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# Differentiating two partial nitrification mechanisms: Inhibiting nitrite oxidizing bacteria activity or promoting ammonium oxidizing bacteria activity

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ABSTRACT

Partial nitrification is usually more easily achieved for high $NH_4^+$ concentration wastewater. In this study, a two-compartment aerobic nitrification reactor was used to raise the $NH_4^+$ concentration in the first aerobic compartment and to enhance partial nitrification for low $NH_4^+$ concentration wastewater. In addition to the regular sludge wastage to maintain sludge retention time (SRT), extra sludge wastage was used to increase the specific biomass $NH_4^+$ load. The experimental results indicated that partial nitrification was closely associated to the specific biomass $NH_4^+$ load. The modelling results indicated that a low SRT was needed to maintain the low biomass concentration and high specific biomass $NH_4^+$ load. The increased AOB (ammonium oxidizing bacteria) activity, instead of the free ammonia (FA) inhibition of NOB (nitrite oxidizing bacteria) was identified to be the main mechanism for partial nitrification in low $NH_4^+$ concentration wastewater treatment with high specific biomass $NH_4^+$ load.

## 1. Introduction

In the partial nitrification (nitritation), the ammonium ($NH_4^+$) oxidation was controlled at nitrite ($NO_2^-$) step, without being further oxidized into nitrate ($NO_3^-$) [1]. The partial nitrification offers several distinctive advantages including oxygen saving in the nitrification process, reduced carbon source requirement in the denitrification process and less sludge production [2]. Partial nitrification is also a requirement for the application of the recently developed autotrophic nitrogen removal by anaerobic ammonium oxidization (ANAMMOX) [3].

Applying oxygen limitation is one of the most widely used techniques for partial nitrification [4]. The rationale behind this operation is that NOB (nitrite oxidizing bacteria) has lower DO (dissolved oxygen) affinity than AOB (ammonium oxidizing bacteria) [5,6]. Therefore, NOB will be washed out under DO limitation. However, recent studies have indicated the certain species of NOB could have higher DO affinity than AOB [7,8], therefore, applying low DO will cause more inhibition in AOB activity than in NOB activity.

Furthermore, the effect of low DO on partial nitrification is also complicated by other NOB inhibiting factors. Free ammonia (FA) inhibition of NOB was considered to another measure or achieving partial nitrification [9]. In studies that relied on FA inhibition on NOB for partial nitrification operation, DO limitation was also applied for a combined inhibition on NOB by FA and low DO [10,11]. It is usually difficult to differentiate the exact contribution of NOB inhibition by low DO or FA. To elucidate the exact contribution of partial nitrification by FA, the DO was supplied in non-limiting condition in this study.

Partial nitrification is usually assumed to be more easily achieved for high $NH_4^+$ load condition [12]. The FA inhibition on NOB was thought to be the main reason; however, besides reducing NOB activity by FA inhibition, the contribution of high $NH_4^+$ concentration on $NO_2^-$ accumulation can also be explained by the increased AOB activity due to the high Monod term value for $NH_4^+$ [13]. No studies have been carried out to elucidate the quantitative contribution of high $NH_4^+$ concentration on $NO_2^-$ accumulation.

Partial nitrification for low $NH_4^+$ concentration wastewater (less than ca. 100 mg N/L) is an important step to implement the recently developed ANAMMOX technique in the municipal wastewater treatment [14]. The bulk liquid $NH_4^+$ concentration in the conventional CSTR (continuously stirred tank reactor) for

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municipal wastewater treatment is usually too low to take advantage of the high $NH_4^+$concentration for partial nitrification. To overcome the problem, the aerobic reactor was split into two compartments. It was expected that the high $NH_4^+$ in the first compartment could enhance partial nitrification by decreasing the NOB activity (by FA inhibition), or increasing the AOB activity (by increasing the value of Monod term for $NH_4^+$). Mathematical model was included to elucidate the exact mechanisms of partial nitrification in the two-compartment reactor.

## 2. Materials and methods

### 2.1. Experimental bioreactor

The schematic layout of the experimental bioreactor was shown in Fig. 1. It includes one anoxic compartment and two aerobic compartments (#1, #2). Each compartment has an effective volume of 2 L. The effective volume of the settlement tank is 1.5 L. The influent flow rate (Q) was maintained at 0.5 L/h, resulting hydraulic retention time (HRT) of 12 h. The recirculation flow ratio ($R_1$ and $R_2$) from #1 and #2 aerobic compartment to the anoxic compartment was varied according to experimental plan shown in Table 1. The sludge retention time (SRT) was adjusted by wasting 400 ml of mixed liquor from the #2 aerobic compartment. As the solids concentration in the #2 aerobic compartment was roughly twice as high as the solids concentration in the other two compartments, the effective SRT was calculated to be 10 d.

Synthetic wastewater was prepared according the recipe from Wu & He [15]. The chemical oxygen demand (COD) and N-$NH_4^+$ ratio was maintained at above 6 to allow complete denitrification in the anoxic tank. The seeding sludge was taken from the aeration tank of the local wastewater treatment plant. The bioreactor was placed in an air-conditioned room with temperature controlled at 25.0 ± 0.8 °C. The DO concentration in both of the aerobic compartments was provided at above 6 mg $O_2$/L to create an oxygen non-limiting condition.

