



Title	Effect of biomacromolecules on calcium carbonate formation and sand solidification induced by urease-based mineralization [an abstract of dissertation and a summary of dissertation review]
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Citation	北海道大学. 博士(工学) 甲第13800号
Issue Date	2019-09-25
Doc URL	<a href="http://hdl.handle.net/2115/75891">http://hdl.handle.net/2115/75891</a>
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Type	theses (doctoral - abstract and summary of review)
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File Information	Thiloththama_H._K._Nawarathna_abstract.pdf (論文内容の要旨)



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## 学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称 博士（工学） 氏名 Thiloththama Hiranya Kumari Nawarathna

### 学 位 論 文 題 名

Effect of biomacromolecules on calcium carbonate formation and sand solidification induced by urease-based mineralization

(ウレアーゼを用いた炭酸カルシウム形成および砂質材料の固化における生体高分子の影響)

Today in the world, lands available for the construction are decreasing day by day due to industrialization and rapid growth of populations. Hence, peoples are moved to use problematic weak grounds for the constructions. Use of these problematic grounds for the construction is an engineering challenge due to associated low bearing capacity and excessive settlement. Hence, proper ground improvement is mandatory before the construction to ensure a safe and economical construction. Available ground improvement techniques have some drawbacks including environmental issues. Mainly, chemical or cement grouts have been used effectively to treat loose sands. However, large amount of CO<sub>2</sub> is released to the environment during the cement production and most of chemicals used for grouting are toxic for the environment. Under these circumstances, a biological approach could be an alternative to the present grouting techniques. Especially, among the bio-mediated soil stabilization techniques, microbially induced carbonate precipitation (MICP) has gained much attention as an efficient and ecofriendly ground improvement technique. A number of laboratory and field experiments have demonstrated that MICP has capability to improve the strength and stiffness of soil effectively. Improving the efficiency and sustainability of MICP is highly required to put the technique to practical use. This thesis addresses the use of biomacromolecules as an additive to MICP to improve the efficiency by enhancing CaCO<sub>3</sub> formation.

In Chapter 1, research background, literature review, research scope and the originality and usefulness of this work are discussed.

In Chapter 2, the effect of synthetic cationic and anionic polypeptides on CaCO<sub>3</sub> formation and sand solidification was examined in MICP method. Poly-L-lysine and poly-glutamate were used as a cationic and an anionic polypeptide, respectively, to elucidate the effect of the polymers on CaCO<sub>3</sub> formation induced by hydrolysis of urea by ureolytic bacteria, *Pararhodobacter* sp. Experiments were conducted in the presence or absence of poly-L-lysine/poly-glutamate under different experimental conditions. Results revealed that poly-L-lysine has positive effect on CaCO<sub>3</sub> formation and gave higher amount of precipitate while no significant effect was found in poly-glutamate addition. Electrostatic interaction between positively charged poly-L-lysine and negatively charged HCO<sub>3</sub><sup>-</sup> ions would lead to the enrichment of HCO<sub>3</sub><sup>-</sup> ions in local regions, resulting in CaCO<sub>3</sub> crystal nucleation and growth. Also, poly-L-lysine drastically changed the morphology of CaCO<sub>3</sub> crystals into ellipsoidal shaped calcite crystals while rhombohedral or polyhedron shaped calcite crystals were dominant without poly-L-lysine. Morphology change would be mainly due to the change in the conformation of poly-L-lysine chain from random coil to  $\alpha$ -helix under alkaline condition. Sand solidification experiments

were conducted with and without poly-L-lysine under different experimental conditions. By adding poly-L-lysine, higher strength was achieved than that of without poly-L-lysine. Positively charged poly-L-lysine could possibly assist the formation of the better bridge between sand particles and cell surface, both of which are negatively charged. And also, morphology change and the higher CaCO<sub>3</sub> precipitation efficiency of the poly-L-lysine have considerable influence on the better cementation.

In Chapter 3, the effect of natural polymers on CaCO<sub>3</sub> formation and sand solidification was discussed. Chitosan, natural cationic polysaccharide, was used for the experiments. CaCO<sub>3</sub> formation catalyzed by ureolytic bacteria was examined as described in Chapter 2 with and without addition of chitosan. Higher amount of precipitate was obtained by adding chitosan compared to that without chitosan. Precipitate with chitosan consisted of precipitated CaCO<sub>3</sub> and chitosan hydrogel. Chitosan formed its hydrogel under alkaline condition, which is induced by the hydrolysis of urea. At lower cell concentrations, rhombohedral calcite crystals was obtained along with chitosan hydrogel while distorted-shaped calcite crystals with rough surface were formed at higher cell concentrations. Chitosan could provide nucleation sites for CaCO<sub>3</sub> crystals to nucleate and growth. Also, distorted shaped of the crystals are due to the binding of the -OH and -NH<sub>2</sub> functional groups of the chitosan on to the surface of the crystals. Sand solidification experiments were conducted by adding chitosan under different experimental conditions. By adding chitosan, strongly cemented sand specimen could be obtained with 38% of strength increment for the top of the sample. Chitosan hydrogel would assist formation of a better bridge between sand particles, leading to better cementation.

In Chapter 4, I introduced a novel fusion protein which can facilitate the organic-inorganic hybrid material formation. Protein was designed for efficient precipitation of CaCO<sub>3</sub> on chitin matrix by introducing calcium binding peptide (CaBP) into chitin binding domain (ChBD). The gene for target fusion protein (CaBP-ChBD) was constructed and the target protein could be successfully expressed in *E. coli*. Chitin binding assay was conducted by introducing insoluble chitin into the CaBP-ChBD. It was found that 80% of the CaBP-ChBD was bound to chitin particles within 3 hours. CaCO<sub>3</sub> precipitation experiments were conducted by hydrolysis of urea using extracted jack bean urease with and without addition of chitin or CaBP-ChBD. Small increment of the precipitate could be found by adding chitin while significant increment (55%) of the precipitate was achieved by adding both of chitin and CaBP-ChBD. The morphology of the crystals changed drastically into dumbbell-shaped crystals in the presence of CaBP-ChBD, where chitin particles were completely covered with CaCO<sub>3</sub> crystals while the particles were uncovered in the absence of CaBP-ChBD. Presence of the basic amino acid residues such as lysine, histidine and arginine in CaBP would facilitate efficient CaCO<sub>3</sub> formation.

All the research outcomes shown in each chapter were summarized in Chapter 5, along with some prospects for future works under above research area.