Efficient option of industrial wastewater resources in cement mortar application with river-sand by microbial induced calcium carbonate precipitation

Yi-Hsun Huang1, How-Ji Chen1, Jyoti Prakash Maity2,3✉, Chien-Cheng Chen4, An-Cheng Sun5 & Chien-Yen Chen2,6✉

The industrial wastewater disposal has been growing attention for environmental protection and resource substitution, current decades. Similarly, the durability enhancement of concrete has increased attention by microbial induced CaCO₃ precipitation (MICP) process (biocalcification). However, ecofriendly utilization of industrial wastewater in concrete formation is unstudied so far. The present study was carried out to evaluate the effect of industrial wastewater on the formation of cement mortar, compressive strength and water absorption. The biocement mortar strength (y) increased ($y = 0.5295x^2 + 1.6019x + 251.05; R^2 = 0.9825$) with increasing percentage of organic wastewater (x) (BM0 – BM100) by MICP, where highest strength (280.75 kgf/cm²) was observed on BM100 (100% wastewater), compared to control (252.05 kgf/cm²). The water absorption (y) of biocement mortar decreases ($y = -0.0251x^2 - 0.103x + 15.965; R^2 = 0.9594$) with increment of wastewater (x) (%) (BM0 – BM100), where a minimum-water-absorption (14.42%) observed on BM100, compared to control (15.89%). SEM micrograph and XRD shows the formation of most-distinctive CaCO₃ crystallization (aragonite/calcite) (acicular, brick shape, massive and stacked structure) inside biocement mortar (BM100), which fills the pores within cement mortar to form a denser structure, by microbial organic wastewater. Thus, present findings implied a cost-effective of MICP technology to improve the concrete properties along with the mitigation of industrial wastewater pollution, which goes some way towards solving the problem of industrial wastewater pollution.

Since the industrial revolution in 18th century, the quality of life of human has improved drastically and the population grows exponentially, worldwide. As a result, the demand for sturdy and comfortable houses has been on the rise. The cement, a most common building material that use for constriction, worldwide. However, manufacturing cement produces a large amount of carbon dioxide that harms the environment1,2. Therefore, the researchers in civil engineering sector have been investigating to extend the service life of cement in building constriction3–6. On the other hand, the researcher reported that the bioremediation process is able to strengthen the structural integrity of buildings and reduces water absorption. Furthermore, the researchers have been trying to report, how organisms repair themselves, and they have been trying to develop ecofriendly bioremediation systems7–9. However, the bioremediation along with repair system has not been reported clearly, so far.

1Department of Civil Engineering, National Chung-Hsing University, Taichung, 402, Taiwan. 2Department of Earth and Environmental Sciences, National Chung Cheng University, 168 University Road, Ming-Shung, Chiayi County, 62102, Taiwan. 3School of Civil Engineering and Surveying and International Centre for Applied Climate Science, University of Southern Queensland, Toowoomba, Australia. 4Department of Biotechnology, National Kaohsiung Normal University, Kaohsiung, 82444, Taiwan. 5Department of Chemical Engineering and Materials Science, Yuan-Ze University, 135 Yuan-Tung Road, Chung-Li, 32003, Taiwan. 6Center for Innovative Research on Aging Society, AIM-HI, National Chung Cheng University, 168, University Rd., Min-Hsiung, Chiayi, 62102, Taiwan. ✉e-mail: jyoti_maity@yahoo.com; chien-yen.chen@oriel.oxon
In natural environment, small sand grains solidify to become sandstone under biogeochemical process (microorganisms, time, and pressure). At adequate calcium concentration, the calcium reacts with carbonate ions to precipitate calcium carbonate that acting as a gluing agent, where the calcium carbonate helps the transformation process of sand solidification. In this process, the microorganisms metabolize and produce urease that transform the urea into ammonium and carbonate ions\(^{10,11}\). This bio-mineralization occurs by microbial induce calcium participation, (MICP) process\(^{12-15}\).

The industrial wastewater is one of the complications to ecofriendly progress in human civilization. Carelessly discharging the wastewater into water bodies are affecting the physical, chemical, and biological changes to the environment, since it (wastewater) is not only harmful to the environment but also to human health. Therefore, industrial sectors are obligated to install comprehensive wastewater processing/treatment system. Such system often requires proper equipment, ecofriendly technology, as well as funding for wastewater treatment to permissible limit, that often increase the production cost\(^{16,17}\). However, the ecofriendly wastewater treatment is a great challenge in present-day research. Thus, it is an urgent need to utilize of wastewater for beneficial purpose of ecofriendly environment pollution management. The investigator utilized the urase enzyme of urealytic microorganism (example: *Sporosarcina pasteurii* and *Bacillus sphaericus*) for MICP process, through which the mineral can precipitate\(^{18-22}\). The organic wastewater of food industries, contains microbial nutrients and a mass amount of microorganism (Example: bacteria), may produces urease in wastewater\(^{23}\). Thus, the urease containing wastewater can be used for MICP process by the possible replacement of the aforementioned microorganism/microorganism/bacteria that may benefits of ecofriendly utilization of industrial wastewater with cost minimization of industrial product\(^{24}\). Furthermore, the utilization of industrial wastewater could reduce the cost for wastewater processing in industries where, the main problem of MICP has always been high-cost.

