Influence of Culture Medium on Cementation of Coarse Grains Based on Microbially Induced Carbonate Precipitation

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Abstract: A main challenge in the large-scale application of the microbially induced carbonate precipitation (MICP) technique includes the low efficiency of the cementation of coarse grains. Actually, in the MICP treatment process, the cementation effect of the bonding points was more important than pore filling due to the large porosity for coarse grains. To achieve a better cementation effect at bonding points between coarse particles, the quick formation and growth of a biofilm is necessary. In this study, an optimized medium was proposed to improve the cementation effects for coarse materials. The optimized medium and other different media were used for bio-cementation tests with MICP. The viable cell concentrations, strengths, microscopic characteristics, biofilm contents, and calcium carbonate (CaCO₃) contents were used to evaluate the bio-cementation and its effects. In bio-cementation tests, the optimized medium led to increased CaCO₃ precipitation at the bonding points and better cementation effects compared to other media. Indeed, the strength of the sample treated with the optimized medium was more than 1.2–4 times higher than that of the values for other media. The advantages of the optimized medium were demonstrated via bio-cementation tests.

Keywords: MICP; coarse materials; optimized medium; biofilm growth; bonding points

1. Introduction

In recent years, bio-cementation or microbially induced carbonate precipitation (MICP) has received the attention of many researchers in geotechnical engineering fields [1–4]. The principle of the MICP technique is that calcium carbonate (CaCO₃) with a cementing function is obtained by calcium ions binding to carbonate ions [5–8]. The implementation potential of the MICP technique is widely recognized [9–13]. For instance, this technique can be applied to enhance soil strength and stiffness [2], reduce soil permeability [14], repair cracks [15–17], improve the mechanical properties of granular soils [16], and control erosion and dust [18–23]. CaCO₃ precipitation can form bridges between granular soil particles, leading to the cementation of loose soil particles into a cohesive and enhanced structure. Although many researchers have studied soil improvement with MICP, limitations exist in the application of this method in the bonding of coarser materials. For coarse materials, MICP treatment is less cost-effective, since it requires a higher number of biochemical treatment cycles to gain acceptable strength [24]. However, coarse materials are common in practical engineering, which limits the extensive application of MICP. To decrease the high investments required for the application of MICP, cost-effective approaches are needed.

In fact, for coarse grains with a large porosity, if bonding points between the coarse particles are cemented well, the solidified sample will exhibit great strength [25]. Compared with the higher number of biochemical treatment cycles, the cementation effect of the bonding points is more important. In order to achieve a better cementation effect at the bonding points between coarse particles, the quick formation and growth of a biofilm is necessary [25]. Precipitated CaCO₃ is attached to the biofilm, with an increasing amount of CaCO₃ precipitation available at the bonding points as the biofilm quickly forms and...
grows, eventually leading to a better cementation effect [26,27]. Biofilms have been used in many applications, such as biobarriers and microbial-enhanced oil recovery [28]. However, there have been few studies focusing on the effect of biofilm growth on bio-cementation to improve the cementation effect. With the aim of promoting biofilm growth, using a suitable culture medium can be an effective method for large-scale applications. Therefore, the medium was optimized in this study for an effective cementation.

Previous researchers have used yeast extract [4,24,29,30], peptone [4], and ammonium sulfate [24,29] as the common nutritional sources for the cultivation of urease-producing bacteria. The same bacterial strain was used in the present work. In addition, several other nutritional sources are available, such as nutrient broth [3] and glucose [15]. In the present study, an optimized medium was obtained. The optimized medium and some other culture media were used, and bio-cementation tests for coarse materials were conducted using these media. The viable cell concentration in the effluent, unconfined compressive strength (UCS) test, scanning electron microscopy (SEM), biofilm growth, and CaCO$_3$ quantification were used to evaluate the bio-cementation effects.

