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Effect of Mn^{2+} andNi^{2+}on Microbially Induced Carbonate Precipitation for Producing Bio-beachrocks

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Abstract

Microbially Induced Carbonate Precipitation (MICP) is a novel soil improvement method with limited application in coastal erosion mitigation; however, challenges remain in the efficiency of using indigenous marine bacteria to strengthen loose beach sediments. This study aims to investigate the influence of nickel (Ni^{2+}) and manganese (Mn^{2+}) metal ions on the efficiency of artificial bio-beachrocks produced using indigenous marine bacteria isolated from erosion-prone coastal areas of Sri Lanka. Small-scale sand column solidification tests were conducted for 14 days, and the unconfined compressive strength (UCS) of treated samples was evaluated. The results indicated that urease activity increased by ~53.4% with 0.01% Ni^{2+}, while 0.01% Mn^{2+} reduced it by ~37.5% compared to the without added ions. Ni^{2+} enhanced urease activity as an essential enzyme cofactor, whereas Mn^{2+} inhibited it through structural interference. The highest UCS was obtained with 0.01% Ni^{2+}, showing a 58.6% increase, whereas Mn^{2+} addition reduced strength by 40%, indicating that UCS is primarily governed by urease-driven cementation. Microstructural observations further confirmed that Ni^{2+} promotes favourable rhombohedral calcite crystal distribution and bonding, while Mn^{2+} leads to poor cementation. Overall, Ni^{2+} enhances MICP efficiency,while Mn^{2+} negatively affects the process.

Keywords

Artificial beachrocks, Biocementation, Metal additives, Sand cementation test, Urea hydrolysis.

1 Introduction

Microbially Induced Carbonate Precipitation (MICP) is an emerging biocementation technique that has gained significant attention in recent years, particularly for applications in erosion control. MICP is a nature-based and environmentally friendly technique, often referred to as urea hydrolysis–based bio-grouting, which utilizes microbial processes instead of conventional chemical or cement-based grouts.In this process, microorganisms induce biochemical reactions in sand that precipitate $CaCO_3$, a natural cement, which fills the pore spaces between sand particles and reduces porosity and particle mobility. Consequently, the treated sediments exhibit increased strength and stiffness (Achal and Kawasaki 2016; Dubey et al. 2023; Gowthaman et al. 2024). There have been limited studies investigating the potential of MICP to strengthen loose beach sediments as an alternative countermeasure for stabilizing beaches vulnerable to coastal erosion (McCutcheon et al. 2017).

Beachrocks form through the natural cementation of unconsolidated beach sediments by calcareous materials and exhibits strong resistance to erosive forces compared with unconsolidated beach sediments. Its durability under wave and wind action highlights its potential as a nature-based model for coastal erosion control (Danjo and Kawasaki 2014; Dubey et al. 2023). There are very limited studies investigating the feasibility and effectiveness of producing artificial bio-beachrocks through MICP, similar to naturally occurring beachrocks, for strengthening loose beach sediments in coastal erosion–prone areas as an alternative coastal erosion control measure using bio-augmentation with indigenous marine bacteria (Danjo and Kawasaki 2016; Dubey et al. 2023). However, indigenous marine bacteria generally exhibit lower urease activity than commercial strains, which can result in limited $CaCO_3$ precipitation and reduced MICP efficiency (Dubey et al. 2023). This study aims to investigate the influence of Ni^{2+} and Mn^{2+} metal ions on the efficiency of artificial bio-beachrocks produced using indigenous marine bacteria isolated from erosion-prone coastal areas of Sri Lanka, through the MICP, initially evaluate through small-scale sand column experiments. Previous studies have reported that metal ions such as Ni^{2+} and Mn^{2+} are essential nutrients for bacteria, and their presence would pose beneficial effects in the performance of the bacteria (Brophy and Nolan2015; Gowthaman et al. 2024).

