid, H_2 gas, and CO_2 [6].The novelty of biohydrogen generation technology using a hybrid anaerobic acidogenesis bioreactor lies in the combination of a suspended growth bioreactor and an attached growth bioreactor using solid media as a cell immobilization medium, thus improving the control of cell biomass washout. Furthermore, the growth of anaerobic bacteria in the acidogenesis phase can be optimized by inhibiting the growth of anaerobic bacteria in the methanogenesis phase, thus optimally supporting the growth of anaerobic bacteria in the acidogenesis phase.This can occur because the growth rate of the biohydrogen-producing acidogenesis bacteria group is much faster than the growth rate of biomethane-producing methanogenesis, namely μ_{max}= 2.0 day^{-1} for the acidogenesis stage and μ_{max}= 0.4 day^{-1} for the methanogenesis stage [7];[8].

Therefore, a breakthrough is needed to separate the growth of acidogenic bacteria to produce biohydrogen and inhibit the growth of methanogenic bacteria. This article aims to examine the steady-state conditions of an anaerobic bioreactor in the acidogenesis phase.

2. METHOD

This research utilized a laboratory-scale system intentionally configured to emulate conditions typically found in real environmental applications. The reactor, having an effective volume of 12.5 L, was operated in a carefully controlled manner to maintain consistency and experimental reliability. Throughout the study, a Hydraulic Retention Time (HRT) of 24 hours was applied to reflect practical operating conditions and to capture the interaction between pollutants and the treatment process. This setup enabled detailed observation of treatment performance, mass transfer behavior, and system stability under representative conditions, while allowing accurate control and monitoring of the main operational variables. The anaerobic hybrid bioreactor system with acidogenesis phase is shown in Figure 1,

As illustrated in Figure 1, the POME wastewater traverses the bioreactor through a series of internal partitions that dictate a sequential down-and-up flow path. This specific hydraulic configuration ensures comprehensive contact time within the treatment zones before the treated liquid finally gravitates into the effluent tank. This additional feed is intended to stimulate and suppress bio-film growth. The stages of research activities in the first year are described as follows:

a) Raw Material Preparation

Palm oil mill wastewater (POME) was taken directly from one of PTPN IV's regional 3 palm oil mills. After that, the POME was measured for Chemical Oxygen Demand (COD), pH, acetic acid, alkalinity, and Volatile Suspended Solids (VSS) levels. The required substrate volume was approximately 9.6 liters, with a sludge-to-water ratio of 1:1 (v/v).

b) Anaerobic Bacteria Breeding and Acclimatization Stage

Nursery aims to grow and develop microbes that will be used in this study. The microorganisms used in this study are mixed cultures derived from cow dung because cow dung contains specific microorganisms [9]. Cow dung is filtered and mixed with palm oil mill wastewater substrate. At this stage, the microorganisms are cultured at room temperature. The resulting culture is then put into an anaerobic digester until it reaches a volume of 12.5 L.

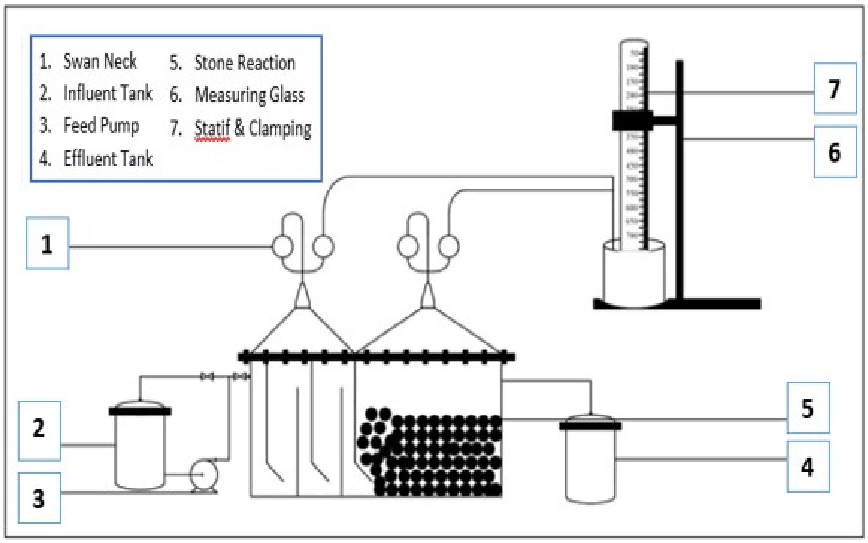

Figure 1: Anaerobic hybrid bioreactor set-up acidogenesis phase

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The next stage of acclimatization aims to enable microorganisms to adapt to the conditions of the wastewater to be processed. Furthermore, the acclimatization process is used for the biodegradation of organic materials in palm oil mill wastewater. The acclimatization process is carried out using the fill and draw method, which involves taking the liquid from the anaerobic digester and adding fresh palm oil mill wastewater in the amount of samples taken daily [9]. The acclimatization process can be stopped if the VSS fluctuation is less than 10%.

c) Bioreactor Start-Up Stage

The biomass from the acclimation process was introduced into the bioreactor. Nitrogen gas was then injected into the system to remove dissolved oxygen from the liquid, and then allowed to stand for three days. This allowed the biomass from the mixed culture to settle.
The POME waste to be processed is then fed into the feed tank. Using a pump, the POME waste is flowed into the bioreactor by controlling the valve opening to obtain a feed flow rate of 0.52 L/hour. The output from the bioreactor is analyzed for parameters such as pH, COD, volatile acid such as acetic acid, alkalinity, biomass concentration, and biogas volume. The start-up process is carried out until a steady state is achieved with COD fluctuations, COD removal efficiency, and a Volatile Fatty Acid (VFA) ratio to relative alkalinity of 10%.

d) Continuous Stage

After steady state was achieved, the process duration was varied with a hydraulic retention time of 24 hours. Samples were taken daily for analysis of pH, COD, volatile acids such as acetic acid, alkalinity, biomass concentration, and biogas volume. The analysis was conducted in accordance with [10].

