



# Partial Nitrification to Nitrite using Activated Sludge Entrapped in Polymerized Gel: Continuous and Batch Operation in an Airlifting Reactor



Lina Chi<sup>1</sup>, Xiaofeng Ye<sup>2\*</sup>, Jun Li<sup>3</sup>, Zhirong Li<sup>1</sup>, Zheng Jiang<sup>4</sup> and Zhenjia Zhang<sup>1\*</sup>

<sup>1</sup>School of Environmental Science and Engineering, Shanghai Jiao Tong University, China

<sup>2</sup>Novozymes (China) Investment Co., Ltd., China

<sup>3</sup>School of Municipal and Environmental Engineering, Shenyang Jianzhu University, China

<sup>4</sup>Faculty of Engineering and the Environment, University of Southampton, UK

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**\*Corresponding author:** Zhengjia Zhang, School of Environmental Science and Engineering, Shanghai Jiaotong University, China. Email: zjzhang@sjtu.edu.cn; Xiaofeng Ye, Novozymes (China) Investment Co., Ltd., Email: xiaofengyip@gmail.com

## Abstract

Partial nitrification to nitrite using activated sludge entrapped in polymerized gel was investigated in an air-lifting reactor in both continuous and batch operations. High-rate partial nitrification to nitrite was achieved under non-limiting dissolved oxygen conditions during the start-up of the reactor. After the stable operation was established, HRT was optimized to achieve the effective ammonium conversion to nitrite and provide the effluent suitable potential for anaerobic ammonium oxidization (ANAMOX). The influences of process parameters, including dissolved oxygen concentrations, temperatures, and initial  $\text{NH}_4^+\text{-N}$  conditions, on partial nitrification kinetics were studied in batch operation. Data of the batch experiments indicate that it is feasible to achieve 95% ammonium removal with dominant nitrite accumulation under non-limiting DO conditions at the temperature of 24 °C or higher. Reaction time affected the nitrite accumulation and the  $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$  ratio in batch operation. These data demonstrate the potential of using immobilized activated sludge for partial nitrification in continuous and sequencing batch reactors and its applicability as the pretreatment for anaerobic ammonium oxidization process.

**Keywords:** Partial nitrification; Nitrite; Activated sludge entrapped in polymerized gel; HRT; Dissolved oxygen

## Highlights

- High rates oxidation of ammonium to nitrite under non-limiting bulk DO conditions
- Optimization of operational parameters for effective partial nitrification
- Dominant nitrite accumulation when 95% of ammonium was removed
- Effective nitrification under low temperature of 8 °C
- Effluent suitable for the ANAMOX by controlling the HRT and the batch reaction time

## Introduction

Due to increasingly stringent effluent discharge requirement, population growth, and the growing desire to reuse wastewater, wastewater treatment plants are expanding all over the world. However, in many countries, especially in metropolitan areas,

limited space is a major challenge for the expansion projects [1]. Various immobilized cell technologies have been developed to address this challenge with minimal additional footprint such as moving bed bioreactors and integrated fixed film in activated sludge process [2-4]. The gel-immobilized activated sludge process is one of the newly developed immobilized cell technologies that has been previously demonstrated to achieve high rates degradation of COD and nitrification in over 11 wastewater treatment plants in Japan and the USA [5-7]. Gel-immobilized biomass can be directly added to aeration basins with additional screens to keep the gel pellets inside the tank [5]. A number of advantages are associated with immobilized-cell treatment systems, including small footprint, high biomass concentration, reduction of excess sludge production, and dissociation of solid and liquid retention times [5,6].

Immobilized activated sludge has potential in nitrification processes due to the retention of biomass, which can overcome

the relatively slower growth rates for nitrifying autotrophs [8]. Biological ammonia oxidation involves two steps: ammonia conversion to nitrite by ammonium oxidizing bacteria (AOB) and the subsequent nitrite conversion to nitrate by nitrite oxidizing bacteria (NOB) [9]. In most cases nitrite seldom accumulates in the environment due to a low  $S_{min}$  (minimum substrate concentration capable of supporting steady-state biomass) value and relatively higher substrate utilization rates of nitrite oxidizers [9,10]. Partial nitrification to nitrite has been attracting increasing attention, not only due to its potential saving of oxygen consumption and electron donor requirement in subsequent denitrifying process, but also because it is the prerequisite for anaerobic ammonium oxidation processes [11-14]. Partial nitrification has been suggested to be the rate limiting step for the combined partial nitrification-ANAMOX process to remove nitrogen via nitrite [15].

