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# The use Immobilized Bacteria-Alginate-Chitin for crack remediation

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**Abstract.** Cracks in concrete allow water and chemicals to enter, a process that may lead eventually to the unwanted damage of the concrete structure. However, this material is that it easily cracks due to its low tensile strength. The potential of self-healing of calcium carbonate precipitating bacteria. The possible application of bacteria to extend the lifetime of concrete is studied. The goal of this project is to incorporate dormant but viable bacteria in the concrete matrix. The research is the healing of cracks in concrete by bacteria immobilization using alginate-chitin. Bacteria were mixed into the gel and both were a concrete mixture. However, as the bacteria-gel matrix showed capable of producing calcium carbonate at pH 7-11, and could precipitate calcium carbonate 0.054 g. Concrete that mixed with bacteria-alginate-chitin 6.3%, showed compressive strength 18.75 Mpa and water absorption 1.6%, after immersion in water for 28 days.

## 1. Introduction

*Concrete* is a substance used for the building which is made by mixing together cement, sand, small stones, and water. This is the most commonly used building material. Concrete also has problems that can reduce its superiority. Among the problems that are often encountered are crack problems that occur in these materials. Cracks in reinforced concrete can arise during pre-construction and post-construction. The causes of concrete cracks occur when making concrete, including the nature of concrete, temperature, iron corrosion, manufacturing processes and material that is not good. The factors that cause concrete cracks that occur after making concrete are environmental influences (the weather) and overloading on concrete construction. Stone and concrete are susceptible to weathering; a breakdown of the mineral matrix leads to increased porosity of the surface and with it brings numerous issues [1]. The way to deal with concrete cracks that are usually done is to give the epoxy injection in the crack section. Concrete cracks are potential to cause corrosion if left unchecked to damage concrete steel reinforcement.

Microbial induced calcite precipitation (MICP) is a microorganism that is able to induce the formation of calcium carbonate, through a metabolic process and biochemical activities [2]. MICP organisms are able to secrete metabolic products as carbonate ( $\text{CO}_3^{2-}$ ) that react with ions calcium ( $\text{Ca}^{2+}$ ) in the environment (i.e. cement), resulting in carbonic salt of calcium ( $\text{CaCO}_3$ ). Therefore a novel technique has been developed by using a selective microbial have metabolic activities promote calcium carbonate precipitation. The calcium carbonate produced can close the concrete crack.

Cell immobilization is coating or capture of cells with organic or inorganic compounds, by binding to the matrix through chemical binding or holding it in the cavity of the supporting material. The



purpose of cell immobilization is to make bacteria more resistant to certain environmental conditions. The immobilized cell is expected to change its activities so that it can be used continuously for the process.

The advantages of using immobilized cells compared to free cells include providing favorable micro-environmental conditions such as contact between cells, gradient nutrient-products, pH gradients for cells, genetic stability, and protecting cells against damage [3]. The weakness of the use of immobilized cells is the obstacle in the process of diffusion both the substrate and the product formed. For living cells, gas growth and evaluation often damage the supporting matrix.

Active immobilization is carried out by two methods, namely the entrapment method and the binding method. The entrapment method is carried out physically in the supporting matrix. Supporting matrices that can be used are polymeric bead (such as agar, alginate, polyacrylamide, chitosan, gelatine, collagen, carrageenan), porous metal screens, polyurethane, silica gel, polystyrene and triacetate cellulose [4]. The polymeric beads requirement is porous enough for the substrate and product.

Alginate is a natural polymer or polysaccharide. Alginate has the ability to bind water and form a gel, its viscosity is high and has good stability. Calcium alginate is most widely used as a matrix in cell immobilization because it is friendly to cells (biocompatibility), ease of preparation and low cost [5]. The effectiveness of the immobilization method is influenced by the pore density of calcium alginate. With the higher concentration of calcium-alginate, the porosity of the gel will be lower so that it will be held in the gel. However, the polymeric beads requirement is porous enough for the substrate and product.

Previous research has shown that *Bacillus* sp. from the river in limestone mountains at Wawolesea Southeast Sulawesi Indonesia are able to precipitate calcium carbonate on their cell constituents. The bacteria are known to have metabolic activities that can convert urea to ammonium and carbonate. The result of a metabolic process causing is local increases the pH and carbonate will occur promote the microbial deposition of calcium carbonate in a calcium-rich environment.

