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Nitrification performance of nitrifying bacteria immobilized in waterborne polyurethane at low ammonia nitrogen concentrations

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Abstract

Suspended and waterborne polyurethane immobilized nitrifying bacteria have been adopted for evaluating the effects of environmental changes, such as temperature, dissolved oxygen (DO) concentration and pH, on nitrification characteristics under conditions of low ammonia concentrations. The results showed that nitrification was prone to complete with increasing pH, DO and temperature. Sensitivity analysis demonstrated the effects of temperature and pH on nitrification feature of suspended bacteria were slightly greater than those of immobilized nitrifying bacteria. Immobilized cells could achieve complete nitrification at low ammonia concentrations when DO was sufficient. Continuous experiments were carried out to discuss the removal of ammonia nitrogen from synthetic micro-pollute source water with the ammonia concentration of about 1 mg/L using immobilized nitrifying bacteria pellets in an up-flow inner circulation reactor under different hydraulic retention times (HRT). The continuous removal rate remains above 80% even under HRT 30 min. The results verified that the waterborne polyurethane immobilized nitrifying bacteria pellets had great potential applications for micro-pollution source water treatment.

Key words: nitrification characteristics; waterborne polyurethane; immobilized nitrifying bacteria; low ammonia concentrations; micro-polluted source water

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Introduction

Chinese government has established more stringent drinking water criterion based on new drinking water quality standards (GB5749-2006) regulating that the concentration of ammonia nitrogen must be below 0.5 mg/L. Effective removal of low ammonia nitrogen from micro-polluted water source has become a hot topic of source water treatment field. In the traditional biological ammonia nitrogen removal process, nitrifying bacteria grow slowly and usually with great loss from water treatment system because the hydraulic retention times (HRT) is too short to achieve solid-liquid separation, especially at a low ammonia concentration environment. Compared with conventional processes, nitrifying bacteria after immobilization can more effectively retain biomasses and can stay in the reactor for unlimited period (Wijffels and Tramper, 1995; Rostron et al., 2001). Nitrifying bacteria show different nitrification characteristics at different ammonia concentrations. However, few studies on environmental factors affecting nitrification at low ammonia concentrations were reported, especially for immobilized cells. The present study used waterborne polyurethane (WPU), a novel support material with excellent mechanical property and chemical stability, to entrap and immobilize nitrifying bacteria. The nitrification characteristics of suspended and immobilized nitrifying bacteria pellets (with the same biomass) were evaluated under different temperatures, dissolved oxygen (DO) concentrations and pH values at low ammonia nitrogen wastewater. The optimum operating conditions were obtained through sensitivity analysis. Additionally, a continuous nitrification test using synthetic wastewater was performed at different hydraulic retention times (HRT) in an up-flow inner circulation reactor with 10% pellets stuffing ratio in volume. The results provided a scientific basis for designing nitrification process at low ammonia concentrations.

1 Materials and methods

1.1 Cultivation of nitrifying bacteria

Activated sludge was used as seed sludge because it was readily available in large amounts from sewage treatment systems. In this research, the nitrifying bacteria obtained from the aeration tank of Minhang Municipal Wastewater

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Treatment Plant, Shanghai, China was utilized as seed sludge for incubating nitrifying bacteria. The composition of culture medium for nitrifying bacteria is listed in Table 1. After 2–3 months of incubation in laboratory, the concentration and number of nitrifying bacteria were about 2500 mg/L and 7.0×10^9 L^{-1} (MPN), respectively.

