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Influencing factors of immobilized bacteria particles for improving denitrification efficiency

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ABSTRACT

The aim of this study was to investigate the factors which influence the denitrification performance of immobilized denitrifying bacteria. Single variable control method was used and it was found that the immobilized denitrifying bacteria particles had the best denitrification performance under these conditions: bacteria addition proportion in the immobilized particles was 15% and with a particle size of 3–4 mm, immobilized particles adding amount was 20%, and using glucose as external carbon sources with carbon–nitrogen ratios of 3.0–3.5 and hydraulic residence time over 6 h under the temperature of 25°C–30°C. This study suggested that a better denitrification effect could be achieved by extending the hydraulic retention time when other environmental factors are difficult to control. The information obtained in this study may help to put the microbial immobilized technology a step forward in wastewater treatment application.

Keywords: Denitrification; Nitrate; Immobilized bacteria; Influence factor

1. Introduction

Denitrification is a biochemical process that denitrifying bacteria convert NO_3^-–N into N_2 under anaerobic conditions [1–3], biological denitrification has become a common technology in wastewater treatment [4,5]. Activate sludge contains a variety of bacteria [6,7] and it is commonly used in wastewater treatment plants for denitrification as a microbial carrier [8,9]. However, activated sludge is easily washed away, so the efficiency of wastewater treatment is greatly affected by water quality fluctuations [10], and some other environmental factors also affect the denitrification performance.

Microbial immobilization technology uses physical or chemical method to confine free bacteria in a specific space and maintain biological activity [11], due to its advantages [12–14] such as large biomass, high wastewater treatment efficiency, and strong impact load resistance, microbial immobilization technology has attracted wide attention from researchers [15–19]. Although there have been many reports on the influence of various parameters in the process of denitrification [20–22], however, most of them are about the denitrification of activated sludge [23,24] or granular sludge [25,26]. At present, there are few reports on the influence factors of immobilized bacteria in denitrification.

In this study, the factors that influence the denitrification of immobilized denitrifying bacteria were investigated, such as sludge added proportion, size and number of immobilized particles, carbon source types, and carbon–nitrogen ratios, temperature, and hydraulic retention time (HRT), etc. Through these investigations, the denitrification process of immobilized denitrifying bacteria particles was optimized, and we hope this study could promote the application of microbial immobilized technology in wastewater treatment.

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2. Material and methods

2.1. Preparation of immobilized particles

A certain amount of cultured sludge which contains denitrifying bacteria [27] was mixed with an appropriate amount of waterborne polyurethane and deionized water, and 1% (w/v) potassium persulfate solution and 0.5% (w/v) N,N,N,N-tetramethylethylenediamine solution were added to initiate polymerization. The mixture was reacted at 27°C ± 2°C for 5–10 min to form a gelatinous solid, then cut into cubes of 3 mm × 3 mm × 3 mm, and cultured in a solution of sodium nitrate and glucose to enable denitrifying bacteria to proliferate in the immobilized particles.

2.2. Experimental setup

Glass beaker was used as a laboratory-scale denitrification bioreactor with an effective volume of 1 L (ϕ = 12 cm, h = 15 cm) in this study, the immobilized particles were mixed evenly in the bioreactor with a constant speed stirrer (Fig. 1). Single variable control method was used to investigate the factors which influence the denitrification performance such as sludge added proportion, size and number of immobilized particles, carbon source types, carbon and nitrogen ratio, temperature, and HRT, etc. To investigate the effects of immobilized particle size on denitrification performance, gel solidified, and cut into different sizes. In this study, the denitrification performance of immobilized particles with particle size <2 mm, 3–4 mm, 5–6, and 6–10 mm was investigated. Cultured sludge [27], as a carrier of bacteria, was often added to immobilized particles to provide bacteria. In order to investigate the influence of sludge added proportion in immobilized particles on denitrification effect, we investigated the sludge added proportion of 5%, 10%, 15%, 20%, 30%, and 40%, respectively. The immobilized particles amount of 5%, 10%, 15%, 20%, 30%, and 40% were studied for investigating the effect of immobilized particles amount on denitrification performance, and the temperature was set at 8°C, 12°C, 20°C, 25°C, 30°C, 35°C, and 40°C, respectively when investigating the influence of environment temperature. In order to investigate the effect of carbon source types on denitrification performance of immobilized particles, methanol, glucose, sodium acetate, starch, and ethanol were used as carbon source, respectively. Specific experimental settings are shown in Table 1.

