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Biocementation of Simulant Martian Regolith

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BIOCEMENTATION OF SIMULANT MARTIAN REGOLITH

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Abstract

Here on Earth biocementation has been extensively studied and holds promise in producing a more energy efficient and environmentally benign alternative construction materials compared to the standard method of production. This research is focused on developing bioprocesses to produce bioconcrete columns using potentially Mars-compatible microalgae and simulated Martian regolith. A marine microalga, *Thraustochytrium striatum* was tested to make Martian regolith-based columns in the presence of $CaCl_2$/urea.

Three different biogrouting methods were investigated including simultaneous, sequential and batch circulation of microalga cell biomass and $CaCl_2$/urea in the columns. The need of post-biogrouting column soaking was studied to develop an understanding of its relationship with unconfined compressional strength (UCS) of the columns. X-ray diffraction (XRD) was used to identify the formation of $CaCO_3$ along with using a scanning electron microscope (SEM) to identify the microstructure of the deposited $CaCO_3$. Carbonite titration analysis and hydraulic conductivity tests were conducted to further characterize the biocemented columns.

T. striatum is capable of biocementation and can produce urease to induce $CaCO_3$ precipitation for regolith columns, with an average $CaCO_3$ concentration of 12.21%±0.79%. Post-biogrouting column soaking has shown to have an inverse relationship with UCS, and therefore is not needed in the process of manufacturing biocemented columns. Removing the biomass from the growth media and resuspending in it DDI water produced a better biogrout solution because the salts within the growth

media reduced the strength of the columns. The average hydraulic conductivity of the biocemented columns was $2.06\pm0.69\times10^{-3}$ cm/s, about 50% less than that of the untreated Martian regolith column. Batch feed biogrout recirculation was found to be the best method of production with producing columns with average UCS values of 732.40±117.84 kPa.

Calcium acetate was used to simulate the anaerobic digestion of food waste in the production of acetic acid, which would further be utilized to dissolve limestone to produce a stable source of calcium for the manufacturing of biocement. The utilization of calcium acetate instead of $CaCl_2$ resulted in a significant decrease in the permeability of the biocemented columns compared to the untreated MMS regolith by 90%. *T. stratum* was able to utilize acetate as a carbon source to precipitate carbonate, instead of using urea through urea hydrolysis. Lastly, the average $CaCO_3$ concentration within the columns produced with calcium acetate was 11.50%±0.34%. Therefore, future research in optimizing the regolith particle size, biomass loading rate, and nutrient loading rate will need to be conducted to improve the UCS of biocemented columns.

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List of Abbreviations

Anaerobic Digestion ..... AD

Artificial Sea Water ..... ASW

Calcium Carbonate..... $CaCO_3$

Calcium Chloride Dihydrate ..... $CaCl_2 \cdot 2H_2O$

Chemical Oxygen Demand ..... COD

Distilled De-Ionized..... DDI

Magnesium Chloride Hexahydrate ..... $MgCl_2 \cdot 6H_2O$

Magnesium Sulfate Heptahydrate ..... $MgSO_4 \cdot 7H_2O$

Microbial Induced Carbonate Precipitation ..... MICP

Mojave Mars Simulant..... MMS

Optical Density ..... OD

Potassium Chloride ..... KCl

Scanning Electron Microscope ..... SEM

Sodium Chloride ..... NaCl

Sodium Hydroxide ..... NaOH

Soil Analysis Support System for Archaeology ..... SASSA

Standard Operation Procedure ..... SOP

Unconfined Compressional Strength ..... UCS

Urease-Producing Microorganisms ..... UPM

Volatile Fatty Acids ..... VFA

X-ray Diffraction ..... XRD

1.0 Introduction

Biocementation, also known as microbial induced carbonate precipitation process (MICP), involves the use of microorganisms to precipitate calcium carbonate ($CaCO_3$) as a binding agent for an eco-friendly alternative compared to regular cement production [1,2]. The formation of $CaCO_3$ is affected by many factors, such as the microorganism, concentration of calcium, dissolved inorganic carbon, pH of the system, the amount of available nucleation sites, and so on [3]. The types of microorganisms that are involved in MICP include photosynthetic organisms, sulfate-reducing bacteria, and some organisms that utilize the nitrogen cycle [4,5]. The most widely studied biocementation microorganisms (e.g., *Sporosarcina pasteurii*) are named urease-producing microorganisms (UPM) that use nitrogen cycle, more specifically urea hydrolysis to generate $CaCO_3$ [3,5–7]. Urea is hydrolyzed into a one to one mole ratio of ammonia and carbamate (Eq. 1); then the carbamate is immediately hydrolyzed to ammonia and carbonic acid (Eq. 2), and finally the ammonia will react with water to form ammonium and hydroxide (Eq. 3) [1–3,5,7–9].

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (3)$$

Simultaneously the carbonic acid will release a hydrogen ion to form bicarbonate (Eq. 4), then another hydrogen ion to form carbonate (Eq. 5) to counter act the production of hydroxide, due to the rise in the pH of the system [1–3,5,7–9]. Meanwhile, the calcium ions in the system will attach to the negatively charged cells [10]. Once the carbonate

precipitates it will attach to the calcium ions to form $CaCO_3$ (Eq. 6) on the outer surface of the cell, which in turn will encapsulate the cell (Figure 1) [10]. Overall, UPM can create biocement in the presence of calcium ion and urea through the mechanism of Eq. 7.

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \tag{4}$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \tag{5}$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \tag{6}$$

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \xrightarrow{UPM} CaCO_3 \downarrow + 2NH_4^+ \tag{7}$$

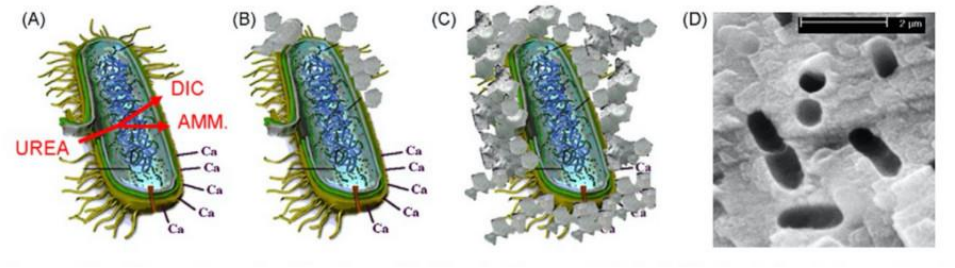

Figure 1. MICP via urease hydrolysis of UPM [10].

Due to a lack of soluble calcium salt distributors on Mars and a need for calcium ions in the production of bio-cement, a process such as anaerobic digestion (AD) can fulfill the needs of producing a stable source of calcium ions. Anaerobic digestion is a biological process used to break down organic matter into volatile fatty acids (VFAs), then convert the VFAs into methane for energy production [11,12]. The four stages of AD are: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is the beginning step of AD in which bacteria use enzymes to break down carbohydrates, lipids, and proteins into sugars, fatty acids, and amino acids. Then the fermentative bacteria will convert the products from hydrolysis into VFAs, alcohol, hydrogen, and carbon dioxide during the process known as acidogenesis. Some of the produced VFAs cannot be used

by methanogenic bacteria, therefore acetogenic bacteria will convert those VFAs into acetate, hydrogen, and carbon dioxide. Within the final step of AD, the methanogenic bacteria use the acetate, hydrogen, and carbon dioxide for the production of methane.

The overall objective of this research is to develop an Earth-independent biocementation process that would be compatible for utilization on Mars in the production of construction materials by using locally available resources (i.e., *in situ* resource utilization) (Figure 2). The production of conventional Portland cement is an energy intensive process that requires a significant amount of heat and produces greenhouse gases. While producing cement with an average the compressive stress of around 8 to 21 MPa depending upon the type of Portland cement produced [13].

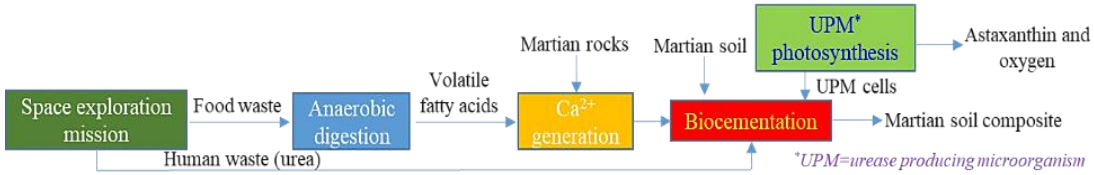

Figure 2. Concept of biocementation to manufacture construction materials on Mars utilizing in-situ resources.

Compared to conventional methods of Portland cement production, the method of biocement production can occur at a lower temperature, and therefore requires a lower input of energy. Along with a lower requirement of energy, the raw materials needed for the production of biocement will be much easier to obtain for the Earth-independent and self-sustaining space exploration mission to Mars. By optimizing the method of biogrout recirculation, the biomass loading rate, and the nutrient loading rate, it will be possible to produce a biocemented column with high compressive stress. Therefore, the main objectives of this research are to (1) compare simultaneously mixed, sequentially mixed,

and batch feed recirculation methods and (2) to utilize simulated AD VFAs for the production of calcium acetate from limestone to compare it with $CaCl_2$ in the column manufacturing process.

2.0 Materials and Methods

2.1 Cultures and growth media

The microorganism used for the research of Martian regolith biocementation was *T. striatum* ATCC 24473 that was purchased from the American Type Culture Collection (ATCC). The stock and seed cultures were prepared by following the protocols provided by the ATCC [14]. The microorganism was grown in a broth made of artificial sea water (ASW), glucose, yeast extract, and peptone. The ASW contains (g/L): 30.0 NaCl, 0.7 KCl, 10.8 $MgCl_2 \cdot 6H_2O$, 5.4 $MgSO_4 \cdot 7H_2O$, and 1.0 $CaCl_2 \cdot 2H_2O$. The concentrations of glucose, yeast extract and peptone were 30.0, 6.0 and 6.0 g/L, respectively. After the chemicals were dissolved into 1.0 L of DDI water, the pH of the broth was adjusted to pH 7.0 using 1 M NaOH followed by autoclave at 121°C for 15 minutes. The cultivation was conducted in 500-mL flasks with 200 mL working volume to which 20 mL seed culture (obtained at mid-log phase of inoculum preparation) was inoculated (1:10, v/v inoculation size) into the fresh broth and incubated at 25 °C with a stirring speed of 140 rpm. After five days of cultivation, *T. striatum* cells were harvested by centrifugation and resuspended in DDI water to make cell "solution" for biocementation.

The Mojave Mars Simulant (MMS) was used in this study to simulate Martian regolith. The MMS was developed using a basalt mined in the western Mojave Desert and were among the suite of test rocks and soils that was used in the development of the

2007-2008 Phoenix Scout and the 2009 Mars Science Laboratory missions [15]. Particle sizes of the selected MMS were all smaller than 1 mm. The chemical composition of the MMS regolith and the average chemical composition of Martian regolith taken during different Mars missions can be seen in Table 1.

Table 1. Comparison of chemical composition for Martian regolith and simulant Martian regolith [15].

Concentrations in wt%	Martian Regolith	Martian Simulant
	Average	MMS
SiO_2	46.46	49.4
TiO_2	0.72	1.09
Al_2O_3	9.53	17.1
Cr_2O_3	0.36	0.05
Fe_2O_3	19.33	10.87
MnO	0.38	0.17
MgO	6.70	6.08
CaO	7.15	10.45
Na_2O	2.70	3.28
K_2O	0.48	0.48
P_2O_5	0.73	0.17
SO_3	4.33	0.1
Loss On Ignition	-	3.39
Total	96.88	99.4

2.2 Cell growth determination

Cell growth was determined by optical density and dry cell biomass weight. The biomass yield was determined by using a BioTek Epoch^2 microplate reader to measure the optical density (OD) of the biomass. After selecting a flask of biomass from the cultivated group, 1 mL of the biomass was added to three individual 1.5 mL centrifuge test tubes. The test tubes were placed into the centrifuge for two minutes at 10,000 rpm.

Once the centrifuge was completed the liquid within the test tubes were discarded and 1 mL of DDI water was added to the test tubes. The test tubes were then mixed using a vortex mixer and place back into the centrifuge to once more be separated. As before the liquid was discarded and 1 mL of DDI water was added to the test tubes, then mixed using the vortex mixer.

To scan for the OD of the biomass, the samples were diluted by a factor of ten within the well plate. Diluting the sample required 180 µL to be added into three different wells followed by 20 µL from each sample added and mixed within the wells using the pipet. The OD was measured at 660 nm, then the data was compared with a standardized curve to find the average amount of biomass within 1 L of the media.

2.3 Calcium acetate production

Based on the research of Lim et al. (2008) and Wang et al. (2014), AD can utilize food waste to produce 24.5 – 25.5 g-COD/L of VFAs with 80% of the VFAs in the form of acetic acid, respectively [16,17]. After converting the concentration of VFAs produced from food waste into acetic acid, assuming that 25 g-COD/L of VFAs were produced, the concentration of acetic acid was 18.52 g/L. Pure acetic acid was used to simulate the VFAs produced during the AD of food waste. Utilized as the calcium source was Espoma Organic Garden Lime, dolomitic limestone, purchased from the local Lowe's with a calcium carbonate concentration of around 50%. To determine the amount of calcium in the calcium acetate solution, the dolomitic limestone was soaked and mixed in acetic acid solution for 24 hours. After mixing for 24 hours, the solution was filtered to remove any remaining solids followed by the addition of sodium bicarbonate to induce the

precipitation of calcium within the solution and filtered again to collect the solids. Once the solids were dried in the oven overnight at 60°C, the samples were weighed and compared to find the optimum ratio of limestone to acetic acid solution.

2.4 Biocementation setup and operation

The reactors used for fabricating Martian regolith columns were designed as seen in Figure 2. The cylindrical tube used to hold and shape the columns was a form of clear acrylic tubing that have an internal diameter of 38.1 mm (1.5 inch) and an outer diameter of 50.8 mm (2 inch). Each of the cylindrical tubes was 127 mm (5 inch) in length with a hole at around 114.3 mm (4.5 inch) for a side stream used to prevent the overflow of biomass. All biomass collected from the side stream was returned to the 150-mL beaker below the reactor for recirculation. The bottom portion of the reactor was made using a PVC cap that had a hole drilled into the center for barbed hose fitting. A piece of tubing was attached to the fitting to help with the drainage of the reactor back into the beaker below.

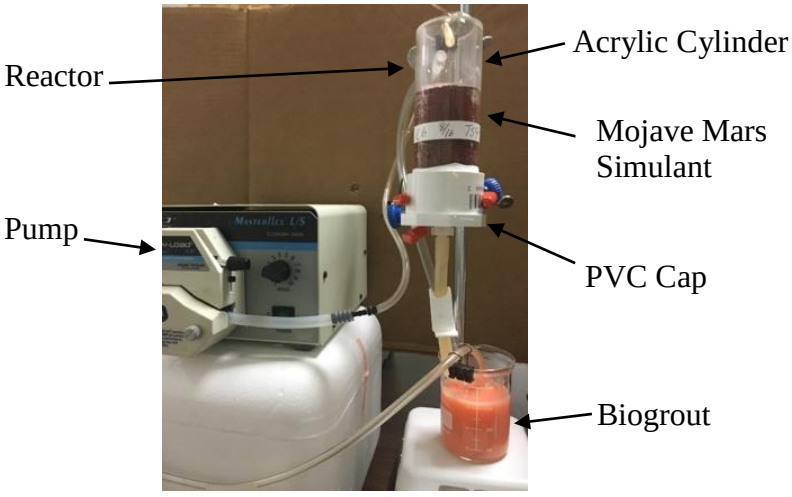

Figure 3. Biocementation setup.

Between the bottle cap and the cylindrical tube was a nylon (40 mesh) filter used to help prevent the loss of regolith. A piece of wax paper (6 in x 3.5 in) was added to the inside of the reactor to help with the removal of the columns in a later step. To make the columns, each reactor was filled with 105.0 g of Martian regolith which was rinsed with 150 mL of DDI water to completely wet the regolith and facilitate the uniform distribution of cell biomass and $CaCl_2$/urea solution during the biogrout recirculation. On top of the regolith is a piece of Scotch-Brite scour pad to aid in distributing the biomass evenly on top of the regolith. To implement the MICP process, the biogrout was placed in the beaker and recirculated through a peristaltic pump to the top of the regolith column (Figure 3). The pumping rate was controlled at 8.0 mL/min.

Once recirculation had finished, the columns were drained of any remaining liquid, then the reactor was flipped upside down in a plastic container to remove the bottom PVC cap. After the columns were air dried upside down for 20 minutes, they were then flipped back upward and were removed from the acrylic cylindrical tube followed by being placed in the oven to dry overnight at 60°C. The finished dry columns with dimensions of 1.5-inch (diameter) by 3-inch (height) were subjected to unconfined compressional strength (UCS) measurements, permeability measurements, scanning electron microscopy (SEM), x-ray diffraction (XRD), and carbonate titration for qualitative analysis of calcium carbonate precipitate in the columns.

2.4.1 Simultaneously mixed and sequentially mixed biogrout recirculation

For simultaneously mixed recirculation, the cell biomass and $CaCl_2$/urea were simultaneously mixed in the beaker and recirculated through the regolith columns for 48

hours. Then, the columns were soaked for 0 (i.e. without soaking) and 4 days. In the sequential recirculation method, cell biomass and $CaCl_2$/urea were sequentially added into the column. Two variables were critical here. The first variable was the time at which $CaCl_2$/urea was added into the reactors circulation stream after the cell biomass was initially recirculated in the column for 3, 6, 9, or 12 hours. The total circulation time for both cell biomass and $CaCl_2$/urea was kept constant of 48 hours. The second variable was the time (0, 2, and 4 days) for the column soaking after $CaCl_2$/urea circulation was stopped.

2.4.2 Batch feeding biogrout recirculation

Five versions of the batch feeding of biogrout were compared. The first version involved a 3-day period of feeding fresh biomass and $CaCl_2$/urea into the reactor each day. The total biomass was divided and added over the span of 3 days. Half of the daily amount of biomass was added first into the reactors and circulated within the reactor for 3 hours. Following the 3 hours, the other half of the daily amount was added into the circulation stream along with a third of the total $CaCl_2$/urea for 21 hours. Once the 3 days of circulation had finished, the columns were soaked for another 3 days. The second version was similar to the first, but the full amount of the daily biomass was added at the beginning and circulated for 3 hours followed by $CaCl_2$/urea circulation (no cell biomass) through the columns. The third version was similar to the first version but it was over a 2-day period instead of a 3-day period for biogrout circulation, with a soaking period of 0 and 4 days. The fourth version involved 100 mL of biomass added each day, half in the beginning and the other half after 3 hours with a fifth of the total $CaCl_2$/urea added for a

21-hour recirculation time period. The process of recirculation continued until the columns clogged, then the columns were removed from the reactors. The fifth version was the same as the fourth version, but the biomass was not removed from the growth media. Therefore, the biogrout for the fifth version consisted of biomass and growth media with no DDI water added.

2.4.3 Calcium acetate biogrout recirculation

This method of biogrout recirculation follows the standard operation procedure discussed further in section 3.3, but calcium acetate solution was used instead of $CaCl_2$ as the calcium source. The columns were produced utilizing three different molar ratios of calcium acetate to urea, which were 1:0, 1:1, and 1:4.53.

2.5 UCS measurement methodology

The UCS was obtained by conducting an unconfined compression test which followed the ASTM C39 standard [18]. The columns had a 1:2 diameter-to-height ratio, therefore the standard suggested a loading rate which corresponded to a stress rate of 0.25±0.05 MPa/s on the columns. This test used a deformation driven scheme to apply the axial compressive load at a constant rate of 1.0 mm/min, which coincided with those used by Cheng et al. (2013) [19]. This load rate produces a strain rate of about 1.3 %/min, which allows for the columns to break within a 2-minute time period.

2.6 Hydraulic conductivity measurement methodology

Following the standards set by ASTM D5856, a falling head permeability test was conducted on the columns to characterize their permeability. Prior to the test, DDI water was flushed through the permeameter to remove any entrapped gases with a pressure of

about 10 kPa. Once the influent pressure was stabilized to about 10 kPa and the effluent pressure was stabilized to atmospheric pressure. Then DDI water was allowed to flow through the column due to the pressure difference while the time and head loss were recorded [20].

