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Isolation and Urease Activity Test of Bacteria for Calcium Carbonate (Calcite) Precipitation (Biocementation) in Soil

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The use of bacterial calcium carbonate (calcite) precipitation (biocementation) has recently become popular as a ground-improvement technique. Ureolytic bacteria having highly urease activities were known to have important roles in calcium carbonate precipitation process. One of our research objectives is to isolate and to select as many as possible such ureolytic bacteria from Indonesian soils to be further utilized for calcium carbonate (calcite) precipitation process in the soil for strengthening the soil structure. Isolation was performed anaerobically in selective media containing 40% urea. Four isolates with different morphologies were purified and coded as TK1, TK2, TK3, and TK4. Each of them was tested for its urease activity either as a pure culture or as a mixture of several cultures. The urease activity was measured based on the ammonia concentration produced in the growth media up to 7 x 24 hours. It was known that isolate TK4 had the highest urease activity on incubation period 6 (D6), whilst a mixture of isolate cultures coded as TKC did not show a better urease activity than the isolate TK4. Hence, it could be concluded that the isolate TK4 was the best candidate to be used for further research on the calcium carbonate (calcite) precipitation process (biocementation) to strengthen the soil structure.

Key words: biocementation, calcite, calcium carbonate, precipitation, urease, ureolytic

Penggunaan presipitasi kalsium karbonat bakteri (*biocementation*) baru-baru ini menjadi populer sebagai teknik perbaikan tanah. Bakteri ureolitik yang memiliki aktivitas urease tinggi diketahui memiliki peran penting dalam proses pengendapan kalsium karbonat tersebut. Salah satu tujuan penelitian kami adalah untuk mengisolasi dan untuk memilih sebanyak mungkin bakteri ureolitik tersebut dari tanah Indonesia untuk digunakan lebih lanjut dalam proses pengendapan kalsium karbonat di dalam tanah untuk memperkuat struktur tanah. Isolasi dilakukan secara anaerobik menggunakan media selektif yang mengandung 40% urea. Empat isolat dengan morfologi yang berbeda dimurnikan dan dikodekan sebagai TK1, TK2, TK3, dan TK4. Masing-masing isolat diuji untuk aktivitas urease baik sebagai kultur murni atau sebagai campuran beberapa kultur isolat. Aktivitas urease diukur berdasarkan konsentrasi amonia yang diproduksi di media pertumbuhan hingga 7 x 24 jam. Diketahui bahwa isolat TK4 memiliki aktivitas urease tertinggi pada periode inkubasi ke-6 (D6), sementara campuran kultur isolat yang dikodekan sebagai TKC tidak menunjukkan aktivitas urease yang lebih baik daripada isolate TK4. Oleh karena itu, dapat disimpulkan bahwa isolat TK4 adalah kandidat terbaik yang akan digunakan untuk penelitian lebih lanjut tentang proses pengendapan kalsium karbonat (biocementation) untuk memperkuat struktur tanah.

Kata kunci: biosementasi, kalsit, kalsium karbonat, presipitasi, urease, ureolitik

Construction on an organic ground involves the risk of bearing capacity failure and excessive settlement (Canakci *et al.* 2015). To prevent such risks, the geotechnical properties of organic soil are improved by ordinary improvement techniques, such as deep mixing with cement or lime, vertical drains, sand columns, and dry jet mixing (Celik and Canakci 2011; Jelisic and Leppanen 2003). Many previous studies have reported that the microbial-induced calcium carbonate precipitation technique is very effective in increasing the shear strength and in decreasing the permeability of sandy and gravelly soil (Canakci *et al.* 2015; Van der Star *et al.* 2011; Van Paassen *et al.* 2010a *and* 2010b; Van Wijngaarden *et al.* 2010). It was presented as a new and environmentally friendly method (Dejong *et al.* 2006) which has an advantage over conventional chemical treatments having a limited injection distance and can be toxic to the environment (Karol 2003). This new method was also known to be cost-effective in comparison to chemical treatments (Ivanov and Chu 2008). Bacterial calcium carbonate precipitation or cementation has been applied to a variety of civil engineering applications, such as to repair cracks in rock and concrete, to improve the bearing capacity, to reduce permeability, to increase dilative tendencies, and to increase the strain stiffness in sand (Al Qabany and Soga, 2013; Dejong, 2013; Martinez *et al.* 2013; Rusu *et al.* 2011).