## 2. Methods

### 2.1. Bacterial strains

Pure cultures (*Bacillus* sp. from the river in limestone mountains at Wawolesea Southeast Sulawesi Indonesia) is inoculated into the nutrient broth (Sigma, USA). Cells were incubated in a shaker incubator 150 rpm, at room temperature. Additionally, cells were grown in 1 day.

### 2.2. Preparation alginate-chitin

Alginate/chitin blend films were prepared by mixing of 2% of alginate and chitin in water and then cross-linked with 4% CaCl<sub>2</sub> solution.

### 2.3. Immobilization process

Matrix calcium-alginate-chitin was prepared by mixing 4% sodium alginate in 100 mL aquadest and 1 g chitin, stirrer 30 min for homogenization. The pellet bacterial was suspended in matrix solution. The matrix solution was dropped into a sterile 0.15 M calcium chloride solution with a pump-syringe. Gelling was completed after 30 min. The beads were stored at 4°C [6].

### 2.4. Urease assay

The rate of hydrolysis of urea to ammonium and carbonate is determined by measuring the enzyme activity of urease. Analysis of urease activity in bacteria using to phenol-hypochlorite assay a method [7]. The bacteria were re-inoculated into urease media and incubated at 37°C. After an interval of 1 day, the culture filtrate was added to potassium phosphate buffer pH 7.0 and Urea 0.1 M. The mixture was incubated at room temperature and addition of phenol nitroprusside and alkaline hypochlorite, re-incubation, and changes are observed.

### 2.5. Estimation of calcium carbonate from bacterial culture

The bacteria were inoculated into calcite precipitation media (urea 20g/L; calcium chloride 49g/L) and incubated at room temperature. Calcite precipitation is measured at regular intervals times.

### 2.6. Preparation of specimen for compressive and split tensile test

The cubes were prepared for concrete mix (sand and cement: 1:1) with variation addition of bacteria. The size of the cubes concrete in shape as 100mm x 100mm x100mm height. The cubes were molded after 2-3 days and subsequently cured in water for 7 and 28 days.

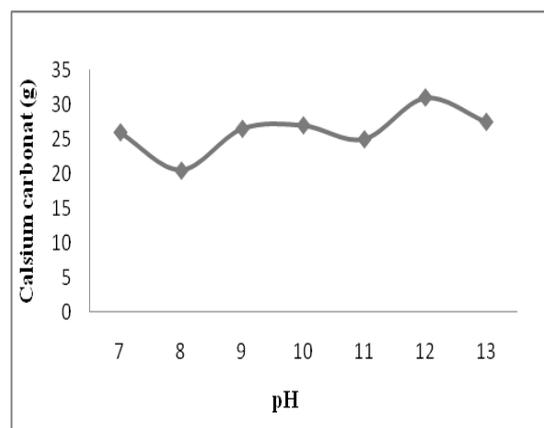
### Results and Discussion

#### 2.7. Immobilization cell

Cell immobilization is the cell of a microorganism that is physically placed inside certain area/space, so that it can maintain its catalytic activity. Cell immobilization is a method of cell containment in an inert material and insoluble like sodium alginate. Cell immobilization is expected can increase cell resistance to environmental changes such as changes in pH or temperature. Cell immobilization method has been developed, one of which is the use of mixture ammonium alginate and chitin.

This study shows that crack concrete healing of bacterial, based on porous sodium alginate-chitin particles loaded with bacterial MICP. The reason for this can be explained by chemical and biological processes in the concrete. When the concrete structure is damaged and water begins to seep through the cracks that appear in the concrete, the bacterial spores will come into contact with water and nutrients. Once activated, the bacteria to produce the urease enzyme. This enzyme will hydrolyze urea into ammonium ions and carbonate ions. Furthermore, the carbonate ion will react with calcium existing in the environment forms calcium carbonate. Hypothesized that calcium hydroxide particles present at the surface of the crack interior [8].

Effect of pH (Figures 1), showed that the optimal pH for both immobilized cells (isolate Wowolesia-T4) to achieve the highest activity were room temperature and pH 12.0. The enhanced stability of immobilized cells may be attributed to the decrease damaged of cell's after the cells were entrapped inside in the chitin-alginate. The experiment showed that the optimal pH 12.0 for both immobilized cells in the alginate-chitin based on urease activity.

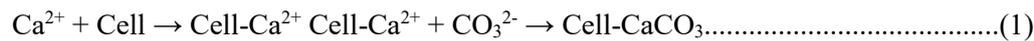


**Figure 1.** Effects of pH on the activity of cell immobilized.

#### 2.8. Urea and $Ca^{+}$ concentrations

The hydrolysis of urea by urease not only increases the pH but also uses it as a nitrogen and energy source [9]. The MICP used in this study was Wawolesea T4 isolate which was able to produce 0.012g/mL calcium carbonate [10]. Wawolesea T4 isolates were identified as *Bacillus* sp. [10].

