Biocementation Mediated By Stimulated Ureolytic Microbes From Brahmaputra Riverbank For Mitigation of Soil Erosion

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Research Article

Keywords: Riverbank erosion, Microbially induced carbonate precipitation (MICP), *Sporosarcina pasteurii*, *Pseudogracilibacillus auburnensis*

Posted Date: February 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-235959/v1>

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Version of Record: A version of this preprint was published at Scientific Reports on July 27th, 2021. See the published version at <https://doi.org/10.1038/s41598-021-94614-6>.

Abstract

Riverbank erosion is a global problem with significant socio-economic impacts. Microbially induced carbonate precipitation (MICP) has recently emerged as a promising technology for improving the mechanical properties of soils. The presented study investigates the potential of native calcifying bacterial communities of the Brahmaputra riverbank for the first time via biostimulation and explores its effect on the mitigation of soil erosion. The ureolytic and calcium carbonate cementation ability of the enriched cultures were investigated with reference to the standard calcifying culture of *Sporosarcina pasteurii* (ATCC 11859). 16S rRNA analysis revealed Firmicutes to be the most predominant calcifying class with *Sporosarcina pasteurii* and *Pseudogracilibacillus auburnensis* as the prevalent strains. The morphological and mineralogical characterization of carbonate crystals confirmed the calcite precipitation potential of these communities. The erosion resistance of soil treated with native calcifying communities was examined via needle penetration and lab-scale flume erosion test. We found a substantial reduction in soil erosion in the biocemented sample with calcite content of 7.3% and needle penetration index of 16 N/mm. We report cementation potential of biostimulated ureolytic cultures for a cost-competitive and environmentally-conscious alternative to current erosion mitigation practices.

1. Introduction

The banks along the worlds' mega-rivers are susceptible to land degradation due to severe soil erosion¹. The natural causes of soil erosion include frequent changes in hydrological conditions, including strong river currents, intense rainfall, and climate change^{2,3}. The land degradation imparts enormous socio-economical vulnerability to the residents of riparian zone⁴. Human interventions such as deforestation and non-engineered construction are further worsening the stability of the vulnerable riverbanks⁵. In India, severe land degradation issues are confronted at the banks of Brahmaputra River, one of the top ten river by discharge in the world⁶.

The existing riverbank erosion control practices are inclined towards rigid structures such as aprons, gabions, and check dams, which can irretrievably damage the riparian ecology by producing the imbalance in sediment inflow and outflow^{5,7}. These structures may drastically impact the features of the river channel and induce floods downstream^{5,8}. Alternatively, riverbank erosion can be controlled by improving the riverbank soil properties by chemical grouting. However, synthetic grout material, including micro-fine cement, epoxy, and silicates has been reported to be toxic for the geo-environment^{9,10} and, therefore, can negatively impact the flora, fauna, and crop productivity of the soil of the riparian zone. One of the options as a sustainable erosion mitigation technique is vegetation along the bank. However, vegetation and their effect on the erodibility of soil are difficult to comprehend and engineer due to their transient life cycle and complex root structures which depend on the vegetation type, nutrition present in the soil, and the surrounding climate¹¹⁻¹⁴. Therefore, an alternative riverbank erosion mitigation strategy with minimum intervention to the riparian ecology is urgently needed.

Nature has been forming sustainable cement for millions of years, as seen in the case of corals, beach rocks, anthills, and cave speleothems^{15,16}. Within this context, microbial induced calcite precipitation (MICP) is proposed by several studies as a potential tool for ecologically sustainable ground improvement technology^{17,18}. The principle of MICP is to utilize the ureolytic bacteria to hydrolyze urea ($\text{NH}_2\text{-CO-NH}_2$), as shown in equation 1. In the presence of any ionic calcium source, calcite is precipitated in the soil pore network to bind the cohesion-less granular soil particles^{19,20} as summarized in equation (2).

There are certain advantages of MICP over existing grouting practices, such as easy permeation through soil media due to the water-like viscosity of the cementation solution and comparatively negotiable influence on the geo-environment²¹. The limitations associated with its prospective field application are ammonium production as a byproduct, non-uniformity of precipitation, and transport of healthy ureolytic bacteria in large quantities to the site^{18,22}. While the potential strategies to negate a higher concentration of ammonium ions and non-uniformity are being investigated comprehensively^{23–28}, the transport of calcifying bacterial culture to the desired site is largely unaddressed. The presence of native ureolytic bacteria and their enrichment on-site can tackle the challenge of bacteria transport^{29,30}.

