

Review

Reinforcement of Recycled Aggregate by Microbial-Induced Mineralization and Deposition of Calcium Carbonate—Influencing Factors, Mechanism and Effect of Reinforcement

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Abstract: Recycled aggregate is aggregate prepared from construction waste. With the development of a global economy and people's attention to sustainable development, recycled aggregate has shown advantages in replacing natural aggregate in the production of concrete due to its environmental friendliness, low energy consumption, and low cost. Recycled aggregate exhibits high water absorption and a multi-interface transition zone, which limits its application scope. Researchers have used various methods to improve the properties of recycled aggregate, such as microbially induced calcium carbonate precipitation (MICP) technology. In this paper, the results of recent studies on the reinforcement of recycled aggregate by MICP technology are synthesized, and the factors affecting the strengthening effect of recycled aggregate are reviewed. Moreover, the strengthening mechanism, advantages and disadvantages of MICP technology are summarized. After the modified treatment, the aggregate performance is significantly improved. Regardless of whether the aggregate was used in mortar or concrete, the mechanical properties of the specimens were clearly improved. However, there are some issues regarding the application of MICP technology, such as the use of an expensive culture medium, a long modification cycle, and untargeted mineralization deposition. These difficulties need to be overcome in the future for the industrialization of regenerated aggregate materials via MICP technology.

Keywords: recycled aggregate; biomineralization; sustainability; reinforcement; recycled concrete

1. Introduction

Since the beginning of the 21st century, with the continuous prosperity of global construction, a large amount of natural building materials has been consumed to construct tall buildings and dense transportation networks. During the past two decades, billions of tons of construction waste have been produced in China, and related statistics show that construction waste accounts for approximately 4 out of 10 urban waste products [1]. In Shenzhen, one of the most developed cities in China, approximately 84% of construction waste has been buried in landfills in recent years [2]. From the perspective of sustainable development, it has great significance to recycle materials in construction waste, which is beneficial for alleviating the shortage of natural construction materials together with the land resources for landfill waste.

The aggregate obtained from the crushing and grading of the building solid waste is called recycled aggregate. The concrete prepared by replacing natural aggregate with recycled aggregate in a certain proportion is called recycled concrete. Many studies have shown that employing recycled aggregate will lead to the degradation of the mechanical properties and durability of recycled concrete [3–5]. When recycled aggregate replaces all natural aggregates, the splitting tensile strength, elastic modulus and compressive

strength of recycled concrete will be greatly reduced [6–8], Guohui [9] found that when the water–cement ratio was 0.44 and the replacement rate of recycled aggregate was 100%, the strength of recycled concrete decreased by nearly 32% compared with that of natural concrete, which was similar to Xuan’s research results [10]. Meanwhile, the chlorine ion diffusion coefficient, carbonization depth and water absorption are all higher than those of natural concrete [11], which indicates that the recycled aggregate has a negative impact on the durability of the recycled concrete. Adessina et al. [12] proposed that there are two main reasons for the degradation of the mechanical properties of recycled concrete, including numerous interface transition zone (ITZs) and old mortar adhering to the aggregate surface. Different from natural aggregate materials, a large amount of adhesive mortar is usually adhered on the recycled aggregate surface, which leads to high water absorption and poor durability of the recycled concrete. Moreover, a more complex ITZ between the fresh mortar and the recycled aggregate was generated in the recycled concrete, as shown in Figure 1 [13].

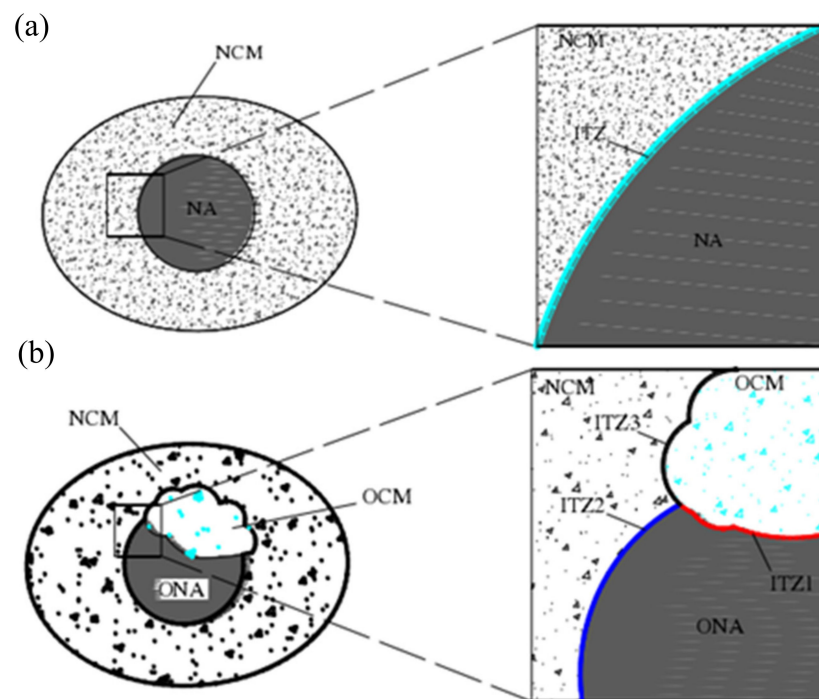


Figure 1. Concrete interface structure [13]: (a) ordinary concrete; (b) recycled concrete; (NA: natural aggregate, ONA: old natural aggregate, NCM: natural concrete mortar, OCM: old concrete mortar, ITZ: interface transition zone).

Compared with natural concrete, recycled concrete has larger porosity, more complex structure and lower apparent density, which leads to the poor mechanical properties and durability of the concrete, limiting its utilization rate. Therefore, to achieve the purpose of recycling recycled aggregate, it is necessary to strengthen and modify the material to improve its performance.

At present, the relatively mature and widely used strengthening technologies of recycled aggregate belong to physical strengthening technology and chemical modification technology. Among them, physical methods strengthen recycled aggregate through a physical process to reduce the water absorption and ITZs by removing the mortar attached to the surface, modifying the particle shape, etc. Commonly used methods include mechanical grinding, particle shaping, heat treatment, and high-pressure pulses [14–17]. Alternatively, chemical methods remove or strengthen the attached mortar on the surface of recycled aggregate through chemical reactions to reduce the water absorption of the aggregate and strengthen the ITZs to improve the mechanical properties and the durability of the aggregate. Common

methods including cement slurry treatment, polymer impregnation, carbonization curing, chemical reagent pre-preg [18–21]. Relevant studies have shown that the above modification methods have problems such as high energy consumption, large equipment wear and tear, complex processes, long time consumption, and secondary pollution to the environment. In recent years, researchers have found that microbially induced calcium carbonate precipitation (MICP), as a basic geochemical cycle process, is eco-friendly, exhibits low energy consumption and low-cost characteristics [22]. If this technology is used as a means to facilitate recycled aggregate, it will have good development prospects.

