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Microbially Induced Calcite Precipitation (MICP) Improved Drilling Fluid Optimization for Gravel Stratum

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Abstract: During the exploration of the gravel stratum, incidents such as wellbore leakage, stuck drilling, and unstable wellbore walls frequently occur. These issues lead to diminished drilling efficiency and prolonged construction timelines, ultimately adversely affecting the core recovery rate, resulting in a significant waste of manpower and material resources. To address the issue of hole collapse during drilling, the microbially induced calcite carbonate precipitation (MICP) technique was employed to enhance the properties of bentonite mud drilling fluids. This study analyzed the effects of three factors, i.e., bentonite, biological solution, and barite powder, on the bentonite mud bio-cementation effectiveness through an orthogonal experiment and response surface methodology (RSM). The biological mechanism was examined using scanning electron microscopy (SEM). The experimental results indicated that optimal formulation was achieved when the mass fraction of bentonite was 13.96%, the biological solution comprised 0.6% xanthan gum and 0.4% carboxymethyl cellulose, and the mass fraction of barite was 25%. This research explores the application potential of MICP in enhancing the rheological properties of bentonite mud drilling fluids, which provides new insights and technical references for optimizing their performance.

Keywords: drilling fluid; orthogonal experiment; response surface methodology; bio-cementation; microstructure; gravel stratum



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1. Introduction

The gravel stratum is composed of gravel particles of different shapes and sizes [1]. Its structure is characterized by large pores and minimal cohesion. During drilling exploration in gravel strata, several challenges frequently arise, including falling blocks, collapsing holes, and the formation of sand bridges, which may impede progress and necessitate additional precautions [2]. In addition, vibration during the drilling process may cause wellbore wall collapse, thereby leading to buried drilling problems and incurring economic losses. To mitigate borehole collapse during drilling, mud circulation is often employed to ensure wellbore stability and prevent issues such as borehole collapse and stuck drilling [3]. Drilling mud plays a vital role in suspending drilling cuttings, cooling drill bits, maintaining stable wellbore walls, and preventing wellbore collapse throughout the drilling process. However, conventional bentonite mud, due to its low viscosity and density, as well as its poor rheological properties, fails to meet the requirements for drilling in gravel strata [4,5]. Therefore, effectively enhancing the rheological properties of drilling fluids is an urgent

challenge that must be addressed. Currently, improvements in drilling issues are primarily focused on three facets: the enhancement of drilling equipment, the refinement of drilling techniques, and the optimization of drilling fluids [6–8]. The optimization of drilling fluids has generally improved their quality and performance by introducing various additives into conventional drilling fluids. Keren et al. [9] demonstrated that the addition of bentonite significantly impacts the rheological properties of drilling fluids. In a paper by Sun and Zhao [10], various scholars utilized foam, polymers, polymer suspensions, and high-density slurries as regulators in laboratory experiments targeting gravel stratum to determine the optimal mud ratios. Au et al. [4] studied the influence of bentonite content on the fundamental properties of mud and concluded that the bentonite content determines the yield stress magnitude of the kaolin–bentonite composite suspensions. However, this approach is neither economical nor environmentally friendly [11]. Experimental investigations on bentonite application in gravelly soil drilling have revealed an optimal range of mud dosages, which have been successfully implemented in practical engineering projects [10,12]. Akpan et al. [13] found that the addition of xanthan gum significantly enhances the viscosity and film-forming properties of mud, thus effectively reducing filtration loss [14]. Sun et al. [10] further analyzed the modification effect of carboxymethyl cellulose sodium (CMC) on mud. Nonetheless, current research predominantly emphasizes enhancing the cementing effect of drilling fluids through various additives, which often result in difficult preparation, environmental pollution, and non-biodegradability [15]. Rheological characteristics are fundamental properties of drilling mud that reflect its flow behavior and film-forming potential in various soil types [16]. The rheological properties of drilling fluids refer to their flow and deformation characteristics under external forces and are typically described by rheological parameters such as rheological curves, plastic viscosity, and yield point [17]. Good rheological properties effectively transport drilling debris, enhance mechanical drilling efficiency [18], and maintain wellbore integrity [19]. A common practice in drilling operations in gravel formations is to use a dispersion-blocking system for drilling muds, which is achieved by adding specific particulate materials as blocking agents to the base drilling fluid. However, this practice often has a significant impact on the environment because of the difficulty in degrading the components of these special particulate materials, which leads to serious environmental pollution issues, and the application costs are relatively high. To address this situation, we propose to introduce MICP technology into dispersion-based drilling fluid systems to improve the wall-consolidation performance of drilling fluids.

The MICP technique essentially utilizes specific urease-producing bacteria found in nature for decomposing urea to generate carbonate ions that react with metal ions in the environment to form carbonate precipitation [20–22]. It effectively reduces greenhouse gas emissions and improves environmental carbon sequestration efficiency [23–27]. Currently, *Bacillus subtilis* is commonly employed as the experimental strain due to its clear reaction mechanism, controllable reaction conditions, robust growth activity, and favorable environmental adaptability [22,26–28]. Chu et al. [29] proposed utilizing the MICP technique to pre-generate calcium carbonate particles, also referred to as “biogrout”, to reinforce coarse sand. Their experimental results demonstrated that this technique effectively reduces the permeability coefficient of coarse sand. Li [30] and Shi et al. [31] found that xanthan gum can delay the bacterial growth rate without affecting bacterial activity. Saracho et al. [32] highlighted the beneficial role of carboxymethyl cellulose in enhancing CaCO_3 generation when the MICP technique is used. Choi et al. [33] showed that the addition of CMC increases microbial calcite precipitation. Through experiments, Jia et al. [34] verified that incorporating montmorillonite effectively enhances CaCO_3 precipitation compared to traditional MICP approaches [35]. The content of solid substances and the viscosity of biological

mud directly influence the effectiveness of mud wall protection [36]. Drilling fluids with higher viscosity can significantly enhance bonding and wall protection in fractured and unstable strata [15,37]. The MICP technique facilitates the generation of calcite crystals, which possess excellent cementation properties. These crystals play a crucial role in forming calcium carbonate within loose and fractured bottom strata, thereby stabilizing the wellbore wall [37]. MICP technology offers a novel approach to addressing wellbore instability in gravel stratum. It could enable the creation of a microbial drilling fluid system that induces calcium carbonate precipitation within gravel stratum during drilling. This would enhance the mechanical strength of the wellbore, improve the integrity of the formation, and provide a new technical solution for effectively stabilizing wellbores in gravel stratum.

