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Experimental Study on Coastal Sediment Reinforcement by Induced Carbonate Precipitation by Different Enzyme Sources

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Abstract: Coastal erosion is increasing worldwide due to the increasing frequency of extreme natural phenomena and excessive human exploitation. In this study, a small model experiment was conducted to investigate the solidification effects of three enzyme sources—soybean urease, freshwater *Bacillus pasteurella*, and seawater domesticated *Bacillus pasteurella*—on coastal sediments and their impacts in a seawater environment. The solidifying effect of different enzyme sources was determined by measuring the mechanical properties and corrosion resistance of the cured specimen model. The influence of solidified seawater in a seawater environment was obtained by measuring the changes in the pH value, calcium ion concentration, and ammonia nitrogen content of solidified seawater. The results show that different enzyme sources have a certain strengthening effect on coastal sediments. The mechanical properties of coastal sediments can be enhanced by increasing the amount of enzyme solution or level of solidification and can effectively resist simulated flow erosion. Comparing the reinforcement effects of different enzyme sources, it can be seen. It was observed that *Bacillus pasteurella* acclimated in seawater had better reinforcement effects than *Bacillus pasteurella* fresh water, and *Bacillus pasteurella* fresh water had better reinforcement effects than soybean urease. In the seawater measurement tests, the solidification of coastal sediments using different enzyme sources led to a decrease in the seawater pH value, and the acidification of seawater dissolved the generated calcium carbonate, increased the concentration of calcium ions in seawater, and produced ammonia nitrogen as a byproduct in the seawater. It was observed that, compared with the other two enzyme source solutions, the seawater-domesticated *Bacillus pasteurella* can better adapt to the high-salt environment of seawater, microbial metabolism is not inhibited, urea decomposition ability is improved, and calcium carbonate production is higher, which can effectively improve the engineering characteristics of coastal sediments and play a positive role in coastal protection and development.

Keywords: MICP; EICP; coastal sediment; anti-erosion; penetration resistance

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1. Introduction

In recent years, with the increase in sea level and the gradual deepening of coastal zone development, the artificial shoreline has gradually increased, and coastal erosion is becoming increasingly serious [1–4]. Coastal erosion is mainly caused by wave erosion and rainfall. Coastal consolidation can effectively reduce the adverse effects of coastal erosion, help restore coastal ecological environments, and improve coastal economic benefits. Traditional measures to address coastal erosion can be divided into four categories: hard structure protection, soft structure protection, land use restriction, and off-site sand response. These traditional measures mainly involve biotechnology-based coastal conservation, which is a soft structure protection measure and emerging technology. This technology is generally considered to be more durable, favorable, and environmentally

friendly than traditional physical and chemical curing technology, as well as more suitable for applications in the special coastal geographical environment [5–8]. At present, the most common approach to biotechnological coastal stabilization is a combination of artificial planting of coastline vegetation and traditional solidification methods [9]. In recent years, microbial-induced carbonate precipitation (MICP) technology has been proposed as a new solidification technology. MICP can be realized in various ways, such as autotrophic and heterotrophic, such as urea hydrolysis, sulfate reduction, and denitrification [10,11]. For soil stabilization applications, a great deal of current research has focused on urea hydrolysis by urea-soluble bacteria because they are widespread in soils, and the process is simple, as shown in Equations (1)–(6).

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+}, \quad (1)$$

$$(NH_2)_2CO + H_2O \leftrightarrow NH_3 + CO_2, \quad (2)$$

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-, \quad (3)$$

$$CO_2 + OH^- \leftrightarrow HCO_3^-, \quad (4)$$

$$Cl^- + HCO_3^- + NH_3 \leftrightarrow NH_4Cl + CO_3^{2-}, \quad (5)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3. \quad (6)$$

The surface of *Bacillus pasteurella* is normally negatively charged, constantly adsorbing Ca^{2+} from nutrient salts and causing it to aggregate on the outer surface; inside microbial cells will produce urease, urea that diffuses from nutrient salts into the cell is broken down, CO_3^{2-} is generated and transported to the cell surface. Due to NH_3 having a much higher solubility than CO_2, pH increases and calcium carbonate is formed when calcium ions are encountered, which is usually precipitated in the form of calcite [12–15]. Some researchers have also proposed directly extracting urease from soybeans to induce calcium carbonate precipitation, a process known as enzymatic-induced carbonate precipitation (EICP). Both of these methods have the advantages of simple construction, less pollution and less disturbance, and they have great potential for applications in the field of coastal foundation solidification. When solidifying coastal sediments, the use of seawater can reduce the cost of biomineralization compared with the use of fresh water to culture bacteria. Therefore, many scholars have explored the MICP process in seawater. In terms of feasibility, Peng et al. and Yu and Ou et al. [16–18] confirmed that the MICP process can also be carried out in a simulated seawater environment. However, when the seawater pH < 10 environments, it will not only inhibit the microbial activity but also inhibit the calcium carbonate production of MICP. In terms of curing methods, in a curing test of marine silt, Fu et al. and Zhao et al. [19,20] found that a mixing reinforcement method could simplify the strengthening process, and a cementing liquid with a low concentration and multiple rounds had better treatment effect. In terms of the influence of the seawater environment on MICP, Wang et al. [21] studied the influencing factors of MICP in a seawater environment and found that a high pH value could accelerate the reaction. Cheng et al., Silva et al., and Ansari et al. [22–24] studied the biomineralization products and mineralization efficiency of different strains under artificial seawater and seawater desalination conditions. At the same time, Xiao et al. [25] studied the domestication of *Bacillus pasteurella* in an artificial seawater environment and obtained strains that were better adapted to seawater. Liu et al. and Li [26–28] studied the effect of adding fibers into MICP on soil, and the results show that fiber reinforcement effectively increased the toughness of the sample. At the same time, scholars also began to study the seawater solidification process of EICP technology.

Zhou et al. [29] studied the extraction of soybean urease in EICP and determined that the optimal ethanol concentration and solid–liquid ratio were 30% and 1:1, respectively. Cao et al. [30] studied the mineralization method of soil reinforced by biostimulation combined with EICP, concluding that biostimulation combined with EICP significantly improved the strength of sand columns. Ahmed et al. [31] conducted EICP treatment on coastal sand and found that the urea hydrolysis rate in seawater-containing solution increased. This was speculated to be caused by the faster urease decay rate in the presence of seawater, but it had no significant impact on the carbonate content and UCS value of coastal sand. Kehinde et al. [32] compared the permeability of EICP and biopolymers (sodium alginate and guar gum) to silica sand, finding that sodium alginate was stronger than EICP or guar gum in reducing the permeability of silica sand. At the same time, researchers also explored the engineering applications of MICP and EICP, such as the study on their resistance to water erosion and rain erosion [28,33–36] sadas.

In conclusion, compared with traditional cement solidification, MICP or EICP can effectively protect the habitat of coastal organisms and reduce the negative impact of coastal buildings without causing significant or even destructive hardening in coastal sediments. To this end, the coastal sediments at the junction of sea and land in the Xiaodonghai area of Sanya were studied. Three urease sources—*Sporosarcina pasteurii*, *Bacillus pasteurella* domesticated in seawater, and soy urease—were selected to solidify coastal sediments. The coastal sediment samples were solidified using surface spraying, and a control group was established to compare the solidified effects of three enzyme sources on the coastal sediments. By monitoring pH, calcium ion concentration, and ammonia nitrogen concentration monitoring, as well as conducting coastal sediment anti-erosion and penetration resistance tests, the effects of three enzyme sources on the chemical composition of seawater, mechanical properties, and anti-erosion ability of coastal sediments were analyzed. The research results can be used as an important reference for future research on coastal erosion protection and bank slope reinforcement.

