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# Optimization of Entrapping Conditions of Nitrifying Bacteria and Selection of Entrapping Agent

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*Abstract*—**Sodium alginate and polyvinyl alcohol were selected as entrapping agents to immobilize nitrifying microbia. Through orthogonal tests, optimal conditions of entrapping nitrifying bacteria by the two agents were determined by ammonia nitrogen removal efficiency. The properties of the two immobilized microbial beads produced under their optimum embedding conditions were compared. Results indicate that the optimum embedding conditions of calcium alginate beads (briefly CA beads) were 10ml of nitrifying bacteria, 4% of sodium alginate content, 3mm in bead diameter and 24-hr crosslinking time. The optimum embedding conditions of polyvinyl alcohol beads (briefly PVA beads) were 10ml of nitrifying bacteria, 10% of PVA content, 3mm in bead diameter and 24-hr crosslinking time. Comprehensive comparisons on mechanical strength, stability, mass transfer property and ammonia nitrogen removal efficiency of the two immobilized microbial beads indicate that polyvinyl alcohol is a better embedding agent for nitrifying bacteria in wastewater treatment compared with sodium alginate.**

***Keywords-immobilized microorganism; embedding agent; nitrifying bacteria; sodium alginate; calcium alginate bead (CA bead); polyvinyl alcohol bead (PVA bead)***

## I. INTRODUCTION

Treatment technology using entrapped microorganisms is a novel biological engineering technology developed in recent years. Compared with the traditional biological engineering technologies, the advantages of this method in wastewater treatment and waterbody remediation are higher biomass, higher treatment efficiency, less space requirement of the processing devices and less sludge production, etc. [1] Microbial immobilization technologies include adsorption method, entrapping method, crosslinking method and medium intercept method. Among the methods, entrapping method, which forms the gel using polymer and embeds the microorganisms in the gel, is most widely used in recent investigations since it is simpler and has less influence on cell viability. [2] To choose the appropriate entrapping agents and determine the optimal entrapping conditions are important in entrapping of microbial agents. Various entraping agents (carriers) were investigated, such as natural polymer gels including AGAR, carrageenan and sodium alginate, etc. and organic synthetic polymer carriers including polyvinyl alcohol, and polyacrylamide, etc. [3] To produce high quality immobilized bacteria requires high quality immobilized agents. High quality entrapping agent should have advantages as good water-soluble, little loss of bacteria activity, good mass transfer property, good mechanical property, good chemical stability, good resistance to biodegradation, cheap in cost and easy shaping, etc. [4] AGAR, carrageenan, sodium alginate, polyvinyl alcohol, polyacrylamide and other carriers commonly used are compared [1-2]. Sodium alginate and polyvinyl alcohol were considered to be easy immobilization, high mechanical strength, small toxicity to organisms, strong resistance to biodegradation and low cost, etc. However, the properties and optimal conditions of the immobilized nitrifying microbial beads by the two embedding agents still need to be evaluated.

Sodium alginate (briefly SA) and polyvinyl alcohol (briefly PVA) were selected as embedding agents. Compared with the microbial beads embedded by only PVA agent, addition of powder activated carbon into PVA can increase the mechanical strength of the embedding agent, expand pore size of beads and improve the efficiency and life of the embedded bacteria. Moreover, the adsorption ability of PVA beads to pollutants can be increased and the immobilized microorganisms have stronger resistance to the change of water quality and stronger ability of decomposing persistent pollutants. [5] Therefore, the mixture of PVA and powder activated carbon (1% in weight) instead of single PVA was used as the embedding agent of nitrifying bacteria in this study.

$CaCl_2$ was used to be the crosslinking agent. Because the $Ca^{2+}$ concentration affects the activity and density of immobilized cells, the concentration of $CaCl_2$ was suggested in a range of 1% to 4%. [6-7] Calcium alginate gel beads (briefly CA beads) and polyvinyl alcohol gel beads (briefly PVA beads) were produced during dosing with sodium alginate and polyvinyl alcohol into calcium chloride solution, respectively. [8]

The main objectives of this investigation are to determine the optimal entrapping conditions of the calcium alginate beads and polyvinyl alcohol beads through ammonia nitrogen removal efficiency of designed orthogonal tests [9] and to evaluate the optimal method of embedding nitrifying bacteria through comprehensive comparisons on embedding difficulty, mechanical strength, stability and cost of the two embedded beads.

