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Article

Response Surface Methodology for Optimizing Cd Removal by Mineralizing Bacterial Strains

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Abstract: Highly efficient cadmium (Cd)-tolerant mineralizing bacterial strains were isolated and screened from farmland soil around abandoned mining areas via isolation and culture methods of mineralizing bacteria. Taxonomic identification of the strains was conducted through morphological observation and 16S rRNA gene sequence analysis, and their adsorption characteristics and mechanisms for Cd^{2+} were investigated. Single-factor experiments were performed to analyze the effects of pH, temperature, urea addition amount, and bacterial solution addition amount on the Cd^{2+} removal rate, and the adsorption conditions were optimized using the response surface methodology (RSM). The results showed that a Cd-tolerant mineralizing bacterial strain, designated as FG43, was isolated and screened, and it was identified as Priestia sp. (GenBank accession number: PX121665). This strain grew well at a Cd^{2+} concentration of 100 mg/L, and its adsorption of Cd^{2+} was mainly extracellular adsorption. The maximum adsorption capacity for Cd^{2+} at 100 mg/L was 21.48 mg/g. Response surface optimization indicated that the order of significance of the factors affecting the Cd^{2+} removal rate was: pH > urea addition amount > temperature > bacterial solution addition amount. Under the optimal conditions (pH = 9, temperature = 30℃, urea addition amount = 22.3 g/L, bacterial solution addition amount = 20.4%), the actual measured value of Cd^{2+} removal rate reached 86.83%. This study provides technical references for the adsorption and immobilization of Cd^{2+} by the mineralizing bacterium Priestia sp. in contaminated water bodies and soils.

Keywords: Cd adsorption; Priestia sp.; response surface optimization; mineralizing bacteria; microbial remediation

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1. Introduction

Cadmium (Cd) is a heavy metal element with high biotoxicity, which can enter the environment through industrial and agricultural activities such as mining, smelting, electroplating, and fertilizer application [1,2]. Cd is difficult to degrade in soil and water bodies, can accumulate through the food chain, seriously threatens ecological security and human health, and causes diseases such as "Itai-itai disease", kidney damage, and cancer [3]. Therefore, developing efficient, economical, and environmentally friendly remediation technologies for Cd pollution has become a research hotspot in the environmental field.

Traditional heavy metal pollution treatment methods (such as chemical precipitation, ion exchange, membrane separation, etc.) have certain effects but suffer from limitations such as high cost, tendency to cause secondary pollution, and high energy consumption [4]. In contrast, microbial remediation technology utilizes the metabolic activities of microorganisms, such as bacteria, fungi, and algae, to adsorb, precipitate, transform, or immobilize heavy metals, offering outstanding advantages such as low cost, simple operation, environmental friendliness, and low risk of secondary pollution [5]. Among them, bacteria are widely studied due to their vast diversity, rapid growth, and strong

adaptability. The mechanisms of heavy metal removal by bacteria mainly include biosorption, such as cell surface complexation, ion exchange, and bioaccumulation [6,7].

Mineralizing bacteria (urease-producing bacteria) are a type of microorganism that can secrete urease to catalyze the decomposition of urea, thereby driving the formation of solid minerals (such as calcium carbonate) from mineral ions like calcium and magnesium in the environment. Their core characteristic is reliance on the "urease-urea" reaction to achieve biomineralization. These microorganisms are widely distributed in nature. In the environmental field, carbonate crystals formed during their formation can immobilize heavy metal ions such as lead (Pb^{2+}), cadmium (Cd^{2+}), and copper (Cu^{2+}) in soil or water bodies through adsorption and encapsulation, reducing their bioavailability (toxicity). Microbial mineralization remediation technology has thus become an emerging research direction in environmental remediation [8,9]. Achal, V et al. screened a Gram-positive bacterium--*Kocuria flava* CR1 from soil, which had excellent tolerance to the heavy metal Cu, achieving a removal rate of 95% for the mineralization remediation of Cu-contaminated soil, and the exchangeable Pb in the solidified soil decreased from 85.4 mg/kg to 14.2 mg/kg [10]. Achal, V et al. used a screened Sr-tolerant urease-producing bacterium to immobilize Sr in soil, and the results showed an Sr removal rate of 80% [11].

However, the adsorption efficiency and mechanisms vary significantly among different bacterial strains, and their adsorption performance is greatly influenced by environmental conditions such as pH, temperature, nutrients, and inoculum size [12]. Therefore, screening mineralizing bacterial strains with high adsorption capacity and systematically optimizing their adsorption conditions are crucial for promoting the practical application of microbial mineralization remediation technology. Response Surface Methodology (RSM) is a statistical method for optimizing multiple factors and their interactions. It can efficiently determine optimal process conditions with the least number of experiments by establishing mathematical models through reasonable experimental design and has been applied to the optimization of microbial fermentation and heavy metal adsorption processes [13-15]. To enhance the application of mineralizing bacteria in Cd environmental remediation, this study aimed to isolate and screen a highly efficient Cd-tolerant mineralizing bacterial strain from a heavy metal-polluted environment, clarify its taxonomic status, study its growth characteristics and adsorption mechanisms under different Cd^{2+} concentrations, investigate the effects of key environmental factors on its adsorption performance, and use RSM to optimize the adsorption conditions, in order to provide an excellent microbial resource and reliable technical parameters for the bioremediation of Cd pollution.

2. Materials and Methods

2.1. Experimental Materials

The test soil samples were collected from farmland soil near an abandoned mine in Wanshan County, Guizhou Province, with the sampling depth being 10-20 cm. The collected target soil samples were placed in sealed bags and stored in a 4°C refrigerator within 24 hours.

Nutrient Broth (NB) medium: Beef extract 3 g, peptone 10 g, sodium chloride 5 g, deionized water 1 L, pH 7.0-7.2. Autoclaved at 121°C for 20 min, and cooled naturally for later use.

Nutrient Agar (NA) medium: Beef extract 3 g, peptone 10 g, sodium chloride 5 g, agar 18 g, deionized water 1 L, pH 7.0-7.2. Autoclaved at 121°C for 20 min, and cooled naturally for later use.

Urea agar medium: Urea 20 g, sodium chloride 5 g, potassium dihydrogen phosphate 2 g, peptone 1 g, glucose 1 g, agar 15 g, deionized water 1 L, pH 7.0-7.2. Urea was sterilized using a 0.22 μm filter, and 2 mL of 0.2% phenol red indicator was added. The base medium was autoclaved at 121°C for 20 min and cooled naturally before adding the filter-sterilized urea solution. Urea liquid medium was prepared without adding agar.

