Microbially included calcite coating layer on concrete surface as inhabitance of coral to produce artificial coral reef

Jian Chu[1], L. Cheng[1], and K. Tun[2]

[1] School of Civil and Environmental Engineering, Nanyang Technological University, Singapore
[2] National Biodiversity Centre, National Parks Board, Singapore

ABSTRACT

The artificial reefs have positive environmental impact due to increase of aquatic biodiversity in coastal areas and enhanced consumption of CO_2 by photosynthetic epibiota. The aim of this study is to establish a new biocalcification method to enhance the creation of coral reefs and other ecosystems of marine epibiota. This paper presents a novel approach for the formation of calcite coating layer on concrete block surface by microbially induced carbonate precipitation (MICP) using urease active bioslurry. The bioslurry is pre-formed urease active crystals consisting of $CaCO_3$ and imbedded urease active bacterial cells. By coating the bioslurry on the surface of concrete blocks followed by submerging in a cementation solution, a mixture of $CaCl_2$, $MgCl_2$ and urea, the loose bioslurry turned into a dense and strong calcite deposit layer. By alternating the usage of the bioslurry and cementation solution, calcite layers with various thickness were established and their bio-compatibilities were also evaluated by monitoring biofilm growth on the calcite surface. This MICP based calcite coating approach has potential to be used in marine environment to facilitate colonization of marine microorganisms, such as corals.

Keywords: biocementation; Bio-slurry; calcite; concrete

1 INTRODUCTION

Coral reefs is one of the most productive and biologically diverse ecosystems on earth. They can act as physical buffers against oceanic currents and waves thus providing a strong habitat for fish and marine organisms. Coral reefs also protect coastlines from being damaged, eroded and flooded by storms via reducing wave action across tropical coastlines. They also can act as cleansing system, which is able to transform, detoxify and sequester wastes produced by human (Moberg and Folke 1999). Coral reefs also supply people with wide range of goods and services such as seafood, recreational possibilities, and coastal protection as well as aesthetic and cultural benefits (Done 1995).

Rising ocean temperature has escalated coral disease outbreaks which results in a recession of coral cover globally (Caldwell et al. 2016; Hughes 1994; Aronson and Precht 2001; Porter et al. 2001). Effects to impede coral degradation have been tried for many decades. Many different materials are proposed to be used in the construction of artificial coral reefs, such as concrete blocks or discarded tires. Although these artificial coral reef have been marginally successful, most of these reefs are still dismally barren compared to the real thing. Recently, a new and promising technology named Biorock establishes cement-like engineering structures and marine ecosystems, often for mariculture of corals, oysters, clams, lobsters and fish in salt water. Over many years of experiments and field tests, it has shown that 1 kWh of electricity will produce about 0.4-1.5 kg of biorock and the yearly growth rate was about 5 cm (Ortega 1989). Although the composition of biorock (brucite, hydromagnesite and limestone) is highly compatible with the marine organisms, the slow growth rate might not be applicable for real practical engineering application. Therefore, there is a need to look for more efficient approach.

A key factor for the growth of coral reefs is the formation of $CaCO_3$ layer as the skeleton for the colonization of corals and associated marine organisms. A new approach to coat the surface of artificial substrates with a layer of $CaCO_3$ using the microbially induced carbonate precipitation (MICP) method is adopted in this study. Using the MICP method, $CaCO_3$ is precipitated due to hydrolysis of urea mediated by halophilic and alkaliphilic urease-producing bacteria (UPB) in solution with calcium ions (Cheng et al. 2013; Chu et al. 2014). According to our previous research, MICP can be used to form calcite layer which can firmly attach on cement-based material surface. However, the traditional submerging method (solid materials are submerged in mixture of bacterial suspension and cementation solution) is inefficient as most calcite crystals formed in aqueous phase are precipitated on the bottom of container.