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Soil bacteria having high urease activities play important roles in calcite precipitation (Dejong *et al.* 2010). The main role of these bacteria in the calcite precipitation process has been to generate an alkaline environment through different physiological actions. The main nutrient and chemical compounds for soil cementation by bacteria contain $NaHCO_3$, NH_4Cl, $CaCl_2$, urea, and a nutrient broth (mixture of peptone, yeast extract, beef extract, and NaCl). Urease (urea amidohydrolase: EC 3.5.1.5) is an enzyme that hydrolyze urea into one mole of carbonate and two moles of ammonia per mole of urea as shown in the following reaction (Burbank *et al.* 2012):

Reaction #1: $(NH_2)_2CO + 2H_2O \longrightarrow CO_3^{2-} + 2NH_3$

Through the hydrolysis of urea, the bacteria then release carbonate ions into soil or water that can bind with calcium to form calcium carbonate and its metastable polymorph, calcite, as seen in the next reaction:

Reaction #2: $Ca^{2+} + Co_3^{2-} \longrightarrow CaCO_3$

Al Thawadi *and* Cord-Ruwisch (2012) and Burbank *et al.* (2012) demonstrated that indigenous ureolytic bacteria could be enriched from a variety of soils and that population of bacteria could precipitate calcite or other calcium carbonate mineral species in quantities sufficient to alter the engineering characteristics of the soil. Several bacteria able to form the calcium carbonate mineral are such as *Bacillus licheniformis* (Helmi *et al.* 2016), *Acinobacter* sp. (Sanchez-Moral *et al.* 2003), *Escherichia coli* (Bachmeier *et al.* 2002), *Microbacterium* sp. (Xu *et al.* 2017), *Myxococcus xanthus* (Gonzalez-Munoz *et al.* 2000), *Agrobacterium tumefaciens* and *Lactobacillus sakei* (Yuliani 2015), also *Bacillus pasteurii* that has been reclassified as *Sporosarcina pasteurii* (Canakci *et al.* 2015). To participate optimally in the hydrolysis of urea for calcite precipitation, the bacteria must constitutively and inducibly produce urease. The purpose of our study is to isolate and select the urease-producing bacteria from Indonesian soils to be used further in our research of biocementation or calcium carbonate precipitation process for strengthening the soil structure.

MATERIALS AND METHODS

Materials used in the research were agar, nutrient broth, D-glucose, NaCl, potassium dihydrogen phosphate, phenol red, urea, potassium citrate, HCl, phenol, ethanol, acetone, NaOH, NaOCl, $(NH_4)_2SO_4$, toluene, filter paper Whatman No. 41, alcohol 70%, aquades, aluminium foil, wrapping, cotton, tissue paper, petri disks, Erlenmeyer flaks, Drugalsky bar, measuring pippets, micropippets, measuring beaker glasses, reaction tubes, autoclave, incubator, colony counter, mixer, pH meter, thermometer, haemocytometer, spectrophotometer UV-VIS Jasco V530, microscope Olympus, laminar air flow, analytical balance, ose needles, and measuring flask.

The biocementation research was performed in Laboratory of Environmental Microbiology, Center for Environmental Technology (PTL, Pusat Teknologi Lingkungan), Agency for Assesment and Application of Technology (BPPT, Badan Pengkajian dan Penerapan Teknologi) in Puspiptek Serpong, Tangerang Selatan, Banten, Indonesia from March until June 2018. The soil sample for this experiment was obtained from the mangrove forest of Tritih Kulon, Cilacap, Central Java. This experiment was conducted using the split plot experimental design. The treatments to be investigated were the isolate factor (I) with six treatment levels as the following:

TK0 = without addition of the isolate as the control treatment

TK1 = with addition of the isolate 1

TK2 = with addition of the isolate 2

TK3 = with addition of the isolate 3

TK4 = with addition of the isolate 4

TKC = with addition of the mixture culture of isolate 1, 2, 3, and 4

and the incubation period factor (D) with eight treatment levels as the following:

D0 = incubation period of 0 x 24 hours

D1 = incubation period of 1 x 24 hours

D2 = incubation period of 2 x 24 hours

D3 = incubation period of 3 x 24 hours

D4 = incubation period of 4 x 24 hours

D5 = incubation period of 5 x 24 hours

D6 = incubation period of 6 x 24 hours

D7 = incubation period of 7 x 24 hours

Such treatments were carried out using the Completely Random Experimental Design which were repeated three times. The parameters measured were the NH_4^+ residue concentration, the pH, the bacterial population density, and the medium temperature.

Bacterial Isolation and Purification. One gram of mud sample taken from the mangrove forest of Tritih Kulon, Cilacap, Central Java was dissolved in 9 mL of distilled water in a test tube. Suspense dilution was carried out until the 10^{-4} dilution level which was then

spread over a plate of solid selective media (Harley *and* Prescott, 1993) consisting of 1 gram of peptone, 1 gram of D-glucose, 2 grams of potassium dihydrogen phosphate, 5 grams of NaCl, 0.012 gr phenol red, 50 mL 40% urea solution, 15 gr agar and distilled water until the volume became 1 L. All ingredients except urea were put in distilled water until the final volume reached 950 mL and heated until dissolved then sterilized using autoclave at 121 C, pressure 2 atm for 15 minutes and chilled. After being chilled, 40 mL of 40% urea solution was added, then sterilized with UV light at a wavelength of 270 nm using UV light at LAFC. According to Gaudy and Gaudy (1981), ultraviolet light with a wavelength between 200 - 300 nm for 15 minutes was bactericidal so that it could be used as a disinfectant.