2. Materials and Methods

2.1. Bacteria

In the present study, a type of ureolytic bacteria was used, namely *Sporosarcina pasteurii* (*S. pasteurii*; ATCC 11859; obtained from the Guangdong Culture Collection Center of China). The changes in cell density can be obtained by monitoring the absorbance (optical density) of the suspension at a wavelength of 600 nm (OD$_{600}$) [6,31]. Therefore, in the present paper, optical density (OD$_{600}$) was used to represent the cell density.

2.2. Granular Column Specimen

Coarse aggregates (2.5–25-mm sieves) and fine aggregates (0.075–2-mm sieves) from the Yangtze River were used to prepare samples for the MICP treatment. The mass percentage of 25% for fine aggregates was also used in the current study. The particle size distribution is presented in Figure 1. All mixtures were classified as poorly graded materials, according to the Unified Soil Classification System [32]. In total, five samples were cemented after reaction(s) catalyzed by the bacteria with five different culture media. Coarse and fine aggregates were sterilized at a high temperature (120 °C) and high pressure (250 kPa) before being placed in polyvinyl chloride (PVC) cylinders, which had an inner diameter of 10 cm and height of 20 cm. The experiments were performed under aseptic conditions, and there was no contamination with other microorganisms.

![Figure 1. Particle size distribution of granular materials.](image-url)
2.3. Culture Media

The culture medium was optimized for bio-cementation. In previous studies, yeast extract, polypeptone, and ammonium sulfate have often been used as nutritional sources for the cultivation of *S. Pasteurii*. [4,24,26]. Hence, yeast extract, polypeptone, and ammonium sulfate were added to the culture medium and their concentrations varied to obtain five distinct media in this study. In addition to these three components, 10.0 g/L of NaCl was also added into the media to provide an adequate salt content for bacterial growth, and the initial pH of the culture medium was 7.0. The NaCl might increase the ionic strength, leading to increased CaCO$_3$ precipitation.

2.4. River Sediments Treatment Procedures

Firstly, air was expelled from the samples through the addition of tap water using the percolation method, until a volume of water equivalent to 150% pore volume was reached, as shown in Figure 2. Subsequently, the bacterial suspension cultured for 48 h was added using the same method, reaching a bacterial suspension volume of 100% of the pore volume. The samples were left to rest for 2 h, which was followed by the addition of 50% of the pore volume of a gelling solution (consisting of a mixture of 1.0-M urea and calcium chloride). The gelling solution was mixed with the remaining culture media. Eventually, the samples had 50% pore volume of bacterial suspension and 50% pore volume of gelling solution. The initial pH value of the gelling solution was 7.0. Incubation for 48 h at 20 ± 2 °C was performed to allow for the production of precipitation. In order to perform the experiments efficiently, the above-mentioned addition cycle was repeated every 48 h to reach a total of six addition cycles. Iqbal et al. [33] stated that calcite precipitates were concentrated at the contact points of the particles, which led to a better improvement of resistance under the saturated condition of 50%. However, the degree of saturation in the treatment procedures for all the samples was 100%; therefore, the results were comparative. The comparison of the results was quantitative.

![Figure 2. Flow chart of the treatment procedures.](image)

2.5. Evaluation of Treatment Effects

Before the treatment cycle, the effluent was collected, and its viable cell concentration (cell/mL) was measured by the plate colony counting method. Studying the viable cell
concentration in the effluent allowed the comparison of the microorganism viability of the strain in different samples.

After 12 days, UCS tests were conducted in accordance with ASTM C617 [34], and the UCS values of the samples were determined to evaluate the cementation effect. The loading speed was constant at 1 mm/min for the duration of the UCS tests.

Following the completion of the UCS tests, the sediments at the bonding points were sampled and subjected to the SEM, and the bonding effects were evaluated via microscopic observation. The sample was rinsed with ionized water and then dried in an oven at 60 °C for 24 h. After that, the sample was gold-sputtered. Several SEM photos were obtained using the apparatus (type: JSM-6300, JEOL company, Tokyo, Japan), which showed the sample treated with the optimized medium or C medium.