Table 1 Composition of feeds for cell growth and test

Chemical	Function	Concentration (mg/L)	
		For cell growth	For test
NH_4Cl	Nitrogen source	153	3.82
$Na_2HPO_4 \cdot 12H_2O$	Phosphate source	46.4	1.16
NaCl	Sodium source	20.5	0.52
KCl	Potassium source	9.6	0.24
$CaCl_2 \cdot 2H_2O$	Calcium source	9.6	0.24
$MgSO_4 \cdot 2\ H_2O$	Magnesium source	33.6	0.84
$NaHCO_3$	Alkalinity control	468	11.7

1.2 Immobilization of nitrifying bacteria in water borne (WPU) gel carrier

Suspensions of incubated nitrifying bacteria were centrifuged to the concentration of 20 g/L, and the concentrate was entrapped in waterborne polyurethane gel carrier. The entrapped concentrate was then mixed with a WPU prepolymer emulsion and a promoter (*N,N,N',N'*-tetramethylenediamine). To initiate polymerization, potassium persulfate, an initiator, was added to the beaker. The mixture was allowed to stand for about 5 min at room temperature of about 25°C. Consequently, WPU immobilized nitrifying bacteria in the form of an elastic gel was obtained. The obtained polymerized gel carrier was cut into 3 mm cubes and then washed thoroughly with distilled water. Before test, the immobilized nitrifying bacteria pellets were cultivated in the medium (Table 1, for cell growth) for four weeks.

1.3 Experimental setup

The batch experiments and continuous experiments were both carried out in an up-flow inner circulation aerated reactor (Fig. 1). The reactor with 18 L operating volume, made of acrylic glass, was fed with synthetic ammonia wastewater (Table 1, for test). An inner circulation tube with two open sides was set in the center of the reactor. Air was supplied through a sintered glass ball fixed at the column bottom. The temperature of water in the reactor was controlled by a temperature controller. The filling fraction of immobilized pellets and free cells with the same biomass in the bioreactor was 10% of reactor volume. Batch experiments focusing on the effect of factors on nitrification process were performed with collecting a sample every 10 minutes. The continuous experiments on removing ammonia nitrogen from synthetic micro-pollute source water (the concentration of ammonia was about 1 mg/L, Table 1, for test) by immobilized nitrifying bacteria pellets were also carried out in the reactor (Fig. 1) with the same stuffing ratio. The synthetic ammonia wastewater was pumped by variable speed peristaltic pumps. HRT was controlled by adjusting the feed flow rate.

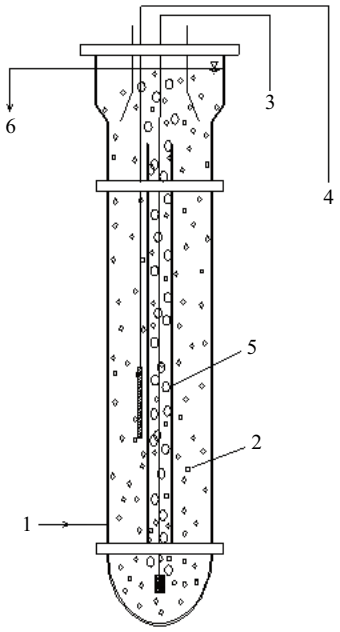

Fig. 1 Diagram of up-flow inner circulation reactor. (1) influent; (2) immobilized pellet; (3) aeration pump; (4) heater and temperature controller; (5) inner circulation tube; (6) effluent.

1.4 Analytical and calculation methods

The DO concentration was measured by dissolved oxygen analyzer (model 830A, Thermo Orin, USA) and pH was measured daily. Temperature of the reactor was monitored using online probe. The concentration of ammonia was determined according to standard methods (APHA, 1995).

The total ammonium nitrogen (TAN) concentration (C_{TAN}, mg/L) was calculated using the following Eq. (1):

$$C_{TAN} = C_{NH_4^+\text{-N}} \tag{1}$$

According to Michaelis-Menten's kinetics equations, at low concentrations of TAN, the concentration is almost negligible in the denominator (Walsh et al., 2010). Ammonia oxidation process resembles a first order reaction. A plot of $\ln C_{TAN}$ vs. time t gives a straight line with a slope of $-k$.

$$\ln C_{TAN_t} = -kt + \ln C_{TAN_0} \tag{2}$$

where, C_{TAN_0} (mg/L) and C_{TAN_t} (mg/L) are the total ammonia nitrogen concentrations at the beginning and the end of each experiment, respectively, and t (hr) is the time of the batch experiment. k is ammonia oxidation rate constant, which has units of 1/time.

