

Article

Study on the Effectiveness of Sulfate Reducing Bacteria to Remove Heavy Metals (Fe, Mn, Cu, Cr) in Acid Mine Drainage

Yanrong Dong ^{1,2,*} , Ziqing Gao ¹, Junzhen Di ¹ , Dong Wang ², Zhenhua Yang ², Yunfeng Wang ¹ and Zhoufei Xie ¹

¹ College of Civil Engineering, Liaoning Technical University, Fuxin 123000, China; gzq904906@163.com (Z.G.); dijunzhen@126.com (J.D.); m1497385455@163.com (Y.W.); xiezhoufei2023@163.com (Z.X.)

² College of Mining, Liaoning Technical University, Fuxin 123000, China; zhangjuanguaoziqing@163.com (D.W.); 471710043@lntu.edu.cn (Z.Y.)

* Correspondence: dongyanrong@lntu.edu.cn

Abstract: Aiming at the problem of environmental pollution caused by heavy metals such as Fe, Mn, Cu, and Cr, *Sulfate Reducing Bacteria* (SRB) were enriched in mining soil. SRB was added to AMD containing different concentrations of Fe, Mn, Cu, and Cr by batch experiments to explore the potential of SRB for treating heavy metals in AMD. Testing combining Scanning Electron Microscopy–Energy Dispersive Spectroscopy (SEM-EDS), X-ray Diffraction (XRD), and X-ray Photoelectron Spectroscopy (XPS) revealed the mechanism by which heavy metal ions were removed by SRB. The results showed that SRB had a strong ability to remove total Fe in the concentration range of 0–300 mg/L, and the removal percentage of total Fe reached to 92.16–93.17%. SRB had a good removal effect on total Cu, in the concentration range of 0–20 mg/L, and the removal percentage of total Cu reached to 79.79–81.80%. SRB had a good removal effect on total Cr as well; SRB activity would probably not be inhibited for total Cr concentrations below 500 mg/L. The ability to solidify different heavy metal ions using SRB was as follows: total Cr > total Fe > total Mn > total Cu. When SRB removed Fe²⁺ there was precipitation at the same time, appearing as black granules. This precipitation consisted mainly of FeS, and contained small amounts of Fe₃(PO₄)₂·8H₂O, FeCO₃, Fe(OH)₂, Fe(OH)₃, and Fe₂S as well.

Keywords: mining area; *Sulfate Reducing Bacteria* (SRB); heavy metal ions; removal mechanism



Citation: Dong, Y.; Gao, Z.; Di, J.; Wang, D.; Yang, Z.; Wang, Y.; Xie, Z. Study on the Effectiveness of *Sulfate Reducing Bacteria* to Remove Heavy Metals (Fe, Mn, Cu, Cr) in Acid Mine Drainage. *Sustainability* **2023**, *15*, 5486. <https://doi.org/10.3390/su15065486>

Academic Editor: Gujie Qian

Received: 15 February 2023

Revised: 10 March 2023

Accepted: 19 March 2023

Published: 20 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

With the development of industrialization and urbanization, a variety of heavy metals are released into the environment, leading to serious environmental pollution [1]. It has been reported that about 40% of lakes and rivers in the world are polluted by heavy metals. The average concentrations of heavy metals such as Cr, Mn, Fe, and Cd in surface water in certain areas are much higher than standards for safe drinking water [2]. The heavy metal content around mining areas is especially likely to reach moderate or severe pollution levels [3]. Among the various problems associated with environmental pollution, acid mine drainage (AMD) released from mining areas is among the most serious. The contents of SO₄^{2−}, Fe, Mn, and Cu in AMD released from the Smolnik mining area in Slovakia have been measured as 14–2902 mg/L, 0.12–322 mg/L, 0.01–25.3 mg/L, and 4–1512 mg/L, respectively, with a pH value of 4–6.4 [4]. The contents of SO₄^{2−}, Fe, Mn, and Cr in AMD released from the Rand mining area in South Africa were 2562 mg/L, 297 mg/L, 71 mg/L, and 0.024 mg/L, respectively, with a pH value of 3.5 [5]. The Fe and Mn contents in AMD released from a mine in South Korea were 78 mg/L and 13 mg/L, respectively, with a pH value of 2.7 [6]. The contents of SO₄^{2−}, Fe, and Mn in AMD released from the Riguang mining area in South Korea were 1997 mg/L, 440 mg/L, and 14.3 mg/L, respectively, with a pH value of 4–6.4 [7]. The contents of SO₄^{2−}, Fe, Mn, and Cu in AMD released from the Williams Brothers mining area in the United States were 100 mg/L, 4.6 mg/L, 1.2 mg/L,

and 74 mg/L, respectively, with a pH value of 3.9 [8]. Overall, there is a high amount of SO_4^{2-} and heavy metal ions (Fe, Mn, Cu, Cr, etc.) in AMD, resulting in AMD being acidic. AMD causes serious heavy metal environmental pollution, and heavy metal ions in water and soil around mining areas tend to seriously exceed environmental and health standards.

Heavy metal pollution has become a global problem to be solved. Heavy metal ions such as Cu, Cr, Mn, Fe, Zn, Cd, and Pb can damage enzyme activity and cell structure, affecting plant growth and metabolic activity [9]. Meanwhile, heavy metal ions can enter into the human body through the food chain. Excessive accumulation of heavy metals in the human body can cause cancer, cardiovascular disease, liver damage, etc. In order to alleviate the toxicity of heavy metal ions to the environment and living organisms, many technologies for repairing heavy metal pollution have emerged. Among them, microbial remediation of heavy metals has attracted wide attention due to its advantages of low cost, high efficiency, environmental friendliness, and simple operation [10]. Microorganisms can change the forms of heavy metals existing in the environment by affecting their chemical or physical properties. Microorganisms can change the presence of heavy metals in the environment by affecting their chemical or physical properties, converting heavy metals into less toxic valence states or reducing their fluidity, thereby changing heavy metals into less toxic forms or reducing their fluidity. This achieves the effect of mineralization or immobilization of heavy metals [11,12]. Microorganisms in the natural environment have mineralization ability; the most widely used mineralization microorganisms in engineering are *Sulfate Reducing Bacteria* (SRB), *Urease-producing Bacteria* (UPB), and *Phosphate Solubilizing Bacteria* (PSB) [13]. Among these, SRB treatment of heavy metals in water has the advantages of high treatment efficiency and no secondary pollution. In particular, SRB reduces sulfate to H_2S by metabolizing organic matter. Then, S^{2-} forms insoluble metal sulfide precipitates with heavy metal ions to remove heavy metal ions from the environment [14]. Therefore, based on its metabolic characteristics, SRB has been widely used in the field of AMD treatment. SRB can efficiently remove heavy metal ions and SO_4^{2-} in AMD [14], and can reduce the environmental pollution caused by AMD. Previous studies of SRB for treating AMD have mainly focused on the use of continuous stirred-tank reactors (CSTR), up-flow anaerobic sludge bed reactors (UASB), fixed-bed reactors (FBR), permeable reaction barriers (PRB), and other types of bioreactors [15]. These bioreactors are usually inoculated with sludge, wetland sediments, or enriched single SRB species [16]. When a site is not suitable for the installation of bioreactors, native SRB enriched from the site are more suitable for treating AMD [17]. A number of studies have attempted to isolate SRB from the sediments of mining areas. However, studies on the sulfate reduction ability or heavy metal removal ability of SRB strains in mining areas remain limited [17].

AMD often contains a large amount of heavy metal ions. Different heavy metal ion concentrations lead to hypoxia during the SRB metabolism, which affects the nucleation morphology of metal sulfide production [18]. Zhang Xin et al. [19] found that high ion concentration can inhibit the growth and metabolic activity of SRB, in turn inhibiting the formation of precipitation products. Mullaugh et al. [20] found that high ionic strength can increase the particle size of CdS. Moreover, they found that in cases of high heavy metal ion content, high ionic strength inhibits intracellular enzyme activity, replaces the cations on the cell membrane, and destroys the integrity of the organelle membrane, resulting in a decrease in microbial activity and even creating conditions that are toxic to microorganisms [21]. When using SRB, different heavy metals have different inhibitory effects. Hao et al. [22] studied the effect of heavy metals on SRB and found that the inhibition degree of different heavy metals on SRB was as follows: $\text{Cu} > \text{Cd} > \text{Ni} > \text{Zn} > \text{Cr} > \text{Pb}$. Cabrera et al. [23] compared the tolerance for heavy metal ions (Cr^{3+} , Cu^{2+} , Mn^{2+} , Ni^{2+} and Zn^{2+}) between two kinds of SRB; their results showed that SRB had different responses to different metals. At the highest tolerance concentration of each heavy metal, the precipitation levels of *Desulfovibrio vulgaris* were as follows: the removal percentage of 15 ppm Cr^{3+} was 24.7%; the removal percentage of 4 ppm Cu^{2+} was 45%; the removal percentage of 10 ppm Mn^{2+} was 60%; the removal percentage of 8.5 ppm Ni^{2+} was 96%; and the removal percentage of

20 ppm Zn^{2+} was 9% [23]. At the highest tolerance concentration of each heavy metal, the precipitation levels of *Desulfovibrio* sp. were as follows: the removal percentage of 15 ppm Cr^{3+} was 25.5%; the removal percentage 4 ppm Cu^{2+} was 71%; the removal percentage of 10 ppm Mn^{2+} was 66.2%; the removal percentage of 8.5 ppm Ni^{2+} was 96.1%; and the removal percentage of 20 ppm Zn^{2+} was 93% [23]. Therefore, the concentration of heavy metal ions has a great influence on the ability of SRB to precipitate heavy metals. Most of the above-mentioned studies focused on the precipitation of heavy metal ions such as Cd^{2+} , Cr^{3+} , Cu^{2+} , Mn^{2+} , Ni^{2+} , Zn^{2+} , and Pb^{2+} by SRB. However, there is a large amount of SO_4^{2-} and heavy metal ions (Fe, Mn, Cu, Cr, etc.) in AMD [4–8]. In particular, the content of iron ions is typically very high. At the same time, the toxicity of Cr^{6+} is much higher than that of Cr^{3+} .

