Enzyme Induced Carbonate Precipitation: Biocemented Sand with Moderate Strength and Reduced Cure Time

A Major Qualifying Project Submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE
in partial fulfillment of the requirements for the Degree of Bachelor of Science

By
Edward Raymond Jacoby V

May, 2024

Advised By
Dr. Nima Rahbar

Background:

Soil column stabilization through calcium carbonate precipitation has been facilitated through the use of microbial and enzymatic accelerators. In the solution urea catalyzes the hydrolysis of carbonate ions of which calcium ions bind with to form the calcium carbonate precipitate. The precipitate acts similarly to cement as a binding agent to increase the stiffness and strength of the soil.

The chemical components involved in the calcium carbonate precipitation process include calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), tris (hydroxymethyl) aminomethane buffer, non-fat milk powder, and the carbonic anhydrase enzyme mixed together in deionized water with access to carbon dioxide (CO_2). The overall process uses free Ca^{2+} and Cl^- ions from dissolved calcium chloride dihydrate, and combines them with HCO_3^{2-} and H^+ ions from the carbonic anhydrase facilitated interaction of H_2O and CO_2. The combination results in products of $CaCO_3$ and HCl; the inclusion of tris buffer is necessary because of this HCl production, which raises the pH and could potentially kill the enzyme.

Study Rationale:

The project aimed to build upon the current research landscape by utilizing the carbonic anhydrase enzyme to facilitate the precipitation of calcium carbonate for soil column stabilization in place of microbes, and urea with urease enzymes. Secondary goals of this project included a reduced curing time when compared to opposing enzymes and microbes, and to produce a competitive compressive strength for the material.

It was hypothesized that in replacing urea and urease enzymes with carbonic anhydrase the cure time could be reduced while compressive strength and calcium carbonate content remain relatively competitive in the current research landscape. Reduced cure times allow for a versatile and applicable product for commercial use.

Materials and Methods:

EICP Treatment Solutions:

EICP solutions were prepared individually in batches of 250 mL, with 1 gram (4 g/L) of non-fat milk powder was included in each batch. Solutions consisted of deionized (DI) water, and a variable amount of calcium chloride dihydrate ($CaCl_2 \cdot H_2O$), carbonic anhydrase enzyme, and tris buffer.

Sample A utilized carbonic anhydrase at a concentration of 33.3mg/L, calcium chloride dihydrate concentration at 1.97 M and the tris buffer at 12.11 g/L. This sample deliberately excluded the non-fat milk powder from the reactants in order to test the impact when used in conjunction with carbonic anhydrase instead of urea and urease enzymes.

Sample B also utilized carbonic anhydrase at a concentration of 33.3mg/L, calcium chloride dihydrate at a concentration of 1.97 M and the tris buffer at 12.11 g/L. This sample included non-fat milk powder to allow strength testing comparisons to sample 4A. Solution 4B was also the solution used during the creation of flexural beam samples.

Soil Treatment:

Sand particles were used as a soil aggregate with a diameter of 75 micrometers, this diameter was chosen to maximize the possible surface area for calcium carbonate precipitation. Samples were created using 350 grams of 75 um sand and 100 mL of their respective solutions. Following mixing, each sample was immediately loaded into a 2 inch (5.08 cm) diameter, 4 inch (10.16 cm) length cylinder in a series of lifts. Between lifts the mixtures were gently tamped and lifts concluded when the soil reached a final height of 4 inches. They were then brought to cure in a $>95\%$ humidity curing room for 48 hours, after which they were oven dried at 60 degrees celsius for four weeks in a modified cylinder mold depicted in Figure 6 found in the appendix. Demolded column samples then received unconfined compressive strength testing

Flexural strength samples were created using molds of 110 mm length by 25 mm width by 12 mm depth. They were constructed by thoroughly mixing 350 grams of 75 um sand with 100 mL of solution. Flexure samples were loaded in a manner identical to the column samples. They then cured in a concrete curing room for 48 hours until being moved to oven dry at 60 degrees celsius for one week. Demolded samples were then subjected to flexural strength testing.