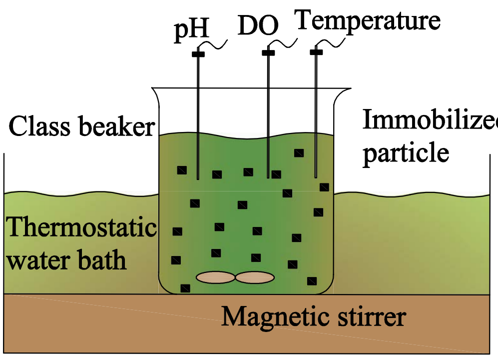

Fig. 1. Schematic diagram of the immobilized bacteria denitrification bioreactor.

The major characteristics of the influent simulated wastewater include KH_2PO_4 0.5 g/L, $MgSO_4$ 0.5 g/L, $ZnSO_4 \cdot 7H_2O$ 0.3 g/L, $CaCl_2 \cdot 2H_2O$ 0.3 g/L, $FeCl_2 \cdot 2H_2O$ 0.2 g/L, NO_3^-–N, and carbon source (chemical oxygen demand, COD). NO_3^-–N and COD were provided by sodium nitrate and glucose, respectively.

2.3. Analytical methods

During the experiment, water samples in influent and effluent were collected and the concentration of NO_3^-–N, NO_2^-–N, and COD were measured daily following the standard methods of water and wastewater analyses [28]. Before the analysis of the above parameters in liquid, samples were membrane filtered (0.45 μm), and all tests were repeated at least twice. Temperature, pH, and DO were determined using a WTW analyzer (Multi3620ids, Germany).

3. Results and discussion

3.1. Effect of immobilized particles size on denitrification performance

The size of particles affects the mass transfer efficiency [29], such as microbial metabolites and nutrient substrate [30]. As can be seen from Fig. 2, the smaller the size of immobilized particles, the higher the nitrate removal efficiency, and the lower the nitrite accumulation, as a result the better the denitrification performance. This is consistent with other studies, Xia et al. [31] reported that nitrification–denitrification rate was negatively related to particle size, and denitrification rate constants increased with decreasing suspended sediment particle size. In this study, when the immobilized particle size was below 4 mm, the nitrate removal rate was over 80%.

With the increase in particles size, the mass transfer resistance of nutrient substrates increases [32], and the NO_3^- transfer path becomes longer, resulting in less NO_3^- and nutrient substrates in the center area of particles [33]. Meanwhile, the larger of the immobilized particles, the smaller the relative specific surface area, and the probability of denitrifying bacteria contacting with the nutrient substrate and NO_3^- in the inner central area of the immobilized particles decreased, so that the denitrification efficiency decreased and the nitrate removal rate was affected [32]. Although the nitrate removal efficiency was better when the immobilized particle size <2 mm, but the particle was too small and easy to lose, which is not conducive to engineering application. When the size of immobilized particles was larger than 5 mm, the effect of wastewater treatment became worse. Considering the influence of mass transfer and the effect of wastewater treatment efficiency, we think that 3–4 mm was the ideal particle size, and the immobilized particles of this size have better mass transfer and permeability, which was more conducive to denitrification.

3.2. Effect of sludge added proportion in immobilized particles on denitrification performance

Microorganisms were the key factors of wastewater treatment [34], and the number of bacteria in immobilized

Table 1
Specific experimental settings of immobilized bacteria particles denitrification influencing factors

Serial number	Particles size (mm)	Sludge added proportion (%)	Particles amount (%)	Temperature (°C)	Carbon source type	C/N ratios	HRT (h)
1	<2, 3–4, 5–6, >6	15	20	27 ± 1	Glucose	3.5	6
2	3–4	5, 10, 15, 20, 30, 40	20%	27 ± 1	Glucose	3.5	6
3	3–4	15	5, 10, 15, 20, 30, 40	27 ± 1	Glucose	3.5	6
4	3–4	15	20	8, 12, 20, 25, 30, 35, 40	Glucose	3.5	6
5	3–4	15	20	27 ± 1	Methanol, glucose, sodium acetate, starch, ethanol	3.5	6
6	3–4	15	20	27 ± 1	Glucose	1, 2, 3, 3.5, 4, 5	6
7	3–4	15	20	27 ± 1	Glucose	3.5	4, 5, 6, 7, 8, 9