Therefore, in this study the enrichment method was used to obtain primary mixed SRB from soil samples in the studied mining area. Based on batch experiments, we inoculated SRB into AMD containing different concentrations of Fe^{2+} , Mn^{2+} , Cu^{2+} , and Cr^{6+} . By combining the changes in OD_{600} , pH, oxidation reduction potential (ORP), electrical conductance (Ec), and the removal effects with respect to SO_4^{2-} , total Fe, total Mn, total Cu, and total Cr, we analyzed the effective reduction potential of heavy metals and sulfate in AMD using SRB. Then, we combined SEM-EDS, XRD, and XPS testing to explore the mechanism by which SRB is able to remove heavy metal ions.

2. Methods and Materials

SRB: The SRB we used was mixed SRB cultured in laboratory. Mixed SRB was enriched using wet soil from the Haizhou open-pit coal gangue mountain in Fuxin City, Liaoning Province ($121^\circ 41' \text{ E}$, $41^\circ 59' \text{ N}$) [24]. Wet soil was inoculated into sterile Starkey medium for anaerobic culture, with the components being 0.5 g K_2HPO_4 , 1.0 g NH_4Cl , 2.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g Na_2SO_4 , 0.1 g $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 1.0 g yeast extract, 4 mL sodium lactate, 0.5 g $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 0.1 g ascorbic acid, and 1 L distilled water, with sterilizing for 30 min at $\text{pH} = 7$, 121°C . Among these components, $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ and ascorbic acid cannot be sterilized at high temperature; therefore, we used a $0.22 \mu\text{m}$ filter membrane to filtrate and sterilize them. When producing H_2S , a large amount of black precipitates emerged in the medium, indicating that the mixed SRB had been cultured [24]. SRB in logarithmic growth phase was used to remove heavy metal ions. There were three replicates for each test setting. SRB was preserved in the method of glycerol tube cryopreservation under sterile operating conditions, adding 500 μL bacterial solution and 500 μL 50% glycerol solution into the sterilized 2 mL cryopreservation tube, then mixing the solution lightly and preserving the mixed solution at -80°C .

Synthetic AMD: AMD was taken from a mining area in Shanxi province following an on-site-survey. After measurement, we found that the pH of the AMD was 5 ± 0.1 and that it contained $2.0 \pm 0.05 \text{ g/L } \text{SO}_4^{2-}$, $0.6 \pm 0.05 \text{ g/L } \text{Na}^+$, $0.04 \pm 0.01 \text{ g/L } \text{Ca}^{2+}$, $0.20 \pm 0.05 \text{ g/L } \text{Mg}^{2+}$, $0.15 \pm 0.02 \text{ g/L } \text{K}^+$, $0.28 \pm 0.02 \text{ g/L } \text{Fe}^{2+}$, $0.018 \pm 0.02 \text{ g/L } \text{Mn}^{2+}$, $0.013 \pm 0.02 \text{ g/L } \text{Cu}^{2+}$, $0.05 \pm 0.01 \text{ g/L } \text{Cr}^{6+}$. Then, combining the measured AMD data and the components of the Starkey medium, we made artificial simulated AMD in the laboratory. The pH value was adjusted by 1 mol/L HNO_3 and 1 mol/L NaOH to make the artificial simulated AMD $\text{pH} = 5$. At the same time, the artificial simulated AMD contained 2.0 g/L SO_4^{2-} , 0.58 g/L Na^+ , 0.04 g/L Ca^{2+} , 0.20 g/L Mg^{2+} , 0.17 g/L K^+ , and a small amount of Cl^- , HPO_4^{2-} , and sodium lactate. To explore the removal efficiency of different heavy metals, namely, Fe, Mn, Cu, and Cr, we added different concentrations of Fe^{2+} into the artificial simulated AMD to form the following AMD solutions: Fe^{2+} 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L; Mn^{2+} 0 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L; Cu^{2+} 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L; and Cr^{6+} 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L. Analytical reagents $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and K_2CrO_4 from Tianjin Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China) were used to prepare the solutions containing Fe^{2+} , Mn^{2+} , Cu^{2+} , and Cr^{6+} listed above. All solutions were

prepared with deionized water, and their ion contents were measured by the instrument to avoid any possible error in the calculations.

Batch experiments were carried out to remove total Fe, total Mn, total Cu, and total Cr by SRB. Among these, the test method of Fe removal by SRB for SRB at logarithmic growth stage was inoculated at 5% inoculum into the AMD solutions containing Fe ion concentrations of 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L. Then, 10 mL of sterilized liquid paraffin was added to the above mixture and the conical flask was sealed with a rubber stopper. The conical flask was placed in a constant temperature oscillation incubator (Changzhou Kaihang Instrument Co., Ltd., Changzhou, China, type HZ-9811K) at 35°C and 150 r/min for oscillation culture. There were three replicates for each test setting. Samples were taken at 1 d intervals. Using sterilised AMD without SRB inoculation as a blank group. The pH value, ORP value, OD₆₀₀ value, and Ec value were measured by PHS-3C pH meter (Shanghai Yidian Scientific Instrument Co., Ltd., Shanghai, China), CT-8022 ORP meter (Shanghai Hechen Energy Technology Co., Ltd., Shanghai, China), and V-1600PC visible spectrophotometer (Shanghai Yuanwang Liquid Level Meter Co., Ltd., Shanghai, China). The samples to be tested were filtered with 0.22 µm filter membrane. The concentrations of SO₄^{2−} and total Fe in the filtered samples were measured by V-1600 PC visible spectrophotometer and Z-2000 flame atomic spectrophotometer (Hitachi society in Japan, Japan), and the removal percentage of SO₄^{2−} and total Fe were calculated. The average value and error of the three groups of repeated tests were used to draw the image. The experimental methods used to remove total Mn, Cu, and Cr by SRB were consistent with that used to removed total Fe by SRB.

The pH value was determined by electrode method (HJ 1147-2020). The SO₄^{2−} concentration was determined by barium chromate spectrophotometry (HJ/T342-2007). Total Fe and total Mn were determined by flame atomic absorption spectrophotometry (GB11911-89). Total Cu was determined by atomic absorption spectrophotometry (GB7475-87). Total Cr was determined by flame atomic absorption spectrophotometry (HJ757-2015).

The precipitation of SRB removing Fe²⁺ was collected and centrifuged at 4000 r/min for 10 min. The precipitation after centrifugation was dried at 90°C in a vacuum drying oven (DZF-6090AB). Then, the dried precipitation was tested by SEM-EDS, XRD, and XPS. The SEM appearance of the sample was detected using a German Zeiss sigma 500 scanning electron microscope, and the chemical substances on the surface of the precipitate were tested by EDS. Phase analysis was carried out by Bruker D8 Advance XRD, and the scanning step of XRD was 5°–90°. The XPS of the precipitate was determined by Kratos AXIS SUPRA XPS.

3. Results and Discussion

3.1. Removal Effect of Total Fe by SRB

It can be seen from Figure 1a that when SRB removed Fe²⁺, the OD₆₀₀ value generally increased first and then tended to become stable. With concentrations of Fe²⁺ of 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L, respectively, the OD₆₀₀ values were 1.02, 1.14, 1.27, 0.83, and 0.70 after adding SRB for 6 days. When the concentration of Fe²⁺ was 100–300 mg/L, the OD₆₀₀ value increased gradually with the increase of Fe²⁺ concentration and finally stabilized at 1.01–1.18, indicating that the appropriate increase of Fe²⁺ concentration in this range is beneficial to the growth, reproduction, and activity of the SRB. When the initial Fe²⁺ concentration was in the range of 300–500 mg/L, the OD₆₀₀ value gradually decreased with the increase of Fe²⁺ concentration and finally stabilized at 0.63–1.18, indicating that the increase of Fe²⁺ concentration in this range inhibits SRB growth. It can be seen from Figure 1b–d that the pH value increased first and then stabilized, while the ORP value decreased when the initial Fe²⁺ concentrations were 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L, respectively. After SRB was added for 8 days, the pH values were 6.87, 6.87, 6.91, 6.40, and 6.15, and the ORP values were −325 mV, −378 mV, −399 mV, −202 mV, and −128 mV, respectively. The Ec values were 3.05 mS/cm, 3.64 mS/cm, 3.77 mS/cm, 3.97 mS/cm, and 4.30 mS/cm, respectively. The process of SRB

metabolizing SO_4^{2-} and organic carbon to generate S^{2-} and HCO_3^- increases the pH value and decreases the ORP value [25]. According to the mechanism by which SRB metabolizing SO_4^{2-} and producing S^{2-} (formula (1)–(3)), we know that when SRB metabolic activity is higher, ORP value decreases significantly [24]. When the initial Fe^{2+} concentration was 100–300 mg/L, the pH value increased significantly, and the ORP value decreased very obviously, indicating that the SRB activity was higher and the metabolic reduction of SO_4^{2-} was greater in this range. In the early stage of cultivation, SRB metabolism was more vigorous, more SO_4^{2-} was reduced, and the Ec value decreased significantly. In the later stage, SRB entered a period of decline, the activity was poor, and the SRB gradually died, resulting in a slight increase in the Ec value.

