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Optimization Study on Microbial Wellbore Sand Control Culture Medium System for Loose Sandstone Reservoirs

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ABSTRACT
To address the high costs and significant pollution issues of traditional sand control technologies in oil fields, and to optimize the economic nature of the culture medium for the microbial-induced calcium carbonate precipitation (MICP) technology, this study screened out Bacillus subtilis from agricultural soil and determined that it grew optimally at 40°C and pH=7. Nine culture media were designed using the single-factor method, and the OD values of the bacteria, the pH of the culture medium, and the amount of calcium carbonate precipitation were compared. The results showed that the No.5 culture medium (sucrose + soaked soybean meal powder) performed the best, with an OD value of nearly 1.2 after 48 hours, a pH of 10, and a calcium carbonate precipitation amount close to the theoretical value of 9 g, which was comparable to the control, and using cheap soybean meal powder instead of peptone significantly reduced costs. This study provides an economically efficient solution for the industrialization of MICP technology for sand control in oil fields.

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I. INTRODUCTION

As global accessible oil resources become increasingly depleted, enhancing oil and gas recovery rates and maintaining wellbore integrity have become key challenges for the sustainable development of the petroleum industry[1]. One of the core difficulties lies in sand control in oil fields. Chemical sand control mainly involves injecting degradable polymers, low-pollution silicates, and other chemical agents into the formation to solidify sand particles or form sand control barriers. Physical sand control mainly uses filter screens, gravel filling, and other physical structures to prevent sand from entering the wellbore. However, traditional chemical sand control is not only costly and prone to environmental pollution, but also may damage the formation's permeability due to residual chemical agents, affecting subsequent production capacity. Physical sand control, although avoiding chemical pollution issues, has limitations such as an easily clogged filter sand structure, poor adaptability to fine sand formations, and high maintenance and replacement costs in the later stage. Both approaches are difficult to balance economic efficiency, environmental protection, and long-term sand control requirements. In this context, the microbial-induced calcium carbonate precipitation technology (MICP) emerges as a unique green biotechnological approach in the field of petroleum engineering. This technology injects urease-producing microorganisms and necessary nutrients into the reservoir. After the microorganisms colonize in the deep part of the reservoir or near the wellbore, their secreted urease will catalyze the hydrolysis of urea in the formation or injected fluid, generating NH_3 and H_2CO_3; ammonia combines with water to produce OH^-, making the environment alkaline, and H_2CO_3 dissociates to release CO_3^{2-}, which combines with Ca^{2+} in the reservoir water to form stable $CaCO_3$[2], thereby achieving multiple goals such as fracture sealing, sand control, and cementing.

The volume of the bacterial solution and nutrient solution required for the sand control process is huge, making the cost and performance of the culture medium the core factor determining the economic feasibility of the MICP technology. Although traditional laboratory culture media can achieve high bacterial yield, they are costly and do not adapt to reservoir conditions. Therefore, it is necessary to optimize the performance of microbial culture media. Through targeted optimization, the applicability of the strain culture media in the sand control field is enhanced. The urease activity and biomineralization efficiency are maximized; exploration of using industrial by-products or cheap agricultural waste as alternative nutrient sources is carried out to reduce the single-well treatment cost.

Currently, after decades of research on MICP technology by experts from both domestic and international sources, many valuable conclusions have been obtained, playing an important role in promoting the development of oil engineering sand control technology. In 2018,Phillips,A.J[3] et al. used a conventional bacterial suspension of Bacillus subtilis and urea-calcium solution in a 310m deep well in Alabama; after 25

