

# Experimental study on the effect of grouting interval on microbial induced calcium carbonate precipitation

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**Abstract:** Microbial induced calcium carbonate precipitation is a kind of environmentally friendly soil improvement technology. Microbial induced calcium carbonate precipitation reaction needs to enhance microorganism and nutrient solution containing urea and calcium salts. Different grouting intervals will get different curing effects.. In this paper, the effect of bio-cemented sand under different grouting time intervals was analyzed from the macroscopic physical and mechanical properties and micropore structures through the soil test and the electron microscope scanning test of the bio-cemented sand. The experimental results show that: with the increase of grouting time interval, the effect of bio-cemented sand was first increased and then decreased, and the 8h was the turning point of trend change. When the time interval was 8h, the permeability coefficient, water absorption, dry density, unconfined compression strength and calcium carbonate content reached  $1.79 \times 10^{-4} \text{m/s}$ , 18.88%,  $1.67 \text{g/cm}^3$ , 0.90MPa and 3.49%, respectively. The scanning pictures show the calcium carbonate precipitation effectively bonded sand particles. The apparent pore content and the average pore diameter under the 8h of the time interval was 12.66% and  $43.690 \mu\text{m}$  respectively, which was smaller than that of the others.

## 1. Introduction

Microbial induced calcium carbonate precipitation was a new environmental friendly soil improvement technology[1]. In the process of the microbial induced calcium carbonate precipitation, urea was hydrolyzed by microbial urease to form ammonium and carbonate ion. The carbonate ions reacted with calcium ions to precipitate as calcium carbonate crystals[2, 3]. The calcium carbonate crystals can bond the sand particles together and provide an improvement to the strength and stiffness of bio-cemented sand[4]. During the process of microbial induced calcium carbonate precipitation, the nutrient solution including urea and calcium salts was required to promote continuous reaction, and calcium carbonate precipitation was continuously generated to increase the strength.[5]. In order to ensure adequate nutrient solution, two kinds of grouting were mainly intermittent and continuous injection[6, 7]. Compared to continuous injection, intermittent injection promoted more uniform distribution of nutrients, thus promoting more uniform precipitation of calcium carbonate[8-10]. Intermittent injection was mainly related to two factors of grouting frequency and interval. With the increase of the grouting times from 2 to 6, the strength of the sand cured with MICP was reached from 0.37MPa to 1.33 MPa[11]. The existing research proved that multiple grouting had a good effect on the solidification effect of bio-cemented sand. However, there was less corresponding study on the time interval of the grouting. In this paper, different grouting time intervals were set up.



Through macroscopic physical and mechanical experiments and micro electric competition scanning experiments, the bio-cemented sand under different grouting time intervals was evaluated comprehensively and analyzed the effect of the time interval of grouting on microbial induced calcium carbonate precipitation.

## 2. Materials and Methods

### 2.1 Materials

**2.1.1 Bacteria.** The type of bacteria used in the present study is *Sporosarcina pasteurii* (DSM 33), a Gram-positive chemoheterotrophic bacteria with rhabditiform cells in the length of 2~3  $\mu\text{m}$ . *Sporosarcina pasteurii* can produce a large amount of precipitation in a short time because of the high urease activity, which is applicable in MICP[12, 13]. The cultivation for bacterial consists of 20 g/L yeast extract, 10 g/L  $(\text{NH}_4)_2\text{SO}_4$ , and 0.13 M tris buffer (pH = 9.0), which are sterilized in an autoclave at 121°C for 20 min prior to mixing them together. The medium with *Sporosarcina pasteurii* is stored in a flask, which is kept in a shaking bed in the rotation speed of 160 rpm and temperature of 30 °C, until the optical density and the urease activity reached 1.5 and 36.5 mM urea/h respectively. Some text.

**2.1.2 Nutrient solution** Nutrient solution is a mixture of the calcium chloride and the urea in equal molar concentration of 0.5 M. Bacteria fixative is 50 mM calcium chloride, in the volume of 1/10 of that of the bacteria.

**2.1.3 Sand** The sand used in this study was a type of CHINA ISO standard sand, a fine sand with uniform particle and poor gradation. The physical properties of the sand are shown in Table 1.

Table 1 The physical properties of CHINA ISO standard sand

Physical Properties	$G_s$	$d_{50}(\text{mm})$	$C_u$	$e_{\text{max}}$	$e_{\text{min}}$
Numerical Value	2.653	0.18	1.56	0.950	0.605

### 2.2 Schedule of experiments

The fixation time was 2h[8], and the cementing solution in the column is allowed to react for up to 12 hours[14]. Thus, the interval of the time interval was 2h, 4h, 6h, 8h, 10h, 12h, respectively.

### 2.3 Methods

**2.3.1 bio-cemented sand test** Permeability of the bio-cemented sand is tested using a falling head method(GB/T50123-1999), and calculated by the equation(1).

$$k = 2.3 \frac{al}{A\Delta t} \log \frac{h_1}{h_2} \quad (1)$$

here  $k$  is the permeability,  $a$  is the cross-section area of water pipe,  $l$  is the height of the sand column,  $A$  is the cross-section area of the sand column,  $\Delta t$  is the time interval,  $h_1$  is the initial head height,  $h_2$  is the terminate the head height.