2.7 XRD measurement methodology

XRD was used to determine the type of $CaCO_3$ precipitated within the columns. The instrument parameters used to run these scans were similar to those used by Wei et al. (2015) with some slight adjustments. Similar to Wei et al. (2015), the accelerating voltage and current were set to 40 kV and 35 mA, respectively [6]. Whereas, the samples were scanned from 20° to 40° (2θ), with a scanning speed of one degree per min.

2.8 Carbonate titration measurement methodology

The method used to determine the percentage of $CaCO_3$ deposited within the columns was found through the Soil Analysis Support System for Archaeology (SASSA). The analysis method provided by SASSA follows that which was developed by Rowell (1994). After the post-biogrout columns were tested via UCS, the broken columns were then used for $CaCO_3$ analysis. Parts from each section were taken and milled into a fine powder using a pestle and mortar. Then 10 mg were measured out and added to a 250-mL conical flask. The regolith sample was subjected to 20 mL of 2 M HCl, followed by boiling the solution for 10 min at 250°C after the reaction has stopped fizzing. Once the flask has cooled down, the solution was then filtered and DDI water was added to the filtrate till the total volume equaled 100 mL. Then 10 mL was added to three different 250-mL conical flask containing 50 mL of DDI water, a few drops of phenolphthalein

indicator solution, and a stir bar. Finally, the solutions were titrated using a 50-mL burette and 0.1 M NaOH, while being stirred using a magnetic stirrer. After reaching pH 10, as indicated by the hot pink color of the solution, the amount of 0.1 M NaOH was recorded and used within the equations given to find the percentage of $CaCO_3$ within the columns [21].

3.0 Results and Discussion

3.1 Confirmation of MICP process

The original regolith and precipitated materials produced from MICP were analyzed by XRD (Figure 4). The XRD patterns found in the MICP produced materials (Figure 4A) matched those of the pure reagent grade of $CaCO_3$ as Calcite (Figure 4B) between 29 to 30 degrees (2θ) and the aragonite standard (Figure 4C) at 33 degrees (2θ). These results validate that *T. striatum* is capable of producing $CaCO_3$ via the MICP process. The variable 2θ on the x-axis of Figure 4 represents the angle at which the x-ray beam was deflected and read by the scanner. The x-ray beam initially makes contact with the sample at angle θ before being deflected. It is shown that raw Martian regolith (i.e., MMS) contains little $CaCO_3$ and MICP produced large amount of $CaCO_3$. The distribution of $CaCO_3$ within the columns was fairly even throughout from top to bottom. There were some cases where one section was slightly higher than the other sections, but for the most part these sections were only off by a small amount. The distribution of the columns will be further discussed in Section 3.5.

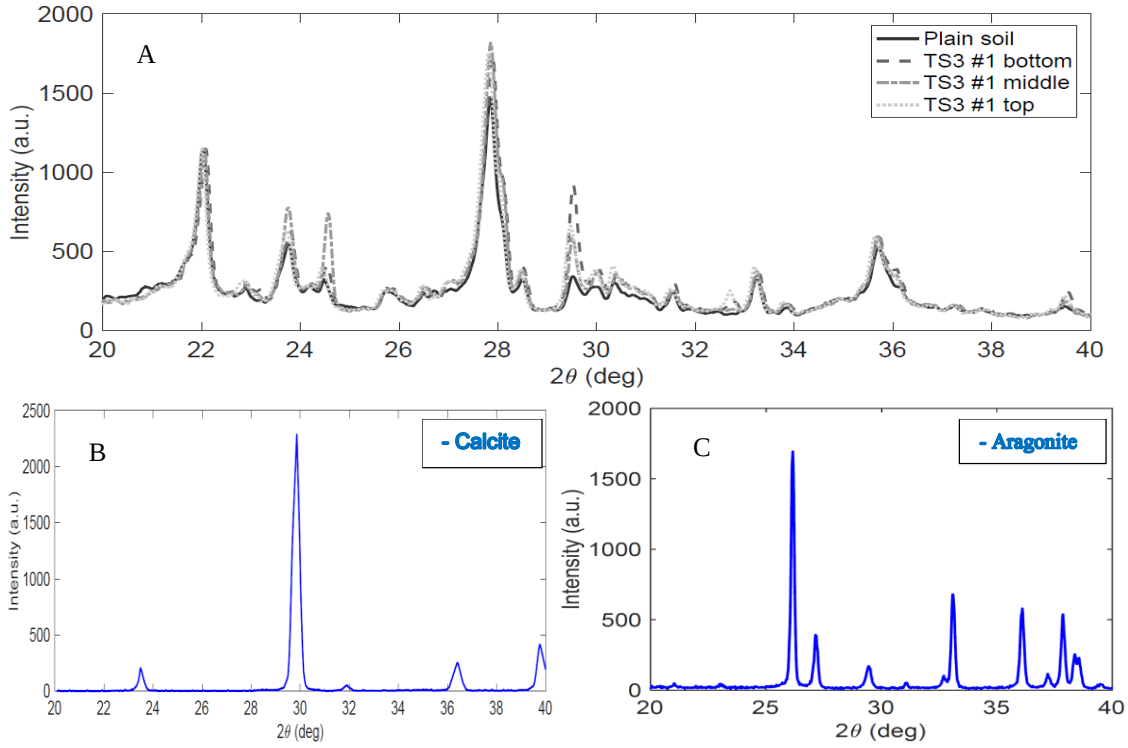

Figure 4. XRD results of the materials at different locations in the columns precipitated from the MICP process (A), the pure reagent grade $CaCO_3$ as Calcite (B), and the aragonite standard (C).

The microstructures of the biocemented columns are shown in Figure 5. After the MICP treatment, regolith particle surfaces were covered with $CaCO_3$ compared with raw regolith particles, and $CaCO_3$ crystals also filled the spaces between the regolith particles (Figures 5A, B). In other areas of the sand columns there was aragonite-like polymorphs deposited onto the regolith and after further investigation (Figure 4C) it was proven that the deposits were aragonite. The polymorphs of the $CaCO_3$ crystal deposits are dependent on the type of UPM, calcium source, and regolith type [22,23]. Further studies will be

needed to study the formation of $CaCO_3$ polymorphs in different MICP processes and the relationship between the polymorphs and properties of treatment regolith columns.

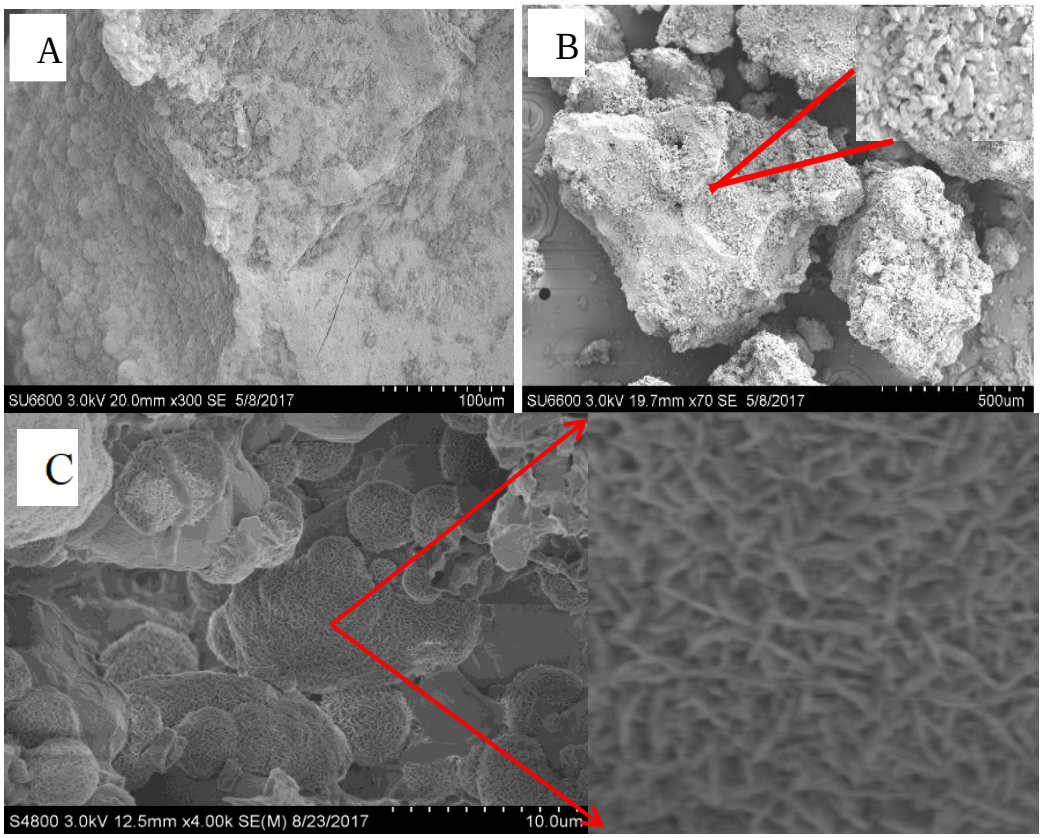

Figure 5. SEM images of raw and biocemented Martian regolith: (A) raw Martian regolith particles; (B) calcite crystals attached on the particle surface and a zoom-in view of calcite crystals (the inset), and (C) aragonite crystals attached on the particle surface and a zoom-in view of aragonite crystals (to the right).

3.2 Simultaneously and sequentially mixed biogrout recirculation

Soaking the post-grouting columns was expected to enhance the UCS results due to the biocementation resulted from the trapped cells. However, after further investigating the effects that soaking had on the strength of the columns, it was determined that there was negative correlation between soaking and column strength. Overall, the strength of the columns decreases as the time for soaking increases after recirculation and before being removed for drying (Figure 6). This can be further seen below in Table 2, when comparing the UCS results to the soaking time for each of the cell biomass circulation hours (i.e. before the nutrients were added). The results shown within the table indicate that the strengths of the columns without soaking on average are about twice as strong as those that soaked for a period of 4 days.

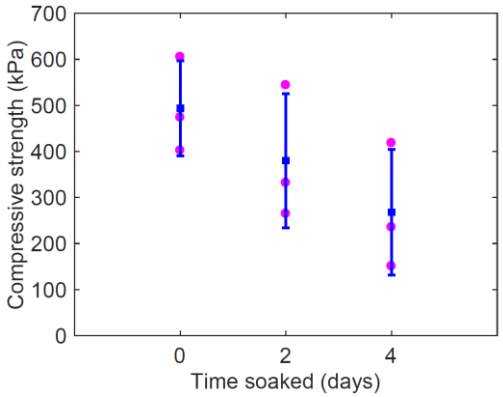

Figure 6. UCS results for 3-hour sequentially mixed reactors versus number of days soaked after completed recirculation.

As shown in Table 2, the cell biomass circulation time had an effect on the UCS results of the columns under the same soaking conditions. Based on the results shown, the optimum cell circulation time before the addition of nutrients within the reactors was 3 hours. Therefore, this gives the microorganisms a chance to penetrate further into the regolith columns before precipitating $CaCO_3$, which lead to a stronger stabilized column.

Table 2. Results for UCS of the columns by simultaneously and sequentially mixed biogrout recirculation.

Cell Biomass Circulation (hr)	Soaking Time	UCS (kPa)
0	No Soaking	365.96±44.25
	4 days	199.4±88.06
3	No Soaking	508.24±83.07
	2 days	453.18±144.24
	4 days	268.01±136.72
6	No Soaking	472.67±72.64
	4 days	337.20±156.91
9	No Soaking	385.93±35.72
	4 days	221.72±45.21
12	No Soaking	408.84±85.06
	4 days	159.34±62.23

3.3 Batch feed biogrout recirculation

Comparing version one with version two, completely separating the biomass circulation from the $CaCl_2$/urea circulation led to a decrease in the UCS of the columns from 290 to 179 kPa based on the average column strength even though the total cell biomass used in the biogrout was the same (Table 3). In the version 1, we found a large amount of $CaCO_3$ precipitation occurred in the beaker and the top of column, which clogged the column and reduced the $CaCO_3$ formation inside the columns, resulting in low UCS. This clogging could be owning to the excessive urease activity, which made $CaCO_3$ precipitation occur too fast outside the columns [24]. Therefore, the third version of biogrout circulation method used an increased daily amount of cell biomass and $CaCl_2$/urea in each circulation and reduced the biogrout circulation time to 2 days in order to mitigate the clogging problem.

Based on the results from version 3, the strength of the columns doubled compared to version 1 (Table 3). The increase in strength of columns could be due to the increased cell biomass distribution within the columns because higher biomass loading compared to version 1 and higher urease activity stayed inside the columns. This allowed for fresher biomass to grow by using the nutrients that was added after the 3-hour biomass circulation, which in turn led to better distribution and strength though out the columns.

Table 3. Results for UCS of the columns by batch feed biogrout recirculation.

Version	Soaking Time	UCS (kPa)
1	3 days	290.43±46.10
2	3 days	179.81±54.62
3	No Soaking	732.40±117.84
	4 days	538.58±210.04
4	No Soaking	728.99±106.17
5	No Soaking	468.09±123.42

To reduce the amount of DDI water needed in the production of the biocemented columns, biogrout made from biomass and growth media was utilized to produce a set of columns and was compared to a set of columns produced with biogrout made of biomass and DDI water. The expected results of this experiment was that the biogrout made with growth media would produce similar results to that of the one made from DDI water. Therefore, both systems were recirculated until no liquid passed through the bottom of the reactors. The system utilizing a growth media based biogrout completed its recirculation period one day earlier than the system using the DDI water based biogrout, even though both systems were fed the same amount of biomass and nutrients each day.

After conducting the UCS test, it was discovered that the columns produced using the growth media biogrout were weaker than those produced with DDI water (Figure 7 A, B).

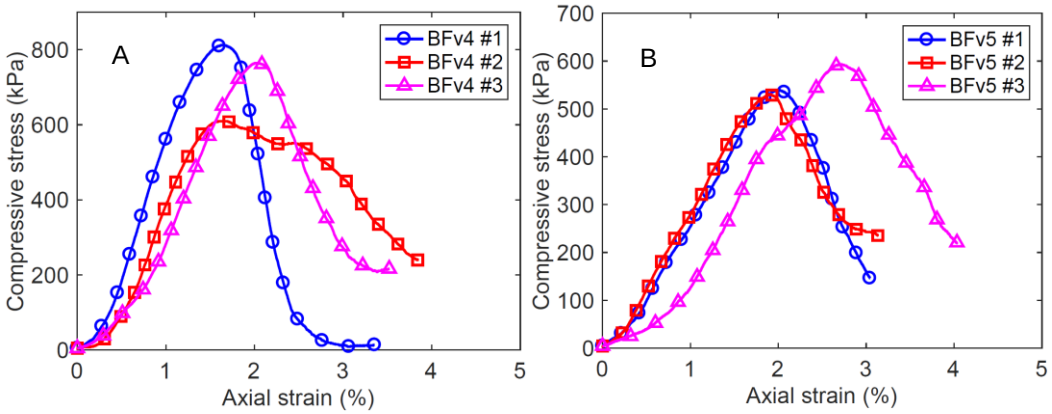

Figure 7. UCS of columns made using batch biogrout circulation methods including DDI water based biogrout (A) and growth media based biogrout (B).

The decrease in strength may be attributed to the salts within the growth media blocking the pour spaces which would normally be occupied by the UPM; this could also be the reason why the system stopped recirculating a day earlier than the DDI water based biogrout column (Figure 7A). The results of Figure 7A at the time were the best results produced utilizing the batch biogrout circulation method, but the method took six days (five for circulation and one for drying) to produce the columns for testing. After further testing the batch biogrout circulation method version 3 without soaking, it took only three days (two for circulation and one for drying) to produce the columns with similar results to those seen in Figure 7A in half the amount of time (Table 3). Therefore, the standard operation procedure (SOP) developed will follow the biogrout recirculation method for version 3 without soaking. Further studies will need to be conducted to optimize these conditions to reduce the amount of time needed to produce the biocement columns.

3.4 Hydraulic conductivity

The hydraulic conductivity of the untreated MMS regolith was about 4.42×10^{-3} cm/s, whereas the results for the biocemented columns following the SOP were 2.06×10^{-3} cm/s (Table 4). Therefore, the results indicated that there was a reduction of more than 50% permeability for the biocemented columns due to the precipitation of $CaCO_3$. However, it was observed that there were notable variations in the permeability of the SOP biocemented columns, with a coefficient of variation of about 33%. There are two possible reasons for this variation. The first possible reason could be due to the variation of $CaCO_3$ concentrations that were precipitated within the columns. The second possible reason would be due to the random distribution of the precipitated $CaCO_3$ within the columns, which would lead to different patterns within the internal structure of the columns for water passage. Both are reasonable explanations for the change of permeability shown in the SOP biocemented columns. Future research will need to be conducted to develop a better understanding of the relationship between the internal structure of the biocemented columns and the distribution of the precipitated $CaCO_3$ within the columns.

Table 4. Hydraulic conductivity results for untreated and treated regolith columns.

ID (two trials each)	Permeability ($\times 10^{-3}$ cm/s)	Mean ($\times 10^{-3}$ cm/s)
Untreated Regolith Column	4.56 4.27	4.42
SOP Regolith Column 1	2.58 2.95	2.77
SOP Regolith Column 2	2.40 2.42	2.41
SOP Regolith Column 3	0.97 0.98	0.98
SOP Regolith Column 4	1.35 1.36	1.36
SOP Regolith Column 5	2.56 2.43	2.49
SOP Regolith Column 6	2.32 2.39	2.36

3.5 Carbonate titration to quantified $CaCO_3$ precipitation

After completing the carbonate titration analysis test for all of the columns produced via the different methods of recirculation, the overall average concentration of $CaCO_3$ precipitated within the columns was 12.21%. Whereas, the average concentrations of $CaCO_3$ for the SOP was 12.65%. While further investigating the concentrations of $CaCO_3$ deposits throughout the columns produced utilizing the current SOP, it was found that the top portion of the columns contained percentages greater than or equal to 12.50, whereas the bottom portion of the columns contained percentages less than or equal to 12.25. The middle portions of the columns $CaCO_3$ concentrations varied significantly, this may be attributed to the random distribution of the regolith grains within the columns and the reduction of biogrout flow through the columns due to the top

portion clogging. Further investigations will need to be conducted to find out the reasons behind this phenomenon. At first it was believed that the microalga was able to produce an adequate amount of $CaCO_3$ within the columns after the biocementation process. Though upon a carbonate titration analysis of the raw MMS regolith, it was found that the calcium concentration of the soil sample was 9.91%. Overall, the calcium oxide concentration of the raw MMS regolith 10.45%, and therefore there is some room for error with the carbonate titration analysis test. Though the main focus of these results was that the microalga deposited only a small portion of $CaCO_3$ within the biocemented columns.

While investigating the relationship between the UCS and $CaCO_3$ concentration of the biocemented columns, it was found that the relationship has the potential to be affected by the concentration of the $CaCl_2$ and the molar ratio of $CaCl_2$ to urea. Based on the results for the columns produced with 17.64 g of $CaCl_2$ following the SOP method, it can be seen that there was an inverse relationship between UCS and $CaCO_3$ concentration (Figure 8A). This trend was also seen in the relationship between UCS and the molar ratio of $CaCl_2$ to urea (Figure 9A). Upon reducing the $CaCl_2$ concentration to 7.2 g, it was seen that there was no relationship between the UCS and the molar ratio of $CaCl_2$ to urea (Figure 9B). While the relationship between the UCS and $CaCO_3$ concentration had a positive correlation (Figure 8B). This positive correlation was mainly due to the biocemented columns produced utilizing a molar ratio of 1:4.53. If this single data point was taken out of the figure (not shown) for the columns produced utilizing 7.2 g of

$CaCl_2$, then the results would have shown that there was no relationship between UCS and $CaCO_3$ concentrations.

