ISSN: 0017-2294

Microbially Induced Calcite Precipitation for Sustainable Agriculture and Construction

Waghmode Meghmala S[1,] , Bhujbal Ravina R[1], Masalkar Swati D[1], Goud Sambhaji A[1], Gunjal Aparna B [2] and Patil Neha N[1]*

3. Affiliations: *1: Department of Microbiology, P. D. E. A.'s Annasaheb Magar Mahavidyalaya, Hadapsar, Pune-411028. Maharashtra, India*

2: Department of Microbiology, Dr. D. Y. Patil Arts, Commerce and Science College, Pimpri, Pune-411018, Maharashtra, India

E-mail ID of the Corresponding author: nehanitinpatil@gmail.com

Abstract

Microbially induced calcite precipitation is attaining a great importance as 'biomimetic' inspiration. Biomineralization is the process by which living organisms carry out reactions that promote mineral precipitation. Bio inspired engineering is futuristic approach for green civil infrastructure with microbial natural phenomenon. This study was done to evaluate the microbially induced calcite precipitation activity of bacteria on strengthening of soil and building material. Ureolytic strains were isolated and identified using biochemical properties as Alcaligenes sp., Bacillus aeolius, Bacillus naganoensis, Bacillus carboniphilus and Bacillus velezensis by 16srRNA sequencing method. Strains found to have the potential of calcite production were grown on calcite precipitation agar and B4 medium. Characterization of calcite was done using stereomicroscopy, scanning electron microscopy, Fourier Transform Infra-Red Spectroscopy and X-ray diffraction techniques. Both circular and hombohedral shapes of calcites were observed with size ranging between 20 to 600 nm. For the utilization of microbially induced calcite precipitation, activity in soil strengthening, sulfur rich soil (80 ppm) was used for field experiment with Zea mays. Maize grown in soil containing Bacillus velezensis, showed elevated vigor index. Concrete brick ameliorated with Bacillus naganoensis and Bacillus velezensis, are herein reported for first time contributing towards increase in crushing load, compressive strength as well as water absorption capacity in comparison to control and bricks meliorated with other strains. This study has concluded that MICP process can be used for sustainable environment.

Keywords: Microbially induced calcite precipitation, bioconsolidation, biomineralization, biogrouting, ureolytic bacteria, vigor index, *Bacillus velezensis*

1.0 Introduction

1.1 Biomineralization:

It is a process in which bacteria produce minerals like carbonates calcium phosphates etc. The synthesis of minerals categorized into two classes, biologically induced mineralization (BIM) and biologically controlled mineralization (BCM). Minerals are synthesized directly at a specific location within or on the cell only under certain conditions in the case of BCM. In case of BIM, the minerals are formed extracellularly as a result of metabolic activity of the organism (1). Minerals known to be formed by means of BIM through passive surface facilitated mineralization includes phosphates, metal sulfates and carbonates. Among all the minerals that have been associated with biomineralization, carbonates are the most observable. Microbially induced calcium carbonate precipitation (MICCP) can be used in

ISSN: 0017-2294

variety of fields ranging from geotechnology, biotechnology to civil Engineering (1). Bio-mediated process of soil improvement has been considered as an innovative and new approach in geotechnical engineering that can be used to avoid liquefaction and landslide in loose sand which usually results in foundation deformation, mortality and economical losses (2).

The introduction of Microbiologically Induced Calcite Precipitation (MICP) took the concrete material technology to a different dimension. The deposited inorganic $CaCO_3$ crystals get attached to the surrounding surfaces. The minute sized bacteria can attach themselves within fine pores and voids; and thereby enhance the structural incorporation of the material. When water seeps through concrete the concrete cracks, it comes in contact with bacteria along with oxygen and calcium-based nutrient, $CaCO_3$ crystals formation seals the cracks. The precipitation of carbonates by urea hydrolysis using ureolytic bacteria is the most easily controlled mechanism of MICCP (Microbiologically Induced Calcium carbonate Precipitation) along with potential to produce high amounts of carbonates within a short period of time. For the duration of microbial urease activity, 1.0 mol of urea is hydrolyzed intracellularly to 1.0 mol of ammonia (NH_3) and 1.0 mol of carbonate, which instinctively hydrolyzes to form additional 1.0 mol of ammonia and carbonic acid as follows (1).

$CO(NH_2)_2 + H_2O \xrightarrow{\text{bacteria}} NH_2COOH + NH_3)$

$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$

These products equilibrate in water to form bicarbonate, 1.0 mol of ammonium and hydroxide ions that give rise to increase in pH.

$H_2CO_3 \rightarrow 2H^+ + 2CO_3^{2-}$

$NH^{3+}H_2O^- \rightarrow NH^{4+} + OH^-$

$Ca^{2+} + CO_2^{3-} \rightarrow CaCO_3$

MICP produces calcite and other calcium carbonate minerals like Vaterite or Aragonite (3).

1.2 Biomineralization process in construction:

Concrete is widely used artificial construction material in world. Though it has many advantages such as low price and ability of being cast in any desirable shape, it has some limitations too. Carbonate precipitation has been investigated extensively due to its wide range of technological implication and is a significant aspect of biomineralization. Applications of carbonate mineralization comprises of bioremediation through leaching, solid phase capture of inorganic contaminants or plugging cementation in rock and other porous media fissures and the production of biomimetic materials (4). Urea hydrolysis is the most efficient and the best way of the carbonate producing reactions, as it is easy to control with the potential to produce high concentrations of carbonate within a short time. It provides alkaline pH and also generates a freely available supply of carbonate for $CaCO_3$ precipitation (5). Now-a-days innovative materials changed the features of concrete.