The bio-cemented samples were dried at 70 °C for 36 hours until their mass-loss ratios (in 24 h) were less than 0.1%, and then cooled to room temperature. The dry density was obtained by measuring the mass, diameter and height of the samples.

The water absorption ratios of the treated samples were calculated by the following equation (2):

$$W = \frac{m_2 - m_1}{m_1} \times 100\% \quad (2)$$

$m_1$  is the moisture-free weight of the sample,  $m_2$  is weight of the sample immersed in water for 24 hours.

Unconfined compression strength (UCS) were tested by press machine, with the axial load in a constant rate of 1.0 mm/min.

The oven-dried sand crushed by a mortar from the treated sand column was washed in HCl solution (0.1 M) to dissolve precipitated carbonates, rinsed, drained, and oven-dried. The Calcite Content, C, was calculated by the equation (3):

$$C = \frac{W_{S1} - W_{S2}}{W_{S2}} \times 100\% \quad (3)$$

$W_{S1}$  and  $W_{S2}$  are the weight of the dry sand before and after washing by HCl, respectively.

**2.3.2. SEM** Microstructure morphology of the bio-cemented sand were scanned by a scanning electron microscope (SEM, FEI Quanta 650), in order to compare changes of microstructure, and then analyze the curing mechanism. SEM images were processed by Image-Pro Plus (IPP) software to compute the apparent pore content, N, and the average pore diameter, D, calculated by the equation(4) and (5), respectively.

$$N = \frac{A_p}{A_t} \times 100\% \quad (4)$$

$$D = \frac{A_p}{M_p} \quad (5)$$

$A_p$ , in  $\mu m^2$ , is the area of all pores in the SEM image,  $A_t$  in  $\mu m^2$ , is the area of the SEM image,  $M_p$  is the number of pores in the SEM image.

### 3. Results

#### 3.1. Geotechnical experiment

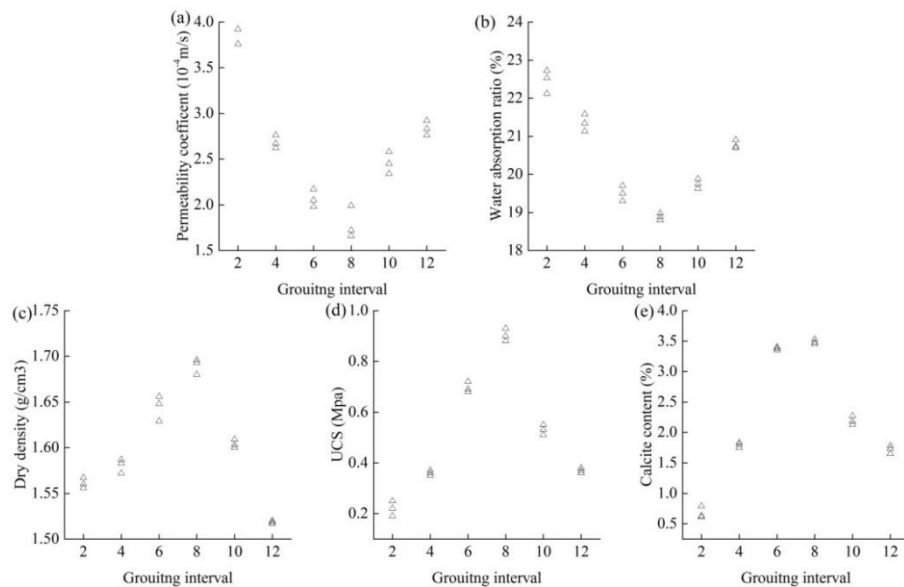


Fig.1 Results of Geotechnical test of bio-cemented sand by different grouting interval  
(a) permeability, (b) water absorption ratio, (c) dry density, (d) unconfined compression strength (UCS), (e) content of calcium carbonate

Geotechnical test results of bio-cemented sand under different grouting time intervals were shown in Fig.1. With the increase of grouting time interval from 2h to 12h, permeability coefficient and water absorption decreased firstly and then increased. Dry density, unconfined compression strength and Calcite content increased firstly and then decreased. When the time interval of grouting was 8h, the permeability coefficient and water absorption ratio of the cemented sand were the smallest, of which was  $1.79\text{E-}04\text{m/s}$  and  $18.88\%$  respectively, the dry density, the unconfined compression strength and the content of calcium carbonate were the largest, of which was  $1.67\text{g/cm}^3$ ,  $0.90\text{MPa}$  and  $3.49\%$  respectively.