Considering the background, the present study focuses on the utilization organic wastewater for MICP process to produce bio cement mortar. The wastewater of food industry was used to produce bio cement mortar. In addition, the mechanical properties such as strength and water absorption, as well as the physiochemical properties of the bio cement mortar by SEM and XRD, were taken under study.

### Results and Discussion

**Microbiologically induced calcite precipitation by industrial wastewater.** Microbiologically induced calcite precipitation and characterization of industrial wastewater. The basic characteristics of industrial water is shown in Table 1. The industrial wastewater was noticed slightly higher pH at 8.40 ± 0.5. COD of wastewater was found significantly high as 1200.10 ± 2.5 mg/l compare to BOD (10.50 ± 0.12 mg/l). This is indicated that the higher amount of oxygen required to chemically oxidize organic compounds compare to the amount of oxygen required to biologically oxidize the organics in the industrial wastewater. The temperature of wastewater was observed 30.90 ± 0.89 °C. The CFU of wastewater was noticed as 10\(^3\) to 10\(^5\) (cfu/ml), where as the urease activity was observed as 0.894 ± 0.01 (mol/l). The results of the varied parameters of urea and Ca(NO\(_3\))\(_2\) with 40 ml of wastewater in different experimental conditions (C\(_1\) – C\(_4\)) (Table 2) shows that the precipitation was increased with the increasing urea and Ca(NO\(_3\))\(_2\) concentration, significantly (y = 0.3568ln(x) + 0.0241; R\(^2\) = 0.9747) (Fig. 1). The precipitation was not occurred in control condition due to absence of urea and Ca(NO\(_3\))\(_2\). Thus, the urea and Ca(NO\(_3\))\(_2\) are essential for microbiologically induced calcite precipitation in wastewater, which are very active in precipitation process. Hammes et al.\(^{24}\) reported that the strain-specific calcification occurred during ureolytic microbial carbonate precipitation by *Bacillus sphaericus*. In another study, the Ca\(^{2+}\) remove from industrial wastewater by MICP process through ureolytic microorganisms\(^{25}\). The precipitate was reported as CaCO\(_3\) which are composed of predominantly calcite crystals with little vaterite crystals by

| Parameter       | Concentration in water | Guideline/Standard (MOEAI\(_D\))\(^{39}\) |
|-----------------|------------------------|------------------------------------------|
| pH              | 8.40 ± 0.5             | 6–9                                      |
| COD (mg/l)      | 1200.10 ± 2.5          | 100                                      |
| BOD (mg/l)      | 10.50 ± 0.12           | 30                                       |
| Color (Pt-Co)   | 51.00 ± 0.05           | 550                                      |
| TSS (mg/l)      | 16.10 ± 0.08           | 30                                       |
| Temp (°C)       | 30.90 ± 0.89           | 35                                       |
| CFU (cfu/ml)    | 10\(^{-7}\)–10\(^{-9}\) | —                                        |
| Urease activity (mol/l) | 0.894 ± 0.01       | —                                        |

**Table 1.** Basic parameter of industrial wastewater. Data represents mean ± SE.

| Chemical/wastewater | Control | C\(_1\) | C\(_2\) | C\(_3\) | C\(_4\) |
|---------------------|---------|---------|---------|---------|---------|
| Wastewater (ml)     | 40      | 40      | 40      | 40      | 40      |
| Urea (M)            | 0       | 1.1     | 1.1     | 1.1     | 1.1     |
| Ca(NO\(_3\))\(_2\) (M) | 0       | 0.1     | 0.3     | 0.5     | 1.1     |

**Table 2.** The varied parameters of urea and Ca(NO\(_3\))\(_2\) with 40 ml of wastewater in different experimental conditions (C\(_1\) – C\(_4\)).

...
Sporosarcina pasteurii strain ATCC 1185926. In the present study, particularly, a rapid precipitation (0.47 g) was occurred within the concentration of 0.3 M of Ca(NO₃)₂, where a maximum precipitation was observed as 0.55 g at concentration of 1.1 M of Ca(NO₃)₂. Therefore, the microbial community in industrial wastewater is effective to precipitate the calcium mineral, naturally.

Characterization of synthesized material by microbiologically induced calcite precipitation. The XRD of the material, synthesized by microbial community of wastewater are shown in Fig. 2. The results indicate that the peak intensities (at 2θ) are observed at 23.06°, 29.3°, 35.98°, 39.41°, 43.17°, 47.49°, 48.5°, 57.42°, 60.99°, and 65.58°, that representing the Miller indices of calcite phase at (012), (104), (110), (113), (202), (018), (016), (122), (208) and (0012), respectively. The peak intensity at 21.09°, 27.24°, 33.16° and 50.27° was observed, which represent the aragonite mineral phases at Miller index of (110), (021), (012), and (132), respectively. The calcite and aragonite are stable forms of calcium carbonate. The present results are comparable with the findings of Torres et al.27 (2013), where the calcite and vaterite are precipitated in different proportions and shapes by several microorganisms in domestic wastewater. Thus, it is confirming that the calcium carbonate precipitation occurs by microbial community of wastewater by MICP process.

