

## Osmotic stress on nitrification in an airlift bioreactor

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### Abstract

The effect of osmotic pressure on nitrification was studied in a lab-scale internal-loop airlift-nitrifying reactor. The reactor slowly adapted to the escalating osmotic pressure during 270 days operation. The conditions were reversed to the initial stage upon full inhibition of the process. Keeping influent ammonium concentration constant at  $420 \text{ mg N L}^{-1}$  and hydraulic retention time at 20.7 h, with gradual increase in osmotic pressure from 4.3 to  $18.8 \times 10^5 \text{ Pa}$  by adding sodium sulphate, the ammonium removal efficiencies of the nitrifying bioreactor were maintained at 93–100%. Further increase in osmotic pressure up to  $19.2 \times 10^5 \text{ Pa}$  resulted in drop of the ammonium conversion to 69.2%. The osmotic pressure caused abrupt inhibition of nitrification without any alarm and the critical osmotic pressure value causing inhibition remained between 18.8 and  $19.2 \times 10^5 \text{ Pa}$ . Nitrite oxidizers were found more sensitive to osmotic stress as compared with ammonia oxidizers, leading to nitrite accumulation up to 61.7% in the reactor. The performance of bioreactor recovered gradually upon lowering the osmotic pressure. Scanning and transmission electron microscopy indicated that osmotic stress resulted in simplification of the nitrifying bacterial populations in the activated sludge as the cellular size reduced; the inner membrane became thinner and some unknown inclusions appeared within the cells. The microbial morphology and cellular structure restored upon relieving the osmotic pressure. Addition of potassium relieved the effect of osmotic pressure upon nitrification. Results demonstrate that the nitrifying reactor possesses the potential to treat ammonium-rich brines after acclimatization.

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**Keywords:** Osmotic stress; Nitrification; Airlift bioreactor; Nitrogen removal; Wastewater treatment

### 1. Introduction

Nitrogenous compounds like ammonium are prevalent in many wastewaters and need to be removed to prevent oxygen depletion and eutrophication of surface waters. Biological nitrogen removal from wastewater using nitrification-denitrification is a well-known and cost-effective treatment process. The first part of this process (nitrification) consists of the oxidation of ammonium to nitrite and finally to nitrate, being carried out by autotrophic ammonia and nitrite oxidizers, respectively. The nitrite and nitrate are then reduced to nitrogen gas by heterotrophic denitrifying bacteria using a carbon source (normally present in the raw wastewater) as the electron donor [1].

Nitrification is commonly the rate-limiting step of the process and nitrifying bacteria are sensitive to environmental factors such as temperature, dissolved oxygen concentration, pH, available substrate, product inhibition, and inhibitory compounds

[2–4], and may be sensitive to the osmotic conditions of aquatic environment where this process occurs.

Water is a basic requirement for all living organisms to carry out normal metabolism and water availability is an important factor affecting microbial growth in nature. Water availability depends on the concentration of solutes such as salts, sugars, or other substances that are dissolved in water. This is because dissolved substances have an affinity for water, which makes the water associated with solutes unavailable to organisms. Water availability is generally expressed in physical terms such as water activity and osmosis [5].

High nitrogen-concentrated streams can also contain large amounts of other ions like chloride (fish canning industry, wet lime-gypsum desulphurization process), sulphate (tannery wastes, antibiotic process, monosodium glutamate process), etc. [6,7] These ions tend to exert high osmotic pressure (OP) on nitrifying microorganisms. Most of the microbial populations are unable to cope with environments of very high osmotic pressure and either die or become plasmolyzed and dormant under such conditions [5]. It is imperative to study the effect of osmotic stress resulting from ionic-strength on the nitrification,

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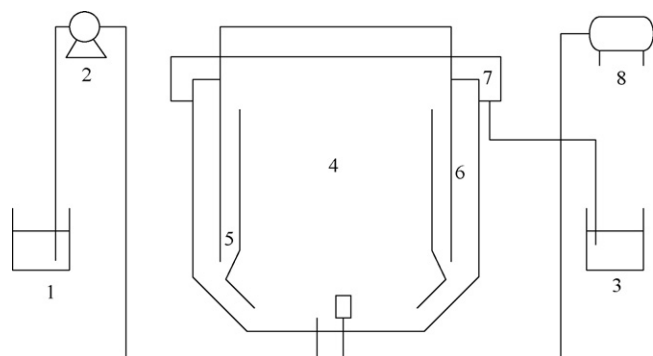


Fig. 1. Scheme of the experimental system. (1) Influent tank, (2) peristaltic pump, (3) effluent tank, (4) riser, (5) downcomer, (6) settler, (7) overflow weir, (8) air pump.

not only from microbiological standpoint but also for the wastewater treatment industry.