2.2. Isolation and Screening of Cd-Tolerant Mineralizing Bacterial Strains

10 g of soil sample was added to a 250 mL conical flask containing 90 mL of sterile water and 20 glass beads, and shaken at 150 rpm, 30°C for 1 h. 1 mL of the soil suspension was pipetted into a test tube containing 9 mL of sterile water, mixed thoroughly by repeated pipetting, and the soil suspension was diluted into different concentration gradients: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. 0.2 mL of each soil suspension dilution was spread onto NA solid medium containing 50 mg/L $CdSO_4$ and incubated at 30°C for 2-3 days. Colony growth on the plates was observed, and single colonies with different morphologies were picked and streaked onto fresh NA medium for purification through successive streaking until pure strains were obtained. The strains were stored on NA slants at 4°C.

The Cd-tolerant strains were streaked onto urea agar medium containing phenol red indicator and incubated at 30°C for 2-3 days. Observation of colony growth and color change; a red color change around the colony indicated a mineralizing bacterium (urease-producing bacterium). The mineralizing bacteria were inoculated into NA medium and stored at 4°C.

2.3. Strain Identification

2.3.1. Scanning Electron Microscopy (SEM) Analysis

A single colony was inoculated into NB liquid medium and incubated with shaking at 35°C until the logarithmic growth phase. 10 mL of bacterial culture was centrifuged at 8000 rpm for 10 minutes, the supernatant was discarded, and the bacterial pellet was collected. The pellet was gently resuspended in phosphate-buffered saline (PBS, pH 7.2-7.4) and washed by centrifugation 2-3 times to remove residual medium. The bacteria were fixed with 2.5% glutaraldehyde fixative at 4°C for 2-4 hours. Dehydration was performed using a graded ethanol series, followed by drying. The sample was mounted on an SEM stub and coated. The stub was placed into the SEM sample chamber for observation.

2.3.2. PCR Amplification and Sequencing Analysis

Molecular identification was performed by PCR amplification and sequencing of the bacterial 16S rDNA fragment. PCR amplification was carried out using universal primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-TACGGCTACCTTGTTACGACTT-3'. The PCR products were sequenced by Guizhou Well-Let Testing Technology Co., Ltd. The sequencing results were compared and searched in the GenBank database, and a phylogenetic tree was constructed using MEGA6.0 software.

2.4. Bacterial Growth Curve under Cd^{2+} Stress

The strain was inoculated into NA liquid medium and cultured at 30°C, 150 rpm for 24 h to achieve a bacterial suspension concentration of 7×10^8 cfu·mL^{-1}. A 5% inoculum was transferred to NB liquid media containing 0 mg/L, 10 mg/L, 50 mg/L, 100 mg/L, and 200 mg/L Cd^{2+}, respectively, and shake-cultured at 30°C, 150 rpm for 72 h. Uninoculated blank medium was used as a control. The absorbance (OD_{600}) of the samples was measured using a spectrophotometer. Each time point had three replicates, and samples were taken periodically for analysis.

2.5. Intracellular and Extracellular Bioaccumulation Capacity

The strain was inoculated into NA liquid medium and cultured at 30°C, 150 rpm for 24 h. A 5% inoculum was transferred to NA medium containing Cd (Cd^{2+} concentrations of 50 mg/L, 100 mg/L, 150 mg/L) and cultured at 30°C, 150 rpm for 72 h. A control without bacterial inoculum was set up. Each treatment had three replicates. After the reaction, the bacterial culture was transferred to a 50 mL centrifuge tube and centrifuged at 8000 rpm

for 10 minutes, and the supernatant was collected. The pelleted bacterial cells were resuspended in 2.5 mL of 0.5 mol/L EDTA elution buffer, mixed thoroughly by pipetting, centrifuged again, and the elution step was repeated three times. The combined supernatants were used to determine the Cd^{2+} content, representing the extracellular adsorption. The eluted bacterial cells were added to a solution of dilute HNO_3 and 0.1% Triton X-100, incubated in a constant temperature water bath at 95°C for 2-3 h until the solution became clear, then centrifuged at 10000 rpm for 10 min. The supernatant was collected to determine the Cd^{2+} content, representing the intracellular adsorption. The centrifuged bacterial cells were freeze-dried and weighed. The Cd^{2+} content in the supernatants was determined by ICP-MS. Each treatment had three replicates.

Total cellular adsorption capacity $Q = [(C_0 - C_e) \times V] / W$

Intracellular or extracellular adsorption capacity $Q_n = (C_n \times V_n) / W$

Where: C_0 is the Cd^{2+} concentration in the supernatant of the control treatment without bacterial inoculum (mg/L); C_e is the Cd^{2+} concentration in the supernatant of the treatment with bacterial inoculum (mg/L); V is the volume of the centrifuged bacterial culture (L); W is the dry weight of the centrifuged bacterial cells (g); C_n is the Cd^{2+} concentration in the elution or digestion solution (mg/L); V_n is the volume of the elution or digestion solution (L).

2.6. Single-Factor Experiments

2.6.1. Preparation of Bacterial Suspension

Bacterial suspension (OD_{600} = 1.0) was pipetted and inoculated under different reaction conditions, with three replicates per sample. The bacterial culture was centrifuged at 8000 rpm for 10 minutes, the supernatant was collected and filtered through a 0.22 μm filter. The Cd^{2+} concentration in the supernatant was determined by ICP-MS, and the Cd^{2+} removal rate (R) was calculated.

$R = (C_0 - C_e) / C_0 \times 100\%$

Where: C_0 is the initial Cd^{2+} concentration in the solution (mg/L); C_e is the residual Cd^{2+} concentration in the solution after the reaction (mg/L). Each experiment was performed in triplicate, and the results are presented as the mean.

2.6.2. Different pH Treatments

Urea liquid medium solutions with an initial Cd^{2+} concentration of 50 mg/L were prepared, and the pH was adjusted to 2.0, 3.0, 5.0, 7.0, 9.0, 11.0 using HCl and NaOH. A 10% bacterial inoculum was added, and the reaction was carried out with shaking at 150 rpm, 30°C for 48 h.

