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Research Article

The Influence of Microbial Agent on the Mineralization Rate of Steel Slag

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Bacteria-based mineralization is a new technique to use the steel slag. In this article, an experimental examination was performed to find out the steel slag advancement by the addition of the microbial agent that has the possibility to accelerate mineralization ability of bacteria. It is observed that, under natural and CO_2 pressure curing conditions, the carbonation rate is significantly raised when microorganisms are added to the steel slag. The increased ratio of microorganisms leads to a better carbonation rate. The reaction products formed by bacteria mineralization were analyzed with the scanning electron microscope (SEM) and X-ray diffraction (XRD), and the amount of reaction products was examined by thermogravimetric analysis. The results show that the compressive strength and carbonation speed rose with the increase in microorganism content. Bacterial could accelerate the rate of carbon sequestration in the mineralization process. The compressive strength of steel slag with 1.5% bacterial could reach up to 51.5 MPa. The micron-sized and roughness mineralization product induced by microorganisms apparently resulted in a denser and compacted structure. The carbon depth increased by 50%, and the content of calcite increased by 3 times. These mineralization products would fill in the pore of steel slag cementitious materials and form the integrated and denser structure which produces more strength.

1. Introduction

Steel slag is rich in both silica and calcium and is a by-product of a process involving high temperature [1]. It could be used as a calcium silicate value-added source. Attempts have been made to use steel slag as a cementitious additional material in cements [2]. Different from ground-granulated blast-furnace (GGBF) slag, the steel slag is not hydraulic or pozzolanic [3]. This is because it is deficient of tricalcium silicates and amorphous SiO_2 components. Contrarily, steel slag has also demonstrated high reactivity to CO_2. This makes it an appropriate slag in mineral sequestration—among the methods suggested for reducing carbon emissions [4]. Steel slag can be stimulated by carbon dioxide to make a strength-contributing carbonate bond matrix [5]. C_3S and C_2S in steel slag could react with the carbon dioxide, encouraging a faster strength increased through the generation of calcium silicate hydrates (CSH) and carbonates ($CaCO_3$) [6].

The investigations of carbonated steel slag for production are inadequate. The compressive strength and immense density of porous slag blocks that were generated by carbonated steel slag curing for about twelve days are 18.4 MPa and 2.4 g/cm^3 [7]. Unconfined compressive strength of carbonated compact by hard-pressed ground slag which contacts CO_2 at a pressure of three bars was 9 MPa [8]. Electric arc furnace slag also could be stimulated by CO_2; the compress strength could accomplish 17 MPa, and its carbon uptake was 11% in 2 h carbonation [9]. When the slaked lime is added to the steel slag, the carbonated steel slag and slaked lime mixed specimen under 0.3 MPa CO_2 curing condition acquires 22.7 MPa compressive strength and 5.3 MPa flexural strength correspondingly [10].

Various microbes in the nature can accomplish the interconversion between CO_2 and $CaCO_3$ [11]. The adaptation from CO_2 to $CaCO_3$ can be used to catch CO_2 in atmosphere. Carbonic anhydrase (CA) could accelerate the interadaptation of CO_2 and HCO_3^- to progress the absorption of CO_2 [12]. Thus, CA can help in the seizure of CO_2 and precipitation of $CaCO_3$. Qian et al. have studied the properties of steel slag such as strength and carbon uptake which were mainly investigated. The result of the research shows that biomineralization could significantly improve the strength and carbon uptake.

In this investigation, one type of bacteria which can generate carbonic anhydrase was included in steel slag-based substances to generate steel slag. Compared to other carbonated steel slag, the bacteria can encourage the inter-adaptation between carbon dioxide from the curing environment and $CaCO_3$. This means that CO_2 might be transmitted to minerals precipitated in pore when reacting fast with soluble Ca^{2+}. The bacteria illustrated exceptional capability to encourage the speed of mineralization.

2. Experiment

2.1. Raw Material. The density of steel slag is 3100 kg/m^3, and the specific area is 510 m^2/kg. Steel slag powder is from Baoye Slag Comprehensive Development Co. Ltd. $Ca(OH)_2$ is from Shanghai Ling Feng chemical agent Ltd. The chemical component of steel slag and slaked lime is shown in Table 1. The particle size of the steel slag was less than 200 μm.

The XRD pattern of steel slag is shown in Figure 1. The main mineral phases in the steel slag are C_2S, C_3S, C_3A, and a large amount of Fe_3O_4 and RO phase which has no hydration performance, so the hydraulic activity of steel slag is rather low.

The modulus of river sand, which was chosen as the aggregate, was 2.34, and the bulk density was 1490 kg/m^3. CO_2 used in the experiment that creates a carbonation environment is of 99% concentration which is produced in the Nanjing Shangyuan industrial gas plant.

2.2. Microbial Preparation. Bacillus mucilaginous used in this experiment was acquired from China Center of Industrial Culture Collection (CCIC). *Bacillus mucilaginous* was cultured in sucrose culture media. The content of purified medium of the strain is shown in Table 2; the pH value adjusted by NaOH is about 8.0. Place the purified medium in the triangle bottle, and sterilize at 121°C for 25 min. Remove the sterilized triangle bottle, and place it in the oven to dry. The purified bacterial strains of carbonic anhydrase were inoculated in the culture medium and cultured in the oscillatory incubators at 30°C for 24 h; the oscillation frequency of the oscillatory incubators was 170 r/min. The culture had an OD600 value of 1.2, the number of bacteria is about 2.62×10^8 cfu·ml^{-1}, and the enzyme activity value is 0.9 U·mmol·L^{-1}. Then, the harvested microorganisms were kept in a refrigerator at 4°C as a stock culture until the microbial was used.