3. RESULTS

3.1 The pH Profile of Start-up Conditions of Anaerobic Hybrid Bioreactor

The pH profile during the start-up phase of the anaerobic hybrid bioreactor is presented in Figure 2. This profile illustrates the initial stabilization behavior of the system under early operational conditions. As illustrated in Figure 2, the pH levels exhibited significant volatility during the initial start-up phase, ranging between a minimum of 4.56 and a maximum of 6.31.

The fluctuation in pH values in the acidogenesis phase of the anaerobic hybrid bioreactor is caused by the formation of volatile acids such as acetic acid, propionic acid, valeric acid, formic acid, butyric acid, CO_2 and H_2 gas, which will affect the overall acidity level of the liquid. At the beginning of the start-up on day 1 to day 69, the pH fluctuation is relatively low. In this condition, if the pH of the liquid is outside the range of 6.8 – 7.4, chemical compounds are added to the bioreactor [11]. However, in this research, the pH was maintained low at pH 6.8 because the biohydrogen formation process took place at the acidogenesis stage.

According to [12] the optimum conditions for the acidogenesis stage occur at a pH range of 5.5 to 6.5. According to [13] at a pH below 6.5, the activity of methane bacteria is very low. Hence, it is estimated that the bacteria

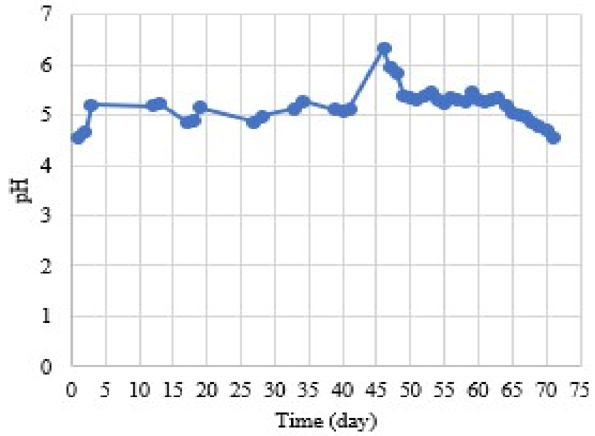

Figure 2: The pH profile of bioreactor start-up conditions

will die due to the biodegradation of the oils and fats contained in the wastewater from palm oil mills through the mechanism–oxidation produces propionic acid and butyric acid in addition to acetic acid. Propionic acid and butyric acid are then converted by a group of acetogenic bacteria into methane gas and CO_2 gas. According to [14], free fatty acids that have an even number of carbons can produce acetic acid and hydrogen, while free fatty acids with an odd number of carbons can produce acetic acid, propionic acid and hydrogen.

Thus, the start-up process of anaerobic hybrid bioreactor for producing biohydrogen has met the optimum conditions for the acidogenesis stage process in the pH range of 4.56 to 6.31 so that it is able to achieve a steady state condition for the anaerobic hybrid bioreactor in the acidogenesis phase.

3.2 VFA Profile of Anaerobic Hybrid Bioreactor Start-up Conditions

The VFA profile of the anaerobic hybrid bioreactor start-up conditions is shown in Figure 3. The fluctuation of volatile fatty acids during the operational process is an indication of the ongoing competition between the acidogenic bacterial group and the methanogenic bacterial group that is shown in Figure 3. The activity of acidogenic bacteria causes the concentration of volatile fatty acids to be high, while the activity of the methanogenic group causes the concentration of volatile fatty acids to be low [15].

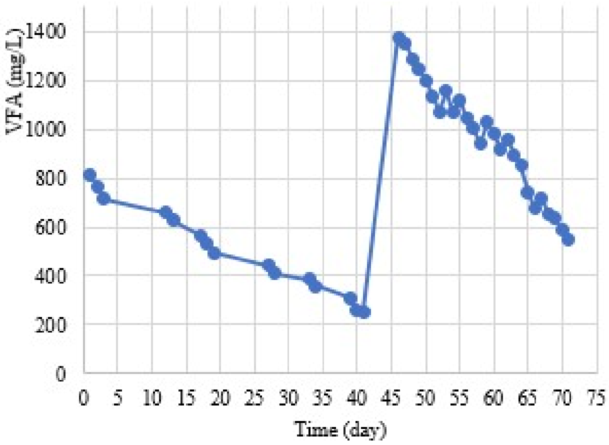

Figure 3: The VFA profile of anaerobic hybrid bioreactor start-up conditions

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On day 1 to day 41, the concentration of volatile fatty acids decreased from 811 mg/L to 254 mg/L, however, there was an increase in volatile fatty acids on day 42 of 1375 mg/L and decreased again from day 43 to day 71. According to [12] that the optimum condition for the acidogenesis stage occurs at a volatile fatty acid concentration of 5458 mg/L and according to [16] that the methanogenesis stage occurs optimally in the range of volatile fatty acid concentrations from 50 to 500 mg/L.

The concentration of volatile fatty acids obtained ranged from 254 mg/L to 1375 mg/L. This range of volatile fatty acid concentrations is relatively low compared to other researchers 2000-10,000 mg/L[17]; 2000-8000 mg/L[12]. However, when reviewed against the substrates used, it turns out that in general previous researchers used substrates containing carbohydrates or containing proteins. This is understandable because substrates containing carbohydrates or proteins are easier to break down compared to substrates containing oils and fats [14].

