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Cross-scale correlations in biocement test samples

Rhett L. Martineau[1], Ph.D., Brandon M. Bradow[2], Matthew J. Tuttle[2], Ph.D., Michael S. Carter[1], Ph.D., Josh A. Mancini[2], Ph.D., and Maneesh K. Gupta[1], Ph.D

[1]Biomaterials Branch, Materials and Manufacturing Directorate, The Air Force Research Laboratory, Wright-Patterson Air Force Base, Ohio, USA; E-mail: rhett.martineau.1@us.af.mil
[2]Biological and Nanoscale Technologies Division, BlueHalo, Dayton, Ohio, USA

ABSTRACT

Cementation by microbes through microbially-induced calcium carbonate precipitation (MICP) shows promise for the construction of cemented structures in an environmentally-friendly, non-energy-intensive manner. Due to the nature of the chemistry, in our case MICP through the ureolytic pathway, biocements are potentially formed in undisturbed soils, creating new paradigms for manufacturing with cement. However, it is not trivial to translate lab-scale experimental results to success in the field or at larger scales. In this work, the results of testing laboratory samples built into multiple form factors are compared with results obtained from small-scale field experiments. Of our tested form factors, we place the most confidence in a laboratory specimen size that is 24 in. deep with a 2.5 in. x 2.5 in. cross section. In this form factor, unconfined compressive strength and calcium carbonate mass fractions can be measured as a function of depth and correlate with laboratory California bearing ratio (CBR) measurements. However, despite a good correlation with laboratory CBR measurements, the laboratory results do not predict cementation depth or hardness in the open flow conditions of small-scale (3 ft. x 3 ft.) field samples.

INTRODUCTION

A large number of publications detail experimental efforts to understand and tame biocementation processes (Dhami et al. 2016; Marzin et al. 2020; Naveed et al. 2020; Omoregie et al. 2017, 2019; Onal Okyay and Frigi Rodrigues 2014). Specific studies related to engineering the soil column cover the gamut of topics ranging from studying the impact of sequential additions of alternating bacterial suspensions and cementation solution(Cheng and Cord-Ruwisch 2012), attempts at using a single combined biomass and cementation solution by starting with a low pH mixture(Cheng et al. 2019), observing the impact on cemented sample uniformity as a function of urease activity within selections of *S. pasteurii* mutants(Konstantinou et al. 2021; Zhao et al. 2019), and studies conducted in field locations where civil engineering techniques were applied to studying the resultant material properties of cemented structures in the field and efficiency of reagent utilization(Gomez et al. 2015). Studies addressing key microbiological aspects of *S. pasteurii* as they relate to the biocementation process are fewer than the civil engineering-focused studies but

are becoming more commonplace(Castro-Alonso et al. 2019; Connolly et al. 2013; Kim and Youn 2016; Ma et al. 2020; Skorupa et al. 2019).

Overview of form factors. Different questions addressing different aspects of the biocementation process may require experiments conducted a different scales. Samples created in form factors that are directly applicable to existing civil engineering standards are highly desirable. Generally speaking, constructing test specimens and properly testing them is costly, and high-throughput methods are desirable but are not described in the literature. The downsides of high-throughput methods include the possibility that the samples are too small to enable the proper characterization of the resultant material properties. Also, the manner in which the samples are constructed in a high-throughput or laboratory setting may not be reflective of the conditions surrounding real-world applications. This paper presents a number of experimental questions that we have addressed, and describes them within the context of the form factors of samples used for experimentation. We present results from the investigations and discuss the validity of results in light of the form factors used to address the questions. Importantly, we present an effort to develop a laboratory procedure based on laboratory form factors that is useful for predicting biocementation performance in the field.

METHODS

Preparation of bacteria. *Sporosarcina pasteurii* (DSM33) was grown in Brain Heart Infusion media at 37 g/L, supplemented with 330 mM urea. Cells were grown in shake flasks under vigorous shaking at 30 °C. After 24-48 hours of growth, cells were pelleted and resuspended into 1 ml 12% sucrose per 1.6 g pellet wet weight. The cell resuspensions were frozen at -80 °C and then lyophilized (LabConco) until dry. The cakes were then pulverized and stored at -80 °C until use. Just prior to use in cementation reactions, cell powders were resuspended in de-ionized water (~18MΩ) at various concentrations, typically 4 g/L.