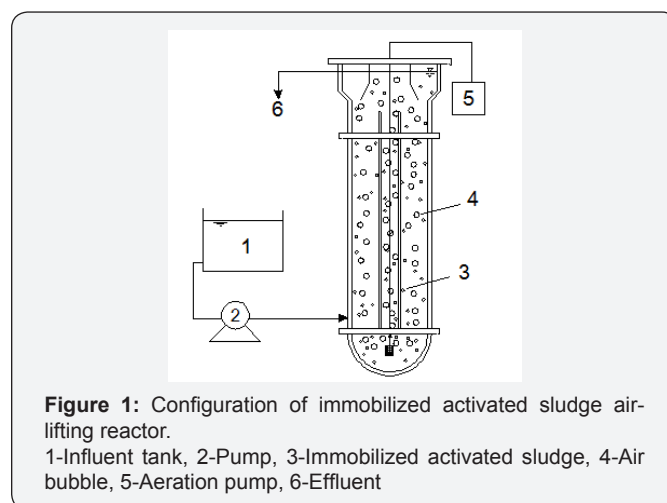
Partial nitrification to nitrite can be achieved by optimizing process parameters, including HRT, dissolved oxygen, temperature and influent ammonium-nitrogen concentration [10,16]. Limiting the dissolved oxygen concentration has been reported to be effective in accumulating nitrite, since the oxygen saturation coefficient of Monod kinetics was significantly lower for ammonium oxidation than that for nitrite oxidation [16-18]. However, DO limitations could potentially sacrifice the overall kinetics of nitrification [19]. Partial nitrification to nitrite has been previously reported at relatively higher temperatures, since AOB have higher growth rate than NOB at elevated temperature ( $>15$  °C) (Sinha & Annachatre [20]); however, limited information is available on the influence of temperature on the nitrite accumulation of immobilized activated sludge system.

In this study, we investigated the partial nitrification performance of immobilized activated sludge in the air-lifting fluidized reactor in both continuous and batch operations. High-rate ammonium conversion to nitrite under non-DO limiting conditions was achieved during the start-up of the reactor. The

influence of HRT on the ammonium oxidation rates and the nitrite accumulation was studied in continuous operation after the stable operation was established. Other factors including dissolved oxygen concentrations, temperatures and initial  $NH_4^+$ -N concentrations were investigated in batch operation to determine appropriate conditions for partial nitrification of immobilized activated sludge. The potential of using partial nitrification immobilized activated sludge process as the pretreatment of anaerobic ammonium oxidation (ANAMOX) is also discussed.

## Materials and Methods

### Experimental set-up



A schematic diagram of the experimental set-up is presented in Figure 1. The air-lifting reactor had a working volume of 18L with 8-10% (v/v) immobilized activated sludge. The air was supplied from the bottom through an air sparger to create inner circulation. Temperature within the reactor was measured on-line and maintained at different levels by a water jacket.

### Waste water composition

**Table 1:** Characteristics of synthetic waste water.

Initial $NH_4^+$ -N Concentration	$NH_4Cl$ (mg/L)	$Na_2HPO_4$ (mg/L)	KCl (mg/L)	$CaCl_2$ (mg/L)	$MgSO_4$ (mg/L)	$NaHCO_3$ (mg/L)	$C_6H_{12}O_6$ (mg/L)
150mg/L	574	171	36	27	62	1755	94
250mg/L	956	290	60	45	103	2925	140
360mg/L	1338	406	84	63	143	6095	202

Synthetic wastewater was composed of  $NH_4Cl$  as the nitrogen source and glucose as the carbon source. Other chemicals such as KCl,  $Na_2HPO_4$ ,  $CaCl_2$ , and  $MgSO_4$  were added as nutrients.  $NaHCO_3$  was also added as a buffering agent. The specific compositions of wastewater at different initial  $NH_4^+$ -N concentrations are shown in Table 1.

### Immobilized activated sludge

Immobilized activated sludge was provided by Hitachi Plant

Engineering and Construction Co. Ltd, Japan. Gel pellets were composed of 10% (w/v) PEG (Polyethylene glycerol), 0.5% (w/v) promoter (N,N,N',N'-tetramethylethylenediamine), 0.25% initiator (potassium persulfate) and around  $10^7$ - $10^9$  cells/ml-pellets (Hashimoto & Sumino [5]). The immobilization process started with dissolving the PEG polymer and the promoter in water, followed by adding activated sludge and the initiator. The polymerized gel was then cut into  $3 \times 3 \times 3$ mm cubes.

## Acclimation and start-up of the reactor

Acclimation was initiated by operating the air-lifting reactor in sequencing batch mode, with a fixed aerobic reaction time of 15 hours. Temperature was controlled at  $28 \pm 1$  °C and DO was kept at  $6.0 \pm 0.5$  mg/L. Influent  $\text{NH}_4^+\text{-N}$  concentrations were increased step-wise from 100 to 300 mg/L within 5 weeks. After the acclimation process, the reactor was then operated in continuous mode to develop nitrite accumulation. DO was kept at 5-6 mg/L in the first stage of continuous operation and then lowered to 1-2 mg/L to increase the nitrite accumulation in the second stage. After stable nitrite accumulation was established, dissolved oxygen was then increased to 3-4 mg/L at the third stage. The influent  $\text{NH}_4^+\text{-N}$  concentration was maintained around 300 mg/L; pH and temperature were controlled at 7.8 and 28 °C, respectively, during continuous operation.

## Continuous experiments

HRT was controlled by adjusting the flow rates of the feed to the reactor. At each HRT, the reactor was operated for a minimum of two weeks to ensure that steady state was achieved; this was also demonstrated by a standard deviation of three consecutive samples of less than 5%.

