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Improvement the characteristics of recycled concrete by isolation and addition *Alkalibacterium iburiense* as abioconcrete bacteria

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ABSTRACT

This research aims to isolate and identify calcite-precipitating bacteria and investigate whether they can be used in concrete to enhance its mechanical qualities and self-healing capabilities. Microbial-induced precipitation of calcium carbonate is a new technique for making cement concrete stronger. The present study aims to compare cement concrete's compressive and split-tensile strengths to those of conventional concrete to examine the possible use of alkaliphilic bacteria to improve its qualities and ability to self-repair hairline cracks in concrete. Through conducting experiments on concrete samples at ages 7, 28, and 56 days, to which the isolated bacteria were added and characterized at the molecular level using the AccuPrep Genomic DNA Extraction kit, amplified, and subjected to agar gel electrophoresis, the sequences were obtained and compared with those in the GenBank database using the BLAST tool in the NCBI-GenBank database. Using PCR and scanning electron microscopy, it was confirmed that the isolated alkaline bacteria had a 99.69% identity rate. The bacteria *Alkalibacterium iburiense* were used at different concentrations of $10^5$and 10^8. Additionally, 2% of recycled coarse aggregate and 10% & 20% was used. It was found that the concrete properties were improved. It was determined that the optimum improvement in mechanical properties was with the addition of bacteria at a concentration of 10^8 and a total recycled aggregate ratio of 10% after 56 days. The compressive, tensile, and flexural strengths increased by 25.75%, 17.27%, and 19.4%, respectively.

Keywords: bacterial concrete, isolated alkaliphilic bacteria, *Alkalibacterium iburiense,* Improving the mechanical properties of concrete, self-heal concrete.

INTRODUCTION

Concrete is a vital building element that serves as the structural foundation for numerous projects worldwide (Ranade *et al.*, 2016; Li, Liu and Guan, 2021; Ding, 2024) Nevertheless, the extended utilization of it unavoidably results in the formation of numerous minuscule cracks (Guo *et al.*, 2021). If not addressed, these cracks have the capacity to gradually expand, posing an increasing risk to the structural stability of structures (Zhang and Li, 2004; Bojórquez *et al.*, 2021).The presence of cracks in concrete is a natural result of environmental conditions, load pressures, and diverse external forces (Şahmaran and Li, 2010; Munawar *et al.*, 2022). Over time, these cracks might undermine the resilience and security of structures, necessitating immediate attention to this matter (Golewski, 2023)

Self-healing concrete is cleverly engineered to fix cracks automatically, reducing the need for extensive physical interventions (Valença *et al.*, 2017). The reason for the creation of this groundbreaking material is its ability to transform the construction sector by substantially decreasing upkeep expenses and improving the durability of the building (Gardner *et al.*, 2018). By incorporating self-healing processes into the concrete matrix, the requirement for heavy human involvement and protracted repair durations can be significantly reduced, thus making a valuable contribution to improved structural durability (Schlangen and Sangadji, 2013).

The utilization of recycled concrete aggregates (RCA) has become a significant method for increasing sustainability in the building field. However, using RCA often results in decreased concrete strength and durability due to the presence of contaminants, old mortar, and the physical and chemical homogeneity of recycled materials.

Recent developments in bio-concrete, or self-healing concrete, provided an innovative solution for solving these issues, improving the performance of concrete created from recycled aggregates. Adding bacteria that can improve the characteristics of concrete is an intriguing strategy in this study after *Alkalibacterium iburiense* was isolated. Because of its capacity to precipitate calcium carbonate ($CaCO_3$) in alkaline environments, which are prevalent in concrete, this bacterium has been found to be a promising candidate for use in bio concrete applications.

Among the bacteria used in self-healing concrete are alkaliphilic bacteria isolated from soils near bridges. In addition to soil, alkaliphilic bacteria can be found in saltwater, guts of insects, deep-sea environments, and artificial settings (Thongaram et al., 2003). One of the microorganisms known to treat concrete cracks is the bacterium *Alkalibacterium iburiense,* which is characterized by its straight rods, Gram-positive, and colonies that are circular, convex, and pale white. It is obligately alkaliphilic with growth at pH 9–12 and an optimal pH 9.5–10.5. Halotolerant that grows best at 3–13% NaCl and distinguished by its cells that grow aerobically and anaerobically.