This study explored the potential of implementing the MICP technique into a dispersed drilling fluid system to prepare microbial drilling fluid, aiming to improve the wall-consolidation performance of drilling muds. The response surface methodology (RSM) was employed to analyze data and optimize the drilling fluid formulation [38]. The center composite design (CCD) method was utilized to analyze the effects of bentonite, biological solution, and barite powder on the rheological properties of drilling mud, including plastic viscosity (PV) (mPa·s), density (g/cm³), and yield point (YP) (Pa). Finally, a quadratic regression equation was developed to fit each factor, leading to the determination of the optimal formulation based on RSM.

2. Materials and Methods

2.1. Test Materials

Table 1 describes the mixing ratios of the culture medium compositions that are utilized as primary experimental materials and the additive admixtures. All experimental reagents utilized in this study, purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, were of analytical grade. Polydimethylsiloxane lotion was employed as an additive to reduce the amount of foam generated during mixing. CMC and xanthan gum are chemical additives that are commonly used in drilling. Chen et al. [23,27] demonstrated that the addition of CMC increased calcium carbonate production in microbial clays. Saracho [28] and Xiao et al. [31] found that xanthan gum hinders bacterial growth without affecting activity.

Table 1. Medium materials and experimental raw materials.

	Raw Materials	Contents
Composition of culture medium (100 mL)	Deionized water	93 mL
	0.5 g/L MnSO ₄ ·H ₂ O solution	2 mL
	Yeast extract	2 g
	NH ₄ Cl	1 g
	1.2 g/L NiCl ₂ ·6H ₂ O solution	2 mL
	1 M/L NaOH solution	3 mL
Other experimental reagents	Bentonite	-
	Barite	-
	Carboxymethylcellulose Sodium (CMC)	-
	Xanthan gum (XG)	-
	Polydimethylsiloxane lotion	-

In this study, *Sporosarcina pasteurii*, which was acquired from the China General Microbiological Culture Collection Center (CGMCC), was utilized as the bacterial culture. The composition of the bacterial activation culture medium was prepared based on the liquid culture medium provided by the CGMCC. The culture medium was sterilized with high-pressure steam at 121 °C for 20 min and then placed on the ultra-clean workbench until cooled [21]. The primary bacterial strain was inoculated into the culture medium and then shaken and cultured at 180 r/min and 30 °C for 24 h [23,31]. The bacteria concentration was expressed by measuring the absorbance of the bacterial solution (turbidimetric method). This principle was mainly based on the fact that the bacterial concentration was directly proportional to the turbidity of the bacterial solution. Therefore, it was also directly proportional to the absorbance. In this paper, the absorbance (OD_{600}) of an ultraviolet visible spectrophotometer (model uv-Molecules, INESA Company, Shanghai, China) at a wavelength of 600 nm was used to determine the bacteria concentration. Following cultivation, the absorbance value of the bacterial solution at 600 nm (OD_{600}) was measured to be 1.396.

2.2. Preparation of Biological Solution

To investigate the combined effect of XG and CMC on bacterial growth activity during MICP, different proportions of XG and CMC were introduced during bacterial culture. The 200 mL culture medium solution was subjected to high-pressure steam sterilization, after which XG and CMC were added in specific proportions. Mixed solutions of 0%:0%, 0.4%:0.2%, 0.6%:0.4%, 0.8%:0.6%, and 0.4%:0.6% were prepared in accordance with the mass of the culture medium. After mixing, 1 mL of bacterial solution was added to every 200 mL of culture medium, which was then placed in a constant-temperature oscillation box for cultivation. The unit urease activity and density OD_{600} of the bacteria were measured.

Bacterial activity was assessed using the conductivity method, and the average conductivity change per minute ($\text{ms} \cdot \text{min}^{-1}$) was recorded using the conductivity meter [28,31,39]. According to Whitfin's empirical function, a conductivity change of $1 \text{ ms} \cdot \text{min}^{-1}$ corresponded to 11 mM of urea hydrolysis per minute [40]. Considering that the bacterial solution was diluted 10 times during the mixing process with urea, the conductivity value was multiplied by 10 to determine the amount of urea hydrolyzed per minute in the tested bacterial solution, which served as a measure of urease activity [40]. During the bacterial culture, the OD_{600} value was measured at regular intervals. When it reached approximately 1.500, the bacterial culture was considered to be complete. Then, the activity was measured after a cultivation duration of 9 h. After the completion of the bacterial culture, the OD_{600} was measured using a visible-light spectrophotometer to characterize the bacterial content. Due to the colorless and transparent nature of xanthan gum and CMC solutions, it was experimentally determined that the addition of these solutions had minimal effect on the determination of OD_{600} .

The patterns of change in activity and OD_{600} were consistent. The highest-unit urease activity and OD_{600} values were observed at the end of the 9 h cultivation period in the culture medium without added admixtures, yielding values of 15.86 U/mL (1 U = 1 μM of urea hydrolyzed/min) and 1.696, respectively. The remaining four groups exhibited a slight decrease in activity compared to OD_{600} as the content increased, but the decrease was not significant, and the activity remained above 11.5 U/mL. When the cultivation time was shorter (4 h), the bacterial concentration in the culture medium without any added substances was the lowest at 0.418. It was speculated that this is due to xanthan gum acting similarly to nutrients in the culture medium, without adversely affecting the bacteria in the short term. In the other four experimental groups, the OD_{600} value increased as the

mixture content in the medium increased, while the activity value remained relatively stable (Figure 1).

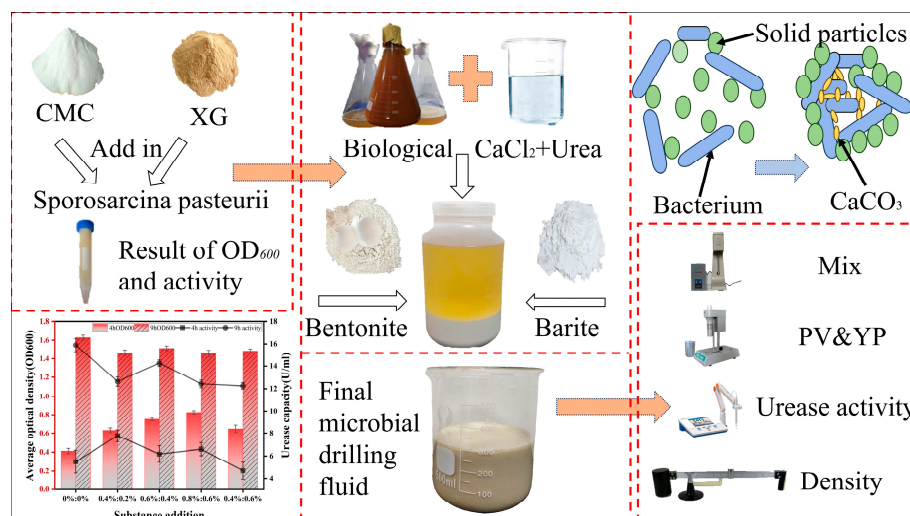


Figure 1. Sample preparation.