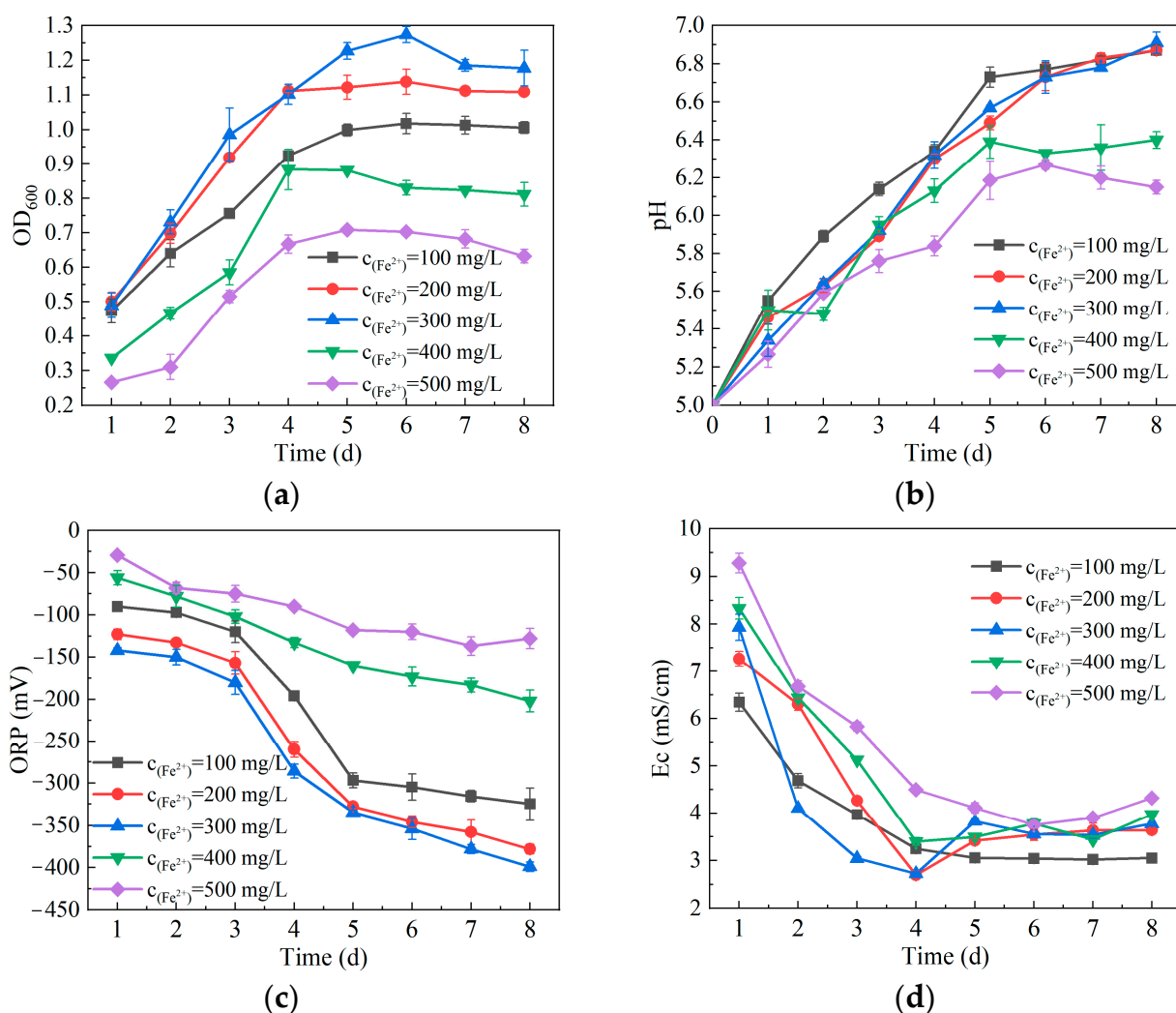
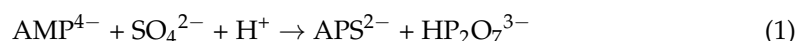


Figure 1. Cont.

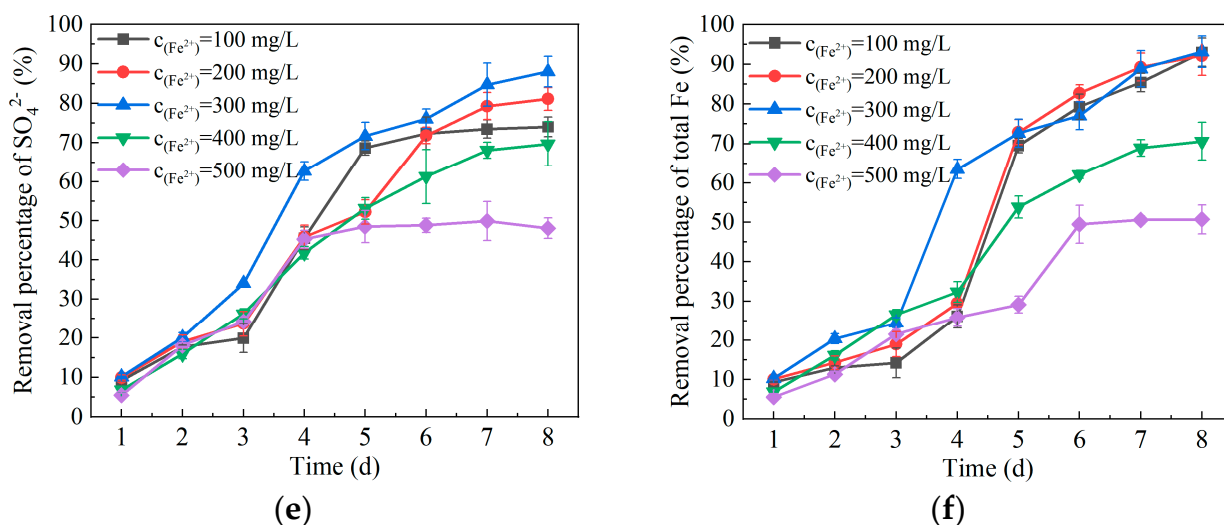


Figure 1. Effect of SRB on Fe ion removal (5% SRB; initial concentrations of Fe^{2+} were 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L). (a) OD_{600} value changes; (b) pH value changes; (c) ORP value changes; (d) Ec value changes; (e) SO_4^{2-} removal conditions; (f) removal percentage of total Fe.

According to Figure 1e, when SRB removed Fe^{2+} , the removal of SO_4^{2-} increased first and then tended to become stable. According to the formula above, SRB was able to effectively metabolize SO_4^{2-} and produce S^{2-} ; in particular, when the SRB activity was high, the conversion rate of SO_4^{2-} was faster. In the early stage, SRB metabolism was high and had strong dissimilatory SO_4^{2-} reduction effect, and the removal percentage of SO_4^{2-} increased rapidly. In the later stage, SRB gradually entered a declining period and the metabolism was slow, with less SO_4^{2-} being reduced; the removal rate of SO_4^{2-} tended to become stable. With Fe^{2+} concentrations of 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L, the removal percentage of SO_4^{2-} after adding SRB for 8 days was 74.05%, 81.13%, 88.05%, 69.7%, and 48.11% respectively. When the concentration of Fe^{2+} was in the range of 100–300 mg/L, the SO_4^{2-} removal rate increased gradually with the increase in Fe^{2+} concentration. It has been reported that the presence of high concentrations of heavy metals can interfere with the biological removal of SO_4^{2-} by SRB [26]. When the concentration of Fe^{2+} was in the range of 300–500 mg/L, the SO_4^{2-} removal rate decreased gradually with the increase in Fe^{2+} concentration. This indicates that increasing Fe^{2+} concentration within the range of 100–300 mg/L can stimulate the growth and metabolism of SRB.

It can be seen from Figure 1f that when SRB removed Fe ions, the removal percentage of Fe ions by SRB generally increased first and then tended to become stable. When the concentration of Fe^{2+} was 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L, the removal percentage of Fe ions reached 92.99%, 92.16%, 93.17%, 70.59% and 50.73%, respectively, after SRB was added for 8 days. When the concentration of Fe ions was in the range of 100–300 mg/L, the removal percentage of Fe ion was more than 92%. This high removal efficiency of Fe^{2+} by SRB is mainly attributed to the low solubility product FeS [27]. The SRB uses its own metabolic activity to make SO_4^{2-} become an electron acceptor, then uses dissimilatory reduction to generate S^{2-} [28]. The resulting S^{2-} and Fe^{2+} in the solution formed precipitation. It has been reported that in addition to removing Fe^{2+} in the form of FeS , SRB can remove it in the form of hydroxide precipitation and carbonates [29]. In addition, the SRB secreted a large amount of extracellular polymeric substance (EPS) during growth, which is useful in the treatment of heavy metal ions by SRB [30]. EPS contains functional groups such as amines, phosphate, and carboxyl and hydroxyl groups, which can enhance the binding ability of cell surfaces to heavy metals [30,31], meaning that SRB can remove Fe^{2+} to an even higher level. It has been reported that the metabolites of SRB are mainly divalent sulfur; divalent sulfur combines with Fe^{2+} to convert to the insoluble

and biologically unavailable FeS [32]. Therefore, when the concentration of Fe ions was in the range of 100–300 mg/L, increasing the concentration of Fe ions facilitated the binding of Fe ions with S^{2-} . When the concentration of Fe ions was over 300 mg/L, the growth and metabolism rate of SRB decreased, which is mainly related to the inhibition of SRB growth by the high concentration of Fe ions. It has been reported that heavy metals can inactivate the enzyme by reacting with the functional groups of SRB, thereby denaturing the protein and competing with the necessary cations to inhibit the activity of SRB [33]. SRB has strong tolerance to Fe, and can remove most Fe. Zhuxiang Liu [27] found that under conditions of $[pH]_0 = 6.0$ and $[SO_4^{2-}]_0 = 2000$ mg/L, the removal percentage of Fe by SRB was 95.2–100%. Bai [34] showed that the tolerance of SRB to Fe concentration was 330 mg/L and the removal percentage of Fe was 85.5%. Mingliang Zhang [35] found that with the addition of other sludge particles that are beneficial to SRB removal of Fe, the removal percentage of Fe was 99.9%. Jong [36] reported that the removal percentage of Fe in an up-flow SRB reactor was more than 82%. Kiran [37] reported that the removal percentage of Fe by SRB was 93.9%. Compared with the above research, the removal effect of SRB on Fe in this study was similar. When the concentration of Fe ions was at 100–300 mg/L, the removal percentage of Fe ion by SRB was more than 92%. The tolerance of SRB to Fe ion concentration was 300 mg/L. When the concentration of Fe ions was 400 mg/L, SRB activity was somewhat inhibited. When the concentration of Fe ions was 500 mg/L, most of the SRB activity was inhibited.

In all, when combining the OD_{600} , ORP value, removal efficient of SO_4^{2-} , and removal of total Fe, we found that SRB had a strong ability to remove Fe ion from 0–300 mg/L, with the removal percentage of Fe ion reaching 92.16–93.17%. When the initial Fe ion concentration was 100–300 mg/L, increasing the Fe ion concentration was able to stimulate the activity of SRB, which was conducive to its growth and reproduction. When the initial Fe ion concentration was 300–500 mg/L, increasing the Fe^{2+} concentration inhibited the growth of SRB. The growth state of SRB was the best when the initial Fe^{2+} concentration was 300 mg/L. The maximum values of OD_{600} , pH value, SO_4^{2-} removal percentage, and Fe ion removal percentage were 1.27, 6.91, 88.05%, and 93.17%, respectively, while the minimum values of ORP and Ec were -399 mV and 2.72 mS/cm, respectively.