2 Results and discussion

2.1 Effects of pH on ammonia oxidation rate constant

Nitrifying bacteria are pH-sensitive organisms (Villaverde et al., 1997). The value of pH is an important factor affecting nitrification. The optimal pH value for nitrification of immobilized pellets and free cells processing at low ammonia concentrations was

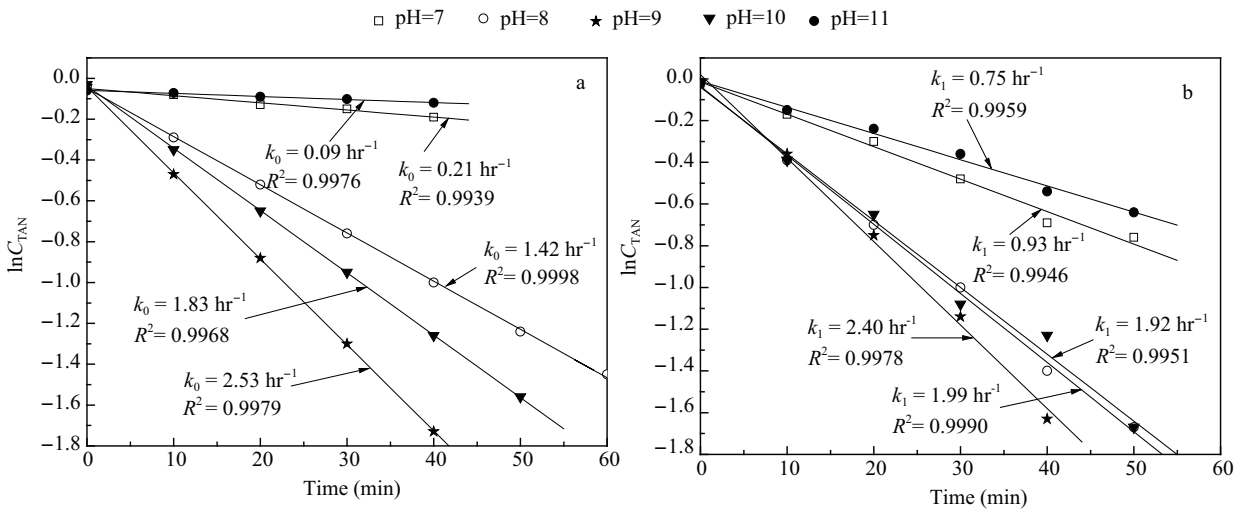

Fig. 2 Ammonia oxidation rate constants of free (k_0) (a) and immobilized (k_1) (b) cells under different pH levels. TAN: total ammonium nitrogen.

investigated under the condition of temperature 25°C, DO 4–6 mg/L. The ammonia oxidation rate constants, k_0 and k_1, were determined from the slope of lnC_{TAN} vs. t as illustrated in Fig. 2. The ammonia oxidation rate constants, k_0 and k_1, under different pH conditions are shown in Fig. 3.

The highest k values were obtained at pH 9 for both groups of bacteria (Fig. 3). Previous publications had reported that optimum pH for nitrification ranged between 7.0 and 8.0 (Jones and Paskins, 1982; Painter and Loveless, 1983; Antoniou et al., 1990). The optimal pH value for bacteria estimated in this experiment was significantly higher than the reported values. The main reason was that the ammonia oxidation rate was determined to a large extent by the free ammonia concentration, which was the major substrate of ammonia oxidation (Groenewega et al., 1994). The free ammonia concentration ($C_{NH_3\text{-free}}$) was estimated using Eq. (3) (Anthonisen et al., 1976).