The results for the unit urease activity and OD_{600} value indicated that as the amounts of xanthan gum and CMC added increased, there was a slight effect on bacterial growth; however, the difference in unit urease activity was not significant. Thus, the addition of mixed powder slowed the bacterial growth rate and prolonged the bacterial culture time, but it had minimal effect on the bacterial growth activity. Simultaneously, it effectively increased the viscosity of the bacterial solution, which created favorable conditions for mud production. Therefore, 0.6% XG and 0.4% CMC were added during the cultivation process.

Urea and CaCl_2 were added in moderate proportions to the cultured *Bacillus subtilis* solution; the urea provided a nitrogen source for biological growth and CaCl_2 supplied calcium for the MICP process. The biological solution with added CMC and XG was utilized as one of the variables, while bentonite and barite powder were used as the other two variables to ensure the basic properties of the slurry and to enhance its viscosity and density. The required biological solution for the experiment was obtained, and rheological parameter testing was conducted according to the experimental plan derived from the response model.

2.3. Response Surface Experimental Design

For multivariate analysis, RSM provides a research approach that employs statistical methods to study parameter interactions [41]. RSM was initially utilized for experimental response modeling and has since been widely adopted for numerical experimental modeling [42]. Essentially, it encompasses a collection of statistical and mathematical techniques used to establish empirical models [43]. Central Composite Design (CCD) is a type of response surface design that can optimize multiple response variables, generate mathematical models, and determine the optimal solution for drilling fluid under various conditions [40,44].

To ensure the fundamental performance of the mud and enhance its viscosity and density, specific amounts of bentonite and barite powder were incorporated into the drilling fluid. Independent variables (bentonite (g), biological solution (mL), and barite powder (g)) were investigated at five levels with three replications in the center domain. Each independent variable was coded at five levels, as detailed in Table 2. A three-factor, five-level response surface design was conducted using a CCD model [38]. The experimental

design of this model includes 8 factorial points, 8 axial points ± 2 distance from the center, and 5 repeated center points, which can be utilized to describe the relationship between the response (i.e., PV, density, and YP) and variables (bentonite, biological solution, and barite powder) [38,43].

Table 2. Experimental range and levels of independent variables.

Variables		Factor Level				
Number	Name	Minimum	Coded Low	Mean	Coded High	Maximum
A	Bentonite	16.36	30.00	47.30	70.00	83.64
B	Biological solution	0.00	0.00	238.07	500.00	670.45
C	Barite powder	0.00	75	91.72	125.00	142.05

2.4. Density and Rheological Parameter Determination

Relative density was measured using a density meter with a volume of 140 m³. The biological mud was mixed using the HTD-3S digital high-speed mixer at a speed of 800 rpm. Following the preparation of the mud, it was transferred to a mud cup, covered, and placed on a bracket. The vernier was adjusted to achieve a horizontal lever, and the left indicator displayed the relative density of the mud [17]. The following formula can also be used for calculation:

$$\rho_x = \frac{m_3 - m_1}{m_2 - m_1} \quad (1)$$

where ρ_x is the relative density; m_1 is the mass of any fixed container; m_2 is the mass of the container filled with clean water; m_3 is the mass of the container filled with drilling mud.

The rheological parameters were primarily measured using a ZNN-D6 rotational viscometer. In this experiment, all results were conducted at consistent ambient temperature and humidity conditions [45,46]. The rheological parameters (i.e., PV and YP) were calculated based on the Bingham plastic fluid model [19]. The Bingham model represents the relationship between shear stress and shear rate:

$$\sigma = \sigma_y + \eta_B \dot{\gamma} \quad (2)$$

where σ is the shear stress of the fluid at different shear rates; σ_y is the resistance to initial flow and represents the stress required to initiate the fluid movement, i.e., the yield point; η_B is the plastic viscosity of the fluid; and $\dot{\gamma}$ is the shear rate of the fluid [19].

The Bingham plasticity model is a widely employed rheological model that can aid in determining the plastic viscosity and yield point of various drilling fluids. This model can predict the minimum force required to initiate fluid flow [17]. The PV is calculated with a rotational viscometer based on Equation (3), while the YP is determined using Equation (4).

$$PV = \varphi_{600} - \varphi_{300}(mP_a \cdot s) \quad (3)$$

$$YP = 0.511 \cdot (\varphi_{300} - PV)(Pa) \quad (4)$$

3. Results and Discussion

3.1. Response Surface Test Results

Table 3 summarizes the operational combinations and corresponding results of the 36 conducted experiments. To minimize the impact of unexpected variability in the observed response, all experimental tests in this study were conducted based on a random arrangement. The statistical significance of the polynomial model was ascertained by

evaluating the coefficient of determination (R^2) and the F-statistic, which represents the ratio of the mean square to the residual mean square of the model. This analysis aided in evaluating the fitting accuracy of the generated polynomial model. Additionally, the significance of the model terms was evaluated using the P-value (probability value) at a 95% confidence level [38]. To determine the interrelationships between the variables, multivariate regression analysis was conducted, and the polynomial model was obtained using the least squares method. The regression coefficients were determined through analysis of variance (ANOVA) [41].

Table 3. Operational combinations of independent variables in the CCD experiment.

