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Bioleaching of vanadium from stone coal vanadium ore by *Bacillus mucilaginosus*: Influencing factors and mechanism

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Abstract: Vanadium and its derivatives are used in various industries, including steel, metallurgy, pharmaceuticals, and aerospace engineering. Although China has massive reserves of stone coal resources, these resources have low grades. Therefore, the effective extraction and recovery of metallic vanadium from stone coal is an important way to realize the efficient resource utilization of stone coal vanadium ore. Herein, *Bacillus mucilaginosus* was selected as the leaching strain. The vanadium leaching rate reached 35.5% after 20 d of bioleaching under optimal operating conditions. The cumulative vanadium leaching rate in the contact group reached 35.5%, which was higher than that in the noncontact group (9.3%). The metabolites of *B. mucilaginosus*, such as oxalic, tartaric, citric, and malic acids, dominated in bioleaching, accounting for 73.8% of the vanadium leaching rate. Interestingly, during leaching, the presence of stone coal stimulated the expression of carbonic anhydrase in bacterial cells, and enzyme activity increased by 1.335–1.905 U. Enzyme activity positively promoted the production of metabolite organic acids, and total organic acid content increased by 39.31 $\mathrm{mg \cdot L^{-1}}$, resulting in a reduction of 2.51 in the pH of the leaching system with stone coal. This effect favored the leaching of vanadium from stone coal. Atomic force microscopy illustrated that bacterial leaching exacerbated corrosion on the surface of stone coal beyond 10 nm. Our study provides a clear and promising strategy for exploring the bioleaching mechanism from the perspective of microbial enzyme activity and metabolites.

Keywords: *Bacillus mucilaginosus*; stone coal vanadium ore; bioleaching; carbonic anhydrase; organic acids

1. Introduction

Vanadium is a valuable metal with excellent physical properties, such as high tensile strength, hardness, and fatigue resistance [1–2]. Vanadium and its derivatives are used in various fields, including the steel industry, metallurgy, pharmaceuticals, ceramics, textiles, and aerospace engineering [3–8]. In recent years, over 85% of vanadium has been used in the production of carbon steel, stainless steel, and ferrovanadium alloys [9–10]. Today, vanadium is recovered as a by-product or coproduct in mineral resources, wherein its content ranges from 0.01wt% to 2wt% [11–12]. Given the low grade of vanadium in minerals and increasing interest in vanadium applications in the chemical and petrochemical industries, energy production, and other sectors, the current primary vanadium resources cannot meet the demand for vanadium. In addition, the increasing demand for vanadium has contributed to increased smelting activity and industrial pollution by-products, resulting in an urgent need for an environmentally friendly technology that can improve the extraction of vanadium from minerals [13–15]. Further treatment or management is necessary to reduce the negative effect of vanadium extraction on the environment [16].

Different industrial technologies for vanadium extraction have been developed. Some traditional processes, such as chlorination roasting, alkali fusion, thermal decomposition, precipitation, water leaching, and acid leaching, require the use of large amounts of acids, alkalis, and other chemical reagents [4,17–19]. This requirement is associated with serious problems, such as environmental pollution and high energy consumption. Therefore, industrial challenges, such as high energy consumption and negative environmental effects, urgently require resolution through the use of other ecological and economic methods to extract vanadium from minerals [12]. In recent years, traditional vanadium extraction has been continuously improved, and new efficient and clean vanadium extraction technologies have been gradually developed. Bioleaching has numerous attractive features in metal extraction from minerals, these features include simple process operations and low investment, energy consumption, and environmental hazards [20–22]. In recent years, bioleaching has been developed into an alternative process for extracting vanadium from minerals [20].

Microorganisms can directly or indirectly interact with metals while possessing strong metal tolerance and the potential for extracting highly valuable metals [17,23–32]. During bioleaching, metals in minerals are converted into a soluble or extractable state by bacteria [33–35]. Silicate bacteria

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are parthenogenic chemoheterotrophic bacteria that can decompose silica–aluminate minerals and release their internal mineral elements. They are typified by *Bacillus mucilaginosus (B. mucilaginosus)*. The release of silicate mineral elements by *B. mucilaginosus* involves two types of leaching: direct and indirect interactions [36–37]. During direct interaction, bacteria adhere to the surfaces of mineral particles through biomechanical action, eroding and then decomposing minerals. During indirect interaction, bacteria secrete various metabolites that dissolve and release metals in minerals through acidolysis and ligand complexation [36–38].

Vanadium leaching by *B. mucilaginosus* has been discussed by researchers. Tian *et al.* [39] enhanced the bioleaching of vanadium-containing shale by chemically inducing *B. mucilaginosus*. Dong *et al.* [40] reported that the surfactant sodium dodecyl sulfate drastically accelerated the adsorption of stone coal vanadium ore by *B. mucilaginosus* during vanadium bioleaching. The vanadium leaching rate of *B. mucilaginosus*-50 reached 18.2%. In addition, Cai *et al.* [41] improved the interfacing behavior of *B. mucilaginosus* on vanadium-bearing shale during bioleaching by adding surfactant and achieved a vanadium leaching rate of 29.12%. Vanadium recovered through biological action could serve as a technical reserve for the green utilization of stone coal vanadium ore in the future.

Enzymatic digestion is an important mode of action in the mechanistic study of the interaction between silicate bacteria and minerals. In addition to their basic regulatory metabolic functions, biological enzymes directly participate in the weathering and dissolution of minerals [42]. Carbonic anhydrase (CA) is a metalloenzyme that is involved in the weathering and microbial leaching of minerals [43–44].

Given that only a few studies on the role of enzymes and other metabolites secreted by bacteria in leaching exist, targeted research must be conducted. Therefore, after evaluating the effects of different influencing factors on bioleaching, this study explored the activity of CA, the production of organic acid metabolites, and the relationship between enzymes and organic acids during leaching. It systematically revealed the mechanism of vanadium bioleaching from stone coal.

