



# Factors affecting the treatment of reject water by the anammox process

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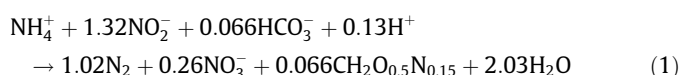
## ABSTRACT

Reject water from a municipal wastewater treatment plant was treated using a stirred tank anammox reactor after being treated by a partial nitrification reactor. The results indicated the variations in the influent  $\text{NO}_2^-$ -N to  $\text{NH}_4^+$ -N ratio had a negative effect on reactor performance, especially when the T-N concentrations were high. Influent total organic carbon concentrations greater than 50 mg/L were proven to have a serious effect on the nitrogen removal efficiencies of the anammox reactor. Observations by scanning electron microscope showed that the surface of the anammox granular sludge was covered by some materials, possibly the effluent SS contained in the partial nitrified reject water. Furthermore, the study of the bacterial composition of the anammox granular sludge showed that the anammox bacterium, Planctomycete KSU-1, was dominant, even during the inhibition phase.

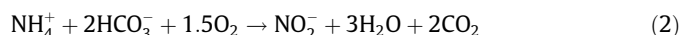
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## 1. Introduction

The use of anaerobic ammonium oxidation (anammox) as an alternative to the conventional nitrogen treatment processes of nitrification and denitrification has received considerable attention since its discovery. In this process, ammonium is oxidized to dinitrogen gas under anaerobic conditions, with nitrite serving as the electron acceptor and carbon dioxide being used as the carbon source for anammox microorganisms involved. In fact, the existence of anaerobic ammonium oxidation was predicted over two decades ago, as shown in Eq. (1). Anammox treatment has the advantage of low operational costs, but the disadvantage of slow growth of anammox bacteria.



Reject water is a high-strength wastewater, in which the low level of organic carbon present is only slightly treated by biodegradation. A high concentration of ammonium contained in reject water must be treated before discharging the effluent to natural bodies. The combination of partial nitrification and anammox provides a novel approach in treating reject water. In the process of partial nitrification, more than half of the wastewater influent ammonium must be partially oxidized to nitrite (Eq. (2)), avoiding the complete oxidation of nitrite to nitrate (Eq. (3)).



Studies have been conducted to evaluate the feasibility of treating leachate with high ammonium concentrations as a pre-parative step for the anammox process (Ganigué et al., 2009), and the successful treatment of livestock wastewaters by anammox has been reported (Molinuevo et al., 2009). However, obtaining stable nitrification efficiency is still a challenge because of variable influent nitrogen and organic matter loads and different environmental conditions (van Kempen et al., 2001; van der Star et al., 2007). Previous studies have demonstrated that the organic loading rate had a definite effect on anammox performance; however, the inhibitory levels remained uncertain (Sabumon, 2007; Wang and Kang, 2005; Molinuevo et al., 2009). An organic matter concentration (based on the chemical oxygen demand (COD)) greater than 300 mg/L was reported to inhibit the anammox process in a reactor fed with milk as an organic carbon source (Chamchoi et al., 2008). Concentrations of 50 mM acetate have also been found to inhibit the anammox process (Dapena-Mora et al., 2007). Finally, COD concentrations greater than 237 mg/L (loading rate of 112 mg COD/L/d) for post-digested effluent and above 290 mg/L (loading rate of 136 mg COD/L/d) for partially oxidized effluent of pig manure effluents have been reported to lead to complete shutdown of the anammox process (Molinuevo et al., 2009).

This study was conducted to further investigate the effects of organic matter concentration on the anammox process in a stirred tank anammox reactor (STAR) fed with reject water from a municipal wastewater treatment plant. Additionally, the effects of variations in influent quality and FA concentrations on

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anammox performance were studied systematically. Finally, batch tests were conducted to confirm the effects of TOC concentrations on the anammox process.

## 2. Methods

### 2.1. Reactor

The configuration of the stirred tank anammox reactor (STAR) is depicted schematically in Fig. 1. The reaction zone was 1.4 L and the stirring speed was maintained between 70 and 120 rpm. To prevent anammox granular sludge from flowing out, a nylon net was used for solid/liquid separation. The working area of the filter was 201 mm<sup>2</sup> and the mesh size was about 1.5 mm. The reactor was covered with black cloth to avoid light inhibition. The reactor temperature was maintained at 33–35 °C using a water jacket. The influent pH was 7.5–8. The influent DO concentration was maintained below 0.5 mg/L by flushing the tank with nitrogen gas (Strous et al., 1997).

### 2.2. Seeding anammox sludge

Anammox sludge that was cultivated in the laboratory in an up-flow column reactor using a nonwoven biomass carrier was used as seed sludge for anammox pellets (Furukawa et al., 2001). The sludge had a mixed liquor suspended solids (MLSS) level of about 3.65 g/L and a volatile MLSS (MLVSS) of 2.90 g/L. After 320 days of operation with synthetic wastewater, partially nitrified reject water was introduced into the STAR. The MLSS and MLVSS of the influent at this time were 10.1 g/L and 8.12 g/L, respectively.