The effect of salts in wastewater on nitrification has been investigated extensively [7–11]. However, presently no report concerning the effect of osmotic pressure on the biological wastewater treatment is available. The objective of this study was to investigate the performance of an airlift reactor for treating nitrogen-concentrated wastewater under high osmotic pressure. Reactor performance, microbial morphology, and cellular structure of the activated sludge prior to and following osmotic stress were compared.

## 2. Materials and methods

### 2.1. Airlift bioreactor

The laboratory-scale internal-loop airlift bioreactor is shown in Fig. 1. It was made of Perspex with a working volume of 10.4 L and height/diameter ratio of 1, and it consisted of four sections: riser, downcomer, gas separator, and settling section. The cross sectional area of riser, downcomer, and settling section were 153.9, 97.4, and 346.4 cm<sup>2</sup>, respectively. The reactor was operated at 30 ± 1 °C in a room equipped with thermostat.

### 2.2. Inoculum

Nitrifying sludge from a lab-scale reactor was used as inoculum for the present study. The mixed liquor volatile suspended solids (MLVSS) of inoculum were 4.5 g L<sup>-1</sup>. The operational parameters of ‘mother’ bioreactor were kept at influent NH<sub>4</sub>-N of 420 mg L<sup>-1</sup>, hydraulic retention time (HRT) 20.7 h, and nitrogen loading rate (NLR) of 486 mg L<sup>-1</sup> day<sup>-1</sup>. The NLR was in the range of common nitrifying activated sludge system.

### 2.3. Synthetic wastewater

The composition of synthetic wastewater is listed in Table 1. For alkalinity and carbon source supplement, the theoretical NaHCO<sub>3</sub> requirement for nitrification (7.1 g as CaCO<sub>3</sub> g<sup>-1</sup> NH<sub>4</sub>-N) was added to the wastewater. In phase III described later, NaHCO<sub>3</sub> was partly or thoroughly replaced by KHCO<sub>3</sub> with equimolar ratio.

Table 1

Composition of synthetic ammonium-containing wastewater, in g L<sup>-1</sup>

Compound	Concentration
KH <sub>2</sub> PO <sub>4</sub>	0.027
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.300
CaCl <sub>2</sub>	0.136
Na <sub>2</sub> SO <sub>4</sub>	0–30.7
NaHCO <sub>3</sub>	1.68–7.78
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.66–3.06
Trace elements I <sup>a</sup>	1.25 mL L <sup>-1</sup>
Trace elements II <sup>b</sup>	1.25 mL L <sup>-1</sup>

<sup>a</sup> Composition of trace elements I (g L<sup>-1</sup>): EDTA 5.00, FeSO<sub>4</sub> 5.00.

<sup>b</sup> Composition of trace elements II (g L<sup>-1</sup>): EDTA 15, ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.43, CoCl<sub>2</sub>·6H<sub>2</sub>O 0.24, MnCl<sub>2</sub>·4H<sub>2</sub>O 0.99, CuSO<sub>4</sub>·5H<sub>2</sub>O 0.25, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 0.22, NiCl<sub>2</sub>·6H<sub>2</sub>O 0.19, Na<sub>2</sub>SeO<sub>4</sub>·10H<sub>2</sub>O 0.21, H<sub>3</sub>BO<sub>3</sub>·7H<sub>2</sub>O 0.014.

### 2.4. Experimental set-up

The reactor was operated in three phases described as follows:

**Phase I-Osmotic stress experiment:** In this phase, the operational parameters were fixed at influent NH<sub>4</sub>-N of 420 mg L<sup>-1</sup>, HRT 20.7 h, sludge retention time (SRT) about 24 days, NLR 486 mg L<sup>-1</sup> day<sup>-1</sup>, pH 7.5–8.5, airflow rate 0.65 L min<sup>-1</sup> and dissolved oxygen (DO) of 1.5–5.0 mg L<sup>-1</sup>. At the same time, the influent sodium sulfate concentration was increased stepwise, with a 710 mg L<sup>-1</sup> (equal to OP of 0.4 × 10<sup>5</sup> Pa) increment per step to raise the OP, until the deterioration of the performance of bioreactor. An adaptation period of four days was allowed at each step before increasing the sodium sulfate to the next higher level.

