

# Responses of Nitrifiers' characteristics to Filling Ratio in IFAS Systems

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**Abstract**—The concentrations of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were measured in batch experiments to study nitrification kinetics in IFAS system with different FR at 0%-50%. The results showed that both of  $\text{NH}_4^+\text{-N}$  oxidation rate ( $R_{\text{NH}_4^+-\text{N}}$ ) and  $\text{NO}_3^-\text{-N}$  production rate ( $R_{\text{NO}_3^--\text{N}}$ ) increased with FR, which respectively increased from 6.3 to 16.9  $\text{mgNH}_4^+\text{-N}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$  and 0.8 to 13.6  $\text{mgNO}_3^-\text{-N}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ . While the highest accumulation concentration of  $\text{NO}_2^-\text{-N}$  fell from 51.8 to 9.0  $\text{mg}\cdot\text{L}^{-1}$ . That was, higher FR instead went against to partial nitrification. Quantitative polymerase chain reaction (q-PCR) analysis revealed that increased FR caused an acceleration of the nitrifiers, which correlated linearly with the nitrification rate. Furthermore, the amount of nitrifiers per unit area in biofilm reached maximum at  $\text{FR} = 30\%$ , and it was also the demarcation point when nitrifiers in biofilm outnumbered that in suspended sludge. Cumulative effect of nitrite occurred in the end of reaction when FR was less than 30%.

**Keywords**—IFAS; filling ratio; nitrification performance; AOB; NOB

## I. INTRODUCTION

In China, the conventional activated sludge (CAS) process is often used to remove COD and  $\text{NH}_4^+\text{-N}$  in wastewater. However, this process presents some shortcomings when exposed to high hydraulic and organic loadings [1]. As the increasingly stringent emission standards of nitrogen, it's of great importance to upgrade the traditional nitrification tank. Integrated fixed-film activated sludge (IFAS) process is a good choice. By adding suspended carriers in the activated sludge, IFAS process cultivate two different biomasses, making sludge concentration be 5-10 times as much as that in CAS [2]. In addition, carriers provide adhesive environment for nitrifiers whose growth rates are relatively low, while the presence of suspended sludge creates conditions for heterotrophic bacteria. As a consequence, IFAS process can achieve high removal rate of both COD and  $\text{NH}_4^+\text{-N}$  even under low temperature [1, 3].

Filling ratio (FR) is one of the most important parameters in IFAS process. Some studies have been carried out on systems adding suspended carriers at different FR to investigate process efficiencies in recent years [4-6]. Whereas the available information remains limited pertaining to the characterization of nitrifying bacteria both in suspended sludge and biofilm as a response to the FR, which make it difficult to put IFAS process into practical applications.

In this study, six IFAS reactors were therefore constructed at different FR from 0% to 50%. Batch

experiments and quantitative polymerase chain reaction (q-PCR) were conducted when reactors ran stably to study nitrification kinetics and population structure of the nitrifiers. The aims of the research were to investigate: i. the effect of FR on nitrification performance in IFAS process; ii. functional relationships between nitrification rates and nitrifying bacteria; iii. variant roles of nitrifiers in suspended sludge and biofilm on system efficiency; iv. why nitrifiers did different responses with FR increasing.

## II. MATERIALS AND METHODS

### A. Experimental systems

Six laboratory-scale sequencing batch IFAS reactors were constructed with a working volume of 15.0L ( $\Phi 190\text{mm} \times 540\text{mm}$ ). Each reactor was equipped with peristaltic pumps (BT100-2J, Longer Pump Co., China), air pumps (IPX4 ACO-9610, Hailea Group Co., China) and a aeration disc (V-30, Hailea Group Co., China), which was fixed at the bottom of the reactor to provide sufficient dissolved oxygen (DO) and fluidize the carriers. Cylinder suspended plastic bio-carriers (SPR-1) were used in this experiment as attached materials for microbes that had a density of  $0.96\text{ g}\cdot\text{cm}^{-3}$  and the effective specific surface area of  $500\text{ m}^2\cdot\text{m}^{-3}$ .

### B. Batch experiments

Batch experiments were performed when systems ran stably. The concentrations of  $\text{NH}_4^+\text{-N}$ , TP and trace elements contained in raw water was the same as that in sequencing batch tests, while COD wasn't added in order to reduce the influence of heterotrophs on the nitrifiers. Liquid samples were taken at regular intervals and immediately filtered through Millipore filter units ( $0.45\mu\text{m}$  pore size) to analyze  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$ .