In this study, a novel approach using preformed urease active crystals, named herein as “bioslurry”, is proposed as a source of urease activity to induce a homogeneous biocementation for surface coating. In contrast to the current usually adopted MICP treatment methods, the newly invented bioslurry treatment

approach involves pasting the bioslurry on solid surface, similar to the traditional plastering method, followed by submerging in cementation solution. The aim of this study is to evaluate the bioslurry calcification method for the establishment of calcite coating on concrete surface to enhance the creation of coral reefs and other ecosystems of marine epibiota.

2 MATERIALS AND METHODS

2.1 Cultivation of bacteria used

The urease active bacteria used in the current study were *Bacillus sp.* strain (DSM 23526), which were cultivated in a sterile aerobic batch growth medium consisting of 20 g/L yeast extract, 10 g/L NH_4Cl and 0.1 mM $NiCl_2$, at a pH value of 9.25. The cultivated bacterial culture was collected after 24 h of cultivation at 28 °C. The optical density (OD_{600}) of collected bacterial culture varied between 2 and 2.5, and the urease activity was approximately 13 U/mL (1 U = 1 mol urea hydrolyzed per minute). The cementation solution used consisted of 0.5 M calcium chloride, 0.5 M $MgCl_2$ and 1 M urea.

2.2 Preparation of bio-slurry

The bioslurry was prepared according to the previous published method (Cheng and Shahin 2016). The bioslurry was obtained by adding specific amounts of urea and calcium chloride (equal moles) into the bacterial culture (100 mL), followed by stirring at a speed of 600 rpm for about 12 h. When the added urea and $CaCl_2$ were completely consumed, which was determined by measuring the amount of produced NH_4^+/NH_3 in the solution, the mixture was allowed to settle for 6 h. After settlement, a clean supernatant was disposed and settled crystals (bioslurry) were then harvested (see Fig. 1).

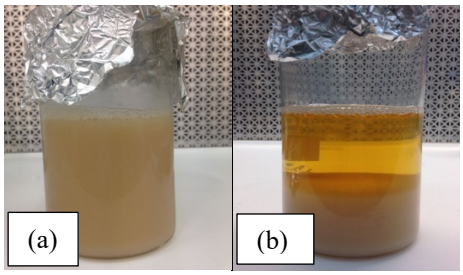

Fig. 1. Photos of bioslurry taken during (a) reaction period and (b) settling period.

2.3 Coating procedure using bioslurry

Procedure of producing microbially induced calcite layer was elaborated in detail as follows:

First, the harvested bioslurry was applied on the surface of concrete blocks (5x5x5 cm). The bioslurry was considerably dense with the liquid percentile of about 10% (w/w). Each coating layers had accounted for 5g of the bioslurry by using a trowel-liked spoon to scoop the dense bioslurry and simply spread it all over the concrete surface, for both vertical and horizontal surfaces (Fig. 2).

Second, the concrete blocks were kept on an open dry environment to let the remained liquid on the concrete surface to evaporate out until nearly dry. The coated bioslurry was flatted to produce a smooth coating layer.

Third, after the coating process was finished, the concrete blocks were submerged in the cementation solution at room temperature (25 ± 1°C) for 24h to allow for a complete cementation reaction.

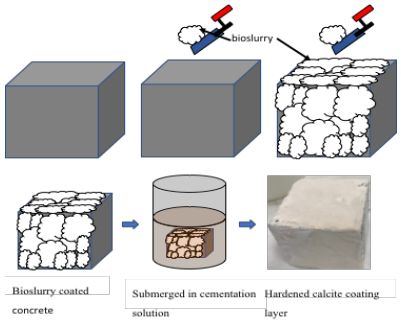

Fig. 2. Schematic of the coated bioslurry induced solid calcite coating layer using submerging method.

2.4 Ex-situ tests of mono species biofilm formation on concrete coated with MICP

Two bacteria strains were tested independently on the MICP bioslurry surface coating treatments and negative controls. Each was grown using a standard microbiological media for 24 hours and the biofilm was then quantified using a fluorescent microscopic analysis. In brief, the biofilm area was measured and optical section taken to provide a 3D model of the total volume of the biofilm growing on the slices.

