An Overview of Biocement Production from Microalgae

Dessy Ariyanti[1], Noer Abyor Handayani[2], Hadiyanto[3]

Chemical Engineering Department, Engineering Faculty of Diponegoro University

Jl. Prof. Soedarto, SH. Kampus Tembalang, Semarang, Indonesia 50239

Tel: 024-7460058/Fax: 024-76480675

[1]dessy@undip.ac.id

[3]noe_boo@yahoo.com

[2]hady.hadiyanto@gmail.com

***Abstract*—The invention of microorganism's involvement in carbonate precipitation, has lead the exploration of this process in the field of construction engineering. Biocement is a product innovation from developing bioprocess technology called biocementation. Biocement refers to $CaCO_3$ deposit that formed due to microorganism activity in the system rich of calcium ion. The primary role of microorganism in carbonate precipitation is mainly due to their ability to create an alkaline environment (high pH and DIC increase) through their various physiological activities. Three main groups of microorganism that can induce the carbonate precipitation: (i) photosynthetic microorganism such as cyanobacteria and microalgae; (ii) sulphate reducing bacteria; and (iii) some species of microorganism involved in nitrogen cycle. Microalgae are photosynthetic microorganism and utilize urea using urease or urea amidolyase enzyme, based on that it is possible to use microalgae as media to produce biocement through biocementation. This paper overviews biocement in general, biocementation, type of microorganism and their pathways in inducing carbonate precipitation and the prospect of microalgae to be used in biocement production.**

***Keywords*— Biocement, Biocementation, Microalgae, $CaCO_3$ precipitation**

I. INTRODUCTION

Construction engineering consumes a huge amount of materials from non-renewable resources, which most of the materials contribute CO_2 emission to the air at their production or application stage. Technology development related to the construction material and their production is necessary, in order to maintain the sustainability and to reduce the production of CO_2 emission. The evidence of microorganism involvement in carbonate precipitation, has lead the development of bioprocess technology in the field of construction material **[1, 2]**.

The precipitation of calcium carbonate ($CaCO_3$) may be performed due to microorganism activity and it produces massive limestone or small crystal forms **[3]**. These deposit of calcium carbonate known as biocement or microbial induced carbonate precipitation (MICP) **[3, 4]**. Biocement has many advantages compared to an ordinary cement, such as: the production process is slightly different with sandstone production, biocement need a much shorter time; it is suitable for in-situ process; raw material of biocement are produced at low temperature, more efficient compared to an ordinary cement which used temperature up to 1500^{o}C in production process; biocement can be used as eco-construction material since it consume less energy and less CO_2 emission in the production process rather than other ordinary cement **[3, 5]**.

The involvement of microorganism in $CaCO_3$ precipitation can be described in three type of mechanism: (i) spontaneous mechanism, usually by photosynthetic microorganism; (ii) through nitrogen cycle; (iii) through sulfur cycle **[1, 6]**. Recently, research and study of biocement production through biocementation still limited to nitrogen cycle mechanism using urease enzyme producing bacteria **[3-7]**. While research using microalgae as media for biocementation still lack in literature, in fact microalgae have a great potency for the objective of biocementation. Microalgae are photosynthetic microorganism, beside that some species of microalgae can produce urea hydrolysing enzyme **[8, 9]**, that can be used to precipitate calcium carbonate. Overview of biocement, biocementation, type of microorganism used in biocementation, mechanism type and potency of microalgae as media in biocement production will briefly described throughout this paper.

II. MICROBIAL INDUCED CARBONATE PRECIPITATION (MCIP)

Calcium carbonate ($CaCO_3$) precipitation is a common phenomenon found in nature such as marine water, freshwater, and soils **[1, 7, 10]**. This precipitation is governed by four key factors: (i) the calcium (Ca^{2+}) concentration, (ii) the concentration of dissolved inorganic carbon (DIC), (iii) the pH (pK2 (CO) = 10.3 at 25^{0}C) and (iv) the availability of nucleation sites **[1, 11]**. Numerous species of microorganism have been detected previously and assumed to be associated with natural carbonate precipitates from diverse environments. The primary role of microorganism in carbonate precipitation is mainly due to their ability to create an alkaline environment (high pH and [DIC] increase) through their various physiological activities **[1, 7]**.