Group Number	Influencing Factors			Response Index		
	A	B	C	PV	Density	YP
1	30.00	5.50	4.00	52.00	1.19	68.406
2	90.00	8.00	6.00	5.00	1.28	0.175
3	70.00	5.50	8.00	64.00	1.19	72.349
4	10.00	8.00	6.00	46.00	1.01	61.905
5	50.00	8.00	6.00	47.00	1.03	65.115
6	50.00	8.00	6.00	68.00	1.21	85.638
7	30.00	10.50	4.00	48.00	1.09	62.905
8	50.00	3.00	6.00	4.00	1.19	2.425
9	30.00	5.50	8.00	63.00	1.11	74.888
10	50.00	8.00	2.00	4.00	1.20	2.864
11	50.00	8.00	6.00	49.00	1.17	57.655
12	70.00	10.50	8.00	49.00	1.17	57.427
13	50.00	8.00	6.00	50.00	1.18	57.497
14	50.00	13.00	6.00	25.00	1.21	23.915
15	50.00	8.00	6.00	48.00	1.17	60.934
16	30.00	5.50	4.00	24.00	1.13	28.436
17	50.00	8.00	6.00	67.00	1.12	88.137
18	30.00	10.50	4.00	50.00	1.17	60.457
19	30.00	10.50	8.00	49.00	1.16	60.822
20	50.00	8.00	6.00	48.00	1.18	60.583
21	30.00	10.50	8.00	47.00	1.17	60.395
22	70.00	10.50	8.00	1.00	1.18	0.176
23	50.00	8.00	6.00	49.00	1.17	60.891
24	70.00	5.50	4.00	51.00	1.18	60.328
25	50.00	8.00	6.00	84.00	1.13	101.412
26	50.00	8.00	6.00	45.00	1.01	58.665
27	30.00	5.50	8.00	58.00	1.17	72.301
28	50.00	8.00	6.00	81.00	1.15	88.478
29	50.00	8.00	6.00	28.00	1.15	39.165
30	70.00	10.50	4.00	29.00	1.24	36.915
31	70.00	10.50	4.00	49.00	1.20	59.978
32	50.00	8.00	6.00	5.00	1.24	1.765
33	50.00	8.00	10.00	45.00	1.16	49.286
34	70.00	5.50	8.00	43.00	1.00	53.785
35	70.00	5.50	4.00	50.00	1.21	59.595
36	50.00	8.00	6.00	51.00	1.25	58.957

Figure 2 presents the correlation analysis heatmap among various factors based on experimental results. From this graph, we observe a strong positive correlation between plastic viscosity (PV) and the biological solution. This indicates that as the amount of biological solution increases, plastic viscosity also rises correspondingly. Conversely, plastic viscosity shows a negative correlation with barite content; that is, higher barite content results in lower plastic viscosity. Additionally, the correlation between plastic viscosity and bentonite content is weak, suggesting that bentonite has a limited direct impact on plastic viscosity. There is a strong positive correlation between density values and barite content, which is expected, as barite is a weighting agent that is commonly utilized to increase the density of drilling fluids. Furthermore, while density values also demonstrate some correlation with bentonite content, this relationship is not as pronounced as the one with barite. Notably, the addition of biological solutions significantly reduces density values, indicating that as biological solution content increases, the density of the drilling fluid decreases. The yield point (YP) is influenced by the combination of bentonite and biological solution content, with the latter having a more pronounced effect. This highlights the biological solution's importance in adjusting the yield point of drilling fluids. To further illustrate these relationships, scatter plots showing the interactions between plastic viscosity, yield point, density, and biological solution content are presented in Figure 2, using biological mud content as a reference. These scatter plots offer concrete visual evidence to reinforce the findings of the correlation analysis discussed above.

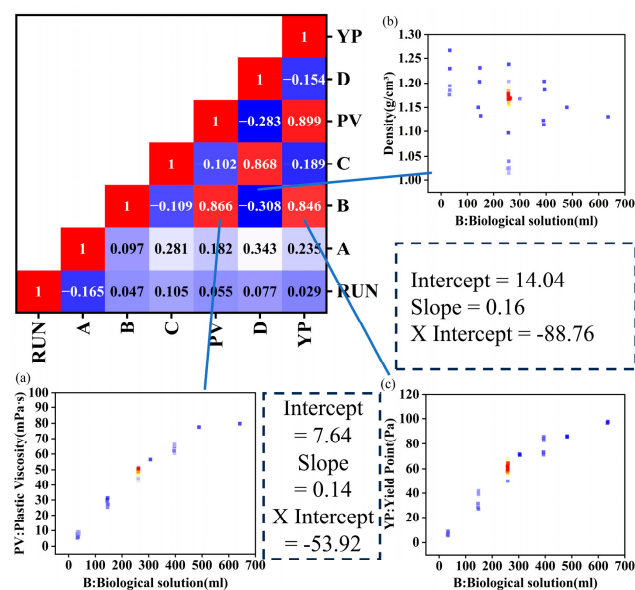


Figure 2. Correlation analysis between various factors and responses. scatter plots: (a) the interactions between B and PV; (b) the interactions between B and D; (c) the interactions between B and YP. (A: Bentonite; B: biological solution; C: barite; PV: plastic viscosity; D: density; YP: yield point. The symbol ‘-’ indicates a negative correlation between variables; “Run” refers to the number of experiments conducted).

3.2. Statistical Analysis

3.2.1. Plastic Viscosity Analysis

Plastic viscosity can be utilized to evaluate drilling fluid’s ability to suspend unstable cuttings, which represents a component of the flow resistance caused by mechanical friction, specifically referring to the shear stress exceeding the yield point [17]. For general drilling fluids, the plastic viscosity primarily depends on the solid concentration within the drilling fluid [15]. In the samples with higher solid content, the increased frictional force between particles leads to enhanced plastic viscosity. Moreover, within the same volume,

smaller particle sizes result in larger surface areas, thereby increasing frictional force and consequently yielding higher PV values.

The experimental results for the first response value of PV were used to fit the model and obtain the best-fitting result through variance analysis. The optimal fitting equation is essentially quadratic [38]. The second-order polynomial mathematical model representing the relationship between the independent variable and the response variable PV (Y1) is presented in Equation (5):

$$Y1 = 49.03 + 1.85A + 36.43B + 0.58C + 0.77AB + 0.18AC - 0.21BC - 0.69A^2 - 8.91B^2 \quad (5)$$

where A represents the bentonite content; B represents the biological solution content; and C represents the barite content. Among them, positive values of coefficients A, B, C, AB, and AC indicated that linear effects increase the PV, while coefficients BC, A^2 , and B^2 exerted negative effects on the increase in PV.

The orthogonal analysis results for the PV response surface quadratic model are demonstrated in Table 4. The model demonstrated good accuracy at a 95% confidence level with an F-statistic of 675.99, indicating the significance and a minimal 0.01% probability of such a high F-statistic resulting from interference. Based on the F-statistic, the influence of the three factors on PV followed the order $B > A > C$. This indicates that the biological solution has a significantly greater impact compared to the contents of bentonite and barite. The analysis yielded an acceptable AP ratio of 103.7526, exceeding the threshold value of four.

Table 4. Analysis of variance results of response value Y1 (PV).

