

# BACTERIALLY INDUCED CALCITE FORMATION AT THE SURFACE OF RECYCLED CONCRETE

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**ABSTRACT.** The construction industry is one of the main sources of greenhouse gas emissions, and portland cement production is responsible for approximately 8% of anthropogenic CO<sub>2</sub> emissions. Microbially induced calcium precipitation (MICP) has the potential to partially replace cement or modify the properties of materials that would otherwise not find use in construction, for example, in concrete recycling. MICP might be an environmentally friendly method to improve the properties of recycled aggregates and form conglomerates from the finest fractions. In this paper, factors influencing MICP's ability to solidify recycled concrete fines are thoroughly investigated. Calcium carbonate precipitate crystals produced by the bacterium *Sporosarcina pasteurii* were analyzed using scanning electron microscopy and energy dispersive spectroscopy.

**KEYWORDS:** Bacteria, MICP, recycled fine aggregate, recycled concrete, material properties.

## 1. INTRODUCTION

Concrete is one of the most widely used building materials. It produces significant amounts of greenhouse gas emissions and consumes a lot of natural resources, especially water [1]. And the use of concrete continues to grow. This calls for immediate solutions. One way to make concrete a more environmentally friendly material is to include recycled materials in its production. However, recycled materials must be used carefully in concrete. Their use can lead to poorer properties and a decrease in the durability of the concrete. This can devalue environmental benefits [2]. The use of recycled aggregates can affect the properties of the final concrete, such as porosity, density, and durability. These differences are attributed to cement mortar that adheres to natural aggregate particles and forms the aggregate itself [3].

The production of cement is a complex process that consumes a significant amount of raw materials and resources and has a negative impact on the environment [4]. The path to reducing the environmental impact of concrete is challenging. The use of other alternative materials that consume less natural resources, are economical and have the potential to act as a binder, would contribute to a more sustainable and healthy environment [5].

Microbially induced calcium carbonate precipitation (MICP) offers a possible solution. Recycled concrete can be modified with MICP to improve its properties for other possible applications, such as aggregate for concrete or biobrick production.

## 2. USE OF BACTERIA IN ENGINEERING APPLICATIONS

### 2.1. MICP PRINCIPLE

Microbially induced carbonate precipitation (MICP) is the collective name for the formation of calcium carbonate minerals, which is produced by the interaction between various metabolic by-products of bacteria and calcium ions [6]. In this process, calcium carbonate serves as both a binder and a filler [7]. In this way, bacteria produce calcium carbonate in various environments around the world, such as soil, groundwater, etc. MICP can be used for engineering applications. Therefore, it is necessary to understand the behavior and life of bacteria.

The calcium carbonate produced by bacteria is produced by its metabolic processes. Each group of bacteria has different metabolic processes. The useful microorganisms in engineering applications use heterotrophic metabolisms and depend on organic carbon sources for their growth [8]. The nitrogen cycle is the most used pathway to achieve calcium carbonate precipitation using bacteria and microorganisms. It involves bacteria using ammonification of amino acids, nitrogen reduction (denitrification), or hydrolysis of urea (ureolysis) [8].

Ureolysis is clearly the most widely used metabolic pathway [9]. In this process, bacteria produce the enzyme urease, which then participates in the hydrolysis of urea CO(NH<sub>2</sub>)<sub>2</sub> to form ammonia NH<sub>3</sub> and carbonic acid H<sub>2</sub>CO<sub>3</sub>. Urea serves as a source of nitrogen [10]. Ammonia and carbonic acid further react with water to form positive ammonium ions NH<sub>4</sub><sup>+</sup> and negative carbonate ions CO<sub>3</sub><sup>2-</sup>. Production of ammonium ions

creates an alkaline environment that promotes precipitation of carbonate. Positively charged calcium ions  $\text{Ca}^{2+}$  bind to the negatively charged bacterial cell wall [8]. As the number of calcium carbonate crystals increases, the cell gradually encapsulates. This causes the bacteria to form a carbonate capsule around itself.

Most ureolytic bacteria are heterotrophic aerobic bacteria and prefer an alkaline environment for their growth [11]. This is beneficial for use in concrete materials that have alkaline conditions within their structure. The use of *Bacillus* species bacteria [12], which are commonly found in soil [13], has proven to be suitable. However, *Sporosarcina pasteurii* has the maximum ureolytic activity, a higher mineral precipitation rate, and rapid growth and is the bacterium most widely used and studied so far [9]. *Sporosarcina pasteurii* is nonpathogenic, highly stable, has great urease activity, and is cost-effective [12]. The frequency of use of ureolytic bacteria is due to their large distribution. They are found in soils and groundwater all over the world [9]. *Sporosarcina pasteurii* is also used in this work.

