

APPLICATION OF NITROSOMONAS AND ANAMMOX COMBINATION IN A REACTOR FOR AMMONIUM REMOVAL IN SWINE WASTEWATER

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ABSTRACT

This study investigated the combination of two kinds of autotrophic bacteria, named Anammox and Nitrosomonas in a reactor to remove ammonium existed in swine wastewater. The reactor which was operated with swine wastewater taken from Dong A pig breeding enterprise (Di An, Binh Duong) had 15 liters volume. The biomass carrier used in this model was made from the synthetic acrylic with attached capacity of 0.5-0.6g-SS/g-material. The sludge was taken from a reactor using partial nitrification/Anammox processes of a previous study at Vietnam Academy of Science and Technology. In 118 days of operation, average influent ammonium was 350mg/l, feeding to three loading rates (LR) 0.47, 0.70, 0.93 kg N-NH⁴⁺/m³/day with flow rate 20, 30, 40 liters, respectively. The highest efficiency was found at the first loading rate 0.47 kg N-NH⁴⁺/m³/day with 91.55 %. The other loading rates 0.70 and 0.93 kg N-NH⁴⁺/m³/day gave lower efficiency, 78.08 and 70.5 %, respectively. VSS at the beginning and the end of the operation were 4g/l and 9.4 g/l, respectively. The optimum pH for the reactor was in range of 7.8 - 8.0.

Keywords: anammox, ammonium, nitrosomonas, swine wastewater.

1. INTRODUCTION

1.1. Biochemical mechanism

Based on investigation results by using isotopic method ¹⁵N, a biochemical mechanism for Anammox process was proposed [1]. The initial studies of Anammox showed that the combination reaction of ammonium and hydroxylamine with hydrazine oxidation reaction took place inside of a "steroid" called Anammoxosome, which can be found in Fig. 1. Anammoxosome is located in cytoplasm, covered by lipid ladderane membrane, and can be wholly separated from Anammox cell. Figure 2 illustrates the process of passing through the intermediate product called hydrazine (N₂H₄) which is implemented by the participation of

hydrazine oxidation (HZO) enzyme. Similar to hydroxylamine oxidoreductase (HAO) enzyme involved in aerobic ammonium oxidation, HZO catalyses reaction to oxidize hydrazine to nitrogen molecule ($G^0 = -288$ kJ/mol). The electrons from this oxidation process ($4e^-$) help deoxidize nitrite to hydroxylamine with the catalysis of an enzyme provisionally called nitrite removing enzyme ($G^0 = -22.5$ kJ/mol). The hydroxylamine produced will react with ammonium to produce a new hydrazine catalyzed by the enzyme Hydrazine hydrolase (HH), ($G^0 = -46$ kJ/mol). The catalytic cycle will be repeated many times.

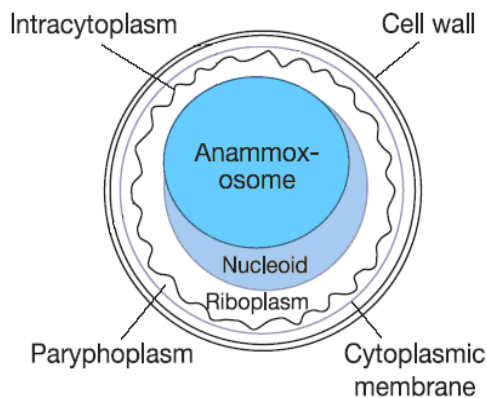


Figure 1. Diagram of components in an Anammox cell [2].

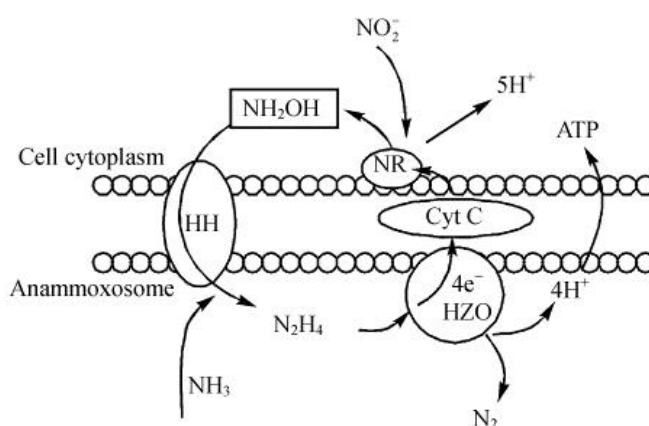


Figure 2. Biochemical mechanism of Anammox [1].



Figure 3. Biomass of Anammox (with a special red colour) [3].

