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# Experimental study on sand solidification with microbial cement

**Ma Fengling[1,2], Li Rong[1,2], Xu Yao[1,2*], Wang Saojiang[1,2], Fang Mingdian[3]**

[1]State Key Laboratory of Simulation and Regulation of Water Cycle in River Basin, China Institute of Water Resources and Hydropower Research， BeiJing 100038;

[2]Key Laboratory of Construction and Safety of Water Project, Ministry of Water Resources, Beijing 100038;

[3]Key Laboratory of Urban Underground Engineering of Ministry of Education, Beijing Jiaotong University, Beijing 100044

*Corresponding Author: xuyao@iwhr.com

**Abstract:** Selecting four types of sand with different ranges of particle diameter, the strength tests of sand solidification with microbial cement are carried out in this paper. With experimental conditions in this paper, most of the strengths of solidified sand with microbial cement are in the range of 1.2~2.7MPa. Particle gradation is an important factor that affects the solidification of microbial cement. And the perfusion method is more suitable for the sand with appropriate porosity and good connectivity which is easy to be perfused, but not for clay and sludge. The stirring molding method is more suitable for clay and sludge which have much content of fine powder. The strength of solidified sand is related to the direction of perfusion; and the strength of its upper part is higher than that of the lower part. Compared with other solidification materials, the solidified sand with microbial cement has no obvious advantages, due to its low strength, many limitations as well as complex construction technology; dnd its application field needs further study and exploration.

## 1. Introduction

The mechanism of microbial induced calcite precipitation (MICP technology) is to provide nutrient substance and calcium salt solution, such as urea, to specific microorganisms, produce carbanion after decomposing nutrients via catalysis of urease produced by microorganisms, create calcium carbonate crystal by combining calcium ion in the surrounding environment, which has filling cementation function, thus making it solidified and stronger.

The mechanism of action of hydration of urea catalyzed by the biological enzyme of microorganisms is expressed by the following reaction equations:

$CO(NH_2)_2$+ $2H_2O$→(microbiological deterioration)→$CO_3^{2-}$+$2NH^{4+}$(improve the pH value of solution)

$Ca^{2+}$+Cell (cell of microorganisms with negative power)→Cell-$Ca^{2+}$

$CO_3^{2-}$+Cell-$Ca^{2+}$→Cell-Ca $CO_3$↓ (bacterial cement)

At present, many experts and scholars have conducted a large amount of experimental study concerning the solidification of microorganism and they have found that MICP technique applied in solidification of sand could improve intensity, rigidity, permeability and anti-liquefaction of the sand, thus rendering the reference for feasibility of micro-organism-solidified sand.

The increasing strength of micro-organism-solidified sand mainly comes from the improvement of cohesion strength of calcium carbonate crystal to soil. And its strength mainly includes the strength of soil skeleton and cementitious strength of calcium carbonate crystal. The strength of soil skeleton is influenced by property of the soil and relevant parameters while the cementitious strength of calcium carbonate crystal mainly depends on the content of calcium carbonate crystal. This paper selects four types of sand with different particle sizes to carry out the test of microorganism solidification and conducts preliminary experimental study on sand solidification with microbial cement.

## 2. Test material and preparation of bacteria solution

### *2.1. Solidified sand*

Four types of natural sand with different particle sizes are used for the solidification test. And those sands were screened by sifter whose sieve mesh was 5mm before the test. The test results of particle analysis for four different types of sand are shown in Figure 1. According to Rules of Geotechnical Testing (SL237-1999), those four types of sand are divided into badly graded sand（SP）, fine-grained sand（SF）, silty sand（SM） and low liquid limit clay（CL）.

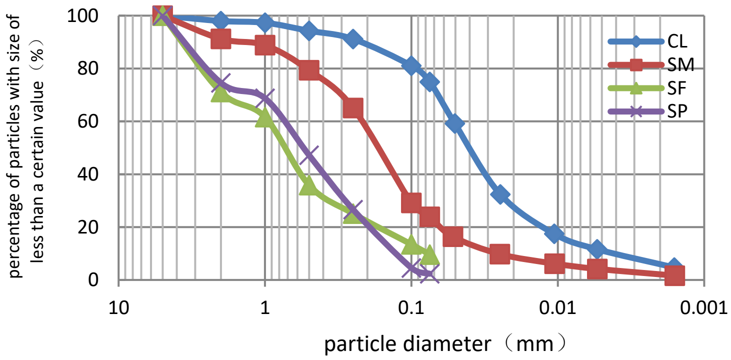


Figure 1. Test results of particle analysis for four different types of sand

### *2.2. Preparation of bacteria solution*

The experimental bacteria was pasteurella sarcina, whose medium composition and concentration are as follows:

1) yeast extract: 20g/L;
2) $(NH_4)_2SO_4$: 10g/L
3) Tris buffer solution: 14.7g/L, used to adjust the pH value of liquid nutrient medium (pH value needs controlling around 9.0.

