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Nitrifying Population Optimization in Municipal Saline Wastewater Treatment

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Abstract A sludge population optimization strategy aims to select the ammonia oxidation bacteria (AOB) by using sodium chloride (NaCl) as a selective inhibitor to nitrite oxidation bacteria (NOB) is applied in biological nitrogen removal process during municipal wastewater treatment using a sequencing batch reactor(SBR). Different salinity tests contributed to the best inhibition salinity chosen were applied. In order to optimize nitrifying microbial communities to get steady nitrite pathway，7.6 g/L salinity and 4 month salt inhibition period were applied. FISH analysis indicated that AOB (*Nitrosospira*) became the dominant nitrifying bacteria and NOB (*Nitrobacter*) had been washed out of the activated sludge. An understanding of salt inhibition mechanism on NOB is also discussed.

Key words nitrifying population optimization；salt inhibition；nitrogen removal via nitrite；municipal saline wastewater；inhibition mechanism

含盐生活污水处理中的硝化菌种群优化

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摘要　为了实现稳定的短程硝化，通过使用 NaCl 作为一种选择抑制剂(只抑制亚硝酸氧化菌(NOB)的生长而不会以抑制氨氧化菌(AOB)的生长)在序批式反应器处理含盐生活污水过程中实现硝化种群的优化。实验考察了不同盐度对 AOB 和 NOB 的抑制程度以及对系统硝化性能的影响，选择 7.6 g/L的盐度作为种群优化的最佳盐度。长期抑制实验实施 4 个月后，亚硝酸盐积累稳定在 95% 以上，短程硝化稳定。利用荧光原位杂交技术(FISH)检测到 AOB (*Nitrosospira*)已经成为硝化菌群的主导菌种，NOB(*Nitrobacter*) 基本检测不出，证明 NOB 已经被淘洗出系统，硝化种群得到优化。同时讨论了盐度对 NOB 的选择抑制机理。

关键词　硝化种群优化；盐度抑制；短程硝化；含盐生活污水；抑制机理
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Biological nitrogen removal (BNR) based on utilizing the function of certain active sludge to remove the target pollutant has been used worldwide. Sludge population optimization strategy aims to select the most desirable organism or a consortium of organism to perform a required function，which is particularly important for nitrification，one of the most important and delicate steps in modern wastewater treatment plants [1].

Faced to the energy crisis，the nitrogen removal through the nitrite pathway (NH_4^+–NO_2^-–N_2) is preferred in many of the plants for the significantly energy saving [2]. The shot-cut nitrogen removal has been successfully achieved for various types of wastewaters in the last few years [3-7]. The principle is to eliminate nitrite oxidizing bacteria (NOB) and make ammonia oxidizing bacteria (AOB) be the dominant population of nitrifiers in nitrification. However，it

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should take a substantial time (in the order of years) to completely remove the NOB using on-line aerobic duration control or other control as the sole selection factor unless an NOB inhibitor is used [8]. Salt, an economical selective inhibitor to NOB rather than AOB, is suggested by Cui et al. [9] and Ye et al. [10] to achieve nitrite pathway quickly in BNR process.

Saline wastewater, rich in both salt (mainly NaCl) and organic matter, are often generated during the manufacture of chemicals and oil and gas recovery operations, professionally known as produced waters and can easily reach into wastewater by many means [11]. In addition to hypersaline-produced water problems, using seawater in the situations which high quality water is not essential, such as toilet flushing, also promote to generate lots of saline wastewaters. A number of researches [12-13] have proved that certain concentration salinity in influent could strongly inhibit the metabolism of functional bacteria and cause a failure to local wastewater treatment plant (WWTP). Nevertheless, there are significant gaps in the current knowledge, especially on the understanding of the salt inhibition mechanism on nitrifiers and how to optimize nitrifying population in saline wastewater treatment process.