Formation and characterization of biocement mortar. Formation of biocement mortar and compressive strength. The organic wastewater, which contain microbial consortium that influences on water adsorption rate and compressive strength of biocement mortar (Fig. 3a–d). The study highlights the effect of organic wastewater on the compressive strength of cement mortar. The effect of organic waste water on compressive strength of cement mortar are shown in Fig. 3a–b. The compressive strength of cement mortar without organic wastewater was 252.05 kgf/cm² at 28 days, where the compressive strength observed as 258.36 kgf/cm², 260.44 kgf/cm², 265.89 kgf/cm², 270.65 kgf/cm² and 280.75 kgf/cm² in the treated group of 20%, 40%, 60%, 80% and 100% organic wastewater, respectively at 28 days incubation. The polynomial relationship (y = 0.5295x² + 1.6019x + 251.05) was observed between compressive strength and increment of wastewater percentage (%) (BM₂₀, BM₄₀, BM₆₀, BM₈₀, BM₁₀₀).
BM60, BM80 and BM100. The regression analysis in between independent variable as wastewater percentage (x) and dependent variable compressive strength (y) reflects a positive polynomial relation ($R^2 = 0.9825$) (i.e. compressive strength increases with the increasing of wastewater percentage). The cement mortar treated with 100% wastewater was observed the highest strength (280.75 kgf/cm²) compared to control after 28 days. Therefore, the strength of biocement mortar and CaCO₃ precipitation were increased with the increasing amount of organic wastewater (Fig. 3a,b). The current findings of compressive strength are comparable with the finding of Chahal et al.18,28, where researchers were indicated the compressive strength was increased in presence of microorganism ($S$. pasteurii). The optimum compressive strength was reported $10^5$ cells/ml whereas the matrix integrity disrupts due to excessive bacterial activity at $10^7$ cells/ml18,28. In another research29, the compressive strength of bacterial concrete was reported to be increased in $10^3$–$10^5$ (cfu/ml), whereas the strength was found to be decreased in/after $10^7$ (cfu/ml), compared to the concrete sample without bacteria. In the present study, $10^{-1}$–$10^3$ (cfu/ml) of bacteria are survived after mixing with cement, which produced the urease enzyme. It is confirming that the reason may be that urease enzyme (produced by bacteria in organic wastewater in Table 1) reacts with urea and

Figure 3. (a) The compressive strength and water absorption of biocement mortar in different samples treated with different percentage (%) of wastewater (BM₀, BM₂₀, BM₄₀, BM₆₀, BM₈₀ and BM₁₀₀). Line and Bar diagram represents mean ± SD, where n = 3. The line of polynomial relationship in between wastewater percentage (%) vs compressive strength and wastewater percentage (%) water absorption of biocement mortar. (b) Change of compressive strength due to CaCO₃ formation in different wastewater cement mortar. (c) Change of water absorption due to CaCO₃ formation in different wastewater cement mortar. (d) Urease activity of different percentage (%) of wastewater with different time in biocement mortar. (e) Urease activity of wastewater + cement and waste water in different time. (f) Porosity vs CaCO₃ formation in different percentage (%) of wastewater (BM₀, BM₂₀, BM₄₀, BM₆₀, BM₈₀ and BM₁₀₀).
calcium nitrate which can produce calcium carbonate precipitation (see XRD of material in previous section)\textsuperscript{22}. The cement mortar provides additional pores (during hydration reaction), where the calcium carbonate is precipitated and fully filled the porosity of biocement mortar. Although the chemical substances contained in organic wastewater which decreases the binding rate of calcium and citrate in hydration reaction as well as produces a retarding effect. However, in addition of organic wastewater totally (100%) that can effectively improve the strength of cement mortar compare to control.

