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**Research Article**

# Short Test Performance of Nitrogen Removal by Anammox Bacteria from Lake Koto Baru, Indonesia, Using Pumice as a Carrier

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**ABSTRACT**
Anaerobic ammonium oxidation (anammox) removes ammonium under anaerobic conditions into nitrogen gas using nitrite as an electron acceptor by anammox bacteria belonging to the phylum *Planctomycetota*. The performance of nitrogen removal depends on the species and operating conditions. The purpose of this study was to compare the performance of anammox bacteria from Lake Koto Baru, Indonesia, in nitrogen removal with *Candidatus Brocadia sinica* at tropical ambient temperature using pumice as a carrier. This experiment utilized two up-flow anaerobic sludge blanket reactors with artificial wastewater containing ammonium and nitrite concentrations of 100 mg-N $L^{-1}$ and a hydraulic retention time of 12 hours for 31 days. Run 1 inoculated with *Candidatus Brocadia sinica*; Run 2 used enriched anammox bacteria from Lake Koto Baru, Indonesia. The concentrations of ammonium, nitrite, and nitrate were analyzed twice a week using Nessler's method, spectrophotometry, and screening UV spectrophotometry, respectively. The best nitrogen removal performance occurs in Run 2 using anammox bacteria from Lake Koto Baru, where the maximum nitrogen removal rate, ammonium conversion efficiency, and nitrogen removal efficiency were 0.395 kg-N $m^{-3}$ $d^{-1}$, 98.12 %, and 92.6 % of 0.341 kg-N $m^{-3}$ $d^{-1}$, 87 %, 79.8 % of Run 1. These results suggest that anammox bacteria from Lake Koto Baru are highly effective for nitrogen removal in tropical climates and are applicable for wastewater treatment systems in similar regions.

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## INTRODUCTION

Anaerobic ammonium oxidation (anammox) (Mulder et al. 1995) oxidizes ammonium into nitrogen gas using nitrite as an electron acceptor under anaerobic conditions by anammox bacteria, as in Equation 1 (Van De Graaf et al. 1996). The anammox process is a more sustainable technology in biological nitrogen removal than the nitrification-denitrification process, due to its high ammonium removal rate, lower oxygen demand, and reduced space requirements in full-scale applications. The anammox process has been successfully applied on a laboratory scale, pilot scale, and large scale to treat ammonium-rich wastewater.

$$NH_4^+ + 1{,}146NO_2^- + 0{,}071HCO_3^- + 0{,}057H^+ \text{ à } 0{,}071CH_2O_{0,5}N_{0,15} + 0{,}986N_2 + 0{,}161NO_3^- + 2{,}002H_2O \quad (1)$$

Anammox biomass has three forms to remove the nitrogen in the reactor. They are granular (Ali et al. 2014a), suspended sludge (Lotti et al. 2014b), and biofilm (Oshiki et al. 2013), with the carrier serving as supporting media. Anammox bacterial granules provide excellent biomass retention, adaptability, and a high capacity to withstand shock loading (Lin et al. 2013). However, this form has a problem with the floating biomass due to nitrogen gas production. A carrier can overcome this flotation of biomass to retain anammox bacteria (Kumar et al. 2017). The carriers derived from natural sources include bamboo charcoal (Chen et al. 2012), palm fiber (Zulkarnaini et al. 2019), and sugarcane bagasse (Zulkarnaini et al. 2021). An inert carrier, such as sand (Strous et al. 1997) and zeolite (Feng et al. 2019), has been widely used as a supporting material for anammox growth. Zulkarnaini and Silvia (2021) reported the growth of anammox bacteria in the pores of the ceramic carrier in a two-inflow anammox biofilm reactor. Another potential carrier that comes from nature is pumice, which contains pores.

Indonesia is situated on the volcanic plate of the Eurasia-Australia-Pacific (Ring of Fire), which makes it possess abundant mineral deposits of non-metallic minerals. Pumice is a natural mineral from volcanoes formed due to volcanic gases and rapid cooling materials. The pumice structure comprises cavities formed by air bubbles trapped in lava during the freezing process (Ridha & Darminto 2016). The Pasak River, located in West Sumatra, Indonesia, is one of the sites where pumice was discovered. It is the residue of sand mining carried out by the local community, with around 1–5 kilograms collected daily and accumulated along the riverbanks. The hollow pumice structure has been used as an adsorbent. Pumice can remove nitrogen as an absorbent in the form of nitrate (Indah et al. 2021) and ammonium (Pratiwi et al. 2019) with an efficiency of up to 40-78%. Based on pumice's potential due to its porous structure, it is readily available in nature and can effectively remove nitrogen. Therefore, this study used pumice as a carrier for anammox bacteria to enhance nitrogen removal.

For the first time in Indonesia, anammox bacteria were successfully cultivated from Lake Koto Baru, Indonesia, using a filter bioreactor (FtBR) operated at tropical ambient temperatures of 35 °C (Zulkarnaini 2024). The results show that the nitrogen removal performance is better and more stable at tropical ambient temperatures than at 35 °C. At a tropical ambient temperature, the microbial community revealed *Candidatus Brocadia fulgida* as the predominant species, with a relative abundance of 20.05% (Zulkarnaini et al. 2025). This report demonstrates that anammox bacteria originating from the native environment outperform those from outside sources. Zulkarnaini et al. (2020) reported a decrease in the performance of the anammox bacteria species *Candidatus Brocadia sinica*, characterized by a change in biomass color from red carmine to reddish-brown when the operation was carried out at tropical ambient temperatures.

