Review of Research on Extraction of Urease from Microorganisms and Plants

Meng Cui1,2,3, Suying Lü1,2 and Huihui Xiong1

1 College of Civil and Structure Engineering, Nanchang Institute of Technology, Nanchang, 330099, China
2 Jiangxi Provincial Engineering Research Center of the Special Reinforcement and Safety Monitoring Technology in Hydraulic & Civil Engineering, Nanchang, 330099, China
3 Jiangxi Province Key Laboratory of Hydraulic & Civil Engineering Infrastructure Security, Nanchang, 330099, China
*Email: 492980622@qq.com

Abstract. Microbially Induced Carbonate Precipitation (MICP) and Enzyme Induced Carbonate Precipitation (EICP) are the two most widely studied new technologies for soil solidification in recent years, both of which can be used to improve the engineering properties of soil. This paper briefly introduces the principle of MICP and EICP technology, and summarizes the application of different kinds of microorganisms and plants urease extracts. Finally, based on the existing research results, different kinds of microorganisms and plants urease extracts were compared from multiple perspectives.

1. Introduction
In recent years, microbially induced carbonate precipitation (MICP) technology uses the mineralization phenomenon occurring in microbial metabolism to generate calcium carbonate, and calcium carbonate has a certain cementation ability. Therefore, it is widely used in repairing concrete cracks [1-2], soil reinforcement [3-4], seepage prevention and leak plugging [5-9], wind-breaking and sand-fixing [10] in the field of engineering construction. Enzyme induced carbonate precipitation (EICP) technology is similar to microbially induced carbonate precipitation technology, and it is also applied to many practical engineering problems [11-13]. There are some differences in the selection of urease extracts between the two strains. The microbial urease extracts are mainly urease-producing bacteria such as Bacillus pasteurii and sporosarcina pasteurii, while the urease extracts from plants are mainly beans and seeds, such as soybean and watermelon seeds.

2. Study on the principle of MICP technology and application of microbial urease extract

2.1. Principle of MICP technology
MICP technology is one of the most common biologically induced mineralization phenomena in nature [14]. It has been suggested that the MICP reaction process usually include the following steps: (1)Formation of alkaline geochemical environment. (2)Formation of relative calcium carbonate saturated liquid. (3)Colloid formation of calcite. (4)Study on bacterial fixation of calcium ions by nucleating calcium carbonate. (5)Diagenetic transformation and crystallization of calcium minerals. (6)The sediments are formed into rocks under the action of cementation and consolidation. MICP is
typically achieved by three microbial metabolic processes: (1) Photosynthetic microorganism. (2) Sulfate reducing bacteria. (3) Microorganisms associated with the nitrogen cycle (ammonification, denitrification and urea hydrolysis). Among them, MICP is commonly used urea-lytic bacteria, which is easy to culture, and easy to control the reaction process of calcium carbonate induction. Simple operation and high efficiency. They can utilize urease produced by their metabolic ability to catalyze urea hydrolysis. \( \text{NH}_3 \) produced by dissolving in water increase the pH in the environment, and resulting in the reaction with \( \text{Ca}^{2+} \) and sufficient carbonate ions \( \text{CO}_3^{2-} \) to form calcium carbonate precipitates. The reaction equations are (1) - (6):

\[
\begin{align*}
\text{CO(NH)_2} + \text{H}_2\text{O} & \rightarrow \text{NH}_2\text{COOH} + 2\text{NH}_3 \\
\text{H}_2\text{O} + \text{NH}_2\text{COOH} & \rightarrow \text{NH}_3 + \text{H}_2\text{CO}_3 \\
2\text{NH}_3 + 2\text{H}_2\text{O} & \leftrightarrow 2\text{NH}_4 + 2\text{OH}^- \\
\text{H}^+ + 2\text{OH}^- + \text{H}_2\text{CO}_3 & \leftrightarrow 2\text{H}_2\text{O} + \text{CO}_3^{2-} \\
\text{Ca}^{2+} + \text{cell} & \rightarrow \text{cell} - \text{Ca}^{2+} \\
\text{cell} - \text{Ca}^{2+} + \text{CO}_3^{2-} & \rightarrow \text{cell} - \text{CaCO}_3 \downarrow
\end{align*}
\]