2.3. Sample Preparation. 5 mix proportions by varying the bacterial ratio are given in Table 3. The water/binder (SS + SL) ratio and sand/binder ratio were fixed at 0.5 and 2.0, respectively. The raw materials were mixed with water for 4 min first and then were cast into moulds of 40 mm × 40 mm × 160 mm. All specimens were demolded after 24 h curing at 70% RH and 20°C. After completing the 1 d hydration curing, samples were cured under different conditions. The standard group samples were cured in 70% RH and at 20°C for 72 h. The carbonation group samples were cured in 0.3 MPa CO_2 curing for 4 h and then were cured in 70% RH and at 20°C for 68 h. The CO_2 concentration in the environment used for curing the standard samples is 450 ppm.

The schematic graphic expression of the mineralization setup is shown in Figure 2. The setup includes the compressed cylinder with 99% purity CO_2 gas, a carbonation chamber, a pressure transducer, and a pressure regulator. The pressure transducer monitors gas pressure, and the regulator could control the chamber pressure at the set value throughout the mineralization process.

2.4. Test Methods. The compressive strength and flexural strength were measured according to Chinese test methods for Portland cement (GB175-2007) [13]. Each property was tested in six samples after curing, and the experimental data were averaged.

Table 1: Chemical compositions (wt.%).

Component	CaO	SiO_2	Al_2O_3	MgO	Fe_2O_3	P_2O_5	SO_3
Steel slag	43.5	13.2	9.2	5.6	17.2	7.4	0.4
Slaked lime	99.8	—	—	—	—	—	—

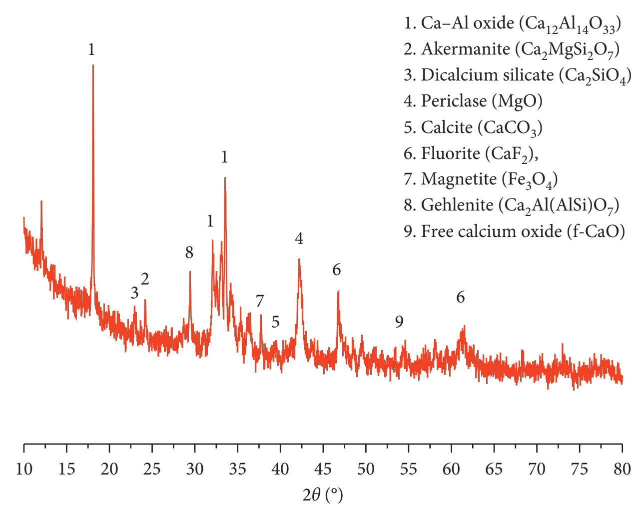


Figure 1: Mineral composition of steel slag.

Table 2: The content of purified medium of the strain.

Sucrose	$NaHPO_4 \cdot 12H_2O$	$MgSO_4$	$CaCO_3$	KCL	$(NH_4)_2SO_4$	Yeast extract
10.00	2.50	0.25	1.00	1.00	0.50	0.20

Table 3: Mix proportions (g).

Mix	SS	SL	Water	Aggregate	Bacterial	Curing
S1	525	105	315	1250	0	Standard
S2	525	105	315	1250	3.15	Standard
S3	525	105	315	1250	6.3	Standard
S4	525	105	315	1250	9.45	Standard
SP1	525	105	315	1250	0	Carbonation
SP2	525	105	315	1250	3.15	Carbonation
SP3	525	105	315	1250	6.3	Carbonation
SP4	525	105	315	1250	9.45	Carbonation

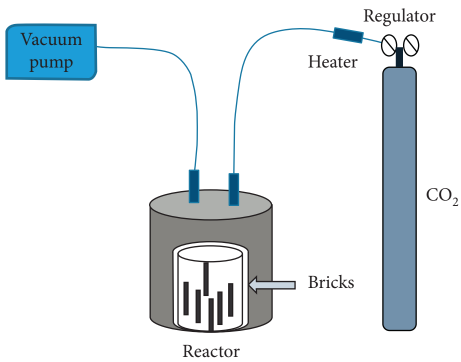


Figure 2: Schematic graphic expression of the mineralization setup.

The micromorphology parameters were measured by using FEI Sirion scanning electron microscope (SEM), mineralogical compositions analysis was conducted by using Bruker D8-Discover X-ray powder diffractometer (XRD), pore structure analysis was conducted by using Micromeritics Autopore IV 9510 mercury porosimetry (MIP), and the thermal analysis on the specimen was conducted by NETZSCH STA449F3 simultaneous thermal analysis meter (DTA/TG), respectively. Ca^{2+} concentration in the supernatant was determined according to the study of Stocks-Fischer et al. [14]. The OD_{400} value was measured with a spectrophotometer (UV-6000PC) every 2 h to characterize microbial growth and reproduction efficiency.

3. Results and Discussion

3.1. Microbial Activity. The OD_{400} value was measured with a spectrophotometer (UV-6000PC) to characterize microbial growth and reproduction efficiency in the in steel slag. The simulated pore solution of steel slag materials is shown in Table 4, and the pH was adjusted to 13.

The OD_{400} value of the bacterial liquid in the simulated pore solution of steel slag materials was tested with time, and the test results are shown in Figure 3. The change of the OD_{400} value directly reflects the amount of bacteria. The initial OD_{400} value is 1.22 in the simulated pore solution of steel slag materials. From 0 to 60 h, the OD_{400} value decreases slowly, and from 60 to 100 h, the OD_{400} value declined accelerated. But at 100 h, the OD_{400} value was still around 1.0. The value dropped less than 20%. *Bacillus* has good adaptability to the high alkaline environment. It can satisfy in the alkaline steel slag materials environment with the internal pH of more than 13.