**Phase II-Relieving osmotic stress experiment:** After phase I, the influent sodium sulfate concentration was decreased stepwise that is 1420 mg L<sup>-1</sup> (equal to OP of 0.8 × 10<sup>5</sup> Pa) per step initially. When the OP was lower than 12 × 10<sup>5</sup> Pa, the concentration was changed to 2840 mg L<sup>-1</sup> (equal to OP of 1.6 × 10<sup>5</sup> Pa) per step. Like phase I, an adaptation period of four days was provided. Other operational parameters were the same as phase I.

**Phase III-Osmotic stress adjustment experiment:** During this period, NaHCO<sub>3</sub> was partly or fully replaced with KHCO<sub>3</sub> (with the same molar amount) as a buffer and inorganic carbon source to study the potential adjustment function of potassium in environment of high osmotic stress. HRT was set at 6.2 h with OP of 19.2 × 10<sup>5</sup> Pa. Other operational conditions were the same as phase I.

### 2.5. Analytical methods

Ammonium, nitrite, and nitrate were determined using the standard methods [12]. The mixed liquor volatile suspended solids determination was performed after filtration of a 50 mL sample of mixed liquor on a glass microfibre filter. Dry weight was determined after the filter was dried for 24 h at 105 °C and weighted on a microbalance. The ash content was calculated after incinerating the dried filter in an oven for 1 h at 550 °C. The DO was measured by JPB-607 dissolved oxygen meter. The OP was calculated by Donnan equilibrium ion distribution and OP equations [13].

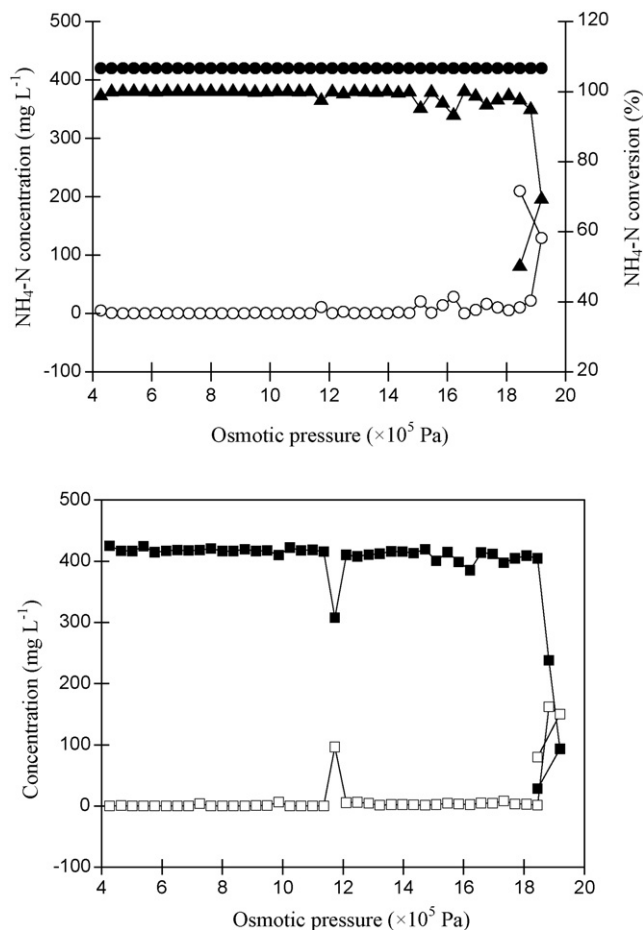


Fig. 2. Performance of nitrifying bioreactor versus osmotic pressure during stage I. (●) Influent  $\text{NH}_4\text{-N}$  concentration, (○) effluent  $\text{NH}_4\text{-N}$  concentration, (▲)  $\text{NH}_4\text{-N}$  conversion, (■) effluent  $\text{NO}_3\text{-N}$  concentration, (□) effluent  $\text{NO}_2\text{-N}$  concentration.