Cementation method. The general approach for cementing soils is as follows: 1) molds were packed with soil, 2) soils were tamped using plastic implements designed to fit into the mold of interest, and the user would apply force over lifts of soil, typically in 2-4 lifts per mold, 3) bacterial powders were suspended in de-ionized water and evenly mixed to avoid clumps, 4) cementation solution was prepared (cementation solution was comprised of 49 g/L calcium chloride dihydrate, 20 g/L urea, 10 g/L ammonium chloride, and 3 g/L nutrient broth), 5) biomass solution was added to the soil column in a volume that was expected to settle to a chosen depth based on the assumed pore volume of the soil, 6) 4-10 volumes of cementation solution were added to the columns based on the targeted volume. Typically, a 2-hour interval separated each fluid addition. This would often require an overnight treatment interval to accommodate the technician's work schedule, with the overnight interval occurring between the 3-5th administration of the cementation solution.

Construction of ¾ in. diameter columns. Cemented cylinders with a ¾ in. diameter and 2-3 in. length were prepared using 3D printed molds. The molds contained two halves of a cylinder, split length-wise with in-printed gasket material forming a liquid barrier between the halves when sealed; two end caps, containing threads that locked the two halves in place when the caps were affixed, and a stainless steel disk (typically 100x100 mesh) pressed against the under-side of the mold assembly when the bottom cap was in place. When the molds were assembled (See **Figure**

1), soil could be retained in place by the mesh disks at the bottom, but bacterial and cementation solutions were free to pass. Typically molds were loaded with 20 grams of soil, resulting in cemented specimens that were approximately 2 in. long. Space above the top of the column and the top of the assembly cap allowed pooling of all cementation solution when percolation was slow, which was observed frequently but not always. Most commonly, samples in this form factor were manufactured using a robotic liquid handler (OpenTrons, OT-2). Demolding samples from the 3D printed columns often resulted in damage, especially for samples with low amounts of cementation. To alleviate this, molds were pre-treated with dry Teflon lubricant sprays, but still samples cemented at the lowest strength levels (in the 10 ft. s of psi) would often break upon demolding.

Construction of 6 in. diameter columns for CBR testing

Samples for CBR testing were made using standard CBR molds (Gilson Company, Inc.) However, the molds needed to be modified to support fluid percolation at reasonable rates. The standard-specified bottom plate holes were opened to 0.25 in. and an additional 8-10 holes were drilled to permit rapid percolation of administered solutions. A 6 in. diameter stainless steel disk (typically 100x100 mesh) was placed in the bottom of the mold, and the mold was filled with approximately 5 in. deep of soil. Biomass and cementation solutions were administered in volumes using the same fill rationale described above for the ¾ in. diameter columns. CBR samples were not demolded, but rather tested in the fixture in accordance with the CBR standard.

