



Anammox biofilm process using sugarcane bagasse as an organic carrier

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ABSTRACT The anaerobic ammonium oxidation (anammox) biofilm process commonly uses various inorganic carriers to enhance nitrogen removal under anaerobic conditions. This study aims to analyze the performance of nitrogen removal in the anammox process using sugarcane bagasse as an organic carrier. The experiment was carried out by using an up-flow anaerobic sludge blanket (UASB) reactor for treating artificial wastewater at room temperature. The reactor was fed with ammonium and nitrite with concentrations of 70-150 mg-N/L and variations in the hydraulic retention time of 24 and 12 h. The granular anammox belongs to the genus *Candidatus Brocadia sinica* that was added as an inoculum of the reactor operation. The experimental stoichiometric of anammox for $\Delta\text{NO}_2^- - \text{N}$: $\Delta\text{NH}_4^+ - \text{N}$ and ΔNO_3^- : ΔNH_4^+ were 1.24 and 0.18, respectively, which is similar to anammox stoichiometry. The maximum Nitrogen Removal Rate (NRR) has achieved 0.29 kg-N/m³.d at Nitrogen Loading Rate (NLR) 0.6 kg-N/m³.d. The highest ammonium conversion efficiency (ACE) and nitrogen removal efficiency (NRE) were 88% and 85%, respectively. Based on these results, it indicated that sugarcane bagasse as organic carriers could increase the amount of total nitrogen removal by provided of denitrification process but inhibited the anammox process at a COD:NO₂⁻-N ratio of 2.02.

KEYWORDS Anammox; sugarcane bagasse; room temperature

1. Introduction

The sources of nitrogen pollution come from domestic and non-domestic wastewater. Non-domestic wastewaters, such as electroplating, fertilizer, and tannery industries, produce the wastewater which contains high ammonium content. The nitrogen pollutions from fertilizer, industrial wastewater, animal and human organic waste, increase the amount of ammonium concentration on the water surface. Together with other nutrients, high nitrogen concentration promotes eutrophication in receiving water bodies.

The process of nitrogen removal from wastewater could be carried out by various physical, chemical, and biological processes. In general, nitrogen removal is physically conducted by the adsorption process, while the biological nitrogen removal is considered for more effective and relatively inexpensive process, also it has been widely used to support physical processes (Ahn 2006). The conventional nitrification-denitrification method is generally applied for nitrogen removal for high carbon/nitrogen (C/N) containing wastewater. In the nitrification-denitrification process, ammonia is oxidized to nitrate under aerobic conditions, then nitrate is converted to dinitrogen gas using organic carbon as an electron acceptor (Jin et al. 2012). However, the con-

ventional nitrification-denitrification increases in operating expenses since it entails complete aeration, additional organic carbon such as methanol, and a large amount of sludge waste (Ali and Okabe 2015).

Anammox was discovered in the 1990s at TU-Delft, Netherlands and nitrite is used as an electron acceptor in converting ammonium to nitrogen gas in this process (Van De Graaf et al. 1996). Anammox is an innovative technology to biological nitrogen removal compared to conventional nitrification-denitrification, due to a higher nitrogen removal rate (NRR), lower operating costs, and no extra space required. In addition, the operation of anammox process is enabled to reduce 64% aeration, no organic carbon required, and less sludge waste (Ali and Okabe 2015).

The anammox process was applied on a laboratory, pilot, and full-scale for ammonium-rich wastewater treatment, such as leachate landfill, industrial and pharmaceutical wastewaters, and other types of wastes (Lackner et al. 2014). Various variations of anammox research have been applied, such as type of reactors, membrane bioreactor (MBR), fluidized bed reactor, sequencing batch reactor (SBR), up-flow anaerobic sludge blanket (UASB) and other modifications (Kumar et al. 2016). However, the UASB reactor has advantages compared to other reactors since no extra space is required and low construction and operating costs (Nathan and Scobell 2012).

TABLE 1 Composition of artificial wastewater.