This paper comprehensively analyzes the research progress of MICP reinforcement technology in recycled aggregate and discusses the influencing factors, strengthening mechanism, strengthening effect, advantages and disadvantages. Some problems and shortcomings existing in current MICP technology are proposed to provide a reference for relevant researchers.

2. Microbially Induced Calcium Carbonate Precipitation (MICP) Technology

Some microorganisms can form mineral precipitates in the Earth's circulation system through a series of physiological activities (respiration, metabolism, reproduction, catalysis, etc.), which is a widely existing complex phenomenon [23]. MICP technology refers to bacterial metabolism producing CO_3^{2-} , which then reacts with Ca^{2+} in soil and concrete to generate CaCO_3 precipitates, filling cracks and pores on the surface of soil and buildings to achieve reinforcement. Due to the good adhesion and stability of the resultant CaCO_3 crystals and excellent compatibility with cement pastes, MICP technology has been widely applied in the fields of solidifying sand soil to reduce soil permeability [24,25], preventing building settlement [26], repairing and reinforcing concrete cracks [27], and repairing historic buildings [28]. There are three different mechanisms of biomineralized precipitation [29]: Curing the soil, preventing building settlement and slope reinforcement, reducing soil permeability, repairing and reinforcing, and renovating historical buildings in areas such as cracks in concrete have been applied.

Research indicates that there are three different deposition biomineralization mechanisms [22].

1. Bioactivity controlled calcium carbonate precipitate—the process of mineralization and precipitation is directly controlled by cell activities. Mineral precipitates are synthesized in cells or at designated locations on cells. The whole process of the nucleation and growth of mineralized products is greatly dependent on biological activities and requires intense mineralization conditions, so it can only be carried out under specific conditions [30,31];
2. Biological metabolic control calcium carbonate precipitate—the process of mineralization precipitation is influenced by cell activities. Biological metabolic reactions related to biofilms cause the accumulation of organic substances or extracellular polymers on the cell surface, leading to the precipitation of calcium carbonate [31];
3. Biologically induced calcium carbonate precipitate—mineralization precipitation is induced by cell activities. The metabolic activities of microorganisms cause changes in the surrounding chemical environment. Metal ions are attracted by the negatively charged CO_3^{2-} generated by respiration on the cell wall surface, which promotes mineral precipitation on the cell wall surface [31,32].

Adherent mortar and microcracks are the reasons for the poor mechanical properties of the recycled aggregate in mortar or concrete. At present, three ways of strengthening recycled aggregate with MICP technology are usually adopted: (1) Direct immersion: the recycled aggregate is directly soaked in nutrient solution containing the appropriate concentration of bacteria for 7–14 days; (2) Two-stage immersion: first, the bacterial liquid cultured under specific conditions is attached to the regenerated aggregate, and then the regenerated aggregate is immersed in the nutrient solution; (3) Spraying: After the bacterial liquid cultured under specific conditions is attached to the recycled aggregate, nutrient solution is sprayed on surface of the aggregate at a regular and quantitative time.

Among them, immersion reinforcement is more widely used. Figure 2, as a schematic diagram, depicts the reinforcement of the recycled aggregate employing MICP technology. Owing to the good fill and cementing effect of biomineralized precipitation on adhered mortar and microcracks, as well as the good compatibility with cement-based materials, this technology has potential for intensified use of recycled aggregates [33]. Dick et al. [34] obtained a layer of dense calcium carbonate crystals created by MICP on degraded limestone by carrying out *Bacillus ureolytic*-driven calcium carbonate precipitation. Their experiments showed that the layer of dense calcium carbonate crystals could effectively reduce the water absorption of limestone. Muynck et al. [35] used bacteria with the number *B. Sphaericus* LMG 22,557 to conduct surface treatment on mortar samples and found that the permeability of samples decreased after biodeposition treatment, and the resistance to carbonation, chloride migration and freeze–thaw increased. The above studies show that the modification treatment has a positive effect on both the performance improvement of limestone (aggregate) and the performance of mortar (adhesive mortar), especially on decreasing water absorption. These experiments show that MICP technology may have a positive effect on intensified recycled aggregates, but the specific strengthening process is affected by the interaction of multiple factors.

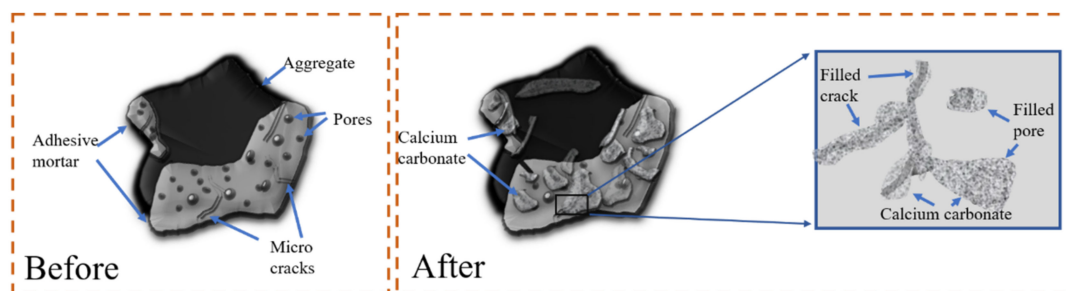


Figure 2. Recycled aggregate enhanced by biodeposition of calcium carbonate.

3. Factors Influencing the Mineralized Sedimentation

The mineralization and precipitation induced by microorganisms is a complex process. A number of factors, such as the type of bacteria, calcium source, medium, pH value, nucleation center location and temperature, and other factors interact together to determine the effect of mineralization precipitation [36,37]. In the following, several important factors affecting the effect of mineralization and deposition are discussed, the modification conditions of recycled aggregate are comprehensively analyzed, and the effect of each influencing factor on the modification of recycled aggregate is discussed.

3.1. Bacteria

Bacteria are ubiquitous in every corner of the planet and make up the majority of the planet's biomass. Bacteria come in a variety of shapes, such as spherical, rod-shaped, and helical shapes. Bacterial cells are approximately one-tenth the size of eukaryotic cells and are usually 0.5–5.0 μm in length [35]. Studies have shown that many bacteria can produce mineralized deposition of calcium carbonate, but not all strains can produce a good strengthening effect on aggregates, depending on whether the strains have a strong negative potential [34]. The negative potential of the cell surface electrical layer is an important factor for bacteria to efficiently adhere to the surface of aggregate. The positive potential of limestone on the surface of recycled aggregate is similar, which makes bacteria with a high negative potential more likely to get more calcium carbonate to settle down on the surface [38]. Comparing the negative potential of the electrical layer on the surface of different species is helpful for mineralization deposition to occur more effectively.

Ureolytic bacteria are widely used in the field of MICP technology. In the reinforcement process, the urease produced by ureolytic bacteria hydrolyzes urea and produces CO_3^{2-} , which generates calcium carbonate precipitates with good cementing ability in an

environment rich in Ca^{2+} to repair and fill the surface defects of recycled aggregate and improve its performance [37]. The urease generation rate has an important effect on the rate of calcium carbonate precipitation. Generally, the bacterial concentration determines the rate of bacteria producing urease. The current study results suggest that most of the concentration of bacteria is scheduled for 10^7 – 10^8 cells/mL, which may be based on the calcium carbonate precipitation rate and the interaction of the urease hydrolysis rate of urea to consider [39,40].