2.0 Material and method

2.1 Isolation and identification of ureolytic bacteria

Ureases (urea amid hydrolases, EC 3.5.1.5) are a group of enzymes available in nature amid bacteria, fungi, algae, plants and invertebrates. Hydrolysis of urea (H_2N-CO-NH_2) yields NH_3 and carbonic acid, which is catalyzed by urease enzyme. The Christensen's Urea agar media was used for isolation of ureolytic bacteria from the rhizospheric soil by spread plate method. Urease producing microorganisms convert urea into NH_3 which is alkaline in nature and due to which pink colored colony occur. Urease producing strains were identified based

ISSN: 0017-2294

on the morphological and biochemical tests. Cultures showing more ureolytic activity were identified using 16srRNA sequencing method.

2.2 Determination of urease activity

Urease activity of the isolates was determined using phenol hypochlorite method (6) and electric conductivity method (5).

2.3 Phenol hypochlorite assay method

The urease activity of bacteria was determined by using phenol hypochlorite assay method. Ammonium chloride (50 to 100 µM) was used as standard. The fermented Christensen's urea broth (250 µl) was added to the mixture containing 1.0 ml of 0.1 M potassium phosphate buffer (pH 8.0) and 2.5 ml of urea (0.1 M). The mixture was incubated for 5 min at 37 °C. Phenol nitroprusside and alkaline hypochlorite were added, 1.0 ml each and incubated at 37 ^{0}C for 25 min. Optical density (OD) was measured at 626 nm and one unit of urease is defined as the amount of enzyme hydrolyzing 1.0 µmol rea/min (6).

2.4 Electric conductivity method

Urease enzyme activity was carried out by using conductivity method. For enzyme assay, 1.0 ml of fermented Christensen's urea broth was added to 9.0 ml of 1.11 M urea solution. Final conductivity was recorded after 30 min of incubation at 20 ^{0}C by electric conductivity meter (NOEQ 660A, India). Urease activity was represented as mS/cm (millisiemens/centimeter) (5).

2.5 Calcium carbonate precipitation

Calcite precipitation agar (CPA) media was used for screening of bacteria which precipitates $CaCO_3$ through ureolysis. It contained nutrient broth, 3.0; urea, 20.0; $CaCl_2.2H_2O$, 28.5; $NaHCO_3$, 2.12; NH_4Cl, 10.0; agar, 15.0 (g/lit). All the media components were autoclaved, except urea which was membrane sterilized. After autoclaving, urea was added. Bacterial cell suspension was spot inoculated on the CPA plates and kept for incubation at 30 ^{0}C for 48 hr. Stereomicroscopic images were taken for 3-7 days (5).

2.6 Calcium carbonate precipitation in broth

For measurement of $CaCO_3$ precipitation in broth, calcite precipitation broth was supplemented with 2% urea and calcium chloride. The broth was inoculated with 2% inoculum and incubated at 30 ^{0}C for 7 days. Precipitated $CaCO_3$ was centrifuged; the pellet was dried in oven at 60 ^{0}C for 3 hr. The dried samples were checked for Fourier Transform Infra-Red (FTIR) Spectroscopy (Perkin Elmer, USA)(5).

2.7 Crystal nucleation site development

Tap water (500 ml) + 100 ml (3 M) concentration of filtered urea + 100 ml inoculum were added and incubated for two weeks at 30 ^{0}C. The deposits of $CaCO_3$ were centrifuged at 4,300 rpm for 15 min. After centrifugation the deposits were kept inside the hot air oven at 35 ^{0}C for 6 hr. The dried samples were checked for FTIR, X-ray Diffraction (XRD) Spectroscopy and scanning electron microscopy (SEM) analysis (SEM-Jeol, Tokyo, Japan) (7).

2.8 Concrete brick preparation using calcite producing bacteria

Brick preparation was done according to standard criteria of cement brick**.** The essential ingredient of concrete block contains cement, aggregate (sand/gravel) and water (Figure 1). These concrete blocks were produced manually. For the production of concrete block, ordinary Portland cement and aggregate (sand/gravel) was mixed in proportion of 1:6. Each brick was made with 2 kg of concrete, 400 g cement (1:6) and enriched with strains which were having the potential to grow in the presence of cement. For control, broth was replaced with water. Bricks were kept undisturbed for 10 days and checked for crushing load and compressive strength using Digital Compressive Testing Machine (2000 KN) (Lawrence and Mayo, India) and water absorption capacity (8). Statistical analysis was done by ANOVA with Tukey HSD using statiskingdom. The experimental results of compressive strength were

ISSN: 0017-2294

compared with control values using ANOVA with Tukey HSD using statiskingdom. A p value of <0.05 was considered as significant.

2.9 Effect of MICP isolates on vigor index of *Zea mays* grown in sulphur rich soil

2.9 (a) Seed germination efficacy of Zea *mays*

For disinfection, seeds were first kept in 1% mercuric chloride ($HgCl_2$) for 3 min and then washed with sterile distilled water (DW) for 15 seconds. Five seeds maize were placed on sterile water agar plates and incubated at room temperature (RT).

2.9 (b) Field assay

Sulfur contaminated soil was used to study the effect of biomineralization process on maize crops. Sulfur content of soil was determined using sodium acetate extraction-barium chloride method (Motsara and Roy, 2008). Five flasks of Luria Bertani (LB) [LB broth casein enzymatic hydrolysate, 10.0; yeast extract, 5.0; NaCl, 10.0 (g/lit)] were inoculated with 5 strains and incubated for 7 days at 30 ^{0}C. Seven squares of 10 x 10 inches were made on the field. Five seeds each of maize were sown into each square and 3-5 g of urea was sprinkled. Enriched LB broths with 5 strains were poured in first five squares. The sixth square was kept as control in which only water was added. The seventh square was kept as control with $CaCl_2$ and urea. After 30 days, the crops were harvested and vigor index was determined (9). Chlorophyll content was also calculated as per the protocol of Maclachlam (10).

