Biocementation mediated by native Carbonic Anhydrase-producing microbes for mitigation of soil settlement.

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Abstract

This study investigated microbially induced calcite precipitation (MICP) as a promising technology for improving the mechanical properties of soils via the carbonic anhydrase (CA) pathway. The aim is to find a method to prevent settlements and increase the bearing capacity of embankment foundation soils, with a particular application being the soft foundation soils of the UK railway network. The CA pathway is of interest as the native CA-producing bacteria can sequester CO_2 from the atmosphere and fix it into different biominerals that can be used to produce biocement. The formed biominerals act as binders between soil particles, increasing the foundation soils stiffness and bearing capacity. An additional environmental advantage of using the CA metabolic pathway is the lack of undesirable by-products, as opposed to the most used method to biocement soils, which adopts the urea hydrolysis route. To achieve the aim of this research, forty CA-producing bacterial isolates from soil layers under a railway embankment in East Anglia, England, UK, were screened and selected using a qualitative CA activity assay. Three of these bacteria expressed high and stable CA enzyme activity and were further characterised by their morphological, molecular, and enzyme profile characteristics. Biostimulation and bioaugmentation were employed to biocement the soil from the embankment with the native CA bacteria. The unconfined compressive strength (UCS) and calcite content of the treated soil were measured. Preliminary results show a substantial increase in soil UCS for bioaugmentation. Although the compressibility characteristics and more advanced shear strength testing are the subjects of future work, the UCS and calcite content results which proved biocementation of the soil, show promise that the CA route can be successful in reducing settlement of existing railway infrastructure, providing a non-disruptive and environmentally friendly alternative to current engineering practices for settlement mitigation.

Keywords: Ground improvement; Soil stabilisation, Carbonic Anhydrase; embankment settlement; CO_2 capture.

1. INTRODUCTION

The shift of population from rural to urban areas has created land scarcity. Over 52 % of the global population lives in urban areas, forcing many places with inferior ground to be used for construction [1]. This has led the construction sector to build new infrastructure, such as housing and transportation, and maintain or expand existing ones on the inferior ground [2,3]. Despite the effectiveness of these ground improvement methods, they are unstainable as they affect the environment [4] and have adverse human health effects [5]. Thus, the development of innovative, eco-friendly, and energy-effective alternative methods of ground improvement that promote sustainability has recently been investigated using biocementation. Biocementation is an ecological process based on the microbial induced carbonate precipitation (MICP) mechanism, which results in the deposition of calcium carbonate. The deposited biomineral from microorganisms improves the mechanical behaviours of treated materials. It has been suggested for liquefaction [6], soil stabilisation [7,8]), erosion and dust suppression [9], organic and expansive soil improvement [10,

11], heavy metal remediation [12] and heritage building restoration [13,14]. This innovative technique has been demonstrated, and thus, a promising approach offers an environmentally friendly alternative to traditional soil improvement cited above.

This study reports the isolation and characterisation of CA-producing bacteria from soils under a railway embankment in East Anglia, England, UK. Because of this, this study applied CA biocementation to a railway embankment in East Anglia, a complex of peat soils, coal ash and sandy clay ash. The area is problematic and has been known for settlement due to wastage of these materials as they are weak, compressible, and highly shrinkable when drained. Both coal ash and sandy soils from the embankments were treated using CA biocementation. The finding of this work may facilitate the development of using CA biocementation as an eco-friendly soil agent. The combined usage of CA biocementation for forming biocements with CO_2 capture would decarbonise the maintenance and construction of railway infrastructure and make it more resilient in the changing climate.

2. MATERIALS AND METHODS

2.1 Soil used

The materials considered in this research study were the soil from the Network Railway embankment from Pricklewillow, East Anglia, United Kingdom (the latitude and longitude are 52.378189N and 0.258608E, respectively). The intrinsic properties and compositions of soils are summarised in Table 1.

