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Comparison of the NH_4^+-N removal ability by *Klebsiella* sp. FC61 in a bacterial suspension system and a bacterial immobilization system

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ABSTRACT

The aim of this study was to compare the removal of ammonia-nitrogen (NH_4^+-N) in a bacterial suspension system (BSS) and a bacterial immobilization system (BIS) using the iron-reducing bacterium *Klebsiella* sp. FC61. The experimental results showed that the pellets in the BIS could adsorb part of the iron ions in the solution. Although the adsorption of Fe^{3+} was stronger than that of Fe^{2+}, single-layer physical adsorption was observed in both cases. Furthermore, the self-inhibition effect of the bacteria from the suspended state to the immobilized state was effectively improved in the BIS, with a maximum NH_4^+-N removal rate (r_{max}) of 0.7241 mg/L/h for the BIS and 0.3732 mg/L/h for the BSS. Moreover, strain FC61 could effectively remove the NH_4^+-N in both the BIS and BSS, while maintaining a lower concentration of iron ions, nitrate, nitrite, and the quantity of microorganism in the solution. The results of response surface methodology showed that the predicted maximum removal ratio of NH_4^+-N in the BIS was 70.31% (actual value = 72.15%), which was higher than that of the BSS at 59.59% (actual value = 61.37%) at the same time point of 48 h. Analysis of bacterial distribution and growth data suggested that removal of NH_4^+-N by strain FC61 would be more favorable in the BIS than in the BSS.

1. Introduction

At present, the exceedances of ammonium nitrogen in underground water is more common, and most of current research is focus on the removal of ammonium nitrogen [1,2]. There are physical methods, chemical methods, biological methods, biochemical methods, physical and chemical methods, and so on. For example, the use of the nitrification characteristics of nitrification bacteria as well as the use of ion exchange technology to remove ammonium nitrogen [3–5]. In the process of removal of ammonium nitrogen, it is restricted by many factors, such as the increase of its own concentration, bacteria in the use of the process will have a certain degree of poison, which will inhibit its removal rate. At the same time in the oxidation process of ammonium nitrogen often has the production of nitrite nitrogen, and the increase of nitrite nitrogen concentration will also restrict its own removal rate, the above phenomenon is known as the substrate self-inhibition [6,7]. And two classical equations of Aiba and Edwards are described in the description of self inhibition [8]. On the other hand, there are many people who use the technology of immobilized embedding to study the removal of ammonium nitrogen, which because of its strong operability, the bacteria are in a stable state, the effect is more prominent, and is widely used in the operation of the experiment [9–11]. Because of its own structural characteristics, the immobilization technology generally has the characteristics of adsorbing heavy metal ions [12]. And the most two famous models for the study of adsorption are Langmuir and Freundlich equations. Langmuir describes quantitatively the formation of a monolayer adsorbate on the outer surface between the solid and liquid phases while the Freundlich equation is applicable for multilayer adsorption [13]. Meanwhile, response surface methodology can be widely used in analytical method because of its scientific and rigorous nature, which can be used to characterize the intrinsic characteristics of relevant data [14]. And in these studies, very few people give a deep comparison of bacterial suspension system and the bacterial immobilization system.

In this study, we evaluated the adsorption characteristics of the immobilized pellets, and then compared the difference in the inhibition of ammonium-nitrogen (NH_4^+-N) and nitrite-nitrogen (NO_2^--N) between the BSS and BIS. Moreover, the differences in growth characteristics of strain FC61 in the BSS and BIS and the optimal conditions were analyzed using response surface methodology (RSM). Finally, the above data were combined with scanning electronic microscopy (SEM) observations to analyze the differences

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between the BSS and BIS, in order to lay a basal foundation for their application in groundwater treatment.

2. Materials and methods

2.1. Media and the strain

All chemical reagents used are analytical grade without further purification. Solutions used in this experiment are prepared using ultra-pure water that is produced using a Milli-Q device (Millipore, USA). The basal medium used in this investigation is as follows: Glucose 0.15 (g/L), NH_4Cl 0.076 (g/L), KH_2PO_4 0.05 (g/L), Fe (III)-citrate 0.13 (g/L), $MgSO_4{\cdot}7H_2O$ 0.05 (g/L), $CaCl_2$ 0.05 (g/L), TE 2 mL. The composition of TE: $MgSO_4{\cdot}7H_2O$ 0.5 (g/L), EDTA 1 (g/L), $ZnSO_4$ 0.2 (g/L), $MnCl_2{\cdot}4H_2O$ 0.1 (g/L), $FeSO_4{\cdot}7H_2O$ 0.15 (g/L), $CuSO_4{\cdot}5H_2O$ 0.5 (g/L), $CoCl{\cdot}6H_2O$ 0.2 (g/L). The final pH of the mediums was adjusted to 7.0 by 1 mol/L NaOH or HCl solution. The strain FC61 was identified as *Klebsiella* sp. iron reducing bacterium with the ability of simultaneous Fe^{3+} reduction and ammonium oxidation under the anaerobic conditions in our previous study and the accession number of strain FC61 is KT860061 [15].

