ECO-Friendly approach in strengthening the different grain size particles by utilizing *Sporsarcina pausterii* NIOT1

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Abstract
A distinct grain size of 600, 425 and 212 µm sand column was developed to test the efficiency of bacterial strain Sporsarcina pausterii NIOT-1. The bacterial strain was effectively able to precipitate calcite crystals between the soil particles through enzymatic hydrolysis of urea which increases ammonium concentration to the maximum of 371.43 mM, 314.54 mM, 302.49 mM, EC of 98.81 ms/cm, 101.84 ms/cm, 101.34 ms/cm and pH of 8.80, 8.90, 8.80 respectively. The synthesized biomaterials have the maximum UCS of 2400 Mpa with 212 µm grain size followed by 425 and 600 µm with 2200 and 2100 Mpa. Scanning Electron Microscopy and XRD results justify the precipitation of $CaCO_3$ bridging the sand particle.

In the study, the maximum UCS attained with fine sand particle size and reduced with the increasing grain size. The current study observed an inverse relationship between grain size and strength. The current green synthesis study also attempts for the feasibility approach to reduce coastal erosion.

Keywords: Grain Size, XRD, UCS, Biomaterials, Electron Microscopy.

Introduction
In the bacterial kingdom, the precipitation of calcite is quite common and it has been slowly commercializing in the name of bio-cementation. Recently, few studies proved the viability of bio-mediated soil improvement applications for effective performance and environmental sustainability[8]. The promising outcomes of these studies have shown more significant potential of exploring a broader application of the technique in geotechnical engineering. In geotechnical engineering, the bio-cementation process is used to enhance the soil properties through the precipitation of calcite using microbial activity and is considered as MICP[4].

Microbial induced calcium carbonate precipitation (MICP) was obtained by urea hydrolysis, denitrification, iron reduction, or sulfate reduction[3]. Though a variety of microbes and different pathways are involved in MICP, the prevailing urea hydrolysis strain *Sporasarcina pasteurii* was utilized in this process due to their potential to produce a large quantity of carbonate within a short period[1]. Therefore, many researchers concentrated mainly on the utilization of *Sporasarcina pasteurii* in various applications like the bioremediation of cracks, strengthening (durability) of concrete and bio-grouting[1].

Few studies are available in strengthening the shoreline using the bio-cementation practice to delimit coastal erosion[5]. The beach sand will consist of various grain sizes and it is well known that the sand grains can impact the strength of the MICP bio-brick[6]. Therefore, the current study was aimed to differentiate the influence of grain size over the strength of the bio-cemented rock. In the present study, the grain sizes of 600, 425 and 212-micron beach sand particles were utilized separately to obtain the sand rock on a laboratory scale because of these types of predominant grain size particles in the coasts of Tamil Nadu[7].

Material and Methods
The urease producing bacterial strain was isolated from calcareous sedimentary rock sample at Ramnad district (9°16.380′N 78°59.478′E), Tamilnadu, India and it has been identified as *Sporosarcina pasteurii* NIOT1 strain (KX097971) which was utilized in this study.

The beach sand was sieved with sieve shaker and various grain sizes were collected separately. Grain sizes such as 212, 425 and 600 µm are available in high quantity to compare to other grain sizes which were utilized to test the influence over the strength of bio-brick. The 10X10 cm container was vertically packed with 600 gm of each grain size (Fig. 1). Triplicate of each grain size particle along with the control was analyzed for concordance in results. The entire setup and the protocol to run this sand plug experiment were carried out by following Kumar et al[5].

Determination of calcium carbonate formation and urease activity during MICP, the parametric changes such as pH, electrical conductivity (EC) and ammonia were monitored at constant time intervals. The concentration of ammonia was determined by Nessler's method[2]. pH and EC were measured using HM digital PH-80 meter (Seoul, Korea) and YSI Pro DO meter (Model: 2030). All parameters were analyzed in triplicate for the concordance of results. To measure the strength of compacted soil samples, the unconfined compressed strength (UCS) analysis was performed according to the test method IS 516:1959.

