APPLICATION OF SUSTAINABLE BIOCEMENT USING EXTRACTED BACTERIAL ENZYME FOR LOOSE SAND IMPROVEMENT

ỨNG DỤNG XI MĂNG VI SINH DỰA TRÊN ENZYME CHIẾT XUẤT TỪ VI KHUẨN CHO MỤC ĐÍCH GIA CỐ ĐẤT CÁT YẾU

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(Received October 19, 2020; Accepted December 20, 2020)

Abstract - Portland cement is a most popular binder used for soil stabilization. However, the cement remained many drawbacks such as consuming high energy and raw material, releasing huge amount of CO_2. The recent study proposed an application of biocement using bacterial enzyme as an alternative binder for stabilizing loose sand soil. A simple method was employed to extract urease enzyme from bacterial cells. The extracted enzyme solution was used to solidify sandy soil via a process of enzyme induced calcium carbonate precipitation. The strength of loose sand after biotreatment could gain up to 1600 kPa, which was comparable to Portland cement (8%) treated sand. In addition, microstructure analysis was used to confirm a formation of calcite mineral from the biocement, in order to enhance strength of sandy soil.

Key words - Biocement; Sand; Bacteria; Urease enzyme, Portland cement

Tóm tắt - Xi măng Portland là loại chất kết dính phổ biến nhất hiện nay được sử dụng trong công tác gia cố nền đất. Tuy nhiên loại xi măng này vẫn còn nhiều nhược điểm như lượng tiêu thụ năng lượng và nguyên liệu thiên rất cao, lượng khí thải CO_2 trong quá trình sản xuất và vận chuyển rất cao. Nghiên cứu này đề xuất việc ứng dụng xi măng vi sinh dựa trên enzyme chiết xuất từ vi khuẩn cho mục đích gia cố đất cát yếu. Phương pháp đơn giản đã được sử dụng để chiết xuất enzyme từ vi khuẩn. Dung dịch enzyme này được sử dụng nhằm mục đích hóa cứng đất cát mềm thông qua quá trình sinh ra kết tủa canxi cacbonat từ phản ứng vi sinh. Cường độ nén của đất cát sau gia cố lên đến 1600 kPa, có thể so sánh được với đất gia cố 8% xi măng Portland. Ngoài ra các phân tích ở kích cỡ vi mô đã chỉ ra rằng xi măng vi sinh có khả năng tạo ra khoáng chất canxit để kết dính các hạt cát lại với nhau nhằm tăng cường độ.

Từ khóa - Xi măng vi sinh; đất cát; vi khuẩn; urê enzyme; xi măng Portland

1. Introduction

Nowadays, human is facing rapid growth of population, fast urbanization, and more development of infrastructures such as major highways, high speed railways, high-rise building and other structures which cause the reduction of availability of soils with desirable characteristics. These result in, civil engineers dealing with soft and weak soils that possess high compressibility and low shear strength and civil engineers are trying to improve their mechanical properties via suitable soil stabilization methods. According to the state-of-art report on ground improvement from Chu et al. [1] there are five categories of ground improvement techniques, which include total of twenty-nine methods. One of the ground improvement method is a admixture grouting which includes methods of particulate grouting, chemical grouting, mixing methods, jet grouting, compaction grouting, compensation grouting. Those methods used Portland cement as a binder to improve engineering properties of weak soil layers. However the cement is dealing with majority drawbacks such as carbon footprint from manufacturing process, quarrying of large amount of raw materials and associated land destruction, release of high pH residuals to the environment [2].

Therefore, civil engineers always find alternative binder materials which can overcome the drawbacks of Portland cement material. Recently, biocement has been widely studied for various potential applications in geotechnical engineering. This method has been proposed to improve the mechanical properties of soil by a process of microbial induced carbonate precipitation (MICP) [3]. A use of alkaliphilic of *Sporosarcina pasteurii* bacteria contain highly urease enzyme activity that is suitable for soil stabilization application. *Sporosarcina pasteurii* bacteria use their own urease to hydrolyze urea by following the reaction shown below:

$$(NH_2)_2CO + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

Then introduction of Ca^{2+} source:

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 + H_2O \quad (2)$$

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + CO_2 + H_2O \quad (3)$$

The bacteria injected into soil matrix is reacting with Urea/Calcium source, in order to induce $CaCO_3$ precipitation which binds soil grains together to increase strength of soil.