2. Materials and methods

2.1. Microorganisms and culture medium

The *B. mucilaginosus* strain utilized in this study was cultured and preserved in the group's laboratory after mutagenesis. The bacteria were cultured in 250 mL erlenmeyer flasks containing 100 mL of liquid culture medium. The culture medium was composed of 10.0 $g\cdot L^{-1}$ sucrose, 3.0 $g\cdot L^{-1}$ $Na_2HPO_4\cdot 12H_2O$, 2.0 $g\cdot L^{-1}$ $(NH_4)_2SO_4$, 0.5 $g\cdot L^{-1}$ $MgSO_4$, 0.1 $g\cdot L^{-1}$ KCl, 0.1 $g\cdot L^{-1}$ $CaCO_3$, and 0.005 $g\cdot L^{-1}$ $FeCl_3$. The initial pH of the medium was adjusted to 7.0 by using 0.5 $mol\cdot L^{-1}$ H_2SO_4.

2.2. Stone coal vanadium ore

The stone coal vanadium ore used in this study was collected from a mine in Hubei Province, China. The particle size of the stone coal samples was reduced to less than 74 μm through crushing and sieving. The content of each element in the sample was determined through X-ray fluorescence spectroscopy. The V_2O_5 content was found to be 1.19wt%. X-ray diffraction revealed that the stone coal samples were mainly composed of quartz and muscovite.

2.3. Bioleaching experiments

The leaching system was 100 mL of medium, and its pH value was stabilized at 7.0 through adjustment with dilute sulfuric acid. The liquid culture was autoclaved and inoculated with *B. mucilaginosus*. Stone coal samples were added to a shake flask, which was placed in an automatic temperature control rotary shaker and incubated. The bioleaching test period was set as 20 d and 1 mL of leachate was collected on 4, 8, 12, 16, and 20 d for the determination of pH and vanadium concentration. Different factors affecting leaching were investigated. The experimental design is shown in Table 1, where the inoculum amount (mL) was the added volume of *B. mucilaginosus* per 100 mL of culture medium.

Table 1. Experimental design of the influencing factor variables for the leaching test

Solid:liquid ratio / ($g\cdot L^{-1}$)	Inoculation amount / mL	Temperature / °C	Carbon source	Sucrose addition amount / ($g\cdot L^{-1}$)	Rotational speed / ($r\cdot min^{-1}$)
10	1	25	sucrose	5	140
20	2	30	glucose	10	160
30	3	35	starch	20	180
40	4	40		30	200

2.4. CA activity test

The bacterial suspension that had been cultured to the logarithmic growth phase was removed and centrifuged at 8000 $r\cdot min^{-1}$ for 10 min. The obtained precipitate was added with 2.0 mL of sterilized deionized water and subjected to intermittent ultrasonic disruption for 5 min. The supernatant obtained through centrifugation at 13000 $r\cdot min^{-1}$ for 10 min was the CA extract. Samples were collected to test enzyme activity.

The CA activity test adopted the colorimetric method reported in a previous study [45]. A working solution was prepared by dissolving 1.8 mg of *p*-nitrophenyl acetate in 0.5 mL of absolute ethanol and 15.6 mg of diethyl malonic acid in 10 mL of phosphate buffer. An equal volume of the working solution was mixed with the crude enzyme extract and reacted in a 30°C water bath for 30 min. The solution was cooled quickly, and its absorbance was measured at 400 nm. *p*-nitrophenol standard solutions with different concentrations were prepared, and their absorbance was measured. A standard curve was thus obtained, as shown in Fig. 1. The absorbance of the solution at 400 nm after the reaction was measured. The concentration of *p*-nitrophenol was obtained by comparing the standard curve of *p*-nitrophenol, and the activity of carbonic anhydrase was calculated.

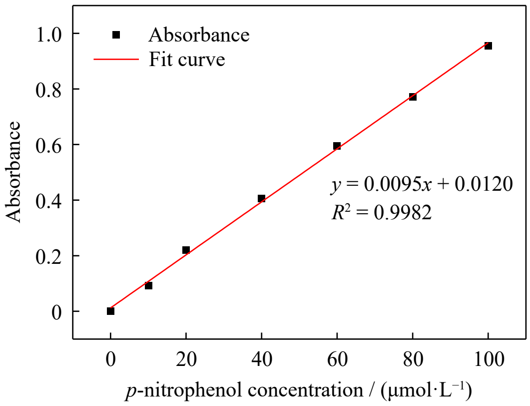

Fig. 1. Standard curve of *p*-nitrophenol.

The relationship between *p*-nitrophenol concentration and absorbance is

$$y = 0.0095x + 0.0120,$$

where y represents the absorbance of the test solution, and x represents the *p*-nitrophenol concentration in the test solution, μmol·L^{-1}.

2.5. Analytical methods

The pH of the bacterial fermentation broth was determined by using a pH meter (S20 Seveneasy). Vanadium concentrations at different bioleaching stages were detected through inductively coupled plasma mass spectrometry (Agilent ICPMS7800). Organic acids in fermentation broth were identified through high-performance liquid chromatography (LC-20AD). The chromatographic column was ZORBAX SB-AQ (5 μm × 250 mm × 4.6 mm), the mobile phase was 1 g·L^{-1} K_2HPO_4 solution and methanol, the flow rate was 1.0 mL·min^{-1}, the column temperature was 40°C, and the test wavelength was 210 nm. The three-dimensional micromorphologies of stone coal before and after bioleaching were detected by using an atomic force microscope (AFM, Dimension Icon). Stone coal samples were analyzed with an X-ray photoelectron spectrometer (XPS, Cougar EVO). A zeta potential analyzer (Bettersize 3000 Plus) was applied to determine the zeta potentials of the bacteria, stone coal, and stone coal surface after bacterial action.

3. Results and discussion

3.1. Vanadium leaching effect of *B. mucilaginosus* under different conditions

The effects of the solid:liquid ratio, bacterial inoculant amount, temperature, carbon source, sucrose addition amount, and dissolved oxygen on the vanadium extraction effect of *B. mucilaginosus* were investigated, as shown in Fig. 2.