RESEARCH OBJECTIVE

Improve the mechanical properties of the concrete by increasing calcium carbonate ($CaCO_3$) precipitation through specific microbial metabolic activity. by isolating *Alkalibacterium* (*Alkalibacterium iburiense*) and incorporating it into concrete.

Enhancement of the mechanical characteristics of concrete at varied bacterial concentrations, various recycled coarse aggregate ratios, and at various ages.

Self-healing Concrete by calcium carbonate precipitate fills the pores and repairs the cracks in the concrete.

MATERIALS AND METHODS

Soil samples were collected from ten different nearby locations in Baghdad for the purpose of isolating alkaline bacteria, at a rate of 50 gm for each sample. The soil samples were placed in sterile zip lock bags, then transferred to the laboratory and placed in the refrigerator until the isolation and diagnosis process was carried out. The modified medium was used, consisting of Nutrient broth medium sterilized using an autoclave at 121 °C for 15 minutes. Urea was prepared by dissolving 100 gm urea in 500 ml distilled water and sterilized by filtration (membrane filter 0.45 µm) because urea decomposes using autoclave. Then 25 ml urea solution was added to 25 ml Nutrient both and 2% Agar medium (Ezzat and Ewida, 2021) was added when needed for solidification. The solidified medium was sterilized using an autoclave at a temperature of 121 °C and a pressure of 1.5 psi for 20 minutes and the pH was adjusted to 9. A series of soil dilutions were prepared by suspending 10 g of soil sample in 90 ml of peptone water (prepared at a concentration of 0.1% peptone).

The soil was mixed with peptone water and then the soil was left to settle. A series of decimal dilutions of the soil filtrate was made as shown in

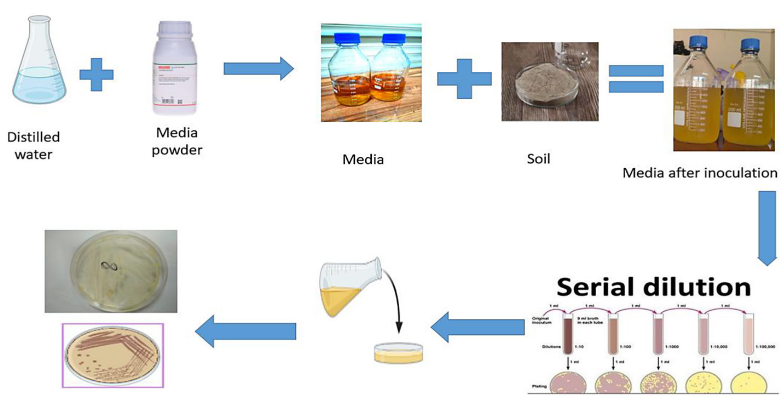

Figure 1. Isolation of bacteria

Figure 1. Spread 501 ml of decimal dilutions onto plates containing Urea agar medium.

The plates were incubated at 37 °C for 24–48 hours. The plates were examined during the incubation period and single colonies were selected. These colonies were re-sprouted on the surface of Urea agar medium in a streaking manner. The cultural properties of the bacteria and their response to Gram stain were studied.

Diagnosis of isolated bacteria

Cultural characteristics – the phenotypic characteristics of colonies of bacterial isolates grown on Urea agar media were recorded in terms of colony shape.

Microscopic examination – microscopic examination of isolated and grown bacterial colonies was performed after 18 hours of growth, and staining was performed with Gram stain to identify the shape and type of bacterial cells in terms of their reaction to Gram stain and examination under a light microscope.

Genetic diagnosis – the isolate was extracted using the Genomic DNA Extraction kit, amplified, and subjected to agarose gel electrophoresis. The NCBI-GenBank database's BLAST tool was then used to obtain the bacterial sequence and compare it with those in the GenBank database. The bacteria were cast into the dishes and returned colony/ml unit; the concentrations of solution bacteria were 10^5 and 10^8 cells/ml (Tawfeeq and Ahmaed, 2023).