Formation of biocement mortar and water absorption. Similar to the compressive strength, the organic wastewater influences on the water absorption capacity of biocement mortar formation. The effects of organic wastewater on water absorption in cement mortar are shown in Fig. 3. The water absorption of biocement mortar was noticed as 15.89% at 28 days without organic wastewater, whereas 15.51%, 15.58%, 15.06% and 14.87% of water absorption in biocement mortar were observed in the treated group of 20%, 40%, 60% and 80% organic waste water, respectively at 28days. The biocement mortar treated with 100% wastewater was observed a water absorption of 14.42% after 28 days. A polynomial relationship ($y = -0.0251 \times 2 - 0.103 \times 15.965$) was observed between water absorption and increment of wastewater percentage (%) (BM\textsubscript{0}, BM\textsubscript{20}, BM\textsubscript{40}, BM\textsubscript{60}, BM\textsubscript{80} and BM\textsubscript{100}). The regression analysis in between independent variable as wastewater percentage (x) and dependent variable water absorption (y) reflects a negative polynomial relation ($R^2 = 0.9594$) (i.e. water absorption decreases with the increasing of wastewater percentage). Thus, the water absorption decreases as the proportion of wastewater increases in the treatment process of biocement mortar formation. Chahal et al.\textsuperscript{28} observed a four-times reduction of water absorption in fly ash concrete with 10\textsuperscript{5} cells/ml of S. pasteurii. In another study, Chahal et al.\textsuperscript{29} reported a maximum reduction of water absorption with 10\textsuperscript{5} cells/ml for 10% silica fume concrete at 91 days; however, concrete with 5% silica fume gave 0.1% water absorption (minimum) at 91 days, which was 0.3% at 28 days. The water-proofing effect was reported to increase with increasing calcium dosages in the presence of Bacillus sphaericus LMG 225 57, whereas for a while the calcium dosage of 17 g Ca\textsuperscript{2+} m\textsuperscript{−2} the water absorption was reported similar to that of untreated cases. In a 50% decrease of the rate of water absorption was reported at a concentrations of 67 g Ca\textsuperscript{2+} m\textsuperscript{−2}. In another report, the surface deposition of calcium carbonate crystals decreased the water absorption by 65% to 90% depending on the porosity of the material by B. sphaericus\textsuperscript{30}. The ureolytic bacteria such as Bacillus sphaericus are able to precipitate CaCO\textsubscript{3} in their micro-environment by conversion of urea into ammonium and carbonate. Thermogravimetric analysis showed that bacteria were able to precipitate CaCO\textsubscript{3} crystals inside the cracks, as a result the permeability of the biocement mortar decreased\textsuperscript{31}. In present study shows that the urease activity plays an important role of the CaCO\textsubscript{3} formation. The urease activity (mol/l) was observed in wastewater, which was increased significantly up to 60 min; however, the activity was decreased a bit with the decreasing concentration of wastewater (Fig. 3d). On the other hand, the urease activity was noticed higher in the mixer of cement with wastewater, compare to only waste water (Fig. 3e). Therefore, urease activity helps to precipitate the calcium carbonate to the mixture of biocement mortar. The water absorption decreased with the increasing of wastewater concentration or CaCO\textsubscript{3} formation (Fig. 3c). Thus, these results reflect the formation and precipitation of calcium carbonate from urea and calcium nitrate in presence of urease from bacteria in organic wastewater. The Fig. 3f shows that the porosity of the biocement mortar decreases with the CaCO\textsubscript{3} precipitation and it is confirmed that the precipitated calcium carbonate effectively fills pores on and within (inside) the surface of the biocement mortar. Therefore, the investigation documents the calcium carbonate precipitation as a result reduction of water absorption on and within biocement mortar, which provides a hopeful solution for durability of cement. Furthermore, the precipitation of calcium carbonate could also fill the pores inside the cement mortar which increases the density and structural strength of the cement mortar.

X-ray diffraction (XRD) analysis of biocement mortar. Figure 4 shows the of XRD result of the cement mortar. The quartz phase was observed the peak intensity at 2\textdegree = 26.63\textdegree, and 68.3\textdegree representing...
the Miller index of (101) and (301), respectively. The peak intensity at $2\theta$ for the value around 29.399°, 39.42°, 43.17°, 60.68°, and 81.5° representing the Miller index of (104), (113), (202), (214), and (2110), respectively for the formation of calcite phase. The observed aragonite and calcite are the products of calcium carbonate, which are formed in biocement mortar, influenced by microbial organic wastewater; and further confirmed of a white powder which is calcium carbonate. The calcite peak intensities of biocement mortar (Fig. 4) treated from BM20 to BM100 are noticed evidently higher compare to BM0, which indicates the addition of organic wastewater is relevant to changes the amount and crystallization form of calcium carbonate. In comparison of compression strength and water absorption, the present result confirms the calcium carbonate precipitation through a biochemical process in presence of urea, calcium nitrate and urease (which generated from microorganisms in wastewater). Furthermore, the precipitated calcium carbonate can fill the pores of cement mortar that formed during cement-hydration reactions.

Morphology of biocement mortar. The morphological signature (SEM micrograph) of the biocement mortar containing 0% (BM0), 20% (BM20), 40% (BM40), 60% (BM60), 80% (BM80), and 100% (BM100) of wastewater are shown in Figs. 5 and 6. SEM-EDX micrograph shows the acicular, massive and stacked calcite structure in cement mortar; in particular, the needle shape, brick shape, and stacks of calcite crystals were observed inside cement mortar. Results shows at a higher proportion of waste water; the crystallization of calcite is more evident/pronounced. The most distinctive calcite crystallization is formed treated with 100% (BM100) waste water, where calcite crystals can fill the pores within the cement mortar to form the denser structure. This result can be mutually confirmed with the results of the strength and water absorption of cement mortar (see previous section). It is clear that the biologically produced calcite, precipitates within the concrete void and block pores/voids, thereby increasing the strength. Ghosh et al.32, reported that a thermophilic anaerobic microorganism increases the compressive strength of 25% in cement mortar in 28 days with the addition of about 10^6 cell/ml of water. The strength improvement was reported.
due to growth of filler material within the pores of the cement–sand matrix by microbial growth and the process of microbiologically induced mineral precipitation. In another research report, the *B. sphaericus* improves strength of cement concrete, where concrete-immobilized bacterial spores and able to seal the cracks by biomineral formation after being revived by water and growth nutrients. The potential crack healing ureolytic bacteria (example *Bacillus sphaericus*) are able to precipitate CaCO₃ in their micro-environment by conversion of urea into ammonium and carbonate; as a results the cracks were filled completely. Sujatha et al. reported a indigenous soil bacteria which enhance the compressive strength of cement mortar by precipitating the calcium carbonate mineral; as 18% of compressive strength was increased with 28 days, where the bacteria transformed soluble organic nutrients into insoluble inorganic calcite crystals (applicable for repair for concrete cracks). Hence, the present investigation reflects a positive direction of the application of microbial consortium of wastewater, which can be applicable and improve the strength, durability and repair of concrete cracks of cement concrete (Fig. 7).