To test the urease activity a liquid selective medium was also prepared just like the solid selective medium, but without the addition of agar. Incubation was carried out at 35 °C for 2 x 24 hours. Bacteria having urease activity will show changes in color on the media from red to orange to red purple around the colonies. Four colonies having different morphologies from the culture were streaked on the selective medium each until pure culture was obtained. After obtaining a pure culture, the isolate was stored in Nutrient Agar (NA) media incubated at 35 C as the isolate stock.

Preparation of Bacterial Isolates for Experiment. Isolates were rejuvenated by transferring the cultures from the old NA media to the new NA media and then incubated at 35 °C for 24 hours. One young growing culture was then put into 10 mL 0.85% NaCl solution, then 1 mL was taken and inoculated into a liquid selective medium and incubated at 35 °C for 2 x 24 hours. Calculation of the number of growing cells was done by using a haemocytometer or a colony counter. Culture with a density of approximately 10^7 cells mL^{-1} was ready to be used as an inoculum. This was in accordance with Alexander's statement (1977) which estimated that the number of soil microbes capable of ammonification was around 10^5 - 10^7 organisms per gram of soil

Urease Activity Test. The reaction tube was filled with 10 mL of a liquid selective medium and added with 1 mL of bacterial isolate then incubated at about 35 °C anaerobically. For the mixture, first take each part of a single isolate and mix it until it is homogeneous and then put 1 mL into the test medium. The treatment was repeated 3 times.

Determination of Urease Activities (Tabatai *and* Bremner 1969 in Iswandi 1989). The urea activity was determined by measuring the ammonia (NH_3) formed during hydrolysis of urea added to the media. Urea activity was expressed in µmol ammonia nitrogen formed within 1 hour in 1 gram of medium sample given the urea at 37 °C. A total of 10 mL of the inoculated bacterial medium was put into a 100 mL Erlenmeyer flask and then added with 15 mL toluene and homogenized. After 15 minutes it was added with 10 mL of 10% urea solution and 20 mL of pH 6.7 buffer citrate solution and shaken. Erlenmeyer flask was clogged with a rubber plug and then incubated in a 37 C incubator for 3 hours. After that, distilled water was added to a volume of 100 mL by keeping the toluene under the 100 mL terra mark on the Erlenmeyer flask and then clogged and shaken well. The suspension was filtered using Whatman paper No. 41, then in the Erlenmeyer flask 1 mL filtrate, 10 mL distilled water, 4 mL Na-phenol solution, 3 mL NaOCl solution were added, shaken and let stand for 20 minutes before being added with distilled water until the volume became 50 mL and shaken again. Light intensity was measured at a wavelength of 590 nm with a Spectrophotometer Jasco V530. The urea activity was calculated based on the standard curve equation whose formula is:

$$\text{Concentration} = \frac{\text{Absorbance} - (-A)}{B}$$

Where A = 0.0010 and B = 0.0107, which is a liner calibration curve with regression coefficient = 0.9941.

RESULTS

From the isolation process of mangrove forest sludge samples from Tritih Kulon, Cilacap, Central Java, four bacterial isolates with urease activity had been obtained. After following the Gram staining procedure as described by Misiolek *et al.* (2018), four bacterial isolates were observed to have different morphological characteristics (Table 1). The four isolates were then given the code names TK1, TK2, TK3 and TK4. Each isolate was then tested on a medium containing 40% urea. Testing on the 40% urea medium was also carried out on the mixture of the four isolates (TKC) to determine its enzymatic ability to transform urea to ammonia (Fig 1).

The graph in Figure 1 can explain that the urease activity of each bacterial isolate was different with one another. The TK0 treatment as a control showed no activity. Isolates TK1, TK4 and TKC had urease activities with the same curve patterns, while TK2 shows similarities of curve patterns with TK3. These isolates showed their urea activities starting at D3,

Table 1 Morphological characteristics of bacterial isolates from Tritih Kulon, Cilacap, Central Java

Isolate Code	Colony Morphology					Gram stain	Cell form	Motility
	Form	Margin	Elevation	Color	Surface			
TK1	Small circular	Entire	Convex	Brownish yellow	Smooth &shiny	-	coccus	Non motile
TK2	Filamentous circular	Filiform	Umbonate (elevated convex)	White	Dry	+	coccus	Non motile
TK3	Irregular	Undulate	Raised (low convex)	Whitish yellow	Smooth & Shiny	+	coccus	Non motile
TK4	Filamentous circular	Entire	Umbonate (elevated convex)	Grey	Dry as powder	+	coccus	Non motile

