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Anaerobic Ammonium Oxidation Performance in Shrimp Pond Wastewater Treatment

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Abstract—High nitrogen in intensive shrimp culture could reduce water quality and environmental carrying capacity. Anaerobic ammonium oxidation (anammox) is a potential technology for nitrogen removal. This research aimed to analyze nitrogen removal performance in a filter bioreactor (FtBR) using sludge from intensive shrimp culture as inoculum. Ammonium and nitrite concentrations of 70 mg-N/L were added to sterilized seawater as substrate and flowed to the reactor with HRT 24 hours. The fast start-up anammox process was achieved within 57 days of the experiment. The maximum nitrogen removal with parameters ACE, NRE, and NRR was 54.83%, 61.29%, and 0.12 kg-N/m^3·d, respectively. The nitrogen stoichiometric ratio NH_4^+-N: NO_2^--N: NO_3^--N was 1:1.42:0.18, close to the stoichiometry of the anammox process. The anammox process can be a new technology for intensive shrimp culture wastewater treatment.

Keywords: Anammox, Nitrogen removal, Shrimp pond.

1. Introduction

The increase in shrimp production has been carried out with super intensive technology that impacts the high amount of waste generated. Nitrogen and phosphate are the main content of super-intensive pond waste that can affect the ecosystem's balance and decrease the environment's carrying capacity. The feed, the most significant input in the pond, has nitrogen retention of about 22%, and the wasted nitrogen is expected to be about 78% in the aquatic environment [1]. Pond waste has exceeded the standard of wastewater quality and has become a pollutant to the environment as a recipient of the waste load. Hence environmentally friendly shrimp farming is needed [2].

The discovery of the anammox process is a revolutionary change in conventional nitrogen removal from wastewater. Currently, many large-scale bioreactors are running using the anammox system worldwide [3]. Some of the advantages of anammox as a wastewater treatment technology are due to its high nitrogen removal capability, does not require aeration, and not require additional carbon sources. Compared with the conventional nitrification-denitrification process, anammox can save up to 50% oxygen demand, 100% organic matter, and nearly 90% waste treatment operational costs [4]. The nitrogen removal process in anammox bacteria can be seen in the following reaction [5]:

$$1NH_4^+ + 1.146NO_2^- + 0.071HCO_3^- + 0.057H^+ \rightarrow 0.161NO_3^- + 0.986N_2 + 0.071CH_{1.74}O_{0.31}N_{0.20} + 2.002H_2O \quad (1)$$

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Anammox bacteria have been found in aquaculture [6], which has high growth potential and high nitrogen removal performance in shrimp culture sediments [7]. Disadvantages in the development of anammox bacteria are slow growth and low biomass. Various studies have reported that the doubling time of these bacteria is 5.3-11 days in both laboratory and full-scale reactors, and the difficulty of isolating anammox bacteria in pure culture [8]. Therefore, an appropriate anammox start-up strategy is needed for waste treatment, such as selecting the type of long-term reactor, the suitable inoculum, and considering critical parameters during the initial development period [8]. The success of the start-up period can be supported by appropriate solid waste inoculation because the waste selection will determine the start-up process's speed. The start-up of the anammox process could be accelerated using inoculation of sludge from operating an anammox reactor [9]. In a shrimp pond environment, identification of anammox bacteria has been successfully carried out in bio-augmented zero water exchange aquaculture ponds [6]. Limited information about enrichment anammox from pond sediment.

This research aimed to start up an anammox process using sludge from intensive shrimp ponds as inoculum. This research could be a basis for the large-scale anammox bioreactors for treating intensive shrimp aquaculture pond waste in nitrogen removal.

2. Method

2.1. Inoculum and Substrate

One liter of sludge was collected from a sedimentation pond (intensive shrimp pond wastewater treatment) with a depth of 0.5-1.0 m below the soil surface. For the start-up process, "food" was supplied to anammox bacteria as a substrate solution. The substrate solution was made from sterilized seawater and flushed with N_2 for 30 minutes to remove dissolved oxygen and was added with $(NH_4)_2SO_4$ and $NaNO_2$ [10,11]. The composition substrate solution (/L seawater) was $(NH_4)_2SO_4$ 330 mg, $NaNO_2$ 345 mg, $KHCO_3$ 500 mg, Mg_2SO_4 300 mg, $CaCl_2.2H_2O$ 180 mg, trace element solutions I, and II [7], [12].

