RNI: MAHENG/2016/68030 **VOLUME 8 (2023), ISSUE 6 : 48-54** **ISSN: 2456-5342 (print)**

MGM UNIVERSITY
JAWAHARLAL NEHRU ENGINEERING COLLEGE

VISTA INTERNATIONAL JOURNAL
ON ENERGY, ENVIRONMENT & ENGINEERING

Production of Biocement from microbes

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A B S T R A C T

Self-healing concrete is a promising new technology that has the potential to revolutionize the construction industry. This type of concrete contains bacteria that are able to precipitate calcium carbonate (CaCO3), which can seal cracks and heal the concrete. The use of self-healing concrete could significantly extend the lifespan of concrete structures and reduce the need for repairs.This paper reports on a study of self-healing concrete that was made using bacteria isolated from alkaline soil. The bacteria were able to precipitate CaCO3 in the presence of urea and calcium chloride. The amount of CaCO3 precipitated varied depending on the strain of bacteria. The researchers also found that the biocement produced by the bacteria was strong and durable.

The use of self-healing concrete has the potential to reduce the environmental impact of the construction industry. Biocement is a more eco-friendly alternative to traditional cement because it does not require the use of high-energy processes. Additionally, biocement can help to sequester carbon dioxide (CO2) from the atmosphere. Overall, this study shows that self-healing concrete is a promising new technology that has the potential to revolutionize the construction industry. The use of biocement could lead to more durable and sustainable concrete structures.

Keywords : *Microbially induced calcium carbonate precipitation (MICP), Self-healing concrete, Biocement, Eco-friendly construction material.*

1. Introduction

Microorganisms serve as a renewable source for numerous novel products with extensive applications in medicine, agriculture, and industrial settings. Therefore, it is crucial to harness their valuable bioproducts to address the ever-increasing challenges. Inspired by nature, scientists have developed self-healing concrete that relies on the metabolic bioactivity of specific bacteria [1]. The advantages of microbial bioactivity have been explored in the preservation of limestone monuments, particularly their ability to induce calcite precipitation under favorable environmental and nutritional conditions [2].

Interestingly, bacteria that induce calcite precipitation hold promise for self-healing concrete. They exhibit greater compatibility with the concrete matrix and are considered environmentally friendly [3-4]. Bacterial-based self-healing concrete has been investigated as a means to extend the service life of concrete structures [5]

Microbially induced calcium carbonate precipitation (MICP) is a process that occurs when microorganisms, primarily bacteria, are provided with suitable substrates, leading to the formation of calcium carbonate ($CaCO_3$) crystals. The $CaCO_3$ formed plays a crucial role in coating surfaces and binding different particles together. MICP can occur under atmospheric pressure and other mild conditions, and it is a natural phenomenon that takes place extensively around the world. This process has coated the surfaces of various natural structures, leaving behind clues about past eras for researchers to uncover. The formation of $CaCO_3$ by microorganisms has been studied through biomimetic approaches and subsequently applied in diverse fields such as construction, environmental protection, geotechnical engineering, and nanotechnology. This $CaCO_3$ precipitation can seal micro-cracks in concrete, extending its service life and reducing the need for maintenance and repair [6-7].

The $CaCO_3$ precipitation during microbial metabolic processes offers several benefits. Utilizing calcifying microorganisms to enhance the mechanical properties of cement allows these microbes to heal cracks from within, resulting in homogeneous repaired materials. Moreover, the biological approach is often more environmentally friendly as it does not require the use of toxic chemicals or high energy consumption, unlike traditional chemical and physical approaches [3].

The present investigation on production of biocement from microbeswill be conducted with following objectives.

a) Isolate and characterize calcite-precipitating bacteria from alkaline soil samples.
b) Optimize the conditions for biocement production using selected calcite-precipitating bacteria.
c) Optimize biocement production using bacteria.
d) Characterize or assess mechanical properties of biocement produced by selected calcite-precipitating bacteria

2. Literature of review:

Numerous studies have demonstrated the effectiveness of MICP in improving the properties of concrete. Some bacterial species, mainly of the genus Bacillus, can induce the precipitation of calcium carbonate ($CaCO_3$) through direct precipitation or ureolytic decomposition of calcium-containing compounds [8].Microalgae, such as Spirulina, Arthrospira *plantensis* (Cyanophyta), *Chlorella* vulgaris (Chlorophyta), Dunaliellasalina, Haematococcus *pluvialis*, Muriellopsis sp., and Porphyridium *cruentum* (Rhodophyta), are also promising candidates for biocement production due to their photosynthetic metabolism [9].

The efficiency of the MICP process is influenced by bacterial concentration, ureolytic activity, and environmental conditions such as urea concentration, pH, and temperature [7].

MICP has the potential for various applications in the construction industry, including soil stabilization, healing of concrete cracks, restoration of limestone surfaces, preventing soil erosions, and treatment of industrial wastewater and removing heavy metals [5].

MICP is a promising technology that can provide sustainable and eco-friendly solutions for enhancing the durability, self-healing capabilities, and environmental performance of concrete structures [10].

3. Methodology:

3.1 Selection and cultivation of calcite producing bacteria from soil:-

1 gm of alkaline soil sample was taken from 10 cm. depth from earth surface. The sample was serially diluted upto 10-8 in 0.85% of NaCl solution and spreadonto the LB agar media (pH was adjusted to 9.5 using NaOH) and kept it toincubate at 37°C for 48 hrs. After 2 days of incubation, colonies were grown and pick up

the desiredcolonies from LB agar plate based on their colour of colonies. Then further selections of desired colonies from different colonies were followedby gram staining.Grams staining of colonies were showed that out of 10 colonies, 8 were rodshape bacilli and 2 were cocci. These colonies are subjected in LB broth for calcite precipitation.Out of those 8 bacterial strains, 3 strains were shown precipitation in LB brothwith urea and calcium chloride. Further identification of those 3 strains was done by different biochemical testslike Urease, Catalase, Citrate, MR and endosperm staining. All 3 strains were positive for Urease, Catalase and Citrate and also sporeforming. These strains have fulfilled the conditions for bio-mineralization (Calcite Precipitation i.e. $CaCO_3$).