## Batch experiments

Batch experiments were conducted in the fluidized reactor after over one month of stable continuous operation. Experiments utilized synthetic wastewater with an initial  $\text{NH}_4^+\text{-N}$  concentration of about 250 mg/L. DO concentrations and temperatures were varied to study their influences on ammonium oxidation kinetics and nitrite accumulation in the batch reactor. The influence of different initial  $\text{NH}_4^+\text{-N}$  concentrations on nitrification kinetics and nitrite accumulation was investigated by operating the reactor at pH=8.0, DO=4 mg/L, and temperature at 24 °C. pH variations in the ammonia oxidation process were minimal due to abundant buffering capacity in the influent.

## Analytical methods

$\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations were measured daily by standard methods (APHA [21]). DO levels were measured twice per day with an oxygen electrode (ORION 830A, Thermo Orion company) pH and flow rates were monitored on a daily basis.

## Results and Discussion

### Acclimation and start-up of partial nitrification continuous reactor

**Acclimation of immobilized activated sludge:** Immobilized activated sludge was stored under anaerobic conditions at 4 °C with little nutrients for 4 months due to international transportation.  $\text{NH}_4^+\text{-N}$  oxidation rates was  $0.28 \text{ kg-N/m}^3\text{-day}$  after long term anaerobic storage and increased to  $1.08 \text{ kg-N/m}^3\text{-day}$  at 35 days, 3.9 times the original rates prior to acclimation (Figure 2). These results suggest that the activity of immobilized activated sludge

can be recovered within five weeks after long time storage under minimal nutrient and low temperature conditions.

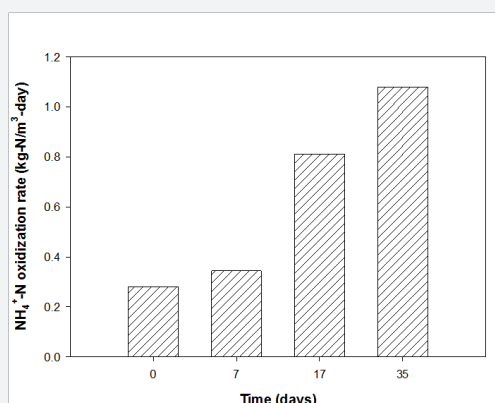


Figure 2:  $\text{NH}_4^+\text{-N}$  oxidation rates at different time intervals during acclimation.

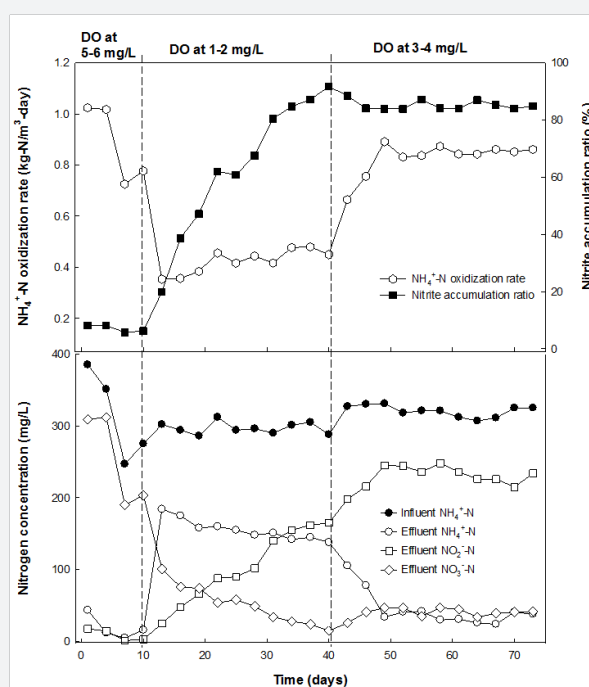


Figure 3:  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$  concentrations of influent and effluent,  $\text{NH}_4^+\text{-N}$  oxidation rate and  $\text{NO}_2^-\text{-N}$  accumulation ratio as functions of time during the start-up of partial nitrification reactor.

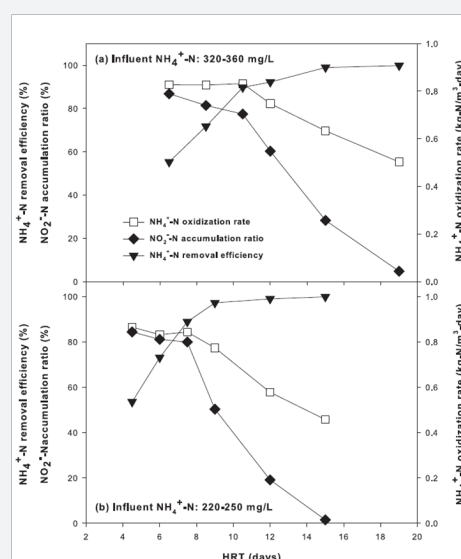
**Start-up of partial nitrification continuous reactor:** Figure 3 shows the  $\text{NH}_4^+\text{-N}$  concentrations in the influent and effluent and  $\text{NO}_2^-\text{-N}$  accumulation ratio at different stages of the start-up process. Immobilized activated sludge effectively converted  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$  during stage one of continuous operation at dissolved oxygen concentration of 5-6 mg/L. Between 5-7% of oxidation products were accumulated in the form of  $\text{NO}_2^-\text{-N}$  at this time. The dissolved oxygen concentration was then decreased to 1-2 mg/L during stage 2 to develop nitrite accumulation.  $\text{NH}_4^+\text{-N}$