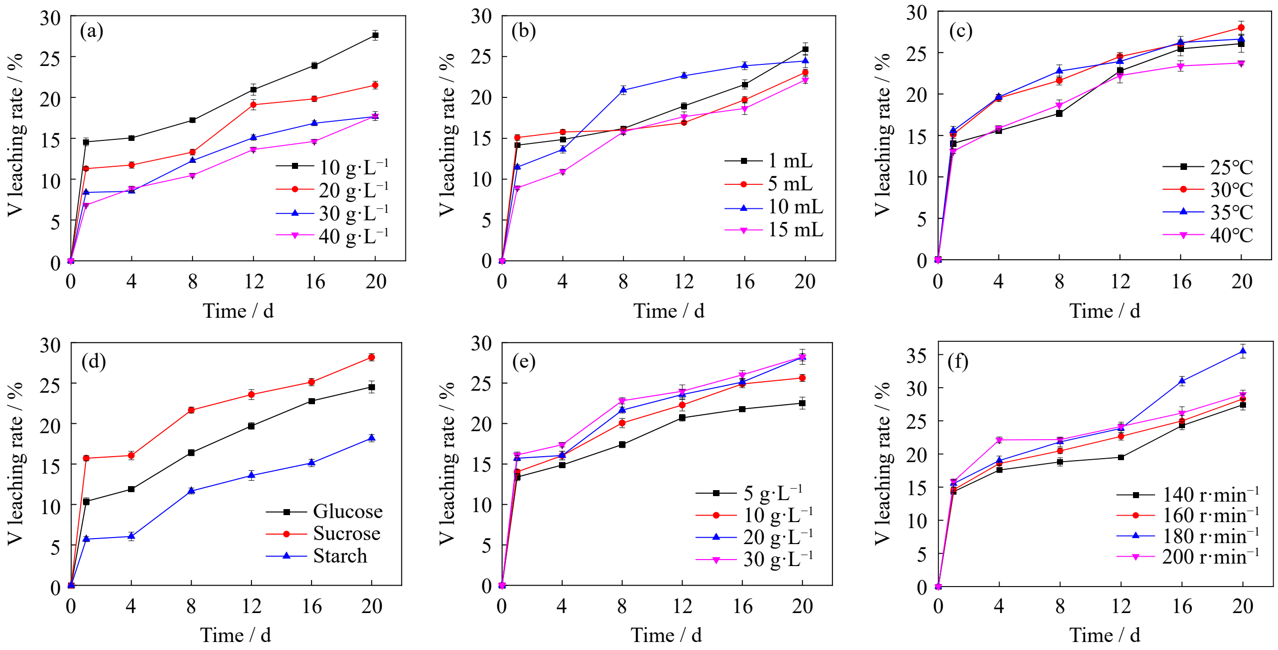

Fig. 2. Vanadium (V) leaching rate of *B. mucilaginosus* under different influencing factors: (a) solid:liquid ratio, (b) bacterial inoculant amount, (c) temperature, (d) carbon source, (e) sucrose addition, and (f) rotational speed.

The results in Fig. 2(a) demonstrate that at the same leaching time, the leaching rate of vanadium decreased with the increase in the solid:liquid ratio, indicating that the excessive concentration of stone coal had an inhibitory effect on the bioleaching of vanadium. With the increased in stone coal dosage, the vanadium leaching rate decreased. When the leaching time was 20 d, the highest vanadium leaching rate of 27.6% was obtained at the solid:liquid ratio of 10 g·L^{-1}. Microbes exert a direct mineral leaching effect by adsorbing on the surfaces of particles. With the increase in mineral addition amount, the number of microorganisms that actually attached to mineral particles decreased, resulting in a decrease in the leaching effect. In addition with the increase in the solid:liquid ratio, the degree of collision and friction between mineral particles increased, and the shear force between mineral particles increased during leaching, resulting in the shedding or damage of the bacteria that had attached to the surfaces of mineral particles; this effect also reduces the leaching effect [46].

Fig. 2(b) shows that the best leaching effect was obtained when the bacterial inoculant amount was 1 mL. The vanadium leaching rate increased gradually with the increase in leaching time, and the leaching rate reached 26.0% on day 20. The bacterial inoculant amount affects the time required for bacterial growth to reach the logarithmic phase in the leaching system. If the bacterial inoculant amount is excessively high, the time to reach the logarithmic phase shortens, but the nutrient consumption required for the growth of the strain increases, resulting in the insufficiency of the nutrients required for the growth and metabolism of the strain; this effect, in turn, influences leaching efficiency [47].

At excessively low temperatures, the bacterial biofilm is in a solidified state, and nutrient transport cannot occur normally, thus hindering the growth of microorganisms [48–49]. When the temperature is excessively high, nucleic acids and proteins in microorganisms are denatured and lose physiological activity [50–51]. Fig. 2(c) shows that with the increase in temperature, the vanadium leaching rate first increased and then decreased during 20 d of leaching. The leaching rate was low when the leaching temperature was 25, 35, and 40°C. The vanadium leaching rate was highest at 30°C, reaching 28.0%.

Fig. 2(d) illustrates the effect of vanadium leaching in the leaching system with three carbon sources: glucose (monosaccharide), sucrose (disaccharide), and starch (polysaccharide). The leaching results show that glucose, sucrose, and starch resulted in vanadium leaching rates of 24.52%, 28.19%, and 18.19%, respectively. The vanadium leaching amount was low when starch was used as the carbon source, and vanadium was continuously dissolved from the stone coal as bacteria consumed energy sources. Bacteria have high glucose and sucrose metabolism, and sucrose could be used as the carbon source for the growth of *B. mucilaginosus*, given its good utilization rate [52].

