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Effect of substrates and free ammonia on kinetic characteristics of nitritation and nitratation by entrapped nitrifiers

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Abstract

In the present study, nitrifying bacteria entrapped in waterborne polyurethane gel was used to investigate the kinetic characteristics of nitritation and nitratation in relation to achieve shortcut nitrification. The nitrite accumulation rate was over 80% during the acclimation period. The following kinetic parameters were experimentally obtained: maximum nitrification rate (v_{max}), half-saturation coefficient (K_S and K_O), and inhibition coefficient (K_{IH}). The bacterial populations were also determined by fluorescence *in situ* hybridization. 73.5% proportion of ammonia oxidizing bacteria (AOB) resulted in a significantly higher ammonia oxidizing rate than nitrite oxidizing rate, which is in agreement with higher V_{max} of nitritation (608.5 mgNl^{-1}-pellet h^{-1}) over nitratation (66.3 mgN l^{-1}-pellet h^{-1}).

Key words

Entrapped nitrifying bacteria, Kinetics, Nitratation, Nitritation, Shortcut nitrifiication

Introduction

Shortcut nitrification of ammonia rich wastewater is now a common practice due to lower required reactor volume, aeration and operational cost (Peng and Zhu, 2006). Shortcut nitrification entails the simultaneous maintenance of ammonia oxidizing bacteria (AOB) activity and inhibition of nitrite oxidizing bacteria (NOB) activity. Shortcut nitrification can be maintained in different ways (Yan and Hu, 2009; van Dongen *et al*., 2001): AOB enrichment, controlling the temperature for AOB and NOB growth, adjustment of dissolved oxygen (DO) during AOB and NOB growth and inhibition of AOB and NOB by free ammonia (FA).

Immobilization techniques can also be used to meet the demands of shortcut nitrification. One such technique is cell entrapment within polymeric gels, which is extensively used for wastewater nitrification (Rostron *et al*., 2001; Li *et al*., 2006). The advantages of entrapped nitrifying bacteria includes protection of cell wash-out, high cell concentration, high nitrification rates, ease of cell separation, and protection from extreme conditions (Li *et al*., 2009; Morita *et al*., 2007). Nitritation and nitratation can be the rate-limiting steps under different environmental conditions. Although some studies (Hill *et al*., 2008) show the possibility of shortcut nitrification by entrapped cells, they do not discuss the roles of nitritation and nitratation by entrapped nitrifying bacteria, nor do they mention the *in situ* bacterial communities within the entrapment matrix, which can achieve effective shortcut nitrification.

In the present study, nitrifying bacteria entrapped in waterborne polyurethane (WPU) gel was used to investigate the kinetic characteristics of nitritation and nitratation in relation to shortcut nitrification. The effects of substrate and DO concentration were investigated, and the kinetics parameters were determined to explain the characteristics of shortcut nitrification by entrapped nitrfiers. The fluorescence in situ hybridization (FISH) technique was also used to analyze the bacterial community and population within the entrapped matrix.

Materials and Methods

Preparation of immobilized nitrifying bacteria : In this study, nitrifying bacteria were entrapped in WPU gel carriers. Seed sludge was obtained from the secondary sedimentation tank of Minhang Municipal Wastewater Treatment Plant and acclimated

for a month by synthetic wastewater. The synthetic wastewater was composed of 153mg NH_4Cl, 468mg $NaHCO_3$, 46.4mg $Na_2HPO_4 \cdot 12H_2O$, 20.5mg NaCl, 9.6mg KCl, 9.6mg $CaCl_2 \cdot 2H_2O$, 33.6mg $MgSO_4 \cdot 7H_2O$, in 1l of tap water. Then, concentrated nitrifying bacteria (20 g l^{-1}) were mixed with WPU prepolymer emulsion, promoter and initiator at room temperature to generate a gel lump. This lump was cut into 3 mm cubes and thoroughly washed with distilled water (Dong *et al*., 2012).