Table 1. Geotechnical Properties of coal ash and soil East Anglia, United Kingdom

Parameter	Sandy silty Clay Soil
Liquid limit: % w/w*	63
Plastic limit: % w/w	33
Plasticity index: % w/w	30
Ash content: % w/w	17.7
Organic matter content: % w/w	3.9
Specific gravity, Gs	1.78
Natural gr moisture content: % w/w	34.5
pH	7.7
Natural CaCO3 Content	4.82

2.1 Isolation and characterisation of carbonic the anhydrase bacteria

The CA-producing bacteria were isolated by placing 1 g of soil in a 15 mL sterile centrifuge tube and adding 10 mL of sterile water. The thoroughly homogenised suspension of soil and sterile water was diluted from 10 to 10,000-fold using sterile water and plated on a broth-peptone agar medium spiked with three mM p-NPA. The p-NPA was used as an indicator for CA-positive-producing bacteria. Colonies of bacteria with the CA-producing ability presented an intense yellow colouration due to the hydrolysis of p-NPA into para-nitrophenol (pNP), as elucidated previously. The cultured plate mediums were placed in an incubator for three days at 30 °C, and about colonies were identified in the plate mediums at the end of the incubation period.

2.2 Microbial growth and CA Production and Characterisation

The CA enzyme was determined colourimetrically as employed by [15] to determine CA activity. The identified bacterial isolates that were observed to have produced intense yellow colouration were further screened for CA enzyme activity in nutrient broth. Briefly, the activity for p-nitrophenyl acetate hydrolysis was determined at room temperature in a reaction mixture (1.35 ml) containing freshly prepared three mM p-nitrophenyl acetate in phosphate buffer (0.13M and pH 7.2). A pure colony was prepared aseptically in 50 mL nutrient broth for the CA enzyme activity assay and incubated at 37°C for 24 h. The reaction was allowed to proceed for 5 minutes, and the

change to A_{348} per min. Then the carbonic anhydrase activity was characterised by the amount of p-nitrophenol produced per unit of time, and enzyme activity was expressed in terms of U.

$$\text{CA activity}\left(\frac{\text{U}}{\text{mL}}\right) = \frac{(\Delta A_{348}\text{T} - \Delta A_{348}\text{B}) \times 1000}{5 \times Volume} \quad (1)$$

Experiments were conducted in triplicate. Microbial growth was measured by UV-vis spectrophotometry (Jenway 6305, Bibby Scientific, Staffordshire, UK) that recorded optical density (OD) readings at 600 nm for 96 h. The temperature would affect the growth and reproduction of CA-producing bacteria and consequently the activity of enzymes, so we set the temperature at 5℃, 15℃, 25℃, 30℃ and 37℃. After sterile inoculation, the medium was cultured at a constant temperature oscillation incubator (120 rpm) at different temperatures for 24 h. Then the OD600 value and carbonic anhydrase activity in the medium solution were measured as described previously.

2.3 Bioprecipitate production and biocementation procedure

The three isolates were used for bioprecipitation using equimolar calcium acetate and Sodium Bicarbonate (0.1, 0.25, 0.5, 0.75, and 1.0 M). The bacterial isolate was precultured for 24 h in a 10 mL nutrient broth medium. Then 1 mL of preculture was inoculated into 100 mL broth to grow the main culture at 37°C for 96 h with continuous aeration at 120 rpm.

2.4 Biocementation of soil

The biocementation procedure consisted of a two-stage injection performed to confine the bacteria (U-1, U-21 and U-26) by injecting to fill the soil column, followed by a cementation solution (calcium Acetate ($Ca(CH_3COO)_2$) and Sodium Bicarbonate ($NaHCO_3$) (0.1M)) second stage. All the cementation solutions were applied at the top of the soil columns and allowed to percolate by gravity. Using a pocket penetrometer, the unconfined compression test (UCS) was used to approximate the strength of the biocemented soil samples of dimensions of 50-mm diameters and 100-mm lengths. A set of control specimens was prepared for comparison purposes, as shown in Table 2.

Bacterial strain	Cementation Solution (M)		Bacterial culture	Curing Time (Days)
	$Ca(CH_3COO)_2$	$NaHCO_3$		
Control	0.1	0.1	-	14 Days
U-1	0.1	0.1	√	14 Days
U-21	0.1	0.1	√	14 Days
U-26	0.1	0.1	√	14 Days

Table 1: Details of different conditions used for the soil biocementation

2.5 Analytical methods

An oven-dried mass of the soil or coal ash samples across the specimen was measured before and after an acid wash (HCl 2M) to determine the calcium carbonate content (Agilent Technologies, Inc., Danbury, CT) with resolution $4cm^{-1}$, range $600\text{-}4000cm^{-1}$; scanning electron microscopy with Thermo Scientific Pharos FEG-SEM (ThermoFisher Scientific, USA) with high vacuum mode, 15KV acceleration voltage, elemental analysis done with EDS (Energy dispersive X-ray detector); and Raman spectroscopy (Horiba Aramis confocal Raman microscope,) with 633nm laser (1% power), 50X objective, 100um pinhole, 600l/mm grating.