Numerous studies have shown that the anammox process operates optimally at warmer temperatures, with 35°C often cited as the ideal condition for achieving maximum nitrogen removal efficiency (Oshiki et al. 2011). However, this presents a significant challenge for wastewater treatment applications, particularly in subtropical and temperate regions, as maintaining such elevated temperatures requires substantial energy input, reducing overall efficiency. In contrast, Lotti et al. (2014a) have demonstrated improved anammox performance at lower temperatures, highlighting the influence of species-specific adaptations. Different anammox bacterial species exhibit varying temperature ranges for optimal nitrogen removal, suggesting that temperature resilience and adaptability are crucial for enhancing the applicability of the anammox process in diverse climatic conditions (Le et al. 2022).

The purpose of this study was to compare the nitrogen removal performance of anammox bacteria originating from Lake Koto Baru, Indonesia, with that of *Candidatus Brocadia sinica*, an anammox bacterium from outside the Indonesian environment, using pumice as a carrier.

## MATERIALS AND METHODS

### Reactor Configuration

This research utilized two 300 mL acrylic up-flow column reactors that operated continuously, filling 20 mL of inoculum and 20 g of pumice. Pumice was filled into reactors to retain anammox biomass from washout as a carrier during the reactor operation. Run 1 was inoculated with *Candidatus Brocadia sinica*. In contrast, Run 2 used anammox biomass enriched from Lake Koto Baru, Indonesia, containing *Candidatus Brocadia fulgida* (20.04%) and *Candidatus Brocadia caroliniensis* (6.20%) in a FtBR (Zulkarnaini et al. 2025).

The substrate was continuously delivered from the tank to the reactor via a peristaltic pump. The tank was connected to a nitrogen-filled gas bag to maintain anaerobic conditions and stabilize atmospheric pressure.

The schematic of the reactor configuration in this study was shown in Figure 1. The reactors were equipped with filters in the bottom and top parts to prevent biomass washout and were covered with aluminum foil to prevent the growth of phototrophic bacteria. The research was conducted for 31 days, with hydraulic retention time (HRT) of 12 hours. Influent and effluent water samples were collected and analyzed twice a week. Ammonium, nitrite, and nitrate concentrations were analyzed using Nessler's method, spectrophotometry, and screening ultraviolet spectrophotometry, respectively. The reactor was operated at ambient temperature in the laboratory without pH adjustment.

### Preparation of pumice

Pumice forms through the expansion of magmatic gases during effusion, giving it a spongy, glassy texture and high internal porosity. Pumice samples from the Pasak River (West Sumatra, Indonesia) were collected for this study. Prior to the experiment, the pumice was repeatedly rinsed with distilled water and air-dried at room temperature. It was then crushed using a basic hammer-type crusher and sieved to a particle size of 8–30 mesh (0.6–2.4 mm) to obtain a uniform material. No acid or base treatment was applied before use.

### Seeding Sludge

Inoculum for Run 1 originated from the up-flow column reactor that cultivated *Candidatus Brocadia sinica* at tropical ambient temperature for 1.5 years. The colour of the biomass changed from red carmine to reddish-brown due to temperature operation at a level lower than optimum, 37 °C (Oshiki et al. 2013). Run 2 was inoculated with enriched anammox bacteria from Lake Koto

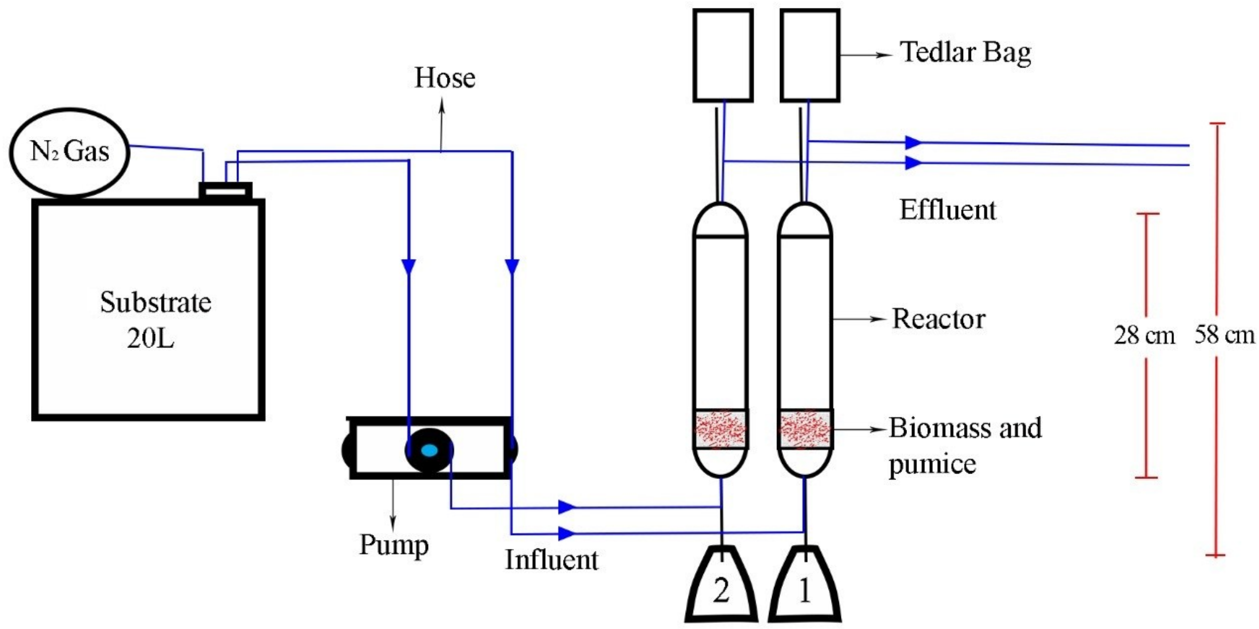


**Figure 1.** Experimental reactor configuration. Both reactors were filled with pumice; Run 1 was inoculated with *Candidatus Brocadia sinica*, and Run 2 was inoculated with anammox bacteria from Lake Koto Baru.