Experimental equipment and analytical methods : A plastic vessel with a working volume of 1 l was used (Fig. 1) filled with 10% (v/v) entrapped nitrifying bacteria gel pellets. A mesh separator at the reactor outlet was used to retain the immobilized pellets. Aeration at the base of the reactor kept the contents well mixed and aerated.

DO concentration and pH were monitored by a DO meter (Orion 830A, Orion, Germany) and pH meter (pH81, YOKOGAWA, Japan). Ammonia nitrogen (NH_4^+–N) and nitrite nitrogen (NO_2^-–N) were determined according to standard methods of APHA, 2005. Nitrification activity can be expressed as

$$v = \frac{\partial S}{\partial t} \quad (1)$$

where v is the nitrification rate (mgN L^{-1}-pellet h^{-1}), S is the substrate concentration of ammonia nitrogen or nitrite nitrogen (mg l^{-1}), and t is the reaction time (h).

Double substrates kinetics : The kinetics of nitrification process mainly depends on the substrate and DO concentration and is normally described by a double-substrate Monod expression (Rongsayamanont *et al*., 2010):

$$v = v_{max}\left(\frac{S}{K_s + S}\right)\left(\frac{DO}{K_o + DO}\right) \quad (2)$$

where v is the nitrification rate for nitritation or nitratation (mgN l^{-1}-pellet h^{-1}), v_{max} is the respective maximum nitrification rate (mgN l^{-1}-pellet h^{-1}), K_s is the half-saturation coefficient for substrate (mgN L^{-1}), and K_o is half-saturation coefficient for oxygen (mgO_2 l^{-1}). For each experiment, the data were fitted to Eq. (2) using a nonlinear regression module in SPSS 14.0 to determine the maximum nitrification and half-saturation coefficient for nitritation and nitratation.

Community analysis of AOB and NOB : Entrapped nitrifying bacteria samples were collected during their maintenance in the acclimation reactor one or two days before they were used in the following shortcut nitrification experiments. The FISH assay was performed according to the methods described by Amann *et al*. (1995). The oligonucleotide probes used in this study are shown in Table 2. Each sample was photographed 20–30 times by a fluorescence microscope (OLYMPUS BX52, Japan) for quantitative analysis (National Institutes of Health, USA).

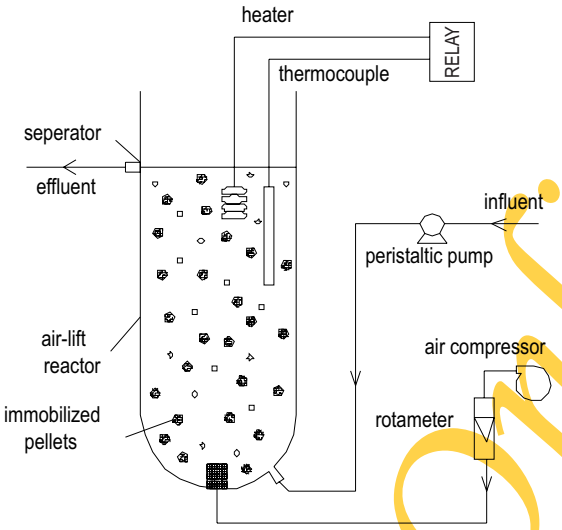

Fig.1 : Schematic diagram of the immobilized cell reactor system

Results and Discussion

The gelled pellets were fed with ammonia enriched synthetic wastewater (Table 1), and after 70 d, more than 95% total ammonia nitrogen was removed. During the acclimation process, the ammonia loading rate gradually improved with increased ammonia concentration and decreased hydraulic retention time based on the nitrification activity of the entrapped