3.2. Calcium Sources

The precipitation of calcium carbonate by biomineralization is mainly three crystal types: calcite, vaterite and aragonite. Among these three crystal types, calcite is the best repair material for recycled aggregate, and the stability of vaterite and aragonite is poor compared with that of calcite. In fact, the type of calcium source influences the mineralization rate of MICP. Therefore, the selection of an appropriate calcium source plays a key role in the modification effect of recycled aggregate. Zhan et al. [41] used calcium nitrate and calcium chloride to explore the role of different calcium sources in the study of modified recycled aggregates. The experimental results showed that the weight gain rate treated with calcium nitrate was 2.15%, while the weight gain rate treated with calcium chloride reached 3.1%. In addition, the data showed that the redesigned aggregate with calcium chloride as the calcium source had the lowest water absorption (1.12) and the highest compressive strength ratio (0.99). These results confirmed that when microorganisms modify aggregates, using calcium chloride as the calcium source could obtain a better strengthening effect than calcium nitrate as the calcium source. Achal et al. [42] further explored the influence of different calcium sources (calcium chloride, calcium oxide, calcium nitrate and calcium acetate) on the mineralization precipitation effect of *Bacillus*. These researchers found little difference in the colony-forming units between the cultures when mineralization experiments were carried out on media that only differed from one another in the source of calcium, but the maximum growth rate of colonies was observed when calcium chloride was used as the calcium source medium. In contrast, the use of calcium oxide as the calcium source medium colony growth condition was not ideal. Urease is an important enzyme leading to calcium carbonate precipitation in microorganisms, and the yield of urease determines the amount of calcium carbonate precipitation when other conditions are almost unchanged. Different calcium sources also showed different effects on urease production by the same bacteria. In a medium with calcium chloride as the calcium source, *Bacillus* spp. CR2 showed the highest urease yield. When the calcium source was calcium nitrate, the urease yield produced by bacteria was second only to calcium chloride. When calcium oxide was the calcium source, the amount of urease produced by bacteria was the lowest. In addition, although most of the precipitates were calcite, when different kinds of calcium sources were used by bacteria for mineralization and precipitation, there was the largest amount of calcite in the medium containing calcium chloride. Therefore, calcium chloride might be the most suitable calcium source for bacterial mineralization and precipitation, which was consistent with previous studies [43].

3.3. Composition of the Culture Medium

Since the culture medium provides nutrients for the process of microbial-induced mineralization and precipitation, the composition and concentration of the medium have an important effect on the rate of microbial metabolism. Muynck et al. [44] took calcium chloride as the calcium source and soaked the specimens in different nutrient solutions for six days to explore the influence of nutrient broth (a complex nutrient that supports rapid bacterial growth) added to the medium on the morphology of calcium carbonate crystals on the surface of mortar. As shown in Figure 3, SEM observation of the mortar surface showed that in the presence of nutrient broth, calcite crystals were almost absent and granular vaterite particles appeared. This observation indicated that medium composition had a

profound influence on the crystal morphology of calcite, which could be attributed to the different actual urease levels under different culture conditions. In fact, a specific medium composition and concentration may directly control the size and form of calcium carbonate generated [44]. Precisely controlling the precipitation process is extremely beneficial for obtaining a better recycled aggregate strengthening effect.

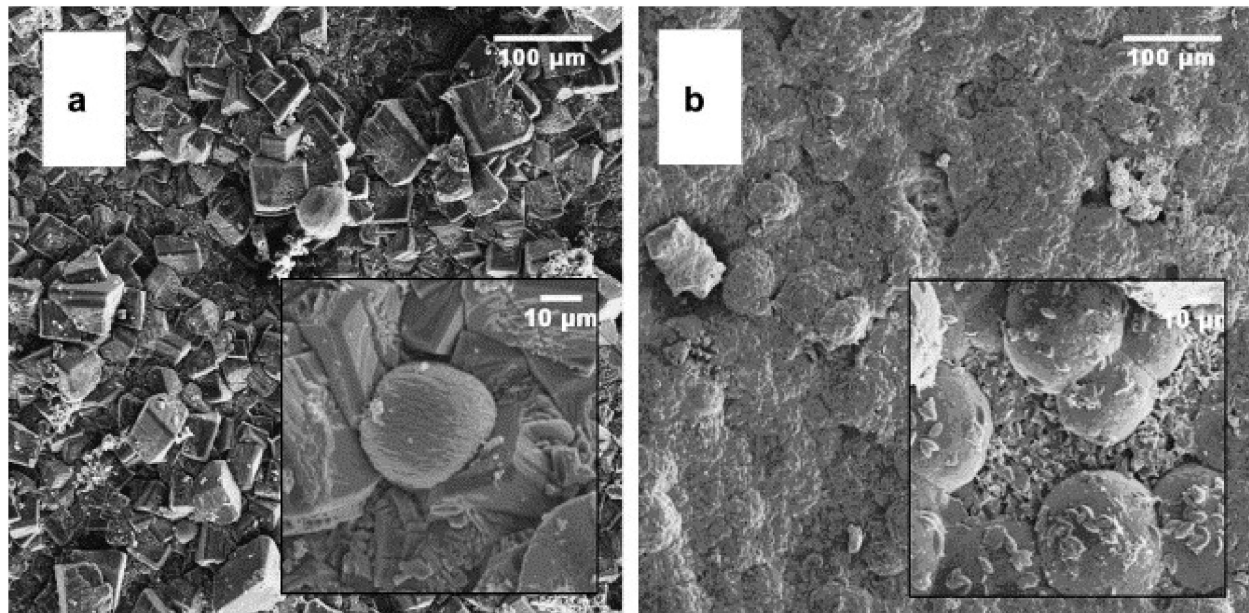


Figure 3. Calcium carbonate crystal morphology on the mortar surface [44]: (a) calcium chloride; (b) calcium chloride and nutrient broth. Reprinted from *Construction and Building Materials*, Bacterial carbonate precipitation as an alternative surface treatment for concrete, Willem De Muynck, Kathelijin Cox, Nele De Belie, Willy Verstraete, 2008, 22, 875–885, with permission from Elsevier.

In the process of recycled aggregate enhancement by microorganisms, standard media usually include a nutrient broth culture medium and yeast extract [45]. To obtain a better precipitation effect or economic value, researchers propose partially replacing the composition of the culture medium.