2. Materials and Methods

2.1. Sea Water

The seawater used for the experiment was collected from the area near the Dadong Sea, Sanya, China, with a pH value of 8.25 and a salt content of 3.6%. Longitude and latitude: 109.50047, 18.22254, located in the intertidal zone. The main ionic components of seawater are shown in Table 1 [21].

Table 1. Ion composition and concentration.

Major Ion	Concentration/(mg·L^{-1})	Major Ion	Concentration/(mg·L^{-1})
Ca^{2+}	426.53	Ba^{2+}	0.12
Mg^{2+}	1219.86	Cl^{-}	18,690.10
Na^{+}	11,078.58	HCO_3^{-}	169.01
K^{+}	410.16	SO_4^{2-}	2769.10

2.2. Physical Characteristics of Coastal Sediments

The coastal sediments used in the test were taken at the coast near Xiaodonghai, Sanya, China. The main strata found at the sampling site are as follows. The coastal sediments are composed of silt, sand, alluvial sand, soil layer, etc., and the basic physical characteristics of the coastal sediments are determined by referring to the Geotechnical Test Regulations (SL237-1999) [37]. Figure 1 shows the grain gradation curve of the coastal sediments, the uneven coefficient, and the curvature coefficient of the coastal sediments, which are extremely poorly mixed.

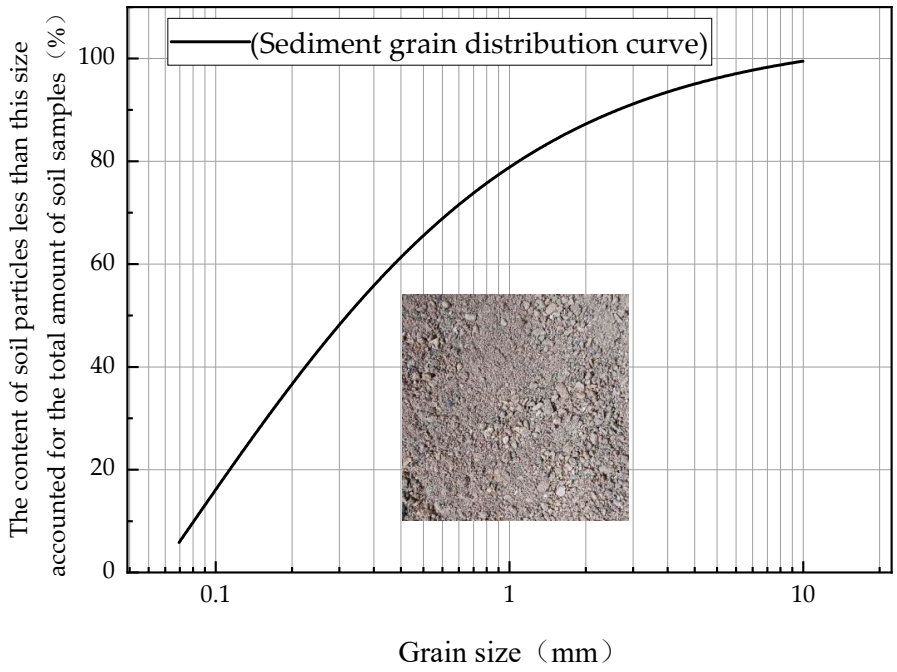

Figure 1. Deposit sediment photo and particle gradation curve.

2.3. Microbial Culture and Soybean Urease Preparation

Escherichia coli ATCC25922 (*Bacillus pasteurella*), purchased from the Microbial Culture Collection Center of Guangdong Province, was selected as the test strain. This is a chemoheterotrophic Gram-positive bacteria and is the most commonly used bacteria in MICP applications in the field of geotechnical engineering. After activation, the bacteria were added to the liquid medium for culture. The medium composition was (per 1000 mL deionized water) urea 20 g (purchased from Aladdin Ltd, Shanghai, China.), peptone 15 g (purchased from aobox biotechnology, Inc, Beijing, China.), soy peptone 5 g (purchased from aobox biotechnology, Inc, Beijing, China.), sodium chloride 5 g (purchased from Xiya Chemical Technology Co., Ltd, Shandong, China), and *NaOH* solution (purchased from Xiya Chemical Technology Co., Ltd., Shandong, China.) was used to adjust the pH of the medium to 7.2–7.5. To ensure suitable for bacterial growth, the medium was placed into the autoclave, sterilizing at 121 °C for 30 min. The medium was incubated at 220 r/min at 30 °C for 36 h. The concentration and urease activity of the bacterial solution was measured.

According to Xiao et al.'s experimental method [25], a three-gradient domestication of the bacterial solution was carried out: 1 mL of *Bacillus pasteurellosis* in conical flask A was added to conical flask B (containing 100 mL of acclimatization medium with 1/3 seawater concentration), and the *Bacillus pasteurellosis* was then removed for use after 36 h of cultivation. Next, 1 mL of the bacterial solution from cone flask B was added to cone flask C (containing 100 mL of acclimatization medium with a concentration of 2/3 seawater) and removed after 36 h of culture for later use. Approximately 1 mL of bacterial solution from cone flask C was added to cone flask D (containing 100 mL acclimatization medium with seawater concentration) and then removed after 36 h of culture, thus completing the three-gradient acclimatization. At this time, the bacteria in cone flask D were the three-gradient acclimated bacteria. Similarly, the concentration of the bacterial solution and the urease activity of the bacterial solution was measured after removal.

The method of soybean urease extraction was as follows: 200 g dried commercial soybean was weighed on a balance, crushed into powder by a wall breaker, dissolved in 1000 mL seawater, fully stirred, and left for 2~3 h. The supernatant in the beaker was filtered through gauze and centrifuged at 3500 r/min and 4 °C for 10 min. The supernatant in the centrifuge tube was the soybean urease solution to be extracted. Finally, the extracted soybean urease solution was stored at 4 °C for later use.

2.4. Determination of Absorbance of Bacterial Solution and Activity of Enzyme Source

Absorbance of bacterial solution was determined using a spectrophotometer (600 nm wavelength), and the concentration of bacterial solution was expressed according to OD_{600} value [14]. The OD_{600} value of the freshwater bacterial solution was 1.203, and the OD_{600} value of the seawater three-gradient acclimated bacterial solution was 1.676 (the seawater was used to zero the measurement).

The enzyme activity was measured by the Thunder Magnetic PXS-270 (conductivity meter) to determine the ability of the bacterial solution to hydrolyze urea. Then, 5 mL bacterial solution was mixed with 45 mL 1.1 mol/L urea solution, and the conductivity meter was used to measure the change in the conductivity of the solution (measured for 5 min). The average conductivity change value for the measured 5 min was multiplied by the dilution ratio (10 times). According to the experimental conclusion of Whiffin [38], the conductivity change in 1 mS/(cm·min) corresponds to the urea hydrolysis amount of 11.1 mmol/min, and the urea hydrolysis amount of bacterial solution or soybean urease per minute (mmol/min) is obtained, which is the initial enzyme activity of bacterial solution or soybean urease. The conductivity change was 7.11 mmol/(L·min) in freshwater bacterial solution, 7.14 mmol/(L·min) in seawater bacterial solution, and 8.22 mmol/(L·min) in soybean urease. To make the results of the three enzyme source treatments comparable, the soybean urease solution and freshwater bacterial solution were diluted so that the urease activity in the three enzyme source solutions was equal.

2.5. Coastal Sediment Solidification and Sea Water Measurement Test

(1) Coastal sediment filling; acrylic material is used to make a sub-regional scour model. Its length is 38 cm × 10 cm × 26.2 cm × 26.2 cm, and its tilt angle is 30° (each groove length is 9 cm × 7.5 cm × 11.5 cm). The flow rate of the circulating water pump was set at 160 L/h. The bottom water flows out from the top layer after passing through the water pump, successively scours each area, and then re-enters the bottom layer. In order to facilitate coastal sediment compaction and the spray of bacterial solution or soybean urease during solidification, the model is tilted so that the finish is level and kept level during scour. The dried coastal sediment was weighed with a balance of 500 g in two layers, added to the model tank, and compacted to a height of 5.5 cm. This step was repeated for the remaining two tanks so that the mass and volume of the coastal sediment in each group were the same.