Without considering the adverse effect of sodium chloride (NaCl), this work pays more attention on how to utilize the natural salt in seawater. This study aimed to utilize NaCl, the mainly salt in seawater, selectively inhibited NOB but not AOB to optimize the nitrifying population by a long period of time, and nitrite pathway could be rapidly achieved during biological nitrification/denitrification process. Both batch and long-term experiments are carried out in this study. Different salinity tests contributed to the best inhibition salinity chosen and the understanding of mechanism of salt inhibition to nitrifying communities were applied in this study. The fluorescence in situ hybridization (FISH) method was performed to monitor the quantitative changes of nitrifying microbial communities in the activated sludge during and after salt inhibition periods. In the meanwhile, the mechanism of salt inhibition on NOB was also discussed.

1 Materials and Methods

1.1 Reactor operation and municipal saline wastewater

A bench-scale sequencing batch reactor (SBR) was inoculated with sludge from a full-scale domestic treatment plant in Beijing. The reactor had a working volume of 10 L. The cycle time was 12 h, which consisted of approximately 7 h aerobic, 3 h anoxic and 2 h settle/decant periods. Constant aeration was applied during the aerobic period. Supplementary external carbon source, acetate (NaAc), was added at the beginning of anoxic phases to achieve denitrification. The hydraulic retention time (HRT) was 24 h. The mixed liquor suspended solids (MLSS) was kept at a level of approximately 2.5 g/L. The sludge retention time (SRT) was controlled at about 15 days.

The wastewater was collected from a wet well located in the Beijing University of Technology on a daily basis. The influent characteristics are listed in Table 1. Commercial crude NaCl salt is added into storage tank to form saline sewage as SBR feed during achieving nitrite build-up. The salinity is adjusted according to experiment design. The activated sludge (AS) for SBR A is delivered initially from the local WWTP return activated sludge line.

1.2 Experiment design

Feeded by the fresh sewage, sludge is acclimated to get complete nitrification-denitrification at controlled conditions in SBR. At the time of batch tests described below: the reactor is achieving a nitrogen removal efficiency of 98% from the fresh domestic wastewater

Table 1　Domestic sewage characteristics for experiment　mg/L

Items	pH	COD	TN	NH_4^+-N	NO_2^--N	NO_3^--N
Min-Max	7.2-7.8	150-305	18.2-81.6	12.6-59.7	0.05-0.25	0.34-1.60
Mean	7.65	215	47.8	38.2	0.15	0.78

containing approximately 40 mg N/L. In each test 400 ml of sludge is transferred into batch reactors. NaCl is added at the beginning resulting in an initial different influent salinity in each reactor. Air is sparged into the reactor at a flow rate of 100 mL/min resulting in an initial DO concentration above 3 mg/L. Aeration is always prolonged each cycle after ammonia is totally oxidized to provide more chances for NOB growing. Three parallel tests are applied respectively at each salinity. The Effect of salt on ammonia removal efficiency (ARE), nitrite accumulation rate (NO_2-N/NO_x-N) $\left(NAR = \frac{NO_2^- - N}{NO_2^- - N + NO_3^- - N}\%\right)$, and specific ammonia uptake rate (SAUR) at different salinity are compared. Microbial population tests are carried out to provide the growth and survival of AOB and NOB at different salinity culture medium. Sludge is taken from the parent SBR with steady nitrification and nitrogen removal. After the best inhibition salinity is chosen, it is applied in SBR for a long term to optimize nitrifying communities. The whole experiment lasts more than 9 months and is divided into 3 phases (Table 2).

1.3 Analytical methods

Most routine chemical analyses (i. e. TN, NH_4^+-N, NO_3^--N, NO_2^--N, Cl^-) were conducted according to the standard methods [4]. pH was measured with a WTW inoLab 340i hand-held instrument. Fluorescence in situ hybridization (FISH) was performed as described in Amann's research [5]. Oligonucleotide probes used in this study were EUBmix [6] for the detection of all Bacteria, NTSPA662 [7] for Nitrospira and NIT3 [8] for Nitrobacter and NSO1225 and NSO190 [9], specific for Betaproteobacterial AOB. The images of FISH samples were captured using an OLYMPUS-BX52 epifluorescence microscope. FISH quantification was carried out by Image-Pros Plus Software, where the relative abundance of the interested bacteria was determined as mean percentage of all bacteria.