Reactor	Artificial Wastewater (mg/L tap water)						Trace Element (ml/L tap water)	
	NH ₄ ⁺ -N	NO ₂ ⁻ -N	KH ₂ PO ₄	MgSO ₄ .7H ₂ O	CaCl ₂ .H ₂ O	KHCO ₃	I	II
UASB	70-150	70-150	27.2	300	180	500	1	1

Source: Van De Graaf et al. (1996)

Inorganic supporting carrier is used to enhance nitrogen removal in anammox biofilm processes, such as non-woven (Gao et al. 2012), string wound filter (Zulkarnaini et al. 2018), and polyvinyl alcohol and sodium alginate (PVA-SA) gel bead (Tuyen et al. 2018). Utilization of inorganic carrier is preferred to prevent the growth of heterotrophic bacteria that uses organic material as electron acceptors. Consequently, the fast growth heterotrophic bacteria eliminate the slow-growing rate (the maximum specific growth rate = 0.0027/h) anammox bacteria which has a doubling time of 10-12 d (Jetten et al. 1998). In addition, carbon as an organic matter is also one of the parameters for the anammox inhibition process. Anammox bacteria is a chemoautotrophic microorganisms that utilize CO₂ as the only carbon source (Strous et al. 1999). Therefore, the composition of substrate contained bicarbonate for the anammox process is important for the cultivation of anammox bacteria. The activity of anammox increased as the influent bicarbonate concentration rose from 1.0 to 1.5 g/L, and inhibited at 2.0 g/L (Liao et al. 2008).

Chen et al. (2012) reported that organic media, such as hollow bamboo sphere media and bamboo charcoal, is enable to accelerate the start-up of the anammox process. The existence of organic carbon could enhance nitrogen removal together with the denitrification process where nitrate converts to nitrogen gas in anaerobic condition. Nitrate is another product of the anammox process instead of nitrogen gas; therefore, a small amount of organic carbon could promote simultaneous anammox-denitrification process for nitrogen removal. The palm fiber has been used as a carrier for nitrogen removal by anammox process with the maximum nitrogen removal rate (NRR) of 0.20 kg-N/m³.d at NLR 0.29 kg-N/m³.d (Zulkarnaini et al. 2019). Therefore, organic material has the potential as a carrier in the anammox biofilm process for reducing the unuseful waste materials including various organic materials that are abundance in the environment.

In Indonesia, sugarcane is one of the major agricultural commodities with a plantation area of about 415.66 thousand hectares (BPS 2018). The agricultural product of sugarcane is used as a raw material in the sugar industry, however, it produces waste in the form of sugarcane bagasse in the production process. Sugarcane bagasse is insoluble because this material consists of cellulose, pentosan, and lignin (Allita et al. 2018). Therefore, in this research, sugarcane bagasse media was used as a carrier for facilitating the formation of biofilm in the nitrogen removal process. In this study, sugarcane bagasse is used in the UASB reactor for removing nitrogen content in wastewater through the anammox process.

2. Materials and Methods

2.1. Preparation of inoculum, organic carrier, and artificial wastewater

Granular anammox *Candidatus Brocadia sinica* from Kanazawa University, Japan, was used as inoculum for start-up of the reactor (Zulkarnaini et al. 2020). Previously, the cultivation anammox reactor was incubated at 35 °C where the growth temperature in the range 25-45 °C (Awata et al. 2013). Before starting the experiment, the bacteria were acclimatized at room temperature for three months. During the acclimatization process, anammox bacteria were fed with ammonium and nitrite.

Sugarcane bagasse in sheet form was used as a carrier to grow anammox biofilm and filled 25% in the reactor's volume. The eight sheets sugarcane bagasse carrier with dimension 15 cm length, 3.2 cm wide, and 0.2 cm thickness were filled vertically into the reactor to increase the contact surface area of the anammox process. Therefore the anammox process is not only occurred at the bottom of the reactor.

The artificial wastewater was designed with medium containing (per L) KHCO₃, 500 mg; KH₂PO₄, 27.2 mg; MgSO₄.7H₂O, 300 mg; CaCl₂.2H₂O, 180 mg; trace element solution I (per liter of distillate water contained EDTA 2Na 6.37 g; FeSO₄ 5 g), and trace element solution II (per liter of distillate water contained EDTA 2 Na 19.11 g; ZnSO₄ 0.241 g; CoCl₂.6H₂O 0.24 g; MnCl₂.4H₂O 0.99 g; CuSO₄.5H₂O 0.25 g; NaMnO₄.2H₂O 0.22 g; NiCl₂.6H₂O 0.19 g; NaSeO₄.10H₂O 0.024 g; H₃BO₄ 0.014 g). The substrate supplemented with (NH₂)₂SO₄ and NaNO₂ was fed into the reactor continuously (Table 1). During the medium preparation, the substrate was purged with nitrogen gas (N₂) for 30 min to remove the oxygen.