Ahmad (2001) [19] obtained a volatile fatty acid concentration of approximately 400 mg/L at a solid retention time (SRT) of 8 hours using glucose as a substrate. Meanwhile, [12] obtained a volatile fatty acid concentration of 5,458 mg/L at a hydraulic retention time of 1 day using palm oil industry wastewater as a substrate, while [6] obtained a volatile fatty acid concentration of 2,026 mg/L at a hydraulic retention time of 2 days using palm oil industry wastewater. Meanwhile, [18] obtained a volatile fatty acid concentration of 13 mg/L at an organic loading of 4.2 kgCOD/m^3-day using a substrate containing long-chain fatty acids (oleic acid). This shows that substrates containing oil and fat have lower volatile fatty acid values compared to substrates containing carbohydrates or proteins.

In previous research, [15] has conducted an acidogenesis process using a two-phase anaerobic fluidized bed bioreactor system, namely a separate acidogenesis phase and a separate methanogenesis phase. In the acidogenesis phase with a hydraulic retention time of 48 hours, a VFA concentration of 2026 mg/L was obtained. Furthermore, [19], has discovered a single-phase anaerobic separator bioreactor system, namely an integrated acidogenesis phase and a methanogenesis phase with a capacity of 10 L which is capable of converting palm oil mill wastewater with a hydraulic retention time of 7.5 hours, a VFA concentration of 234 mg/L was obtained.

In addition, [2] has conducted an acidogenesis process using a two-phase polyethersulfone-based anaerobic membrane bioreactor system with a hydraulic retention time of 20 hours in the acidogenesis phase, obtaining a VFA concentration of 1420 mg/L. [20] has conducted an acidogenesis process using a two-phase polypropylene-based anaerobic membrane bioreactor system with a hydraulic retention time of 24 hours with a VFA concentration of 520 mg/L.

Therefore, the volatile fatty acid concentration during the acidogenesis phase of the anaerobic bioreactor startup process proceeded well because the concentration value was relatively high compared to the optimum conditions in the methanogenesis phase. The stability of the volatile fatty acid concentration can be influenced by alkalinity, which acts as a buffer for the volatile fatty acids, thus stabilizing the system's pH.

3.3 Alkalinity Profile of Start-up Conditions of Anaerobic Hybrid Bioreactor

The alkalinity profile of the anaerobic hybrid bioreactor start-up conditions is shown in Figure 4. It can be seen in Figure 4, the alkalinity fluctuation during the start-up process is an indication of the reaction between acetic acid as a volatile fatty acid and the alkalinity formed in the system. This reaction causes the alkalinity concentration to be high, while the volatile fatty acid concentration becomes low. On day 1 to day 71, the alkalinity concentration ranged from 760 mg/L to 1860 mg/L. According to [16], the optimum alkalinity concentration is in the range of 2000 to 3000 mg/L. The alkalinity formed in wastewater comes from CO_2 which combines with water to form carbonic acid. Furthermore, the carbonic acid dissociates to form hydrogen ions and bicarbonate ions. These ions act as buffers [21].

According to [12] that the optimum condition for the acidogenesis stage occurs at an alkalinity concentration of 2105 mg/L and according to [16] that the methanogenesis stage occurs optimally in the alkalinity concentration range of 2000 to 3000 mg/L. Thus, the alkalinity value during the start-up process of both the acidogenesis phase bioreactor and the methanogenesis phase bioreactor cannot occur at optimum conditions.

The alkalinity concentration obtained ranged from 760 mg/L to 1860 mg/L. This alkalinity concentration range is relatively low compared to other researchers [15]. However, when reviewed against the substrates used, it turns out that in general previous researchers used substrates containing carbohydrates or containing proteins. This is understandable because substrates containing carbohydrates or proteins are easier to decompose than substrates containing oils and fats [14].

Therefore, the alkalinity concentration during the acidogenesis phase of the anaerobic bioreactor startup process proceeded smoothly because it was relatively low compared to the optimum conditions during the methanogenesis phase. Volatile fatty acids can influence the stability of this alkalinity concentration, resulting in relatively low fluctuations.

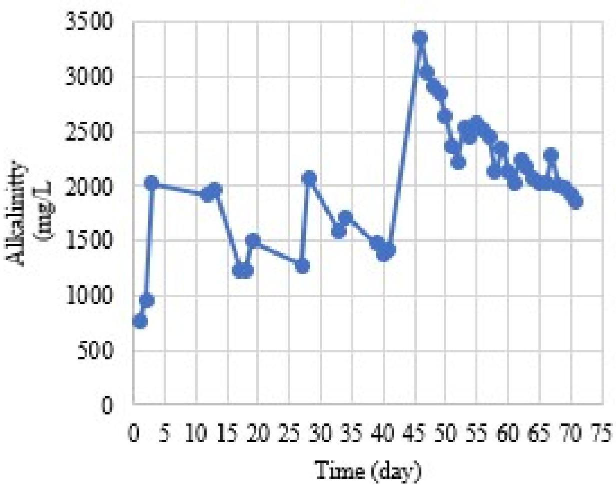

Figure 4: Alkalinity profile of start-up conditions of anaerobic hybrid bioreactor

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3.4 The VFA-Alkalinity Ratio Profile of Bioreactor Start-up Conditions

The VFA-Alkalinity ratio profile of the anaerobic hybrid bioreactor start-up conditions is shown in Figure 5. Figure 5 shows that the ratio of volatile fatty acids to alkalinity during the start-up process in the acidogenesis phase bioreactor was obtained in the range of 0.29 to 0.48. The stability of the anaerobic hybrid bioreactor can be seen from the relationship between acetic acid and alkalinity. According to [21] that the optimum ratio of volatile fatty acids to alkalinity is below 0.4 so that the anaerobic hybrid bioreactor system has high stability. This study shows high stability because the ratio of alkalinity and volatile fatty acids obtained is smaller than 0.4.