There are limited studies on soil erosion mitigation with MICP; however, most of these studies are based on the bio-augmentation approach with foreign bacteria *Sporosarcina pasteurii* (ATCC 11859). Salifu *et al.*³¹ reported the effect of tidal cycles on different soil slope angles treated with 18 cycles of MICP treatment solution of 0.7 M cementation solution³¹. The study reported that calcite filled in 9.9% pore volume was effective in controlling soil erosion against 30 tidal cycles on the steep slope (53°). Jiang and Soga³² investigated seepage-induced internal erosion for gravel-sand mix in a novel designed device considering *Sporosarcina pasteurii* (ATCC 6452) and reported cementation concentration higher than 0.4 M reduce the erosion to a negligible level³². Wang *et al.*³³ investigated hydraulic flume and erosion function apparatus (EFA) based study on erosion considering PVA-based modified cementation solution and observed non-uniform calcite precipitation with 1 M cementation solution via surface percolation strategy and suggested spraying of biocementation as an alternative application strategy. Later, Jiang *et al.*³⁴ investigated rainfall-induced erosion simulations for various treated soil 0.2, 1 and 2.0 M by spraying of cementation solution four times and observed that 0.2 and 1.0 Molar cementation solution treated soils were substantially more resistant against rainfall-induced erosion when compared to 2 M treated soil. One of the recent studies by Clarà Saracho *et al.*³⁵ investigated the influence of tangential erosion by utilizing erosion function apparatus (EFA) and reported that treatment of soil sample with one pore volume of 0.08 M biocementation solution for 10 days brought values down to a negligible level for a tangential flow of 0 to 0.185 m/s³⁵.

As soil is rich in microbial diversity with approximately 10^9 - 10^{12} microorganisms per kilogram of soil nearby the ground surface³⁶, any supplemented foreign bacteria has to compete with the native microorganisms for their survival in the new environment^{29,30,37}. Hence, utilizing the indigenous microorganisms over the bio-augmentation approach for soil improvement has definite advantages such as minimal intervention to native biodiversity and reducing the cost of bacterial transport.

With this background, we investigated the erosion mitigation potential of the native bacterial communities of the riverbank site in the Assam valley of the Brahmaputra River by conducting (a) Enrichment of ureolytic calcium carbonate precipitating microbial communities, (b) Isolation and identification of the ureolytic bacteria from the natural riverbank site, (c). Comparison of their biocementation potential in terms of the urease activity and calcium utilization rate with *Sporosarcina pasteurii* (SP), (d). Evaluation of soil strength improvement by the needle penetration test, and (e). Investigation on the influence of incremental cementation level on the soil erodibility with a laboratory scale hydraulic flume erosion test.

2. Experimental Summary

Soil samples were collected from the Brahmaputra riverbank (26°10'50" N; 91°41'26" E) near the Indian Institute of Technology, Guwahati, Assam campus as shown in figure 1. The Brahmaputra river is an anabranching trans-boundary mega-river flowing in the south –Asian countries, including China, India, and Bangladesh³⁸. Severe erosion with a rate of 80 square kilometers per year is reported at the Brahmaputra riverbank in the Assam valley along with the demolition of 2500 villages and 18 towns, impacting the livelihood of half a million people³⁹. During the twentieth century (1912-1996), a total of 2358 square kilometer area from the Brahmaputra riverbank was eroded, and 1490 square kilometer of the area was accreted as fill, resulting in approximate 868 square kilometers of net land degradation in the Assam valley along the 630 km length of the Brahmaputra river⁴⁰. Another study reported a loss of 35.5% loss of the land in the world's largest river island, " Majuli," situated in the upper reach of the Brahmaputra river in Assam⁶. Therefore, the protection of these banks is paramount for social, economic, and environmental sustainability. For comparison, another soil sample was collected from a nearby natural vegetative slope (26°11'05" N; 91°41'32" E) inside the Indian Institute of Technology, Guwahati campus in the vicinity of the riverbank. The topsoil (1 cm) was removed and the soil beneath was collected in a sterile tube from both sites and kept in an ice bucket for isolation purposes. The soil was collected separately for geotechnical classification. The evaluated engineering properties of soil following ASTM standard methods for soil classification is shown in table 1⁴¹⁻⁴³.

Table 1 soil properties

Properties	Site 1 (Brahmaputra Riverbank)	Site 2 (natural slope near IIT Guwahati-core 4)
Coordinates	26°10'50"N; 91°41'26"E	26°11'05"N; 91°41'32"E
pH	7.5	8
Environmental Temperature	32±3°C	32±3°C
Specific Gravity	2.7	2.65
D ₆₀ (mm)	0.15	0.3
D ₃₀ (mm)	0.12	0.15
D ₁₀ (mm)	0.09	0.016
Coefficient of uniformity (C _u)	1.67	18.75
Coefficient of Curvature (C _c)	1.06	4.68
USCS classification	SP (poorly graded fine sand)	SC (Well graded sandy Clay)
Maximum Dry Density (kN/m ³)	15.36	16.75

After collecting the soil, a series of experiments were performed to enrich, isolate, and characterize the biocementation potent microorganisms. A summary of the work-plan can be comprehended by the flow chart of Fig. 2. After the isolation and characterization of the native bacteria, the best isolate was selected for soil treatment. The treated soil specimens with the best performing ureolytic strain were evaluated by the needle penetration resistance and flume erosion tests. The microstructural analysis of the precipitates and the treated soil sample was conducted to evaluate the morphology and mineralogy of the precipitates and the treated soil.

