Polymer modified microbial induced carbonate precipitation: Novel approach to densify the loose sand

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Microbial induced carbonate precipitation (MICP) has gained much attention recently as an ecofriendly approach to treat the problematic grounds. In MICP, calcium carbonate ($CaCO_3$) is produced artificially by hydrolysis of urea using enzyme urease. In the current research, the effect of the cationic polysaccharide, chitosan on the MICP process was investigated. $CaCO_3$ precipitation experiment was conducted with and without chitosan by the hydrolysis of urea using ureolytic bacteria *Pararhodobacter* sp. SO1 in the presence of $CaCl_2$. Morphology and the polymorphism of the samples were analyzed by scanning electron microscopy and X-ray diffraction. In the presence of chitosan, higher amount of precipitate was obtained than that of without chitosan. Without chitosan, rhombohedral crystals were dominant while with chitosan distorted crystal agglomerations combined with the chitosan hydrogel were observed. Further, sand solidification experiments were conducted in the presence and absence of chitosan. With chitosan, strongly cemented sand specimen could be obtained.

1 INTRODUCTION

Bio grouting has been recognized as an efficient and environmentally friendly technique to treat the weak soils in contrast to the other grouting methods which are harmful for the environment. Further, most interesting point is bio-grouts have a possibility to increase the cohesion with the nearby materials, then increase the mechanical strength of the whole system (Li et al., 2015). Among the bio grouting methods, microbial induced carbonate precipitation (MICP) has gained much attention recently as an ecofriendly and sustainable approach to treat the problematic weak grounds.

MICP is a bio-geochemical process which is driven by enzyme urease. Urease is a multi-subunit, nickel containing enzyme and can be found in some bacterial species and plants (Maroney and Ciurli, 2014). Urease enzyme has a capability of hydrolysis of urea and produce ammonium and carbamate ions. Carbamate ions spontaneously transferred into ammonia and carbonic acid and then in the presence of calcium ions (Ca^{2+}), $CaCO_3$ is formed (Amarakoon and Kawasaki, 2017; Fujita et al., 2017; Mortensen et al., 2011; Dejong et al, 2010) as given in the equations (1-3).

$$CO(NH_2)_2 + H_2O \longrightarrow H_2NCOO^- + NH_4^+ \quad (1)$$

$$H_2NCOO^- + H_2O \longrightarrow HCO_3^- + NH_3 \quad (2)$$

$$Ca^{2+} + HCO_3^- + NH_3 \longrightarrow CaCO_3 + NH_4^+ \quad (3)$$

It has been proven that MICP method has capability to improve the soil properties very effectively and efficiently. Remarkable increment of the strength and stiffness have been reported for the MICP treated sand (Montoya and DeJong, 2015; Cheng et al., 2013). Also, interestingly MICP has capability to increase the soil strength while maintaining the sufficient permeability which is required during the biocementation process (Paassen, 2009; Ivanov et al., 2010).

Due to the higher performance of the MICP process, researchers are now looking for the possible techniques to improve the efficiency of the MICP process further. Use of organic materials is one of the most interesting approach and could increase the efficiency of the MICP process more effectively. The concept is called as the polymer modified MICP. Since it is a recently identified trend, only little number of previous research works can be seen under the polymer modified MICP (Wang and Tao, 2018; Nawarathna et al., 2018). This study is the first to address the effects of chitosan on the $CaCO_3$ crystallization and MICP process using the ureolytic bacterium *Pararhodobacter sp.* SO1. The *Pararhodobacter sp.* SO1 is a gram-negative bacterium which is locally isolated from the beach sand in Nago, Okinawa, Japan (Danjo and Kawasaki, 2013). Chitosan is one of the most abundant and inexpensive cationic natural polysaccharides.

2 METHODOLOGY

2.1 *Preparation of bacterial cell culture*

The bacterial cells were pre-cultured in Zobell 2216E medium (polypeptone 5.0 g/L, yeast extract 1.0 g/L, $FePO_4$ 0.1 g/L in artificial seawater, pH 7.6–7.8; 5 mL) by shaking at 30 °C and 160 rpm for 24 h. Then, main culture was prepared by adding 1 mL of pre-culture into 100 mL of fresh Zobell 2216E medium and kept in the shaking incubator under the same conditions as those used for the pre-culturing, for 48 h. By centrifugation of the bacteria culture (10 °C, 6300 ×g, 5 min), cells were collected and resuspended in sterilized water to adjust the cell concentration (OD600 =1). Bacteria cell concentration was measured as an optical density value measured at 600 nm wave length (OD600) by using UV-visible spectroscopy.