Thus, the relationship between volatile acids and alkalinity is an indicator of process stability. The ratio between volatile acids and alkalinity at the start-up acidogenesis phase of the anaerobic hybrid bioreactor was carried out under optimum conditions. This figure indicates that the process was stable during the start-up of the acidogenesis phase of the anaerobic hybrid bioreactor, thus achieving steady-state conditions.

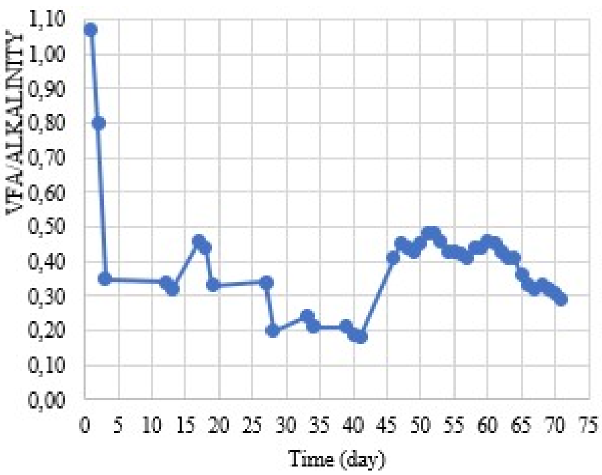

Figure 5: Start-up VFA-alkalinity ratio profile anaerobic hybrid bioreactor

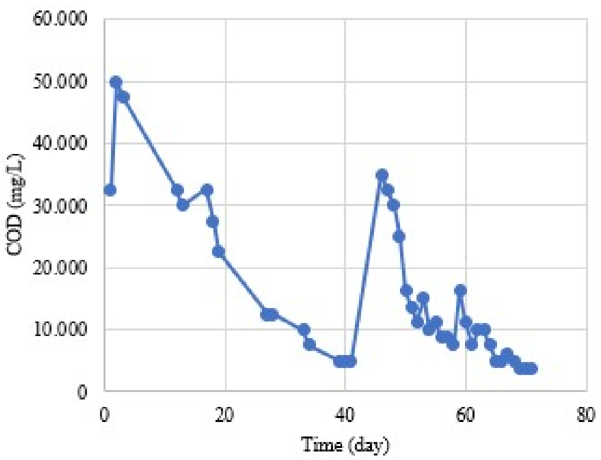

Figure 6: The COD profile start-up condition anaerobic Hybrid Bioreactor

3.5 The COD Profile Bioreactor Start-up Conditions

The COD profile of the start-up conditions of the Anaerobic Hybrid Bioreactor is shown in Figure 6. Figure 6 shows that the COD concentration during operation at operating conditions start-up relatively fluctuated with the lowest COD concentration at a concentration of 3750 mg/L. These results indicate that anaerobic bacteria are able to degrade organic compounds contained in palm oil mill wastewater into biogas so that the COD concentration is low at 3750 mg/L from a feed concentration of 50,000 mg/L. The decrease in COD concentration indicates that anaerobic microorganisms in the anaerobic hybrid bioreactor are able to be highly active in processing the wastewater used [22]. This decrease also proves that the formation of a layer of microorganisms on the attached media (in this case using stone media) takes place and is followed by biodegradation of complex organic compounds that produce biogas. This biodegradation will affect the resulting COD concentration, so if the COD concentration is low.According to [23] during the start-up period, the anaerobic hybrid bioreactor remains in a non-steady state or also known as a quasi-steady state until the biofilm fully develops. The process can be said to be complete when it has reached a steady state, which is characterized by a fluctuation in COD concentration of 10%

According to [15], during the start-up stage, the bioreactor remained in a non-steady state until the bio-film fully developed. The results of this study showed that COD concentrations began to remain constant from day 69 to day 71, indicating that a steady state (non-steady state) had been achieved. [21]and [24] explained that the degradation of organic compounds in the anaerobic process includes 4 stages, namely the hydrolysis stage, the acidogenesis stage, the acetogenesis stage, and the methanogenesis stage. In the start-up period, these four stages occur simultaneously in a bioreactor so that during the steady state condition, COD concentration fluctuations occur, but after the steady state condition is reached, the COD fluctuations are relatively low. In previous research, [15] has conducted an anaerobic process using a two-phase anaerobic fluidized bed bioreactor system, namely a separate acidogenesis phase and a separate methanogenesis phase. In the acidogenesis phase using a hydraulic retention time of 48 hours, the effluent COD was obtained at 20.8 g/L. Furthermore, [19] has found a single-phase anaerobic baffle bioreactor system, namely an integrated acidogenesis phase and a methanogenesis phase with a capacity of 10 L which is capable of converting palm oil mill wastewater using a hydraulic retention time of 7.5 hours, obtaining a COD concentration of 0.67 g/L.

In addition, Ahmad et al [2] have conducted an acidogenesis process using a two-phase polyether-sulfone-based anaerobic membrane bioreactor system using a hydraulic retention time of 20 hours with a COD removal efficiency of 59 - 61%, obtaining a COD concentration of 7.2 g/L. [20] have conducted an acidogenesis process using a two-phase polypropylene-based anaerobic membrane bioreactor system using a hydraulic retention time of 24 hours, obtaining a COD concentration of 4.4 g/L.

Thus, the decrease in COD concentration during the start-up process of the anaerobic bioreactor in the acidogenesis phase took place well because the COD concentration decreased from 50,000 mg/L to 3,750, which indicates that the biodegradation process of the palm oil mill wastewater substrate to produce biogas was relatively good until a steady

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state was reached in the anaerobic hybrid bioreactor in the acidogenesis phase.

