

TREATMENT OF HIGH-STRENGTH AMMONIA CONTAINING WASTEWATER USING PARTIAL NITRITATION SYSTEM WITH BIOLOGICAL SELECTOR

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ABSTRACT

In this paper, one partial nitrification system with biological selector (PNBS) was first evaluated in treating high-strength ammonia containing wastewater. Nitrite production rate (NPR) of $8.25 \text{ kg N m}^{-3} \text{ d}^{-1}$ was achieved with influent $\text{NH}_4\text{-N}$ concentration 2000 mg l^{-1} and HRT 3.2 h, which is, to our knowledge, the highest NPR to be treated successfully by a nitrification reactor. A genetic analysis of the organisms in the PNBS revealed an abundance of *Nitrosomonas* sp. ENI-11, uncultured bacterium clone: AnDHS-3, *Nitrosomonas* sp. ENI-11 and *Nitrosomonas eutropha* enriched in the PNBS reactor.

Keywords: Nitrification; Biological selector; High-strength; Ammonia containing wastewater; Nitrite oxidizing bacteria;

1. Introduction

ANAerobic AMMonium OXidation (ANAMMOX) process has proven to be one promising technology due to high efficiency, low running cost and reasonable footprint comparing to conventional biological nitrogen removal process (Kuenen, 2008). The number of full-scale installations has substantially increased in the past three to five years since the first full-scale partial nitrification–anammox reactor in Rotterdam (Van der Star *et al.*, 2007). In order to achieve nitrogen removal, about 56% ammonia in the wastewater should only be oxidized to nitrite by ammonia oxidizing bacteria (AOB) in an anammox process. Following, anammox bacteria convert nitrite and residual ammonia into nitrogen gas under anaerobic conditions. However, nitrite oxidizing bacteria (NOB), mutualistic bacteria of AOB, can oxidize nitrite to nitrate, which cannot be degraded any further by anammox process. Therefore, the growth and activity of NOB should be strictly controlled in anammox process.

High temperature, low dissolved oxygen (DO), and free ammonia are usually suggested to inhibit the growth of NOB (Fux *et al.*, 2002). SHARON (Single reactor system for High activity Ammonium Removal Over Nitrite) process has been developed in view of different growth rate of AOB and NOB under high temperature, and reasonable sludge retention time (SRT) is controlled in the reactor (Hellings *et al.*,

1998). Subsequently, although high nitrogen loading rate up (NLR) to $10 \text{ kg m}^{-3} \text{ d}^{-1}$ was reported in one full-scale anammox reactor, NLR was only achieved at $0.8 \text{ kg m}^{-3} \text{ d}^{-1}$ in the nitrification reactor due to the short SRT (Van der Star *et al.*, 2007). In some cases, low DO was adopted to inhibit the growth and activity of NOB (Sun *et al.*, 2014). However, low DO decreases the matrix transfer rate of AOB, which is the key factor for high rate wastewater treatment. It was reported that the growth of NOB would be limited when free ammonia reached $0.1\text{--}10 \text{ mg l}^{-1}$ in the reactor (Jianlong and Ning, 2004). Thus, pH adjustment was used to control the free ammonia in the reactor (Qiao *et al.*, 2008). Nonetheless, NOB could accustom to high free ammonia condition following the cultivation period, therefore, combined method such as low DO is also adopted in real wastewater treatment. As a result, reducing NLR is often adopted for anammox process. In order to reduce the footprint of anammox process, increasing NLR was urgent for nitrification reactor.

In this study, one new designed partial nitrification system with biological selector (PNBS) was used to study the maximum nitrogen loading rate for nitrification process. Genetic analysis using the 16S rRNA gene was employed to characterize the microbial population of the bacteria in the reactor.