There are three main groups of microorganism that can induce the carbonate precipitation: (i) photosynthetic microorganism such as cyanobacteria and microalgae; (ii) sulphate reducing bacteria; and (iii) some species of microorganism involved in nitrogen cycle **[1, 6, 7]**. The most common MCIP phenomena appeared in aquatic environments is caused by photosynthetic microorganisms **[6, 12]**. Photosynthetic microorganisms use CO_2 in their metabolic process (eq. 1) which is in equilibrium with HCO3- and CO_3^{2-} as described in eq. 2. Carbon dioxide consumed by photosynthetic microorganisms shift the equilibrium and resulting the increment of pH (eq. 3) **[6]**. When this reaction occurs in the present of calcium ion in the system, calcium carbonate is produced as described at chemical reaction in eq. 4 **[7].**

$$CO_2 + H_2O \rightarrow (CH_2O) + O_2 \qquad (1)$$

$$2HCO_3^- \leftrightarrow CO_2 + CO_3^{2-} + H_2O \qquad (2)$$

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^- \qquad (3)$$

$$Ca^{2+} + HCO_3^- + OH^- \leftrightarrow CaCO_3 + 2H_2O \qquad (4)$$

The precipitation of calcite ($CaCO_3$) can also be induced by heterotrophic organism. This microorganism produces carbonate or bicarbonate and modified the system so that the carbonate precipitation may occur **[1]**. Abiotic dissolution of gypsum ($CaSO_4.H_2O$) (eq. 5) causes system rich of sulfate and calcium ion. In the presence of organic matter and the absence of oxygen, sulphate reducing bacteria (SRB) can reduce sulphate to H_2S and HCO_3^- as described in eq. 6 **[1, 6]**. When the H_2S degasses from the environment, pH of system will increase and the precipitation of calcium carbonate will occur **[1]**.

$$CaSO_4.H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 2H_2O \qquad (5)$$

$$2(CH_2O) + SO_4^{2-} \rightarrow HS^- + HCO_3^- + CO_2 + H_2O \qquad (6)$$

Currently urease enzyme activity in most of microorganism metabolism process has been used as a tool to induce the precipitation of calcium carbonate **[9, 12]**. The hydrolysis of urea by urease enzyme in heterotrophic microorganism will produce carbonate ion and ammonium. This mechanism will result system with higher pH and rich of carbonate ion **[12]**. One mole of urea hydrolysed intracellularly to one mole ammonia and one mole carbamate (eq. 7), which spontaneously hydrolysed to one mole ammonia and one mole carbonic acid (eq. 8). Ammonia and carbamate subsequently equilibrate in water to form bicarbonate and 2 moles of ammonium and hydroxide ions as described in eq. 9 and 10 **[2]**.

$$CO(NH_2)_2 + H_2O \rightarrow H_2COOH + NH_3 \qquad (7)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \qquad (8)$$

$$2NH_3 + 2H_2O \rightarrow 2NH^{4+} + 2OH^- \qquad (9)$$

$$2OH^- + H_2CO_3 \rightarrow CO_3^{2-} + 2H_2O \qquad (10)$$

Total reaction:

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH^{4+} + CO_3^{2-} \qquad (11)$$

The presence of calcium ion in the system will lead to the calcium carbonate precipitation once a certain level of supersaturation is reached. The calcium carbonate precipitation mechanism induced by urease enzyme activity illustrated in Figure 1.

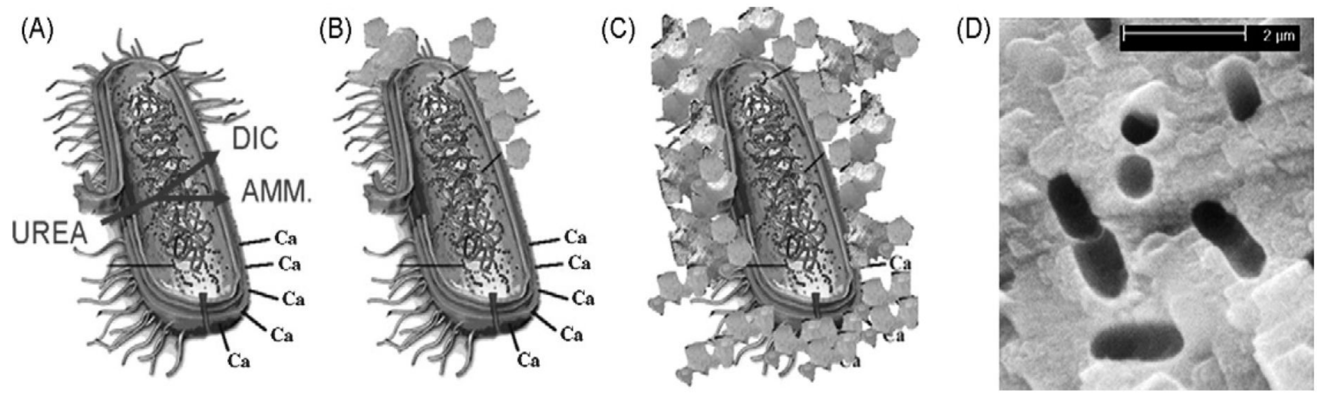

Fig. 1 Illustration of calcium carbonate precipitation mechanism induced by urease enzyme activity in microorganism **[7].**