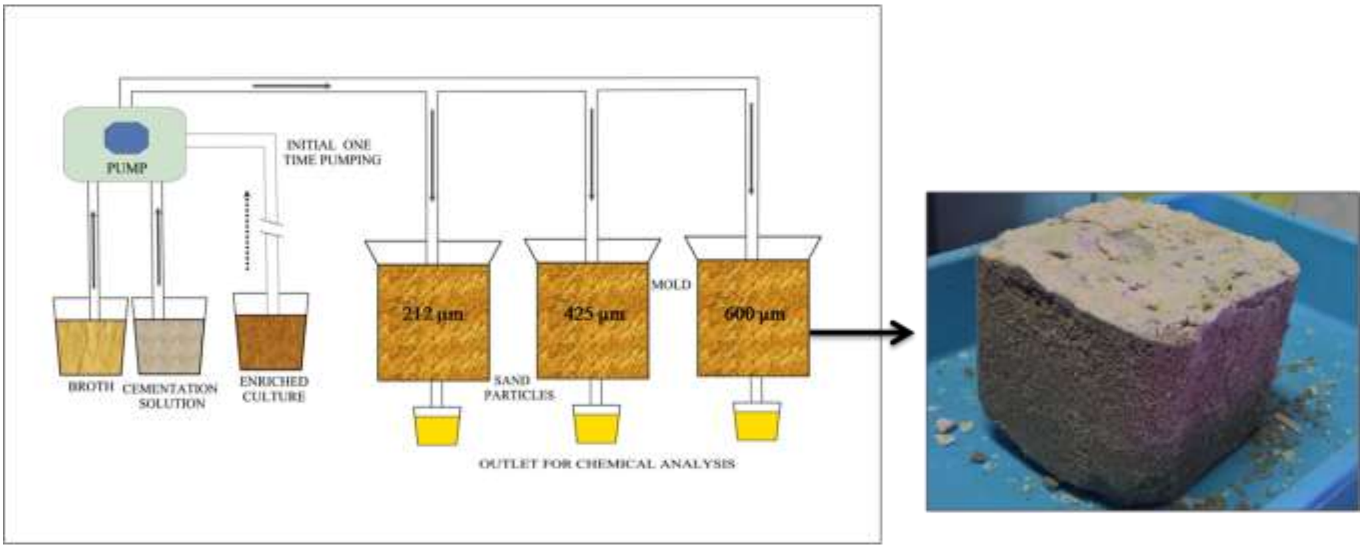

Figure 1: Schematic diagram representing the different sand particle size MICP experimental setup

The calcium carbonate content was estimated using the gravimetric acid washing (2 M HCl) technique after the completion of UCS by following Mahawish et al[6] procedure.

The SEM (Jeol JSM IT500, Tokyo, Japan) and P-XRD (Model: SmartLab SE X-Ray; Make: Rigaku, Japan) (Scanning range -20° to 100° 2θ) were performed on composite samples to visualize and confirm the precipitation of calcite. The XRD sample results were identified with the help of standards established by the International Centre for Diffraction Data. PAST 3 software was utilized for the statistical analysis.

Results and Discussion

The cementation process within the soil granules can be monitored only by estimating ammonia, electrical conductivity and pH as ammonia and carbonate are the bye-products of urea hydrolysis.

pH, EC and Ammonia: The pH was regularly monitored every 24 hours and it was varied from 8.80 to 8.00 with an average of 8.46±0.27 in 600 µ; 8.90 to 7.54 with an average of 8.27±0.42 in 425 µ and 8.80 to 8.10 with an average of 8.46±0.22 in 212 µ (Fig. 2A). The pH of the control sand column was constantly maintained below 8.0 without any alteration.

The EC of the effluent was varied from 118.9 to 77.7 ms/cm with an average of 98.81±10.22 ms/cm in 600 µ; 113.6 to 74.1 ms/cm with an average of 101.84±10.84 ms/cm in 425 µ and 113.0 to 76.6 ms/cm with an average of 101.34±10.43 ms/cm in 212 µ (Fig. 2B). EC for the control was maintained around 55 ms/cm invariably without any major variations.

The effluent ammonia concentration varied from 371.43 to 60.23 mM with an average of 191.66±97.51 mM in 600 µ; 314.54 to 55.43 mM with an average of 168.73±55.43 mM in 425 µ and 302.49 to 95.45 mM with an average of 165.52±63.13 mM in 212 µ (Fig. 2C). There were no traces of ammonia in the control. Our experiment results were also similar to many studies conducted by different researchers[5,6]. During the current study, no significant difference was observed in ammonia concentration between the triplicates (ANOVA, $P < 0.05$).

The alteration in the ammonia concentration, EC and pH of each day in the effluent of the experimental setup is compared to control endorsing the cementation process within the soil. The variation in the concentration of ammonia was due to the variation in microbial activity[9]. Percolations, extended reaction time, exposure to a high concentration of calcium chloride are some factors that may influence microbial activity. The variation in the pH and EC was directly proportional to the concentration of ammonia.