2.2. Reactor configuration and operation condition

The 300 mL acrylic UASB reactor was conducted for this experiment. The substrate was delivered through the bottom of the reactor using a peristaltic pump. To assure anaerobically condition, the tank of the substrate was

TABLE 2 Variation of influent concentration and hydraulic retention time.

Period	HRT (h)	NH ₄ ⁺ -N (mg/L)	NO ₂ ⁻ -N (mg/L)	NLR (kg-N/m ³ .d)
I	24	70	70	0.14
II	12	70	70	0.28
III	12	150	150	0.60

equipped with a gas bag containing N₂ gas. The reactor operated in three periods, with various concentrations of artificial wastewater, 70-150 mg-N/L (NH₂)₂SO₄ and NaNO₂, and hydraulic retention time (HRT) of 24 h and 12 h. The operation condition can be seen in Table 2.

2.3. Calculation of nitrogen removal performance

Anammox reactor performance was calculated based on nitrogen balance. Ammonium Conversion Efficiency (ACE,%), Nitrogen Removal Efficiency (NRE,%), Nitrogen Loading Rate (NLR, kg-N/m³.d) and Nitrogen Removal Rate (NRR, kg-N/m³.d) were calculated based on the Equation 1, 2, 3, and 4 (Zulkarnaini et al. 2019).

$$ACE(\%) = \frac{inf [NH_4^+ - N] - eff [NH_4^+ - N]}{inf [NH_4^+ - N]} \times 100\% \quad (1)$$

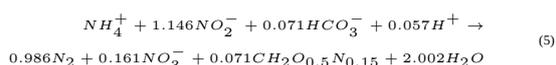
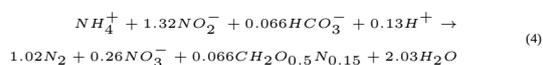
$$NRE(\%) = \frac{[NH_4^+ - N]_{in} + [NO_2^- - N]_{in} - [NH_4^+ - N]_{out} - [NO_2^- - N]_{out} - [NO_3^- - N]_{out}}{[NH_4^+ - N]_{in} + [NO_2^- - N]_{in}} \times 100\% \quad (2)$$

$$NRR(kg - N/m^3 \cdot d) = \frac{[NH_4^+ - N]_{in} + [NO_2^- - N]_{in} - [NH_4^+ - N]_{out} - [NO_2^- - N]_{out} - [NO_3^- - N]_{out}}{HRT} \times \frac{24}{1000} \quad (3)$$

3. Results and Discussion

3.1. Anammox stoichiometry

Anammox process in the reactor can be observed from the molar ratio of ΔNO₂⁻-N/ΔNH₄⁺-N to produce N₂ and NO₃⁻ (Hu et al. 2017), based on the stoichiometry of the anammox process defined by Strous et al. (1997) (Equation 4) and Lotti et al. (2014) (Equation 5).



The ratio of nitrite consumption to ammonium consumption (ΔNO₂⁻-N: ΔNH₄⁺-N) and the ratio of nitrate production to ammonium consumption (ΔNO₃⁻-N: ΔNH₄⁺-N) were calculated from the conversion of ammonium, nitrite, and produced nitrate. The average of the ratio ΔNO₂⁻-N: ΔNH₄⁺-N obtained from the experiment at stable period (12-75 d operation) was 1.16 molar. Based on Figure 2, the ratio ΔNO₂⁻-N: ΔNH₄⁺-N is similar to the ratio defined by Lotti et al. (2014). Reduction of ammonium and nitrite simultaneously indicated that one of the products of the anammox process, instead of nitrogen gas, is a small amount of nitrate (NO₃⁻) (Tsushima et al. 2007). In Figure 3, it showed the ratio of ΔNO₃⁻-N: ΔNH₄⁺-N

