



# High-efficient nitrogen removal by coupling enriched autotrophic-nitrification and aerobic-denitrification consortiums at cold temperature



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## HIGHLIGHTS

- Autotrophic nitrification and aerobic denitrification consortiums coupled at 10 °C.
- High specific nitrifying/denitrifying rate (8.85 and 32.93 mg N/(g SS h)) at 10 °C.
- High-efficient TN removal at COD/N 4 and DO 1.5–4.5 mg/L by mixed consortium.
- Main functional microbes identified as *Nitrosomonas* sp. and *Pseudomonas* sp.

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## ABSTRACT

This study paid particular attention to total nitrogen removal at low temperature (10 °C) by excellent coupling of enriched autotrophic nitrifying and heterotrophic denitrifying consortiums at sole aerobic condition. The maximum specific nitrifying rate of the nitrifying consortium reached 8.85 mg N/(g SS h). Further test in four identical lab-scale sequencing batch reactors demonstrated its excellent performance for bio-augmentation in potential applications. On the other hand, the aerobic denitrifying consortium could achieve a specific denitrifying rate of 32.93 mg N/(g SS h) under dissolved oxygen of 1.0–1.5 mg/L at 10 °C. Coupling both kinds of consortiums was proved very successful for a perfect total nitrogen (TN) removal at COD/N of 4 and dissolved oxygen of 1.5–4.5 mg/L, which was hardly reached by any single consortium reported previously. The encouraging results from coupling aerobic consortiums implied a huge potential in practical treatment of low-strength domestic wastewater (200–300 mg/L COD) during wintertime.

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## 1. Introduction

Excess nitrogen in the natural receiving water bodies, which is usually caused by insufficient treatment of discharging water in wastewater treatment plants (WWTPs), may contribute much to eutrophication in surrounding environment (Zhang et al., 2011). In order to strengthen the nitrogen removal efficiencies in most biological treatment facilities, the factors which led to deterioration of simultaneous nitrification and denitrification (SND) were deeply investigated for years, among which the seasonal temperature drops of temperate zones in winter were commonly

recognized as a key inhibitor. Zilouei et al. (2006) indicated that low temperature had a drastic effect on the growth of bacteria, resulting in low removal performances for ammonia and chemical oxygen demand (COD). The inhibition of nitrifying microorganisms, including ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), was reported at 10 °C or similar temperatures (Gu et al., 2012; Kim et al., 2006; Rodriguez-Caballero et al., 2012; Siripong and Rittmann, 2007). Meanwhile, Carrera et al. (2003) also suggested that the dependence for denitrifiers on temperature was rising below 10 °C.

Nitrification in cold environment has drawn increasing attentions currently. One way to achieve bioaugmentation of nitrifying consortium was through continuous seeding (Head and Oleszkiewicz, 2004). However, the nitrate concentration was commonly raised up in the bioaugmented system, leading to a limited

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application in practical treatment facilities. In order to realize total nitrogen (TN) removal under low temperature, nitrification and denitrification must be considered together. Recently, some bacteria capable of both heterotrophic nitrification and aerobic denitrification had been isolated (Joo et al., 2005; Zhang et al., 2011). Through single aeration stage, these promising microorganisms could convert ammonia and nitrate into its gaseous form (Yao et al., 2013a). Since no anaerobic and anoxic phases are involved, they have huge advantages in reducing the volume of biological treating system and decreasing the energy required by operation. However, further efforts must be made to minimize carbon source input for efficient nitrogen removal in low-strength domestic wastewater (Yao et al., 2013a; Zhang et al., 2011).

This paper focuses on unraveling the limitations for cost-effective total nitrogen removal at low temperature through a successful coupling of newly enriched psychrotrophic autotrophic nitrifying consortium and our previously cultivated aerobic denitrifying consortium (Yao et al., 2013a) at 10 °C. It was proved that the novel coupled consortium not only greatly enhanced nitrogen removal in sequencing batch reactors (SBRs) but also significantly reduced energy consumption and carbon source input, which would be of primary importance for potential practical applications.

## 2. Methods

### 2.1. Enrichment and characterization of autotrophic nitrifying consortium

The seed sludge was obtained from a well-established SBR. In order to strengthen the nitrifiers' activity and accelerate the growth rate of the consortium at 10 °C, enrichment was conducted in 5, 25 and 50 L SBRs (Table 1) successively. The feeding wastewater contained  $\text{NH}_4\text{Cl}$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{NaHCO}_3$ . The system was maintained at pH 7.0–7.5. With an initial 2500 mg/L mixed liquor suspended solids (MLSS), the 5 L enrichment SBR was well operated for 14 days. The whole system was expanded to 25 L and later 50 L for the sake of better growth and rapid proliferation. In step 3, the concentration of  $\text{NaHCO}_3$  was raised to 1.0 g/L to balance the strong acid generated by increasing intensity of nitrification. Every 24 h-cycle began with 0.5 h feeding, followed by 22 h aeration, 1 h settling and 0.5 h drainage. The drainage rate was 80%, leading to a hydraulic retention time (HRT) of 28.8 h. The feeding and drainage phase was achieved by a peristaltic pump. Aeration rate was controlled through the flow

**Table 1**

Enrichment process and specific nitrifying rate (SNR) of autotrophic nitrifying consortium.