3. Results And Discussion

3.1 Biostimulation of ureolytic communities

3.1.1 Enrichment The native communities of the soils were successfully grown in the enrichment media (NB5U). The cultivated communities after two subcultures were serially diluted (10⁻² to 10⁻⁶) and were spread with a sterile loop over Nutrient agar plates supplemented with 2% urea. Later, 36 morphologically distinct single colonies were obtained on the urea agar base plates based on visual observation.

3.1.2 Isolation, identification, and characterization of the ureolytic isolates Out of 36 isolated bacteria, six isolates (BS1, BS2, BS3, BS4, LS1, and LS2) were selected after checking for the urease activity test on the urea agar base (UAB) plate. These selected

isolates turned the color of the UAB plate from orange to pink within 12 hours. The biochemical characterization (details in supplementary data, table 2) of the isolates revealed that all the isolates are Gram-positive. All the isolates were rod-shaped, endospore-forming, urease, and oxidase positive. All the isolates were not able to utilize the Lysine and ONPG contrary to *Sporosarcina pasteurii* (SP). The 16s rRNA sequence revealed the isolates as close relatives of SP. The details of the identified isolates provided by NCMR (details in supplementary data, table 1). A further investigation of the isolated sequence was done via the NCBI database. The sequences were submitted to the GenBank database of the NCBI (National Center for Biotechnology Information) under the accession number MW024144 to MW024149. The BLAST analysis revealed that these strains are novel strains of the *Sporosarcina* family. We found that the isolate BS1 and BS2 had 96.62% (coverage 100%) and 96.22% (coverage 99%) identity with *Sporosarcina sibirinis* (NCBI accession number NR 134188). BS3 had 98.8 % identity (coverage 97%) with *Sporosarcina pasteurii* (NCBI accession number NR 104923). BS4 and LS1 had 97.4% (coverage 99%) and 97.37 % identity (coverage 100%) with *Sporosarcina soli* (NCBI accession number NR 043527). Contrarily, LS2 was found to be related to the *Pseudogracilibacillus* family. LS2 was observed to be closely related to *Pseudogracilibacillus auburnensis* P-207 with 97.06% identity (96% coverage). Based on these findings, the Phylogenetic tree was constructed with bootstrap (1000 replicates) considering the reference sequences obtained from the BLAST analysis, as shown in figure 3. Similar observations were made at Graddy et al.³⁰, where the majority of the isolated strains (47 out of 57) from bio-stimulation soil tank were found to be strains of the *Sporosarcina* genus. It is worth noting that the soil enrichment media for stimulation was rich in urea, similar to Gomez et al.⁴⁴ and Graddy et al.³⁰, which is conditional stress for selective stimulation of ureolytic microorganisms. Moreover, the isolated strains were screened based on the morphology and qualitative urease activity.

3.2 Evaluation of biocementation potential of the isolated strains

3.2.1 Growth and pH

The various parameters of the biocementation potential of the isolated strains in comparison with *Sporosarcina pasteurii* (SP) have been plotted in figure 4. The growth characteristics of the isolates in NBU media and pH during growth have been represented in figures 4 (a) and 4 (b). The initial pH of the growth media is kept at 7.5. It was observed that the pH of the growth media rises to 9.5 within 24 hours of growth, indicating that these strains favor an alkaline environment to grow similar to SP 45. All the isolates start growing when the pH of the media rises to 8.5 or above. Isolate LS2 was observed to have slower growth when compared with other isolates. This can be explained as LS2 belongs to different genera (*Pseudogracilibacillus*).

3.2.2 Specific urease activity

The specific urease activities of the isolates were found to be comparable with SP (shown in figure 4c). Based on the provided NBU media and growth condition, the specific urease activity of SP is found to be 173.44 mM urea hydrolyzed h⁻¹ (OD₆₀₀)-⁻¹, which is around 2.9 mM urea hydrolyzed min⁻¹ (OD₆₀₀)-⁻¹. The specific urease activity of the isolate BS3 was observed to be maximum as 186.6 mM urea hydrolyzed h⁻¹ (OD₆₀₀)-⁻¹ during a growth period of 24 hours and pH > 9. Consortia also demonstrated significant urease activity as 160 mM urea hydrolyzed h⁻¹ (OD₆₀₀)-⁻¹ at a growth period of 48 hours. The maximum ureolytic activity in BS1 was observed after 72 hours of growth with a value of 106.67 mM urea hydrolyzed h⁻¹ (OD₆₀₀)-⁻¹. Maximum specific urease activity of the isolate BS2, BS4, and LS1 was observed to be 160.2, 120, and 173.4 mM urea hydrolyzed h⁻¹ (OD₆₀₀)-⁻¹ respectively after a growth duration of 48 hours. LS2 demonstrated the maximum specific urease activity of 146.4 mM urea