Scanning Electron Microscope: The calcite crystals precipitated by the isolate *Sporosarcina pasteurii* NIOT1 were observed under SEM. The rhombic and irregularly shaped crystals are of dominant morphology precipitated by the isolate *Sporosarcina pasteurii* NIOT1 (Fig. 3). Different authors also visualized a similar kind of precipitation using different microbes for precipitations[10].

XRD Analysis: The XRD analysis results visualize the high precipitation of calcium carbonate within the sand column (Fig. 4) and its correlation with SEM images. In a few studies conducted by the researchers, a similar kind of calcium carbonate peak was obtained[10]. The current study clearly states that the microbe *Sporosarcina pasteurii* NIOT1 has a high capacity in precipitating calcium carbonate devoid of grain size.

UCS: The strength of the materials will always decide the destiny of usage. The MICP mediated bio-cemented soil particles of varying size were subjected to unconfined strength analysis. The unconfined strength of the different particle sizes was 2100, 2200 and 2400 Mpa related to 600, 425 and 212 microns respectively (Fig. 5). In the case of control, there is no compactness in sand particles. The current results admit that the strength of the bio-cemented soil particle was inversely proportional to soil particle size as the maximum was observed with 212-micron soil granules. This is because the improved and closer bridging contacts between the soil and precipitated calcium carbonate improving the strength compared to coarser soil particles with relatively larger sizes.

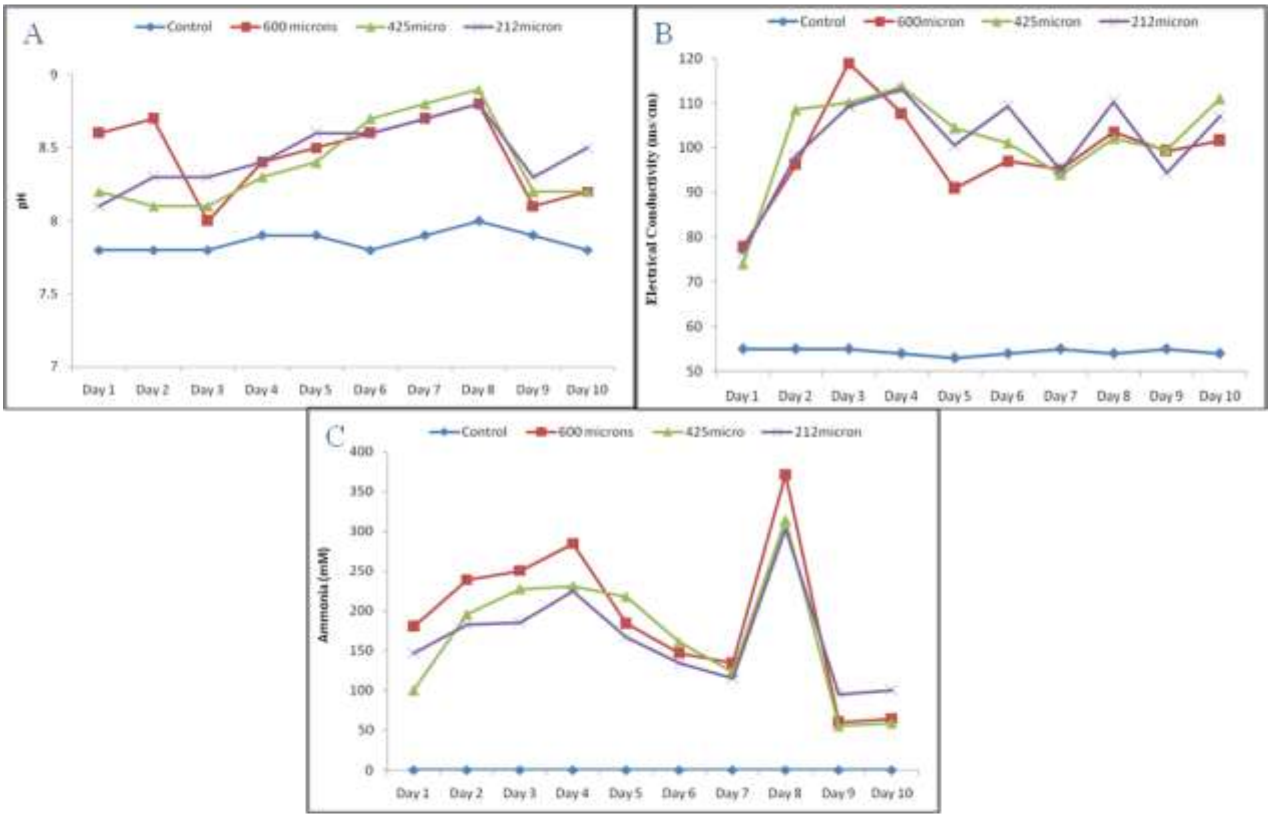

Figure 2: Variation in the Sand Column (A) pH, (B) Electrical Conductivity and (C) Ammonia during the study period