2 Results

Fig. 1 shows the batch experiment results of different salinity effect on nitrification behavior and performance. In order to overall evaluate the method, six batch experiments are conducted under aerobic conditions using a range of salt concentrations from 5 to 25 g/L to compare the effects of different salinity on nitrification behavior and performance. It is important to mention that salt addition influences the process of biodegradation. It is clear from Fig. 1 that higher salt concentration results lower ammonia removal performance and specific ammonia uptake rate (SAUR). NH_4-N can not be totally oxidized when salt concentration is over 10 g/L and SAUR decreases sharply when salinity is above 5 g/L. With a contrast, the nitrite accumulation rate increases quickly and keeps above 85% when salinity is over 7.5 g/L. Higher salt concentration is detrimental to both AOB and NOB but lower salt concentration only strongly inhibits NOB.

Based on the above results, 7.6 g/L influent salinity is applied in a SBR to achieve nitrogen removal via nitrate pathway (Phase Ⅲ). The nitrification process changes form complete nitrification-denitrification to nitrite pathway after adding salts in SBRA apparent in the first week. The NH_4-N removal efficiency almost keeps above 98% during salt addition which indicates AOB activity isn't influenced much by 7.6 g/L salt level (Fig. 2). As soon as salt is added, nitrite accumulation rate increases rapidly to 95% and after 3 weeks nitrite becomes the only production by the end of aeration (Fig. 3). Salt inhibition is applied more than 4 months to optimize the population of nitrifiers.

Table 2 Design and planning of experiments

Operation	Procedure	t/d	Salinity/($g \cdot L^{-1}$)	SBR
Phase Ⅰ	Acclimated and complete N-D	0-163	0	10 L
Phase Ⅱ	Batch and microbiological tests for salinity chosen	93-163	0-25	Batch reactors
Phase Ⅲ	Optimizing nitrifiers under salt inhibition	164-285	7.6	10 L

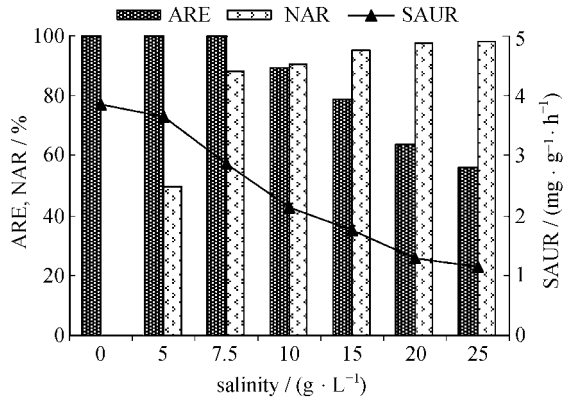


Fig. 1　Effect of salt on ammonia removal efficiency (ARE), nitrite accumulation rate (NAR) and specific ammonia uptake rate (SAUR) at different salinity

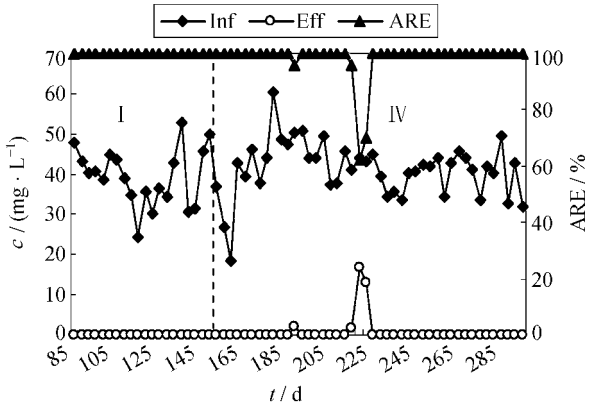


Fig. 2　Concentration of influent and effluent NH_4-N and its removal efficiency before and after salts addition at 7.6 g/L salinity in SBR A during long term inhibition test