2.6.3. Different Temperature Treatments

Urea liquid medium solutions with an initial Cd^{2+} concentration of 50 mg/L and pH 7.0 were prepared. A 10% bacterial suspension was added, and the reaction was carried out with shaking at 150 rpm at 20°C, 25°C, 30°C, 35°C, and 40°C, respectively, for 48 h.

2.6.4. Different Urea Addition Amount Treatments

Liquid medium solutions with urea addition amounts of 2 g/L, 5 g/L, 10 g/L, 20 g/L, and 30 g/L were prepared, with an initial Cd^{2+} concentration of 50 mg/L and natural pH. A 10% bacterial suspension was added, and the reaction was carried out with shaking at 30°C, 150 rpm for 48 h.

2.6.5. Different Bacterial Inoculum Size Treatments

In urea liquid medium solutions with an initial Cd^{2+} concentration of 50 mg/L and pH 7.0, bacterial suspensions were added at inoculum sizes of 2%, 5%, 10%, 15%, 20%,

and 30%, respectively. The reaction was carried out with shaking at 30°C, 150 rpm for 48 h.

2.7. Response Surface Optimization Design

Based on the single-factor experiments, the four factors of pH, temperature, urea addition amount, and bacterial inoculum size were taken as independent variables, and the Cd removal rate was taken as the response value. A Box-Behnken design was used to analyze the four factors and optimize the optimal adsorption reaction conditions for Cd. The experimental factors and level design are shown in Table 1.

Table 1. Factors and Level values of Box-Behnken design.

Code	Factor	Level(-1)	Level(0)	Level(1)
A	pH	7	9	11
B	Temperature (℃)	25	30	35
C	Urea addition amount(g/L)	10	20	30
D	Bacterial inoculum size(%)	15	20	25

2.8. Data Processing

Excel 2023 and OriginPro 2025 software were used for data organization and chart creation. Design Expert 8.0.6 software was used for significance analysis and response surface model analysis.

3. Results and Analysis

3.1. Isolation and Identification of Cd-Tolerant Mineralizing Bacterial Strain

As shown in Figure 1, strain FG43 colonies were light yellow, circular, with smooth edges. When cultured on urea medium, it turned red. Scanning electron microscopy showed that the bacterial cells were rod-shaped, 5-8 μm in length. Gram staining was negative. 16S rRNA sequence comparison results indicated that strain FG43 had a 98% similarity with Priestia aryabhattai (Figure 2), and the GenBank accession number is PX121665. Based on morphological observation, physiological and biochemical characteristics, and gene sequence analysis, strain FG43 was identified as *Priestia sp*.

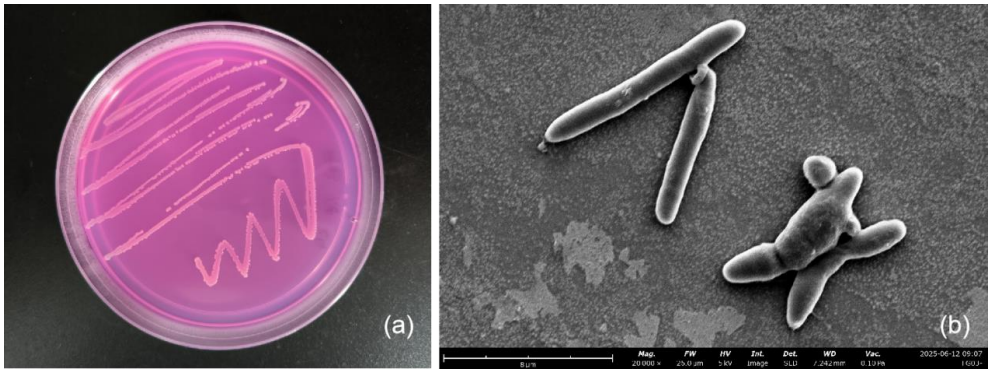

Figure 1. Colony morphology(a) and scanning electron microscope (SEM) image(b) of strain FG43.

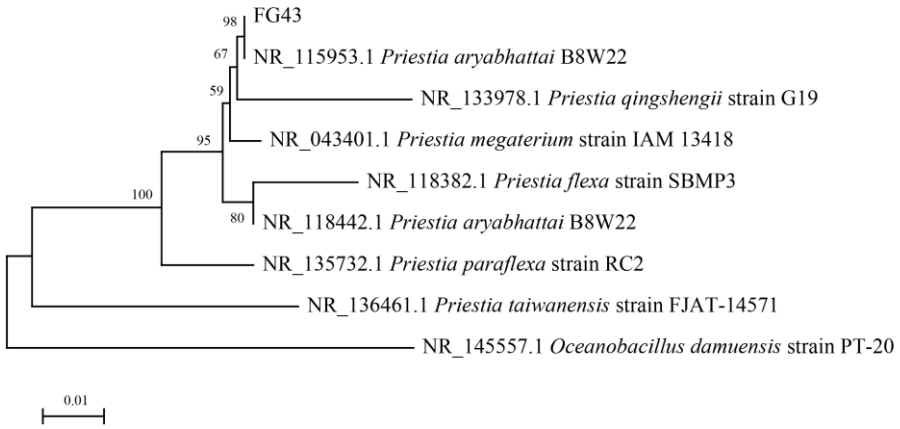

Figure 2. Phylogenetic tree analysis of the 16S rRNA gene sequence of strain FG43.

3.2. Growth Curve of the Strain under Different Cd^{2+} Concentrations

Figure 3 shows the growth curves of strain FG43 cultured for 120 h under Cd^{2+} concentrations of 0, 10, 50, 100, and 200 mg/L. It can be seen from Figure 3 that increasing Cd^{2+} concentration significantly inhibited the growth of the strain, and the degree of inhibition was concentration-dependent. In the control group with 0 mg/L Cd^{2+}, the strain grew well, with OD_{600} reaching its peak at 72 h, followed by the stationary phase. Low Cd^{2+} concentration (10 mg/L) had little effect on growth, and the OD_{600} trend was similar to the control group, with a slightly lower peak (OD_{600}=2.19), indicating that the strain had a certain tolerance. Medium to high Cd^{2+} concentrations (50-200 mg/L) significantly delayed growth and reduced the maximum biomass. When the Cd^{2+} concentration was 50 mg/L, the OD_{600} at 72 h was only 1.38, much lower than the control group; at Cd^{2+} concentrations of 100 mg/L and 200 mg/L, the peaks were only 0.87 and 0.38, respectively, and no obvious logarithmic growth phase was observed. Cd stress also delayed the time for the strain to enter the logarithmic growth phase. The control group entered the logarithmic phase at 12 h (OD_{600}=0.63), while the 200 mg/L group had an OD_{600} of only 0.15 at the same time point, with growth almost stagnant.