3.2. Removal Effect of Total Mn by SRB

It can be seen from Figure 2a that at 1–5 d there was a significant increase in the OD_{600} value related to the proliferation of SRB. At 6–8 d, the OD_{600} value decreased significantly, indicating that this portion of the of SRB had died. When the concentrations of Mn^{2+} were 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L, the OD_{600} values were 0.86, 0.98, 1.02, 1.08, and 0.74, respectively, after adding SRB for 4 days. When the concentration of total Mn was 10–40 mg/L, the OD_{600} value gradually increased with the increase of Mn^{2+} concentration and stabilized at 0.83–1.06 on the fifth day, indicating that an appropriate increase of Mn^{2+} concentration in this range was beneficial to stimulate SRB reproduction. When the concentration of Mn^{2+} was 50 mg/L, the OD_{600} value increased slightly, indicating that Mn^{2+} at 50 mg/L inhibited the growth of SRB. It can be seen from Figure 2b–d that when the concentration of Mn^{2+} was 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L, after adding SRB for 8 days, the pH values were 7.35, 7.38, 7.13, 6.99, and 6.41, respectively, the ORP values were -363 mV, -371 mV, -348 mV, -368 mV, and -214 mV, respectively, and the Ec values were 3.05 mS/cm, 3.35 mS/cm, 3.77 mS/cm, 3.97 mS/cm, and 4.32 mS/cm, respectively. In comparison with the initial pH value, the pH value increased, indicating that SRB was able to grow and metabolize in the above concentrations of Mn^{2+} solution to produce alkaline substances. When the concentration of Mn^{2+} was 10–40 mg/L, the pH value increased significantly and the ORP value and Ec value decreased and then stabilized, indicating that the SRB activity was better in this range. When the concentration of Mn^{2+} was 50 mg/L, the pH value increased slightly while the ORP value and Ec value decreased first and then increased, indicating that the

ability of SRB to reduce SO_4^{2-} to S^{2-} decreased; its activity was significantly inhibited, and a portion of the SRB died.

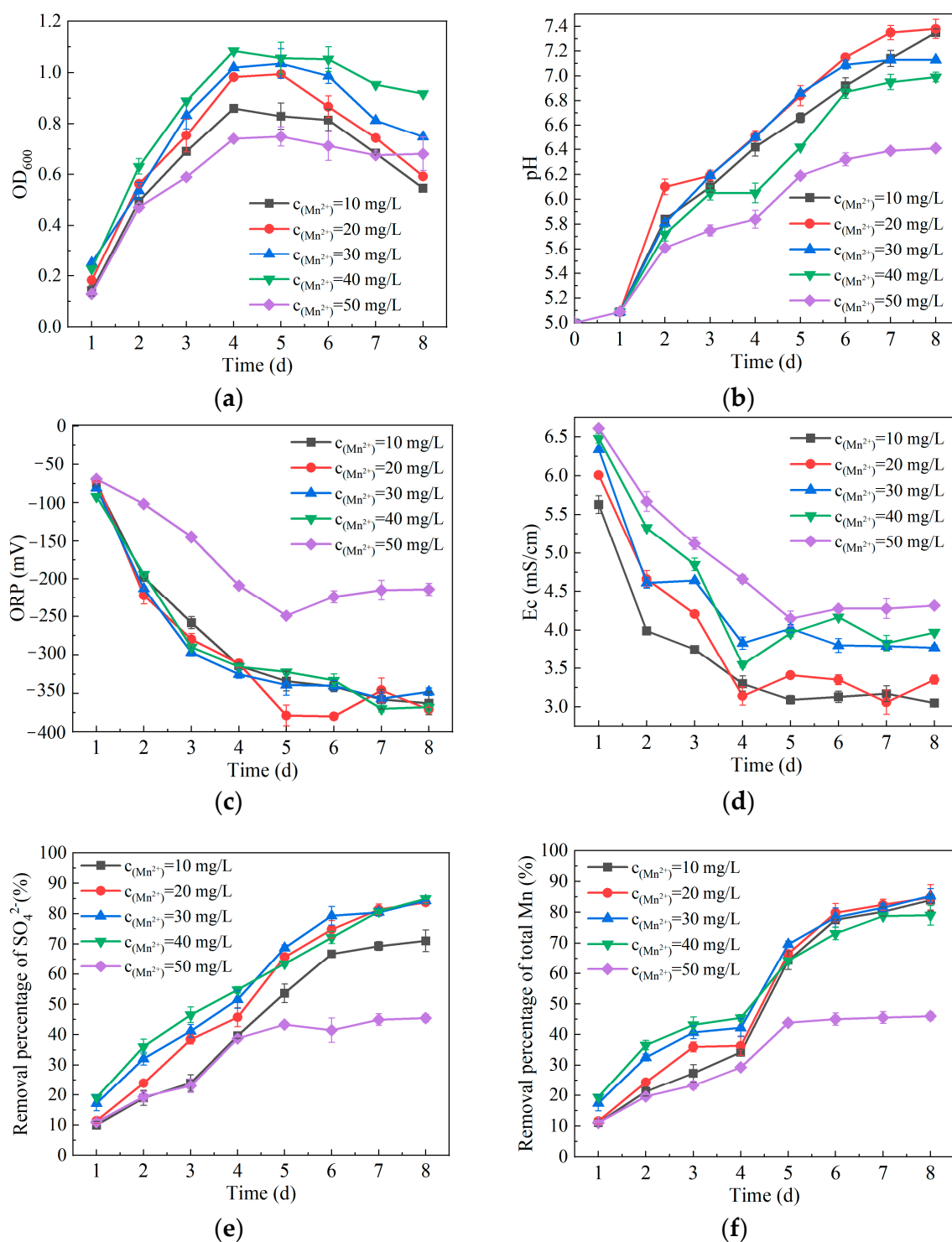


Figure 2. Effect of SRB on total Mn removal (5% SRB; initial Mn^{2+} concentrations were 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L, respectively). (a) OD_{600} value changes; (b) pH value changes; (c) ORP value changes; (d) Ec value changes; (e) SO_4^{2-} removal conditions; (f) removal percentage of total Mn.

It can be seen from Figure 2e,f that in the early stage the SRB metabolism was exuberant, dissimilatory reduction of SO_4^{2-} was higher, and the removal rate of SO_4^{2-} increased

rapidly. In the later stage the SRB gradually entered a period of decline, and its metabolism was slow. At the same time, the reduction of SO_4^{2-} was less, and the removal rate of SO_4^{2-} tended to be stable. From these results, it is apparent that an appropriate increase of the Mn^{2+} concentration in the range of 0–40 mg/L increases the activity of SRB, while 50 mg/L Mn^{2+} inhibits SRB reduction of SO_4^{2-} . With Mn^{2+} concentrations of 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L, the removal percentage of SO_4^{2-} was 71.09%, 83.77%, 84.33%, 84.98%, and 45.43%, respectively, after SRB was added for 8 days. At 1–4 d, the removal percentage of total Mn was relatively low. At 4–8 d, the removal percentage of total Mn increased from 34.21%, 36.32%, 42.25%, 45.47%, and 29.27% to 83.99%, 84.84%, 85.40%, 79.06%, and 46.01%, respectively. The K_{sp} of MnS was larger, and the pH value of the initial precipitation was higher [38]. In the early stage of SRB, the removal percentage of total Mn was low, and the removal percentage of total Mn was high in the later stage. The main reason for this was that the amount of MnS produced by low S^{2-} concentration and low pH value in the early stage was less. Mn ions were mainly combined with CO_3^{2-} produced in the process of SRB reducing SO_4^{2-} to form MnCO_3 [39]. It has been reported that the key to improving the removal of total Mn by SRB is the pH [40]. After 5 days of SRB treatment, the pH value increased significantly. Therefore, the total Mn removal percentage increased significantly in the later stage, and Mn was removed in the form of MnS. Zhang Mingliang [35] have reported that it is difficult to remove manganese by SRB immobilized particles, and found a removal efficiency of 42–99%. The results of our study are consistent with the above results, for the removal efficiency of SRB on Mn^{2+} , although the removal efficiency is not good. Bai et al. [34] reported that the removal efficiency of manganese by SRB and iron-reducing bacteria is lower than that of other heavy metals, finding a removal efficiency of only 20–57%. Compared with the removal effect of Fe, we found that the removal effect of Mn by SRB was significantly lower than that of Fe. Mainly, this is because $K_{sp}(\text{MnS}) > K_{sp}(\text{FeS})$ and because the adsorption of Mn^{2+} by microorganisms is poor [38]. SRB had a better removal efficiency on Mn^{2+} in low concentrations, while higher concentration inhibited the reduction efficiency of Mn^{2+} by SRB.

In all, SRB had strong ability to remove Mn^{2+} in the concentration range of 0–40 mg/L, and the removal percentage was 79–86%. When the concentration of Mn^{2+} was 50 mg/L, SRB growth was inhibited. When the concentration of Mn^{2+} was 40 mg/L, the growth state of SRB was the best. The maximum values of OD_{600} , pH value, SO_4^{2-} removal percentage, and Mn^{2+} removal percentage were 1.08, 6.99, 84.98%, and 79.06%, respectively, while the minimum values of ORP value and Ec value were -370 mV and 3.55 mS/cm, respectively.

3.3. Removal Effect of Total Cu by SRB

It can be seen from Figure 3a that the OD_{600} value increased first and then decreased when SRB removed Cu^{2+} . The decrease in the OD_{600} value in the later stage was related to the decline of SRB. When the concentration of Cu^{2+} was 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L, the OD_{600} values were 0.95, 0.93, 0.83, 0.59, and 0.51, respectively, after SRB was added for 5 days. When the initial Cu^{2+} concentration was 10–20 mg/L, the OD_{600} value was larger. When the concentration of Cu^{2+} was 20–50 mg/L, the OD_{600} value decreased gradually with the increase of Cu^{2+} concentration, and Cu^{2+} in this concentration range inhibited the growth of SRB. It can be seen from Figure 3b–d that when the concentration of Cu^{2+} was 10–20 mg/L, the ORP value decreased first and then stabilized. When the concentration of Cu^{2+} was 30–50 mg/L, the ORP value decreased first and then increased, and the increase of ORP value was related to the death of a portion of the SRB. In addition, high concentrations of Cu^{2+} were able to inhibit and poison the SRB. It can be seen from Figure 3e,f that when SRB removed total Cu, the removal rate of SO_4^{2-} and total Cu generally increased first and then tended to become stable. At 1–4 d, SRB metabolism was vigorous and the removal of SO_4^{2-} and total Cu is obvious. At 4–8 d, SRB gradually entered a declining period, and the removal percentage of SO_4^{2-} and total Cu tended to be stable. When the concentration of total Cu was 10–20 mg/L, the removal percentages of SO_4^{2-} and total Cu by SRB were stable at 78.78–80.76% and 79.79–81.80%, respectively.