oxidization rates dropped from 0.8 to 0.35-0.5kg-N/m<sup>3</sup>-day. Nitrite concentrations in the effluent concurrently increased to 160mg/L; the NO<sub>2</sub><sup>-</sup>-N accumulation ratio increased from below 10% at 10 days to over 90% at 40 days. Despite high nitrite accumulation, NH<sub>4</sub><sup>+</sup>-N concentration stayed above 100mg/L in the effluent due to inefficient NH<sub>4</sub><sup>+</sup>-N oxidization rates under oxygen-limited conditions. Thus, at the third stage, dissolved oxygen concentrations were further increased to 3-4mg/L to stimulate ammonium removal. NH<sub>4</sub><sup>+</sup>-N oxidization rates were significantly improved from 0.3-0.5 to above 0.8kg-N/m<sup>3</sup>-day. Nitrite accumulation ratio decreased slightly but could be maintained above 80%.

Effective oxidization of NH<sub>4</sub><sup>+</sup>-N to NO<sub>2</sub><sup>-</sup>-N was achieved in continuous operation with NH<sub>4</sub><sup>+</sup>-N oxidization rates of 0.8-0.9kg-N/m<sup>3</sup>-day and over 80% of all products accumulated in the form of nitrite, even though the activated sludge immobilized inside the gel had not been pre-selected for ammonium oxidizing bacteria. Limiting DO concentrations has been previously demonstrated to be an effective strategy in accumulating nitrite in the nitrification process [10,18,22], which was successfully applied in the immobilized activated sludge system to achieve partial nitrification to nitrite in this study; however, the ammonium oxidization kinetics were also inhibited under DO-limitation conditions. Increasing dissolved oxygen concentrations from 0-1.5mg/L to 3-4mg/L improved the ammonium oxidization rates by approximately 60-70%, while only decreased NO<sub>2</sub><sup>-</sup>-N accumulation ratio by around 10% during stage 3. These data suggested that partial nitrification to nitrite was feasible in the immobilized activated sludge reactor under non-limiting dissolved oxygen conditions, which could be important to achieve dominant nitrite accumulation in the effluent while maintaining efficient oxidization of NH<sub>4</sub><sup>+</sup>-N.

### Influence of HRT on NH<sub>4</sub><sup>+</sup>-N removal and NO<sub>2</sub><sup>-</sup>-N accumulation

NH<sub>4</sub><sup>+</sup>-N removal efficiency was improved significantly, but the nitrite accumulation ratio declined as the HRT increased from 4 to 15 hours and from 6 to 19 hours for influent NH<sub>4</sub><sup>+</sup>-N concentrations of 220-250mg/L (Figure 4a) and 320-360mg/L (Figure 4b), respectively. For influent NH<sub>4</sub><sup>+</sup>-N concentrations of 220-250mg/L, only 50-80% NH<sub>4</sub><sup>+</sup>-N removal was achieved when the HRT was less than 8 hours. Nitrite composed 80% of the oxidization products. Increasing the HRT to 8 hours improved NH<sub>4</sub><sup>+</sup>-N removal to 85% without negatively influencing the NO<sub>2</sub><sup>-</sup>-N accumulation (83%). NH<sub>4</sub><sup>+</sup>-N oxidization rates remained almost constant at 0.86-0.83kg-N/m<sup>3</sup>-day when the HRT was maintained at 4, 6 and 8 hours. Increasing HRT to 10 hours continuously improved the NH<sub>4</sub><sup>+</sup>-N removal to over 98% but decreased the nitrite accumulation ratio to about 50%. Additional HRT increases did not change the NH<sub>4</sub><sup>+</sup>-N removal yet significantly decreased the NO<sub>2</sub><sup>-</sup>-N accumulation ratio in the effluent and NH<sub>4</sub><sup>+</sup>-N oxidization rates of the reactor. Similar trends were observed with the influent NH<sub>4</sub><sup>+</sup>-N concentration of 320-360mg/L, except that it required around 2-4 hours longer of HRT to reach similar levels of NH<sub>4</sub><sup>+</sup>-N removal and nitrite accumulation.



**Figure 4:** NH<sub>4</sub><sup>+</sup>-N removal efficiency, NH<sub>4</sub><sup>+</sup>-N oxidization rate, and NO<sub>2</sub><sup>-</sup>-N accumulation ratio as functions of HRT for influent NH<sub>4</sub><sup>+</sup>-N concentrations of 320-360 (a) and 220-250mg/L (b).