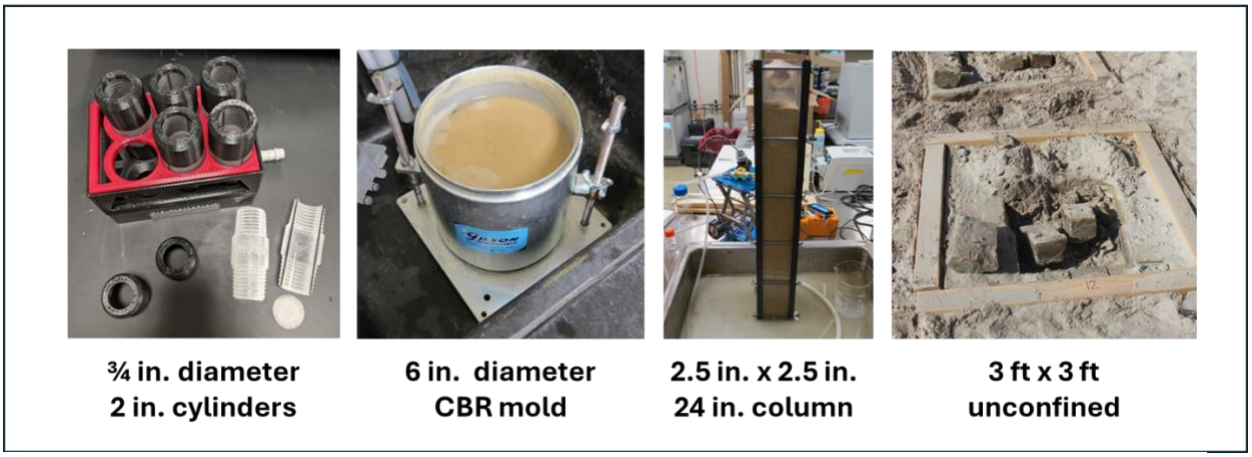

Figure 1. Biocementation in various form factors.

Construction of 24 in. columns for UCS testing, depth profiling. Cemented samples up to 24 in. long, with a 2.5 in. x 2.5 in. cross section, were manufactured using custom molds constructed of black acetal, acrylic, and neoprene rubber gasket material. The pieces were assembled using either stainless steel threaded rods and wing nuts or nylon hardware. The acrylic faces allowed direct observation of fluid percolation through the column. Demolding of the samples from the square profiles with break-away walls on all sides was minimally disruptive, such that samples that were weakly or even completely uncemented (such as negative controls) could be demolded without damaging the cast specimen. Usually the columns were incompletely filled to prevent loss of solutions when percolation was slow.

Construction of 3 ft. x 3 ft. field samples. Samples in the field were constructed under open-flow conditions. Wooden frames were formed to mark the targeted areas in the soil by screwing wood boards together with inner dimensions of 3 ft. x 3 ft. The frames were loosely placed over the target ground. Solutions were added using backpack sprayers at a delivery rate of 0.6 gallons per minute. The wooden boards were tightly attached to each other such that when administration rates exceeded percolation rates, pooling was observed and the solution was retained until it drained through the soil column. Samples in the field were constructed on a strict 2-hour interval.

Experiments

Two experimental blocks were tested as shown in **Table 1**.

Table 1. Experimental questions

	Experimental question	Form factor	Treatments	Replicates
A	Does biocement require curing over time, or is the reaction nearly instantaneous?	3/4 in. diameter, 2 in. long cylindrical column	Time post-curing, ranging from immediately after manufacturing until 180 days out	3
	What is the impact of increasing amounts of biomass in the biocementation reaction?	3/4 in. diameter, 2 in. long cylindrical column	Biomass concentration in 2x increases over 3 levels	12
	Are nutrient broth and ammonium chloride necessary in the cementation solution?	3/4 in. diameter, 2 in. long cylindrical column	Standard cementation solution, standard without nutrient broth, standard without ammonium chloride, standard without ammonium chloride but with 2x Ca++	6
	Under a constant dose, does the concentration of reagents impact cementation strength?	3/4 in. diameter, 2 in. long cylindrical column	6 x 8 ml CS @ 65.3 g/L $CaCl_2 \cdot 2H_2O$ 8 x 8 ml CS @ 49 g/L $CaCl_2 \cdot 2H_2O$ 10 x 8 ml CS @ 39.2 g/L $CaCl_2 \cdot 2H_2O$ 12 x 8 ml CS @ 32.6 g/L $CaCl_2 \cdot 2H_2O$	6
	Does the salt concentration in the biomass suspension and cementation solutions impact the strength of cementation?	3/4 in. diameter, 2 in. long cylindrical column	Components (biomass and cementation solution) suspended in DI water, tap water, and brackish water	12
	Does the biomass concentration impact the strength vs. depth profile?	2.5 in. x 2.5 in. square, 24 in. long column	Biomass concentration in 2x increases over 3 levels	1
B	Is it possible to use laboratory measurements of calcium carbonate precipitation in CBR and 24 in. deep columns to predict the CBR in the field?	2.5 in. x 2.5 in. square, 24 in. long column; CBR mold (6 in. diameter cylinder)	Standard cementation solution and biomass solutions poured to an expected depth of 2 in. based on a 40% assumed pore volume in the form factor of interest (6 mls for the 3/4 in. diameter columns; 370 ml for the CBR mold; 82 ml for the 24 in. column, 17 liters for the 3 ft. x 3 ft. open field)	3 for lab CBR; 1 for all others