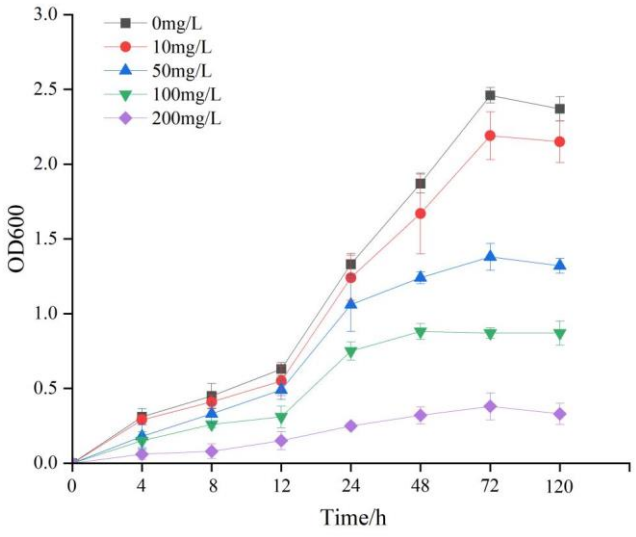

Figure 3. Growth curves of strain FG43 under different Cd^{2+} concentrations.

3.3. Bioaccumulation Capacity of the Strain for Cd

The total adsorption capacity, extracellular adsorption capacity, and intracellular adsorption capacity of strain FG43 for Cd under different Cd^{2+} concentrations were measured to analyze its bioaccumulation capacity. As shown in Figure 4, strain FG43 exhibited significant adsorption capacity for Cd, with extracellular adsorption being the primary mechanism. As the Cd^{2+} concentration increased from 50 mg/L to 100 mg/L, the total adsorption capacity of the strain significantly increased from 13.13 mg/g to 21.48 mg/g, extracellular adsorption increased from 10.25 mg/g to 17.62 mg/g, and intracellular adsorption increased from 2.88 mg/g to 3.86 mg/g, showing synchronous growth, indicating that the bioaccumulation capacity of the strain did not reach saturation within this concentration range. However, when the Cd^{2+} concentration continued to rise to 150 mg/L, both the total adsorption capacity and intracellular adsorption capacity decreased slightly, while the extracellular adsorption capacity remained basically unchanged, indicating that a Cd^{2+} concentration of 150 mg/L might inhibit bacterial activity, weakening the intracellular accumulation mechanism. Within the experimental concentration range, the proportion of extracellular adsorption was much higher than that of intracellular adsorption (about 78%-85%), indicating that the removal of Cd by this strain mainly relies on extracellular adsorption (such as cell wall surface complexation, precipitation, etc.) rather than active intracellular uptake.

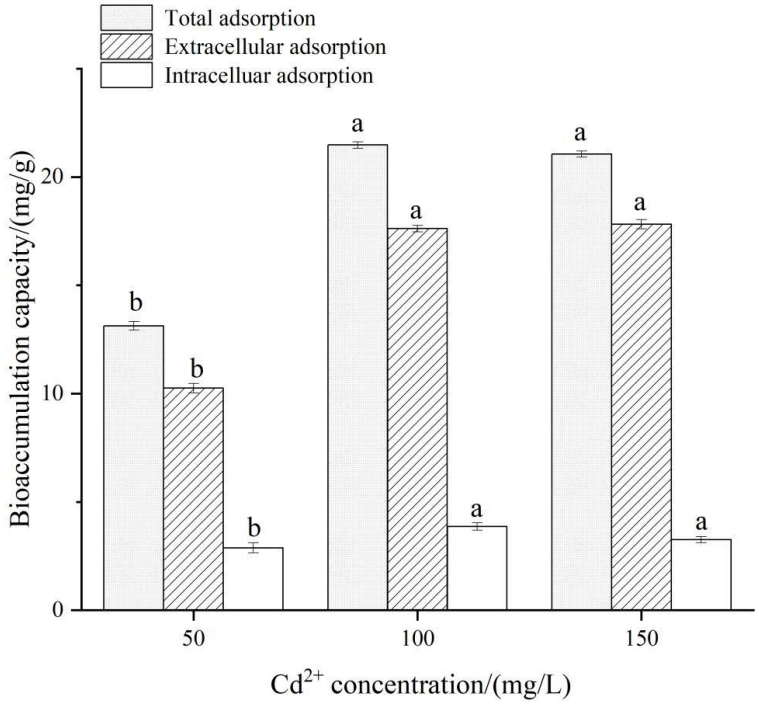

Figure 4. Bioaccumulation capacity of Cd^{2+} by strain FG43 at different concentrations of Cd^{2+}.

3.4. Single-Factor Experiment Results

3.4.1. Effect of Different pH Values on Cd^{2+} Adsorption by the Strain

As shown in Figure 5, the Cd^{2+} removal rate by the strain was significantly affected by pH, with the optimal pH range being pH 7 to pH 9 (neutral to weakly alkaline), where the removal rate was highest, approximately 79%. Under strongly acidic conditions (pH 3), the removal rate dropped sharply to 21.87%, which might be due to the protonation of the cell surface at low pH, reducing the adsorption sites for metal ions. Under strongly alkaline conditions (pH 11), the removal rate also decreased to 57.95%, which might be related to the formation of Cd hydroxide precipitates or effects on bacterial activity.

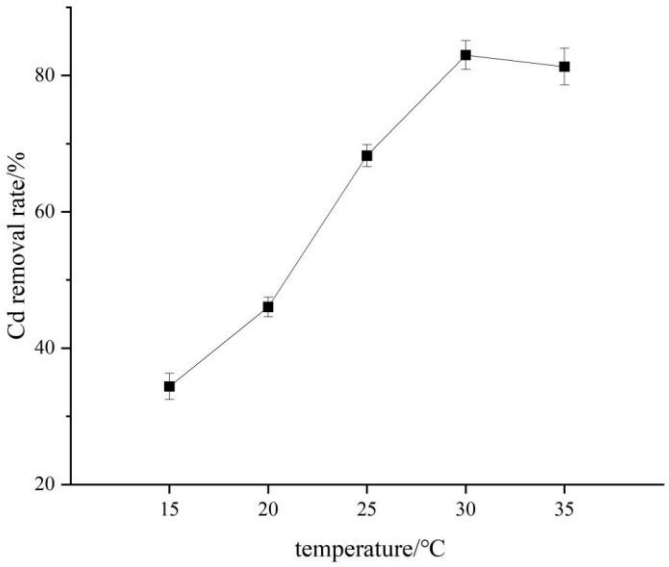

Figure 5. Changes in Cd^{2+} removal rate under different PH conditions.