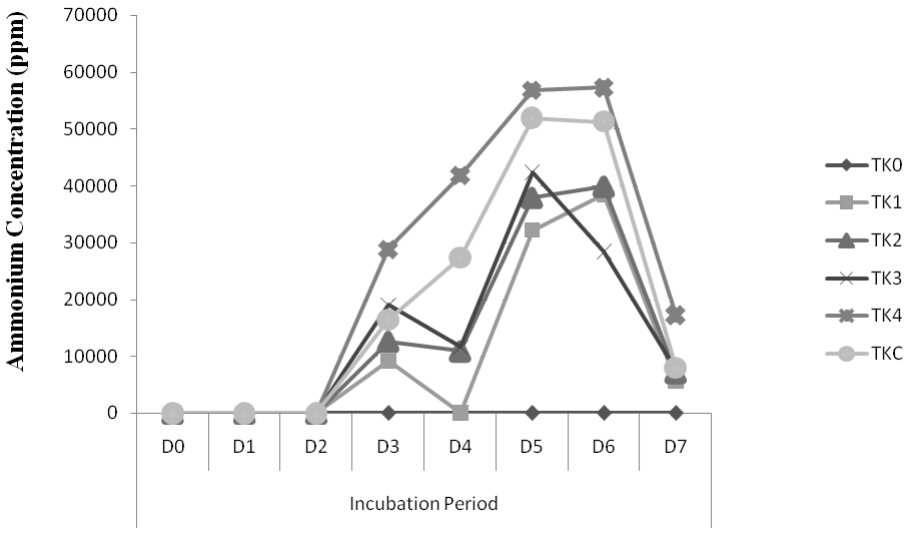

Fig 1 The Urease Activity of bacterial isolates from Tritih Kulon, Cilacap, Central Java expressed as the concentration of Ammonium (NH_4^+) detected from the medium.

which was a measurement on the 3rd day, because at the second day measurement there was no ammonia detected in the medium of each isolate. In general, all isolates reached the peak of urease activity in D5 - D6 which at that time TK4 had the highest activity of 56.82 ppm at D5 and 57.32 ppm at D6.

Based on the data in Figure 1 it can also be seen that the tolerance limit for the ammonia accumulation of each isolate differed from 38.57 ppm from TK1 on D6 to 57.32 ppm from TK4 on D6. Figure 1 also explains that TK4 was an isolate that had the highest urease activity. This isolate began to show urease activity in D3, ie measurements on the third day with 28.86 ppm ammonia levels and reached its peak activity on D6 with measured ammonia levels of 57.32 ppm. TK4 isolates were coccus-shaped bacteria which in solid media showed gray TK4 colonies forming a regular circle of filamentous and surface-like powder. Gram staining on all these isolates showed that this isolate TK4 was Gram positive.

In general, the bacterial isolates had decreased urease activity in D6 and D7 however TK3 began to experience a decrease in activity at D5. This decrease was suspected due to several things, among others, because bacterial cells still needed time to adapt to their new environment or bacteria still use N nutrient sources other than urea, peptone, which was also found in the media. This could be seen in TK2 which continued to experience cell growth which continued to increase from 8.5×10^{11} CFU mL^{-1} on D3 to 1.4×10^{12} CFU mL^{-1} on D4 (Fig 2) although there is a change in the pH value from 5.2 on D3 to 5.6 on D4 (Fig 3 pH value). While in TK3 there were also indications that even though the urea activity decreases, there was still growth in the cells. This could be seen from the increase in the number of cells from 1.5×10^{11} CFU mL^{-1} on H3 to 4.3×10^{12} CFU mL^{-1} on D4. But at this TK3 the pH value decreased from 5.2 on D3 to 4.8 on D4.

Graph of cell growth in Figure 2 shows that in D5 and D6 the highest number of bacteria was seen, when compared to the Graph of Urease Activity in Figure 1 it could be seen that the number of cells and urease

activity were directly proportional. This indicated that an increase in cell numbers would increase urease activity. At D7 there was a decrease in the number of cells which in turn was followed by a decrease in urease activity seen from a decrease in ammonia concentration on the medium.

The graph in Figure 2 also shows that TK4 was an isolate that had the highest cell number during this urease activity test. This isolate was the best growth isolate. With this high cell number, the urea activity was also the highest. However, when viewed from the ability per individual cell of each colony, seen from the comparison of the number of cells with urea activity, TK2 and TK3 were better than TK4. Likewise with TKC, a mixture of these four isolates had much better urease activity than isolate TK4, because with far fewer cells but TKC urease activity was not much different from TK4. In general, however, isolate TK4 was an isolate that had the highest urease activity because in the same incubation time the isolate was able to convert urea to ammonia in the highest concentration.

During the treatment there was a change in pH value (Fig 3). There was a decrease in the pH value from D0 to D2 which might be caused by bacteria using the simplest nutrition first, namely glucose as a carbon source contained in the medium. In D3 there was an increase in pH due to an increase in ammonia levels in the medium. But in D7 when the pH value was the highest value during the treatment, it actually decreased the measured ammonia level. This could be caused by the increase in pH affecting the growth and performance of bacterial urease.

The graph of temperature changes (Fig 4) showed that the longer the test time caused the temperature of the medium increased. In the urease activity test, the temperature increase had been caused by an exogenic reaction. Temperature increase was generated from cellular reactions, namely from the use of ATP and $NADPH + H^+$ to $NADP^+$. This reaction occurred when urea was hydrolized to ammonia. In this urease test, each isolate began to experience a decrease in activity ranging from 31 °C to 32 °C and it could also be explained that the urease enzyme produced by each isolate was optimum at the temperature range of 30 °C, because at this temperature the hydrolysis process of urea to ammonia reached the peak activity.