$$C_{NH_3\text{-free}} = \frac{17}{14} \times \frac{C_{TAN} \times 10^{pH}}{K_b/K_w + 10^{pH}} \quad (3)$$

where, K_b is the dissociation constant of ammonia and K_w is the ionization constant of water. The $C_{NH_3\text{-free}}$ increased with the pH increasing from 7 to 11, but the concentrations were far below the threshold concentration (6–8 mg/L) when the inhibition of ammonia oxidation began (Stein et al., 1997). The increase in free ammonia concentrations within a certain range firstly enhanced the ammonia oxidation rate. At pH 10, k_0 and k_1 both remained relatively high values. When the pH further rose to 11, k_0 and k_1 decreased drastically. This phenomenon was due to high concentration of OH^- and low availability of inorganic carbon (Villaverde et al., 1997). Compared with ammonia oxidation process at high ammonia nitrogen concentrations, nitrification was more likely to complete with increasing pH value appropriately at low ammonia nitrogen concentrations.

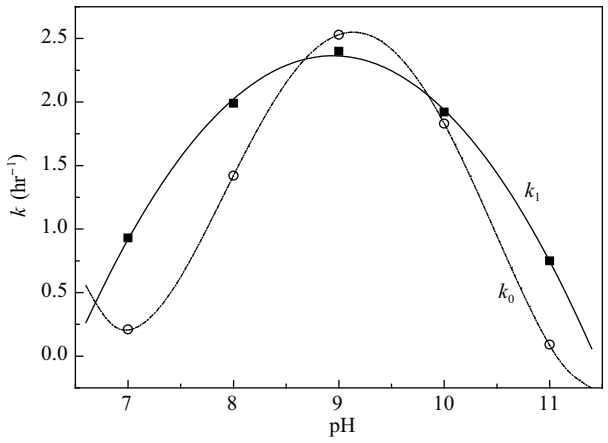

Fig. 3 Effects of pH on ammonia oxidation rate constants.

The optimal pH for nitrification of immobilized and free was similar at low ammonia nitrogen concentrations (Fig. 3). In contrast, the immobilized system showed a wider operating range of optimal pH values. The smooth lines in Fig. 3 were drawn to connect the data points using nonlinear curve fitting method. Fitting polynomials are the following Eqs. (4) and (5):

$$k_0: Y = 0.10X^4 - 3.80X^3 + 51.33X^2 - 301.92X + 653.39 \qquad R^2 = 0.9999 \quad (4)$$

$$k_1: Y = -0.38X^2 + 6.84X - 28.20 \qquad R^2 = 0.9986 \quad (5)$$

At higher or at lower pH values, the activity of immobilized cells became less sensitive from polynomials. The ammonia oxidation rate constant of the immobilized cells, k_1, maintained more than 70% of the maximum value at pH 7 or 11, whereas the free cells decreased about 90% at pH 7 or 11. In addition, the activity of immobilized cells could maintain at a wider pH range than free cells due to a buffering capacity by the carrier WPU.

The results indicated that the optimal pH value of ammonia oxidation was about 9 for both immobilized and free cells at low ammonia nitrogen concentrations. An alkaline environment was helpful for through nitrification in wastewater treatment system with a low ammonia nitrogen concentration. The results also showed that immobilized cells were less sensitive to pH and more suitable for wastewater with the wide pH fluctuations treatment than free cells.

2.2 Effect of temperature on ammonia oxidation rate constant

The ammonia oxidation rate constants of free and immobilized cells at different temperatures are shown in Fig. 4. The correlation between temperature and nitrification rate constant virtually followed zero-order. Linear equations were as following Eqs. (6) and (7):