(2) Enzyme source and nutrient salt spraying: Factors that impact the curing effect are very complex. In this study, after the activities of the three enzyme sources are unified, the relationship between enzyme source dosage and curing days (nutrient salt spraying times) is studied, as shown in Table 2.

Table 2. Experiment scheme.

Batch	Sample Number	Spray Quantity of Freshwater Bacterial Solution mL	Amount of Seawater Acclimated Bacteria Solution Sprayed mL	Spraying Amount of Soybean Urease mL	Single Spraying Amount of Nutrient Salt mL/Number of Nutrient Salt Sprays (Days of Curing)	Seawater Injection Volume mL
0	0–0	0	0	0	0	480
1	1–10	10	10	10	10/1	460
	1–30	30	30	30	30/1	420
	1–60	60	60	60	60/1	360
2	2–10	10	10	10	10/2	450
	2–30	30	30	30	30/2	390
	2–60	60	60	60	60/2	300
3	4–10	10	10	10	10/4	430
	4–30	30	30	30	30/4	330
	4–60	60	60	60	60/4	180

Similarly, in order to make the results comparable, in the experiment, the mole ratio of $CaCl_2$ (purchased from Xiya reagent) and urea (purchased from Aladdin Ltd.) was 1:1, 1 mol/L calcium chloride was mixed with 1 mol/L urea in equal volume, and the concentration of nutrient salt after mixing is 1 mol/L (That is, 0.5 mol/L calcium chloride and 0.5 mol/L urea in the nutrient salt), was formulated according to the test result found by Wang et al. [21]. The curing test was carried out using a spraying method. The specific operation steps are as follows (as shown in Figure 2): First, bacterial solution (MICP), seawater-acclimated bacterial solution and soybean urease (EICP) were uniformly sprayed on the surface of coastal sediments and left for 12 h. Next, nutrient salts in the same volume as the enzyme source solution are uniformly sprayed on the surface of the coastal sediment, and then the spray nutrient salt is every 24 h for reinforcement according to the number of nutrients grouped in the experiment until the required number of nutrient salt sprays was completed.

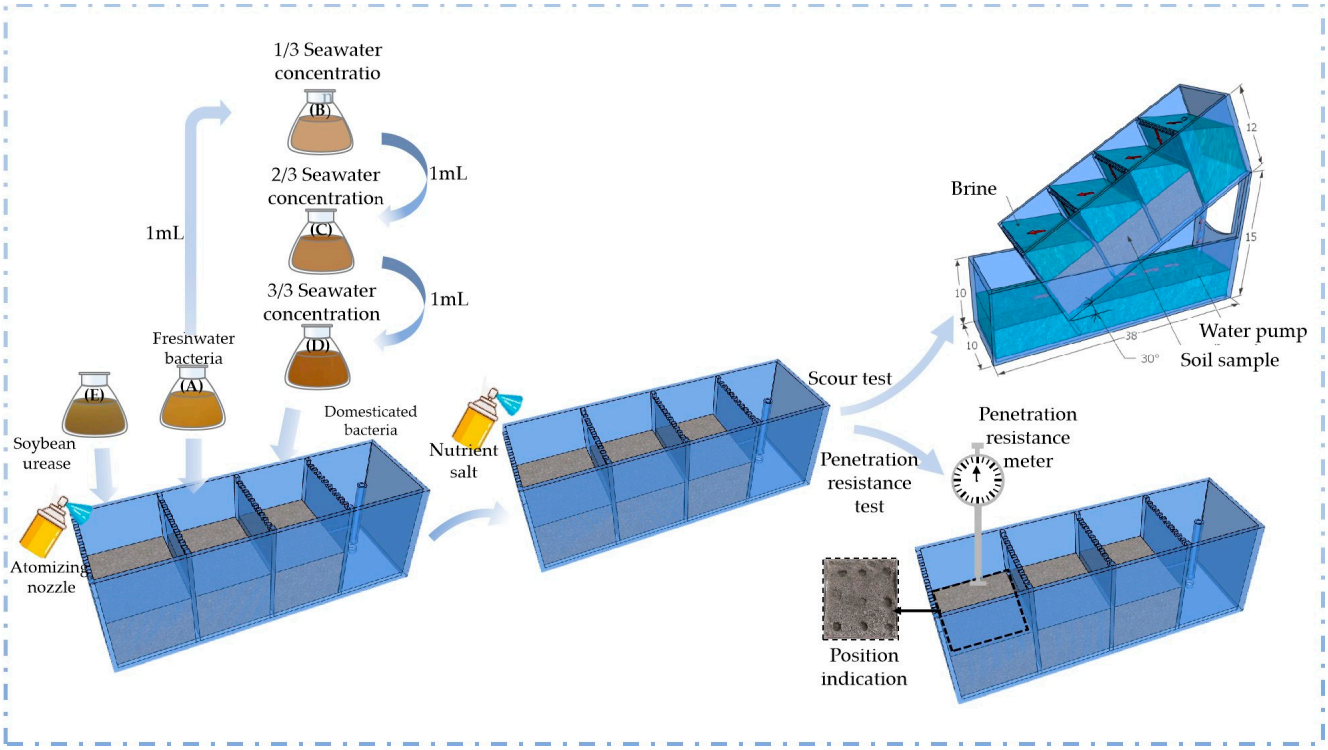

Figure 2. Scour and penetration resistance test.

(3) Seawater injection and the determination of pH, calcium ion concentration and ammonia nitrogen: microbial urease curing is relatively green and environmentally friendly, but it still has an impact on seawater pH and ion environment. Therefore, this experiment mainly explores the effects of different enzyme sources curing seawater pH, calcium ion, and ammonia nitrogen content. In order to avoid the impact of water flow during water injection, the peristaltic pump was used to slowly inject seawater (Rate: 100 mL/min). Due to the limitation of the capacity of the experimental tank, only 480 mL solution can be added to each tank after adding sediment. After spraying the corresponding enzyme source solution and nutrient salt of each group, according to Table 2, the remaining volume is the amount of injected seawater. After the final nutrient spray, let it sit for 24 h before adding seawater. In the experiment PXS-270 (ion meter), PCa-1-01 (calcium ion electrode), and 232-01 (reference electrode) were used. The ion meter needs to be calibrated with a standard solution (10^{-1}, 10^{-4} mol/L calcium chloride solution) before each measurement. Then, use a standard solution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} mol/L calcium chloride solution) to measure pX and the corresponding potential value, draw a relationship curve between pX and

potential, measure the potential value each time, and then obtain the pX value according to the relationship curve. PHS-25 (pH meter) and E-201-C (pH composite electrode), before each use of the pH meter, buffer solutions with pH values of 4.00, 6.86, and 9.18 should be used for calibration, and measurements should be made after stirring evenly each time. The ammonia nitrogen (free NH_3 and NH_4^+ in solution) was detected by the Luhengbio-ammonia nitrogen detection test paper. Calcium ion concentration, ammonia nitrogen concentration, and pH values were measured at 0 min, 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 2 h, 3 h, 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h after seawater injection. In order to reduce measurement errors of ammonia nitrogen and calcium ions, 1 mL of seawater was diluted to a reasonable range using deionized water.

2.6. Coastal Sediment Scour and Penetration Resistance Test

(1) Coastal sediment scour test: Because the purpose of this test is not to simulate the real scour process of coastal seafloor sediment after solidification but only to evaluate the effectiveness of the three enzyme sources against scour, the real scour rule of seafloor coastal sediment was not taken into account. After the sea water measurements were carried out, the model was placed level, and the pump was turned on to start the flushing process, which was recorded using a camera. According to the preliminary test results, the groups treated with different enzyme sources and the control group tended to be stable after 4 h, and the scour shape did not change significantly to reach equilibrium. Therefore, all groups of cameras were recorded 4 h after the scour ended.