In total 3 types of concrete samples: control, thin coating (approx. 1 mm), thick coating (approx. 3 mm) were tested. The concrete slice samples sterilized by soaking in 70% Ethanol and washing with PBS and additional UV step to remove all existing biofilm and bacteria. The containers were also sterilized by wiping with 70% Ethanol followed by UV step. Incubation of microbes was conducted at 37 ºC, 80 rpm (enough to agitate the culture medium without splashing out of the container) for 24 hours. Two types of strains eYFP and GFP were used. The bacteria were cultivated at M9 media with additional Casamino acid.

2.5 In-situ tests of marine colonization on concrete coated with MICP

Having successfully established the bioslurry induced calcite layer, the surface modified concrete blocks were sent to the marine laboratory of NParks, Singapore, for further testing of coral establishment. These samples were placed on real seabed.

2.6 Scanning electron microscope (SEM) analysis

A microscopy analysis was conducted on the coating layer. Before conducting the microscopy investigation, the coating layer was flushed with tap water and dried at

50 °C for 24 h. The microscopy investigation was carried out using the scanning electron microscope (SEM).

3 RESULTS AND DISUCSSIONS

3.1 Optimization of bioslurry

In the current study, the bioslurry produced from 200 mM cementation solution had two times higher urease activity (U/g) compared to that produced from the 400 mM cementation solution (Fig. 3). For the same concentration of $CaCl_2$/urea solution, higher urease activity of bioslurry was obtained when the urease activity of the bacterial culture was higher (Fig. 3). The total urease activity produced depends on the urease activity of the bioslurry and the amount of bioslurry produced under different conditions. In the current study, it shows that the highest total urease activity of about 1169 U was obtained when the undiluted bacterial culture (0.13 U/mL) and 400 mM $CaCl_2$/urea solution was used. This is in line with the previous studies (Cheng and Shahin 2016). The highest urease activity enables a maximum calcite production, hence an efficient coating process.

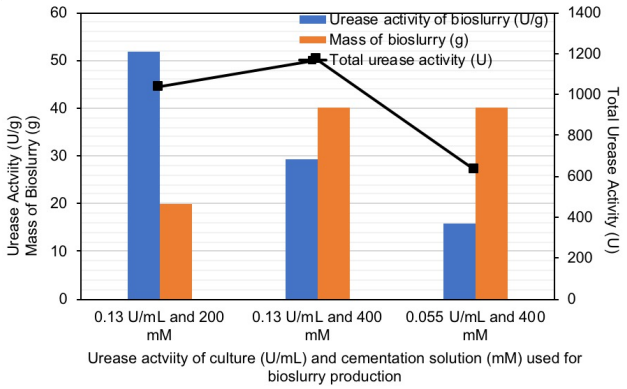

Fig. 3. Bioslurry generation using different concentrations of bacterial culture and cementation solution ($CaCl_2$/Urea).

3.2 Bioslurry induced carbonate coating layer

Dense bioslurry (with solid content of about 20-25%) was used to coat the concrete block on both vertical and horizontal surfaces. The coating on vertical surface was only possible using a dense and thick bioslurry. Such a sticky bioslurry material could firmly adheres on the vertical surface of a concrete block even submerged in solution. Most of the liquid in the bioslurry was removed before applied the bioslurry on the concrete surface. Immediately after the coating process, the concrete block was submerged in the cementation solution. As the bioslurry was condensed prior to coating, no evaporation was needed so that the time of coating process was significantly reduced. Overall this method produced a coating layer of about 1-3 mm (Fig. 4).

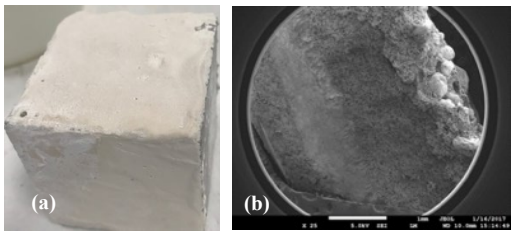

Fig. 4. Thick dense bioslurry-induced coating layer (a) on both vertical and horizontal surface and (b) SEM images of bioslurry induced carbonate layer.

