

## Effects of Influent Substrate Concentration on the Performance and Kinetics of Anammox in a Bio-sand Filter

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### Abstract

A bio-sand filter was used to investigate the effects of influent substrate concentration ( $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$ ) on the performance of anaerobic ammonium oxidation (anammox), and a nitrogen removal kinetic model for anammox was developed. At a constant hydraulic loading rate of 1.0 m/d and an influent  $\text{NO}_2^-\text{-N}/\text{NH}_4^+\text{-N}$  concentration ratio of 1.3, along-the-column water quality analysis showed that the apparent removal rate constants of both  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  decreased with increasing influent substrate concentration, indicating that the substrate mass-transfer driving force was relatively sufficient at low substrate concentrations and that the substrate removal rate per unit biofilm area was higher. The removal kinetic models obtained by fitting were as follows:  $S_h/S_0 = \exp(-0.6244S_0^{-0.714}h)$  for  $\text{NH}_4^+\text{-N}$  and  $S_h/S_0 = \exp(-0.7633S_0^{-0.7178}h)$  for  $\text{NO}_2^-\text{-N}$ . The average relative errors between the calculated and measured values were below 10%. The model can be used for effluent quality prediction and filter bed height control, providing a theoretical basis for the design and operational regulation of anammox bio-sand filter processes.

### Keywords

Substrate Concentration; Bio-sand Filter; Anammox; Kinetics; Mathematical Model.

### 1. Introduction

Anaerobic ammonium oxidation (anammox), discovered in the mid-1990s by Delft University of Technology (the Netherlands) in a denitrifying fluidized-bed reactor, is a novel biological nitrogen removal pathway in which anammox bacteria, under anaerobic conditions, oxidize ammonium ( $\text{NH}_4^+\text{-N}$ ) directly to nitrogen gas using nitrite ( $\text{NO}_2^-\text{-N}$ ) as the electron acceptor [1]. Compared with the conventional nitrification-denitrification process, the anammox process requires no aeration, no external organic carbon source, and produces low sludge yields, with significant greenhouse gas reduction benefits. It has therefore become a research hotspot in the field of biological nitrogen removal from wastewater in recent years [2-4].

Since the discovery of anammox, research has mainly focused on its discovery and bacterial identification, enrichment cultivation and reactor start-up, influencing factors and enhancement strategies, and microbial community structure analysis [5-8]. The factors affecting anammox are numerous, mainly including influent substrate concentration, pH, temperature, dissolved oxygen (DO), hydraulic retention time, salinity, and inhibitory substances [9-13]. Among these,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$ , as the direct reaction substrates of anammox, exert the most significant influence on anammox activity through their concentration levels and ratio. On the one hand, an appropriate substrate concentration is a prerequisite for maintaining the normal metabolism of anammox bacteria; on the other hand, when  $\text{NO}_2^-\text{-N}$  exceeds a certain concentration, it can exert significant inhibition on anammox bacteria, whereas excessively low substrate concentrations make it difficult to maintain a

sufficient specific growth rate of the bacterial consortium, easily causing biomass washout and system instability [14, 15]. Therefore, clarifying the quantitative relationship between substrate concentration and the nitrogen removal rate of the anammox process is of great importance for the optimized operation of reactors.

With the rapid development of mathematical models in the field of wastewater treatment, it has become common practice to use mathematical models to analyze the migration and transformation of pollutants and to guide process design and operational optimization. Since the 1950s, foreign scholars have successively proposed representative steady-state models based on growth-decay theory, such as the Eckenfelder model, the McKinney model, and the Lawrence-McCarthy model, and subsequently developed dynamic models such as the Andrews inhibition model, the Activated Sludge Model series (ASM), biofilm models, and anaerobic digestion models [16]. In recent years, as wastewater discharge standards have become increasingly stringent, nitrogen removal has become a priority in the field of wastewater treatment, and mathematical models for nitrogen removal in wastewater treatment processes have accordingly become a research hotspot. Kinetic studies are an important approach for investigating the migration and transformation of pollutants within bioreactors, and are of great theoretical and practical significance. However, existing anammox kinetic studies have mostly focused on suspended activated sludge systems, and the model parameters obtained can hardly be directly applied to fixed-bed biofilm systems. The bio-sand filter, a wastewater treatment unit characterized by simple configuration, low operational energy consumption, and convenient management, is widely used in rural domestic sewage, aquaculture wastewater, and small-scale decentralized wastewater treatment, and is a typical fixed-bed biofilm system [17]. In the filter column, biofilm grows attached to the filter media, and the mass transfer of substrates within the biofilm on the media surface differs markedly from that in suspended systems; consequently, its removal kinetic characteristics are also distinctive. At present, research on the effects of influent substrate concentration on the anammox process in bio-sand filters, as well as on the along-the-column kinetic behavior, remains scarce.