3.4.2. Effect of Different Temperatures on Cd^{2+} Adsorption by the Strain

As shown in Figure 6, temperature had a significant effect on the Cd^{2+} removal rate. The optimal growth and adsorption temperature for the strain was 30°C, with a removal rate of 82.99%. At lower temperatures (15-25°C), the removal rate increased with rising temperature, consistent with the pattern of increasing microbial metabolic activity with temperature. When the temperature rose to 35°C, the removal rate slightly decreased to 81.3%, indicating that excessively high temperatures might begin to inhibit the physiological activity of the strain.

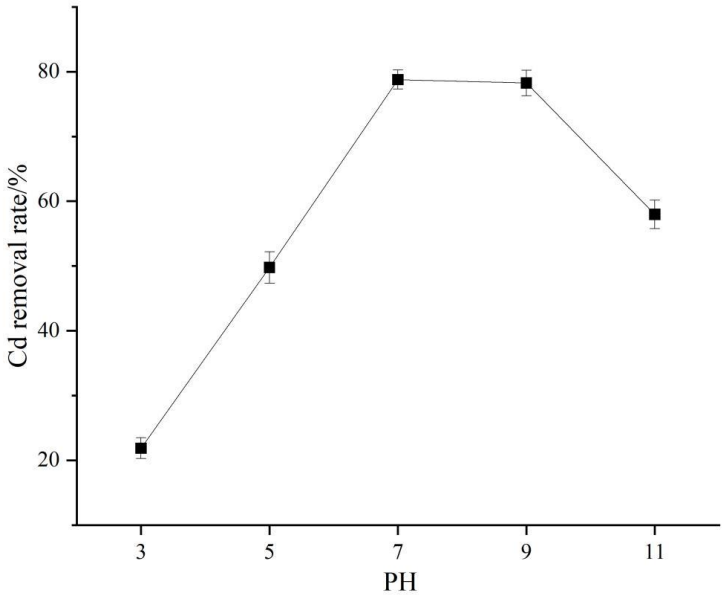

Figure 6. Changes in Cd^{2+} removal rate under different temperature conditions.

3.4.3. Effect of Different Urea Addition Amounts on Cd^{2+} Adsorption by the Strain

As shown in Figure 7, urea, as a nitrogen source, significantly affected Cd removal efficiency. When the addition amount increased from 2 g/L to 10 g/L, the removal rate increased from 70.16% to 82.88%, indicating that sufficient nitrogen source promoted the growth and metabolism of the strain, thereby enhancing its adsorption capacity. When the addition amount was 20 g/L and 30 g/L, the Cd^{2+} removal rate stabilized and slightly decreased, indicating that the nitrogen source was no longer a limiting factor, and excessive addition might slightly inhibit the strain due to factors such as osmotic pressure. 10 g/L was the better choice for urea addition amount.

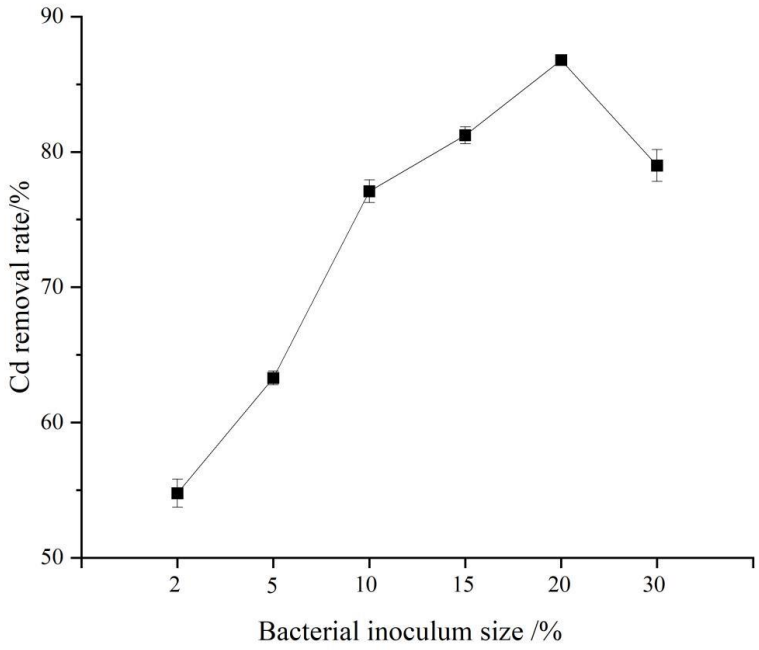

Figure 7. Changes in Cd^{2+} removal rate under different Urea addition amount.

3.4.4. Effect of Different Bacterial Inoculum Sizes on Cd^{2+} Adsorption by the Strain

As shown in Figure 8, the Cd^{2+} removal rate significantly increased with the increase of bacterial inoculum size, reaching the highest removal rate of 86.78% at a 20% inoculum size. This indicates that increasing the biomass can enhance the total adsorption capacity. When the inoculum size was 30%, the removal rate decreased to 79%, which might be due to intensified nutrient competition or insufficient dissolved oxygen caused by excessive cell density, which in turn inhibited the overall metabolic activity.

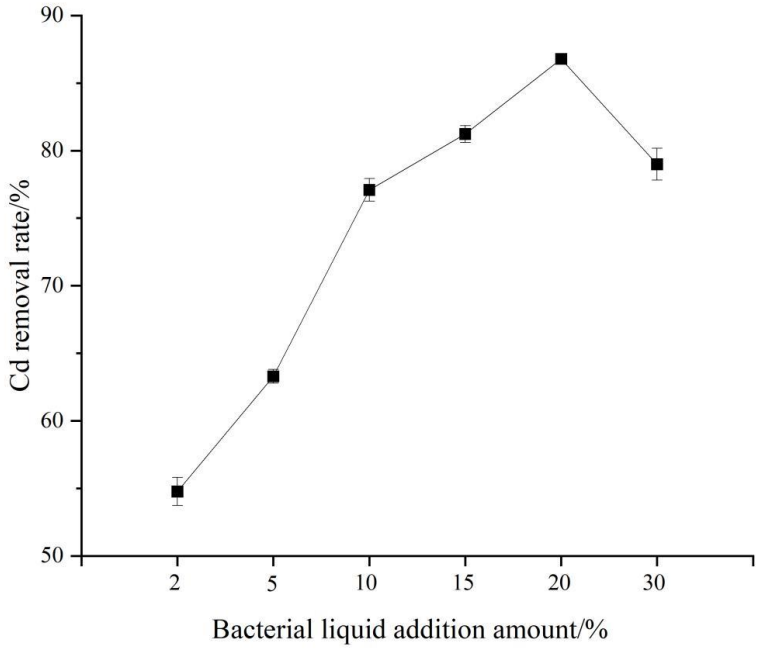

Figure 8. Changes in Cd^{2+} removal rate under different Bacterial inoculum size.