However, the MICP method has several disadvantages such as limited to deep soil due to constrain of bacterial growth and transport in sub soil; The reduction of pore spaces in fine soils prevents movement of microbes (microbes can pass through pore throats smaller than approximately 0.4 μm [4]; Complicated condition of cultivation and storage. Thereby, a newer ureolysis method has been studied, in which the use of nano-scale and water-soluble urease enzyme also can induce carbonate precipitation. The enzyme induced calcium carbonate precipitation (EICP) has been studied to reduce the permeability of porous media [5], to improve the mechanical properties of sand samples [6], [7], [8]. These studies used either commercial urease or plant-derived enzyme. The commercial urease is very expensive for geotechnical applications, whereas plant-derived enzyme

is plants requires time (plant growing) and space and it is produced in small amounts.

This study proposed an in-house extraction method of urease enzyme from living bacteria. This method was able to produce high activity enzymes, in large quantities via shortest and simplest extraction technique. The extracted bacterial enzyme was employed to stabilize loose sand to improve strength which compared with chemical stabilization soil using conventional Portland cement. A series of mechanical testing, microstructure analysis such as a Scanning Electron Microscope (SEM), energy dispersive X-ray spectroscopy (EDS), and cost comparison were conducted to evaluate compressive strength and potential applications of a new biocement binder using extracted bacterial enzyme.

2. Materials and Methods

2.1. Materials

Bacterial strain used an isolated strain of *Sporosarcina pasteurii* ATCC-6453 which was purchased from the American Type Culture Collection (Manassas, VA). These bacteria were cultured in a laboratory under sterilized aerobic batch conditions in an ammonium yeast (NH4 -YE) medium, on a shaker with 160 rpm of shaking speed, at 30 °C, for 48 hours. Table 1 shows the content of the NH4 -YE medium.

Table 1. *Component of the ATCC medium: 1376 Sporosarcina pasteurii NH4-YE medium*

Constituents	Amount
Yeast extract	20 g
Ammonium Sulfate $(NH_4)_2SO_4$	10 g
0.13 M Tris buffer (pH 9.0)	1 L

Testing sand was Ottawa silica sand as described in ASTM C778. The grain sizes of sand were in between 0.6 mm (sieve #30) and 0.85 mm (sieve #20) with a mean grain size of 0.73 mm. Its specific gravity was 2.65. The maximum and minimum void ratio of testing sand were 0.742 and 0.502, respectively. The cement binder used the mixture of cement type I/II contains 90 – 95 % of Portland cement and other chemicals as gypsum (~5%), magnesium oxide (~2%), limestone (<3%), flue dust (<1.5%), and quartz (<0.3%). Chemical solution for biocement included urea and calcium chloride with a concentration of 0.3 M by 1:1 ratio.

2.2. Methods

2.2.1. Enzyme extraction process

The viable cells were used for enzyme extraction were *Sporocarcina pastuerii* (ATCC 11859). Bacteria were cultivated in the NH4 -YE medium during 48 hours to achieve 12.5 mM/min of urease activity. The culture was sit directly in the sonication bath (BRANSONIC 220 ultrasonic cleaner with 117 Volts; 125W; 50/60kHz). Two categories condition of sonication were a continuous running sonication and a running & cooling sonication. For the continuous running sonication, there were 3 periods of running time such as 0, 40, and 80 mins. In case of the running & cooling sonication, the sonication process was paused every 10 mins to let the solution cooling down to room temperature. The total mins of running & cooling sonication process are 60 mins. When the sonication process was finished, the solution was collected for a centrifuge step at 5500 RCF during 20mins to separate fraction of cells and urease. The optical density, temperature, and urease activity were measured after lysing process of bacteria cells [9].

2.2.2. Sand column preparation and treatment

Sand column was packed in a PVC column (5 cm of diameter and 10cm of height) using a moisture tamping method. The dry sand was mixed with distilled water to achieve a moisture content of 5%. To achieve a similar void ratio (e~0.60) within all samples, predetermined amounts of soil were compacted into 10 layers of equal thickness (10mm per layer).

The commercial cement stabilized sand columns used 4% and 8% by weight of Portland cement. The moisture pre-mixed cement and sand with 7% of water were packed in the PVC column followed the moisture tamping method. The stabilized cement sand columns were wrapped by plastic bags and cured at room temperatures for 7 days before performing other tests.