$$k_0: Y = 0.10X - 0.36 \qquad R^2 = 0.9935 \qquad (6)$$

$$k_1: Y = 0.13X - 1.06 \qquad R^2 = 0.9914 \qquad (7)$$

The result indicated that k_0 and k_1, were significantly accelerated when the temperature increased from 10°C to 30°C. For free cells, k_0 at 30°C was eleven times of that at 10°C and six times of that at 20°C. The results coincided with the effect of temperature (10°C vs. 30°C) on the nitrification rate coefficients (Yamaguchi et al., 1996). In the literature, the values of nitrification rate coefficients at 30°C for the corresponding first-order and zero-order reaction were 9 to 15 times and 2 to 4 times larger than that at 10°C, respectively. In general, nitrification at high ammonia concentrations was a zero-order reaction and a first order reaction at low concentrations. The results indicated that temperature had a greater impact on nitrification of suspended bacteria in low concentrations than at high concentrations of ammonia nitrogen (Bae et al., 2002). For immobilized cells, k_1 at 30°C was four times of that at 10°C, which was well accorded with the classical temperature effect found by Downing and Hopwood (1964). The reported nitrification rate doubled as temperature rose by 10°C over the range of 5–30°C (Downing and Hopwood, 1964). At 40°C, the free ammonia concentration was 0.167 mg/L and still far below inhibitory concentration. The rate constant slightly decreased at temperature above 40°C , which was possibly due to harmful effect of high temperature on the activity of nitrifying bacteria. Thus, the optimal temperature was about 30°C for both free and immobilized cells. Furthermore, immobilized nitrifying bacteria retained high activity even under low temperatures (Fig. 4). Jones and Hood (1980) pointed out that the nitrification was almost inhibited if temperature was less than 10°C. When the temperature was 10°C, k_1 at 10°C was three times of k_0 at 10°C. The optimum temperature range, which showed as a relatively broad concave curve, was found to be between 25 and 35°C for the cells entrapped in WPU gel pellets. The results implied that the stability of bacteria against temperature was increased after immobilization in the WPU gels.

2.3 Effect of DO on ammonia oxidation rate constant

The ammonia oxidation rate constants of free and immobilized cells under various DO concentrations were investigated at 25°C (Fig. 5). The coefficients of determination were above 0.99 in all sets of experiments. Linear equations are as the following Eqs. (8) and (9):

$$k_0: Y = 0.50X + 0.36 \qquad R^2 = 0.9999 \qquad (8)$$

$$k_1: Y = 0.60X - 0.09 \qquad R^2 = 0.9993 \qquad (9)$$

Since dissolved oxygen is a co-substrate in the nitrification reaction, its concentration will affect the reaction in a dual-limitation manner (Jayamohan et al., 1988, Bae and Rittmann, 1996). As shown in Fig. 5, k_1 was much lower than k_0 under DO = 1 mg/L and these two values both increased as DO increased. k_0 increased 2.8 times as DO increased from 1 to 4 mg/L. k_1 increased even more, giving a 4.8 times higher value when DO concentration increased from 1 to 4 mg/L. Consequently, k_0 and k_1 values were similar under DO = 4 mg/L. The reason was that the oxygen transfer resistance within the immobilized cells pellets tended to lower the activity of nitrifying bacteria. The dissolved oxygen concentration had different effects on ammonia oxidation rate constants of immobilized cells compared with temperature and pH values. In this study, k_1 was affected more sensitively by decreasing DO. The sufficient dissolved oxygen in the water treatment system could enhance nitrification efficiency of immobilized cells pellets under low ammonia concentrations.

2.4 Parameter analysis

Table 2 summarizes the key results of our experiment. The optimum operation condition in each set of experiment was underlined in Table 2, where pH = 9, DO = 4 mg/L, and temperature = 30°C for both groups of bacteria. When all of the optimum conditions were met, the ammonia