EICP Solution	Test No.	Peak Strength (N, MPa)	Mean Strength (N, MPa)	$CaCO_3$ %	Mean $CaCO_3$ %
Sample A	1 (1.1)	656.6, 0.47	397.6, 0.28	3.0%	1.6%
	1 (1.2)	235.9, 0.16		0.2%	
	2	300.2, 0.20		*	
	3	N/A		0	
Sample B	1	612.4, 0.44	767.0, 0.41	8.2%	5.3%
	2	428.5, 0.21		3.2%	
	3	1200, 0.59		4.5%	
	4	N/A		0	

** Compromised sample*

Table 1. Unconfined Compressive Strength and $CaCO_3$ Content Results

EICP Solution	Test No.	Peak Strength (N, kPa)	Mean Strength (N, KpA)	$CaCO_3$ %	Mean $CaCO_3$ %
Sample B	1	4.1, 33.8	2.82, 28.3	3.2%	2.3%
	2	1.6, 22.7		1.8%	
	3	N/A		2.0%	

Table 2. Flexural Strength Beam Testing and $CaCO_3$ Results

Carbonate Content Measurement:

The percentage carbonate content of each sample was measured through acid digestion. Approximately 15% - 30% of each sample was taken from failed samples and submerged in a 4M hydrochloric acid solution. The amount of mass lost after submerging, rinsing, and oven drying is equal to the assumed mass of calcium carbonate. From this value the percentage calcium carbonate content can was calculated, seen in Tables 1 and 2.