### 3. Results

#### 3.1. Reactor performance under osmotic stress

During the process operation of the first seven months (phase I), the experiment was conducted by raising the osmotic pressure from  $4.3 \times 10^5$  to  $19.2 \times 10^5$  Pa to elucidate the influence of osmotic stress on the performance of internal-loop airlift-nitrifying reactor while keeping the influent  $\text{NH}_4\text{-N}$  at  $420 \text{ mg L}^{-1}$ . Fig. 2 illustrates the variations in ammonium removal with increasing OP. At  $\text{OP} < 14.7 \times 10^5$  Pa, removal capacity of the reactor was  $486 \text{ mg N L}^{-1} \text{ day}^{-1}$  and the effluent ammonium was lower than minimum quantifiable value. Thus, ammonium removal was as high as 100% and almost all the influent ammonium was utilized by nitrifying bacteria. At  $\text{OP}$  of  $11.7 \times 10^5$  Pa, DO concentration was lower than  $1.5 \text{ mg L}^{-1}$  because of aerator malfunction, ammonium removal percentage dropped to 97.4%, still maintaining a high level, and finally resumed to 100% after replacement of the aerator. It seems that  $\text{OP}$  levels of less than  $14.7 \times 10^5$  Pa did not inhibit the nitrification.

Upon further increase in  $\text{OP}$  ( $14.7 \times 10^5$ – $18.8 \times 10^5$  Pa) resulted in a slight decrease in ammonium removal efficiency

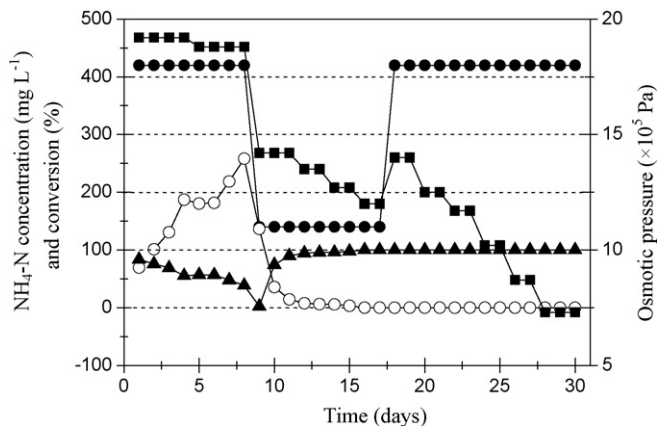


Fig. 3. Evolution of the performance of nitrifying bioreactor during stage II. (●) Influent  $\text{NH}_4\text{-N}$  concentration, (○) effluent  $\text{NH}_4\text{-N}$  concentration, (▲)  $\text{NH}_4\text{-N}$  conversion, (■) osmotic pressure.

that is 93–100%, suggesting a minor negative impact exerted by  $\text{OP}$  upon nitrification. However, the removal percentage dropped to 69.2% suddenly for samples exposed to  $\text{OP}$  of  $19.2 \times 10^5$  Pa and removal capacity decreased to  $336 \text{ mg N L}^{-1} \text{ day}^{-1}$ . This implies that nitrification was badly inhibited with an  $\text{OP}$  as high as this level and the inhibition may be paroxysmal, with a critical level of  $18.8$ – $19.2 \times 10^5$  Pa. Following inhibition, the removal efficiency kept on deteriorating to 50.0% finally while removal capacity dropped to  $243 \text{ mg N L}^{-1} \text{ day}^{-1}$ , even though the  $\text{OP}$  was lowered to  $18.5 \times 10^5$  Pa.

Data in Fig. 2 also indicates that the influent ammonium was completely converted into nitrate ( $\text{NH}_4^+ \rightarrow \text{NO}_3^-$ ) without obvious nitrite accumulation in the situations with  $\text{OP}$  value less than  $18.5 \times 10^5$  Pa. One exception was a nitrite accumulation up to 23.9% of nitrification products caused by aerator malfunction at  $\text{OP}$  of  $11.7 \times 10^5$  Pa mentioned above. However,  $\text{OP}$  value greater than  $18.5 \times 10^5$  Pa resulted in partial nitrification with nitrite as the dominant product (up to 61.7%).