Therefore, in this study, a successfully started and stably operated bio-sand filter was selected as the research object. Under the conditions of a fixed hydraulic loading rate and a fixed substrate ratio ( $\text{NO}_2^- \text{-N}/\text{NH}_4^+ \text{-N}=1.3$ ), the effects of different influent substrate concentrations on the along-the-column nitrogen removal performance were systematically investigated; the migration and transformation of nitrogenous pollutants within the system were analyzed; a quantitative relationship between influent substrate concentration and the apparent removal rate constant was established; and a kinetic model applicable to the anammox process in bio-sand filters was constructed and validated. The results are expected to provide a scientific reference for the engineering design and operational regulation of such reactors.

## 2. Materials and Methods

### 2.1. Experimental Setup

The experiment was conducted on a laboratory-scale bio-sand filter that had been successfully started and had been stably operating anammox. Its structure is shown in Fig. 1. The filter column was made of PVC, with a total height of 70 cm and an inner diameter of 7 cm. From bottom to top, the column consisted of a support layer, a filter media layer, and a buffer layer. The buffer layer and the support layer were both filled with gravel, each 2.5 cm thick, located above and below the filter media layer, respectively, with a particle size of 5-18 mm. The filter media layer was 50 cm thick and consisted of three types of filter media: natural river sand, shell sand, and zeolite sand, all with a particle size of 0.5-1.0 mm, uniformly mixed at a volume ratio of 6:3:1. The combination of the three media takes into account specific surface area, porosity, and alkalinity supply: river sand is cheap and readily available, providing the main

attachment surface for biofilm; shell sand, composed mainly of calcium carbonate, slowly dissolves and releases alkalinity to neutralize the acidity produced by the anammox process, maintaining a stable pH in the reaction zone; zeolite sand has a good selective adsorption capacity for  $\text{NH}_4^+\text{-N}$ , which buffers influent fluctuations and slowly releases  $\text{NH}_4^+\text{-N}$  during biofilm regeneration, enhancing the system's shock resistance. The filter media layer was inoculated with aerobic nitrifying sludge and heterotrophic denitrifying sludge at a volume ratio of 1:2.

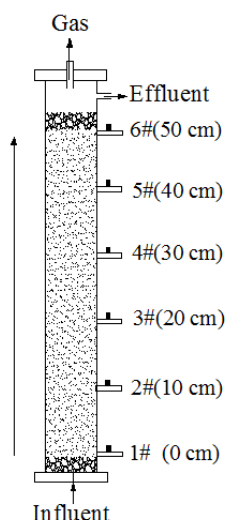


**Fig 1.** Schematic diagram of anammox bio-sand filter

The filter column was operated in upflow mode. The influent was fed at a constant flow rate from the bottom by a peristaltic pump, uniformly distributed through the gravel distribution layer, and then flowed upward through the filter media layer. Sampling ports were installed on the side wall of the column at 10 cm intervals, six in total from bottom to top, corresponding to the bottom of the filter media layer ( $h=0$ ) and positions 10, 20, 30, 40, and 50 cm above the bottom, enabling along-the-column sampling. The temperature in the reaction zone was maintained at 20-30 °C, and the hydraulic loading rate was kept constant at 1.0 m/d.

## 2.2. Influent Water Quality

Synthetic wastewater was used in the experiment, with  $\text{NH}_4\text{Cl}$  and  $\text{NaNO}_2$  providing  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$ , respectively. The influent  $\text{NO}_2^-\text{-N}/\text{NH}_4^+\text{-N}$  ratio was set at 1.3, close to the theoretical stoichiometric ratio of anammox, 1.32:1 (in terms of  $\text{NO}_2^-\text{-N}/\text{NH}_4^+\text{-N}$ ). Trace elements were added according to the literature [18]. The pH was controlled at 7.0-8.2. Prior to feeding, the influent was purged with nitrogen gas to reduce the DO concentration below 3 mg/L. The concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  were adjusted as required by the experiment.

## 2.3. Experimental Scheme

At a fixed hydraulic loading rate of 1.0 m/d and a  $\text{NO}_2^-\text{-N}/\text{NH}_4^+\text{-N}$  ratio of 1.3, the influent  $\text{NH}_4^+\text{-N}$  concentration was set sequentially at 25, 20, 15, 10, and 5 mg/L, corresponding to  $\text{NO}_2^-\text{-N}$  concentrations of 32.5, 26.0, 19.5, 13.0, and 6.5 mg/L, designated as T1-T5, respectively. Each operating condition ran stably for more than 3 days. After the along-the-column effluent quality remained continuously stable, samples were taken to investigate the effect of influent substrate concentration on the along-the-column nitrogen removal performance and to establish the kinetic model. After model construction, five additional verification conditions (T6-T10) were set, with influent  $\text{NH}_4^+\text{-N}$  concentrations of 6.3, 12.1, 17.5, 22.7, and 24.2 mg/L, respectively. The model was validated by comparing the experimental measured values with the model calculated values.