Response	F-Statistic	R ²	p	Adj.R ²	AP	SD	PRESS
PV (Y1)	675.99	0.9957	<0.0001	0.9943	103.7526	1.56	153.65

To evaluate the accuracy of the model, a diagnostic chart comparing predicted and actual values was utilized, which confirmed the acceptable accuracy of the model [41]. In cases where the fitting degree of the model is insufficient, continued optimization of the response surface could yield adverse consequences [44]. The appropriate distribution of data points near the line represents the model's ability to predict response values [43]. Figure 3d illustrates the diagnostic graph comparing the predicted and actual PV values. The experimental design points were distributed diagonally, which indicated that the actual PV values align with the predicted values of the model. According to Table 4, the R^2 and Adj. R^2 values were similar, with a difference of less than 0.2, which indicated that the generated mathematical model can effectively reflect the relationship between mud viscosity and various factors and can predict the optimal viscosity.

Figures 3e and 4f demonstrated that the residuals obtained from the operating results conform to the basic assumption of the error term, with an R^2 of the residuals fitted at 0.95684. It is evident that the error is within an acceptable range, and there is no violation of the assumptions of independence or constant variables [36]. In addition, the interaction between various influencing factors and PV is illustrated in Figure 3, where the addition of each material ultimately increases the solid content. Figure 3a–c depict the relationship between plastic viscosity (Y1) and bentonite (A), biological solution (B), and barite powder (C). Figure 3a,c show that as the containment of the biological solution increased, the PV value gradually increased and exhibited a rapid increase in the range of 0 mL to 200 mL. The additional amount significantly influenced the PV value. However, in the range of 200 mL to 400 mL, while the PV value continued to increase, the growth rate gradually slowed down, and the curvature of the surface decreased. Figure 3 illustrates that bentonite

positively affects the increase in PV value, but the effect is not as significant compared to biological solutions. The barite content had a minimal effect on the PV value.

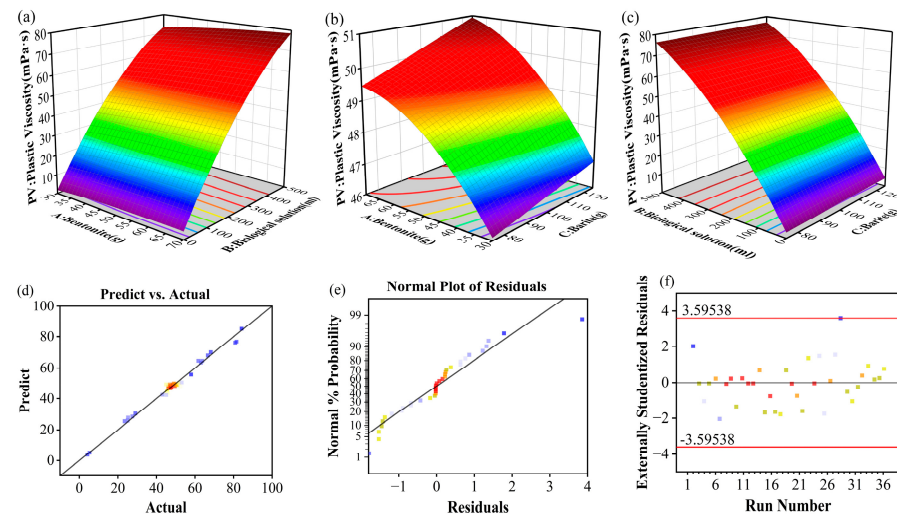


Figure 3. Three-dimensional response surface plots for various factors: (a) A and B, (b) A and C, (c) B and C; (d) diagnostic chart of predicted and actual values of plastic viscosity; (e) the normal graph of residuals; (f) residual and running chart.

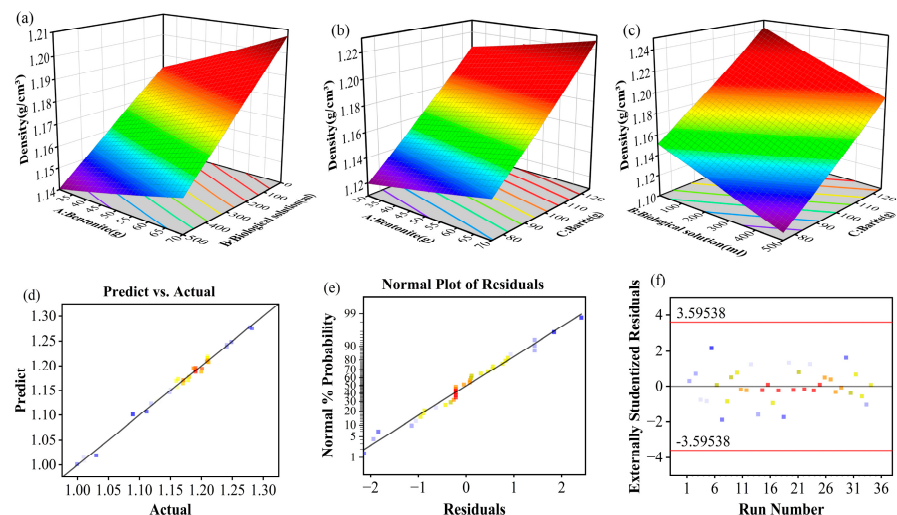


Figure 4. Three-dimensional response surface plots of various factors: (a) A and B, (b) A and C, (c) B and C; (d) diagnostic chart of predicted and actual densities; (e) the normal graph of residuals; (f) residual and running chart.

3.2.2. Density Analysis

The fitting equation derived from the variance analysis on density is presented in Equation (6):

$$Y_2 = 1.1714 + 0.0096A - 0.0240B + 0.0439C - 0.0021AB - 0.0004AC - 0.0018BC + 0.0004A^2 - 0.0001B^2 + 0.0015C^2 \quad (6)$$

where positive values of coefficients A, C, BC, and C^2 indicate that linear effects increase the density, while coefficients B, AB, AC, A^2 , and B^2 exert negative effects on the increase in density.

The orthogonal analysis results of the response surface quadratic model are displayed in Table 5. The F-statistic of the model was 408.09, indicating significant results. The

p -values of A, B, and C were all less than 0.0001, designating them as important model terms. The importance of the three factors on the response results was as follows: $C > B > A$. Barite C had a significant impact on density, while biological solution and density had a relatively minor effect. The R^2 value for this response was 0.9930, which was close to 1. The Adj. R^2 value was 0.9905, which was in good agreement with the R^2 value. The AP ratio was 81.7644, exceeding 4, which was acceptable. A ratio greater than four was expected and indicated model differentiation, which suggested that the model can guide the design space defined by CCD [38].

Table 5. Analysis of variance results of response value Y2 (density).