### C. Quantitative polymerase chain reaction (q-PCR)

DNA was extracted from biofilms sampled from six reactors and amplified according to primer features of nitrifiers listed in table. I[7]. Reaction mixtures ( $50\mu\text{L}$  final volume) contained  $1\times$ Gold PCR buffer ( $150\text{mM}$  Tris-HCl,  $\text{pH} = 8.0$ ,  $500\text{mM}$  KCl),  $1.5\text{mM}$   $\text{MgCl}_2$ , 5% dimethylsulfoxide,  $200\mu\text{M}$  of dNTPs,  $20\text{pM}$  of each primer, 1U of AmpliTaq Gold polymerase and  $100\mu\text{g}$  bovine serum albumine [6]. The temperature profile for PCR reaction was as follows [8]: initial denaturation at  $95^\circ\text{C}$  for 3min; 40 cycles of denaturation at  $95^\circ\text{C}$  for 15s, annealing at  $56^\circ\text{C}$  for 30s, extension at  $72^\circ\text{C}$  for 30s, the second extension at  $80^\circ\text{C}$  for 30s; and final reparation

extension at 72°C for 7min. The final PCR products were cleaned and concentrated using Amicon Ultra-0.5ml Centrifugal Filters.

TABLE I. THE PRIMER SEQUENCES OF AOB, AOA AND NOB

Species	Primer Sequence
AOB	amoA-1F(5'-GGGGGTTTCTACTGGTGGT-3') amoA-2R(5'-CCCCTCKGSAAGCCTTCTTC-3')
AOA	Arch-amoAF(5'-STAATGGTCTGGCTTAGACG-3') Arch-amoAR(5'-GCGGCCATCCATCTGTATGT-3')
NOB	Nitrospira EUB338f(5'-ACTCTACGGGAGGCAGC-3') Ntspa0685M(5'-CGGGAATTCCGCGCTC-3')
	Nitrobacter EUB338f(5'-ACTCTACGGGAGGCAGC-3') NIT3(5'-CCTGTGCTCCATGCTCCG-3')

### III. RESULTS AND DISCUSSION

#### A. Nitrification performance in IFAS systems

The batch tests were operated to assess the nitrification performance in IFAS systems (fig. 1). Calculated sum values of  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N concentrations were almost equal to the oxidized  $\text{NH}_4^+$ -N concentration, demonstrating that almost no denitrification occurred in all reactors. It was mainly due to the high DO concentration ( $\text{DO} \geq 2.0 \text{ mg}\cdot\text{L}^{-1}$ ) controlled throughout the experiment.

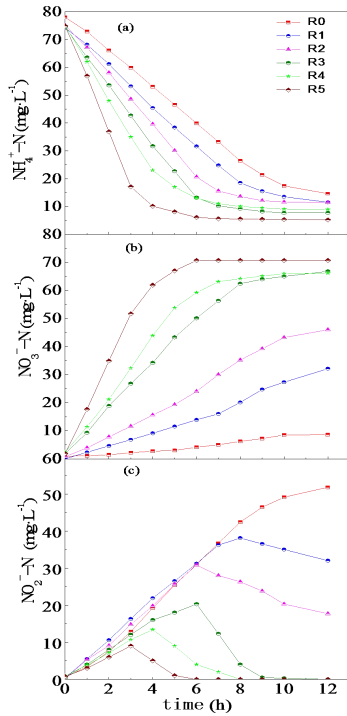


Figure 1. Variations in  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N concentration during one typical batch test in six IFAS systems: (a) reduction of  $\text{NH}_4^+$ -N; (b) production of  $\text{NO}_3^-$ -N; (c) accumulation of  $\text{NO}_2^-$ -N.