DISCUSSION

The unmeasured ammonia at D0, D1, and D2 did not mean that there was no urea transformation to ammonia at all, but it was suspected that the activity was still low or the bacteria still needed time to adapt because moreover it was seen from the growth or number of cells still in the lag phase, so that the resulting ammonia was small and not detected by the device used, namely Spectrophotometer Jasco V530 which has a limit of ammonia detection to a concentration of 0.1 ppm.

Alexander (1977) explained earlier that bacteria able to hydrolyze urea to ammonia and used it as a source of energy for growth were mostly in the form of coccus, including the genus *Micrococcus*. Schlegel and Schmidt (1994) had classified bacteria capable of using $NH4^+$ as electron donors and called them lithotrophic organisms which, according to Stanier *et al.* (1984) the results of ammonia metabolism are glutamate acid, asparagine and glutamine, all three of which are direct protein starters. Payne (1980) and Fardiaz (1994) added that NH_4^+ as a result of the reaction of the enzyme urease was used for the formation of amino acids which were then converted to form cell mass, the formation of cell membranes and enzymes. According to Alexander (1977), the speed of decomposition of urea into ammonia and its accumulation and immobilisation depended on the type of microbe, type of substrate, and its environment.

Hardiyanto (2002) stated that collaboration between microorganisms would accelerate the degradation of organic compounds, but in this study TKC which was a mixed treatment of isolates TK1, TK2, TK3, and TK4 turned out to have lower urease activity than isolate TK4. This might be due to the interaction of antagonism between these microorganisms. In relation to antagonism one type of bacteria will destroy or inhibit the growth of other bacteria so that the population decreases. Like the previous statement of Atlas and Bartha (1998), the microbes living together in one environment will interact between populations. These interactions can be positive (neutral, commensalism, synergism, or mutualism) and negative (antagonism, competition and predation) which can cause the destruction of some microbial populations. Positive interactions will improve the life ability of some microbial populations.

In the soil there is a dynamic process in the release of nitrogen from the organic N form through microbial decomposition called ammonification. At the same time, there is an inorganic N binding which is transferred to organic N by microbial activity. Because of this microbial activity in nature, the level of ammonia and nitrate in the media becomes non-

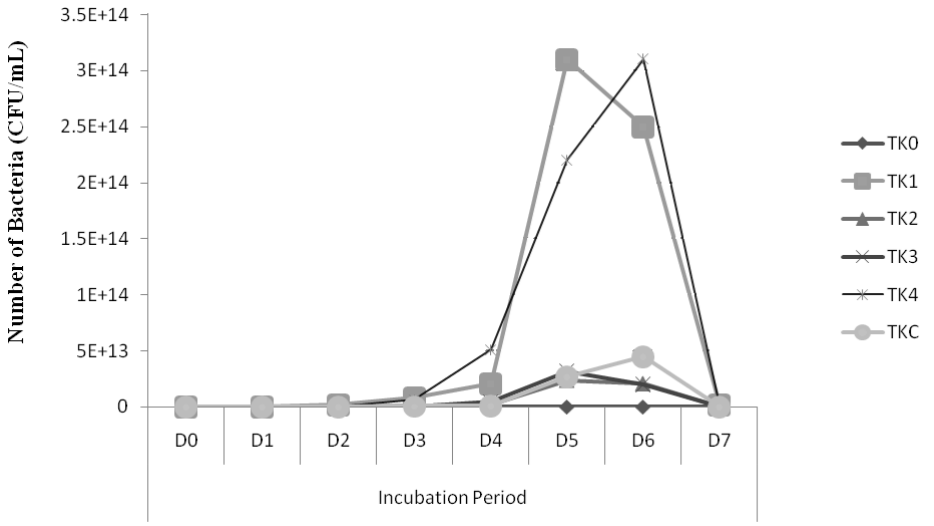

Fig 2 Growth of bacterial isolates from Tritih Kulon, Cilacap, Central Java during the urease activity testing.

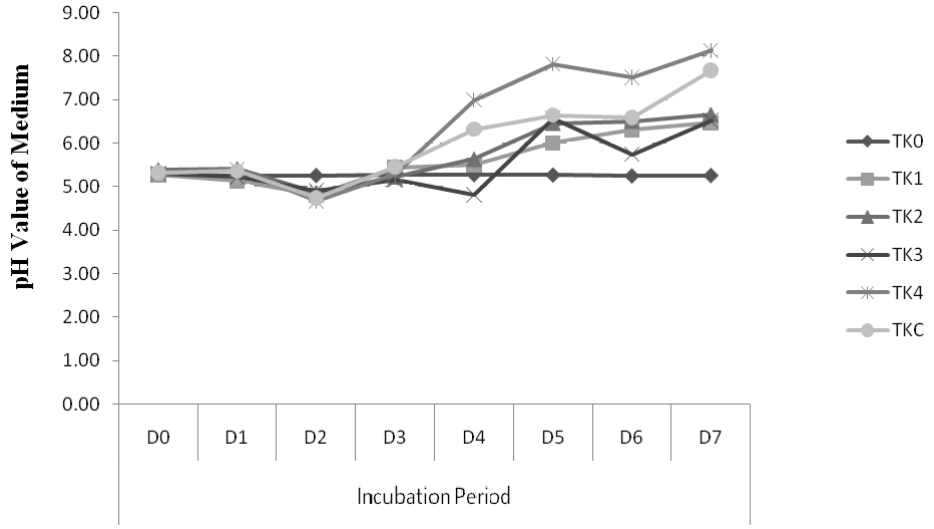

Fig 3 pH value of the medium during the urease activity testing of bacterial isolates from Tritih Kulon, Cilacap, Central Java.

