Factors Affecting Bio-cemented Typical Silt from Middle and Lower Reaches of Yellow River

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Abstract. Microbial-induced calcite precipitation (MICP) technique takes advantage of the metabolic process of bacteria bonding soil particles together with an environmentally-friendly and sustainable manner. In this study, the silt from reaches of Yellow River was used for bio-grouting. The effects of temperature, pH, inoculum size and revolving speed on urease activity were studied by single factor method for Sporosarcina pasteurii. Moreover, the effects of different nutrient concentrations on the stabilization for the typical silt were evaluated. Highest urease activity could be obtained under temperature of 32 ℃, inoculum size of 5%, pH of 8 and speed of 180 r/min. Besides, higher concentration of calcium salt could generate more calcium carbonate effectively, and calcium carbonate content can reach to 261 mg/g with uniaxial compressive strength of 1.7 MPa.

1. Introduction

Bio-grouting reinforcement technology uses a kind of urease-producing microorganism to hydrolyze urea to form carbonate ions and ammonia ions. Free carbonate ions combine with calcium ions under alkaline conditions to form a precipitated calcium carbonate precipitate in this process. It is a new green reinforcement technology with low energy consumption, low emissions and low pollution. It has been successfully applied in the field of slope instability protection, dam body seepage prevention and foundation soil reinforcement [1]. Based on the mechanical strength test of microbial-treated soil [2], the foundation stability reinforced by bio-grouting can be significantly improved.

Most of the Huang fan District in Henan belongs to the alluvial plain of the Yellow River. The terrain is flat and open, and the surface layer is the Quaternary new alluvial silt. Silt is poor in structure and easily liquefied by vibration. When silt is used as a bearing layer, it is easy to be disturbed with the structure and cause the project to fail. This paper studies the effect of microbial grouting to strengthen silt. The core of microbial induced calcium carbonate deposition technology is how to obtain stable microbial urease activity [3]. Sporosarcina pasteurii from the United States and S. pasteurii from Germany belong to the same strain and can produce urease [4-5]. Although many scholars have studied S. pasteurii from the American Collection of Cultures (ATCC, No. 11859) [6-8] and obtained its optimal growth conditions, the strain is expensive. The price of S. pasteurii provided by the China General Microbiological Culture Collection Center is only 1/4 of that. Although they belong to the same species in biology, their optimal growth conditions have changed due to the...
Influence of the ecological environment of their origin. At present, little research has been done on the optimal growth conditions of \textit{S. pasteurii}.

In this paper, \textit{S. pasteurii} from Germany was adopted. The effects of temperature (27–37 °C), pH (6–10), rotational speed (130–200 r/min) and inoculation amount (1%–5%) on growth conditions for this strain were studied by single factor analysis. Besides, the effect of different nutrient concentration (0.5–1.1 mol/L) on the reinforcement of typical silt, which is taken from middle and lower reaches of Yellow River (Zhengzhou area), was also studied.

2. Test materials and methods
\textit{S. pasteurii} used in this experiment (Strain number 1.3687) is purchased from China General Microbiological Culture Collection Center. The medium consisted of 1 g/L peptone, 3 g/L beef extract, and 20 g/L urea, with the initial pH of 8.0. Urease activity in the culture medium was measured by conductivity method [8]. The urease activity of each single factor test was measured every 2 hours and 24 monitoring points were obtained for each type of test.

Silt in the middle and lower reaches of the Yellow River was selected as the object of bio-grouting reinforcement technology. The soil grain sizes distribution curve of the silt is shown in Figure 1. The basic physical indicators are shown in Table 1. The void ratio ($e$) is controlled to 0.6 and the water content of the sample is set to 10%. The sample preparation was carried out with a mold made of plexiglass. The diameter and height of the sample are 3 cm and 6 cm respectively. The peristaltic pump is used for pressure grouting. The grouting speed is 0.5 mL/min. The grouting material is a mixture of calcium acetate and urea. In order to reduce the loss of clay particles in samples under grouting pressure, sample ends are sealed with gauze and filter paper.