It was not hard to find that both of  $R_{\text{NH}_4^+-\text{N}}$  and  $R_{\text{NO}_3^--\text{N}}$  markedly increased with FR, which respectively increased from 6.3 to 16.9  $\text{mg}\text{NH}_4^+-\text{N}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$  and 0.8 to 13.6  $\text{mg}\text{NO}_3^--\text{N}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ . Such variations indicated that higher FR could improve the nitrification performance,

especially do more benefit to  $R_{\text{NO}_3^--\text{N}}$ . The highest accumulation concentration of  $\text{NO}_2^-$ -N exhibited negative correlation with FR as indicated by correlation coefficient  $R^2$  of 0.975 (fig. 3a), which further confirmed that increased FR was adverse to nitrite accumulation. Also, IFAS system in this experiment could accomplish exhaustive nitrification once  $\text{FR} \geq 30\%$ .

#### B. Functional relationship between nitrification rate and nitrifying bacteria

The concentrations of AOA, AOB and NOB (Nitrobacter and Nitrospira) were analyzed using q-PCR to reveal nitrifiers' responses to FR. As shown in the fig. 2, AOB was the main undertaker in ammonia oxidation process, yet AOA concentration was practically negligible. The linear correlation analysis (fig. 3b, 3c and 3d) indicated that AOB and NOB were well correlated with  $R_{\text{NH}_4^+-\text{N}}$  and  $R_{\text{NO}_3^--\text{N}}$  ( $R^2 = 0.889$  and  $0.959$ ), respectively, and AOB/NOB exhibited good correlations with the highest accumulation concentration of  $\text{NO}_2^-$ -N ( $R^2 = 0.902$ ). Therefore, the varying numbers of AOB, NOB and their ratio as a result of FR were valid for estimating different nitrification performance.

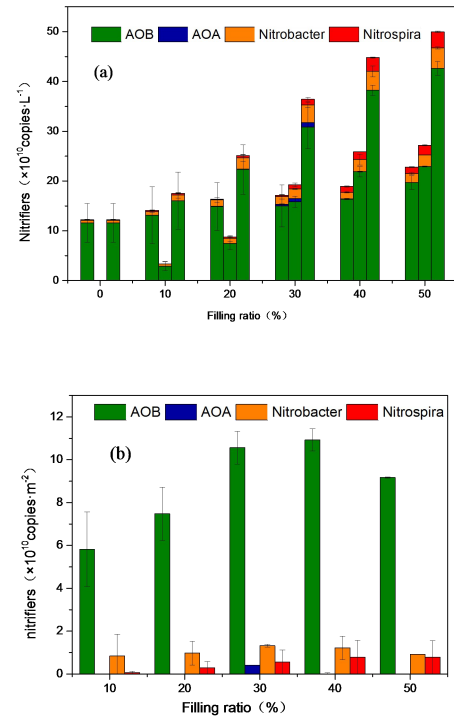


Figure 2. (a) Nitrifiers' concentration in suspended sludge, biofilm and IFAS systems at  $\text{FR} = 0\%-50\%$ ; (b) nitrifiers' concentration per unit area in biofilm at  $\text{FR} = 0\%-50\%$ .

#### C. Responses of nitrifiers' characteristics to FR

##### 1) Activities of AOB and AOA

The linear correlations of nitrifiers' concentration and FR were listed in fig. 4. Compared with AOB, AOA occupied little amount in six reactors. The reason was that DO was always controlled above  $2 \text{ mg}\cdot\text{L}^{-1}$  to fluidize carriers and inhibit denitrification, which seemed adverse to growth of AOA [9]. However, the amount of AOA

raised significantly at FR = 30%, accounting for 3.69% of ammonia oxidizing microbes. It was hypothesized that DO and substrate concentration gradient caused by proper thickness of biofilm played a part [10], in other words, it was favorable to cultivate AOA at FR = 30%.

The amount of AOB was always over 80% of nitrifying bacteria, and was positively linearly correlated with FR both in suspended sludge and biofilm ( $R^2 = 0.932$ ,  $0.955$ , fig. 4a). Notably, unlike upward trend at other FR, the amount of AOB per unit area in biofilm declined contrary at FR = 50%. This was probably related to carriers' fluidization. Gjaltema et al [11] demonstrated that although the total surface area of carriers increased with FR, shear stresses on biofilm became greater and detachment enhanced when FR was more than 50%. It conformed to the phenomenon in this study that nitrifiers per unit area in biofilm declined at FR = 50%. Moreover,

