ISSN: 2710-0588

Preparation and Properties of Pine Powder-Bacillus pasteurii Microspheres

Yuwen Feng[1], Sheng Fu[2] and Haijun Li[1, *]

[1]School of Green Mining and Resource Engineering, Liaoning Petrochemical University, Fushun 113001, China

[2]School of Green Mining and Resource Engineering, Liaoning Petrochemical University, Fushun 113001, China

[*] Corresponding Author

Abstract

Aiming at the problems of low survival rate and weak mineralization activity of microorganisms in the high- alkali and high- shear environment of concrete, pine powder and nanomaterials were used as composite carriers to prepare Bacillus pasteurii microspheres by embedding method. The optimal culture conditions of microorganisms were determined by single factor experiments, the mix ratio of microspheres was optimized by orthogonal design, and systematic characterization was carried out from physical properties, mechanical strength, microscopic morphology and phase composition. The results show that the growth and urease activity of Bacillus pasteurii are optimal when the culture temperature is 30℃, initial pH=7, inoculation amount is 2%, and culture time is 24h; the optimal mass ratio of pine powder, nanomaterials and bacterial solution is 1:1:3, at this time, the average particle size of microspheres is 2.1±0.3mm, the embedding rate is 91.6%, the 24h bacterial burst release rate is less than 8.5%, and the mass loss rate after shaking for 24h is only 2.35%; SEM and XRD confirm that the microspheres form a porous composite structure, where microorganisms colonize well and can induce the formation of calcite-type calcium carbonate. The microspheres can effectively protect the bacteria and meet the application requirements of concrete self-healing.

Keywords

Pine Powder; Bacillus pasteurii; Microspheres; Embedding Method; Mineralization Performance; Concrete Self-healing.

1. Introduction

Microbially Induced Calcium Carbonate Precipitation (MICP) is a green and efficient approach for self-healing of concrete cracks. However, Bacillus pasteurii is easily inactivated in the concrete mixing shear and high-alkali pore fluid environment, which limits its engineering application [1-2]. Immobilization embedding technology can provide shelter, nutrition and mass transfer channels for microorganisms through carriers, thereby improving the tolerance of bacteria [3-4].

Pine powder has the advantages of wide source, renewability, developed porous structure and good biocompatibility, which can be used as a natural carrier for microorganisms; the sodium alginate- calcium chloride crosslinking system has good spheroidization, non-toxicity and moderate strength, which is suitable for microbial embedding [5]. In this paper, pine powder + nanomaterials were used as composite carriers, sodium alginate as gel matrix, and Bacillus pasteurii as repair strain to prepare microbial self- healing microspheres. The optimal

ISSN: 2710-0588

preparation process and performance parameters were determined to provide a material basis for subsequent microbial concrete experiments.

2. Experimental Materials and Methods

2.1. Experimental Materials

Microorganism: Bacillus pasteurii (ATCC11859);

Carrier: 200-mesh Pinus sylvestris var. mongolica pine powder, with moisture content of 8.5% and water absorption rate of 180%;

Gel materials: Sodium alginate (BR), Calcium chloride (AR);

Nanomaterials: nHA (biochemical reagent);

Culture medium: LB liquid medium, beef peptone solid medium;

Reagents: Urea, sodium hydroxide, tryptone, yeast extract, etc. (all are analytical pure)

2.2. Experimental Instruments

Electric thermostatic incubator, air bath shaker, high-pressure steam sterilizer, ultraviolet spectrophotometer (N5S), scanning electron microscope (SU8000), X-ray diffractometer (D8 Advance), etc.

2.3. Experimental Methods

2.3.1. Culture of Bacillus pasteurii and Determination of Optimal Conditions

LB liquid medium was prepared, sterilized at 121℃ for 30min, and urea was added by filtration sterilization. Single factor experiments were set up with temperature (20~40℃), initial pH (6~10), inoculation amount (1%~5%) and culture time (6~48h) as variables, and OD_{600} and urease activity were used as evaluation indicators to determine the optimal culture conditions.