Table 1 : Summary of kinetic parameters

Process	Symbol	Definition	Unit	Parameter value (R^2)
Nitritation	$v_{max,S,AOB}$	Maximum nitrification rate of AOB for ammonia	mgN l^{-1}-pellet h^{-1}	608.5±11.1 (0.977)
	$K_{S,AOB}$	Half-saturation coefficient of AOB for ammonia	mgN l^{-1}	0.94±0.26 (0.977)
	$v_{max,DO,AOB}$	Maximum nitrification rate of AOB for oxygen	mgN l^{-1}-pellet h^{-1}	540.1±10.1 (0.969)
	$K_{O,AOB}$	Half-saturation coefficient of AOB for oxygen	mgO_2 l^{-1}	2.07±0.10 (0.969)
Nitratation	$v_{max,S,NOB}$	Maximum nitrification rate of NOB for nitrite	mgN l^{-1}-pellet h^{-1}	66.3±0.77 (0.997)
	$K_{S,NOB}$	Half-saturation coefficient of NOB for nitrite	mgN l^{-1}	7.27±0.47 (0.997)
	$v_{max,DO,NOB}$	Maximum nitrification rate of NOB for oxygen	mgN l^{-1}-pellet h^{-1}	62.6±0.77 (0.960)
	$K_{O,NOB}$	Half-saturation coefficient of NOB for oxygen	mgO_2 l^{-1}	1.46±0.06 (0.960)

pellets.

After acclimation, the nitrite accumulation rate was higher than 80% and increased with increased ammonia loading rate. By the end of acclimation, the nitrite accumulation rate (NO_2^-/NO_x^- ×100%) was >95%, which indicated that nitrite was discharged from the reactor before being oxidized to nitrate. Thus, the acclimated entrapped pellets maintained nitritation process with high nitrite accumulation rate.

Two tests on nitritation and nitratation were performed to investigate the effect of substrate concentration. For nitritation process, the initial TAN nitrogen concentration of synthetic water was 0, 3, 5, 10, 20, 40, 80, 150, 300 mg l^{-1}, respectively. For nitratation process, the initial nitrite nitrogen concentration was 0, 5, 10, 20, 40, 80, 120, 150 mg l^{-1}, respectively.

At 0–5 mg l^{-1} TAN for nitritation and 0–40 mg l^{-1} NO_2^-–N for nitratation, the AOB and NOB growth and nitrification rates depended on the substrate concentration (Fig. 2). When the substrate concentrations were higher than 5 mg l^{-1} TAN for nitritation and 40 mg l^{-1} NO_2^-–N for nitratation, the nitrification rates reached maximum and were independent of the substrate concentration. The constant v_{max} for nitritation (608.5 mgN l^{-1}-pellet h^{-1}) was higher (Table 1) than that for the corresponding nitratation (66.3 mgN l^{-1}-pellet h^{-1}), which was in agreement with the observation that amount of AOB in entrapped