2. Materials and methods

The PNBS system includes aeration zone and biological selector (Fig. 1). The cross-sectional areas of the aeration zone were $25 \times 20 \text{ cm}$, and the height of effluent port was 100 cm (total volume 50 l). A biological selector with volume of 10 l was used for AOB selecting and recycling. DO under 0.5 mg l^{-1} was controlled to inhibit the growth of NOB in the biological selector, and recycling rate of 400% was adopted to select the sludge of aeration zone. One mixer was set on the upper part of the biological selector, which was used for agitating the settling sludge. The feed solution was introduced to the updraft section with a peristaltic pump (Baoding Longer Precision Pump ZG-600). Air was supplied using an air pump at the bottom. pH was controlled by addition of alkali ($0.5 \text{ mol l}^{-1} \text{ NaHCO}_3$ and $0.5 \text{ mol l}^{-1} \text{ Na}_2\text{CO}_3$) solution. Reactor temperature was controlled by using one heater.

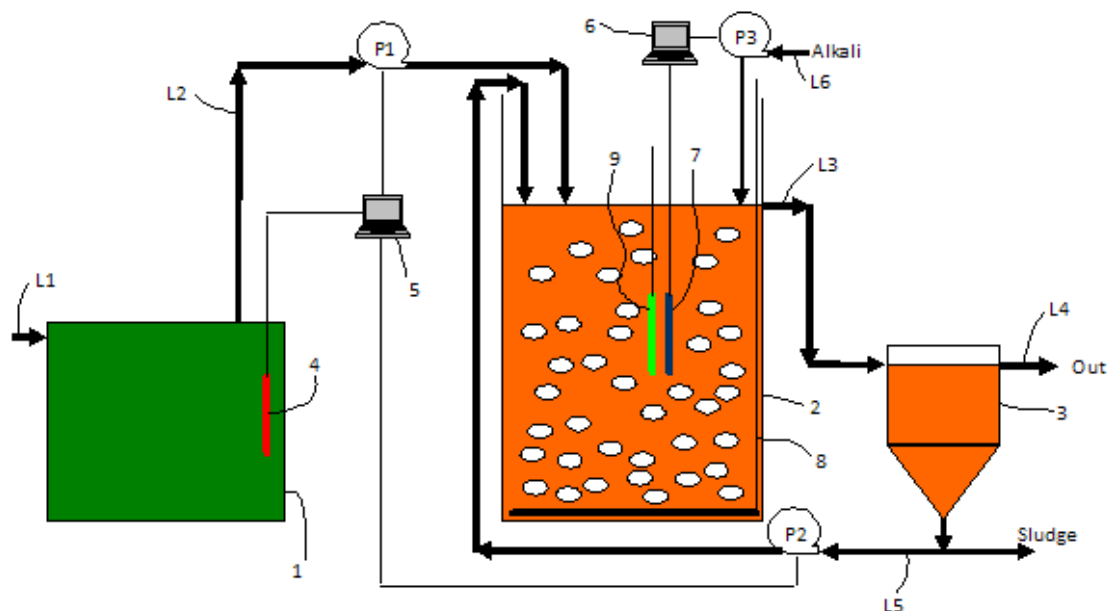


Figure 1. Schematic view of PNBS system

(Symbols: L1 Influent; L2 Influent pipe; L3 Aeration zone out-pipe; L4 Effluent pipe; L5 Sludge-recycle pipe; L6 Alkali pipe; 1 Adjustment tank; 2 Aeration zone; 3 Biological selector; 4 Ammonia online analyzer; 5 Pump flow controlling panel; 6 Alkali pump flow controlling panel; 7 pH online analyzer; 8 Air blow; 9 DO online analyzer; P1 Influent pump; P2 Sludge-recycle pump; P3 Alkali pump)

The PNBS system was inoculated with activated sludge from a running ceramic membrane reactor in Guilin, China, and the initial mixed-liquor suspended solids (MLSS) concentration was 2000 mg l⁻¹. The reactor was fed with synthetic wastewater. Nutrient medium consisted of 500-2000 mg-N l⁻¹ NH₄HCO₃, 50 mg l⁻¹ KH₂PO₄, 200 mg l⁻¹ MgSO₄·2H₂O, 300 mg l⁻¹ CaCl₂·2H₂O, and 1 ml of trace element solution I and II (Qiao *et al.*, 2008).