3.5. Response Surface Experiment Results and Significance Analysis

Based on the single-factor experiment results, pH (A), temperature (B), urea addition amount (C), and bacterial inoculum size (D) were selected as the four independent variables, and the Cd^{2+} removal rate was the response value. A 4-factor, 3-level Box-Behnken design was created using Design-Expert 8.0.6 for response surface analysis to determine the optimal experimental conditions. The BBD experimental design and results are shown in Table 2. Regression fitting of the data in Table 2 yielded the regression equation for Cd^{2+} removal rate (R) against the response variables A, B, C, D: $R = 86.692 + 1.561A + 0.828B + 1.572C + 0.958D - 1.57AB + 0.0425AC - 0.075AD - 1.428BC - 0.15BD - 0.95CD - 3.136A^2 - 4.611B^2 - 3.262C^2 - 4.114D^2$

Table 2. Results of Box-Behnken design.

Run	A(pH)	B(Temp, °C)	C(Urea, g/L)	D(Inoculum, %)	Cd^{2+} Removal Rate(%)
1	9	25	10	20	74.5
2	9	25	20	15	76.4
3	9	30	30	15	80.2
4	7	25	20	20	74.3
5	9	25	30	20	81.2
6	7	35	20	20	79.2
7	7	30	20	15	77.6
8	9	30	20	20	87.25
9	9	30	10	15	76.6
10	9	35	30	20	80.85
11	11	30	10	20	79.87
12	9	30	20	20	87.58
13	11	25	20	20	81.6
14	11	30	20	15	79.5

15	9	30	10	25	80.1
16	9	35	20	25	78.9
17	9	30	30	25	79.9
18	11	30	20	25	81.7
19	7	30	10	20	76.5
20	7	30	30	20	80.22
21	11	35	20	20	80.22
22	9	35	10	20	79.86
23	9	30	20	20	86.59
24	7	30	20	25	80.1
25	9	25	20	25	78.5
26	9	30	20	20	86.77
27	11	30	30	20	83.84
28	9	35	20	15	77.4
29	9	30	20	20	85.27

The variance analysis results in Table 3 show that the quadratic polynomial model was extremely significant ($P < 0.0001$), indicating a significant relationship between the independent variables and the response value. The correlation coefficient R^2 of the model was 0.966, indicating small experimental errors and a good fit between the experimental values and the predicted values of the regression equation. Moreover, the lack-of-fit term $P = 0.4806 > 0.05$, indicating that the lack-of-fit test for the equation was not significant, and this regression equation can be used to analyze and predict the experimental results. The primary terms A, B, C, and D, the interaction terms AB and BC, and the quadratic terms A^2, B^2, C^2, and D^2 all reached significant levels. The order of influence of the experimental factors on the Cd^{2+} removal rate (R) was: pH (A) > Urea addition amount (C) > Temperature (B) > Bacterial inoculum size (D) .

Table 3. Results of variance analysis of Box-Behnken design.

Source	Sum of Squares	df	Mean Square	F value	P-valueprob>F
Model	348.31	14	24.88	28.43	<0.0001
A	29.23	1	29.23	33.40	<0.0001
B	8.22	1	8.22	9.39	0.0084
C	29.64	1	29.64	33.87	<0.0001
D	11.02	1	11.02	12.59	0.0032
AB	9.86	1	9.86	11.27	0.0047
AC	7.225	1	7.225	8.255	0.9289
AD	0.23	1	0.23	0.26	0.8749
BC	8.15	1	8.15	9.31	0.0086
BD	0.09	1	0.09	0.1	0.7532
CD	3.61	1	3.61	4.12	0.0617
A2	63.77	1	63.77	72.87	<0.0001
B2	137.89	1	137.89	157.55	<0.0001
C2	69.01	1	69.01	78.85	<0.0001
D2	109.80	1	109.80	125.46	<0.0001
Residual	12.25	14	0.88		
Lack of Fit	9.11	10	0.91	1.16	0.4806
Pure Error	3.14	4	0.78		
Cor Total	360.56	28			

Response surface plots and contour plots (Figures 9, Figures 10, Figures 11, Figures 12, Figures 13, Figures 14) were drawn to explore the relationships between the significant interaction terms AB and BC in the regression equation. From the figures, it can be seen

that all six response surface plots are smooth surfaces opening downward, indicating that the maximum Cd^{2+} removal rate can be found within the designed factor level range. In the contour plots, the AB interaction was highly significant (P=0.0047<0.01), indicating that pH has a greater influence on Cd^{2+} removal rate than reaction temperature. Within the optimal pH range, the Cd^{2+} removal rate increased significantly with increasing reaction temperature. The BC interaction was highly significant (P=0.0086<0.01), indicating that reaction temperature has a greater influence on Cd^{2+} removal rate than urea addition amount. Within the optimal reaction temperature range, the Cd^{2+} removal rate increased significantly with increasing urea addition amount.

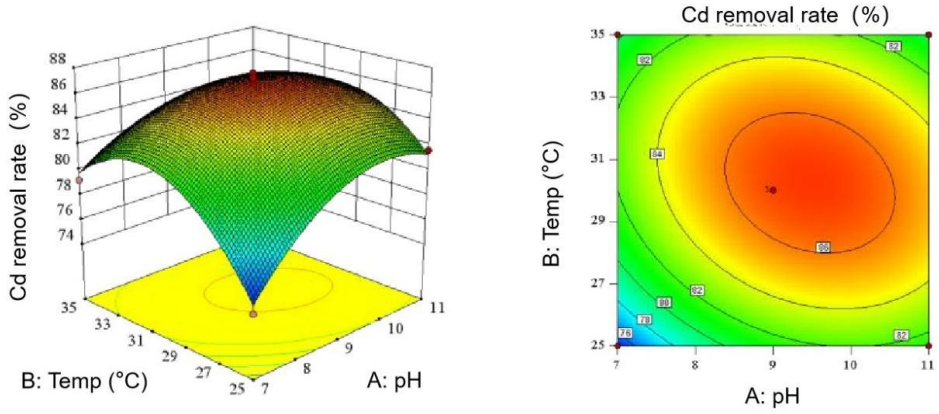

Figure 9. Interactive response surface and contour of pH value (A) and temperature (B).