Baru, Indonesia, using FtBR and had already operated at tropical ambient temperature for one and a half years (Putra et al. 2020). For the reactor's start-up, 20 mL of anammox biomass and pumice were added to each reactor.

### Composition of Artificial Wastewater

The artificial wastewater, prepared per liter of groundwater, contained 500 mg $KHCO_3$, 27.2 mg $KH_2PO_4$, 300 mg $MgSO_4 \cdot 7H_2O$, 180 mg $CaCl_2 \cdot 7H_2O$, 1 mL each of trace element solutions I and II, and 100 mg $L^{-1}$ nitrogen supplied from both $NaNO_2$ and $(NH_4)_2SO_4$ (Zulkarnaini et al. 2024).

### Calculation of Nitrogen Removal Performance

The nitrogen removal performance of the anammox process was evaluated following the nitrogen balance method described by Zulkarnaini et al. (2019). They were the nitrogen removal rate (NRR, kg-N $m^{-3}$ $d^{-1}$), nitrogen removal efficiency (NRE, %), and ammonium conversion efficiency (ACE, %).

## RESULTS

### Stoichiometry of the anammox process

The anammox process was evaluated using the stoichiometric reaction shown in Equation (1). The nitrite-to-ammonium removal ratio ($\Delta NO_2^-$-N/$\Delta NH_4^+$-N) and the nitrate-to-ammonium consumption ratio ($\Delta NO_3^-$-N/$\Delta NH_4^+$-N) were determined based on the quantities of ammonium oxidized, nitrite removed, and nitrate produced, respectively.

The average ratio of the $\Delta NO_2^-$-N/$\Delta NH_4^+$-N obtained at a stable Run 1 and Run 2 was 1.53 and 1.09, respectively. Run 2 was near the stoichiometry of the anammox process of 1.146, where the anammox process was predominant from the beginning of reactor operation. Higher nitrite oxidation occurred in Run 1. It could be the activity of the denitrification process, where part of the nitrite and nitrate is converted to nitrogen gas under anaerobic conditions in the presence of organic carbon. In various anammox reactors, the $NO_2^-$-N/$NH_4^+$-N removal ratio typically ranges from 0.5 to 4, depending on reactor configuration, operating conditions, and substrate concentration (Ahn 2006). The comparison of the $\Delta NO_2^-$-N/$\Delta NH_4^+$-N ratio from the experiment is shown in Figure 2.

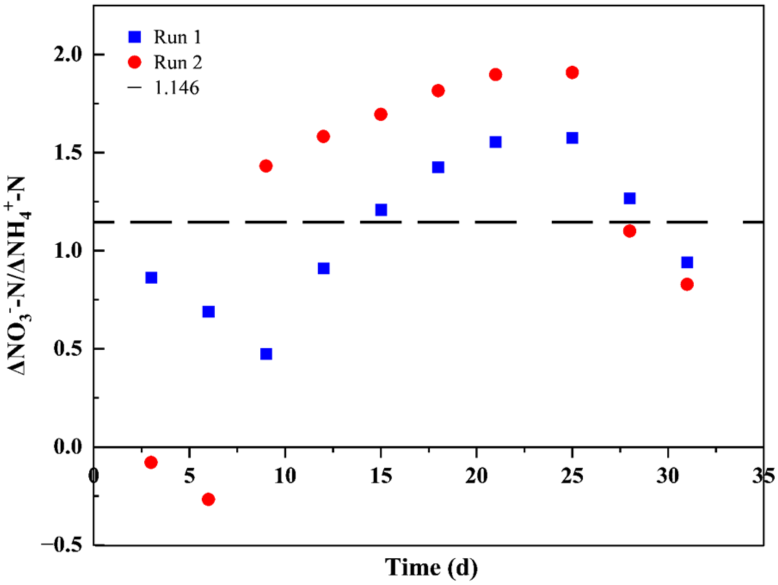


**Figure 2.** Stoichiometry ratio of $\Delta NO_3^-$-N/$\Delta NH_4^+$-N compared with anammox stoichiometry of 1.146 (Lotti et al. 2014b).

Anammox process produces a small amount of $NO_3^-$ instead of $N_2$. 1 mole of $NH_4^+$ produces 0.161 $NO_3^-$. The average $\Delta NO_3^-$-N/$\Delta NH_4^+$-N ratio values obtained in this study were 0.05 for Run 1 and -0.14 for Run 2. Both ratios were lower than the anammox stoichiometry. Figure 3 shows the ratio of $\Delta NO_3^-$-N/$\Delta NH_4^+$-N during the study. Lower nitrate concentrations can indicate that nitrate was removed due to other processes, such as denitrification. Produced nitrate could be adsorbed by pumice and retained in the reactor; then, denitrification takes place in the pumice.