3.2. Acceleration of Calcium Ion Deposition. Figure 4 shows the change rule of Ca^{2+} concentration with reaction time during the bacterially induced calcium carbonate deposition. The change of calcium concentration in solution directly reflects the formation rate of carbonate ions and the rate of carbonate mineralization. The initial concentration of Ca^{2+} was 50 mmol/L in the simulated pore solution of steel slag materials. In the control test, the calcium ion concentration remained basically the same, there was no carbonate ions

Table 4: Simulated pore solution of steel slag materials.

$Ca(OH)_2$	NaOH	KOH	K_2SO_4	pH
0.05 mol/L	0.25 mol/L	0.54 mol/L	0.003 mol/L	13.54

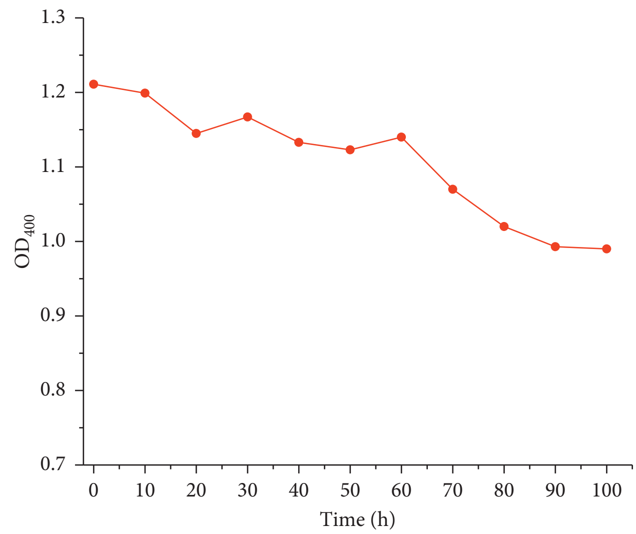


Figure 3: Survival capacity of bacterial in simulation of pore solution of steel slag materials.

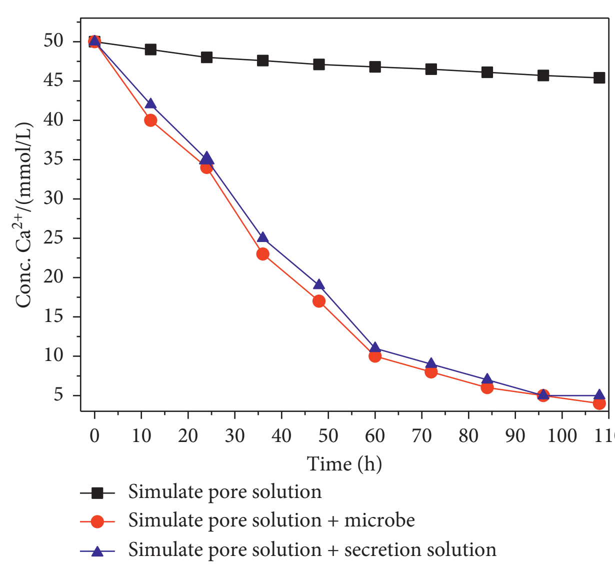


Figure 4: Change of Ca^{2+} concentration with time.

formed in the simulated pore solution of steel slag materials, the carbon dioxide hydrate reaction rate was low, and it is hard to generate more carbonate ions in a short period of time and unable to form mineral deposits. Microbial secretion is obtained by centrifugation of the microbial culture medium, and the microbial cells were removed. The calcium ion concentration decreased significantly under the effect of microorganism, and the calcium ion basically completely reacted at about 90 h. The carbon dioxide hydration reaction rate increased significantly, more carbonate was generated in a relatively short time, and carbonate mineral deposition was generated when reacting with calcium ions. The decrease rate of calcium ion concentration in the culture medium was slightly higher than that in the secretion, and the bacteria with a certain negative charge were first coupled with free Ca^{2+} in the solution, which was regarded as nucleation site and promoted mineral deposition [15].

Figure 5 show the calcium carbonate deposition induced by bacterial in the simulated pore solution of steel slag. The surface and inside of calcium carbonate accumulates with a large number of bacteria. The bacteria exist in the steel slag with certain negative charge, and the bacteria could provide a nucleation site for mineralized deposition, and the spheroid and crystallinity of calcite are formed in steel slag. Meanwhile, bacteria could generate a particular enzyme which can accelerate the generation of carbonate anions through appropriate enzymatic action, so the total amount of $CO_3{}^{2-}$ and internal mineralization product generated speed was higher. Bacteria accelerate calcium ion deposition obviously.

3.3. Strength. The effects of the bacterial powder ratio on the strength of carbonated and uncarbonated steel slag are shown in Figure 6. The strength of uncarbonated samples increases with the bacterial powder ratio; when the adding quantity reached the maximum at the 1.5%, the compressive strength of S4 reached to 6.5 MPa; however, when the adding quantity is 0, the compressive strength of S1 is only 1.2 MPa. When 1.5% of bacterial powder is added, the compressive strength could improve about 5 times. The same tendency is observed in the case of carbonated steel slag. When the adding quantity reached 1.5%, the compressive strength of SP1 reached to 51.5 MPa, while the carbonated steel slag without bacterial powder was 33.8 MPa, the compressive strength improved about 50%.

3.4. Carbon Area. Obviously some area formed purple-red on response surface of mortar after phenolphthalein solution. Cross-section staining experiments were done after curing under different conditions immediately. The mineralization area of steel slag cementing materials is shown in Figure 7. The experimental results proved the promotion of mineralization by microorganisms, and bacterial has obvious effect on the mineralization of steel slag cementing materials. The mineralization area of steel slag with the bacterial was bigger than the samples under the same mineralization process only. Bacterial could obviously accelerate the rate of carbon sequestration of steel slag and the mineralization process. Meanwhile, the process of mineralization accelerated with the increasing of curing pressure, the speed growth particularly.