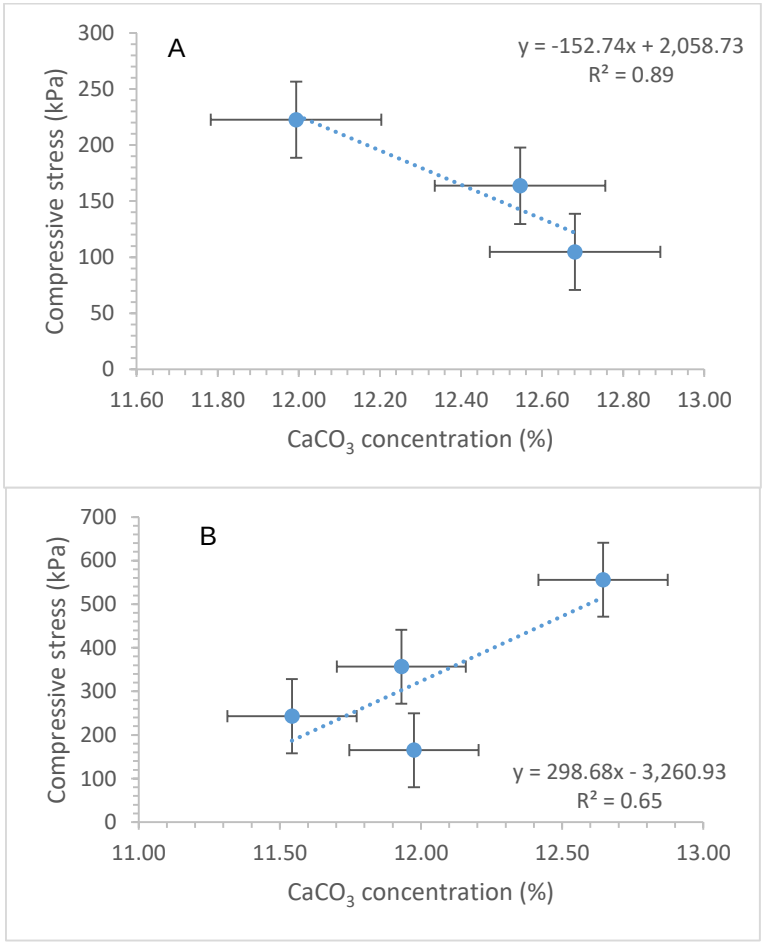

Figure 8. Results for UCS and $CaCO_3$ concentrations for columns produced utilizing 17.64 g of $CaCl_2$ (A) and 7.2 g of $CaCl_2$ (B).

The reason why this single point caused such a large change in the figure was due to this set of columns had produced an average UCS value of 555.95kPa and a maximum UCS value of 815.72 kPa. It was also noticed while comparing the UCS values to the $CaCO_3$ concentrations that there were columns that had the same $CaCO_3$ concentrations values, but a significant difference in UCS values. An example of this would be columns that have a $CaCO_3$ concentration of 12.27%, but a difference in UCS values of 263.14

kPa. The main cause of this difference could be attributed to the internal structure of the columns. A difference in the internal structure of the biocemented columns would have led to a difference in the placements of the $CaCO_3$ deposits within the columns; therefore leading to the difference in the strengths of the columns during the UCS tests.

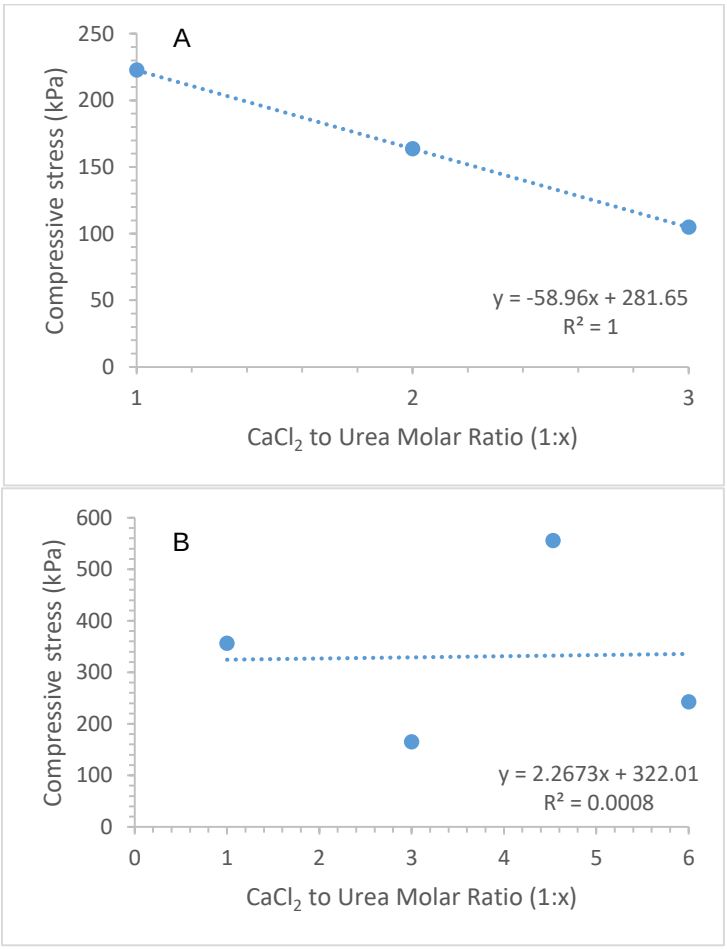

Figure 9. Results for UCS at different $CaCl_2$ to urea molar ratios for columns produced utilizing 17.64 g of $CaCl_2$ (A) and 7.2 g of $CaCl_2$ (B).

During the investigation of the relationship of UCS and the molar ratio of $CaCl_2$ to urea, it was found that for columns made with 17.64 g of $CaCl_2$ that the pH of the system, measured from the biogrout, stayed at around a pH of 7.0 for both after the first and second day of circulation. Whereas, the pH of the columns made utilizing 7.2 g of

$CaCl_2$ increased with the increase in urea. After the first day of circulation all methods utilizing different ratios had a pH of 7.0, but after the second day of circulation the pH of the systems increased except for the method that had a molar ratio of 1:1. This showed that the increase in urea lead to an increase in urease production as well as an increase in the pH of the system. Though this does not explain why there was no increase in the pH of the system due to an increase in urea for the columns made with 17.64 g of $CaCl_2$. The reason why there was no increase in the pH of the system may be due to the large amount of $CaCl_2$ inhibiting the microalgae, and therefore inhibiting the process of urease production. Overall, the inhibition of the microalgae may be the reason why the columns made utilizing 17.64 g of $CaCl_2$ were fairly weaker when compared to the columns made utilizing 7.2 g of $CaCl_2$.

3.6 Calcium acetate

Through simulating AD of food waste by utilizing pure acetic acid to degrade limestone into a calcium acetate solution will help to develop an understanding to prove if AD is a viable solution to solve the calcium source dilemma for the Mars mission. Based on the results found in the batch feed biogrout recirculation method and carbonate titration tests, it was originally believed that by utilizing the SOP and a molar ratio of 1:4.53 that it was possible to produce columns of the same structural strength by utilizing a calcium acetate solution instead of $CaCl_2$. During the column manufacturing process there was a significant amount of urease activity by the end of the first day, therefore the majority of the calcium carbonate had precipitated either in the beaker containing the biogrout or on top of the Scotch-Brite scour pad used for distribution of the biomass

instead of inside the column. To mitigate the issue, the columns were manufactured with the molar ratios of 1:1 and 1:0. Reducing the amount of urea within the manufacturing process would reduce the amount of urease activity, which would allow for the biomass to have the opportunity to precipitate within the columns. The columns produced at a molar ratio of 1:0 were utilized as a control method with the belief that by using the acetate within the calcium acetate solution as a carbon source the microalgae would produce the carbonate needed instead of via urea hydrolysis.

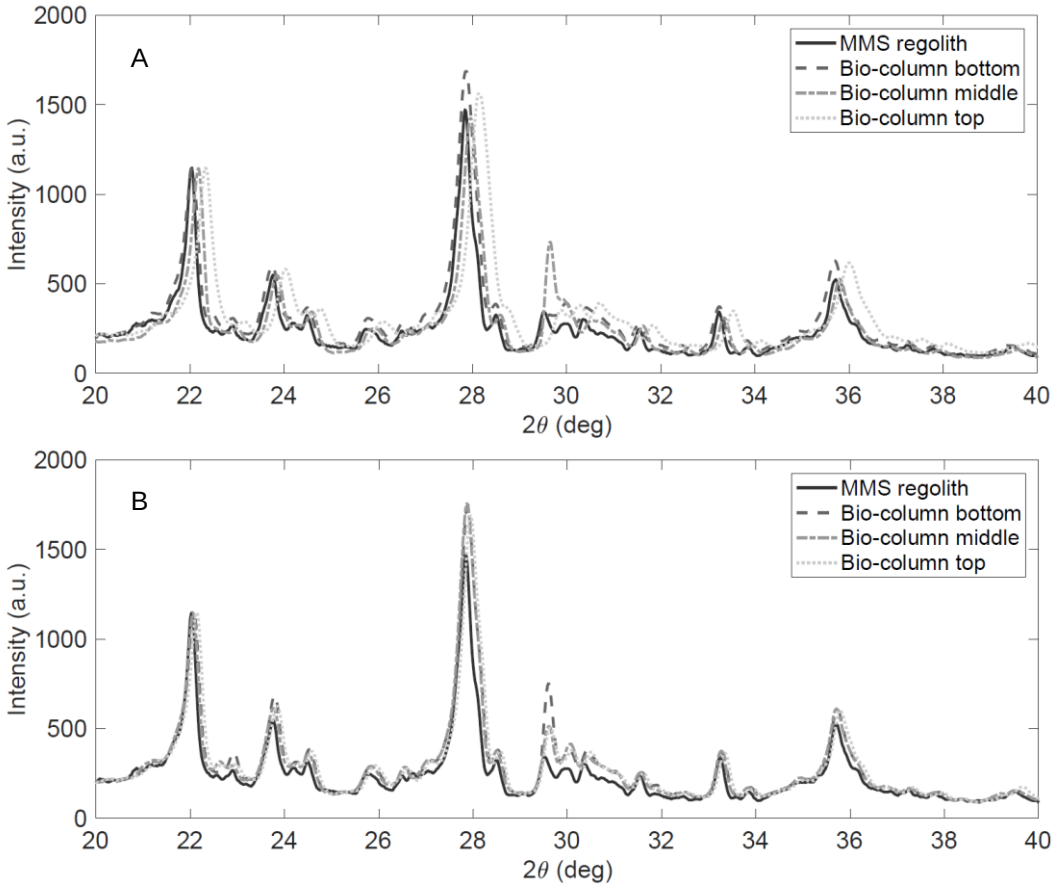

Figure 10. XRD results of biocemented columns made with calcium acetate at molar ratios of (A) 1:0 and (B) 1:1.

The XRD results of the biocemented columns produced utilizing a calcium acetate solution at the molar ratios of 1:0 and 1:1 can be seen in Figure 10. Overall,

similar to the XRD results of the columns produced with $CaCl_2$ (Figure 4), there was calcite detected at 29-30 degrees (2θ) and aragonite at 33 degrees (2θ). The XRD results for the 1:0 molar ratio columns prove that the microalgae were able to precipitate $CaCO_3$ without utilizing urea hydrolysis. By utilizing the acetate form the simulated AD of food waste as a carbon source to produce carbonate instead of using urea for urea hydrolysis, this can help reduce the issue with the ammonia emissions during the biocementation manufacturing process. Though further investigations will need to be conducted on the utilization of acetate versus urea as a carbon source in the production of carbonate with the microalgae.

Table 5. UCS results for various molar ratios of calcium acetate to urea.

Molar Ratio	UCS (kPa)
1:0	N/A
1:1	237.80±46.14
1:4.53	168.11±85.19

Overall, the compressive strength of the columns produced utilizing the calcium acetate solution were low (Table 5) compared to the columns manufactured with $CaCl_2$. As for the columns produced utilizing the 1:0 molar ratio, all specimens collapsed after 30 minutes of being removed from the reactor and placed within the oven for drying (Figure 11). The collapse of the 1:0 molar ratio columns may be attributed to the low levels of $CaCO_3$ precipitation within the columns (Figure 12). Compared to the other molar variations, the 1:0 molar ratio columns contained the lowest concentration of $CaCO_3$. Whereas, the 1:4.53 molar ratio columns contain the highest concentration of $CaCO_3$, but had a lower compressive strength compared to the 1:1 molar ratio columns.

Figure 11. Calcium acetate 1:0 molar ratio columns after drying for 24 hours at 60°C.

The difference in the strength of the two variations may be attributed to the distribution of the $CaCO_3$ within the different portions of the columns. The columns that were weak had a large difference between the distribution of $CaCO_3$ within the top, middle, and bottom portions of the columns. Whereas, the columns with a much smaller difference in $CaCO_3$ concentrations within the different portions were stronger. Though further investigations will need to be conducted to clarify this phenomenon.

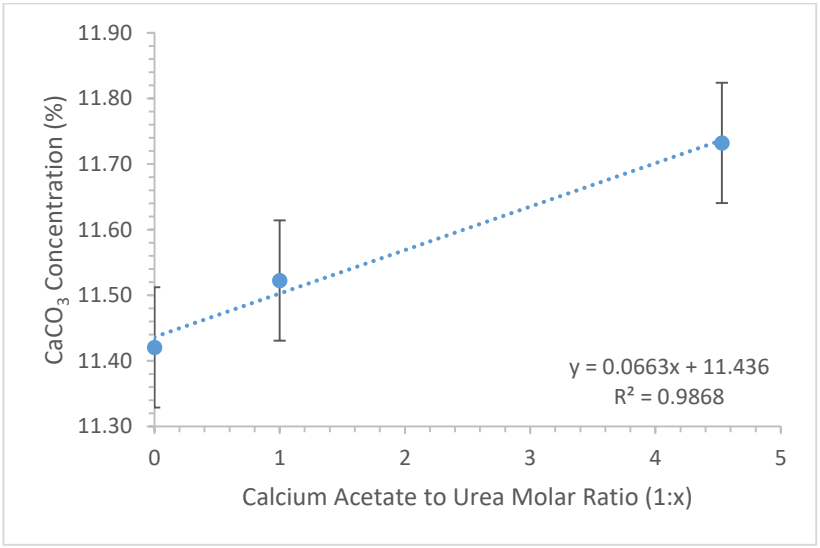

Figure 12. Results for $CaCO_3$ concentrations at different molar ratios of calcium acetate to urea.

Each of the three variations of the molar ratios were subjected to hydraulic conductivity tests. Based on the results of the tests (Table 6), there was a reduction of more than 90% permeability of the columns produced utilizing the calcium acetate solution compared to the untreated MMS regolith. Though based on the previous results, the precipitation of $CaCO_3$ within the columns was less than those of the columns produced utilizing the SOP and $CaCl_2$. Therefore, the decrease in permeability cannot be attributed to just the precipitated $CaCO_3$. Other reasons for the decrease in the permeability may be due to the internal structure of the columns and any remaining unused calcium acetate. Though to clarify this phenomenon, further tests will need to be conducted to find the relationship between the internal structure of the columns, the precipitated $CaCO_3$, and the calcium acetate solution utilized for the production of the biocemented columns.

Table 6. Hydraulic conductivity results for biocemented columns produced utilizing calcium acetate solution.

ID (two trials each)	Permeability ($\times 10^{-4}$ cm/s)	Mean ($\times 10^{-4}$ cm/s)
AD 1:0 Regolith Column 1	0.66 0.60	0.63
AD 1:0 Regolith Column 2	0.48 0.44	0.46
AD 1:0 Regolith Column 3	0.89 0.82	0.86
AD 1:1 Regolith Column 1	0.53 0.55	0.54
AD 1:1 Regolith Column 2	0.86 0.81	0.84
AD 1:1 Regolith Column 3	1.32 1.16	1.24
AD 1:4.53 Regolith Column 1	3.79 3.44	3.62
AD 1:4.53 Regolith Column 2	0.28 0.26	0.27
AD 1:4.53 Regolith Column 3	1.93 2.14	2.04

4.0 Conclusions

In this study, bioprocesses were developed to produce bioconcrete columns utilizing potentially Mars-compatible microalgae and simulated Martian regolith. The tested microalga, *T. striatum*, can function as a UPM for the biocementation of Martian regolith. In the presence of $CaCl_2$ and urea, the urease activity of *T. striatum* can generate $CaCO_3$ in the form of calcite and aragonite on the surface regolith grains and bind grains to form strong regolith-stones, as evidenced by XRD analysis and SEM images. Biogrout recirculation methods had significant impacts on the properties of the regolith columns. It was found that the batch feed biogrout recirculation method of cell biomass and

$CaCl_2$/urea achieved higher UCS compared to the simultaneously and sequentially mixed biogrout recirculation methods.

The relationship between the strength of the columns and soaking time were found to have an inverse relationship. Therefore, post-biogrout circulation column soaking is not necessary. The removal of the biomass from the growth media via centrifugation is important due to the decrease in the strength of the columns from biogrout made with growth media instead of DDI water. The hydraulic conductivity of the SOP biocemented columns had a reduction of more than 50% permeability compared to the simulated Martian regolith. The average concentration of $CaCO_3$ found precipitated within the biocemented columns was 12.21%±0.79%.

Utilizing calcium acetate instead of $CaCl_2$ resulted in a significant decrease in the permeability of the biocemented columns compared to the untreated MMS regolith by 90%. *T. stratum* was able to utilize acetate as a carbon source to precipitate carbonate, instead of using urea through urea hydrolysis. The average $CaCO_3$ concentration within the columns produced with calcium acetate was 11.50%±0.34%. Overall, *T. stratum* has proven to be a promising urease producing microbial for biocementation. Lastly, further research will need to be conducted on optimizing the regolith particle size, biomass loading rate, and nutrient loading rate.

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Appendix

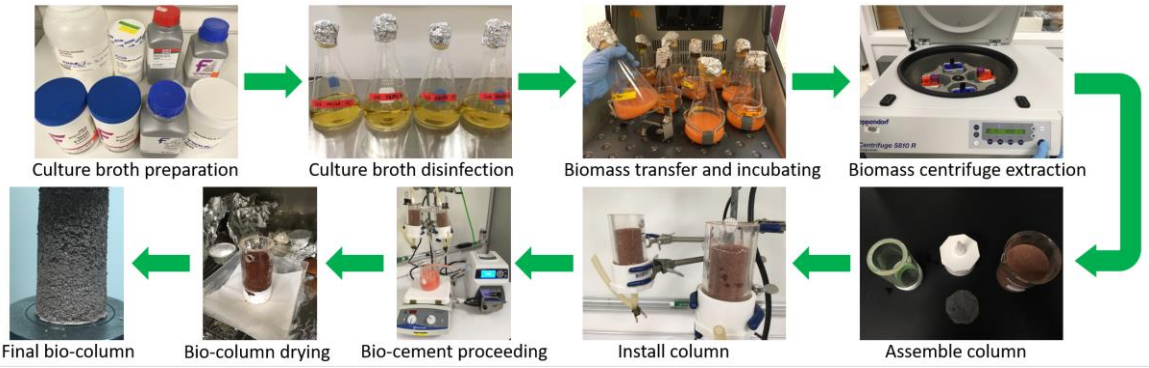

Figure A.1. Flow diagram of the general biocementation manufacturing process.

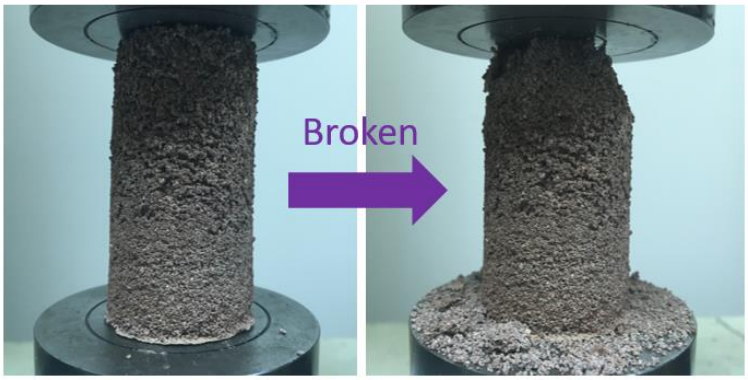

Figure A.2. Example of UCS test.