## 2.4. Model Development

For a fixed-bed biofilm reactor, when the substrate flows in plug flow through the filter bed and the reaction follows first-order kinetics, the relationship between the influent  $\text{NH}_4^+\text{-N}$  or  $\text{NO}_2^-\text{-N}$  concentration ( $S_0$ , mg/L) and the effluent concentration at filter height  $h$  ( $S_h$ , mg/L) can be expressed as an exponential decay function:

$$\ln \frac{S_h}{S_0} = -mh \quad (1)$$

where  $m$  is the apparent removal rate constant, a comprehensive parameter related to the filter structure, filter media properties, influent flow rate, substrate concentration, biofilm characteristics, and environmental conditions. When the filter structure, filter media properties, biofilm characteristics, and environmental conditions are fixed,  $m$  varies with the influent substrate concentration  $S_0$  and the hydraulic loading rate  $q$ . In this study, the hydraulic loading rate was fixed at 1.0 m/d. Referring to empirical relationships,  $m$  can be expressed as a power function:

$$m = kS_0^\alpha \quad (2)$$

where  $k$  is a coefficient related to the influent substrate concentration and hydraulic loading rate, and  $\alpha$  is a characteristic constant related to the influent substrate concentration. The sign and magnitude of  $\alpha$  reflect the direction and degree of the influence of substrate concentration on the apparent removal rate:  $\alpha > 0$  indicates that the removal rate increases with substrate concentration (substrate limitation type), whereas  $\alpha < 0$  indicates that the removal rate decreases with increasing substrate concentration (mass-transfer limitation or substrate inhibition type).

Substituting Eq. (2) into Eq. (1) gives:

$$\frac{S_h}{S_0} = e^{-kS_0^\alpha h} \quad (3)$$

Exponential fitting of the  $S_h/S_0$ - $h$  data for each operating condition yields the corresponding  $m$  values. Power-function fitting of  $m$  against  $S_0$  then determines the parameters  $k$  and  $\alpha$ , yielding an along-the-column removal kinetic equation incorporating an influent substrate concentration correction term.

## 2.5. Analytical Methods

Water samples were filtered through 0.45  $\mu\text{m}$  membranes prior to analysis.  $\text{NH}_4^+\text{-N}$  was determined by the Nessler's reagent spectrophotometric method (HJ 535-2009), and  $\text{NO}_2^-\text{-N}$  by the N-(1-naphthyl)-ethylenediamine spectrophotometric method (GB 7493-87). Each water sample was analyzed in triplicate, and the average value was taken, with the relative deviation controlled within 5%.

## 3. Results and Discussion

### 3.1. Along-the-column Effluent Characteristics

With the hydraulic loading rate fixed at 1.0 m/d, the influent  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  concentrations were changed simultaneously, and the  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  concentrations ( $S_h$ ) at various heights ( $h$ ) along the filter column were analyzed. The results are shown in Table 1.

**Table 1.** Variation of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  concentrations under different filter heights

| Test | $S_h/(\text{mg/L})$      | $h/\text{cm}$ |      |     |     |     |     |
|------|--------------------------|---------------|------|-----|-----|-----|-----|
|      |                          | 0             | 10   | 20  | 30  | 40  | 50  |
| T1   | $\text{NH}_4^+\text{-N}$ | 25.0          | 13.4 | 6.9 | 4.2 | 2.3 | 1.5 |
|      | $\text{NO}_2^-\text{-N}$ | 32.5          | 17.2 | 9.1 | 5.6 | 2.6 | 1.7 |
| T2   | $\text{NH}_4^+\text{-N}$ | 20.0          | 9.8  | 4.7 | 2.3 | 1.0 | 0.5 |
|      | $\text{NO}_2^-\text{-N}$ | 26.0          | 12.6 | 6.0 | 3.1 | 1.3 | 0.5 |
| T3   | $\text{NH}_4^+\text{-N}$ | 15.0          | 6.2  | 2.5 | 1.1 | 0.2 | 0.1 |
|      | $\text{NO}_2^-\text{-N}$ | 19.5          | 7.9  | 3.3 | 1.5 | 0.1 | 0.0 |
| T4   | $\text{NH}_4^+\text{-N}$ | 10.0          | 2.9  | 0.8 | 0.2 | 0.0 | 0.0 |
|      | $\text{NO}_2^-\text{-N}$ | 13.0          | 3.8  | 1.1 | 0.3 | 0.1 | 0.0 |
| T5   | $\text{NH}_4^+\text{-N}$ | 5.0           | 0.7  | 0.1 | 0.0 | 0.0 | 0.0 |
|      | $\text{NO}_2^-\text{-N}$ | 6.5           | 0.9  | 0.1 | 0.0 | 0.0 | 0.0 |