Similarly, to evaluate the biofilm growth at the bonding points between coarse particles, the biofilm between two coarse particles was scraped, and the plate colony counting method was used again to determine the colony-forming units (CFU). In addition, the bonding points between coarse particles were chosen after the UCS tests. The CaCO$_3$ content at the bonding point was measured using the gravimetric acid washing (2-M HCl) technique [4]. Their dry mass was measured before and after the acid wash. The difference between the mass of the samples was considered to indicate the mass of precipitated CaCO$_3$, and the CaCO$_3$ content was obtained by dividing the mass of calcium carbonate by the mass of the sediments. Triplicate samples were prepared to obtain the average CaCO$_3$ content levels.

3. Results and Discussion

3.1. Preparing Different Culture Media

For various building materials with different particle sizes, the pore sizes for bacterial growth and calcium carbonate precipitation varied greatly. Biomass evolution with time in the pore network was primarily controlled by the growth, decay, and attachment of the biomass [35–37], as shown in Equation (1):

$$ R^{bio} = \mu \varepsilon w C^{bio} - k_{dec,1} \left( 1 + \frac{m^2_{H^+}}{K_{pH}} \right) \varepsilon w C^{bio} - k_{att} S^{bio} C^{bio} $$

where $\mu$ is the growth rate coefficient of the biomass or biofilm, calculated as shown in Equation (2). $\varepsilon w$ is the volume fraction of the water phase, and $C^{bio}$ (kg/m$^3$) is the concentration of the biomass. $k_{dec,1}$ is the endogenous decay rate constant, $m_{H^+}$ is the molality of proton, and $K_{pH}$ is an empirical constant. $k_{att}$ refers to the attachment coefficient, and $S^{bio}$ (m$^2$) is the available specific area for biomass attachment in a pore element, which can be obtained based on the pore sizes and volume fraction of the water phase [38]. On the right-hand side of Equation (1), the terms denote the growth of the biomass, the decay of the biomass, and the first-order kinetics of the biomass attachment, respectively [38].

As shown in Equation (2), nutrient and oxygen are very important for biomass growth.

$$ \mu = k_{\mu} \frac{C^n}{k_n + C^n} \frac{C^0}{k_0 + C^0} $$

where $k_{\mu}$ is the maximum substrate utilization rate constant, $C^n$ (kg/m$^3$) and $C^0$ (kg/m$^3$) are the mass concentrations of the nutrient and oxygen, respectively, and $k_n$ and $k_0$ are the half-saturation constants.

Once the biomass concentrations in the pore elements were obtained, the biofilm volume fractions were determined, according to Equation (3) [39]:

$$ R^f = \mu \varepsilon w \rho^f - \left( k_{dec,1} + k_{dec,2} \frac{\max(0, r_{prec}) M^c}{\rho^c (1 - \varepsilon^c)} \right) \varepsilon w \rho^f + k_{att} S^{bio} C^{bio} $$
where $\rho_f$ is the biofilm density, and $\epsilon^f$ is the volume fraction of the biofilm. The growth rate coefficient ($\mu$) and the endogenous decay coefficient ($k_{dec,1}$) are the same for both the biofilm and the suspended biomass [39], while $k_{dec,2}$ is the second decay rate constant due to the calcite precipitation [37,40]. $\rho^c$ (kg/m$^3$) is the calcite density. $M^c$ is the molecular weight of calcite, and $r_{prec}$ (mol·m$^{-3}$·s$^{-1}$) is the calcite precipitation rate.

To take into account the effects of possible mass transfer limitations and complex nucleation mechanisms on the CaCO$_3$ precipitation rate at the pore element scale [41–44], the equation for the CaCO$_3$ precipitation rate was slightly revised based on the equation described in Reference [45], as shown in Equation (4), where $\beta$ is a constant correction coefficient; $k_g$ is the growth rate constant, which is derived empirically and depends on the growth mechanism, mineral type, and temperature [46–49]; and $n$ is the kinetic order. $S$ is the supersaturation, which is based on the concentration of calcium and carbonate ions and biomass according to the study in Reference [47].