2 Methodology

The bacteria used in this study were *Pseudidiomarina marina*, a ureolytic marine microorganism isolated from coastal sands along the erosion-pronesouthern to western coastlines of Sri Lanka. The isolation process of the bacteria can be found in the previous work (Surangani et al. 2025). The bacteria were cultivated in 100 mL of Zobell 2216E liquid medium (pH 7.8, 30 °C, 180 rpm) under four conditions: Case 1 without added ions, Case 2 with Ni^{2+} (0.01%), Case 3 with Mn^{2+} (0.01%), and Case 4 with both Ni^{2+} (0.01%) and Mn^{2+} (0.01%).Microbial cell growthof each case was determined by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer at 24 h intervals. Microbial urease activity of each case was evaluated by suspending the cells in a urea solution and measuring the amount of enzyme required to hydrolyze 1 μmol of urea per minute (U/mL) following the method of Fujita et al. (2017). The $CaCO_3$ precipitation of each case was investigated through aqueous solution tests using 10 mL test tubes. Each tube contained 0.5 mL of 5 mol/L urea, 0.5 mL of 5 mol/L $CaCl_2$, and 3 mL of sterilized distilled water. One mL of bacterial suspension collected at the peak growth stage of each case was added to the respective tubes. An additional control tube without bacterial culture (Case c) was also prepared.

The sand used in this experimental study was Toyoura sand. Toyoura sand is commercially available and, according to the Unified Soil Classification System (USCS) (ASTM 2017), it is classified as poorly graded fine sand with a mean particle diameter (D_{50}) of 0.20 mm. Sand columns for each case were prepared in vertically positioned syringes (30 mm diameter, 60 mm height). Silicone valves were fitted at the bottom to collect outlet solutions, and non-woven fabric was placed to prevent sand loss. Sand was placed in three equal layers, and each layer was tamped 20 times to achieve an average dry density of 1.5 ± 0.05 g/cm³.A two-phase percolation method was adopted. Initially, 20 mL of bacterial culture was injected into the columns at a rate of about 10 mL/min and retained for 2 h to allow bacterial attachment to sand particles. In the second phase, 20 mL of cementation solution was injected every 24 h and was allowed to percolate for 14 days, with bacterial reinoculation on the 8th day (bio-dosing) to maintain urease activity. Both the bacterial culture and the cementation solution were introduced from the column surface and allowed to percolate under gravity. After the treatment, 20 mL distilled water was flushed through each column, followed by oven drying at 60 °C for 48 h before

demolding and further analysis. The amount of $CaCO_3$ precipitated was determined from the mass difference before and after the MICP treatment using (Eq. 1):

$$CCC = \frac{m_2 - m_1}{m_1} \times 100 \quad (1)$$

where m_1 is the initial dry mass of sand before treatment and m_2 is the dry mass of the sand column after treatment.

The needle penetration test was conducted according to Japanese Geotechnical Society standards (JGS 2012) to estimate unconfined compressive strength (UCS) of treated sand columns. Tests were performed only at a depth 3 cm from the column surface. Penetration resistance (N) and penetration depth (mm) were recorded, and UCS was estimated using the regression relationship proposed by Ulusay (2014). Microstructural observations were conducted using scanning electron microscope (SEM). Small block samples (~2 mm) were collected, coated with gold for 20 s, and then examined under the SEM.