NO₂-N was measured by the colorimetric method according to Standard Methods (APHA, 1995). NH₄-N was measured by the phenate method according to Kanda (1995). Total nitrogen (TN) was determined by the persulfate method (APHA, 1995) using the UV spectrophotometric screening method (APHA, 1995) for quantification of TN as NO₃-N (the oxidization product of the persulfate digestion). NO₃-N (of the original sample) was determined by calculation of the difference of TN and the sum of NO₂-N and NH₄-N. The pH was measured by using a pH meter (9010, Jenco, USA), and dissolved oxygen (DO) was measured by using a DO meter (6010, Jenco, USA).

During the operation, the sludge was taken out from the PNBS reactor. The sludge samples were first ground with a pestle under liquid nitrogen.

2.1. DNA extraction and PCR amplification

Meta-genomic DNA was extracted using an ISOIL kit (Wako, Osaka, Japan) according to the manufacturer's instructions. Amplification of the 16S rRNA gene was performed with Phusion High-Fidelity DNA polymerase (FINNZYMES, Finland) using conserved eubacterial primers 6F (forward primer: 5'-GGAGAGTTAGATCTTGGCTCAG-3') and 1492r (reverse primer: 5'-GGTTACCTTGTTACGACT-3'). PCR was carried out according to the following thermocycling parameters: 30 s initial denaturation at 98 °C, 25 cycles of 10 s at 98 °C, 30 s at 51 °C, 20 s at 72 °C and 5 min final elongation at 72 °C. The amplified products were electrophoresed on a 1% agarose gel and extracted fragments were purified using Wizard SV Gel and PCR Clean-Up System (Promega, U.S.A.).

2.2. Cloning and sequencing of the 16S rRNA gene

The purified fragments were ligated into the EcoRV site of pBluescript II KS+ (Stratagene, USA) and *Escherichia coli* DH10B was transformed using the constructed plasmids. White colonies including the insert were randomly chosen and the plasmids were extracted by the alkaline method. The nucleotide sequences were determined with a 3130xl genetic analyzer and a BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, USA). The sequences determined in this study were compared with the sequences in the nr database using the basic local alignment search tool program (BLAST) on the NCBI web site.

2.3. Denaturing gradient gel electrophoresis (DGGE)

Partial 16S rRNA gene was amplified by PCR with a eubacterial primer set, 1055F- 1392R.

The extracted meta-genomic DNA and cloned plasmids were used as templates for the sample and markers in the DGGE, respectively. The amplified fragments were purified and combined with the GC-clamp (5'-CGCCCGCCGCGCCCGCGCCCGTCCCGCCGCCCCGCCCCG-3') at the 5' termini by a second PCR using a primer set, 357F with GC-clamp and 534R. The products were resolved by DGGE for 14 h at 90 V at 60 °C using the DCode system (Bio-Rad, Hercules, CA, U.S.A.). An 8% polyacrylamide gel with a 30-65% denaturing gradient was used, where 100% denaturant was defined as 7 M urea and 40% formamide. The gel was stained with SYBR-Gold (Invitrogen, U.S.A.) and visualized using the FLA-2000 system (Fuji Photo Film, Tokyo, Japan).

3. Results

A nitrogen loading rate (NLR) of 0.8 kg N m⁻³ d⁻¹ was used at the startup of the PNBS reactor, followed by gradual increases in the loading as the AOB population was enriched. The time courses of the levels of nitrogenous compounds over the course of the study are shown in Fig. 2. In this study, an effluent NO₂-N to NH₄-N above 1.32 was considered as the achievable goal for the start-up of the PNBS reactor in order to provide appropriate substrate for the following anammox reactor (Mulder *et al.*, 1995).