#### 3.2. Reactor performance during osmotic stress relieving phase

Osmotic stress relieving experiment (phase II) lasted for more than 30 days; it was performed to revive the performance of the bioreactor. During initial nine days (Fig. 3),  $\text{OP}$  in the influent was decreased from  $19.2 \times 10^5$  Pa through  $7.3 \times 10^5$  Pa keeping influent  $\text{NH}_4\text{-N}$  fixed at  $420 \text{ mg L}^{-1}$  and  $\text{HRT}$  at 20.7 h.  $\text{OP}$  values of  $19.2 \times 10^5$ – $18.5 \times 10^5$  Pa resulted in the elevation of effluent  $\text{NH}_4\text{-N}$  in the range of 180.4–258.3  $\text{mg L}^{-1}$ . Consequently, the ammonium removal efficiency went down from 57.0 to 38.5%. The effluent nitrite concentration persistently increased to be more than 60% of the total nitrite and nitrate, thus partial nitrification was achieved. The data suggested that detrimental effects resulting from osmotic stress did not cease with the passage of time though  $\text{OP}$  was decreased, showing hysteresis phenomenon.

To restore the reactor performance, the influent  $\text{NH}_4\text{-N}$  concentration was incrementally reduced to  $140 \text{ mg L}^{-1}$  with  $\text{OP}$  of  $14.2 \times 10^5$  Pa and  $\text{NLR}$  was decreased from 486 to

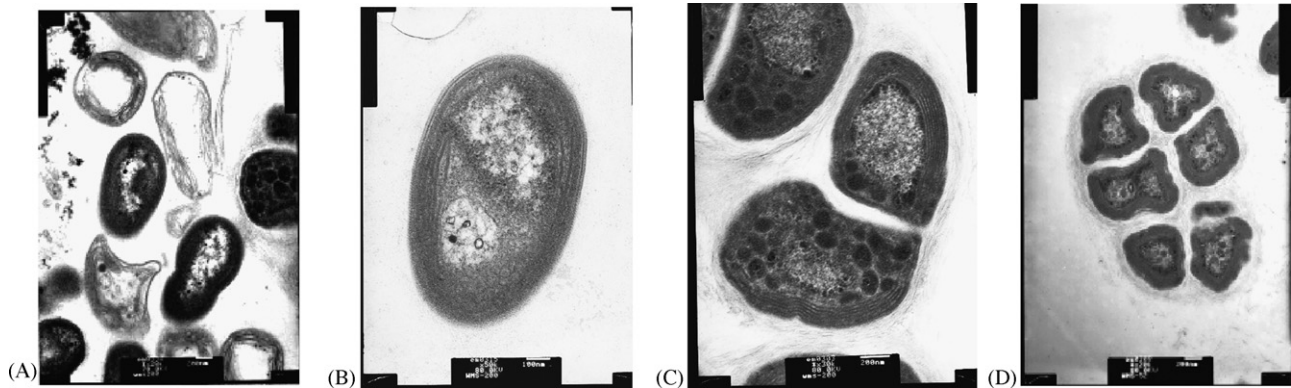


Fig. 4. TEM (transmission electron microscope) photograph of nitrifying bacteria in activated sludge (A) (30,000 $\times$ ) at osmotic pressure of  $19.2 \times 10^5$  Pa, (B) (50,000 $\times$ ) before osmotic stress, (C) (30,000 $\times$ ) after osmotic stress, and (D) (20,000 $\times$ ) after relieving osmotic stress.

$162 \text{ mg L}^{-1} \text{ day}^{-1}$  at constant HRT of 20.7 h. Under these conditions, ammonium removal elevated from 2.4 to 89.8%. Afterwards OP was continuously reduced to  $12.0 \times 10^5$  Pa, with a constant influent substrate level; as a result ammonium removal percentage reached to 99.9%, that is recovery of reactor performance. Later influent ammonium-nitrogen concentration was elevated to the level used during osmotic stress, while the OP was reduced from  $14 \times 10^5$  to  $7.3 \times 10^5$  Pa. The bioreactor worked steadily with a removal rate of 100% achieving complete nitrification. These operational parameters indicated that the osmotic stress on nitrification was over.

### 3.3. Microbial composition and cell structure

Fig. 4(A) shows the transmission electron microscope (TEM) photograph of nitrifying bacteria found in activated sludge inhibited by OP of  $19.2 \times 10^5$  Pa. Under the osmotic stress of this level, the cellular structure of some microbes was disrupted, which confirmed inhibition displayed by the reactor performance.