2.10 Determination of soil urease activity

Soil urease activity was done by using the protocol of Tabatabai. The method was based on hydrolysis of urea to NH_3 due to urease activity (11).

3.0. Result and Discussion

3.1 Isolation and identification of ureolytic bacteria

Rhizospheric soil was used for the isolation of ureolytic strains. Out of the 10 isolates, total 5 isolates were found to be ureolytic and further studied for MICP activities. These isolates were identified based on the morphological and biochemical characters (Table 1).

Table 1: Biochemical characteristics of the isolates:

Tests	Isolates				
	SSR1	SSR2	SSR3	SSR4	SSR5
Gram character	Gram -ve cocci	Gram +ve rod	Gram +ve rod	Gram +ve short rod	Gram +ve rod
Motility	Motile	Motile	Motile	Motile	Motile
Sugar fermentation					
i) Glucose	A, G	A, G	-	-	A, G
ii) Mannitol	A, G	A, G	-	-	-
iii) Xylose	A, G	A, G	-	-	A, G
iv) Lactose	-	-	-	-	-
Nitrate reduction	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+
Gelatin	+	+	+	+	+
Growth at varied conc. of NaCl (%)					
3	+	+	+	+	+
5	+	+	-	+	+
7	+	-	-	+	+
10	-	-	-	-	+
Isolate identified	*Alcaligenes* sp.	*Bacillus aeolius*	*Bacillus naganoensis*	*Bacillus carboniphilus*	*Bacillus velezensis*

ISSN: 0017-2294

A: Acid, G: Gas, +: Positive, -: Negative

3.2 16srRNA sequencing method of the microbial isolates

The microbial isolates were identified to be *Alcaligenes* sp., *Bacillus aeolius, Bacillus naganoensis, Bacillus carboniphilus* and *Bacillus velezensis*.

16s rRNA sequence of *Bacillus velezensis* is found to be:

>NMTTAATGCGTTAGCTGCAGCACTAAGGGGCGGGAAACCCCCTAACACT
TAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCC
CACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGG
TGTTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCTCTTCT
GCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCA
CATCAGACTTAAGAAACCGCTGCGAGCCTTTACGCCCAATAATTCCGGACAACG
CTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCTTTCTGGT
ACCGTCAAGGTGCCGCCCTATTTGAACGGCACTTGTTCTTCCCTAACAACAGAGC
TTTACGATCCGAAAACCTTCATCACTCACGCGGCGTTGCTCAGACTTTCGTCCATT
GCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCC
AGTGTGGCCGATCACCCTCTCCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTG
GTGGTGAGCCGTTACCTCACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTG
TGGTAGCCGAAGCCACCTTTTATGTCTGAACCATGCGGTTCAAACAACCATCCGG
TATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATCCCAGTCTTACAGGCAGG
TTACCCACGTGTTACTCACCCCGTCCGCCGCCGCTAACATCAGGGAGCAAGCTCC
CATCTGTCCGCTCGTCCGCATGTATTAGGCACGCCGCCAGCGTTCGTCCTGAGCC
AGGA

Based on the report of basic local alignment search tool, bacteria showed 99 % similarity with *Bacillus velezensis.*

3.3 Determination of urease activity

Phenol hypochlorite assay and electric conductivity based method was or urease activity.

3.3.1. Phenol hypochlorite assay

In the medium, the ammonium ions react with the phenol hypochlorite to form greenish blue color indophenol, which absorbs light at 626 nm proportional to initial urea concentration. According to the absorbance of phenol hypochlorite assay, ammonia released during the reaction was calculated by using standard graph of ammonium chloride.

$$Urea + 2H_2O \xrightarrow{Urease} 2NH_3 + CO_2 \quad (6)$$

One mole of urea gets hydrolyzed into two molecules of NH_3. Hence, according to the graph of enzyme unit verses time of incubation, *Bacillus naganoensis* showed continuous increase in enzyme unit; whereas *Bacillus aeolius* and *Bacillus carboniphilus* showed decreased in enzyme activity after some period of time. *Bacillus velezensis* and *Alcaligenes sp*. showed constant urease enzyme activity (Figure 1).

ISSN: 0017-2294

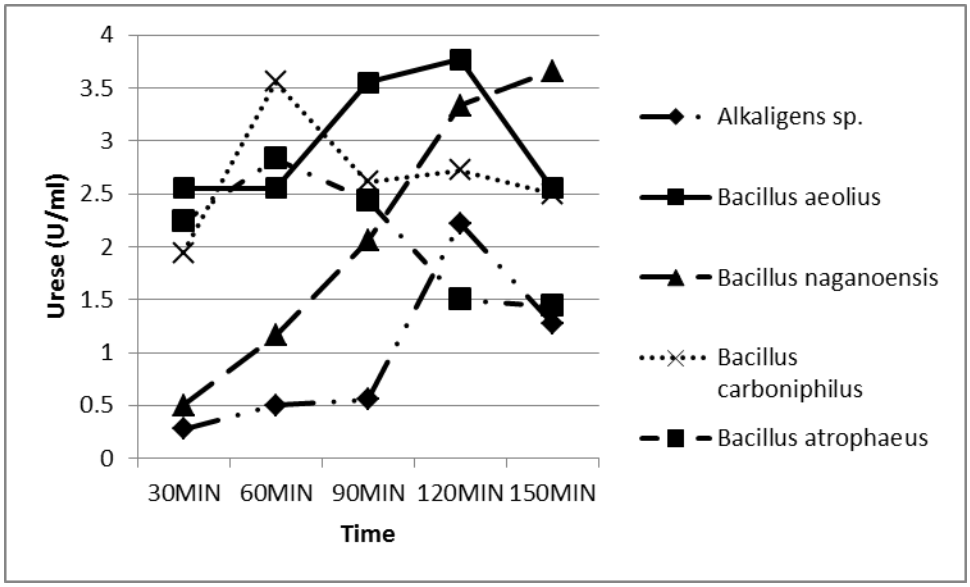


Figure 1: Urease activity of isolates using phenol hypochlorite assay.

