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High rate partial nitrification treatment of reject wastewater

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Partial nitrification (PN) treatments on reject wastewater were carried out. Dissolved oxygen concentration was limited by controlling air flowrate, which was the main operational strategy in this study. Stable PN performance was obtained during continuous operation for 80 days, with a maximum nitrogen loading rate (NLR) of 4.2 kg-N m^{-3} day^{-1} and ammonium conversion rate of 2.1 kg-Nm^{-3} day^{-1}. The production of nitrite oxidizers was assumed to be responsible for the nitrogen loss in the reactor. The ratios of NO_2^-–N/ (NO_2^-–N + NO_3^-–N) were always above 99.9%, and BOD removal efficiencies were also stable at around 70% even if a sharp increase in NLR was applied during the stable period. Additionally, bacterial consortia analysis showed ammonium-oxidizing bacteria were the dominant microorganisms, which provided evidence for the long-term stable performance of this PN reactor. During the experiment, sludge setting properties deteriorated due to the absence of a biomass carrier. The stable performance of partial nitrification from reject wastewater demonstrated the feasibility of the operation strategy in this study.

[**Key words:** Partial nitrification; Reject wastewater; DO concentration; Anammox]

Nitrogen pollution has become a major concern in environment protection and increasing efforts have been directed at improving and discovering techniques for reducing the amount of nitrogen in wastewater. Historically, sequential nitrification and heterotrophic denitrification have typically been applied for nitrogen removal, as well as the removal of organic matter, from waste water. However, high operational costs, due to the oxygen supply and organic carbon requirements, have hampered wider applications of nitrification and denitrification for nitrogen removal. Recently, a new segment of the nitrogen cycle, anaerobic ammonium oxidation (Anammox), was discovered by Mulder et al. (1). The anammox process does not require external carbon, in contrast to the conventional heterotrophic denitrification process. Partial nitrification can produce a suitable influent to an anammox reactor for NH_4^+–N removal from reject water in domestic wastewater treatment. Thus, partial nitrification of ammonium to nitrite is the principal factor for successful application of a shortcut nitrogen-removal system in combination with the anammox process (2). The excessive accumulation of nitrite inhibits anammox bacteria to a large extent. From a practical point of view, the critical factor in the partial nitrification process is the ammonia conversion rate (ACR). The effluent ratio of NH_4^+–N/NO_2^-–N of 1:1.32 was considered as a suitable ratio for the subsequent anammox reaction.

The dissolved oxygen (DO) concentration plays a key preliminary role in successful application of PN. When the DO concentration was below 1.0 mg/L, the growth rate of ammonium oxidizers was 2.6 times faster than that of nitrite oxidizers (3). DO control is difficult to implement smoothly compared with other operational strategies, such as pH control and temperature control. The sludge characteristics may also vary under a limited DO concentration.

In this study, the treatment performance of a PN reactor operated by strictly controlling the DO concentration for 80 days was evaluated and the effect of free ammonia (FA) and free nitrous acid (FNA) were also investigated. Microscope observation and DNA analysis were applied to evaluate the characteristics of sludge and the bacteria shift in our reactor.

MATERIALS AND METHODS

Seed sludge and reject wastewater The PN reactor was inoculated with activated sludge from a lab-scale fill-draw reactor and the initial mixed-liquor suspended solids (MLSS) concentration was 3 g/L. Reject wastewater was obtained from the Kumamoto East Wastewater Treatment Plant and was used as influent wastewater in this study. The characteristics of the reject wastewater were pH 8.0, 50–100 mg/L SS, 180–280 mg/L COD, 150–210 mg/L BOD, 800–1000 mg/L NH_4^+–N, 30–70 mg/L NO_2^-–N and NO_3^-–N not detectable.