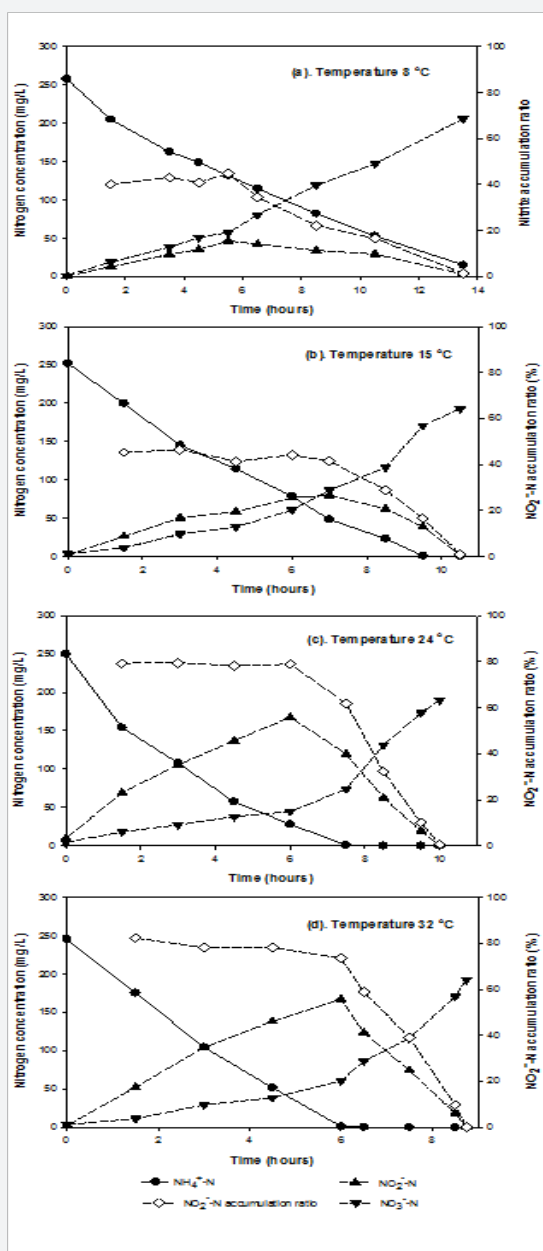
These results demonstrate the importance of HRT in achieving effective ammonium oxidization to nitrite using immobilized activated sludge. Running the reactor with a short hydraulic retention time could not effectively remove NH<sub>4</sub><sup>+</sup>-N in the effluent despite high NH<sub>4</sub><sup>+</sup>-N oxidization rates. When the HRT was too long, NH<sub>4</sub><sup>+</sup>-N oxidization rates and nitrite accumulation were sacrificed; at full-scale, longer hydraulic residence times translate into larger effective volumes and larger footprints. In this study, the data showed that the HRT could be controlled at around 8 hours for influent NH<sub>4</sub><sup>+</sup>-N of 220-250mg/L, and 10 hours for influent NH<sub>4</sub><sup>+</sup>-N of 320-360mg/L to achieve high rates of ammonium removal (NH<sub>4</sub><sup>+</sup>-N oxidization rates exceeded 0.8kg-N/m<sup>3</sup>-day) with about 80% oxidation products accumulated in the form of nitrite. Meanwhile, controlling the HRT in the partial nitrification immobilized activated sludge reactor could also potentially provide an effluent suitable for anaerobic ammonium oxidization processes. An NH<sub>4</sub><sup>+</sup>-N/NO<sub>2</sub><sup>-</sup>-N ratio close to 1:1 was previously reported for optimal anaerobic ammonium oxidization process [6,15]. HRT can be controlled around 9 and 13 hours for influent NH<sub>4</sub><sup>+</sup>-N concentrations of 220-250 and 320-360mg/L, respectively, to keep NH<sub>4</sub><sup>+</sup>-N/NO<sub>2</sub><sup>-</sup>-N ratio close to 1:1 in the effluent.

### Factors affecting the NH<sub>4</sub><sup>+</sup>-N removal and NO<sub>2</sub><sup>-</sup>-N accumulation

The influences of dissolved oxygen, temperature, and initial NH<sub>4</sub><sup>+</sup>-N concentration on NH<sub>4</sub><sup>+</sup>-N oxidization kinetics and NO<sub>2</sub><sup>-</sup>-N accumulation were investigated in batch operation and discussed as below.

**Influence of dissolved oxygen on NH<sub>4</sub><sup>+</sup>-N removal and NO<sub>2</sub><sup>-</sup>-N accumulation:** Figure 5 presents the concentrations of NH<sub>4</sub><sup>+</sup>-N, NO<sub>2</sub><sup>-</sup>-N and NO<sub>3</sub><sup>-</sup>-N and the nitrite accumulation ratio over time under different DO conditions. When DO was controlled

at 2mg/L, it took 13 hours until  $\text{NH}_4^+\text{-N}$  concentration reached below 10mg/L. Nitrite was the major oxidation product (levels exceeded 80%) until 13 hours, and was then oxidized to nitrate. Increasing DO to 4mg/L shortened the time from 13 hours to 6-7 hours for  $\text{NH}_4^+\text{-N}$  concentrations to decrease below 10mg/L.  $\text{NO}_2^-\text{-N}$  composed around 60-80% of the oxidation products at 6-7 hours, and then was oxidized to  $\text{NO}_3^-\text{-N}$  after the depletion of  $\text{NH}_4^+\text{-N}$ . When DO was controlled at 6mg/L, it took around 6-7 hours for  $\text{NH}_4^+\text{-N}$  concentration to reach below 10mg/L; however, the  $\text{NO}_2^-\text{-N}$  accumulation ratio was significantly lower than those at DO concentrations of 2 and 4mg/L.