At the early stage of leaching, the growth and metabolism of microorganisms mainly depend on the nutrients in the medium. In the middle and late stages of leaching, the leaching system lacks energy substances. This poor nutritional environment causes the bacteria to reuse the organic acids and extracellular polysaccharides produced by their own metabolism. This behavior affects leaching efficiency. Increasing the amount of added sucrose and supplementing energy substances can maintain good bacterial growth and metabolic activity and improve leaching efficiency. The vanadium leaching rate in the four leaching systems increased with sucrose addition, as shown in Fig. 2(e). After 20 d of leaching, the vanadium leaching rates in the presence of 5, 10, 20, and 30 $g·L^{-1}$ sucrose were 22.52%, 25.63%, 28.19%, and 28.24%, respectively. Sucrose is an important energy source for the growth and metabolism of compound mutant bacteria during leaching. The results showed that the sucrose addition amounts of 20 and 30 $g·L^{-1}$ resulted in similar leaching rates, indicating that 20 $g·L^{-1}$ of sucrose can provide the energy required for bacterial growth. Therefore, 20 $g·L^{-1}$ was selected as the best sucrose addition amount.

B. mucilaginosus is an aerobic microorganism with a high critical oxygen concentration and requires sufficient oxygen supply during culture. The amount of dissolved oxygen in bacterial leaching is usually determined by the rotation speed of the shaker (the rotation of the shaker drives the air flow in the conical flask, thereby changing the dissolved oxygen content of the medium). Fig. 2(f) shows that as the shaking speed increased, vanadium leaching first increased and then decreased. After 20 d of leaching, the vanadium leaching rates at 140, 160, 180, and 200 $r·min^{-1}$ were 27.4%, 28.3%, 35.5%, and 29.0%, respectively. The dissolved oxygen content of the medium increased as the rotation speed of the shaker increased within a certain range. At the same time, the rotation of the shaker could evenly distribute nutrients and promote the growth of microorganisms. However, an excessively high rotation speed is unconducive to nutrient adsorption by microorganisms, affecting the use of microbial nutrients and thus influencing the leaching effect. Therefore, the optimal rotation speed for compound mutant leaching is 180 $r·min^{-1}$.

3.2. Vanadium bioleaching in contact and noncontact leaching modes

Bioleaching experiments were conducted in contact and noncontact modes to investigate the effect of bacteria and their metabolites on stone coal. In the contact leaching system, bacteria and metabolites can directly act on minerals, whereas in the noncontact leaching system, stone coal can only come into contact with small-molecule metabolites and cannot be directly used by bacteria. The pH changes and vanadium leaching effects of the two leaching modes are given in Fig. 3.

Fig. 3(a) shows that the two groups had the same pH change trend. Specifically, their pH levels first decreased, then increased slightly, and finally remained stable. The contact leaching system had a lower pH and higher vanadium leaching rate than the noncontact leaching system. The lowest pH value of 3.86 in the contact leaching group was observed on day 5, which was lower than that of the noncontact group (pH = 3.98) for 5 d. Fig. 3(b) shows that the microbial leaching effect and vanadium leaching rate were higher in the contact mode than in the noncontact mode. With the prolongation of leaching time, the difference in the vanadium leaching rate between the two groups gradually increased. Leaching in the noncontact mode mainly depends on the metabol-

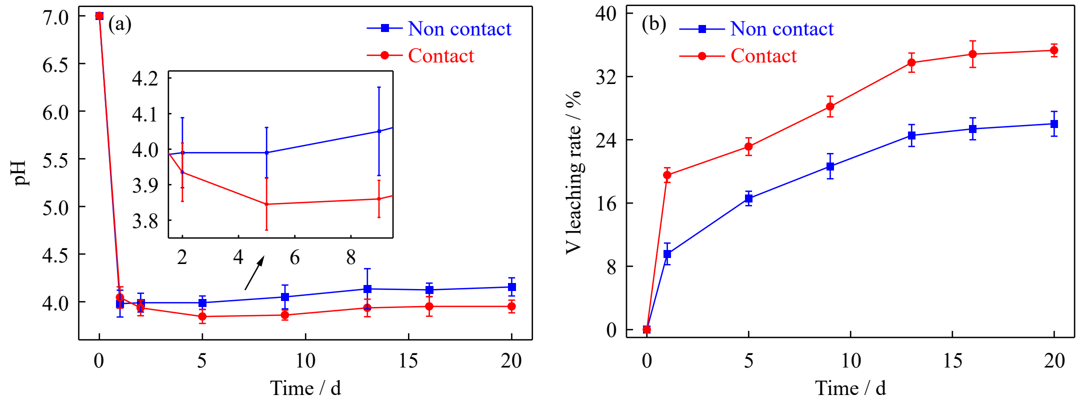

Fig. 3. Changes in (a) pH and (b) vanadium leaching rate in contact and noncontact microbial leaching systems.

ites of the bacteria in the leaching system. By contrast, in contact leaching, bacteria can adsorb on the surface of stone coal to utilize energy substances in addition to the metabolites produced by the decomposition of stone coal, thus maintaining high physiological activity [36,53].

Bacteria, extracellular polymers, and minerals can form complexes, creating a good microenvironment that promotes material exchange and component dissolution [54]. Contact leaching involves the direct effect of bacteria and the indirect effect of metabolites. After 20 d of leaching, the cumulative vanadium leaching rate of the contact group reached 35.5% due to the direct effect of bacteria and the indirect effect of metabolites. By contrast, the noncontact group achieved a vanadium leaching rate of 26.2%, which was due only to the indirect influence of metabolites. In accordance with the vanadium leaching efficiency in different leaching systems, the indirect and direct effects contributed approximately 73.8% and 26.2% to the vanadium leaching rate, respectively, indicating that the indirect effect had a prominent contribution to vanadium leaching. The metabolites of *B. mucilaginosus* played a leading role in microbial leaching.

3.3. Relationship between CA activity and organic acid production in *B. mucilaginosus*

According to previous studies [42,55], available exogenous substances and environmental factors affect the expression of CA and regulation of enzyme activity in *B. mucilaginosus*. They also play a key role in the induced generation of precipitates and dissolution of metal ions in minerals. Therefore, in this experiment, stone coal vanadium ore was used as the exogenous mineral, and the pattern of the changes in CA activity in bacteria and the trend of corresponding environmental pH changes during different incubation periods were investigated. The results are depicted in Fig. 4.