Calcium ions in the solution are attracted to microorganism cell wall due to the negative charge of the latter. After the addition of urea to the system, microorganism convert urea to dissolved inorganic carbon (DIC) and ammonium (AMM) and released it to the environment (A). The presence of calcium ion cause the supersaturation condition and precipitation of calcium carbonate in microorganism cell wall (B). After a while, the whole cell becomes encapsulated by calcium carbonate precipitate (C). As whole cell encapsulated, nutrient transfer becomes limited and resulting in cell death. Image (D) shows the imprints of microorganism cell involved in carbonate precipitation **[7].**

III. BIOCEMENTATION

Biocementation is a process to produce binding material (biocement) based on microbial induced carbonate precipitation (MICP) concept. This process can be applied in many fields such as construction, petroleum, erosion control, and environment. Application in construction field include wall and building coating method, soil strengthening and stabilizing, and sand stabilizing in earthquake prone zone **[2].**

In application, the precipitation of calcium carbonate (biocement) is combined with other supporting material such as sand. The patented method of producing biocement can be seen in Figure 2 **[6, 13]**.

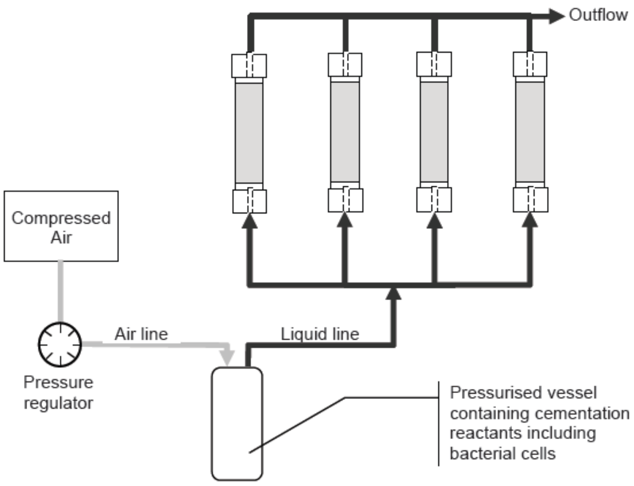

Fig. 2 Injection method of cementation liquid (contain calcium/urea solution and bacterial cell) in biocementation **[6, 13]**.

Biocementation illustrated in Figure 2 uses heterotroph bacteria Bacillus pasteurii with urea hydrolysis mechanism. The cementation process occurs in pipe columns filled with commercial sand contained silica. Urea/calcium solution and bacteria solution were mixed immediately and put in the pressurized vessel to be injected to the sand core in pipe column for several time until the sand core fully saturated. Biocementation takes about 24 hours to complete the reaction, after that the biocement were dried in temperature of 60^{o}C **[6]**.

Biocementation is also developed in the process of biological mortar production, crack in concrete remediation and production of bacterial concrete **[2, 11]**. Table 1 shows overview of various construction materials made from biocementation.

In general, mortar refers to ”ready to use” binder material contained a binder, and sand or aggregate. Biological mortar consists of three main components such as limestone powder, nutrient and bacterial paste **[2]**. Biocementation applied in concrete rift remediation and the production of bacterial concrete has been investigated **[14]**. Specimen of crack in concrete filled with biocement shows the significant increment of strength and stiffness value compared with specimen without biocement **[2, 14]**.

IV. MICROORGANISM USED IN BIOCEMENTATION

Theoretically, calcium carbonate precipitation occur in nature following several process such as: (i) abiotic chemical precipitation from saturated solution due to evaporation, temperature increase and/or pressure decrease; (ii) production of external and internal skeleton by eukaryotes; (iii) CO_2 pressure derivation under effect of autotrophic processes (photosynthesis, methanogenesis); (iv) fungal mediation; (v) heterotrophic bacterial mediation **[1]**. Most of the mentioned processes above are mediated by microorganism. Both photosynthetic and heterotrophic microorganisms have natural ability to induce the precipitation of calcium carbonate. There are huge amount of microorganism in many type of species spreads throughout the world.

Recently, advanced research in this field still limited to the use of urease enzyme produced by bacteria. Other types of microorganism have also a potency to be developed as media for biocementation such as photosynthetic microorganism. Table 2 shows several species which is already investigated as media in calcium carbonate precipitation **[7]**.

