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Short communication

Carbon and nitrogen removal through "*Candidatus* Brocadia sinica"-dominated simultaneous anammox and denitrification (SAD) process treating saline wastewater

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HIGHLIGHTS

- Simultaneous C and N removal was achieved by SAD process under saline condition.
- "*Candidatus* Brocadia sinica" had good tolerance to salinity in SAD process.
- Percentage of nitrogen removal by anammox was higher than that by denitrification.
- Modified Boltzmann model could analyze the effect resulting from salinity.

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ABSTRACT

"*Candidatus* Brocadia sinica"-dominated simultaneous anammox and denitrification (SAD) process was used to treat nitrogen-rich saline wastewater. The reactor was operated at 30 ± 0.5 °C with influent pH of 7.5 ± 0.1. '*Ca.* B. sinica' could adapt to high saline surroundings after 42 cycles' operation. With 100% seawater, nitrogen removal rate and organic removal rate were 0.71 kg/(m^3·d) and 0.27 kg/(m^3·d), respectively. Both were closed to those without seawater. Independent of salinity, percentage of nitrogen removal by anammox was higher than that by denitrification during the whole treatment period. '*Ca.* B. sinica' had good tolerance to salinity. However, maximum removal rate of NH_4^+-N declined with growing salinity. Modified Boltzmann model was proper to analyze the effect resulting from salinity on the maximum removal rate of NH_4^+-N. Granular sludge characterized by anammox granule embedded in denitrifying granule might play an important role in salt tolerance of anammox bacteria.

1. Introduction

With rapid economic growth, increasing emission of wastewater tends to aggravate environmental pollution. These industrial effluents, apart from having a high nitrogen concentration, are also characterized by elevated salinity [1]. Some industrial wastewater, like saline mustard tuber wastewater, also contained high concentration of COD besides nitrogen [2]. Complex compositions make these kinds of wastewater more difficult to treat. Anammox process, regarded as a cost-effective and environment-friendly way to removal nitrogen from wastewater, with 60% decrease in oxygen demand, 100% decrease in organic carbon source demand and less or no global warming gas production compared to conventional nitrification-denitrification related nitrogen removal processes [3,4], had shown a potential to treat nitrogen-rich saline wastewater [5–7]. However, long acclimatization was needed for anammox bacteria to adapt to saline surroundings. Nitrogen removal by salt-tolerant anammox species is an alternative strategy. The "*Candidatus* Kuenenia stuttgartiensis" was reported to have good salt tolerance and can be the dominant anammox species after salt acclimatization [8,9]. Besides, the salt tolerance of "*Candidatus* Brocadia sinica" was even higher than that of '*Ca.* K. stuttgartiensis' [10]. Apart from salt, COD also has inhibitory effect on anammox performance [12]. However, COD supply could enhance total nitrogen (TN) removal efficiency in SAD process due to denitrification [12,13].

The '*Ca.* B. sinica' was widespread in wastewater treatment where

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organic compounds, ammonia and nitrate were all present [11,12]. Therefore, it can be well cooperated with denitrifying bacteria. Recent studies indicated that simultaneous anammox and denitrification (SAD) process was an effective way to achieve carbon and nitrogen removal [12–14]. However, they mainly focus on the effect resulting from organic matter or C/N under freshwater condition [12–14]. Limited information is available on SAD process treating saline wastewater.

In this work, '*Ca.* B. sinica'-dominated SAD process was operated to treat nitrogen-rich saline wastewater. Carbon and nitrogen removal performance was evaluated at various influent salinities. The individual contributions of anammox and denitrification to nitrogen removal were quantitatively determined. In addition, kinetic analysis was performed to study inhibitory characteristics under saline condition.

2. Materials and methods

2.1. Reactor configuration and operation

A sequencing batch reactor (SBR) (Fig. S1) made of polymethyl methacrylate was used in this study. The reactor was double-jacketed with an effective volume of 7.0 L. Its temperature was controlled at 30 ± 0.5 °C by a water bath with water recirculation through the outer chamber. The reactor was covered with a black cloth to prevent potential growth of phototrophic micro-organisms. The operating mode of the reactor consisted of 0.5 h influent feeding, 7 h anoxic stirring reaction, 0.5 h sludge settling and 0.5 h effluent discharging. The seeding sludge were taken from another lab-scale SBR reactor, which had been operated for three years with influent NH_4^+-N, NO_2^--N and chemical oxygen demand (COD) concentrations of 80, 160 and 300 mg/L, respectively. Our previous study indicated that the dominant anammox species was '*Ca.* B. sinica' which accounted for 96% [12]. The concentration of mixed liquor suspended solids (MLSS) was around 11 g/L after inoculation in the studied reactor.