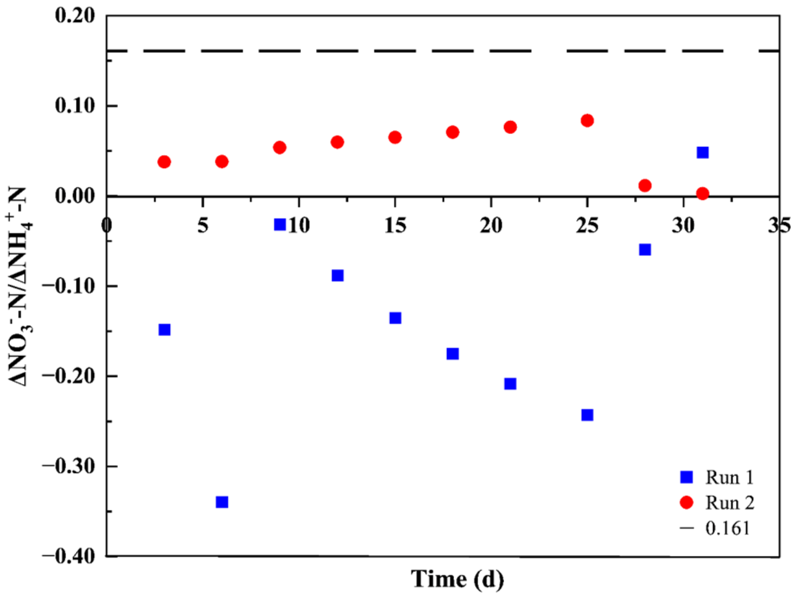


**Figure 3.** Stoichiometry ratio of $\Delta NO_3^-$-N/$\Delta NH_4^+$-N compared with anammox stoichiometry of 0.161 (Lotti et al. 2014b).

## Performance of Reactors

The performance of the anammox process in the up-flow column reactor was shown in Figures 4-5. Both reactors were supplied with ammonium and nitrite at concentrations of 100 mg-N $L^{-1}$ each, with a hydraulic retention time (HRT) of 12 hours. Based on ammonium removal efficiency, the start-up process was classified into four stages: the cell lysis phase (effluent ammonium concentration exceeding the influent), the lag phase (gradual decrease in effluent ammonium), the transition phase (moderate rise in NRR), and the activity elevation phase (rapid increase in NRR). (Chen et al. 2016; Lulrahman et al. 2022). During the cell lysis phase, inhibition causes decay of anammox biomass, resulting in elevated ammonium levels in the effluent. As microorganisms adapt to the new environment, ammonium concentrations gradually decline, followed by a further reduction driven by the enhanced bioactivity of the anammox microorganisms.

### Performance of Run 1

Cell lysis phase (days 1–8). As shown in Figure 4(a), the effluent ammonium concentration exceeded the influent, resulting in a negative ammonium removal efficiency. This increase in ammonium was likely due to the lysis of dead anammox biomass. The decay of previously dormant bacteria, leading to cell lysis and the conversion of organic nitrogen to ammonium, may have been triggered by changes in substrate concentration during the experiment as well as conditions from the preceding cultivation process.

At the beginning of operation Run 1, in which *Candidatus Brocadia sinica* was the inoculum and pumice as the carrier, there was no ammonium removal in Run 1. On the contrary, the ammonium concentration in the effluent was higher than in the influent. This increase was nearly double the concentration in the influent.

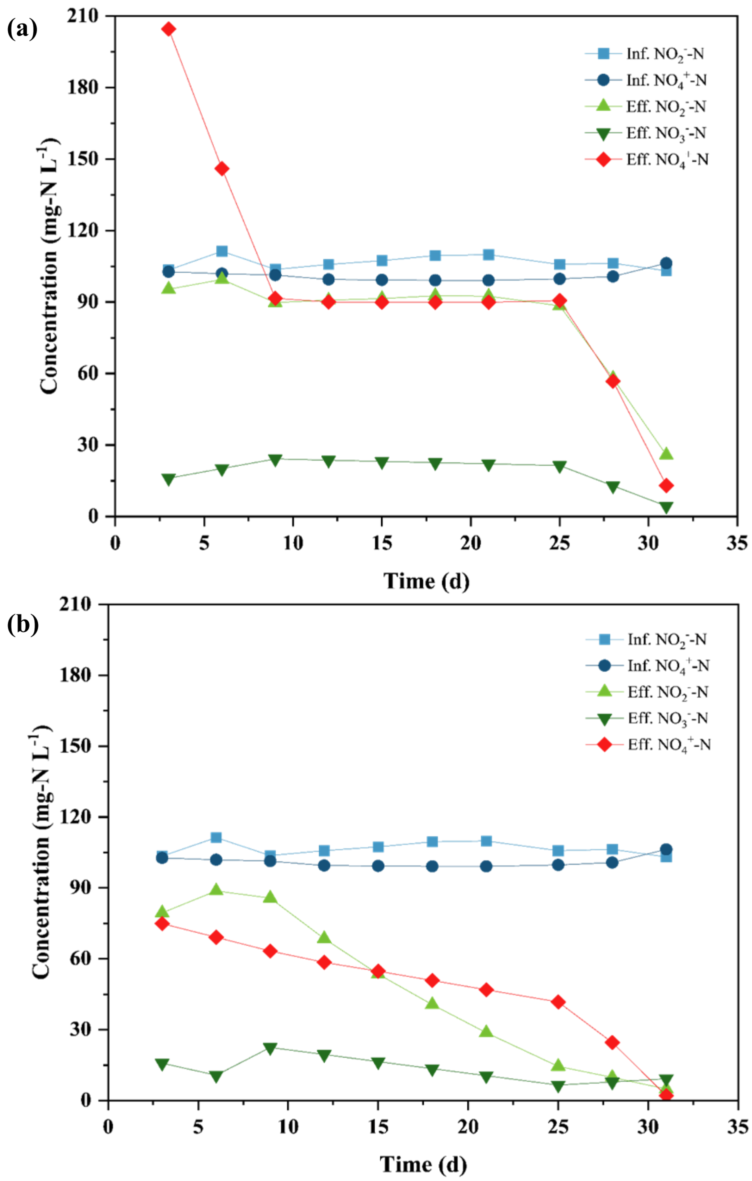


**Figure 4.** Nitrogen profile of influent and effluent in (a) Run 1 and (b) Run 2.

Anammox activity occurred during the lag phase (days 9-25), where ammonia and nitrite were removed simultaneously, and nitrate was produced. Anammox bacteria adapt to environmental changes over a period of up to 25 days of reactor operation. During the adaptation process, the anammox bacteria undergo a duplication process, with a doubling time of seven days for *Candidatus Brocadia sinica.* Anammox bacteria, classified as slow-growing bacteria, require a considerable amount of time to recover after complete inhibition.