**Figure 5:**  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  concentrations, and  $\text{NO}_2^-\text{-N}$  accumulation ratio as functions of time at different dissolved oxygen concentrations (a. DO of 2mg/L; b. DO of 4mg/L; c. DO of 6mg/L).

**Table 2:**  $\text{NH}_4^+\text{-N}$  removal, oxidation rates, and  $\text{NO}_2^-\text{-N}$  accumulation ratio at different dissolved oxygen concentrations.

DO (mg/L)	$\text{NH}_4^+\text{-N}$ Removal (%)	$\text{NH}_4^+\text{-N}$ Oxidization Rates (kg-N/m <sup>3</sup> -day)	$\text{NO}_2^-\text{-N}$ Accumulation ratio (%)
2	95	0.65	86
4	95	0.9	68
6	95	1.01	50

$\text{NH}_4^+\text{-N}$  oxidation rates were obtained using a linear regression method to fit the first 6 data points of  $\text{NH}_4^+\text{-N}$  concentration. Since the values of coefficient determination ( $R^2$ ) in the regression were all above 0.98, the nitrification process of immobilized activated sludge virtually followed zero order kinetics. Table 2 summarizes the  $\text{NH}_4^+\text{-N}$  oxidation rates and nitrite accumulation ratios when  $\text{NH}_4^+\text{-N}$  removal reached above 95% ( $\text{NH}_4^+\text{-N}$  concentration < 15mg/L). Increasing DO concentration from 2 to 4mg/L significantly improved the  $\text{NH}_4^+\text{-N}$  oxidation rates by 38%, suggesting that partial nitrification was limited by dissolved oxygen as a co-substrate. Even though the dissolved oxygen profile inside the PEG gel is not sufficiently understood, it is possible that increasing the bulk dissolved oxygen concentrations from 2 to 4mg/L was beneficial for bacteria encapsulated inside the gel to uptake more oxygen, which led to increased  $\text{NH}_4^+\text{-N}$  oxidation rates. The  $\text{NO}_2^-\text{-N}$  accumulation ratio decreased from 87% to 80% and then to 66% when dissolved oxygen increased from 2 to 4 and then to 6mg/L, respectively. These results are consistent with several published results of bio film partial nitrification reactors (Okabe et al. [15]). Okabe et al. demonstrated that both nitrite and nitrate production rates increased with increased air flow rates, and nitrate production was not significant when air flow rate/Ammonium loading rate was kept below 0.15 [(m<sup>3</sup>/day)/(kg-N/m<sup>3</sup>-day)] (Okabe et al. [15]).

**Influence of temperature on  $\text{NH}_4^+\text{-N}$  removal and  $\text{NO}_2^-\text{-N}$  accumulation:** Figure 6 shows the concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$  and the nitrite accumulation ratios over time under different temperature conditions. When temperature was controlled at 8 °C, 45% of the maximum oxidation products accumulated as nitrite for the first 5.5 hours with 50% removal of  $\text{NH}_4^+\text{-N}$ . Nitrite and ammonium were simultaneously oxidized to nitrate after 5.5 hours, and little nitrite accumulation was observed at the end of the batch experiment. Similar trends were observed at 15 °C. 42% of maximum oxidation products stayed in the form of nitrite at 7 hours with 81%  $\text{NH}_4^+\text{-N}$  removal. Increasing the temperature to 24 and 32 °C increased nitrite production.  $\text{NO}_2^-\text{-N}$  composed 75% of the oxidation products, with above 95%  $\text{NH}_4^+\text{-N}$  removal at 24 and 32 °C.

Table 3 summarizes the  $\text{NH}_4^+\text{-N}$  oxidation rates and  $\text{NO}_2^-\text{-N}$  accumulation ratios when  $\text{NH}_4^+\text{-N}$  removal exceeded 95% ( $\text{NH}_4^+\text{-N}$  concentration < 15mg/L) at different temperatures. Increasing temperature from 8 to 32 °C improved the  $\text{NH}_4^+\text{-N}$  oxidation rates by 139%. When temperatures were maintained at 8 and 15 °C, nitrite accumulation was not significant; less than 20% of oxidation products were in the form of nitrite when over 95%

of  $\text{NH}_4^+\text{-N}$  removal was achieved. Due to slower growth rates of Nitrosomonas than Nitrobacter at relatively lower temperatures, it is difficult to achieve 95% ammonium oxidation to nitrite under lower temperatures [13,23]; however, at 8 °C immobilized activated sludge can oxidize  $\text{NH}_4^+\text{-N}$  at the rate of 0.5kgN/m<sup>3</sup>-day, which is higher than a number of reported results [10,24]. It is also possible to combine this process with an aerobic ammonium oxidation at 15 °C by controlling the batch reaction time at 6 hours, which gave a  $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$  ratio of 1:1 in the reactor (Figure 6a) [6,15].