When the concentration of total Cu was 30 mg/L, the removal percentages of SO_4^{2-} and total Cu by SRB were 68.14% and 69.01%, respectively. When the concentration of total Cu was 40–50 mg/L, the removal percentages of SO_4^{2-} and total Cu by SRB were 45.74–49.61% and 46.32–50.25%, respectively. According to the removal of total Cu and SO_4^{2-} , when the concentration of Cu ions was low, the metabolic activity of SRB was relatively high. However, when the concentration of Cu ions increased, the metabolic activity of SRB decreased significantly. This indicates that Cu ions inhibit the activity and growth metabolism of SRB.

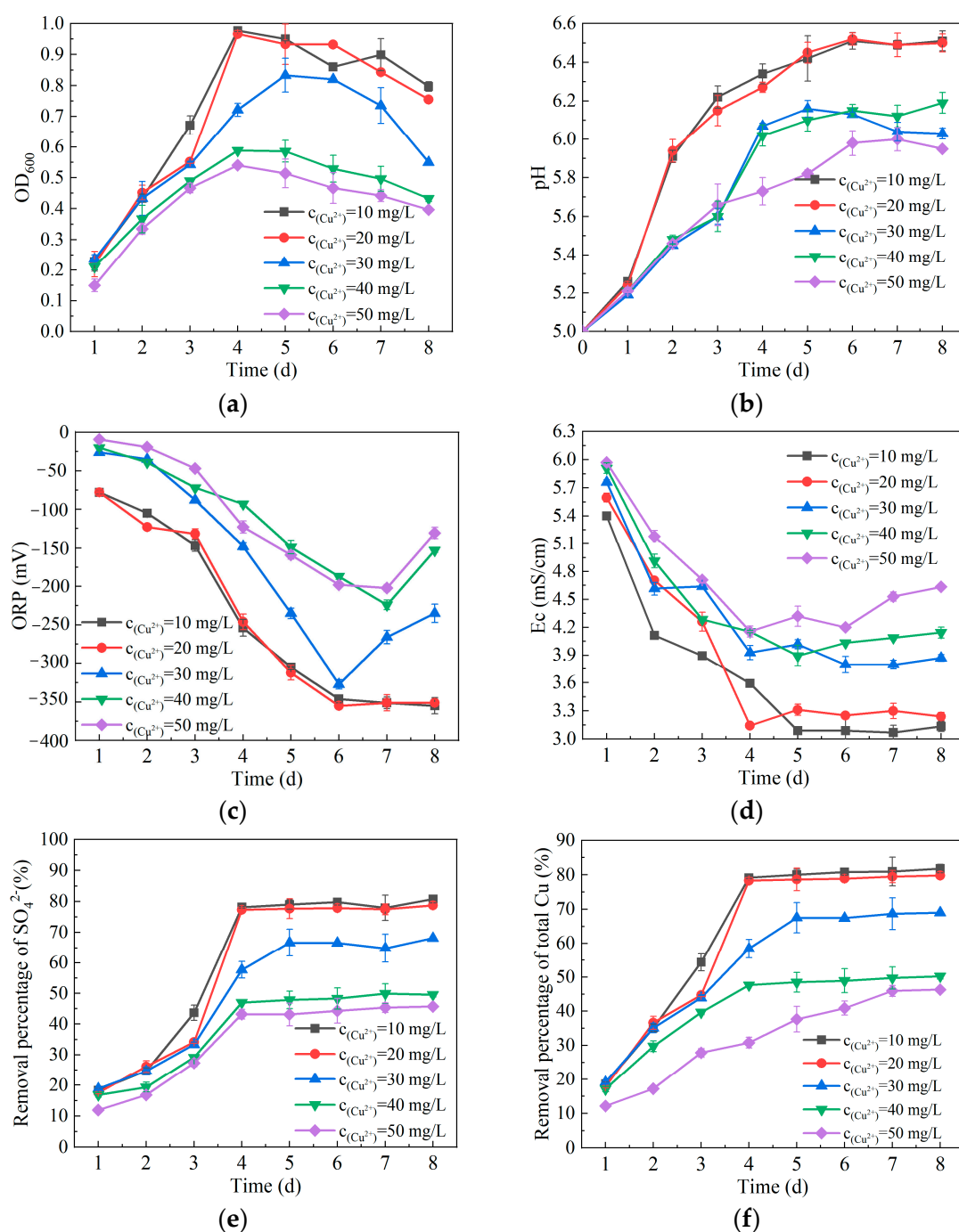


Figure 3. Effect of SRB on total Cu removal (5% SRB; initial Cu^{2+} concentrations were 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, and 50 mg/L, respectively). (a) OD_{600} value changes; (b) pH value changes; (c) ORP value changes; (d) Ec value changes; (e) SO_4^{2-} removal conditions; (f) removal percentage of total Cu.

Therefore, it can be seen that SRB had a good removal effect on 0–20 mg/L total Cu, with the removal percentage of total Cu being 79.79–81.80%. When Cu^{2+} exceeded 20 mg/L, the activity of SRB was inhibited. It has been reported that Cu^{2+} can poison SRB and inhibit the ability of SRB to reduce sulfate [41]. Hwang et al. [16] found that 9.9 ± 4.6 mg/L Cu^{2+} inhibited the activity of SRB, though it did not cause SRB death. This is mainly because when SRB is exposed to concentrations of Cu^{2+} which are not conducive to its growth, the formation of EPS is increased, and the resulting negatively charged functional groups of EPS can bind to Cu^{2+} [42]. When the concentration of Cu^{2+} was 20 mg/L, the maximum values of OD_{600} , pH value, SO_4^{2-} removal percentage, and Cu^{2+} removal percentage were 0.97, 6.52, 78.78%, and 79.79%, respectively, while the minimum values of ORP value and Ec value were -355 mV and 3.14 mS/cm, respectively.

3.4. Removal Effect of Cr Ion by SRB

It can be seen from Figure 4a that the OD_{600} value increased first and then decreased when SRB removed Cr^{6+} . The decrease in OD_{600} value was related to the decline of SRB. When the concentration of Cr^{6+} was 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L, the OD_{600} values were 1.02, 1.08, 1.03, 0.97, and 0.99, respectively, after SRB was added for 5 days. When the concentration of Cr^{6+} was 100–200 mg/L, the OD_{600} value was larger. It can be seen from Figure 4b–d that after 8 days of SRB addition, the pH value, ORP value, and Ec value were 7.07–7.40, -317 – -333 mV, and 2.84 – 2.95 mS/cm, respectively. It can be seen from Figure 4e,f that when SRB removed total Cr, the removal rate of SO_4^{2-} and total Cr generally increased first and then tended to become stable. The removal efficiency of SO_4^{2-} and total Cr in different concentrations of total Cr by SRB was similar. On the 8th day, the removal rates of SO_4^{2-} and total Cr in different concentrations by SRB were stable at 71.06–78.37% and 83.51–87.21%, respectively. Wang et al. [43] showed that the maximum removal percentage of 10 mg/L Cr^{6+} by SRB was 93.75% under optimal growth conditions. The removal of Cr^{6+} by SRB that we found is significantly higher than that found by Wang [43]. At 1–5 d, SRB metabolism was strong and the removal of SO_4^{2-} was obvious. At 3–8 d, SRB metabolism accumulated S^{2-} , and Cr^{6+} was mainly removed by converting to Cr^{3+} through direct reaction with S^{2-} and redox reactions. [44]. It has been reported that SRB can remove Cr in the form of hydroxide as well [45]. Among the different ions, the toxicity of Cr^{3+} is much lower than that of Cr^{6+} . SRB removes Cr^{6+} and Cr^{3+} in the form of sulfides and hydroxides, thereby effectively reducing Cr^{6+} pollution.

Therefore, combining the removal efficiency of OD_{600} , ORP value, and removal efficiency of SO_4^{2-} and Cr^{6+} , we found that SRB has a good removal effect on Cr^{6+} . When Cr^{6+} concentration was below 500 mg/L, it did not inhibit the activity of SRB. The tolerance of Cr^{6+} concentration for the SRB isolated by Xilin Li [46] was 500 mg/L. For the strain of SRB isolated by Liu Changhong [47], growth was best when the concentration of Cr^{6+} was 300 mg/L, and the removal rate of Cr^{6+} by SRB was 75%. In comparison with the above research, the removal percentage of 300 mg/L Cr^{6+} by the mixed SRB in this study was increased by 9%. Cabrera et al. [23] showed that the removal percentage of 15 ppm Cr^{3+} by *Desulfovibrio vulgaris* could reach 24.7%, and the removal percentage of 15 ppm Cr^{3+} by *Desulfovibrio* sp. could reach 25.5%. Haodong Wang et al. [43] found that the maximum removal percentage of 10 mg/L Cr^{6+} could reach 93.75%. In comparison with the above studies, in this study the removal percentage of Cr by mixed SRB was improved significantly. Moreover, the Cr ion we used to configure the artificial simulated AMD was Cr^{6+} , which has toxicity far in excess of Cr^{3+} . These results indicate that the SRB in this study is able to efficiently remedy Cr pollution.