**Conclusion**

The industrial wastewater (10³–10⁵ cfu/ml) was applied to enhance the durability of biocement mortar such as compressive strength, water absorption by microbial-induced calcium carbonate precipitation (MICP) (biocalcification). The ‘strength’ of biocement mortar increased (R² = 0.9825) and ‘water absorption’ of biocement mortar decreases (R² = 0.9594) with the increasing percentage (%) of organic wastewater by MICP process. The highest ‘strength’ (280.75 kgf/cm²) and lower ‘water absorption’ (14.42%) was noticed in addition of 100% wastewater after 28 days. Morphological study reveals the acicular, massive and stacked calcite structure in cement mortar samples; in particular, the needle shape, brick shape, and stacks of calcite crystals were observed inside cement mortar. XRD analysis indicated the formation of calcium carbonate (aragonite and calcite) in biocement mortar which influences by hydrolysis of urea, catalyzes by microbial enzyme of urease in MICP process using microbial organic wastewater. The crystallization of calcite is more evident/pronounced in higher proportion of waste-water. The most distinctive calcite crystallization is formed in the samples of 100% (BM₁₀₀) waste water, where calcite crystals fills the pores within the cement mortar to form the denser structure. Thus, the findings implied a cost-effective of MICP technology to improve the permeability of concrete and thereby enhancing the life of concrete structures along with the mitigation of industrial wastewater pollution, which also goes some way towards solving the problem of industrial wastewater pollution.

**Methods**

**Characterization of industrial wastewater.** The food industrial wastewater was collected from “Grape King Bio” company (wastewater release 8462 tons per month) and used to produce biocement mortar by MICP process. The basic wastewater parameter such as pH (HI 9828 Multiparameter, HANNA, Taiwan), COD (Chemical Oxygen Demand) (mg/l) (NOVA-60, MERCK), BOD (mg/l) (Biological Oxygen Demand) (NOVA-60,
MERCK), Color (Pt-Co), TSS (mg/l) (Total suspended solids) (HI 9828 Multiparameter HANNA, Taiwan), temperature (°C) (HI 9828 Multiparameter, HANNA, Taiwan) and colony-forming unit (CFU) was measured during sample collection, and stored properly for further use. The urease activity was measured immediately after sampling following the procedure of Chen et al.14.

**Experimental procedure of MICP process.** The urea, Ca(NO3)2 and food industrial wastewater were used for CaCO3 precipitation by MICP process. The schematic experimental conditions are shown in Table 2. Urea (final concentration 1.1 M) was mixed with different concentration of Ca(NO3)2 (0.1 M, 0.3 M, 0.5 M and 1.1 M) considering the final volume 40 ml by food industrial wastewater. Mixture was incubated for 24 h at 30 °C, with shaking at 120 rpm for precipitation. The precipitates were collected by centrifuging at 5000 rpm and dry at 50 °C for 3 days. The dry powder was weighted by gravimetric method and store for further study. The chemical character synthesized powder particle was measured by XRD analysis. The urease activity in wastewater was measured following the procedure of Chen et al.14.

**Preparation of biocement mortar.** A standard Portland cement (produced by “Taiwan Cement”; Type-I, specific weight: 3.15) (Table 3) and natural river sand (Table 4; Fig. 8) was used for biocement mortar experiment. Both of natural water (as control) and industrial wastewater was used for the formation of biocement mortar considering the ratio or proportion as 0.6 [water to cement (W/C)] (Table 5). Since the formation of pores in cement mortar by cement-hydration reactions are small to survive8,31,35 the microorganisms, it is necessary a significant larger pore size within the cement mortar for MICP process in building materials5,8,31,35. Therefore, in the present study, the river sand (<0.075 mm) (grains size distribution is shown in the Fig. 8) was used into the mortar to form larger pores that could improve the survival of microorganisms for MICP. The biocement mortar was prepared using fixed concentrations of urea and Ca(NO3)2 at 1.1 M (consider as per standardized results of the highest precipitation in MICP process from section “Experimental procedure of MICP process”), while 40% of river sand was used in mortar. The industrial wastewater was used in the range of 20–100% (with 20% interval), where a control experiment (BM0) was design with 100% natural water. The different composition of cubic shapes of biocement mortar (BM0, BM20, BM40, BM60, BM80 and BM100) were prepared to optimized the MICP within the biocement mortar cube. In each composition (as per Table 3) of biocement mortar, the Portland cement and natural sand was mixed with low speed (140 ± 5 rpm) for 1 min, and then the natural water and organic wastewater was mixed as well as stirred for 1.5 minutes before switched to medium speed (285 ± 10 rpm) for 1 min. The mixture was cast in a 125 cm3 (5 cm × 5 cm × 5 cm) cube mold for 24 hours with the water-cement ratio (W/C) of 0.6. After demolding, the cubic sample preserved for 28 days at 70 ± 2% RH and 20 °C ± 2 °C for further study. The urease activity of different percentage (%) of wastewater in different set of samples (BM0, BM20, BM40, BM60, BM80 and BM100) were measured following the procedure of Chen et al.14. Also, the urease activity of the mixer of cement and waste water was estimated. The porosity of the biocement mortar was measured following the procedure of Emamian and Eskandari-Naddaf36.