Increase in OP lead to a change in microbial morphology as evident in Fig. 5. After osmotic stress, the microbial diversity in sludge was narrowed with a reduction in cell size; the inner cell membrane was partly damaged along with reduction in its number of layers (Fig. 4(B) and (C)). Many blurred granular inclusions were prominent in the cells (Fig. 4(C)).

Fig. 4(D) shows the TEM micrograph of nitrifying bacteria present in activated sludge after revival from osmotic stress. At this stage, majority of nitrifying bacteria lived in encysted form assuming irregular shape with recovered inner membrane having increased number of layers. The granular inclusions formed during phase I were diminished.

### 3.4. Effect of potassium ions on nitrification during osmotic stress

Table 2 shows the effects of potassium addition on ammonium conversion at various  $\text{KHCO}_3/\text{NaHCO}_3$  ratios. At OP value of  $19 \times 10^5$  Pa, influent  $\text{NH}_4^+\text{-N}$  of  $420 \text{ mg L}^{-1}$ ,  $0.06 \text{ mol L}^{-1}$   $\text{KHCO}_3$  and  $\text{NaHCO}_3$  buffer, with  $\text{KHCO}_3/\text{NaHCO}_3$  ratios of 0(0/0.06), 2(0.04/0.02), and 3(0.045/

$0.015) \text{ mol mol}^{-1}$ , the ammonium removal efficiency was  $57.55 \pm 2.92$ ,  $64.87 \pm 1.43$ , and  $70.60 \pm 4.19\%$  (mean  $\pm$  standard deviation), respectively. It implies that the ammonium removal efficiency increased with increasing potassium levels. This finding indicates that addition of potassium ion can partially alleviate the inhibition caused by osmotic stress.

The effects of  $\text{K}^+$  to alleviate the osmotic stress were investigated in detail. An experiment was carried out to study the effects of  $\text{KHCO}_3$  addition during nitrification. The data listed in Table 3 shows that an increase in  $\text{KHCO}_3$  content resulted in the elevated ammonium removal efficiency along with a

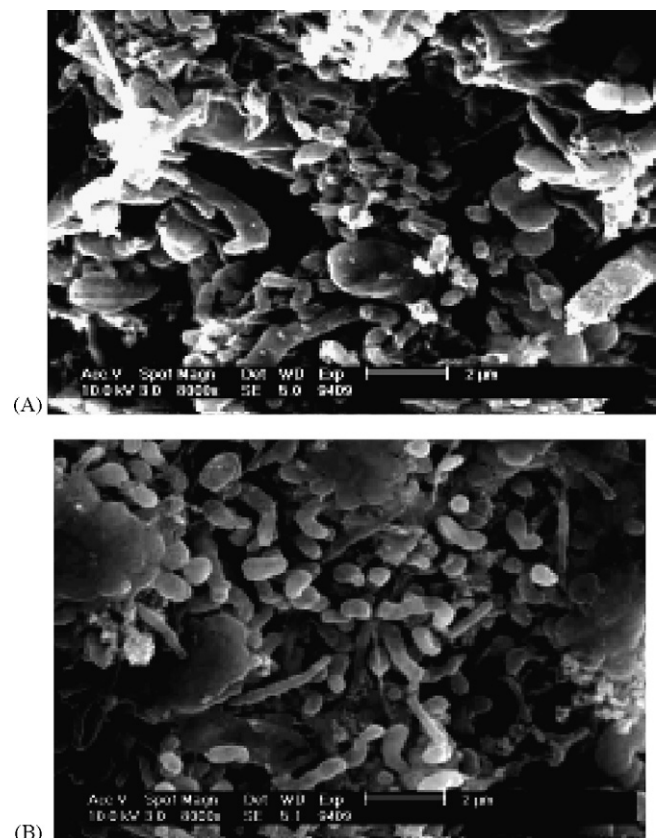


Fig. 5. Scanning electron microscope (SEM) photograph of nitrifying bacteria in activated sludge before (A) and after (B) osmotic stress (8000 $\times$ ).