Preparation process: firstly, each single solution was prepared based on certain concentration; the solution extracted from yeast and Tris buffer solution were sterilized for 20 min under high-pressure steam sterilizer with the temperature of 120℃; after disinfection by ultraviolet light on sterile operating table, the nutrient medium was prepared by mixing those three solutions uniformly according to the above concentration; the activated bacteria was inoculated to the nutrient medium according to 5% concentration; the solution was moved to oscillatory incubator whose speed is 190r/min with the temperature of 30℃, until the muddy occurred (about 12 hours); bacterial optical density (OD600) was measured by using spectrophotometer (being controlled within the range 0.8-0.9).

The nutrient solution in the test was the mixed solution of urea with 0.5mol/L concentration and calcium chloride and the ratio between urea and calcium chloride mole was 1:1.

## 3. Test of sand solidification

### *3.1. Test of sand solidification via the perfusion method*

#### *3.1.1. Test method*
This test adapted two mould test moldings. (1) 50mL medical syringe was used to be the trial model and Φ30mm×60mm cylinder sample was prepared. The sand was scanned by 3mm sieve and per-blended with optimal- water -consumption tap water. Permeable stone with 2.5mm was placed in the bottom and sand was put into the mould by 4 or 5 times. The ramming was inserted appropriately to keep bulking and the other permeable stone was used for capping. (2) PVC trial model was used and Φ50mm×100mm cylinder sample was prepared The optimal- water -consumption tap water was added into the sand for preblend, the sand was put into the mould by 4 or 5 times and appropriate ramming was inserted to keep bulking.

In the test, deionized water more than 1 times the pore volume of the sample was injected from the top of sample, thus eliminating the excessive air bubble in sample. The bacterial solution with more than 1 times the pore volume of the sample was injected slowly by handmade from the top of sample. It flowed out of from the bottom and stewed about 2 hours. Then the nutritive medium with 1 times the pore volume of the sample was injected and it stewed about 10 hours. The processes above were a circulate and there were 16 circulates in this test. In the process of test, Φ30mm×60mm sample was place in the constant temperature water bath at 30 ℃ while Φ50mm×98mm sample was placed at room natural environment (the environment temperature is 0-12℃). The processes of the test were shown in Figure 2.

Figure 2. Tests of sand solidification with the perfusion method

#### *3.1.2. Test results*
During the process of perfusion test, bacteria solution were cultivated twice in the morning and night of everyday. The cultivated time for each time was about 12 hours. The test result of optical density value of bacteria was in the range of 0.844~0.966 and its average value was 0.891.

1) Φ30mm×60mm test result of sample strength

The sample continued 16 circulates of perfusion and it was kept moisturizing curing. Strength tests of sample were carried out at 3-day and 10-day curing ages, respectively. The test results could be seen in the Table 1. Due to the uneven strength of upper and lower part, when testing, the lower part of the sample was broken and this part was to detect the strength of the lower sample. And the upper one was used to carry out the other test and it was the strength of the upper.

From the results of the test, it is found that: (1) Because of the small porosity, it is hard for clay to provide living space for microorganisms. With low permeability, clay is not conducive to the injection and mixing of bacteria and mixture. Therefore, it strength was low, about 0.8MPa. The solidifying strength of other sand is within the range of 1.2MPa~2.7MP, whose solidification effect is excellent. With relatively small grain size, the inter-particle pore of fine-grained sand particles is easy to be filled with calcium carbonate crystals and its solidification effect is high, about 2.5MPa. Therefore, grain composition is an important influence factor for solidification of microorganisms. Perfusion method for the solidification of microorganism is more suitable for the sand with appropriate pore size and good connectedness. Perfusion method is suitable for clay that has smaller grain size and porosity, instead of sludge. (2) The strength of sand solidification is related with the perfusion direction and the upper strength of sand column is higher than that of lower part. (3) After shopping perfusion, the sample continued to keep moisturizing and curing and its strength could be further improved. This suggests that at constant temperature of 30℃, the bacterial solution after 3 days and 10 days could still survive and proceed mineralization reaction.

Table 1 Strength test results of Specimen of Φ30mm×60mm with the perfusion method

| Type of sand | Part of specimen | Diameter of specimen （cm） | Height of specimen （cm） | Compressive strength （MPa） | Age of specimen |
|---|---|---|---|---|---|
| SP | Lower part | 2.9 | 6.0 | 1.85 | Test after 3 days' moisturizing maintenance after stopping injection |
| SP | Upper part | 2.9 | 3.6 | 1.88 | |
| SF | Lower part | 2.9 | 6.0 | 1.21 | |
| SF | Upper part | 2.9 | 5.1 | 2.26 | |
| CL | Lower part | 2.9 | 6.0 | 0.782 | |
| CL | Upper part | 2.9 | 3.3 | 0.833 | |
| SP | Lower part | 2.9 | 6.0 | 2.06 | Test after 10 days' moisturizing maintenance after stopping injection |
| SP | Upper part | 2.9 | 3.6 | 2.11 | |
| SF | Lower part | 2.9 | 6.0 | 1.56 | |
| SF | Upper part | 2.9 | 3.6 | 2.70 | |
| SM | Lower part | 2.9 | 6.0 | 2.50 | |
| SM | Upper part | 2.9 | 3.6 | 2.51 | |

2) Φ50mm×100mm test result of sample strength

The sample continued 16 circulates of perfusion and it was kept moisturizing curing. Strength tests of sample were carried out at 3-day curing ages. The test results could be seen in the Table 2. Since the strength of sample is relative low and the mould is difficult to remove, the lower part all was broken during the process of removing. Therefore, the lower part of the sample was cut and the upper was used for the test.