3 Results and discussion

3.1 Effect of Mn^{2+} and Ni^{2+} on microbial cell growth, urease activity and calcium carbonate precipitation

The cell growth and urease activity of *Pseudidiomarina marina* for all cases are shown in Fig. 1(a–c). In all cases, a similar growth pattern was observed, where the cell concentration rapidly increased to $OD_{600} \approx 3.0–4.0$ within 3 days, followed by a gradual transition to the stationary phase. The highest growth was observed in the presence of Mn^{2+}, followed by the condition without added ions. The lowest growth occurred in the presence of Ni^{2+}, while the combined presence of Ni^{2+} and Mn^{2+} resulted in growth higher than Ni^{2+} alone but lower than Mn^{2+} alone (Fig. 1a). During normal aerobic metabolism, bacteria generate reactive oxygen species (ROS), including superoxide (O_2^-), which can damage proteins, DNA, and cell membranes. To mitigate this oxidative stress, bacteria produce enzymes such as superoxide dismutase (SOD), which converts superoxide into less harmful molecules. The activity of SOD requires metal cofactors such as Fe, Mn, or Cu/Zn. The availability of suitable metal ions can therefore influence the efficiency of oxidative stress resistance and consequently bacterial growth. Different bacteria require different metal ions for their enzymes to function properly (Brophy and Nolan 2015). In this study, we hypothesize that the marine bacterium *Pseudidiomarina marina* preferentially utilizes Mn^{2+} as a cofactor for oxidative stress protection, which may explain the enhanced growth observed in Mn^{2+}-supplemented culture. The reduced growth observed in Ni^{2+}-supplemented cultures is attributed to its toxic effects on bacterial metabolism. Ni^{2+} disrupts metal-dependent enzymatic functions by competing with essential cofactors such as Mn^{2+}, leading to metabolic disruption, increased cellular stress, and reduced energy availability for cell proliferation. In cultures containing both Ni^{2+} and Mn^{2+}, growth reflects a balance between nickel toxicity and manganese's protective role. While Mn^{2+} partially mitigates these effects by supporting enzyme function, overall growth remains intermediate (Marques et al. 2023).

The effect of metal ions on urease activity showed that 0.01% Mn^{2+} (Case 3) had a negative impact, causing ~37.5% decrease, whereas 0.01% Ni^{2+} (Case 2) significantly increased activity by ~53.4% compared to Case 1. Urease is a dinickel enzyme containing two Ni^{2+} ions at its active site, which are essential for its structural stability and catalytic function. Therefore, the availability of Ni^{2+} directly influences both urease synthesis and enzymatic activity. Supplementation of Ni^{2+} in the growth medium facilitates the incorporation of nickel into the urease active site, leading to the formation of more active enzymes and consequently higher urease activity.Mn^{2+} exhibits an inhibitory effect on urease activity, similar to other metal ions such as Zn^{2+}, Cu^{2+}, Co^{2+}, and Ag^+. Mn^{2+} can interact with sulfur-containing groups in urease, altering the enzyme structure and partially blocking the active site, thereby reducing the ability of urease to hydrolyze urea (Maroney and Ciurli 2014; Gowthaman et al. 2024).The combination of Ni^{2+} and Mn^{2+} (Case 4) decreased urease activity by 38.8% compared to Ni^{2+} alone (Case 2), likely due to the inhibitory effect of Mn^{2+} (Gowthaman et al. 2024).Although higher bacterial cell density generally leads to increased urease enzyme production (Dubey et al. 2023), the Mn^{2+}-amended culture showed lower urease activity than the Case 1 despite relatively

higher bacterial growth. This suggests that the inhibitory effect of Mn^{2+} on enzyme functionality reduces the urease activity,even when bacterial cell density is higher.

The mass of $CaCO_3$ precipitate in the aqueous solution tests are shown in Fig. 1d. The highest precipitation occurred in Case 2, while Case 3, containing Mn^{2+}, showed reduced $CaCO_3$ mass. $CaCO_3$ precipitation depends on both urease activity (which produces carbonate) and bacterial cells (which act as nucleation sites for crystal growth) (Dubey et al. 2023).Ni^{2+}-amended cultures showed a pronounced increase in precipitation due to enhanced urease activity, whereas Mn^{2+} resulted in reduced precipitation due to inhibition of urease activity.