Table 2  
Effect of potassium ion on ammonium conversion under osmotic stress ( $\text{mg L}^{-1}$ ) at influent  $\text{NH}_4\text{-N}$  of  $420 \text{ mg L}^{-1}$  and HRT of 6.2 h

	$\text{KHCO}_3/\text{NaHCO}_3 = 0 \text{ (mol mol}^{-1}\text{)}$		$\text{KHCO}_3/\text{NaHCO}_3 = 2 \text{ (mol mol}^{-1}\text{)}$		$\text{KHCO}_3/\text{NaHCO}_3 = 3 \text{ (mol mol}^{-1}\text{)}$	
	Effluent $\text{NH}_4\text{-N}$ concentration	$\text{NH}_4^+\text{-N}$ conversion (%)	Effluent $\text{NH}_4\text{-N}$ concentration	$\text{NH}_4\text{-N}$ conversion (%)	Effluent $\text{NH}_4\text{-N}$ concentration	$\text{NH}_4\text{-N}$ conversion (%)
First day	165.6	60.6	155.0	63.1	101.0	76.0
Second day	175.4	58.2	139.9	66.7	147.0	65.0
Third day	173.2	58.8	147.9	64.8	113.4	73.0
Fourth day	178.9	57.4	151.5	63.9	132.0	68.6
Fifth day	198.4	52.8	143.5	65.8	124.0	70.5
Mean value	178.3	57.6	147.6	64.9	123.5	70.6

Table 3  
Effect of potassium ion on performance of nitrification under osmotic stress ( $\text{mg l}^{-1}$ ) at different influent  $\text{NH}_4\text{-N}$  and constant HRT of 6.2 h

	Influent $\text{NH}_4\text{-N}$ concentration	Effluent			$\text{NH}_4\text{-N}$ conversion (%)	$(\text{KHCO}_3/\text{NaHCO}_3) / (\text{mol mol}^{-1})$
		$\text{NH}_4\text{-N}$	$\text{NO}_2^- \text{-N}$	$\text{NO}_3^- \text{-N}$		
First day	503.8	170.9	120.1	212.7	66.1	0/1
Second day	585.7	101.0	62.3	422.4	82.8	0/1
Third day	587.3	122.2	0.34	464.8	79.2	0/1
Fourth day	583.0	58.8	0.66	537.3	89.9	1/1
Fifth day	596.8	37.5	0.41	547.5	93.7	1/1
Sixth day	596.6	22.9	0.02	573.6	96.2	1/1
Seventh day	645.7	5.5	0.07	640.2	99.2	1/0
Eighth day	648.5	0.22	0.00	648.2	99.9	1/0
Ninth day	646.3	0.25	0.00	645.2	99.9	1/0

decreasing levels of nitrite in oxidized products, confirming the finding described above.

#### 4. Discussion

The present experiment was performed to elucidate the effects of OP upon nitrification process in a lab-scale internal-loop airlift-nitrifying reactor. The reactor performance was not obviously affected by its exposure to OP up to  $18.8 \times 10^5 \text{ Pa}$ , equal to about  $30 \text{ g NaCl L}^{-1}$  that is in the range of salt concentration mentioned in the literature [8,10,11,14,15]. Such behavior might be explained through an adaptation model, saline-resistant nitrifying bacteria may develop in conditions with an increment of  $710 \text{ mg L}^{-1}$  sodium sulfate per step. The disturbance in balance between the number of freshwater and saline-resistant nitrifying bacteria can also be another possible explanation. Chen et al. [14] reported a shift in the dominant species of non-saline-resistant ammonia oxidizers, such as *Nitrosomonas europaea* lineage and *Nitrosomonas eutropha*, to saline-resistant species such as *Nitrosococcus mobilis* lineage when the salt concentration was increased from 16.5 to  $30 \text{ g NaCl L}^{-1}$ .

In present study, inhibition caused by osmotic stress at OP levels above  $19.2 \times 10^5 \text{ Pa}$ , took place without any alarm. It is different from the results reported in some literatures suggesting that the inhibition caused by salinity occurred gradually [14–16]. The high OP level might have approached the upper limit of bacterial metabolism beyond which abrupt inhibition of nitrifying ability took place during present work.

The osmotic stress relieving experiment was also conducted according to an adaptation model, the reactor performance recovered in 30 days and the negative influence due to a rapid decrease in OP was relieved (Fig. 3). Earlier work showed that rapid variations in salt concentration caused immediate release of cellular constituents; moreover, a decrease in salt concentration caused more severe negative impacts on microorganisms than an increase in salt concentration [17].