hydrolyzed h-1 (OD600)-1. The observed order of specific urease activity at 24 hours of growth period is BS3>SP>Consortia>LS1>BS2>BS4>LS2>BS1. As the urease activity of the strains depends on the growth media, urea content, and environmental conditions such as pH and Temperature⁴⁶, we considered the conditions at the riverbank at the time of isolation, and the pH and temperature of the growth media were set at 7.5 and 37 degrees Celsius. The specific urease measured by the electrical conductivity method is reported to be between 3 to 9.7 mM urea hydrolyzed min⁻¹ (OD600)-1 in yeast-extract urea media at pH 7 and temperature 30 degree Celsius⁴⁵. It is reported around 5 mM urea hydrolyzed min⁻¹ (OD600)-1 in the nutrient broth urea (2%) media at a temperature of 25 degrees Celsius⁴⁶. The comparative analysis of the urease activity (measured by electrical conductivity method) was done considering SP as positive control in this study. The maximum specific urease activities of all isolates were found to be in a range of 106.67 to 186.67 mM urea hydrolyzed h-1 (OD600)-1 (1.78 to 3.11 mM urea hydrolyzed min⁻¹ OD600-1), which indicates that all of the isolated strains are capable of biocementation^{32,45}.

3.2.3 Calcium utilization and carbonate precipitation potential

It was experimentally observed that the depletion of the supplemented soluble calcium in the precipitation media (PM) was corresponding to the ureolytic activities of the isolated strains. It took 48 hours for the 1% bacteria (OD600=1) to approximately utilize all the soluble calcium chloride (50 mM) in the precipitation media (PM) to precipitate carbonate crystals (Figure 4d). Within 12 hours of the inoculation period, BS3 was able to utilize 75% of the supplied calcium, while SP was able to utilize only 62.5% of the soluble calcium. The order of the calcium utilization potential in the isolates was observed as BS3≥LS2>L.S.1>Consortia>SP>BS4>BS2>BS1 during the inoculation period. Contrarily, LS2, despite being a comparatively slow urease producing bacteria, was able to utilize calcium ions at par with other isolates. Negligible changes were observed in the soluble calcium concentration of the control group eliminating the possibility of abiotic precipitation. The carbonate precipitation rate for each isolate (1% at OD600=1) for the 50 mM cementation media is plotted in figure 4 (e). The isolate BS3 with maximum ureolytic activity (specific urease activity 186.6 mM urea hydrolyzed min⁻¹ OD600-1) precipitated the highest carbonate crystals after 96 hours of the incubation period. BS3 precipitated 438 mg/100 ml of carbonate crystals, which is around 87.66% precipitation from the total supplied CaCl₂, while precipitation with SP was quantified as 389 mg/100 ml (78%). The precipitation in consortia was observed to be 407 mg/100ml (81%), which is slightly higher than SP. Precipitation in other isolates was found to be significantly lower than isolate BS3. Isolate BS1 and BS2 precipitated 334 mg/100ml (67%) and 343 mg/100ml (69%) of carbonate crystals respectively, whereas isolate LS1 and LS2 precipitated around 357 mg/100 (71%) ml of carbonate crystals each. Isolate BS4 precipitated minimum carbonate crystals 292 mg/100 ml (58%). No precipitation was observed in the negative control set. Low concentrations of bacterial cells (1%) were considered in this experiment to slow down the urea hydrolysis in order to differentiate the calcium utilization potential of the isolated strains. This approach was modified from Dhami et al.⁴⁷, and our results show agreement with their finding where 1% of SP cells depletes the 25 mM of CaCl₂ within 24 hours. It was observed that all the isolates took approximately 48 hours to deplete the 50 mM CaCl₂. The depletion of soluble calcium concentration was rapid in the initial 24 hours in all the isolates. After 48 hours, the residual soluble calcium was observed to be in the range of 2.5-5 mM in all the isolates (except BS1 and BS2), which might be due to loss of nutrients or super-saturation condition in the precipitation

media 45. The carbonate precipitation and calcium utilization by the ureolytic cells are also impacted by the metabolites during the growth and metabolic activities. As maximum precipitation was recovered with the isolate BS3, the isolate BS3 was selected for further soil treatment.

3.2.4 Microstructure analysis of the precipitates

The FESEM images of the carbonate crystal precipitated from BS3 was investigated further. The shape of the precipitated crystals was observed to be rhombohedral and trigonal (Figure 5 a). The average size of the crystals was observed in a range of 25 to 50 microns. The entrapped bacteria and rod-shaped bacterial imprints were identified (Figure 5b), indicating that the bacteria acted as a nucleation site 19. The smaller crystals were observed to coagulate in layers to develop larger calcite crystals. The entrapped bacteria were noticed on the grown and coagulated calcite crystal in figure 5 (c). After taking the FESEM image (figure 5a) of the precipitate, EDX analysis was conducted, and the elemental composition suggested an abundance of calcium, carbon, and oxygen, which indicates the presence of calcium carbonate crystals (details provided supplementary data, figure 1). XRD analysis was conducted to confirm the mineralogy of the precipitates, and the majority of the observed peaks of the XRD plot belonged to calcite, which is consistent with the observation of rhombohedral crystal shapes in the FESEM image. The XRD analysis also suggested an insignificant presence of aragonite in the precipitates.