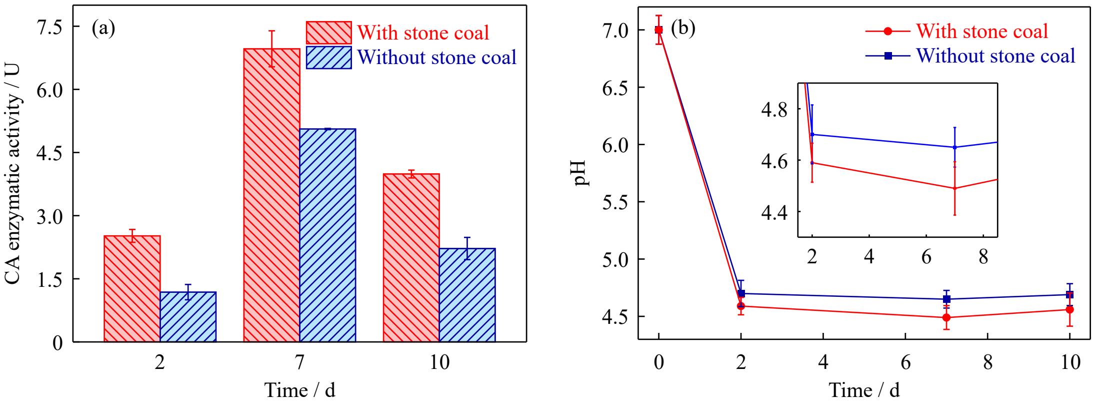

Fig. 4. Changes in (a) CA activity and system (b) pH of *B. mucilaginosus* at different incubation times.

The results in Fig. 4(a) show that bacterial CA activity in the culture system was consistently higher in the presence of stone coal than in the absence of stone coal. At 2, 7, and 10 days of leaching, the enzyme activity of the group with stone coal increased by 1.335, 1.905, and 1.770 U, respectively, compared to the group without stone coal. This result indicates that the presence of stone coal facilitates the increase in intracellular CA activity and suggests that exogenous minerals and adverse environments can stimulate CA expression in microorganisms. When the bacterial culture time was extended from 2 to 10 d, CA activity first increased and then decreased because, at the beginning of the incubation period, the presence of sufficient nutrients led to a weak demand for bacteria to perceive stone coal, and the expression of the cynT gene (Table S1 and Fig. S1) of CA function is weak. With the consumption of nutrients in the culture system and the increase in the demand for nutrients by the cells inside stone coal, the cynT gene of CA in bacteria executes an effective role and enzyme activity is enhanced, stimulating the leaching and dissolution of minerals by bacteria [42,55]. With the prolongation of incubation time, the metabolic activity of bacteria and leaching of minerals gradually weakened, and enzyme activity then decreased.

Combined with Fig. 4(b), the pH decreased rapidly, recovered briefly, and reached its lowest value on day 7 of incubation. This trend is consistent with the pattern of CA activity. The pH values of the systems with and without stone coal eventually decreased by 2.51 and 2.35, respectively. The view that CA catalyzes CO_2 hydration to promote H^+ production has been confirmed [38]. In this experiment, two sets of experimental systems (one with and one without stone coal) were established, and bacterial cultures were selected in different periods to test the production of four organic acids. The results are shown in Fig. 5.

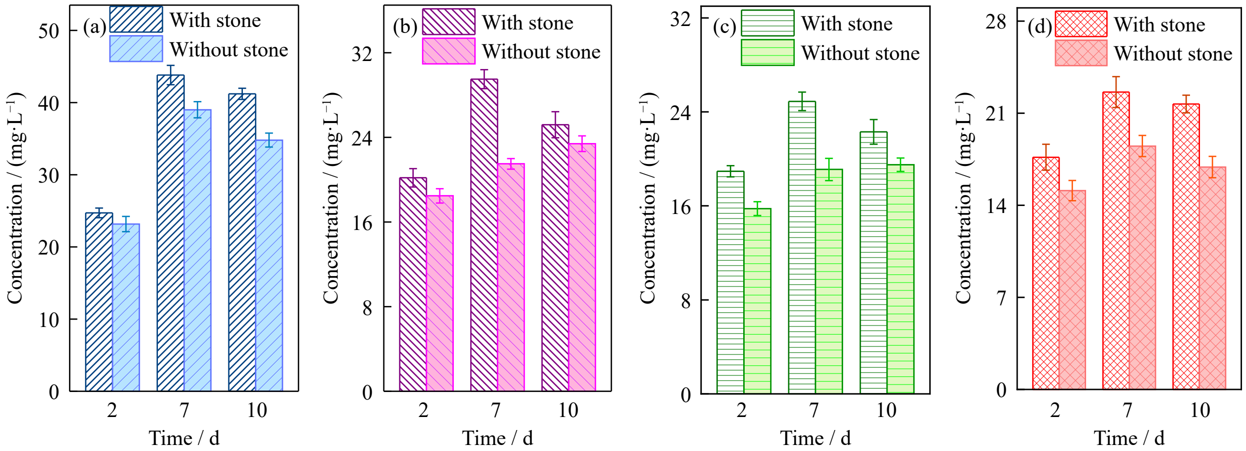

Fig. 5. Concentration of organic acids produced by *B. mucilaginosus* in different incubation periods: (a) malic acid, (b) citric acid, (c) tartaric acid, and (d) oxalic acid.

As shown in Fig. 5, the total content of all four organic acids produced by bacteria increased and then decreased throughout the incubation cycle. In the culture systems with stone coal, the total organic acid content was highest after 7 d of leaching and had increased by 39.31 $mg\cdot L^{-1}$ compared with that after 2 d of leaching. Among the metabolites, malic acid had the highest production level of 43.8 $mg\cdot L^{-1}$ after 7 d of incubation, followed by citric acid and tartaric acid. Oxalic acid showed the lowest production level among the organic acids. The contents of the four organic acids increased by 19.09, 9.32, 5.95, and 4.95 $mg\cdot L^{-1}$, respectively, compared with leaching for 2 d. Combining these results with the change trend of pH in the culture system presented in Fig. 4 revealed that with the prolongation of time, pH levels decreased first and then increased slightly. This trend corresponded to the change trend of organic acid content in the system containing stone coal illustrated in Fig. 5.