The start-up period for the PNBS reactor was considered to be from day 0 to day 5 (Fig. 2), during which time the influent $\text{NH}_4\text{-N}$ (500 mg l^{-1}) and HRT (15 h) was remained constant. The corresponding effluent $\text{NO}_2\text{-N}$ to $\text{NH}_4\text{-N}$ achieved on day 5 was 4.1 corresponding to zero $\text{NO}_3\text{-N}$ production. Subsequently, from day 6 to day 108 (Fig. 2), NLR was increased stepwise to $1.2 \text{ kg N m}^{-3} \text{ d}^{-1}$, $1.7 \text{ kg N m}^{-3} \text{ d}^{-1}$, $2.1 \text{ kg N m}^{-3} \text{ d}^{-1}$, $3.3 \text{ kg N m}^{-3} \text{ d}^{-1}$, $5.0 \text{ kg N m}^{-3} \text{ d}^{-1}$, $7.5 \text{ kg N m}^{-3} \text{ d}^{-1}$, $10 \text{ kg N m}^{-3} \text{ d}^{-1}$ and $15 \text{ kg N m}^{-3} \text{ d}^{-1}$ to probe the treatment potential of the PNBS reactor. On day 95, the influent $\text{NH}_4\text{-N}$ concentration and NLR was increased to 2000 mg l^{-1} and $15 \text{ kg N m}^{-3} \text{ d}^{-1}$, respectively, which were the highest levels in this study. Under these conditions, the effluent $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations were 1100 mg l^{-1} and 830 mg l^{-1} , respectively. Overall, the reactor could operate with a stable effluent $\text{NO}_2\text{-N}$ to $\text{NH}_4\text{-N}$ of over 1.32.

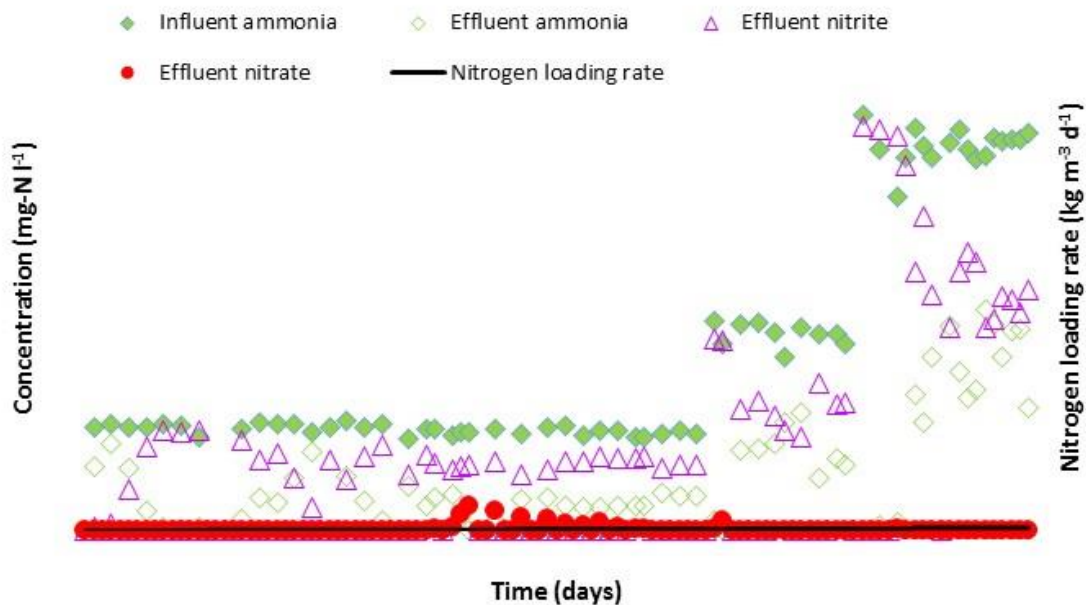


Figure 2. Reactor performance during the study

Table 1. Homology search results for 16S rRNA gene sequences of the main bacterial members in the community

Phylum	Taxon	Identity (%)	Number of clones					
			Day 0	Day 20	Day 40	Day 60	Day 80	Day 108
β -proteobacteria	<i>Nitrosomonas sp. ENI-11</i>	98-100	3	12	15	25	30	32
	<i>Uncultured bacterium clone: AnDHS-3</i>	100	1	1	1	2	2	2
	<i>Nitrosomonas sp. ENI-11</i>	95	1	1	1	1	1	1
	<i>Nitrosomonas eutropha</i>	98	1	1	1	1	1	1
Bacteroidetes	<i>Uncultured soil bacterium clone bacnit28</i>	96	10	8	8	6	6	4
	<i>Uncultured bacterium clone DSBR-B022</i>	99	22	15	12	5	3	1
	<i>Uncultured bacterium clone KD3-77</i>	91	10	6	4	5	2	1
	<i>Uncultured bacterium clone NR.4.002</i>	90	2	1	1	1	1	1