(2) Coastal sediment penetration resistance test: In order to explore the bearing capacity of three enzyme sources on the coastal sediment surface after solidification, the penetration resistance WXGR-3.0/4.0 miniature penetration instrument (needle type) was used in this study. A penetration resistance WXGR-3.0/4.0 miniature penetration instrument (needle type) purchased from Geotechnical Instrument Co., Ltd Changzhou, China with a penetration depth of 6 mm, a maximum range of 5300 kPa, and a resolution of $\pm$1%F.S was used. When measuring penetration resistance, solidified samples parallel to the scour test were prepared according to Table 2 and saturated with seawater, but no scour test was carried out. After 72 h, the penetration resistance was measured. The average value of the penetration resistance at 9 equidistance points was measured, as shown in Figure 2, and represents the group of penetration resistance.

3. Test Results and Analysis

3.1. Effects of Three Enzyme Sources on Seawater

(1) Change in seawater pH: After injection into seawater, the pH of most test groups steadily decreased, and pH changes in different enzyme sources were similar because the reaction mechanism of the three different enzyme sources was almost the same before injecting seawater urea was decomposed into NH_3 and CO_2 by urease and CO_2 was dissolved in water to form carbonic acid and combined with nutrient salt Ca^{2+} to form calcium carbonate. With the increase in time, the pH of seawater decreased, as shown in Figure 3. It can be seen from the figure that the pH change rate becomes exceptionally faster within three hours of seawater injection, indicating that different enzyme sources react with nutrient salts before seawater is added, and the metabolites dissolve in seawater and volatilization of ammonia after seawater injection, causing a decrease in seawater pH. After three hours, the pH decline rate gradually stabilized. With increases in the amounts of enzyme source solution and nutrient salt, the number of metabolic products increased, causing an increase in the rate of pH decline. In different amounts of enzyme source solution and different curing times, the pH decline rate of the soybean urease group was faster than that of the other two groups after injecting seawater. The results show that soybean urease metabolism was fast, and the nutrient utilization rate was high. In the 60 mL cementitious quartic group (Corresponds to sample numbers 4–60 in Table 2, the same below), the sum of the amount of enzyme source solution and nutrient salt was 300 mL, while the injection of seawater was only 180 mL, resulting in a direct decrease in pH. With

the hydrolysis of urea, the pH of the solution OH^- increased, and the slow increase in soybean urease also indicated its fast metabolism and high nutrient utilization rate. In general, the pH of different enzyme sources is close to 7.2–7.6 after seawater injection.

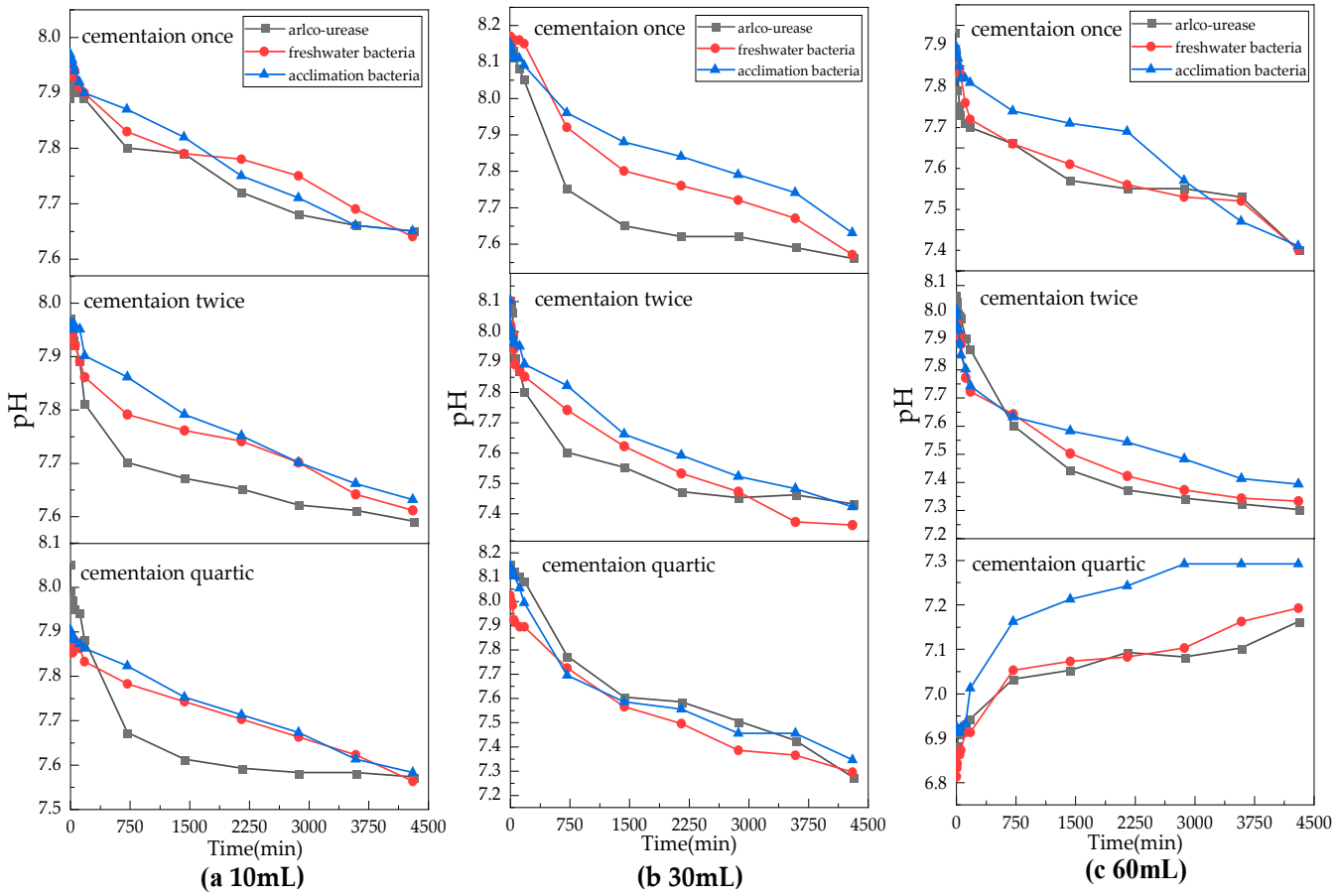

Figure 3. Change curve of seawater pH after reaction of different enzyme sources. (The amount of enzyme source injected: (**a**) 10 mL; (**b**) 30 mL; (**c**) 60 mL).