The typical cycles of the N pollutant degradation with pH profile before and after salt addition are compared in Fig. 4 and Fig. 5. As known Ammonia Valley point (A) appeared on pH profile indicates the ammonia is totally oxidized [6]. The corresponding ammonia profile is also confirming the end-point of nitrification. Before the salt is added in the system, nitrate concentration, by the end of aeration, is up to 18.7 mg/L and the nitrite is 10.3 mg/L (Fig. 4). After it changes to nitrite pathway system, nitrite becomes the only production by the end of nitrification (Fig. 5). The prolonged aeration doesn't increase the concentration of nitrate. It indicates that even under advantageous growing conditions NOB is totally inhibited and gradually washed out of the system.

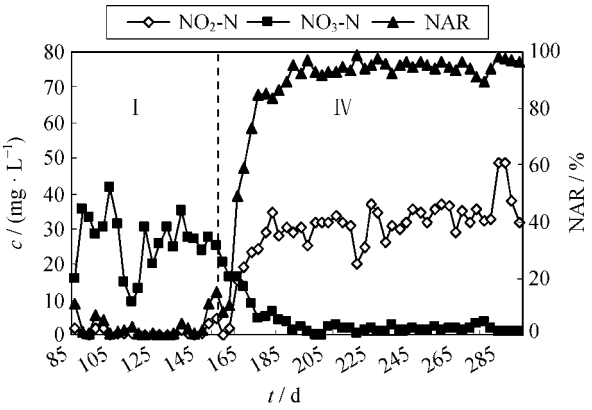


Fig. 3　Concentration of effluent NO_2-N and NO_3-N and the nitrite accumulation rate (NAR) at the end of aeration before and after salts addition at 7.6 g/L salinity in SBRA during long term inhibition test

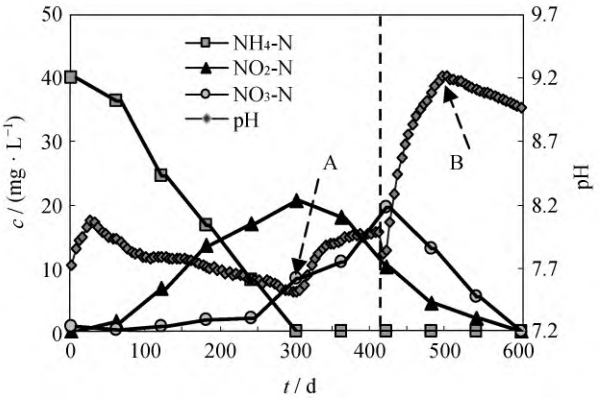


Fig. 4　Typical cycle of N pollutant degradation and feature points on pH profile (A&B) through complete nitrification-denitrification progress under prolonged aerated condition (at the 90 day)

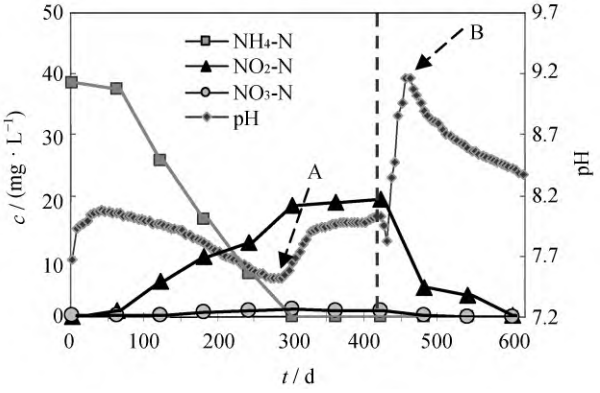


Fig. 5　Typical cycle of N pollutant degradation and feature points on pH profile (A&B) through nitrite pathway under prolonged aerated condition (at the 191 day)