3. RESULTS AND DISCUSSION

3.1 Isolated strain with CA enzyme production

The CA enzyme's microbial growth, CA activity, and gram stain were studied to understand the selected bacteria. The microbial growth shows similar growth characteristics as previously isolated strains showing microbial growth's lag, exponential, stationary, and death phases. The microbial growth curve shown in Figure 1a showed a luxurious increase for the first 24 h showing the exponential phase for the three strains. The chosen strains reached the maximum growth at a different time: 24 h for U-1, whereas U-21 and U-26 were observed at 48 h. Figure 3b shows the measurements of the CA activity of each bacterial isolate. The quantitative analysis of the CA enzyme activity showed that U-1, U-21, and U-26 showed higher specific CA activity values of 2.31, 0.94, and 1.53 U/ml, respectively. The three strains have higher CA activity in comparison to previous isolated CA-producing bacteria: *B. schlegelii* (0.0453 U/mL) isolated from garden soil [16] and *B. altitudinis* (0.695 U/mL) isolated from mangrove sediments [17]. However, the three isolated from our study are less in CA activity than Bacillus sp. AP6 produced 5.61 EU mL CA in the purified fraction used for biocementation and Bacillus mucilaginosus [18], well known for CO2 capture. These results show that the new isolates can be used for CA biocementation. In this study, the sequence analysis of the three isolates belongs to the genera *Bacillus*. In previous investigations, Bacilli sp is generally tolerant to high CO_2 concentrations [19]. These characteristics make the new isolates the right candidate for biocementation and carbon sequestration applications.

To further understand the mechanism by which the isolate secretes the CA enzyme, the intracellular enzymes and extracellular enzymes were determined, as shown in Figure 3d. The difference is that intracellular enzymes are produced within the cell, and extracellular enzymes are produced outside the cell. This result indicates that the isolate can secrete intracellular and extracellular CA activity. One advantage of this observation is that the strains can be used as sources of crude enzymes for bioaugmentation purposes. Similar results were reported for a study showing apparent differences in soil bacteria's intracellular and extracellular CA activities. This result confirms this suggestion.