Unconfined compressive strength testing. It was generally necessary to flatten the top surfaces of samples before strength testing. Diamond bladed saws were effective at creating smooth cuts in strongly cemented samples but left broken and uneven surfaces in weaker samples. Manually machining the surface with a metal file was useful at all strengths and was the method generally used. For square profile samples, the samples could be placed on their sides and measured directly but were still typically machined with flat sides in a cubic aspect ratio. ¾ in. diameter cylinders were separated into a top and a bottom section, filed into an approximate aspect ratio of 1:1. Field samples were cut out of the ground with a concrete saw and then filed down manually into cubic structures of varied aspect ratios, but typically of a 1:1 ratio.

California Bearing Ratio testing. It was not possible to construct samples per the CBR standard (ASTM D1883), but cemented samples in the CBR forms were tested according to the standard.

Measurement of calcium carbonate content. Calcium carbonate content was assessed using thermogravimetric analysis. (TGA, TA Instruments). 25-50 mg of soil were placed into platinum pans and subjected to heat ramps up to 1000 °C. Mass losses within the initial 200 °C ramp were attributed to water and organic content. Mass losses at ~ 600 °C were attributed to calcium carbonate degradation and CO_2 off-gassing.

RESULTS

Does biocement require curing over time? Figure 2A shows that there is no clear evidence that continued hardening of the biocemented samples occurs over time. For these experiments, no careful attention was paid to the moisture content of the sample at the time of testing. Presumably, the moisture content was highest at the first timepoint and then decreased as time passed. A more thorough investigation of the impact of time would require a larger number of experiments to be run and for care to be taken regarding the moisture content of specimens at the time of testing. But when rapid testing of experimental conditions is desirable, it is not expected that a wait period is needed between iterations.

What is the impact of increasing amounts of biomass on the biocementation reaction? An initial experiment with dried biomass powders indicated that strong cementation was achievable when adding 0.5 g per ¾ in. diameter column. To assess the impact of biomass concentration on cementation performance, that concentration was compared in 2-fold changes. The resultant strength, as assessed by UCS measurement, show a decreasing strength in the top portions of the columns as the biomass load was increased. The same trend was not observed in the lower portions. In addition to concentration impacting the resultant strength, the variability within each treatment condition also changed with changing biomass loading. The greater the biomass loading in these specimens, the lower the variability in strengths in the specimens, most notably in the top portions.

Are nutrient broth and ammonium chloride necessary in the cementation solution? Nutrient broth and ammonium chloride are common ingredients in the cementation solutions used in literature reports. Their addition has been justified by the assumption that these components are helpful for bacterial growth and thus promote greater cementation in soils. Their impact on dry formulations is presented in **Figure 2C**. The best performance using lyophilized biomass was still observed with standard cementation solution, based on the maximum observed strength. This is despite that fact that the viability of lyophilized powders was 1-2 orders of magnitude poorer than fresh cells as measured by cfu/mass (data not shown.) Removal of nutrient broth appeared to cement the column more uniformly but not as strongly in the strongest locations. Removal of nutrient broth also appeared to reduce the variability between replicates. Removal of ammonium chloride also seemed to reduce the strength of columns, whether or not additional calcium was added to the solutions, although it is clear from this data that both the strength and the distribution of that strength is impacted by the composition of the cementation solution. Follow on experiments that more powerfully resolve strength over depth, for example in 24 in. columns, are recommended for answering the question of the impact of cementation solution composition on strength and uniformity.

Under a constant dose, does the concentration of calcium chloride impact cementation? The concentration of reactants could impact the rate of calcium carbonate formation and thus high concentrations might be expected to promote gradient properties in the finished columns. As well, lower concentrations of calcium could result in slower crystal growth, impacting material properties. Generally speaking, the final strength appears (**Figure 2D**) to fall off as a function of increasing concentration. The best conversion efficiency was observed when the same dose was administered over 12 treatments at lower concentration.

