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## Nitrogen Removal by Anammox Biofilm Column Reactor at Moderately Low Temperature

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### Abstract

The anaerobic ammonium oxidation (anammox) as a new biological approach for nitrogen removal has been considered to be more cost-effective compared with the combination of nitrification and denitrification process. However, the anammox bioreactors are mostly explored at high temperature (>30°C) in which temperature controlling system is fully required. This research was intended to develop and to apply anammox process for high nitrogen concentration removal at ambient temperature used for treating wastewater in tropical countries. An up-flow biofilm column reactor, which the upper part constructed with a porous polyester non-woven fabric material as a carrier to attach the anammox bacteria was operated without heating system. A maximum nitrogen removal rate (NRR) of 1.05 kg-N m<sup>3</sup> d<sup>-1</sup> was reached in the operation days of 178 with a Total Nitrogen (TN) removal efficiency of 74%. This showed the biofilm column anammox reactor was successfully applied to moderate high nitrogen removal from synthetic wastewater at moderately low temperature.

*Keywords: Anammox, biofilm column reactor, ambient temperature, nitrogen removal*

### Abstrak (Indonesian)

Anaerobic ammonium oxidation (anammox) sebagai salah satu pendekatan baru secara biologi untuk menghilangkan nitrogen dianggap lebih murah biayanya dibandingkan dengan kombinasi proses nitrifikasi dan denitrifikasi. Namun, bioreaktor anammox kebanyakan dieksplorasi pada suhu tinggi (> 30°C) dimana diperlukan sistem pengendalian suhu. Penelitian ini bertujuan untuk mengembangkan dan menerapkan proses anammox untuk menghilangkan nitrogen dengan konsentrasi tinggi pada suhu lingkungan yang digunakan untuk pengolahan air limbah di negara tropis. Dalam penelitian ini, dipakai sebuah Reaktor kolom biofilm *up-flow*, dimana pada bagian atas kolom ditempatkan bahan poliester *non-woven* berpori sebagai pembawa untuk menempelkan bakteri anammox, telah dioperasikan tanpa sistem pemanas. Laju penghilangan nitrogen maksimum (NRR) sebesar 1,05 kg-N m<sup>3</sup> d<sup>-1</sup> telah dicapai pada hari operasi ke 178 dengan efisiensi total penghilangan nitrogen (TN) sebesar 74%. Hal ini menunjukkan bahwa reaktor kolom biofilm anammox telah berhasil diterapkan untuk menghilangkan nitrogen dengan konsentrasi cukup tinggi dari air limbah sintesis pada suhu yang cukup rendah.

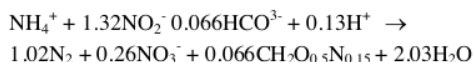
### INTRODUCTION

Nitrogen pollution from industrial wastewater, domestic wastewater and agricultural wastewater streams is recently considered as one of critical environmental problems for water resource protection. The discharge of untreated wastewater containing high nitrogen concentration is known to be one of the causes of eutrophication and oxygen depletion as a result of an abundance of nitrates and phosphates in surface water [1].

Excessive use of fertilizers in the agricultural sector and daily human activities are the main sources of nutrient pollution. Nitrogen pollution has become an important problem in many countries around the world, including Indonesia as one of developing countries with a lot of agricultural activity. Indonesia also has many fertilizer industries to support their various crop production. Treatment of nitrogen from industrial wastewater has become an emerging issue for environmental protection in Indonesia.

Conventionally, ammonium nitrogen from wastewater has been removed by a combination of biological process between nitrification and denitrification. This system requires an oxygen supply as electron acceptor during nitrification process. Besides an additional carbon source must sometimes be supplied for anoxic denitrification. Thus, this leads to increase the operational cost of the full-scale treatment plant.

Recently, anaerobic ammonium oxidation (anammox) has been recommended as a new biological approach for ammonia removal from wastewater. Anammox is based on the utilization of ammonium nitrogen ( $\text{NH}_4^+$ -N) as an electron donor under anaerobic condition for nitrite ( $\text{NO}_2^-$ -N) reduction resulting dinitrogen ( $\text{N}_2$ ) gas as the final product. This process is described on equation [2] below.



Compared to the conventional nitrification and denitrification process, the partial nitrification-anammox process is considered to be more cost-effective due to less required oxygen demand and no extra additional carbon source [3]. In the partial nitrification and anammox process, half of ammonium is partly oxidized to nitrite and subsequently anammox bacteria oxidize anaerobically the ammonium using nitrite to dinitrogen gas. However, in the practice, an anammox reactor system with highly efficient biomass retention and with high mass transfer system is entailed due to extremely slow growth rate of anammox bacteria with a doubling time of about 11 days [4]. Besides, up to now the reported anammox systems in the literature were mostly operated at 30–37°C [5] [6].