2.2. Wastewater characteristics

Synthetic feed was prepared with seawater taken from Jiaozhou Bay of Qingdao City (Shandong Province, China). Varied salinities (Table 1) were achieved by adding different contents of seawater. In this study, influent was determined according to our previous study with slight modification [12]. In detail, the influent NH_4^+-N and NO_2^--N were 80 ± 10 mg/L and 145 ± 20 mg/L, respectively. Additionally, influent COD, provided by glucose, was around 300 ± 20 mg/L. Influent pH was 7.5 ± 0.1. The trace elements were also added according to previous study [12].

2.3. Sample pretreatment and analysis

Samples were withdrawn at the beginning and end of each operation cycle and centrifuged at 6000 rpm and 4 °C for 30 min. The concentration of NH_4^+-N, NO_2^--N, NO_3^--N and COD in supernatants were analyzed according to the standard methods [15]. Besides, MLSS was also analyzed according to the standard methods [15]. Oxidation-reduction potential (ORP) and pH were measured by using relevant probes coupled with an S20 K ORP meter and an FE20 pH meter (Mettler Toledo). The maximum removal rate of ammonia and nitrite ($V_{ammonia}$ and $V_{nitrite}$) were achieved from the maximum slope of the curves described by nitrogen removal performance in a typical cycle.

Table 1
Salinity in wastewater containing different content of seawater.

Seawater (%)	10	20	30	40	50	60	70	80	100
Salt (g/L)	3.5	7.0	10.5	14	17.5	21	24.5	28	35
Cl^- (g/L)	1.9	3.8	5.7	7.6	9.5	11.4	13.3	15.2	19

The percentages of nitrogen removal by anammox and denitrification were calculated according to Li et al. [12].

2.4. Kinetic analysis

The modified Boltzmann model [Eq. (1)] was used to indicate potential performance and recovery performance of anammox reactor [16]. Eq. (2) was used to calculate inhibition rate resulting from salinity on anammox bacteria.

$$I = I_{max} + \frac{I_{min} - I_{max}}{1 + e^{(s-s_c)/s_d}} \quad (1)$$

$$I\,(\%) = \frac{V_{control} - V}{V_{control}} \times 100 \quad (2)$$

where I represents inhibition rate (%); I_{min} represents minimum I (%); I_{max} represents maximum I (%); s represents seawater content (%); s_c represents center value (%); s_d represents constant value of seawater content (%); $V_{control}$ represents maximum removal rate under freshwater condition (kg/(m^3·d)); V represents maximum removal rate at a typical seawater content (kg/(m^3·d)).

3. Results and discussion

3.1. Performance of the SAD process treating saline wastewater

Independent of salinity, NO_2^--N could be removed almost completely during the whole process (Fig. 1a&b). Compared to NO_2^--N removal, salinity greatly affected NH_4^+-N removal. Nitrogen were removed mainly via anammox and denitrification in SAD process, which indicated that salinity had little effect on denitrification compared to anammox. When seawater content was 60%, NH_4^+-N removal efficiency decreased sharply to only 71.18%. Accordingly, nitrogen removal rate (NRR) was 0.67 kg/(m^3 d) which was decreased 8.47% compared to that acquired without seawater (0.732 kg/(m^3 d)) (Fig. 1c). By contrast, Yang et al. [7] reported that activity of anammox bacteria was completely inhibited when salt concentration increased from 14 g/L to 20 g/L. Similar results were also found by Malovanyy et al. [1]. However, '*Ca.* B. sinica'-dominated SAD process had good salt tolerance in this study. After acclimation, NH_4^+-N removal efficiency grew when seawater content increased from 60% to 100%. The NRR was achieved as high as 0.71 kg/(m^3·d) at 100% seawater. Accordingly, the mean organic removal rate (ORR) was 0.27 kg/(m^3·d). Both NRR and ORR were similar with that of without seawater. Simultaneous and efficient carbon and nitrogen removal could be achieved through '*Ca.* B. sinica'-dominated SAD process treating nitrogen-rich saline wastewater.

The routes of nitrogen removal were analyzed over the whole test period. The percentages of nitrogen removal by anammox and denitrification were presented in Fig. 1d. Interestingly, the percentage of nitrogen removal by anammox (P_{ana}) was higher than that by denitrification (P_{den}) during the whole treatment period. The P_{ana} decreased after seawater addition, and then increased with growing seawater content. It peaked at 66.74% when seawater content was 40%. P_{den} was enhanced by salinity in most test periods. This result also indicated that salinity had little inhibitory effect on denitrification compared to anammox. P_{den} reached its highest value (47.2 ± 5.26%) at 60% seawater. However, it was still lower than the least value of P_{ana}. When seawater content was 100%, P_{ana} was 65.74 ± 4.82%.

3.2. Nitrogen removal in a typical operating cycle

To explore the synergistic mechanism of anammox and denitrification, nitrogen removal performance within different operating cycles were analyzed. The test were operated at the end of each salinity gradient. As presented in Fig. 2, both NH_4^+-N and NO_2^--N were