As shown in Table 1, the  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  concentrations decreased stepwise along the filter column height, with a fast decay rate at first and a slower one later. In the lower part of the column (0-20 cm), where substrate concentrations were high, the mass-transfer driving force was large and the removal rate was high. As the substrates were consumed along the column, the mass-transfer driving force weakened, the removal rate gradually decreased, and the curve flattened out, showing a typical concentration distribution characteristic of plug-flow biofilm reactors [19].

A comparison of the different operating conditions shows that at the same filter height, the higher the influent  $\text{NH}_4^+\text{-N}$  or  $\text{NO}_2^-\text{-N}$  concentration, the higher the residual  $\text{NH}_4^+\text{-N}$  or  $\text{NO}_2^-\text{-N}$  concentration in the effluent, i.e., the greater the substrate residual ratio ( $S_h/S_0$ ). For example, at  $h=20$  cm, the  $\text{NH}_4^+\text{-N}$  residual ratios for conditions T1-T5 were 27.6%, 23.5%, 16.7%, 8.0%, and 2.0%, respectively, increasing markedly with influent concentration. This indicates that with a fixed biomass (biofilm amount), a higher influent substrate concentration means a greater substrate load entering the reactor per unit time; the portion exceeding the biofilm's capacity can only remain in the effluent at a higher concentration. To maintain the same effluent concentration, a greater filter bed height or longer contact time is required. Thus, at relatively high influent substrate concentrations, the existing filter height is insufficient for anammox to proceed fully-this is the core problem that the model aims to solve.

The removal rates at the column effluent ( $h=50$  cm) were further calculated for each condition. At an influent  $\text{NH}_4^+\text{-N}$  of 25 mg/L, the removal rates of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  were 94.0% and 94.8%, respectively. When the influent  $\text{NH}_4^+\text{-N}$  concentration decreased to 15 mg/L, the removal rates of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  rose above 99%. When the influent  $\text{NH}_4^+\text{-N}$  concentration was below 10 mg/L, the effluent  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  concentrations were both below the detection limit, with removal rates approaching 100%. These results demonstrate that the bio-sand filter performs well in treating wastewater with medium and low nitrogen concentrations. Even under the most unfavorable condition (T1), the effluent  $\text{NH}_4^+\text{-N}$  (1.5 mg/L) still met the Grade 1A limit ( $\text{NH}_4^+\text{-N} \leq 5$  mg/L) of the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants (GB 18918-2002) [20]. It should be noted, however, that the influent in this experiment was synthetic wastewater containing no organic matter; in practical engineering, organic carbon would promote the proliferation of heterotrophic bacteria, which may compete with anammox bacteria for substrates and living space, so a safety margin should be reserved in engineering applications.

A stoichiometric analysis of substrate consumption along the column and for the whole column (expressed as  $\Delta\text{NO}_2^-\text{-N}/\Delta\text{NH}_4^+\text{-N}$ ) shows that the whole-column consumption ratios for the five conditions were stable between 1.30 and 1.31; along the column, the consumption ratios in the

various height intervals mostly fluctuated between 1.1 and 1.6, with the mean close to 1.3, basically consistent with the influent ratio. The classic anammox stoichiometric relationship is:  $\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.15}\text{N}_{0.15} + 2.03\text{H}_2\text{O}$  [21]. In this experiment,  $\text{NH}_4^+$ -N and  $\text{NO}_2^-$ -N were removed synchronously with a consumption ratio close to the theoretical value, indicating that anammox was the dominant pathway of biological nitrogen removal in the bio-sand filter [22], and the contribution of other nitrogen removal pathways such as short-cut nitrification can be neglected. Meanwhile, the slight surplus of  $\text{NO}_2^-$ -N (the ratio of 1.3 was slightly below the theoretical value of 1.32) ensured the full utilization of  $\text{NH}_4^+$ -N, and the phenomenon that the effluent  $\text{NO}_2^-$ -N residual was slightly higher than  $\text{NH}_4^+$ -N corroborates this.