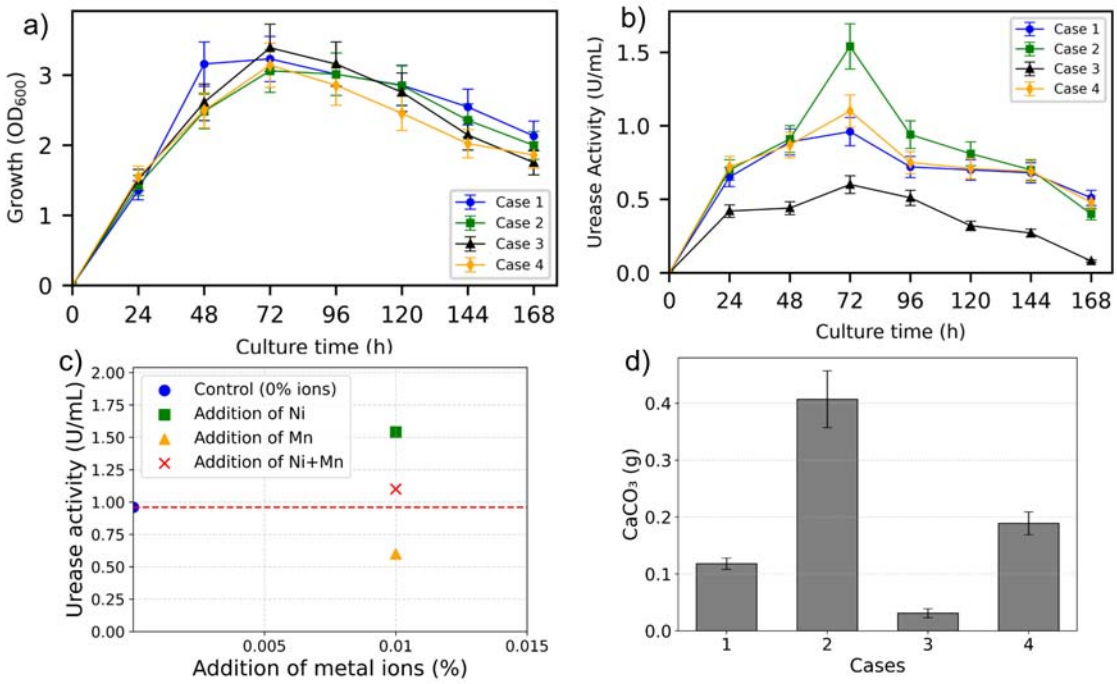


Fig. 1a) Microbial cell growth;b) microbial urease activity; c) addition of metal ions on the urease activity; d) mass of calcium carbonateproduced in aqueous solution.

3.2 Effect of Mn^{2+} andNi^{2+}on small-scale sand column tests

The appearance of the treated sand columns after drying is shown in Fig. 2a. Most columns exhibited moderate cementation, except for Case 3, which showed fragmentation at the top due to weak cementation. Case c represents the untreated sand column. The UCS values for each case, estimated using a needle penetration device, are presented in Fig. 2b. The results indicate that Case 2, which contained Ni^{2+} ions, exhibited the highest strength, showing a ~58.6% increase compared to the other cases. In contrast, Case 3, which included Mn^{2+} ions, showed a 40% reduction in strength compared to Case 1.The precipitation content (Fig. 2c) showed that the UCS values were in good agreement with the amount of mineralized calcium precipitate in each case. Notably, Case 2 achieved both the highest UCS and the highest precipitation content. The results suggest that the increase in UCS is primarily attributed to the amount of cementing material formed within the sand matrix, which depends on urease activity. To further examine the effect of metal ion addition on (i) urease activity and (ii) cement content, the urease activity of Cases 1–4 was plotted against their precipitation content (Fig. 2d). The results indicate that the addition of trace amounts of Ni^{2+} enhances urease activity, thereby promoting the formation of calcium carbonate responsible for strength improvement. Previous studies (e.g., Gowthaman et al. 2024) have also reported a strong correlation between UCS and the quantity of precipitated cementing minerals such as calcium carbonate. In contrast, the presence of Mn^{2+} showed an inhibitory effect, resulting in lower urease activity, reduced precipitation, and consequently lower strength. Further studies are recommended to investigate the relationship between Ni^{2+} concentration and the resulting biological and mechanical improvements.

SEM images of the treated sand columns are presented in Fig. 3. Strength improvement depends not only on the amount of $CaCO_3$ but also on crystal shape, size, spatial distribution, and bonding efficiency (Danjo and Kawasaki 2014; Dubey et al. 2023).