An interesting point regarding the HZO enzyme of Anammox bacteria is that its structure is similar to that of HAO in *Nitrosomonas* bacteria, i.e. it contains cytochrome C (cyt C) with heme C nucleus which is able to absorb strong light at wavelength $\lambda = 468$ nm (similar to P460 of HAO) [1]. Because the central ions of these haemium are iron ions (Fe^{II} and Fe^{III}), the Anammox bacteria are red in color gathering at high concentrations. As shown in Fig. 3, appearing red colour in activated sludge is a good indication of the presence of Anammox bacteria.

1.2. Microbiology of Anammox process

Based on sequence analysis of the 16S rDNA, three genera of Anammox bacteria were discovered, including *Brocadia*, *Kuenenia* and *Scalindua*. These three genera share an ancestor, but are far apart in evolution, similarity is smaller than 85 %, while they are clearly similar in phenotype: slower growth rates are the same, having Anammoxosome with a lipid ladderance membrane [4].

Brocadia is the name of the ammonium removal pilot station where the *Candidatus Brocadia Anammoxidans* bacterium was discovered for the first time at Gist-Brocades, the Netherlands [1]. In 2000, the *Anammox* bacterium were found in the RBC (Rotating Biological Contactors) treatment system in Stuttgart (Germany) was identified as a new kind (less than

90 % similar to *Candidatus Brocadia Anammoxidans*) and was named *Candidatus Kuenenia stuttgartiensis* [4]. Anammox bacteria was also found in natural ecosystems of the Black Sea where have low dissolved oxygen in water. The results of the 16S rDNA sequence analysis show that 87.9 % and 87.6 % are similar to those of *Kuenenia* and *Brocadia*, which means that the discovered bacterium belongs to another genus and is named *Candidatus Scalindua sorokinii* [4].

2. MATERIALS AND METHODS

The wastewater input used to operate the model was taken from the outlet of the UASB tank in the pig manure wastewater treatment system of Dong A pig breeding factory in Di An district, Binh Duong province with the capacity of 150 m³/day. The data for the analysis of input wastewater is summarized in Table 1. The wastewater is taken daily to operate the model. Samples were taken twice a day to analyze pH, COD, N-NH₄⁺, N-NO₂⁻, N-NO₃⁻, and P-PO₄³⁻ throughout the experiment period.

Table 1. Characteristics of swine wastewater after Anaerobic tank.

No.	Param	Unit	Range	Avg
1	pH	-	7.7 – 8.4	8.0
2	COD	mg/l	116 – 216	160
3	N-NH ₄ ⁺	mg/l	307 – 401	350
4	N-NO ₂ ⁻	mg/l	0 – 0.6	0.3
5	N-NO ₃ ⁻	mg/l	1.0 – 2.2	1.5
6	Total P	mg/l	14 – 25	18
7	t ^o	°C	28 – 32	30
8	DO	mg/l	0.1 – 0.2	0.1

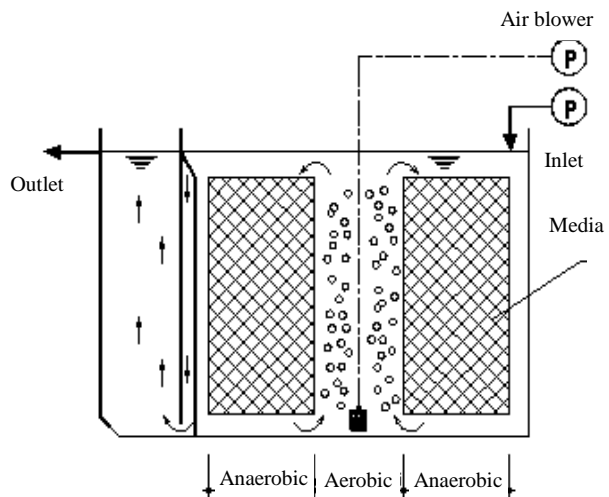


Figure 4. Model of reaction tank and settlement tank.

Table 2. Operation of the reactor.

Phase	Day	Loading rate kgN-NH ₄ ⁺ /m ³ /day
Preparation	4	-
Starting	24	-
Operation	90	-
The 1 st LR	30	0.47
The 2 nd LR	30	0.70
The 3 rd LR	30	0.93

As shown in Fig. 4, the wastewater was fed into the reaction chamber from bottom, flowing through the media, passing the wall to reach to the settlement tank, then went out via a tube.

Sludge was obtained from the pig manure wastewater treatment system by the nitrification /anammox combination of a previous study at the Institute of Tropical Biology [5]. The sludge is put into the measuring cylinder, then fed to the reaction chamber 4 liters. The sludge sample was analyzed for VSS (4000 mg/l). The use of this type of mud is to reduce the system start-up time because the liquor contains *Nitrosomonas* bacteria and Anammox has adapted to swine wastewater.