To reduce the cost of culture medium, researchers have focused on waste containing rich nutrients such as chicken manure wastewater, corn milk, lactose mother liquor and activated sludge from the agriculture, dairy and baking industries [46]. Grabiec et al. [40] chose two kinds of media to culture bacteria in the process of exploring microbe-induced carbonate precipitation: one was a standard medium containing a meat extract and peptone, and the other was a whey medium. The results showed that the bacterial concentration reached 9.0×10^6 CFU mL⁻¹ after 48 h of culture in the standard medium, and the highest bacterial concentration reached 3.7×10^5 CFU mL⁻¹ after 48 h of culture in the whey medium, which suggested that whey could be a promising alternative medium for *Pasteuria*. Achal et al. [45] cultured *Pasteurella* in three different media (lactose mother medium, nutrient medium, yeast extract medium) for 30 h and further explored the feasibility of lactose mother liquor as an alternative nutrient source for *Pasteurella* in intensified recycled aggregate concrete using the MICP technique. These authors found that the growth status of bacteria in different media was similar, and there was no significant difference in bacterial concentration. Then, they tested the urease activity of three kinds of culture media, and the results showed that although the lactose mother liquid medium was slightly inferior to the other two kinds of culture media, the effects of the three kinds of calcium carbonate precipitation and urease activity in the medium change curve were similar. These results provide reliable evidence for the use of lactose mother liquor instead of standard medium in bacterial culture.

The cost of lab-grade bacterial culture media could reach 60% of the total cost in the process of using MICP technology to enhance recycled aggregate [46]. It is obvious that a standard medium with a high cost can have a good effect on the process of MICP, but the high cost of this technique makes it a considerable challenge to apply in the field of engineering. This issue should be mitigated to find suitable substitutes for nutrients to reduce the economic cost of media for the industrialization of mineralized precipitation technology.

3.4. Effect of pH

The pH of the environment has an important influence on the process of MICP. In the natural world, a variety of microorganisms can create an alkaline environment for the formation of calcium carbonate through various physiological activities, such as photosynthesis, urinase, ammonification, denitrification, sulfate reduction, anaerobic sulfide oxidation and methane oxidation [47]. A study showed that pH has a double effect on calcium carbonate precipitation. Taking *Pasteurella* as an example, the most suitable growth pH was approximately 9.5. The specific urease activity of *Pasteurella* increased with increasing pH, and the urease activity reached the peak value when the pH was approximately 8, but the precipitation amount of calcium carbonate only reached the maximum value when the pH value was 9.5 under the combined action of a high specific urease activity and bacterial number [45]. The optimum pH for mineralization and precipitation is slightly different with various microorganisms because the process needs to occur under alkaline conditions. However, after the pH reaches its peak value of 9.5, the amount of calcium carbonate precipitation decreases sharply as the pH value increases. Qiu et al. [48] explored the influence of pH on the process of MICP. The experimental results indicated that the amount of calcium carbonate precipitation showed a tendency to rise and then fall as the pH of the medium increased. When the pH = 7, the amount of calcium carbonate precipitation in the medium was only 0.016 g, and when the pH of the medium rose to 9, the mass of calcium carbonate precipitation reached a peak of 0.111 g, which was about 10 times the mass of pH = 7. After that, with the rise of pH, the amount of calcium carbonate precipitation decreased. When pH = 10.5, the mass of precipitation was 0.050 g.

The cement provides an alkaline environment for the growth of bacteria, which is conducive to the formation of calcium carbonate precipitation during the reinforcement of the recycled aggregate. In the current research on the enhancement of recycled aggregate by MICP technology, most researchers left the nutrient solution pH in the optimum range of bacterial metabolism. Zeng et al. [49] cultivated *S. pasteurii* DSM 33 strains with hydrochloric acid and sodium hydroxide to adjust the medium pH value to 9.5. Xu et al. [50] chose to adjust the pH of the medium to 7.0 when using the same bacteria from different sources in the experiment.

3.5. Availability of the Nucleation Center

Nucleation center effectiveness for the continuous stability of calcium carbonate formation is very important. There are three different mechanisms of biomineralization precipitation, as illustrated above. The mechanism type of bioactivity controlling calcium carbonate precipitate is highly dependent on the cell metabolism and precipitation process, which makes the mineralization and deposition process take a long time. However, because strengthening the aggregate requires substantial calcium carbonate precipitation, this mechanism is not suitably used for modifying recycled aggregate. The latter two mechanisms (biological metabolic control calcium carbonate precipitate and biologically induced calcium carbonate precipitate) can provide a more controllable precipitation process, so the MICP technique usually chooses biologically metabolic and biologically induced strains to induce mineralizing precipitation [32,51].

Critical saturation is the state in which the maximum amount of precipitation is induced by microorganisms under a given condition. The enhancement of recycled aggregate using MICP technology requires a series of delicate operations. In this process,

the nucleation center affects the state and degree of nucleation, and the saturation state greatly affects the reinforcement effectiveness. Electron acceptor is the essence of nucleation centers. Tobler et al. [52] proposed that electron acceptors, such as oxygen in *Pasteurella*, play an important role in microbial-induced mineralization precipitation. Taking ureolytic bacteria as an example, although ureolytic activity is not dependent on oxygen, the growth of microorganisms and urease activity are limited by the availability of electron acceptors. To confirm the influence of oxygen on the aggregate modification effect when MICP technology was used to strengthen the recycled aggregate, Zhu Yaguang [53] selected different oxygen supply concentrations and soaking positions to modify the recycled aggregate. The experimental results showed that when the concentration of calcium peroxide was 15 g/L and the soaking position was in the middle of the culture medium, the water absorption rate of recycled aggregate decreased by 40.4%, the crushing index decreased by 19.7%, and the porosity was significantly improved. A large number of calcium carbonate crystals filled the cracks of aggregate, and the modification effect of aggregate was significantly improved. The urease activity may be limited by the existing enzymes in the inoculation of the aerobic culture. To overcome the problem of insufficient urease activity during mineralization precipitation induced by microorganisms, native populations can be stimulated by the injection of a growth medium rich in electron acceptors or urease to make mineralization occur efficiently and continuously [54].

Mineralization precipitation is a passive process that protects cells from harmful calcium concentrations for a short period of time, in which microorganisms are spontaneously involved [55]. It has been reported that almost all bacteria can induce the precipitation of calcium carbonate [56]. However, the requirement of mineralization precipitation is different for various bacteria. Similarly, the number of electron acceptors provided differs sharply. It is crucial to continue to explore the biological activities of strains in the laboratory to obtain the most effective nucleation centers.

3.6. Temperature

Temperature always affects the whole process of biological growth and reproduction. Different bacteria have different degrees of alkali tolerance and different pH values to achieve the best precipitation effect, but most of the bacteria show similar choices at the appropriate temperature. Kim et al. [57] explored the influence of different temperatures on calcium carbonate deposition in *S. saprophyticus* and *S. pasteurii*. The results showed that the two kinds of bacteria were very sensitive to temperature changes during the precipitation process, but both of them showed the best precipitation amount at 30 °C. The effect of temperature on bacterial activity usually varies from species to species, but the cell activity of most bacteria is limited below 5 °C [58]. In addition, temperature also has a profound influence on the rate of urease promoting urea hydrolysis. Low temperature will reduce urease activity, while high temperature will deactivate urease. In the range of 5–37 °C, urease activity will increase exponentially with increasing temperature [59]. Under the condition of good cell activity, the process of urease-catalyzed urea hydrolysis is highly dependent on temperature. Suitable temperature can improve the rate of bacterial calcite precipitation and the ability of bacterial strains to form crystals. Keeping the precipitation process occurring at an appropriate temperature is conducive to obtaining a satisfactory treatment effect.