**Assessment of biocement mortar properties.** Estimation of compressive strength of biocement mortar. The measurement of compressive strength of cubic biocement mortar (BM0, BM20, BM40, BM60, BM80 and BM100) were conducted according to CNS1010 R3032. The measurement of cubic biocement mortar was conducted with 3 replicates; repeated for 3 times (YS/5001–25 T, YENSTRON, Taiwan). Center of the samples is placed in the compression testing machine for testing and the compression load is increased at a speed of 0.5 mm/min until the sample can no longer sustain the compression, and the structural integrity is damaged. To calculate the compressive strength of the sample, the maximum load was recorded, and divided by the cross-section area of the sample.
Estimation of water absorption of biocement mortar. The change of water absorption by CaCO₃ precipitation that may occurs by the MICP process within the biocement mortar cube and fills the pores of the cement mortar samples. The water absorption test was conducted on cement mortar samples (BM0, BM20, BM40, BM60, BM80 and BM100) following the procedure of ASTM C64238. The measurement of water absorption of the biocement mortar cube was carried out by drying the biocement mortar cube to a constant temperature at 110 °C in an oven, and the gravimetric weights were measured at 24 h intervals until the mass balance between initial and final weight less than 0.5%. The dry biocement mortar cubewas then immersed in water at 21 °C for 48 h, and after taking out, the surface was wiped dry, and the mass of the saturated substance after the immersion was calculated.

Table 3. Physical property of cement mortar (CNS 61 R2001, 2011; CNS 1078 R3039, 2011)⁴⁰,⁴¹.

| Parameter                         | Test results | Specification |
|-----------------------------------|--------------|---------------|
| Specific weight (m²/kg)           | 3.15         | CNS 61/CNS 1078⁴⁰ |
| Fineness (air permeability test) (m²/kg) | 352          | min.280       |
| Initial set (min)                 | 215          | min.45        |
| Final set (min)                   | 286          | max.375       |
| Soundness (autoclave expansion test) (%) | 1            | max.0.80      |
| Air content (specific mass) (%)   | 7.4          | max.12        |
| SiO₂ (%)                          | 20.21        |               |
| Fe₂O₃ (%)                         | 2.97         |               |
| Al₂O₃ (%)                         | 5.35         |               |
| CaO (%)                           | 60.55        |               |
| MgO (%)                           | 3.94         |               |
| SO₃ (%)                           | 2.51         |               |
| Loss on ignition (%)              | 1.3          |               |

Table 4. Physical property of natural river sand (CNS 486 A3005, 2015)⁴² (Fineness modulus = 2.76; Specific weight = 2.64; 24-hour water absorption = 0.9%).

| Screen number | Size (mm) | Percentage of accumulated residue | Percentage of screening |
|---------------|-----------|-----------------------------------|-------------------------|
| #4            | 4.75      | 2.75                              | 97.25                   |
| #8            | 2.36      | 21.94                             | 78.06                   |
| #16           | 1.18      | 36.16                             | 63.84                   |
| #30           | 0.6       | 51.91                             | 48.49                   |
| #50           | 0.3       | 72.69                             | 27.31                   |
| #100          | 0.15      | 90.91                             | 9.09                    |
| #200          | 0.075     | 100.00                            | 0.00                    |

Figure 8. Grains size distribution chart of Sand.
Table 5. Different experimental conditions (BM_{0} – BM_{100}) in varied parameters for biocement mortar.

| Type                  | BM_{0} | BM_{20} | BM_{40} | BM_{60} | BM_{80} | BM_{100} |
|-----------------------|--------|---------|---------|---------|---------|----------|
| Cement (g)            | 81.78  | 81.78   | 81.78   | 81.78   | 81.78   | 81.78    |
| Water (g)             | 49.068 | 39.254  | 29.441  | 19.627  | 9.814   | 0        |
| Waste water (g)       | 0      | 9.814   | 19.627  | 29.441  | 39.254  | 49.068   |
| Sand 40% (g)          | 130    | 130     | 130     | 130     | 130     | 130      |
| Urea (g)              | 3.24   | 3.24    | 3.24    | 3.24    | 3.24    | 3.24     |
| Ca(NO_{3})_{2}.4H_{2}O (g) | 12.74 | 12.74   | 12.74   | 12.74   | 12.74   | 12.74    |
| W/C                   | 0.6    | 0.6     | 0.6     | 0.6     | 0.6     | 0.6      |
| Water                 | 100%   | 80%     | 60%     | 40%     | 20%     | 0%       |
| Wastewater            | 0%     | 20%     | 40%     | 60%     | 80%     | 100%     |

The percentage of water adsorption was calculated as follow as. Water absorption (%) = \((C-A)/(C-D) \times 100\); where A is the weight (g) of the oven dried sample in air; C is the weight (g) of sample after immersion and boiling; and D is the apparent weight (g) of sample in water after immersion and boiling.