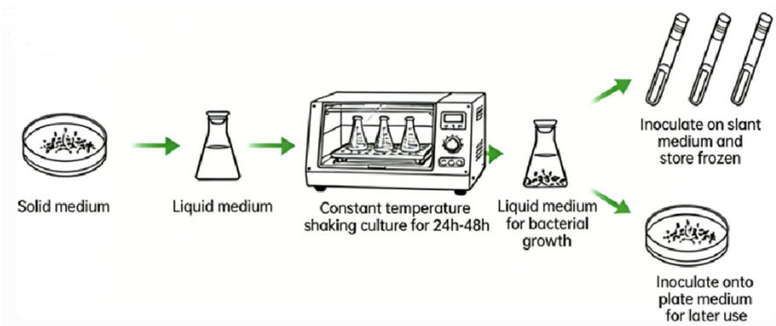

Figure 1. Flowchart of bacterial culture

2.3.2. Preparation of Pine Powder-Microorganism Microspheres

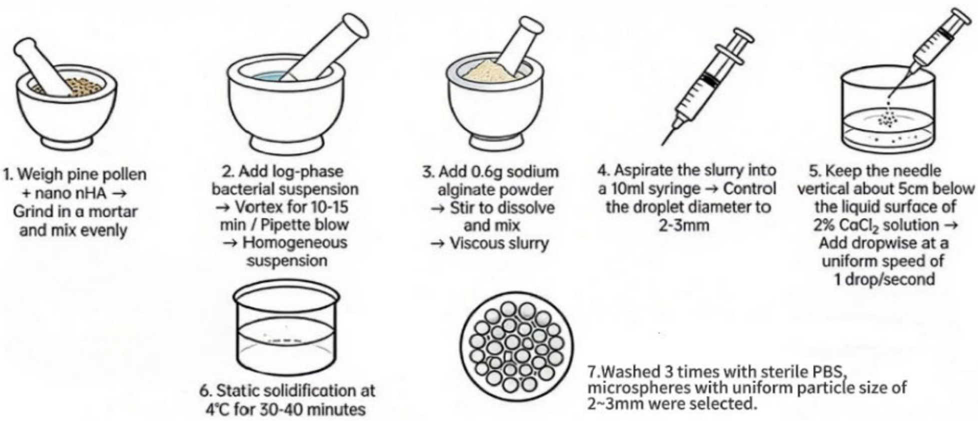

Figure 2. Preparation flowchart of pine wood powder-Bacillus pasteurii microspheres

ISSN: 2710-0588

The sodium alginate-calcium chloride drop embedding method was adopted:

1. Pine powder and nanomaterials were ground and mixed uniformly, and logarithmic phase bacterial solution was added for vortex oscillation;

2. Sodium alginate was added and stirred to form a uniform slurry;

3. The mixture was dropped into 2% $CaCl_2$ solution with a syringe and solidified at 4℃ for 30~40min;

4. Washed 3 times with sterile PBS, and microspheres with uniform particle size of 2~3mm were selected.

2.3.3. Performance Testing of Microspheres

Spheroidization: Appearance, roundness, particle size uniformity; Mechanical strength: Shaki ng at 150rpm for 24h, calculating mass loss rate; Embedding rate: Determining free bacteria by OD_{600} method, calculating embedding rate; Activity recovery: Determining urease activity after culturing for 24h; Microscopic characterization: Observing morphology by SEM and analyzing phase composition by XRD.

3. Results and Analysis

References are cited in the text just by square brackets [1]. (If square brackets are not available, slashes may be used instead, e.g. /2/.)

3.1. Optimal Culture Conditions of Bacillus pasteurii

3.1.1. Culture Time

The growth of bacteria showed a typical S-shaped curve: 0~6h lag phase, 6~18h logarithmic phase, 18~36h stationary phase, and decline phase after 36h. The OD_{600} and urease activity reached the peak at 24h, which was determined as the optimal culture time.

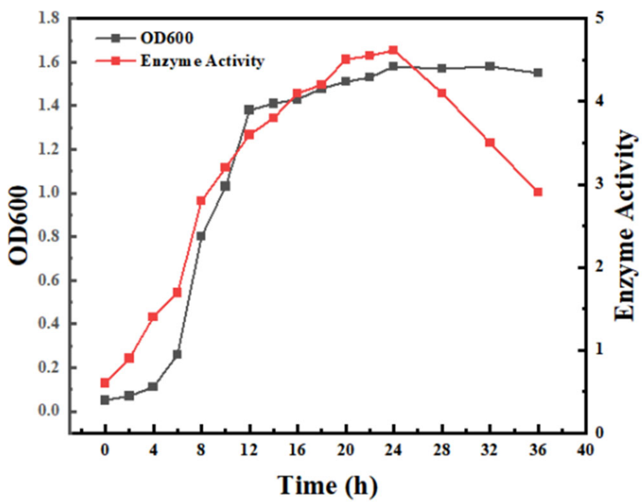

Figure 3. Bacterial growth curve and enzyme activity curve

3.1.2. Initial pH

When pH=7, OD_{600}=1.482 and urease activity=4.505 $mmol \cdot min^{-1}$, which were significantly higher than those of other groups; when pH>10, the bacterial activity decreased sharply. The optimal initial pH was determined to be 7.

3.1.3. Culture Temperature

The urease activity was the highest at 30°C (4.598$mmol \cdot min^{-1}$), and the viable bacteria metabolism was vigorous; the bacterial concentration was high at 25°C but the enzyme activity

ISSN: 2710-0588

was low, and high temperature at 40℃ inhibited growth. The optimal culture temperature was determined to be 30℃.