injections of calcium solution and 10 injections of bacterial solution, the injection rate decreased from 0.29m³/h (1.28gpm) to less than 0.023m³/h (0.05gpm),with a pressure attenuation rate less than 18%, and 125μm fractures were sealed better than traditional materials; they believed that the low viscosity of MICP could target small channels. In 2020, Cheng[4] et al. used MICP to make bio-bricks, treating quartz sand at 50% saturation; the strength was 9MPa (2 times full saturation), the water absorption rate was 10%,and 8 times salt corrosion loss was 0.5g; they believed that partial saturation MICP could improve the efficiency of calcite precipitation. In 2021, Kirkland, C.M[5] et al. conducted MICP experiments in a failed water injection well in Indiana, developing a direct injection system, using "frozen bacteria resuscitation+biological reactor expansion" to increase the production of Bacillus subtilis, with salt water interval injection of microbial and urea-calcium solution (flow rate 3.4-1.4gal/min); the result was that it reached 1384psi/1.4gal/min in 3 days, close to the historical level (1400psi/1gal/min), confirming that it can seal fractures and thief layers; they believed that it is suitable for large-volume scenarios with restricted reactant transportation. In 2021, Muhammed, A.S[6] et al. focused on the improvement of EICP sand soil, with 3 cycles of 1M cement; the UCS reached 1712kPa, calcite 8.21%, porosity from 23.66% to 4.42%, and the UCS and calcium carbonate index were correlated (R^2 = 0.9762); they believed that multiple cycles could improve the uniformity of cementation. In 2022, Zhong, M[7] et al. studied sandstone MICP with Bacillus subtilis; when the permeability was less than 66mD, the effect declined (bacterial blockage at the injection end), and the strength increased by 10.05% after treatment; the calcite in the sandstone was in rhombic hexagonal shape (spherical in the shake flask); they believed that the "free calcite sealing + biological membrane mineralization" dual mode should be paid attention to. In 2022, Alidoustsalimi, N[8] et al. designed a reactor with Bacillus subtilis, alternating injections to build a porous calcite structure; the strength was optimal when using 60-mesh powder during alternating injections, SEM showed that the calcite bridged and the pores were uniform; they believed that it could replace natural rock cores and provide a low-cost solution. In 2024, Du, Z.H[9] et al. conducted gradient acclimation of Bacillus subtilis in drilling fluid with high temperature and strong alkalinity; its OD_{600} in CMC was 1.373, urease was 1.98, and the sealing strength was 1.232MPa (excellent XG system); they believed that it could relieve well wall instability. In 2025, Chen Qian and Hu Qizhi[10] et al. conducted MICP curing experiments with Xiamen standard sand, using 3 particle sizes and 4 cementing liquids; The results showed that the optimal combination was a particle size of (0.25 - 1mm) + 1 mol/L cementing fluid (with an intensity of 10 MPa). The smaller particle size (0.075 - 0.25mm) had the weakest strength, and when the cementing fluid concentration was greater than 1 mol/L, the strength decreased. Larger particle size was prone to brittle fracture. It was concluded that the preferred choice for engineering should be the particle size + 1 mol/L cementing fluid. In 2025, Prongmanee[11] et al. improved the MICP process: using the supernatant of Bacillus subtilis carbonate ammonium and NaOH to synthesize Na_2CO_3 (with an optimized purity of 93.82%), as an activator to prepare geopolymer mortar, with a 28-day strength of 34 MPa at room temperature; it was believed that this could solve the problems of geopolymer high-temperature curing and MICP ammonium salts. In 2025, Deng[12] et al. used MICP + tall fescue to reinforce the coral island calcareous sand slope; the result was that the 0.5 mol/L cementing fluid plus 4 cycles of recirculation water increased by 56.72%, and the J12-1 group (with OD_{600} = 2.2 and 0.1 mol/L cementing fluid) had the best wind erosion resistance (with a 10 m/s wind speed loss of 1.85%); it was believed that the mechanism of synergy was surface cementation + root anchoring, providing an ecological solution. In 2025, Zhao Zhifeng and Han Shaoheng[13] et al. explored the MICP peptone formula: a 1:1 low-purity bovine pancreatin - soybean peptone combination was the optimal (with bacterial liquid OD_{600} = 2.48 and urease 9.11 mmol/(L·min)), with a soil strength of 1068 kPa and a cost reduction of 64%; it was believed that this could replace high-purity peptone and reduce the cost of MICP. However, the optimization research of MICP culture media for petroleum engineering applications is still insufficient, and most studies still use the standard formula, lacking in-depth understanding and systematic optimization of the nutritional requirements under actual reservoir conditions.

This research focuses on the problem of sand production from wellbores in loose sandstone reservoirs. With the MICP (microbial induced carbonate precipitation) technology as the core, based on the original peptone culture medium, through the optimization of carbon and nitrogen sources in different nutrient solutions, a cost-effective and highly mineralizing microbial nutrition system was screened under simulated reservoir conditions. This research is expected to provide an economical, efficient and adaptable biological solution for the efficient development of oil and gas reservoirs using MICP technology, laying a core foundation for promoting the industrial application of this technology.

II. EXPERIMENTAL MATERIALS AND METHODS

1. The Sample of Soil

From the farmland in a certain village that frequently applies nitrogen fertilizer.

2. Experimental Drugs and Reagents

The drugs and reagents used in this experiment are listed in Table 1 below.

Table 1: List of Experimental Drugs and Reagents

Name	Chemical formula	Purpose
Peptone	/	Cultivating bacterial strains
Beef extract powder	/	Cultivating bacterial strains
Yeast powder	/	Cultivating bacterial strains
Agar powder	/	Cultivating bacterial strains
Soybean meal powder	/	Cultivating bacterial strains
Yeast extract	/	Cultivating bacterial strains
Corn starch powder	/	Cultivating bacterial strains
Glucose	$C_6H_{12}O_6$	Cultivating bacterial strains
Sodium chloride	NaCl	Cultivating bacterial strains
Potassium dihydrogen phosphate	KH_2PO_4	Cultivating bacterial strains
Sucrose	$C_{12}H_{22}O_{11}$	Cultivating bacterial strains
Syrup	$C_6H_{12}NNaO_3S$	Cultivating bacterial strains
Ammonium sulfate	$21(NH_4)_2SO_4$	Cultivating bacterial strains
Magnesium sulfate	$MgSO_4$	Cultivating bacterial strains
Urea	H_2NCONH_2	Screening of bacterial strains
Calcium chloride	$CaCl_2$	Form solid precipitates

3. Experimental instruments

The experimental equipment used in this chapter is shown in Table 2.

Table 2: Main Experimental Equipment

Instrument Name	Model number	Manufacturer
Syringe	10-1000uL	Lianhua Technology
High-pressure steam sterilization oven	XFH-50MA	Zhejiang Xinfeng Medical Devices Co., Ltd.
Electronic pH meter	PHS-3C	Shanghai Yueping
Incubator/Dehydrator	PH-050A	Shanghai Yiheng Scientific Instrument Co., Ltd.
Ultra-clean workbench	SW-CJ-2FD	Shanghai Lichen Technology Co., Ltd.
Electronic balance	JA2003	Shanghai Lichen Technology Co., Ltd.
Constant temperature incubator	THZ-100	Shanghai Yiheng Scientific Instrument Co., Ltd.
Constant temperature oscillator	THZ-98A	Shanghai Yiheng Scientific Instrument Co., Ltd.
Test tube	5ml	Sichuan Shu Guo Glass Co., Ltd.
Beaker	200ml	Sichuan Shu Guo Glass Co., Ltd.
Funnel	125ml	Sichuan Shu Guo Glass Co., Ltd.