Comparing the two experimental groups revealed that organic acid production in the system containing stone coal was remarkably higher than that in the system without stone coal. This result, when combined with the change in CA activity in *B. mucilaginosus*, demonstrated that the presence of stone coal stimulated the increase in CA activity in *B. mucilaginosus*. This effect, in turn, promoted the release of H^+ in the reversible hydration reaction, increased the acidity of the surrounding microenvironment, and accelerated the dissolution of stone coal. At the same time, the increase in cell membrane permeability promoted the absorption of inorganic salt ions from the environment, and bacteria obtained nutrients from minerals to accelerate their growth and reproduction, further promoting the production of organic acids and thereby accelerating mineral dissolution [42,56]. Therefore, stone coal may be an exogenous substance that stimulates bacterial metabolism to produce organic acids by increasing CA activity [38].

The above phenomenon is attributed to the catalysis of CO_2 hydration by CA to produce HCO_3^- and H^+; HCO_3^- participates in the biosynthesis of malonyl coenzyme A, which is essential for lipid metabolism in bacterial cell membranes [42–44,56]. H^+ produced by the reaction can replace cations in the mineral lattice, leading to the leaching of metal elements, and the release of metal ions is accelerated by the enhanced acidity of the external environment that promotes mineral dissolution [42–44]. Thus, CA promotes microbial leaching through a dual approach: improving the fluidity of bacterial cell membranes and attacking the mineral crystal structure (Fig. 6).

3.4. AFM

The change in mineral surface morphology can reflect the corrosion of minerals by bacteria. Detecting the microscopic morphology of the stone coal before and after bacterial leaching can help understand the effect of microbial leaching on the structural evolution of minerals. Compared with traditional electrochemical methods, AFM has higher resolution and imaging accuracy and can obtain clearer three-dimensional images to show the original appearance of samples. In this experiment, the raw stone coal before leaching, the stone coal sample in the middle of leaching, and the stone coal leach residue after leaching were cleaned, dried, and subjected to AFM. The results are shown in Fig. 7.

Fig. 7 depicts the three-dimensional micromorphology of the stone coal surface before and after leaching by *B. mucilaginosus*. The images of three-dimensional morphology in Fig. 7 show that the degree of corrosion on the surfaces of the stone coal samples in different leaching periods differed. The

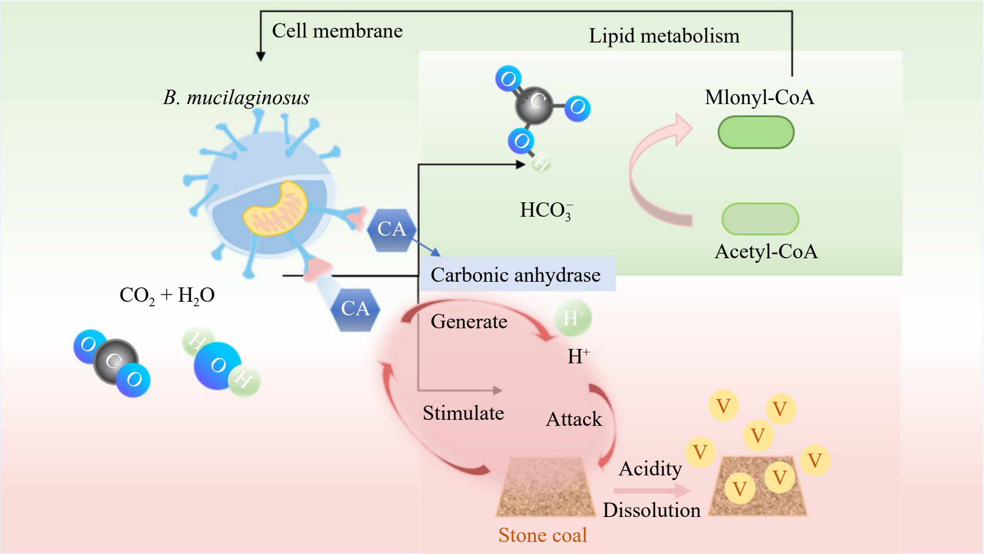

Fig. 6. Schematic of the CA-catalyzed reaction.

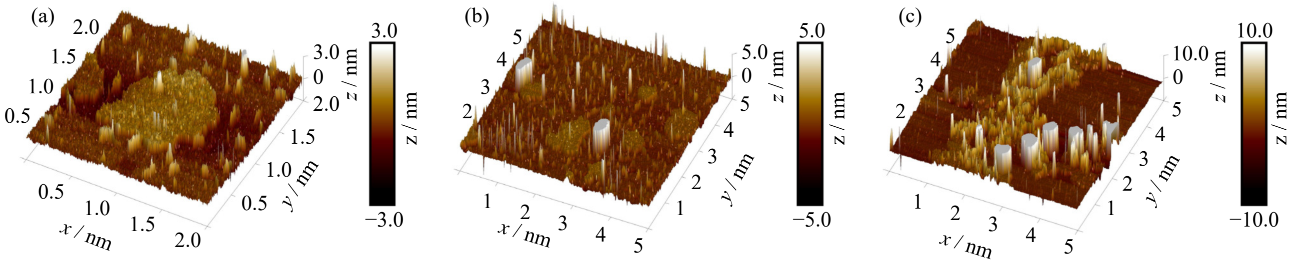

Fig. 7. Three-dimensional AFM maps of stone coal samples in different leaching periods: (a) original stone coal, (b) stone coal after 10 d of bioleaching, and (c) stone coal after 20 d of bioleaching.

surface of the original ore was smooth and flat, with undulations of less than 3 nm. At this time, the sample had not yet been subjected to biomechanical action. Therefore, its surface texture was uniform. After 10 d of leaching, the surface of the stone coal was damaged at numerous sites and became uneven due to decomposition by bacterial leaching, and the fluctuation degree was approximately 5 nm. After 20 d of microbial leaching, the corrosion degree of the surface of the stone coal leaching residue increased, and corrosion became observable. Protrusions and several scattered corrosion pits were observed. The degree of fluctuation was remarkable and even exceeded 10 nm. The leaching action of bacteria decreased the surface flatness and increased the roughness of stone coal samples. After 20 d of bacterial action, the surfaces of the mineral particles changed from flat and smooth to rough, with numerous fissures and corrosion pits.