Table 1. Data table of the activity of mineralizing bacteria by initial PH values of different culture media

PH value	6	7	8	9	10
OD_{600}	1.340	1.482	1.405	1.326	1.064
Urease activity value ($mmol \cdot min^{-1}$)	3.321	4.505	3.087	2.345	1.512

Table 2. Data table of Mineralizing bacteria activity at different culture temperatures

Temperature (℃)	20	25	30	35	40
OD_{600}	1.403	1.598	1.504	1.396	0.897
Urease activity value ($mmol \cdot min^{-1}$)	2.987	3.507	4.598	3.391	1.643

3.1.4. Inoculation Amount

The enzyme activity was the highest when the inoculation amount was 2% ($4.654 mmol \cdot min^{-1}$); when the inoculation amount was more than 3%, it was easy to enter the decline phase in advance. The optimal inoculation amount was determined to be 2%.

Table 3. Table of the Effects of Different Inoculation Amounts on the Activity of Mineralized Bacteria

inoculation amount (%)	1	2	3	4	5
OD_{600}	1.431	1.527	1.580	1.426	1.315
Urease activity value ($mmol \cdot min^{-1}$)	2.364	4.654	3.187	2.756	2.549

In summary, the optimal culture conditions of Bacillus pasteurii are: 30℃, pH=7, inoculation amount 2%, culture time 24h.

3.2. Optimal Mix Ratio of Microspheres

aking the ratio of pine powder: nanomaterials: bacterial solution as variables, 5 groups of mix ratios were set up, and the results are shown in Table 1.

Table 4. Performance Comparison of Microspheres with Different Mix Ratios

Group No.	Mix Ratio (Pine Powder Nanomaterials Bacterial Solution)	Embedding Rate/%	Mass Loss Rate/%	Urease Activity/ ($mmol \cdot min^{-1}$)
A	1:1:4	76.24	13.35	68.3
B	1:1:3	91.60	2.35	85.7
C	3:1:3	68.45	16.83	52.1
D	2:1:3	80.12	10.24	60.5
E	0.5:1:3	65.33	18.00	48.9

ISSN: 2710-0588

Group B had the best comprehensive performance:

• High roundness and uniform particle size (2.1±0.3mm);

• Embedding rate of 91.6%, sufficient bacterial load;

• High mechanical strength, mass loss rate of only 2.35% after shaking;

• Fast activity recovery and the highest urease activity.

The optimal mix ratio was determined as: pine powder: nanomaterials: bacterial solution = 1:1:3, and the sodium alginate addition amount was 2%.

3.3. Microscopic Morphology and Phase Analysis of Microspheres

3.3.1. SEM Microscopic Morphology

Blank group: Dense structure, obvious agglomeration, few pores, and no microbial characteristic morphology;

Group B (optimal mix ratio): Good particle dispersion, porous surface, a large number of short rod-shaped Bacillus pasteurii attached uniformly, and some bacterial surfaces covered with nano-scale calcium carbonate crystals, which proved the success of microbial colonization and mineralization