3.6 COD Removal Efficiency Profile of Bioreactor Start-up Conditions

The COD Removal Efficiency Profile of the Anaerobic Hybrid Bioreactor start-up conditions is shown in Figure 7. The highest COD removal efficiency was obtained at 93% (Figure 7). According to [25] an anaerobic bioreactor with a removal efficiency of 90% is a system capable of degrading organic components well. The removal efficiency in this study was relatively higher compared to the removal efficiency obtained by [26] with the same bioreactor system, but using oil palm frond cell immobilization media and also higher compared to the bioreactor system using oil palm empty bunch cell immobilization media [27].

Figure 7 shows that from day 1 to day 71 there was an increase in COD removal efficiency. The COD removal efficiency continues to increase during the start-up period until a steady state is reached. The increase in COD removal efficiency is thought to be due to the higher concentration of biomass surrounding the carrier particles. The increase in biomass concentration on the carrier particles is accelerated by continuously introducing feed. Given the relatively high COD removal efficiency (93% in bioreactor I and 93% in bioreactor II) at start-up (steady state) conditions, palm oil industry wastewater can be processed using an anaerobic fluidized bed bioreactor.

In the research of [28], processing molasses wastewater using an anaerobic bioreactor with stone media obtained the highest COD removal efficiency of 55%. Meanwhile, [29], processing palm oil mill wastewater obtained the highest COD removal efficiency value for empty oil palm bunches and oil palm fronds media of 73.3% and 77%. In previous research, [15] has conducted an anaerobic process using a two-phase anaerobic fluidized bed bioreactor system, namely a separate acidogenesis phase and a separate methanogenesis phase. In the acidogenesis phase, a hydraulic retention time of 48 hours was used with a COD removal efficiency of 57%. Furthermore, according to Ahmad (2001) [19], has discovered a single-phase anaerobic separator bioreactor system, namely an integrated acidogenesis phase and a methanogenesis phase with a capacity of 10 L.

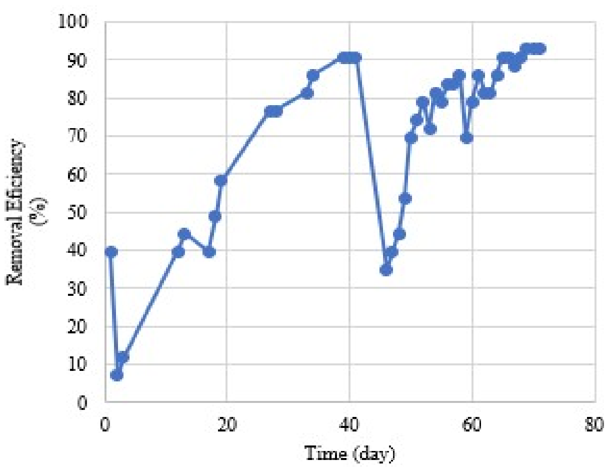

Figure 7: The COD removal efficiency profile for anaerobic hybrid bioreactor start-up conditions

That is capable of converting palm oil mill wastewater using a hydraulic retention time of 7.5 hours with a COD removal efficiency of 75% [19].

In addition, [2] have conducted an acidogenesis process using a two-phase polyether-sulfone-based anaerobic membrane bioreactor system using a hydraulic retention time of 20 hours with a COD removal efficiency of 59 - 61%, obtaining a COD concentration of 7.2 g/L. [20] have conducted an acidogenesis process using a two-phase polypropylene-based anaerobic membrane bioreactor system using a hydraulic retention time of 24 hours with a COD removal efficiency of 60.7%.

Thus, the COD removal efficiency during the start-up process of the anaerobic bioreactor in the acidogenesis phase was good because the COD removal efficiency was obtained at 93%, which indicates that the biodegradation process of the palm oil mill wastewater substrate to produce biogas was relatively good until the steady state of the anaerobic hybrid bioreactor in the acidogenesis phase was achieved.

3.7 TS Profile of Anaerobic Hybrid Bioreactor Start-up Conditions

The TS profile of the start-up conditions of the anaerobic hybrid bioreactor is shown in Figure 8. Figure 8 shows that the total solids content of the output during the start-up process in the acidogenesis phase bioreactor tended to decrease from a concentration of 41.2 g/L to 18.5 g/L on day 1 to day 41 of observation, but increased on day 42 to 41.5 g/L. Furthermore, starting from day 43, there was a decrease until day 71 to 30.1 g/L.

In Figure 8 shows that the Total Solids (TS) concentration has a decreasing tendency during the start-up period and conversely the COD removal efficiency has a tendency to increase. This is because the organic solids from palm oil mill wastewater are degraded by anaerobic bacteria to form biogas, thus reducing the organic solids in the effluent. Fluctuations in the solids content, both total solids (TS), from day 70 onwards were relatively small. This condition indicates that the steady statehas been achieved. An interesting thing to study is the relatively better biodegradation capability of the acidogenesis phase anaerobic hybrid bioreactor towards organic solids.

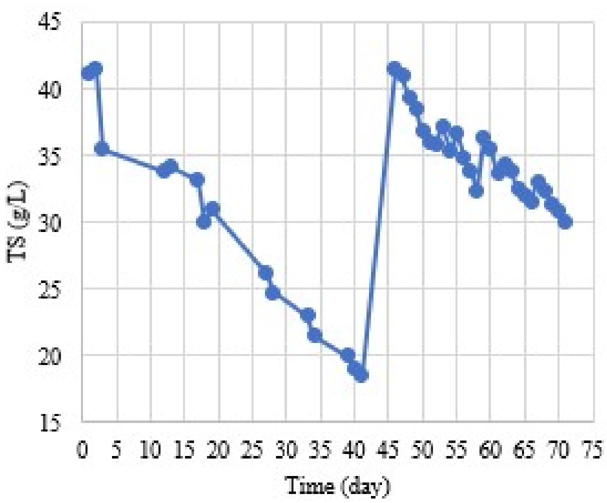

Figure 8: The TS profile of bioreactor start-up conditions

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This is because the organic solids carried out of the bioreactor are relatively small, resulting in a higher concentration of anaerobic biomass in the system. The biodegradation of organic solids indicates that the anaerobic bacteria present in the acidogenesis phase anaerobic hybrid bioreactor are able to reproduce.