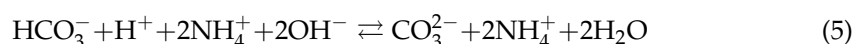
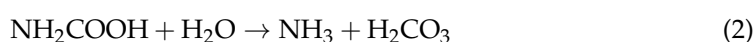
4. Reinforcement Mechanism

On the one hand, the mineralization induced by microorganisms is controlled by the metabolism of microorganisms. On the other hand, mineralization follows the physicochemical rule of mineral nucleation. The mechanism of inducing calcium carbonate precipitation by different microorganisms is slightly different. Microorganism species are accustomed to divide into ureolytic bacteria and nonureolytic bacteria according to the metabolic manner. Hereby, the mechanism of microbially strengthening reclaimed aggregate is according to the type of bacteria.

4.1. Mineralization Mechanism of Ureolytic Bacteria

Bacteria have the largest ratio of the surface area to volume in all forms of life and have a negative charge on the cell surface [60,61]. The large surface area and negative charge enable the bacterial surface to adsorb dissolved metal ions. Taking *Bacillus ureolyticus* globules as an example [34,62,63], *Bacillus* grows in a suitable nutrient solution concentration of urea, and calcium salt produces urease, which catalyzes urea hydrolysis in the nutrient solution, generating carbamic acid ester and ammonia, as illustrated in reaction Equation (1).

Carbamate is unstable and spontaneously hydrolyzes to produce ammonia and carbonic acid (H_2CO_3), as shown in Equation (2). The interaction between ammonia and carbonic acid will lead to an increase in the environmental pH value and form CO_3^{2-} . The reactions are shown in Equations (3)–(5).



The negative charge carried by the cell wall of *Bacillus* spontaneously adsorbs Ca^{2+} in the nutrient solution. Finally, the generated carbonate and calcium ions form calcium carbonate precipitates on the cell wall, which serve as nucleation sites. The reaction is shown in Equations (6) and (7).



The process of formation of calcium carbonate crystals by bacterial mineralization and precipitation on the cell wall is depicted in Figure 4 [64].

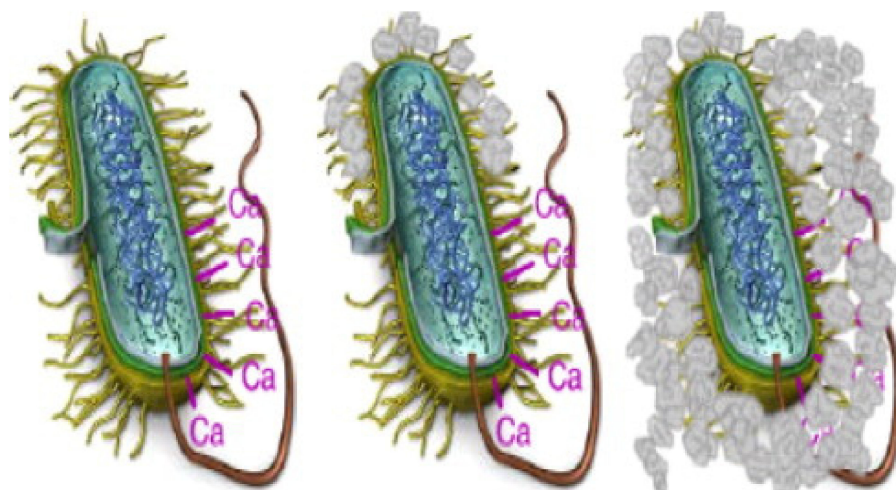


Figure 4. Process of formation of calcium carbonate by bacteria on cell wall [64]. Reprinted from Construction and Building Materials, Effect of ureolytic bacteria on concrete properties, Rafat Siddique, Navneet Kaur Chahal, 2011, 25, 3791–3801, with permission from Elsevier.

The water absorption rate and weight gain directly show the effect of mineralized precipitation. The precipitated particles fully cover the surface area of the aggregate and filling the surface pores will reduce water absorption. Wang et al. [65] used *Bacillus sphaer-*

icus LMG22257 and different soaking methods to strengthen the aggregate (a—no soaking treatment; b—one basic soaking treatment; c—two basic soaking treatments). The results showed that the surface of the aggregate after two soaking treatments was completely covered by dense particles, which were a spherical aragonite type and a hexagonal calcite type of calcium carbonate. In addition, bacterial imprints could be clearly seen on the particles, which indicated that the production of precipitation depended on the physiological activities of bacteria.

Ureolytic bacteria, as a way to strengthen recycled aggregates, have attracted the attention of many researchers due to their advantages of simplicity, high efficiency and low energy consumption. However, ureolytic bacteria will release the unwanted byproduct NH_4^+ in the process of urea decomposition, and NH_4^+ salts and their conversion products may cause problems such as water contamination, aggregate discoloration, or metabolic competition among bacterial communities [32,66].

4.2. Mineralization Mechanism of Nonureolytic Bacteria

To avoid the generation of NH_3 in the process of mineralization, some researchers proposed using nonureolytic bacteria to induce the mineralization precipitation of CaCO_3 to strengthen the recycled aggregate. Then, the mineralization mechanism of nonureolytic bacteria was introduced.

4.2.1. Fermentation of Fatty Acids

Among the nonureolytic bacteria, some bacteria rely on their metabolism to complete the mineralization and precipitation process. After the metabolism of basophilic carbonate mineralizing bacteria, the organic acids in a nutrient solution react with CO_2 generated in the metabolic process to form CaCO_3 precipitates in an alkaline environment. Notably, different types of organic carbon sources have a significant effect on the type and morphology of the precipitated crystals. Common organic carbon sources include a variety of organic acids such as calcium lactate, calcium acetate, calcium glutamate and sodium gluconate [67,68].

Taking calcium lactate as the calcium source, the mineralization process can be expressed as Equation (8).



Taking calcium glutamate as the calcium source, the mineralization process can be expressed as Equation (9).



Among them, R_xNH_y is an amine compound.

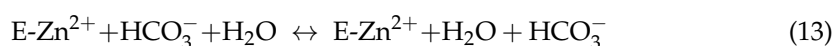
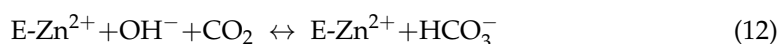
When calcium lactate is the calcium source, the precipitate is calcite. However, when calcium glutamate is the calcium source, the precipitation produced is mainly vaterite, and there is a small amount of calcite crystals. Xu et al. [68] believed that the existence of a small amount of calcite crystals is caused by the instability of vaterite. Plummer et al. [69] pointed out that among the three main crystal forms of CaCO_3 (calcite, vaterite, and aragonite), vaterite is the most unstable crystal. When the temperature is higher than 3 °C, it gradually transforms into more stable calcite or aragonite. Moreover, vaterite has strong solubility and low density, so it is not suitable for filling the porous parts on the surface of recycled aggregate. Qian et al. [70] found that pH plays an important role in the mineralization process. Equations (10) and (11) indicate that mineralization and precipitation of calcite only begin when the solution is alkaline.