![Figure 1. The soil grain sizes distribution curve of silt.](image)

| Soil sample name | Void ratio ($e$) | Water content ($w_1$) (%) | Liquid limit ($w_L$) (%) | Plastic limit ($w_P$) (%) | Plasticity index ($I_p$) | Specific gravity of soil grain ($G_s$) |
|------------------|------------------|---------------------------|--------------------------|---------------------------|--------------------------|-----------------------------------|
| Silt             | 0.6              | 10%                       | 19.82                    | 14.6                      | 5.22                     | 2.61                              |

3. Test content

3.1. Optimization of microbial growth conditions
The liquid medium and the solid medium are prepared before the activation of the strain. The components of the liquid medium are urea, peptone, and beef extract (prepared according to the mass ratio: 3:5:20). The urea needs to be sterilized by a disposable sterilization filter, and the other mediums are sterilized at 121 °C. The solid medium was formed by adding 1.5% agar to the sterilized liquid medium solution. After the strain was activated, it was re-cultured at 30 °C for 16 h, and then stored in a refrigerator at 4 °C. A single colony was picked from the deposited solid plate and placed in a 5 mL medium, placed in a shaking incubator (180 r/min), and cultured at 30 °C for 16 h. When the 5mL liquid test tube medium appears turbid, start to expand the culture liquid. The urease activity
of the expanded culture medium was tested by conductivity method. In order to optimize the suitable growth conditions of the bacterium, the single factor control method was used to study the urease activity of expanded culture medium by temperature (27-37 °C), pH value (6-10), inoculums size (1%-5%) and shaking table speed (130-200 r/min). Table 2 for details.

Table 2. Optimization of microbial growth conditions.

| Group | Variable                  | Invariant                      |
|-------|---------------------------|--------------------------------|
| I     | temperature(°C)           | 27, 30, 32, 34, 37             |
| II    | pH                        | 6, 7, 8, 9, 10                 |
| III   | inoculum size(%)          | 1, 2, 3, 4, 5                  |
| IV    | Rotating speed(r/min)     | 130, 150, 180, 200             |

3.2. Microbial grouting
The urease activity of the microbial grouting was 11 U/mL, and the nutrient concentration of the 1*-4* sample was 0.7, 0.9, 1.1, and 0.5 mol/L, respectively. The 5* sample was a control with no microbial solution. Firstly, injecting 50 mL of bacterial solution (urease activity was 11 U/mL), followed by 300 mL of nutrient salt (mixture of calcium acetate and urea). All samples were immersed in deionized water for 48 h and then dried at 70 °C for 36 h [4]. UCS tests at a loading rate of 1 mm/min were carried out for all the samples. After UCS tests were completed, all fractions of each bio-cemented sample were collected and mixed with excessive 2 M HCl, and then the final residues were collected by filter paper and were dried at 105 °C for 24 h. The mass of calcium carbonate was calculated through the result of the sample before and after treatment.

4. Test results and discussion

4.1. Effects of various factors on microbial growth conditions
The change trend of urease activity of bacterial liquid at different temperatures is shown in figure 2. During the period of 0–8 h, the urease activity of the strain increased gradually. At the stage of 8–14 h, the urease activity of the liquid was stable. After 14 h, as the medium is continuously absorbed by the microorganisms, the growth of the microorganisms is inhibited, and the urease production is gradually reduced. From the comparison of different temperatures, the higher the temperature, the higher the urease. At the temperature of 32 °C, the urease activity of the *S. pasteurii* was the highest, with the highest value 11.1 U/mL. After the temperature exceeded 32 °C, the urease activity of the bacterial liquid was significantly restricted (at 37 °C, the urease activity value was 6.882 U/mL). Increasing the temperature by 2 °C to 34 °C, the strain's stable urease activity value changed from 11.1 U/mL to 7.992 U/mL. The urease activity was decreased from 11.1 U/mL to 7.77 U/mL, when the temperature was reduced to 30 °C on the basis of 32 °C. Thus, the temperature has a great influence on the growth trend of the *S. pasteurii*, which needs to be strictly controlled. When the temperature is 32 °C, it is especially beneficial for grouting microorganisms in the later stage.
As shown in Figure 3, the metabolism of the S. pasteurii was rapid in the 0–6 h period, and the urease production was changed from 0 U/mL to 6.882 U/mL. The environment of the strain changed from acidic to alkaline (pH=6–10), and the urease value of the metabolic output of the strain increased gradually from 6.882 U/mL (pH=6) to 8.214 U/mL (pH=8). When the surrounding alkaline gradually increased to a pH of 10, the urease activity decreased to 7.326 U/mL (Figure 3). It can be seen that the growth of the S. pasteurii tends to be alkaline environment. The optimum pH value for grouting is 8–10, and when the pH is 8, the urease production is the most (8.214 U/mL).

Cheng et al. [10] found that when the initial pH values of the medium were 8, 9 and 10, the yield of calcite induced by microbial organisms was relatively close. When the initial pH value of the medium was 7, the yield of calcite induced by microbial sedimentation is significantly reduced. The yield is significantly reduced. This paper also validates this view, which is consistent with the findings of the Hammes et al. [11].