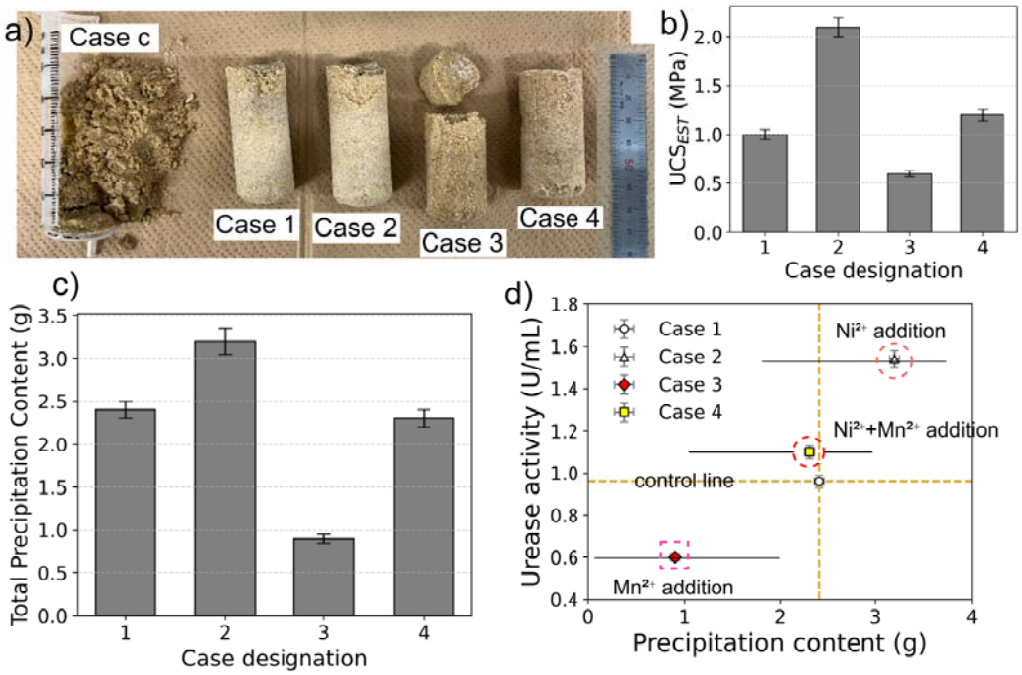


Fig. 2a) The appearance of sand columns after MICP treatment; b) UCS of sand columns (estimated by the needle penetration test); c) Average precipitation quantities of calcium carbonate in treated columns; d) Urease activity against the precipitation content of calcium carbonate-intense view on the effect of metal ions addition.

$CaCO_3$ precipitated at inter-particle contact points forms effective bridges between soil grains, which significantly enhance UCS (Dubey et al. 2023). SEM analysis of the Ni^{2+}-amended samples revealed numerous small crystals, moderate cement coatings around the sand particles (Fig. 3c), strong inter-particle $CaCO_3$ bridges (Fig. 3a), and limited pore filling, which enhanced the filtering effect, collectively resulting in higher soil strength. In contrast, the Mn^{2+}-amended samples exhibited larger crystals, weak cement coatings surrounding the sand particles, very thin inter-particle $CaCO_3$ bridges, and minimal pore filling, resulting in lower soil strength (Dubey et al. 2023). The precipitates were observed as rhombohedral calcite crystalline structures.