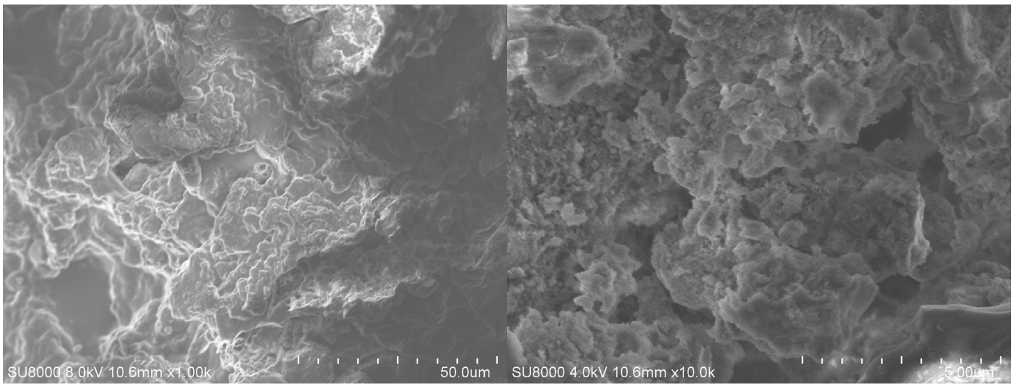

Figure 2. Microscopic morphology of the microspheres in the blank group

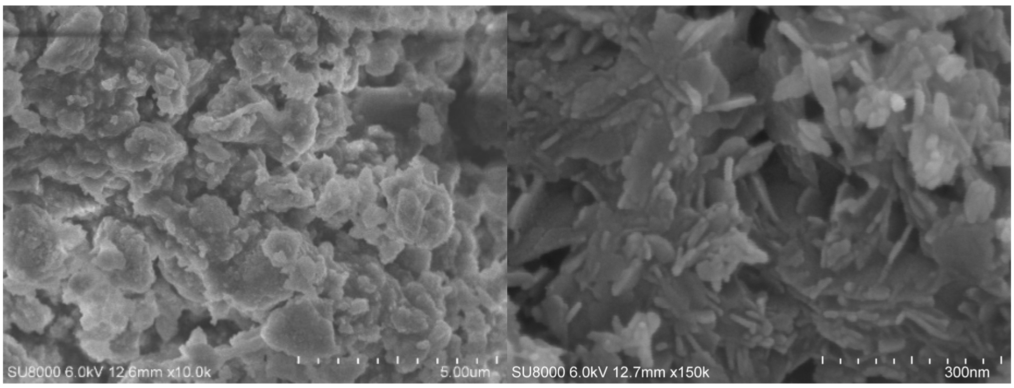

Figure 3. Microscopic morphology of group microspheres

3.3.2. XRD Phase Analysis

Blank group: Shows characteristic peaks of pine wood powder cellulose (2θ=16.4°, 34.6°), no peak of calcium carbonate; Group B: Distinct calcite type calcium carbonate characteristic peaks were observed at 2θ=29.4°, 39.5°, and 43.2°, which were highly consistent with the PDF#05-0586 standard card, confirming the microbial induction of high-crystallinity $CaCO_3$.

ISSN: 2710-0588

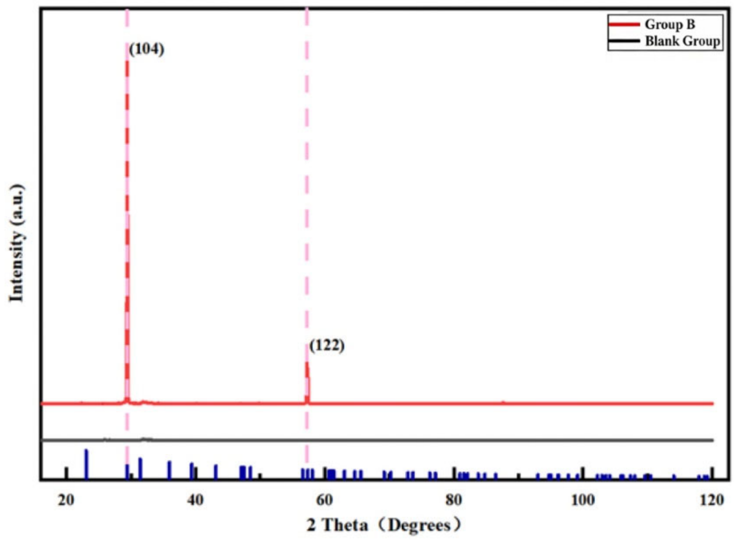

Figure 4. XRD analysis diagrams of the blank group microspheres and the B group microspheres

4. Conclusion

1. The optimal culture conditions of Bacillus pasteurii are: temperature 30℃, initial pH=7, inoculation amount 2%, culture time 24h, at which the bacterial concentration and urease activity reach the best match.

2. The optimal preparation ratio of pine powder-Bacillus pasteurii microspheres is: pine powder: nanomaterials: bacterial solution = 1:1:3, sodium alginate concentration 2%, solidified with 2% $CaCl_2$ for 30~40min. The microspheres have an embedding rate of 91.6%, high mechanical strength, mass loss rate of only 2.35% after 24h shaking, and excellent sustained-release performance.

3. SEM and XRD confirm that the microspheres have a porous composite structure, where microorganisms colonize well and can stably induce the formation of calcite-type calcium carbonate, which meets the requirements of concrete self-healing for carrier strength, bacterial protection and mineralization ability.

References

[1] Qian C X, Feng J H, Su Y L: Improving Early Mechanical Properties and Self-healing Effect of Cement-based Materials by Microbially Induced Calcium Carbonate Precipitation, Materials Review, Vol. 33 (2019) No.12, p.1983-1988. (In Chinese)

[2] *Cheng W F: Study on the Performance of Microcapsule-Embedded Microbial Self-healing Concrete Cracks*, (Master, Shenzhen University, China 2016).

[3] *Ren X H: Preparation of Immobilized Mixed Bacteria Carrier and Its Experimental Study in Organic Wastewater Treatment*, (Master, Yanshan University, China 2018).

[4] *Zhao X Z: Wood Powder and Lignocellulose-Polymer Composites*, (Master, Hefei University of Technology, China 2019).

[5] WANG J Y, SOENS H, VERSTRAETE W, et al. Self-healing concrete by use of microencapsulated bacterial spores[J]. Cement and Concrete Research, Vol. 56 (2014), p.139-152.