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Development of bacterial consortia from traditional lime mortar for the preparation of sustainable and strength-enhanced concrete: A solution for heavy metal toxic concrete

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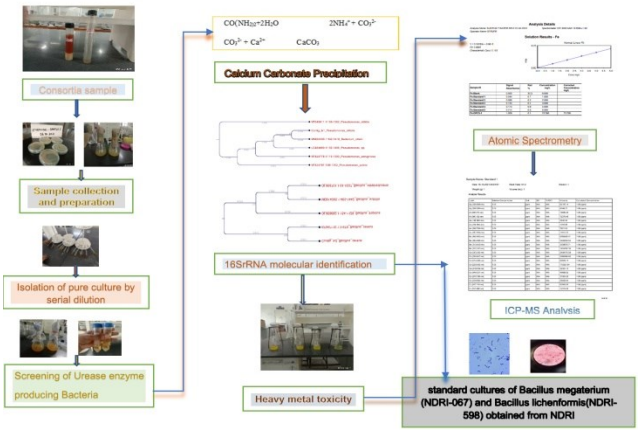
Graphical abstract



Abstract

The microbial consortia incorporated in bio concrete should have traits such as high alkali resistance and the ability to form endospores to overcome the mechanical and chemical stresses produced during concrete preparation. The primary objective of our work is to segregate and identify the calcium carbonate precipitating microorganisms with a strong urease activity, compare their potential for the preparation of bio concrete, and evaluate its performance, durability, and effects on heavy metal toxicity. Bacteria, algae, protozoans, yeasts, and fungi are predominantly reported in the consortia of microbes in a construction sample, making it an excellent source of wild microbial species and strains which have adapted to the environment of the construction place which consists of a consortium of limestone, fine sand, and curing water. Consortia of organisms were isolated from fermented lime mortar and curing construction water, which was recognized using the 16S rRNA gene sequencing technique. Atomic Absorption Spectrometry [AAS] and Inductively Coupled Plasma Mass Spectrometry [ICP-MS] were carried out to analyze the deduction of heavy metal toxicity present in the produced bio concrete. The results of strength and toxicity tests were compared with bio-concrete produced from standard cultures of *Bacillus megaterium (NDRI-067) and Bacillus licheniformis (NDRI-598)* obtained from the National Dairy Research Institute (NDRI) and compared with conventional concrete.

Keywords: Bio concrete, calcite precipitation, consortium, endospore-forming, heavy metal, micro-organisms, urease activity

1. Introduction

Concrete is a vital mixture used in almost all modern structures. As it ages it is prone to the creation of micro cracks. Micro cracks in concrete provide a way for water to flow into them which causes them to widen further threatening the integrity of the structure. It also accelerates the corrosion of vital structural reinforcements which results in the reduction of the strength and durability of vital structural elements. Many techniques are available to fix these cracks before the structural integrity is compromised but most of these rely on toxic chemicals and are quite expensive even if we ignore the fact that they are a potential harm to the environment the society (Zhang, *et al.*, 2020). Microbiologically Induced Calcite Precipitation (MICP) is deemed to be a viable alternative to traditional methods for closing in the formed micro-cracks in concrete MICP technique is quite cost-effective and bio-friendly and does not lag behind traditional healing methods. The urease enzyme plays an important role in the precipitation of calcite it catalyzes the formation of CO_2 and Ammonia from Urea which results in an increase in Ph which is considered a prerequisite for the microbial precipitation of calcite (Sharma *et al.*, 2017). Our work involves isolating and identifying $CaCO_3$ bacteria from wild sources and analyzing the CS and healing properties of the concrete while also comparing the toxicity of the prepared bio concrete with other types of concrete (Wiktor, 2011 and Huynh, 2017). The precipitates of calcite were analyzed using SEM analysis techniques and XRD technique which showed that the precipitates were in the form of vitriol

crystals. (Zhu *et al.*, 2020). Further, an FTIR test was performed to confirm the presence of $CaCO_3$ in the formed precipitate. Heavy Metal Toxicity analysis such as AAS and ICP-MS were performed on the samples labeled OPC-01, CFS-02, BCS-03, LSS-04**,** OPC-01 (Ordinary Portland Cement), CFS-02 (Cement with Flyash Sample) BCS-03 (Bacterial Consortia Sample) and LSS-04 (Limestone sample). Development of microbial consortia from traditional lime mortar and identify the calcium carbonate precipitating microorganisms. Consortia of organisms were isolated from fermented lime mortar and curing construction water, which was recognized using the 16S rRNA gene sequencing technique.