## II. MATERIALS AND METHODOLOGY

### A. Experimental Strains and Analysis Method

Strains used in this study were nitrifying bacteria. After cultivation and enrichment, nitrifying bacteria concentration were approximately $7.25 \times 10^8$cfu/ml.

The amount of nitrifying bacteria was determined by method of dilution plate notation [10].

### B. Processing Methods of Immobilized Cells

Embedding agent was dissolved by distilled water under heating. After the embedding agent solution cooled to around 20℃, nitrifying bacteria solution was added into and mixed evenly with the cooled embedding agent solution. Then, the mixture was added into crosslinking agent (4% $CaCl_2$ solution) drop by drop using a pipette to process embedded nitrifying bacteria beads. After crosslinking for around 20 hours, the processed beads were taken out, washed with distilled water two to three times, and dried the surface moisture with filter paper for use.

## III. RESULTS AND DISCUSSION

### A. Optimal Entrapping Conditions of CA beads

DESIGN OF ORTHOGONAL EXPERIMENTS AND THE EXPERIMENTAL RESULTS FOR CA BEADS

| | Factors and Levels | | | | $NH_4^+$-N removal efficiency in 24 hr (%) |
|---|---|---|---|---|---|
| | SA content (%) | embedding bacteria (ml) | bead diameter (mm) | crosslinking time (hr) | |
| 1 | 2 | 0.1 | 3 | 12 | 15.43 |
| 2 | 2 | 1 | 5 | 24 | 28.61 |
| 3 | 2 | 10 | 7 | 36 | 33.18 |
| 4 | 4 | 0.1 | 5 | 36 | 12.57 |
| 5 | 4 | 1 | 7 | 12 | 23.85 |
| 6 | 4 | 10 | 3 | 24 | 47.78 |
| 7 | 6 | 0.1 | 7 | 24 | 11.09 |
| 8 | 6 | 1 | 3 | 36 | 20.69 |
| 9 | 6 | 10 | 5 | 12 | 32.23 |
| $K_1$ | 77.22 | 39.09 | 83.90 | 71.52 | |
| $K_2$ | 84.21 | 73.14 | 73.41 | 87.48 | |
| $K_3$ | 64.02 | 113.19 | 68.13 | 66.45 | |
| R | 6.730 | 24.700 | 5.260 | 7.013 | |

Orthogonal tests with four factors and three levels were used to determine the optimal entrapping conditions of CA beads. Four factors were sodium alginate content, embedding bacteria content, diameter of beads and crosslinking time. Average removal efficiency of ammonia nitrogen in 24 hours was used as the evaluation criterion. The levels of four factors were referred to the related researches [6, 11-13]. The design of orthogonal tests and the experimental results for CA beads are shown in Table I.

Experimental results of Table I show that $NH_4^+$-N removal efficiency in 24 hours of the No. 6 test is the highest. The corresponding levels of the four investigated factors of the No. 6 test should be the optimal conditions for CA bead processing, i.e. 4% of sodium alginate content, 10ml of embedding bacteria, 3mm in bead diameter and 24-hr crosslinking time.

K values in Table I reflect the relation of each factor with $NH_4^+$-N removal efficiency under the same levels of other factors. A larger K value indicates a better level of the corresponding factor. Based on the K values in Table I, the optimal conditions of proceeding CA beads are 4% of sodium alginate content ($K_2$), 10ml of embedding bacteria ($K_3$), 3mm in bead diameter ($K_1$) and 24-hr crosslinking time ($K_2$). This conclusion is well conform to the experimental result of the No. 6 test.

R values in Table I reflect changing amplitude of experimental results with the change of investigated factors. A larger R value indicates a significant influence of the factor on the criterion. Based on the R values, the importance in series (from major to minor) of the four investigated factors are embedding bacteria content, crosslinking time, sodium alginate content and bead diameter. That is embedding bacteria content, which is the primary factor, has the most significant influence on $NH_4^+$-N removal efficiency in the four factors.