PCR products were sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer, by Macrogen Corporation – Korea. The results were received by email and then analyzed using geneious software.

The mixing and testing for bio-concrete

Cement, fine aggregate, coarse aggregate, recycled coarse aggregate (with two percentages, 10% & 20%), and media (with two concentrations 10^5&10^8 cells /ml bacteria *Alkalibacterium iburiense*) instead of water are the main materials used to make concrete mixes. The weights of the essential components: 380 kg/m³ of Cement, 787 kg/m³ of fine aggregate, 1024 kg/m³ of coarse aggregate and media (165 L/m³) (Figure 2).

The concrete samples were tested for compressive strength using 150 × 150 × 150 mm cubes, tensile strength using cylinders with a height of 200 mm and a diameter of 100 mm, and flexural strength using 100 × 100 × 400 mm beams. These were examined at ages 7, 28, and 56 days.

One non-destructive testing (NDT) method for determining how quickly ultrasonic waves pass through a material is ultrasonic pulse velocity (UPV). Concrete and other building materials are frequently evaluated for quality and integrity, and their mechanical qualities are estimated.

SEM test was conducted during 24–48 hours from culturing bacteria to observe the bacterial aggregation after 48 hours at a temperature of 37 °C To see how bacteria affected the concrete structure, a SEM analysis was also performed on two concrete samples – one containing bacteria and the other not – 28 days after casting.

An SEM test was conducted to observe the bacterial aggregation after 48 hours at a temperature of 37 °C. A very small amount of the sample was withdrawn and coated with a layer of gold, and the SEM device was used at several magnifications (from 250 to 120,000 times).

Figure 2. Components of concrete mix

RESULTS AND DISCUSSION

Cultural characteristics:

The process of isolating any microorganism depends on the selective medium used to isolate the bacteria initially. Eight isolates appeared as pale white colonies, and the colony shape was circular and convex on the surface of the Urea agar medium when grown at a temperature of 37 °C for 48 hours. This result was consistent with what was mentioned by (Sato *et al.*, 2016) about the cultural properties of Alkalibacter isolates, as urea provides a selective pressure to ensure the exclusion of other species.

Microscopic characteristics:

Microscopic examinations showed that the bacteria have Gram-positive cells in the form of single, motile rods (Figure 3), which is consistent with what was mentioned by (Nakajima *et al.*, 2005) about the microscopic characteristics of *Alkalibacterium* bacteria.

Molecular diagnosis

The obtained isolate was genetically identified based on the 165rRNA gene. The genetic material (DNA) of the isolate was extracted and its purity was confirmed, as it showed the presence of a single clear molecule, indicating the association of the primers with the desired gene, as presented in Figure 4. The molecular size of the amplification results was estimated as (1500) base pairs.

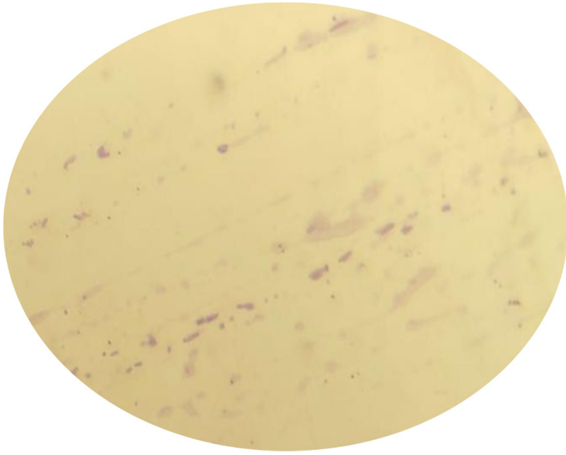

Figure 3. Gram staining of the cell morphology for bacterium *Alkalibacterium* sp.

IMPACTS OF INTEGRATING ALKALIBACTER INTO CONCRETE

Upon adding media to the mixture after isolation, the *Alkalibacterium iburiense* bacteria, in addition to their self-healing effectiveness, improved the qualities of concrete, particularly its compressive, splitting, and flexural strength.