(2) The change in seawater calcium ion concentration: The Ca^{2+} pair was measured after seawater injection, as shown in Figure 4. The Ca^{2+} concentration of the cementitious quartic group, except for 60 mL, other groups increased with time. It can be seen that the Ca^{2+} concentration after injection of different enzyme sources into seawater corresponded to the pH in Figure 3 in the same group at the same time. Before seawater injection, MICP or EICP reactions occur between different enzyme source solutions and nutrients. When the calcium ions provided by nutrients are almost exhausted, microorganisms will continue to produce urease and carbonate. When seawater is injected, the free calcium ions in seawater will be rapidly utilized, so when t = 0 min, the measured calcium ion concentration is lower than the calcium ion concentration in Table 1. As microorganisms metabolize, the pH of seawater continues to decline, which causes the calcium carbonate attached to the surface of microorganisms to begin to dissolve, and, thus, the calcium ion concentration in seawater begins to rise. When t = n, the calcium ion concentration is greater than that in seawater because most of the calcium ions are additionally provided by the calcium chloride solution in the sprayed nutrient salt. In addition, when injecting seawater, a peristaltic pump is used to slowly inject seawater, while t = 0 min is measured when seawater injection is completed, which also provides time for microorganisms to utilize seawater calcium ions to generate calcium carbonate. H^+ reacts with $CaCO_3$ in the solution to form soluble $Ca(HCO_3)_2$ Equation (7). The Ca^{2+} concentration in the solution is increased, which is consistent with the experimental results of Daniella et al. [39] and Fei [40]. It can be seen that the rate of

decrease in the pH of seawater after the soybean urease reaction is fast; therefore, the Ca^{2+} concentration increase rate of seawater after soybean urease reaction is accelerated, and the Ca^{2+} concentration of marine aqueous solution after soybean urease solidification is higher than that of the other two enzyme source solutions. In the sample in the 60 mL cementitious quartic group, the calcium ion concentration is three to six times the initial value, which is due to the excessive calcium ion in the added nutrient salt, resulting in the inability of microorganisms to fully utilize it. Subsequently, it is mixed with the injected seawater, resulting in an increase in the concentration of calcium ions in the solution. Ca^{2+} concentration decreased because the pH of this group increased. The urea decomposition product CO_2 reacted with OH^- to form HCO_3^- Equation (8), and HCO_3^- reacted with OH^- and Ca^{2+} to form calcium carbonate, in seawater solution Equation (9).

$$CaCO_3 + H^+ \rightarrow Ca^{2+} + HCO_3^-, \tag{7}$$

$$CO_2 + OH^- \leftrightarrow HCO_3^-, \tag{8}$$

$$HCO_3^- + OH^- + Ca^{2+} \rightarrow H_2O + CaCO_3(s). \tag{9}$$

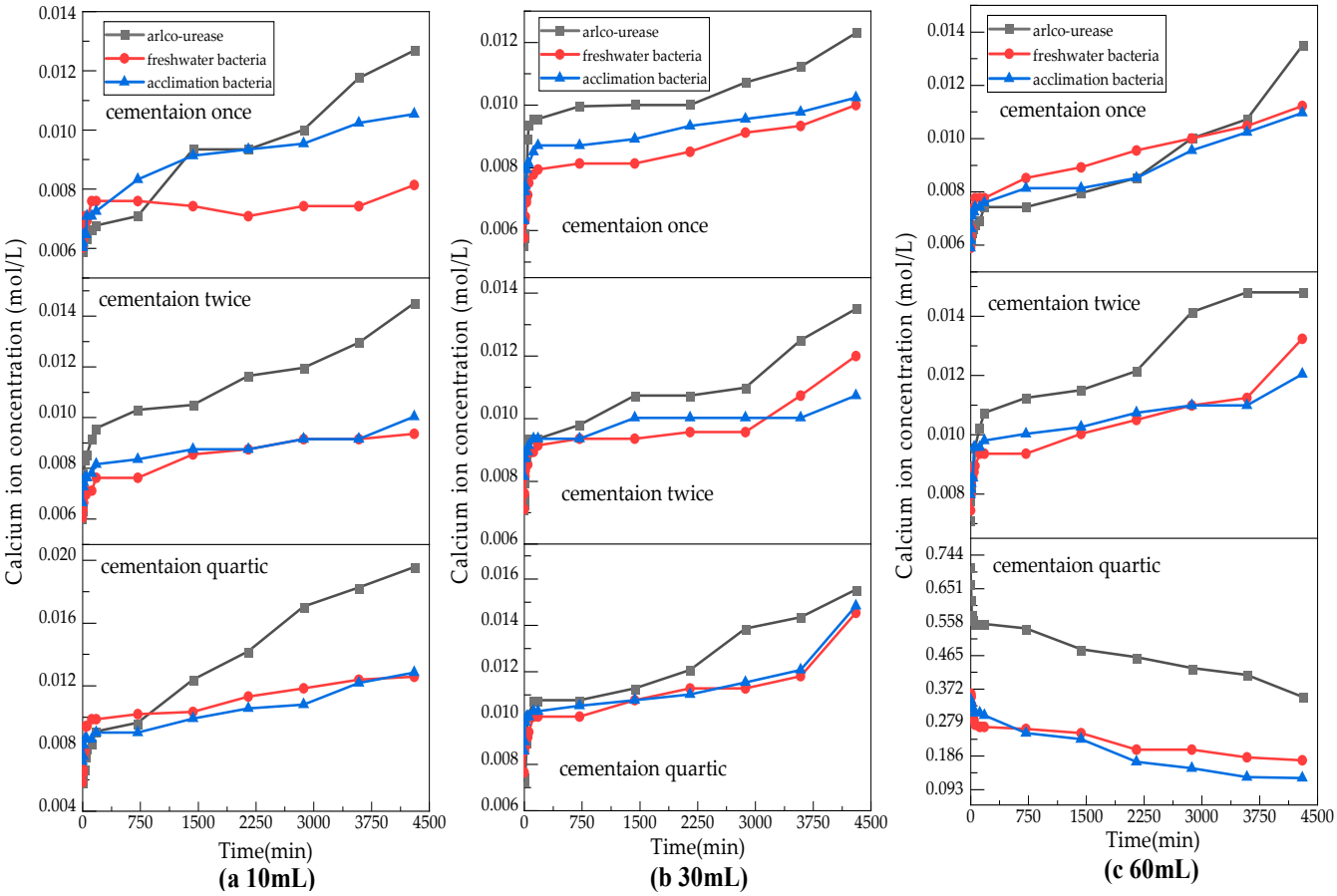

Figure 4. Change curve of seawater calcium ions after different enzyme sources. (The amount of enzyme source injected: (**a**) 10 mL; (**b**) 30 mL; (**c**) 60 mL).

The amount of calcium carbonate produced after 72 h can be roughly expressed as the sum of the injected seawater Ca^{2+} content and the nutrient salt Ca^{2+} content minus the Ca^{2+} content measured after 72 h. Divide the decrease in calcium ion concentration in the solution by the total amount of the solution (480 mL), and then multiply by the relative molecular weight of calcium carbonate to obtain the calcium carbonate production

amount. This calculation method may be interfered with by organic precipitation of calcium hydroxide or calcium, so there is some error. As shown in Figure 5, although the nutrient content of each group with the same consolidation times remains the same, the total amount of calcium carbonate precipitation produced should be the same as the chemical conversion efficiency, but the calcium carbonate content produced is different. The calcium carbonate content after soybean urease solidification is lower than that of the other two enzyme source solutions. It further indicates that the decrease in pH will reduce the content of calcium carbonate that has been generated, and pH will affect the final curing effect. However, in the 60 mL cementitious quartic group, the production of calcium carbonate in the domesticated bacteria solution was higher than that of the other two enzyme source solutions, indicating that the domesticated bacteria solution could better adapt to the high-salt environment of seawater and become fully reactive.

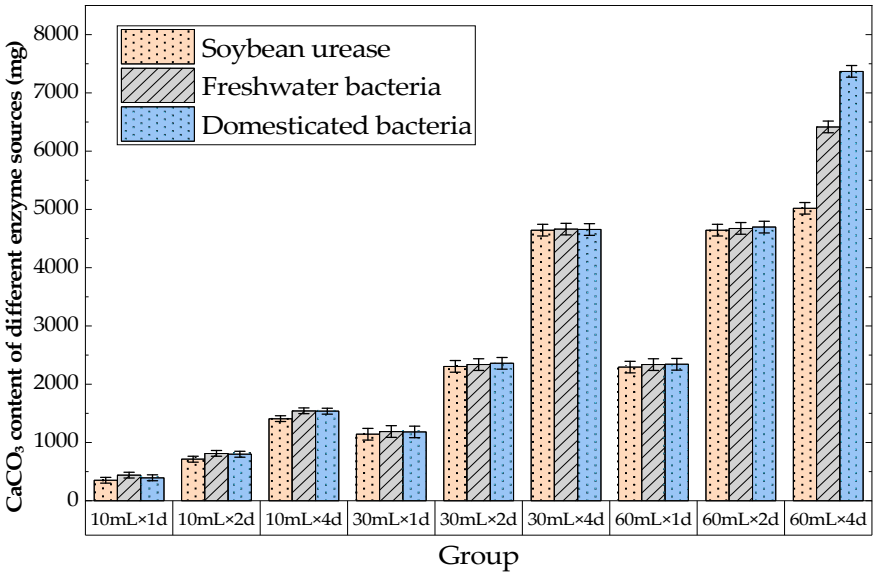

Figure 5. Calcium carbonate production 72 h after seawater injection.