Does the salt concentration of biocementation solutions impact cementation strength? As both bacterial surfaces and soil surfaces are on balance negatively charged, percolation of bacteria through soil is expected to depend upon the ionic strength and potential charge screening in the aqueous medium. To test the impact of ionic strength from various water sources, de-ionized water, tap water, and brackish water from a coastal sound were tested for their impact on cementation. The salt content of the de-ionized water was essentially 0, whereas the tap water had an estimated salt content of ~0.1 g salt/L, and the brackish water contained approximately 15 g salt/L. There was little to no impact in changing the salt content of the suspension media as measured by UCS measurements of the top and bottom portions as shown in **Figure 2E**. Both the dried biomass and the cementation solutions were made using the experimental salt solutions.

Does the biomass concentration impact the strength vs. depth profile? As strength gradients were apparent in 2 in. columns in samples made with various biomass loads, the impact of biomass concentration was tested again using the 2.5 in. x 2.5 in. x 24 in. long columns. A similar span of biomass concentrations was used, covering a 4-fold change in concentration. The experiment was conducted at *n=1*, and depth resolution in the columns was complicated by the requirement to extract 2.5 in. cubes from the columns with uneven top surfaces. The results in **Figure 2F** indicate that strength profiles in the cemented columns vary considerably between treatment conditions. For the highest concentration samples, a greater depth of cementation is observed. However, as the concentration varied over the tested range, the strength vs. depth profile within columns varied. For the columns with the lowest biomass loading, strength was highest towards the top of the column. For the column with the highest biomass loading, this trend was inverted; the highest strength was observed towards the bottom of the column. Although these observations were made on *n=1* columns, the results indicate that the depth profiles of the cemented columns can vary dramatically by biomass loading, with a more complex range of qualitative responses than might be expected.

Is it possible to use laboratory measurements of calcium carbonate to predict CBR performance in the field? Figure 3 shows a number of correlations obtained on lab samples which are designed to enable prediction of CBR in the field. First, samples exhibiting a range of CBRs were created by adding increasing numbers of cementation solution administrations (**Figure 3A)**. The CBR response of lab samples to the number of administrations of cementation solution is linear over 0-10 administrations, achieving a CBR of approximately 40% in beach sand. **Figure 3B** shows TGA measurements on the samples with varied CBRs, with calcium carbonate mass fractions ranging from roughly 1-2.5% for CBRs in the 10-40% range. Next, measurements of calcium carbonate precipitation vs. depth were taken under the same conditions using the 24 in. long columns (**Figure 3C)**. Using the TGA vs. CBR correlation obtained previously, the expected CBR as a function of depth is calculated and plotted as shown in **Figure 3D**. Thus, it is expected

that adding the scaled volume in the field (which is also expected to penetrate down 2 in. based on direct filling of the pore space), should result in soil strength improvements at least 7 in. deep.