3.3 Application of native communities on riverbank soil and its influence on soil strength

3.3.1 Needle penetration resistance of treated soil

The average NPI (N/mm) for different cases has been shown in Figure 6 (a). With one bio cementation cycle treatment, the consortia treated soil sample (Consortia-BC1) demonstrated higher value of NPI (5.15 N/mm) than SP-BC1 (4.19 N/mm) and BS3-BC1 (4.64 N/mm). The increase in the biocementation cycle treatment significantly improved the needle penetration resistance. Sample BS3-BC2 showed 116% improvement with the NPI value of 10.03 N/mm when compared to one cycle treated sample BS3-BC1. A similar trend was observed in the sample BS3-BC3 (NPI= 16.12 N/mm), which showed around 347% improvement in NPI when compared to sample BS3-BC1. From the needle penetration test, it was evident that the soil properties improve significantly with the increased level of biocementation cycles, indirectly indicating an improvement in the soil erosion resilience. Since non-uniformity is one of the undesired traits of MICP, a contour was plotted corresponding to the 25 points NPI, as shown in figure 6 (b). The contrasting color difference in the contours of the samples BS3-BC1, BS3-BC2, and BS-BC3 clearly demonstrates the stark difference in the strength of treated samples. The non-uniformity in the strength of treated soil crust of sample BS3-BC2 and BS3-BC3 can also be realized with the contrasting color gradient of the NPI contours. Since the rate of penetration has insignificant influence on the test results⁴⁸, the needle penetration test is recommended for quick testing of the strength of the stabilized soils and soft rocks. As a large number of tests can be conducted due to the small diameter of the needle without destroying the sample, the needle penetration test is a better alternative to evaluate the local grain bonding in the biocemented soil than bulk strength properties like unconfined compressive strength and calcite content. The response of the needle penetration resistance in terms of nominal strain (ratio of penetration to rod diameter) also indicated that the measured responses are independent of needle diameter for a small range, i.e., 1 to 3 mm^{49,50}. A portable penetrometer of Maruto. Co. Ltd. (needle maximum diameter 0.84 mm at 12mm from the tip) have been correlated with high confidence value to conventional physicochemical parameters such as unconfined compressive strength (UCS), elasticity modulus, and elastic wave velocity

in several studies^{48,49}. In our setup, we have utilized a similar configuration chenille 22 needle with (maximum diameter 0.86 mm at 9 mm from the tip) and a penetration rate of 15 mm/minute for measuring the strength properties of cemented soil. Adopting the UCS and NPI correlation suggested by Ulusay et al.⁴⁸, the UCS of samples BS3-BC1, BS3-BC2, and BS3-BC3 are around 1.67 MPa, 3.4 Mpa, and 5.3 Mpa.

3.3.2 Erodibility test in the hydraulic flume

To investigate the influence of hydraulic current on different levels of biocementation, all the treated samples were exposed to hydraulic current gradually varying from gentle flow (0.06 m/s) to five times of the critical erosion velocity (0.75 m/s) in a 45-minute duration test and were compared with control (untreated soil). The results of the erosion tests are plotted in figure 7. As expected, with an increase of biocementation cycles, i.e., calcite content, the fluvial erosion reduced substantially. Approximately 7.3% of calcite content resulted in negligible erosion (12% mass loss) when compared to the control, i.e., untreated sand (56% mass loss). One biocementation cycle treatment (sample BS3-BC1) produced an average of 2.5% of calcite, reducing the soil loss to 31%. Sample BS3-BC2 with 4.93% calcite content resulted in 22% soil mass loss during the flume erosion test. From the visual observation of the soil specimen after the erosion test, it was evident that the soil particles start bonding with an increased level of bio cementation. A tough crust was formed on the top of BS3-BC2 and BS3-BC3 the samples, which got eroded with the fluvial current. Insignificant aggregation was observed in the sample BS3-BC1. However, with two and three cycles of biocementation treatment (BS3-BC2 and BS3-BC3), the biocemented soil particles (BCS) were evidently noticed (photos are shown in supplementary data, figure 4). Clarà Saracho et al.³⁵ addressed the erosion due to tangential flow (similar to river current) by treating the soil specimen with for ten pore volume of low concentration of cementation media (0.02 M to 0.1 M) by injection strategy and tested the specimen in the flow velocity ranging from 0.035 to 0.185 m/sec for 120 minutes in a modified erosion function apparatus (EFA). The study concluded that the treatment with 0.08M cementation media (calcite content varying from 1.2 to 4%) resulted in negligible erosion in the stated test conditions. In this study, we have considered spraying of bio-cementation media (0.5 M), assuming the convenience of field application. We found that 7.3% of calcite content was required to control the soil erosion substantially in the test flow range (0.06 m/s to 0.75 m/s). The partial loss of soil occurred as the top layer of the sample was not well bonded due to the recurring disturbances induced by spraying.