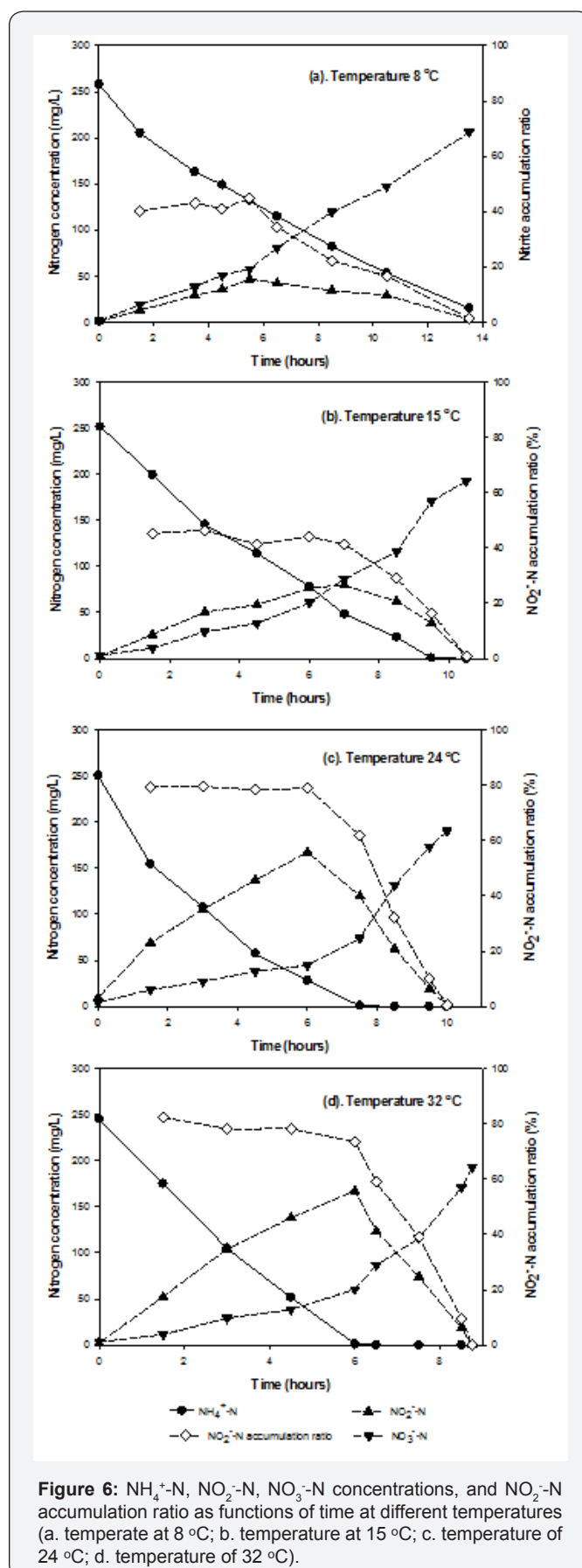
**Table 3:**  $\text{NH}_4^+\text{-N}$  removal, oxidation rates, and  $\text{NO}_2^-\text{-N}$  accumulation ratio at different temperatures.

DO (mg/L)	$\text{NH}_4^+\text{-N}$ Removal (%)	$\text{NH}_4^+\text{-N}$ Oxidization Rates (kg-N/m <sup>3</sup> -day)	$\text{NO}_2^-\text{-N}$ Accumulation ratio (%)
8	95	0.51	1.5
15	99	0.78	17
24	95	1.01	70
32	99	1.23	73

**Table 4:**  $\text{NH}_4^+\text{-N}$  removal, oxidation rates, and  $\text{NO}_2^-\text{-N}$  accumulation ratio at different initial  $\text{NH}_4^+\text{-N}$  concentrations.

Initial $\text{NH}_4^+\text{-N}$ Concentrations (mg/L)	$\text{NH}_4^+\text{-N}$ Removal (%)	$\text{NH}_4^+\text{-N}$ Oxidization Rates (kg-N/m <sup>3</sup> -day)	$\text{NO}_2^-\text{-N}$ Accumulation Ratio (%)
50	95	0.88	45
150	95	1.15	51
250	97	1.12	78
350	98	1.2	82