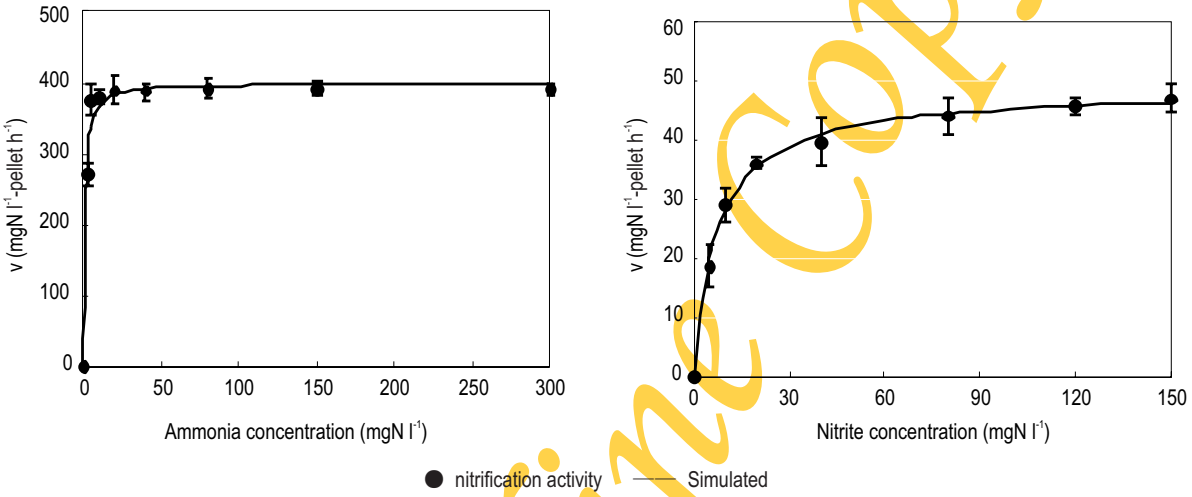

Fig. 2 : Nitrification rate of entrapped nitrifiers as a function of total ammonia concentration and nitrite concentration (a) nitritation; (b) nitratation

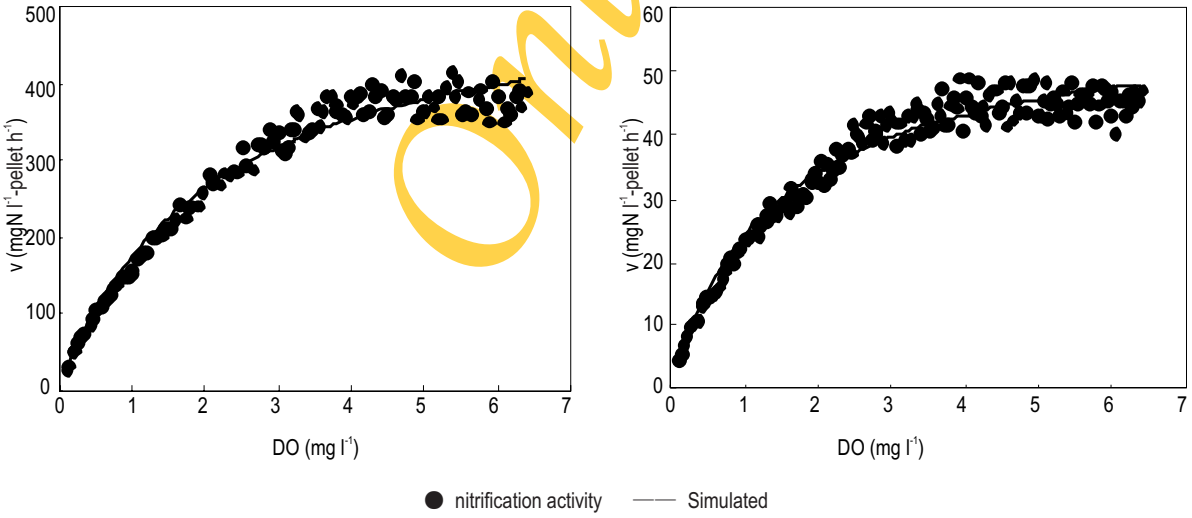

Fig. 3 : Nitrification rate of entrapped nitrifiers as a function of DO concentration (a) nitritation; (b) nitratation

pellets were higher than those of NOB. The fact that the value of half saturation coefficients for ammonia ($K_{S,AOB}$, 0.94 mg l^{-1}) was much lower than that for nitrite ($K_{S,NOB}$, 7.27 mg l^{-1}) suggested that AOB had better substrate affinity than NOB to maintain shortcut nitrification.

However, due to internal substrate transfer limitation within the entrapment gel, substrate transfer from bulk solution into gel matrix would slow down the nitrification activity per unit biomass and reduce substrates affinity of all bacteria. Thus, the v_{max} values of both AOB and NOB were a bit lower than those recently observed for the suspended nitrifiers (Carrera *et al*., 2004), whereas the half-saturation coefficients were higher (Laanbroek *et al*., 1994).

An initial ammonia nitrogen concentration of 150 mg l^{-1} and an initial nitrite nitrogen concentration of 100 mg l^{-1} were chosen for these nitritation and nitratation tests, respectively.