2.2. Application of microbial urease extract

Boquet et al. [15] found that bacteria induced calcium carbonate deposition in soil as early as 1973. Dhami et al. [16] isolated 6 urease bacteria from soil, and among them: B. megaterium [17], the urease activity was the highest. The second B. thuringiensis, B. cereus [18], L. fusiformis and B. subtilis [19]. At present, most urease producing bacteria are selected for urease extract in microbially induced carbonate precipitation technology. The types of urease producing bacteria are different, and the most commonly used urease type bacteria are Bacillus pasteurii and sporosarcina pasteurii. The application of different urea-soluting bacteria was studied as follows:

2.2.1. Bacillus pasteurii. Zhao [20] selected ATCC 11859 Bacillus pasteurii as the strain, and the study showed that when the bacterial concentration increased from 0.3 to 0.6, the compressive strength of soil increased by 200% as well. Chu et al. [5] measured through experiments that the bending strength and unconfined compressive strength of soil treated with Bacillus pasteurii solution were up to 256kPa and 932kPa respectively. Jing et al. [21] conducted tests under the same indoor test and consolidation conditions, and found that the shear strength and unconfined compressive strength of sand were inferior to those of medium sand, which were 76% and 46% respectively. Zhao et al. [22] selected Bacillus Pasteurii DSMZ from Germany as the strain and found that it is most appropriate to treat Marine silt under the conditions of bacterial solution OD600 of 1.2, and cementing solution concentration of 0.5mol/L, also and beside interval time of 12h, temperature of 20°C and multiple rounds of cementing as well. Wiktor et al. [2] selected Bacillus barbili liquid and calcium lactate as concrete self-healing agent to enhance mineral precipitation on the surface of cracks and make cracks heal with a maximum width of 0.46mm. Wang et al. [23] fixed Bacillus pasteurii and nutrient solution on the sample surface with AGAR as solid carrier. After 7 days of curing, the capillary water absorption rate was reduced to 15% compared with samples not treated by bacterial solution.

2.2.2. Sporosarcina pasteurii. Liang et al. [24] used the Dutch DSM Group’s sporosarcina pasteurii as the test strain and calcium nitrate as the calcium source, which showed high strength and good effect of solidified sand column. Han et al. [25] used the number ATCC 11859 as urease extract of sporosarcina pasteurii, and the treatment of bacterial liquid and three different calcium salts could improve the mechanical properties of the liquefiable sand, among which calcium acetate had the best effect. Jonkers [26] added calcium lactate to the bio-cement as a calcium ion source, and finally produced a cement based on the self-repair of sporosarcina pasteurii, which has high strength and tolerance.

2.2.3. Bacillus sphaericus. Demuyck et al. [27] used Bacillus sphaericus no. LMG 22557 as urease extract, and treated mortar samples with bacterial liquid and calcium source, their water absorption
rate was nearly five times less than that of untreated samples as well. Wang et al. [28] compared the cracking samples healed by ammonia ester immobilized bacteria with silica gel immobilized bacteria using Bacillus sphaericus as strain, and found that the cracking samples healed by ammonia ester immobilized bacteria had higher healing efficiency, i.e. 60% strength recovery rate and low permeability of $10^{-10}$ to $10^{-11}$ m/s. Yuan et al. [29] used diatomite loaded with Bacillus sphaericus to repair concrete cracks, after soaking in 28-day nutrient solution for four groups of cement net slurry, and the incorporation of microorganisms improved the compactness of cement net slurry and reduced the water absorption rate compared with specimens without microorganisms.

2.2.4. Other urease bacteria. Dejong et al. [17] used Bacillus megacephoris as the strain, and the permeability of the soil was reduced by using the bacterial solution. Khaliq et al. [19] directly added Bacillus subtilis into concrete to produce crack healing in concrete, and the results showed that the bacteria immobilized in the graphite nanosheet had the best effect on the presplit samples at 3 and 7 days, while the bacteria immobilized in the lightweight aggregates had the best effect on the presplit samples at 14 and 28 days. Kumari et al. [18] used Bacillus cereus isolated from the Yangtze River near Chongming County, Shanghai, China, to repair the soil contaminated by heavy metals, and the content of Cr(VI) in the soil after restoration was low, also and beside only 0.34-0.65 mg/kg.