$$r_{prec} = \beta \frac{\rho_c}{M_c} \cdot 4\pi \cdot [k_g \cdot (S - 1)^n]^2$$  \hspace{1cm} (4)

On the basis of Equation (4), bacteria can serve as the nucleation site, and CaCO$_3$ will be produced around the bacteria. These parameters and empirical constants were derived from the study in Reference [9]. In the process of solidification, the relationship between the volume fractions of calcite ($\epsilon^c$), the water phrase ($\epsilon^w$), and the biofilm ($\epsilon^f$) is given in Equation (5). Moreover, with the increase in solidification time, the volume fraction of the water phase gradually decreased, and the sum of the volume fractions of calcite and the biofilm reached 1.

$$\epsilon^c + \epsilon^w + \epsilon^f = 1$$  \hspace{1cm} (5)

Overall, the volumes of pores can be calculated in advance, as shown in Equation (6), where $V$ is the volume of the samples, and $e_{ave}$ is the average void ratio. On the basis of the volume of pores and Equations (1) and (3)–(5), the required initial biomass concentration and growth rate can be obtained.

The initial biomass concentration can be transformed into the optical density (OD$_{600}$) via Equation (7) [50]. The parameters in these equations are defined in Table 1.

$$V_{pore} = V \frac{e_{ave}}{1 + e_{ave}}$$  \hspace{1cm} (6)

$$\text{Concentration of cells/mL} = 8.59 \times 10^7 \times \text{OD}_{600}^{1.3627}$$  \hspace{1cm} (7)

Table 1. Parameters in the model.

| Parameter                                      | Value                      | Reference |
|------------------------------------------------|----------------------------|-----------|
| Maximum substrate utilization rate $k_s$      | $4.1667 \times 10^{-5}$ l/s | [51]     |
| Half saturation constant of oxygen $k_o$      | $2.0 \times 10^{-5}$ g/L   | [37]     |
| Half saturation constant of nutrient $K_N$    | $7.99 \times 10^{-4}$ g/L  | [52]     |
| Endogenous decay rate $k_{dec,1}$             | $3.18 \times 10^{-7}$ g/L  | [52]     |
| Empirical parameter $K_{pH}$                  | $6.15 \times 10^{-10}$     | [39]     |
| Biomass attachment rate $k_{att}$             | $6.15 \times 10^{-7}$ mm/s | [39]     |
| Decay rate due to calcite precipitation $k_{dec,2}$ | 1.0                       | [53]     |
| Biofilm density $\rho^f$                      | 2.0 g/L                   | [53]     |
| Calcite density $\rho^c$                      | 2710 g/L                  | [37]     |
| Constant coefficient $\beta$                 | 0.1                       | [39]     |
| Molecular weight of calcite $M^c$             | 100.09 g/mol              | [39]     |
| Kinetic order $n$                             | 2                         | [47]     |

The initial biomass growth rate was driven by the concentrations of the nutrients in the medium after the culture of the bacteria. Therefore, the optimized medium has a suitable initial biomass concentration and growth rate after the culture that could
ensure the good cementation effects of the coarse materials. The medium can be optimized based on the calculations. According to the characteristics of the sample columns, the initial total volume of the pores could be calculated \((4.158 \times 10^{-4} \text{ m}^3)\), and then, based on Equations (1) and (3)–(5), the required initial biomass concentration was obtained \((\text{OD}_{600} = 1.68)\). In the pre-experiment, the concentrations of the yeast extract, polypeptide, and ammonium sulfate varied greatly to prepare different media, which were used for the culture of \(S.\ pasteurii\), and the biomass concentrations were measured after 48 h. According to the authors’ pre-experiment, a similar biomass concentration could be obtained at 30 °C for 48 h (Table 2) with the initial concentrations of the nutrients in the medium (20-g/L yeast extract, 10-g/L polypeptide, and 5-g/L ammonium sulfate). Therefore, the medium was defined as the optimized medium.