When DO < 4 mg l^{-1}, the nitrification rate for nitritation and nitratation was limited by DO concentration (Fig.3). However, when DO > 4 mg l^{-1}, the nitrification rate was constant and reached its maximum. Similar to the effects of substrate concentration, v_{max} for nitritation (540.1 mgN l^{-1}-pellet h^{-1}) was higher than that for the corresponding nitratation (62.6 mg N l^{-1}-pellet h^{-1}). Whereas, the oxygen half saturation coefficient for nitritation (2.07 mgO_2 l^{-1}) was a bit higher than that for nitratation (1.46 mgO_2 l^{-1}), which indicated that under DO limiting conditions, the v_{max} for nitritation was reduced more than that for nitratation because of lower oxygen affinity of AOB than NOB. The results were different from those reported for suspended nitrifiers systems (Rongsayamanont *et al*., 2010; Laanbroek *et al*., 1994). It seemed that under low DO which intensified oxygen transfer limitation, the activity of entrapped AOB would be more suppressed than the activity of entrapped NOB. When DO concentration was low, ammonia oxidization was limited. However, when DO concentration was high, the activity of AOB decreased and the activity of NOB increased, so both nitrite accumulation and ammonia oxidization weakened.

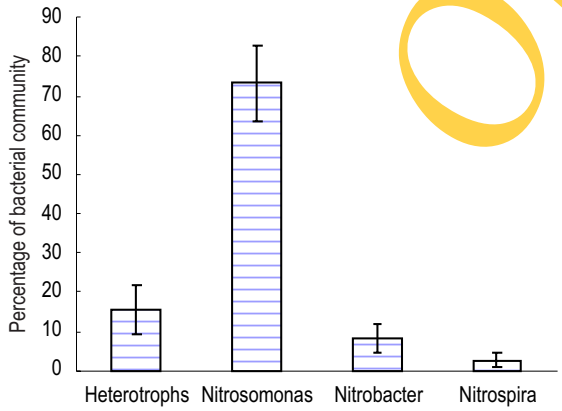

Fig. 4 : Bacterial community structures of immobilized pellets by FISH

The results showed that a win-win situation of high nitrification rate and shortcut nitrification would be achieved by keeping DO within 2~4 mg l^{-1}. Although oxygen is often the limiting substrate in nitrifying systems, it might not be an efficient method by using DO limitation as a sole control strategy in this study.

The FISH method was used to determine the species and quantity of AOB and NOB in the entrapped pellets. The AOB and NOB fractions were determined from the relative areas of the Nse1472 probe as well as the combination of Ntspa 662 and NIT3 probes to the EUB338 probe. The fraction of heterotrophs was estimated from the remaining relative area other than those of AOB and NOB.

Fig. 4 shows that the fraction of *Nitrosomonas europaea* in the entrapped pellets was 73.5% ± 9.7%. Due to high ammonia oxidation activity and high nitrite tolerance of *N. europaea*, it was usually found to be the absolutely dominant AOB (Ahn *et al*., 2008; Dytczak *et al*., 2008). On the other hand, *Nitrobacter* spp. were the dominant NOB (8.2% ± 3.6%) followed by *Nitrospira* (2.6% ± 1.8%). *Nitrobacter* spp. are known to have higher nitrite oxidation activity than *Nitrospira*. Therefore, *Nitrobacter* spp. are classified as *r*-strategists that thrive under high nitrite concentrations and *Nitrospira* spp. are regarded as *K*-strategists that thrive under low nitrite concentrations (Schramm *et al*., 1999). Coexistence of *Nitrobacter* and *Nitrospira* in the high-nitrite-fluctuation environment of nitrifying reactors has been reported by Kim and Kim (2006). The results of the present study showed that AOB were far more abundant than NOB (10.8% ± 4% total population of *Nitrosomonas* and *Nitrospira*) in the entrapped pellets. Apparently, nitritation activity was also much higher than nitratation activity. This suggests that shortcut nitrification mostly depended on the ratio of AOB and NOB. After acclimation with ammonia wastewater, AOB required an overwhelming proportion of biomass to maintain stable shortcut nitrification.

Acknowledgments

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