3.3.3 Microstructure of the biocemented samples

To investigate the influence of different biocementation levels on the microstructure of the treated sand grains, FESEM imaging was conducted for light biocemented samples (BS3-BC1) and heavy biocemented samples (BS3-BC3). While calcite crystals were observed to be growing on the grooves of sand grains in the light biocemented sample (BS3-BC1), bridging of sand grains with rhombohedral crystals was observed in the heavy biocemented sample (BS3-BC3) as shown in figure 8. The effective calcite bridging between sand grains increases the frictional and cohesive property of sand grains^{51,52}, which improves the erosion resilience of the soil. Bacterial imprints were observed in both cases. Further EDX analysis on a bridged sand grain (supplementary data, figure 5) suggested an abundance of silicon and oxygen on the sand grains with a trace amount of chlorine and calcium. This indicates the presence of residual calcium chloride on silica grains. The EDX analysis on the grain bridge indicated the presence of calcium, carbon, and oxygen, suggesting CaCO₃ precipitation. XRD analysis on treated and untreated sand confirmed the precipitation of calcite. Most of the peaks correspond to quartz (silica). In the biocemented

sand sample, a visible peak of calcite was observed at around 29 degrees of 2 θ (Details in supplementary data, figure 6).

4. Conclusions

Biostimulation of ureolytic carbonate precipitating communities from Brahmaputra riverbank soil was successfully conducted in this study. The majority of the isolates belonged to *Sporosarcina* genera highlighting the prevalence of these strains. One of the isolates (BS3) was found to have marginally better biocementation potential than the conventionally used *Sporosarcina pasteurii* (ATCC 11859) in terms of specific urease activity and calcium utilization. This implicates that the enrichment of native bacteria has definite advantages, along with reduced bacterial transport costs. This study provides promising evidence of the potential of native ureolytic communities from riverbank sites in their ability to mitigate soil erosion. Further, with needle penetration resistance tests, we were able to quantify the improvement of soil strength with the number of biocementation cycles, i.e., calcite content. However, the distribution of needle penetration resistance was found to be non-uniform, indicating the poor distribution of the precipitated calcite with spraying strategy. The assessment of soil erodibility was conducted in the hydraulic flume, and it was observed that with an increase in the calcite content in treated soil specimens, the eroded soil mass loss decreased substantially. With microstructural analysis (FESEM, EDX, and XRD), the morphology and the mineralogical composition of precipitated crystals were investigated. The calcite crystal growth in the sand grooves and bridging of sand grains was observed as the cause of increased soil strength in the biocemented soil samples. In conclusion, native bacterial communities were found to be effective in mitigating soil erosion.

The river ecology is very sensitive to environmental changes, and the practiced rigid-structures to mitigate the riverbank erosion are observed to be harmful in the long run ⁵. In this study, we proposed MICP treatment as a potential eco-friendly erosion control technique with native bacteria so that there is a minimum intervention to the river ecology. However, there are numerous concerns for its field application. The alkalinity of growth media and ammonia generation are major concerns for the river ecology and geo-environment. There is a high possibility of ammonia being diluted to negligible levels at the riverbanks; however, this can be threatening to the flora and fauna of the riparian zone. Therefore, a detailed investigation of ammonia dilution or removal strategies will be required, which is not addressed in our study. As non-uniform soil improvement was observed with spraying, a systematic approach for uniform distribution of calcite with a low concentration of cementation media and continuous application by spraying or injection is recommended. There are chances of the cementation media getting diluted in the saturated zones of the riverbanks. Therefore, it is essential to consider the application of the MICP for erosion control only in the low flood seasons to vulnerable riverbanks. A detailed investigation of MICP treatment of the riverbank soil, based on their suction characteristics, will be critically useful to design the field application strategy. Further investigations can be conducted on actual field site conditions for a longer duration of time to investigate the potential of this technology in the long term.

5. Methods

5.1 Enrichment of ureolytic communities from the collected soils

Around 10 g of soil from the riverbank and natural slope was procured to the laboratory in sterile containers. One gram of the collected soil was inoculated into the 100 ml enrichment media containing 13 g/l of Nutrient Broth (NB) and 5% Urea in a shaking incubator at 37° C and 120 rpm for five days. This was followed by two subcultures of the enriched communities into new enrichment media. Confirmation of the ureolytic capability of these communities was verified by inoculating the bacterial culture into urea agar base (Christensen, 1946) Petri dishes wherein change in color was recorded within 12 hours. The details of each of the media (sterile) are reported in Table 2.

5.1.1 Isolation, identification, and characterization of the isolates

After the enrichment of ureolytic communities, we attempted to isolate the efficient ureolytic cultures from the consortia. For this, the enriched consortia were serially diluted and inoculated on NBU agar plates and single colonies were obtained. These colonies were then screened for qualitative urease production on urea agar plates⁵³. Six highly ureolytic cultures were screened. This was followed by the identification of these cultures via 16S rRNA genomic sequencing. The 16S rRNA sequencing was conducted at NCMR (National Centre for Microbial Research, India). The sequences of the isolated strains were analyzed and compared with the highly similar sequences available on the NCBI database utilizing nucleotide BLAST. The DNA sequences of the isolated strains were aligned with the reference sequences of the obtained highly similar strains by the MUSCLE algorithm and the phylogenetic tree was constructed by the neighbor-joining method using Mega-X software⁵⁴.