during this studies, the average of ΔNO₃⁻-N: ΔNH₄⁺-N obtained was 0.18, relatively similar to Lotti et al. (2014). At the beginning of the operation, the ratio of ΔNO₃⁻-N: ΔNH₄⁺-N was higher than stoichiometry of anammox reaction. This result tends to decrease and be lower than the stoichiometric until the end of the experiment. It could be a simultaneous anammox-denitrification process for nitrogen removal where nitrate produced by the anammox process is converted into N₂ in the presence of organic carbon originated from sugarcane bagasse. In agreement with the previous report that the application of sugarcane bagasse enhanced the nitrogen removal, thus this process can be developed for wastewater treatment (Li et al. 2016).

The difference of value ratio in Strous and Lotti's stoichiometry is occurred due to the different anammox process operation, such as the type of reactor, species of anammox bacteria, the form of biomass (granule or biofilm) and temperature in operation. Strous et al. (1997) used SBR with granular bacteria at an optimum temperature of 32-33 °C. In comparison, Lotti et al. (2014) used an MBR with biofilm-shaped bacteria at a temperature of 30 °C. This study is relatively similar to Lotti et al. (2014), although it used a different UASB reactor, both are continuous operation.

In this study, the temperature also affects the anammox process. The range of temperature during the whole

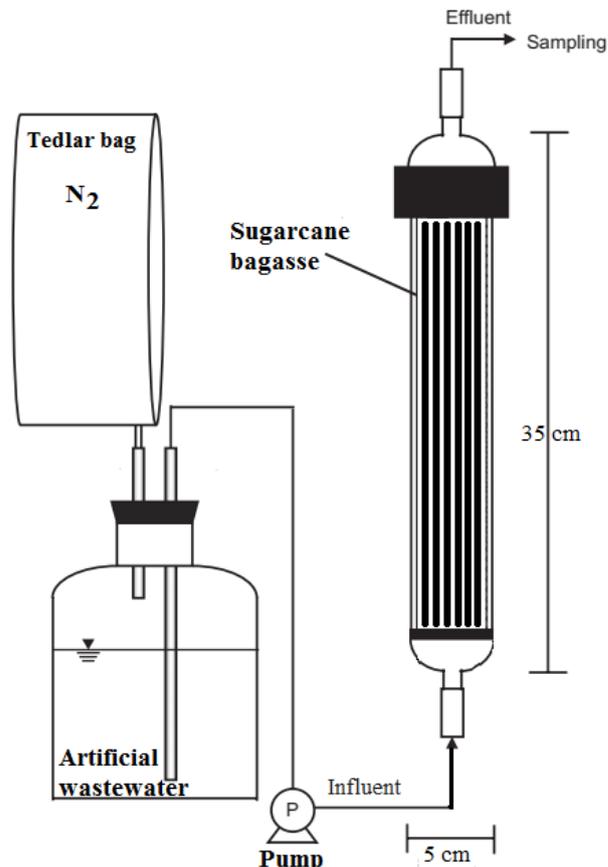


FIGURE 1 Reactor configuration of UASB anammox biofilm.

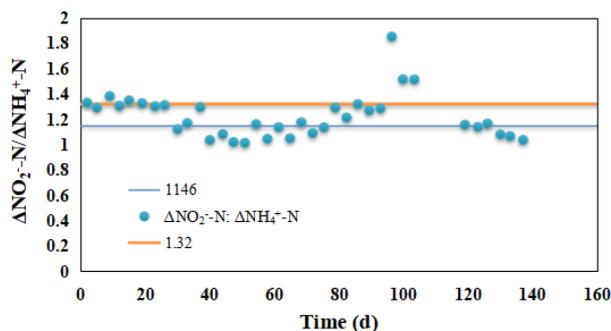


FIGURE 2 Ratio of $\Delta\text{NO}_2^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$ compared to the stoichiometry of anammox. The ratio of 1.32 defined by Strous et al. (1997) and 1.146 by Lotti et al. (2014).