3.3 Mono species biofilm formation on concrete coated with MICP

It was found that using the bacterial strain of eYFP, the biofilm volume on the thin-coat or thick-coat samples was larger than the control coupons, which can be visualized Fig. 5 and Fig. 6. It can also be found that the biofilm area on the thick-coat sample was relatively larger than other two methods suggesting that the MICP coating layer to some extent might facilitate the growth of eYFP biofilm. The images of biofilm were shown in Fig. 6.

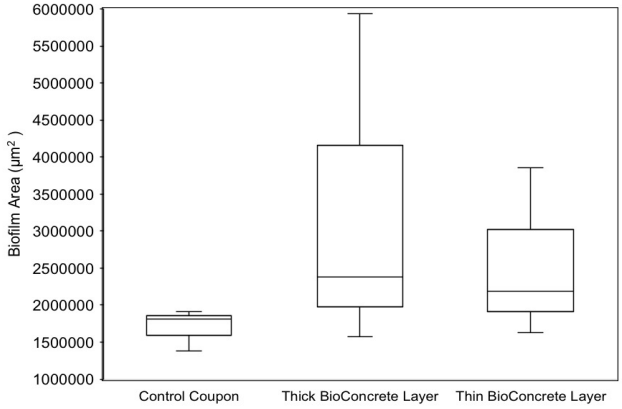

Fig. 5. eFYP biofilm area on three different samples.

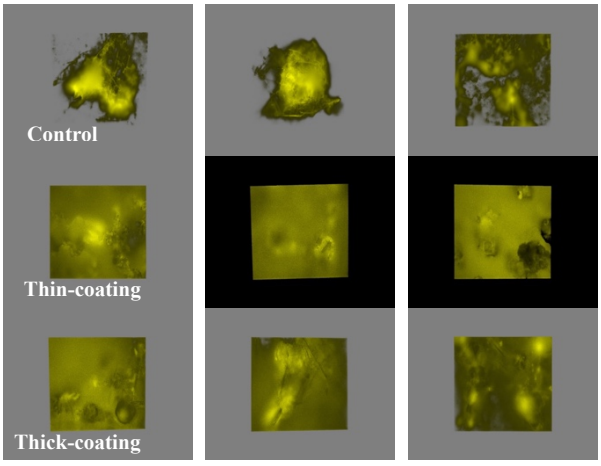

Fig. 6. Images of eFYP biofilm growth on three different samples.

The GFP bacterial strain mono biofilm has also been tested for its growing on the control, thin-coat or thick-coat samples. Similar result was found which shows the MICP coated sample might result in a higher amount

surface area coated with biofilm compared with control samples (Fig. 7). Although some preliminary results were obtained, more comprehensive statistical analysis is needed in the future study.

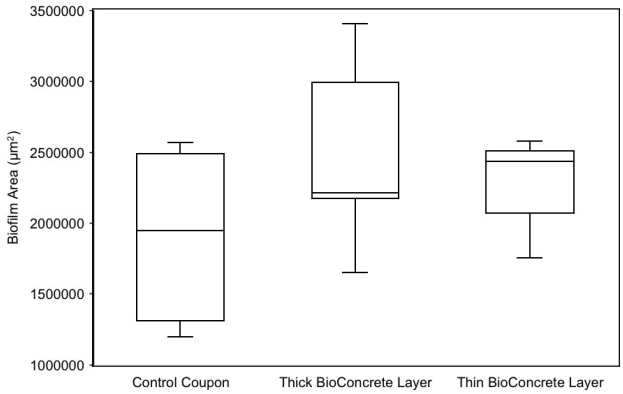

Fig. 7. GFP biofilm area on three different samples.

3.4 In-situ tests and observation

Several prepared bioslurry coated concrete samples were placed on the seabed in a marine lab for continuous monitoring of marine larvae colonization. Although the test is still ongoing, some preliminary conclusions can be drawn from the observation. Coating of purple furry stuff of cyanobacteria was found on all samples including control and bioslurry coated concrete block, as shown in Fig. 8. However, those with bioslurry seemed to have less of cyanobacteria and more of purple flat patches, which are the coralline algae. The coralline algae was the ones that are important for establishing primary layers before corals grow. Continuous monitoring and in-depth analysis of marine larvae species is still on going.