The screened isolates were further characterized based on their biochemical properties, growth characteristics, urease activity, and calcium utilization potential. The biochemical properties of the isolates were evaluated using the Microbact 12 A, 12B, and 24 E kit. The isolates were grown in the NBU media for four days at 37°C and 180 rpm in a shaking incubator to evaluate growth characteristics and urease activity. The performance of isolates was compared with the comprehensively used SP (*Sporosarcina pasteurii* ATCC 11859) and the artificial consortia. The artificial consortia were created by mixing an equal proportion of the isolated strains from site 1.

The urease activity (mM urea hydrolyzed per hour) of the bacterial isolates was evaluated following the electrical conductivity method⁵⁵. For assay of urease activity, 1 ml of bacteria is mixed with 9 ml of 1.11 M urea, and the change in electrical conductivity was measured for 5 minutes at room temperature (25±3 °C). The variation in electrical conductivity rate (mS per cm per minute) is measured and the urease activity is calculated taking the dilution factor into account⁵⁶. The ratio of the urease activity and the bacterial optical density (OD₆₀₀) is defined as the specific urease activity (mM urea hydrolyzed h⁻¹ OD₆₀₀⁻¹).

5.2 Analytical methods for evaluation of biocementation potential of the isolates

The biocementation potential of the efficient bacterial isolate was evaluated following a modified method from Dharmi *et al.*⁵⁸ 1% overnight grown bacterial culture ($OD_{600}=1$) was added in the precipitation media (PM) consisting of 1g/l Nutrient Broth, 2% urea, and 50 mM of $CaCl_2$ in a shaking incubator at 37°C and 120 rpm. A negative control set consisting of only flask cementation media was also observed for the test duration to check the possibility of abiotic precipitation.

The soluble calcium depletion was evaluated by measuring soluble Ca^{2+} for 48 hours by the EDTA titration method¹⁹. For evaluation of soluble Ca^{2+} , 10 ml of the inoculated cementation media was taken out at different time intervals and centrifuged at 4°C and 8000 rpm for 5 minutes. The supernatant was supplemented with 0.5 ml of 5N sodium hydroxide and few drops of hydroxy-naphthol blue indicator and the mixture was titrated against the 250 mM EDTA. The required EDTA to change color from pink to blue was noted and compared with a standard calibration plot of $CaCl_2$ solutions from a range (5-100 mM). The performance of isolates was compared with comprehensively used SP (*Sporosarcina pasteurii* ATCC 11859) and the consortium. A negative control set consisting of only precipitation media was also observed for the test duration to check the possibility of abiotic precipitation.

At the end of four days, the precipitates were collected on Whatman no. 1 filter paper and washed with sterile water as per the protocol of Dharmi *et al.*⁴⁷. The precipitates were air-dried after filtration at room temperature for 48 hours, weighed, and analyzed for gravimetric, microstructural, and mineralogical analysis.

5.3 Soil sample preparation

Fine sand equivalent to Brahmaputra riverbank sand was procured from Cook Industrial Minerals, Western Australia. The sand was filled at a 40% relative density of 1.6 g/cc in the containers. For the needle penetration test and calcite content measurements, the soil samples were prepared in Petri dishes of diameter 5 cm and depth 1 cm. After the preliminary investigation on the penetration resistance improvement, samples for the hydraulic flume erosion tests were prepared in a container of length 9.25 cm, width 5.8 cm, and depth 4 cm. Separate samples were made for flume erosion test and needle penetration test as the Needle penetration prior to hydraulic erosion can damage the bio cemented crust formed on the top surface. The soil specimens were treated with 0.5 M of equimolar cementation media, as suggested by Jiang and Soga³² and Porter *et al.*⁵¹. The spraying strategy for the treatment was considered as it is a convenient strategy for the field application. As per the recommendation of Wang *et al.*³³, non-uniform calcite precipitation was observed with surficial percolation strategy and spraying strategies were suggested as a viable alternative. For uniform precipitation, the biocementation solutions were supplied in three steps, which include the application of fixation solution, ureolytic bacteria solution, and cementation solution, as suggested by Harkes *et al.*²⁸. In the current study, one biocementation cycle (BC1) is completed by spraying one pore volume of fixation solution, one pore volume of bacterial solution (at optical density ≥ 1), and one pore volume of the cementation solution (two times) consecutively after the retention period of 24 hours for each step. Similarly, samples with two and three

biocementation cycles (BC2 and BC3) treatments were prepared to investigate improved soil properties. Details of all the sample prepared for evaluation has been summarized in table 3.