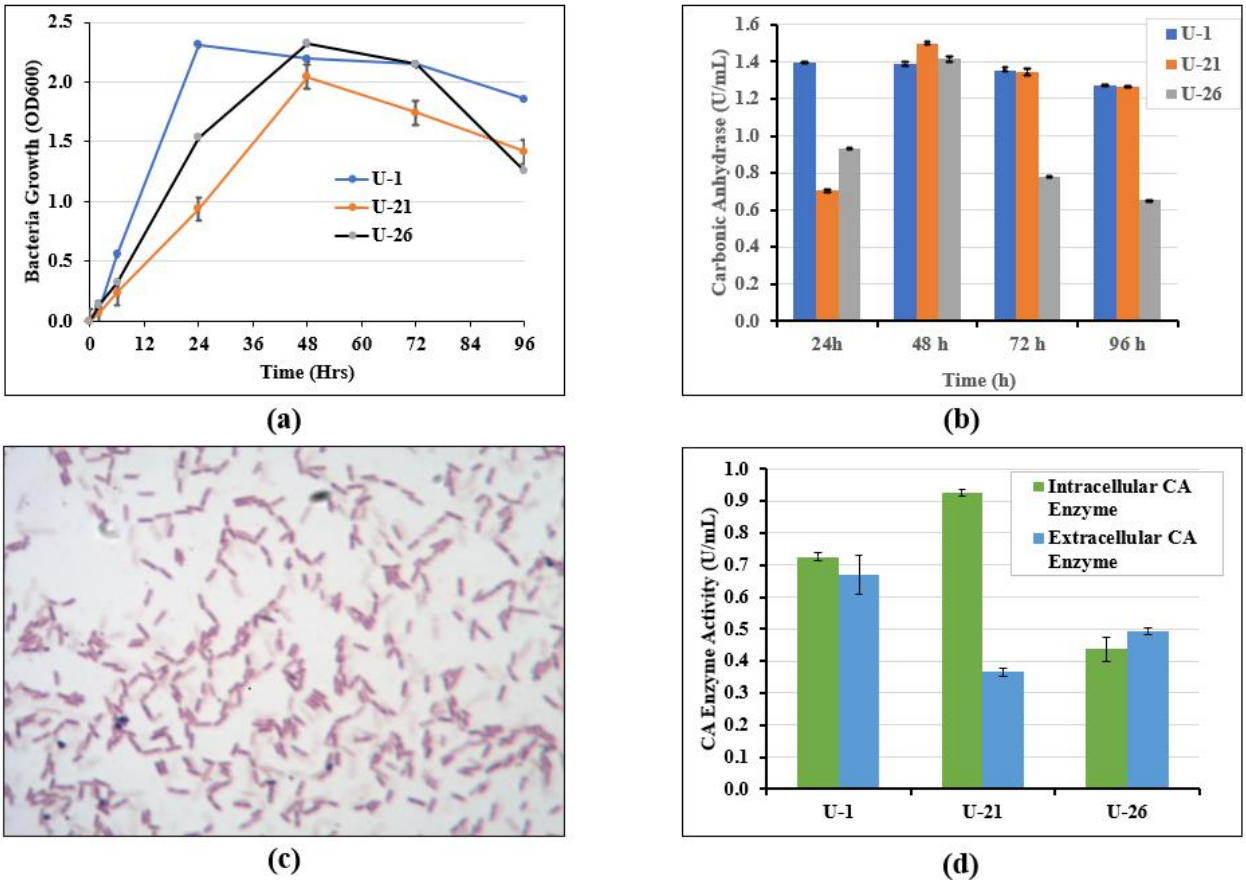


Figure 1: (a) Microbial growth curve (b) Carbonic anhydrase activity of three isolates with time (c) Typical gram-stained image of three selected isolate

3.2 Calcium carbonate bioprecipitation

The calcium carbonate bioprecipitation was investigated to understand the ability of the three selected strains to precipitate Calcium carbonate from the solution. The bacterial suspicion of OD600 of 0.5 was supplemented with different initial concentrations of $NaHCO_3$ and $Ca(CH_3COO)_2$. Figure 2 shows the amount of deposited Ca^{2+} at different initial equimolar of $Ca(CH_3COO)_2$ and $NaHCO_3$ for U-1, U-21, and U-26. An increase in Ca_{2+} deposited bioprecipitate increased as the concentration increased. The increase in deposited Ca_{2+} is due to bacterial cells acting as a nucleation site for the $CaCO_3$ crystallisation (Achal & Pan, 2011). However, when the equimolar concentration was above 1.0 M, no marginal increase was observed between a purely chemical reaction and when bacteria was used. This observation could be considered the upper limit of cementation solution for the CA biocementation because of a retarding effect of the Ca^{2+} ion. Previous studies have reported retarding impact on MICP occurs due to the inhibition or loss of enzyme activity under high concentrations of metal ions. Han-Jiang and team reported similar findings in the ureolytic pathway, who reported a much higher upper limit of 2.5M equimolar of urea and $CaCl_2$ (Lai et al., 2021). (Lai et al., 2021)