4.Culture medium

Mineralized bacteria liquid culture medium:2g of disodium hydrogen phosphate,20g of urea,5.0g of peptone,5.0g of sodium chloride,2g of beef extract,0.1g of glucose,1000mL of deionized water, pH=7.2-7.4,sterilized at 121℃ for 20 minutes under high temperature.

Mineralized bacteria solid culture medium:20g of agar,2g of potassium dihydrogen phosphate,20g of urea,5.0g of peptone,5.0g of sodium chloride,2g of beef extract,0.1g of glucose, phenol red reagent,1000mL of deionized water, pH=7.2-7.4,sterilized at 121℃ for 20 minutes(add urea after sterilization and cooling).

5. Microbial strain screening

Urease-producing bacteria are usually found in the soil of farmlands that frequently apply nitrogen fertilizers. However, various microorganisms in the soil grow in a mixed community manner. To isolate the urease-producing strains, it is necessary to separate and purify the target mineralizing bacteria from the soil through biological experiments. In this experiment, the characteristic of urease-producing bacteria decomposing urea is utilized to screen the target bacterial strain. The specific steps are as follows：

1. Domestication: Remove the larger impurities from the collected soil. Soak the sampled soil with a 5 mol/L high-concentration urea solution every day for five days, so that only the bacterial communities that can grow in an environment with high-concentration urea remain in the soil.
2. Enrichment: Prepare 200 ml of mineralized bacterial liquid medium. Weighing 2 g of pre-treated soil and adding it to 100 ml of the medium, Cultivate it in a constant temperature shaker at 40℃ and 140 r/min for 24 hours. Taking 2 ml of the bacterial liquid from the conical flask and conduct another liquid enrichment culture to reduce the interference from miscellaneous bacteria.
3. Dilution plating: Perform aseptic operations in a laminar flow hood. Prepare sterilized deionized water and dilute the bacterial solution to 10^{-5}, 10^{-6}, … ,10^{-9} times of the original concentration. Pour about 1/3 of the solid medium into the petri dish. After the medium cools and solidifies, take 100 μl from each concentration for plating. When the bacterial solution on the surface is completely spread out, invert the medium in a constant temperature incubator at 40°C and cultivate until obvious microbial colonies grow. Then removing it and placing it in the liquid medium. Take it out when it grows to the logarithmic phase.

The selected strains were subjected to proliferation culture. The strains were inoculated at a 2% ratio from the freeze-dried tubes into the mineralized bacterial liquid culture medium. Two bottles were inoculated and labeled as KH-1 and KH-2. The cultures were cultivated in a constant temperature shaker at 40°C and 140 r/min. Samples were taken every 4 hours to measure the pH value and OD_{600} value. The microbial bacterial solution concentration is the most intuitive indicator to characterize the bacterial growth activity. The higher the bacterial concentration, the higher the cell activity. The bacterial strain concentration can be expressed by measuring the absorbance of the bacterial solution (optical density method), which is based on the extension application of Lambert-Beer's law scattering: bacterial cells as suspended particles will cause scattering of incident light; the higher the bacterial concentration, the stronger the scattered light, and the weaker the light intensity passing through the bacterial solution, and the greater the absorbance, so the absorbance is positively correlated with bacterial concentration; among them, at a wavelength of 600 nm, the scattering effect of bacterial cells on light is dominant, and the light absorption of the cells themselves is relatively weak, which can avoid the situation of "light absorption masking scattering signals", making the change in absorbance more purely reflect the difference in bacterial concentration[14].

6. Bacterial strain performance test

The selected strains were subjected to proliferation culture. The strains were inoculated from the freeze-dried tubes into the mineralized bacterial liquid culture medium at a 2% ratio. Four temperature gradients of 30, 40, 50 and 60°C were set for the strain cultivation and the OD values of the culture medium at different times were recorded. The growth curves of the strains under different temperatures were plotted. Meanwhile, three groups of culture media with different pH values (pH = 5.5, 7, 10) were set up. Samples were taken at regular intervals to measure the pH values, and the growth curves of the strains under different pH values were plotted.

7. Design of the optimization plan for the culture medium

The optimization of the culture medium system was studied using the single-factor control variable method. By changing the carbon and nitrogen sources in the culture medium, the growth conditions of the strains under different carbon and nitrogen sources were analyzed. The formulation of the culture medium and the experimental scheme are shown in Tables 3 and 4.

Table 3: Culture Medium Formula

Name	Recipe
Culture medium No. 1 (control)	Peptone 6g/L, Sodium Chloride 5g/L, Potassium Phosphate Dihydrate 2g/L
Culture medium No. 2	Sucrose 10g/L, ammonium sulfate 4g/L, sodium chloride 5g/L, potassium dihydrogen sulfate 2g/L
Culture medium No. 3	Sucrose 10g/L, (soaking) soybean meal powder 12.85g/L, sodium chloride 5g/L, potassium dihydrogen phosphate 2g/L
Culture medium No. 4	Sucrose 10g/L, corn starch dry powder 13g/L, sodium chloride 5g/L, potassium dihydrogen phosphate 2g/L
Culture medium No. 5	Sucrose 6g/L, (soaked) soybean meal powder 12.85g/L, sodium chloride 5g/L, potassium dihydrogen phosphate 2g/L
Culture medium No. 6	Syrup 6g/L, Corn Starch Powder 13g/L, Sodium Chloride 5g/L, Potassium Dihydrogen Phosphate 2g/L
Culture medium No. 7	Corn starch dry powder 4g/L, ammonium sulfate 2.9g/L, sucrose 2.61g/L, sodium chloride 5g/L, potassium dihydrogen phosphate 2g/L
Culture medium No. 8	Sucrose 1.72g/L, Yeast Extract 6.8g/L, Corn Starch Powder 6.58g/L, Sodium Chloride 5g/L, Potassium Dihydrogen Phosphate 2g/L
Culture medium No. 9	Sucrose 2.25g/L, yeast extract 4g/L, corn starch powder 4g/L, urea 0.763g/L, sodium chloride 5g/L, potassium dihydrogen phosphate 2g/L