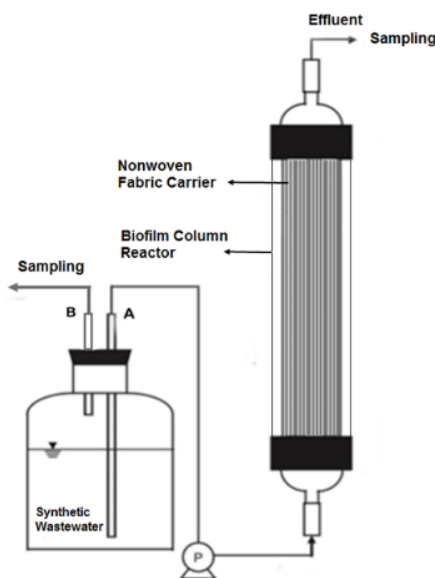
It is therefore important to find a suitable anammox reactor system capable of retaining the biomass as effective as possible and with a highly efficient substrate transfer for treating high ammonium nitrogen concentration at moderately low temperature (25–27°C). This research has opened an operational window for treating fertilizer wastewater with high ammonium concentration applied in tropical countries. In many tropical countries, the availability of sunlight is abundant. It is possible to develop a bioreactor system which equipped with microorganism living in moderate low temperature, without any water heating systems. By using the appropriate bacteria and reducing additional cost caused by heating apparatus, the low cost bioreactor system could be improved. The objective of this research is to develop and to apply anammox process for high nitrogen concentration removal

at ambient temperature used for treating wastewater in tropical countries, such as Indonesia.

## MATERIALS AND METHODS

### Reactor

An up-flow biofilm column reactor with an effective volume of 1.65 L was operated where the upper part was constructed with a porous polyester non-woven fabric material (Ohyapile, Japan) as a carrier to effectively attach the Anammox bacteria. The column in Anammox reactor was inoculated with KSU-1 strain from Osaka University and operated at ambient tropical temperature without any water heating systems (25–27°C). The reactor was always enclosed with a black vinyl sheet to prevent the inhibition of the growth of photosynthetic bacteria [7]. Peristaltic pumps (Watson Marlow, 500 series) were used to control the feed inflow rate. The reactor was run without recycling and the effluent was driven by gravity. Samples were taken from the inflow and overflow lines regularly to analyze nitrogen reduction.



**Figure 1.** Schematic research equipment of biofilm column reactor

### Feeding Media

The bioreactor's influent was a synthetic wastewater consisted of  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{NaNO}_2$ ,  $\text{NaHCO}_3$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and EDTA. All of

The chemicals were supplied by Merck. Concentrations of ammonium and nitrite were increased stepwise over the ranges. The Anammox enrichment medium had a bicarbonate-buffering agent instead of phosphate, given the possible toxicity of the latter.

### Analytical methods

Ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) and Nitrite nitrogen ( $\text{NO}_2^-\text{-N}$ ) was determined with the colorimetric method and nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ) with the ultraviolet spectrophotometric screening method [8]. pH and DO was measured using a pH meter (F55, Horiba Ltd, Japan) and a DO meter (OM-51, Horiba Ltd, Japan).

## RESULT AND DISCUSSION

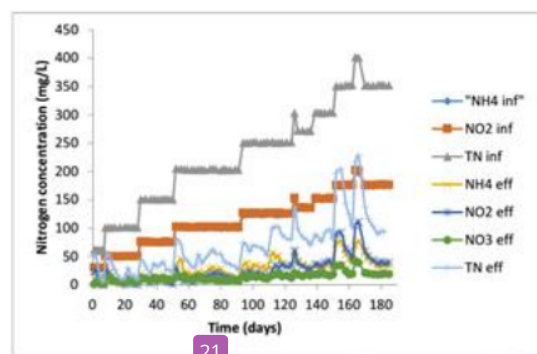


Figure 2. Evolution of the nitrogen concentration in the influent and effluent along the operational period.

The biofilm column in anammox reactor has been operated for 185 days. The reactor was continuously fed with ammonium and nitrite with a ratio of 1:1.1. The time course of influent and effluent nitrogen concentrations shown in Figure 2. As can be observed from this figure, during the operation, the influence of Total Nitrogen concentration had increased up to 350 mg/L (with 174 mg/L  $\text{NH}_4^+\text{-N}$  and 176 mg/L  $\text{NO}_2^-\text{-N}$ ) at the end of the experiment. Meanwhile, Hydraulic Retention Time (HRT) had decreased from 24 hours in the beginning until 6 hours in the day of 145 of total operations (Figure 3).

The reactor was started up with an influent Total Nitrogen concentration of 60 mg/L resulting a feed loading rate of 0.06  $\text{kg-N m}^{-3} \text{d}^{-1}$ . In early starting up period, the biomass inoculated into reactor was easy to wash out due to unwell attached of biomass. This shows a slower process during the start-up phase compared to the anammox reactor

studied by Ma et al. [9]. In their study, a hybrid reactor was installed and performed during day 1-39 resulting a rapidly increasing of the Nitrogen Loading Rate (NLR) from 0.35 to 1.2  $\text{kg-N m}^{-3} \text{d}^{-1}$  [9]. It is mainly because of temperature difference applied in this project. In our study, the biofilm column reactor was not equipped with a temperature controller, resulting the temperature varied from 25 to 27°C.