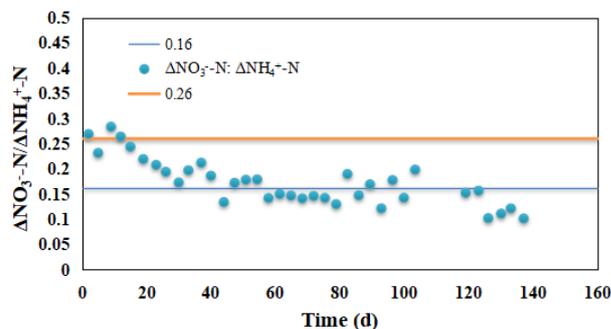


FIGURE 3 Ratio of nitrate production to ammonium consumption ($\Delta\text{NO}_3^- \text{-N} / \Delta\text{NH}_4^+ \text{-N}$). The ratio of 0.26 defined by Strous et al. (1997) and 0.16 by Lotti et al. (2014).

operation was 23–28 °C, in the range activity of anammox bacteria of 25–45 °C where the optimum performance of *Candidatus Brocadia sinica* was conducted at 35–40 °C (Oshiki et al. 2011). Therefore, the lower temperature operation will decrease the performance of anammox for removing nitrogen. However, the result of the nitrogen conversion ratio indicated the anammox process has occurred in the reactor.

3.2. Profile of nitrogen concentration in UASB reactor operation

The anammox process took place from the beginning of the study, where the ammonium and nitrite conversion occurred under anaerobic conditions. In this study, anammox bacteria of the *Candidatus Brocadia sinica* species were used as inoculum since it was adapted to environmental temperatures in Indonesia. The use of pure anammox bacteria accelerates the time of the start-up anammox process in nitrogen removal in wastewater compared to using activated sludge containing various types of organisms. The abundance of anammox bacteria in the environment is very small compared to other bacteria due to the slow growth rate. The experience of the world's first large-scale anammox reactor in the Netherlands took more than 3.5 years for the start-up process with high nitrogen removal performance (van der Star et al. 2007).

In the first three weeks of reactor operation, ammonium and nitrite concentration were relatively stable in the range 41–49 mg–N/L and 32–40 mg–N/L, respectively. Simultaneous conversion of ammonium and nitrite in anaerobic conditions without organic carbon is an indication of the anammox process, which distinguishes it from the nitrification-denitrification process and takes place in aerobic process. One of the disadvantages of selecting this anammox bacteria is classified as slow growth rate bacteria, thus generating the anammox process is also getting slower. The doubling time of anammox bacteria is between 7–14 d. However, the growth time of *Candidatus Brocadia sinica* can be accelerated to 2.1 d using an up-flow column reactor for cultivation with high substrate loading rates at 37 °C (Zhang et al. 2017).

Then, the ammonium and nitrite concentrations be-

gan to drop simultaneously from 40.48 mg–N/L and 29.73 mg–N/L on day 23 to 26.75 mg–N/L and 23.08 mg–N/L on day 40, respectively. The conversion performance of ammonium and nitrite was stable for 14 d. From day 54 effluent concentrations were in the range 23–29 mg–N/L and 19–26 mg–N/L. The process that undergoes a growth process besides nitrogen conversion by consuming ammonium using nitrite as an electron acceptor was carried out by anammox bacteria. Based on Equation 5, 1 mole of ammonium and 1.146 mole nitrite generate 0.071 mol biomass. Low biomass production is also one of the advantages of anammox processes that produce less biomass, $0.071\text{CH}_2\text{O}_{0.5}\text{N}_{0.15}$, as shown in Equation 5. However, the activity of nitrogen conversion is determined by the amount of anammox biomass. Therefore, more anammox biomass is required in order to increase the conversion process. In the reactor, the anammox bacteria carried out the growth process for two weeks following the doubling time of the anammox bacteria, which is 7–14 d.

After multiplying of anammox bacteria, there was a rapid decrease in the concentration of ammonium and nitrite. Almost all ammonium and nitrite were lost from the reactor. At the end of the first period, the concentrations of ammonium and nitrite reached the lowest levels of 8.67 mg–N/L and 3.74 mg–N/L, respectively. At this stage, the anammox process achieved the maximum performance where almost all substrates were consumed into nitrogen gas. Prevented the limitation of the substrate, which is the food of the growing anammox bacteria, the hydraulic retention time (HRT) decreased from 24 h to 12 h.