2. Materials and methods

2.1. Sample collection and preparation

Construction samples were collected from Coimbatore, India. A mixture of Limestone, jaggery, haritaki, and sand which are necessary for construction was taken for the preparation of Limestone mortar. Curing water was also collected from the construction site. The samples were properly labelled and transferred to the laboratory.

2.2. Isolation of pure culture by serial dilution

Two bacterial species were isolated from the fermented mixture and construction water samples were also obtained from a local construction site at Coimbatore, Tamilnadu.1 ml or 1 g of each sample was transferred to an enrichment nutrient media and incubated at 37 °C for 2 to 3 days. Serial dilution was performed on the sample cultures, which were inoculated onto the Urea agar with an optimum pH of 9.4, which includes Urea (20 g/l), Sodium bicarbonate (2.12 g/l), Ammonia chloride (10 g/l), Calcium chloride hydrate (25 g/l), and Nutrient broth (3.0 g/l). The plates were incubated for 2 to 3 days at 37°C (Huynh *et al.* 2017). After incubation, bacterial colonies with crystallized precipitation were identified and transferred to Urea broth. Endospore staining was performed to identify spore formation. The endospores will be green, whereas the vegetative cells will be red or pink. The $CaCO_3$ precipitation of the chosen isolates was investigated using nutrient broth containing 2% urea, and calcium chloride (NB-U/Ca). 30 ml of this broth was inoculated with 0.6 ml of isolated inoculum and incubated for 7 days at 30°C, 130 rpm. Nutrient agar is a multifunctional prepared media for growing a wide variety of bacteria and fungi. Bacteria can be grown in both solid (Agar) and liquid (Broth) mediums (broth). Below 45°C, agar solidifies, giving a solid surface for bacteria to develop. (Almutairi and Helal, 2020).

2.3. Screening of urease enzyme producing bacteria

The agar tubes(urea) containing phenolic red (0.018 g/l) as a pH indicator were utilized for the qualitative urease assay. The identified bacterial species were transferred aseptically into agar tubes(urea) and cultured for 3 to 5 days at 37°C. Every day, the test tubes were analyzed for a change in coloration, indicating that the activity of the urease enzyme is present (Andalib R A *et al.*, 2016). Culture consortia of all the selected isolates grown in a nutrient broth were centrifuged at 8000g for 15 min. The pellets were homogeneously mixed after being suspended in deionized water. The suspension's Optical Density (OD_{600}) for the conductivity test was determined and adjusted to 1.0. The test to analyze the conductivity was carried out using a mixture containing 1 M urea solution (10 ml), deionized water (8 ml), and cell suspension (2 ml). Blank was utilized with 2 ml of deionized water as a control (Sharma *et al.*, 2017). For calibration purposes, the conductivity of 1 M urea solution, deionized water, and isolation conductivity was compared to *B. megaterium NDRI.* The urease activity assay was performed using the conductivity method from (Krishnapriya *et al.*, 2015) The urease reaction resulted in an increase in conductivity by hydrolyzing urea into conductive ionic components. 9.0 ml of 1.11 M urea solution was combined with 1.0 ml of bacterial broth culture for the enzyme experiment (Nutrient Broth -Urea). The final conductivity readings were taken with an electrical conductivity meter at 20°C after 5 minutes (Yahya *et al.*, 2019).

2.4. Calcium carbonate precipitation test

Calcite precipitation was analyzed by inoculating bacterial isolates with calcium chloride in a urea broth. For 3–5 days, the test tubes were incubated at 37°C and 130 rpm (Krishnapriya *et al.*, 2015). The broth was centrifuged at 8000g for 15 minutes after 5 days to produce pellets. The pellet was weighed after drying for 24 hours at 80°C to limit the dry mass of the precipitate. Bacillus megaterium and Bacillus licheniformis isolates were examined for $CaCO_3$ precipitation to confirm the bacteria's precipitation of calcium carbonate, (Seifan *et al.*, 2017). The cultures were allowed to incubate for 7 days and $CaCO_3$ was constructed and weighed (Abdelhamid *et al.*, 2020) The equations below summarize the formation of calcium carbonate by bacteria (Xu J *et al.*, 2015).

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3$$

2.5. Preparation of bioconcrete

Bio-concrete was produced using cement, fine aggregate, coarse aggregate, and broth-containing microbes with optimum cell concentrations of 10^3, 10^5, 10^7 cells/ml to identify the effects of varying bacterial concentrations on its Compressive Strength (CS). As for the conventional mixture M40 N/mm^2(without fibre and bacteria) mixing procedures are carried out as per codes.