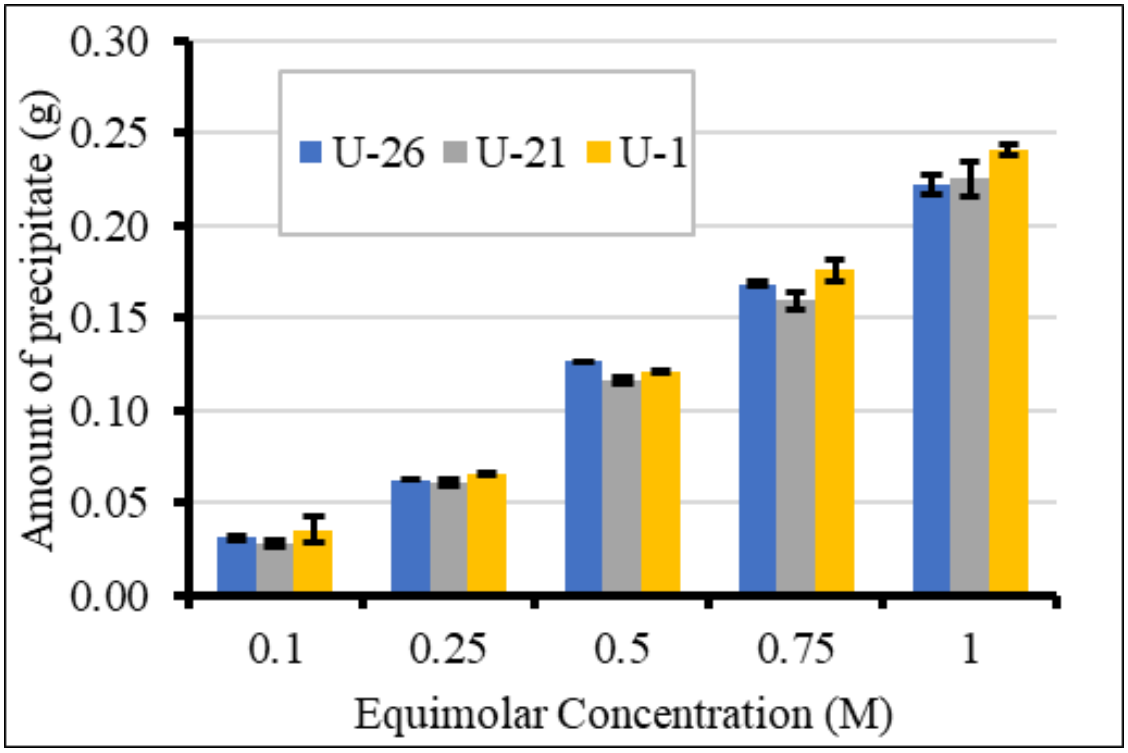


Figure 2: Amount of deposited Ca2+ at different initial equimolar of Ca(CH3COO)2 and NaHCO3 for U-1, U-21, and U-26

Furthermore, to confirm the microbial calcium carbonate, bioprecipitation by U-1, U-21, and U-26 was analysed by SEM. Fig. 6 clearly shows that the changes in initial concentration influence the morphology, size and shape of calcite crystals. The purely chemical reaction of $Ca(CH_3COO)_2$ and $NaHCO_3$ yielded mainly rhombohedral calcite crystals that were dominant in the precipitate. In contrast, those produced by the mediation of the selected CA-producing bacteria were mostly spherical calcite crystals. At higher concentrations of 1.0 M, the crystal morphology was irregular for all the cases, mainly due to the impact retardation effect elucidated above.