### B. Optimal Entrapping Conditions of PVA Beads

DESIGN OF ORTHOGONAL EXPERIMENTS AND THE EXPERIMENTAL RESULTS FOR PVA BEADS

| | Factors and Levels | | | | $NH_4^+$-N removal efficiency in 24 hr (%) |
|---|---|---|---|---|---|
| | PVA content (%) | embedding bacteria (ml) | bead diameter (mm) | crosslinking time (hr) | |
| 1 | 8 | 0.1 | 3 | 18 | 24.15 |
| 2 | 8 | 1 | 5 | 24 | 29.37 |
| 3 | 8 | 10 | 7 | 30 | 31.06 |
| 4 | 10 | 0.1 | 5 | 30 | 22.39 |
| 5 | 10 | 1 | 7 | 18 | 27.82 |
| 6 | 10 | 10 | 3 | 24 | 42.65 |
| 7 | 12 | 0.1 | 7 | 24 | 19.87 |
| 8 | 12 | 1 | 3 | 30 | 22.42 |
| 9 | 12 | 10 | 5 | 18 | 36.75 |
| $K_1$ | 84.57 | 66.42 | 89.22 | 88.71 | |
| $K_2$ | 92.85 | 79.62 | 79.50 | 91.89 | |
| $K_3$ | 79.05 | 110.46 | 78.75 | 78.90 | |
| R | 4.61 | 14.68 | 3.49 | 5.34 | |

Orthogonal tests with four factors and three levels were used to determine the optimal entrapping conditions of PVA beads. Four factors were PVA content, embedding bacteria content, diameter of beads and crosslinking time. Average removal efficiency of ammonia nitrogen in 24 hours was used as the evaluation criterion. The levels of four factors were referred to the related researches [12-14]. The design of orthogonal tests and the experimental results for PVA beads are shown in Table II.

The analysis method of Table II is the same as that of Table I. According to the K values in Table II and the experimental results of 24-hr ammonia nitrogen removal efficiency, the optimal conditions of proceeding PVA beads are 10% of PVA content, 10ml of embedding bacteria, 3mm in bead diameter and 24-hr crosslinking time (the conditions of the No. 6 test).

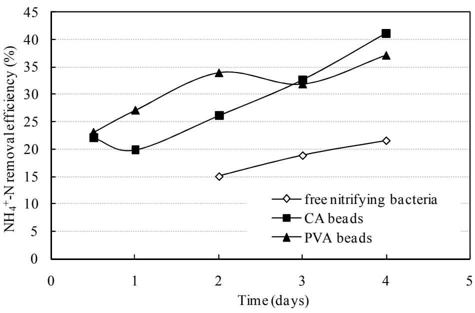
Based on the R values, the importance in series (from major to minor) of the four investigated factors are embedding bacteria content, crosslinking time, PVA content and bead diameter.

### C. Comparisons of the Two Immobilized Cells

CA beads and PVA beads were processed based on the methods described in section II-B under their optimal entrapping conditions obtained in section III-A and III-B. 25g CA beads and 25g PVA beads, in which 10ml nitrifying bacteria was embedded respectively, were taken to conduct the following tests.

*1) Comparisons of degradation property*

25g CA beads (10ml nitrifying bacteria embedded), 25g PVA beads (10ml nitrifying bacteria embedded) and 10ml free nitrifying bacteria were dosed into 150ml synthetic wastewater (with its quality close to the polluted water of Waihuan River, Tianjin), respectively. To speed up the degradation process, the experiment were conducted under around 25℃ and well mixing. Ammonia nitrogen removal efficiency against time was shown in Figure 1.




Removal efficiency of $NH_4^+$-N against time

Figure 1 shows that the free nitrifying bacteria had higher ammonia nitrogen removal efficiency compared with the immobilized nitrifying bacteria in the first two days of dosing into wastewater. This is believed the mass transfer of the free bacteria was better than that of the immobilized bacteria in the first two days. However, the immobilized nitrifying bacteria with PVA agent had similar ammonia nitrogen removal efficiency with the free nitrifying bacteria after 3-day reaction. After 4 days, the ammonia nitrogen removal efficiency of the immobilized PVA beads reached to around 41%, higher than that of the free nitrifying bacteria (around 37%). The phenomenon indicates that nitrifying bacteria embedded in PVA could play its full role after a period of adaptation. Moreover, in the embedding process, the dissolution of nitrifying bacteria was not found.