At the activity elevation phase (days 26–31), the anammox process recovered, with a rapid nitrogen removal rate, where both ammonium and nitrite concentrations in the effluent decreased rapidly from 90.60 to 13.00 mg-N $L^{-1}$ and from 88.4 to 25.76 mg-N $L^{-1}$, respectively. This decrease in ammonium and nitrite concentration indicates the high activity of anammox bacteria. In the anammox process, nitrite functions as an electron acceptor in the conversion of ammonium to nitrogen gas. Figure 4(a) shows the change in nitrogen concentration in Run 1 during the study.

Reactor performance or capability is obtained from changes in nitrogen concentration during the study (Figure 5). Reactor performance was evaluated based on nitrogen loading rate (NLR), NRR, ACE, and NRE. The reactor was fed with NLR 0.40 kg-N $m^{-3}$ $d^{-1}$ during the entire reactor operation. The NRR in the reactor increases gradually from -0.180 kg-N $m^{-3}$ $d^{-1}$ to 0.341 kg-N $m^{-3}$ $d^{-1}$. The negative NRR at start-up was due to the production of ammonium from the dead biomass. At the end of reactor operation, NRR achieved 0.341 kg-N $m^{-3}$ $d^{-1}$.

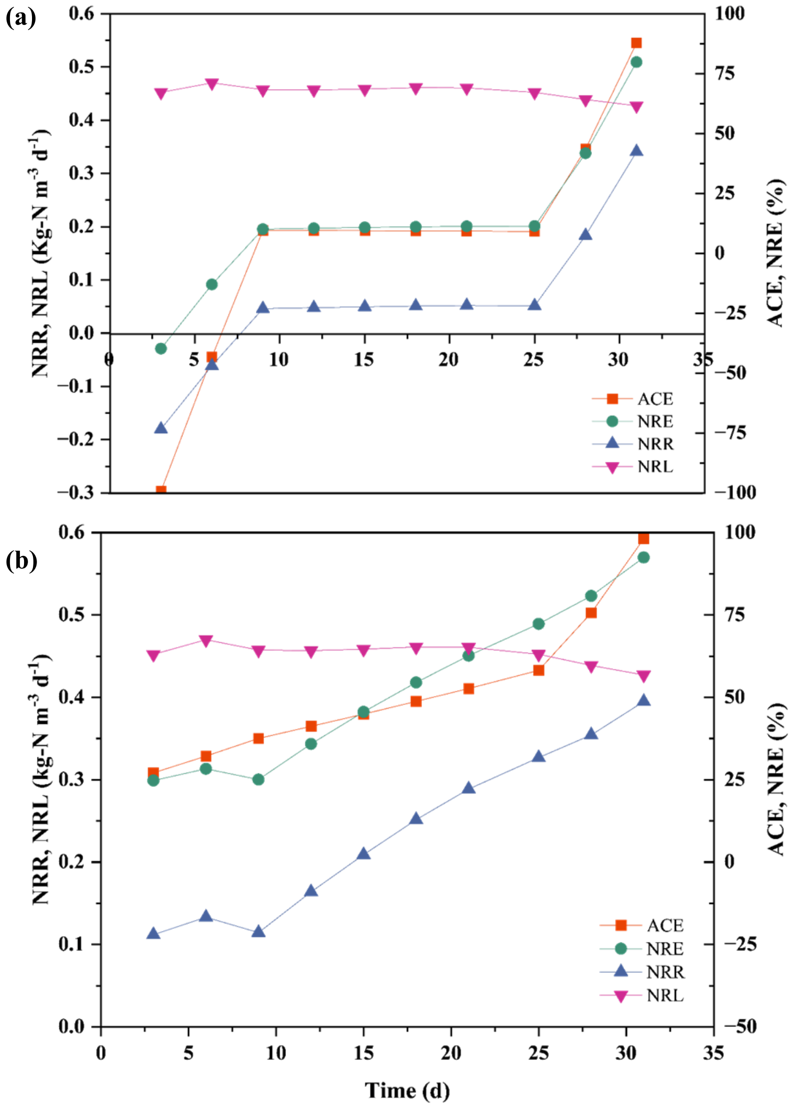


**Figure 5.** Performance of nitrogen removal in (a) Run 1 and (b) Run 2.

ACE and NRE calculated the reactor's efficiency. ACE was negative, at -99 %, at the beginning of the study, due to ammonium production from decaying anammox biomass. However, ACE continued to increase until the end of the test. On day 31, ACE achieved 88%. The overall nitrogen efficiency was expressed in NRE, with an optimum of 79.8%.

Figure 6 shows the change in biomass color in Run 1. The biomass in the reactor is a reddish-brown color. This color is slightly pale compared to the color characteristics of the anammox bacteria. The colour of anammox biomass is bright red or dark red. The color of the biomass is an indicator of the visibility and performance of anammox bacteria. If the biomass's color changes from red to pale red or black, it is an indicator of reduced activity of the anammox bacteria.