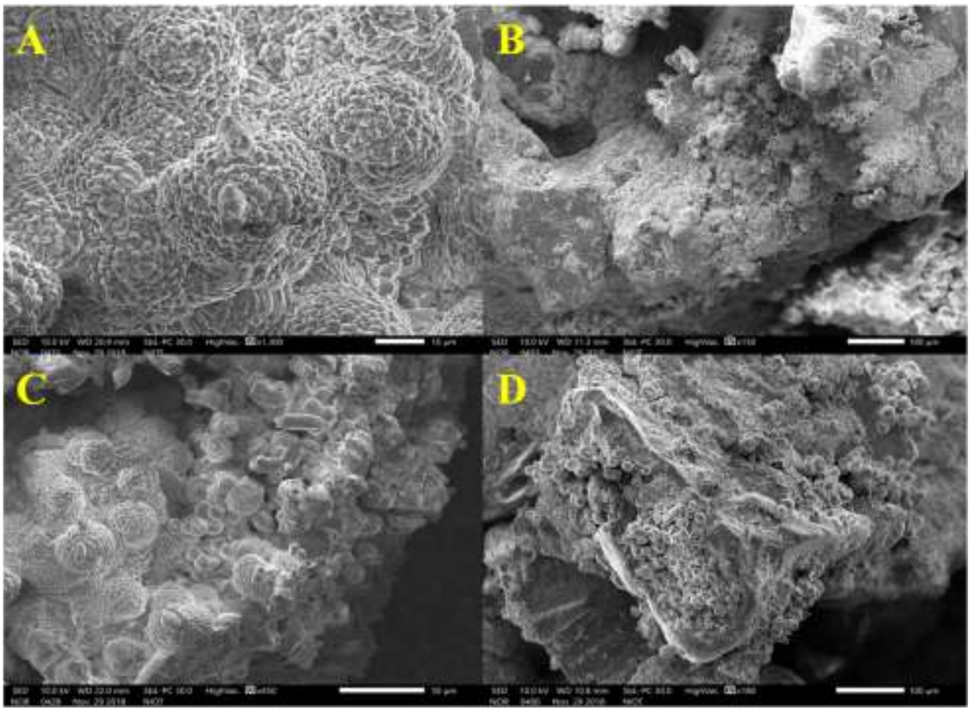

Figure 3: Scanning Electron Images of Carbonate particles precipitated over the sand particles. B and D showing calcite precipitation on sand particles, A and C showing different shapes (cubic, polygonal, rhomboidal, spherical and irregular) of precipitated calcite particles

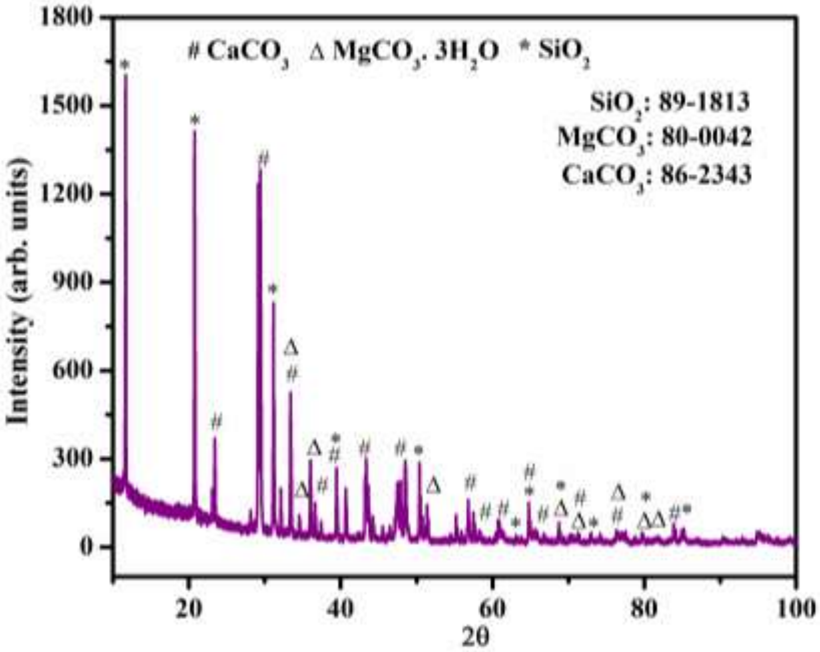

Figure 4: XRD results visualizing the precipitation of Calcium Carbonate ($CaCO_3$) in sand column