### 3.2. Nitrogen Removal Kinetics

Equation (1) was used to perform exponential fitting of the  $S_h/S_0$ - $h$  relationships, and the results are shown in Fig. 2 and Table 2. As shown in Fig. 2, the  $S_h/S_0$ - $h$  fitting curves for  $\text{NH}_4^+$ -N and  $\text{NO}_2^-$ -N show essentially the same trend, with the two almost overlapping, reflecting the synchronous consumption of the two substrates. The coefficients of determination ( $R^2$ ) for all conditions were greater than 0.99, indicating that the exponential decay function describes well the variation of substrate concentration along the column in this plug-flow biofilm filter, and that characterizing the apparent removal process by first-order kinetics is reasonable.

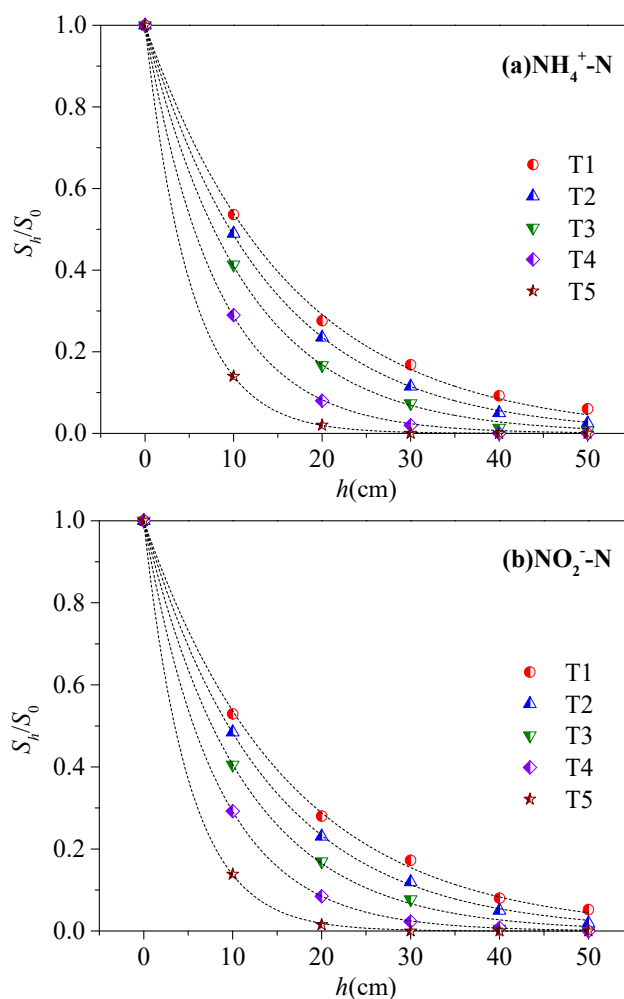


**Fig 2.** Fitting curves of  $S_h/S_0$  variation with  $h$

**Table 2.** Kinetic parameter  $m$  values of the fitting curves

| Test | N                               | $S_0$ /(mg/L) | Fitting equation        | $m$     | $R^2$  |
|------|---------------------------------|---------------|-------------------------|---------|--------|
| T1   | NH <sub>4</sub> <sup>+</sup> -N | 25.0          | $S_h=25e^{-0.06169h}$   | 0.06169 | 0.9991 |
|      | NO <sub>2</sub> <sup>-</sup> -N | 32.5          | $S_h=32.5e^{-0.0622h}$  | 0.06220 | 0.9999 |
| T2   | NH <sub>4</sub> <sup>+</sup> -N | 20.0          | $S_h=20e^{-0.07217h}$   | 0.07217 | 0.9999 |
|      | NO <sub>2</sub> <sup>-</sup> -N | 26.0          | $S_h=26e^{-0.07279h}$   | 0.07279 | 0.9999 |
| T3   | NH <sub>4</sub> <sup>+</sup> -N | 15.0          | $S_h=15e^{-0.08921h}$   | 0.08921 | 0.9996 |
|      | NO <sub>2</sub> <sup>-</sup> -N | 19.5          | $S_h=19.5e^{-0.09011h}$ | 0.09011 | 0.9999 |
| T4   | NH <sub>4</sub> <sup>+</sup> -N | 10.0          | $S_h=10e^{-0.12482h}$   | 0.12482 | 0.9999 |
|      | NO <sub>2</sub> <sup>-</sup> -N | 13.0          | $S_h=13e^{-0.12324h}$   | 0.12324 | 0.9999 |
| T5   | NH <sub>4</sub> <sup>+</sup> -N | 5.0           | $S_h=5e^{-0.19665h}$    | 0.19665 | 0.9999 |
|      | NO <sub>2</sub> <sup>-</sup> -N | 6.5           | $S_h=6.5e^{-0.19851h}$  | 0.19851 | 0.9999 |

The  $m$  values in Table 2 reveal two clear regularities. First, the apparent removal rate constants of NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N are very close to each other (relative deviations all below 3%), which corroborates the stoichiometric characteristic that the two substrates are consumed synchronously at a ratio of 1.3:1 under the same condition. This indicates that the  $m$  value truly reflects the intrinsic activity of anammox in the co-metabolism of the two substrates, rather than the individual degradation rate of a single substrate [23]. Second, the  $m$  values decrease monotonically with increasing influent substrate concentration: when the influent NH<sub>4</sub><sup>+</sup>-N increased from 5 to 25 mg/L, the  $m$  value decreased by 68%; the  $m$  value of NO<sub>2</sub><sup>-</sup>-N also decreased from 0.19851 to 0.06169, with essentially the same trend and magnitude.