*2) Comparisons of mechanical strength*

25g CA beads or 25g PVA beads together with approximately 500ml physiological saline were put into 1000ml beakers, respectively. To shorten the period of mechanical strength test, the disturbance to the immobilized CA beads and PVA beads was conducted by an aeration system. The changes of bead shape were examined at different time intervals (data were abbreviated) to evaluate the resistance of the two immobilized microbial beads to mechanical strength.

Results shows that either of the two immobilized microbial beads had good resistance to mechanical strength in the beginning six days. After that, PVA beads showed an obviously better resistance to mechanical strength than that of CA beads. The shape of PVA beads processed under its optimal entrapping conditions changed little during one month aeration. However, the shape of CA beads changed obviously after around 15 days.

*3) Comparisons of stability*

25g CA beads and 25g PVA beads were put into 250ml synthetic wastewater, respectively. To shorten the period of the dissolution tests, the two beads together with the wastewater were put into a shaking table. Nitrifying bacteria were found to be dissolved into solution from CA beads. The dissolution was faster and more obvious with increasing of the embedded bacteria. However, the dissolving phenomenon was not found in the dissolution test of PVA beads.

Another 25g CA beads and 25g PVA beads were put into 250ml distilled water, respectively. pH of the two solutions were adjusted to different values and the changes of the immobilized CA and PVA beads were observed. The test results show that CA beads can be stable for approximately a month under pH of less than 7; while were dissolved immediately under pH of higher than 10. However, different pH values had little effect on PVA beads.

The aforementioned two tests indicate PVA beads have better stability than CA beads.

*4) Comparisons of mass transfer*

The same amount of CA beads and PVA beads were put into the same volume of inert red ink solution. The beads were examined at different time intervals to identify the mass transfer of the two immobilized nitrifying bacteria beads.

After 30min steep, the center of the CA beads became red entirely. However, only part of the PVA beads became red after 30min steep. Even though PVA beads turned red entirely after 120 minutes, the color of some PVA bead center was very light. This indicates that CA beads had better mass transfer performance compared with PVA beads.

*5) Comprehensive comparisons of the two beads*

Comprehensive comparisons of the CA and PVA immobilized nitrifying bacteria beads are showed in Table III.

COMPREHENSIVE COMPARISONS OF PERFORMANCES BETWEEN THE TWO IMMOBILIZED MICROBIAL BEADS

| | CA beads | PVA beads |
|---|---|---|
| $NH_4^+$-N removal efficiency | low | high |
| Mechanical strength | good | very good |
| Stability | bad | good |
| Mass transfer | good | bad |
| Difficulty of preparation | easy | difficult |
| Cost | cheap | very cheap |

Because nitrifying bacteria is aerobic microbe, aeration is generally required in nitrification reaction. Therefore, the immobilized nitrifying bacteria beads should have good nitrifying capacity and high resistance to aeration. After comprehensive comparison shown in Table III, PVA beads have higher nitrifying efficiency, higher strength and higher resistance to aeration. Embedding nitrifying bacteria into PVA agent is an ideal immobilization method.

## IV. CONCLUSIONS

1. The optimum conditions of embedding nitrifying bacteria into CA beads were 10ml of nitrifying bacteria, 4% of sodium alginate, 3mm in bead diameter and 24-hr crosslinking time.
2. The optimum conditions of embedding nitrifying bacteria into PVA beads were 10ml of nitrifying bacteria, 10% of PVA, 3mm in bead diameter and 24-hr crosslinking time.
3. Embedding of nitrifying bacteria into CA beads and PVA beads were processed under their optimum conditions. Comprehensive comparisons on mechanical strength, stability, mass transfer and ammonia nitrogen removal efficiency of the two beads indicate that polyvinyl alcohol is a better embedding agent for nitrifying bacteria compared with sodium alginate.

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