### 3.2. SEM

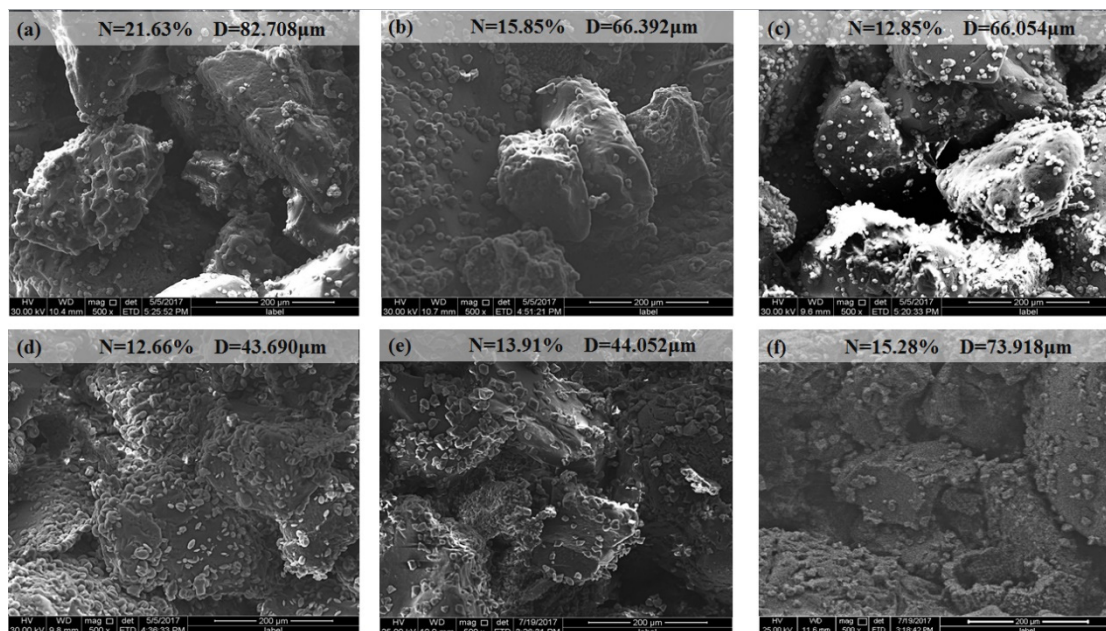


Fig.2 SEM pictures of different grouting time intervals

Pictures in Fig 2 were SEM images of different grouting time intervals. Figs.2 (a) ~ (f) correspond to the time interval from 2h to 12h. The amount of calcium carbonate precipitation was not the same at different grouting time intervals. The calcium carbonate content of the bio-cemented sand with a time interval of 8h was more than that of the others. The apparent pore content and the average pore diameter under the 8h of the time interval was  $12.66\%$  and  $43.690\mu\text{m}$  respectively, which was smaller than that of the others. No matter how much time the interval was, the particles precipitated by calcium carbonate were smaller and scattered in the bio-cemented sand.

### 4. Discussion

When the time interval was 2h, the less calcium carbonate content could be seen in Fig. 2, the larger permeability coefficient, the larger water absorption ratio, the smaller dry density, the smaller unconfined compressive strength and the smaller calcium carbonate content could be seen in Fig. 1. That was mainly because the time interval was too short, bacteria couldn't completely fixed in pores. With the supplement of nutrient solution later, some bacteria were washed away. Thus, there were less calcium carbonate and larger pores in bio-cemented sand column.

When the filling interval was 8h, most of bacteria were fixed between pores, providing nucleation sites for the formation of calcium carbonate precipitation, and played an important role in biological mineralization and soil agglomeration stability [15]. The curing effect under the 8h of the time interval was better than others. The characteristics of the bio-cemented sand column with the 12h of the time interval were similar to that of the bio-cemented sand column at the interval of 2h, but the reasons

were different. When the time interval was too long, the nutrient solution was insufficient, which made it impossible to produce calcium carbonate.

The calcium carbonate precipitation with different time interval in the bio-cemented sand column was not uniform distribution. The main reasons were the following: (i) the time interval was too short, the bacteria couldn't be fixed and couldn't provide effective nucleation sites; (ii) the time interval was too long, the nutrition was supplement producing less calcium carbonate precipitation; (iii) the bacteria and nutrient solution through the sand in the free infiltration of the reaction fluid by gravity, leading to the existence of an area not permeable, and the process of microbial induced calcium carbonate precipitation.

## 5. Conclusion

Through the experimental study of the effect of the bio-cemented sand particles under different grouting intervals, the following conclusions were obtained from the macroscopic mechanics and microstructure analysis: with the increase of the time interval of the grouting, the effect of the biological solidified sand first increased and then decreased. And the 8 hour was the turning point of the trend change. When the interval of the grouting time was 8, the strength of the sand column after the solidification was larger. The content of calcium carbonate was more. Meanwhile, the filling time interval of 8 h could shorten the curing time effectively under the premise that the curing effect was good.

## Acknowledgements

Authors would gratefully like to acknowledge the support provided by the National Key Research and Development Program of China (2017YFB0903700, 2017YFB0903703), the Chinese Postdoctoral Science Foundation (2017M622635), and the Natural Science Fund of Guangdong Province (2017A030310244, 2016A030310345).

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