In the whole reactor process, the nitrate does not supplement into the substrate, only ammonium and nitrite. The nitrate contents were detected in the samples effluent with relatively constant concentrations in the range of 7.6–9.6 mg–N/L. The reactor operation was designed in an anaerobic condition and intended into the nitrogen conversion process which is carried out by the anammox process and nitrification inhibition. Based on stoichiometry in Equation 5, the anammox process produces a small nitrate instead of nitrogen gas. Thus, the nitrate produced is a product of the anammox process; the presence of nitrate in anaerobic conditions is an indicator of the anammox pro-

cess.

In the second period with the 12 h HRT, there was a further increase in the ammonium and nitrite concentrations in the effluent to 30.60 mg-N/L and 19.96 mg-N/L, respectively. Shock loading occurred when the NLR was increased from 0.14 kg/m³.d to 0.28 kg/m³.d. Anammox bacteria supplied with a double loading rate as a result of the nitrogen conversion process back to the initial conditions up to day 93. Effluent concentrations of ammonium and nitrite were in the range 28-31 mg-N/L and 17-22 mg-N/L, respectively. A rapid decrease in nitrogen conversion in period two occurred again on day 96, where the ammonium and nitrite concentrations in effluent increased to 47.95 mg-N/L and 29.31 mg-N/L, respectively. This condition was stable up to day 103. This phenomenon was unusual since anammox bacteria will adapt to the shock loading that occurs and returns to be stable after one month, then the nitrogen conversion performance will increase again. There could be other parameters that cause these to be happened. If the nitrate concentration was observed in the second period, there was a decrease in the concentration from 8.79 mg-N/L to 5.22 mg-N/L. This is consistent with an increase in ammonium and nitrite, which indicates a decrease in the activity of anammox bacteria. Nitrate concentrations in the effluent were stable until the 89 d with a range of 6.2-8.4 mg-N/L. However, there was a drastic decrease on day 93 from 6.94 mg-N/L to 4.7 mg-N/L, which also indicated a decrease in anammox activity.

One of the reasons for decreasing nitrogen removal activity is the inhibition of the anammox process due to the presence of organic carbon from sugarcane bagasse, which is used as a carrier for anammox bacteria. The next step is to measure the COD to confirm the hypothesis and determine the next steps to overcome the inhibition that occurs.

On day 96, COD analysis was carried out from observe the reactor's C/N ratio. Anammox bacteria have a high ability to convert ammonium from wastewater containing low C/N. Figure 6 shows the C/N ratio is higher than the inhibition limit, and it can be ascertained that the inhibition process occurred in the reactor in the presence of organic carbon. COD reaches 140.8 mg/L, while the nitrite concentration is 70 mg/L so that the COD/NO₂⁻-N ratio becomes 2.02, which exceeds the inhibition threshold. In the next seven days, the maintenance of the reactor operation was carried out at the pump due to the instability of the flow rate, where the substrate flow was slower than the desired HRT of 12. The concentration of ammonium and nitrite further increased to 150 mg-N/L in the influent for overcoming the inhibition due to the high organic matter content in the reactor.

After the concentration of ammonium and nitrite increased up to 150 mg-N/L, shock loading occurred again. Ammonium and nitrite concentrations raised to 83.61 mg-N/L and 75.43 mg-N/L at the beginning of period three operation. Unlike the previous conditions, there was a simultaneous decrease in ammonium and nitrite concentrations and an increase in nitrate production until the end of re-

actor operations. The anammox process was successfully recovered by lowering the C/N ratio below the inhibition threshold.

3.3. Nitrogen removal performance

From the beginning of the operation, nitrogen removal was achieved with ammonium and nitrite removal and the presence of produced nitrates. It showed that the anammox process had already taken place in to the reaction. In addition, NRR is a key parameter to the anammox process. The calculation of NRR was shown in Equations 3. NRR value indicated that the level of nitrogen removal calculated based on the difference in the value of loading nitrogen with nitrogen concentration in the effluent divided by retention time. During 75 d with 24 h HRT, the NRR value gradually increased with the Nitrogen Loading Rate (NLR) 0.14 kg-N/m³.d. The NLR value is the loading of nitrogen during the operation of the anammox process.