## 2.2. INFLUENCING FACTORS FOR MICP

The amount of calcium carbonate precipitated depends on the type of bacteria, their activity and metabolism [14]. During ureolysis, bacteria produce the enzyme urease, which accelerates the hydrolysis of urea. Therefore, bacteria with high ureolytic activity are selected for MICP. These are bacterial strains of the *Bacillus* strain or *Sporosarcina pasteurii* [15]. Carbonate precipitation is then carried out by hydrolysis of urea to ammonia and  $\text{CO}_2$  [15]. Different types of bacteria can produce different amounts of urease and precipitate calcium carbonate at different speeds [9].

High concentrations of bacteria ( $10^6$  to  $10^8$  cells) increase the amount of  $\text{CaCO}_3$  precipitated. The bacterial surface serves as a nucleation site for precipitation of  $\text{CaCO}_3$  [9]. However, concentrations of bacteria that are too high lead to a rapid and excessive reaction, followed by blocking the surface pores and poor distribution of the bacterial solution in the material, or even disrupting the material due to internal stresses. Therefore, it is always necessary to find the optimum bacterial concentration for each specific application.

The concrete structure is a highly alkaline environment with a pH of 12–13. This does not create very suitable conditions for bacteria, and they remain in the form of spores with such a high pH [16]. The optimal pH value for urease and bacteria is around 8. But the pH range for its activity is larger. A pH of 7–10 has been reported [9]. If the concrete is disturbed (microcracks, pores), the pH of the surrounding area will drop. This drop causes bacterial spores to wake up and begin their activity [16].

Bacteria also need nutrients for their growth, life, and metabolic activities [12]. A sufficient concentration of nutrients provides bacteria with a source of

energy [15]. Heterotrophic organisms need a source of carbon and nitrogen. Consumption of organic acids affects the precipitation of minerals [15]. Ureolysis uses urea as a source of carbon, nitrogen, and energy [9], but peptone, yeast extract, or calcium lactate can be used as an alternative and cheaper carbon source [13, 15].

The microstructural properties, morphology, regularity, and durability of the final material formed by MICP are also influenced by the source of free calcium ions in the surrounding area of bacteria [7, 17]. Calcium salts, such as  $\text{CaCl}_2$ , are suitable calcium sources for the formation of  $\text{CaCO}_3$  crystals [12]. The ideal calcium source and optimal concentration are different for each strain of bacteria [9].

There are many factors that influence the course of MICP, and they need to be studied very carefully. Each bacteria will have different requirements for optimal conditions in different environments. The exact behavior of the bacteria must be known if MICP is to become a true engineering method to modify the properties of materials.

## 3. USE OF MICP

The uses of MICP in engineering applications are many. Bacteria can be added to the concrete to improve its properties. Bacterial spores and nutrients can be added directly or encapsulated in capsules. The spores remain inactive due to the high pH in the concrete (pH 12–13). However, when microcracks or pores are formed, the pH drops slightly (a pH value of 11 is sufficient) and moisture penetration occurs. The dissolution of nutrients creates suitable conditions for bacteria. The bacteria then regenerates and can heal microcracks and pores with calcium carbonate [18]. This can reduce the permeability and absorption of water and increase the compressive strength and durability of the structure [15]. Bacteria can also restore the mechanical properties of concrete and thus ensure self-healing of concrete [9]. By adding bacteria with the ability to precipitate calcium carbonate to concrete, it can reduce maintenance costs and, together with suitable additives, it can partially replace cement [17]. At the same time, bacteria create an alkaline environment in their surroundings sufficient to protect the reinforcement from corrosion [18].

MICP bacteria can also be used to improve soil properties. Precipitated calcium carbonate can improve soil erosion resistance or slope stabilization [8].  $\text{CaCO}_3$  acts as a binder that increases soil strength and stiffness and reduces its permeability [14]. It is a gentle, cheap, and sustainable procedure compared to conventional means [9]. Bacteria can also be used to remove and immobilize heavy metals and radionuclides from the soil [9, 17]. Using MICP, soluble heavy metals in the soil are stabilized into insoluble forms. Metal ions bind to  $\text{CO}_3^{2-}$  and replace  $\text{Ca}^{2+}$  cations [17]. Lead, copper, cadmium, chromium, arsenic, and many others can be immobilized in the

soil [9]. Otherwise, these heavy metals would have to be removed from the soil through complex technological processes.