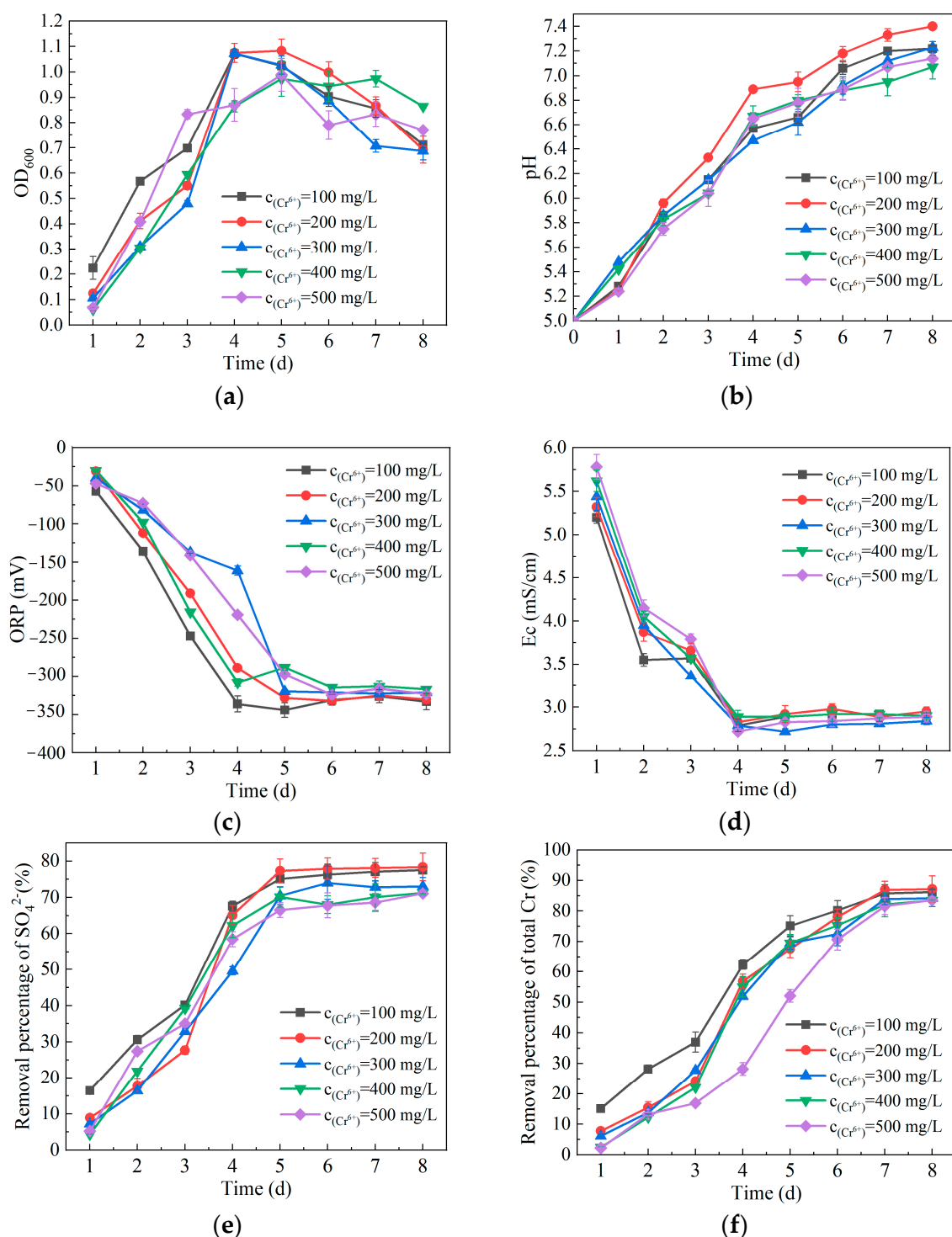


Figure 4. Effect of SRB on total Cr removal (5% SRB; initial Cr^{6+} concentrations were 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L, and 500 mg/L, respectively). (a) OD_{600} value changes; (b) pH value changes; (c) ORP value changes; (d) Ec value changes; (e) SO_4^{2-} removal conditions; (f) removal percentage of total Cr.

3.5. Analysis of Mechanism for Removing Fe Ions by SRB

In order to explore the mechanism by which SRB removes heavy metals, Fe ion removal was taken as an example for detection and analysis. The precipitate remaining after removing Fe ions by SRB was collected and vacuum dried for SEM-EDS, XRD, and XPS tests. The results are shown in Figures 5 and 6.

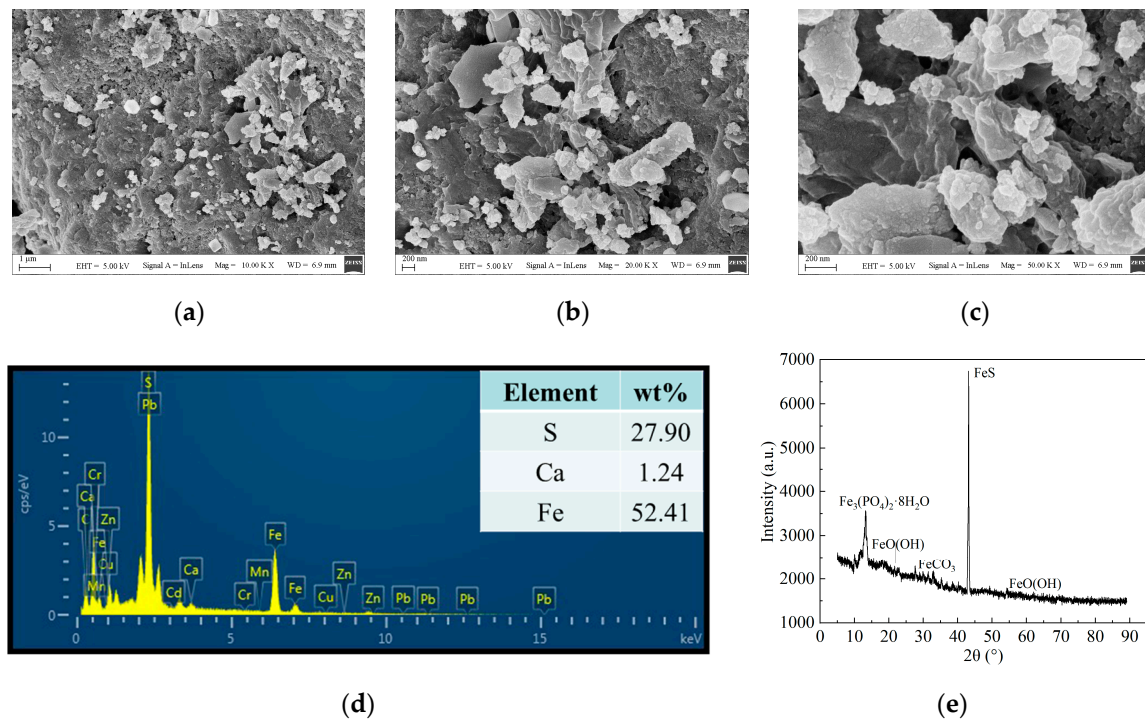


Figure 5. SEM-EDS and XRD patterns of the precipitate formed by SRB removal of Fe ions: (a) SEM magnified 10,000 times; (b) SEM magnified 20,000 times; (c) SEM magnified 50,000 times; (d) EDS; (e) XRD.

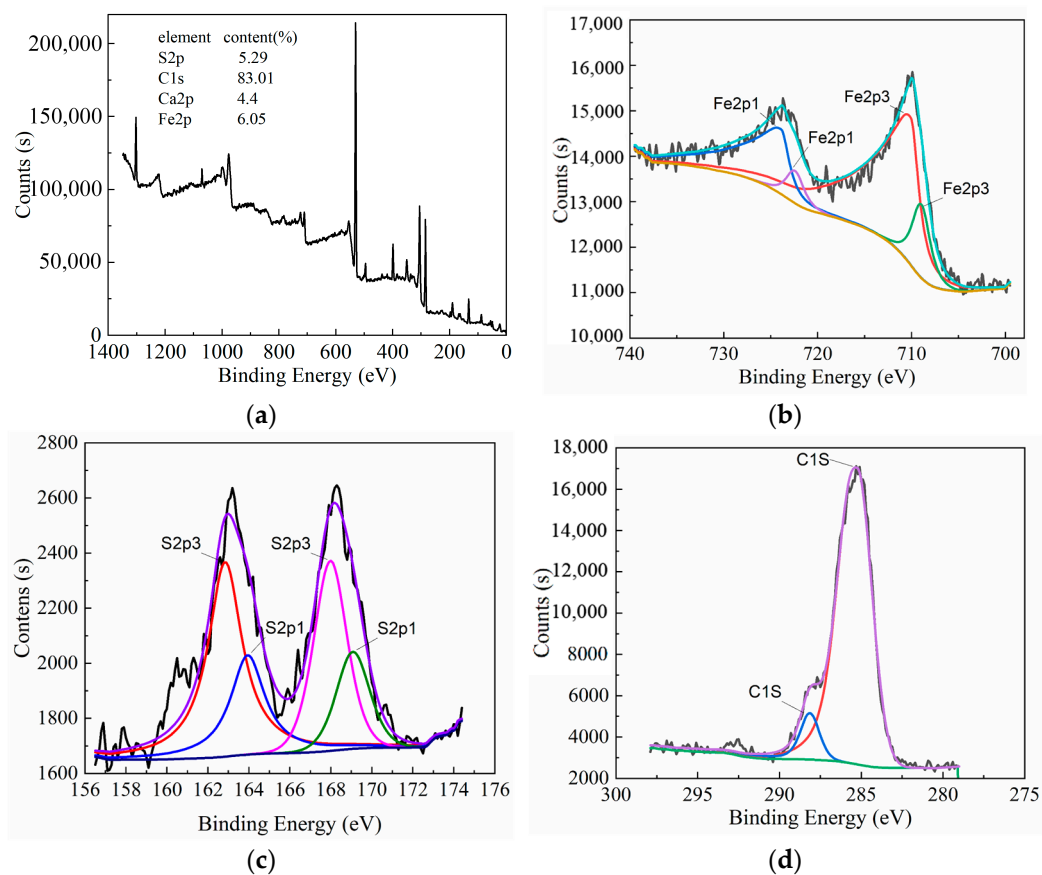


Figure 6. XPS of Fe ion removed by SRB: (a) XPS survey; (b) XPS of Fe; (c) XPS of S; (d) XPS of C.