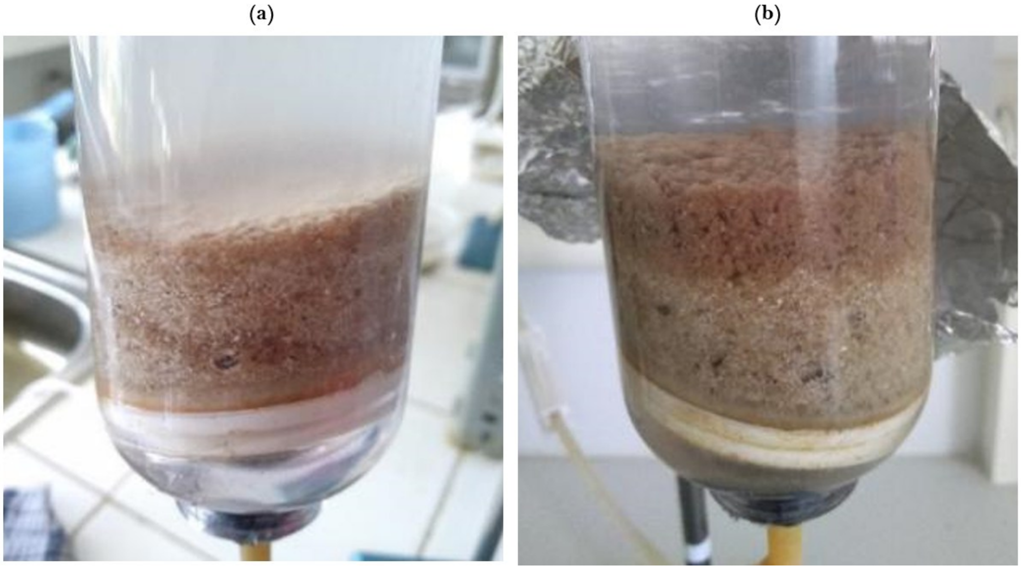


**Figure 6** Anammox biomass Run 1, (a) day 1, pumice and anammox biomass mixed as carrier and inoculum, (b) day 31, anammox biomass flows into the upper as a sludge blanket.

## Performance of Run 2

Run 2 was a reactor containing *Candidatus Brocadia fulgida* and *Candidatus Brocadia caroliniensis* with pumice as a carrier. These bacteria are also the first reported anammox bacteria to be found in Indonesia. These bacteria are enriched using FtBR with an inoculum sourced from Talago Koto Baru Lake, Tanah Datar, West Sumatra, at tropical ambient temperatures.

Lag Phase (days 1–9), Run 2 does not pass through the cell lysis phase, as well as Run 1, which experiences inhibition and adaptation to changes in the reactor operating environment. However, it is immediately during the lag phase that anammox bacteria adapt to the change in the new environment. The Anammox process has been in operation since the beginning of reactor operation, where both ammonium and nitrite have been removed. Even operated at a lower concentration than the previous operation, 100 mg-N $L^{-1}$, but the nitrogen loading rate was higher than the previous one due to decreased HRT from 24 to 12 hours.

The transition phase (days 10–25) shows higher nitrogen removal in Run 2. The ammonium concentration decreased gradually from 74.9 mg-N $L^{-1}$ to 41.7 mg-N $L^{-1}$ in the effluent on day 25. Likewise, the effluent's nitrite concentration decreased gradually from 79.46 mg-N $L^{-1}$ to 14.5 mg-N $L^{-1}$.

During the activity elevation phase (days 26-31), ammonium and nitrite concentrations decreased rapidly to 2.0 mg-N $L^{-1}$ and 4.9 mg-N $L^{-1}$, respectively, by the end of the experiment. Figure 4(b) shows the change in nitrogen concentration in Run 2. The NLR in the reactor remained stable throughout the experiment, with a value of ± 0.40 kg-N $m^{-3}$ $d^{-1}$. Then, NRR increased gradually from 0.112 kg-N $m^{-3}$ $d^{-1}$ to 0.395 kg-N $m^{-3}$ $d^{-1}$ on day 31.

Ammonium was oxidized from the beginning of reactor operation, with an ACE of 27% on day three and increasing gradually to a maximum of 98.12% in the last experiment. Likewise, NRE was stable during the first ten days of operation, ranging from 24.74% to 28.31%, and then increased rapidly, reaching a maximum of 92.47% at the end of the experiment. Run 2 performance in nitrogen removal is the best among both reactors. The nitrogen removal performance in Run 2 was shown in Figure 5(b).

The reactor's biomass is bright red, consistent with the characteristics described by Ali et al. (2014b). The color of the biomass is an indicator of the visibility and performance of anammox bacteria. The red color indicates the development of beneficial bacteria and can also be observed in the good performance of Run 2 in removing nitrogen.

Based on the quantity of bacterial biomass, it has increased from the first day. At the beginning of reactor operation, the anammox biomass inoculated into the reactor was not yet in a granular form. On day 31, it was clear that the anammox bacteria had formed granules. The granules are on the surface of the pumice. The influent flow pushes anammox biomass, causing the granular anammox to be at the top of the pumice.

Similar to Run 1, Run 2 also contained gas bubbles during reactor operation. It indicates the formation of nitrogen gas ($N_2$) resulting from chemical/metabolic reactions during the anammox process. An illustration of the visual changes that occurred in Run 2 is shown in Figure 7.