3.3.2. Electric conductivity method

Urease assay using conductometer was also done. Once the enzyme was added to substrate, the conductivity was recorded as zero time. Thereafter, it was recorded after 30 min. The urease activity by conductivity is shown in Table 2. The conductivity continuously increased with time in a positive proportional relation with urease activity. It showed stability and consistency in the rate of conductivity. The rate of enzyme activity in *Alcaligenes* sp., *Bacillus naganoensis* and *Bacillus velezensis* found to increase with time. Increased conductivity was due to generation of ionic products of NH^{4+} and CO_3^{2-} from non-ionic substrate, urea. The regular increase in conductivity with time is attributed to the regular hydrolysis of urea, which is confirmed by the calculation of correlation coefficients (R) for all tests. The high correlation coefficients indicated strong positive linkage between conductivity and urea hydrolysis (Table 2, Figure 2).

Table 2: Electric conductivity of urease enzyme (mS/cm):

Isolates	Urease activity by conductivity (mS/cm) against time (min)							Correlation Coefficient (R)
	0	30	60	90	120	150	180	
Alcaligenes sp.	1700	1710	1760	1750	1810	1910	1920	95.18
Bacillus aeolius	1290	1300	1370	1380	1390	1430	1440	0.9654
Bacillus naganoensis	1500	1530	1560	1600	1610	1670	1620	0.9181
Bacillus carboniphilus	1280	1300	1340	1360	1370	1410	1370	0.8988
Bacillus velezensis	1250	1360	1400	1380	1450	1490	1480	0.9279

ISSN: 0017-2294

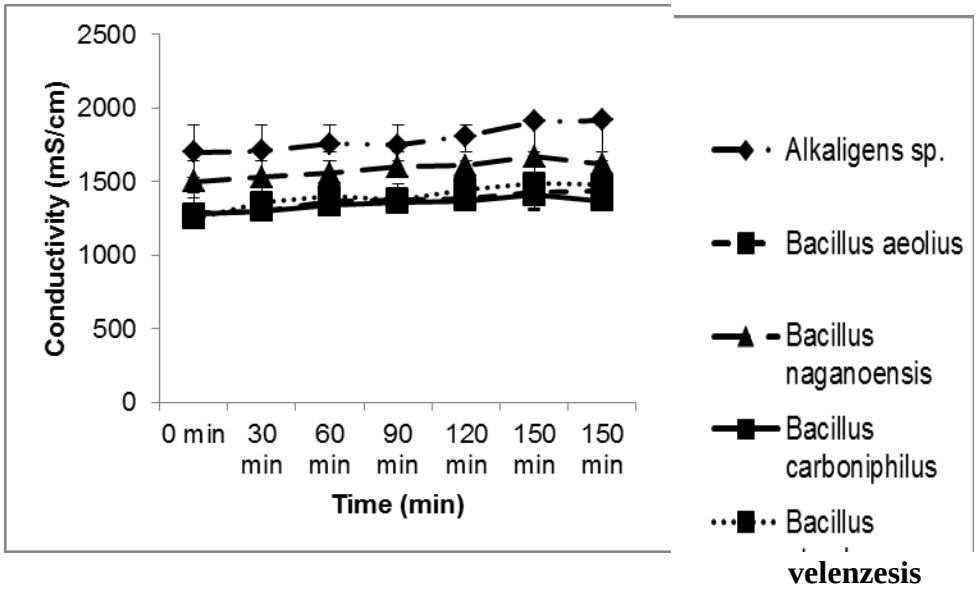


Figure 2: Urease activity of isolates by electric conductivity method.

3.4 Calcium carbonate precipitation on agar plate

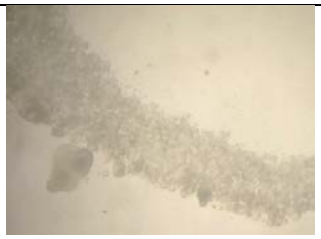
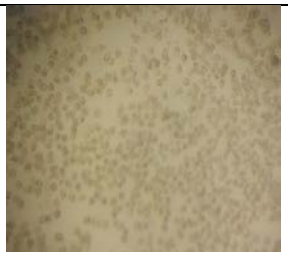
Calcite precipitation agar plate was used for CaCO3 precipitation. These isolated colonies showed white precipitation within round the growth area of colonies. On CPA media plates *Alcaligenes sp*. and *Bacillus aeolius* showed rhombohedral crystals of $CaCO_3$. *Bacillus naganoensis, Bacillus carboniphilus* and *Bacillus velezensis* showed circular crystals with size variations (Table 3, Figure 3).

Table 3: Shape of crystals under stereomicroscope grown on different agar medium.

Isolates	Agar Medium		
	CPA containing $CaCl_2$	CPA containing $CaCO_3$	B4
Alcaligenes sp.	Rhombohedral	Rhombohedral	Sugar Cube
Bacillus aeolius	Rhombohedral	Circular	Oval
Bacillus naganoensis	Circular	Small Circular	Circular
Bacillus carboniphilus	Small Circular	Large Circular	Circular
Bacillus velezensis	Circular	Circular	Circular

CPA: Calcite precipitate agar, $CaCO_3$: Calcium carbonate

.