The phenomenon that the  $m$  value decreases with increasing influent substrate concentration can be explained from the following aspects. (a) From the mass-transfer perspective, the substrate transfer rate within the biofilm is jointly controlled by the bulk liquid concentration and the intramembrane diffusion rate. At low influent concentrations, the main substrate removal zone is concentrated in the biofilm in the lower part of the column, the mass-transfer distance is short, and the apparent removal rate is high. As the influent concentration increases, to consume more substrate, the reaction zone extends upward, and the substrate must diffuse to deeper filter layers or into the interior of thicker biofilm before being utilized; the mass-transfer resistance increases, and the along-the-column apparent removal rate constant decreases. (b) From the reaction kinetics perspective, the substrate utilization rate of anammox bacteria does not increase linearly with substrate concentration; its specific activity shows a saturation trend, and at high concentrations the removal capacity per unit biofilm area approaches a limit, causing the apparent first-order fitted  $m$  value to decrease. (c) The potential inhibitory effect of nitrite should not be ignored. The highest influent NO<sub>2</sub><sup>-</sup>-N in this experiment reached 32.5 mg/L. Although no obvious activity inhibition was observed (removal rates were still above 94%), long-term high-concentration feeding is unfavorable for system stability. The negative value of  $\alpha$  is a comprehensive manifestation of the above coupled mass-transfer-reaction control and the potential substrate inhibition effect.

Using  $S_0$  as the abscissa and  $m$  as the ordinate, power-function fitting (log-log linear regression) was performed according to Eq. (2), and the results are shown in Fig. 3.

The fitting relationships for NH<sub>4</sub><sup>+</sup>-N and NO<sub>2</sub><sup>-</sup>-N are given in Eqs. (4) and (5), respectively:

$$m = 0.6244S_0^{-0.714} \quad (4)$$

$$m = 0.7633S_0^{-0.7178} \quad (5)$$

The  $R^2$  values of both fitting equations are greater than 0.998, indicating a good power-function relationship between  $m$  and  $S_0$ , and good applicability of the power-function model to this system. The values of  $\alpha$  in Eqs. (4) and (5) (-0.714 and -0.7178) are both negative and very close

to each other, once again showing that the apparent removal rate constants of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  follow the same variation pattern with influent concentration, i.e., the removal rate is negatively correlated with the influent substrate concentration.

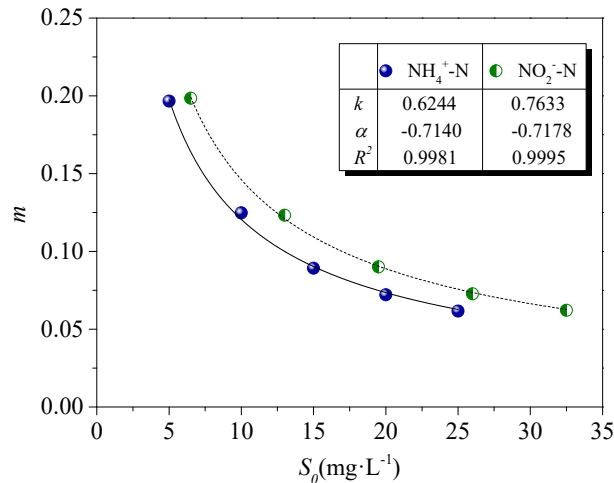


**Fig 3.** Fitting curve and parameters of  $S_0$  and  $m$

Substituting Eqs. (4) and (5) into Eq. (3), the along-the-column removal kinetic equations for  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  in the anammox system are obtained as Eqs. (6) and (7), respectively:

$$\frac{S_h}{S_0} = e^{-0.6244 S_0^{-0.714} h} \quad (6)$$

$$\frac{S_h}{S_0} = e^{-0.7633 S_0^{-0.7178} h} \quad (7)$$

It can be seen from Eqs. (6) and (7) that  $S_h/S_0$  and  $h$  follow a base- $e$  exponential function relationship, and the influent concentration correction term  $S_0^\alpha$  is introduced into the exponent, enabling the model to reflect the variation of the removal rate constant under different influent substrate concentrations. This is the key feature distinguishing this model from constant-coefficient first-order kinetic models. It should be noted that this model is an apparent kinetic model; the parameters  $k$  and  $\alpha$  comprehensively reflect the coupled effects of filter media properties, biofilm amount, hydraulic conditions, and substrate concentration. Its applicable conditions are: hydraulic loading rate of 1.0 m/d,  $\text{NO}_2^-\text{-N}/\text{NH}_4^+\text{-N} \approx 1.3$ , and temperature 20-30 °C. When the operating conditions change significantly, the parameters should be recalibrated.