3. RESULTS

3.1. Ammonium removal efficiency

Figures 5, 6 and 7 illustrate N-NH₄⁺ removal efficiency at loading rates of 0.47; 0.70; 0.93 kg N-NH₄/m³/day, respectively.

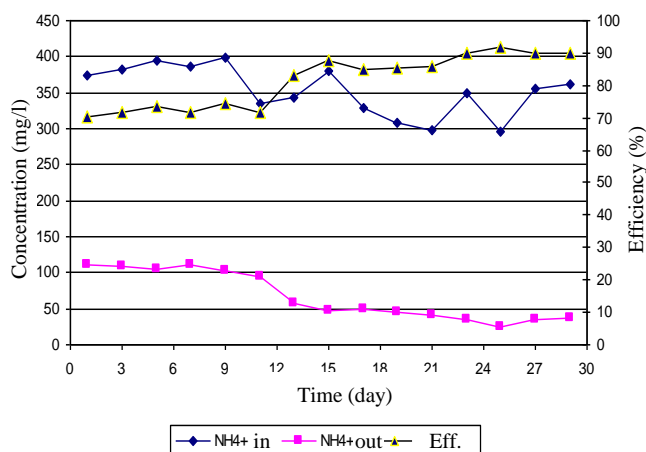


Figure 5. NH₄⁺ removal eff. at LR of 0.47 kg N-NH₄/m³/day.

As mentioned above, because the sludge for the experiment with Nitrosomonas and Anammox has already adapted to swine wastewater. In addition, in starting up phase, the model was run with a loading rate which is nearly equal to the expected loading rate in the experiment. Therefore, the model reached a relatively high efficiency (70.51 %). After 15 days of operation, the efficiency started to rise from 71.56 % to 83 %. The efficiency got the highest at 91.55 %. As observed, the efficiency of the model at 0.74 kgN-NH₄/m³/day just fluctuated around 90 %.

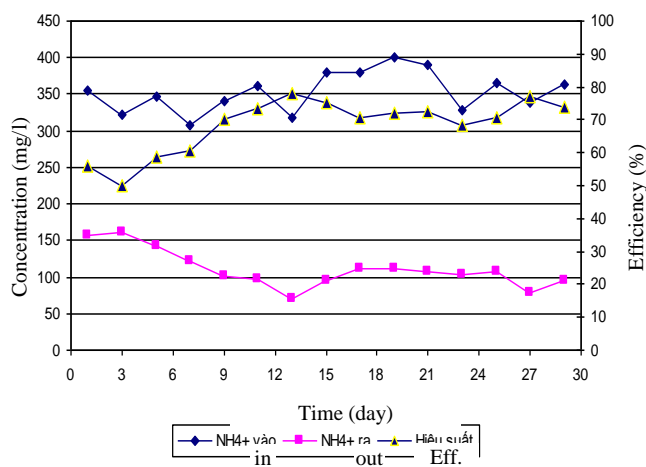


Figure 6. NH₄⁺ removal eff. at LR of 0.7 kg N-NH₄/m³/day.

As presented in Fig.6, when the loading rate was increased 1.5 times more than the previous one, the treatment efficiency was relatively low (50.16 %). The reason is that bacteria need a little time to adapt to the new environment. After 15 days of adaptation, the efficiency increases from 60.59 to 70.09 %, then the efficiency continue to go up but it is not steady. The efficiency of 78.08 % is reached when the model run in 30 days and this efficiency is stable.

Just like when increasing the loading rate in the previous experiment, it took some time for the bacteria to adapt, resulting in relatively low efficiency (52.52 %) for initial time. In addition, the loading rate of this experiment was relatively high, thus requiring a longer adaptation time than the previous two loading rates. In 20 days in a row, the performance was slow, unstable, and in the range of 52.52 % to 57.48 %. After 30 days of operating, efficiency reached 70.5 %.

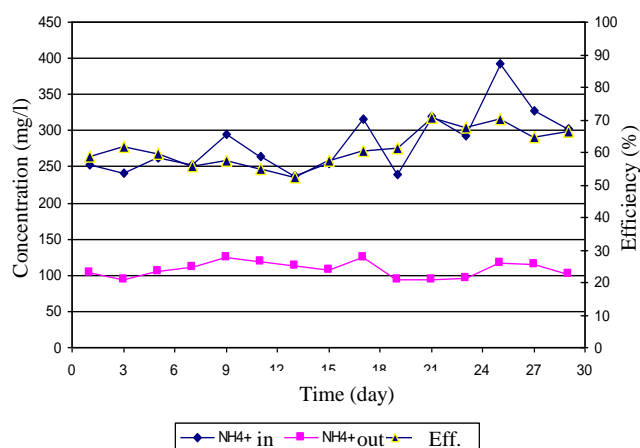


Figure 7. NH_4^+ removal eff. at LR of 0.93 kg N- $\text{NH}_4/\text{m}^3/\text{day}$.

