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Immobilization of ammonia-oxidizing bacteria by polyvinyl alcohol and sodium alginate

Yuwei Dong[a,b,*], Yanqiu Zhang[b], Baojun Tu[a,b]

[a] Xuzhou University of Technology, Xuzhou, PR China
[b] China University of Mining and Technology, Xuzhou, PR China

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ABSTRACT

Ammonia-oxidizing bacteria were immobilized by polyvinyl alcohol (PVA) and sodium alginate. The immobilization conditions and ammonia oxidation ability of the immobilized bacteria were investigated. The following immobilization conditions were observed to be optimal: PVA, 12%; sodium alginate, 1.1%; calcium chloride, 1.0%; inoculum concentration, 1.3 immobilized balls/mL of immobilized medium; pH, 10; and temperature, 30 °C. The immobilized ammonia-oxidizing bacteria exhibited strong ammonia oxidation ability even after being recycled four times. The ammonia nitrogen removal rate of the immobilized ammonia-oxidizing bacteria reached 90.30% under the optimal immobilization conditions. When compared with ammonia-oxidizing bacteria immobilized by sodium alginate alone, the bacteria immobilized by PVA and sodium alginate were superior with respect to pH resistance, the number of reuses, material cost, heat resistance, and ammonia oxidation ability.

Introduction

Ammonia-oxidizing bacteria belong to one of the physiological subsets of the nitrifying bacteria family.[1] These bacteria play a role in the first rate-limiting step of nitrification[2,3] and in the oxidation of amines to nitrites and are widely used in the denitrification of industrial wastewater and soil.[4,5] However, because ammonia-oxidizing bacteria are autotrophic, they have a long generation time, slow growth rate, and high sensitivity, and they can easily be eliminated.[6] To address these limitations, cell immobilization technology has been developed.[7] Immobilized cell technology offers distinct advantages and combines liquid fermentation and immobilized enzymes. Immobilized cells exhibit improved catalytic activity, reduced production time, low production cost, high yield, and extensive application prospects.[8,9] Many raw materials and synthetic polymers such as sodium alginate, polyacrylamide, agar, and polyvinyl alcohol (PVA) have been extensively applied in cell immobilization.[10–12] In the

* *Corresponding author at:* Xuzhou University of Technology, Xuzhou, PR China.
E-mail: 258210902@qq.com (Y. Dong).

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present study, ammonia-oxidizing bacteria were immobilized by PVA and sodium alginate to improve their characteristics and applications.

Materials and methods

Strains

The ammonia-oxidizing bacteria used in this study were screened from activated sludge collected from a sewage treatment plant at China University of Mining and Technology (Xuzhou, China) and exhibited 98% homology with *Nitrosomonas* sp. GH22.

Culture media

The simulation sewage medium (pH 8.0–8.2) comprised 4 g/L $(NH_4)_2SO_4$, 1 g/L K_2HPO_4, 0.5 g/L $MgSO_4$, 2 g/L NaCl, 0.4 g/L $FeSO_4$ and 10 g/L $CaCO_3$. The immobilized medium (pH 7.0–7.2) contained 0.8 g/L $(NH_4)_2SO_4$, 1 g/L K_2HPO_4, 0.5 g/L $MgSO_4$, 0.4 g/L $FeSO_4$ and 10 g/L $CaCO_3$.[13,14]

Preparation of immobilized ammonia-oxidizing bacteria

The ammonia-oxidizing bacteria were cultured in simulated sewage medium. After the culture was allowed to stand for a certain period, the supernatant was discarded. Subsequently, PVA and sodium alginate were added to the culture at a PVA/sodium alginate culture ratio of 3:1 (v/v). The culture mixture was added to calcium chloride using an injector, and the bacteria were immobilized at 4 °C for 6 h. Subsequently, the immobilized bacteria were washed with deionized water and inoculated into 250–1000 mL of immobilized medium at a concentration of 1–2 immobilized balls/mL of immobilized medium at 30 °C and 100 r/min for 16–20 days.

Determination of nitrate nitrogen and ammonia nitrogen contents

The nitrite nitrogen content was determined by α-naphthylamine spectrophotometry and phenol disulfonic acid spectrophotometry, whereas the ammonia nitrogen content was determined using phenol disulfonic acid.

Results

Effect of PVA concentration on the formation of immobilized balls

To determine the effect of PVA concentration on the formation of immobilized balls, the following conditions were applied: 1% sodium alginate; 2.0% calcium chloride; and 6.0%, 8.0%, 10.0%, 12.0%, and 14.0% PVA.

The results showed that at PVA concentrations less than 10%, the immobilized balls were transparent and soft, whereas at concentrations higher than 14.0%, the immobilized balls were opaque, hard, and trailing. A PVA concentration of 12% produced immobilized balls that showed better transparency and hardness.

Effect of sodium alginate concentration on the formation of immobilized balls

To determine the effect of sodium alginate concentration on the formation of immobilized balls, the following conditions were employed: 2.0% calcium chloride; 12% PVA; and 0.8%, 0.9%, 1.0%, 1.1%, and 1.2% sodium alginate.

The results showed that at sodium alginate concentrations less than 1.0%, the immobilized balls were transparent and soft, whereas at concentrations higher than 1.2%, the immobilized balls were opaque, hard, and trailing. A sodium alginate concentration of 1.1% produced immobilized balls that showed better transparency and hardness.

Effect of calcium chloride concentration on the formation of immobilized balls

To determine the effect of calcium chloride concentration on the formation of immobilized balls, the following immobilization conditions were employed: 1.1% sodium alginate, 12% PVA, and 1.0%, 2.0%, 3.0%, 4.0%, and 5.0% calcium chloride.

The results showed that at calcium chloride concentrations higher than 2%, the immobilized balls were opaque, hard, and trailing, whereas at a concentration of 1.0%, the immobilized balls exhibited better transparency and hardness.

Effect of sodium alginate concentration on the ammonia nitrogen removal ability of immobilized ammonia-oxidizing bacteria

To determine the effect of sodium alginate concentration on the ammonia nitrogen removal ability of immobilized ammonia-oxidizing bacteria, the following immobilization conditions were employed: 1.0% calcium chloride; 12% PVA; 0.8%, 0.9%, 1.0%, 1.1%, and 1.2% sodium alginate; and 1.3 immobilized balls/mL of immobilized medium. The reaction was performed in a 250-mL shake flask containing 250 mL of immobilized medium of pH 8.0 maintained at 30 °C and 120 r/min for 16 days. The ammonia nitrogen removal ability of the immobilized ammonia-oxidizing bacteria under the above-mentioned conditions is shown in Fig. 1.

The concentration of sodium alginate was observed to affect the hardness of immobilized cells. The higher the concentration of sodium alginate was, the greater the strength of immobilized cells became. However, if the concentration was too high, immobilized cells would not grow, and if the concentration was too low, immobilized balls would break. Furthermore, the nitrite nitrogen concentration was observed to increase with increasing cultivation time. The optimal sodium alginate concentration was 1.1%, which produced the highest nitrite nitrogen concentration.

Effect of PVA concentration on the ammonia nitrogen removal ability of immobilized ammonia-oxidizing bacteria

The higher the concentration of polyvinyl alcohol was, the greater the strength of the immobilized cells became. However, increased viscosity made the operation more difficult and detrimental to matrix transfer and the growth

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