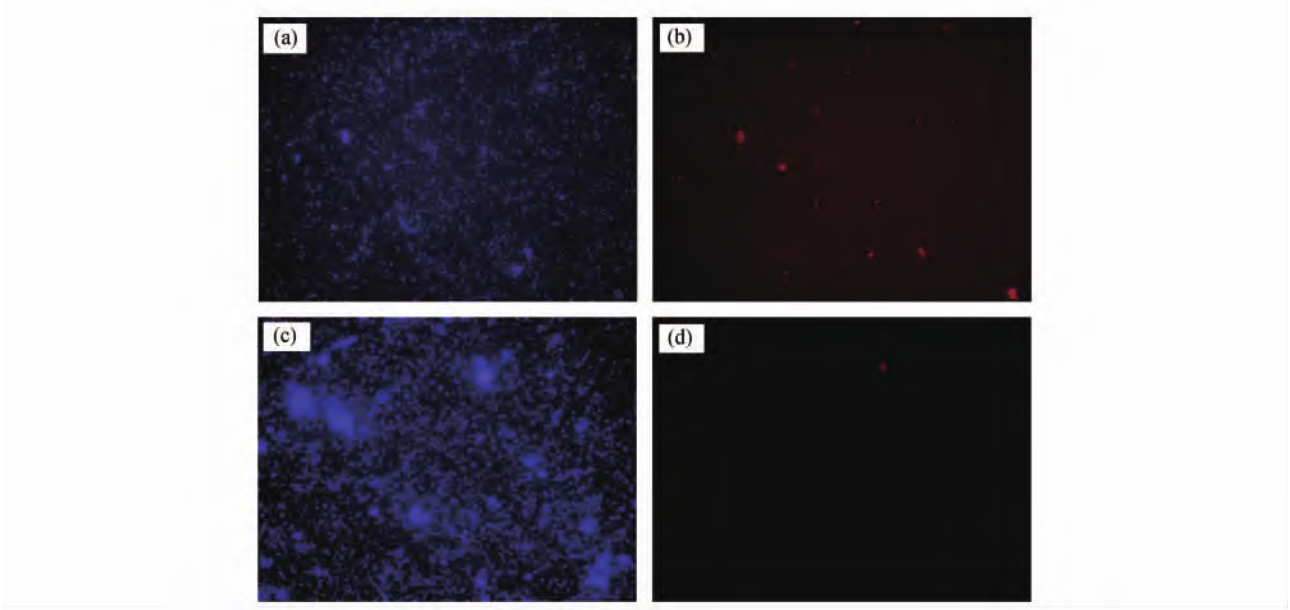
FISH analysis results (Fig. 6) show that after 4 months inhibition AOB (*Nitrosospira* spp.) has become the dominant nitrifying bacteria of this system. NOB (*Nitrobacter* spp.) has been washed out of the activated sludge. It's important to mention that the *Nitrobacter* is the dominant specie of NOB in this system while *Nitrospira* is rarely to be found. This is also reported by Chen et al. [20] who observe that *Nitrobacter* is the only species of NOB, as NaCl concentration is lower than 16.5 g/L and it completely disappears as NaCl concentration increases up to 30 g/L. It indicates that the nitrifying microbial communities are optimized through salt inhibition at 7.6 g/L salinity after 4 months.

3 Discussion

The results reported above show a clear effect of salt on the NOB and also on the nitrite accumulation processes. It is, however, unclear how these inhibitory effects occurred and which steps in the metabolic pathways are affected. Salt inhibition effects on nitrification of synthetic wastewater are investigated by Kargi and Dincer [12]. It is reported that a non-competitive inhibition affecting both the maximum rate and the saturation constants are found to be suitable for salt inhibition of nitrification while saturation constant is more adversely affected. Measures [21] reported that the growth rate of non-halophilic bacteria diminished at increasing salt concentrations, due, in part, to the fact that a fraction of the substrate was consumed in the synthesis of the so-called compatible solutes. It also indicated that the enzyme's activities were reduced at increasing salt concentration.