Table 4: Experimental Plan Table

Bacterial strain type	Type of culture medium	Culture medium dosage/ml	Environmental temperature / °C	Experimental period /h	Amount of $CaCl_2$ solution (0.9 mol/L) / ml
Clostridium pasteurianum	Culture medium 1-9	200	30-40	48	100

Taking 2% bacterial solution and inoculating it into different culture media, Cultivate it at 40℃ with a speed of 140 r/min in a shaking incubator for 12 hours (the cultivation time should be the logarithmic growth phase). Take several 10 ml sterilized test tubes, mark them as 0h, 2h, 4h, …, 48h respectively. Pour 5 ml mineralized bacterial liquid culture medium into each tube and add 2% of the previously grown bacterial solution. Shake gently and then cultivate under the same conditions. Take the corresponding test tubes at the marked time points and measure the OD value according to the time point. Select 600 nm as the measurement wavelength, use the sterilized liquid culture medium without inoculation as the blank control for calibration. Shake the bacterial solution to be measured thoroughly and drop it into the colorimeter for OD value measurement (each sample should be measured at least 3 times and the average value should be taken). Plot the growth curve with the cultivation time as the abscissa and the OD value as the ordinate. Analyze the characteristics of the growth curve. At the same time, use an electronic pH meter to measure the pH value of the bacterial solution, record it and analyze it.

Taking the mineralizing bacteria that have grown to the logarithmic growth stage under different culture media, and take a certain volume of the experimental group and control group culture media respectively, and add them to separate sterile beakers. Use a pipette gun to slowly and steadily drip the same amount of $CaCl_2$ solution into the beakers. After slow stirring, pour the reaction suspension into a funnel equipped with pre-dried and weighed filter paper. Through filtration, obtain the filter paper with precipitates, and place it in a drying oven to dry until constant weight. Put the dried samples in a desiccator to cool to room temperature. Weigh the total weight of the samples, subtract the weight of the filter paper, and then you can obtain the weight of calcium carbonate.

IV. EXPERIMENTAL RESULTS

3.1 Microbial strain screening

After conducting the cultivation and enrichment of urease bacteria in the soil, the growth concentrations of the bacterial cultures were compared, and the target bacterial strains with good growth conditions were preliminarily screened. In this experiment, the cell count was characterized by the absorbance (OD_{600}) at 600nm wavelength using a UV spectrophotometer.

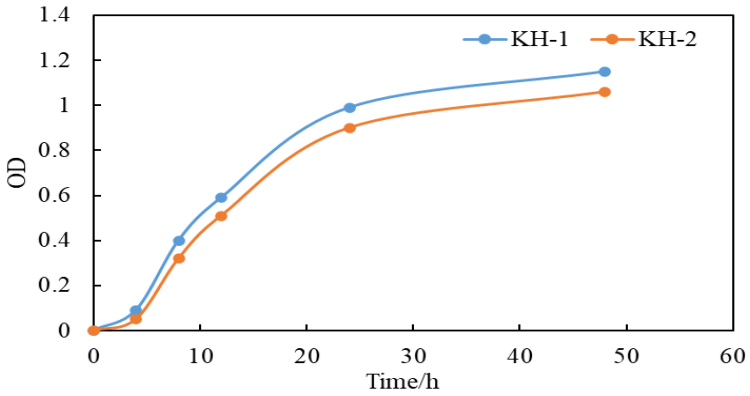


Figure 1: Graph of OD value of culture medium versus time

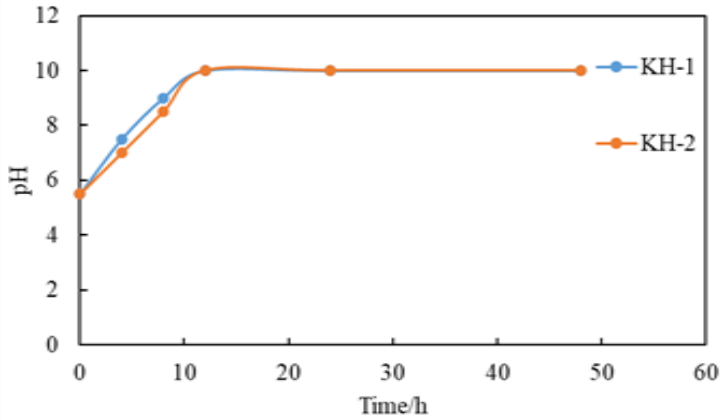
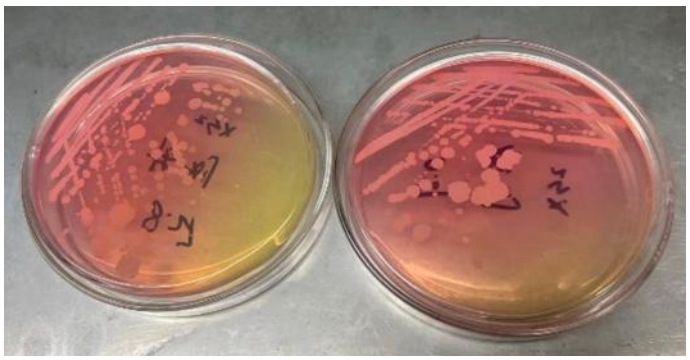