Therefore, the significant decrease in TS concentration during the start-up process of the anaerobic bioreactor in the acidogenesis phase indicates the ability of anaerobic bacteria to biodegrade the palm oil mill wastewater substrate to produce relatively good biogas until the steady state of the anaerobic hybrid bioreactor in the acidogenesis phase is reached.

3.8 VSS Profile of Anaerobic Hybrid Bioreactor Start-up Conditions

The VSS profile of the start-up conditions of the Anaerobic Hybrid Bioreactor is shown in Figure 9. Figure 9 shows that the biomass concentration, represented by volatile suspended solids (VSS) at start-up conditions, tended to decrease until it reached a concentration of 2.97 g/L. The decrease in the effluent VSS concentration indicates that the bioreactor's ability to immobilize cells on solid media is relatively high, allowing anaerobic bacteria to adhere to the cell immobilization media. The implication is that the higher the cell concentration in the bioreactor, the faster the degradation process of palm oil mill wastewater into biogas. This is interesting to review because the anaerobic bacteria introduced have been well acclimatized to the wastewater used. As the bacteria adapt during the start-up process of the anaerobic hybrid bioreactor, it shows that most anaerobic bacteria are resistant to the operating conditions. The surviving anaerobic bacteria will later become the precursors of bacteria capable of degrading palm oil mill wastewater.

Figure 9 shows that significant biomass loss occurred at the start-up stage. This was due to the anaerobic bacteria adapted to the anaerobic hybrid bioreactor being partially capable of forming flocs/granules, while others were unable to form flocs. Anaerobic bacteria that did not form flocs were carried away by the outflow and measured as VSS from the bioreactor outlet.

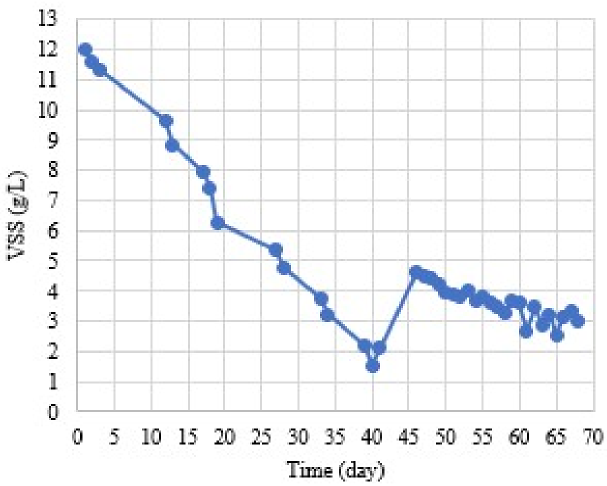

Figure 9: The VSS profile of anaerobic hybrid bioreactor start-up conditions

An interesting observation is that a biomass loss percentage of less than 10% indicates very low biomass loss, indicating that the system is very good at retaining biomass [30]. In addition, Total Suspended Solids (TSS) were relatively higher than VSS. In general, Total Suspended Solids (TSS) was approximately 40% greater than volatile suspended solids (VSS). This is because anaerobic biomass solids contain other solids besides organic solids derived from anaerobic bacteria. Using the VSS measurement method, volatile organic solids and other organic solids were measured.

In previous research,[15]has conducted an acidogenesis process using a two-phase anaerobic fluidized bed bioreactor system, namely a separate acidogenesis phase and a methanogenesis phase with a capacity of 25 L. In the acidogenesis phase with a hydraulic retention time of 48 hours, a VSS of 7.8 g/L was obtained. Furthermore, [19], has found a single-phase anaerobic baffle bioreactor system, namely an acidogenesis phase with an integrated methanogenesis phase with a capacity of 10 L which is capable of converting palm oil mill wastewater with a hydraulic retention time of 7.5 hours, a VSS of 0.85 g/L was obtained. According [2] have conducted an acidogenesis process using a two-phase polyether-sulfone-based anaerobic membrane bioreactor system with a capacity of 20 L with a hydraulic retention time of 20 hours, obtaining an MLSS concentration of 2.0-2.5 g/L. In addition, [20] have conducted an acidogenesis process using a two-phase polypropylene-based anaerobic membrane bioreactor system with a capacity of 20 L with a hydraulic retention time of 24 hours, obtaining an MLSS concentration of 4.8 g/L.

Thus, the acidogenesis phase anaerobic hybrid bioreactor is able to retain biomass and can prevent biomass loss (wash-out) due to being carried away by the flow, because this bioreactor has a ratio of biomass retention time to hydraulic retention time that is much greater compared to a perfectly mixed bioreactor system (CSTR, continuous stirred tank reactor). According to [31] that the advantages of anaerobic hybrid bioreactors include easy operation, shorter start-up time compared to anaerobic filter bioreactors, not easily clogged compared to anaerobic filters, UASB and fluidization reactors, does not require sludge recycling, does not require stirring and simple construction.