It can be seen from the SEM images in Figure 5 that a black granular precipitate was formed when SRB removed Fe ions, with finer particles attached to the surface. The internal particles are closely agglomerated and the surface has a porous structure. It can be seen from the EDS diagram in Figure 5 that the precipitates formed by SRB removal of Fe ions mainly include Fe, S, and Ca, with element contents of 52.41%, 37.90%, and 1.24%, respectively, indicating that the precipitate is mainly FeS and contains a small amount of other forms of Fe salt and Ca salt precipitations. It can be seen from the XRD pattern in Figure 5 that the precipitates generated by SRB removal of Fe^{2+} mainly include FeS, $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, FeCO_3 , and $\text{FeO}(\text{OH})$.

The XPS full spectrum scan diagram of the precipitate formed by SRB removal of Fe^{2+} is shown in Figure 6a, with the absorption peaks corresponding to Fe, S, C, and O at the binding energies near 711.23 eV, 163.72 eV, 285.3 eV, and 531.12 eV, respectively. The respective contents of S2p, C1s, and Fe2p are 5.29%, 83.01% and 6.05%. It can be seen from the fine scanning spectrum of Fe in Figure 6b that Fe mainly has four peaks at FeCO_3 at 708.94 eV, Fe_3O_4 at 723.38 eV, FeS at 710.16 eV, and $\text{Fe}(\text{OH})_2$, $\text{Fe}(\text{OH})_3$, as well as a few Fe_2O_3 at 710.58 eV. Among these, FeCO_3 , Fe_3O_4 accounts for 19.8% of the total iron, $\text{Fe}(\text{OH})_2$, $\text{Fe}(\text{OH})_3$ and Fe_2O_3 account for 39.5% of the total iron, and FeS accounts for 40.7% of the total iron. The above results show that in addition to the production of FeS after treatment by SRB, a small amount of iron is oxidized to Fe_2O_3 , Fe_3O_4 , $\text{Fe}(\text{OH})_3$, etc. It can be seen from Figure 6c that FeS, Fe_2S_3 , and FeS_2 at 159.71 eV, 162.18 eV, and 163.28 eV of the S2p spectrum, respectively, account for 50.6% of the total sulfur, while SO_3^{2-} at 167.08 eV and SO_4^{2-} at 168.18 eV account for about 49.4% of the total sulfur. It can be seen from Figure 6d that carbon appears near 284.8 eV in the C1s spectrum, accounting for 89.54% of the total carbon, and CO_3^{2-} appears near 287.71 eV, accounting for 10.46% of the total carbon, indicating that a small amount of carbon is oxidized after the reaction, forming a small amount of FeCO_3 . In summary, when SRB removes Fe^{2+} , the resulting precipitate is mainly FeS, as well as smaller amounts of $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, FeCO_3 , $\text{Fe}(\text{OH})_2$, $\text{Fe}(\text{OH})_3$, Fe_2S , etc.

4. Conclusions

Aiming at the problem of environmental pollution caused by heavy metals such as Fe, Mn, Cu, and Cr, the enrichment method was used to obtain primary mixed SRB from soil samples in a heavy mining area. Based on batch experiments, SRB was inoculated into AMD containing different concentrations of Fe^{2+} , Mn^{2+} , Cu^{2+} , and Cr^{6+} . Combined with the changes of OD_{600} , pH, ORP, and Ec values and the removal effects of SO_4^{2-} , Fe^{2+} , Mn^{2+} , Cu^{2+} , and Cr^{6+} , the effective reduction potential of SRB on sulfate and heavy metals in acid mine drainage was analyzed. SRB had a strong ability to remove Fe ions at 0–300 mg/L, and the removal percentage of Fe ions reached 92.16–93.17%. When the initial Fe ion concentration was 300 mg/L, the growth state of SRB was the best. The maximum values of OD_{600} , pH value, SO_4^{2-} removal percentage, and Fe^{2+} removal percentage were 1.27, 6.91, 88.05%, and 93.17%, respectively, while the minimum values of ORP and Ec were −399 mV and 2.72 mS/cm, respectively. SRB had a strong ability to remove Mn^{2+} at 0–40 mg/L, and the removal percentage was 79–86%. When the concentration of Mn^{2+} was 40 mg/L, the growth state of SRB was the best, and the maximum removal percentages of SO_4^{2-} and Mn^{2+} were 84.98% and 79.06%, respectively. SRB had a good removal effect on Cu^{2+} at 0–20 mg/L, and the removal percentage of Cu^{2+} was 79.79–81.80%. When Cu^{2+} exceeded 20 mg/L, the activity of SRB was inhibited. When the concentration of Cu^{2+} was 20 mg/L, the maximum removal percentages of SO_4^{2-} and Cu^{2+} were 78.78% and 79.79%, respectively. SRB had a good removal effect on Cr ions, and when the Cr ion concentration was below 500 mg/L, the activity of SRB was not inhibited. The removal percentages of SO_4^{2-} and Cr ions at 100–500 mg/L Cr ion by SRB were stable at 71.06–78.37% and 83.51–87.21%, respectively. SRB can metabolize SO_4^{2-} to form a heavy metal sulfide-based precipitation. The immobilization ability of SRB for different heavy metal ions was as follows: $\text{Cr}^{6+} > \text{Fe}^{2+} > \text{Mn}^{2+} > \text{Cu}^{2+}$. We explored the mechanism by which SRB removes

heavy metal ions using a combination of SEM-EDS, XRD, and XPS tests. It was found that the precipitate formed by SRB removal of Fe^{2+} is a black granular precipitate consisting mainly of FeS , and contains a small amount of $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, FeCO_3 , $\text{Fe}(\text{OH})_2$, $\text{Fe}(\text{OH})_3$, Fe_2S , etc.

Author Contributions: Conceptualization, Y.D.; methodology, Y.D.; validation, Y.D. and Z.G.; formal analysis, Y.D. and Z.G.; investigation, Y.D., Z.G., J.D., D.W., Z.Y., Y.W. and Z.X.; data curation, Y.D. and Z.G.; writing—original draft preparation, Y.D., Z.G., J.D., D.W., Z.Y., Y.W. and Z.X.; writing—review and editing, Y.D.; visualization, Z.Y.; supervision, Z.G.; project administration, Y.D., J.D. and D.W.; funding acquisition, Y.D., J.D. and D.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Discipline Innovation Team of Liaoning Technical University (LNTU20TD-01, LNTU20TD-21), Liaoning Province Natural Science Foundation Doctoral Project (23-1015), Key Projects of Liaoning Provincial Department of Education (LJKZ0324), the National Natural Science Foundation of China (41672247), the Innovation and Entrepreneurship Training Program for College Students (S202210147014, X202210147078, X202210147079). Liaoning Provincial Department of Education Basic Research Project (Youth Project) (LJKQZ20222319).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data could be provided if necessary.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Ojuederie, O.B.; Babalola, O.O. Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review. *Int. J. Environ. Res. Public Health* **2017**, *14*, 1504. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zamora-Ledezma, C.; Negrete-Bolagay, D.; Figueroa, F.; Zamora-Ledezma, E.; Ni, M.; Alexis, F.; Guerrero, V.H. Heavy metal water pollution: A fresh look about hazards, novel and conventional remediation methods. *Environ. Technol. Innov.* **2021**, *22*, 101504. [\[CrossRef\]](#)
- Chen, L.; Zhou, M.; Wang, J.; Zhang, Z.; Duan, C.; Wang, X.; Zhao, S.; Bai, X.; Li, Z.; Li, Z.; et al. A global meta-analysis of heavy metal(loid)s pollution in soils near copper mines: Evaluation of pollution level and probabilistic health risks. *Sci. Total Environ.* **2022**, *835*, 155441. [\[CrossRef\]](#)
- Demcak, S.; Balintova, M.; Holub, M. Monitoring of the abandoned mine Smolnik (Slovakia) influence on the aquatic environment. *IOP Conf. Ser. Earth Environ. Sci.* **2020**, *444*, 012010. [\[CrossRef\]](#)
- Alegbe, M.J.; Ayanda, O.S.; Ndungu, P.; Nechaev, A.; Fatoba, O.O.; Petrik, L.F. Physicochemical characteristics of acid mine drainage, simultaneous remediation and use as feedstock for value added products. *J. Environ. Chem. Eng.* **2019**, *7*, 103097. [\[CrossRef\]](#)
- Lee, G.; Han, J.; Jang, M.; Kim, M. Long-term treatment of acid mine drainage by alkali diffusion ceramic reactor: Simultaneous metal removal mechanisms. *Chemosphere* **2022**, *298*, 134186. [\[CrossRef\]](#)
- Cheong, Y.; Hur, W.; Yim, G.; Ji, S.; Seo, E. Evaluation of Neutralization-Precipitation Process of Acid Mine Drainage using a Process Simulator. *J. Korean Soc. Miner. Energy Resour. Eng.* **2016**, *53*, 1–9. [\[CrossRef\]](#)
- Clyde, E.J.; Champagne, P.; Jamieson, H.E.; Gorman, C.; Sourial, J. The use of a passive treatment system for the mitigation of acid mine drainage at the Williams Brothers Mine (California): Pilot-scale study. *J. Clean. Prod.* **2016**, *130*, 116–125. [\[CrossRef\]](#)
- Edelstein, M.; Ben-Hur, M. Heavy metals and metalloids: Sources, risks and strategies to reduce their accumulation in horticultural crops. *Sci. Hortic.* **2018**, *234*, 431–444. [\[CrossRef\]](#)
- Maity, J.P.; Chen, G.-S.; Huang, Y.-H.; Sun, A.-C.; Chen, C.-Y. Ecofriendly Heavy Metal Stabilization: Microbial Induced Mineral Precipitation (MIMP) and Biomineralization for Heavy Metals within the Contaminated Soil by Indigenous Bacteria. *Geomicrobiol. J.* **2019**, *36*, 612–623. [\[CrossRef\]](#)
- Ayangbenro, A.S.; Babalola, O.O. A New Strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents. *Int. J. Environ. Res. Public Health* **2017**, *14*, 94. [\[CrossRef\]](#) [\[PubMed\]](#)
- Liu, P.; Zhang, Y.; Tang, Q.; Shi, S. Bioremediation of metal-contaminated soils by microbially-induced carbonate precipitation and its effects on ecotoxicity and long-term stability. *Biochem. Eng. J.* **2021**, *166*, 107856. [\[CrossRef\]](#)
- Han, L.; Li, J.; Xue, Q.; Chen, Z.; Zhou, Y.; Poon, C.S. Bacterial-induced mineralization (BIM) for soil solidification and heavy metal stabilization: A critical review. *Sci. Total Environ.* **2020**, *746*, 140967. [\[CrossRef\]](#) [\[PubMed\]](#)
- Su, Z.; Li, X.; Xi, Y.; Xie, T.; Liu, Y.; Liu, B.; Liu, H.; Xu, W.; Zhang, C. Microbe-mediated transformation of metal sulfides: Mechanisms and environmental significance. *Sci. Total Environ.* **2022**, *825*, 153767. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kaksonen, A.H.; Puhakka, J.A. Sulfate Reduction Based Bioprocesses for the Treatment of Acid Mine Drainage and the Recovery of Metals. *Eng. Life Sci.* **2007**, *7*, 541–564. [\[CrossRef\]](#)