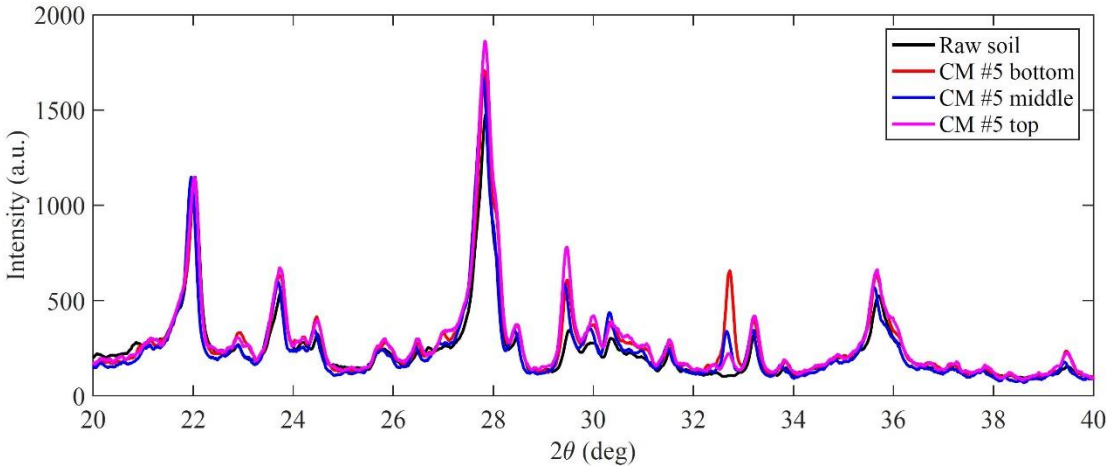

Figure A.3. XRD results of the materials at different locations in the, simultaneously mixed biogrout recirculation, columns precipitated from the MICP process.

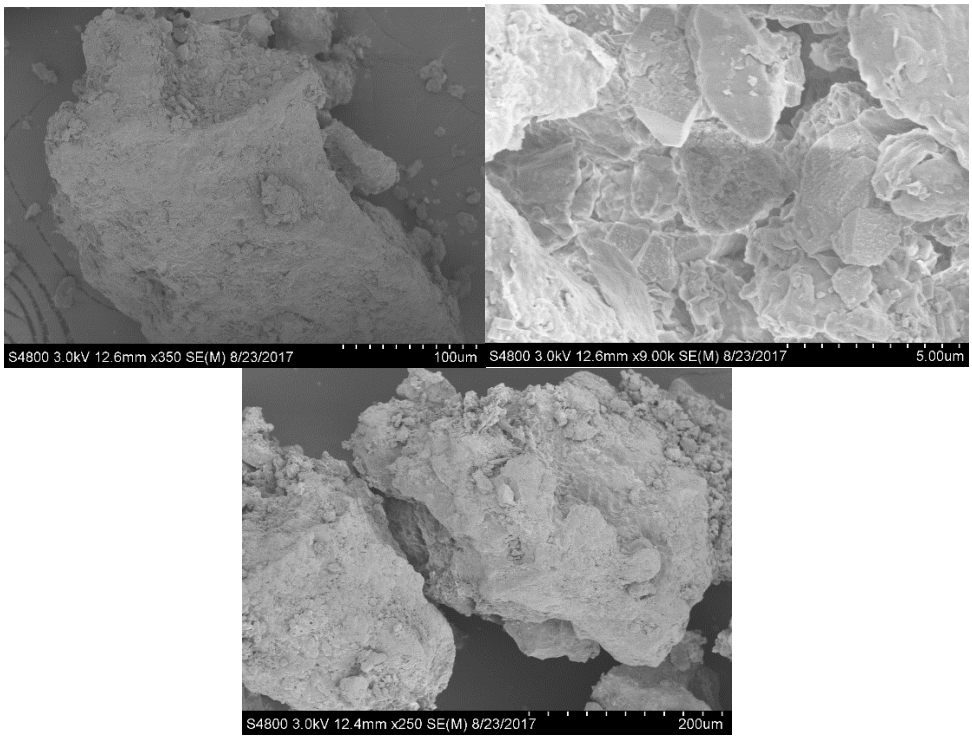

Figure A.4. SEM images from bottom portion of simultaneously mixed biogrout recirculation column.

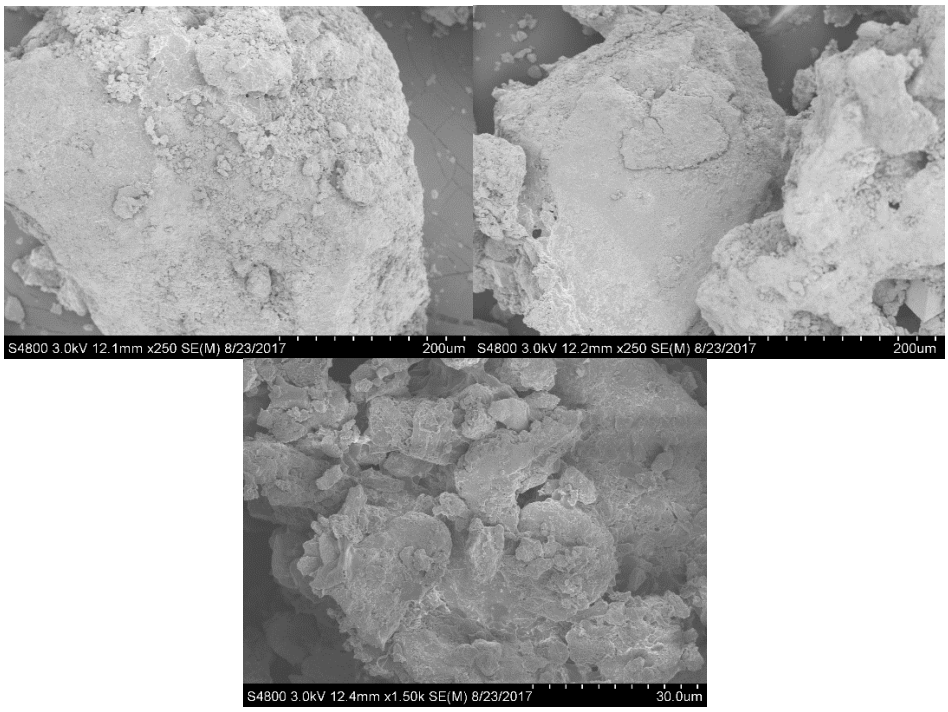

Figure A.5. SEM images from middle portion of simultaneously mixed biogrout recirculation column.

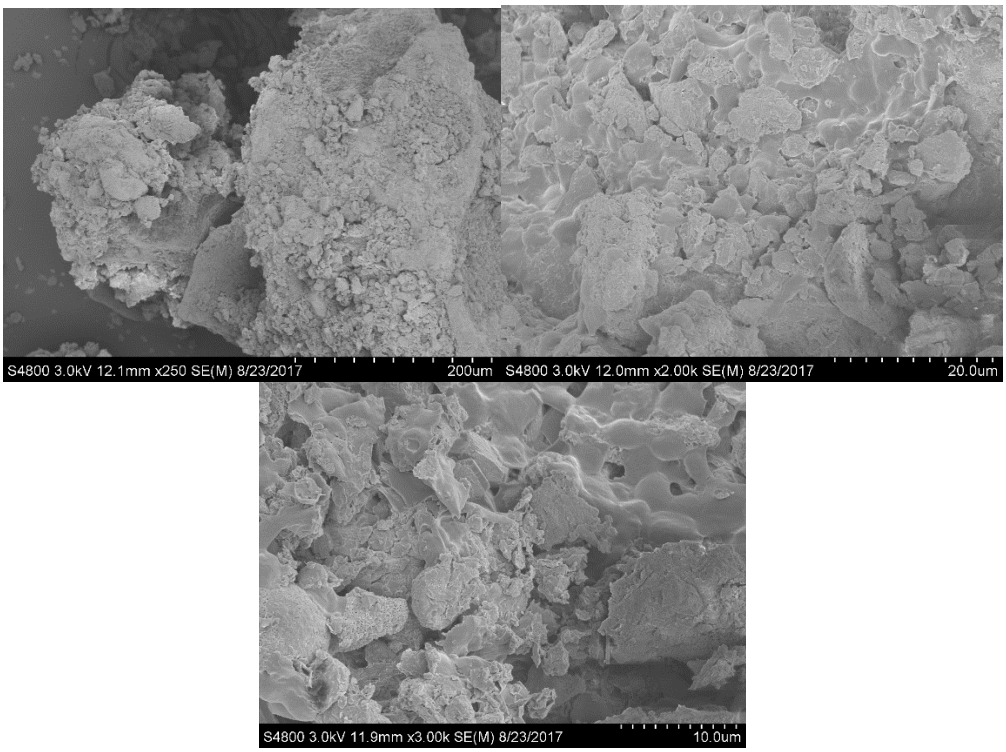

Figure A.6. SEM images from top portion of simultaneously mixed biogrout recirculation column.

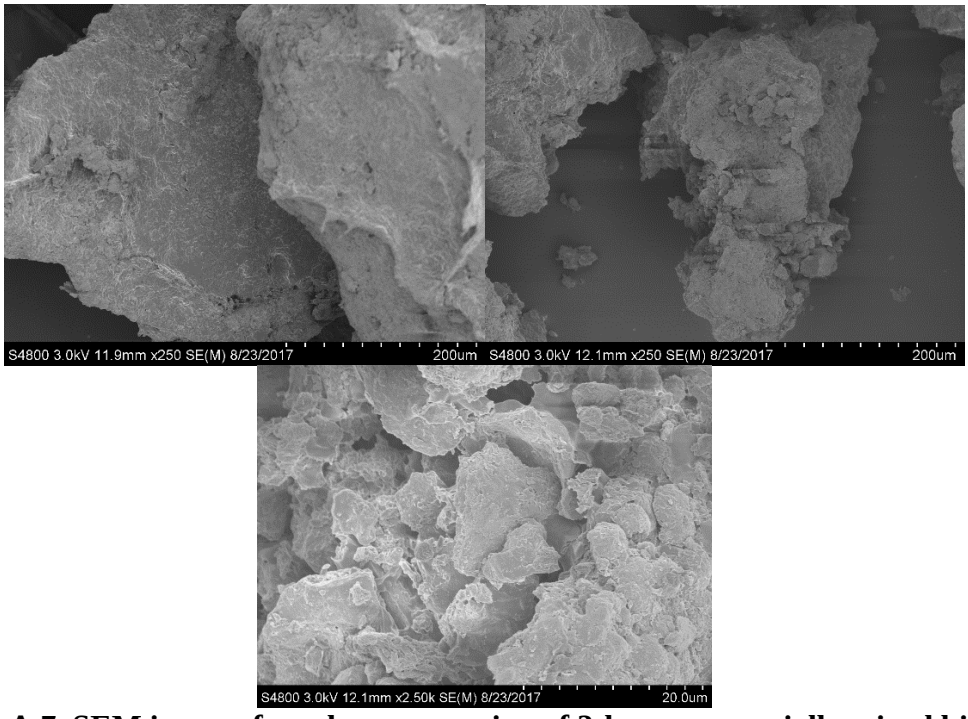

Figure A.7. SEM images from bottom portion of 3-hour sequentially mixed biogrout recirculation column.

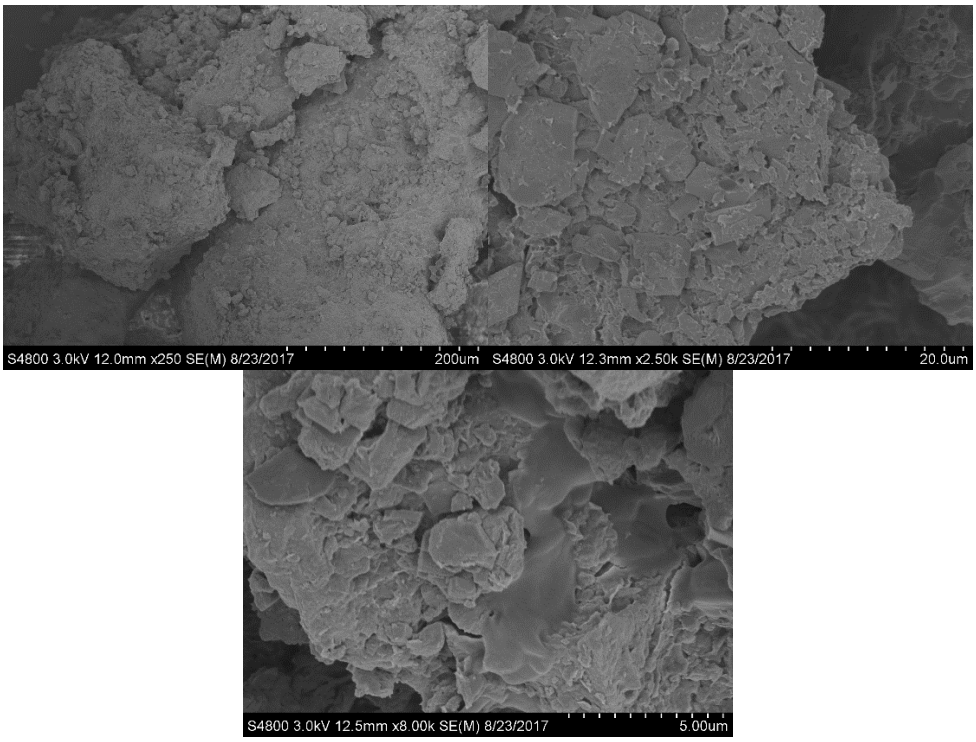

Figure A.8. SEM images from middle portion of 3-hour sequentially mixed biogrout recirculation column.

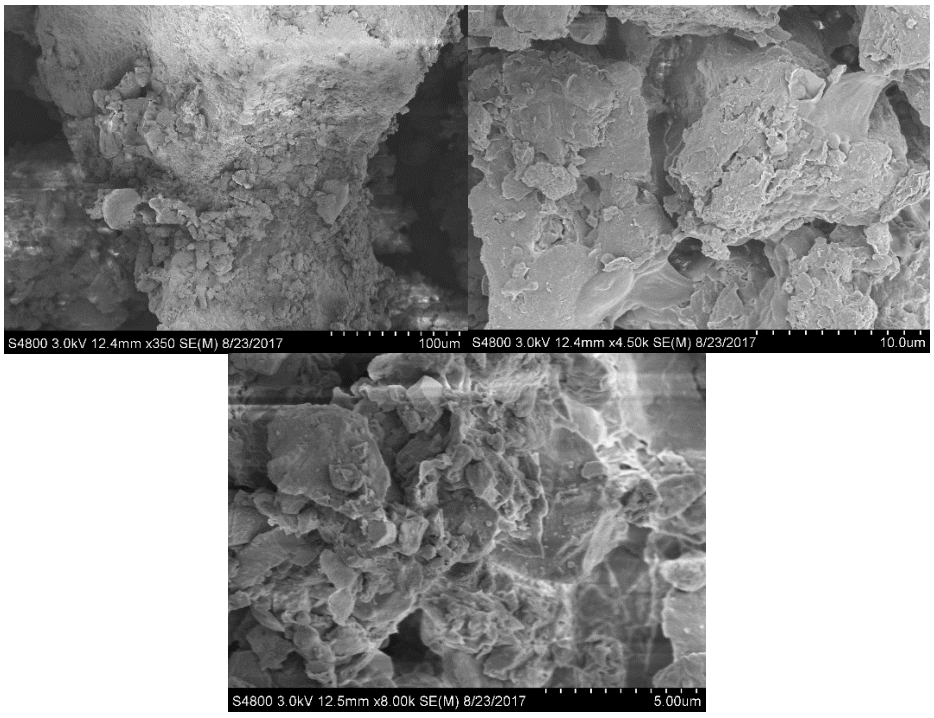

Figure A.9. SEM images from top portion of 3-hour sequentially mixed biogrout recirculation column.

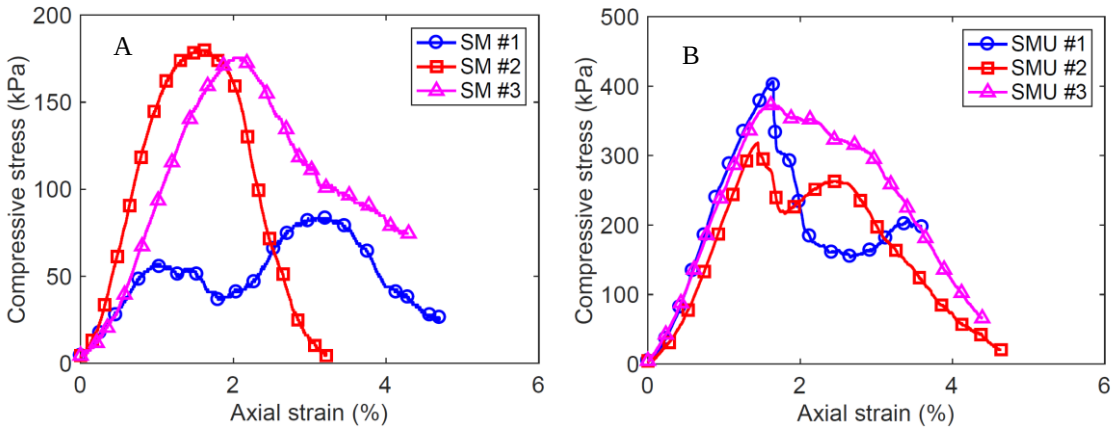

Figure A.10. UCS results for simultaneously mixed biogrout recirculation columns with (A) four days of soaking and (B) no soaking.

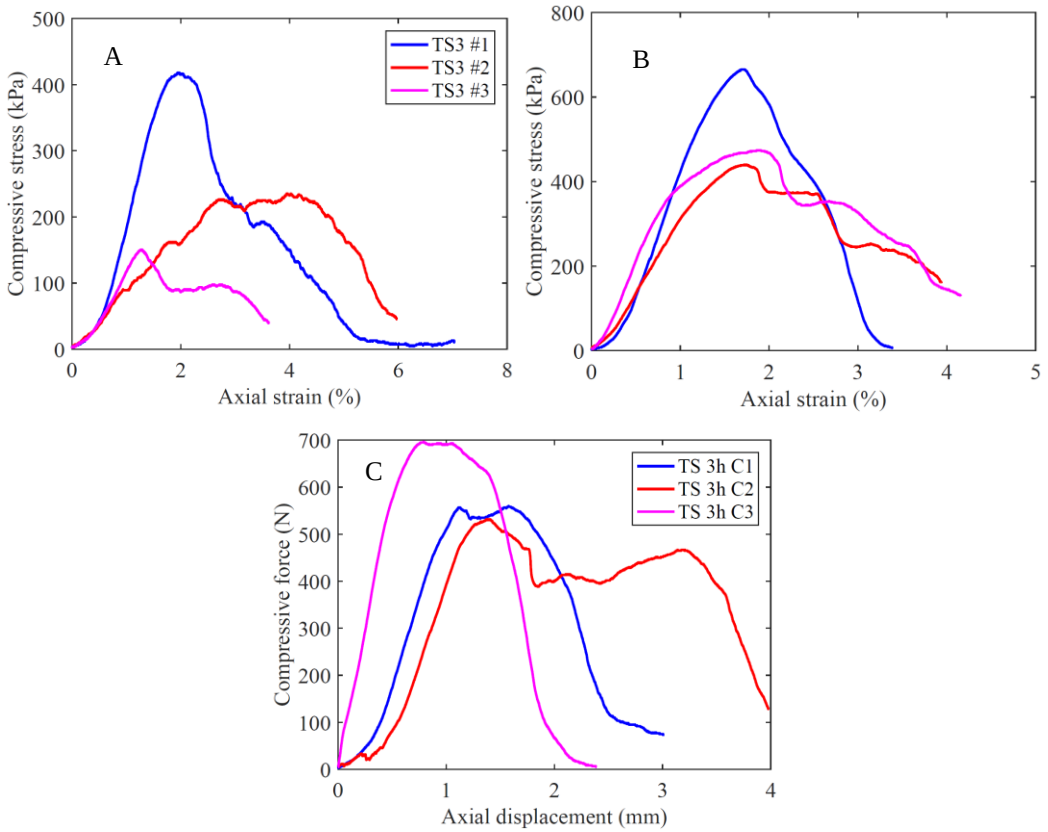

Figure A.11. UCS results for 3-hour sequentially mixed biogrout recirculation columns with (A) four days of soaking, (B) two days of soaking, and (C) no soaking.