Figure 2: the relationship between the pH value of the culture medium and time

In Figure 1, both curves exhibit the typical growth characteristics of microorganisms: rapid growth in the early stage and stable maintenance in the later stage. The OD values of both KH-1 and KH-2 media increased rapidly in the early stage, indicating that the bacteria were actively proliferating and efficiently

accumulating biomass. In the later stage, the curves gradually became flat and entered a stable phase, with the biomass maintained at a high level and the growth metabolism reaching a dynamic equilibrium. In Figure 2, the pH values of the KH-1 and KH-2 media increased significantly with the growth time in the early stage, and the urease secreted by the microorganisms continuously catalyzed the hydrolysis of urea to produce ammonium ions. In the later stage, the pH remained at a relatively high level of around 10, which not only reflects that the microorganisms can continuously secrete sufficient urease but also indirectly confirms that the bacteria have sufficient biomass to support this efficient metabolic process. In summary, the growth and pH curve trends fully demonstrate that the selected microorganisms not only have a fast growth rate and sufficient biomass accumulation, but also can continuously and efficiently carry out metabolic activities, and their overall growth and metabolic performance are excellent.

After diluting and spreading the bacterial solution onto a plate, a colony with a white and smooth surface and a red color around the growth area was picked and inoculated into a liquid culture medium for further growth. After 24 hours of cultivation, samples were taken for NCBI homology testing. Comparing with the NCBI data, it was found that the obtained strain was Bacillus subtilis, and the NCBI accession number was MZ165021.



The selected Bacillus subtilis

The growth curves of the strain under different temperatures and pH were analyzed. From Figure 3, it can be seen that under the condition of 40℃, the growth advantage of the strain was significant. The OD value of the culture medium continued to increase with the growth time of the strain. The OD value of the culture medium was close to 1.3 around 48 hours. Under this temperature condition, not only was the growth rate of the strain fast, but also the biomass accumulation of the culture medium was the highest. The growth trend of the 30℃ group was relatively close to that of the 40℃ group. The OD value of the culture medium reached approximately 1.2 in the later stage. The growth performance was good. Under the condition of 50℃, the growth of the culture medium had a relatively slow OD value increase. At 48 hours, it was only about 0.8. The growth rate of the strain and the biomass accumulation capacity of the culture medium were both weaker than those of the 30℃ and 40℃ groups. Under the condition of 60℃, it had a significant inhibitory effect on the growth of the strain. The OD value in the later stage was only about 0.6. The growth rate of the strain was significantly lower than that of other temperature groups. Through analysis, it can be concluded that 40℃ is the optimal temperature for the growth of the strain.

As shown in Figure 4, from the curve trend, the bacterial strains in the neutral environment with pH = 7 have a good growth condition. The OD value of the culture medium continuously and rapidly increases with the cultivation time. The OD value approaches 1.2 when the bacterial strain growth time is 48 hours, and slightly decreases when the growth time is 120 hours. Under this growth condition, not only does the growth rate of the strain increase rapidly, but the concentration of the bacterial strain in the culture medium also reaches a relatively high level. The growth condition of the bacterial strain in the alkaline environment with pH = 10 is weaker compared to the neutral environment with pH = 7. The growth rate and bacterial strain concentration of the culture medium are both weaker than those in the neutral environment with pH = 7. However, the OD value of the culture medium shows a stable upward trend under the condition of pH = 10, and the OD value of the culture medium reaches approximately 0.9 when the growth time is 48 hours, slightly decreases when the growth time is 120 hours, indicating that the bacterial strain can still grow well in the alkaline environment. The growth rate of the bacterial strain in the acidic environment with pH = 5.5 is the slowest. It has a significant inhibitory effect on the bacterial growth in the early stage. When the growth time is 48 hours, the OD value of the culture medium is only about 0.7. When the growth time exceeds 48 hours, the growth rate of the bacterial strain begins to increase again. When the growth time is 120 hours, the OD value of the culture medium can also maintain around 1.1. The biomass accumulation ability of the culture medium under pH = 5.5 is significantly lower than that in the neutral and alkaline environments. Therefore, the bacterial strain grows slowly in the

acidic environment initially but continues to grow stably. In the alkaline environment, the bacterial strain can also maintain stable growth. The growth is best in the neutral environment.

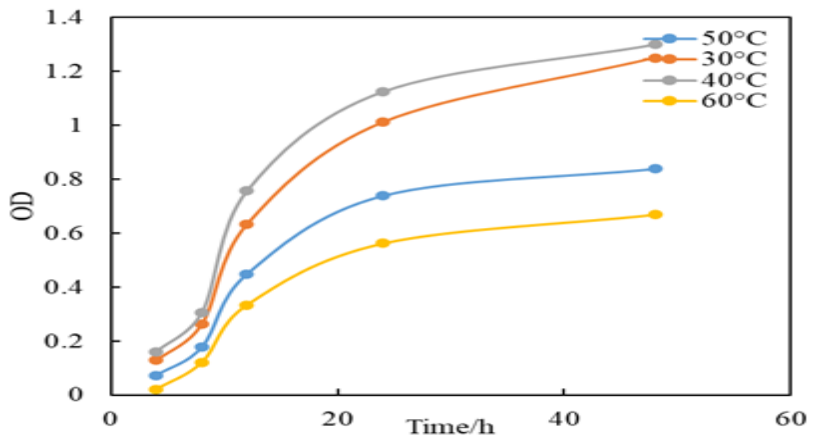


Figure 3: the relationship between OD values of the culture medium and time at different temperatures

