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> Friday, April 29, 2022 Roberts Hall 312 2:00–3:00 p.m.

Improving pH and temperature stability of urease for ureolysisinduced calcium carbonate precipitation

Ureolysis-induced calcium carbonate (CaCO3) precipitation (UICP) is a promising technology that takes advantage of urea hydrolysis. During UICP, the enzyme urease hydrolyzes urea, and in the presence of calcium (Ca2+), calcium carbonate can precipitate. This process is also known as biomineralization, and the enzyme urease can be found in several bacterial and plant cells. UICP can solve engineering challenges and potentially replace traditional Portland cement in certain applications.

To develop well founded biomineralization for engineering applications such as sealing leakage pathways around wells for CO2 sequestration, urease must be active, but many biotechnological applications are limited by physicochemical conditions (temperature, pH, toxic compounds, etc.). While utilizing biomineralization for applications in the subsurface, urease may be challenged by elevated temperatures and pH values that are significantly different from the pH optima of urease.

Hence, *Sporosarcina pasteurii* and jack bean meal (JBM) ureolytic activities were investigated while simulating potential environmental stresses such as high temperature and extreme pH conditions. Mathematical models were developed to determine reaction and inactivation constants at elevated temperature conditions.

Urease was extracted from *S. pasteurii* cells by sonication, and its activity was assessed by exposing the enzyme to temperatures between 50-80 °C. *S. pasteurii* urease exhibited ureolytic activity and thermal inactivation similar to JBM urease. Both *S. pasteurii*- and JBM-ureases were found to have reduced stability at elevated temperatures (> 50 °C) and pH values further away from circumneutral pH conditions, i.e., at pH < 5 and pH > 9. A preliminary analysis revealed that the cost of producing bacterial ureases can be lower than for JBM urease, and that costs can be reduced further using alternative nutrient sources for bacterial growth.

Improvements in urease stability relative to temperature and pH exposure were also achieved. For instance, while suspended urease did not demonstrate any residual activity after exposure to pH 4.1 at 60 °C for one-hour, immobilized urease remained active. These studies suggest that UICP technology may be used in a broad range of applications.

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