the amount of AOB in suspended sludge was very close to that in biofilm at FR = 30%, above which AOB in biofilm would outnumbered. That was, the sludge dominating  $R_{NH_4^+-N}$  changed from suspended state to biofilm with FR increasing. The concentrations of AOA, AOB and NOB (Nitrobacter and Nitrospira) were analyzed using q-PCR to reveal nitrifiers' responses to FR. As shown in the fig. 2, AOB was the main undertaker in ammonia oxidation process, yet AOA concentration was practically negligible. The linear correlation analysis (fig. 3b, 3c and 3d) indicated that AOB and NOB were well correlated with  $R_{NH_4^+-N}$  and  $R_{NO_3^--N}$  ( $R^2 = 0.889$  and  $0.959$ ), respectively, and AOB/NOB exhibited good correlations with the highest accumulation concentration of  $NO_2^- - N$  ( $R^2 = 0.902$ ). Therefore, the varying numbers of AOB, NOB and their ratio as a result of FR were valid for estimating different nitrification performance.

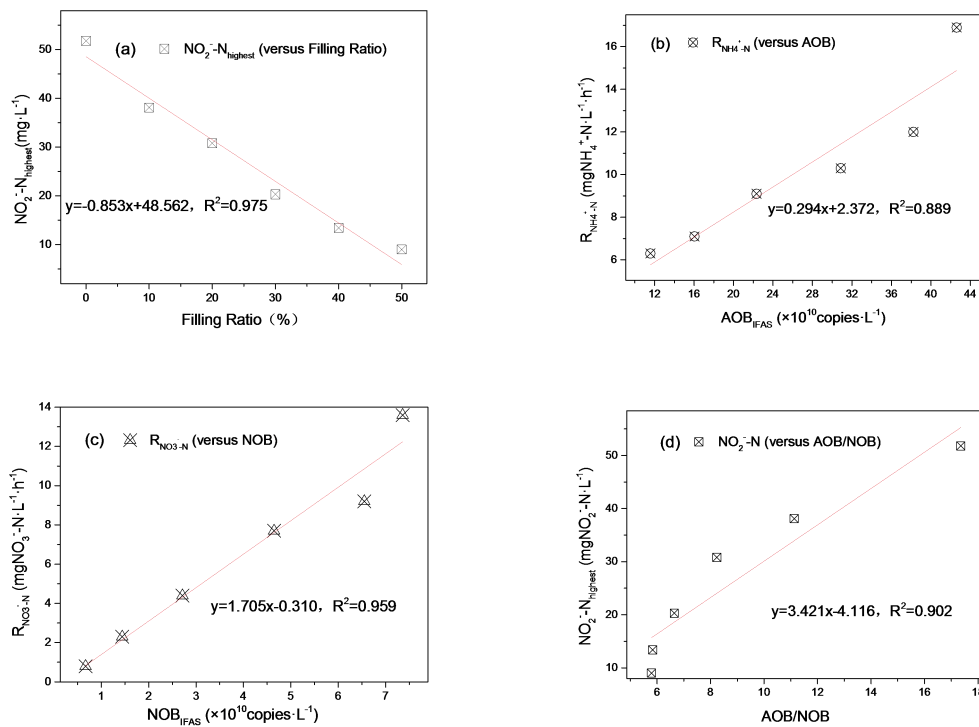


Figure 3. (a) Relationship between  $NO_2^- - N_{highest}$  and FR; (b) relationship between  $R_{NH_4^+-N}$  and  $AOB_{IFAS}$ ; (c) relationship between  $R_{NO_3^--N}$  and  $NOB_{IFAS}$ ; (d) relationship between  $NO_2^- - N_{highest}$  and AOB/NOB.

## 2) Activities of NOB

The quantity of Nitrospira in biofilm was well positively correlated with FR ( $R^2 = 0.979$ , fig. 4d), whereas Nitrobacter was trending downward when FR exceeded 40%, illustrating that higher FR was more conducive to culture Nitrospira. Schramm et al [12] determined the population structure of nitrifying bacteria by combined use of microsensors and fluorescence in situ hybridization (FISH), and probed Nitrospira in the microaerobic zone of biofilm, i.e. between 150 and 300  $\mu m$  away from the membrane, even out-competing Nitrobacter. Authors then tested concentrations of DO,  $NO_3^- - N$ ,  $NO_2^- - N$  in the biofilm, and implied that it was the affinity for DO, not  $NO_2^- - N$  that played a selective role. Because the