MICP can be used to treat heavy metal-contaminated wastewater or water [9]. Using the metabolic processes of bacteria,  $\text{CO}_2$  can be sequestered in the soil from the air during the formation of  $\text{CaCO}_3$  [17].

Like concrete or soil, bacteria can also work in aggregates. MICP can improve the properties of aggregates, fill voids and pores, and improve their strength [19]. This effect can be used to improve the properties of recycled aggregate. Compared to natural aggregate, recycled crushed concrete has worse properties – strength, absorbency, and porosity. With MICP, all these properties of recycled concrete can be improved. Properties are modified in the area of the ITZ zone between the aggregate and the old cement mortar. The surface of the recycled aggregate becomes stronger and can better bond to the new cement matrix [19]. This can be achieved in different ways [20]:

- The recycled aggregate can be directly immersed in the bacterial solution. However, this method risks a rapid reaction between bacteria and the nutrient solution, which could block pores and cracks on the surface of the aggregate and prevent bacteria from penetrating deeper layers. It is more suitable for fine-grained aggregates. The process is faster.
- The recycled aggregate is first impregnated with a bacterial solution with spores and then a nutrient solution is added. This ensures a more homogeneous distribution of bacteria throughout the aggregate structure. The method is also suitable for larger pieces of aggregate, but the process takes longer.

Modified recycled aggregate can be used as a bacterial carrier for self-healing concrete [19]. If bacterial spores are added to the concrete mix, their viability may be reduced during the concrete mixing and cement hydration process. Encapsulation of bacterial spores in aggregate can increase their chance of survival and they only become activated when the concrete is disturbed. Concretes with aggregates treated in this way may have reduced water absorption capacity, increased flexural strength, and resistance to carbonation [21].

Microbially precipitated calcium carbonate may not only serve as a filler. It can also be used in bonding materials. Using this principle, biobricks can be formed from sand, bacteria, and nutrients [17]. By mixing these ingredients, a strong bond can be formed between the grains of the filler material. The final product is a sandstone-like material [22]. MICP offers the production of bricks at low temperatures and low cost [7]. Biobricks do not have the strength of conventional bricks (the strength of biobricks is around 1300 kPa [22]), but their production is environmentally friendly, compatible with other materials and has good properties even in contact with water [23].

Their water absorption is similar to that of conventional bricks [22]. Repeated application of a bacterial solution improves the properties of biobricks, as does the addition of other ingredients [24]. The addition of fibers creates load-bearing bridges between the bonded material and the precipitates, which improve compressive strength, ductility, erosion resistance, and reduce water absorption [25].

#### 4. CALCITE PRECIPITATES AT SURFACE OF FINELY GROUND RECYCLED CONCRETE

To use MICP to modify the properties of the material or produce new materials, the course and precipitation process of  $\text{CaCO}_3$  must be investigated at the microscale. In this work, the focus is on evaluating the activity of cultured bacteria and their ability to precipitate calcium carbonate in finely ground materials. The resulting products were investigated by SEM and EDS analysis.

For this purpose, materials prepared from recycled concrete drainage trough and finely ground silica sand were used. The recycled drainage trough was prepared from unreinforced precast concrete with a lower amount of cement matrix. Portland cement rich in alite was used to produce it, which provided a rapid increase in strength and stiffness. Its original strength was C20/25, and its age was 4 years. This concrete was then prepared by crushing and grinding.

The ureolytic bacterium *Sporosarcina pasteurii* was used and cultured at 28 °C in a culture medium with 20 g/l urea. The pH value of the solution was 7.3. The cells were then suspended in a physiological solution. The solution was then mixed with the prepared recycled finely ground material. After 8–12 hours of sedimentation, a cementing solution consisting of 4 g/l of nutrient medium, 2.8 g/l  $\text{CaCl}_2$ , and 20 g/l urea was added to the sample. The solution had a pH of  $6.8 \pm 0.2$ . After every 24–36 hours, physiological solution or cementing solution was added, always alternately. After 6 cycles, the samples were left at 28 °C for 7 days and then dried at 65 °C. After this process, partially consolidated samples of uniform material were formed but without significant strength. The material could be crushed between the fingers.

In Figure 1, the silica sand sample after the MICP process can be seen. The sample contains grains of bonded material but is still loose, with no overall cohesion, and with a minor strength of the individual grains.

In Figure 2 the recycled concrete sample after the MICP process can be seen. The sample was joined into individual pieces of material. However, the material did not have significant strength or cohesion, and even after light manipulation, it began to disintegrate into the grains shown in the photograph.



FIGURE 1. Silica sand sample after MICP process.