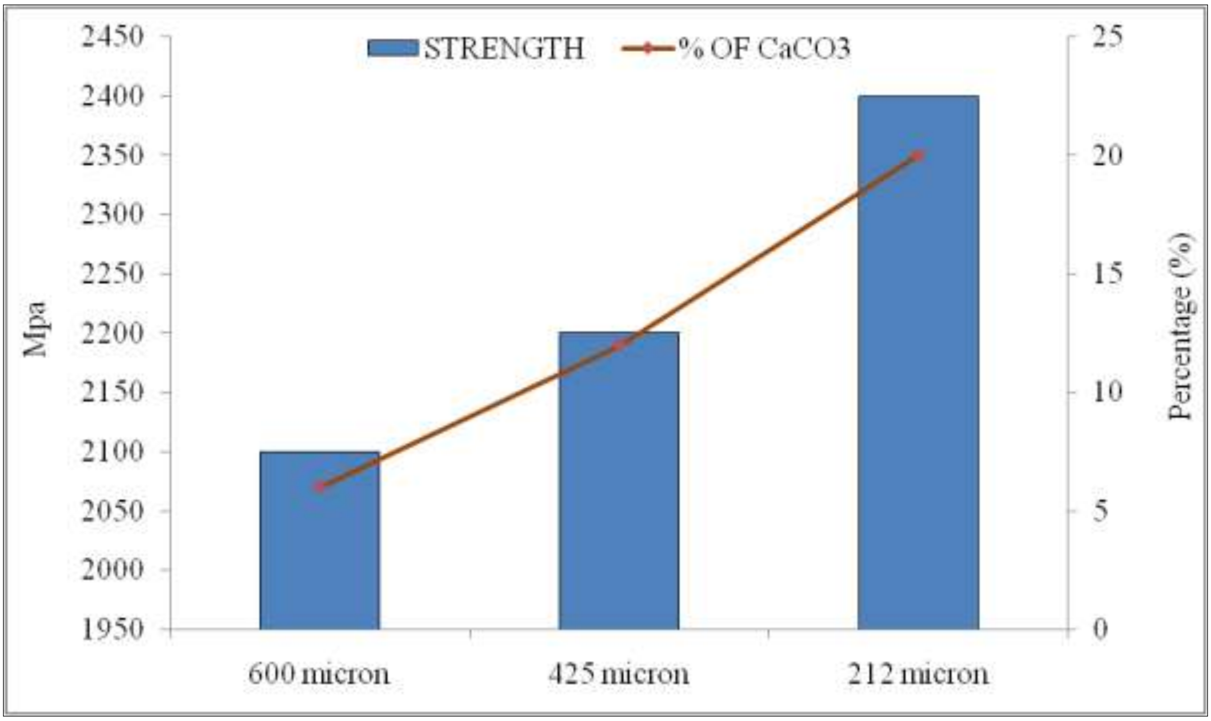

Figure 5: Variation in the Compressive strength and % of $CaCO_3$ precipitation in different particle size

Similarly, Mahawish et al[6] also recorded a minimum strength with the 100% coarser soil compared with a mixed proportion of coarser and finer granules. Researchers worldwide identified that the increase in calcium carbonate precipitation positively influences a significant increase in strength of the particle[6]. Likewise, in our study, the maximum precipitation of calcium carbonate and strength were observed in 212µ soil particles (Fig. 5). The current study proved that particle size has also been added among the critical parameters in the cementation process.

Conclusion

A laboratory MICP experiment was conducted to test the influence of particle size over the strength and to find its efficiency to suppress coastal erosion. In this study, the maximum calcite precipitation and strength were observed in 212µ particles followed by 425µ and 600µparticles. From the study, it is evident that particle size is also one of the significant components that greatly influences consolidation strength. The study also clearly suggests that the particle size is inversely proportional to the material's strength (UCS).

The study also concludes that the strain *Sporosarcinapasteurii* NIOT1 has the better advantage even with the coarser particles which was also evident by the SEM and XRD analysis. This study also supports the necessity of MICP in compacting beach sand particles which are otherwise prone to washing away and erosion by coastal dynamics.

Acknowledgement

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