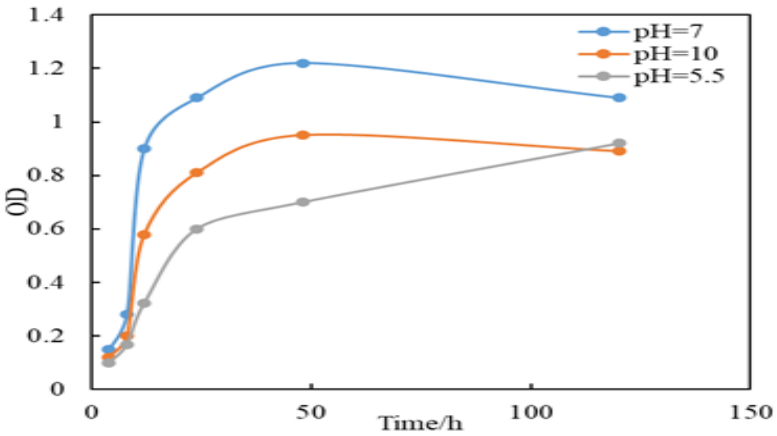


Figure 4: Relationship between OD value of the culture medium and time at different pH

By studying the relationship between the OD value of the Bacillus subtilis obtained and the growth time, it is helpful to grasp the growth rhythm of the strain and summarize the growth pattern of the strain. As shown in Figure 5, during the cultivation process of the strain, there are significant differences in the OD value at different time periods. Based on these differences, the strain cultivation process can be divided into four stages. During the cultivation process, from 0 to 4 hours is the first stage, which is the lag phase, and the growth is relatively slow; from 4 to 12 hours is the second stage, which enters the logarithmic phase. During this stage, the culture medium is rich in nutrients, the metabolic products are few, and the dissolved oxygen is sufficient, and the bacteria are in the logarithmic growth phase; the bacterial concentration shows a rapid upward trend; from 12 to 48 hours is the third stage, which enters the stable phase; after 12 hours, the nutrients are exhausted, resulting in a decrease in the reproduction rate, and the metabolic products and dissolved oxygen are insufficient, thereby intensifying the inhibition of bacterial growth. In addition, the strain regulates its own growth cycle, ultimately causing the bacterial growth to enter the stable phase. After 48 hours, it is the fourth stage, where the nutrients are depleted and the bacterial growth enters the senescence phase, and the cell metabolic activity significantly decreases. This indicates that this microorganism can rapidly achieve high activity and high bacterial concentration within a short period of time (especially during the logarithmic growth phase), and can efficiently meet the experimental demand for a large number of bacteria, providing a key reference for the establishment of sampling time for subsequent experiments related to the replacement of the culture medium for this strain.

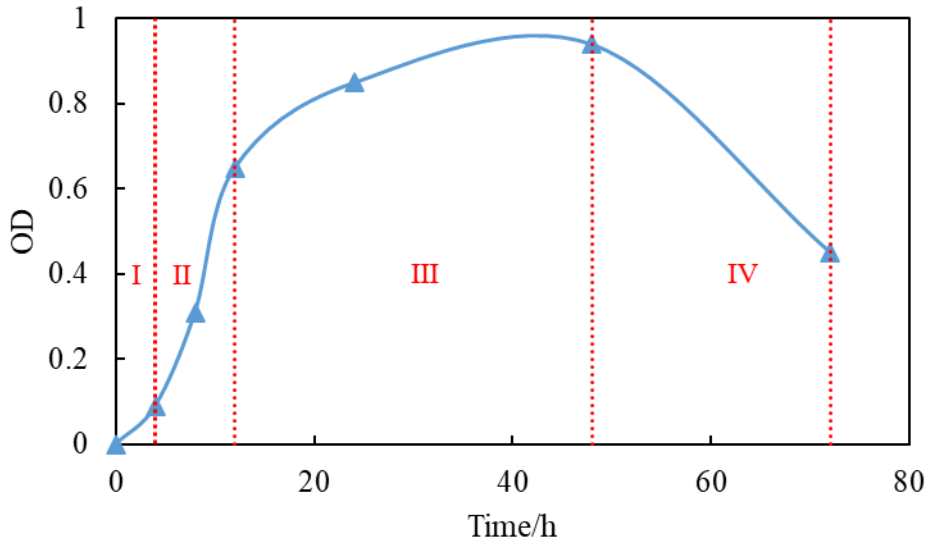


Figure 5: OD values at different times

In conclusion, this strain exhibits the best growth performance at a temperature of 40℃. It can maintain growth in an alkaline environment. In acidic conditions, the growth rate is initially inhibited. However, after the microorganism metabolizes and produces a large amount of urease to improve the surrounding acidic environment, the growth state returns to normal. In a neutral environment with a pH of 7, the growth performance is the best. The microorganism can reach its maximum activity in a short period of time, resulting in a large number of bacteria, and the concentration of the strain reaches its peak around 48 hours of growth.

3.2 Optimization of the culture medium system

According to the experimental plan in Section 2, the performance of the bacterial strains was analyzed. The results are as follows. The 1st to 9th culture media were used to cultivate the selected mineralizing bacteria, and the growth ability of microorganisms, urea decomposition effect, and mineralization effect under different culture times of different culture media were analyzed. After processing the data, the results are shown in Figures 6, 7, and 8.