It is possible that individual microorganisms can produce ammonia as a result of enzymatic hydrolysis of urea to create ammonium ( $\text{NH}_4^+$ ) and carbonate ( $\text{CO}_3^{2-}$ ). Ammonium around the cell and increase the pH, and subsequently inducing reaction  $\text{CO}_3^{2-}$  with calcium in the environment, produce calcium carbonate precipitation [11]. The metabolic / reaction stages: First, urea is hydrolyzed to carbonate and ammonia [12]. Microbial cell surfaces have negatively charged and acted as scavengers for cations ( $\text{Ca}^{2+}$ ), in environments microorganisms act as ideal crystal nucleation sites binding onto their cell surfaces [13], readily available for  $\text{CaCO}_3$  precipitation [14]. Actually, the  $\text{Ca}^{2+}$  is not likely utilized by metabolic processes.



The amount of  $\text{CaCO}_3$  precipitation depends on ion calcium ( $\text{Ca}^{2+}$ ) concentrations from hydrolyzes urea [15, 16].

### 2.9. Compressive strength

Precipitation of calcium carbonate induced by microbes (MICP) is a new technique that can cause a decrease in the porosity of concrete structures, and offer higher strength. The next impact is increased durability, corrosion resistance, and reduced water permeability. This condition is caused by MICP is a bio-geochemical process induces precipitation of calcium carbonate in the concrete matrix so that it will close the concrete cracks.

The compressive strength test results revealed that there is an increase for all the samples in which immobile isolate *Wowolesia-T4* in alginate-chitin, compared with control (Table 1). A significant increase of 100% and 120% was observed for free cell (not immobilization) concentrations of  $1.75 \times 10^7$  and  $3.50 \times 10^7$  cells/ml respectively, for 28 days compressive strength. The strength of concrete of immobile isolate *Wowolesia-T4* was incorporated. It can be observed that the increase in compressive strength, but weaker compressive strength than microbes not immobilized, is about 8% and 60% for a concentration of  $1.75 \times 10^7$  cells/ml and  $3.50 \times 10^7$  cells/mL respectively (Table 1).

The above results, it can be observed that there is an initial early strength gain for the first seven days. The improvement in compressive strength by isolate *Wowolesia-T4* free and immobile, 100 % and 8% respectively. This data shows the precipitation capability of calcium carbonate in non-immobilized microbes is better so that the compressive strength of the concrete becomes high. This condition is probably caused by immobilized microbes not mixed homogeneously in the concrete mixture so that not all cracks can be covered by calcium carbonate. In concrete mixed with microbes not immobilized could be to bio-mineralization of  $\text{CaCO}_3$  on the cell surfaces and cause the pores concreated to be covered, i.e pore-filling in the specimens.

Tables 1, it can be noted that there is an increase in compressive strength for 7 days compared to 28 days in both cases. This may be, which enabled high pH level to provide nourishment and buffering action to grown microbial cells in the concrete. The high pH in the concrete, increased porosity of the surface and ability water to enter through the cracked pores, caused microbial cells were able to grow fast and production calcite. Calcium carbonate will be formed subsequently filling the pores and reduction in porosity [17].

The final compressive strength concrete for mixture adding free cell systems was higher than immobilized cells. The compressive strength decreased by 27.27% for concrete with immobilized cell chitin-alginate. A low yield has been observed in compressive strength with immobilized cells, which might be due to problems because entrapped cells may not have effective contact with essential nutrients in the broth or the cells may be inhibited by the product [18]. The porosity of gel chitin-alginate, caused cations ( $\text{Ca}^{2+}$ ) are prevented from reacting with calcium in their environment. The form of immobilized cells becomes granules, causing mixing with cement and sand on the concrete making to be not homogeneous.

**Table 1.** Compressive strength for wild strain Wawolesea-T4 incorporated mortar.

Days	Control	Compressive strength (MPa)			
		1.75 x 10 <sup>7</sup> cell/mL		3.50 x 10 <sup>7</sup> cell/mL	
		Free cell	Immobilized cell	Free cell	Immobilized cell
7th	8.59	9.38	10.94	11.72	11.8
28th	11.72	23.44	11.8	25.78	18.75

### Acknowledgments

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