Too many metabolites will lead to an increase in the decreasing rate of pH; therefore, in practice, the pH can be adjusted to ensure that the nutrients are fully utilized and the generated calcium carbonate is prevented from dissolving.

(3) Changes in ammonia nitrogen content in seawater: free NH_3 and NH_4^+ reflect the decomposition of urea in seawater and can be used to study the effects of different enzymatic sources on seawater eutrophication after solidifying seabed coastal sediments, as shown in Figure 6. The amount of enzyme source solution solidification and ammonia nitrogen content in seawater increased simultaneously, and in the 60 mL cementitious quartic group, the ammonia nitrogen content reached a maximum after 2880 min (freshwater and seawater bacterial solution was 21 g/L; soybean urease solution was 18 g/L). Firstly, because the ammonia nitrogen content in seawater was close to saturation, the stimulating and weaker gas, NH_3, could be detected in the test. Secondly, due to the decrease in seawater pH, the increase in metabolites was no longer suitable for microbial survival. In 60 mL cementitious quartic group, the ammonia nitrogen content significantly increased faster than that in the other eight groups within 750 min of injection due to the high reactivity between enzyme source solution and nutrient salt before injection into seawater. The urea decomposition ability of the domesticated bacterial solution was higher than that of the other two enzymatic solutions in the six groups of experiments when curing was carried out two times and four times, while the urea decomposition ability of the three groups of experiments decreased when the number of curing was carried out once, indicating that the domesticated bacterial solution with multiple curing times had a better urea decomposition

ability and could better adapt to the seawater environment. The optimal consolidation times will be further explored in future tests.

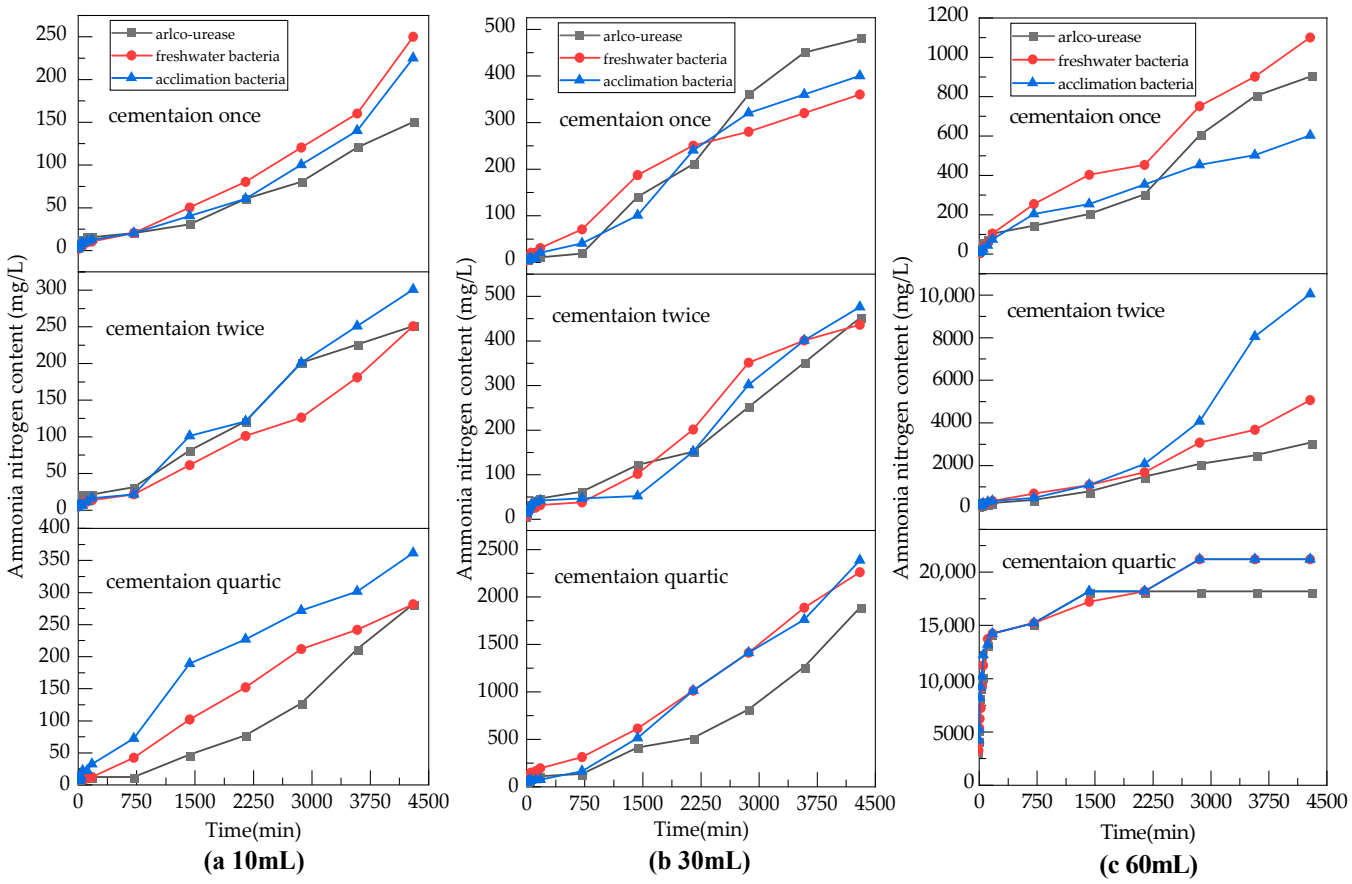

Figure 6. Change curve of seawater ammonia nitrogen content after different enzyme sources. (The amount of enzyme source injected: (**a**) 10 mL; (**b**) 30 mL; (**c**) 60 mL).

All three enzymatic sources are cured in a manner based on the urea hydrolysis reaction, which produces a large amount of the byproduct NH_4^+, which can be spontaneously removed from seawater by the volatilization of NH_3 and microbial oxidation to nitrite or nitrate. The natural seawater environment is conducive to the spontaneous NH_4^+ removal process, but a high NH_4^+ content in seawater can cause seawater eutrophication. It is generally believed that NH_3 in the atmosphere is very harmful to the human body when higher than 10 $\mu g/m^3$ [41]. Therefore, in the practical application process, the emission of NH_4^+ and NH_3 can be reduced by artificially adding nitrifying and denitrifying bacteria or chemical methods.

3.2. Erosion and Penetration Resistance of Coastal Sediments

(1) Analysis of coastal sediment scour tests: This experiment does not adopt the method of Liu using a propeller to create harmonic scour sediment [33] and is different from Fang's simulation of surface runoff scour sediment [35], but directly uses water scour to test the anti-erosion ability of sediment. The captured images show changes in the coastal sediment profile over time. The flow against the wall scours the coastal sediment and generates shear stress, so the coastal sediment changes considerably only near the current wall. In the control group, untreated coastal sediments were scoured by currents, as shown in Figure 7. In these images, it can be clearly seen that the untreated coastal sediments formed a groove along the wall under erosion by the current, while the alluvial coastal sediments moved and accumulated downslope. The erosion of coastal sediments significantly changed within 30 min of the experiment beginning. Then, the further erosion

and deposition gradually changed, and the distribution of the coastal sediments maintained a relatively balanced state after 4 h. After treatment with different enzyme sources, the scour process was similar to the control group, but the grooves formed by scour were reduced to different degrees compared with the control group.