### 3.3. Validation of the Kinetic Model

To verify the accuracy and applicability of the model, while keeping the hydraulic loading rate at 1.0 m/d, the influent  $\text{NH}_4^+\text{-N}$  concentration was adjusted to 6.3, 12.1, 17.5, 22.7, and 24.2 mg/L (corresponding to influent  $\text{NO}_2^-\text{-N}$  concentrations of 8.2, 15.7, 22.8, 29.5, and 31.5 mg/L), designated as T6-T10. The along-the-column effluent  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  concentrations were measured, and the measured values ( $S_{h,e}$ ) were compared with the model calculated values ( $S_{h,t}$ ) from Eqs. (6) and (7). The results are shown in Fig. 4.

As shown in Fig. 4, under all five verification conditions, the model calculated values of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  are in high agreement with the along-the-column measured values; the two curves basically coincide, with no systematic overestimation or underestimation. Quantitative analysis shows that the average relative errors of the  $\text{NH}_4^+\text{-N}$  concentrations for conditions T6-T10 were 4.56%, 4.49%, 3.68%, 4.00%, and 5.65%, respectively, and those of the  $\text{NO}_2^-\text{-N}$

concentrations were 2.23%, 5.05%, 3.85%, 4.23%, and 6.21%, respectively. The average relative errors of all conditions did not exceed 10%. This error level is within the acceptable range for environmental engineering kinetic models (generally, errors below 10-15% are considered to have engineering applicability), and the error distribution shows no obvious correlation with influent concentration, indicating that the model has stable prediction accuracy throughout the entire experimental concentration range ( $\text{NH}_4^+\text{-N}$  5-25 mg/L), with no overfitting phenomenon in which the model is valid only near the fitting points.

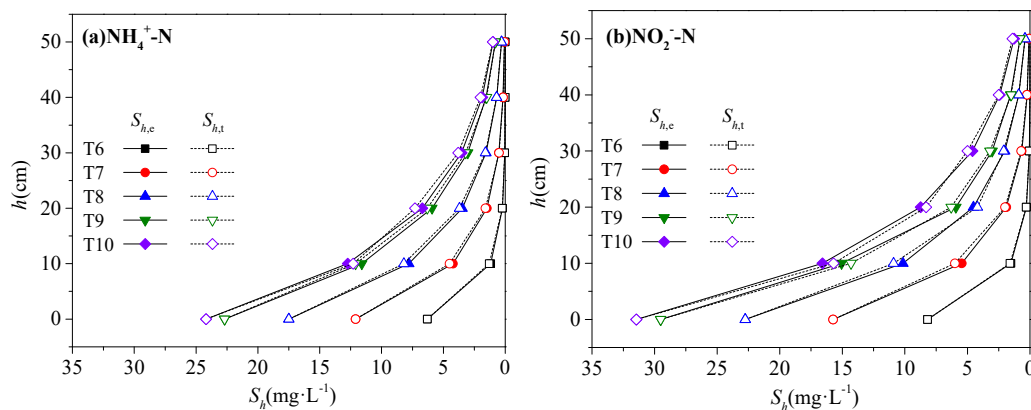


Fig 4. The  $S_{h,e}$  and  $S_{h,t}$  values under different filter height

The average relative errors of the verification conditions are at a relatively low level, mainly due to two aspects. First, the concentration gradient of the modeling conditions T1-T5 covered the concentration range of the verification conditions, and the model adopts a power-function form with clear physical meaning for the  $m$ - $S_0$  relationship, giving it good extrapolation capability. Second, the exponential fitting of  $S_h/S_0$ - $h$  itself had  $R^2$  greater than 0.99, laying a solid foundation for the model. The relatively larger errors in individual conditions (e.g.,  $\text{NO}_2^-\text{-N}$  in T9 and T10) may be related to fluctuations of intramembrane mass-transfer conditions at high substrate concentrations and amplified nitrite measurement errors (in the low-concentration range), but they do not affect the overall applicability of the model. In summary, the kinetic model constructed shows good accuracy and applicability.