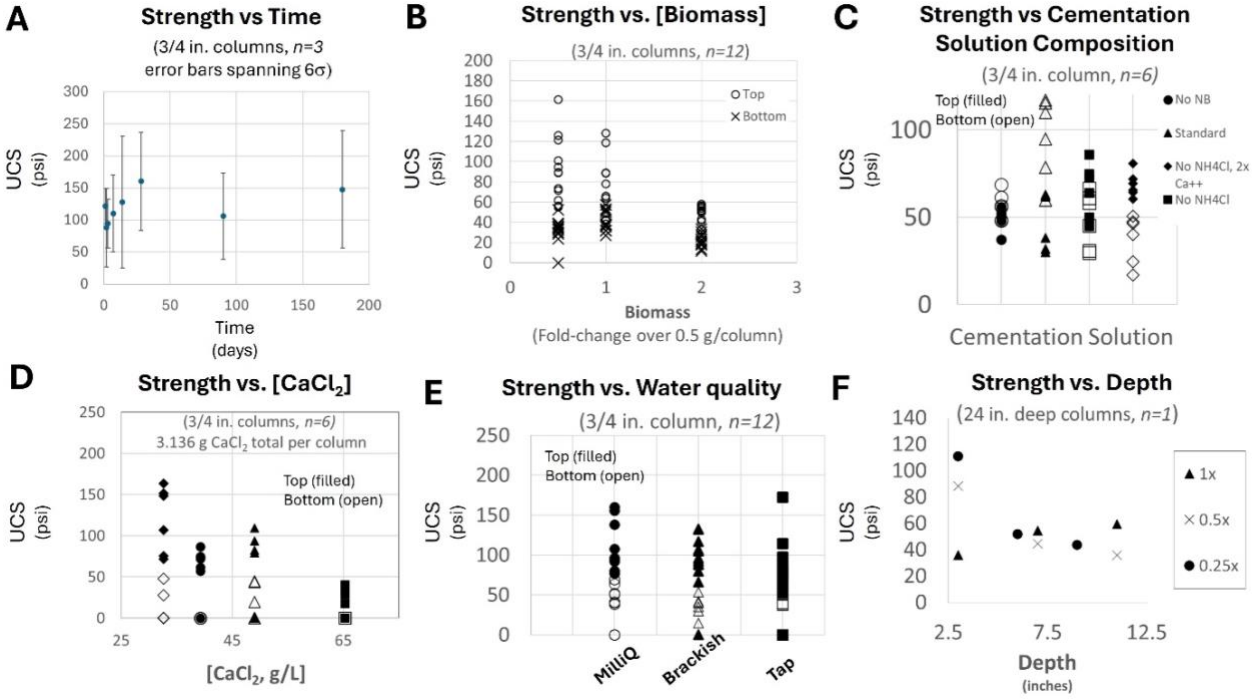

Figure 2. Biocementation results. Panels A-F show, respectively: the impact of cure time on unconfined compressive strength, the impact of biomass concentration on unconfined compressive strength, the results of modifying a standard literature medium, the impact of reagent concentration on the resultant strength of specimens, the impact of various water salinities used during cementation, and strength vs. depth as measured in 24 inch columns under varied biomass concentration, showing a more complex response than B.

Panel 3E shows the collection of the field CBRs on plots built with scaled biocementation reagents. **Panel 3F** overlays the lab CBRs vs. treatments by comparison to the field CBRs as measured on the top surface. For the lower administration numbers (6 and 9 cementation solution administrations), the lab and field CBR measurements are in approximate agreement. For the field sample with 12 cementation solution administrations, the resultant CBR is lower than the lab experiments would predict. It should be noted, however, that a 12-administration CBR sample was not prepared in the laboratory. Also, (not shown), CBR measurements attempted at 3 in. depth in the field samples indicated no soil strengthening at depth, in contrast to predictions from laboratory experiments. Finally, the untreated native soil exhibited CBRs between 5-9%, suggesting that as few as 6 administrations was sufficient to significantly strengthen the soil.

CONCLUSIONS

A number of experiments were performed using the various form factors, with some form factors presumably producing data that capture aspects important to understanding biocementation generally, whereas other form factors only produce a snapshot in the tested phenomena and may

in fact be misleading. Case in point, studying cementation aspects that vary by depth may not be effective using the ¾ in. and 2 in. deep cylindrical columns. Likewise, predicting field performance even in larger format CBR molds or 24 in. deep columns is not straightforward. In general, large numbers of samples could be constructed in the ¾ in. diameter columns, making them excellent for certain questions where depth of percolation may not matter, although assembly of the columns, demolding and cleaning the columns, manually shaping the produced specimens, and testing by UCS or TGA was still laborious. It was experimentally determined that the noise in the specimens often required *n=12* replicates to get good resolution of results between samples, allowing each build run to test only 2-4 treatment conditions.