The biocement treated sand using EICP method employed a two-phase percolation circulation treatment methods [10]. A piece of 3 M Scotch Brite scouring pad was placed at each end of the sample as a filter and also facilitate drainage and avoid calcite precipitation on the top surface of the soil column as shown in Figure 1. The bacterial enzyme solution was circulated for 3 hrs using a peristaltic pump (3-5ml/mins). The mixed chemical solution then was used and circulated for 9-12 hrs. Afterward, soil sample was fluxed by water during 2 hrs to remove soluble byproducts. When one cycle treatment (enzyme and urea/calcium chloride solution) had been finished, the column was treated again with new enzyme and chemical solutions. The treatment cycle was repeated for 8 and 16 days which equals to 8 and 16 cycles of treatment.

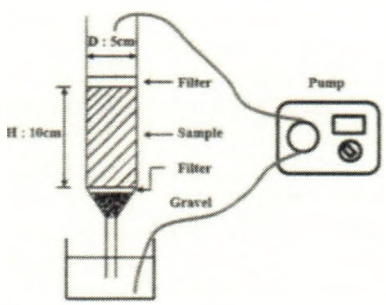

Figure 1. *Schematic of a treatment cycle [10]*

2.2.3. Testing methods

The stabilized sand columns were tested unconfined compressive strength (UCS) followed ASTM (1996) standard D4219-08 with 2mm/min of loading rate. After completing the UCS test, the sub-samples of biocement treated sand were collected for later measurement of calcium carbonate content (CCC) and microstructure analysis. The calcium carbonate content was determined by an acid-rinsed method which was mentioned by Feng &

Montoya [11]. The microstructure analysis included SEM and EDS were performed to evaluate the formation of calcite crystals due to EICP method.

The optical density of bacterial cells was measured spectrophotometrically at 600 nm (OD600). The temperature of sonicated solution was measured during the sonication process. The urease activity of culture and extracted enzyme was examined by a conductivity meter followed a method which was mentioned by Chu et al. [12].

3. Results and discussions

3.1. Enzyme extraction

Figure 2 shows the relationship between the urease activity of the extracted enzymes, running time, and optical density. The measurement results shows that longer sonication time provided higher urease activity. However, the urease activity had a constant trend after 40 mins of sonication run. The highest urease activity of continuous running method was approximate 20 mM/min. An increase in urease activity value corresponded with reduction in optical density of culture cells. This indicated that the cells were lysed to release urease, thereby disappeared cells reducing culture density.

For the "run-cool" method, the temperature of culture was around 34°C due to the pausing step in this method. The temperature of solution from the "run-cool" method was much lower than that from the "continuous" method (e.g. ~55°C). Keeping the solution at low temperature using "run-cool" method can produce enzyme with the highest urease activity which was relative higher than from continuous running method at 80 mins. Such controlled environment resulted in urease activity 2 times higher than that of original culture prepared with conventional MICP method (25 vs 12.1 mM/min). According to the experimental results, this suggested using the "run-cool" sonication method for 60mins to achieve high urease activity of extracted enzyme solution.

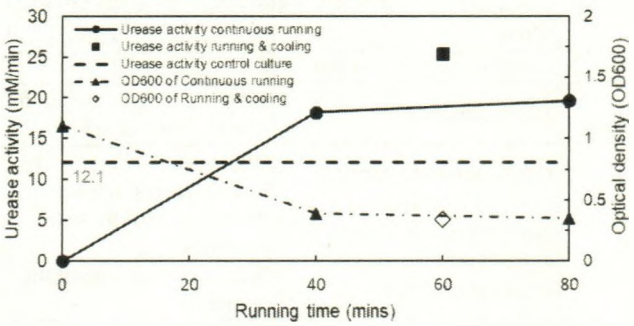

Figure 2. *Urease activity vs Sonication time vs Optical density*

3.2. Unconfined compressive strength

The sandy soil columns were stabilized using either biocement or Portland cement at low and high levels of treatment. After achieving a certain degree of treatment, the PVC molds were carefully removed to collect samples for the unconfined compressive test and calcium carbonate measurement. Figure 3 shows testing results of UCS between biocement and Portland cement sand at two levels of treatment.

Figure 3(a) presents the failure pattern of samples that was captured immediately after the compressive test. It should be noted that the Portland cement sand displayed a darker color due to a grey color of cement binder, whereas biocement sand had a lighter color. As can be seen, the failed samples due to compressive load generated cracks from the top to bottom within both cemented samples. It indicated that the samples were subjected under a uniform treatment. In general, it is not difficult to pack the uniformity Portland cement sand sample because of the use of mixing method, but there was a non-uniform issues for the biocement treated sample resulted from percolation method. The non-uniform of biocement samples using MICP method showed that the top part was higher strength than the bottom part, which was mentioned by L. A. Van Paassen et al. [13]. However, this study used the EICP method to treat sand columns, in which the enzyme with nano scale and water-soluble properties would migrate through samples to precipitate calcium carbonate equally from top to bottom parts.