3.9 Biogas Volume Profile of Anaerobic Hybrid Bioreactor Start-up Conditions

The biogas volume profile for the start-up conditions of the anaerobic hybrid bioreactor is shown in Figure 10. The biogas production at the start of the start-up process was very low, around 120 mL/day that depicted in Figure 10. However, as the start-up process progressed, biogas production increased. On the 71st day, biogas production reached 1,860 mL/day. Furthermore, from the 68th to the 71st day, biogas production remained relatively constant at around 1,790 mL/day. The increase in biogas production during the start-up process indicates that anaerobic bacteria were relatively effective in degrading palm oil mill wastewater into biogas. According to [32], the pKa value of acetic acid is 4.75 and butyric acid pKa 4.82. At pH 4, acetic acid is in an undissociated form in the media. In line with the above, it can be seen that the efficiency of organic material removal is getting lower. The lower efficiency of organic material removal indicates that increasing organic loading causes the

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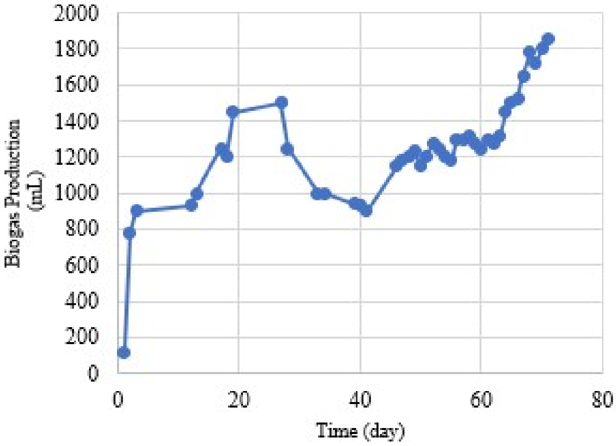

Figure 10: The biogas volume profile for start-up conditions of anaerobic hybrid bioreactor

methane gas formed to decrease, conversely CO_2 gas increases. This is understandable because with a high organic loading, it is essentially the same as increasing the feed flow rate so that the growth of the methanogenic bacteria group is disrupted. This disruption occurs because more organic material is broken down into acetic acid, resulting in the accumulation of acetic acid and inhibiting the growth of the methanogenic bacteria group, so that the acidogenic bacteria group grows well.

In previous research, [15] has conducted an acidogenesis process using a two-phase anaerobic fluidized bed bioreactor system, namely a separate acidogenesis phase and a separate methanogenesis phase with a hydraulic retention time of 48 hours, producing a biogas volume of 24,000 mL/day. Furthermore, [19], has discovered a single-phase anaerobic baffle bioreactor system, namely an integrated acidogenesis phase and a methanogenesis phase with a capacity of 10 L which is capable of converting palm oil mill wastewater with a hydraulic retention time of 7.5 hours to obtain a biogas volume of 12,600 mL/day.

Moreover, Ahmad et al (2002) [2] conducted an acidogenesis process using a two-phase polyether-sulfone-based anaerobic membrane bioreactor system with a hydraulic retention time of 20 hours, producing a biogas volume of 310 L/day. Then, Ahmad et al (2003) [20] has conducted an acidogenesis process using a polyether-sulfone-based anaerobic membrane bioreactor system with a hydraulic retention time of 48 hours with a biogas volume of 134000 mL/day. According [19] has found a single-phase anaerobic baffled bioreactor system with a capacity of 10 L which is capable of converting palm oil mill wastewater with good performance with an organic loading rate of 3.2 g/L-day with a hydraulic retention time of 20 hours with a biogas production of 5600 mL/day.

Furthermore, the biogas production during the start-up process demonstrates the biodegradation capability of palm oil mill wastewater using an acidogenesis-phase anaerobic hybrid bioreactor to produce biogas. Biogas production exhibits a progressive increase throughout the start-up phase of the anaerobic hybrid bioreactor. This upward trend continues until the acidogenesis-phase reactor reaches a steady-state condition.

3.10 Steady Condition of Anaerobic Hybrid Bioreactor

The steady state conditions of the anaerobic hybrid bioreactor in the acidogenesis phase are shown in Table 1. It can be seen in Table 1, the steady state was achieved after the start-up process lasted for 68 days, however, to ensure that the start-up process had run well, its operation was tested until it reached 71 days. During the period from day 68 to day 71, fluctuations were not significant so that it was determined that the duration of the anaerobic bioreactor start-up process until the steady state was reached was 71 days. The results obtained were slower than those carried out by [30] Grobicki and Stuckey (1991), but longer than those of other researchers as presented in Table 2.

Table 1: Steady state conditions of anaerobic hybrid bioreactor

No.	Parameter	Unit	Mark
1.	Bioreactor volume	L	12.5
2.	Hydraulic retention time	hour	24
3.	COD Charges	mg/L-hour	2,240
4.	Feed flow rate	L/hour	0.52
5.	pH	Unit	7
6.	COD influent	mg/L	53,850
7.	Effluent COD	mg/L	3,750
8.	COD removal efficiency	%	93.03
9.	Alkalinity	mg/L	1920
10.	Volatile fatty acids (VFA)	mg/L	589
11.	VFA/Alkalinity Ratio	-	0.307
12.	TS	g/L	30.77
13.	TSS	g/L	9.37
14.	TVS	g/L	24.07
15.	VSS	g/L	3.14
16.	Biogas Volume	L	1,793
17.	Processing time	Day	71

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Table 2: Steady-state process of anaerobic bioreactor with various wastes-water

Types of Bioreactors	Waste	Organic Load (kg/m^3-day)	Organic Removal Efficiency (%)	Duration (day)	References
BUFAN	Palm oil	-	88	48	Arief, 1992 [33]
BUFAN two stages	Palm oil	1.6 – 2.4	93	70	Ahmad, 1992 [15]
HBAR	Pig farm	-	-	45	Yang and Chou, 1985 [31]
ABR	Synthetic	1.2	83-84	25	Grobicki and Stuckey, 1991 [30]
ABR	Scotch Whisky	2.2	-	90	Boopathy et al., 1988 [34]
MABR	Pig farm	-	-	50	Boopathy and Sievers, 1991 [35]
BIOPAN	Palm oil	1.83	93	68	Faisal, 1994 [36]
BIOPAN	Palm oil	6.37	92	69	Retnowati, 1996 [37]
BIOPAN	Oils and fats	0.8	88	40	Ahmad, 2001 [19]
BIOHAN Acidogenesis Phase	Palm oil	2.24	93	71	This research

Information:
DSA = semi-continuous anaerobic digester; BUFAN = anaerobic fluidized bed bioreactor; HABR = hybrid anaerobic baffled reactor; ABR = anaerobic baffled reactor; MABR = modified anaerobic baffled reactor; BIOPAN = anaerobic baffled bioreactor; BIOHAN = anaerobic hybrid bioreactor.