Experimental setup and operational strategy The experiment was carried out in a laboratory-scale rectangular reactor with an effective volume of 5 L (Fig. 1). The reactor had downdraft and updraft sections in a parallel upright arrangement. The cross-sectional areas of the downdraft and updraft parts of the reactor were 110×110 mm and 110×30 mm, respectively, and the height of effluent port was 320 mm (total volume 6.2 L). A settling tank with volume of 2.5 L was used for sludge sedimentation and recycling. The feed solution was introduced to the updraft section with a peristaltic pump (Cassette Tube Pump SMP-21). Air was supplied using an air pump at the bottom, serving both to mix and oxygenate the wastewater. The air flow rate was changed along with increments in the nitrogen loading rate (NLR).

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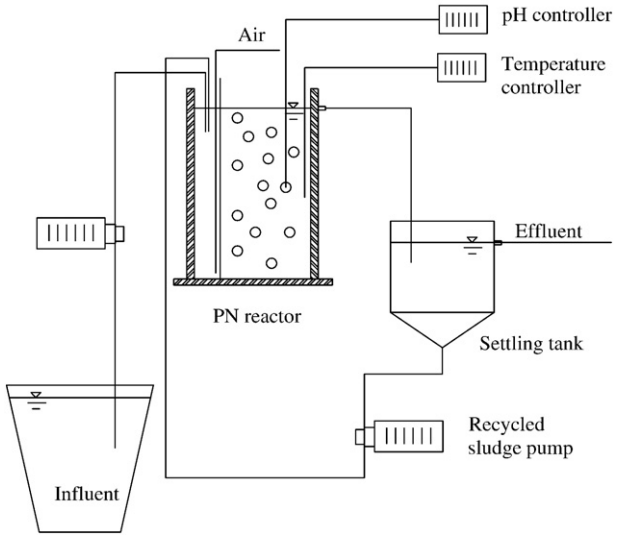

FIG. 1. Schematic of the PN reactor.

Temperature was maintained at 26 ± 4 °C throughout the study and pH was controlled at 7.6 ± 0.1 by addition of acid (1 N HCl) and alkali (1 N NaOH) solution.

Analytical methods NH_4^+–N was measured by the modified phonate method described by Kanda involving use of o-phenyl phenol as a substitute for liquid phenol (4). NO_2^-–N and NO_3^-–N were determined by the colorimetric method. pH was estimated using a pH meter (B-211, Horiba, Japan), while DO was measured with a digital portable DO meter (D-55, Horiba, Japan). MLSS analysis was performed by drying at 105 °C in an evaporating dish. FA and FNA concentrations were calculated by equilibrium as follows:

$$NH_3(mg/L) = 17/14 \times \text{Total ammonia as } N(mg/L) \times 10^{pH} / \left[\exp(6344/(273 + T)) + 10^{pH}\right] \quad (1)$$

$$HNO_2(mg/L) = 47/14 \times NO_2^- - N(mg/L) / \left[\exp\left(-2300/(273 + T) \times 10^{pH}\right)\right] \quad (2)$$

DNA extraction and PCR amplification The sludge samples used for DNA extraction were stored at −20 °C prior to analyses. The sludge sample was first ground with a pestle under liquid nitrogen. Meta-genomic DNA was extracted using an ISOIL kit (Wako, Osaka, Japan) according to the manufacturer's instructions. The amplification of 16 S 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, 20 s at 51 °C, 35 s at 72 °C, and 5 min final elongation at 72 °C. The amplified products were purified using a Wizard SV Gel and PCR Clean-Up System (Promega, USA).

Cloning and sequencing of 16 S rRNA gene The purified fragments were ligated into the *Eco*RV 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 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 (BLAST) program on the NCBI web site.

RESULTS

Nitrification performance of the PN reactor The operational parameters and treatment results are summarized in Table 1. Fig. 2 shows the daily changes in NLR and ACR. During the entire period, the PN reactor was fed with a mixture of reject wastewater and tap water. The percentage of tap water in the feed solution was progressively increased (dilution times are shown in Fig. 2).