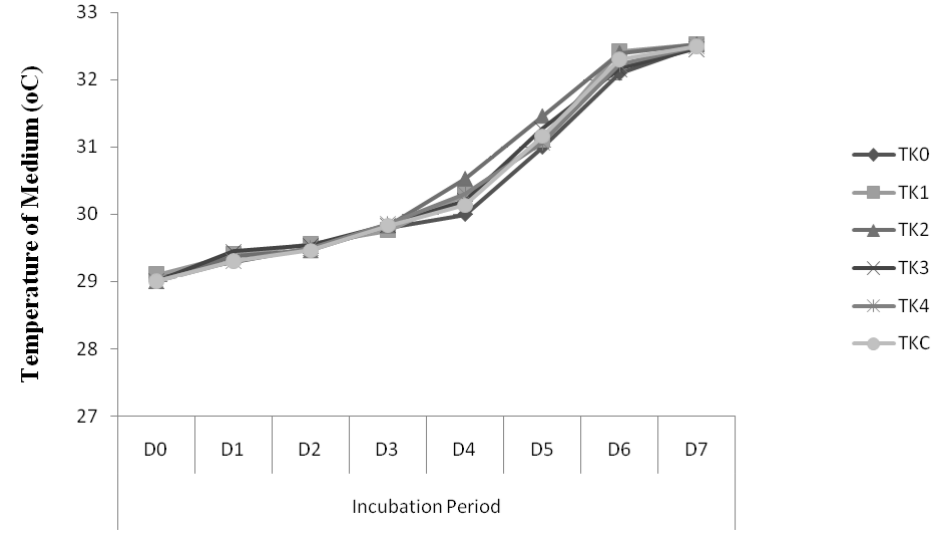

Fig 4 Temperature of the medium during the urease activity testing of bacterial isolates from Tritih Kulon, Cilacap, Central Java.

constant. In the wild when inorganic nitrogen is added to the environment, some will be transferred into organic bonds while others will be released in an inorganic form (Stanley 1995). David *et al.* (1999) stated that ammonia concentration in soil was 1.25 mg m^{-3} while according to Stevenson (1959) in Stanley (1995), the equivalent ammonia concentration per kg of soil was about 5 to 15 ppm.

The process of nitrogen immobilization involves combining NH_4^+ into amino acids. This depends on the growth of microbial cells. NH_4^+ ions are immobilized or accumulated in the medium depending on the need for nitrogen for microorganisms to grow. Bacteria need nitrogen to form cytoplasmic components and peptidoglycan cell walls. Cellular nitrogen levels in bacterial constant cells are at the ratio of C:N between 3:1 to 5:1 (Paul and Clark, 1989). Meanwhile, according to Elsas *et al.* (1997), the transformation of several pulses in the soil such as urea, depending on soil microbes, and these microbes produce urease to hydrolyze urease to ammonium carbamate.

The decrease in cell numbers was thought to be due to the results of metabolism in the form of ammonia which accumulated in the medium which was toxic to microorganisms. Like the statement of Brock and Madigan (1994), the excessive accumulation of ammonia as a result of metabolism was toxic to microorganisms. Normal bacterial growth was limited by the availability of nutrients and the results of toxic metabolism in the medium and environmental conditions such as pH and temperature. Temperature and medium pH at D7 were the highest temperature and pH during the urease activity test. This caused a decrease in growth speed and even microbial cell death. Another thing that caused a decrease in growth speed was the depletion of nutrients in the medium, especially the content of C and N. Yulinah *et al.* (1990) stated that the main factors that determine growth were nutrition. Growth or increase in cell mass indicates the presence of nutrients that can be consumed by cells. If nutrients are reduced, growth will decrease and if nutrients are depleted, the cell growth will stop. In this study, the reduced number of cells caused a decrease in urease activity because with fewer cells, the amount of enzyme produced was reduced.

Whilst, the decrease of pH value for TK3 might be caused by TK3 using glucose in the medium first to grow before using other compounds, so that the results of glucose metabolism in the form of acid resulted in a decrease of the medium pH and this fermentation of glucose caused an increase in temperature. Glucose is as simple as the simplest so it can be directly absorbed and most easily metabolized by bacteria. Hadiutomo (1990) stated that chemoorganotrophs especially bacteria would use simple carbohydrates in the form of glucose because these compounds are the simplest and most easily degraded compared to other compounds. In an anaerobic atmosphere, glucose will be fermented to produce acids that can reduce the pH value in the bacterial growth medium. Wijendra (1989) stated that urea underwent a breakdown and produced an amine group which when reacting with water would become NH_4OH which was alkaline so that during the treatment time there would be an increase in pH value.