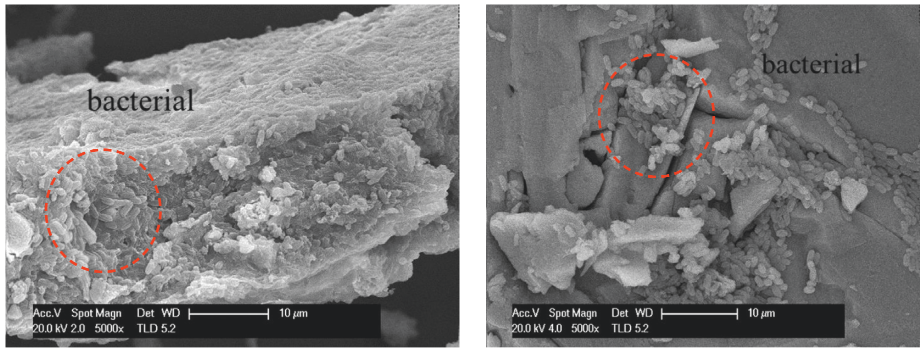
The effects of the bacterial on the carbide area are shown in Figure 8. The carbonation depth increases with the additive amount of bacterial. Under natural curing conditions, when the adding quantity reached up to 1.5%, the carbonation depth of S4 reached to 6.1 mm; however, the carbide area of S1 was only 2.3 mm. Carbonation depth could increase about 2 times. The same tendency is observed in the case of carbonation curing condition. When the adding quantity is 1.5%, the carbide area of SP1 could reach 18.3 mm; while the carbonated steel slag without bacterial was 11.8 mm, the carbonation depth improved about 50%. There was a positive correlation between carbonation depth and strength, and the increase ratio of carbonization depth is approximately equal to the increase ratio of strength.

3.5. Mineralogical Composition. The XRD patterns of microbial mineralized steel slag and carbide steel slag without microorganism can be observed in Figure 9. Constituent minerals $CaCO_3$, C_2S, gehlenite, and Fe_3O_4 were found in the XRD patterns. Carbon dioxide activated the converted part (C_2S and $CaOH_2$) into calcium carbonate. The major difference between XRD patterns of microbial mineralized and mineralized without microorganism is the intensity of calcium. The calcium diffraction peaks of microbial mineralized steel slag cementing materials were increased, and it indicates that the crystallization degree of calcium carbonate and magnesium carbonate is increased and that was the reason why the strength of microbial mineralized steel slag cementing materials is far higher than the mineralized steel slag cementing materials without microorganism.

3.6. Thermal Analysis. As shown in Figures 10(a) and 10(b), the TG curves display the total weight loss of the mixture of $Ca(OH)_2$ and $CaCO_3$. It demonstrates that certainly a number of characteristics peak and overall weight loss increased with the bacterial. As the figures show the TG curives of microbial mineralized steel slag and carbide steel slag. From the TG curve, two lightness peaks are experiential at approximately 400–550°C as well as 600–800°C, which represent dehydration of $Ca(OH)_2$ and de-carbonation of $CaCO_3$ correspondingly, in addition to their respective weight losses of 4.87%, 2.70% and 3.28%, 8.50%, respectively. The content of calcium carbonate is 6.1% and 19.3%, respectively, and the content of calcium carbonate increased by 300%. The principal strength of microbial mineralized steel slag source is the mineralization of $Ca(OH)_2$ as well as the calcium silicate under mineralization state. With the increase of CO_2 curing pressure, calcium utilization of mineralized steel slag also increased. The addition of microorganisms might significantly accelerate mineralization reaction. The chemical procedure of mineralization sequestration reaction might be illustrated as shown in the following equations:

$$\begin{aligned} CO_2 + H_2O &\longrightarrow H_2CO_3 \\ Ca(OH)_2 + H_2CO_3 &\longrightarrow CaCO_3 + 2H_2O \\ 3CaO \cdot 2SiO_2 \cdot 3H_2O + 3H_2CO_3 &\longrightarrow 3CaCO_3 + 2SiO_2 \\ &\quad + 6H_2O \\ 2CaO \cdot SiO_2 \cdot 4H_2O + 2H_2CO_3 &\longrightarrow 2CaCO_3 + 2SiO_2 \\ &\quad + 6H_2O \end{aligned} \tag{1}$$

3.7. Pore Structure Analysis. In the three-dimensional CT scan, different colors represent different pore size and the



(a)

(b)

Figure 5: Calcium carbonate deposition induced by bacterial in the simulated pore solution of steel slag.