16. Hwang, S.K.; Jho, E.H. Heavy metal and sulfate removal from sulfate-rich synthetic mine drainages using sulfate reducing bacteria. *Sci. Total Environ.* **2018**, *635*, 1308–1316. [[CrossRef](#)] [[PubMed](#)]
17. Kieu, H.T.; Muller, E.; Horn, H. Heavy metal removal in anaerobic semi-continuous stirred tank reactors by a consortium of sulfate-reducing bacteria. *Water Res.* **2011**, *45*, 3863–3870. [[CrossRef](#)] [[PubMed](#)]
18. Hou, Q.; Fang, D.; Wang, D.; Liang, J.; Zhou, L. Integration of cascaded aeration and neutralization for the treatment of acid mine drainage: Insights into the formation of jarosite. *Hydrometallurgy* **2021**, *206*, 105755. [[CrossRef](#)]
19. Zhang, X. He Mechanism and Environmental Significance of Sulfate-Reducing Bacteria Reducing and Decomposing Sulfate Minerals. Master's Thesis, Hefei University of Technology, Hefei, China, 2015.
20. Mullaugh, K.M.; Luther, G.W. Growth kinetics and long-term stability of CdS nanoparticles in aqueous solution under ambient conditions. *J. Nanoparticle Res.* **2010**, *13*, 393–404. [[CrossRef](#)]
21. Blanca, M.; Gonzalez-Silva; Briones-Gallardo, R.; Razo-Flores, E.; Celis, L.B. Inhibition of sulfate reduction by iron, cadmium and sulfide in granular sludge. *J. Hazard. Mater.* **2009**, *172*, 400–407. [[CrossRef](#)]
22. Hao, T.W.; Xiang, P.Y.; Mackey, H.R.; Chi, K.; Lu, H.; Chui, H.K.; van Loosdrecht, M.C.; Chen, G.H. A review of biological sulfate conversions in wastewater treatment. *Water Res.* **2014**, *65*, 1–21. [[CrossRef](#)] [[PubMed](#)]
23. Cabrera, G.; Perez, R.; Gomez, J.M.; Abalos, A.; Cantero, D. Toxic effects of dissolved heavy metals on *Desulfovibrio vulgaris* and *Desulfovibrio* sp. strains. *J. Hazard. Mater.* **2006**, *135*, 40–46. [[CrossRef](#)] [[PubMed](#)]
24. Dong, Y.; Di, J.; Yang, Z.; Zhang, Y.; Wang, X.; Guo, X.; Li, Z.; Jiang, G. Study on the Effectiveness of Sulfate-Reducing Bacteria Combined with Coal Gangue in Repairing Acid Mine Drainage Containing Fe and Mn. *Energies* **2020**, *13*, 995. [[CrossRef](#)]
25. Baumgartner, L.K.; Reid, R.P.; Dupraz, C.; Decho, A.W.; Buckley, D.H.; Spear, J.R.; Przekop, K.M.; Visscher, P.T. Sulfate reducing bacteria in microbial mats: Changing paradigms, new discoveries. *Sediment. Geol.* **2006**, *185*, 131–145. [[CrossRef](#)]
26. Barbosa, L.P.; Costa, P.F.; Bertolino, S.M.; Silva, J.C.; Guerra-Sa, R.; Leao, V.A.; Teixeira, M.C. Nickel, manganese and copper removal by a mixed consortium of sulfate reducing bacteria at a high COD/sulfate ratio. *World J. Microbiol. Biotechnol.* **2014**, *30*, 2171–2180. [[CrossRef](#)] [[PubMed](#)]
27. Liu, Z.; Li, L.; Li, Z.; Tian, X. Removal of sulfate and heavy metals by sulfate-reducing bacteria in an expanded granular sludge bed reactor. *Environ. Technol.* **2018**, *39*, 1814–1822. [[CrossRef](#)]
28. Zhang, M.; Wang, H.; Han, X. Preparation of metal-resistant immobilized sulfate reducing bacteria beads for acid mine drainage treatment. *Chemosphere* **2016**, *154*, 215–223. [[CrossRef](#)]
29. Dvorak Darryl, H.; Hedin, R.S.; Edenborn, H.M.; Pamela, E.M. Treatment of Metal-Contaminated Water Using Bacterial Sulfate Reduction: Results from Pilot-Scale Reactors. *Biotechnol. Bioeng.* **1991**, *40*, 15236. [[CrossRef](#)]
30. Lu, X.; Zhen, G.; Ni, J.; Hojo, T.; Kubota, K.; Li, Y.Y. Effect of influent COD/SO₄^{2−} ratios on biodegradation behaviors of starch wastewater in an upflow anaerobic sludge blanket (UASB) reactor. *Bioresour. Technol.* **2016**, *214*, 175–183. [[CrossRef](#)] [[PubMed](#)]
31. Sabumon, P.C. Development of enhanced sulphidogenesis process for the treatment of wastewater having low COD/SO₄^{2−} ratio. *J. Hazard. Mater.* **2008**, *159*, 616–625. [[CrossRef](#)]
32. Nancucheo, I.; Rowe, O.F.; Hedrich, S.; Johnson, D.B. Solid and liquid media for isolating and cultivating acidophilic and acid-tolerant sulfate-reducing bacteria. *FEMS Microbiol. Lett.* **2016**, *363*, 83. [[CrossRef](#)]
33. Kikot, P.; Viera, M.; Mignone, C.; Donati, E. Study of the effect of pH and dissolved heavy metals on the growth of sulfate-reducing bacteria by a fractional factorial design. *Hydrometallurgy* **2010**, *104*, 494–500. [[CrossRef](#)]
34. Bai, H.; Kang, Y.; Quan, H.; Han, Y.; Sun, J.; Feng, Y. Treatment of acid mine drainage by sulfate reducing bacteria with iron in bench scale runs. *Bioresour. Technol.* **2013**, *128*, 818–822. [[CrossRef](#)]
35. Zhang, M.; Wang, H. Preparation of immobilized sulfate reducing bacteria (SRB) granules for effective bioremediation of acid mine drainage and bacterial community analysis. *Miner. Eng.* **2016**, *92*, 63–71. [[CrossRef](#)]
36. Jong, T.; Parry, D.L. Removal of sulfate and heavy metals by sulfate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. *Water Res.* **2003**, *37*, 3379–3389. [[CrossRef](#)] [[PubMed](#)]
37. Kiran, M.G.; Pakshirajan, K.; Das, G. Heavy metal removal from multicomponent system by sulfate reducing bacteria: Mechanism and cell surface characterization. *J. Hazard. Mater.* **2017**, *324*, 62–70. [[CrossRef](#)] [[PubMed](#)]
38. Tabak, H.H.; Scharp, R.; Burckle, J.; Kawahara, F.K.; Govind, R. Kawahara & Rakesh Govind. Advances in biotreatment of acid mine drainage and biorecovery of metals: 1. Metal precipitation for recovery and recycle. *Biodegradation* **2003**, *14*, 423–436. [[CrossRef](#)] [[PubMed](#)]
39. Hedrich, S.; Johnson, D.B. Remediation and selective recovery of metals from acidic mine waters using novel modular bioreactors. *Environ. Sci. Technol.* **2014**, *48*, 12206–12212. [[CrossRef](#)]
40. Simate, G.S.; Ndlovu, S. Acid mine drainage: Challenges and opportunities. *J. Environ. Chem. Eng.* **2014**, *2*, 1785–1803. [[CrossRef](#)]
41. Martins, M.; Faleiro, M.L.; Barros, R.J.; Verissimo, A.R.; Barreiros, M.A.; Costa, M.C. Characterization and activity studies of highly heavy metal resistant sulphate-reducing bacteria to be used in acid mine drainage decontamination. *J. Hazard. Mater.* **2009**, *166*, 706–713. [[CrossRef](#)]
42. Wang, F.; Huang, W.; Guo, C.; Liu, C.Z. Functionalized magnetic mesoporous silica nanoparticles: Fabrication, laccase adsorption performance and direct laccase capture from *Trametes versicolor* fermentation broth. *Bioresour. Technol.* **2012**, *126*, 117–122. [[CrossRef](#)]
43. Wang, H.; Li, Q.; Yang, Q. Kinetics and thermodynamics of Cr(VI) removal from wastewater by SRB. *Environ. Sci. Technol.* **2018**, *41*, 37–42. [[CrossRef](#)]

44. Liu, C.; Chen, T.; Zhou, Y.; Jin, J. Treatment of simulated Cr (VI)-containing wastewater by rape stalk fixed bed based on sulfate reducing bacteria. *Acta Mineral. Sin.* **2011**, *31*, 742–749. [[CrossRef](#)]
45. Guo, J.; Kang, Y.; Feng, Y. Bioassessment of heavy metal toxicity and enhancement of heavy metal removal by sulfate-reducing bacteria in the presence of zero valent iron. *J. Environ. Manag.* **2017**, *203*, 278–285. [[CrossRef](#)] [[PubMed](#)]
46. Li, X.; Fan, M.; Liu, L.; Chang, J.; Zhang, J. Treatment of high-concentration chromium-containing wastewater by sulfate-reducing bacteria acclimated with ethanol. *Water Sci. Technol.* **2019**, *80*, 2362–2372. [[CrossRef](#)] [[PubMed](#)]
47. Liu, C.; Jin, L.; Xue, Y. Preliminary study on the reduction of Cr(6+) by Bacillus Cr23. *Miner. Rock Geochem. Bull.* **2011**, *30*, 265–269. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.