2.2. Reactor Configuration

This research was conducted using a filter bioreactor (FtBR). FtBR successfully cultivated anammox bacteria from Lake Koto Baru, Indonesia [13]. The result of cultivation was determined by observing the nitrogen removal on the substrate. The bioreactor was made from a polypropylene filter housing with a volume of 1.5 liters and a string wound filter cartridge of 0.5 μm as the carrier. The reactor was covered with aluminum foil to prevent the growth of photosynthetic bacteria. A Tedlar bag containing N_2 was connected to a substrate tank to maintain anaerobic conditions. Peristaltic pumps were used to flow substrate to the reactor continuously according to the Hydraulic Retention Time (HRT) of 24 hours [8].

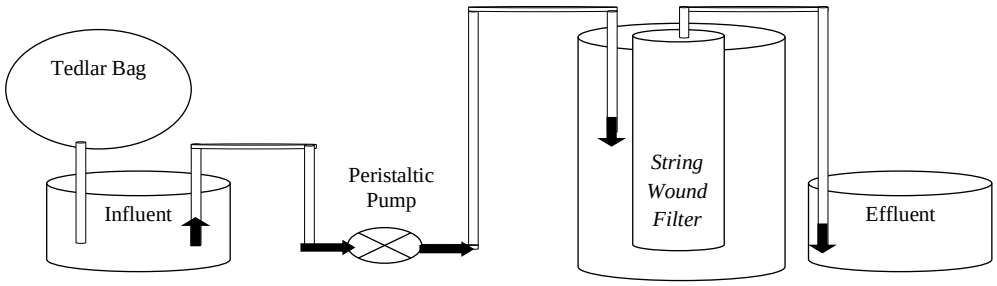

Figure 1. Configuration of FtBR

2.3. Data Analysis

Water samples were taken from the influent and effluent of FtBR once a week. The performance of the reactor can be seen from the nitrogen profile of influent and effluent [14]. Ammonium, nitrite, and nitrate concentrations were analyzed using Nessler, spectrophotometry, and Brucine method. Performance of nitrogen removal calculated based on parameters of ammonium conversion efficiency (ACE, %), nitrogen removal efficiency (NRE, %), nitrogen loading rate (NLR, kg-N/m^3·d), and nitrogen removal rate (NRR, kg-N/m^3·d) [15] .

3. Result and Discussion

The profile of the influent and effluent concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N in FtBR are shown in Figure 2.

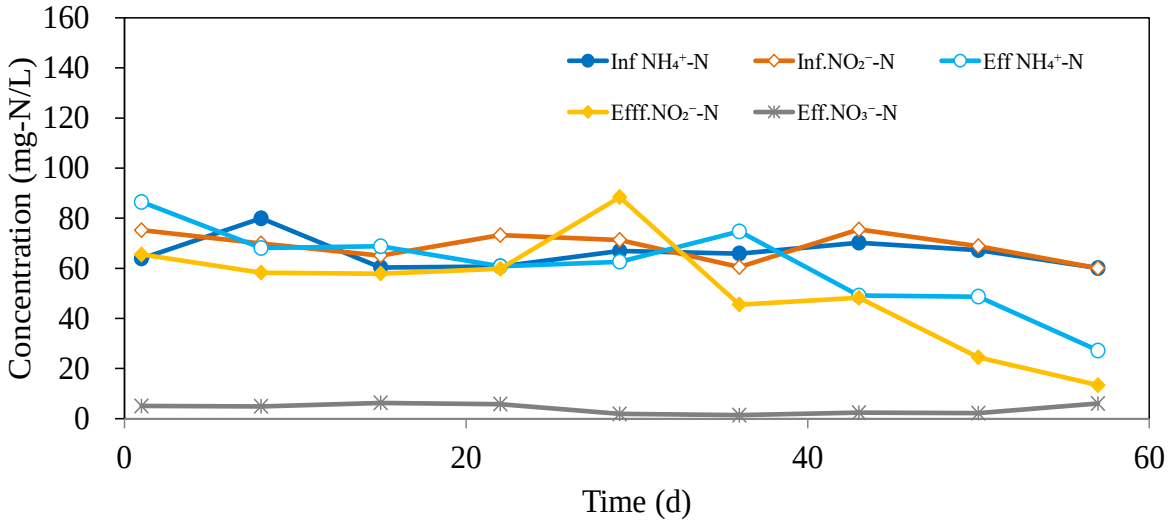

Figure 2. Nitrogen Profile

In the first week of operation of the reactor, the ammonium effluent showed an ammonium concentration of 86.46 mg-N/L, which was higher than the influent ammonium concentration of 63.91 mg-N/L. The higher ammonium effluent value was caused by the process in the adaptation phase, where cell lysis occurred and the breakdown of organic nitrogen into ammonia. In the initial development research on the anammox reactor, it was also found that ammonium concentrations were higher in the early weeks than the influent [14,15,16]. Furthermore, the ammonium effluent showed almost the same value as the influent for 30 days. On day 36, the effluent value increased from 62.62 mg-N/L to 74.71 mg-N/L. Then gradually, the concentration of ammonium effluent decreased until day 57 from 74.71 mg-N/L to 27.15 mg-N/L. This data is the lowest effluent data from the reactor during this study.