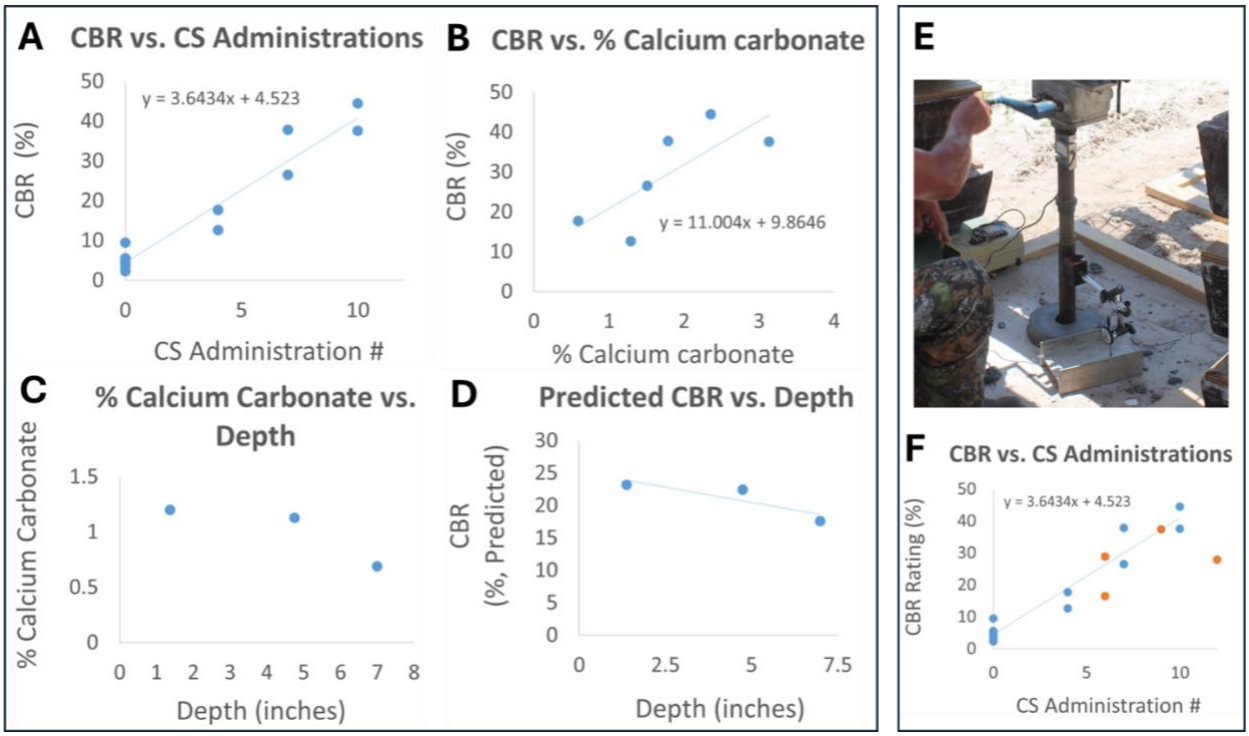

Figure 3. Experiments designed to enable field prediction of CBR strengths: A, CBR vs. cementation solution (CS) administration number is collected in the laboratory. B, the calcium carbonate content of the specimens is correlated to CBR. C, the calcium carbonate concentration is measured in samples constructed in 24 in. columns as a function of depth. D, using the CBR vs. % calcium carbonate relationship, the measured calcium carbonate values in the 24" columns can be used to predict CBR at depth in the field. E shows the field CBR measurement process, and F overlays the field measurements (orange) onto the lab measurements (blue).

The 24 in. columns were also laborious to handle and resulted in the consumption of large amounts of material. However, we observed generally that results that were collected in this form factor created a more complete picture of the results of treatment modifications on the cementation process. Studies at depth could be specifically undertaken in this form factor, and most experimental conditions impacted cementation depth in some fashion, or creation of gradient properties in the samples, which could easily be observed in the 24 in. column form factor.

Experiments done using the CBR mold were somewhat ambiguous, as construction of the samples could not follow the CBR standard, and thus the results cannot be taken directly as if the standard had been followed, eliminating the key advantages of adhering to the standard.

Finally, an effort to use laboratory observations to predict field performance was only partially successful, with resultant CBR measurements agreeing with lab measurements at the lower administration numbers only, and then only at the surface of the plots.

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