Medium Isolates	CPA containing calcium chloride	CPA containing calcium acetate	B4
Alcaligenes sp.			

ISSN: 0017-2294

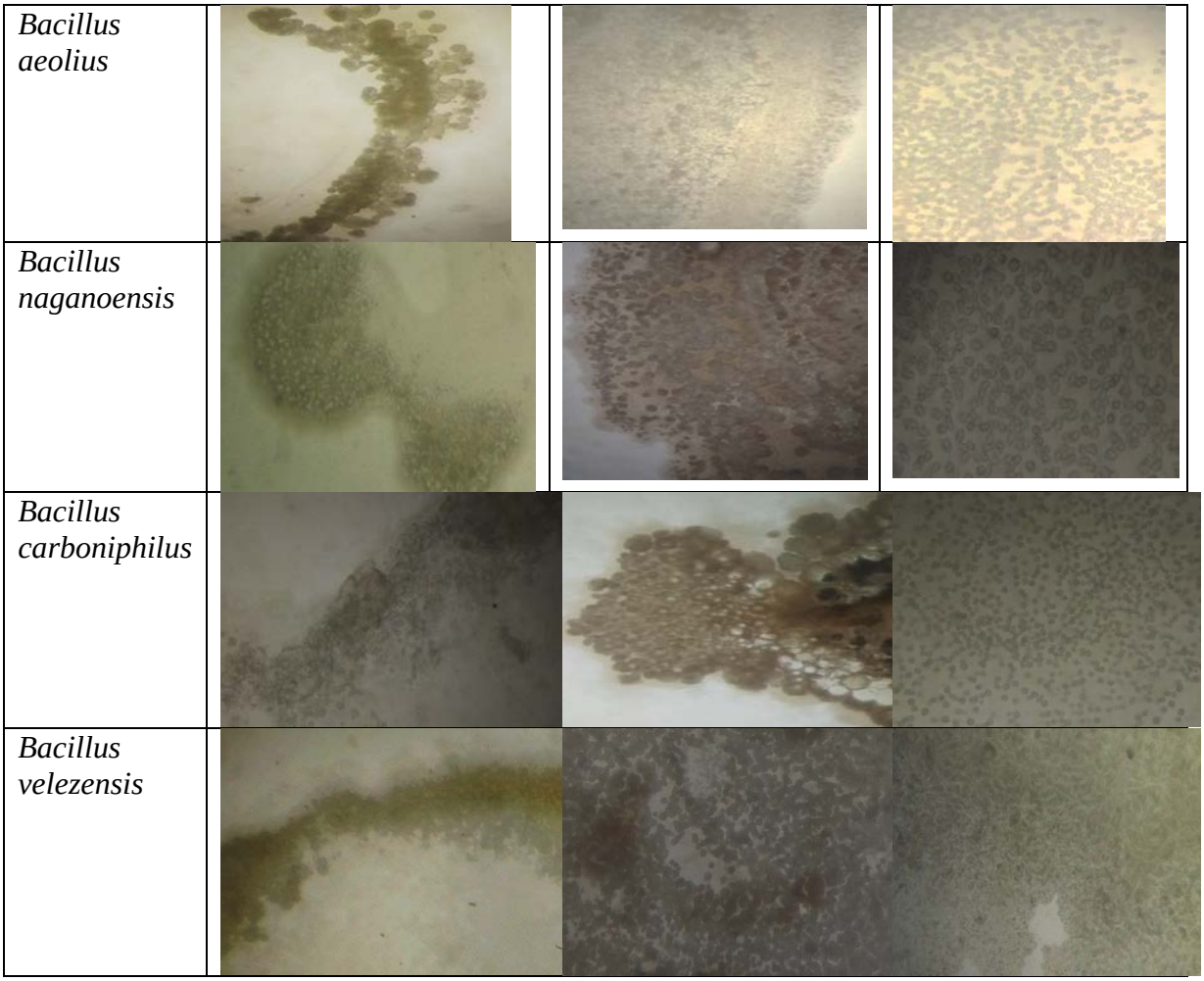


Figure 3: Calcite on calcite precipitation agar and B4 medium under stereomicroscope

3.5 Characterization of crystals

3.5.1. FTIR analysis

The FTIR Spectroscopy was used for qualitative analysis from the characteristic frequencies, which provides information to identify chemical constituents in the compound (Figure 4).