2.2 *$CaCO_3$ precipitation*

$CaCO_3$ precipitation was achieved by adding bacteria into the substrate solution containing urea (0.3 mol/L) and $CaCl_2$ (0.3 mol/L). Reaction mixture (10 mL) was kept in shaking incubator under 30 °C and 160 rpm for 24 h. $CaCO_3$ precipitation was separated from the supernatant by centrifugation (24 °C, 14200 ×g, 10 min) and after oven drying (90 °C, 24 h) dry weight of the precipitate was measured. Experiments were conducted with (0.03 %) and without chitosan under two different bacteria concentrations (OD_{600} = 0 – 0.05). Chitosan solution (1%) was prepared by completely dissolving the chitosan powder in 1% acetic acid solution and neutralizing to pH 6.8 by using 0.1 M NaOH solution. Morphology of the $CaCO_3$ precipitate was analyzed for uncoated samples by scanning electron microscopy (SEM; acceleration voltage – 5 kV).

2.3 *Sand Solidification in syringe*

Laboratory scale model experiments were conducted using 35 mL syringe (mean diameter = 2.5 cm and height 7 cm) in-order to check the effect of the chitosan on sand solidification. Commercially available Mikawa sand was used for the experiment and it is a uniformly graded sand with the mean diameter of D_{50} = 0.6 mm and particle density is equal to 2.66 g/cm^3. oven dried (110 °C, 48 h) sand (40 g) was filled into the syringe as 3 layers and 20 hammer blows were given to each layer. After initial set-up, bacteria suspension (16 mL, OD600 = 1) was injected into the syringe and leave it 5-10 min for the addiction of the bacteria to sand particles. Then drained out from the outlet, maintaining 2 mL of the solution above the surface.

After that, cementation solution (20 mL; 0.3 M urea, 0.3 M $CaCl_2$, 0.02 M sodium hydrogen carbonate, 0.2 M ammonium chloride, and 3 g/L nutrient broth) was added into the syringe and solution drained out from the outlet, keeping 2 mL of the solution above the surface to maintain the sand in saturated condition.

Experiments were conducted with and without addition of chitosan and curing time was 14 days. Bacteria were injected on the first day and again after the 7 days while cementation solution was injected daily. In the case of experiment with chitosan, chitosan was injected to the syringe on the 11th day. After 14 days, sand samples were recovered from the syringe and unconfined compressive strength (UCS) of the samples were measured using needle penetration device.

All experiments were done in triplicate and mean value was plotted. Standard deviation was used to represent the error bars.

3 RESULTS AND DISCUSSION

3.1 *Effects of chitosan on $CaCO_3$ precipitation*

Variation of the amount of precipitate with and without chitosan under different bacteria concentration is given in Fig. 1. It can be seen that, the amount of the precipitate has increased with the bacteria concentration. In MICP, $CaCO_3$ formation efficiency is directly proportional to the urease activity of the bacteria (Qabany et al, 2012). Fujita et al. (2017) found that urease activity of the *Pararhodobacter* sp. SO1 increase with the increase of cell concentration. Increase of the urease activity with the cell concentration led to the formation of higher amount of precipitate at higher cell concentration.

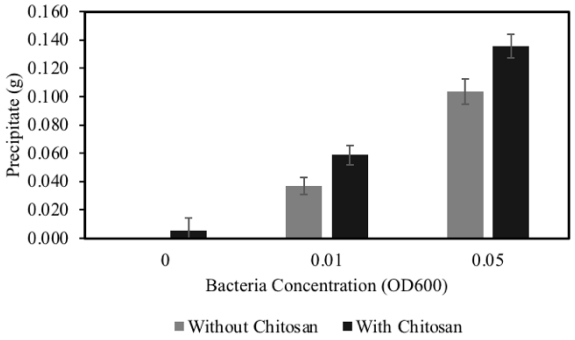

Fig. 1 Variation of the amount of precipitate with the bacteria concentration (OD600), with and without chitosan