2.6. 16SrRNA molecular identification

Identification of the isolated cultures was done using the 16S rRNA sequencing Technique. BLAST software was run on the isolated DNA sequences to identify the individual species.

3. Characterization analysis

3.1. Atomic absorption spectrometry

Atomic Absorption Spectrometry was performed to identify the concentration levels of 5 toxic heavy metals in

OPC-01, CFS-02, BCS-03, and LSS-04. 1.0 g of each examined sample was pre-prepared and carefully transferred to a 100 mL Teflon tube with 7.0 mL HNO_3 and 21 mL HCL. After the reaction, the liquid was filtered, and distilled water was added to make it up to 50 mL.

3.2. ICP-MS analysis

ICP-MS was used to find the concentration levels of 5 metals in OPC-01, CFS-02, BCS-03, and LSS-04 samples. The samples were prepared by grinding them into a fine powder through a 75-mesh sieve for similarity, set in a plastic flask. 0.20 grams of the samples were taken into a container and placed in a microwave-pressure vessel. 10 mL concentrated HNO_3 and 0.10 mL HCL was added, and the trials were dissolved using a microwave power progressively rising to 500W for 40 min. The solutions were dissolved in 100 ml of water after freezing. The material was carried out by boiler with 12 ml of aqua regia for 40 min and then purified to dry condition. The dissolved 50 ml of water was used to add 25 ml of strong HCL and 2.5 ml of HNO_3 to the heated deposit.

4. Results and discussion

4.1. Electrical conductivity assay

Table 1 shows the urease assay's electric conductivity at various time intervals.

Pseudomonas otitidis (IS I) and Bacillus cereus (IS II) were chosen for usage in concrete due to their strong urease activity. The standard culture, *Bacillus megaterium (067) & Bacillus licheniformis (598)* (ICAR -National Dairy Research Institute, Karnal, India), was utilized in concrete to compare the findings with the isolated strains (Li. Q *et al.*, 2015). *B. megaterium and B. licheniformis* precipitated calcite and it is found that *megaterium* precipitated the most calcite (1.34g), followed by IS I, IS II, BM (067), and BL (598) NDRI. Karnal was both calcite precipitating calcite, which plugs micro fractures and endospores in concrete (Konopacka-Lyskawa, D.2019). IS 2 produced the smallest amount of calcite. IS 1, IS 2, BM(067), and BL(598) NDRI, Karnal are both calcite precipitating calcite, which plugs micro fractures and endospores in concrete. IS 2 produced the smallest amount of calcite.

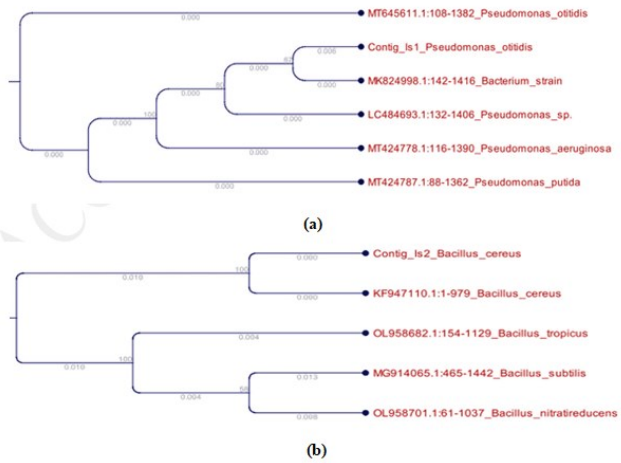


Figure 1. joining phylogenetic tree based on bacterial 16S rRNA sequence data along with sequences available in GenBank database for (a) Isolated Species 1 (b) Isolated Species 2

Table 1 Electric Conductivity Assay for Quantitative Urease

Time Intervals (mS/min)	Isolated Culture		Standard Culture from NDRI	
	Pseudomonas otitidis (IS I)	***Bacillus cereus (IS II)***	***Bacillus megaterium (067)***	***Bacillus licheniformis (598)***
0	138.6	122.8	300	320
350	140.5	136.4	315	340
3800	148.6	142.6	364	372
6688	152.6	147.2	380	388

Table 2 Toxic metal concentration levels by AAS "PPM"

	Toxic metal concentration levels "PPM"				
Sample ID	**Magnesium (Mg)**	**Iron (Fe)**	**Copper (Cu)**	**Lead (Pb)**	**Nickel (Ni)**
OPC -01	11.6	24.792	0.309	1.34	3.251
CFS -02	11.25	23.506	0.145	0.251	3.119
BCS -03	10.55	21.310	0.102	0.173	0.169
LSS -04	11.15	23.900	0.152	0.147	3.250