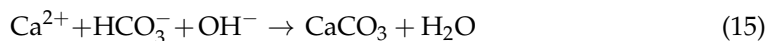
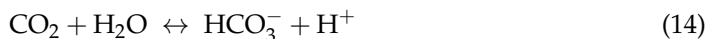
Notably, basophilic carbonate mineralizing bacteria rely on their metabolism for mineralization, and precipitation does not produce NH_3 in the process of producing calcium carbonate, but the mineralization process depends on the rate of metabolism. A variety of factors, such as the strain type, medium composition, temperature, pH, and concentration of O_2 , play a significant role in the metabolic process of bacteria, which makes it harder for the laboratory to control mineralized precipitation.

4.2.2. Carbonic Anhydrase Catalysis

Carbonic anhydrase bacteria promote the mineralization precipitation of CaCO_3 by its ability to capture CO_2 . Carbonic anhydrase is a metal enzyme widely found in nature, and its active center has a zinc atom necessary for catalysis. The carbon dioxide hydration reaction is the most important biological function for carbonic anhydrase [71]. During the hydration reaction, CO_2 reacts in the activity center of the enzyme. Reactions are shown in Equations (12) and (13) [72]:



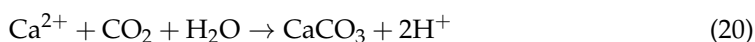
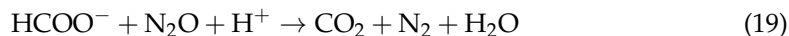
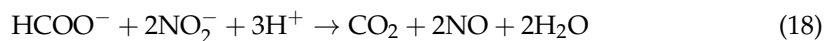
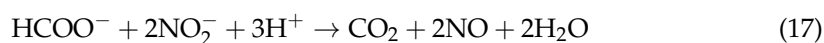
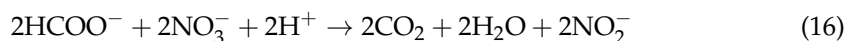
In fact, the rate constant of the first-order reaction of at 25°C is 0.037 s^{-1} without a catalyst, and the reaction rate can be almost seven orders of magnitude higher after carbonic anhydrase catalysis [73]. The excellent catalytic ability of carbonic anhydrase has attracted the attention of researchers. Qian et al. [74] proposed applying carbonic anhydrase bacteria to achieve the MICP technique and predicted that carbonic anhydrase bacteria could induce and accelerate the precipitation reaction of calcium carbonate in alkaline environments on cement surfaces to achieve protection and repair effects on defective surfaces. The reaction process is shown in Equations (14) and (15):



Compared with the process of mineralization induced by ureolytic bacteria, using carbonic anhydrase bacteria to mineralize and deposit calcium carbonate to repair recycled aggregates is not only harmless and nontoxic but also absorbs CO_2 from the air as the reaction raw material, which is relatively friendly to the environment. On the one hand, the carbonic anhydrase catalytic process is greatly affected by pH, temperature, and CO_2 concentration, which limit the mineralization rate. On the other hand, the reversibility of the carbon dioxide hydration reaction leads to the constant generation of CO_2 in the whole precipitation process, which increases the porosity of the precipitation products and then affects the performance of recycled concrete in the later stage [75].

4.2.3. Denitrification

Biological reduction of NO_3^- occurs when microorganisms oxidize organic matter. Nitrifying reducing bacteria use NO_3^- instead of O_2 as electron acceptor to produce calcium carbonate precipitation in the nutrient solution. The reaction process is shown in Equations (16)–(20) [76,77].

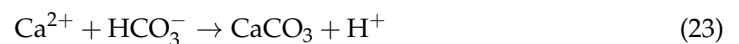
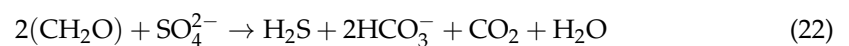
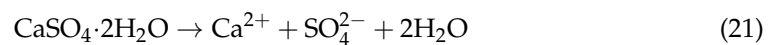


As electron acceptor in most mineralization reactions, O_2 plays an important role in the mineralization process. At present, soaking is the main strengthening method for MICP regeneration aggregate, and the poor solubility of O_2 in solution limits the number of electron acceptors in solution. Nitrifying reducing bacteria can produce calcium carbonate deposits in anoxic environment, which is undoubtedly a significant advantage for strengthening recycled aggregate [78]. In addition, it can survive in an environment lacking nutrients and produce calcium carbonate deposits, which may also reduce the cost of the aggregate strengthening process.

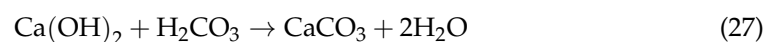
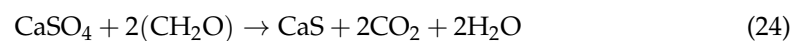
4.2.4. Sulphur Cycle

Mondal et al. [77]. pointed out that, as a non-ureolytic bacterium, sulfate reducing bacteria have two ways to mineralize and deposit calcium carbonate.

Sulfate reducing bacteria can reduce sulfate to H_2S with the production of HCO_3^- in an organic-rich and oxygen-free environment. The separation of H_2S from the environment will lead to the rise of pH in the surrounding environment, and the rise of pH will further promote the generation of calcium carbonate precipitation. The reaction process is shown in Equations (21)–(23) [79].



In addition, there is another mineralization process of sulfate reducing bacteria. Sulfate-reducing bacteria can also reduce calcium sulfate to calcium sulfide. After further reaction of reaction products in aqueous solution, mineralization and deposition of calcium carbonate is completed. The reaction process is shown in Equations (24)–(27) [80].



Similar to nitrate reducing bacteria, sulfate reducing bacteria also do not rely on O_2 as an electron acceptor. However, a difficult problem to solve is that sulfate reducing bacteria release H_2S gas during mineralization, which is not environmentally friendly. In addition, the mineralization process needs to be carried out under the conditions of rich nutrients, and the pH will constantly change, which is not conducive to the uniform and stable production of calcium carbonate.

5. Reinforcement Effect of Recycled Aggregate

Researchers are most concerned with whether the performance of recycled aggregate and recycled aggregate concrete (mortar) can be improved regardless of how the aggregate is strengthened. Singh et al. [81] discussed the strengthening effect of reclaimed aggregate in detail. These investigators studied the modification effect of recycled aggregate by ureolytic bacteria, nonureolytic bacteria and nanomaterials. They point out that the optimal strengthening period of recycled aggregate was 14 days. After 14 days of modification treatment, the increase in specific gravity was found to be 29% and 30% as well as the percentage decrease in water absorption was found to be 42.9% and 64.3% in Non-Ureolytic (*B. cohnii*) and Ureolytic bacteria (*B. megaterium*) modified recycled aggregate, respectively. Both biological and bio-nanocomposite strengthening had obvious strengthening effects on the ITZ of the recycled aggregate. Figures 5 and 6 show the ITZ of natural aggregate concrete and the ITZ of recycled aggregate concrete after modification, respectively.