Compressive strength test

Bio concrete's compressive strength, while variable depending on the type of bacteria, shows promise for sustainable construction applications. Ongoing research aims to enhance strength. The effect of bacterial content on the compressive strength of concrete mixes incorporating coarse

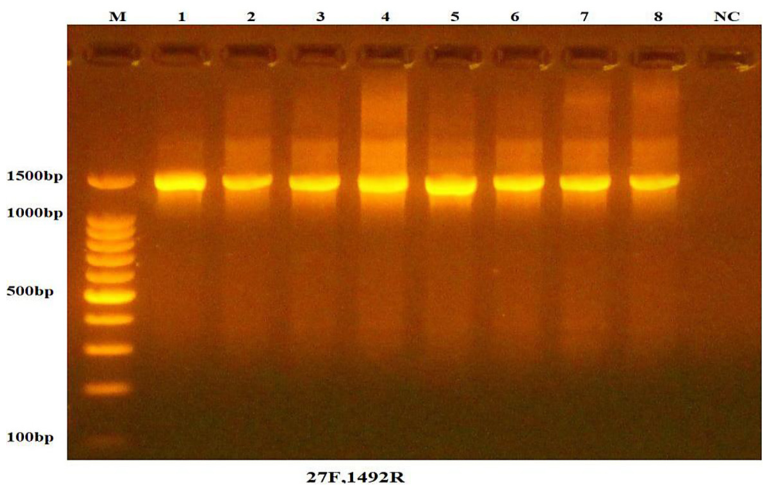

Figure 4. Results of the amplification of *16s RNA gene* of Unknown bacterial species were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker.
Lanes 1–8 resemble 1500bp PCR products

recycled aggregate for all ages is shown in Figures 3 and 4. For concrete samples with two different bacterial concentrations and two different percentages of coarse recycled aggregate, the percentage of improvements in compressive strength relative to the control specimen are 5.2–8.8%, 12.9–18.66%, and 18.82–25.75% for ages 7, 28, and 56 days, in concentrations 10^5 and 10^8 respectively and two percentages coarse recycled aggregate 10% and 20% as shown in Figure 5.

This increase in strength is caused by the biological mineralization. process, in which bacteria assists in the precipitation of calcium carbonate, which fills in the concrete's holes and cracks and leads to a denser, more durable material (Chahal et al., 2012a, 2012b).

Split-tensile strength

Figure 6 shows the split-tensile strength of the concrete specimens, which were treated with bacteria. It is apparent that the split-tensile strength of the bio-cured concrete is higher than that of the traditional concrete specimens. It shows how bacterial content affects the split-tensile strength of concrete mixtures containing coarse recycled aggregate at various ages. For concrete samples containing two different bacterial concentrations and two different percentages of coarse recycled aggregate, the split-tensile strength gains relative to the control specimen are. 3.2–6.2%, 6.2–11.42%, and 12.82–17.27 at ages 7, 28, and 56 days displayed in Figure 7. Because of the substantial activity of bacterial concrete for 56 days, in concentrations of 10^5 & 10^8, respectively, and two percentages of coarse recycled aggregate, 10% & 20% men, calcium carbonate precipitation was biochemically produced, increasing the load-resisting ability (Hussein *et al.*, 2019; Banchhor *et al.*, 2025)

Flexural -tensile strength

Concrete flexural testing measures the flexural strength and hardness of concrete beams. The test consists of applying a load to the beam until failure, then measuring the load and

