Experimental study on enzyme-induced calcium carbonate precipitation at high temperatures

Étude expérimentale sur les précipitations de carbonate de calcium induites par les enzymesà haute température

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ABSTRACT: The research of Microbially Induced Carbonate Precipitation (MICP) is mostly carried out at normal temperature. However there are still some areas with higher temperature, such as oil fields, mining areas, hot springs and others. The realization of reinforcement at high temperatures is a new breakthrough for biosolidification technology. In this article, the cheap plant urease was selected as the object to study its feasibility of sand consolidation under high temperatures. The urease was extracted from soybeans to study the effect of high temperature conditions on the tolerance of soybean urease was studied. In addition, the yield of calcium carbonate was studied using precipitation experiments. The results showed that the urease activity would increase with the improvement of temperature, and the urease activity would be gradually lost when the temperature is higher than 65℃. Furthermore, the precipitation was also closed related to temperature. Increasing the amount of urease at the inactivated temperature can ensure the precipitation. In this paper, the effect of high temperature on the EICP reaction was studied, which laid the foundation for the application research of EICP curing technology under high temperatures.

RÉSUMÉ : La recherche de la Précipitation de Carbonate Induite Microbiennement (MICP) est principalement réalisée à température normale. Cependant, il existe encore des zones à température plus élevée, telles que les champs pétrolifères, les zones minières, les sources chaudes et autres. La réalisation de renfort à haute température est une nouvelle avancée pour la technologie de biosolidification. Dans cet article, l'uréase végétale bon marché a été choisie comme objet pour étudier sa faisabilité de consolidation du sable sous des températures élevées. L'uréase a été extraite du soja pour étudier l'effet des conditions de température élevée sur la tolérance de l'uréase de soja. De plus, le rendement en carbonate de calcium a été étudié en utilisant des expériences de précipitation. Les résultats ont montré que l'activité uréase augmenterait avec l'amélioration de la température et que l'activité uréase serait progressivement perdue lorsque la température est supérieure à 65 ℃. De plus, les précipitations étaient également fermées en fonction de la température. L'augmentation de la quantité d'uréase à la température inactivée peut assurer la précipitation. Dans cet article, l'effet de la température élevée sur la réaction EICP a été étudié, ce qui a jeté les bases de la recherche d'application de la technologie de durcissement EICP à haute température.

KEYWORDS: Enzyme induced carbonate precipitation, soybean urease, high temperature.

1 INTRODUCTION

Microbially Induced Carbonate Precipitation (MICP) is a popular technique which reinforce soil with microorganisms that can produce urease in nature. In this technique, urea can be decompose into to CO_3^{2-} accelerately by urease, and the cementitious and filling calcium carbonate can be produced by the combination of CO_3^{2-} and Ca^{2+} in the surrounding environment which play the effect of strengthening the soil (Chu & Ivanov & He 2011, Sun & Miao & Tong 2018). MICP is of latent applied value on sand reinforcement (Zarghaam & Mijo & Frank 2019), foundation treatment (Van Paassen 2009), concrete repair (Jonkers & Thijssen & Muyzer 2019), seepage blocking (Cuthbert & McMillan & Handleysidhu 2013) and other fields. The core of MICP technology is the urease produced by microbial metabolism. However the cultivation of bacteria is often restricted by many environmental factors, and it also induces additional costs. Therefore, urease is directly used to replace the bacteria in MICP technology for solidification research by some scholars. The technology is called Enzyme induced carbonate precipitation (EICP) (Tung & James & Bora 2018, Abdullah & Hamed & Edward 2018).

The growth of microorganisms and the synthesis of urease will be influenced by the variety of environment and then it will affect the curing effect of MICP (Nemati & Greene & Voordouw 2004, Whiffin & Van Paassen & Harkes 2007). Nowadays, the research of MICP is mostly carried out at room temperature or low temperature (Sun & Miao & Tong 2019, Sun & Miao & Wu 2019), but there are still some areas with higher temperature in reality, such as oil extraction areas, mining areas, hot springs, etc. Compared with microorganisms, enzymes are more adaptable to temperature changes, and still have a certain activity at relatively high temperatures, so that biomineralization has the potential in high temperature environments. Therefore, the urease extracted from soybean was chosen to study the influence of high temperature environment on urease activity, tolerance and calcium carbonate yield, which provided guidance for the application of EICP in high temperature environment.

2 MATERIALS AND METHODS

In the paper, the urease was extracted from soybean. Soybean solution (100g/L) was prepared and placed in the refrigerator. 24 hours later, soybean solution was centrifuged at a speed of 3000 r/min for 15min. And the supernatant was used as the urease solution to conduct experiments.

2.1 *Measurement of urease activity*

The urease activity could be obtained indirectly by measuring conductivity of solution per minute, as the amount of urea hydrolyzed was in proportional to the change on the conductivity of solution (Whiffin 2004). With 3ml of urease solution combining with 27ml of urea solution (1mol/L), the change on conductivity in 10min was monitored via electrical conductivity meter. The average change on conductivity per minute (mS/min) was calculated, which was defined as urease activity in the paper.