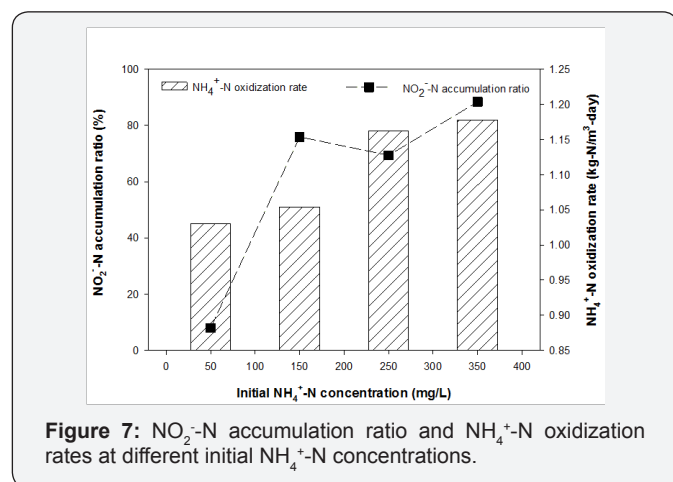
**Influence of initial  $\text{NH}_4^+\text{-N}$  concentration on  $\text{NH}_4^+\text{-N}$  removal and  $\text{NO}_2^-\text{-N}$  accumulation:** Table 4 presents the  $\text{NH}_4^+\text{-N}$  oxidation rates and  $\text{NO}_2^-\text{-N}$  accumulation ratios when  $\text{NH}_4^+\text{-N}$  concentration reached below 15mg/L for different influent  $\text{NH}_4^+\text{-N}$  concentrations, ranging from 50 to 350mg/L. Increasing the initial  $\text{NH}_4^+\text{-N}$  concentration from 50mg/L to 150mg/L increased the  $\text{NH}_4^+\text{-N}$  oxidation rates from 0.88 to 1.15kg-N/m<sup>3</sup>-day, which remained relatively constant as the initial  $\text{NH}_4^+\text{-N}$  concentration increased from 150 to 350mg/L. Nitrite accumulation was more significant at higher initial  $\text{NH}_4^+\text{-N}$  concentrations. When over 95% of ammonium removal was achieved, 78-82% of oxidation products stayed in the form of nitrite at initial  $\text{NH}_4^+\text{-N}$  concentrations of 250 and 350mg/L; while only 45-51% products accumulated as nitrite when initial  $\text{NH}_4^+\text{-N}$  concentrations were 50 and 150mg/L. These results suggested that effective partial nitrification to nitrite can be achieved with initial  $\text{NH}_4^+\text{-N}$  concentration exceeding 150mg/L in the immobilized activated sludge air-lifting reactor; this is consistent with previous findings that nitrite accumulation was more significant at higher initial  $\text{NH}_4^+\text{-N}$  conditions in suspended activated sludge system [10]. Increased  $\text{NH}_4^+\text{-N}$  concentrations resulted in higher free ammonium concentrations, which have been reported to inhibit the activity of nitrite oxidizing bacteria more severely than that of ammonium oxidizing bacteria (Antoniou et al. [25]).



**Figure 6:**  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  concentrations, and  $\text{NO}_2^-\text{-N}$  accumulation ratio as functions of time at different temperatures (a. temperate at 8 °C; b. temperature at 15 °C; c. temperature of 24 °C; d. temperature of 32 °C).

**Practical implication of batch experiments:** Batch experiments investigated could potentially provide relevant information for future utilization of immobilized activated sludge in sequencing batch reactors (SBR). It has been shown in this study that it is possible to keep effluent  $\text{NH}_4^+\text{-N}$  concentrations low (<15mg/L) with most oxidization products present as  $\text{NO}_2^-\text{-N}$  by controlling the batch reaction time in SBR operations to treat high strength ammonium wastewater (initial  $\text{NH}_4^+\text{-N}$  concentration >150mg/L) when the temperature was above 15 °C. Dissolved oxygen concentrations maintained at 4mg/L maximized  $\text{NH}_4^+\text{-N}$  oxidization rates without affecting nitrite accumulation. For example, a batch aeration time of 7 hours with an influent  $\text{NH}_4^+\text{-N}$  concentration of 250mg/L, DO of 4mg/L, and temperature of 24 °C effectively removed ammonium to below 1mg/L with over 65% oxidization products accumulated in the form of  $\text{NO}_2^-\text{-N}$  (Figure 6b).

In addition, it is promising to connect the immobilized activated sludge partial nitrification reactor with anaerobic ammonium oxidization processes. Effluent  $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$  ratios can be maintained at 1:1 by optimizing the batch reaction time for different operating conditions. For example, the batch process can be operated for 3 hours to treat influent  $\text{NH}_4^+\text{-N}$  concentrations of 250mg/L at 24 °C to maintain a  $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$  ratio of 1:1 with only 10% of total nitrogen in the form of nitrate in the effluent (Figure 6c). Even at low temperatures of 15 °C, it is possible to reach the ideal  $\text{NH}_4^+\text{-N}/\text{NO}_2^-\text{-N}$  ratio of 1:1 in the effluent by extending the batch reaction time to 6 hours (Figure 7).



## Conclusion

The potential of using immobilized activated sludge for partial nitrification to nitrite was investigated in an air-lifting reactor operated in both continuous and batch modes. We have successfully achieved partial nitrification to nitrite under non-DO limiting conditions, while maintaining high oxidization rates. Even though only 80% of oxidization products stayed in the form of nitrite when 95% of ammonium removal was achieved, the nitrification rates of close to  $1\text{kg-N}/\text{m}^3\text{-day}$  were achieved under non bulk dissolved oxygen limiting conditions. We also demonstrate that the immobilized activated sludge partial

nitrification process could be potentially linked to anaerobic ammonium oxidization operated either continuously or as a batch reactor. Since the ammonium conversion to nitrite was considered as the rate-limiting step for the Partial nitrification-ANAMOX process, the high rates conversion of ammonium to nitrite using immobilized activated sludge was of special interest to potentially debottleneck the kinetic limitation of partial nitrification process and promote the full-scale application of Partial nitrification-ANAMOX for nitrogen removal.

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