Characterization of synthesized material. The crystallinity of MICP synthesized powders and biocement mortar cube was analyzed by XRD (Shimadzu XRD-6000) with CuKα radiation (\(\lambda = 0.15418 \text{ nm}\)) at 40 kV and 30 mA. The angle was set to 20–80°, with two degrees (20) per minute. Morphological study of the biocement mortar cube particles was conducted by Field-Emission Scanning Electron Microscope (FE-SEM) analysis (TOPCON-ABT-150S, Japan) with a coating (Pt) operated at 0.1–30 kV.

References

1. Edvardsen, C. Water permeability and autogenous healing of cracks in concrete. ACI Mater. J. 96, 448–54 (1999).
2. Hearn, N. Self-sealing, autogenous healing and continued hydration: what is the difference? Mater. Structure. 31, 563–7 (1998).
3. Chahal, N. & Siddique, R. Permeation properties of concrete made with fly ash and silica fume: Influence of ureolytic bacteria. Constr. Build. Materials. 49, 161–174 (2013).
4. Pacheco-Torgal, F. & Labrincha, J. A. Biotech cementitious materials: Some aspects of an innovative approach for concrete with enhanced durability. Constr. Build. Materials. 67, 344–352 (2014).
5. Sikerr-Beltran, M. G., Jonkers, H. M. & Schlangen, E. Characterization of sustainable bio-based mortar for concrete repair. Constr. Build. Materials. 88, 374–84 (2015).
6. Wiktor, V. & Jonkers, H. M. Field performance of bacteria-based repair system: Pilot study in a parking garage. Case Studies in Construction. Materials. 2, 11–17 (2015).
7. De Muynck, W., Verbeken, K., De Belie, N. & Verstraete, W. Influence of urea and calcium dosage on the effectiveness of bacterially induced carbonate precipitation on limestone. Ecol. Eng. 36, 99–111 (2010).
8. Jonkers, H. M., Thijsen, A., Muyzer, G., Copuroglu, O. & Schlangen, E. Application of bacteria as self-healing agent for the development of sustainable concrete. Ecol. Eng. 36, 230–235 (2010).
9. Ramachandran, S. K., Ramakrishnan, V. & Bang, S. S. Remediation of concrete using micro-organisms. ACI Mater. J. 98, 3–9 (2001).
10. Knorre, H. & Krumbein, K. E. Bacterial calcification. Riding, E. E. & S. M., Awramik, S. M. (Eds.), Microbial Sediments, Springer–Verlag, Berlin, 25–31 (2000).
11. Rivadeneyra, M. A., Parraga, J., Delgado, R., Ramos-Cornemanzana, A. & Delgado, G. Bimineralization of carbonates by Halobacillus marismortui in solid and liquid media with different salinities. FEMS Microbiol. Ecol. 48, 39–46 (2004).
12. Bachmeier, K. F., Williams, A. E., Warmington, J. R. & Bang, S. S. Uracil activity in microbiologically-induced calcite precipitation. J. Biotech. 93, 171–181 (2002).
13. Bang, S. S., Galinat, J. K. & Ramakrishnan, V. Calcite precipitation induced by polyurethane-immobilized Bacillus pasteurii. Enzyme Microb. Technol. 28, 404–409 (2001).
14. Chen, H. J., Huang, Y. H, Chen, C. C., Maity, J. P. & Chen, C. Y. Microbial Induced Calcium Carbonate Precipitation (MICP) Using Pig Urine as an Alternative to Industrial Urine. Waste and Biomass Valorization, https://doi.org/10.1007/s12649-018-0324-8 (2018).
15. Maity, J. P., Chen, G. S., Huang, Y. H., Sun, A. C. & Chen, C. Y. Ecofriendly heavy metal stabilization: Microbial induced mineral precipitation (MIMP) and bioneralization for heavy metals within the contaminated soil by indigenous bacteria. Geomicrobiology Journal. 36(7), 612–623 (2019).
16. Garcia-Garcia, G., Woolley, E. & Rahimifard, S. Optimising Industrial Food Waste Management. Procedia Manufacturing. 8, 432–439 (2017).
17. Sivaperumal, P., Kamala, K. & Rajaram, R. Chapter Eight - Bioremediation of Industrial Waste Through Enzyme Producing Marine Microorganisms. Adv. Food Nutr. Research. 80, 165–179 (2017).
18. Chahal, N., Siddique, R. & Rajor, A. Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of concrete incorporating silica fume. Constr. Build. Mater. 37(1), 645–651 (2012a).
19. Dhami, N. K., Reddy, M. S. & Mukherjee, A. Improvement in strength properties of ash bricks by bacterial calcite. Ecol. Engineering. 39, 31–35 (2012).
20. Kim, H. K., Park, S. J., Han, J. I. & Lee, H. K. Microbially mediated calcium carbonate precipitation on normal and lightweight concrete. Constr. Build. Materials. 38, 1073–1082 (2013).
21. Perriot, B. & Mastronel, G. Conservation of monumental stones by bacterial biomineralization. Microbiology Today. 30, 113–114 (2003).
22. Siddique, R. & Chahal, N. K. Effect of ureolytic bacteria on concrete properties. Constr. Build. Materials. 25(10), 3791–3801 (2011).
23. Varalakshmi & Devi, A. Isolation and characterization of urease utilizing bacteria to produce biocement. J. Environ. Science, Toxicol. Food Technol. 8(4), 52–57 (2014).
24. Flammes, F., Boon, N., De Villiers, J., Verstraete, W. & Siciliano, S. D. Strain-Specific Ureolytic Microbial Calcium Carbonate Precipitation. Appl. Environ. Microbiology. 69(8), 4901–4909 (2003a).
25. Hammes, F., Seka, A., De Knijf, S. & Verstraete, W. A novel approach to calcium removal from calcium-rich industrial wastewater. *Water Res.* 37(3), 699–704 (2003b).