At the beginning of the experiment, NRR was 0.049 kg-N/m³.d. The low NRR value was due to anammox bacteria having a slow growth rate compared to other types of bacteria such as AOB and NOB. The increased of NRR values always occurred until day 75, with a maximum NRR value. However, on day 96, NRR is decreased up to 0.11 kg-N/m³.d and slowly rose to 0.15 kg-N/m³.d until the end of period two. On day 123 (period three), ammonium and nitrite influent concentrations were increased to 150 mg-N/L, consequently raising NRR to 0.261 kg-N/m³.d at NLR 0.6 kg-N/m³.d.

Substrate concentrations consist of ammonium and nitrite with each concentration of 70-150 mg-N/L. The efficiency of ammonium removal (ACE) is also one of the parameters to prove that the anammox process has taken place. Based on Equation 1, ACE is calculated based on the percent of the difference between the ammonium loading and the ammonium in the effluent divided by the ammonium loading concentration. At the start of the reactor, only 33% of the ammonium was oxidized. With increasing time, the efficiency of ammonium conversion also increased. This result is revealed in the data of Figure 4, whereby on day 37 ACE had reached 55%. Until day 75, the efficiency increased again to reach the maximum ACE in 24 h HRT, 88%. In contrast, beginning the 12 h HRT, ACE drops rapidly to 57% and ranged from 55-61% until day 93. On day 96, the ACE drops again to 31% and rose to 56% after day 119. On day 120, the influent concentration was increased to 150 mg-N/L, and ACE decreased to 44%, then increased gradually to 55% at end of reactor operation.

The nitrogen removal efficiency (NRE) shows the accuracy in nitrogen removal expressed in percent. The NRE value is calculated based on the percent of the difference between loading nitrogen and nitrogen obtained at the effluent per concentration of ammonium loading. NRE value was also higher by increasing operating time. At the beginning of the operation, NRE was only 34%, and there was an increase in the following days. The maximum efficiency with 24 h HRT obtained on day 75 reached 85%.

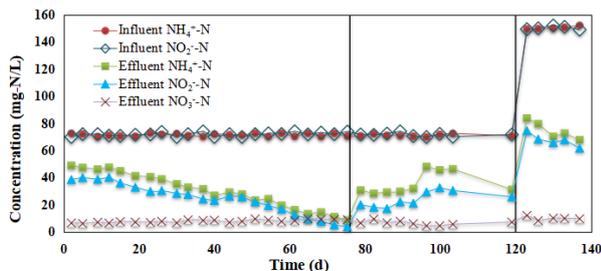


FIGURE 4 Profile of nitrogen during the whole operation of UASB anammox biofilm reactor.

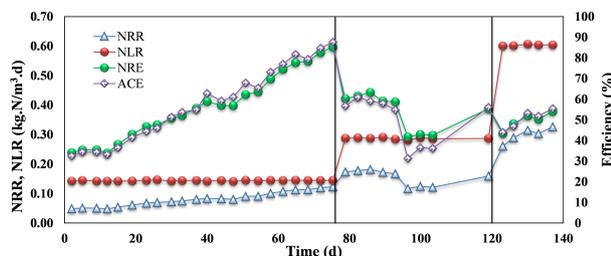


FIGURE 5 Nitrogen removal performance of UASB anammox biofilm reactor.

While the efficiency decreased after HRT was reduced to 12 h, the NRE value obtained was 60% and ranged from 59-62% until day 93. However, on day 96, the NRE value dropped dramatically to 42%. On day 120, the influent concentration was increased to 150 mg-N/L, and then, NRE increased gradually to 54% at the end of period three.