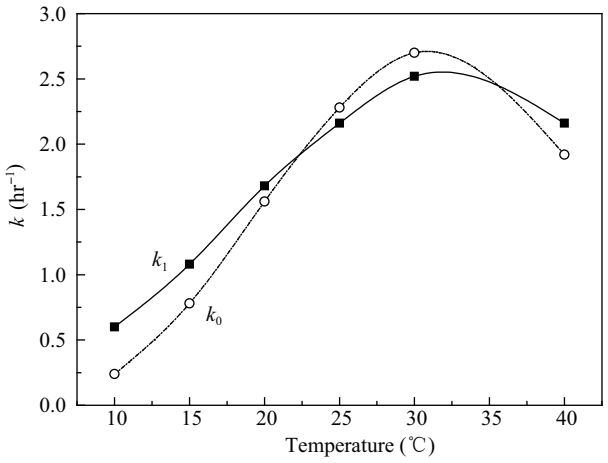

Fig. 4 Effects of temperature on ammonia oxidation rate constants.

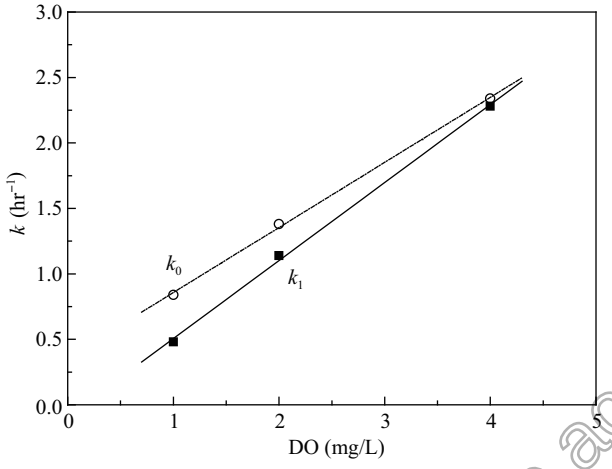

Fig. 5 Effects of DO on ammonia oxidation rate constants.

oxidation rate constants, k_0 and k_1, were above 2.28. It will be recognized that the data sets for pH = 9 and temperature = 25°C had identical experimental conditions and obtained very similar results, demonstrating that these experiments were highly reproducible. The results revealed that the ratio k_1/k_0 was close to or higher than 1.0 in most experimental conditions. This showed bacteria immobilized in WPU maintained considerable nitrification activity relative to suspended bacteria in many cases.

In order to evaluate some factors affecting the k values of free and immobilized cells, the concept of sensitivity was introduced using the following Eq. (10) (Wang, 1993).

$$S(y, x_i) = \frac{\partial y/\partial x_i}{y/x_i} \tag{10}$$

According to the previous fitting Eqs. (4)–(9), sensitivity of factors on free (S_0) and immobilized cells (S_1), were calculated in each case. The sensitivity of pH was significantly higher than DO and temperature, and DO was slightly higher than temperature (Table 2). At the same time, S_0 was higher than S_1 in most cases. The results indicated that the immobilized cells were less sensitive to environmental change than the free cells. The operations in low sensitivity range were conducive to system stability and maintaining high removal ammonia rate. Immobilized pellets would not be washed out as free cells due to being retained within the reactor by network isolation barrier. Thus, gel entrapment enables prolonged biomass retention times even under short hydraulic retention times. All these phenomena suggested that the immobilized pellets were suitable for ammonia nitrogen removal at low concentrations from micro-polluted source water.

2.5 Continuous treatment of micro-polluted water with WPU immobilized pellets

Continuous removal of ammonia nitrogen at low concentrations from synthetic micro-polluted drinking water source using immobilized nitrifying bacteria pellets was investigated in an up-flow inner circulation reactor (10% pellets stuffing ratio in volume). The system was operated continuously under the condition of temperature 30°C, pH = 9, DO = 4 mg/L based on nitrification characteristics of immobilized bacteria. After start-up of the experiment, the ammonia concentration in the effluent decreased quickly and the ammonia removal efficiency reached a steady level in the first 7 days. The optimal HRT was then obtained by increasing flow rate. The HRT was set in the range of 10–60 min, as shown in Fig. 6. During the 60 days of operation, the removal rate for ammonia nitrogen remained above 87% with 60 min of HRT, dropped to 68% with 10 min of the HRT. The effluent ammonia concentration under HRT 10 min was below 0.5 mg/L, conforming to the drinking water quality standards. No recognizable destruction of pellets was observed at the end of the experiment over the 60-day operation period. These results demonstrated that WPU prepolymer was an ideal material for immobilizing nitrifying bacteria. The WPU immobilized nitrifying bacteria pellets were verified to possess high ammonium removal efficiency in micro-polluted water treatment.