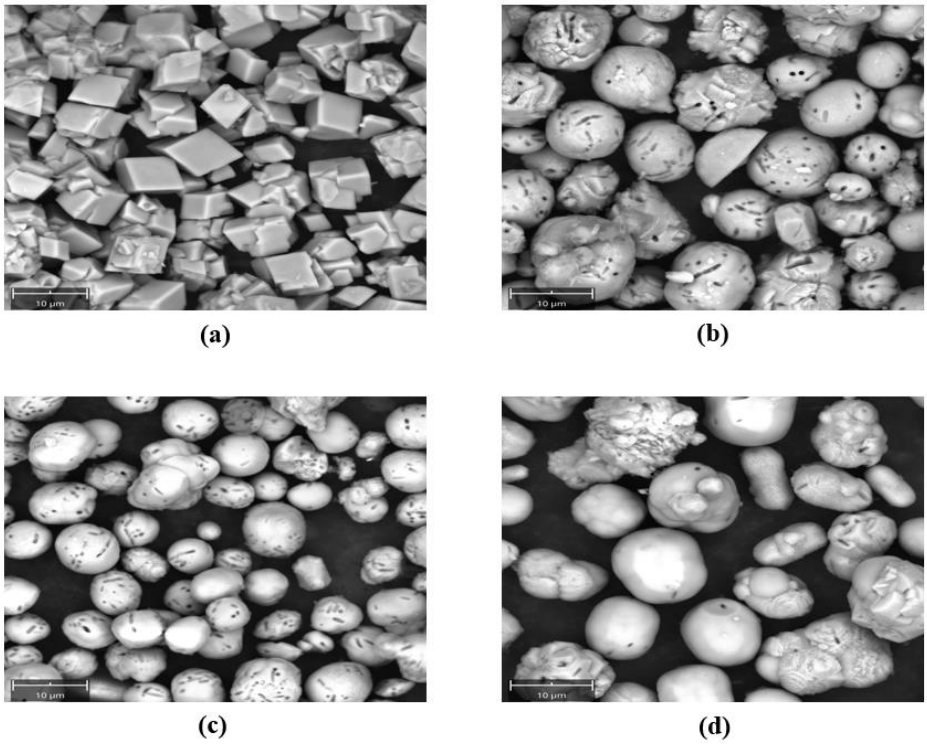


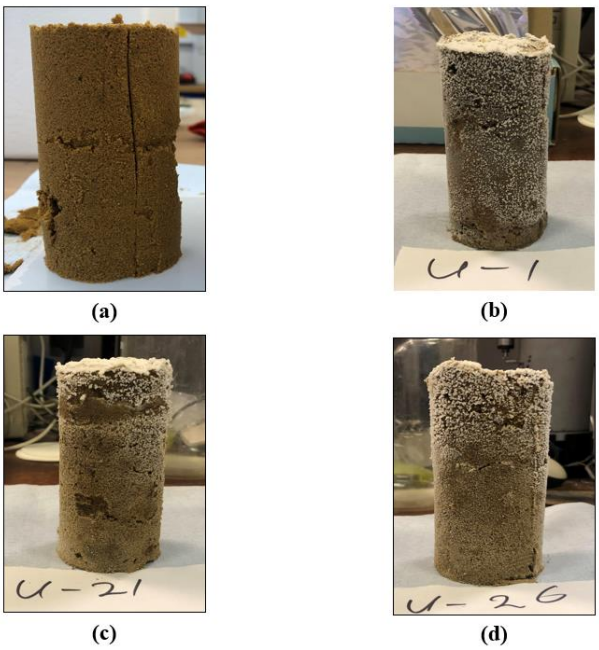
Figure 3: SEM images of U-1, U-21, and U-26 at different equimolar Ca(CH3COO)2 and NaHCO3

3.3 Biocementation of soil

Table 3 shows untreated and treated soil samples' estimated UCS, $CaCO_3$, and moisture content. The control specimen had no strength, whereas the soil was biocemented by U-1. U-21 and U-26 estimated UCS values of 1.0 Mpa, 1.5 Mpa and 1.5 MPa, respectively. The soil without treatment has a background content of 4.82, whereas the treated has increased calcium carbonate due to CA-producing bacteria's action. Furthermore, the moisture content was almost uniform, ranging from 20 to 23%, whereas the untreated had high moisture content. The decrease in moisture could probably be the filling of voids by the calcite mineral that precipitated between soil voids. Figure 4 Biocemented images of soil biocemented using water only, U-1, U-21 and U-26 CA-producing bacteria. Therefore, the increased mechanical strength of biocemented soil has the potential to prevent settlement along the railway embankment. This increase in strength would additionally increase the bearing capacity of embankment foundation soils, with a particular application being the soft foundation soils of the UK railway network. The CA pathway is of interest as the native CA-producing bacteria can sequester CO_2 from the atmosphere and fix it into different biominerals that can be used to produce biocement. The formed biominerals act as binders between soil particles, increasing the foundation soil's stiffness and bearing capacity. An additional environmental advantage of using the CA metabolic pathway is the lack of undesirable by-products, as opposed to the most commonly used method. More advanced testing for settlement is a subject of ongoing work.

Table 2: Estimated UCS, $CaCO_3$ Content and Moisture Content of untreated and treated samples

	UCS (MPa)	**$CaCO_3$ Content (%)**			**Moisture Content (%)**		
		Top	Middle	Bottom	Top	Middle	Bottom
Control	0.0	4.82	4.82	4.82	22	25	28
U-1	1.0	7.55	5.34	3.53	23	21	21
U-21	1.5	7.55	6.34	3.5	22	20	20
U-26	1.5	8.01	6.70	4.5	22	20	20



(a) (b) (c) (d)

Figure 4: Biocemented images of soil (a) Biocementation using water only (b) U-1 CA-producing bacteria (c) U-21 (d) U-26 CA-producing bacteria.

4. CONCLUSIONS

In this research, we employed microbial-induced calcite precipitation (MICP) as an alternative method to prevent the settlement of inferior soils using the carbonic anhydrase pathway. This pathway would reduce the CO2 emitted to the atmosphere, and the CA-producing bacteria capture CO2. We isolated and selected the carbonic anhydrase-producing bacteria with higher CA activity named U-1, U-21 and U-26. These stains were characterised and found to increase the mechanical strength of the soil. The UCS and calcite content results proved biocementation of the soil, showing promise that the CA route can successfully reduce settlement of existing railway infrastructure, providing a non-disruptive and environmentally friendly alternative to current engineering practices for settlement mitigation. In addition, microbiologically produced calcium carbonate is benign to the environment compared to aggressive chemical reagents used in the construction industry.

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