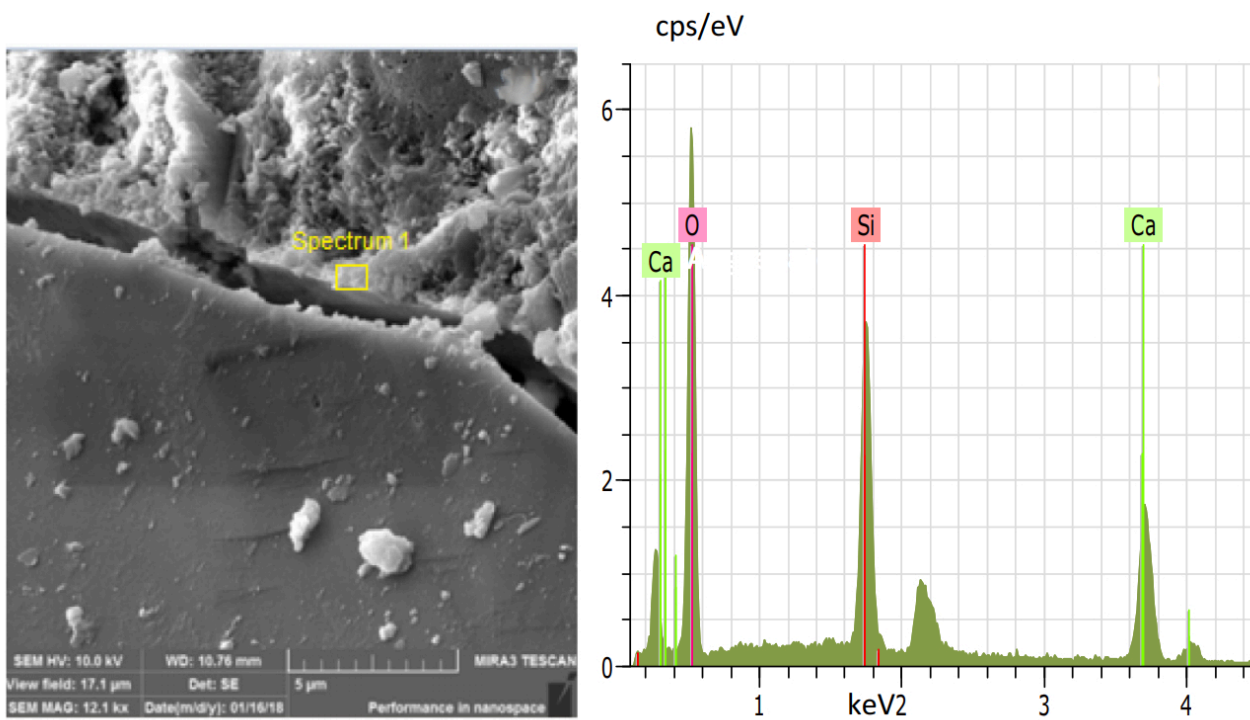


Figure 5. Microstructure diagram of interface transition zone of natural aggregate concrete [81]. Reprinted from Construction and Building Materials, Studies on performance enhancement of recycled aggregate by incorporating bio and nano materials, L.P. Singh, Vishakha Bisht, M.S. Aswathy, Leena Chaurasia, Sanjay Gupta, 2018, 181, 217–226, with permission from Elsevier.

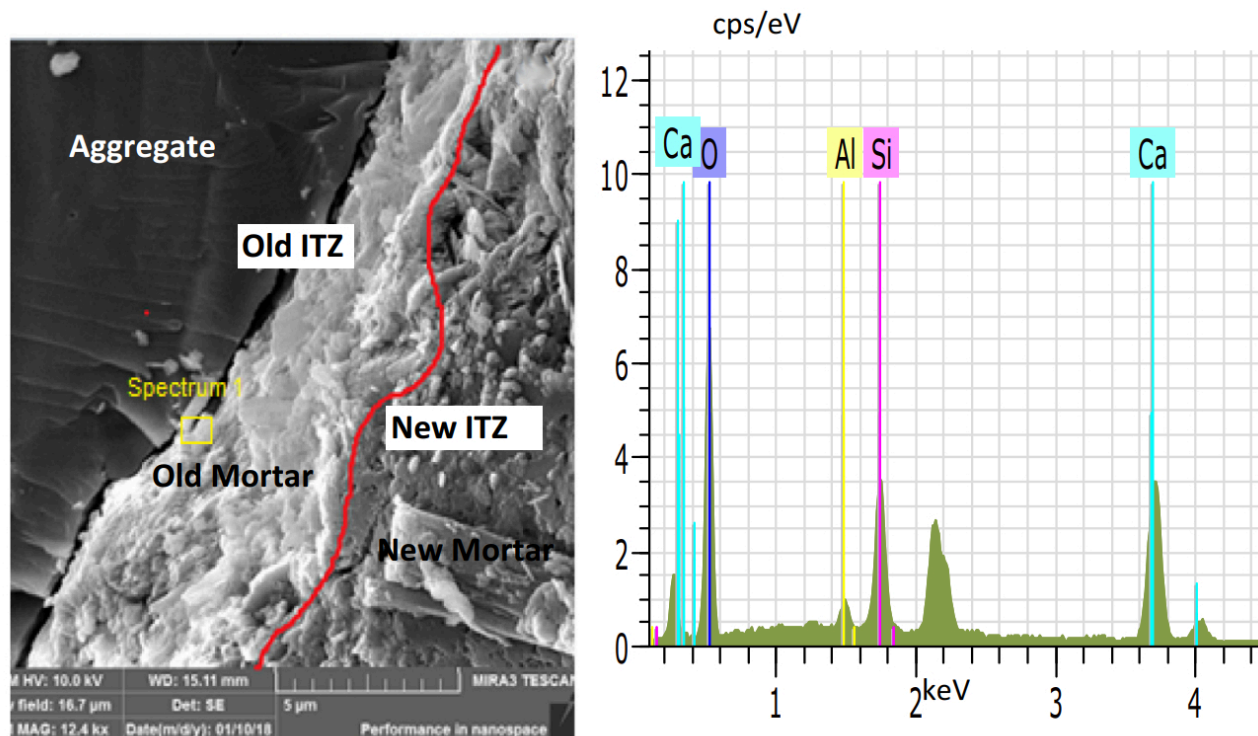


Figure 6. Microstructure diagram of interfacial transition zone of recycled aggregate concrete [81]. Reprinted from Construction and Building Materials, Studies on performance enhancement of recycled aggregate by incorporating bio and nano materials, L.P. Singh, Vishakha Bisht, M.S. Aswathy, Leena Chaurasia, Sanjay Gupta, 2018, 181, 217–226, with permission from Elsevier.