	Working volume (L)	Initial ammonia (mg/L) <sup>a</sup>	MLSS (g/L) <sup>a</sup>	TSS (g) <sup>a</sup>	SNR <sup>a,b</sup>	Nitrification ratio <sup>a,c</sup>
Step 1	5	119	3.083	15.415	4.83	14.89
Step 2	25	116	0.731	18.275	7.57	5.52
Step 3	50	125	0.642	32.100	8.85	5.68

<sup>a</sup> The values in the column were obtained from the ending cycle of each step.

<sup>b</sup> The unit of specific nitrifying rate was  $\text{mg NH}_4\text{-N}/(\text{g suspended solid h})$ .

<sup>c</sup> The unit of nitrification ratio was  $\text{mg NH}_4\text{-N}/(\text{L h})$ .

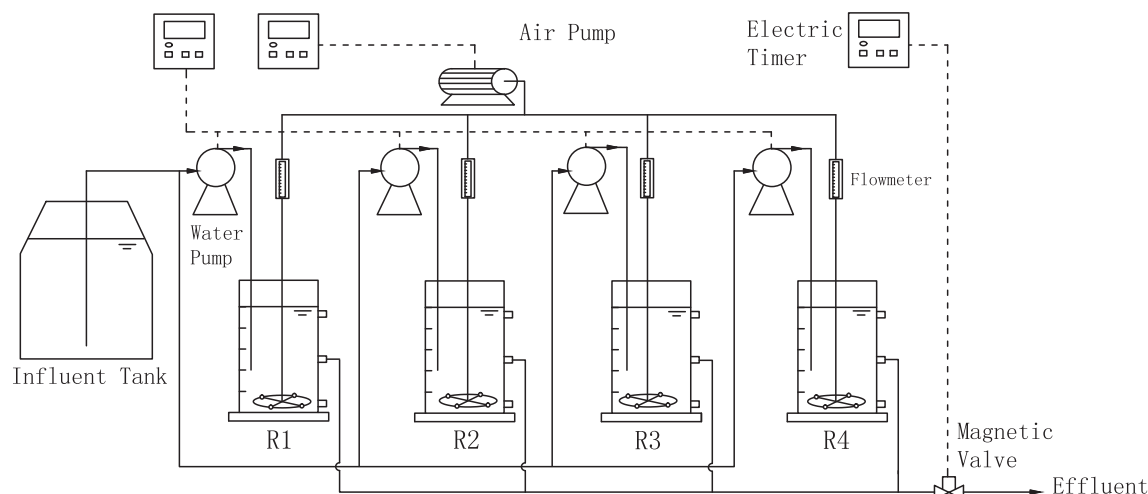
meter, supplying the dissolved oxygen (DO) of 4.1–12.6 mg  $\text{O}_2/\text{L}$ . Six aerators were fixed on the bottom to make the bubbles distributed uniformly. No sludge was discharged during the entire enrichment phase.

During the step 1, samples were taken every 1–3 h for the determination of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ . From step 2 to step 3, only influent and effluent were collected for analysis considering the system was stably operated.

### 2.2. Bioaugmentation of enriched nitrifying consortium

The bioaugmentation of autotrophic nitrifying consortium towards normal systems was conducted in four identical 5 L SBRs (R1, R2, R3 and R4), as shown in Fig. 1. All were inoculated with the normal sludge obtained from Langfang municipal wastewater treatment plant (Hebei, China) on September 20th, 2012. With an initial MLSS of 3000 mg/L, the system experienced four 6-h cycles, including 0.5 h feeding, 4 h aeration, 1 h settling and 0.5 h decanting of the 2.5 L supernatant. The synthetic influent wastewater contains (per liter): 0.255 g sodium acetate (200 mg/L COD), 0.191 g  $\text{NH}_4\text{Cl}$ , 0.044 g  $\text{KH}_2\text{PO}_4$ , 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.006 g  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.5 g  $\text{NaHCO}_3$ . The HRT was 12 h and sludge retention time (SRT) was around 15 days. Aeration rate was well controlled by four aerators at the bottom, leading to dissolved oxygen (DO) of 5.8–7.1 mg/L. After two weeks, as the MLSS of all four SBRs gradually increased to ca. 3500 mg/L, the ammonia concentration in the effluent of all reactors reached a stable condition (around 20 mg  $\text{NH}_4\text{-N}/\text{L}$ ).

In order to evaluate the bioaugmentation performance of nitrifying consortium in the cold circumstances, enriched autotrophic nitrifying consortium was added into R2, R3 and R4, the amount to each was determined by:



**Fig. 1.** Configuration of SBRs for bioaugmentation by autotrophic nitrifying consortium.

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