3.2 Preparation of biocement mixture:

The next step is to prepare the biocement mixture.

200 mL of LB broth which contains 2 % of urea and calcium chloride was preparedin three different conical and inoculated with three selected bacterial strains.These mixtures were kept for incubation in shaking incubator in 37°C at 150 rpm for 10 – 15 days.After 15 days the white crystalline precipitation was observed at the bottom whichwas the final product.

3.3 Harvesting:

Harvesting was done by the filtration of broth with Whatman filter paper and the filtered white precipitate was dried at 60°C in hot air oven.

3.4 Chemical verification of precipitated calcite:

Precipitated calcite was verified by chemical methods. Acetic acid was addedin dried calcite powder under fume hood and observed for effervescence as acetic acid down into carbonic acid which further releases CO2. Calcite powder was boiled and added in HCl, as HCl dissolves calcite powder

4. Results and discussions :

4.1 Results :

The morphological characteristic and the biochemical result of the isolated 10 different Bacillus species from soil as shown in below Table 1.

Table 1 Morphological characteristic of isolated Bacillusspp.

Strain No.	Colony morphology	Colony shape	Gram staining
1	White, irregular	Bacilli	Positive
2	White, round	Cocci	Positive
3	White, circular	Bacilli	Positive
4	White, irregular	Cocci	Positive
5	White, irregular	Bacilli	Positive
6	White, irregular	Cocci	Positive
7	White, irregular	Bacilli	Positive
8	White, round	Bacilli	Positive
9	White, irregular	Cocci	Negative
10	White, circular	Bacilli	Positive

Table 2 Biochemical and Morphological characteristic of isolated Bacillus spp.

Strain No.	Biochemical Analysis			
	Urease	**Catalase**	**Citrate**	**Methyl Red**
1	Positive	Positive	Positive	Negative
3	Positive	Positive	Positive	Negative
10	Positive	Positive	Positive	Negative

4.1.1 Precipitation of biocement:

Table 3.Strains 1, 3, and 10 were selected for precipitation, which exhibited high urease activity.

Strain No.	Quantity of precipitation (in g.)
1	≈0.341
3	≈1.117
10	≈0.063

4.1.2 Biochemical analysis and characteristics of calcite precipitating Bacillus species,

Fig.1 Urease test

Fig.2 Methyl red test

Fg.3 Citrate test

Fig.4 Catalase test

Fig.5 Filtration of preciptatedbiocement.

Fig.6 Morphology of precipitated biocement

Fig.7 Effervences after addition of acetic acid and HCl

4.2 Discussions :

The self-healing ability of concrete has been demonstrated by certain bacterial species, primarily those belonging to the genus Bacillus. This remarkable phenomenon is attributed to the precipitation of calcium carbonate ($CaCO_3$), which occurs either through direct precipitation or ureolytic decomposition of calcium-containing compounds. This process effectively seals micro-cracks, restoring the integrity and durability of concrete structures. In this comprehensive review, we delve into the intricacies of microbially induced calcium carbonate precipitation (MICP), unraveling its mechanisms and applications.

The MICP process hinges on the pivotal role of urease, an enzyme produced by a diverse range of microorganisms. These urease-producing microorganisms efficiently hydrolyze urea, a compound commonly present in concrete mixtures. The hydrolysis of urea liberates ammonium ions (NH^{4+}) and carbonate ions (CO_3^{-2}), which combine to form calcium carbonate ($CaCO_3$) in the presence of calcium ions (Ca^{2+}). This $CaCO_3$ precipitation leads to the formation of calcite crystals, which fill and seal micro-cracks in concrete.

MICP presents a promising, eco-friendly alternative to conventional remediation technologies employed to address environmental challenges in diverse fields. Unlike traditional methods that often involve the use of harsh chemicals and energy-intensive processes, MICP harnesses the natural metabolic processes of microorganisms to achieve its remediation goals. This environmentally conscious approach aligns with the growing global movement towards sustainable practices.

The versatility of MICP extends to a wide range of applications, encompassing the removal of heavy metals and radio nuclides from contaminated environments, the enhancement of construction materials by promoting their durability and strength, and the sequestration of atmospheric CO_2 through bio-mineralization processes. These applications underscore the transformative potential of MICP in addressing environmental concerns and promoting sustainable practices.

In conclusion, MICP emerges as a ground breaking technology with the potential to revolutionize various fields. Its ability to self-heal concrete structures, remediates contaminated environments, and enhance construction materials highlights its versatility and effectiveness. As we strive towards a more sustainable future, MICP stands poised to play a pivotal role in safeguarding our environment and ensuring the longevity of our infrastructure.

5. Conclusions:

Microorganisms can be used to produce a strong and renewable building material with minimal impact on the environment. This material, called biocement, is made by stimulating native soil bacteria to connect soil particles through a process called microbially induced calcite precipitation (MICP). Biocement can be used to remediate cracks in building materials, rocks of different strengths development and regain strength within a month. It also enhances the durability of bricks by increasing compressive strength and reducing their permeability. Additionally, biocements and biogrouts are used mainly for geotechnical works such as soil strengthening, sealing, desaturation, and soil particles aggregation.

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