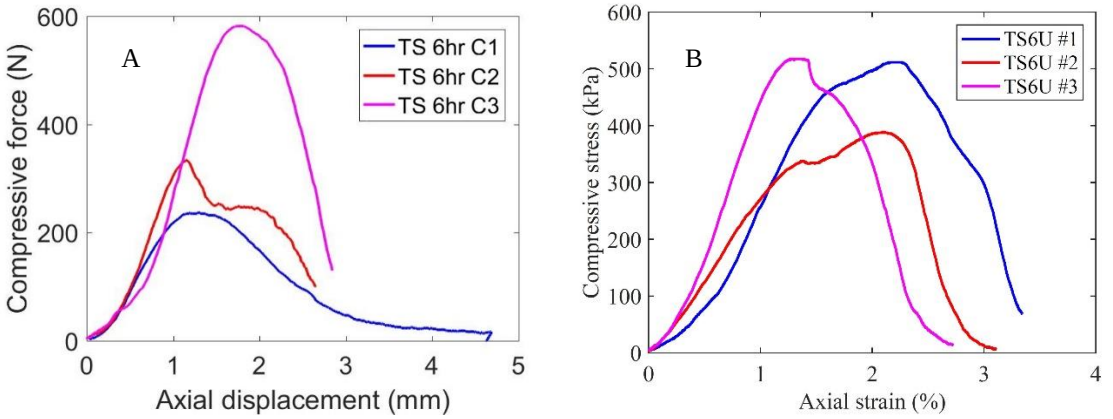

Figure A.12. UCS results for 6-hour sequentially mixed biogrout recirculation columns with (A) four days of soaking and (B) no soaking.

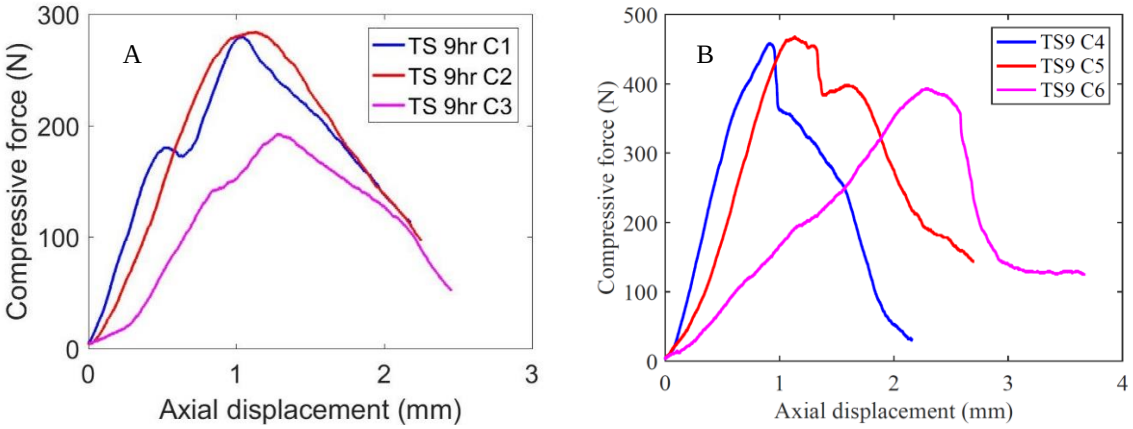

Figure A.13. UCS results for 9-hour sequentially mixed biogrout recirculation columns with (A) four days of soaking and (B) no soaking.

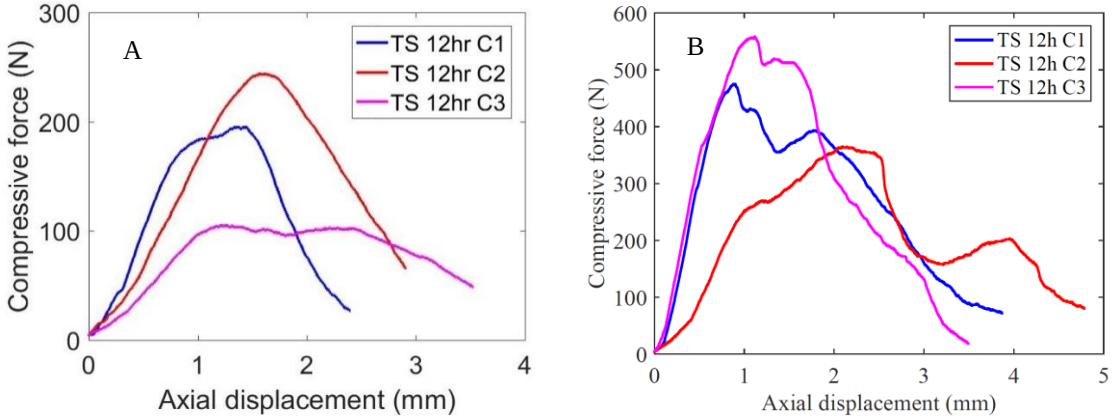

Figure A.14. UCS results for 12-hour sequentially mixed biogrout recirculation columns with (A) four days of soaking and (B) no soaking.

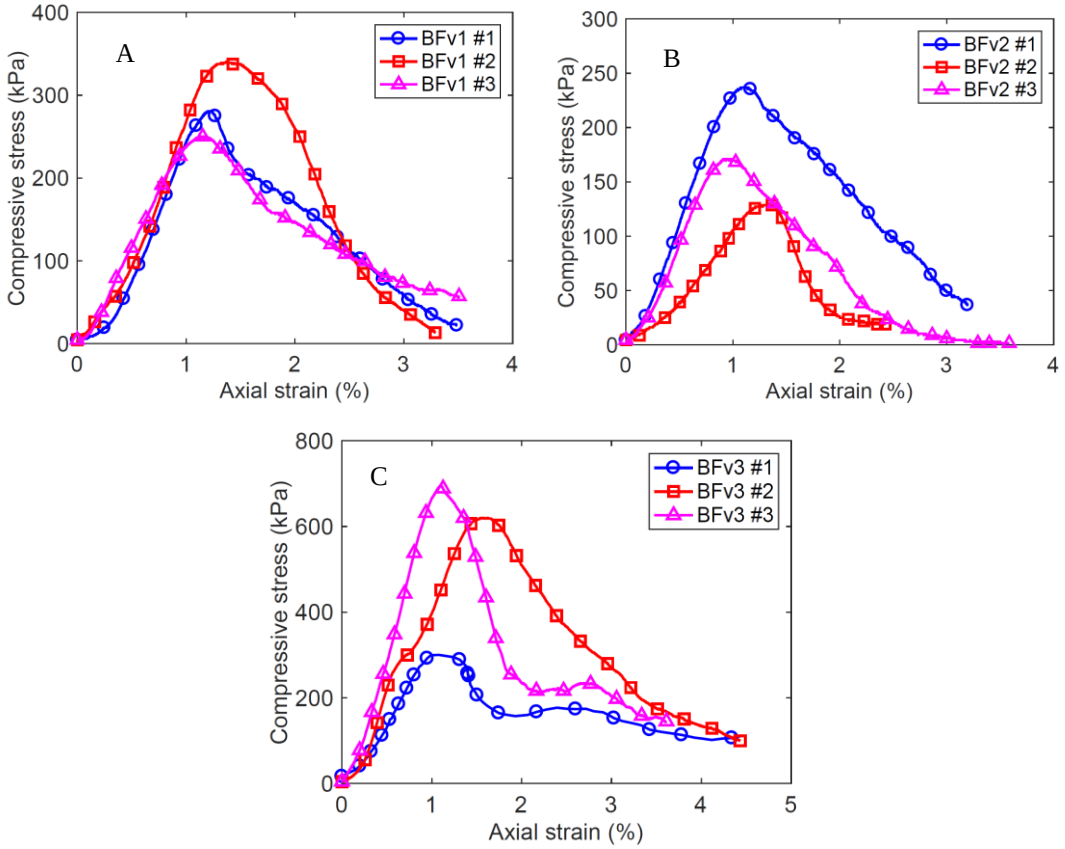

Figure A.15. UCS results for batch feed biogrout recirculation (A) version one, (B) version two and (C) version three columns.

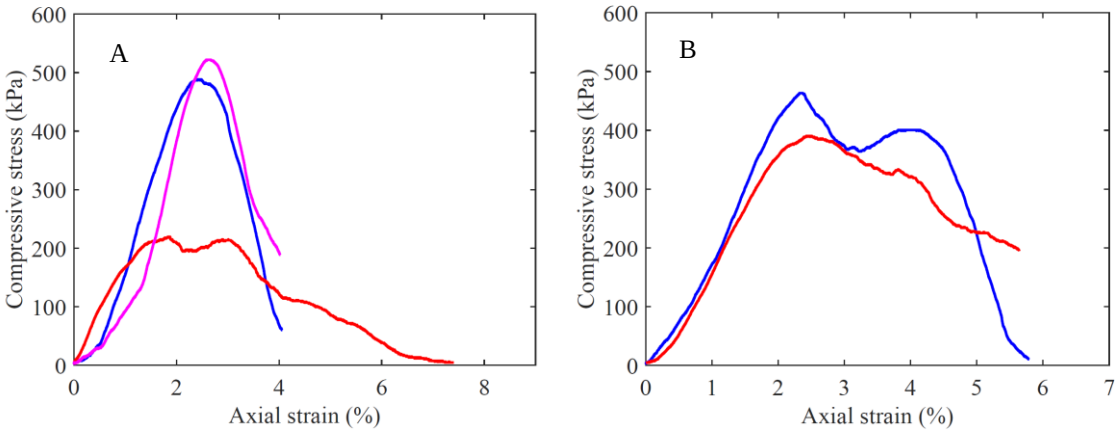

Figure A.16. UCS results for batch feed biogrout recirculation version three columns without soaking, utilizing (A) old plastic disk distributor and (B) new Scotch-Brite scour pad distributor.

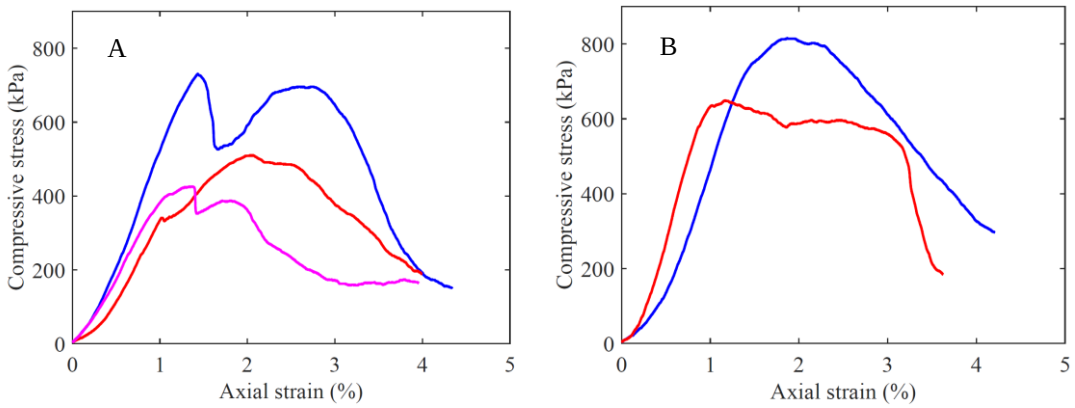

Figure A.17. UCS results for batch feed biogrout recirculation version three columns utilizing wax paper for column removal with (A) four days of soaking and (B) no soaking.

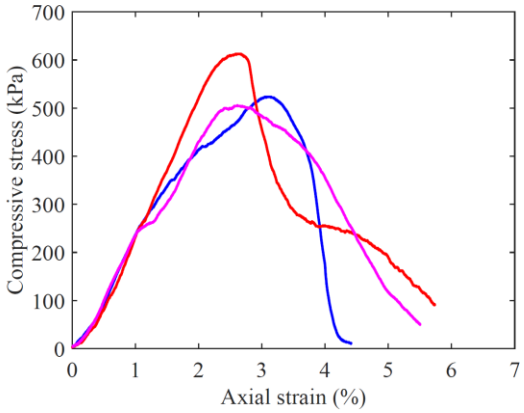

Figure A.18. UCS results for batch feed biogrout recirculation version three columns, without soaking, utilizing both wax paper for column removal and new Scotch-Brite scour pad distributor.

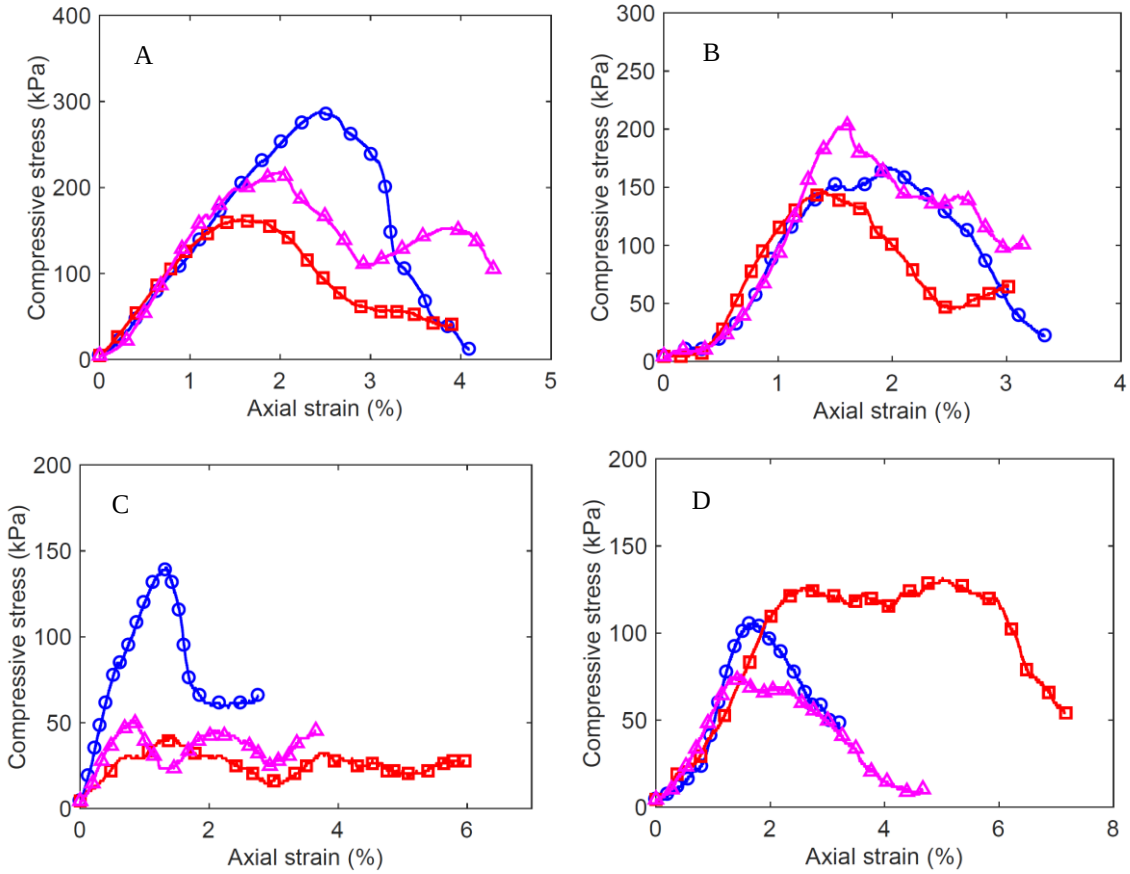

Figure A.19. UCS results for SOP columns produced with 17.64 grams of $CaCl_2$ at molar ratios of (A) 1:1, (B) 1:2, (C) 1:2, and (D) 1:3.

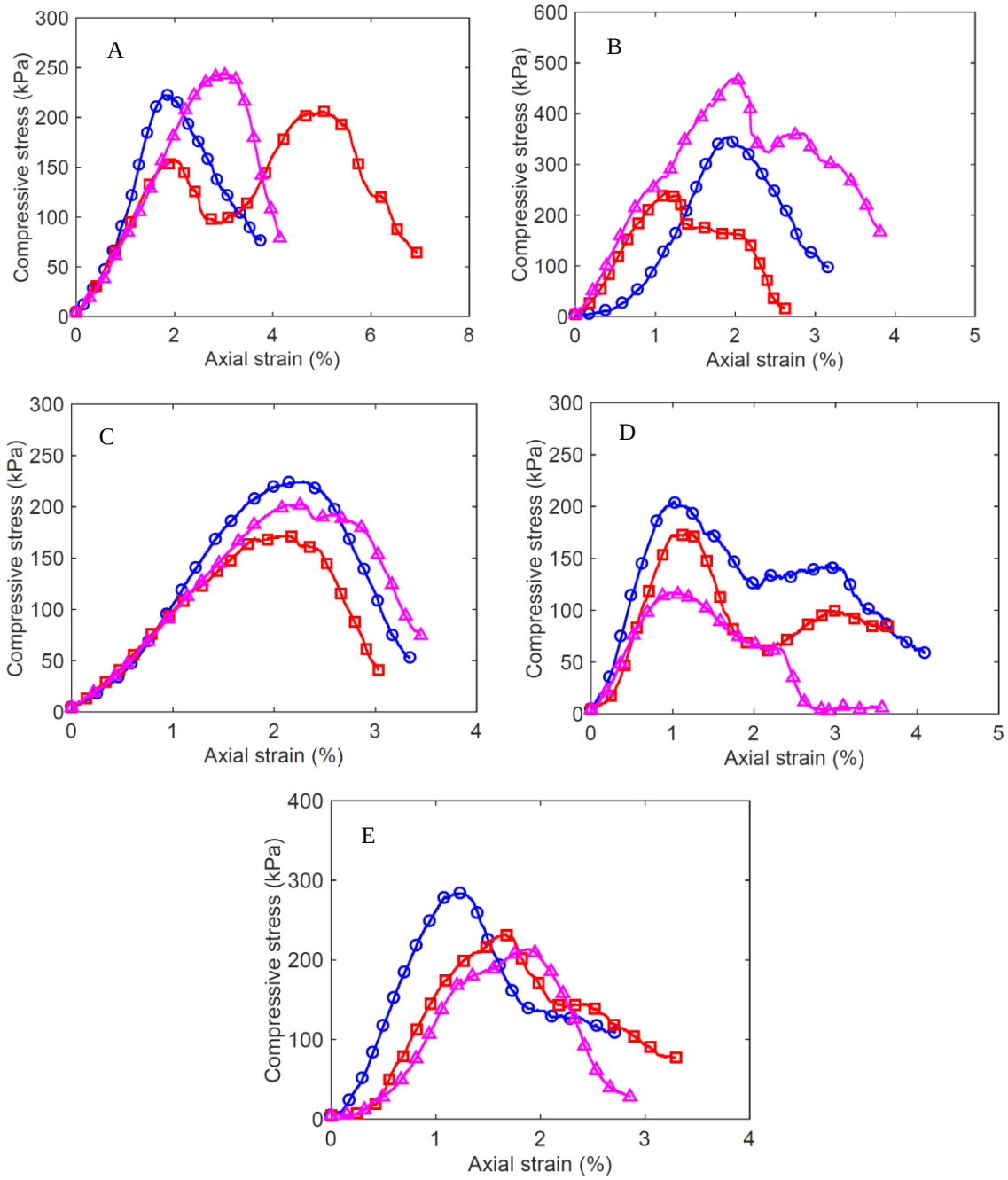

Figure A.20. UCS results for SOP columns produced with 7.2 grams of $CaCl_2$ at molar ratios of (A) 1:1, (B) 1:1, (C) 1:3, (D) 1:3, and (E) 1:6.

Table A.1. UCS and carbonate titration results for simultaneously mixed biogrout recirculation columns with four days of soaking and no soaking.

UCS (kPa)	235.36	323.07	83.33	179.81	175.42	406.4	318.69	372.78
Method	CM	CM	CM	CM	CM	CMU	CMU	CMU
Column	C1	C2	C1	C4	C5	C1	C2	C3
Date	14-Jun	14-Jun	26-Jun	5-Jul	5-Jul	16-Aug	16-Aug	16-Aug
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)								
Test 1	15.7	17.5	14.2	12.9	16.2	16.0	18.1	16.4
Test 2	15.8	18.0	13.0	12.4	16.1	16.3	17.9	16.0
Test 3	15.5	17.4	13.0	12.6	15.8	16.5	17.6	16.4
Average	15.67	17.63	13.40	12.63	16.03	16.27	17.87	16.27
NaOH (mol)	1.57E-03	1.76E-03	1.34E-03	1.26E-03	1.60E-03	1.63E-03	1.79E-03	1.63E-03
HCl (mol) (100 mL solution)	1.57E-02	1.76E-02	1.34E-02	1.26E-02	1.60E-02	1.63E-02	1.79E-02	1.63E-02
HCl (mol) (react with soil)	2.43E-02	2.24E-02	2.66E-02	2.74E-02	2.40E-02	2.37E-02	2.21E-02	2.37E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.22	1.12	1.33	1.37	1.20	1.19	1.11	1.19
$CaCO_3$ (%) (air-dried soil)	12.18	11.19	13.31	13.70	12.00	11.88	11.08	11.88
Average (mean value)	12.48					11.61		
Standard Deviation (sample)	1.02					0.46		

Table A.2. UCS and carbonate titration results for 3-hour sequentially mixed biogrout recirculation columns with four days of soaking and two days of soaking.