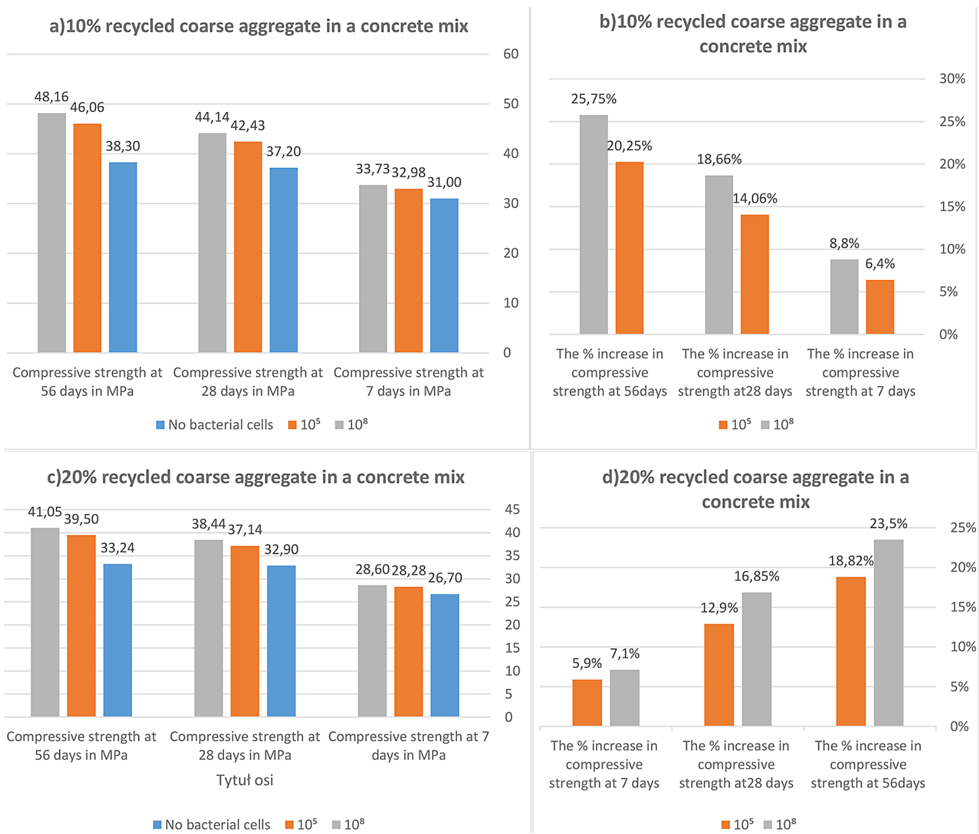

Figure 5. Results of compressive strength of concrete mixtures containing 10% and 20% recycled coarse aggregate where: (a) The value of compressive strength (with 10% recycled), (b) the percentage improvement in compressive strength compared to the mix without bacteria (with 10% recycled), (c) the value of compressive strength (with 20% recycled), (d) the percentage improvement in compressive strength compared to the mix without bacteria (with 20% recycled)