Nitrite accumulation due to partial nitrification during osmotic stress is beyond expectation. Nitrite accumulation usually can be obtained by disequilibrating the activities or numbers of ammonia oxidizers and nitrite oxidizers [18]. The disequilibrium in numbers occurs when ammonia oxidizers outgrow nitrite oxidizers in a mixed culture nitrification system through control of HRT and SRT in a chemostat at temperature higher than  $25^\circ \text{C}$  [3]. Differences in the oxidation activities can be created, for example, by a selective inhibition of the nitrite oxidizers activity through pH control or/and DO [19–21]. The operational conditions in present study are not suitable for nitrite accumulation, which is confirmed by the bioreactor performance before osmotic stress. It can be deduced that the ammonia oxidizers are less susceptible to osmotic stress than nitrite oxidizers. This was in accordance with the results of earlier investigations [10,22], which indicated that nitrite oxidizers are more negatively affected by high salt concentrations. While comparing N-removal over nitrate, and N-removal over nitrite such as SHARON (nitrification and denitrification via nitrite) and ANAMMOX (anaerobic ammonium oxidation with nitrite to form nitrogen gas), the advantages of N-removal over nitrite

include lower aeration and organics costs for nitrification and denitrification, and less sludge production [3,23–25]. These advantages are even more striking in case of effluents with high ammonium and low organic contents. The nitrite accumulation under high OP may propose a new strategy to reach partial nitrification, especially in the case of salty wastewater treatment.

Fig. 4 shows that some granular inclusions developed in cells during osmotic stress but disappeared later when the osmotic pressure was relieved. This phenomenon indicates that these inclusions perhaps are related to the adaptation of nitrifying bacteria to the variations in OP. As far as we know, these inclusions have not been reported in nitrifying bacteria elsewhere. Further study should be undertaken to elucidate their structure and function.

The results indicated that addition of potassium ions could alleviate the inhibition caused by osmotic stress. When microbes grow in a medium with a high OP, they can obtain water from environment only by lowering their cellular osmotic potential.  $K^+$  is a kind of compatible solutes that can be pumped into the cell from the environment to lower the cellular osmotic potential and it does not cause inhibition to metabolic processes within the cell. In the case of high OP,  $K^+$  can be accumulated from the environment to adjust cytoplasmic water activity [5,26,27]. It was proposed that  $KHCO_3$ , a substitute for  $NaHCO_3$ , could be used as buffer and carbon source during nitrification under osmotic stress. Techno-economic analysis should be carried out to have an insight into an alleviation of osmotic stress inhibition caused by potassium ions and the higher cost for  $KHCO_3$  addition compared with  $NaHCO_3$ .

It appears that nitrifying bacteria can perform under halophilic conditions and high osmotic pressure; further work is necessary to explicate the physiological basis of their osmotic pressure tolerance mechanisms. Overall, these data advocate that the airlift bioreactor can adapt to handle ammonium-rich solutions with high osmotic pressure as often produced by fish canning industries [6], the wet lime-gypsum desulphurization process [7] and regeneration of ion exchange columns [28] but careful monitoring of the DO concentration, pH, and other operational parameters over a longer time is required.

## 5. Conclusions

The main conclusions from present research are as follows:

- Based on an adaptation model, the nitrifying sludge in an internal loop airlift bioreactor could cope with OP as high as  $18.8 \times 10^5$  Pa. The nitrifying reactor possesses the potential to treat ammonium-rich brines after appropriate acclimatization.
- The inhibition of nitrification under the influence of osmotic pressure is paroxysmal, with a critical level of  $18.8$ – $19.2 \times 10^5$  Pa in present study.
- Nitrite oxidizers are more sensitive to osmotic stress than ammonia oxidizers, leading to nitrite accumulation in the reactor.
- The reactor was able to regain 100% of its bioactivity gradually by relieving osmotic stress through gradual decrease

in the salt concentration. Lowering substrate concentration appeared to favor the recovery process.

- Osmotic stress leads to changes in microbial diversity and cellular structure, which revived after relieving osmotic stress.
- Because of the microbial ability to adjust cytoplasmic water activity, potassium ions in wastewater could partially alleviate the inhibition caused by osmotic stress.

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