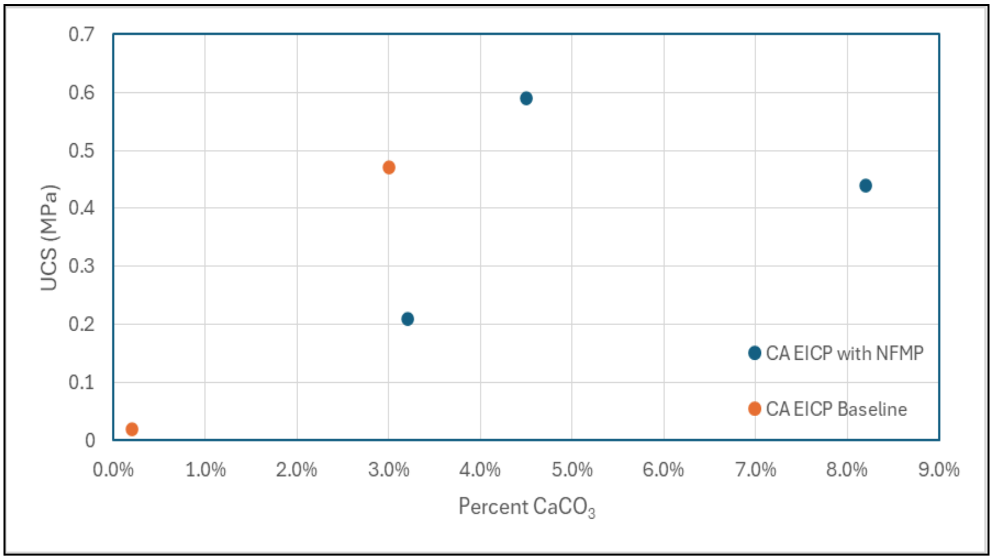

Figure 1. Percent $CaCO_3$ vs Unconfined Compressive Strength

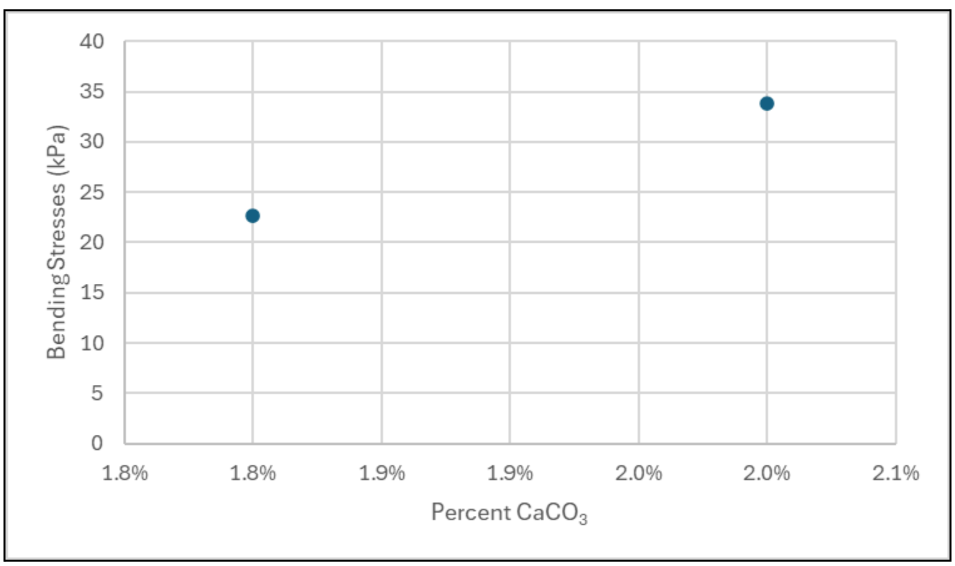

Figure 2. Percent $CaCO_3$ vs Bending Stress

Results and Analysis:

Following soil treatment samples experienced both measurable strength and stiffness changes. Immediate descriptions following demolding of the sample were relatively similar between the top ⅓ and bottom ⅔ of the cylinder. However, while the sample looked visibly similar throughout the entire length the strength was measurably higher in the top layer of the column. Samples A & B had noticeably different unconfined compressive strength testing results with each sample having one compromised cylinder. Solution B was then chosen to be used during the flexural strength sample creation.

Baseline samples, sample A, achieved an average unconfined compressive strength of 0.28 MPa and a maximum strength of 0.47 MPa. Whereas samples created with non-fat milk, powder, sample B, achieved an average unconfined compressive strength of 0.41 MPa, and a maximum strength of 0.59 MPa. Beam samples utilized the mixture including non-fat milk powder and obtained an average flexural strength of 28.3 kPa. These results are visible in depth in both Tables 1 and 2, as well as Figures 1 and 2.

Carbonate precipitation through carbonic anhydrase was similar to various other enzyme induced carbonate precipitation studies, and had lower carbonate content percentages than microbe induced carbonate precipitation. The precipitation percentage range and strength are outlined in Figure 3, visible by the blue rectangle labeled "Carbonic anhydrase enzyme, Single Treatment". By visualizing the datasets the studies can be compared quickly and accurately. In this figure, from (Almajed, A. Et al, 2019), the CaCO3 percentage of various EICP and MICP studies are graphed against their obtained compressive strength values. The strength values from the carbonic anhydrase based EICP reactions lie in the same range as most other EICP studies found in the figure, thus both the strength and CaCO3 results have been labeled moderate in comparison the research landscape.