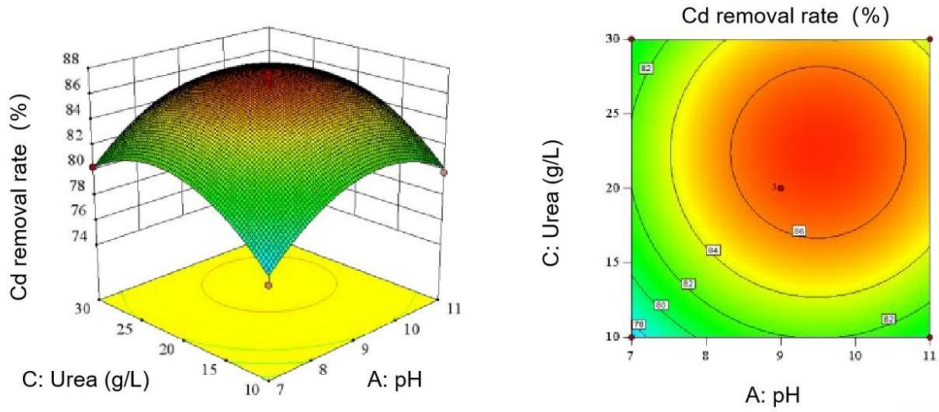

Figure 10. Interactive response surface and contour of pH value (A) and urea addition amount (C).

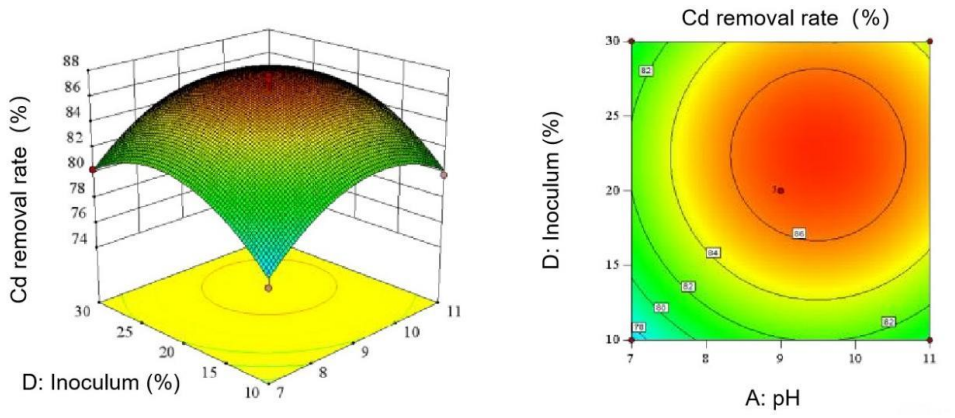

Figure 11. Interactive response surface and contour of pH value (A) and bacterial inoculum size (D).

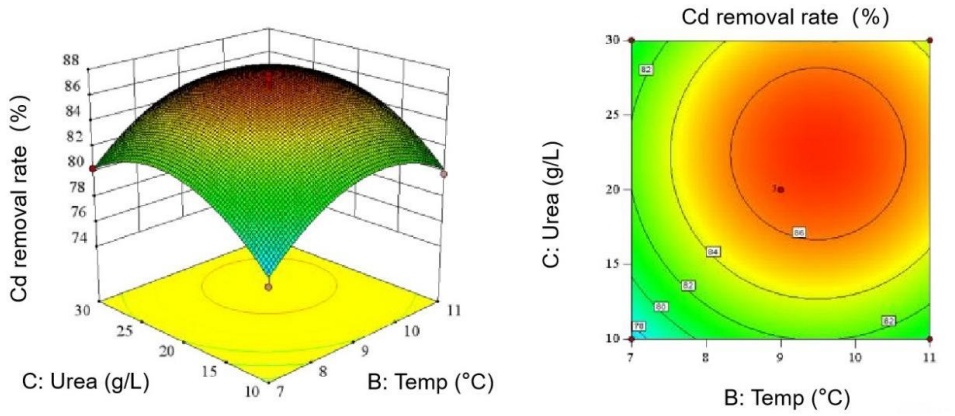

Figure 12. Interactive response surface and contour of temperature (B) and urea addition amount (C).

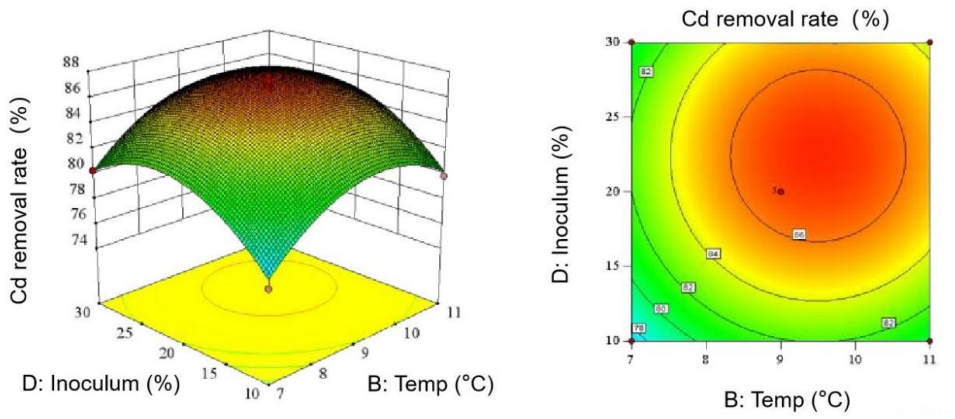

Figure 13. Interactive response surface and contour of temperature (B) and bacterial inoculum size (D).