In biocementation, microorganism that used as media should meet the specific requirement, since the process create a high pH in the environment and involving high concentration of calcium ion. For example, in biocementation based on urea hydrolysis, the process will produce high concentration of ammonium and not all type of microorganism can survive in such condition. Based on that, the selected of microorganism should meet the criteria such as: (i) have a high urease enzyme activity; (ii) ammonium and calcium ion tolerable; (iii) not pathogenic **[6]**.

V. PROSPECT OF MICROALGAE IN BIOCEMENTATION

Microalgae are very prospective to be used in biocementation, due to its photosynthetic metabolism. Algae’s species like Spirulina, Arthrospira plantensis (Cyanophyta), Chlorella vulgaris (Chlorophyta), Dunaliella salina, Haematococcus pluvialis, Muriellopsis sp., Porphyridium cruentum (Rhodophyta) basically are autotrophic microorganisms that live through photosynthetic process, same as plants in general **[17-19]**.

TABLE 1
OVERVIEW OF VARIOUS CONSTRUCTION MATERIALS MADE FROM BIOCEMENTATION

Application	**Microorganism**	**Metabolism**	**Solution**	**Reference**
Biological mortar	*Bacillus cereus*	oxidative deamination of amino acids	Growth media (peptone, extract *yeast*, KNO_3, NaCl) + $CaCl_2.2H_2O$, *Actical*, *Natamycine*	(Muynck et al., 2010)
Crack in concrete remediation	*Bacillus pasteurii*	Hydrolysis of urea	Nutrient broth, urea, $CaCl_2.2H_2O$, NH_4Cl, $NaHCO_3$	(Santhosh et al., 2001)
	Bacillus sphaericus	Hydrolysis of urea	Extract *yeast*, urea, $CaCl_2.2H_2O$	(Belie, 2010)
Bacterial concrete	*Bacillus pasteurii*	Hydrolysis of urea	*Nutrient broth*, urea, $CaCl_2.2H_2O$, NH_4Cl, $NaHCO_3$	(Santhosh et al., 2001)

TABLE 2
SEVERAL SPECIES WHICH ALREADY INVESTIGATED AS MEDIA IN CALCIUM CARBONATE PRECIPITATION **[7]**

Type of microorganism	**System**	**Chrystal type**	**Reference**
Photosynthetic organism			
Synechococcus GL24	Meromictic lake	Calcite $CaCO_3$)	(Douglas and Beveridge, 1998)
Chlorella	Lurcene Lake	Calcite ($CaCO_3$)	(Dittrich, 2004)
Sulfate reducing bacteria			
Isolate SRB LVform6	Anoxic hypersaline lagoon	Dolomite (Ca(Mg) CO_3)	-
Nitrogen cycle			
Bacillus pasteurii	Urea degradation in synthetic medium	Calcite ($CaCO_3$)	(Mc.Connaughey, 2000)
Bacillus cereus	Ammonification and nitrate reduction	Calcite ($CaCO_3$)	(Castanier et al., 1999)

As was previously described, the photosynthetic metabolism can induce the precipitation of calcium carbonate.

Several types of microalgae use urea hydrolysis mechanism to fulfil their nitrogen needs. For example, Chorella, sp utilizes urea as a nitrogen source; urea is hydrolysed by urease or urea amidolyase enzyme to produce ammonia and bicarbonate **[20]**. The activity of urease enzyme also can induce the precipitation of calcium carbonate **[9, 12]**.

Microalgae are type of renewable resources that easily cultivated, so that its availability as raw material can be maintained properly. Besides that, microalgae are easy to grow especially in tropical area like Indonesia, where many non-agricultural landfills can be utilized as a raceway pond for microalgae cultivation and sunlight is not a problem. As a tropical country, Indonesia also has a good temperature and water with high mineral contained which is very suitable for microalgae cultivation.

VI. CONCLUSION

Biocement is product innovation in material field that can be produce naturally using microorganism such as bacteria and microalgae. Microalgae have a great potential to be developed as media for biocement production through biocementation. Microalgae metabolism activity such as photosynthesize and hydrolysing urea can create the alkaline environment (pH and DIC elevation), so that calcium carbonate precipitation occurs in the presence of calcium ion in the system. On the other side, microalgae also part of renewable resources that is easily cultivated especially in tropical area like Indonesia, so that its availability as raw material can be maintain properly.

Further research needs to be done, primary to the theme related to suitable type of microalgae and mechanism use in biocement production through biocementation, and also the optimum condition to produce good quality of biocement.

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