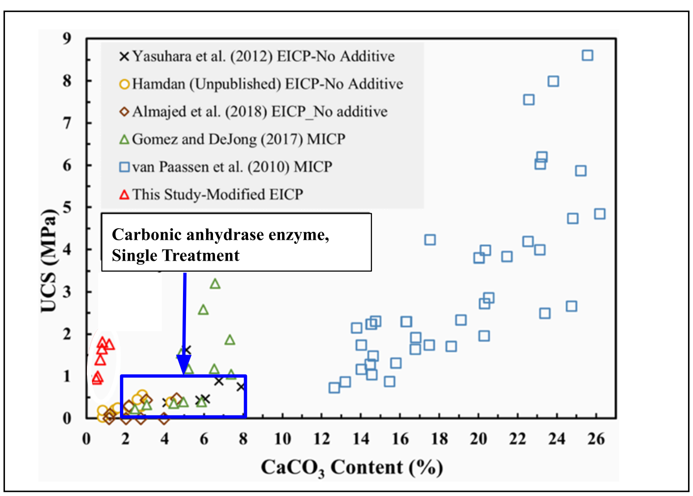

Figure 3. Carbonic Anhydrase results overlaid on a graph from Enzyme Induced Biocementated Sand with High Strength at Low Carbonate Content (Almajed, A. Et al, 2019)

Conclusion

The carbonic anhydrase enzyme is effective in facilitating the precipitation of calcium carbonate for the purpose of an EICP reaction. Through the enzymatic process samples were properly cemented together in a manner similar to comparable studies. These samples were then able withstand moderate strengths and stresses in both compression and flexure before failure. The resulting amounts of carbonate relative to the mass of the sample were also moderate in comparison to the research landscape.

The performance of carbonic anhydrase as an EICP enzyme was on par with the original hypothesis. After balancing the chemical formula to the desired ratio and generating the required concentrations the final samples resulted in competitive strengths, stresses, and carbonate content. These samples had maximum stress of 0.59 megapascals and an average of 0.41 megapascals in compression, and a maximum 33.8 kilopascals and an average of 28.3 kilopascals in flexure. Though with further research and testing it is believed that this value can be further increased.

Acknowledgements

I would like to express sincere gratitude to my advisor and professor Dr. Nima Rahbar. His knowledge, expertise, and constant encouragement helped propel me towards the completion of this capstone project. I would also like to extend heavy gratitude to Affiliate Research Scientist Shuai Wang for being generous with his time, his past experiences, and his knowledge of Enzymatic Construction Materials to guide me toward the path of success. Additionally, I would like to thank Sara Heidarnezhad, Pardis Pourhaji, Dalton Vassallo, and Compaore Alassane for their assistance and willingness to provide guidance. Finally, I would like to thank lab manager Russel Lang for his approachability and desire to help me succeed. Without the help from each of these sources the completion of this project would not have been possible.