| Name            | Concentration of Yeast Extract | Concentration of Polypeptide | Concentration of Ammonium Sulfate | Optical Density (OD\(_{600}\)) |
|-----------------|-------------------------------|------------------------------|----------------------------------|------------------------------|
| Optimized medium| 20 g/L                        | 10 g/L                       | 5 g/L                            | 1.68                         |
| A medium        | 20 g/L                        | 10 g/L                       | 0 g/L                            | 1.72                         |
| B medium        | 20 g/L                        | 20 g/L                       | 5 g/L                            | 2.12                         |
| C medium        | 33.63 g/L                     | 33.63 g/L                    | 0 g/L                            | 2.79                         |
| D medium        | 5 g/L                         | 5 g/L                        | 5 g/L                            | 1.21                         |

To compare the effect of nutrients on the bio-cementation effects, ammonium sulfate was not added as the A medium. The B medium had a larger concentration of polypeptide (20 g/L) to study the influence of polypeptide. The growth characteristics (growth rate) would be greatly affected by the amount of bacterial culture added to the liquid culture medium [16]; therefore, the bacteria in the present study were inoculated with identical \(\text{OD}_{600}\) values \((\text{OD}_{600} = 1.0)\). The optical density \((\text{OD}_{600})\) with different media after 48-h culture at 30 °C can be seen in Table 2.

3.2. Bio-Cementation of Coarse Materials

3.2.1. Viable Cell Concentration in Effluent

The MICP technology has the potential to be used extensively because of the cementing characteristics of \(\text{CaCO}_3\) precipitation [34]. In order to determine whether the optimized medium offers advantages for the bio-cementation of coarse materials, an analysis of the cementation effect is needed. Therefore, sand solidification tests with MICP were often conducted in previous studies [12,14,55]. Prior to each treatment cycle, the viable cell concentration (cell/mL) of the effluent was measured using the plate colony counting method, as shown in Figure 3. The viable cell concentrations in an effluent with the optimized medium and A medium were similar. After 8 days, the viable cell concentrations increased, which was due to less new bacterial cells being attached on the grain surface, resulting in more viable bacterial cell being left in the effluent. The phenomenon was also observed in Reference [56]. If the viable cell concentration (cell/mL) of the effluent was higher, there was two reasons. The first reason was that more nutrients left in the medium accelerated the biomass growth during the 48-h reaction period. This reason was demonstrated by measuring growth curves over time obtained from cultures, as shown in Figure 4. The second reason was due to decreased attached biomass in the river sediments matrix. This reason will be further analyzed in the “Calcium carbonate quantification” section. The growth of viable cells in the effluent of media A, C, and optimized increased more after 8 days, because more bacteria attached on the grain surface before 8 days, resulting in a smaller space left for later attachment.
Figure 3. Viable cell concentrations in the effluent for various media.

Figure 4. Growth curves over time for various media.

For the B medium, the viable cell concentrations in effluent were always slightly larger compared with those observed for the optimized and A media, since polypeptone with a high concentration might provide more nutrients for biomass growth [4]. The viable cell concentration in the effluent with D medium was the smallest, only around $4 \times 10^7$ cell/mL. For the D medium, most of the nutrients were consumed before 48 h, which indicated that there were not sufficient nutrients in the bacterial suspension for the bacterial growth and reproduction and biofilm growth between the two treatment cycles. Meanwhile, the viable cell concentration in the effluent did not fluctuate greatly, since the slow biofilm growth did not markedly affect the attachment of bacterial cells on the grain surface [38].

For the C medium, the viable cell concentrations in the effluent were larger than those observed for the other four media. The reason behind this might be that, after the cultivation of the bacteria, enough nutrients remained in the medium, which deserves further study. Furthermore, after 8 days, the increasing range of the viable cell concentration in the effluent for the C medium was also relatively larger, indicating that quick biofilm growth resulted in less new cells being attached on the grain surface.
3.2.2. UCS

The sample treated with optimized medium exhibited the largest strength (at about 420 kPa) (Figure 5), which was nearly two times higher than those exhibited by the samples treated with the C medium and 4.5 times greater than that of the sample treated with the D medium (only 92 kPa). The B medium and C medium had more nutrients in the medium than the optimized medium and A medium, but the samples treated with the B medium and C medium had worse cementation uniformity, eventually leading to smaller strengths than the sample with the optimized medium and A medium.