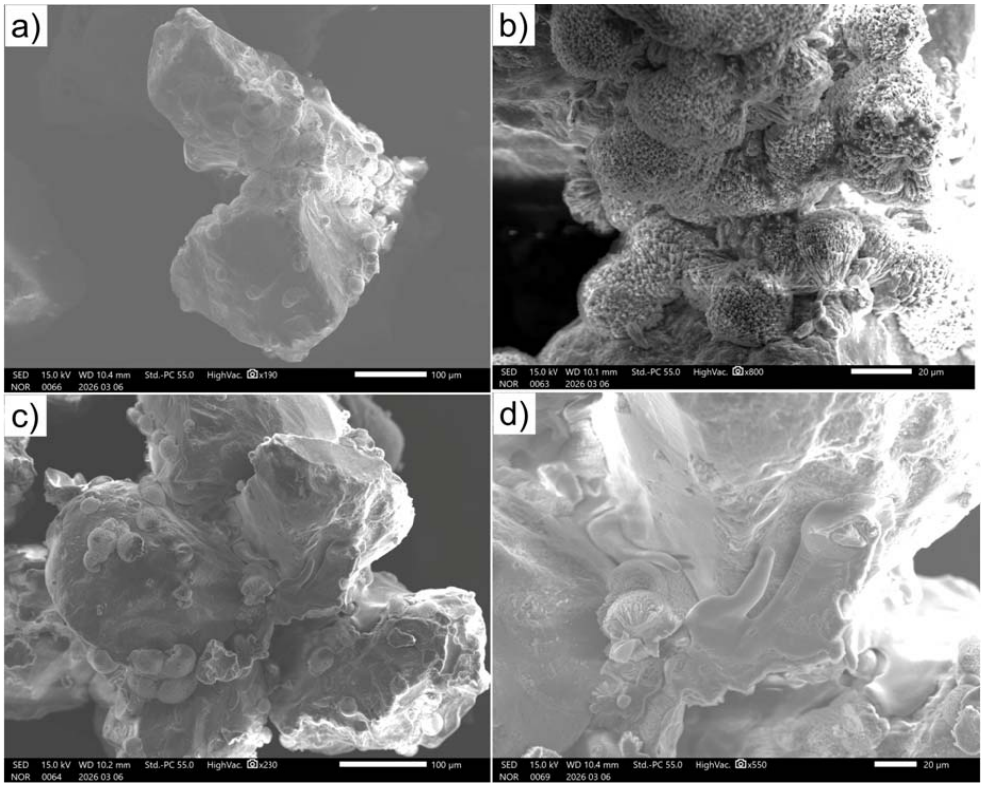


Fig. 3 SEM images showing: a) rhombohedral calcite crystallization at particle–to-particle contact point and on particle surfaces; b) closer view of the rhombohedral calcite showing many small crystals and a few larger crystals; c) cement coating the sand grains, binding the particles at contact points, and filling the pore spaces; d) closer view of cement binding.

4 Conclusion

This study aimed to investigate the influence of Ni^{2+} and Mn^{2+} metal ions on the efficiency of artificial bio-beachrocks produced through MICP using indigenous marine bacteria isolated from erosion-prone coastal areas of Sri Lanka, which was initially evaluated through small-scale sand column experiments. The results demonstrate that the type and balance of metal ions play an important role in the microbial growth and urease activity of *Pseudidiomarina marina*. Mn^{2+} enhanced bacterial growth, likely by improving oxidative stress resistance, whereas Ni^{2+} suppressed growth due to its toxic effects on metabolic processes. In contrast, Ni^{2+} significantly increased urease activity as an essential cofactor of the enzyme, while Mn^{2+} inhibited its activity through structural interference. The combined presence of Ni^{2+} and Mn^{2+} resulted in intermediate behaviour due to competing effects. Consequently, $CaCO_3$ precipitation was highest in Ni^{2+}-amended condition, demonstrating that urease activity, rather than bacterial growth, is the dominant factor controlling MICP efficiency. The highest UCS was achieved in the sand column treated with a culture medium containing 0.01% Ni^{2+}, showing about a 58.6% increase compared to the case without metal ions addition. In contrast, the addition of Mn^{2+} ions resulted in a 40% reduction in strength. SEM analysis showed that Ni^{2+} promoted numerous small rhombohedral calcite crystals, stronger particle bonding, and better cement distribution, leading to higher strength, whereas Mn^{2+} led to larger rhombohedral calcite crystals with weak bonding and poor pore filling, leading to lower strength. Overall, Ni^{2+} is beneficial for enhancing MICP efficiency, whereas Mn^{2+} negatively impacts the process under the studied conditions.

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