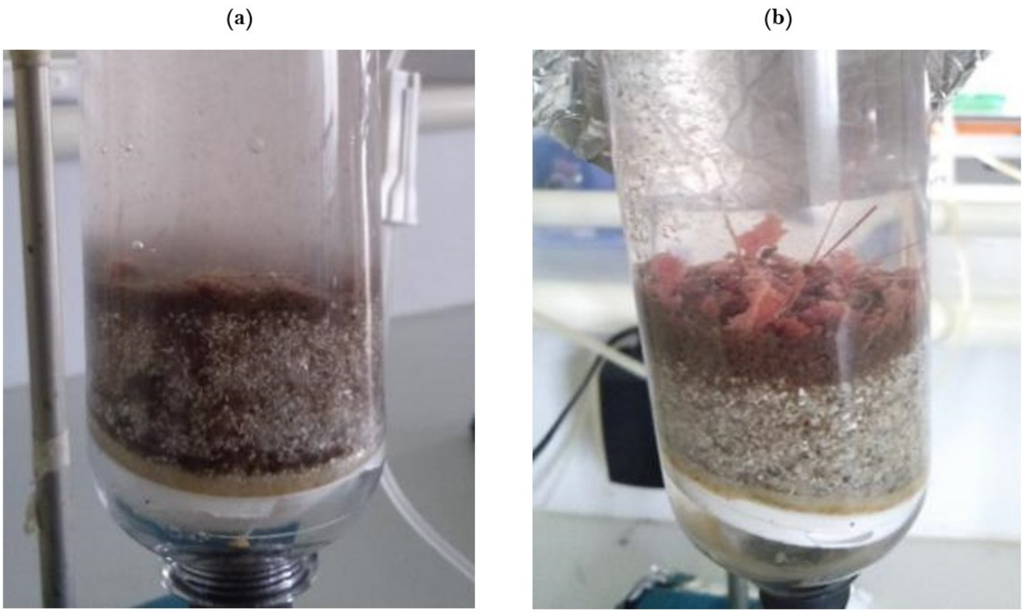


**Figure 7** Anammox biomass Run 2, (a) day 1, the mixed pumice and anammox biomass generated bubbles of nitrogen gas as an indicator of a rapid anammox process (b) day 31, bright red anammox biomass grown.

## DISCUSSION

The temperature operated lower than 37 °C, high nitrite concentration, and inoculum quality all influenced the anammox process activity during anammox reactor operation. The anammox process occurred at the beginning of both reactor operations, but it differed in the predominant process. In Run 1, another process could involve nitrogen conversions, such as denitrification and organic degradation, that release ammonium. Inhibition and anammox biomass lysis occurred due to the high concentration of supplied nitrite.

The maximum NRR obtained in this study was higher than that reported in previous studies (0.196 kg-N $m^{-3}$ $d^{-1}$) conducted by Zulkarnaini et al.

(2020) using the same reactor type, HRT, and granular biomass without a carrier. The use of pumice enhanced NRR and improved nitrogen removal. In up-flow column reactor operations, challenges remained, such as seed sludge loss caused by the poor settleability of flocculent sludge. Pumice offers a promising solution, as its porous structure supports the attachment of anammox biomass while enhancing sludge settleability and activity.

### Effect of pumice on nitrogen removal

The effect of adding pumice can be seen in Run 1, compared to Zulkarnaini et al. (2020), who used 20 mL of biomass from the *Candidatus Brocadia sinica* species as an inoculum, which was conducted at a tropical ambient temperature with an NRE of 77% and an ACE of 82%. Meanwhile, Run 1 with the same parameters improved NRE values by 79.80% and ACE by 87.77% in a shorter time of 31 days. Based on this comparison, the addition of pumice can improve nitrogen removal performance in the anammox process.

Previously, sand was used in the fluidized bed reactor, where the anammox process was first identified. In this study, pumice was used instead of sand. Two porosity levels can be distinguished when pumice is used as a filter bed material: the porosity of the pumice itself and the porosity of the filter bed. As a result, smaller particles are kept in the pores of the pumice, while larger particles are kept in the filter bed. As a result, clogging occurs more slowly, and the bed's volume is utilised more effectively in a pumice bed than in a sand bed.

In comparison to a sand bed, a pumice bed has more porosity and a larger capacity for particle deposition. The pore was filled with growing anammox biomass. It became thicker, as observed by Zulkarnaini and Silvia (2021), who noted that pores of the ceramic carrier, 1 mm in size, were filled with anammox biomass throughout the carrier's depth.

Pumice stone has been widely applied for nitrogen removal in various nitrogen compounds in different water or wastewater treatments. Helard et al. (2020) utilized pumice as an adsorbent in a fixed-bed column for nitrate removal, achieving a total removal efficiency of 31.42% and an adsorption capacity of 1,394 mg $g^{-1}$ in groundwater. Additionally, pumice has been applied in biofilter tanks for nitrogen removal in recirculating aquaculture systems through the nitrification–denitrification process (Pungrasmi et al. 2016). The interaction and transformation of nitrogen compounds (ammonium, nitrite, and nitrate) are thought to occur through surface adsorption, followed by diffusion into the pumice pores, with hydrogen bonding and electrostatic attraction serving as the primary mechanisms of adsorption (Heibati et al. 2014; Edwin et al. 2023). Therefore, ammonium and nitrite, as substrates for anammox bacteria, will be retained in pumice, where 60% nitrogen removal occurs in the bottom part of the upflow anaerobic sludge blanket (UASB) reactor (Zulkarnaini et al. 2023). The use of pumice as a carrier increased the nitrogen removal in the UASB reactor.

Anammox bacteria naturally tend to form granules. Granulation begins with the presence of nuclei or bio-carriers that facilitate microbial attachment. Cell adhesion to these particles marks the initial stage of biofilm development, which then progresses into thick, dense biofilms on clusters of inert carriers (Yang et al. 2019). Such carriers should possess properties like a high specific surface area and adequate hydrophobicity to enhance sludge aggregation. In reactors with suspended biomass, pumice serves as an effective support medium, promoting anaerobic granule formation, improving biomass sedimentation, preventing washout, and enhancing nitrogen removal efficiency. Given the slow growth rate of anammox bacteria, using pumice as a carrier for attached growth can accelerate start-up and improve reactor performance.