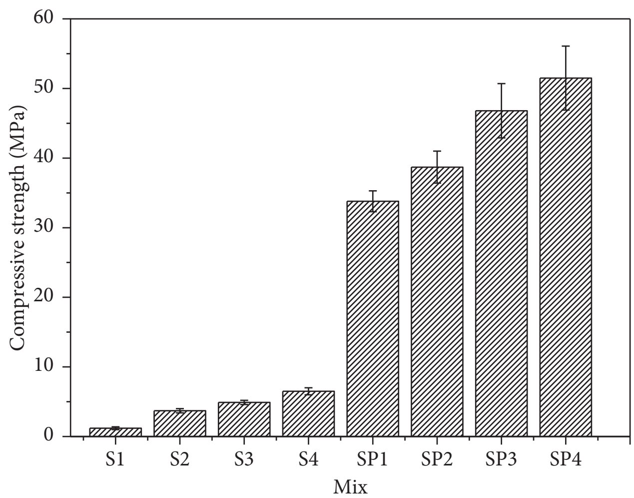
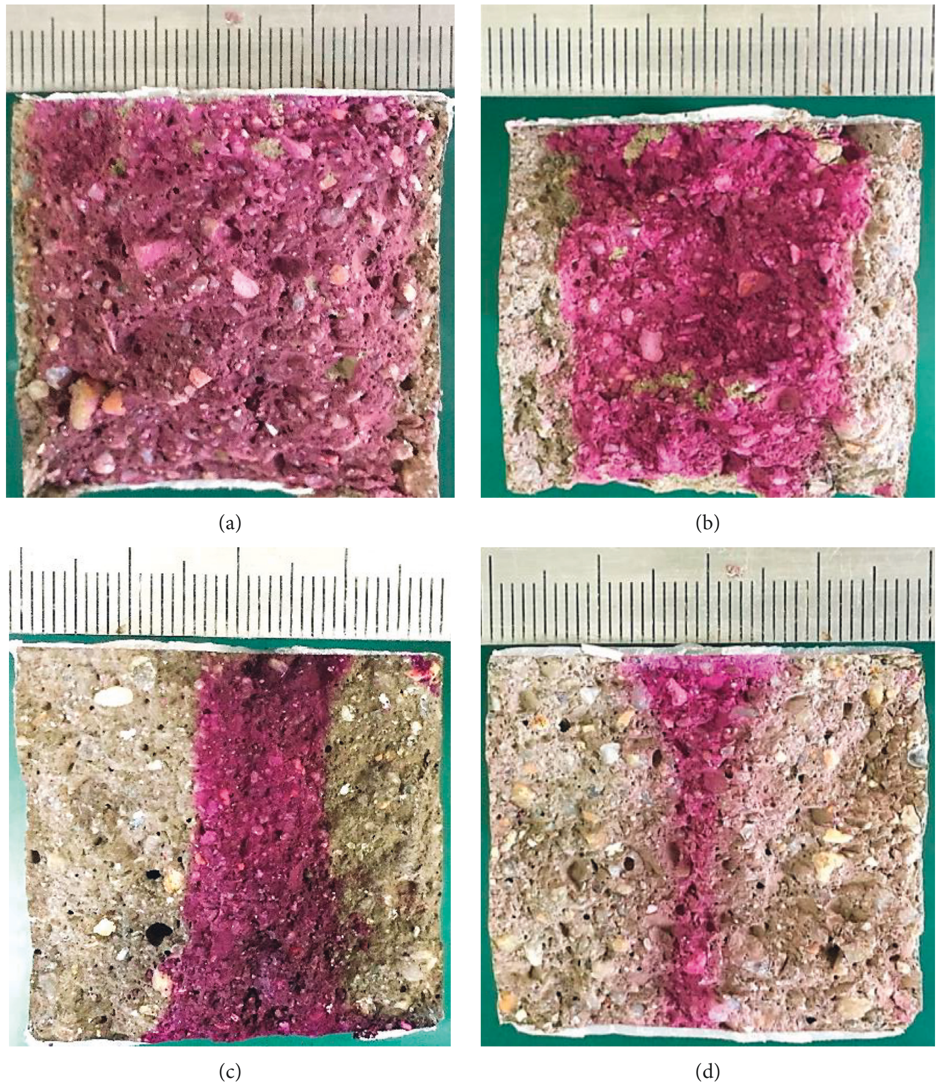


Figure 6: Strength of steel slag.

pore distribution. The results of pore size distribution showed the total porosity that the pore diameter was between 0.1 mm and 1 mm in Figure 11, and the samples of the natural curing group, carbonized group, and the microbial mineralization group were 1.25%, 1.39%, and 1.80%. Since the molar volume of calcium carbonate is 11.8% larger than that of calcium hydroxide, the calcium carbonate crystals will gradually fill the pores in the steel slag. The porosity inside the microbial mineralization group is lower; the pore size is relatively small, and the distribution is more uniform; the microbial mineralization optimized the pore structure. It can be seen from the figures that the porosity of the edge zone in the three groups is smaller than the internal part. In the microorganism mineralization group, the pore size distribution is the most uniform between edge and internal regions. The carbonated samples showed the uneven distribution and larger porosity.

3.8. Microstructure Analysis. SEM and EDS analyses of the samples curing under the carbonated condition are shown in Figures 12 and 13, and Table 5. As SEM images show, the overall evaluation of the morphology of the samples indicates a suitable and uniform distribution of particles of calcite ($CaCO_3$) crystal composites, the internal structure of carbonated steel slag with microbes is compacted, calcite crystal formed in high pressure arranges closely, crystallinity is better, and crystalline size is greater, which contributed high strength. EDS showed that, in the internal structure of carbonated steel slag, calcite distributed uniformly in the binding material which contributed the strength obviously. Meanwhile, there was hexagonal prism shape crystal formed in the samples; EDS showed that calcium hydroferrocarbonate ($C_3A \cdot FeCO_3 \cdot 11H_2O$) has formed during the hydration of carbonated steel slag, and it may contribute to the increase of the compressive strength in later ages [16].

The surface morphology parameters of calcite particles were observed by the atomic force microscope. The surface of calcite formed by the microbiological method and the carbonation method is show in Figure 14. Microbial mineralization production presents various textures with many grain boundaries and dislocations, and the surface is relatively rough. The enhancement of the strength of the steel slag cementitious materials by biomineralization not only the improves the mineralization rate, but also the difference of the morphology and surface state of the biomineralized products, which is also a reason for the enhancement of the strength of the microbial mineralized steel slag cementitious materials.