### 3.4. Engineering Application Analysis

The kinetic model established can be used to guide the design and operation of the anammox process in bio-sand filters. Under the conditions of an influent hydraulic loading rate of 1.0 m/d,  $\text{NH}_4^+\text{-N}$  concentration of 5-25 mg/L, and  $\text{NO}_2^-\text{-N}$  concentration of 6.5-32.5 mg/L, Eqs. (6) and (7) can be used to predict the concentrations and removal of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  in the reactor effluent at different filter heights.

For example, when the influent  $\text{NH}_4^+\text{-N}$  concentration is 16 mg/L, Eq. (6) gives the effluent  $\text{NH}_4^+\text{-N}$  concentrations at filter heights of 10, 20, 30, 40, and 50 cm as 6.8, 2.9, 1.2, 0.5, and 0.2 mg/L, respectively. The corresponding influent  $\text{NO}_2^-\text{-N}$  concentration is 20.8 mg/L, and Eq. (7) gives the effluent  $\text{NO}_2^-\text{-N}$  concentrations at the same heights as 8.8, 3.7, 1.6, 0.7, and 0.3 mg/L, respectively. At filter heights of 30, 40, and 50 cm, the simultaneous removal rates of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  exceed 92%, 96%, and 98%, respectively. Thus, the system achieves high nitrogen removal efficiency at relatively low influent substrate concentrations; a filter layer of about 30 cm can meet high nitrogen removal requirements, and the 50 cm design has ample margin.

When the influent  $\text{NH}_4^+\text{-N}$  concentration is 24 mg/L, the model predicts the effluent  $\text{NH}_4^+\text{-N}$  concentrations at filter heights of 10, 20, 30, 40, and 50 cm to be 12.6, 6.6, 3.5, 1.8, and 1.0 mg/L, respectively. The corresponding influent  $\text{NO}_2^-\text{-N}$  concentration is 31.2 mg/L, and the effluent  $\text{NO}_2^-\text{-N}$  concentrations at the same heights are predicted to be 16.4, 8.6, 4.5, 2.4, and 1.2 mg/L,

respectively. At filter heights of 30, 40, and 50 cm, the simultaneous removal rates of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  exceed 85%, 92%, and 96%, respectively. Compared with the low-concentration condition, the filter layer required to achieve the same removal rate is markedly thicker: at a concentration of 16 mg/L, a 30 cm filter layer achieves a 92% removal rate, whereas at 24 mg/L a 40 cm filter layer is needed to reach 92%. This indicates that the nitrogen removal efficiency of the system decreases at high influent substrate concentrations, because in a stably operating reactor the biomass is in dynamic equilibrium and the substrate removal capacity per unit filter volume is limited; excessively increasing the influent substrate concentration will make the system unable to consume the load, resulting in higher residual substrate concentrations in the effluent.

The above regularities have direct guiding significance for engineering operation. The model can be used to predict the effluent status of the anammox reactor under different influent  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  concentrations, and forward-looking regulation can be carried out accordingly. In particular, when the influent substrate concentration is too high or the ratio is imbalanced (e.g., the effluent  $\text{NO}_2^-\text{-N}/\text{NH}_4^+\text{-N}$  from an upstream partial nitrification (PN) reactor deviates from 1.3 due to unstable operation, or the concentration surges due to instantaneous shock loads), measures can be taken in advance—such as operating multiple anammox reactors in parallel, temporarily reducing the influent flow rate, modifying the packing structure, or adding chemicals (e.g., ferrous salt precipitation)—to avoid irreversible inhibition of anammox bacteria by high-concentration substrates and ensure long-term stable operation of the system [24, 25]. In addition, the model can be used to back-calculate design parameters: given the target effluent concentration and influent concentration, the required filter height can be solved from  $h = \ln(S_0/S_h)/(k \cdot S_0^\alpha)$  using Eqs. (6) and (7), providing a basis for filter column design.

## 4. Conclusion

In the anammox bio-sand filter system, the concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  decrease exponentially along the filter height and are removed synchronously; the whole-column  $\Delta\text{NO}_2^-\text{-N}/\Delta\text{NH}_4^+\text{-N}$  remains stable at 1.30–1.31, indicating that anammox is the dominant nitrogen removal pathway in the system. The apparent removal rates of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_2^-\text{-N}$  decrease with increasing influent substrate concentration, essentially reflecting the combined effects of increased biofilm mass-transfer resistance, saturation of substrate utilization, and potential nitrite inhibition. The average relative errors between the calculated and measured values of the constructed anammox along-the-column nitrogen removal kinetic model are all below 10%, showing high prediction accuracy. It can serve as an effective tool for effluent quality prediction and filter height design of anammox bio-sand filters. Combined with model-based predictions, proactive regulation of high-concentration or imbalanced-ratio influent can prevent the system from shock-induced instability.

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