It can be seen from the figures that natural aggregate concrete had an ITZ with a width of 580 nm, while the transition zone between the recycled concrete aggregate and used mortar was only 170 nm. Energy dispersive X-ray spectrometry (EDX) also showed that the content of calcium and silicon was increased, which confirmed that the microstructure of the reinforced aggregate was denser.

A large amount of recycled fine aggregate can be produced in the process of recycling recycled aggregate. To date, most physical or chemical strengthening treatment methods for recycled fine aggregates have not obtained optimistic results. When adding microorganisms to modify recycled aggregates, the experimental data on the enhancement of regenerated aggregate by MICP technology may be optimistic. Take the research results of Xu et al. [82] as an example, When they explored the effect of microbial mineralization deposition on the regenerated fine aggregate, they found that compared with the control group, the compressive strength and flexural strength of recycled mortar made of recycled fine aggregate treated by microorganisms were generally improved. The 28 days compressive strength and flexural strength of the recycled mortar specimen increased by 27.78% and 34.59%, respectively. The nanoindentation results show that the elastic modulus and microhardness of the interfacial transition zone can be increased by more than 100%. These studies indicated that the MICP technique also has great potential for enhancing reclaimed fine aggregates.

Zeng et al. [49] studied the influence of microbially induced carbonate precipitation on the performance of recycled aggregate concrete and modified the concrete model by using different calcium source addition methods and rotating treatment methods. These coworkers suggested that regenerated aggregates with better performance can be obtained by soaking them in bacteria, modifying them in a sterile medium, and then adding calcium ions in batches. When the modified aggregate was used in concrete, the compressive strength of the modified aggregate concrete was increased by 6.3% compared with that of the unmodified aggregate concrete. The results of the microhardness test in the ITZ showed the reason for the improvement in mechanical properties. For recycled aggregate concrete without modification, the microhardness of the new and old transition zones is similar, but for recycled aggregate concrete after modification, the microhardness of the new ITZ is approximately 16.5% higher than that of the old ITZ. This increase indicated that the mineralized precipitation of microorganisms could provide a more favorable binding force between the reclaimed aggregate and cement-based materials. Xu et al. [50] tested the durability of modified concrete when exploring the coupling effect of carbonation and bioprecipitation in concrete surface treatment. These authors found that the resistance to chloride ion penetration of MICP-modified concrete increased by 6.2%, while carbonization followed by the MICP technique increased the resistance to chloride ion penetration by 10.5%. Muynck et al. [44] believed the reason for the improved ability to resist chloride ion penetration was that on the one hand, the density of the aggregate surface precipitation of calcium carbonate crystals could effectively reduce the capillary water absorption rate and gas permeation. On the other hand, irregular calcium carbonate crystals covering the aggregate surface could improve the bonding force between the aggregate and cement based materials. Therefore, the performance of recycled concrete could be improved.

To intuitively describe the modification effect of the MICP technique on recycled aggregate, Table 1 compares several cases of recycled aggregate before and after strengthening:

Table 1. Effect of reinforcement treatment on properties of reclaimed aggregate.

Classification of Aggregate	Strengthening Effect										References
	Recycled Aggregates					Recycled Mortar and Recycled Concrete					
	Bibulous Rate (%)		Porosity (%)		Percentage Increase in Mass (%)	Compressive Strength (MPa)		Flexural Strength (MPa)		Percentage Reduction of Chloride Ion Permeability Coefficient (%)	
	Before	After	Before	After		Before	After	Before	After		
Coarse aggregate	5–5.5	4.5–5	15	10	0.8	44–54	51–76	-	-	6.2–10.5	[50,65,72]
Fine aggregate	24	16.5	-	-	2.7	29 12	38 13.7	2.65 1.8	3.6 3.32	-	[49,82,83]

A large number of studies showed that MICP technology used to strengthen recycled aggregate could achieve a significant strengthening effect, and the properties could be significantly improved whether coarse aggregate or fine aggregate was used. However, it should be noted that both the mechanical properties and durability of recycled aggregate concrete (mortar) also need to be further improved. Feng et al. [83] used *Sporosarcina pasteurii* bacteria to strengthen recycled fine aggregates, and the compressive strength and flexural strength of the recycled mortar increased by 14.3% and 84.5%, respectively. However, there is still a sharp difference between the compressive strength and flexural strength of natural mortar. The flexural strength was approximately 39.6% of natural mortar, and the compressive strength was only approximately 28.5%. Similarly, the research results of Muynck et al. [43] on the durability of concrete also indicated that although the MICP technique could effectively improve the chloride ion penetration resistance of recycled concrete, it is still difficult to apply it to structural buildings. At the same time, a large number of studies have shown that the optimal period for the MICP technique to achieve a significant strengthening effect on recycled aggregate is soaking in nutrient solution for continuous mineralization for 14 days (at least 7 days) [32,47,81]. The long period of strengthening also greatly limits the application of the MICP technique.

6. Conclusions and Prospect

This paper reviewed the influencing factors, strengthening mechanism and effectiveness, and advantages and disadvantages of using the MICP technique to strengthen recycled aggregate. Through a systematic and comprehensive analysis of numerous studies, the following conclusions were drawn:

1. As a relatively new and popular strengthening method in recent years, the MICP technique for modifying recycled aggregate is favored by researchers due to its environmental protection and low energy consumption performance. This technique can obtain a good result for improving the properties of recycled aggregate and concrete, especially microproperties. However, its mineralization period is too long, and almost all studies have shown that it takes more than 7 days to achieve significant effects [48,81]. It is difficult to reduce the cost of a culture medium due to the high requirements of a biological culture [45]. All of these factors have limited the development of this technique.
2. Microbial mineralization and deposition occur under the combined action of many factors, such as bacteria, calcium source, medium composition, pH value, nucleation center location, and temperature. At present, studies on modified aggregates mostly focus on the influence of calcium source, medium composition and pH value. In fact, as a process of biological growth and metabolism, comprehensive exploration of the optimal range of influencing factors should be the first step before the modification of aggregates. For example, when selecting different strains, the influence of different pH values on the experimental results should be determined to achieve the best precipitation effect. In particular, there is still no good solution for fixing the nucleation site on the weak spot of the aggregate surface, and the influence of these factors should be considered first to obtain a better modification effect.
3. The mechanism of mineralization precipitation of different types of bacteria is slightly different. Generally, it is the process of generating calcium carbonate. When using the MICP technique to modify recycled aggregate, researchers should choose the most suitable strain according to their own needs and laboratory conditions to achieve the best modification effect.
4. According to current studies, it is difficult to overcome the disadvantages of using the MICP technique, such as consumption of time and lack of pertinence, to strengthen recycled aggregates. In future studies, researchers could try to combine some physical or chemical strengthening methods with the MICP technique for composite strengthening of recycled aggregate according to the mechanism and the advantages and

disadvantages of different strengthening methods, which may be the development tendency of reinforcing recycled aggregate in the future.

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