### 2.2. Experimental plans

Before taking samples for measurement, the bioreactor was operated for 20 days of assimilation period. Then the bioreactor was operated according to the experiment plan shown in Table 1. During the experiment period, the $NH_4^+$, $NO_2^-$ and $NO_3^-$ concentration in the influent, anoxic compartment and two aerobic compartments were measured regularly. The pH and $NH_4^+$ concentration in the influent were varied to examine the effect of FA on NOB inhibition.

The changes in pH, influent $NH_4^+$ concentration and recirculation ratio were also necessary to provide enough data excitation for the mathematical model calibration [16].

At the end of period A, in addition to the regular sludge wastage to maintain the SRT, an extra portion of biomass solids was wasted to further decrease the biomass concentration and increase the specific biomass $NH_4^+$ loading rate. This allowed the high $NH_4^+$ loading conditions to be investigated for low $NH_4^+$ concentration wastewater.

**Table 1**
Experimental plan and operational conditions.

| Period | A | B | C |
|---|---|---|---|
| Date (d) | 0–23 | 24–42 | 43–58 |
| Recirculation ratio $R_1$ | 1 | 1 | 2.5 |
| Recirculation ratio $R_2$ | 1 | 1 | 1 |
| Influent TAN (mg N/L) | 50.0 ± 4.6 | 105.0 ± 6.8 | 105.0 ± 6.8 |
| pH in the First compartment | 7.5 ± 0.2 | 8.7 ± 0.2 | 8.85 ± 0.15 |

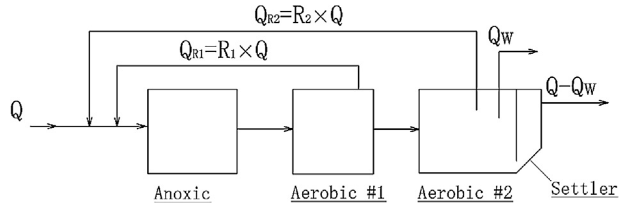

**Fig 1.** Schematic layout of the experimental bioreactor.

### 2.3. Batch experiment for the measurement of the maximum specific growth rate for AOB and NOB

The oxygen uptake rate (*OUR*) was measured to calculate the maximum specific growth rate for AOB and NOB ($\mu_{AOB}$ and $\mu_{NOB}$, $d^{-1}$). The OUR was measured using the respirometer shown by Wu et al. [17]. The OUR measured at time *t* under ammonium and DO non-limiting condition ($OUR_{NH}(t)$) can be expressed by the following equation [18]:

$$OUR_{NH}(t) = e^{t(\mu_{AOB}-b_{AOB})}OUR_{NH}^0 \tag{1}$$

$OUR_{NH}(t)$ is expressed by the exponential function of $OUR_{NH}^0$ (the initial OUR after ammonium addition). By curve fitting the measured OUR to the simulated OUR and assuming the decay rate $b_{AOB}$ to be 5% of $\mu_{AOB}$, $\mu_{AOB}$ can be estimated. Similarly, the $\mu_{NOB}$ can be estimated by the following equation:

$$OUR_{NO2}(t) = e^{t(\mu_{NOB}-b_{NOB})}OUR_{NO2}^0 \tag{2}$$

where, $OUR_{NO2}(t)$ is the OUR at time t after nitrite addition; $OUR_{NO2}^0$ is the initial OUR after nitrite addition.

### 2.4. Sample analysis method

The pH and DO were measured by HACH pH electrode and LDO oxygen sensor respectively. VSS, COD, $NH_4^+$, $NO_2^-$ and $NO_3^-$ concentration were measured according to the standard method [19].

### 2.5. Mathematical modelling

#### 2.5.1. Model kinetics and parameters

The ASM (activated sludge model) type of model was included to simulate the reactor shown in Fig. 1 [16]. The model had 4 particulate components including AOB ($X_{AOB}$), NOB($X_{NOB}$), heterotrophic bacteria ($X_H$) and inert material ($X_I$); and 6 soluble components including DO ($S_O$), $NH_4^+$ ($S_{NH}$), $NO_2^-$ ($S_{NO2}$) and $NO_3^-$ ($S_{NO3}$) and soluble COD (Ss). The complete model stoichiometric matrix and kinetic rate expressions were shown in the Supporting information Table S1. The maximum specific growth rate for AOB and NOB were determined by respirometer shown in Section 2.3. The FA inhibition constant for NOB ($K_{IA}$) was determined from model calibration. Other parameter values was taken from the literature and shown in Table S2.

The growth rates AOB or NOB were expressed by the following equation:

$$\frac{dX_{AOB}}{dt} = \mu_{AOB}\frac{S_{NH}}{K_{S,NH}+S_{NH}}\frac{S_O}{K_{O,AOB}+S_O}X_{AOB} \tag{3}$$

$$\frac{dX_{NOB}}{dt} = \mu_{NOB}\frac{S_{NO2}}{K_{S,NO2}+S_{NO2}}\frac{S_O}{K_{O,NOB}+S_O}\frac{K_{IA}}{K_{IA}+FA}X_{NOB} \tag{4}$$