Response	F-Statistic	R^2	p	Adj. R^2	AP	SD	PRESS
Density (Y2)	408.09	0.9930	<0.0001	0.9905	81.7644	0.0064	0.0020

Figure 4 illustrates the interaction between various influencing factors and the density of drilling fluid. Figure 4a–c show the relationships between bentonite (A), biological solution (B), barite powder (C), and density (Y2). The density of drilling fluid was positively correlated with the contents of bentonite and barite. The addition of bentonite resulted in a slight increase in density, though the effect was not significant. Conversely, the addition of barite effectively increased the density. There was a negative correlation between biological solution and density value, and the addition of biological solution significantly decreased the density of drilling fluid. An increase in biological solution reduces the density; however, it may slightly increase the density at lower concentrations. This result aligned with expectations, as barite possessed a very high density (typically $4.3 \times 10^3 \text{ kg/m}^3$) and functioned as a density enhancer in water-based drilling fluids [47].

Figure 4d presents a diagnostic chart of predicted and actual densities, where points above the diagonal indicate overestimation, while points below the diagonal indicate underestimation [44,48]. The actual response values were relatively consistent with the predicted values, which indicates that the model provides a good estimate. The predicted values closely aligned with the actual experimental value, and the model yielded a high degree of fit, which can be utilized for analysis and prediction in this experiment. Figure 4e,f reveal that the R^2 value of the residual fitting is 0.98115, and the error term follows a normal distribution.

3.2.3. The Yield Point Analysis

The yield point is an important component of the flow resistance of drilling fluid, which is also referred to as yield value. It reflects drilling fluid's ability to form structures during laminar flow and indicates the drilling fluid's capacity to carry and suspend drilling cuttings [17]. These forces arise from the negative and positive charges on the surface of clay particles, electrolytes, and polymers within the drilling fluid [9,19].

The experimental results for the third response value, i.e., YP, were used to fit the model and obtain the best-fitting result through analysis of variance. The second-order polynomial mathematical model representing the relationship between independent variables A, B, and C and the response variable YP (Y3) is expressed in Equation (7):

$$Y3 = 59.6259 + 5.6167A + 44.2132B - 1.3064C + 2.8236AB + 0.04338AC + 0.4196BC - 0.5138A^2 - 12.1055B^2 - 0.0110C^2 \quad (7)$$

where positive values of coefficients A, B, AB, AC, and BC indicate that the linear effect increases the yield point, while coefficients C, A^2 , B^2 , and C^2 decrease the yield point.

The orthogonal analysis results of the response surface quadratic model of YP are displayed in Table 6. The F-statistic of the model was 654.37, which indicates that the model is significant at a 95% confidence level, and the probability of such a large “model F-statistic” was only 0.01% due to interference [43]. According to the F-statistic, the order of influence of the three factors on plastic viscosity was $B > A > C$, which showed that the biological solution exerts a much greater influence than barite and bentonite contents. Figure 5d shows a diagnostic graph comparing the predicted and actual values of YP. The experimental design points were distributed diagonally, indicating that the actual YP values align with the predicted values of the model [41]. Table 6 demonstrated that the R^2 and Adj. R^2 values are similar, with a difference of less than 0.2. Figure 5e,f indicate that the mathematical model is reliable, and the residual distribution exhibits normality. The R^2 value of the residual fitting was 0.97109, which revealed that the error is not significant.

Table 6. Analysis of variance results of response value Y3 (YP).

Response	F-Statistic	R^2	p	Adj. R^2	AP	SD	PRESS
YP (Y3)	654.37	0.9956	<0.0001	0.9941	97.6378	1.99	283.51

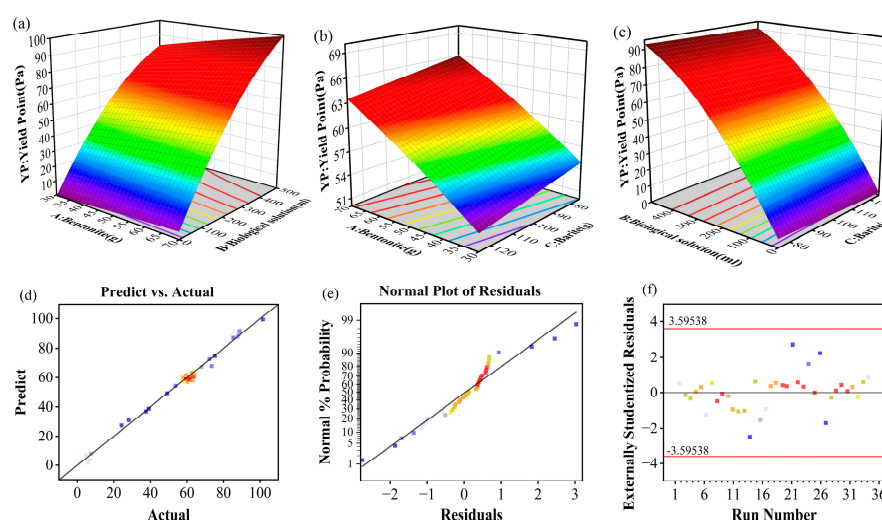


Figure 5. Three-dimensional response surface plots of various factors: (a) A and B, (b) A and C, (c) B and C; (d) diagnosis chart of predicted and actual values of yield point; (e) the normal graph of residuals; (f) residual and running chart.

Figure 5a–c show that for both bentonite and biological solutions, YP increased as the amount of content increased. When the content of the biological solution was low, the interaction effect of bentonite on YP was relatively minor. As the content of the biological solution increased, the effect of bentonite on the YP value gradually became significant. An increase in barite content led to a decrease in YP value; however, the magnitude of the change was relatively small. When the bentonite content was maintained at a constant level, the YP value initially increased rapidly along with the content of the biological solution before the growth rate slowed down; overall, however, it underwent an increasing trend.

3.3. Analysis of Optimization Results

3.3.1. Model Validation

Given that the primary properties of drilling fluids include density and rheological properties, separate optimization analyses were conducted for density and rheological properties. Under the premise of meeting quality requirements while ensuring economic and environmental protection, the optimal solution was selected to determine the optimal

concentration of each material, so as to provide the best microbial drilling fluid formulation suitable for drilling applications in loose formations. The optimal ratio of bentonite mud under the conditions of maximum viscosity, maximum density, and minimum yield point was obtained with the aid of the optimization function in the Design-Expert 12.0.3.0 software. Specifically, the optimal content was as follows: 50 g of bentonite, 200 mL of biological solution, and 120 g of barite. At this point, the predicted viscosity, density, and yield point of mud were 40.174 mPa·s, 1.223 g/cm³, and 46.584 Pa, respectively. The results obtained from three sets of repeated tests are presented in Table 7. The actual test results matched well with the predicted values derived from the response surface-based model, validating the accuracy of the model's predictions. This confirmed that the results for plastic viscosity, density, and yield point predicted by the response surface are correct and that the response model results are of practical reference value. The b-value of the dynamic-plastic ratio roughly reflect the strength of mud shear dilution. The b-value obtained through testing met the requirements with the standards. [49].