Salt has much stronger inhibitory effects on the biosynthesis processes of *Nitrobacter*, this could be due to the selective inhibition of which could immediately bind to an enzyme that responsible for a crucial step in carbon transformation, and reduce its affinity to the substrate hence block further biosynthesis. There would be three possible scenarios if NaCl would have inhibited the growth of *Nitrobacter*.

1) Salt inhibits the nitrite oxidoreductase, which carries out the oxidation of the nitrite to nitrate. As shown in Fig. 7, the oxidation of nitrite to nitrate (by the membrane bound enzyme nitrite oxidoreductase) produces reducing equivalents (NADH), which are subsequently oxidized. The flow of electrons through the electron transport chain, leads the translocation of protons (H^+) from the inside to the outside of the membrane [22]. The nitrite oxidoreductase is inhibited by high-levels of salt concentration, as the *Nitrobacter*



(a) (b): DAPI staining (blue) and AOB with probe NSO190 (red); (c) (d): DAPI staining (blue) and NOB with probe NIT3 (red)

Fig. 6 FISH results of AOB and NOB in SBRA at the 282 day after salt inhibition

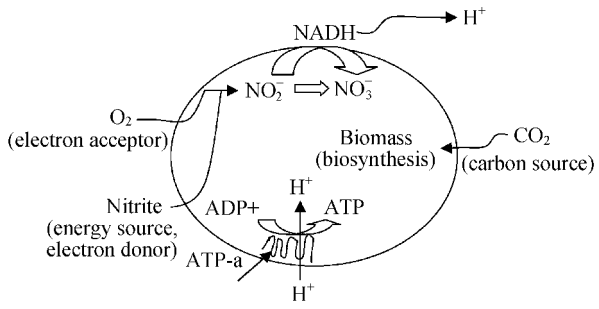


Fig. 7 Simplified mechanisms involved in a nitrite oxidizing bacterial cell

recover its activity with the decrease of salt concentration.

2) Salt increase the osmotic pressure inner cells but also decrease the affinity of O_2 which could severely affect NOB rather AOB [23]. They concluded that the high nitrite accumulation of the nitrification could be due to the effect of mass transfer limitation, as the experiments are performed with biomass flocs, and to the exclusion of the effect of the granular sludge in the analysers. The affinity of O_2 to *Nitrobacter*, as the electron acceptor during the nitrite oxidizing process, decreased with higher salt levels, which also limited the electron transportation.

3) Salt inhibits the ATP synthase (the ATP synthesis process). With the salt inhibition, the increased demand of energy by energy consuming processes would lead to the accumulation of ATP, which would lower the intracellular ADP concentration [24]. This would slow down the ATP synthesis and subsequently reduced the *Nitrobacter* growing rate.

4 Conclusions

It is apparent that *Nitrosomonas* has much higher level of tolerance to salt comparison to *Nitrobacter*, which may contributed significantly to the elimination of NOB from systems treating low C/N saline wastewater through partial nitrification. The key findings are:

1) Salt levels above 10 g/L will apparently decrease the ammonia removal efficiency. Salinity between but 5 and 10 g/L is a good selective range for elimination of NOB from systems.

2) After 7.6 g/L salinity and 4 month salt inhibition period was applied, above 95% nitrite accumulation was achieved steadily. FISH results demonstrated NOB had been washed out of the activated sludge.

3) The strategy to utilize salt inhibition at low salinity has been shown as an efficient way to achieve nitrogen removal via nitrite pathway quickly and steadily. The nitrifying microbial communities could be optimized through long term salt inhibition.

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