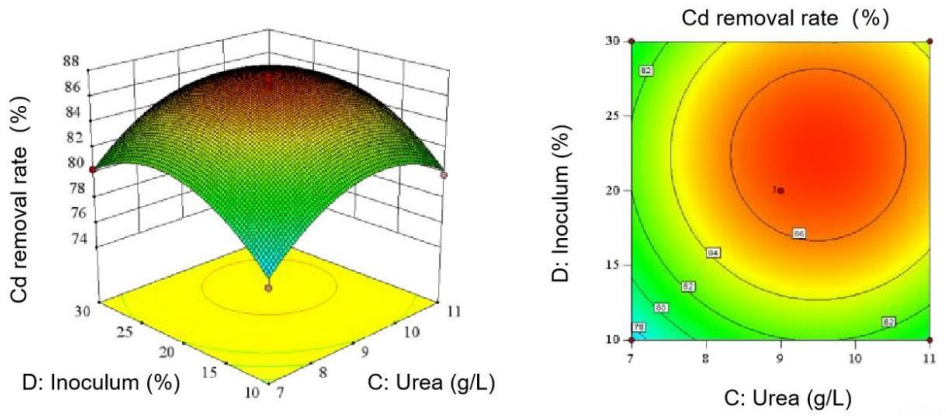

Figure 14. Interactive response surface and contour of urea addition amount (C) and bacterial inoculum size (D).

3.6. Verification of Optimal Reaction Conditions

Software analysis determined the optimal reaction conditions for Cd removal under these experimental conditions as follows: pH = 9.495, temperature = 30.054°C, urea addition amount = 22.276 g/L, bacterial inoculum size = 20.439%. Under these conditions, the model predicted value for Cd^{2+} removal rate was 87.11%. Considering practical operational feasibility, the values were adjusted as follows: pH = 9, temperature = 30°C, urea addition amount = 22.3 g/L, bacterial inoculum size = 20.4%. Through three repeated verification experiments, the average measured Cd^{2+} removal rate was 86.83%, which is close to the model predicted value, with a relative error of 0.32%. This indicates that the Cd^{2+} removal conditions optimized through the BBD experimental design are reliable.

4. Discussion

This study successfully isolated a Cd-tolerant bacterial strain FG43 from a polluted environment, which was identified as *Priestia sp*. based on morphological, physiological, biochemical, and 16S rRNA gene sequence analysis. This strain exhibited strong tolerance and adsorption capacity for Cd^{2+}. The adsorption of Cd^{2+} by strain FG43 primarily occurred through an extracellular adsorption mechanism, a process that may involve complexation by cell wall surface functional groups (such as carboxyl, phosphate, amino groups), ion exchange, and electrostatic adsorption. The results of this study showed that the extracellular adsorption accounted for a relatively high proportion under different Cd^{2+} concentrations, which is similar to the adsorption characteristics of *Bacillus sp* [16]. As a passive process not dependent on energy, extracellular adsorption has the advantages of fast speed and less susceptibility to toxicity, which might be the key reason why the target strain maintained a certain removal capacity even at high Cd^{2+} concentrations. When the Cd^{2+} concentration increased to 150 mg/L, both the total adsorption capacity and intracellular adsorption capacity decreased, indicating that high Cd^{2+} concentration might inhibit bacterial activity, damaging the structure and function of the cell membrane, thereby affecting active transport and other intracellular accumulation mechanisms [17].

Environmental factors significantly affected the adsorption efficiency of the target strain. pH was a key factor influencing adsorption, with the optimal range being pH 7-9. Under acidic conditions (pH=3), protonation of the bacterial cell surface led to an increase in positive charges, generating electrostatic repulsion with the positively charged Cd^{2+} ions, reducing adsorption sites. Under strongly alkaline conditions (pH=11), Cd^{2+} tends to form hydroxide precipitates. Although this can remove part of the Cd, it might cover the

bacterial surface sites or affect bacterial activity, leading to a decrease in the biosorption rate. Strain FG43 had the maximum immobilization effect on Cd^{2+} at pH=9. Temperature regulates the adsorption process by affecting the activity of metabolic enzymes and membrane fluidity, with 30°C being the optimal temperature for this strain. Urea, as a nitrogen source, significantly promoted strain growth and adsorption performance, indicating that sufficient nitrogen source is a prerequisite for the synthesis of biomass, enzymes, and cell wall components. Furthermore, urea hydrolysis might alter the local microenvironment pH and introduce CO_3^{2-}, indirectly promoting the formation of insoluble precipitates such as $CdCO_3$, i.e., the Microbially Induced Carbonate Precipitation (MICP) process, which might be another potential mechanism for enhancing Cd removal. The optimal bacterial inoculum size was 20%, and the decrease in removal rate with excessive inoculation might be related to nutrient competition, insufficient dissolved oxygen, and cell aggregation [18].

This study used Response Surface Methodology to optimize the adsorption conditions. The established model was highly significant ($P < 0.0001$, $R^2 = 0.966$) and reliable. Model analysis revealed the order of influence of various factors on the Cd^{2+} removal rate as: pH > Urea addition amount > Temperature > Bacterial inoculum size. Among them, the interactions between pH and temperature (AB), and temperature and urea addition amount (BC) were particularly significant, indicating that these parameters need to be regulated synergistically in practical applications to achieve the best results. Verification experiments under the optimized conditions confirmed the effectiveness and practicality of RSM in optimizing microbial adsorption conditions for heavy metals.

Compared with relevant domestic and international studies, strain *Priestia sp*. FG43 achieved a high Cd^{2+} removal rate of 86.83% under optimal conditions, and its adsorption capacity reached 21.48 mg/g under 100 mg/L Cd^{2+} conditions, which is superior to some reported strains [19-21].

5. Conclusion

A highly efficient Cd-tolerant bacterial strain, *Priestia sp*. FG43, was successfully isolated and identified. This strain exhibited significant tolerance and adsorption capacity for Cd^{2+}. The adsorption of Cd^{2+} by strain FG43 is primarily an extracellular passive adsorption process. Its adsorption capacity for Cd^{2+} increased with the initial concentration but decreased at 150 mg/L, possibly due to toxicity inhibition. A reliable predictive model was established through Response Surface Methodology optimization. After optimization and verification, under the optimal conditions of pH=9, temperature=30°C, urea=22.3 g/L, and bacterial inoculum size=20.4%, the Cd^{2+} removal rate reached 86.83%.

In summary, strain *Priestia sp*. FG43 is a promising biological remediation material for Cd pollution, particularly suitable for the remediation of wastewater or soil in neutral to weakly alkaline environments.

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