In this study, the seed sludge used in the PNBS reactor was drawn from a running MBR reactor. Samples were collected on day 0, day 20, day 40, day 60, day 80 and day 108 (Table 1). The genetic analysis showed that the share of *Nitrosomonas*. increased from 12% to 83.72% at the genus level. *Nitrosomonas sp. ENI-11*, *Uncultured bacterium clone: AnDHS-3*, *Nitrosomonas sp. ENI-11* and *Nitrosomonas eutropha* was dominant in the PNBS reactor. *Uncultured soil bacterium clone bacnit28*, *Uncultured bacterium clone DSBR-B022*, *Uncultured bacterium clone KD3-77* and *Uncultured bacterium clone NR.4.002*, were identified as co-existing bacteria in the PNBS reactor. Although these bacteria were also reported by other researchers (Yamamoto *et al.*, 2006), their functions are largely unknown.

4. Discussions

As shown in Fig. 2, the influent $\text{NH}_4\text{-N}$ concentration was increased to 2000 mg l^{-1} with NLR at $15 \text{ kg N m}^{-3} \text{ d}^{-1}$ (process hydraulic retention time of 3.2 h) near the end of the study. To our knowledge, this is the highest influent $\text{NH}_4\text{-N}$ concentration to be successfully treated to date (Table 2). Highly concentrated nitrogen wastewater is usually diluted prior to treatment in nitrification reactor (Yamamoto *et al.*, 2011). However, this practice is forbidden by law for wastewater treatment in China. Accordingly, the PNBS reactor as demonstrated here could have a very effective application for naturally occurring high-strength wastewaters.

Table 2. Comparison of possible influent $\text{NH}_4\text{-N}$ concentration and NLR for different reactors in wastewater treatment

Reactor type	Reactor volume (l)	NLR ($\text{kg m}^{-3} \text{ d}^{-1}$)	Influent $\text{NH}_4\text{-N}$ concentration (mg l^{-1})	References
Continuous activated sludge system	25	9.3	1400	Torà <i>et al.</i> (2014)
Granular airlift reactor	150	1.75	700	Torà <i>et al.</i> (2013)
Internal-loop airlift reactor	3.8	1.58	1484	Xing <i>et al.</i> (2013)
PNBS	50	15	2000	Present work

The predominant bacteria in this study are *Nitrosomonas sp. ENI-11*, *Uncultured bacterium clone: AnDHS-3*, *Nitrosomonas sp. ENI-11* and *Nitrosomonas eutropha*, which is a population balance same as others cases (Yamamoto *et al.*, 2006). Yamamoto *et al.*, (2011) reported that only 15% of the total clones were identified as *Nitrosomonas* in one nitrification reactor. In this study, *Nitrosomonas* was enriched at 83.72%. Accordingly, the achieved NLR is near to 7 times of the NLR reported by Yamamoto *et al.*, (2011). Thus, it is considered that the PNBS reactor used in this study can enrich *Nitrosomonas* effectively by which the NLR can be readily increased. Above that, PNBS reactor can provide appropriate conditions for *Nitrosomonas*, therefore, high NLR might be achieved by enhancing the activity of *Nitrosomonas*.

5. Conclusions

One PNBS reactor was used to investigate the treatment potential of ammonia containing wastewater. NLR was increased stepwise to $15 \text{ kg N m}^{-3} \text{ d}^{-1}$ with the influent $\text{NH}_4\text{-N}$ concentration of 2000 mg l^{-1} . The effluent $\text{NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations were 1100 mg l^{-1} and 830 mg l^{-1} , respectively. The reactor could operate with a stable effluent $\text{NO}_2\text{-N}$ to $\text{NH}_4\text{-N}$ of over 1.32. The results got in this study suggested that PNBS reactor can enrich and provide appropriate conditions for *Nitrosomonas*.

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