As shown in Figure 4, when the pH is 8, the temperature is 32 °C, the shaking speed is 180 r/min, and the inoculum size is 5%, the urease activity of expanded culture medium of S. pasteurii was higher (8.436 U/mL). The inoculum amount was in the range of 1%–5%, the urease activity increased with the increase of inoculum size, but within 6–14 h, the urease activity value of the strains was basically stable. The urease activities at 3%, 4%, and 5% inoculation were close, which were 8.01 U/mL, 8.201 U/mL, and 8.245 U/mL, respectively. It can be seen that under the amount of unit medium, the urease activity of the strain increases with the increase of the inoculum, but the increase rate reduces. When the inoculum size was 5%, the stable urease activity value was the highest (8.436 U/mL). The growth of S. pasteurii was inhibited by insufficient space because the medium was gradually fully filled with S. pasteurii in the range of 1%–5% inoculum size. Therefore, it is more appropriate to select the inoculum size of the strain as 5%.

As shown in Figure 5, when the culture speed of the cultured bacterium of the S. pasteurii was 180 r/min, the urease activity of the bacterium was the highest (8.214 U/mL). When the culture medium of
S. pasteurii was cultured for 10 h, the growth of the strain was the strongest, the metabolism was the fastest, and the urease activity was the highest. When the culture time was 10-15 h, the growth of the strain reached equilibrium. After 15 h, the growth of the strain began to decline, and the production of urease decreased significantly (Figure 5).

In summary, the optimal culture medium for the culture of the strain is cultured at 32 °C, the pH of the culture solution is 8, the inoculum is 5%, and the speed is 180 r/min.

4.2. Analysis of silt grouting effect

As shown in Figure 6, the calcium carbonate content in the silt sample after microbial grouting increased significantly, and the content of calcium carbonate formed by bio-gelation gradually increased with the increase of microbial grouting concentration. When the concentration of microbial grouting increased from 0.5 mol/L to 0.7 mol/L, the calcium carbonate content increased from 115 mg/g to 144 mg/g. When the microbial concentration increased from 0.7 mol/L to 0.9 mol/L and 0.9 mol/L to 1.1 mol/L, the calcium carbonate content increased by 225 mg/g and 261 mg/g, respectively. The increase of microbial grouting concentration leads to an increasing trend of calcium carbonate content. When the concentration is in the range of 0.7~0.9 mol/L, the increase of calcium carbonate content is 81 mg/g. When the grouting concentration is 0.5~0.7 mol/L and 0.9~1.1 mol/L, the amount of calcium carbonate is 29 mg/g and 36 mg/g, respectively. It can be seen that when the grouting concentration is in the range of 0.7~0.9 mol/L, the rate of increase of calcium carbonate induced by microorganisms is the largest. If the microbial grouting concentration is continued to increase, the calcium carbonate content increases slowly. This phenomenon indicates that the concentration of calcium ions in the nutrient salt should not be too high. When the concentration of calcium ions in the grouting is higher than 0.9 mol/L, it will inhibit the urease activity of S. pasteurii, which is not conducive to the formation of calcium carbonate.

Figure 6. Relationship between nutrient salt concentration and calcium carbonate content.

As shown in Figure 7, as the nutrient salt concentration increases, the uniaxial compressive strength increases from 0.5 MPa to 1.7 MPa. Consistent with the above conclusions, with the increase of nutrient salt concentration, the uniaxial compressive strength of the sample also increased, and the maximum value is 1.7 MPa. The uniaxial compressive strength of the sample not injected with the microbial solution was 30 kPa, and the highest uniaxial compressive strength of the microbial grouting sample (5") was 56.6 times that of the uninjected (1"). It can be seen that microbial grouting can significantly improve the compressive strength of the soil and can achieve a good strengthening effect on the silt in the middle and lower reaches of the Yellow River.

5. Conclusion

In the experimental study, when the urease activity value is 10 U/mL and the calcium salt concentration is 1.1 mol/L, the strength of the silt can be significantly improved, and the uniaxial compressive strength can reach 1.7 MPa at the maximum. Urease produced by microbial
metabolism is the dominant factor in inducing the formation of calcium carbonate, which affects the strength of the soil. The specific conclusions are as follows:

1) When the temperature is 32 °C, the strain can produce a higher amount of urease. In addition, the growth of the strain is alkaline, and the optimum pH is 8. The microbial concentration and the vibration speed also have an effect on the metabolism of the microorganism. The inoculum size of 5% and the rotation speed of 180 r/min are suitable.

2) The culture time is very important for obtaining stable urease, usually 6~14 h. During this period, the metabolism of the strain is relatively stable, which is the best time for microbial grouting.

3) The concentration of grouting nutrient salt is an important factor affecting the reinforcement effect of silt. The calcium carbonate content has a linear relationship with the uniaxial compressive strength. The calcium ion concentration should be lower than 0.9 mol/L. When the concentration of nutrients is more than 0.9 mol/L, the formation rate of calcium carbonate will be reduced.

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