3.5. XPS

XPS was performed on stone coal samples before and after bacterial leaching to examine the changes in the binding energy of elements on their surfaces. The results are given in Fig. 8 and Table 2.

Fig. 8 shows the electronic binding energy of the different elements present on the surfaces of stone coal samples. Fig. 8(a) and (b) illustrates that after peak separation, the 2p orbital spectra of elemental vanadium contained two peaks at 531.0 and 514.3 eV that corresponded to V_2O_5 and V^{5+}, respectively, indicating that elemental vanadium in stone coal was mainly present in the form of V^{5+} [57]. Fig. 8(c) and (d) shows the spectrum of elemental silicon, which had a Si 2p electron binding energy of 99.3 eV. Fig. 8(e) and (f) presents the 2p orbital spectrum of elemental aluminum, which was processed to obtain the peak attributed to Al_2O_3 with a binding energy of 71.4 eV. Comparing the XPS spectra of the ore samples before and after bacterial leaching revealed that the signals of vanadium and aluminum elements weakened after leaching, and the intensity of their corresponding peaks decreased. This conclusion is also reflected in Table 2. The corresponding peaks of different elements were integrated. The calculated peak area is the relative content of the elements on the mineral surface. Table 2 shows the relative content percentages of different elements on the surfaces of stone coal before and after leaching. The results reveal that the relative atomic percent content of vanadium decreased by 3.8% from 15.4% to 11.6%, indicating that *B. mucilaginosus* can attack silicate minerals, dissolve mineral components, and affect the valence and binding morphology of the elements on the surfaces of the minerals. These effects result in a decrease in the relative content of metallic elements on the surface of the minerals, thus enabling the release of metallic vanadium from stone coal.

3.6. Zeta potential analysis

Bioleaching leads to the attachment of numerous bacteria

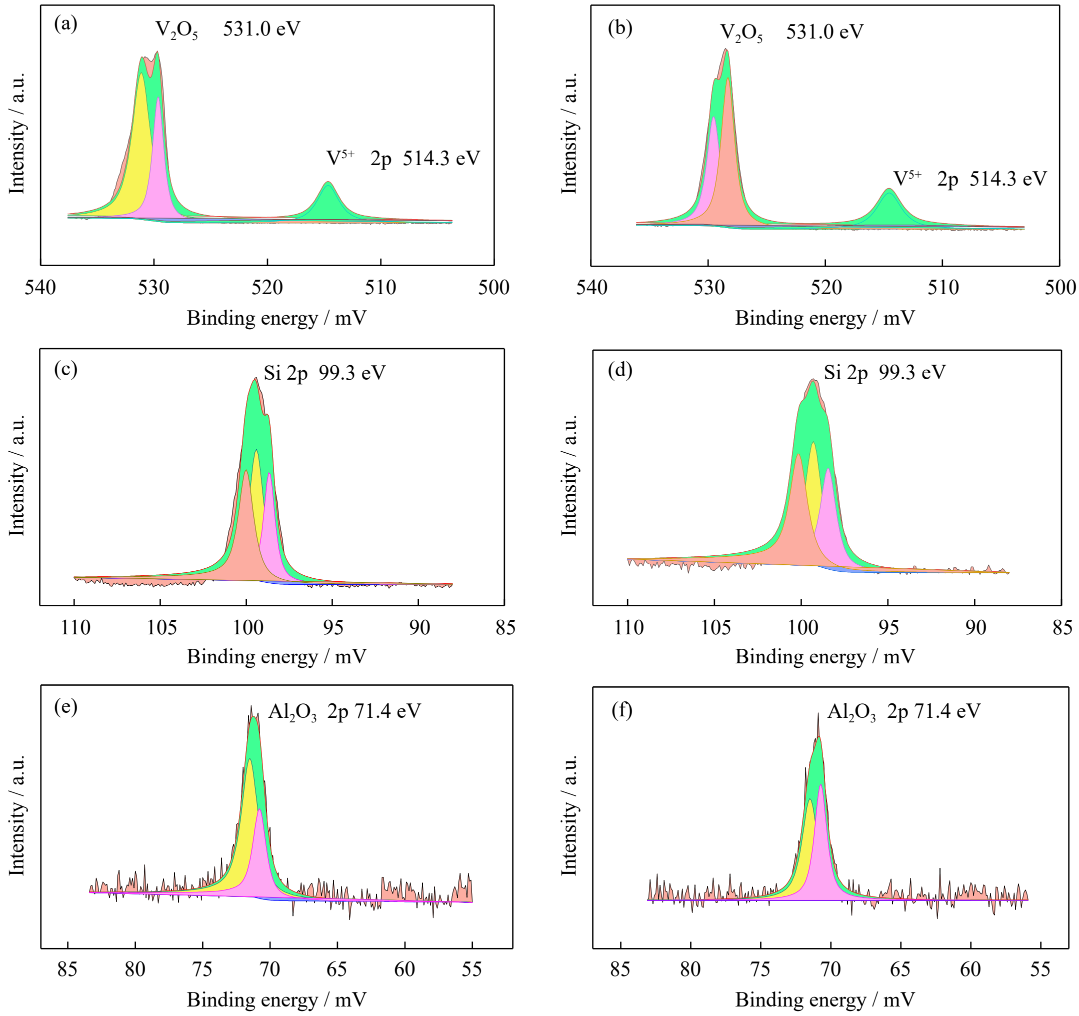

Fig. 8. XPS spectra of stone coal vanadium ore before and after leaching by *B. mucilaginosus*: (a, c, e) the electron binding energy spectra of vanadium, silicon, and aluminum on the surfaces of stone coal samples before leaching; (b, d, f) the electron binding energy spectra of vanadium, silicon, and aluminum on the surfaces of stone coal samples after leaching.