Table 7. Numerical and actual optimization results of response surface.

Factor	Y1 (mPa·s)	Y2 (g/cm ³)	Y3 (Pa)
Estimate	40.174	1.223	46.584
Experimental value 1	40	1.23	48.545
Experimental value 2	40	1.23	50.078
Experimental value 3	41	1.23	49.056

3.3.2. Characterization of Biological Mud

The mud cake was obtained by subjecting the optimally formulated drilling fluid to a gas pressure of 0.69 MPa for 30 min at a recorded filtration loss of 5 mL in accordance with the API standard. The resulting mud cake was dried using the Critical Point Dryer, Tousimis Autosamdri-815 Series, Rockville, MD, USA, and the samples were subsequently gold-sputtered using the Ion Sputterer, Hitachi High-Technologies Corporation, Tokyo, Japan.. Finally, the samples were subjected to scanning electron microscope (SEM) testing with the SU8010 Field Emission Scanning Electron Microscope, Hitachi High-Technologies Corporation, Tokyo, Japan to observe their microstructures.

Figure 6 displays the energy-dispersive spectroscopy (EDS) spectrum, with a small image in the upper-right corner displaying the SEM image of the test material. Figure 6a,c depict samples incorporated with biological solution, while Figure 6b,d represent ordinary mud samples without added biological solution. In Figure 6a,c, characteristic peaks corresponding to calcium (Ca), sulfur (S), and barium (Ba) are evident. Notably, there is a significant increase in the peaks for elemental calcium compared to those of the untreated samples shown in Figure 6b,d. This suggests a marked rise in calcium content in the mud samples treated with the biological solution, potentially due to calcium being released from the solution or due to its reaction with the mud cake samples, aligning with the experimental expectations. In contrast, the changes in other elements aside from calcium were relatively minor. Specifically, in Figure 6b,d, the atomic ratio of sulfur (S) to barium (Ba) is 1, consistent with the atomic ratio of the molecular formula for barium sulfate, the primary component of the selected barite powder. This indicates that the large granular material with a smooth surface seen in the sample image is the barite component.

Additionally, experimental observations revealed that the addition of the biological solution caused the slurry to undergo volumetric expansion during high-speed mixing, leading to a decrease in density. Given that colloidal substances tend to expand, it is supposed that this phenomenon resulted from the introduction of the biological media and colloidal materials.

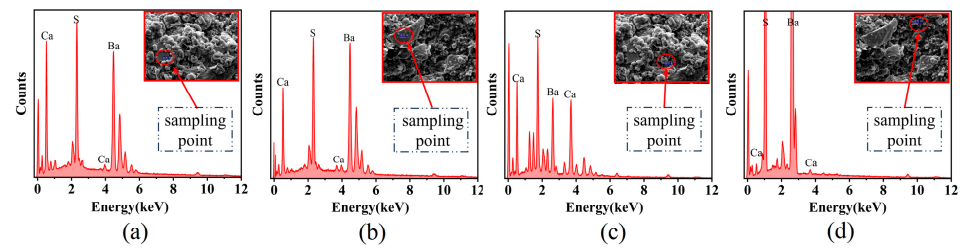


Figure 6. EDS spectrum of biological mud (a–d).

Figure 7 illustrates the mud cake samples obtained from the previously described experiments. Figure 7a reveals that the generated calcium carbonate crystals are uniformly distributed on the sample surface, with particle sizes ranging from approximately 1 to 5 μm . Under the influence of microbial solidification, abundant calcium carbonate crystals can be recognized on the surfaces and interfaces of solid particles and fibers, leading to a compact structure [29,34]. The small particle sizes of calcium carbonate solid satisfy the requirement that a smaller solid phase in drilling fluid yields a larger specific surface area, thereby increasing friction force and YP. The better the rheological properties of drilling fluid, the more favorable it is for engineering applications. It is speculated that the irregular shape of calcium carbonate crystals is due to the recombination effect on the calcium carbonate crystals produced by biological reactions during high-speed stirring. Distinct fibrous connections can be observed in Figure 7b,c, where Figure 7b exhibits a ribbon-like connection that aligns with the typical microscopic appearance of xanthan colloid. The locally enlarged image in Figure 7c shows a number of small solid particles distributed along the fiber connectors formed by the colloid, presumed to be calcium carbonate precipitates. The figure illustrates a large number of clustered calcium carbonate precipitate crystals at both ends of the connecting colloid, with additional crystal distribution on the connecting body. This indicates that the addition of colloid components provides more nucleation sites in the mud, resulting in uniform precipitation distribution and increased YP. The interaction between colloids and small solid particles forms a combination in the fluid, creating large solid clusters that contribute to an increase in the PV of the drilling fluid. This finding corroborates the conclusions reported by Mandala et al. and Bayas, who state that “there is a synergistic effect between the two biopolymers, leading to the formation of stable associations that increase PV” [15,36,50,51].

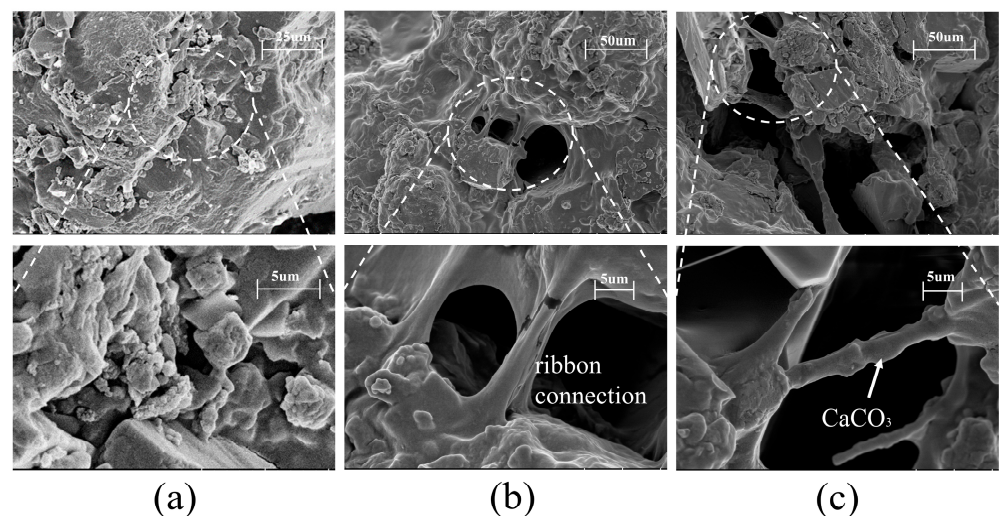


Figure 7. Microscopic morphology diagram of biological mud: (a) Calcium carbonate distribution; (b) ribbon-like connection; (c) calcium carbonate distribution on the fibrous connector.