The activity of each organism is affected by the pH of the environment to achieve the maximum activity of an enzyme that is required by an environment with a certain optimum pH range. The pH value of the medium directly affects microbial cellular activity and affects growth (Yulinah *et al.* 1990). According to Timothius (1993), pH affected the action of enzymes and changed cell permeability. Ions H^+ and OH^- ions are the most mobile ions, therefore a small change in the concentration of H^+ ions and OH^- ions can cause major changes in the cellular system of microorganisms. According to Schlegel and Schmidt (1994), urea decomposing bacteria were more suitable to live in environments with slightly alkaline pH (pH values 6 to 8) and if there was an increase in pH it would cause interference with the cell system which could cause cell death. The decrease in urease activity of TK3 since D5 could occur due to several factors, among others due to a decrease in the number of bacterial cells which was triggered by a fairly high increase in pH and ammonia accumulation in a medium that was already outside the tolerance limit for bacteria.

Temperature will affect all processes that occur in microorganisms and will limit their reproduction. High temperatures will denaturate proteins and microorganism enzyme systems, while low temperatures will deactivate all cellular systems (Hawker and Linton, 1979). Temperature is one of the factors that greatly affect growth, propagation and resistance of microorganisms. Girindra (1993) explained that most enzymes would experience decreased activity at 45 °C and would lose their activity altogether at 55 °C, but Lehninger (1990) argued that the loss of biological functions of enzymes was due to the influence of specific temperatures for each enzyme. Suriawiria stated (1986) that in the reaction of metabolism a chemical reaction would occur, then the increase in temperature to a certain value could

increase the reaction, but beyond that limit would cause the cessation of the cellular process. According to Sutedjo (1997), bacterial urease activity would reach a maximum at 30 °C. Bacteria having urease activity would experience decreased activity at temperatures below 30 °C and above 35 °C , however there would still be certain types of bacteria that were able to grow and move up to 45 °C.

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REFERENCES

Al Abany A, Soga K. 2013. Effect of chemical treatment used in MICP on engineering properties of cemented soils. Geotechnique. 63(4): 331-339.

Alexander M. 1977. Introduction to Soil Microbiology. John Wiley & Sons, New York.

Al-Thawadi S, Cord-Ruwisch R. 2012. Calcium carbonate crystal formation by ureolytic bacteria isolated from Australian soil and sludge. J. Adv. Sci. Eng. Res. 012(2): 12-26.

Atlas RM, Bartha R. 1998 Microbial Ecology, Fundamental and Application. The Benjamin/Cumming Publishing, Co., California.

Bachmeier KL, Williams AE, Warmington JR, Bang SS. 202. Urease activity in microbiologically-induced calcite precipitation. J. Biotechnol. 93(2): 171-181.

Brock TD, Madigan MT. 1994. Biology of Microorganism. Prentice Hall Inc., New Jersey.

Burbank MB, Weaver TJ, Williams BC, Crawford RL. 2012. Urease activity of ureolytic bacteria isolated from six soils in which calcite was precipitated by indigenous bacteria. Geomicrobiol. J. 29: 389-395.

Canakci H, Sidik W, Kilic IH. 2015. Effect of bacterial calcium carbonate precipitation on compressibility and shear strength of organic soil. Soils & Foundations 55(5): 1211-1221.

Celik F, Canakci H. 2011. Shear strength properties of organic soil with sand column. In: Proceedings of the International Balkan Conference on Challenges of Civic Engineering. EPOKA University Tirane, Albania.

David MS, Jeffrey JF, Peter GH, David AZ. 1999. Principle and Applications of Soil Microbiology. Prentice Hall Inc., New Jersey.

Dejong JT, Fritzges MB, Nusslein K. 2006. Microbially induced cementation to control sand response to undrained shear. J. Geotech. Geoenviron. Eng. 132:1381-1392.

Dejong JT, Mortensen BM, Martinez BC, Nelson DC. 2010. Bio-mediated soil improvement. Ecol. Eng. 36(2): 197-210.

Dejong JT. 2013. Biogeochemical processes and geotechnical applications: progress, opportunities and challenges. Geotechnique. 63(4): 287-301.

Elsas JD, Jack TT, Elisabeth, MHW. 1997. Modern Soil Microbiology. Marcel Dekker Inc., New York.

Fardiaz S. 1993. Analysis of Food Microbiology. Raja Grafindo Persada, Jakarta.

Gaudy AE, Gaudy ET. 1981. Microbiology for Environmental Scientist and Engineer. McGraw-Hill International, Auckland.

Girindra A. 1993. Biochemistry I. Gramedia Pustaka Utama, Jakarta.

Gonzalez-Munoz MT, Checkroum KB, Aboud A, Arias JM, Rodriquez-Gallego M. 2000. Bacterially induced Mg-calcite formation: Role of Mg^{2+} in development of crystal morphology. J. Sedimentary Res. 70: 559-564.

Hadiutomo. 1990. Basic Microbiology First Edition. Erlangga, Jakarta.