![UCS values for solidified columns.](image)

The strengths of the samples in this study were smaller than that reported in Reference [24]. According to the ASTM national standards, for soil samples, the largest particle size should be smaller than one-sixth of the specimen diameter, which should be 16.7 mm [57], but 25-mm aggregate in a 100-mm core would be acceptable for concrete testing [58]. When the coarse particles had smaller sizes, it is hard to say that the overall strength of the treated samples was attributed to the formation of bonding points between coarse particles rather than the filling of fines between those coarse particles. Therefore, coarse particles with larger sizes were used to conveniently study the cementation effects of the bonding points. It was because the pores could not be filled completely by bacteria and precipitation that the cementation effect of the bonding points contributed to the development of strength. The smaller strengths of the samples in this study were also because the coarse particles had larger sizes. In addition, aggregates up to 25 mm in a 100-mm-diameter column could potentially lead to nonuniform packing and, hence, smaller strength. A minimal unconfined compressive strength of 345 kPa is required to ensure the sufficiency in soil stabilization [59].

3.2.3. Scanning Electron Microscopy

Figure 6a shows that, in response to the optimized medium treatment, compared with the coarse particle surface, a large number of CaCO\(_3\) crystals was produced between the coarse particles. Near the bottom corners, a sediment particle seemed to be broken, exposing a layered structure. This pattern was more likely to be a layered silicate mineral such as muscovite. Under flowing conditions, it may be more likely for bacteria to attach at contact points due to the physical straining of cells, and in partially saturated conditions, it may occur due to liquid hold-up at the contact points. However, in this experiment, the entire pore volume was saturated with a high concentration of bacterial suspension, and a 2-h static period (no flow) provided time for the bacterial cells to attach uniformly.
over the entire surface. Therefore, several minerals and bacterial biomass were deposited on the surfaces of the coarse particles.

![SEM images of samples treated with optimized medium](image1)

**Figure 6.** SEM images of samples treated with (a) the optimized medium (magnification = 500×), (b) C medium (magnification = 500×), and (c) optimized medium (magnification = 40,000×).

Through observation, more CaCO₃ crystals in the sample with the C medium treatment could be seen on the surfaces of the coarse particles, as shown in Figure 6b. In fact, the SEM images for the optimized media and the C media both showed CaCO₃ almost everywhere. To study the effect of various media on biofilm growth and evaluate the cementation effect in the bonding points between coarse particles, the amounts of biofilm and contents of CaCO₃ should be investigated. There are a large number of works demonstrating the production of calcium carbonate via XDS or any other X-ray diffraction techniques [14,24,60]. In this work, the existence of calcium carbonate was also demonstrated via XRD (Figure 7). The CaCO₃ crystals was verified with the micrograph of Figure 1 in the study of Uenishi and Matsubara [61].

![XRD figure](image2)

**Figure 7.** XRD figure of the sample treated with the optimized medium.

### 3.2.4. Biofilm Growth

Based on Equations (1) and (3), when sufficient nutrients and oxygen are available, the concentration of the suspended biomass increases; meanwhile, the biofilm grows gradually. Therefore, more nutrient resources in the medium resulted in a higher growth rate of the bacterial cell density, as well as the biofilm. The content of the biofilm is not easily quantified, and there is a lack of consensus among the diversity of techniques used to
grow and study biofilms [62]. The plate colony counting method used in this present study to determine the CFU presents several limitations [63]; for example, a subpopulation of biofilm cells can be viable but nonculturable and will not be detected by the CFU approach. However, this method is the most widely used technique to estimate biofilm cell viability, and the requirements of the experiments can be fulfilled by most platforms. Moreover, the sample treated with the optimized medium had the largest strength, and the formation of bonding points between coarse particles was attributed to the optimized medium-formed biofilm. During the 48-h incubation period, bacterial growth and reproduction, biofilm growth, and biomineralization occurred [38]. The biofilm was sampled, and the viable cell concentration method was used to show the biomass concentrations in the bonding points, as shown in Figure 8. The measuring points were chosen around the bottom, which can be used to better show the cementation effect in the bonding points.