(a) (b) (c) (d)

Figure 7: Carbon area of steel slag. (a) S1. (b) S4. (c) SP1. (d) SP4.

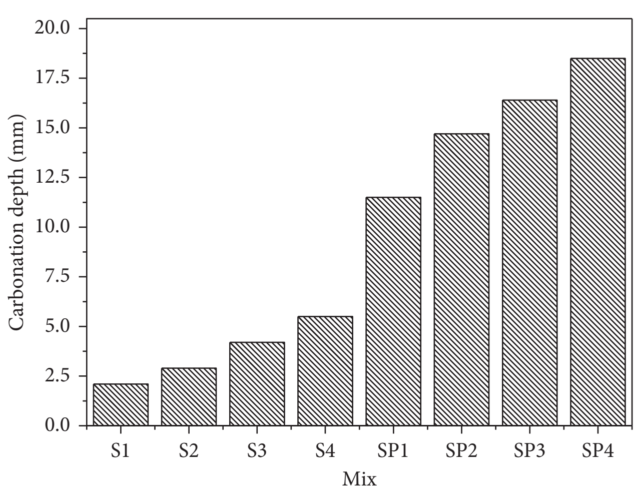


Figure 8: Carbonation depth of steel slag.

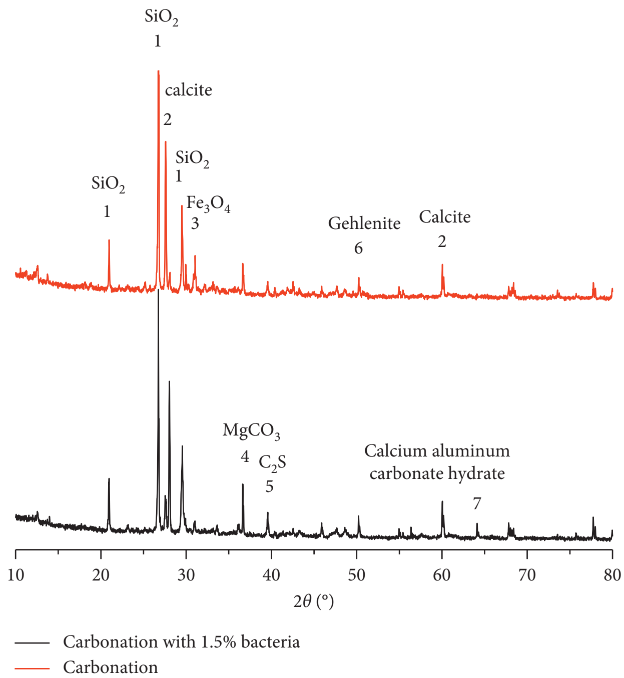
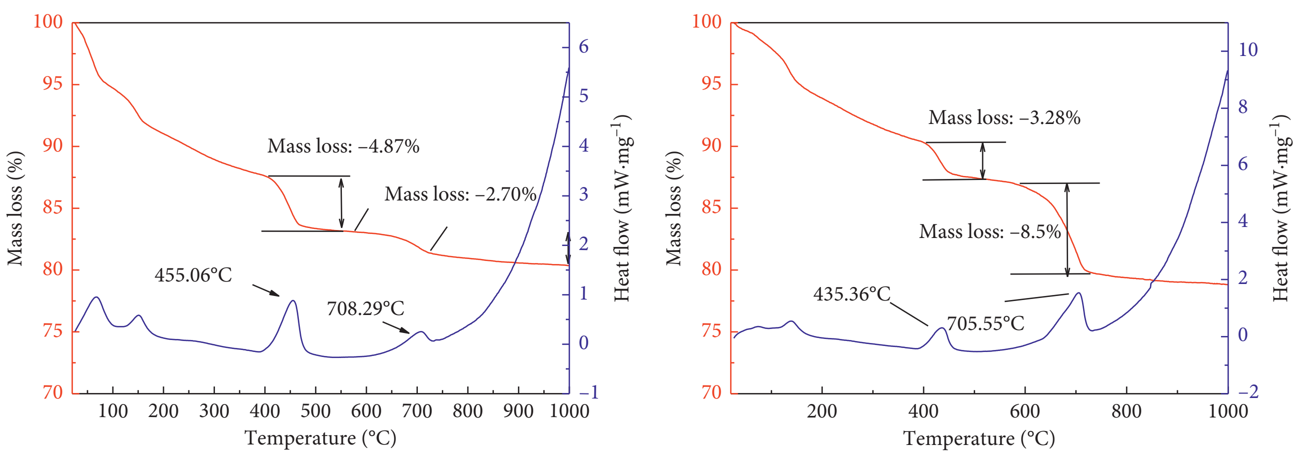
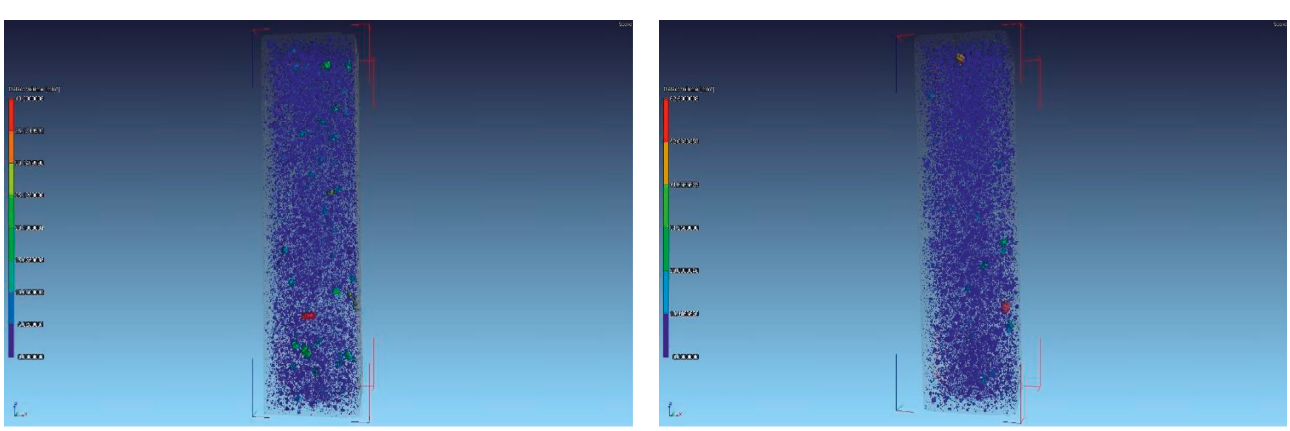