ISSN: 0017-2294

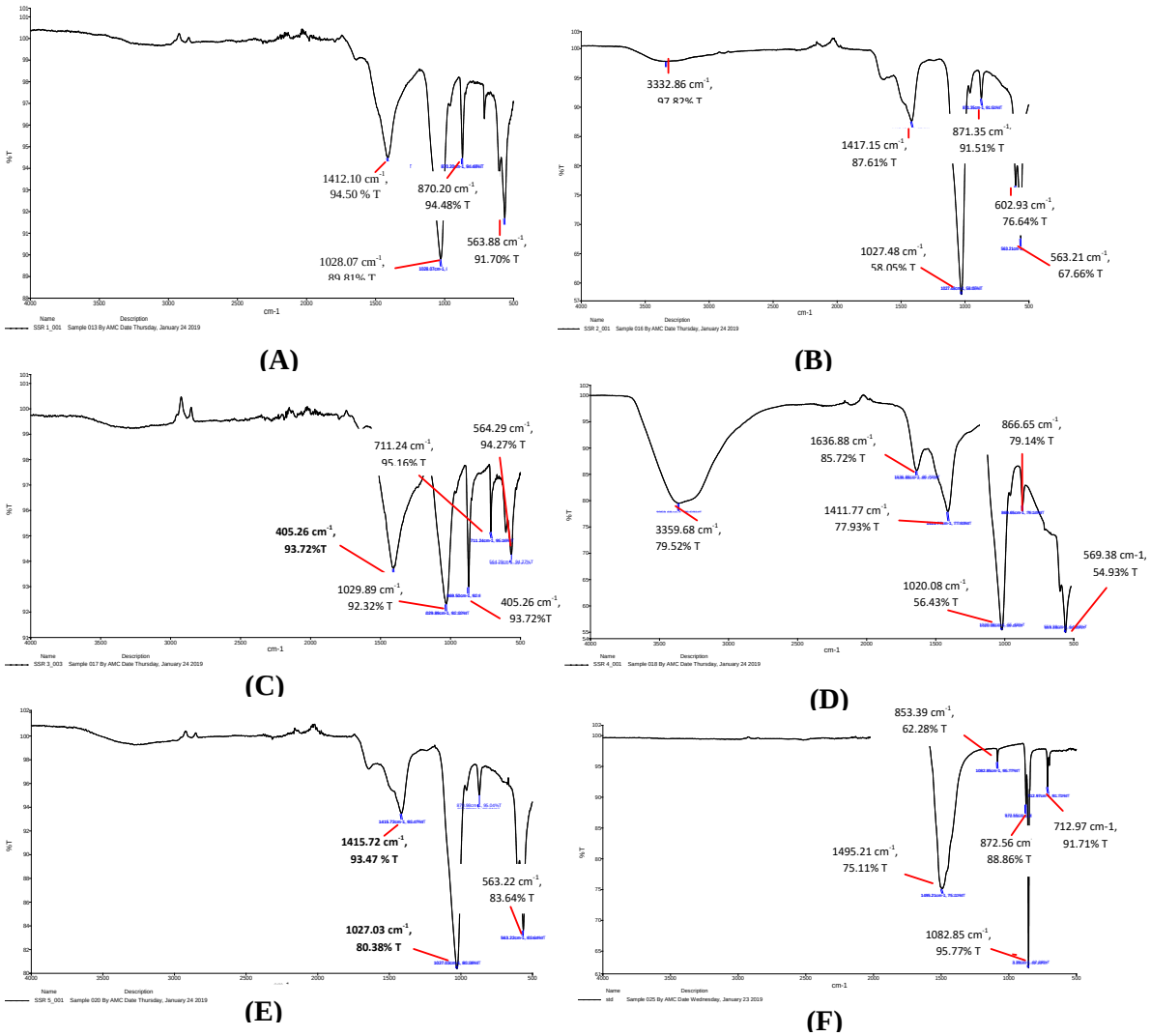


Figure 4: FTIR of Calcite crystals produced by (A) Alcaligenes sp. (B) *Bacillus aeolius* (C) *Bacillus naganoensis* (D) *Bacillus carboniphilus* (E) *Bacillus velezensis* (F) Standard $CaCO_3$.

FTIR spectra were obtained in regime 4000-550 cm^{-1}. The FTIR spectrum of pure $CaCO_3$ showed the presence of strong band center around 1495 cm^{-1}. Characteristic of C-O stretching mode of carbonate together with a narrow band around 873 cm^{-1} of the bending mode. Sharp band at 873 cm^{-1} is assigned to $CaCO_3$. Strong band (C-O) at 1444 cm^{-1} is related with $CaCO_3$ bond. The (C=O) bond 1415 cm^{-1} is associated with calcium chloride (12).

3.5.2. XRD analysis

To ensure that the crystals produced by *Bacillus aeolius* and *Bacillus velenzensis* using crystal nucleation site development method were indeed $CaCO_3$ mineral polymorph, they were examined using x-ray diffraction (Figure 5).

ISSN: 0017-2294

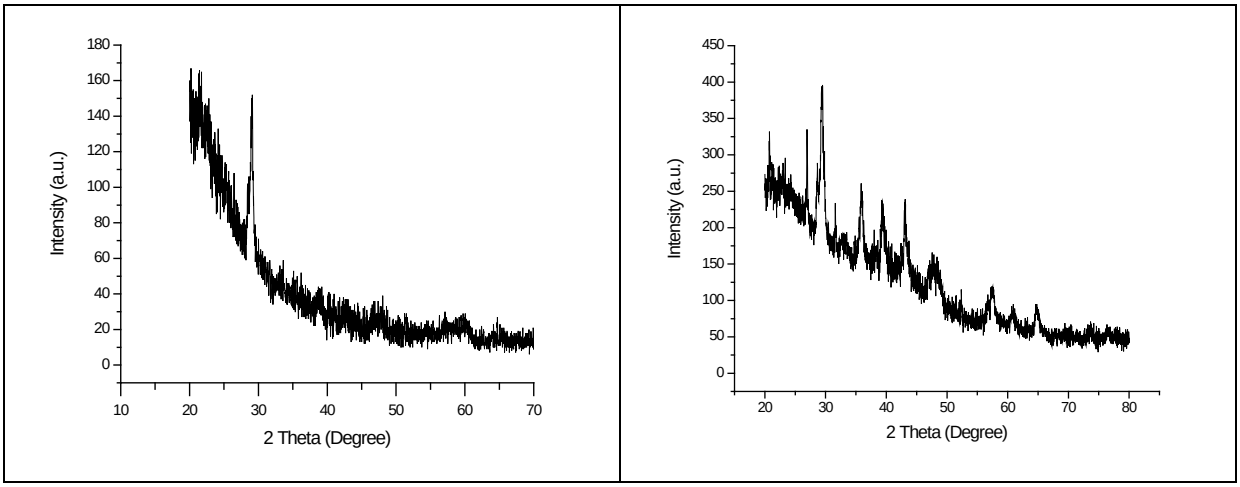


Figure 5: XRD graph of $CaCO_3$ crystals produced by (A) *Bacillus aeolius* and (B) *Bacillus velezensis*.