According to Figure 3(b), the low level of treatment samples provided significantly lower strength than from high level treatment for both biocement and Portland cement sand. The UCS of biocement sand sample was relatively higher than that of Portland cement sand at both levels of treatment (630 kPa vs. 430 kPa at low treatment; and 1600 kPa vs. 1450 kPa at high treatment). An increase in the number of treatment cycles in biocement sand resulted in the significant improvement of calcium carbonate content (1.99% vs 7.89%), in order that the UCS of biocement sand also was enhanced by a factor of 2.45. The UCS of biocement sand treated by bacterial extracted enzyme was comparable with results reported by H. Yasuhara et al. [14] (~ 400-1600 kPa), in which they used commercial plant enzyme. This suggests that the bacterial extracted enzyme used for EICP would stabilize loose sand to achieve a similar unconfined strength as EICP treatment using commercial plant enzyme, as well as Portland cement treatment.

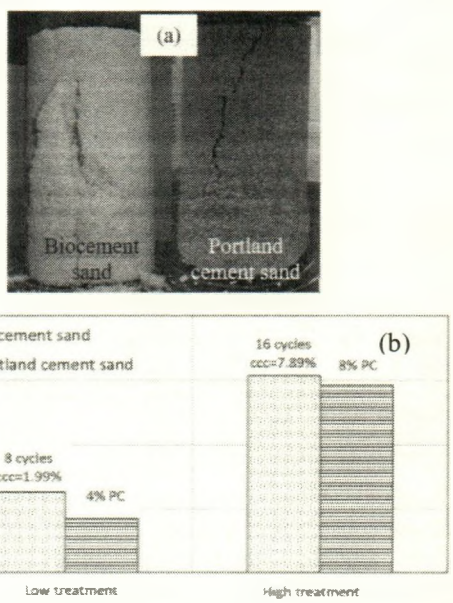

Figure 3. *Comparison between biocement sand and Portland cement sand: (a) Failure pattern after compression, (b) UCS*

3.3. SEM and EDS analysis for biocement sand

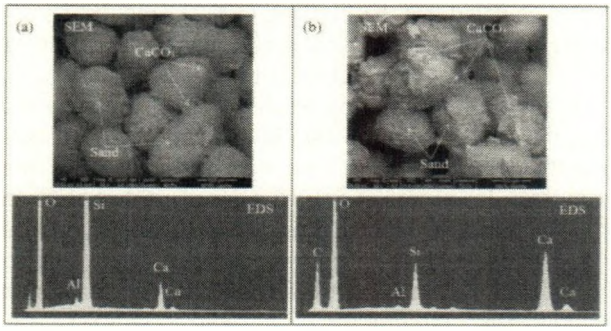

Figure 4. *Microstructure analysis for biocement sand: (a) 8 cycles of treatment, (b) 16 cycles of treatment*

The sub-sample of biocement sand was collected after compression test for the microstructure testing including SEM and EDS. Analyzing microstructure of stabilized sand would confirm the formation of calcium carbonate precipitation due to EICP process. It is visible in SEM image Figure 4(a) that calcium carbonate deposited on sand particles at various locations after 8 cycles of treatment. The contact point between two sand grains was bridged by calcium carbonate precipitation. The bridges of $CaCO_3$ were dominantly observed in the sand matrix, in order to increase the strength of loose sand. When sand was treated at the higher level (i.e. 16 cycles), the $CaCO_3$ precipitation was deposited not only at void spaces but also covering sand grains (see SEM in Figure 4(b)). The significant high density calcium carbonate observed in 16 cycles treatment biocement sand corresponded to the high level of CCC of that sample (e.g. 7.89%). The thick layer of $CaCO_3$ connected sand grains, along with a lumpy shape of sand grains resulted from covered $CaCO_3$, would enhance the strength gain of biocement sand sample. This was in line with UCS results in Figure 3, in which the increase in CCC (from 1.99% to 7.89%) resulted in the improvement in strength (from ~630 kPa to ~1600 kPa) of biocement sand.