In order to explore the tolerance of soybean urease, urease activity was measured by heating at different time and different environmental temperatures. The soybean urease was heated in a temperature environment of 45℃—85℃ within 3 hours, and its urease was measured at different time.

2.2 Precipitation experiments

The cementitious calcium carbonate induced by urease was the key to the reinforcement of EICP. Therefore, precipitation experiments at different temperatures were carried out to study the effect of different temperatures on the yield of calcium carbonate.

Supernatant of soybean solution (100g/L) was diluted 5 times with water, one volume of solution was diluted with four volumes of water, and urease solution with a concentration of 20% could be obtained. Similarly, 50% and 100% were obtained to experiment. In precipitation experiments, 15ml uraese solution and 15ml gelling solution (mixture of 0.67 M calcium acetate and 1.0 M urea solution (Abdullah & Hamed & Edward 2018)) were mixed resulting in a total solution volume of 30mL, and placed in a temperature environment of 25℃, 45℃, 65℃ and 85℃ for the experiment.

After the precipitation reaction, the precipitate was washed with deionized water and the mass of $CaCO_3$ were obtained indirectly by the carbon dioxide gas method (Debendra & Hideaki & Naoki 2015). The schematic representation of the experiment was shown in Fig. 1.

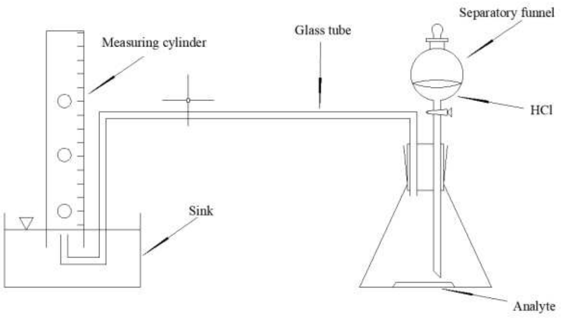

Figure 1. The testing device of precipitation yield

CaCO3 neutralizes acids to give the salts of calcium and CO2 gas. The volume of the evolved CO2 gas has a direct relation to the amount of CaCO3 consumed during the neutralization reaction. The mass of the calcite can be quantified by measuring the volume of the evolved gas. The mass of calcium carbonate produced is calculated by the volume of carbon dioxide produced. The calculation formula is shown in Eq. (1).

$$pV = nRT \tag{1}$$

Where V is volume of gas, p is atmospheric pressure, T is Kelvin temperature, n is amount of substance, R is constant.

3 RESULT

3.1 *The effect of temperature on tolerance of urease*

The activity of soybean urease heated in a temperature environment of 45℃—85℃ within 3 hours was shown in Fig. 2.

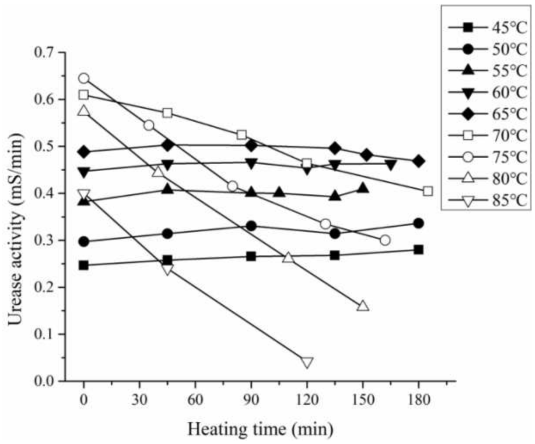

Figure 2. Time-dependent changes of urease activity at different temperatures

From Fig. 2, there was almost no change in the activity of urease with the extension of heating time in the range of 45℃-65℃, and the activity increased with the temperature developing. In addition, the urease activity gradually decreased with the increase of heating time when the temperature was higher than 65℃, and the higher temperature, the more obvious downward trend.

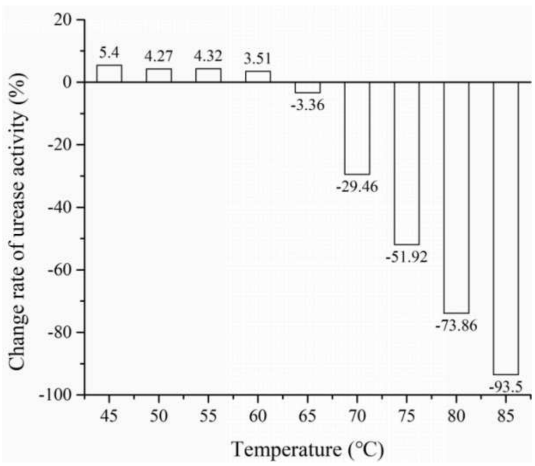

Figure 3. Activity change rate of soybean urease after heating at different temperatures for 2 hours

From Fig. 3, the activity of soybean urease hardly changed after heating for two hours when the temperature was not higher than 65℃. However, the tolerance of soybean urease decreased significantly when the temperature was high than 65℃. And as the temperature increased, the activity decreased more.