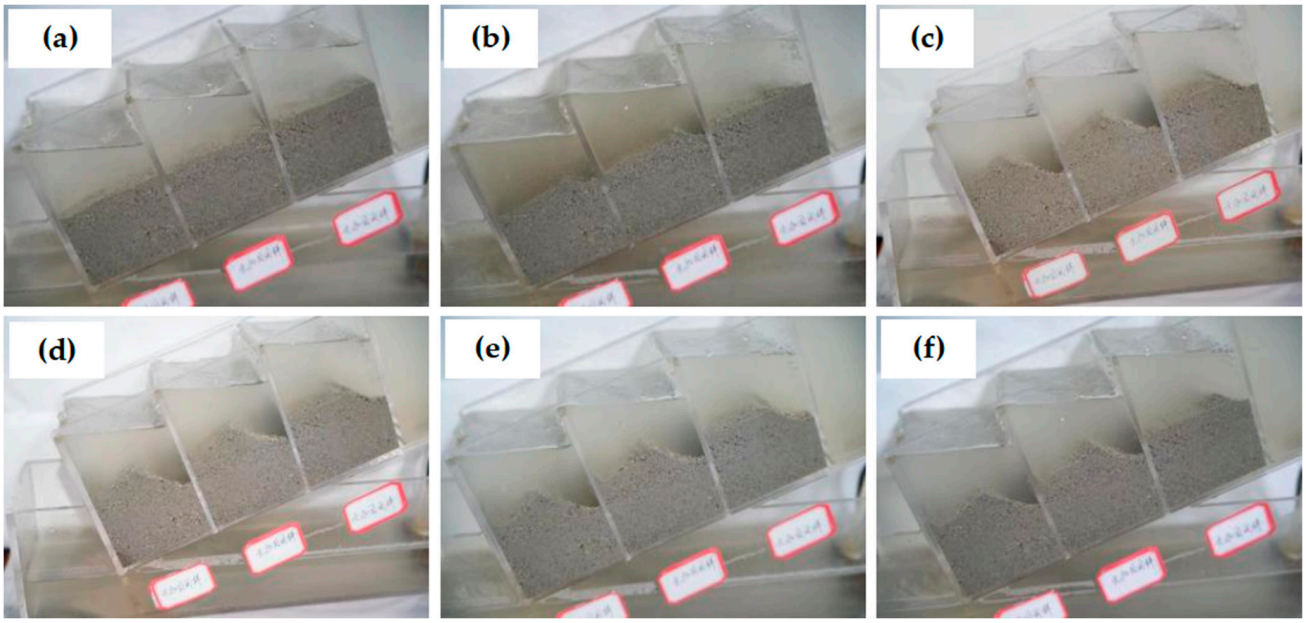

Figure 7. Change in untreated coastal sand scouring (Scour time: (**a**) 0 min; (**b**) 1 min; (**c**) 30 min; (**d**) 1 h; (**e**) 2 h; (**f**) 4 h).

The images were compared after 4 h of scouring with different enzyme sources, as shown in Figure 8. The soybean urease solution, freshwater bacterial solution, and acculturated bacterial solution were added to three test tanks in each group from left to right. It was observed that different enzyme sources had a low anti-scour ability of coastal sediments under low nutrient salt content and low curing times. In groups 1–10 (See Table 2, the same below), the three enzyme sources formed significant impact grooves after scouring, which could not cure the surface of coastal sediments. Horizontally, the same amount of different enzyme source solutions improved the curing effect by increasing curing times. Vertically, the same consolidation times can also improve the curing effect by increasing the content of different enzyme source solutions. The surface cementation of coastal sediments can clearly be observed in groups 2–10, but the thin cementation layer causes the water to flow through and scour the unconsolidated coastal sediments below to form cavities. With the increase in the amount of enzyme source solution and consolidation, this situation improves. The consolidated layer reached a certain thickness in 2–60 so the water only scoured the surface of the coastal sediment but failed to scour the unconsolidated coastal sediment. There were no longer significant changes in the coastal sediment for the scour of 4–60 groups. Different from Li and Ning, which simulated rain scour, this experiment adopts simulated water scour, but the conclusions obtained are consistent with the results that their MICP can greatly improve the surface hardening ability and anti-erosion ability [28,36]. The penetration resistance analysis is described in detail later.

(2) Analysis of coastal sediment penetration resistance: The penetration resistance test was carried out on the parallel group without a scour test. Since the penetration resistance of the untreated coastal sediment was lower than the minimum range of the micro penetration resistance meter (14 kPa), it was not explored. As shown in Figure 9, it can be seen that there is a corresponding relationship between each group of penetration resistance and its 4 h scour image, that is, with the increase in penetration resistance, the scour grooves

are reduced. At low enzyme source content (10 mL), prolongation of curing times could not effectively improve the penetration resistance of coastal sediments. The penetration resistance significantly increased after groups 2–30, and reached the maximum in groups 4–60 (soybean urease: 764.5 kPa, freshwater bacterial solution: 2903.2 kPa, acclimated bacterial solution: 3348.6 kPa). Therefore, the penetration resistance can be improved by increasing the amount and curing times of the three enzyme source solutions in this experiment. The penetration resistance of acclimated bacterial solution in the six groups of 2d and 4d tests was higher than those of the other two enzyme source solutions. This result was consistent with the results obtained for the determination of ammonia nitrogen content. These results demonstrate that the acclimated bacterial solution could better adapt to seawater and achieve better results at multiple solidification times, which further reflected the effectiveness of the acclimated bacterial solution. Although the three enzyme source solutions shown in Figure 9 have almost the same anti-scouring ability on the surface of coastal sediments after solidification, the penetration resistance of soybean urease is significantly lower than that of the other two enzyme source solutions, mainly for two reasons. Firstly, the content of calcium carbonate in coastal sediments solidified by soybean urease solution is low; secondly, the analysis of coastal sediments after solidification shows that soybean urease-induced calcium carbonate precipitation (EICP) solidifies evenly and can penetrate deep into the coastal sediment. However, it also reduces the content of calcium carbonate precipitation on the surface of the coastal sediment, while microbe-induced calcium carbonate precipitation (MICP) mainly concentrates on the shallow surface, which makes the surface of the coastal sediment more compact. The observed phenomenon is consistent with Chu et al.'s previous study on the uneven distribution of calcium carbonate precipitates formed by MICP [8,33,42–44].