The most interesting point is higher amount of precipitate could be obtained with the addition of chitosan. Precipitate with chitosan mainly has two components, precipitated $CaCO_3$ and the precipitated chitosan hydrogel. Chitosan forms its hydrogel under the alkaline conditions since the dissolution coefficient of the chitosan is approximately 6.5 (Liu et al, 2015; Kumirska et al, 2015). In the current reaction mixture, pH is around 7.1 due to formation of

the ammonia as a byproduct of the urea hydrolysis. This weak alkaline environment forms a favorable condition for the chitosan to form its hydrogel by hydrophobic interactions (Rami et al, 2014). Further, chitosan upgrades the $CaCO_3$ nucleation and growth. Ca^{2+} ions are embedded into the chitosan hydrogel and produce nucleation sites for the $CaCO_3$ crystals to nucleate and growth by following acid-base interaction (Greer et al, 2017).

Morphology of the $CaCO_3$ crystals in the presence of the chitosan was analyzed by SEM and given in Fig. 2. With the increase of the bacteria concentration morphology of the crystals has changed from rhombohedral crystals to distorted shape crystal agglomerations. Chitosan hydrogel can be seen clearly in lower bacteria cell concentrations due to the lower rate of $CaCO_3$ formation. However, in higher cell concentrations, due to higher rate of $CaCO_3$ formation crystal agglomerations were formed. Also, due to adsorption of the chitosan into the growing crystal faces distorted crystal faces were created. Nawarathna et al (2018) found that, without any additives fewer number of good rhombohedral crystals are formed under lower bacteria cell concentrations and at higher bacteria cell concentrations larger number of smaller crystals are formed due to the presence of larger number of nucleation sites. In the MICP process bacteria cells themselves act as nucleation site for $CaCO_3$ crystals to nucleate and growth.

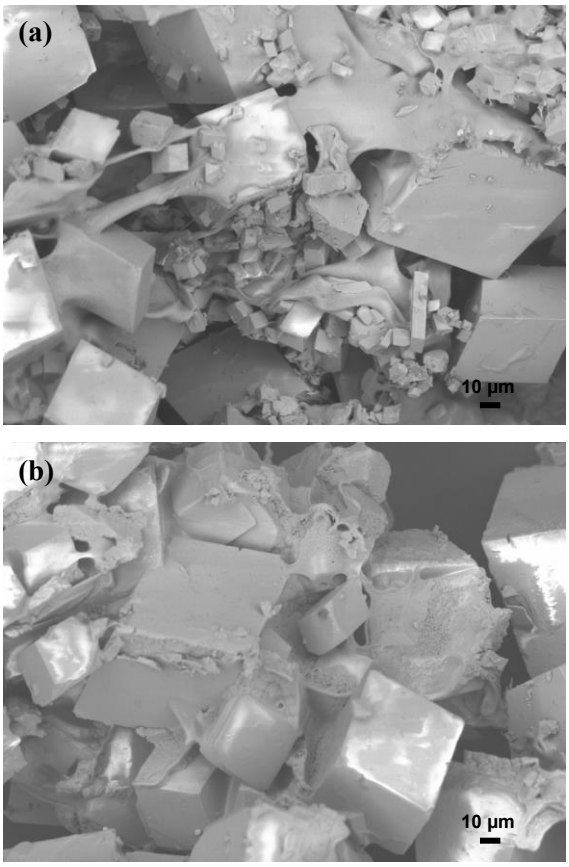

Fig. 2 SEM images of the precipitate with chitosan under different bacteria concentrations (OD600) (a) 0.01 (b) 0.05

3.2 *Effect of chitosan on sand densification*

Laboratory scale sand solidification experiments were conducted with and without chitosan and after 14 days of curing, UCS values of the top, middle and bottom of the sample were measured. Fig. 3 shows the obtained UCS values for the samples with and without chitosan.