Table 2 Details of the media used for enrichment, isolation, and cementation

Media/Components	Identifier	Nutrient Broth	Urea	Calcium Chloride
Enrichment media	NB5U	13 g/l	5%	NA
Growth Media	NBU	13 g/l	2%	NA
Precipitation Media	PM	1 g/l	2%	50 mM
Fixation Media	FM	-	-	25 mM
Cementation Media	CM	1 g/l	500 mM	500 mM

Table 3 Details of soil samples prepared

Sample Identifier	Bacteria	Biocementation treatment cycles
SP-BC1	Sporosarcina Pasteurii	1
Con-BC1	Consortia	1
BS3-BC1	Isolate BS3	1
BS3-BC2	Isolate BS3	2
BS3-BC3	Isolate BS3	3
Control	No Bacteria	-

5.3.1 Needle penetration test

Needle penetration test was performed at a penetration rate of 15 mm/minute for 5 mm depth, and the ratio of force to depth (N/mm) has been reported as needle penetration index (NPI) as suggested by Ulusay *et al.*⁵⁰ and mapped for 25 points. The Chenile needle #22 with a diameter of 0.86 mm, was mounted on a universal testing machine (Shimadzu AGS-X). First, the average needle penetration indices of samples SP-BC1, BS3-BC1, and Consortia-BC1 were compared to investigate the influence of different strains and the same level of biocementation on soil strength improvement. Later, to quantify the improvement in soil strength, needle penetration resistance was investigated for samples treated with native bacteria-based two and three biocementation cycles (BS3-BC2 and BS3-BC3).

5.3.2 Erodibility test for a bed slope in Hydraulic flume

The sample treated with the most suitable native strain at a different level of biocementation (BS3-BC1, BS3BC2, and BS3-BC3) were further tested and compared with the untreated sand on the hydraulic flume-based erosion test. The soil erosion tests were performed in a 12 m long, Armfield Engineering Ltd. S5

tilting flume with the glass walls 320 mm high and 300 mm wide (internal breadth). The treated soil containers were plugged into an acrylic bed slope in the flume (Supplementary data, figure 2). The samples were saturated and then the flow velocity was increased stepwise at intervals of a 5-minute time interval (Supplementary data, figure 3). This stepwise incremental flow methodology was considered based on the study by Clarà Saracho *et al.*³⁵, as the critical flow velocity is expected to increase with the biocementation treatment levels. The flow was operated using the control panel and velocity was measured on three different points near the sample using a pulse velocity meter. The critical flow velocity for the riverbank soil calculated from Briaud' (2008) suggested equation⁵⁹ was found to be 0.15 m/s.

$$V_c = 0.35(D_{50})^{0.45} \quad (3)$$

Here, V_c is the critical flow velocity required to initiate the erosion and D_{50} is the mean diameter of the cohesionless soil. The samples were installed 6 meters downstream in the hydraulic flume.

5.3.3 Microstructural analysis and Calcite content of the treated soil

The ZEISS Neon 40 EsB dual FESEM/FIBSEM instrument equipped with Field Emission Scanning Electron Microscopy (FESEM) and Energy-dispersive X-ray spectroscopy (EDX) was used for the microstructural investigation. The air-dried treated soil specimens were mounted on the aluminum stub for its microstructural and elemental composition analysis. The samples were coated with a 10 nm platinum coating. For mineralogical analysis of the precipitates, all the samples were micro-ionized (particle size <10 microns) and analyzed by X-ray diffraction (XRD) on Bruker D8 advanced diffractometer with Nickel filtered Cu-K α radiation varying 2θ from 5° – 100° with a step size of 0.013°. The phase identification was made utilizing COD and ICDD databases with Bruker EVA.

The calcite content of the treated soil was measured by the acid washing method. 5-gram treated soil was collected in triplicates from the crust after the flume erosion test and washed with 20 ml of 1 M HCl. The calcite content was determined based on the gravimetric difference between the collected soil sample (5g) and retained weight of the acid-washed soil on Whatman filter grade 1⁶⁰.

5.4 Statistical Analysis

All samples were analyzed in triplicates, and the average was compared except for the needle penetration test and flume erosion test. The data were analyzed by one-way analysis of variance (ANOVA). For the needle penetration test, the tests were performed at 25 points on the crust and the average value with the standard deviation is reported.

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Figures

Figure 1

Soil collection site at Brahmaputra riverbank

Figure 2

Schematic for the experiments in the current study

Figure 3

Neighbor-joining phylogenetic tree based on the 16S rRNA sequence of the isolates and reference sequence from the GenBank database (NCBI)

Figure 4

(a). Growth characteristics, (b). pH, (c). specific urease activity, (d). calcium utilization rate, and (e). carbonate precipitation rate of the isolates and consortia

(a)

(b)

(c)

Figure 5

FESEM images of the calcite precipitated from BS3 (a). Coagulated crystals (b) Bacterial imprints, (c). Entrapped bacteria on the precipitates

Figure 6
 Comparison of the Needle penetration resistance (N/mm) of the treated soil specimen (a). average values & (b) the contours

Figure 7

Weight of the eroded soil (%) after the flume erosion test

Figure 8

FESEM images of the treated sand grains (a). Calcite crystal growing on the sand grains on BS3- BC1 sample (b). Calcite bridging in BS3- BC3 samples

Supplementary Files

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