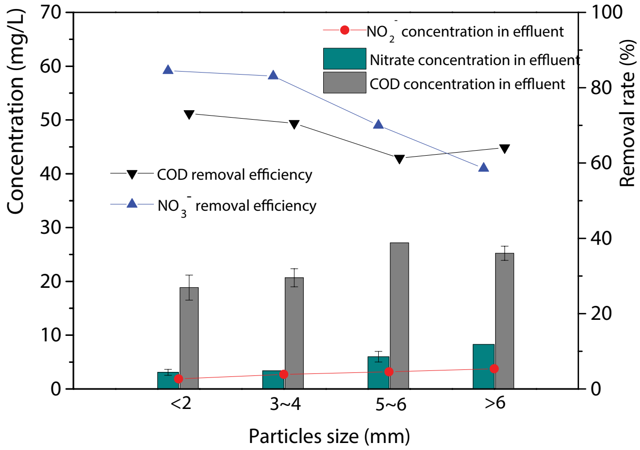

Fig. 2. Changes of concentration and removal efficiency under different immobilized particles size.

particles directly affects the efficiency of denitrification. As can be seen from Fig. 3, within the range of 5%–20%, the higher the denitrification sludge proportion in the immobilized particles, the higher the nitrate removal rate, and the better denitrification performance. When the proportion of sludge in the immobilized particles exceeded 20%, the nitrate removal efficiency did not change significantly.

According to the investigation in this study, in a certain range, with the increase of the denitrifying sludge proportion, the number of denitrifying bacteria in the immobilized particles increased, and the denitrification efficiency of immobilized particles increased. When the number of bacteria in the immobilized particles increased to a certain extent, the wastewater treatment efficiency was no longer significantly improved because the space inside the particles was limited and bacteria could not proliferate indefinitely. When there was too much sludge in the immobilized particles (>30%), the particle density will be increased which will affect the physical properties such as mass transfer and particle strength. When the sludge was too less (<10%), the biomass in the immobilized particles was insufficient, which affects the efficiency of wastewater treatment. Therefore, the sludge addition proportion was 10%–15%, which not only meets the needs of wastewater treatment, but also does not affect the physical properties of immobilized particles.

3.3. Effect of immobilized particles amount on denitrification performance

When the sludge added proportion in immobilized particles was determined, the number of immobilized particles directly affected the biomass of microorganisms in the reactor. Fig. 4 shows that the higher removal rate was achieved when the number of immobilized particles increased. These results demonstrated that better denitrification performance achieved with the increase of immobilized particles. When the number of particles was less than 10%, the denitrification performance was poor and the wastewater treatment effect was not ideal. when the amount was too large (>40%), it will affect the flowing state of immobilized particles in the

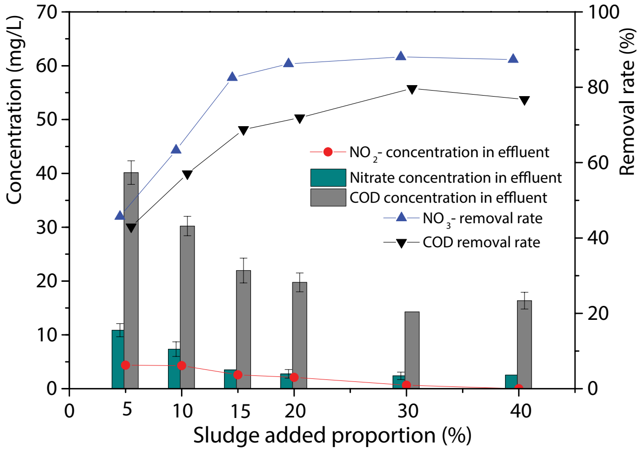

Fig. 3. Changes of concentration and removal efficiency under different sludge added proportion in immobilized particles.

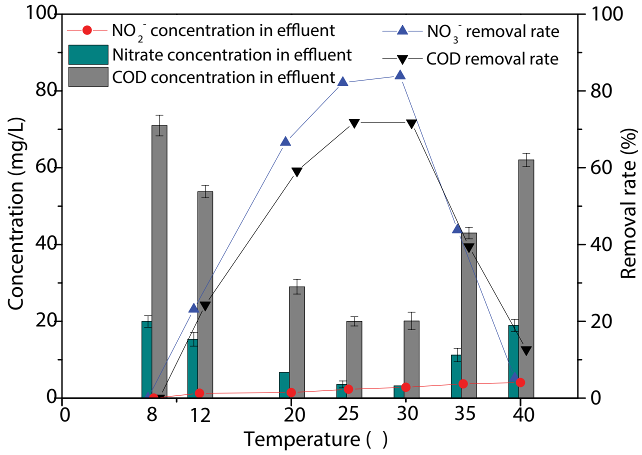

Fig. 5. Changes of concentration and removal efficiency under different Environment temperature.