Figure 9: XRD patterns of mineralized steel slag.



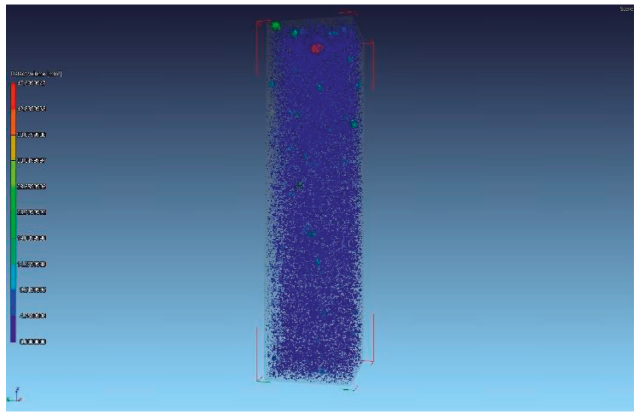
(a) (b)

Figure 10: (a) TG-DSC curve of steel slag under 0.3 MPa CO_2 curing for 4 h. (b) TG-DSC curves of microbial mineralized steel slag under 0.3 MPa CO_2 curing for 4 h.



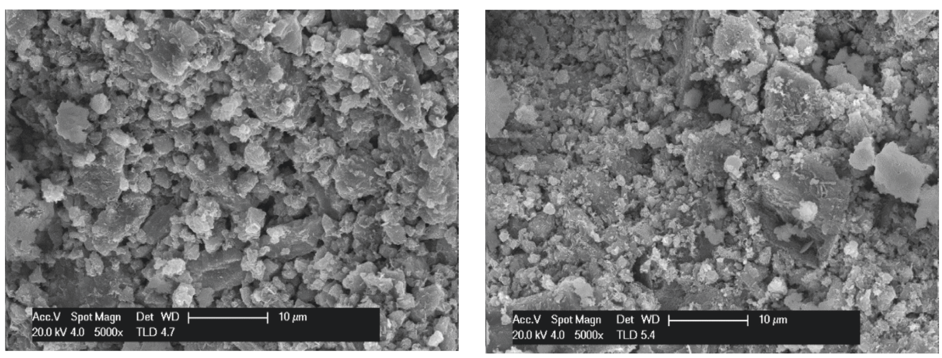
(a) (b)

Figure 11: Continued.



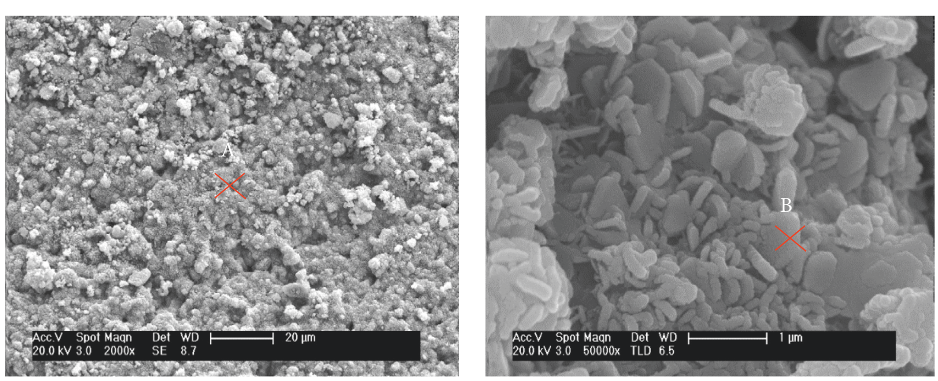
(c)

Figure 11: CT scan of steel slag cementitious materials. (a) Natural curing without microorganism. (b) Carbonation without microorganism. (c) Microorganism mineralization.



(a) (b)

Figure 12: The SEM image of steel slag. (a) Without bacterial. (b) With bacterial.



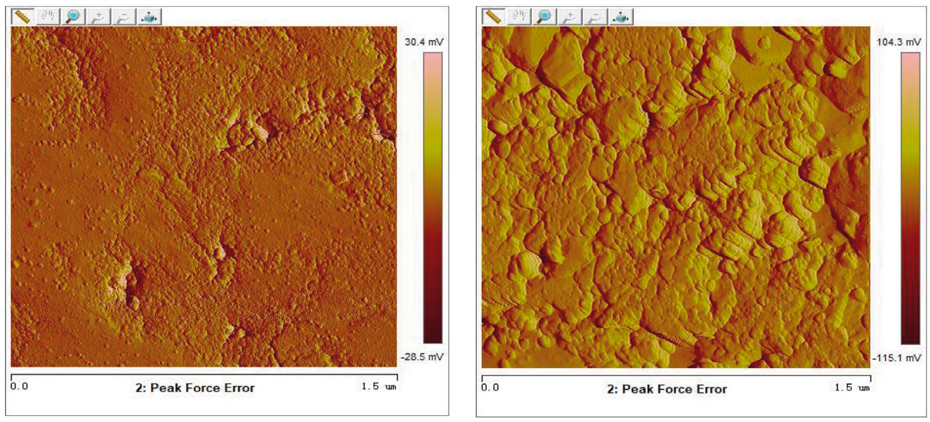
(a) (b)

Figure 13: EDS analysis of points A and B. (a) Calcium carbonate. (b) Calcium hydroferrocarbonate.