Table 2 shows that the steady-state process is considered complete after the pH and COD concentration in each bioreactor are relatively stable. This condition can be said to have reached a steady state. The results of this research indicate that the steady-state process lasted for 71 days. Under these conditions, the COD removal efficiency was 93%. According to Chen et al. [11], the steady-state conditions of an anaerobic fluidized bed bioreactor with synthetic wastewater feed containing 10,000 mg/L COD were carried out for 60 days. After 60 days, the activated carbon bed particles were covered by a layer of bio-film, while according to [15] stated that the steady-state conditions of an anaerobic fluidized bed bioreactor using synthetic wastewater feed lasted for 56 days. Under these conditions, the sand bed particles were completely covered by bio-film. Other researchers, [38] reported that the steady-state conditions of an anaerobic fluidized bed bioreactor using synthetic wastewater feed lasted for 55 days. Under these conditions, COD removal efficiency was obtained at around 90% with a loading rate of 5.3 kg COD/m³day.

The relatively short time required to reach steady state is supported by several factors, including the characteristics and nutritional content of the wastewater. Waste water containing sufficient nutrients and minerals will accelerate the achievement of steady state bioreactor. Waste water with a balanced nutrient content between carbon, nitrogen and phosphorus with a COD: N: P ratio of 100: (10-1): (5-1) can accelerate the achievement of steady state bioreactor, while mineral elements that are very necessary in the growth period of anaerobic bacteria include iron (Fe), nickel (Ni), cobalt (Co) and molybdate (Mo) [39]. The nutritional and mineral content above is mostly found in the wastewater of the palm oil mill used.

Another factor that accelerates the time to achieve steady state bioreactor is the source of the inoculum used. The inoculum used in this process anaerobic hybrid bioreactor acidogenesis phase comes from anaerobic seeds derived from cow dung because cow dung contains various *Clostridium sp* bacteria [19]. The selection of anaerobic seeds used is based on the source of the seeds themselves because seeds derived from cow dung alkimatized with the same waste water will accelerate the stage of achieving steady state compared to seeds derived from installations that process different wastewater [39]; [40]. Whilts, according to [40], the amount of inoculums that can provide high activity and adaptation is 10% to 30%. The amount of inoculums used in this anaerobic hybrid bioreactor is 10% with a batch inoculation technique. [41];[42] stated that inoculation techniques also affect the process of achieving a steady state bioreactor. Inoculation techniques that are often used include batch inoculation and continuous inoculation. Continuous inoculation is not profitable because it requires a large number of seeds. In addition, the cultivation of anaerobic bacteria in the process of achieving a steady state anaerobic bioreactor is very dependent on the process conditions and the organic loading technique chosen.

The optimum conditions for non-methane processes (hydrolysis, acidogenesis and acetogenesis) range from pH 5.0 to pH 6.5 while the temperature for the mesophilic bacterial group ranges from 33 to 37 ^{0}C [25]. Meanwhile, the organic loading that is commonly used includes the low organic loading rate approach and the high organic loading rate. Low organic loading is more profitable than high organic loading because it does not require the addition of alkali compounds during the process. However, the excess of high organic loading also needs to be considered because the process occurs more quickly. The low loading limit is below 1 kgCOD/kg-VSS-day ([41]; [40]; [39].

Table 2 shows that the acidogenesis phase anaerobic hybrid bioreactor in this research with the same system in processing wastewater from the palm oil industry ([36]; [37] showed that COD removal in this research was relatively high, but the time to reach steady state was much longer, namely 71 days. This is because at pH 6 the activity of extracellular enzymes needed in the hydrolysis process can be optimally increased so that more substrates from the hydrolysis reaction are formed. This dissolved substrate is very useful for the growth of anaerobic bacteria and the formation of products and this substrate easily enters the cell membrane of anaerobic bacteria.

4. CONCLUSION

Based on the research results on the start-up process of the anaerobic hybrid bioreactor, the following conclusions can be drawn:

1. The acidogenesis phase in the anaerobic hybrid bioreactor reached steady state after 71 days. Although this duration was longer than in other researchers [30]; [33]; [15]; [31]; [34]; [35]; [36] and [37], the COD removal efficiency achieved was higher.
2. The acidogenesis phase of the anaerobic hybrid bioreactor reached steady state under a hydraulic retention time of 24 hours and an organic loading rate of 2.24 kg COD/m³·day, achieving a COD removal efficiency of 93% and producing an effluent with a COD concentration of 3,750 mg/L.
3. The stability of the anaerobic hybrid bioreactor system in the acidogenesis phase is relatively good with a volatile fatty acid to alkalinity ratio of 0.3.
4. The acidogenesis phase of the anaerobic hybrid bioreactor effectively minimizes biomass washout, as evidenced by the relatively low effluent VSS of 3,100 mg/L. As a result, the loss of anaerobic biomass is limited, allowing the system to maintain a relatively high concentration of anaerobic bacteria.
5. The steady-state condition of the acidogenesis phase anaerobic hybrid bioreactor produced 1,793 L of biogas in a process time of 71 days. This indicates that anaerobic bacteria are capable of converting palm oil mill wastewater into biogas with a hydraulic retention time of 1 day.

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