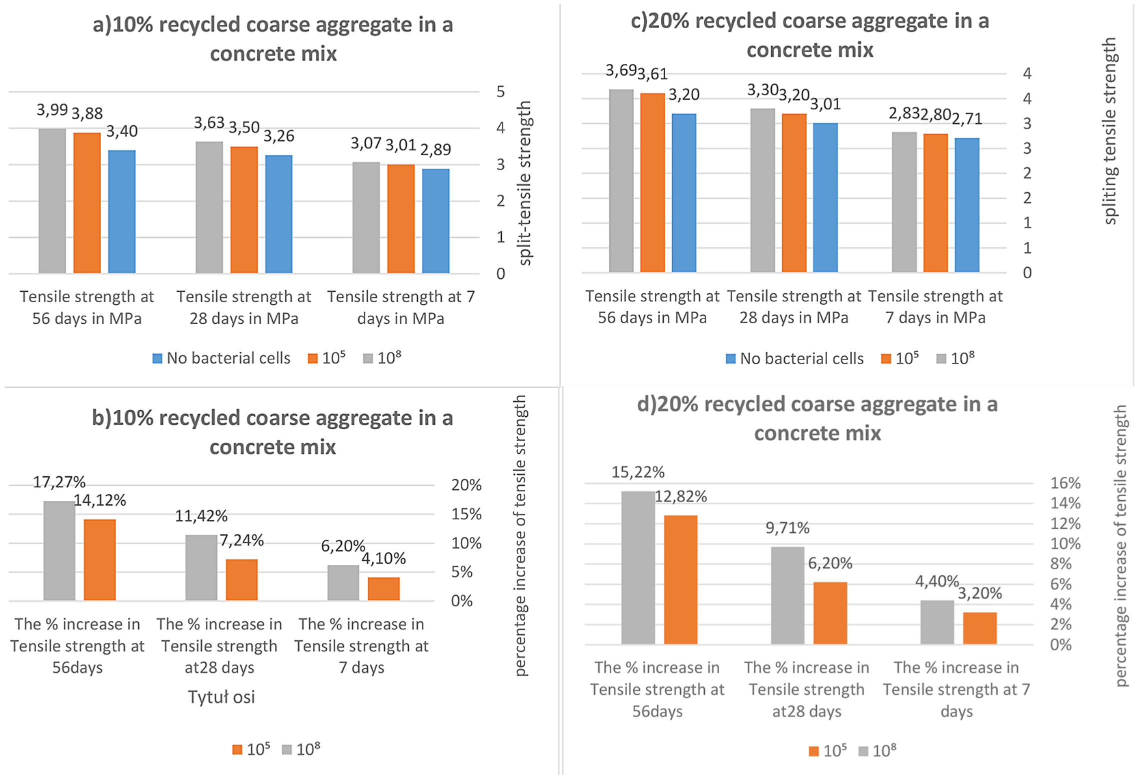

Figure 6. The results of split-tensile test were: (a) the value of split-tensile strength (with 10% recycled), (b) the percentage improvement in split-tensile strength compared to the mix without bacteria (with 10% recycled), d) value of split-tensile strength (with 20% recycled), b) Percentage improvement in split-tensile strength compared to the mix without bacteria (with 20% recycled)

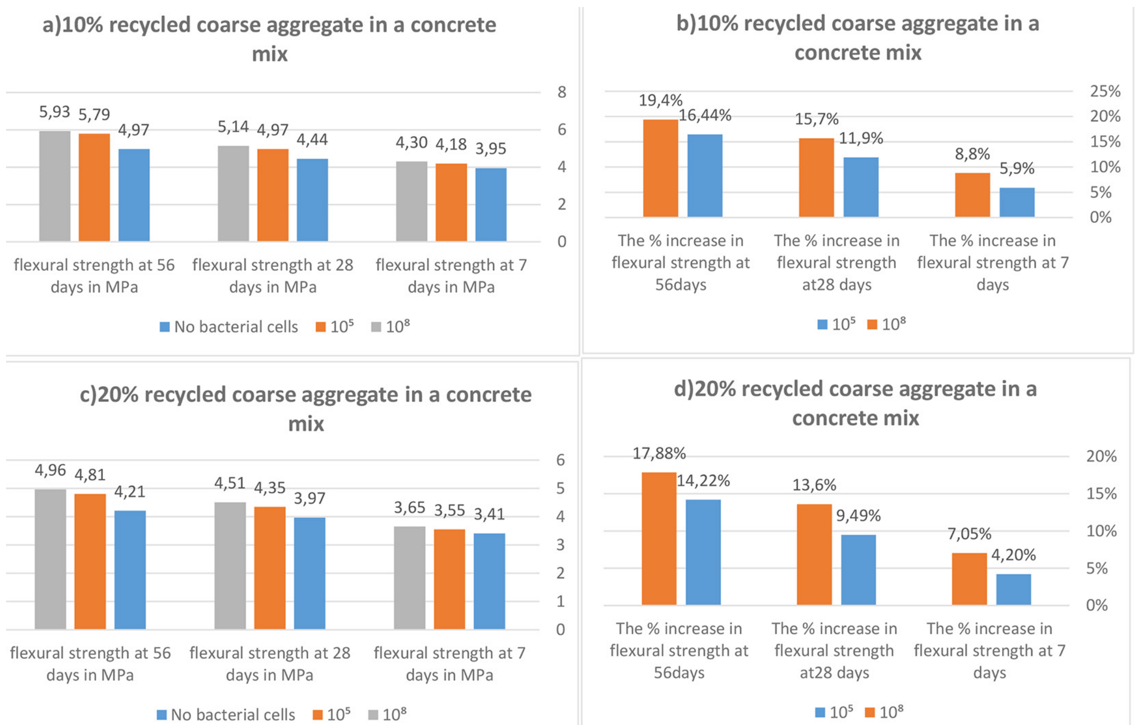

Figure 7. The results of flexural test were: a) the value of flexural strength (with 10% recycled), b) the percentage improvement in flexural strength compared to the mix without bacteria (with 10% recycled), d) value of flexural strength (with 20% recycled), b) percentage improvement in flexural strength compared to the mix without bacteria (with 20% recycled)