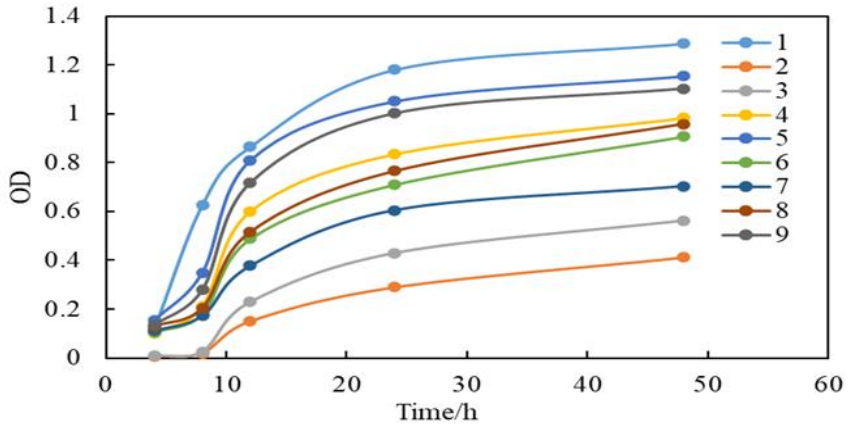


Figure 6: Graph showing the average OD values of culture medium 1-9 over time

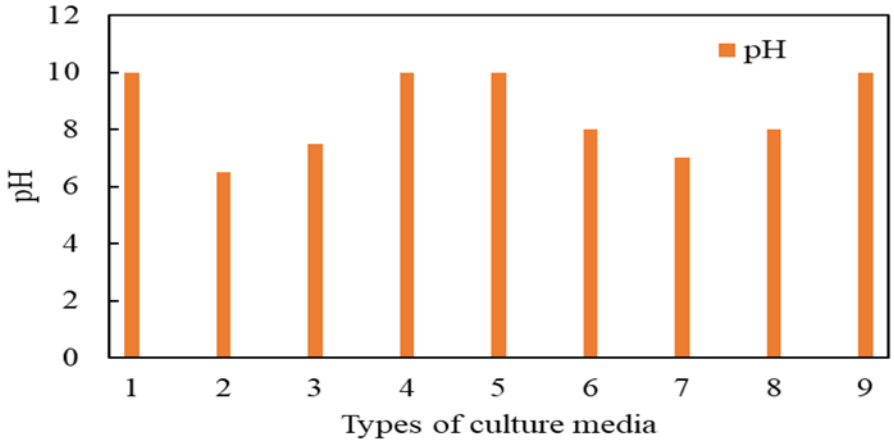


Figure 7: the relationship between the average pH of culture medium 1-9 and growth time

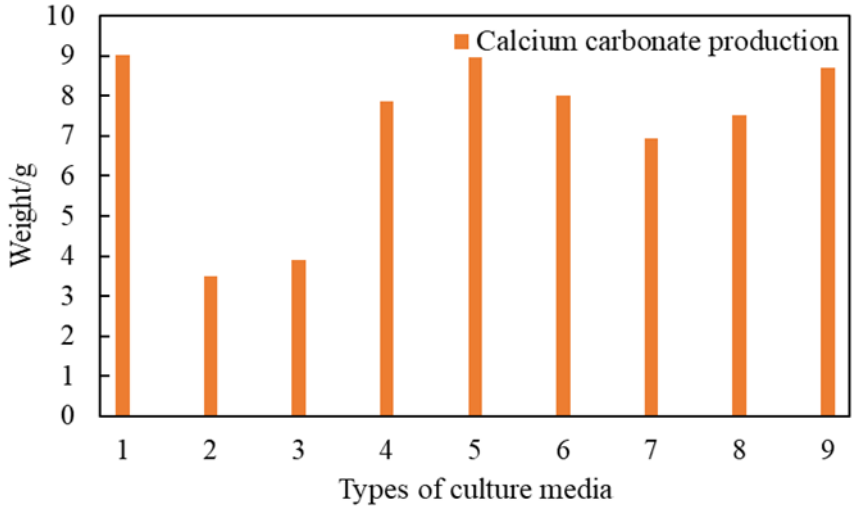


Figure 8: Comparison Chart of Calcium Carbonate Precipitation Amount in Cultures 1-9

According to the formula of substance quantity：

$$C=A\times V \qquad (1)$$

Here, C represents the amount of substance, A represents the concentration of the solution, and V represents the volume of the solution[15]。

The volume of the $CaCl_2$ solution is 100 mL, and the molar amount of $CaCl_2$ is 0.9 mol/L × 0.1 L = 0.09 mol. Since $CaCl_2$ completely dissociates in the solution as one Ca^{2+} and two Cl^- ions, that is, 1 mol of $CaCl_2$ can provide 1 mol of Ca^{2+}, the molar amount of Ca^{2+} in the solution is equal to the molar amount of $CaCl_2$, which is 0.09 mol. According to the reaction equation for the formation of calcium carbonate, as shown in formula (2),:

$$Ca^{2+}+CO_3^{2-}\rightarrow CaCO_3\downarrow \qquad (2)$$

The reaction between Ca^{2+} and CO_3^{2-} produced by the hydrolysis of urea occurs in a 1:1 ratio to form $CaCO_3$. According to formula 1, substituting the data gives: 0.09 mol × 100.9 g/mol ≈ 9.08 g. Since in the experiment, the result needs to be simplified to serve as the benchmark for evaluating the mineralization capacity, it is approximately 9 g.

As shown in Figures 6, 7, and 8, for the 1st, 5th, and 9th culture media, when the growth time was 48 hours, the OD values of the culture media were high, the pH value reached 10, and the calcium carbonate precipitation amount approached the theoretical value of 9g, meeting the performance requirements of the MICP technology. Among them, the 1st control group culture medium was used as the performance benchmark. The OD value of the culture medium at 48 hours was approximately 1.2 or higher, and the growth rate of the microorganism was the fastest, and the biomass accumulation of the culture medium was the most. The pH value of the culture medium reached 10, and the calcium carbonate precipitation amount generated by the culture medium was close to 9g, precisely matching the theoretical value; the 5th culture medium was the only replacement group that matched the performance of the control group. At 48 hours, the OD value was

approximately 1.2, almost no difference from the control, and the pH value reached 10, indicating that the urease activity was not affected by the substitution of soybean meal powder for peptone and the use of sucrose as the carbon source. The calcium carbonate precipitation amount of the culture medium was also close to 9g. The 9th culture medium performed slightly worse than the 5th, with an OD value of approximately 1.1 at 48 hours, a slightly lower biomass, and a pH value of 10, with a calcium carbonate precipitation amount of approximately 8.5g, close but not reaching the theoretical value. The overall performance was excellent but slightly inferior to the 5th culture medium.