Nitrite plays a vital role in the anammox process as an electron acceptor. Influent and effluent nitrite for 22 days of the study showed almost the same value. On day 29, the effluent increased rapidly from 59.77 mg-N/L to 88.42 mg-N/L. Then the effluent concentration in the reactor showed a gradual and stable decrease until day 57. The rapid increase in nitrite can be a limiting factor in the activity of anammox bacteria [18]. The lowest nitrite value was obtained on day 57 at 13.35 mg-N/L.

The effluent concentration of nitrate in this study was relatively stable. The lowest nitrate was obtained at 1.35 mg-N/L, while the highest was 6.25 mg-N/L. Nitrate is a by-product of the anammox process, which indicates ammonium oxidation in anaerobic conditions by anammox bacteria but reduced or in the absence of nitrate indicates a denitrification reaction in the reactor [19].

Figure 3 shows the results of nitrogen removal performance, including ACE, NRE, NRR, and NLR.

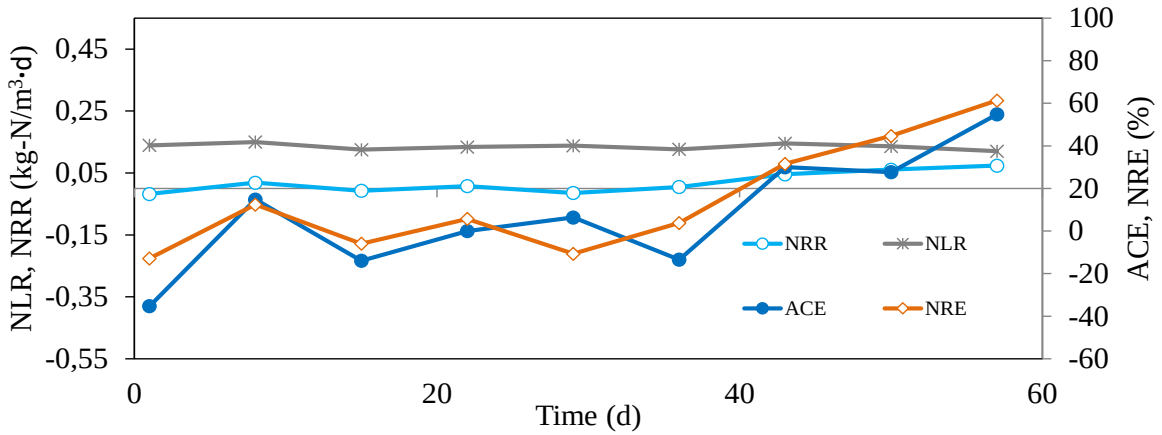

Figure 3. Nitrogen Removal Performance

At the beginning of reactor operation by 36 days, NRR was close to zero because the level of removal could not be observed clearly. This condition was due to the adaptation of the inoculum to the given substrate and reactor configuration. Adaptation and sifting of microbe in inoculum determine the level of NRR at the initial stage in the reactor [20]. After day 36, the NRR increased from 0,005 kg-N/m^3·d to 0,074 kg-N/m^3·d at the end of the study.

The efficiency was shown by ACE and NRE, which led to negative results for 36 days. On days 36 to 57, ACE and NRE increased significantly. NRE increased from 3.80% to 61.29%. At the same time, ACE increased from -13.40% to 54.83%. This increase in efficiency shows anammox's performance from the lysis stage to the exponential stage. This increasing trend was an anammox stage from cell lysis to exponential characterized by efficiency values above 50% [17].

The results obtained for the stoichiometric ratio were 1:1.42:0.18 which were close to the stoichiometry of the anammox process 1:1,32:0.26 [21] and 1:1,146:0,071 [5]. In research on other marine anammox bacteria, slightly different stoichiometric results were obtained of 1: 1.07 for the enrichment of several samples from the sea [22], coastal sediments 1:1,28:0,24 [18] and enriched Hiroshima Bay sediment was 1:1,21:10,15 [20].

4. Conclusion

After 57 days of reactor operation, the maximum nitrogen removal performance with parameters ACE, NRE, and NRR for the reactor was 54.83%, 61.29%, and 0.12 kg-N/m^3·d. Nitrogen stoichiometric ratios were NH_4^+-N: NO_2^--N: NO_3^--N 1:1.42:0.18, indicating the anammox process occurred in FtBR. Anammox can be a new method for developing intensive shrimp culture waste treatment.

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