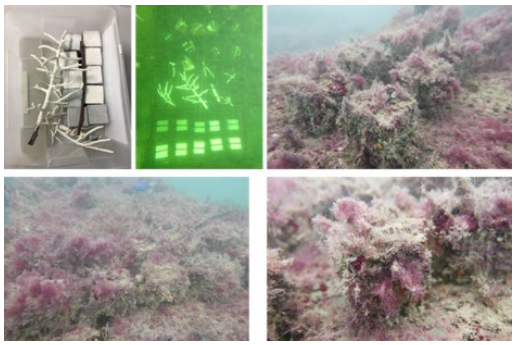

Fig.8. Images of in-situ test of bioslurry-coated concrete block in 5 months.

4 CONCLUSIONS

This paper presents a novel approach for the formation of calcite coating layer on concrete block surface by microbially induced carbonate precipitation (MICP) using urease active bioslurry. By alternating the usage of the bioslurry and cementation solution, calcite layers with various thickness were established and their bio-compatibilities were also evaluated by monitoring biofilm growth on the calcite surface.

Two bacteria strains were tested independently and the results indicate that the bioslurry coated concrete facilitates the biofilm growth comparing to the uncoated concrete. The bioslurry modified concrete blocks were also placed in the real marine environment. After 5 months coating of purple furry stuff of cyanobacteria were found on all samples. However, those treated with bioslurry seemed to have less of cyanobacteria and more of purple flat patches, which were the coralline algae. The coralline algae is the one that is important for the establishment of primary layers before corals grow.

This novel MICP based calcite coating approach has the potential to be used in marine environment to facilitate colonization of marine microorganisms, such as corals.

ACKNOWLEDGEMENTS

The authors would like to thank the financial supported by Grant No MOE2015-T2-2-142 provided by the Ministry of Education, Singapore, and Dr Stephen Summers and his team for their assistance with laboratory experiments of mono specie of biofilm growth. The assistance of Mr Koh Kwan Siong from the National Parks Board, Singapore, is also gratefully acknowledged.

REFERENCES

Aronson, R.B. and Precht, W.F. (2001). White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia, 460, 25-38.

Caldwell, J., Heron, S., Eakin, C. and Donahue, M. (2016). Satellite SST-based coral disease outbreak predictions for the Hawaiian archipelago. Remote Sensing,8(2), 93-107.

Cheng, L., Cord-Ruwisch, R. and Shahin, M.A. (2013). Cementation of sand soil by microbially induced calcite precipitation at various degrees of saturation. Canadian Geotechnical Journal, 50(1), 81–90.

Cheng L. and Shahin, M.A. (2016). Urease active bioslurry: a novel soil improvement approach based on microbially induced carbonate precipitation. Canadian Geotechnical Journal, 53(9), 1376-1385.

Chu, J., Ivanov, V., Naeimi, M., Stabnikov, V. and Liu, H.L. (2014), Optimization of calcium-based bioclogging and biocementation of sand. Acta Geotechnica, 9, 277–285.

Done, T.J. (1995). Ecological criteria for evaluating coral reefs and their implications for managers and researchers. Coral Reefs, 14(4), 183-192.

Hughes, T. (1994). Catastrophes, phase shifts and large-scale degradation of a Caribbean coral reef. Science, 265,1547-1551.

Moberg, F., and Folke, C. (1999). Ecological goods and services of coral reef ecosystems. Ecological Economics, 29(2), 215-233.

Ortega, A. (1989). Basic Technology: Mineral Accretion for Shelter. Seawater as a Source for Building. MIMAR 32: Architecture in Development, Concept Media Ltd No. 32, pp. 60-63.

Porter, J., Dustan, P., Jaap, W., Patterson, K., Kosmynin, V., Meier, O., Patterson, M. and Parsons. M. (2001). Patterns of spread of coral disease in the Florida Keys. Hydrobiologia, 460, 1-14.