Table 2. Relative atomic percent contents of vanadium, silicon, and aluminum on the surface of stone coal vanadium ore before and after leaching by *B. mucilaginosus*

Sample	V / at%	Si / at%	Al / at%
Stone coal vanadium ore before bioleaching	15.40	60.50	24.10
Stone coal vanadium ore after bioleaching	11.60	69.30	19.10

to mineral surfaces, and biosorption leads to changes in the nature of the charges on the surfaces of bacteria and minerals [58]. The change in charge drives the biochemical reaction between minerals and bacteria. In this experiment, zeta potential tests were performed on stone coal before and after the action of *B. mucilaginosus* to investigate the effect of bacteria on the surface electrochemical properties of stone coal. The results are presented in Fig. 9.

Fig. 9 depicts the zeta potentials of the surfaces of *B. mucilaginosus* and stone coal before and after bioleaching. The isoelectric points of *B. mucilaginosus* and the original stone coal were located at pH values of approximately 3.0 and 3.8, respectively. The surface of bacteria was negatively charged when the pH of the leaching system was greater than 3, and minerals were positively charged when the pH of the leaching system was less than 3.8. These opposing electrical prop-

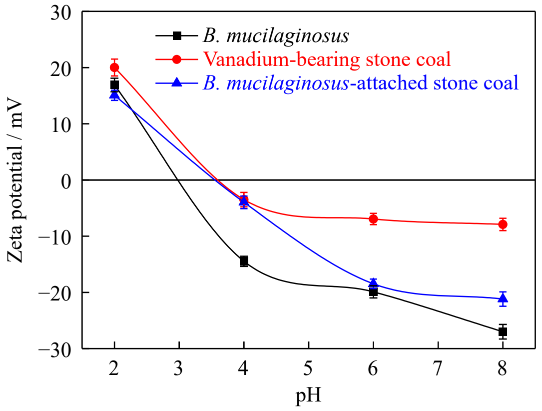

Fig. 9. Change in the zeta potentials of bacteria and stone coal before and after the action of *B. mucilaginosus*.

erties prompted electrostatic adsorption between bacteria and minerals, and the isoelectric point decreased to pH = 3.6 after the interaction between stone coal and bacteria. The change in the surface potential of stone coal and bacteria in the leaching system was mainly induced by biosorption. Strong electrostatic attraction enhances the adsorption affinity between minerals and bacteria, resulting in changes in the electrokinetic behavior of stone coal; these changes subsequently drive the adsorption behavior between bacteria and stone coal [57,59].

With the occurrence of biosorption, the number of bacteria on the surface of stone coal, the electric quantity carried by bacteria and the surface of stone coal, and the charge properties of bacteria and stone coal increased, contributing to the lowering of the isoelectric point of the stone coal after adsorption on stone coal; the continuous changes in charges drove the biochemical reaction [60]. However, given that the surface of stone coal cannot be fully covered by bacteria, the isoelectric point of the adsorbed bacteria on the surface of stone coal could not coincide with that of the bacteria in the leaching system and only approached it [58]. The trend of zeta potentials on bacterial and stone coal surfaces suggests that biosorption could induce changes in the surface charges of minerals as well as interfacial properties. These changes shortened the micro distance between bacteria and minerals, thus facilitating microbial leaching.

4. Conclusions

B. mucilaginosus, which can destroy the crystal structure of silicate minerals, was selected to investigate its leaching effect on stone coal vanadium ore and identify its metabolites. The results of this work showed that the best leaching effect on stone coal vanadium ore was achieved with a solid : liquid ratio of 10 g·L^{-1}, a bacterial inoculant amount of 1 mL, a temperature of 30°C, a sucrose addition amount of 20 g·L^{-1}, and a shaker speed of 180 r·min^{-1}. After 20 d of leaching under these conditions, the vanadium leaching rate reached 35.5%. The indirect effect had a prominent contribution of approximately 73.8% to vanadium leaching by *B. mucilaginosus*. In addition, during leaching, the presence of stone coal stimulated the expression of CA in bacterial cells, and enzyme activity increased by 1.335–1.905 U. Consistent with the change trend of CA activity in bacteria, the contents of malic, oxalic, citric, and tartaric acids produced by bacteria first increased and then decreased during the whole leaching period. The increase in enzyme activity increased the total organic acid content by 39.31 mg·L^{-1}, which decreased the pH of the leaching system with stone coal by 2.51. Enzyme activity positively promoted the production of organic acid metabolites. The increase in the acidity of the microenvironment around stone coal favored mineral dissolution and leaching.

Microstructural observation and XPS revealed that after 20 d of bacterial leaching, the surface of the stone coal leaching residue became severely corroded, and its roughness exceeded 10 nm. The relative vanadium atomic percent content on the surface of stone coal before and after leaching decreased by 3.8% from 15.4% to 11.6%. In addition, the electrostatic adsorption of stone coal and bacteria was induced by their opposing electrical charges. Zeta potential tests revealed that the isoelectric point of stone coal decreased from 3.8 to 3.6, the electronegativity of the stone coal surface increased, and the isoelectric point of stone coal was close to that of bacteria. This work explored the mechanism underlying the bacterial leaching of stone coal by investigating the enzymatic activity and metabolites involved in the leaching of stone coal. It can provide a theoretical basis for the regulation and mechanistic study of the microbial leaching of stone coal vanadium ore.

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Conflict of Interest

Hai Lin is an editorial board member for this journal and was not involved in the editorial review or the decision to publish this article. All authors do not have competing interests to declare.

Supplementary Information

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