Startup period: during this period (0–14 days), the reactor was operated to cultivate ammonia-oxidizing bacteria (AOB) and to inhibit nitrite-oxidizing bacteria (NOB). Initially, the reactor was started at a low NLR of 0.3 kg-N m^{-3} day^{-1} (400 mg/L of NH_4^+–N, 20 mg/L of NO_2^-–N), with a hydraulic retention time (HRT) of 24 h. Air was supplied to the reactor at a flow rate of 0.3 L/min for controlling the reactor DO concentration to inhibit NO_3^-–N production. Effluent NH_4^+–N and NO_3^-–N concentrations were decreased gradually accompanying the decline in DO concentration (0.7 ± 0.2 mg/L of DO) in the PN reactor, demonstrating the occurrence of nitrification as well as the inhibition of NOB in the reactor.

Acclimation and stable running period: From day 15 to day 48, the influent NH_4^+–N concentration was fixed at 460 mg/L and HRT was shortened to increase the NLR. The airflow rate was increased correspondingly to ensure sufficient oxygen supply under the high NLR and the residual DO concentration in the reactor was under 0.1 mg/L. Shortening HRT did not lead to negative effects in reactor performance except for a short period of adjustment in the effluent NO_2^-–N/NH_4^+–N ratio. The maximum ACR and nitrite production rate (NPR) obtained during this period were 1.16 kg-N m^{-3} day^{-1} and 1.12 kg-N m^{-3} day^{-1}, respectively, under a NLR of 2.62 kg-N m^{-3} day^{-1}. The average effluent NO_2^-–N/(NO_2^-–N + NO_3^-–N) ratio was always more than 99%. This suitable NO_2^-–N/NH_4^+–N ratio and low NO_3^-–N concentration were appropriate for a subsequent anammox process.

Stepwise increase in NLR: After obtaining satisfactory PN treatment results, the influent flow rates and TN concentration were simultaneously increased in a step. A maximum NLR of 4.2 kg-Nm^{-3} day^{-1} was achieved on day 68, with an influent NH_4^+–N concentration of 600 mg/L and HRT of 3.5 h. The DO concentrations in

TABLE 1. Operational parameters and treatment performance of the PN reactor.

Parameters	Unit	Phases				
		0–14	15–40	41–48	49–62	63–80
Inflow rate	L/day	3–15	25	27	30	35
Airflow rate	L/min	0.3–1	1.5	2.2	3.3	3.6
HRT	hours	25.5	17 ± 1.5(26)	12.5	11	7.25
NLR	kg-Nm^{-3} day^{-1}	0.2–1.4	*2.1 ± 0.2(26)	2.5 ± 0.1(8)	3.3 ± 0.1(14)	4.0 ± 0.2(18)
Inf. pH				8.0–8.5		
Eff. pH				7.5–7.6		
Inf. Alkalinity	mg$CaCO_3$/L			1450–2200		
Eff. Alkalinity	mg$CaCO_3$/L			600–950		
Inf. BOD_5	mg/L			130–190		
Eff. BOD_5	mg/L			40–70		
Inf.TN. Conc.	mg/L		460 ± 30(26)		560 ± 20(40)	
Eff.NH_4^+-N.Conc.	mg/L	30–320	200 ± 25(26)	200 ± 10(8)	230 ± 20(14)	255 ± 15(18)
Eff.NO_2^-–N.Conc.	mg/L	50–270	200 ± 20(26)	220 ± 10(8)	270 ± 10(14)	275 ± 15(18)
Eff.NO_3^-–N.Conc.	mg/L	16–122	<1.0	<1.0	<1.0	<1.0
TN removal	%	10–25	10 ± 5(26)	10 ± 2(8)	12 ± 3(14)	12 ± 5(18)
Eff.NO_2^-–N/NH_4^+–N		0.2–3.5	1.0 ± 0.2(26)	1.1 ± 0.08(40)		

Note: average concentrations ± standard deviation (n).