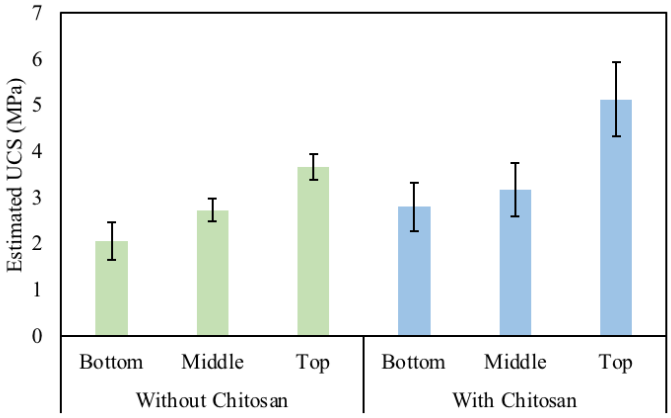

Fig. 3 Estimated UCS values of the sand specimens with and without chitosan

In both of the cases, UCS value decrease from top to bottom due to the accumulation of bacteria at the top of the sample compare with the bottom of the sample. Higher urease activity at the top of the sample led to higher precipitation and higher cementation at the top of the specimen and gradually decrease along the length of the sample. Also, *Pararhodobacter* sp. SO1 is an aerobic bacterium (Khan et al, 2015) and bacterial activity of aerobic bacteria mainly depend on the oxygen concentration of the surrounding environment. Therefore, lack of the oxygen at the bottom of the sample would be a reason for the lower precipitation and cementation at the bottom of the sand column. By adding chitosan, well cemented sand column with higher UCS value could be obtained compared with the case of without chitosan. 40 % of strength increment could be achieved at the top of the sample by adding chitosan than without chitosan.

As mentioned earlier, chitosan forms its hydrogel under the alkaline condition. This hydrogel assisted to form a better bridge between sand particles and filled the pore spaces efficiently. Therefore, better cementation and strength was achieved. Efficient filling of pore spaces and formation of the bridge between the sand particles are essential to have a better cementation and efficient solidification of the loose sand (Harkes et al, 2009). During the experiment, 11th day was selected to inject chitosan in-order to prevent the early formation of chitosan hydrogel. Introduction of chitosan at the begging will be led to formation of hydrogel initially and prevent the further penetration of the cementation solution

through the sand specimen. Therefore, it will reduce the efficient formation of $CaCO_3$ as well as cementation between the sand particles. Fig. 4 shows, how chitosan hydrogel helps to achieve a better cementation.

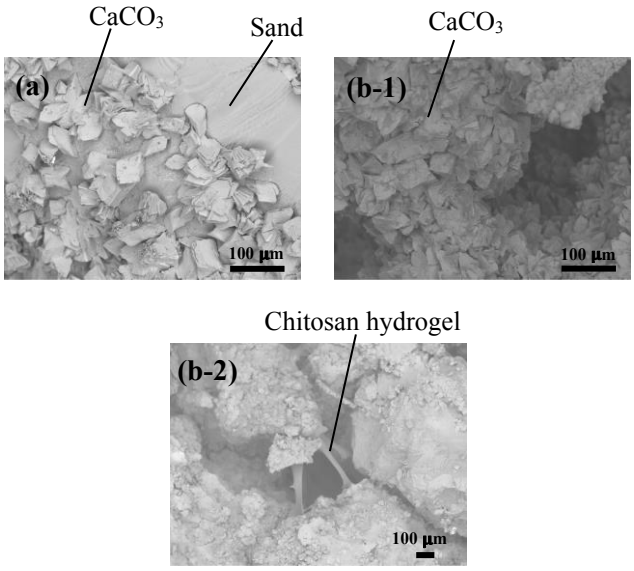

Fig. 4 SEM images of the solidified sand specimens (a) with chitosan (b-1,2) without chitosan

4 CONCLUSION

Effects of the chitosan on the sand densification and the $CaCO_3$ formation was investigated by using the MICP process. Higher amount of precipitate could be obtained by adding chitosan than that of without chitosan. Chitosan upgrade the $CaCO_3$ formation by providing more nucleation sites. Due to the acid-base reaction, Ca^{2+} ions are attached into the chitosan hydrogel and produced nucleation sites for the $CaCO_3$ crystals to nucleate and growth. Further in the presence of chitosan, chitosan hydrogel also precipitated with the $CaCO_3$. Distorted shape crystal agglomerations were formed in the presence of chitosan while without chitosan rhombohedral crystals were dominant. Most interestingly, strongly cemented sand column could be obtained by adding chitosan than the conventional method. Formed chitosan hydrogel assisted to create a better bridge between the sand particles and led to achieve a better strength.

ACKNOWLEDGMENTS

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