![Images of the viable cell concentration method showing the biofilm growth (10^6 times dilution).](image)

**Figure 8.** Images of the viable cell concentration method showing the biofilm growth (10^6 times dilution).

The contents of the biofilm in the samples treated with the optimized medium were the largest. For the A medium, the concentration of ammonium sulfate was smaller than that in the optimized medium, which might be the reason why the biofilm content of the samples treated with the A medium was smaller than with the optimized medium. The content of the biofilm in the sample treated with the A medium was a little larger than the samples treated with the B medium. It might be because the effect of polypeptone on biofilm growth was smaller than the effect of yeast extract on biofilm growth. High-concentration nutrients resulted in bacterial suspension with high concentrations of cells, which significantly impacted the cementation uniformity [4,16]. For the C medium, the content of the biofilm was much smaller, which indicated that less bacterial cells were attached on the grain surface. The biofilm content of the samples treated with the D medium was the smallest because of the much lower concentrations of nutrients.

### 3.2.5. Calcium Carbonate Quantification

In the current study, a great number of coarse aggregates (75%) was used, and precipitated CaCO₃ could not fill the pores; thus, the higher strength was attributed to the better cementation effect of the bonding points between the coarse particles. The better cementation effect was a result of the higher amount of precipitated CaCO₃. According to Figure 8, the optimized medium resulted in greater biofilm growth and larger strength. A linear relationship was observed between the OD₆₀₀ and ureolysis rate with *S. pasteurii* [64]. However, there was no linear relationship between the biofilm and ureolysis rate in previous studies; therefore, calcium carbonate quantification was then conducted to demonstrate that the optimized medium would lead to better cementation effects.

The distribution of the CaCO₃ content along the bio-cemented granular columns is shown in Figure 9. Except for the C medium, the samples treated with the other four media exhibited good cementation uniformity. The CaCO₃ content of the samples treated with the D medium was much smaller, since the D medium had less nutrients for biomass growth and biofilm growth and was not the optimum medium for *S. pasteurii*, which was consistent with the results of the biofilm contents. The CaCO₃ content of the sample treated with the optimized medium reached almost 10%, indicating that there was much more CaCO₃ as compared to the other four columns. Many factors can impact the type and amount
of CaCO$_3$ precipitation, such as the rate of urea hydrolysis, urea and calcium dosages, and amino acids [65–69]. In the present study, the nutrients in the medium affected the biofilm growth, which also affected the amount of CaCO$_3$ precipitation. For the decrease in CaCO$_3$ with the depth in the sample with medium C, the reason might be the decreased oxygen concentrations in the bottom or the larger inhibition effect on transport due to bacterial suspension with high-concentration cells.

![Calcium carbonate content distribution.](image)

Figure 9. Calcium carbonate content distribution.

4. Conclusions

The low efficiency of coarse aggregates for bio-cementation is one of the challenges in the large-scale application of MICP. For various building materials with different particle sizes, the initial biomass concentration and biomass growth rate can be controlled. As a consequence, the optimized medium (20.0-g/L yeast extract, 10.0-g/L polypeptone, 5.0-g/L ammonium sulfate, and 10.0-g/L NaCl) was used to increase the cementation effect of the coarse materials. Furthermore, the tests were conducted with the bacterium grown in different culture media. The samples treated with the optimized medium exhibited the highest UCS value. The optimized medium can promote biofilm growth and increase CaCO$_3$ precipitation at the bonding points between the coarse particles, eventually leading to better cementation effects. The practicability of the optimized medium was demonstrated by the bio-cementation test, which provides a solid foundation for soil stabilization and can be applied in the civil and material engineering fields. The transfer to a natural environment is a major step before the final conclusion about the value of the used method; therefore, further field experiments and studies will be conducted.

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