XRD analysis was used for determination of $CaCO_3$ crystals. 0-450 Intensity (a.u.) and 2Θ (Degree) range from 20 to 90^0 were selected to analyze the crystal orientation. XRD analysis confirms the presence of these multiple mineral forms, including Calcite, Vaterite and Aragonite which is only transiently found in nature. According to XRD pattern, peak was observed at 2Θ values of 39^0, 72.35^0, 29.34^0, 29.52^0 in *Bacillus aeolius* whose crystal size ranging from 28-500 nm and in case of *Bacillus velezensis* peak observed at 2Θ values of 29.52^0, 43.06^0, 57.50^0, 20.78^0 whose crystal size ranges from 100-630 nm (13).

3.5.3. SEM analysis

Carbonate crystals structure were different in size and showed rhombohedral structure of $CaCO_3$. The SEM image of biocement producing *Bacillus velezensis* on the surface of their cell are shown in Figure 6.

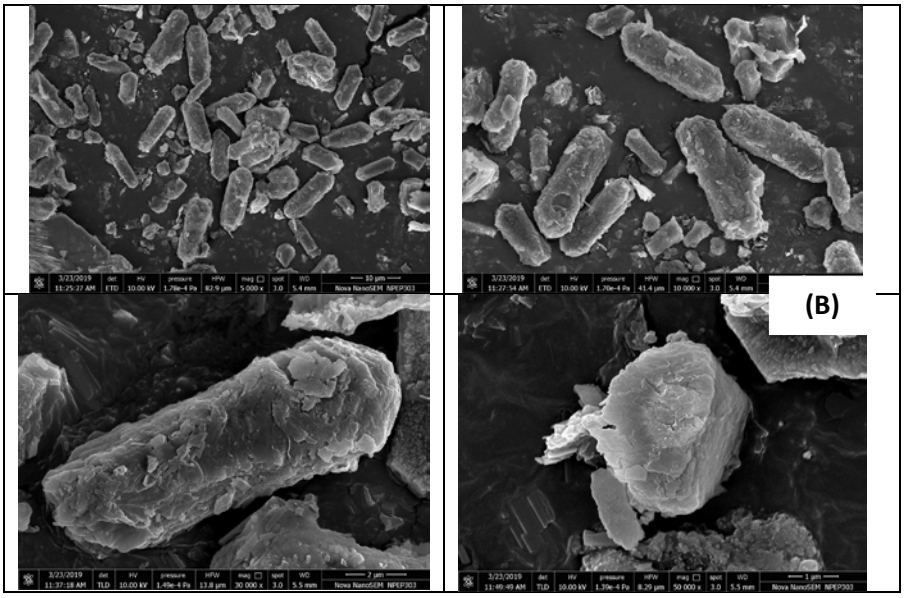


Figure 6: SEM images of calcite crystals produced by *Bacillus velezensis*

3.6 Cement brick preparation using calcite producing bacteria

Out of the five strains, only 3 strains viz, *Alcaligenes* sp., *B. naganoensis* and *B. velezensis* were found to grow and produce calcite in the presence of cementitious mixture. Hence, these 3 strains were used for preparing cement brick which were further tested for preparing

ISSN: 0017-2294

cement brick and also checked for crushing load, compressive strength and water absorption capacity. After checking the crushing load, compressive strength and water absorption capacity of bricks, it was found that the bricks made by using the two organisms *Bacillus naganoensis* and *Bacillus velezensis* are stronger than control. *Bacillus velezensis* as inoculum in cement reparation was found to be more effective as it reduced water absorption capacity and increased compressive strength (Table 4).

Table 4: Crushing load and compressive strength of bricks:

Sample	Weight of Block (gm)	Area of Block (mm^2)	Crushing load (KN)	Compressive Strength (N/mm^2)	Water absorption capacity (%)
Control (X1)	2400	19089	152.2	7.97	14.00
Brick using *Alcaligenes* sp.(X2)	2400	16559	134.0	8.09	2.87
Brick using *Bacillus naganoensis*(X3)	2400	17679	160.0	9.05	2.10
Brick using *Bacillus velezensis* (X4)	2400	17084	185.0	10.83	1.96

For statistical analysis, the experimental results of compressive strength were compared with control values using ANOVA with Tukey HSD using statiskingdom. A p value of <0.05 was considered as significant. Analysis of variance with Tukey's procedure suggested there were significant differences for the mean based on 2.38198e-12 p-value and 170.290627 F statistic value. The means of the following pairs are proved to be significantly different between x1-x3 (p-value= 2.76834e-7, M= 1.09), x1-x4 (p-value=6.38534e-12, M=2.25), x2-x3 (p-value=0.00000130361,M=0.97), x2-x4(p-value=1.33634e-11, M=2.14), x3-x4 (p-value= 1.01013e-7,M=1.17) pairs using Tukey HSD (honestly significant difference). Increase in the compressive strength suggesting biocement as biodesign material has been reported (14).

3.7 Effect of MICP isolates vigor index of Zea *mays* grown in sulphur rich soil

3.7.1. Seed germination efficacy of Zea mays

After 6 days, the seeds were grown and seed germination efficacy was calculated and found to be 100%.

***3.5.2.* Field assay**

After 30 days, the crops were harvested from the field. Root and shoot length were measured and vigor index was calculated (Table 5). Crops grown in *Bacillus velezensis* treated soil showed significant vigor index (Table 5).