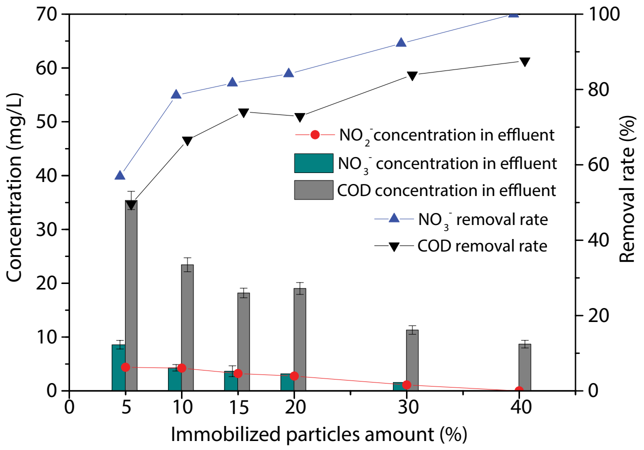

Fig. 4. Changes of concentration and removal efficiency under different immobilized particles amount.

reactor, and increase the friction between immobilized particles, further affect the life of immobilized particles. Therefore, the immobilized particles amount was 10%–20% can meet the actual needs of wastewater treatment.

3.4. Effect of temperature on denitrification performance

Environmental temperature affects the physiological activity and enzyme activity of microorganisms [35,36], and the optimum temperature can accelerate the enzymatic reaction in microorganisms [37]. Therefore, within a certain range, the denitrification performance of immobilized particles was directly related to the temperature. According to Fig. 5, the temperature has a significant effect on the denitrification performance of immobilized particles. When the temperature was lower than 20°C, the removal rate of nitrate was low, and the denitrification effect of immobilized particles was poor, especially when it was lower than 8°C, immobilized particles almost did not work. When the temperature was 25°C or 30°C, the nitrate removal efficiency was higher, which was consistent with the optimal environmental temperature of denitrifying bacteria.

It was reported that when the temperature was too low [38], the bacteria was in a dormant state and the denitrifying bacteria almost stopped their life activities. When the temperature exceeded 30°C, the denitrification performance of immobilized particles decreased, and bacteria inactivation will affect the efficiency of wastewater treatment, and the high temperature will have a certain impact on the structure of immobilized materials. This study found that when the immobilized bacteria were at 40°C for a long time, the hardness of the immobilized bacteria decreases and the immobilized bacteria were easy to break, which was not conducive to its application. Through this study, we suggest that the optimal temperature of denitrification immobilized particles was 25°C–30°C.

3.5. Effects of carbon source types on denitrification performance

Most denitrifying bacteria were heterotrophic denitrifying bacteria [39], which used organic carbon for denitrification through different respiratory pathways [21,40]. Organic carbon sources were used as electron donors in denitrification and for cell growth of denitrifying bacteria [41]. In the process of heterotrophic denitrification, different types of carbon sources showed different denitrification efficiency. It can be seen from Fig. 6 that the denitrification efficiency of immobilized particles varies greatly with different carbon sources. When glucose was used as the carbon source [42], nitrate removal was more complete and the effect of wastewater treatment was better.

Denitrification takes a long time to come into effect when methanol was used as a carbon source, meanwhile, methanol was toxic to some microorganisms [43], which is not conducive to the growth of denitrifying microorganisms. Since ethanol was not toxic, it often used as a substitute for methanol [44], but this study found that immobilized bacteria using ethanol as an additional carbon source for denitrification was not ideal. The denitrification performance of the reactor using starch as a carbon source was poor in this study. We suspected that it might be related to the large molecular

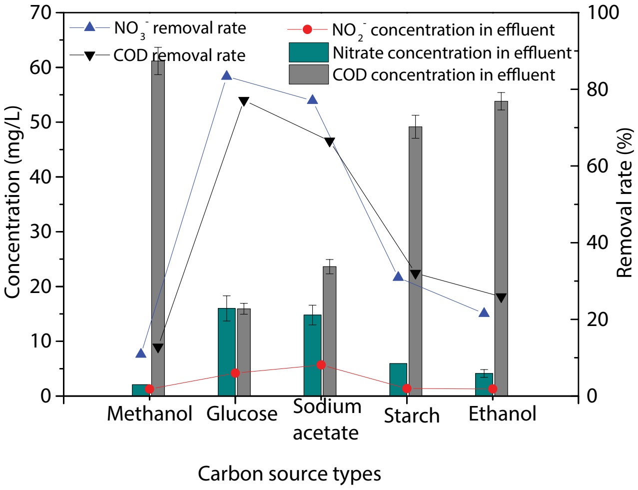

Fig. 6. Changes of concentration and removal efficiency under different carbon source types.