It cloud be inferred that, soybean urease could maintain activity in an environment where the temperature was not higher than 65℃. And its activity would gradually lost, but it also needed to take a certain time, when the temperature was higher than 65℃. Therefore, it was possible to use soybean urease to perform EICP experiments in high temperature environments.

3.2 Precipitation experiments at different temperatures

The amount of calcium carbonate produced was measured to investigate the effect of temperature on the yield of calcium carbonate. Precipitation experiments at different temperature was shown in Fig. 4.

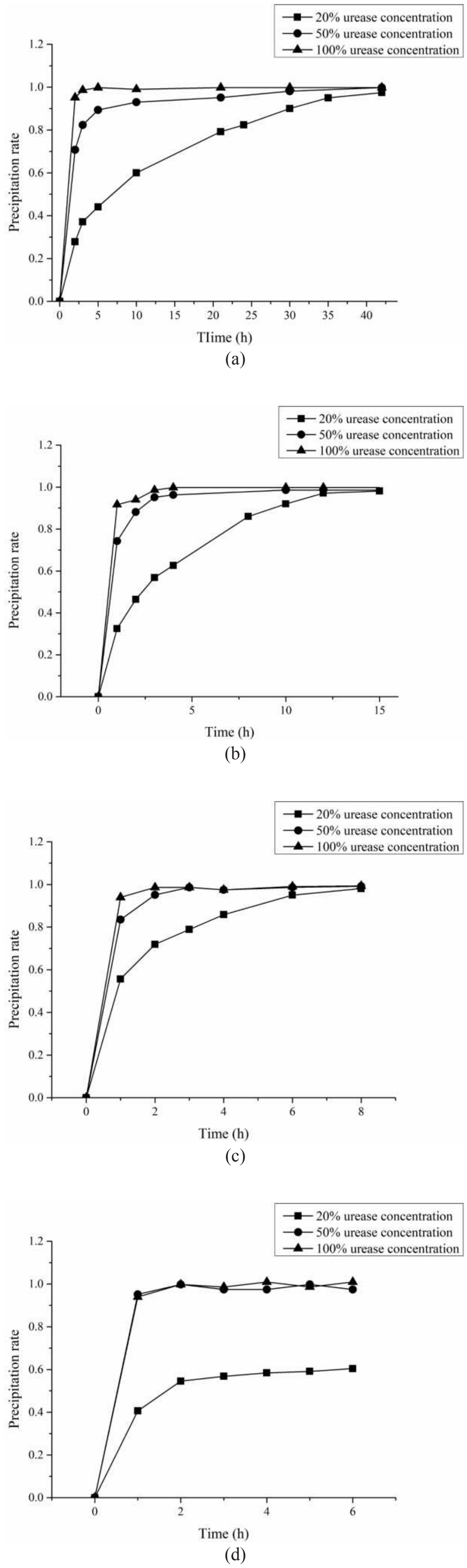

Figure 4. Precipitation experiments at different temperatures (a) 25℃, (b) 45℃, (c) 65℃, (d) 85℃.

As seen in Fig. 4, when the temperature is 25℃, 45℃, 65℃ the concentration of urease had no effect on the final production of precipitation, but as the concentration of urease increases, the rate of formation of precipitation also increased. It was mainly because it had little effect on the tolerance of urease when the temperature was below 65℃, the yield of precipitation was not affected. At the meanwhile, the reaction rate would be accelerated with the increase in the concentration of urease.

At 85°C, the final yield of precipitation with a urease concentration of 20% was about 0.6, while the yields of 50% and 100% were close to 1. This was because the gradual loss of urease activity in a high temperature environment of 85℃. The experimental group with a concentration of 20% had a slower reaction rate due to the low content of urease. The urease activity was completely lost and the reaction was stopped before the urea was completely hydrolyzed. Therefore, the amount of precipitation produced increased after a period of time and then stopped. Due to the increase of urease concentration, the reaction rate of the experimental groups with the concentration of 50% and 100% increased. The urea was completely hydrolyzed before the urease activity was completely lost, so the final rate of precipitation production was not affected.

In summary, the reaction rate of EICP might be influenced by temperature and concentration of urease. The rate of precipitation generation could be controlled by adjusting the concentration of urease when the EICP curing reaction was carried out in a high temperature environment. It was an effective method to achieve and optimize EICP in a high temperature environment.

4 CONCLUSIONS

In this section the most significant results of your work should be summarized.

In this paper, soybean urease was used in EICP research the under high temperature environment. The conclusions are as follows：

(1) The tolerance of soybean urease will be influenced by the high temperature environment. It is manifested that the urease activity will gradually lose under the condition of high temperature when the temperature is higher than 65°C. And with the increase of temperature, the loss of urease activity is more and the inactivation time is shorter.

(2) Within a certain temperature range (below 65°C), the increase in temperature can promote the formation of calcium carbonate. An excessively high temperature environment will affect the final yield of precipitation when the urease concentration is low. But this problem can be improved by appropriately increasing the urease concentration.

5 ACKNOWLEDGEMENTS

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