UCS (kPa)	418.1	235.36	150.57	331.84	543.82	264.6	665.15	440.02	473.65
Method	TS3	TS3	TS3	TS3.2ds	TS3.2ds	TS3.2ds	TS3.2ds	TS3.2ds	TS3.2ds
Column	C1	C2	C3	C4	C5	C6	C1	C2	C3
Date	17-Jul	24-Jun	24-Jun	5-Jan	5-Jan	5-Jan	27-Sep	27-Sep	27-Sep
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	16.8	15.2	14.4	17.9	17.8	17.7	14.7	16.7	17.6
Test 2	16.2	15.4	14.3	17.7	16.1	17.2	15.5	17.0	17.7
Test 3	16.8	14.9	14.0	17.4	16.9	18.1	16.0	17.1	17.6
Average	16.60	15.17	14.23	17.67	16.93	17.67	15.40	16.93	17.63
NaOH (mol)	1.66E-03	1.52E-03	1.42E-03	1.77E-03	1.69E-03	1.77E-03	1.54E-03	1.69E-03	1.76E-03
HCl (mol) (100 mL solution)	1.66E-02	1.52E-02	1.42E-02	1.77E-02	1.69E-02	1.77E-02	1.54E-02	1.69E-02	1.76E-02
HCl (mol) (react with soil)	2.34E-02	2.48E-02	2.58E-02	2.23E-02	2.31E-02	2.23E-02	2.46E-02	2.31E-02	2.24E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.17	1.24	1.29	1.12	1.15	1.12	1.23	1.15	1.12
$CaCO_3$ (%) (air-dried soil)	11.71	12.43	12.90	11.18	11.54	11.18	12.31	11.54	11.19
Average (mean value)	12.35			11.68					
Standard Deviation (sample)	0.60			0.57					

Table A.3. UCS and carbonate titration results for 3-hour sequentially mixed biogrout recirculation columns with no soaking.

UCS (kPa)	402.01	473.65	605.21	491.19	466.34	611.06
Method	TS3U	TS3U	TS3U	TS3U	TS3U	TS3U
Column	C1	C2	C3	C1	C2	C3
Date	5-Jan	5-Jan	5-Jan	7-Aug	7-Aug	7-Aug
Soil Mass (g)	10.00	10.00	10.00	10.00	10.01	10.00
NaOH (mL)						
Test 1	17.7	17.7	17.8	15.8	13.0	16.2
Test 2	18.7	17.9	17.6	15.4	15.0	15.6
Test 3	18.7	17.9	16.70	15.3	16.0	15.4
Average	18.37	17.83	17.37	15.50	14.67	15.73
NaOH (mol)	1.84E-03	1.78E-03	1.74E-03	1.55E-03	1.47E-03	1.57E-03
HCl (mol) (100 mL solution)	1.84E-02	1.78E-02	1.74E-02	1.55E-02	1.47E-02	1.57E-02
HCl (mol) (react with soil)	2.16E-02	2.22E-02	2.26E-02	2.45E-02	2.53E-02	2.43E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.08	1.11	1.13	1.23	1.27	1.21
$CaCO_3$ (%) (air-dried soil)	10.83	11.09	11.33	12.26	12.67	12.15
Average (mean value)	12.36					
Standard Deviation (sample)	0.27					

Table A.4. UCS and carbonate titration results for 6-hour sequentially mixed biogrout recirculation columns with four days of soaking and no soaking.

UCS (kPa)	207.59	292.37	511.65	295.3	192.97	342.08	388.86	309.92
Method	TS6	TS6	TS6	TS6	TS6	TS6	TS6U	TS6U
Column	C2	C2	C3	C1	C2	C3	C1	C2
Date	5-Jul	17-Jul	17-Jul	20-Jun	20-Jun	20-Jun	18-Aug	18-Aug
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)								
Test 1	15.3	14.2	15.4	15.1	16.8	13.4	16.3	16.2
Test 2	14.5	14.1	14.6	15.8	17.0	12.5	15.0	17.7
Test 3	14.5	14.6	14.9	15.3	16.6	13.3	15.6	17.5
Average	14.77	14.30	14.97	15.40	16.80	13.07	15.63	17.13
NaOH (mol)	1.48E-03	1.43E-03	1.50E-03	1.54E-03	1.68E-03	1.31E-03	1.56E-03	1.71E-03
HCl (mol) (100 mL solution)	1.48E-02	1.43E-02	1.50E-02	1.54E-02	1.68E-02	1.31E-02	1.56E-02	1.71E-02
HCl (mol) (react with soil)	2.52E-02	2.57E-02	2.50E-02	2.46E-02	2.32E-02	2.69E-02	2.44E-02	2.29E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.26	1.29	1.25	1.23	1.16	1.35	1.22	1.14
$CaCO_3$ (%) (air-dried soil)	12.63	12.86	12.53	12.31	11.61	13.48	12.20	11.44
Average (mean value)	12.57						11.82	
Standard Deviation (sample)	0.62						0.53	

Table A.5. UCS and carbonate titration results for 9-hour sequentially mixed biogrout recirculation columns with four days of soaking and no soaking.

UCS (kPa)	245.59	249.98	169.58	402.01	410.79	345
Method	TS9	TS9	TS9	TS9U	TS9U	TS9U
Column	C1	C2	C3	C4	C5	C6
Date	3-Jul	3-Jul	3-Jul	16-Aug	16-Aug	16-Aug
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)						
Test 1	15.5	14.6	15.7	17.4	16.9	16.3
Test 2	15.3	14.9	15.5	16.3	16.7	17.1
Test 3	15.7	14.8	15.4	16.4	17.3	17.0
Average	15.50	14.77	15.53	16.70	16.97	16.80
NaOH (mol)	1.55E-03	1.48E-03	1.55E-03	1.67E-03	1.70E-03	1.68E-03
HCl (mol) (100 mL solution)	1.55E-02	1.48E-02	1.55E-02	1.67E-02	1.70E-02	1.68E-02
HCl (mol) (react with soil)	2.45E-02	2.52E-02	2.45E-02	2.33E-02	2.30E-02	2.32E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.23	1.26	1.22	1.17	1.15	1.16
$CaCO_3$ (%) (air-dried soil)	12.26	12.63	12.25	11.66	11.53	11.61
Average (mean value)		12.38			11.60	
Standard Deviation (sample)		0.22			0.07	

Table A.6. UCS and carbonate titration results for 12-hour sequentially mixed biogrout recirculation columns with four days of soaking and no soaking.

UCS (kPa)	171.04	214.89	92.1	416.63	320.15	489.73
Method	TS12	TS12	TS12	TS12U	TS12U	TS12U
Column	C1	C2	C3	C1	C2	C3
Date	12-Jul	12-Jul	12-Jul	9-Aug	9-Aug	9-Aug
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)						
Test 1	16.0	15.0	17.0	19.6	16.8	17.5
Test 2	15.9	14.7	16.2	19.0	16.6	17.3
Test 3	15.8	14.9	16.2	18.5	16.7	17.2
Average	15.90	14.87	16.47	19.03	16.70	17.33
NaOH (mol)	1.59E-03	1.49E-03	1.65E-03	1.90E-03	1.67E-03	1.73E-03
HCl (mol) (100 mL solution)	1.59E-02	1.49E-02	1.65E-02	1.90E-02	1.67E-02	1.73E-02
HCl (mol) (react with soil)	2.41E-02	2.51E-02	2.35E-02	2.10E-02	2.33E-02	2.27E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.21	1.26	1.18	1.05	1.17	1.13
$CaCO_3$ (%) (air-dried soil)	12.06	12.58	11.78	10.49	11.66	11.34
Average (mean value)		12.14			11.17	
Standard Deviation (sample)		0.41			0.60	

Table A.7. UCS and carbonate titration results for batch feed biogrout recirculation version one and two columns.

UCS (kPa)	280.68	340.62	249.98	238.28	130.11	171.04
Method	BFv1	BFv1	BFv1	BFv2	BFv2	BFv2
Column	C1	C2	C3	C1	C2	C3
Date	19-Jul	19-Jul	19-Jul	25-Jul	25-Jul	25-Jul
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)						
Test 1	15.9	16.1	13.9	16.7	16.8	16.5
Test 2	16.0	16.4	13.7	17.0	15.8	16.4
Test 3	16.1	16.3	13.6	16.6	16.0	16.9
Average	16.00	16.27	13.73	16.77	16.20	16.60
NaOH (mol)	1.60E-03	1.63E-03	1.37E-03	1.68E-03	1.62E-03	1.66E-03
HCl (mol) (100 mL solution)	1.60E-02	1.63E-02	1.37E-02	1.68E-02	1.62E-02	1.66E-02
HCl (mol) (react with soil)	2.40E-02	2.37E-02	2.63E-02	2.32E-02	2.38E-02	2.34E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.20	1.19	1.31	1.16	1.19	1.17
$CaCO_3$ (%) (air-dried soil)	12.01	11.88	13.15	11.63	11.91	11.71
Average (mean value)		12.35			11.75	
Standard Deviation (sample)		0.70			0.15	

Table A.8. UCS and carbonate titration results for batch feed biogrout recirculation version three columns.

UCS (kPa)	300.05	619.83	695.85
Method	BFv3	BFv3	BFv3
Column	C1	C2	C3
Date	31-Jul	31-Jul	31-Jul
Soil Mass (g)	10.00	10.00	10.00
NaOH (mL)			
Test 1	14.2	16.4	15.9
Test 2	14.1	15.8	15.8
Test 3	14.2	15.6	16.0
Average	14.17	15.93	15.90
NaOH (mol)	1.42E-03	1.59E-03	1.59E-03
HCl (mol) (100 mL solution)	1.42E-02	1.59E-02	1.59E-02
HCl (mol) (react with soil)	2.58E-02	2.41E-02	2.41E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.29	1.20	1.21
$CaCO_3$ (%) (air-dried soil)	12.93	12.05	12.06
Average (mean value)		12.35	
Standard Deviation (sample)		0.51	

Table A.9. UCS and carbonate titration results for batch feed biogrout recirculation version three columns utilizing wax paper for column removal with four days of soaking and no soaking.

UCS (kPa)	730.94	510.19	425.4	815.72	649.07
Method	BFv3W	BFv3W	BFv3W	BFv3UW	BFv3UW
Column	C4	C5	C6	C1	C3
Date	1-Nov	1-Nov	1-Nov	18-Sep	18-Sep
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00
NaOH (mL)					
Test 1	16.3	16.5	18.1	16.3	15.2
Test 2	16.4	15.5	17.9	15.7	14.1
Test 3	16.6	15.8	17.2	15.7	14.7
Average	16.43	15.93	17.73	15.90	14.67
NaOH (mol)	1.64E-03	1.59E-03	1.77E-03	1.59E-03	1.47E-03
HCl (mol) (100 mL solution)	1.64E-02	1.59E-02	1.77E-02	1.59E-02	1.47E-02
HCl (mol) (react with soil)	2.36E-02	2.41E-02	2.23E-02	2.41E-02	2.53E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.18	1.20	1.11	1.21	1.27
$CaCO_3$ (%) (air-dried soil)	11.80	12.05	11.14	12.06	12.68
Average (mean value)		11.66		12.37	
Standard Deviation (sample)		0.47		0.44	

Table A.10. UCS and carbonate titration results for batch feed biogrout recirculation version three columns without soaking, utilizing new Scotch-Brite scour pad distributor and wax paper for column removal.

UCS (kPa)	463.41	390.32	523.35	612.52	505.81
Method	BFv3UF	BFv3UF	BFv3UFW	BFv3UFW	BFv3UFW
Column	C2	C3	C1	C2	C3
Date	20-Sep	20-Sep	25-Sep	25-Sep	25-Sep
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00
NaOH (mL)					
Test 1	16.9	14.1	16.0	14.2	14.8
Test 2	17.7	13.9	15.9	13.9	13.8
Test 3	16.8	14.0	15.8	14.6	14.4
Average	17.13	14.00	15.90	14.23	14.33
NaOH (mol)	1.71E-03	1.40E-03	1.59E-03	1.42E-03	1.43E-03
HCl (mol) (100 mL solution)	1.71E-02	1.40E-02	1.59E-02	1.42E-02	1.43E-02
HCl (mol) (react with soil)	2.29E-02	2.60E-02	2.41E-02	2.58E-02	2.57E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.14	1.30	1.21	1.29	1.28
$CaCO_3$ (%) (air-dried soil)	11.44	13.01	12.06	12.90	12.85
Average (mean value)	12.23		12.60		
Standard Deviation (sample)	1.11		0.47		

Table A.11. UCS and carbonate titration results for batch feed biogrout recirculation versions four and five columns.

UCS (kPa)	812.8	609.6	764.56	374.24	530.66	539.43	302.61	593.52
Method	BFv4U	BFv4U	BFv4U	BFv5U	BFv5U	BFv5U	BFv5U	BFv5U
Column	C1	C2	C3	C1	C3	C1	C2	C3
Date	2-Oct	2-Oct	2-Oct	9-Oct	9-Oct	27-Oct	27-Oct	27-Oct
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)								
Test 1	15.4	16.6	15.8	18.3	16.0	15.1	15.9	17.6
Test 2	15.5	15.8	15.7	18.4	16.2	15.0	17.2	17.9
Test 3	15.2	16.6	16.2	18.2	14.8	15.0	17.0	17.9
Average	15.37	16.33	15.90	18.30	15.67	15.03	16.70	17.80
NaOH (mol)	1.54E-03	1.63E-03	1.59E-03	1.83E-03	1.57E-03	1.50E-03	1.67E-03	1.78E-03
HCl (mol) (100 mL solution)	1.54E-02	1.63E-02	1.59E-02	1.83E-02	1.57E-02	1.50E-02	1.67E-02	1.78E-02
HCl (mol) (react with soil)	2.46E-02	2.37E-02	2.41E-02	2.17E-02	2.43E-02	2.50E-02	2.33E-02	2.22E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.23	1.18	1.21	1.09	1.22	1.25	1.17	1.11
$CaCO_3$ (%) (air-dried soil)	12.33	11.85	12.06	10.86	12.18	12.50	11.66	11.11
Average (mean value)		12.08				11.66		
Standard Deviation (sample)		0.24				0.69		

Table A.12. UCS and carbonate titration results for SOP columns.

Average UCS (kPA)	555.95																													
UCS (kPa)	703.16			815.72			649.07			223.67			450.26			445.87			497.04			676.85			657.84			440.02		
Method	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP	SOP
Column	C2.T	C2.M	C2.B	C2.T	C2.M	C2.B	C3.T	C3.M	C3.B	C1.T	C1.M	C1.B	C1.T	C1.M	C1.B	C2.T	C2.M	C2.B	C3.T	C3.M	C3.B	C1.T	C1.M	C1.B	C3.T	C3.M	C3.B	C2.T	C2.M	C2.B
Date	29-Nov	29-Nov	29-Nov	1-Nov	1-Nov	1-Nov	1-Nov	1-Nov	1-Nov	13-Nov	13-Nov	13-Nov	29-Nov	29-Nov	29-Nov	13-Dec	13-Dec	13-Dec	29-Nov	29-Nov	29-Nov	13-Dec	13-Dec	13-Dec	13-Dec	13-Dec	13-Dec	13-Nov	13-Nov	13-Nov
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)																														
Test 1	14.8	16.0	15.6	15.0	12.5	16.3	12.9	16.7	16.1	13.9	14.8	12.6	15.2	14.5	14.7	12.4	13.8	13.2	15.3	15.8	16.2	15.6	15.0	14.9	16.7	14.2	14.9	15.4	16.3	15.8
Test 2	15.1	15.8	15.5	14.9	13.0	16.1	13.5	16.4	16.2	13.9	14.9	12.6	14.8	14.2	13.6	12.7	14.1	12.7	14.3	15.8	16.4	15.3	14.0	14.0	16.6	14.2	14.5	15.2	15.6	15.5
Test 3	14.9	16.1	15.6	14.1	13.1	16.0	13.3	16.6	15.8	13.7	14.8	12.8	15.2	14.2	14.3	12.2	14.2	13.2	15.2	15.6	16.5	14.2	14.9	13.3	14.5	14.0	13.8	15.0	15.3	15.2
Average	14.93	15.97	15.57	14.67	12.87	16.13	13.23	16.57	16.03	13.83	14.83	12.67	15.07	14.30	14.20	12.43	14.03	13.03	14.93	15.73	16.37	15.03	14.63	14.07	15.93	14.13	14.40	15.20	15.73	15.50
NaOH (mol)	1.49E-03	1.60E-03	1.56E-03	1.47E-03	1.29E-03	1.61E-03	1.32E-03	1.66E-03	1.60E-03	1.38E-03	1.48E-03	1.27E-03	1.51E-03	1.43E-03	1.42E-03	1.24E-03	1.40E-03	1.30E-03	1.49E-03	1.57E-03	1.64E-03	1.50E-03	1.46E-03	1.41E-03	1.59E-03	1.41E-03	1.44E-03	1.52E-03	1.57E-03	1.55E-03
HCl (mol) (100 mL solution)	1.49E-02	1.60E-02	1.56E-02	1.47E-02	1.29E-02	1.61E-02	1.32E-02	1.66E-02	1.60E-02	1.38E-02	1.48E-02	1.27E-02	1.51E-02	1.43E-02	1.42E-02	1.24E-02	1.40E-02	1.30E-02	1.49E-02	1.57E-02	1.64E-02	1.50E-02	1.46E-02	1.41E-02	1.59E-02	1.41E-02	1.44E-02	1.52E-02	1.57E-02	1.55E-02
HCl (mol) (react with soil)	2.51E-02	2.40E-02	2.44E-02	2.53E-02	2.71E-02	2.39E-02	2.68E-02	2.34E-02	2.40E-02	2.62E-02	2.52E-02	2.73E-02	2.49E-02	2.57E-02	2.58E-02	2.76E-02	2.60E-02	2.70E-02	2.51E-02	2.43E-02	2.36E-02	2.50E-02	2.54E-02	2.59E-02	2.41E-02	2.59E-02	2.56E-02	2.48E-02	2.43E-02	2.45E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.25	1.20	1.22	1.27	1.36	1.19	1.34	1.17	1.20	1.31	1.26	1.37	1.25	1.29	1.29	1.38	1.30	1.35	1.25	1.21	1.18	1.25	1.27	1.30	1.20	1.29	1.28	1.24	1.21	1.23
$CaCO_3$ (%) (air-dried soil)	12.55	12.03	12.23	12.68	13.58	11.95	13.40	11.73	12.00	13.10	12.60	13.68	12.48	12.86	12.91	13.80	13.00	13.50	12.55	12.15	11.83	12.50	12.70	12.98	12.05	12.95	12.81	12.41	12.15	12.26
Average per Column (%)		12.27			12.73			12.37			13.12			12.75			13.43			12.17			12.72			12.60			12.27	
Average (mean value)	12.65																													
Standard Deviation (sample)	0.40																													

Table A.13. UCS and carbonate titration results for SOP columns produced with 17.64 grams of $CaCl_2$ at a 1:1 molar ratio.