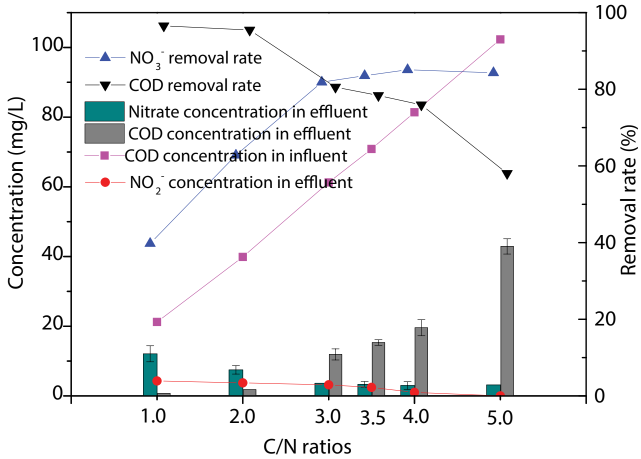

Fig. 7. Changes of concentration and removal efficiency under different carbon–nitrogen ratios.

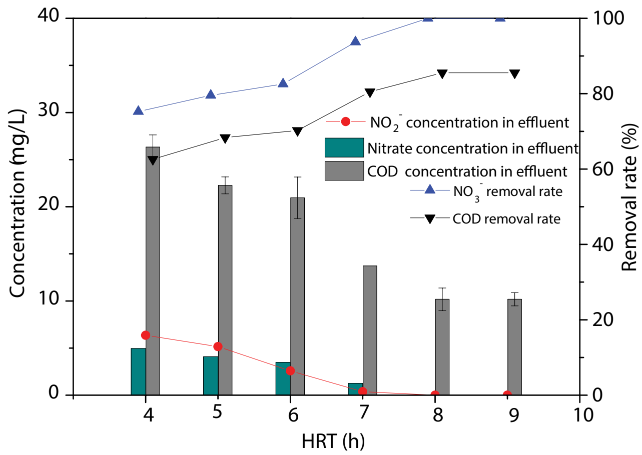

Fig. 8. Changes of concentration and removal efficiency under different hydraulic retention time.

weight of starch, which was not easy to pass through the pores of immobilized particles. In this study, the denitrification effect of the reactor with sodium acetate was second only to that of glucose, which could be used as a substitute for glucose. In the practical application of immobilized particles, we suggest that the best carbon source should be added according to different water quality, and the organic matter with no toxicity or low toxicity should be selected as the carbon source for denitrification.

3.6. Effect of carbon–nitrogen ratios on denitrification performance

Organic carbon was an essential material and energy source for the growth and metabolism of heterotrophic denitrifying bacteria [43,45], when biodegradable organic carbon compounds in wastewater were insufficient, additional carbon sources were needed to make up for the lack of carbon sources in influent [20]. In this study, the denitrification performance of immobilized particles was investigated when the influent carbon–nitrogen ratios were 1.0, 2.0, 3.0, 3.5, 4.0, and 5.0 with glucose as the added carbon source and nitrate concentration of 20 mg/L. It was found that (Fig. 7) nitrate removal efficiency increased with the increase of carbon–nitrogen ratio, and the removal efficiency increased slowly when it exceeded 3.5. Suitable C/N ratios can make the denitrification process run economically and efficiently [46]. Excessive C/N ratio was easy to cause resource waste; meanwhile, denitrification cannot be fully carried out and denitrification efficiency was reduced with low C/N ratios [47]. In this study, it was found that C/N ratio was 3–3.5 could meet the demand of denitrification.

3.7. Effect of HRT on denitrification performance

HRT affects the contact time between wastewater and immobilized particles [48], it determines the treatment effect of wastewater by affecting the reaction time, and it plays a very important role in the denitrification process [36,49]. In this study, the effect of HRT at 4, 5, 6, 7, 8, 9 h was investigated. As can be seen from Fig. 8, with the extension of HRT, the nitrate removal efficiency increased, and all the nitrate of 20 mg/L could be removed by denitrification after about 8 h. It was found that when other environmental factors were not easy to control, better denitrification effect could be achieved by extending the HRT.

4. Conclusions

Through the adoption of single-variable control method, this study found that the immobilized denitrifying bacteria particles had the best denitrification performance under these conditions: bacteria addition proportion in the immobilized particles was 15% and with a particle size of 3–4 mm, immobilized particles adding amount was 20%, and using glucose as external carbon sources with carbon–nitrogen ratios of 3.0–3.5 and hydraulic residence time over 6 h under the temperature of 25°C–30°C. When other environmental factors are difficult to control, a better denitrification effect could be achieved by extending the

HRT. We hope this study could promote the application of immobilized technology in wastewater treatment.

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