Previous studies have shown that ACE values reached 97.3%, and NRE reached 97% in 21 h HRT using the Up-flow Biofilter (UBF) reactor (Jin et al. 2008). It also has been proved that the anammox process could remove ammonium up to ACE 92% and NRE of 94% using the UASB reactor. When it compared to this study, nitrogen removal has been observed (Ma et al. 2013). The maximum NRR was 0.12 kg-N/m³.d at NLR 0.14 kg-N/m³.d for HRT 24 h and 0.17 kg-N/m³.d at NLR 0.28 kg-N/m³.d for HRT 12 h. NRE and ACE are also directly proportional to NRR, always increasing with time. The maximum NRE and ACE values were 85% and 88%, respectively, in 24 h HRT and decreased 55% and 56%, respectively, in 12 h HRT. The decrease in NRR, NRE, and ACE after reduced HRT to 12 h was due to shock loading, which remarkably increased the pollutant load. However, the anammox process can be applied in the tropical area and proved that anammox bacteria can easily adapt to new environments by acclimatization. However, the decline of bacteria performance in nitrogen removal that occurred on day 96 was caused by high levels of organic matter (COD), thus disrupting the anammox process. Therefore, the influent concentration increased to 150 mg-N/L to compensate for the high organic content.

3.4. Inhibition of anammox process

One inhibiting factor of the anammox process is the level of organic matter (COD). Chamchoi (2008) reported that

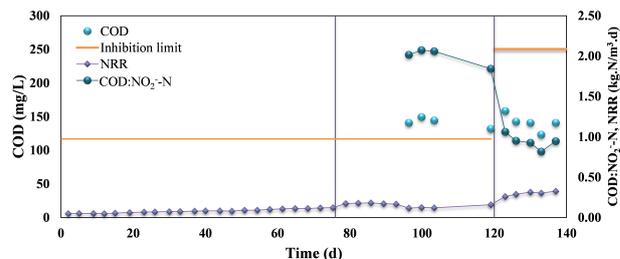


FIGURE 6 Inhibition of anammox process by COD from degradation of sugarcane bagasse.

COD levels that exceed 300 mg/L cause inactivation of the anammox process. This is similar to Molinuevo et al. (2009) where COD concentrations exceeding 292 mg/L inhibited the total anammox process. However, according to Tang et al. (2010), the anammox process begins to be hampered when the COD:NO₂-N ratio is 1.67, while the anammox process almost does not occur when the ratio of COD:NO₂-N is 2.92. On day 96, nitrogen removal performance decreased significantly and anammox bacteria experienced partial death indicating the color changed on the sugarcane bagasse. The morphological colors of anammox bacteria with the best performance are bright red or dark red, while bacteria with pale red to black color indicate that there is a decrease in activity or death of bacteria (Ali et al. 2013). After measuring the COD on day 96, a COD value of 140.8 mg/L with a COD:NO₂-N ratio of 2.02 was obtained (Figure 6). The ratio exceeds the inhibition limit as described by Tang et al. (2010), therefore decreased in nitrogen removal performance. However, this inhibition process did not occur from the beginning of the study until day 95 of reactor operation. COD concentration was lower than 99.7 mg/L, which could enhanced nitrogen removal via the coexistence of denitrification and anammox (Chen et al. 2016). It assumed that the higher COD concentration was affected by the degradation of bagasse media. This result was supported by Ristianingsih (2018), where 53% cellulose content in sugarcane bagasse was decomposed during research and caused high levels of COD in reactor effluents. To overcome this obstacle from COD, the influent concentration was increased to 150 mg/L to compensate for COD, which ranged from 132-158.4 mg/L, so that the COD:NO₂-N ratio becomes one.

4. Conclusions

Based on these results, the nitrogen removal using anammox process with the Up-flow Anaerobic Sludge Blanket (UASB) reactor can be concluded by the reaction with a stoichiometric ratio of ΔNO₂⁻-N:ΔNH₄⁺-N and ΔNO₃⁻-N:ΔNH₄⁺-N was 1.24 and 0.18, respectively. The highest performance of nitrogen removal obtained by NRR optimum value of 0.29 kg-N/m³.d on HRT 12 h and NLR 0.60 kg-N/m³.d. The highest ACE and NRE on HRT 24 h reached 88% and 85%, respectively. The anammox pro-

cess can take place in the area in the temperature range 23–28 °C using sugarcane bagasse as the organic carrier. The application of sugarcane bagasse could enhance nitrogen removal by provided denitrification process, also could be inhibited at certain COD concentration.

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Authors' contributions

ZLK, PS designed the study. AA carried out the laboratory work. AA, ZLK, PS analyzed the data. AA, ZLK wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The author declare that they have no competing interest.

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