deflection (ASTM C78, 2019). The addition of microorganisms into concrete has emerged as a unique way to enhance its durability and mechanical qualities. Specifically, the addition of bacteria has been shown to improve the flexural strength of concrete, as displayed in the figure. According to research, the bacteria can use a mechanism called microbial induced calcite precipitation (MICP) to create calcite, a naturally occurring cementitious material (De Muynck et al., 2008). This calcite production can increase the concrete's mechanical qualities, such as its flexural strength, by filling in its cracks and pores. The flexural strength of concrete can be increased by up to 25% by adding bacteria, according to research (Jonkers et al., 2010; Yan et al., 1999). The enhanced microstructure and decreased porosity of the concrete are responsible for this improvement.

Ultrasonic pulse velocity test

To ensure the effectiveness of bacteria in the self-healing process of concrete, an ultra-test was conducted. Cubes measuring 150 × 150 × 150 mm were cast, and after 28 days, a load was applied to create a crack. The ultra test was performed before and after applying the load to confirm the occurrence of the crack. The model was left for another 28 days (making it 56 days old), and the test was conducted again. It was observed that the speed had increased, indicating that the crack had reduced and the self-healing process had occurred (Raut et al., 2019) (Figure 8).

Scanning electron microscope

Analysis of scanning electron microscopy (SEM) was performed, to determine the fundamental differences between the microstructures of control concrete and bacterial concrete that contains *Alkalibacterium*. Samples were extracted from both bacterial and non-bacterial concrete specimens in each category. To conveniently understand bio-mineral deposits, SEM imaging is being done.

SEM images revealed that the bacteria *Alkalibacterium iburiense*, as rod-shaped cells of size, were dispersed within the sample as shown in Figure 9. Finer particles, presumably sand, were found. The results confirmed that most of the bacterial cells were able to survive and form within the sample of concrete. Cracks and voids were also observed within the concrete of the untreated concrete while the cracks contained small, clumped spores of *Alkalibacterium iburiense* embedded within the cement matrix.

SEM confirmed that the inclusion of bacteria can enhance the microstructure of bio concrete through calcite precipitation. Nasser *et al.* (2022), verified that the SEM clearly showed that the treated samples had concrete cavities filled in. The calcite crystals were rhomboidal and needle-like. By filling the mortar matrix's pores, calcite improves the mortar's impermeability and micro crack sealing (Siddique *et al.*, 2016).

As a result of the deposition of $CaCO_3$, a product of bacterial metabolic processes (Lee and Park, 2018), it has been shown in the bio-concrete experiments that all concrete mixtures improved in varying proportions (Nightingale

Figure 8. Ultrasonic pulse velocity test

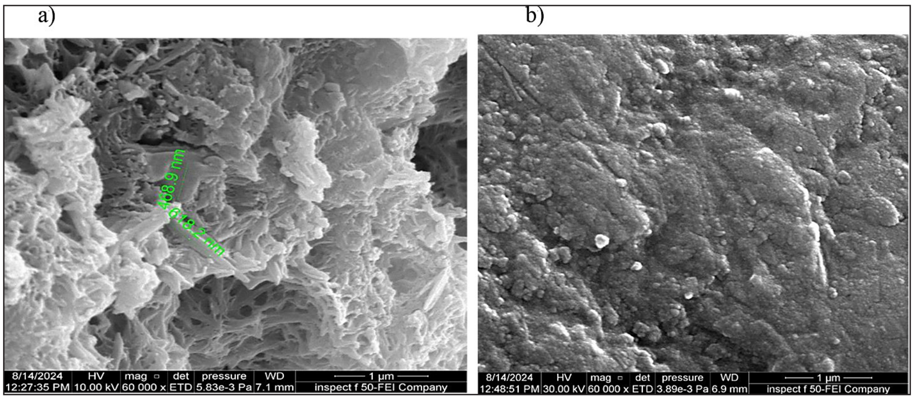

Figure 9. The scanning electron microspore images (SEM) of the concrete: (a) with *Alkalibacterium,* (b) without *Alkalibacterium*