Comparing EDS results between Figs. 4 (a)&(b), the level of Ca mineral in the biocement sand with 16 cycles of treatment was much higher than in the sample treated at 8 cycles. The peak of Ca mineral form Figure 4(b) was approximately double of that from Figure 4(a). The notably higher peak of Ca in the high level of treatment sample confirmed that the increase in number of EICP treatment would led more $CaCO_3$ precipitation in the sand matrix that contributed to enhancing strength of loose sand.

3.4. Comparison of biocement with other conventional soil treatment methods

Table 2 presents a comparison of biocement with other conventional cement binders such as Portland cement, ultrafine-cement, chemical binders. In term of injection energy, the biocement only required a very low energy, likely to chemical binder, due to very low viscosity of injection solutions that allowed biocement gravity migrated into soil matrix. In contrast, the energy requirement of using Portland cement was very high because the Portland cement itself cannot seep into soil as water, thereby the methods of either high pressure injection or heavily mixing need to be applied for the Portland binder.

Although ultrafine-cement and chemical binder had similar advantages with biocement, they are very expensive and unfriendly environment, in particular chemical materials were banned from some countries due to their toxic. Therefore, both types of binder were not popular in construction materials as Portland cement.

Table 2. *Comparison of various construction methods/binders* [17]–[19]

Method/Binder	**Portland cement**	**Biocement**	**Ultrafine cement-based grout**	**Chemical grout**
Injection energy	Very high energy for pressure injection (1.7 – 6.8 MPa) Soil cement mixing	Very low energy for pressure injection, gravity seepage (0.001MPa)	Moderate energy for pressure injection (0.6 – 1.8 MPa)	Very low energy for pressure injection (0.05 – 0.07 kPa/m of depth)
Materials	PC + water	Micro-organism, Urea+$CaCl_2$	Ultrafine cement + water	Chemical
Advantages	Strong, durable, mature technology	Low carbon footprint Possible use waste materials	Strong, durable	Strong, quick isolation, various product options
Disadvantages	Large carbon footprint, Disruption of local ecosystem, High pressure, disruption of soil structure Disposal problems	Ammonia by product Less field-scale studies	Large carbon footprint, Using chemical admixture, Raw material is 5 times more expensive than PC Disposal problems	High cost Environmental impacts (toxic), banned by some countries Washout and durability problems
Average UCS (MPa)	2 – 20 (jet grouting) 0.5 – 2 (soil cement mixing)	0.5 – 10 (depend on number of treatments)	0.5 – 10 (depend on W/C ratio and % admixture)	0.8 (acrylic polymer-treated sand)
Estimate cost for 1m^3 soil (USD)	150	150 – 400	450	430

Portland cement is the most popular binder material using in construction projects because of its low cost, strong bonding, durable properties, and mature technologies. However, it remained many disadvantages to environments. For example, producing of Portland cement required burning large amount of natural raw material, consuming very high energy, which released huge amount of carbon into atmosphere. In addition, construction methods of Portland cement stabilized soil, such as the high pressure injection and mixing, also demanded much higher energy for operation than low pressure injection of biocement. Therefore, in term of sustainable materials, the

biocement is a potential sustainable and environmental friendly binder for construction materials.

However, the cost of biocement is relatively higher than the Portland cement due to the raw material cost of culture. Most of recent studies conducted in laboratory scale have used lab-grade chemical/substrates which were very high cost, thereby increasing overall cost of biocement [15]. One of newest large scale biocement study already focused on the use of low-grade chemical and industry-scale bacterial cultivation for reducing biocement cost [16]. Therefore once the cost reducing, the biocement would be the potential alternative binder for construction material, in which the material would meet requirement of economic and sustainable aspects.

4. Conclusions

In summary, this paper presented a technique, "run-cool" sonication, for the extraction of urease enzyme from bacterial cells. The in-house "run-cool" sonication would provide the enzyme solution with highest urease activity, comparing to continuous method as well as to the original culture. The biocement using EICP method with extracted urease could significantly improve the strength of loose sand. The enhancement of strength in sand resulted from calcium carbonate precipitated at the contact points of sand grains. The microstructure analysis indicated that higher level of treatment provided higher calcium carbonate content which contributed to the increase in strength of samples. The UCS of biocement sand was slightly higher than Portland cement sand. Therefore, biocement using EICP with bacterial enzyme proposed in the recent study might be a potential alternative binder for construction. Although biocement is a green and sustainable material, it still need to conduct more studies to reduce the cost when applying for large scale of construction projects.

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