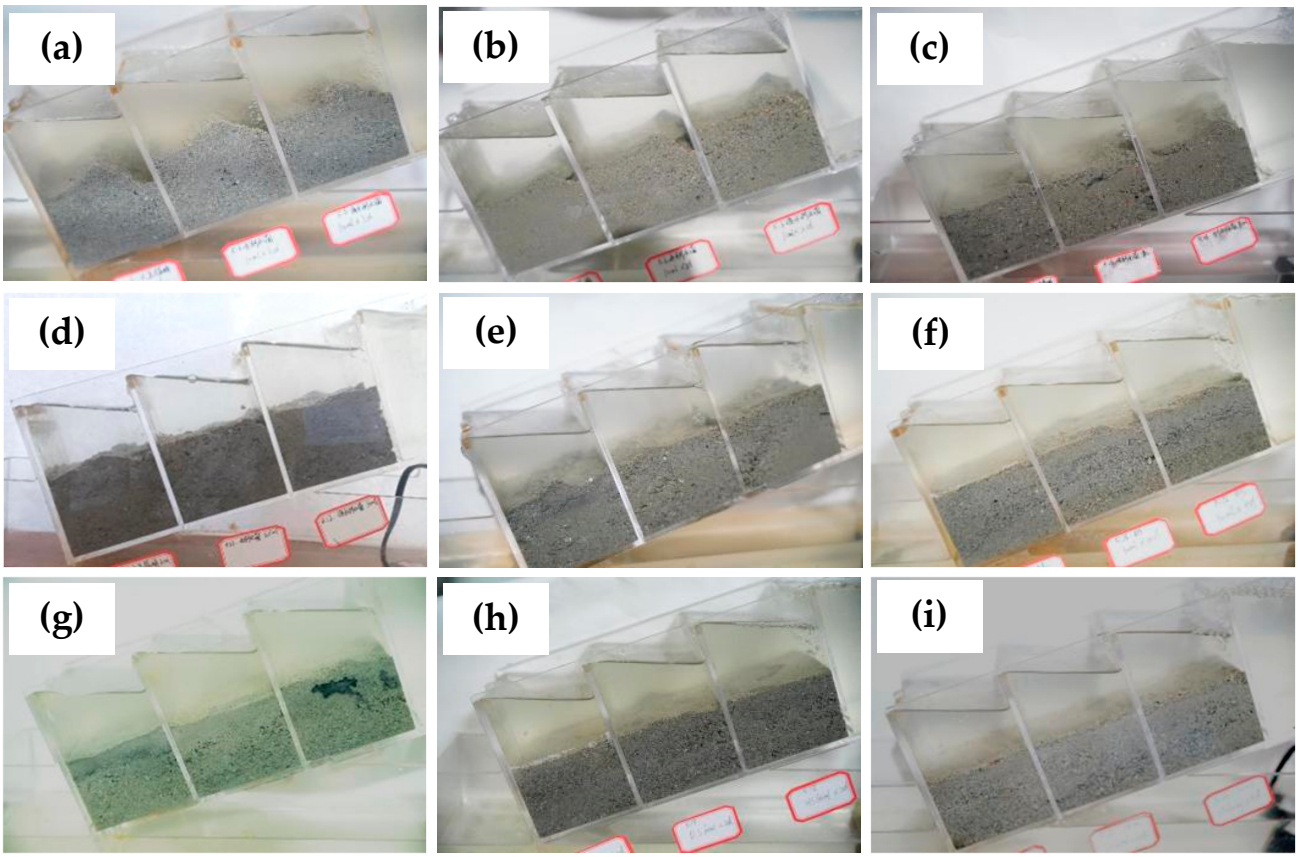

Figure 8. A 4 h wash-out plot after curing of different enzyme sources. (Corresponding sample number in Table 2: (**a**) 1–10; (**b**) 2–10; (**c**) 4–10; (**d**) 1–30; (**e**) 2–30; (**f**) 4–30; (**g**) 1–60; (**h**) 2–60; (**i**) 4–60).

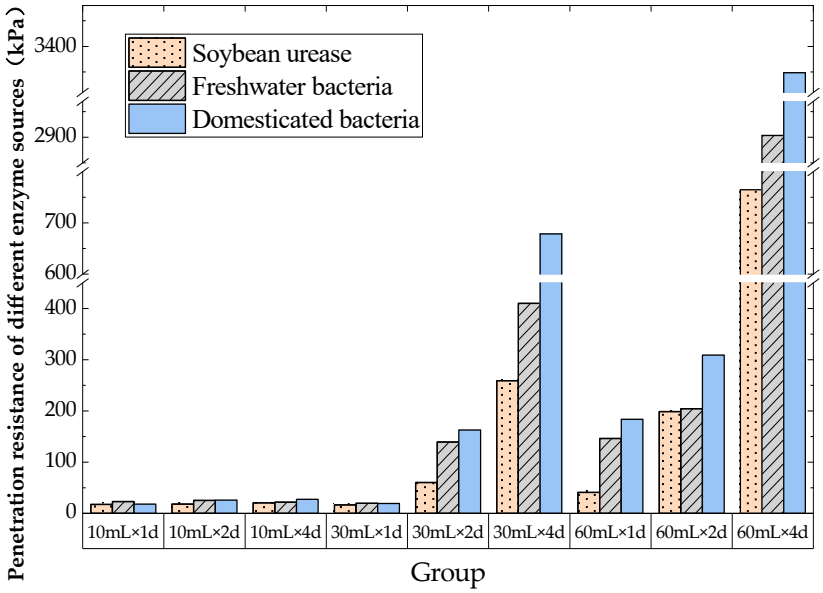

Figure 9. Penetration resistance measured value.

4. Discussion

(1) Under the same enzyme activity conditions, the effect of seawater acclimated bacterial solution on the solidification of submarine coastal sediments is better than that of freshwater bacterial solution and soybean urease solution. This technology can be used as a method to solidify the shallow surface of coastal sediments and prevent erosion. The effect of the seawater-acclimated bacterial solution is more significant, providing new ideas for economic and environmental protection to improve coastal sediment foundation in nearshore engineering. However, since this test only compared the curing effects of different enzyme sources and did not take into account various complex factors in the real environment, further tests and summary rules are needed before the actual application of this technology, especially in the controllability of curing space, durability, the treatment process of ammonia nitrogen pollution, and other aspects.

(2) The maximum number of curing times set in this test is four, and further study is needed to initiate longer curing times in order to obtain the optimal number of consolidation times of coastal sediments and, thus, provide a reference for evaluating the durability of coastal sediments. Caution should be exercised when applying MICP or EICP on coastal beaches and dunes. If the reinforcement is excessive, it will make the coastal sediment extremely hard, which may damage the coastal ecosystem and affect the implementation of coastal projects. In the future, more research is needed to establish the threshold of consolidation beyond which dune hardening becomes apparent.

5. Conclusions

In this paper, a small-scale model experiment was conducted to study the solidifying effect of different enzyme sources on coastal sediments and the effect on the seawater environment. The mechanical properties and anti-scour properties of cured model samples were enhanced via different enzyme sources. By measuring the change in pH value, calcium ion concentration, and ammonia nitrogen content of seawater after solidification with different enzyme sources, it was found that the reinforcement of samples with different enzyme sources would have a certain impact on the seawater environment. Specific conclusions are as follows:

(1) The simulated scour test showed that the three enzyme source solutions all improved the scour resistance of the coastal sediment surface layer, and the scour resistance was related to the amount of enzyme source solution and the number of curing times. The scour resistance increased with the increase in the amount of enzyme source solution and

the number of curing times. However, this does not reflect the relationship between the curing strength of the three enzyme sources. Since only the surface of coastal sediments can be solidified by spray curing, the water flow will break through the surface of coastal sediments and form cavities when the amount of enzyme source solution and a number of curing times are low.

(2) According to the results of the micro-penetration resistance test, the three enzyme sources could not effectively resist scouring by increasing the curing times under the condition of low enzyme source solution quantity (10 mL group), and the penetration resistance of coastal sediments was not significantly enhanced. The effect of the acclimated bacterial solution on coastal sediments was better than that of the freshwater bacterial solution, and the effect of the freshwater bacterial solution on coastal sediments was better than that of soybean urease under a large amount of enzyme source solution.

(3) The solidification of seafloor coastal sediments by three enzyme sources—soybean urease, *Bacillus pasteurella* freshwater, and *Bacillus pasteurella* acclimated to seawater—resulted in the decrease in seawater pH, and the pH tended to be between 7.2 and 7.6 under different enzyme source solution amounts and curing times. The acidification of seawater dissolved the generated calcium carbonate and increased the concentration of calcium ions in seawater. Among them, the pH of soybean urease after solidification is lower than that of the other two enzyme sources, resulting in a higher dissolution of calcium carbonate.

(4) Compared with the other two enzyme source solutions, the liquid phase of the three types of acclimated bacteria in seawater can better adapt to the high-salt environment of seawater, and microbial metabolism is not inhibited, thus improving the urea decomposition ability and producing higher calcium carbonate.

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