Hardiyanto A. 2002. Effect of Urea and KH_2PO_4 addition on biodegradation of Naphthalene (C10H8) using mix cultures of *Pseudomonas*, *Alcaligenes*, *Bacillus*, and *Aerobacter* (Thesis). Faculty of Biology, University of General Soedirman, Purwokerto.

Harley JP, Prescott LM. 2002. Laboratory Exercises in Microbiology. McGraw-Hill, Boston.

Hawker LE, Linton AH. 1979. Microorganism, Function, Form and Environmental. Edward Arnold Publisher Ltd., London.

Helmi FM, Elmitwalli HR, Elnagdy SM, El-Hagrassy AF. 2016. Calcium carbonate precipitation induced by ureolytic bacteria *Bacillus licheniformis*. Ecol. Eng. 90: 367-371.

Iswandi A. 1989. Practical Guidance for Soil Biology. Center of Inter-University, IPB Bogor.

Ivanov V, Chu J. 2008. Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. Rev. Environ. Sci. Biotechnol. 7: 139-153.

Jelisic N, Leppanen M. 2003. Mass stabilization of peat in road and railway. Construction Geotech. Special Publ. 1201: 552-561.

Karol RH. 2003. Chemical Grouting and Soil Stabilization. Marcel Dekker, New York: 558.

Lehninger. 1990. Principles of Biochemistry. First Edition, Erlangga, Jakarta.

Martinez BC, Dejong JT, Ginn TR, Mortensen BM, Barkouki TH, Hunt C, Tanyu B, Major D. 2013. Experimental optimization for microbial induced carbonate precipitation for soil improvement. ASCE J. Geotech. Geoenviron. Eng. 139(4): 587-598.

Misiolek K, Popielski P, Affek K. 2018. Preliminary research for identification of bacteria useful in microbially induced calcium carbonate precipitation. E3S Web of Conferences 44, 00115. https://doi.org/10.1051/e3sconf/20184400115.

Paul EA, Clark FE. 1989. Soil Microbiology and Biochemistry. Academic Press Inc., New York.

Payne JW. 1980. Microorganism and Nitrogen Source Transport and Utilization of Amino Acid, Peptides, Protein and Related Substrates. John Wiley & Sons Inc., New York.

Rusu C, Cheng X, Li M. 2011. Biological clogging in Tangshan sand columns under salt water intrusion by *Sporosarcina pasteurii*. Adv. Mater. Res. 250-253: 2040-2046.

Sanchez-Moral S, Canaveas JC, Laiz L, Saiz-Jimenez C, Bedoya J, Luque L. 2003. Biomediated precipitation of calcium carbonate metastable phases in hypogean environments: A short review. Geomicrobiol. J. 20(5): 491-500.

Schlegel HG, Schmidt K. 1994. General Microbiology. UGM Press, Yogyakarta.

Stanier RY, Edelberg EA, Ingraham J. 1984. Microbial World. Bharata Aksara, Jakarta.

Stanley AB. 1995. Soil Nutrient Bioavailability. John Wiley & Sons Inc., New York.

Suriawiria U. 1986. Water Microbiology. Alumni, Bandung.

Sutedjo MM. 1997. Soil Microbiology. PT Rineka Cipta, Jakarta.

Timothius KH. 1993. Basic Microbiology. Christian University Satya Wacan, Salatiga.

Van der Star WRL, Van Wijngaarden WK, Van Paassen LA, Van Baalen LR, Van Zwieten G. 2011. Stabilization of Gravel Deposits using Microorganism. Amsterdam: 85-90.

Van Paassen LA, Daza MC, Sorokin DY, Van der zon W, Van Loosdrecht MC. 2010. Potential soil reinforcement by biological denitrification. Ecol. Eng. 36: 168-175.

Van Paassen LA, Ghose, Ranajit, Van der Linden TJM, Van der Star WRL, Van Loosdrecht MCM, 2010. Quantifying biomediated ground improvement by ureolytic large-scale biogrout experiment. J. Geotech. Geoenviron. Eng. 136(12): 1721-1728.

Van Wijngaarden WK, Vermolen FJ, Van Meurs GAM, Vuik C. 2010. Modelling biogrout: a new ground improvement method based on microbial-induced carbonate precipitation. Trans. Porous Med. http://dxdoi.org/10.1007/s11242-010-9691-8.

Wijendra FX. 1989. Effect of nitrogen addition on the variations of color and pigmen number of Angkak coloring substrate (Thesis). Faculty of Biology, University of General Soedirman, Purwokerto.

Xu G, Li D, Jiao B, Li D, Yin Y, Lun L, Zhao Z, Li S. 2017. Biomineralization of a calcifying bacterium *Microbacterium* sp. GM-1. Electronic J. of Biotechnol. 25: 21-27.

Yuliani E. 2015. Study on effectivity of sandy soil structure improvement using bioclogging and biocementation methods. J. Watering Technique. 6(2): 167-174.

Yulinah E, Liang OB, Sukandar U, Kisman S. 1990. Growth and Ecology of Microbe. Teaching Material. PAU Biotechnology, IPB Bogor.