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Appendix

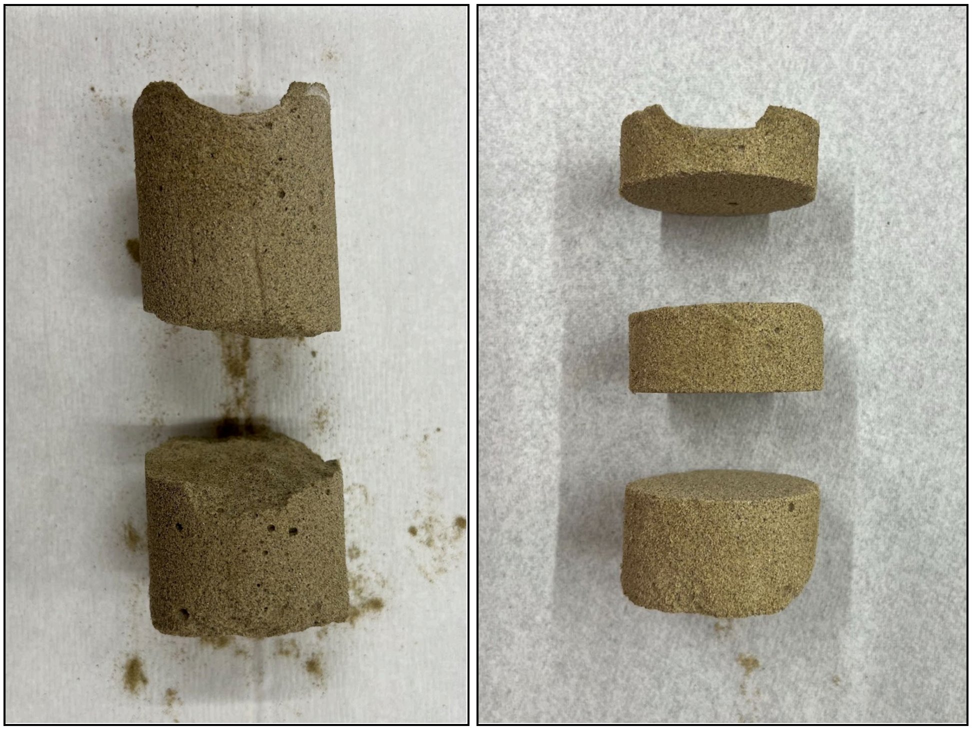

Figure 4. Baseline Soil Column Pre Compression Test

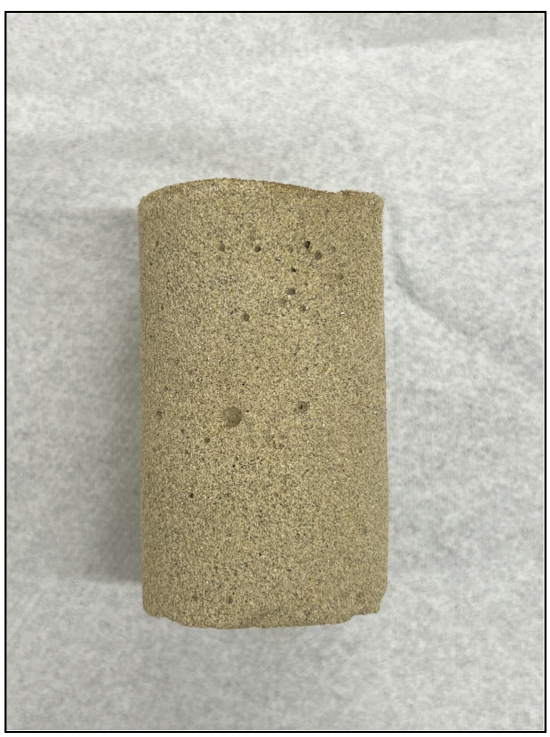

Figure 5. Soil Column with Non-fat Milk Powder Pre Compression Test

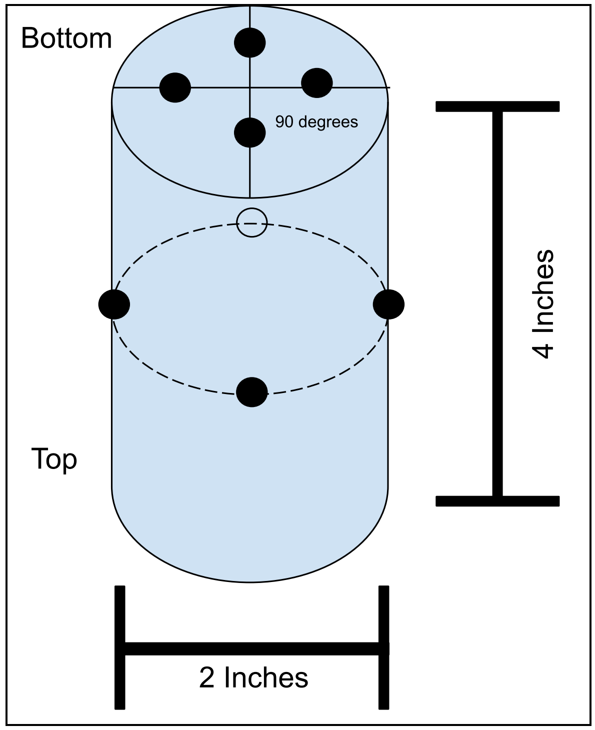

Figure 6. Model of the Modified Cylindrical Mold