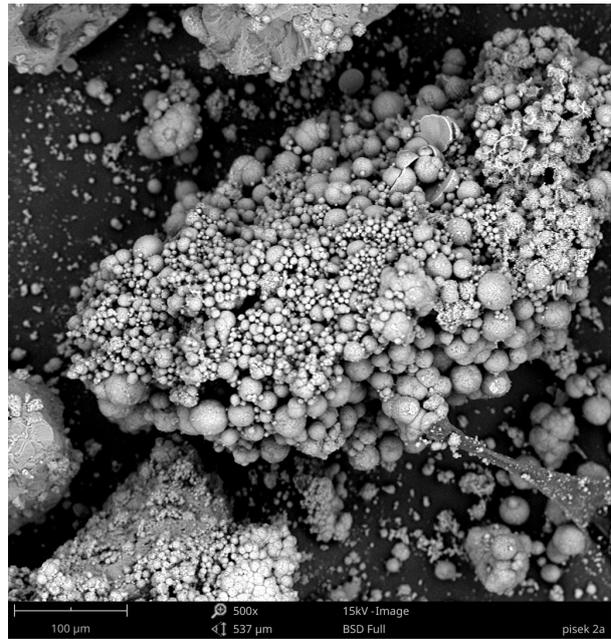
FIGURE 2. Sample of recycled concrete after the MICP process.

### 5. SEM ANALYSIS

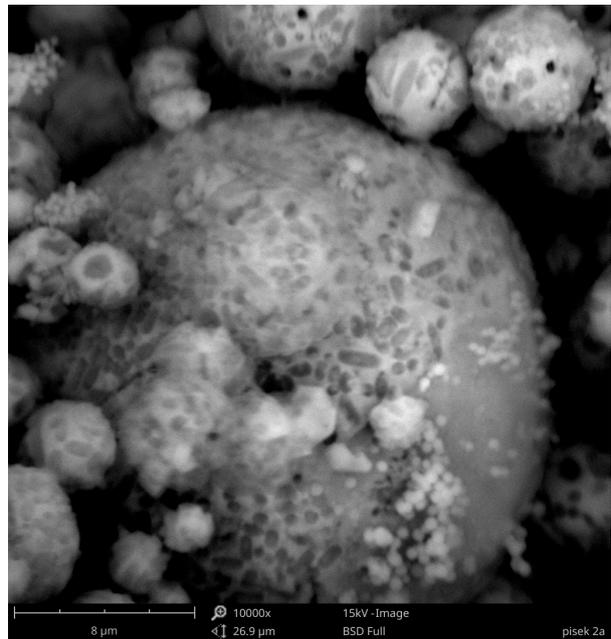
After drying, a portion of the sample was ground again into a fine powder, which was then analyzed using a scanning electron microscope. The powder was deposited on the adhesive surface of the target.

SEM images of silica sand samples after the MICP process can be seen in Figure 3a. The densely distributed spherical precipitates of  $\text{CaCO}_3$  can be seen. The entire sand grains are encased in crystals and it is almost impossible to find a pure sand grain. The  $\text{CaCO}_3$  precipitates reach a size of  $35 \mu\text{m}$ . Figure 3b shows a detail of a  $\text{CaCO}_3$  precipitate grain with bacterial cavities. This proves the microbiological origin of the crystals.

Figure 4 shows SEM images of recycled concrete samples after the MICP process. The spherical precipitates of  $\text{CaCO}_3$  are uniformly distributed on the



(A). Silica sand sample.



(B).  $\text{CaCO}_3$  precipitate.

FIGURE 3. SEM images of silica sand sample and  $\text{CaCO}_3$  precipitate.

sample in addition to the ground concrete grains. The  $\text{CaCO}_3$  precipitates reach a size of  $25 \mu\text{m}$ .

### 6. EDS ANALYSIS

In silica sand, it was verified that the spherical formations on the surface of all grains are composed of  $\text{CaCO}_3$ . Calcium and carbon content, a small amount of chlorine, and other elements. The residual chlorine is from the  $\text{CaCl}_2$  solution; the remaining elements (especially sodium, magnesium, and silicon) are the original aggregates and impurities. At the same time, a small amount of nitrogen was found. Nitrogen could