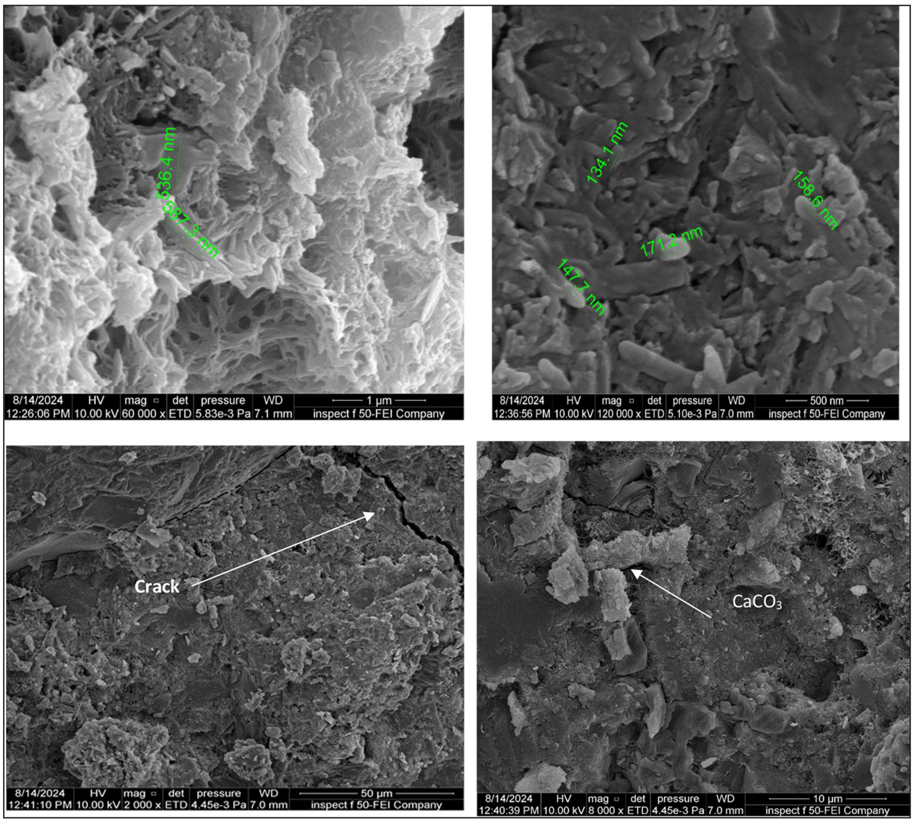

Figure 10. SEM images of control (A, A′) and bacterial of *Alkalibacterium iburiense* samples at 120000x and showing elements of $CaCO_3$ after 30 days

et al., 2018) and are deemed good; their strength (compressive, tensile, and flexural) improved, and cracks were healing (Figure 10). Since bacteria are categorized as harmless and are simple to extract and add to concrete, this process is seen as both environmentally beneficial and safe from a health perspective. In the majority of the world's countries, the method of using microorganisms as a concrete repair and improver is constantly developing.

CONCLUSIONS

Bacterial direct addition led to greatly increasing the mechanical properties (compressive strength, splitting tensile strength and flexural strength. The percentage improvement in strength over the reference mix varies based on the percentage of coarse recycled aggregate, the age of the concrete, and the quantity of bacteria. Practical experiments demonstrated that a combination comprising 10% CRA produced greater improvement results than a mix containing 20% CRA. In terms of concrete mix age, 56 days provided the highest improvement rate, followed by 28 days and finally 7 days. As a result, the older the concrete, the higher the improvement rate. In terms of bacterium concentration, it was discovered that 10^8 cell/ml was more effective than 10^5 cell/ml.

It was also noted that the self-healing process of concrete occurred through results of SEM examination and ultrasound tests. The healing of concrete is attributed to the deposition of $CaCO_3$ and the closing of cracks through the metabolic process of bacteria.

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