26. Okwadh, G. D. O. & Li, J. Optimum conditions for microbial carbonate precipitation. *Chemosphere* 81(9), 1143–1148 (2010).

27. Torres, A. R. *et al.* Precipitation of carbonates by bacteria isolated from wastewater samples collected in a conventional wastewater treatment plant. *Int. J. Environ. Sci. Technol.* 10, 141–150 (2013).

28. Chaah, N., Siddique, R. & Rajor, A. Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete. *Constr. Build. Mater.* 28, 351–356 (2012b).

29. Andalib, R. *et al.* Optimum concentration of Bacillus megaterium for strengthening structural concrete. *Constr. Build. Mater.* 118, 180–193 (2016).

30. De Muynck, W., Debrouwer, D., De Belie, N. & Verstraete, W. Bacterial carbonate precipitation improves the durability of cementitious materials. *Com. Conc. Res.* 38(7), 1005–1014 (2008).

31. Van Tittelboom, K., De Belie, N., De Muynck, W. & Verstraete, W. Use of bacteria to repair cracks in concrete. *Cem. Conc. Research.* 40, 157–166 (2010).

32. Ghosh, P., Mandal, S., Chattopadhyay, B. D. & Pal, S. Use of microorganism to improve the strength of cement mortar. *Cem. Conc. Research.* 35(10), 1980–1983 (2005).

33. Gavimath, C. C. *et al.* Potential application of bacteria to improve the strength of cement concrete. *Int. J. Adv. Biotechnol. Res.* 3(1), 541–544 (2012).

34. Sujatha, S. *et al.* Soil Bacteria for the Strength Enhancement of Cement Mortar. *J. Civ. Eng. Research*, 4(2A), 51–54 (2014).

35. Wang, J. *et al.* X-ray computed tomography proof of bacterial-based self-healing in concrete. *Cem. Conc. Composites.* 53, 289–304 (2014).

36. Emamian, S. A. & Eskandari-Naddaf, H. Effect of porosity on predicting compressive and flexural strength of cement mortar containing micro and nano-silica by ANN and GEP. *Constr. Build. Materials.* 218, 8–27 (2019).

37. CNS 1010 R3032: Chinese National Standards, Method of Test for Compressive Strength of Hydraulic Cement Mortars (Using 50 mm or 2 in. Cube Specimens). http://www.cnsonline.com.tw/?node=result&generalno=1010&locale=zh_TW (2011).

38. ASTM C642. Standard test method for Density, Absorption and voids in Hardened concrete. In: Annual book of ASTM standards ASTM C642, 4(2) (1997).

39. MOEAIDB: (Release water standard, Republic of China January 22, 103 Executive Yuan Environmental Protection Department Environmental Protection Department No. 1030005842) https://www.moea.gov.tw/iplw/tucheng/park/file20.pdf (2018).

40. CNS 1078 R3039: Chinese National Standards, Method of test for chemical analysis of hydraulic cement. http://www.cnsonline.com.tw/?node=detail&generalno=1078&locale=en_US (2011).

41. CNS 61 R2001: Chinese National Standards, Portland cement. http://www.cnsonline.com.tw/?node=detail&generalno=61&locale=zh_TW (2011).

42. CNS 486 A3005: Chinese National Standards, Method of test for sieve analysis of fine and coarse aggregates. http://www.cnsonline.com.tw/?node=detail&generalno=486&locale=zh_TW (2015).

Acknowledgements

Authors would like to thank Ministry of Science and Technology (Taiwan) for financial support (MOST 108-2811-M-194-510, MOST 107-2811-M-194-006 and MOST 108-2116-M-194-006).

Author contributions

Chien-Yen Chen, How-Ji Chen and Jyoti Prakash Maity designed the study; Yi-Hsun Huang, analyzed the data; Jyoti Prakash Maity and Chien-Yen Chen wrote the manuscript; Chien-Cheng Chen and An- Cheng Sun revised and suggestion of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to J.P. Maity or C.-Y. Chen.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020