Average UCS (kPA)	222.69								
UCS (kPa)	217.81			162.27			287.99		
Method	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1
Column	C3 T	C3 M	C3 B	C2 T	C2 M	C2 B	C1 T	C1 M	C1 B
Date	22-Jan	22-Jan	22-Jan	22-Jan	22-Jan	22-Jan	22-Jan	22-Jan	22-Jan
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	16.9	16.7	17.1	16.9	15.5	16.7	14.2	15.5	17.2
Test 2	16.9	16.4	16.7	17.6	15.2	17.3	13.3	15.2	16.8
Test 3	15.3	16.2	16.2	15.1	15.8	16.4	13.9	14.5	17.5
Average	16.37	16.43	16.67	16.53	15.50	16.80	13.80	15.07	17.17
NaOH (mol)	1.64E-03	1.64E-03	1.67E-03	1.65E-03	1.55E-03	1.68E-03	1.38E-03	1.51E-03	1.72E-03
HCl (mol) (100 mL solution)	1.64E-02	1.64E-02	1.67E-02	1.65E-02	1.55E-02	1.68E-02	1.38E-02	1.51E-02	1.72E-02
HCl (mol) (react with soil)	2.36E-02	2.36E-02	2.33E-02	2.35E-02	2.45E-02	2.32E-02	2.62E-02	2.49E-02	2.28E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.18	1.18	1.17	1.17	1.23	1.16	1.31	1.25	1.14
$CaCO_3$ (%) (air-dried soil)	11.83	11.80	11.68	11.75	12.26	11.61	13.11	12.48	11.43
Average per Column (%)	11.77			11.87			12.34		
Average (mean value)	11.99								
Standard Deviation (sample)	0.30								

Table A.14. UCS and carbonate titration results for SOP columns produced with 17.64 grams of $CaCl_2$ at a 1:2 molar ratio.

Average UCS (kPA)	124.99																
UCS (kPa)	204.66			146.19			166.65			140.34			40.93		51.17		
Method	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2	SOP 1:2
Column	C3 T	C3 M	C3 B	C2 T	C2 M	C2 B	C1 T	C1 M	C1 B	C4 T	C4 M	C4 B	C5 T	C5 M	C6 T	C6 M	C6 B
Date	6-Feb	6-Feb	6-Feb	6-Feb	6-Feb	6-Feb	6-Feb	6-Feb	6-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)																	
Test 1	13.9	15.5	17.1	11.1	15.7	15.7	14.6	13.5	15.2	9.4	16.6	19.0	12.7	14.6	10.4	15.9	15.6
Test 2	13.1	15.1	16.0	11.8	16.3	16.0	14.6	13.4	14.7	10.7	16.2	19.7	13.1	14.1	11.2	15.2	15.2
Test 3	14.0	14.2	16.1	12.2	16.4	15.5	13.6	13.6	14.1	9.9	16.4	19.6	13.1	14.8	11.3	15.2	15.8
Average	13.67	14.93	16.40	11.70	16.13	15.73	14.27	13.50	14.67	10.00	16.40	19.43	12.97	14.50	10.97	15.43	15.53
NaOH (mol)	1.37E-03	1.49E-03	1.64E-03	1.17E-03	1.61E-03	1.57E-03	1.43E-03	1.35E-03	1.47E-03	1.00E-03	1.64E-03	1.94E-03	1.30E-03	1.45E-03	1.10E-03	1.54E-03	1.55E-03
HCl (mol) (100 mL solution)	1.37E-02	1.49E-02	1.64E-02	1.17E-02	1.61E-02	1.57E-02	1.43E-02	1.35E-02	1.47E-02	1.00E-02	1.64E-02	1.94E-02	1.30E-02	1.45E-02	1.10E-02	1.54E-02	1.55E-02
HCl (mol) (react with soil)	2.63E-02	2.51E-02	2.36E-02	2.83E-02	2.39E-02	2.43E-02	2.57E-02	2.65E-02	2.53E-02	3.00E-02	2.36E-02	2.06E-02	2.70E-02	2.55E-02	2.90E-02	2.46E-02	2.45E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.32	1.25	1.18	1.42	1.19	1.21	1.29	1.33	1.27	1.50	1.18	1.03	1.35	1.28	1.45	1.23	1.22
$CaCO_3$ (%) (air-dried soil)	13.18	12.55	11.81	14.16	11.95	12.15	12.88	13.26	12.68	15.02	11.81	10.29	13.53	12.76	14.53	12.30	12.25
Average per Column (%)		12.51			12.75			12.94			12.37			13.15		13.02	
Average (mean value)	12.79																
Standard Deviation (sample)	0.30																

Table A.15. UCS and carbonate titration results for SOP columns produced with 17.64 grams of $CaCl_2$ at a 1:3 molar ratio.

Average UCS (kPA)	104.77								
UCS (kPa)	76.02			131.57			106.72		
Method	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3
Column	C3 T	C3 M	C3 B	C2 T	C2 M	C2 B	C1 T	C1 M	C1 B
Date	29-Jan	29-Jan	29-Jan	29-Jan	29-Jan	29-Jan	29-Jan	29-Jan	29-Jan
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	14.6	15.0	14.8	14.0	14.7	15.3	12.3	15.2	16.6
Test 2	14.0	14.9	14.7	14.3	14.4	14.8	12.3	15.5	16.5
Test 3	14.8	14.5	14.9	14.6	14.4	14.2	12.6	15.4	16.6
Average	14.47	14.80	14.80	14.30	14.50	14.77	12.40	15.37	16.57
NaOH (mol)	1.45E-03	1.48E-03	1.48E-03	1.43E-03	1.45E-03	1.48E-03	1.24E-03	1.54E-03	1.66E-03
HCl (mol) (100 mL solution)	1.45E-02	1.48E-02	1.48E-02	1.43E-02	1.45E-02	1.48E-02	1.24E-02	1.54E-02	1.66E-02
HCl (mol) (react with soil)	2.55E-02	2.52E-02	2.52E-02	2.57E-02	2.55E-02	2.52E-02	2.76E-02	2.46E-02	2.34E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.28	1.26	1.26	1.29	1.28	1.26	1.38	1.23	1.17
$CaCO_3$ (%) (air-dried soil)	12.78	12.61	12.61	12.86	12.76	12.63	13.81	12.33	11.73
Average per Column (%)	12.67			12.75			12.62		
Average (mean value)	12.68								
Standard Deviation (sample)	0.06								

Table A.16. UCS and carbonate titration results for SOP columns produced with 7.2 grams of $CaCl_2$ at a 1:1 molar ratio.

Average UCS (kPA)			356.70						
UCS (kPa)		353.77			469.26			247.06	
Method	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1	SOP 1:1
Column	C1 T	C1 M	C1 B	C3 T	C3 M	C3 B	C2 T	C2 M	C2 B
Date	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	13.6	15.9	19.0	15.1	16.6	15.0	15.4	17.2	18.0
Test 2	12.3	15.5	18.7	14.3	16.3	15.2	15.5	17.4	18.6
Test 3	13.1	16.1	18.7	15.0	17.3	15.5	14.9	17.5	18.7
Average	13.00	15.83	18.80	14.80	16.73	15.23	15.27	17.37	18.43
NaOH (mol)	1.30E-03	1.58E-03	1.88E-03	1.48E-03	1.67E-03	1.52E-03	1.53E-03	1.74E-03	1.84E-03
HCl (mol) (100 mL solution)	1.30E-02	1.58E-02	1.88E-02	1.48E-02	1.67E-02	1.52E-02	1.53E-02	1.74E-02	1.84E-02
HCl (mol) (react with soil)	2.70E-02	2.42E-02	2.12E-02	2.52E-02	2.33E-02	2.48E-02	2.47E-02	2.26E-02	2.16E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.35	1.21	1.06	1.26	1.16	1.24	1.24	1.13	1.08
$CaCO_3$ (%) (air-dried soil)	13.51	12.10	10.61	12.61	11.64	12.40	12.38	11.33	10.79
Average per Column (%)		12.07			12.22			11.50	
Average (mean value)					11.93				
Standard Deviation (sample)					0.93				

Table A.17. UCS and carbonate titration results for SOP columns produced with 7.2 grams of $CaCl_2$ at a 1:3 molar ratio.

Average UCS (kPA)	165.19								
UCS (kPa)	204.66			173.96			116.94		
Method	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3	SOP 1:3
Column	C4 T	C4 M	C4 B	C5 T	C5 M	C5 B	C6 T	C6 M	C6 B
Date	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb	19-Feb
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	15.8	17.4	18.3	16.7	15.8	13.9	15.5	17.0	16.6
Test 2	15.0	16.7	18.3	16.3	16.0	13.8	14.0	16.6	17.2
Test 3	15.3	16.8	18.1	16.9	15.0	13.9	14.4	16.4	16.3
Average	15.37	16.97	18.23	16.63	15.60	13.87	14.63	16.67	16.70
NaOH (mol)	1.54E-03	1.70E-03	1.82E-03	1.66E-03	1.56E-03	1.39E-03	1.46E-03	1.67E-03	1.67E-03
HCl (mol) (100 mL solution)	1.54E-02	1.70E-02	1.82E-02	1.66E-02	1.56E-02	1.39E-02	1.46E-02	1.67E-02	1.67E-02
HCl (mol) (react with soil)	2.46E-02	2.30E-02	2.18E-02	2.34E-02	2.44E-02	2.61E-02	2.54E-02	2.33E-02	2.33E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.23	1.15	1.09	1.17	1.22	1.31	1.27	1.17	1.17
$CaCO_3$ (%) (air-dried soil)	12.33	11.53	10.89	11.70	12.21	13.08	12.70	11.68	11.66
Average per Column (%)	11.58			12.33			12.01		
Average (mean value)	11.97								
Standard Deviation (sample)	0.67								

Table A.18. UCS and carbonate titration results for SOP columns produced with 7.2 grams of $CaCl_2$ at a 1:6 molar ratio.

Average UCS (kPA)	243.16								
UCS (kPa)	283.6			232.44			213.43		
Method	SOP 1:6	SOP 1:6	SOP 1:6	SOP 1:6	SOP 1:6	SOP 1:6	SOP 1:6	SOP 1:6	SOP 1:6
Column	C1 T	C1 M	C1 B	C2 T	C2 M	C2 B	C3 T	C3 M	C3 B
Date	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb	26-Feb
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	18.2	18.2	19.2	16.1	18.0	18.9	16.2	15.7	16.4
Test 2	17.6	17.3	18.2	15.0	17.9	19.0	14.5	15.4	15.5
Test 3	17.6	17.3	18.2	15.0	17.6	18.5	14.8	15.7	15.3
Average	17.80	17.60	18.53	15.37	17.83	18.80	15.17	15.60	15.73
NaOH (mol)	1.78E-03	1.76E-03	1.85E-03	1.54E-03	1.78E-03	1.88E-03	1.52E-03	1.56E-03	1.57E-03
HCl (mol) (100 mL solution)	1.78E-02	1.76E-02	1.85E-02	1.54E-02	1.78E-02	1.88E-02	1.52E-02	1.56E-02	1.57E-02
HCl (mol) (react with soil)	2.22E-02	2.24E-02	2.15E-02	2.46E-02	2.22E-02	2.12E-02	2.48E-02	2.44E-02	2.43E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.11	1.12	1.07	1.23	1.11	1.06	1.24	1.22	1.21
$CaCO_3$ (%) (air-dried soil)	11.11	11.21	10.74	12.33	11.09	10.61	12.43	12.21	12.15
Average per Column (%)	11.02			11.34			12.26		
Average (mean value)	11.54								
Standard Deviation (sample)	0.73								

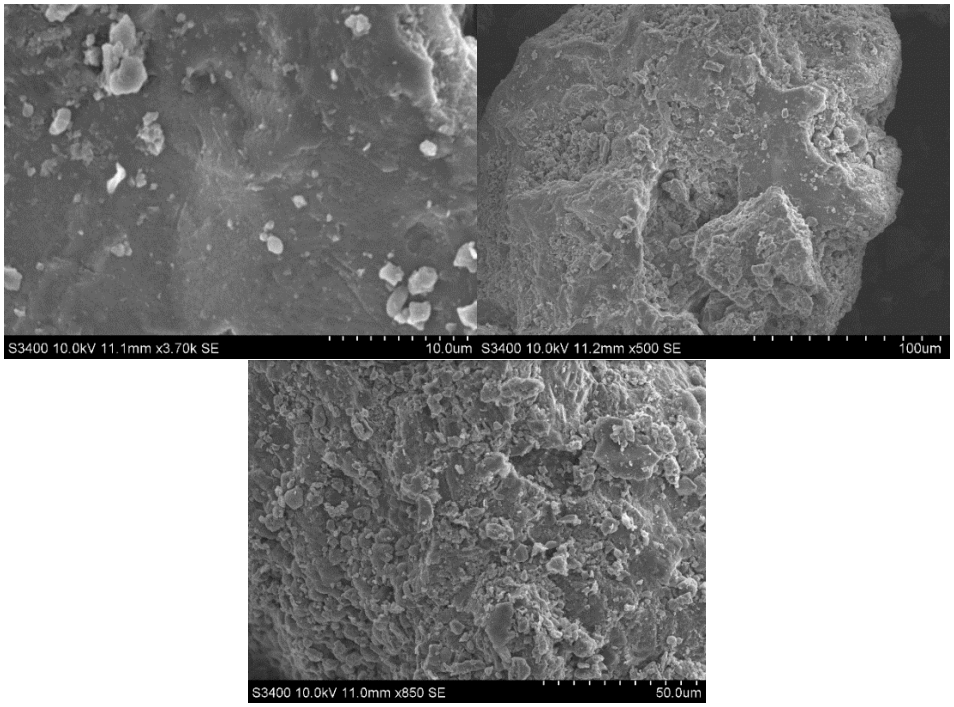

Figure A.21. SEM images from bottom portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:0.

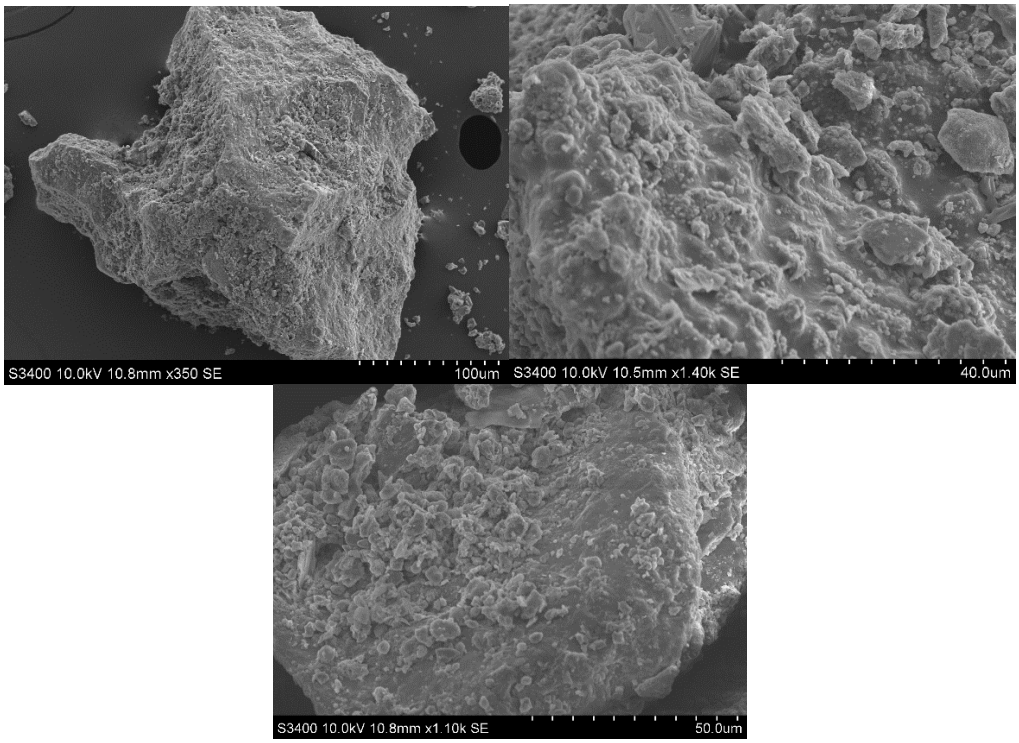

Figure A.22. SEM images from middle portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:0.

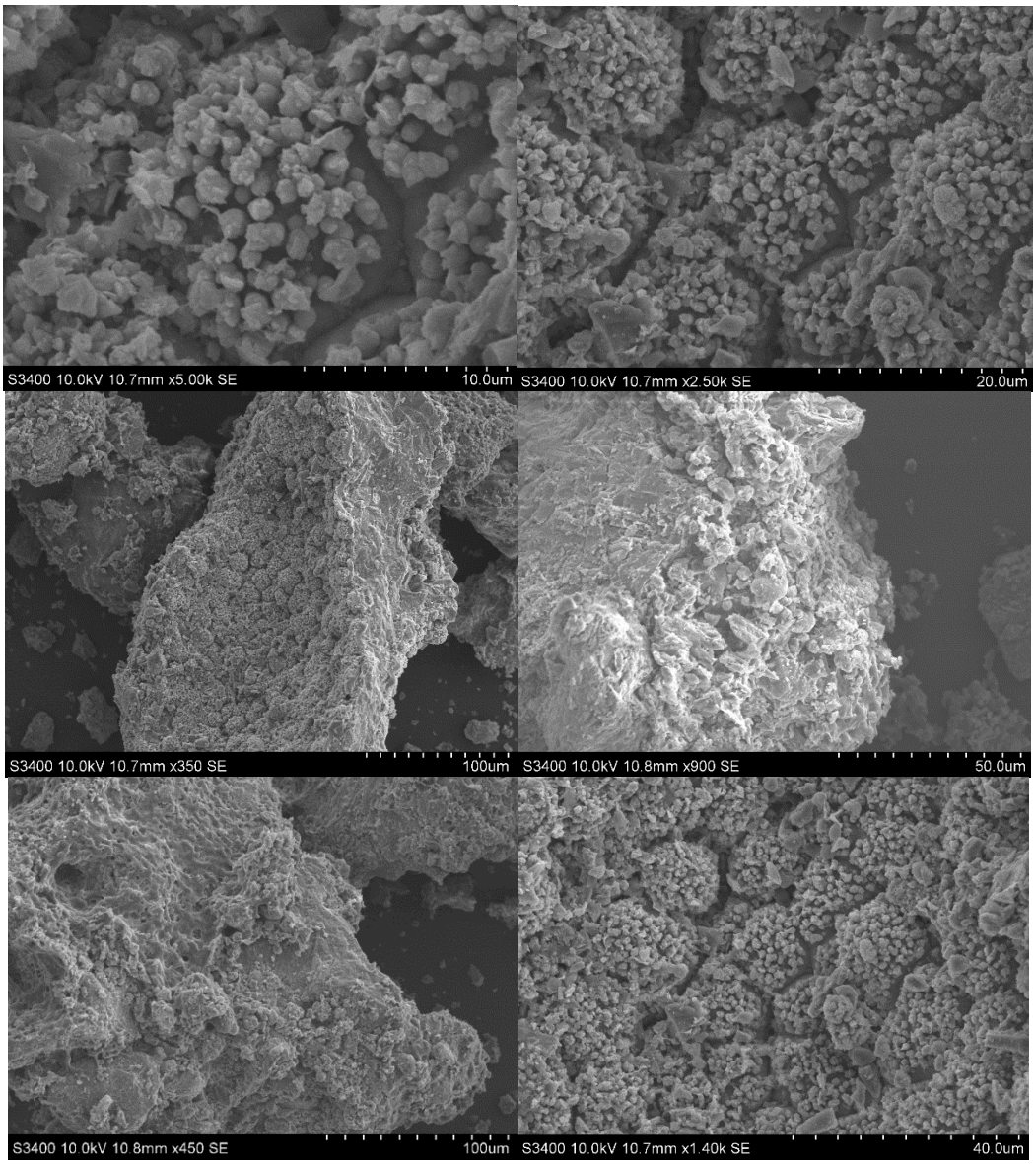

Figure A.23. SEM images from top portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:0.