Figure 1 shows the BLAST result of the 16S rRNA sequence from the Isolated species (Raíssa *et al.*, 2016). Species were identified via sequencing techniques. High-scored (98.98%) bacterial strains such as *Pseudomonas otitidis strain HR-2(MT645611.1), Bacterium strain BS1810(MK824998.1), Pseudomonas aeruginosa strain XT211(MT424778.1), Pseudomonas putida strain TIMI1011(MT424787.1) and Pseudomonas sp. FJFR621(LC484693.1*). In this study, *Pseudomonas otitidis strain HR-2(MT645611.1) was used.* BLAST result of the 16SrRNA sequence from the Isolated species II. High-scored (98.98%) bacterial strains such as *B. cereus strain HKG201(KF947110.1), B. tropicus strain Tr5(OL958682.1), B. subtilis strain BKLC2(MG914065.1) and B. nitratireducens strain Tp8 (OL958701.1).* These two calcium-precipitating bacterial strains were used for our further research work.

4.2. Reduction of heavy-metal toxicity [AAS and ICP-MS]

The following Tables 2 and 3 illustrate the Toxic metal concentration levels of AAS and ICP-MS.

Table 3 Heavy metal concentrations by ICP-MS in $mgkg^{-1}d^{-1}$

Heavy metal concentrations "$mgkg^{-1}d^{-1}$"					
Sample ID	**Chromium (Cr)**	**Iron (Fe)**	**Cadmium (Cd)**	**Lead (Pb)**	**Nickel (Ni)**
OPC -01	35.00	2.14	1.252	8.96	23.40
CFS -02	29.12	1.98	0.293	7.14	18.74
BCS -03	24.14	0.98	0.124	6.94	19.18
LSS -04	6.56	1.24	0.942	6.00	21.22

Tables 2 and 3 show the Copper and Nickel concentrations of the OPC-01 were 0.309 and 3.251 ppm. The heavy metal concentration and sources of heavy metal were identified by using AAS analysis (Duan T., Liu, S., Wang, D. *et al.*, 2020). Magnesium and Iron were detected in all four samples (OPC-01, CFS-02, BCS-03, LSS-04) (Brykov *et al.*, 2013), (Khadanga, 2022) The standard sample OPC-01 had the following values. Iron is found in the highest concentrations with a value of 24.792 ppm compared to existing samples. The highest concentration level in Mg was identified as 11.6 ppm, while the lowest level is identified as 10.55 ppm in BCS-03. The concentration of lead was 1.34 ppm which is a major toxic metal present in the conventional sample (Ravipati E, Mahajan N., *et al.*, 2019). The Ni contents reported through ICP-MS had a concentration value of 23.40 $mgkg^{-1}d^{-1}$ and the lowermost value of 6.48$mgkg^{-1}d^{-1}$ found in sample OPC-01 compared with CFS-02, BCS-03, and LSS-04. For the Fe level in the samples, the uppermost value of 24.792 $mgkg^{-1}d^{-1}$ was found in the sample OPC-01 whereas the lowest value of 21.310 $mgkg^{-1}d^{-1}$ was in BCS-03. The toxic elements of Cd and Pb in the samples were noted at higher values of 1.252 $mgkg^{-1}d^{-1}$ and a lower value of 0.124 $mgkg^{-1}d^{-1}$ was found in BCS-03. (Almutairi and Helal, 2020). Heavy Metal Toxicity analyses of AAS and ICP-MS models are shown in Figure 2.

Figure 2. AAS and ICP – MS Models

5. Conclusion

Organisms were isolated from the microbial consortia due to their strong urease activity. 16S rRNA gene sequencing technique was used to identify individual species, and the two bacterial isolates were identified as *Pseudomonas otitidis and Bacillus cereus.* They were used for bio-concrete preparation. Studies on the optimization of the parameters in the production of bio-concrete could yield even better results. Heavy Metal Toxicity analyses such as AAS and ICP-MS were performed on the various concentration levels of heavy metals Magnesium (Mg), Iron (Fe), Copper (Cu), Lead (Pb), and Nickel (Ni) to reduce the toxicity using the isolated species. The microbial consortia were able to reduce the heavy metal toxicity of the concrete produced without compromising its strength. Future studies involve the comparison of consortia with standard cultures of *Bacillus megaterium (NDRI-067) and Bacillus licheniformis (NDRI-598)* used in standard bio concrete.

Conflicts of Interest

The authors declare that they have no known competing interests in the publication of this research work.

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