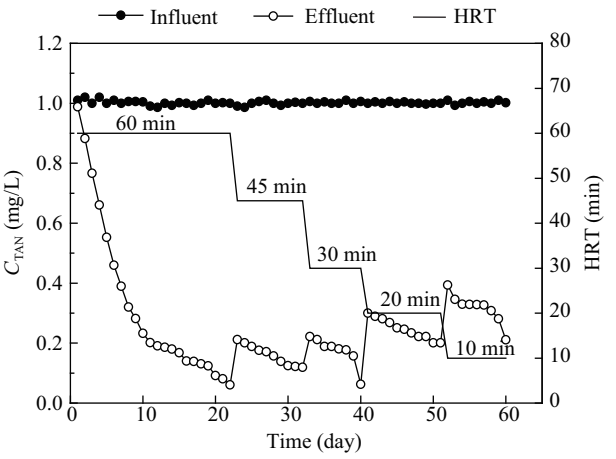

Fig. 6 Ammonia nitrogen removal with WPU immobilized pellets by changing HRT.

3 Conclusions

Nitrification at low ammonia nitrogen concentrations was more likely to complete appropriately with increasing pH, DO and temperature. Sensitivity analysis suggested that the effects of temperature, pH and DO on the ammonia oxidation rate constants were significantly different. The pH value had greater impact on ammonia oxidation rate

Table 2 Summary of the experimental results

Parameter		Initial $C_{NH_3\text{-free}}$ (mg/L)	k_0 (hr^{-1})	k_1(hr^{-1})	k_1/k_0	S_0	S_1
pH	7	0.007	0.21	0.93	4.43	–156	11.4
	8	0.066	1.42	1.99	1.4	–30.7	3.06
	9	0.443	2.53	2.40	0.95	–34.8	0
	10	1.034	1.83	1.92	1.05	–83.7	–3.96
	11	1.195	0.09	0.75	8.33	–2402	–22.3
DO (mg/L)	1	0.066	0.84	0.48	0.57	0.59	1.24
	2	0.066	1.38	1.14	0.83	0.72	1.05
	4	0.066	2.34	2.28	0.97	0.85	1.05
Temprature (°C)	10	0.022	0.24	0.6	2.5	4.08	2.13
	20	0.047	1.56	1.68	1.08	1.26	1.52
	30	0.092	2.70	2.52	0.93	1.09	1.52
	40	0.167	1.92	2.16	1.13	2.04	2.37

k_0, k_1 are the ammonia oxidation rate constants of free and immobilized nitrifying bacteria, respectively. S_0 and S_1 are the sensitivity of factors on free and immobilized nitrifying bacteria, respectively.
The numbers with underline is the optimum operation condition.

constants than temperature followed by DO. The optimum operational condition was pH 9, DO 4 mg/L and temperature 30°C when the initial ammonia concentration was 1 mg/L. Under the conditions of adequate oxygen, immobilized pellets had better nitrification characteristics and retained its nitrification activity under lower temperatures, wider pH values than free cells. Continuous treatment of micro-polluted water showed that the removal rate of ammonia nitrogen remains above 80% using WPU immobilized pellets with HRT 30 min. The effluent water was in line with the national standards even under HRT 10 min. The WPU immobilized nitrifying bacteria pellets showed high removal ability of ammonia nitrogen from wastewater at low concentrations, with stability for long-term operation. Thus, this convenient immobilized nitrifying bacteria method is promising for micro-polluted source water treatment applications in long-term operation.

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