### 2.3. Feed wastewater and experimental procedures

The anammox reactor operation process could be divided into two periods. During the first period, the reactor was fed with synthetic wastewater consisting of different concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup>: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (75–380 mg/L), NaNO<sub>2</sub> (75–380 mg/L), KHCO<sub>3</sub> (125 mg/L), KH<sub>2</sub>PO<sub>4</sub> (54 mg/L), FeSO<sub>4</sub>·7H<sub>2</sub>O (9 mg/L), EDTA-2Na (5 mg/L) and (2 ml/L) of concentrated mineral salt solution (Strous et al., 1998). The ammonium to nitrite molar ratio in the feeding media was fixed at 1.0. After 320 days of continuous operation, the nitrogen loading rate (NLR) increased to the maximum 9.52 kg/m<sup>3</sup>/d (Li et al., 2010). During the second period, reject water obtained from the Kumamoto East Wastewater Treatment Plant was treated in a PN reactor before being fed into the reactor. The characteristics of the reject water are as follows: TOC concentrations, 50–150 mg/L; BOD<sub>5</sub> concentrations, 150.1–200.8 mg/L; COD concentrations, 178.1–274.3 mg/L; NH<sub>4</sub><sup>+</sup>-N concentrations, 428.0–1210.5 mg/L; NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N concentrations, 0; SS, 40–100 mg/L; pH, 8.16–9.37.

### 2.4. PN treatment

Fig. 1 also shows a diagram of the PN reactor. The PN reactor had a working volume of 5.3 L, updraft and downdraft sections in a parallel upright arrangement, and was aerated via a peristaltic pump at the bottom (Cassette Tube Pump SMP-21) that was controlled using a Rota meter. The reactor was equipped with acrylic fiber biofringe media (BF, NET Co., Japan), which was used as the biomass carrier to provide a good environment for microbial growth. A settling tank with a volume of 2.5 L was used for sludge sedimentation and recycling. The settled sludge was gently mixed and returned to the aeration tank from the bottom of the settling tank at a 100% recycling rate. The reactor temperature was maintained at 19–32 °C and the pH was controlled using the alkalinity of the influent without the addition of acid.

### 2.5. Batch tests

Completely closed vials with a total volume of 120 mL were used to conduct the anammox batch tests in the dark. The biomass concentration (VSS) at the beginning of the experiment was about 0.5 g/L. The pH and temperature were fixed at 7.5 and 35 ± 1 °C, respectively. The head space of the vial was replaced with N<sub>2</sub> gas. The serum bottles were sealed tightly with butyl rubber caps. The TOC concentrations were changed by adding different concentrations of KHCO<sub>3</sub> and yeast extract, respectively. Variations in the NH<sub>4</sub><sup>+</sup>-N concentrations were periodically monitored during the incubation period.

### 2.6. Analytical methods

NO<sub>2</sub><sup>-</sup>-N, NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N were measured according to the American Public Health Association standard methods (APHA, 1995). Specifically, NO<sub>2</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N were measured using colorimetric methods and NO<sub>3</sub><sup>-</sup>-N was analyzed by UV spectrophotometry. The pH was measured with a pH meter (B-211, Horiba, Japan), while DO was measured using a digital portable DO meter (D-55, Horiba). TC (total carbon) and TOC (total organic carbon) were measured using a TOC analyzer (TOC-V CPH, Shimadzu, Japan). The concentrations of free ammonia (FA) and free nitrous acid (FNA) were determined as a function of pH, temperature, and total ammonium as nitrogen (TAN) for FA or total nitrite (TNO<sub>2</sub>) for FNA (Anthonisen et al., 1976).

### 2.7. SEM observation

The anammox granular sludge was observed using a scanning electron microscope (SEM). The specimens were prepared as follows. Samples were fixed with 2.5% glutaraldehyde solution and 1% osmic acid solution, after which they were washed with phosphate buffer. Next, the samples were subjected to sequential ethanol dehydration (including 10%, 30%, 50%, 70%, 90%, 95% and 99.5%

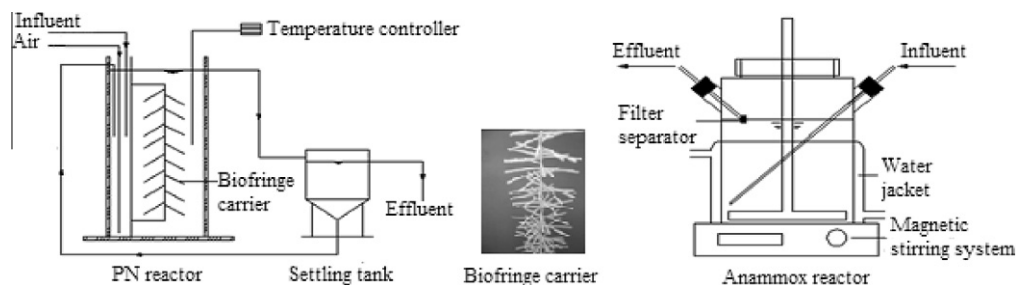


Fig. 1. Configuration of the reactors used for partial nitrification (PN) process and continuous anammox treatment.

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