The presence of gas bubbles in the reactor indicates the formation of nitrogen gas ($N_2$) due to a chemical/metabolic reaction that occurs in the anammox process (Figure 6(b)). Pumice's porous and amorphous characteristics produce a substantial surface area and skeletal structure with open channels that allow ions and water to move into and out of the crystal structure. The released dinitrogen gas ($N_2$) bubbles prevent the anammox biomass from floating, a common phenomenon in the anammox process. Granular anammox has two types of internal channels, closed and open channels. In a closed channel, the anammox-produced $N_2$ gas is trapped in the granule and will float, resulting in a decrease in efficiency and biomass washout. Whereas granular material with open channels remains precipitated at the bottom of the reactor, granular material remains precipitated at the bottom of the reactor. The use of pumice can improve biomass retention and increase the efficiency of nitrogen removal.

## Temperature dependence

The temperature of reactor operation influenced the anammox process, which was conducted at a tropical ambient temperature, ranging from 22 to 28 °C. In contrast, the optimum temperature for *Candidatus Brocadia sinica* bacteria is 37 °C. Even though the species has already adapted to tropical ambient temperature for 1.5 years, the lower temperature reduces the performance of bacteria in the ammonium removal process. The changes in the environment caused some bacteria to die. The reddish-brown color of the biomass in Run 1 is evident. The biomass of freshwater anammox species in their optimum state should be red carmine. The bacterial death causes cell lysis and the breakdown of organic nitrogen into ammonium. Therefore, the ammonium concentration increases in the effluent. This phenomenon was also reported by Lulrahman, Silvia and Zulkarnaini (2022) during the start-up of the anammox process to cultivate anammox bacteria using sludge from Penjalinan estuary as inoculum. The increasing ammonium in the effluent only occurred within one week of reactor operation. Then, the ammonium concentration in the effluent decreased to 91.6 mg-N $L^{-1}$. A small nitrite concentration was removed during this phase, indicating the anammox process was completely inhibited.

In a batch experiment with a limited ammonium concentration, Strous et al. (1999) reported that the anammox process was inhibited entirely by a nitrite concentration of more than 100 mg-N $L^{-1}$. When the operational reactor conditions change, shock loading occurs due to a higher concentration and lower HRT. Consequently, anammox bacteria undergo cell death.

## Potential anammox research and application in a tropical country

The first discovery of anammox bacteria in tropical environments in Indonesia using a novel FtBR is expected to enhance research on anammox in Indonesia and Southeast Asia in the future. Likewise, the application of anammox bacteria to treat domestic wastewater and industrial wastewater, such as tofu waste and industrial fertiliser wastewater.

Effluents from industrial and municipal wastewater treatment plants frequently fail to meet Indonesian effluent quality standards for the nitrogen parameter. Consequently, eutrophication is a common issue in Indonesia's aquatic environment (Komala et al. 2024). Eutrophication, caused by excessive nutrients, primarily nitrogen and phosphorus, can lead to oxygen depletion, fish mortality, foul odors, and toxicity, as observed in Lake Koto Baru (Putra et al. 2020). Efforts have been proposed to apply the anammox process to address these issues (Wijaya & Soedjono 2018). However, despite being discovered over 30 years ago and implemented in full-scale reactors globally, the application of anammox in Indonesia remains limited due to the scarcity of related research.

There is significant potential to implement the anammox process for wastewater treatment and eutrophication control in tropical environments by utilizing indigenous anammox bacteria. In parallel, developing large-scale anammox reactors could complement or replace existing systems, such as wetlands in the fertilizer industry. Applying the anammox process to domestic and industrial wastewater treatment in tropical countries, particularly Indonesia, could support compliance with national effluent quality standards and help prevent eutrophication —a widespread issue in tropical regions caused by nutrient overloading in water bodies.

This study offers critical insights into the potential of native anammox bacteria from Lake Koto Baru to provide an efficient and sustainable solution for nitrogen removal in wastewater treatment, particularly in tropical regions. By utilizing a local bacterial strain, this research supports the development of regionally adapted environmental technologies that can enhance the performance and economic feasibility of nitrogen removal processes in tropical climates. The successful use of pumice as a carrier in these reactors also highlights its potential as an accessible and cost-effective medium for biofilm development in anammox-based treatment systems. Furthermore, the comparative analysis with *Candidatus Brocadia sinica* demonstrates the viability of indigenous strains, contributing to the broader application of anammox processes in environmental engineering and sustainable water management solutions.

## CONCLUSIONS

The use of pumice as a carrier significantly enhances nitrogen removal in both reactors, demonstrating its effectiveness as a biofilm support material for anammox bacteria. The comparison between the two reactors revealed that the enriched anammox bacteria from Lake Koto Baru in Run 2 significantly outperformed *Candidatus Brocadia sinica* in Run 1, with Run 2 achieving a higher nitrogen removal rate (0.395 kg-N $m^{-3}$ $d^{-1}$), ammonium conversion efficiency (98.12 %), and nitrogen removal efficiency (92.6 %) compared to Run 1's performance (NRR: 0.341 kg-N $m^{-3}$ $d^{-1}$, ACE: 87 %, NRE: 79.8 %). These findings indicate that the anammox bacteria sourced from Lake Koto Baru exhibit robust nitrogen removal capabilities in tropical conditions, making them highly suitable for wastewater treatment applications in such climates. The results also highlight the potential for utilizing indigenous bacterial strains to optimise nitrogen removal processes, contributing to more sustainable and locally adapted wastewater treatment solutions.

## AUTHOR CONTRIBUTION

Z.Z. M.Z. designed the research and supervised all the processes. M.Z. collected and analyzed the data and wrote the manuscript. R.A. revised the manuscript.

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## COMPETING INTERESTS

There is no competing interests.

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