concentrations of  $NO_2^- - N$  and DO in this range were respectively about 400-500  $\mu M$  and 0-15  $\mu M$ , according to the  $K_m$  ( $NO_2^- - N$ , 60-600  $\mu M$ ) and  $K_m$  (DO, 62-256  $\mu M$ ) of Nitrobacter, a competitive advantage for Nitrospira on the basis of oxygen affinity would prevail over. In this study, Nevertheless, DO always maintained about 2-4  $mg \cdot L^{-1}$  in bulk solution, while the highest accumulation concentration of  $NO_2^- - N$  declined with FR from 38.1 to 9.0  $mg \cdot L^{-1}$ , so it was more likely the concentration gradient of  $NO_2^- - N$  in biofilm at various FR that made for such changes. Besides, the change trends of Nitrobacter and Nitrospira in suspended sludge was in accordance with that in biofilm. Due to the relatively low growth rate of NOB, we speculated that this part increment was mainly caused by the shedding of biofilm.

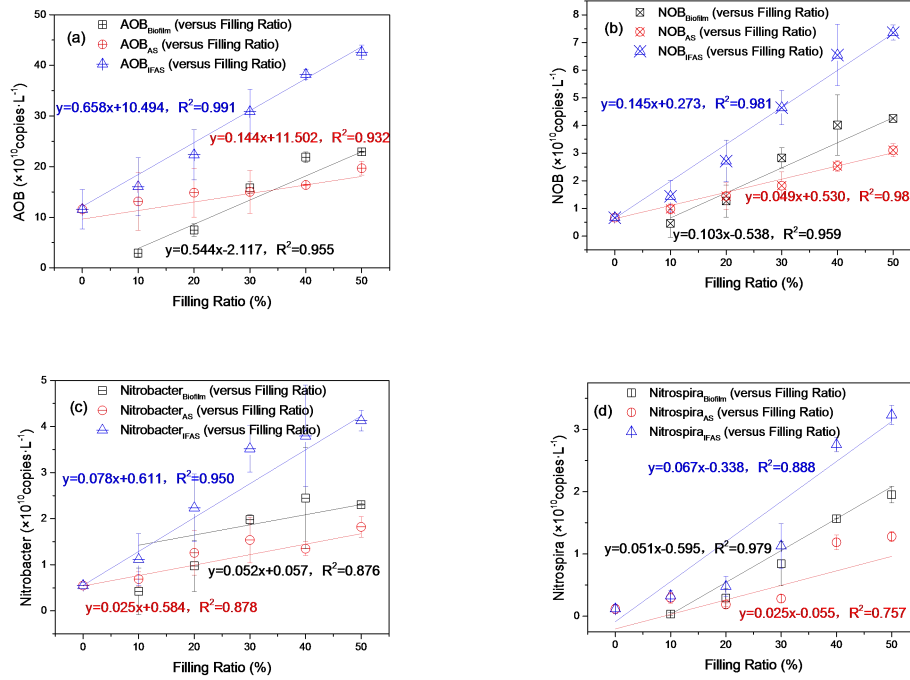


Figure 4. Correlation analysis between nitrifiers' concentration and FR in suspended sludge, biofilm and IFAS systems: (a) relationships between AOB and FR; (b) relationships between NOB and FR; (c) relationships between Nitrobacter and FR; (d) relationships between Nitrospira and FR.

#### IV. CONCLUSIONS

The results demonstrate that: i. IFAS process is an efficient biological treatment to remove  $NH_4^+-N$ , and nitrification performance improved with FR increasing from 10% to 50%. Higher FR did more increment to  $R_{NO_2^- - N}$  than  $R_{NH_4^+ - N}$ , resulting in inferior performance at partial nitrification; ii. linear analysis showed that increasement of AOB and NOB is the fundamental reasons to improve  $R_{NH_4^+ - N}$  and  $R_{NO_3^- - N}$ , while accumulation concentration of  $NO_2^- - N$  mainly depended on AOB/NOB; iii. the amount of nitrifiers per unit area in biofilm reached maximum at FR = 30%, and it was also the demarcation point when nitrifiers in biofilm outnumbered that in suspended sludge; iv. the change of total effective surface area and substrate concentration gradient in biofilm caused by FR was the main reason to influence nitrifiers' concentration.

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