From the test results, we could know that (1) under the experimental condition, basically, the clay has no solidification strength and the upper strength of other sand is in the range of 1.4MPa~2.2MPa. (2) the fine-grained soil used two compactness to carry out solidification strength test. When being loaded, No. Φ50-SF2 the sample was packed with dense packing mash. Therefore, with the increasing of compactness, the solidification strength of fine-grained soil increases, the value of which is 4.10MPa. The whole strength is improved dur to the large compactness and high strength of skeleton structure.

Table 2 Strength test results of Specimen of Φ50mm×100mm with the perfusion method

| Type of sand | Part of specimen | Diameter of specimen （cm） | Height of specimen （cm） | Compressive strength （MPa） | Age of specimen |
|---|---|---|---|---|---|
| SP | Upper | 5.0 | 5.2 | 2.20 | Test after 3 days' moisturizing maintenance after stopping injection |
| SF | Upper | 5.0 | 5.1 | 2.04 | |
| SF | Upper | 5.0 | 5.2 | 4.10 | |
| SM | Upper | 5.0 | 5.0 | 1.38 | |
| CL | Upper | 5.0 | 5.0 | 0.068 | |

Note: the compactness of No. Φ50-SF1 and Φ50-SF2 is different and their inventories are 375g and 400g respectively.

### *3.2. Test of sand solidification via the stirring molding method*

When using the stirring molding method of microbial solution to solidify the sand, the bacteria solution and nutrient medium with 1:1 ratio was mixed and added into the sand, whose addition is 1.0 time, 1.5 time, 2.0 time and 2.5 time of optimum water content in sand. Then the sand was stirred after adding intermixture. It was poured into No. Φ5mm×10mm sample about 4-5times and inserted until compactness. Then, after it was kept moisturizing and curing for 5 days at the room temperature (15~20℃), the strength was tested. The results could be seen in Table 3.

Based on the test results, it is shown that the poorly graded sand was broken when the mold was removed due to its less content of fine powder and low strength. The solidification strength of clay is highest, whose value is 0.576MPa and the solidification strength of silty sand and fine-grained soil sand are 0.163MPa and 0.239MPa respectively. The amount of intermixture increases. Despite increasing microorganism, the strength declines and even disperses due to the increasing water consumption. Stirring molding method is more suitable for sludge and clay with much content of fine powder.

Table 3 Strength test results of Specimen of Φ50mm×100mm with the stirring molding method

| Type of sand | Mixed solution sddition | Diameter of specimen （cm） | Height of specimen （cm） | Compressive strength （MPa） | Age of specimen |
|---|---|---|---|---|---|
| SP | 1.5 | | The specimens broke up | | Test after 5 days' moisturizing maintenance after stopping injection |
| SP | 2.0 | | The specimens broke up | | |
| SP | 2.5 | | The specimens broke up | | |
| SF | 1.0 | 5.0 | 10.0 | 0.239 | |
| SF | 1.5 | 5.0 | 10.0 | 0.097 | |
| SF | 2.0 | | The specimens broke up | | |
| CL | 1.5 | 5.0 | 10.0 | 0.576 | |
| CL | 2.0 | 5.0 | 10.0 | 0.224 | |
| CL | 2.5 | 5.0 | 10.0 | 0.122 | |
| SM | 2.0 | 5.0 | 10.0 | 0.163 | |

## 4. Conclusions

Under the experimental conditions in the paper, most of the strengths of solidified sand with microbial cement are in the range of 1.2~2.7MPa. Particle gradation is a significant factor that influences the solidification of microbial cement. And the perfusion method is more suitable for the sand with appropriate porosity and good connectivity which is easy to be perfused; while the stirring molding method is more suitable for clay and sludge which have much content of fine powder. The strength of solidified sand is related to the direction of perfusion; and the strength of its upper part is higher than that of the lower part.

Compared with other solidification materials, the solidified sand with microbial cement has no obvious advantages, due to its low strength, many limitations as well as complex construction technology; and its application field needs further study and exploration.

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**About the author**

First Author: Ma Fengling (1965-), Master, mainly engaged in the study of hydraulic concrete material, E-mail：756066473@qq.com；*Corresponding Author: Xu Yao (1982-), Ph.D., mainly engaged in the study of hydraulic structure and material, E-mail：xuyao@iwhr.com.

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