3.3.3. Biodegradability and Heavy Metal Detection Results

The drilling fluid prepared based on the optimized process formulation has been proven to meet the requirements of national standards regarding physical, chemical, and microbiological indicators. It does not contain heavy metals and falls within the permissible safety range established by national regulations. The drilling fluid underwent tests for biodegradability, and heavy metal elements (cadmium, chromium, lead, and arsenic) were assessed separately.

According to the Technical Specification for Environmental Performance Evaluation of Drilling Fluids, a DXY-3 biological toxicity tester was employed to monitor the BOD₅ (Biochemical Oxygen Demand, an important indicator of organic matter pollution in water that is indirectly represented by the dissolved oxygen consumed by microbial metabolism) and COD (Chemical Oxygen Demand, a chemical method for measuring the amount of reducing substances that need to be oxidized in water samples) of the biological mud drilling fluid. The results, presented in Table 8, are in accordance with the “Identification Standard for Hazardous Wastes” and the “Technical Specification for Environmental Performance Evaluation of Drilling Fluid”.

Table 8. Results of biodegradability testing and heavy metal content testing of drilling fluid.

Parameters	Test Content (mg/L)	Biodegradable BOD ₅ /COD	Standard
COD _{CR}	9758	0.426	>5%
BOD ₅	4156		
Element Name	Test result (mg/kg)	Standard value (mg/kg)	
Pb	0.9500	<1000	
Cr	2.0600	<1000	
AS	1.9100	<75	
Cd	0.0151	<20	
Hg	0.0019	<25	

3.3.4. Analysis of Cost Advantages

The raw materials for microbial drilling fluid are easily sourced and readily available. The unit cost of producing 1 kg of microbial drilling fluid can be calculated as Table 9:

Table 9. Cost of microbial drilling fluid.

Raw Materials		Price	Cost per Kilogram (CNY)
Biological solution	Bentonite	0.5 CNY/kg	1×10^{-3}
	Composition of culture medium	12 yuan/L	2.4
	Carboxymethylcellulose sodium (CMC)	20 CNY/kg	1.6×10^{-3}
	Xanthan gum (XG)	19 CNY/kg	2.28×10^{-2}
	Barite	0.44 CNY/kg	1.056×10^{-1}
Final cost		2.531 CNY/kg	

We have selected other types of drilling fluids that are top sellers in the market and calculated their unit prices, as shown in the Table 10:

From the table above, it is evident that the unit cost of microbial drilling fluid is significantly lower than that of other drilling fluids on the market. This shows that microbial drilling fluid has a clear cost advantage. In practice, microbial drilling fluid is particularly suitable for gravel formations with larger pores, which are more sensitive to pressure changes. However, before this fluid can be utilized, attention must be given

to the cultivation of microbial fluids. Currently, the expansion of primary bacteria still requires laboratory work. Some researchers are focusing on the induced domestication of microbial strains to identify those better suited for deep-well environments. With ongoing advancements in culture technology, it is expected that bacterial fluids will soon be available directly on the market, avoiding the need for laboratory processes. This would broaden the potential applications of biological mud significantly.

Table 10. Cost of other types of drilling fluids.

Type	Price per Kilogram
Lianyungang Chemical Drilling Fluid A	7.4 CNY/kg
Shandong Compound Drilling Fluid B	4.8 CNY/kg
Shandong Chemical Fiber Drilling Mud C	8.8 CNY/kg
Xi'an Chemical Mud D	3.92 CNY/kg

4. Discussion

This study evaluated the optimal preparation formulation for biological mud using the CCD and explored the mechanism by which bio-cementation improves drilling fluid performance at the microscopic level. Analyses of the experimental results yielded the following conclusions:

1. The analyses of the rheological and density test results of drilling fluids containing different amounts of biological solution indicated that the PV of biological drilling fluids increased with the addition of biological solutions, exhibiting a growth rate that initially increased and then decreased. The density increased monotonically with the addition of barite, while the increased rate of YP exhibited a trend of first increasing and then decreasing as the biological solution content increased. Interactions among various factors were evident, and the effect of biological solutions on density was manifested as an increase in response to low content and a decrease in response to high content, which was significantly influenced by the interaction. The influence of bentonite and barite on YP exhibited a trend of first increasing and then decreasing; as such, they could generally offset each other. However, as amount of biological solution increased, the influence of bentonite on the YP gradually became significant.
2. Based on the response surface optimization results, the formulation containing bentonite, biological solution, and barite in quantities of 50 g, 200 mL, and 120 g, respectively, was optimal, and the rheological parameters of drilling fluid under this formulation were obtained through experimental measurements. The plastic viscosity, density, and yield point were 40.174 mPa·s, 1.223 g/cm³, and 46.584 Pa, respectively, all of which met the regulatory requirements and expected standards.
3. The experimental results indicate that the addition of colloidal substances does not affect the growth activity of *Bacillus subtilis*. The microstructure of the mud cake samples obtained from the experiment was analyzed using SEM. The SEM and EDS analyses demonstrated that the addition of colloidal substances does not hinder the formation of solid calcium carbonate precipitates and facilitates the formation of fibrous and ribbon connections within the mud, improving the plastic viscosity of the mud. The generated small calcium carbonate solids contributed to the increased yield stress of the drilling fluid. After inspection, the mud was found to comply with national environmental protection requirements, and its heavy metal content and biodegradability met the standard requirements. The unit cost of the mud was deemed to be lower, indicating its cost advantage in terms of pricing.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance.
API	American Petroleum Institute.
CCD	Central Composite Design.
CGMCC	China General Microbiological Culture Collection Center.
CMC	Carboxymethyl cellulose sodium.
EDS	Energy-dispersive spectroscopy.
MICP	Microbially induced calcite carbonate precipitation.
OD_{600}	The absorbance value of the bacterial solution at 600 nm.
PV	Plastic viscosity.
P-value	Probability value.
R^2	Coefficient of determination.
RSM	Response surface methodology.
SEM	Scanning electron microscopy.
XG	Xanthan gum.
YP	Yield point.

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