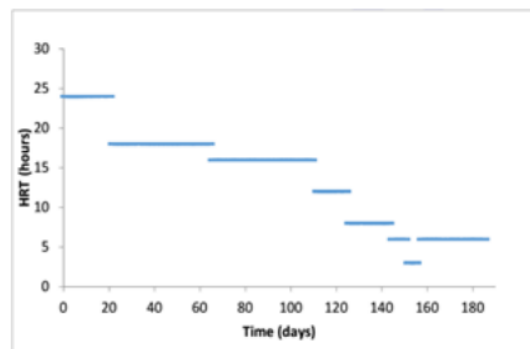
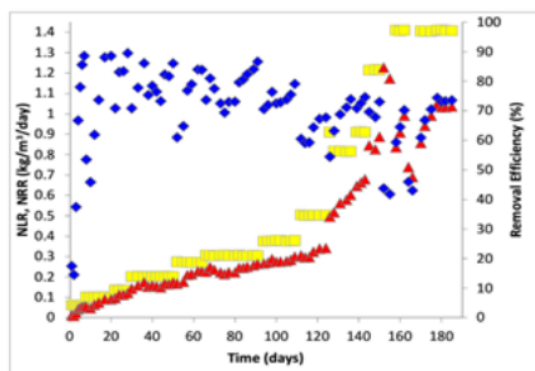


Figure 3. Time course of HRT during reactor operation

As can be seen in Figure 4, as the capacity of nitrogen removal of the system going up after a successful start-up, both the ammonium and nitrite concentrations in the influent flows were gradually increased from 60 to 350 mg/L. Besides, from days 121 to 145, the HRT was reduced from 12 to 6 hours. Consequently, the NLR was doubled from 0.6 to 1.4  $\text{kg-N m}^{-3} \text{d}^{-1}$ . During this period, a relatively high TN removal efficiency average was 75.3%. This value was lower compared with the maximum nitrogen removal based on anammox stoichiometry due to  $\text{NO}_2^-\text{-N}$  limitation. In addition, a maximum Nitrogen Removal Rate (NRR) of 2.05  $\text{kg-N m}^{-3} \text{d}^{-1}$  was reached by 178-day operation with a TN removal efficiency of 74%. Values of NRR treated in this research are lower than those previously referred by Ma et al. of 5.72  $\text{kg-N m}^{-3} \text{d}^{-1}$  in a UASB reactor treating low strength wastewater at 30°C [10]. Lower and uncontrolled temperature applied in this study could be addressed as the cause of decreased anammox duplicity. However, the value of NRR lower than this experiment was reported by Zekke et al. [11] working in an UASB reactor at 20°C with 0.5  $\text{kg-N m}^{-3} \text{d}^{-1}$  of NRR.



**Figure 4.** Time course of total NLR, NRR and Nitrogen removal efficiency ( $\diamond$  : TN removal efficiency;  $\square$  : NLR;  $\Delta$  : NRR)

However, higher total nitrogen removal efficiency achieved was desired. It was reported in a recent study by Reza and Cuenca (2016) that nitrogen removal began 140 days after start-up. In their study about simultaneous biological removal of nitrogen and phosphorus in a vertical bioreactor,  $\text{NH}_3\text{-N}$ ,  $\text{NO}_2^-$  and  $\text{NO}_3\text{-N}$  concentrations in the effluent were unsteady for approximately 240 days of the reactor operation. The nitrogen removal reached steady state conditions as concentrations were stable and consistent from day 240 until the end of the experiments (day 350) delivering above 90% total nitrogen removal efficiency [12]. The biofilm column reactor has been successfully operated for treating nitrogen from synthetic wastewater at moderately low temperature in this research, but the experimental result has shown total nitrogen removal efficiency of 74% was reached by 178-day operation. It might be because of the operation time was not long enough to allow the bacteria to get the optimum biomass for nitrogen removal. At 178 days of reactor operation probably the steady state condition of nitrogen removal has not been reached. Extended of reactor operation time was needed to increase total nitrogen removal.

## CONCLUSION

Nitrogen removal treatment of synthetic wastewater by using anammox biofilm column reactor was successfully operated. In this study, the application of anammox process for treating the wastewater at moderately low temperatures of 25-27 °C was utilized without any water heating systems. Total nitrogen removal efficiency of 74% in 178 days of reactor operation with a maximum NRR of 1.05

$\text{kg-N m}^3 \text{d}^{-1}$  was reached. This biofilm column reactor could be a suitable system for nitrogen removal using anammox biomass at ambient tropical temperature. The results showed that the operating cost of operating anammox biofilm column reactor could be reduced

## ACKNOWLEDGMENT

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