2.2. Experimental setup

BSS represents the bacterial suspension system, while BIS represents the bacterial immobilization system. In the course of the study in this paper, the BSS and BIS are in the same growth environment as well as the control group and three parallel groups. The bottles are 280 mL containing 250 mL medium, and all of the mediums are prepared by heating the solution to 121 °C in 30 min and immediately cooling it to room temperature under an anoxic atmosphere, and all the bottles are placed in an incubator at a constant temperature of 30 °C. The pellets in the BIS are made as follows: 2% (m/v) sodium alginate (SA) was mixed with strain FC61 or without it as 1:1 (v/v), then injected it into 2% (m/v) $CaCl_2$ with a syringe needle of diameter 2 mm to form a homogeneous pellets slowly and evenly. In this experiment, calcium alginate pellets which are added to the bottles in the BIS are 0.25 g (dry weight). The ratio of wet/dry for pellets is 9.92 ± 0.83. All operations are under sterile conditions.

2.3. Analytical methods for the experimental data

The concentration of Nitrate-N and Fe^{2+} are measured by the method of N-(1-naphthyl)-1, 2-diaminoethane dihydrochloride and 1,10-phenanthroline mono- hydrate [16]. The concentration of Fe^{3+} in the solution is evaluated as the difference between dissolved Fe^{2+} after and before reduction with excess hydroxylamine hydrochloride. Bacterial growth is determined by monitoring the optical density at 600 nm (OD_{600}) by spectrophotometry (DR 5000, HACH, USA) and pH (HQ11d, HACH, USA). The NH_4^+-N removal ratio is calculated using the formula $(1 - C_n/C_0) \times 100\%$. C_0 is the initial concentration of NH_4^+-N. C_n is the final concentration of NH_4^+-N at n hour.

2.4. Analytical methods for the dynamics of adsorption and self-inhibition

Adsorption equilibrium is studied by the method of shaking bottles: the bottles are 280 mL containing 250 mL medium with different concentration of Fe^{3+} and Fe^{2+} in solution, and 0.25 g (dry weight) calcium alginate pellets are added to the bottles containing with 48 h in the oscillator, the oscillation speed was 120 rpm. The equilibrium adsorption concentration (C_e) of Fe^{3+} and Fe^{2+} in solution can be obtained and according to the formula:

$$q_e = (C_0 - C_e) \cdot V/W \quad (1)$$

The q_e also can be calculated. Where the C_0 (mg/L) and C_e (mg/L) are the initial and equilibrium concentration of Fe^{3+} or Fe^{2+}, respectively. V is the volume (mL) of the solution and W is the mass (g) of calcium alginate pellets used in this study. And the models of Langmuir and Freundlich are used to evaluate the adsorption characteristics of the pellets, the equations are as follows:

$$\text{Langmuir adsorption } q_e = K_f C_e^{1/n} \quad (2)$$

$$\text{Freundlich adsorption } q_e = q_{max} \cdot K_1 \cdot C_e/1 + K_1 \cdot c_e \quad [17,18] \quad (3)$$

where q_e is the solid phase adsorbate concentration in equilibrium (mg/g), C_e is the equilibrium concentration (mg/L), K_l and K_f are the Langmuir and Freundlich constant, q_{max} is the maximum adsorption capacity for a single molecular layer on the surface of the pellets (mg/g), n is the Freundlich exponent.

Two equations are used in the comparison of the self inhibition models, which are as follows:

$$\text{Edwards } r = \frac{r_{max} S}{K_s + S + S^2/K_i} \quad (4)$$

$$\text{Aiba } r = r_{max}(\exp(-S/K_i) - \exp(-S/K_S)) \quad [19] \quad (5)$$

In this investigation, r is the removal rate of NH_4^+-N or NO_2^--N (mg/L/h), r_{max} is the maximum removal rate which is fitted by the equations (mg/L/h), K_S and Ki are the half-saturation and inhibition constant, respectively (mg/L), S is the concentration of NH_4^+-N or NO_2^--N (mg/L).

2.5. Response surface experimental design

In order to compare the difference of NH_4^+-N removal between the BSS and BIS under the same conditions, the time of measuring the sample is set at the same time point of 48 h. And three effective variables are setup: pH (4–8), temperature (20–40 °C), the concentration of initial Fe^{3+} (5–35 mg/L). Specific experimental was setup as shown in Table 3. Design-Expert (version 8.06) software is used for the statistical design of experiments and data analysis. The response variable (NH_4^+-N removal ratio, %) are fitted with a full quadratic model:

$$Y = \beta_0 + \sum \beta_i \alpha_i + \sum \beta_{ii} \alpha_i^2 + \sum \beta_{ij} \alpha_i \alpha_j. \quad (6)$$

where Y is the response variable, i, j, β_0, β_i, β_{ii}, and β_{ij} are coefficients of the intercept, linear, square, and interaction effects, α_i and α_j are coded independent variables.

2.6. Methods for studying the characteristics of strain FC61 in the BSS and BIS

At the time point of 48 h, the sample is taken out from the bottle of the BSS, and the sample is centrifuged at 9000 rpm 5 min, then the supernatant is discarded, and 2.5% glutaraldehyde fixed liquid is poured into the centrifuge tube. After 4 h, centrifugal supernatant is discarded, adding the solution of phosphoric acid buffer. And the samples are dehydrated using a series of different concentrations of ethanol (30%, 50%, 70%, 80%, 90%, 100%) followed by drying, finally the isoamyl acetate is added until the sample has been dried which can be observed by using the scanning electron microscope (SEM). Similarly, at the same time point of 48 h, the pellets are taken out from the BSS, followed by the residual water is filtered. Other operations are the same as the bacteria in the suspended state.