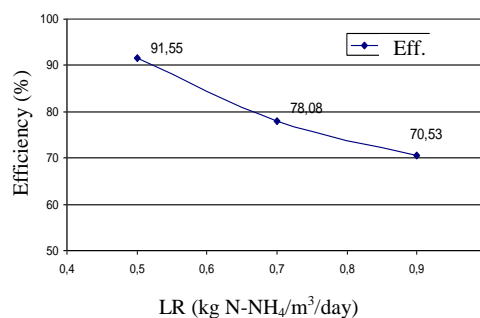


Figure 8. Eff. of three different LR.s.

3.2. Comparison of different three loading rates

Figure 8 illustrates efficiency of three LR.s. At a loading rate of 0.47 kg N- $\text{NH}_4/\text{m}^3/\text{day}$ the process yielded the highest efficiency, 91.55 %. At higher loading rates the treatment efficiency begins to decrease, the efficiency is 70.41 % at 0.93 kg N- $\text{NH}_4/\text{m}^3/\text{day}$ load. This result is consistent with a study of Lieu PK, using a model of combination of partial nitrification and anammox, which used acrylic fiber media and leachate. In his study, a loading rate of 0.6 and 0.1 N- $\text{NH}_4^+/\text{m}^3/\text{day}$ give efficiencies nearly 90 and 80 %, respectively [6].

3.3. COD, NO_2^- and NO_3^- concentrations

Table 3 presents experimental results for COD, NO_2^- and NO_3^- concentrations.

Table 3. COD, NO_2^- and NO_3^- concentrations.

Contents	Influent (mg/l)	Effluent (mg/l)
COD	116 - 216	110 - 210
NO_2^-	0 - 0.6	2.0 - 5.4
NO_3^-	1.0 - 2.2	11.8 - 17.6

The experiment results in the accumulation of nitrite, which means that nitrification (Nitrosomonas bacteria participation) took place in the reactor. If the amount of nitrite produced continues to be degraded i.e. oxidized to nitrate, the nitrate concentration produced after the reaction must be high, while the fact that the amount of nitrate generated here is negligible. It is assumed that if denitrification occurs in the anaerobic zone (Anammox zone), the amount of COD at the outlet must be significantly reduced as nitrate degradation is an anaerobic process that requires organic carbon source, while COD outlet changed very little, so it is possible to eliminate the traditional denitrification process. Thus, the traditional denitrification does not exist in the reactor, nitrite accumulation, nitrite and ammonium concentrations in effluent are

low. In summary, it is possible to confirm that this is Nitrification – Anammox combination process, in which two groups of bacteria Nitrosomonas and Anammox participate.

3.4. Comparison of treatment efficiency with other studies

The model operates efficiently, stably with high ammonium treatment efficiencies at loading rates of 0.47 - 0.70 kgN-NH₄⁺/m³/day. At higher loads, performance is reduced but still acceptable. Compared with the loads of authors who previously studied, the loading rate in the experimental model in this study was moderate. As Ahn and his colleagues study swine wastewater at a load of 1.36 kg N-NH₄⁺/m³/day, the treatment efficiency is 80 % [7]. M. Strous studied synthetic wastewater, with a loading capacity of 0.2 - 2.0 kg N - NH₄⁺ /m³/day, respectively, reaching 90 % and 57 %, respectively [8]. Jetten et al. studied the effluent from the slurry, loaded with 0.48 - 2.6 kg N - NH₄⁺/m³/day, respectively with a treatment efficiency of 90 % and 47 % [9].

4. CONCLUSIONS

From experimental results, the combination ability of two groups of Nitrosomonas and Anammox bacteria in one reactor to treat ammonium in swine waste water was determined. Because these two groups of bacteria have different physiological characteristics, the use of the media (sticky biological model) is appropriate for these two groups to work together in one reactor. The ammonium treatment efficiency is rather high, the ammonium concentration after treatment meets the standard TCNV 5945 - 2005, column B.

The appropriate loading rate which is applied to this technology for treating ammonium in swine waste water is 0.5-0.7 kgN-NH₄⁺/ m³/day. With the advantages that were analyzed in the theory and the results from the experiment, it was found that the combination of Nitrosomonas and Anammox in one reactor was appropriate for treatment of nitrogen in swine waste water as well as rich ammonium wastewater.

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