Table 5: Chlorophyll content and vigor index of plants:

Treatment	Avg shoot length (cm) ± SD	Avg root length (cm) ± SD	Chl (mg/g)		Seed germination (%)	Vigor Index	Urease production (U/ml)
			Chl A	Chl B			
Alcaligenes sp. + urea+ $CaCl_2$	9.3±0.4	13.7±0.4	0.0236	0.0026	100	930	2300

ISSN: 0017-2294

Bacillus aeolius + urea+ $CaCl_2$	9.4±0.4	16.1±0.4	0.0018	0.0018	100	940	2550
Bacillus naganoensis + urea+ $CaCl_2$	8.1±0.4	15±0.4	0.0008	0.00008	100	810	2310
Bacillus carboniphilus + urea+ $CaCl_2$	8.6±0.4	14.4±0.4	0.003	0.0003	100	860	2300
Bacillus velezensis + urea+ $CaCl_2$	7.6±0.4	19.1±0.4	0.002	0.002	100	760	2670
Control (Water)	7.1±0.4	14.3±0.4	0.0012	0.0012	100	710	2620
Control ($CaCl_2$ + urea)	7±0.4	11.67±0.4	0.0014	0.0014	100	700	1867

SD: Standard deviation

3.8 Urease activity of soil

Bacillus naganoesis and Alcaligenes sp. showed less urease activity, whereas *Bacillus aeolius, Bacillus carboniphilus* and *Bacillus velezensis* treated soil showed higher urease activity. Solidification of sand is dependent upon urease distribution and its activity which is important to avoid erosion (15). Relationship between calcite production and urease activity has been reported in mutated strain of *Sporosarcina pasteurii* for its applicability as construction structure to fill the gaps (6).

4.0 Conclusion

- Concrete brick ameliorated with *Bacillus naganoensis* and *Bacillus velezensis* can have application in construction i.e., in the making of buildings. These concrete bricks will be cheap and have good properties in comparison to the cement bricks. These microorganisms are reported for the first time in making of the concrete bricks.
- The microorganisms *Alcaligenes* sp., *Bacillus aeolius, Bacillus naganoensis, Bacillus carboniphilus* and *Bacillus velezensis* showing calcite precipitation were found to increase the plant growth. So, these microorganisms can be used for sustainable agriculture which will be eco-friendly and economical.

Competing Interest

The authors declare that no competing interest exists among them.

Ethical statement

Authors declare that there is no violation of ethical regulations occurred during their work.

ISSN: 0017-2294

References

1 Reddy, M. Sudhakara. "Biomineralization of calcium carbonates and their engineered applications: a review." Frontiers in microbiology 4 (2013): 314.
2. Umar, Murtala, Khairul Anuar Kassim, and Kenny Tiong Ping Chiet. "Biological process of soil improvement in civil engineering: A review." Journal of Rock Mechanics and Geotechnical Engineering 8, no. 5 (2016): 767-774.
3. Ivanov, Volodymyr, and Viktor Stabnikov. "Optimization and design of construction biotechnology processes." In Construction Biotechnology, pp. 235-260. Springer, Singapore, 2017.
4. Li, Meng, Xiaohui Cheng, and Hongxian Guo. "Heavy metal removal by biomineralization of urease producing bacteria isolated from soil." International Biodeterioration & Biodegradation 76 (2013): 81-85.
5. Hammad, Ibtisam A., F. N. Talkhan, and A. E. Zoheir. "Urease activity and induction of calcium carbonate precipitation by Sporosarcina pasteurii NCIMB 8841." Journal of Applied Sciences Research 9, no. 3 (2013): 1525-1533.
6. Chahal, Navneet, Anita Rajor, and Rafat Siddique. "Calcium carbonate precipitation by different bacterial strains." African Journal of Biotechnology 10, no. 42 (2011): 8359-8372.
7. Ramanathan, G., T. Vinodh Kumar, R. Rama, and R. Vijayalalitha. "Isolation of cement degrading bacteria and screening of their efficacy for biocementation." J. Pharm. Chem. Biol. Sci 3 (2016): 518-527.
8. Morsali, Saeed, Gamze Yucel Isildar, and Azade Tahni. "The application of bacteria as a main factor in self-healing concrete technology." Journal of Building Pathology and Rehabilitation 4, no. 1 (2019): 1-6.
9. Abdul-Baki, Aref A., and James D. Anderson. "Vigor determination in soybean seed by multiple criteria 1." Crop science 13, no. 6 (1973): 630-633.
10. Maclachlan, Sally, and Saul Zalik. "Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley." Canadian Journal of Botany 41, no. 7 (1963): 1053-1062.
11. Tabatabai, M. A., and J. M. Bremner. "Assay of urease activity in soils." Soil Biology and Biochemistry 4, no. 4 (1972): 479-487.
12. Galván-Ruiz, Miguel, Juan Hernández, Leticia Baños, Joaquín Noriega-Montes, and Mario E. Rodríguez-García. "Characterization of calcium carbonate, calcium oxide, and calcium hydroxide as starting point to the improvement of lime for their use in construction." Journal of Materials in Civil Engineering 21, no. 11 (2009): 694-698.
13. Shafiu Kamba, Abdullahi, Maznah Ismail, Tengku Azmi Tengku Ibrahim, and Zuki Abu Bakar Zakaria. "Synthesis and characterisation of calcium carbonate aragonite nanocrystals from cockle shell powder (Anadara granosa)." Journal of Nanomaterials 2013 (2013).
14. Lee, Chungmin, Hyesun Lee, and Ok Bin Kim. "Biocement fabrication and design application for a sustainable urban area." Sustainability 10, no. 11 (2018): 4079.
15. Imran, Md Al, Shuya Kimura, Kazunori Nakashima, Niki Evelpidou, and Satoru Kawasaki. "Feasibility study of native ureolytic bacteria for biocementation towards coastal erosion protection by MICP method." Applied Sciences 9, no. 20 (2019): 4462.