For the 4th, 6th and 8th culture media, at a growth time of 48 hours, the OD values were moderate, lower than those of the control group, and the pH values were mostly 8. The calcium carbonate precipitation amount of the culture media was approximately 8g. Although these media could support microbial growth and mineralization, their performance was not optimal and the optimization effect was limited. Among them, the OD value of the 4th culture medium at 48 hours was approximately 0.9, and the pH value reached 10. The urease had a relatively superior ability to hydrolyze urea, but the bacterial concentration was insufficient. The calcium carbonate precipitation amount of the medium was approximately 8g. The 6th culture medium had an OD value of approximately 0.9, a pH value of 8, and the urease had a moderate ability to hydrolyze urea. The calcium carbonate precipitation amount of the medium was approximately 8g. The mineralization effect was slightly inferior to that of the control group. The 8th culture medium had an OD value of approximately 0.95. The early growth was acceptable but the later growth was weak. The pH value was 8 and the calcium carbonate precipitation amount of the medium was approximately 7.5g. These three types of culture media could only meet basic requirements and did not have significant optimization advantages.

The OD values of the 2nd, 3rd and 7th culture media were much lower than those of the control group at 48 hours, with lower pH values, weak or almost no ability to hydrolyze urea by urease, and only 3.5-7g of calcium carbonate precipitation, which was far below the theoretical value. The microbial growth was inhibited and the mineralization ability was severely insufficient. There was no optimization value. Among them, the OD value of the 2nd culture medium was only about 0.4, with a pH value of 6. This might be due to the acidic environment inhibiting growth and urease activity. The calcium carbonate precipitation of the 3rd culture medium was approximately 3.5g; the OD value of the 3rd culture medium was about 0.5, with a pH value of 7.5, and the urease had weak ability to hydrolyze urea. The calcium carbonate precipitation of the 3rd culture medium was approximately 4g; the OD value of the 7th culture medium was about 0.7, with a pH value of 7. The urease had extremely weak ability to hydrolyze urea, and the calcium carbonate precipitation of the 7th culture medium was approximately 7g. The bacterial growth in these three types of culture media was slow, the urease activity was low, and they could not support effective mineralization. The optimization value was low.

In summary, in the medium replacement system, strain No. 5 of Bacillus subtilis showed the fastest growth rate, the best urea hydrolysis effect, and the strongest ability to induce calcium carbonate precipitation. It performed comparably to the control group medium in all aspects. Moreover, compared to the control group medium, strain No. 5 medium uses inexpensive agricultural by-products (soybean meal powder) to replace expensive peptone, and uses low-cost sucrose as the carbon source, significantly reducing the raw material cost of the medium and making the formula more cost-effective. In the process of sand control in the petroleum industry, it can effectively reduce expenses, achieve cost reduction and efficiency improvement, and enhance the recovery rate.

V. EXPERIMENTAL CONCLUSION

This study aimed to address the economic and effectiveness bottlenecks faced by the microbial induced calcium carbonate precipitation (MICP) technology in sand control applications. A systematic experiment was conducted on the culture medium of the core functional strain Bacillus subtilis. Through the combination of strain screening, performance testing, comparison of culture medium replacement system parameters, and verification of mineralization effects, the following conclusions were drawn：

(1) Successfully screened and identified the target mineralizing strain. A strain with mineralization effect was successfully isolated from the soil, and it was identified and compared with NCBI. The result showed that the identified strain was Bacillus subtilis.

(2) Conduct performance tests of the bacterial strain. Through gradient experiments under different temperatures and pH levels, it was found that the growth activity of this strain is significantly influenced by environmental factors. 40°C is the optimal temperature for the strain. At this temperature, the bacterial concentration (OD value) can reach above 1.3 after 48 hours of growth, with a fast growth rate and the strongest biomass accumulation ability. Under the 40°C condition, the growth advantage of the strain is significant. The growth trend under the 30°C condition is relatively similar to that under the 40°C condition, with good growth performance but the OD value of the culture medium is slightly lower compared to the 40°C condition. Under the 50°C condition, the growth of the culture medium shows a relatively slow increase, reaching only about 0.8

after 48 hours. The 60°C condition significantly inhibits the growth of the strain, with the growth rate being significantly lower than that of other temperature groups.

(3) Set up a medium replacement system, with No. 1 medium as the control group. Analyze the relationship between OD, pH value and calcium carbonate precipitation content of the 2-8 medium and the growth time. It is concluded that the average OD value of No. 5 medium reaches 1.154 at 48 hours, close to the control group. The concentration of the strain and the growth rate are the best among all the replacement groups. Moreover, at 48 hours, the pH value of the medium reaches 10, the same as the control group, indicating that the urease activity of the strain is not affected by the replacement of carbon and nitrogen sources, and can effectively decompose urea to provide an alkaline environment. After adding $CaCl_2$ solution, the amount of calcium carbonate precipitation is close to 9g, significantly higher than other replacement groups, indicating that its ability to induce calcium carbonate precipitation is the best, the same as the control group. From an economic effect perspective, compared with the control medium, No. 5 medium has the same bacterial concentration, urea hydrolysis effect and mineralization ability as the control group, providing performance guarantee for on-site application. However, No. 5 medium replaces peptone with soybean meal powder and uses sucrose as the carbon source, significantly reducing the cost of the medium, achieving cost reduction and efficiency improvement in the sand control process.

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