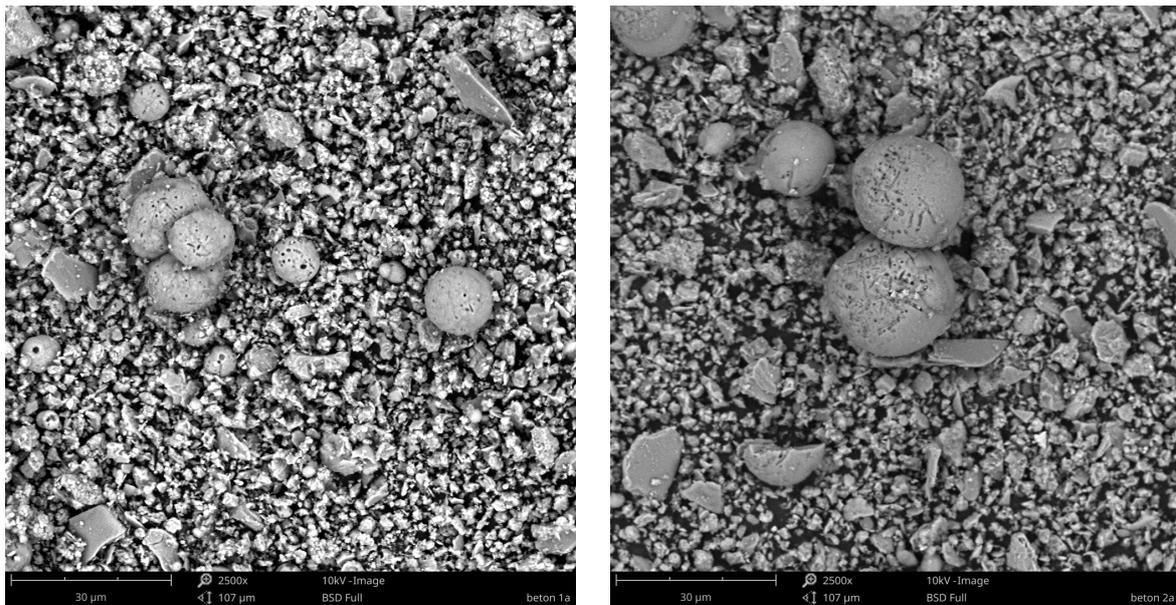


FIGURE 4. SEM images of a sample of finely ground recycled concrete and  $\text{CaCO}_3$  precipitate.

show by-products of bacterial activity.

Elemental analysis of recycled concrete samples verified that the spherical formations contained calcium, carbon, and trace amounts of chlorine. This shows that it is a  $\text{CaCO}_3$  crystal with residual chlorine from the  $\text{CaCl}_2$  solution. However, other elements such as sodium, magnesium, silicon, and iron were detected in the crystals, which were from the original concrete or impurities. The finely ground material around the spherical grains was determined to be the original concrete grains (content of silicon, aluminum, and other elements, especially potassium and magnesium) and the original aggregate grains (high silicon content and lower calcium and aluminum content).

Samples exposed to the bacteria were compared with samples of the original material. Formations containing a combination of the elements calcium, oxygen, and carbon are not present in the original material. In the study by Nguyen et al. [13], similar results were obtained when identifying bacterial products in concrete. As a result, it was concluded that the spherical formations in the samples are the product of bacteria and consist of calcium carbonate.

## 7. CONCLUSIONS

The study investigates the possibility of binding fine-grained concrete particles with  $\text{CaCO}_3$  precipitates produced by bacteria. The preliminary study shows that silica sand samples allow for a higher precipitation rate of  $\text{CaCO}_3$  than those composed of recycled concrete. However, the recycled concrete was bonded to form a more compact material with higher strength. This difference is mainly attributed to the different grain size distribution. If the particles are too fine (sized as bacteria = 1.3–4.0  $\mu\text{m}$ ), the bacteria cannot proliferate and do not form enough calcium carbonate crystals. If the particles are too coarse, larger and

weaker crystals form. The grain size of the original material has a significant effect on the MICP process. At the same time, the recycled concrete underwent a secondary hydration process, making the samples stronger and more cohesive.

The MICP process for binding recycled concrete has not been explored to date. It is necessary to find optimal conditions and suitable bacteria species that are efficient, economical, use ecological substances, and are safe for humans and the environment. Recycled concrete and silica sand were used for performance experiments with finely ground materials. Attention was paid to the composition and shape of the MICP products and the cohesion of the newly formed material. Calcium carbonate crystals in different volumes and sizes were found in the materials. The grain size distribution of the starting material is crucial for the progress of MICP and the properties of the resulting material. For recycled concretes, the effect of MICP and secondary hydration must be identified and assessed.

These results will be used in future experiments and further analyses that will be performed with different combinations of our sample materials. Our future work will focus on the most effective use of the bacterium *Sporosarcina pasteurii* in improving the properties of recycled concrete and bonding it into a compact material.

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