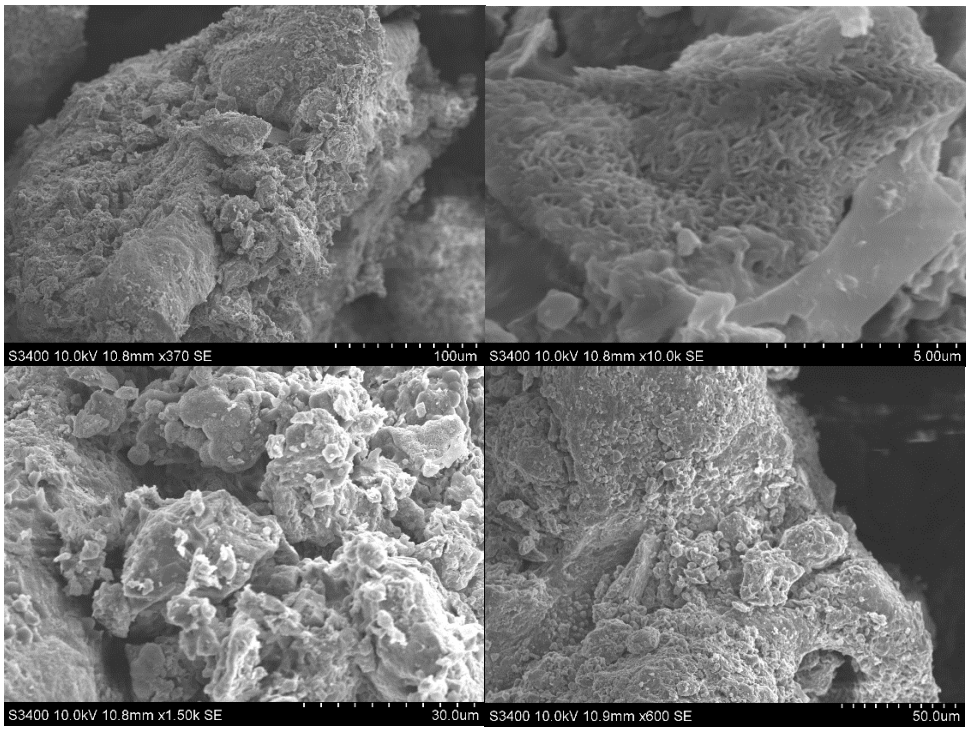

Figure A.24. SEM images from bottom portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:1.

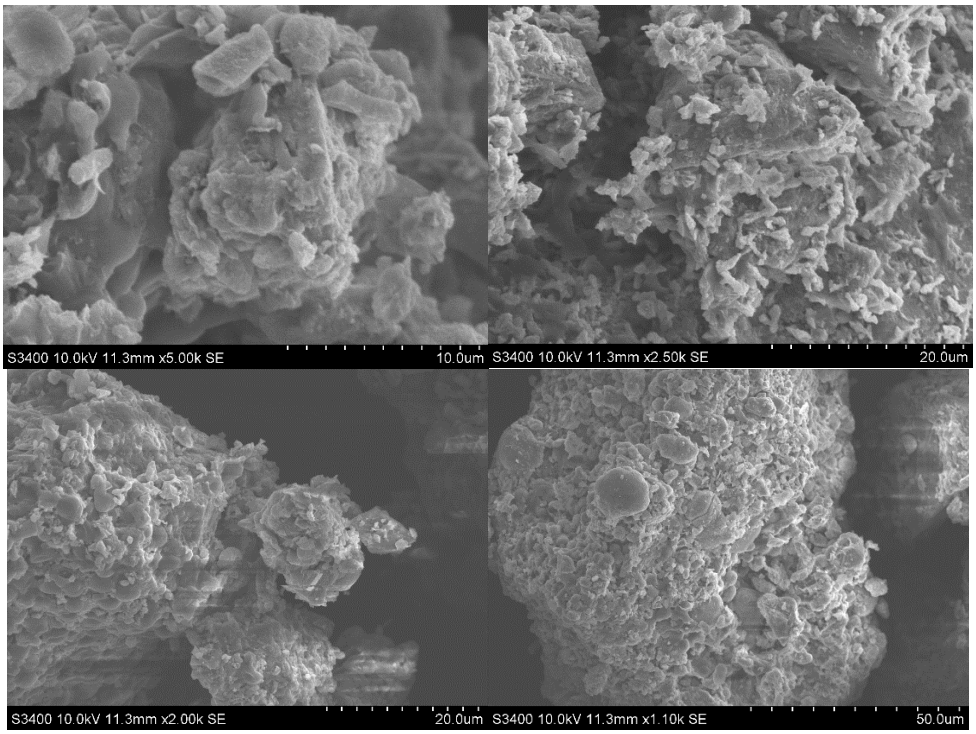

Figure A.25. SEM images from middle portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:1.

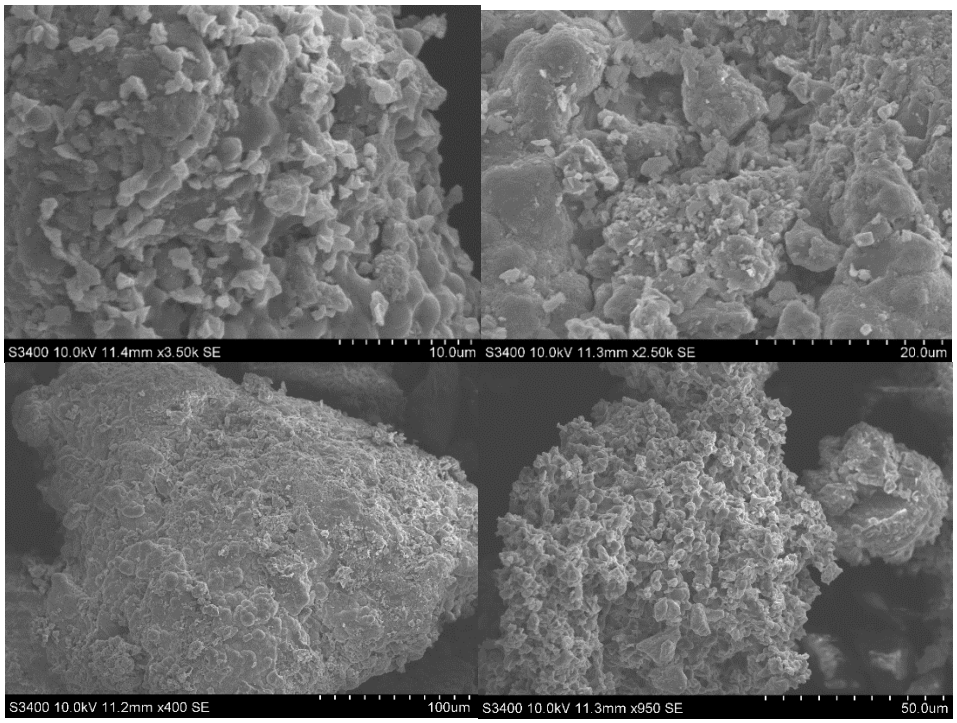

Figure A.26. SEM images from top portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:1.

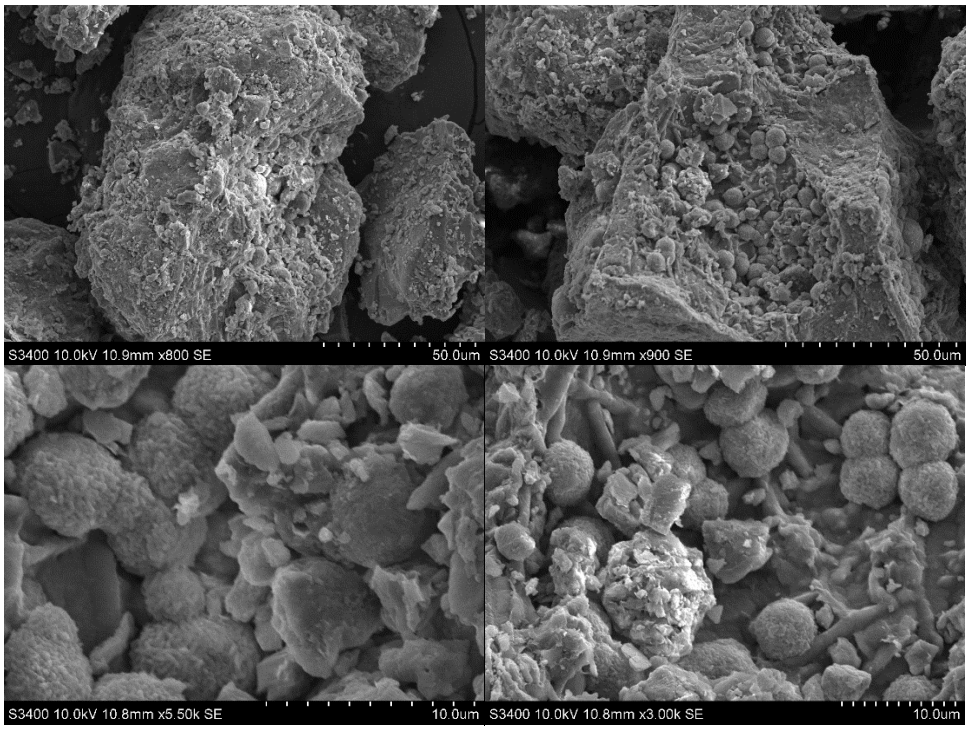

Figure A.27. SEM images from bottom portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:4.53.

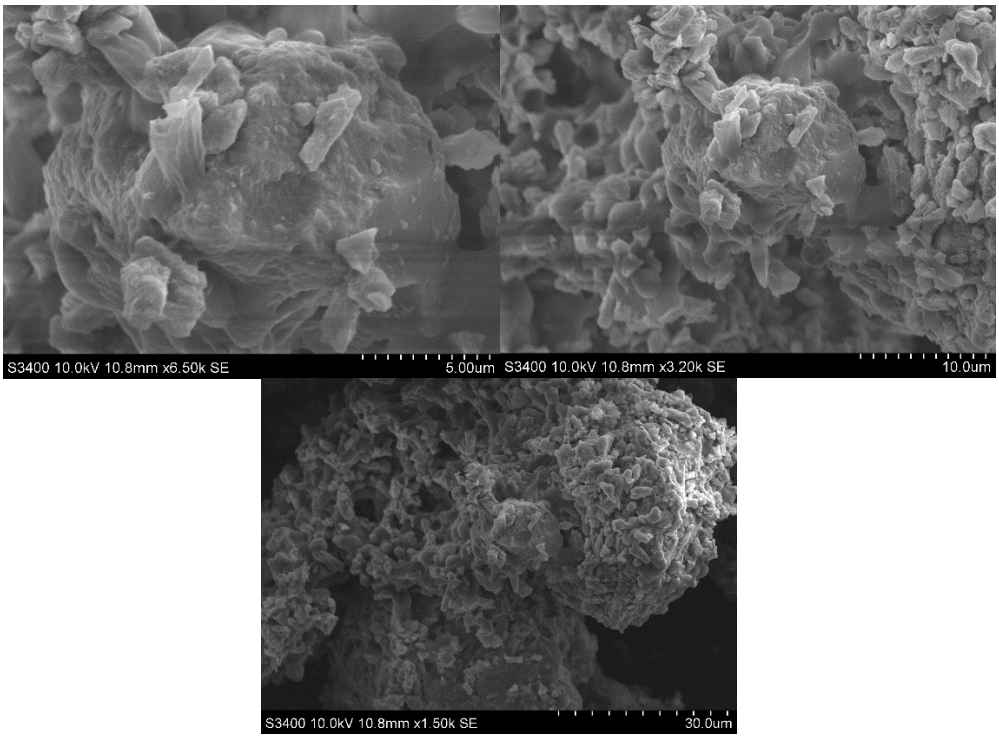

Figure A.28. SEM images from middle portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:4.53.

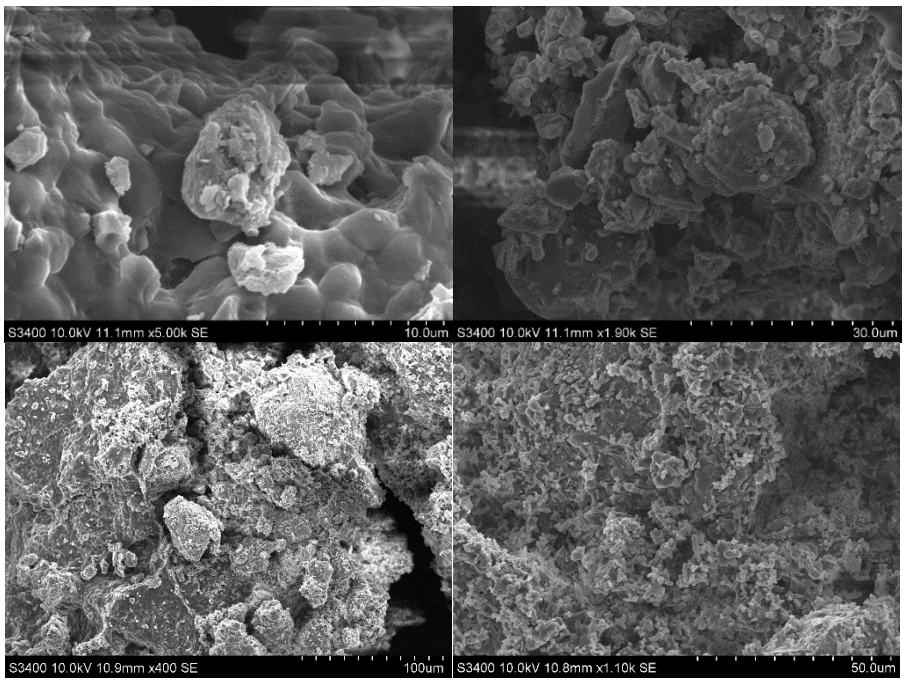

Figure A.29. SEM images from top portion of SOP column produced utilizing calcium acetate at a molar ratio of 1:4.53.

Table A.19. UCS and carbonate titration results for SOP columns produced utilizing calcium acetate at a molar ratio of 1:0.

Average UCS (kPA)	N/A								
UCS (kPa)	N/A			N/A			N/A		
Method	AD 1:0	AD 1:0	AD 1:0	AD 1:0	AD 1:0	AD 1:0	AD 1:0	AD 1:0	AD 1:0
Column	C4 Top	C4 Mid	C4 Bot	C5 Top	C5 Mid	C5 Bot	C6 Top	C6 Mid	C6 Bot
Date	1-May	1-May	1-May	1-May	1-May	1-May	1-May	1-May	1-May
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	17.1	15.9	15.7	17.4	17.8	15.9	18.5	19.0	17.2
Test 2	17.5	16.2	15.7	16.3	18.1	16.2	18.6	19.0	17.3
Test 3	17.9	16.5	15.7	16.5	17.8	16.0	18.5	18.5	17.1
Average	17.50	16.20	15.70	16.73	17.90	16.03	18.53	18.83	17.20
NaOH (mol)	1.75E-03	1.62E-03	1.57E-03	1.67E-03	1.79E-03	1.60E-03	1.85E-03	1.88E-03	1.72E-03
HCl (mol) (100 mL solution)	1.75E-02	1.62E-02	1.57E-02	1.67E-02	1.79E-02	1.60E-02	1.85E-02	1.88E-02	1.72E-02
HCl (mol) (react with soil)	2.25E-02	2.38E-02	2.43E-02	2.33E-02	2.21E-02	2.40E-02	2.15E-02	2.12E-02	2.28E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.13	1.19	1.22	1.16	1.11	1.20	1.07	1.06	1.14
$CaCO_3$ (%) (air-dried soil)	11.26	11.91	12.16	11.64	11.06	12.00	10.74	10.59	11.41
Average per Column (%)	11.78			11.57			10.92		
Average (mean value)	11.42								
Standard Deviation (sample)	0.45								

Table A.20. UCS and carbonate titration results for SOP columns produced utilizing calcium acetate at a molar ratio of 1:1.

Average UCS (kPA)	237.80								
UCS (kPa)	290.91			214.89			207.59		
Method	AD 1:1	AD 1:1	AD 1:1	AD 1:1	AD 1:1	AD 1:1	AD 1:1	AD 1:1	AD 1:1
Column	C1 Top	C1 Mid	C1 Bot	C2 Top	C2 Mid	C2 Bot	C3 Top	C3 Mid	C3 Bot
Date	1-May	1-May	1-May	1-May	1-May	1-May	1-May	1-May	1-May
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	17.4	18.0	18.2	15.1	17.2	17.4	17.4	17.0	16.7
Test 2	16.5	17.5	17.5	15.3	17.3	17.7	16.9	16.6	16.6
Test 3	16.5	17.9	17.3	15.6	17.4	17.4	17.1	16.5	16.4
Average	16.80	17.80	17.67	15.33	17.30	17.50	17.13	16.70	16.57
NaOH (mol)	1.68E-03	1.78E-03	1.77E-03	1.53E-03	1.73E-03	1.75E-03	1.71E-03	1.67E-03	1.66E-03
HCl (mol) (100 mL solution)	1.68E-02	1.78E-02	1.77E-02	1.53E-02	1.73E-02	1.75E-02	1.71E-02	1.67E-02	1.66E-02
HCl (mol) (react with soil)	2.32E-02	2.22E-02	2.23E-02	2.47E-02	2.27E-02	2.25E-02	2.29E-02	2.33E-02	2.34E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.16	1.11	1.12	1.23	1.14	1.13	1.14	1.17	1.17
$CaCO_3$ (%) (air-dried soil)	11.61	11.11	11.18	12.35	11.36	11.26	11.44	11.66	11.73
Average per Column (%)		11.30			11.66			11.61	
Average (mean value)	11.52								
Standard Deviation (sample)	0.19								

Table A.21. UCS and carbonate titration results for SOP columns produced utilizing calcium acetate at a molar ratio of 1:4.53.

Average UCS (kPA)	168.11								
UCS (kPa)	261.67			147.65			95.02		
Method	AD 1:4.53	AD 1:4.53	AD 1:4.53	AD 1:4.53	AD 1:4.53	AD 1:4.53	AD 1:4.53	AD 1:4.53	AD 1:4.53
Column	C1 Top	C1 Mid	C1 Bot	C2 Top	C2 Mid	C2 Bot	C3 Top	C3 Mid	C3 Bot
Date	16-Apr	16-Apr	16-Apr	16-Apr	16-Apr	16-Apr	16-Apr	16-Apr	16-Apr
Soil Mass (g)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH (mL)									
Test 1	16.5	15.9	17.4	16.3	16.0	18.5	15.7	18.5	15.5
Test 2	16.7	16.2	17.2	16.4	15.4	18.6	16.2	17.7	14.9
Test 3	16.1	15.3	17.0	16.0	15.6	18.5	16.2	17.8	15.0
Average	16.43	15.80	17.20	16.23	15.67	18.53	16.03	18.00	15.13
NaOH (mol)	1.64E-03	1.58E-03	1.72E-03	1.62E-03	1.57E-03	1.85E-03	1.60E-03	1.80E-03	1.51E-03
HCl (mol) (100 mL solution)	1.64E-02	1.58E-02	1.72E-02	1.62E-02	1.57E-02	1.85E-02	1.60E-02	1.80E-02	1.51E-02
HCl (mol) (react with soil)	2.36E-02	2.42E-02	2.28E-02	2.38E-02	2.43E-02	2.15E-02	2.40E-02	2.20E-02	2.49E-02
$CaCO_3$ (g/mol)	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1	100.1
$CaCO_3$ (g) (react with HCl)	1.18	1.21	1.14	1.19	1.22	1.07	1.20	1.10	1.24
$CaCO_3$ (%) (air-dried soil)	11.80	12.11	11.41	11.90	12.18	10.74	12.00	11.01	12.45
Average per Column (%)		11.77			11.61			11.82	
Average (mean value)	11.73								
Standard Deviation (sample)	0.11								

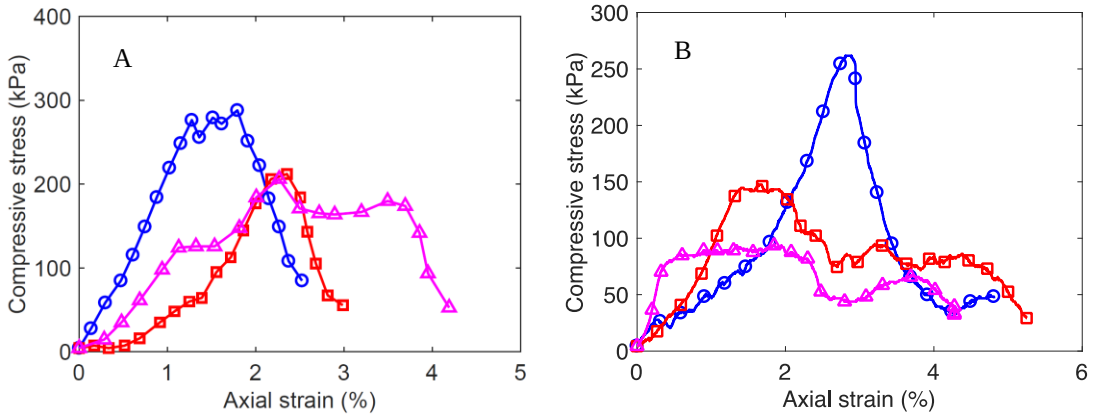

Figure A.30. UCS results for SOP columns produced utilizing calcium acetate at the molar ratios of (A) 1:1 and (B) 1:4.53.