3. Study on the principle of EICP technology and application of plant urease extract

3.1. Principle of EICP technology

Enzyme Induced Carbonate Precipitation (EICP) uses free urease to catalyze urea hydrolysis. Urease is a catalyst for hydrolysis of urea. Urea hydrolysis refers to the chemical reaction of urea hydrolysis under the condition of aqueous solution, and the reaction products form ammonia ion and carbonate ion. The carbonate ions formed by the reactants react with positively charged calcium ions to form calcium carbonate deposits.

3.2. Application of plant urease extract

In recent years, the use of plant-derived urease has been considered as an alternative method for urea-solting bacteria or marketing pure enzymes [30]. The most commonly used plant extract is soybean. Here are the applied studies of different beans and watermelon seeds:

3.2.1. Jack Bean. Almajed et al. [31] used Jack bean as urease extract in three test methods based on EICP technology, and found that the addition of 4g/L skim milk powder made UCS of Ottawa sand reach unprecedented strength. Park et al. [32] found in the experiment that after curing for 3 days, also and beside the unconfined compressive strength of the sand treated with urease extracted from Jack bean increased by 10 times to 317kpa as the urea content increased continuously. Moghal et al. [33] selected Jack bean as urease extract, and found through experiments that 1M urea, and 0.67M calcium chloride, and 3g/L plant urease along with 4g/L skim milk powder were used to treat heavy metals in soil, and the treatment effect of heavy metals in soil was the best.

3.2.2. Soybean. Lee et al. [34] used soybean as urease extract and found that when urea-calcium chloride concentration was less than 1.5M, curing time had no significant effect on UCS enhancement, but when urea-calcium chloride concentration was 3M, the strength would increase with the increase of curing time. Yuan et al. [35] extracted urease from soybean based on traditional EICP technology and improved it by adding three different organic materials, and the improved soil strength was about 4 times higher than that of the original soil and 25-33% higher than that of the EICP method without organic materials. Gao et al. [36] used soybean as urease extract, and through the triaxial consolidation undrained test, the mechanical properties of the treated silt were significantly improved compared with that of the untreated silt.

3.2.3. Watermelon Seeds. Dilrukshi et al. [37] used watermelon seeds as urease extract in this paper,
and the study showed that after adding 3.912U/mL urease and 0.7mol/L calcium chloride-urea solution into sand and curing for 14 days, UCS was 3Mpa, when 0.877U/mL urease and 0.3mol/L and 0.50mol/L calcium chloride-urea solution were added to the sand. UCS was 1.5-2.0Mpa at 25℃. The strength of sand can be improved by changing different parameters.Chen et al. [38] studied the redistribution of urease in beans and melon seeds common in China, and watermelon seeds also had high pulse enzyme activity, among which Xincheng No. 1 watermelon seeds had higher pulse enzyme activity than Sumi No. 1. Imran et al. [39] used watermelon seeds as urease extract and added urease and cementing solution to sand samples, and the unconfined compressive strength of sand increased significantly with the promotion of concentrated solution.Javadi et al. [40] extracted urease solution from watermelon seeds, which could induce soil calcium carbonate content to be 64% of the theoretical maximum content.

4. Conclusion
In recent years, the application of different kinds of microorganisms and plant urease extracts has solved a large number of practical engineering problems. On the basis of existing results, the different kinds of urease extracts are compared through multi-angle analysis. (1) From the perspective of PH environment: Bacillus pasteurii and sporosarcina pasteurii than other bacteria can survive in high alkaline environment more. (2) Urease activity: B.megaterium had the highest urease activity in microbial urease extract, followed by B.thuringiensis, B.cereus, L. fusiformis and B. subtilis. Bacillus Pasteuris had the strongest urease activity among known species. The urease activity of plant urease extract was the highest in jack bean and soybean. The urease activity of sporosarcina pasteurii was 14 times of that of jack bean, and 100 times of that of soybean. (3) Urease production: The urease production of sporosarcina pasteurii was the most in microorganism, and the urease production of jack bean was the most in plant, and the urease production of the whole species was more than that of plant. (4) Time and economic cost: microorganisms produce more urease, and selecting microbial extracts can save time and cost compared with plant urease extracts when large-scale urease solution is needed. (5) Soil curing effect: MICP technology has better curing effect, while EICP technology needs to be mixed with organic matter to achieve ideal curing effect.

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