Table 5: Element content (%).

Element	Ca	Fe	O	C	Al	Si
A	45.74	3.75	35.91	14.55	0	0
B	22.02	14.56	41.41	13.29	4.84	3.87

3.9. Mechanism of Microbial Mineralization. The formation process of the mineralization product in steel slag cementing materials is shown in Figure 15. In the mineralization process of the steel slag cementing materials, the positively charged calcium ions are adsorbed on the surface of the



(a) (b)

Figure 14: The surface of calcite. (a) Carbonation. (b) Mineralization by microorganism.

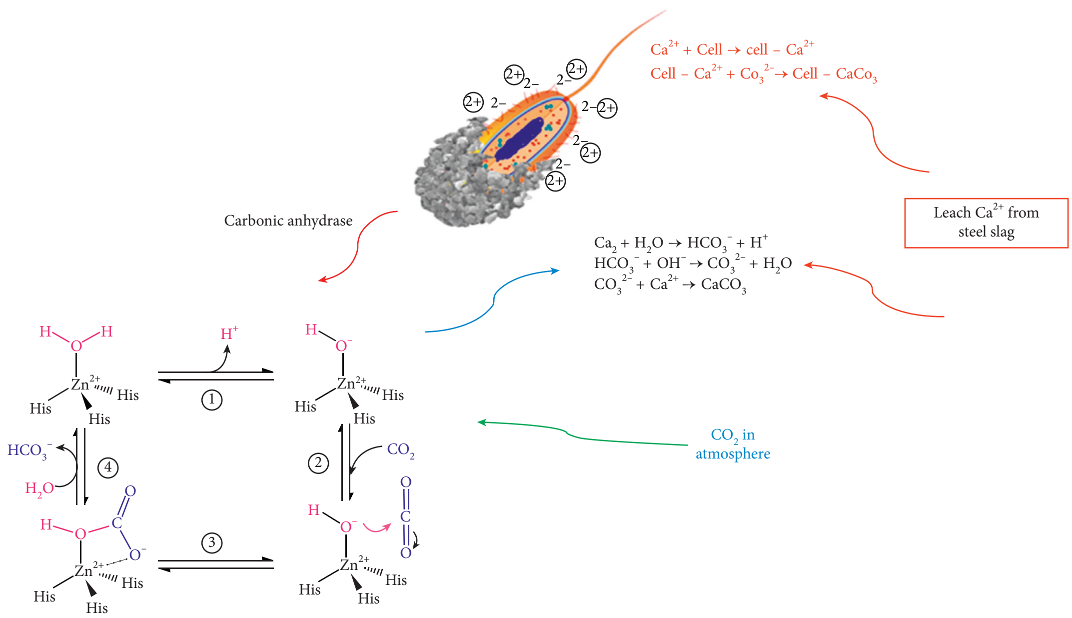


Figure 15: Formation process of biomineralization product in steel slag.

negatively charged microbial bacteria. Bacteria could provide the nucleation site for mineralized deposition, and the spheroid and crystallinity of calcite are formed in steel slag. Meanwhile CO_2 could diffuse and form CO_3^{2-} with water, and CO_3^{2-} would react with the free Ca^{2+} inside the samples and generate $CaCO_3$. After mineralization, these reaction products could fill in the pores, and the pore size is relatively smaller. Under the natural curing condition, the concentration of CO_2 gas in steel slag is low, and the formation rate of CO_3^{2-} is slower. The adding of microorganism in the steel slag could generate a particular enzyme [17] which can accelerate the generation of carbonate anions through appropriate enzymatic action [18], so the total amount of CO_3^{2-} and internal mineralization product generated speed were higher than those of the specimens without bacteria, so the strength of mineralization specimens was much higher [19].

4. Conclusion

The compressive strength of steel slag with 1.5% bacterial could reach up to 51.5 MPa. The micron-size and roughness mineralization product induced by microorganism apparently resulted in a denser and compacted structure, and the porosity reduced by 50%. The carbon depth increased by 50%, and the content of calcite increased by 3 times. There was a positive correlation between carbonation depth and strength, and the increase ratio of carbonization depth is approximately equal to the increase ratio of strength. These mineralization products would fill in the pore of steel slag cementitious materials and form the integrated and denser structure which produces more strength. The role of microorganisms is loading and acting as catalyst, and it could transport a continuous stream of carbon dioxide into the body inside the carbonization reaction; meantime, the secretion of the enzyme can significantly accelerate the hydration rate of carbon dioxide and form more CO_3^{2-} inside the steel slag. Ca^{2+} has a higher probability when combined with CO_3^{2-} to generate $CaCO_3$, and the compressive strength and carbonation speed of steel slag rised with the increasing of microbes. Bacterial obviously accelerates the rate of carbonation of steel slag in the mineralization process. Microbes could induce the deposition of $CaCO_3$ efficiently with the dissolution of Ca^{2+} in the steel slag-slaked lime mixture. The calcium silicates in steel slag and $Ca(OH)_2$ will mineralize the crystals into $CaCO_{3,}$ and the mineralization will fill in the gap to optimize pore structure and increase strength.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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