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Junzhen Di (✉ dijunzhen@126.com)

Liaoning University of Engineering and Technology

Yangyang Jiang

Liaoning University of Engineering and Technology

Mingjia Wang

Liaoning University of Engineering and Technology

Yanrong Dong

Liaoning University of Engineering and Technology

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Experimental Study on Single Factor, Orthogonal and Characteristics of SRB Particles Fixed by Biologically Activated Lignite

Junzhen Di^{1,*}, Yangyang Jiang¹, Mingjia Wang¹ & Yanrong Dong¹

Abstract: Aiming at the problems of insufficient supply of carbon source and toxic effect of heavy metal ions in the treatment of acid mine wastewater (AMD) by sulfate reducing bacteria (SRB), immobilized particles were prepared with Rhodopseudomonas spheroides, SRB and lignite as the main raw materials, and the optimal ratio of immobilized particles was determined based on single factor test and orthogonal test. The adsorption experiment of immobilized particles was carried out under the optimal ratio, the reaction kinetic process and adsorption capacity of immobilized particles for different ions were analyzed, and the action mechanism was studied. The results show that lignite not only has good adsorption performance, but also can be used as the carbon source of SRB after degradation by Rhodopseudomonas spheroides, so as to solve the problems of low removal efficiency of AMD by SRB and insufficient supply of carbon source. When the dosage of lignite, Rhodopseudomonas spheroides and SRB is 3%, 10% and 10% respectively, and the particle size of lignite is 200 mesh, the overall treatment effect is the best. The removal rates of SO_4^{2-} , Zn^{2+} and Cu^{2+} are 83.21%, 99.59% and 99.93% respectively, the pH is increased to 7.43, the release of COD is 523 mg/L, and the ORP number is -134 mV. The reduction process of SO_4^{2-} by immobilized particles conforms to the pseudo first-order kinetics, the isothermal adsorption of Zn^{2+} more conforms to the Freundlich isothermal adsorption equation, and the adsorption kinetics of Zn^{2+} more conforms to the pseudo second-order kinetic model.

Keyword: Acid mine drainage; Sulfate reducing bacteria; Lignite; Immobilization technology; Rhodopseudomonas spheroides

1 Introduction

Acid mine wastewater is produced with mining activities. Because of its acidity and high concentration of heavy metal ions, it will have a serious impact on the environment^{1,2}. At present, the most commonly used methods to deal with AMD mainly include physical method, chemical method and biological method. Physical and chemical methods have problems such as high cost or unsatisfactory treatment effect, so microbial method has been widely studied. SRB is the most commonly used, but in the actual treatment process, due to the inhibition of AMD low pH, the toxicity of heavy metal ions and the lack of carbon source in the later stage, the treatment effect of SRB is not ideal¹. Lignite has large specific surface area and rich oxygen-containing functional groups. Heavy metal ions are easy to combine with oxygen-containing functional groups on the surface of lignite; As a coal dissolving bacterium, Rhodopseudomonas spheroides can degrade lignite for SRB for growth and metabolism. Therefore, using the biodegradable lignite as the carbon source of SRB can improve the pH of water and reduce the toxicity of acid and heavy metal ions to SRB²⁻⁴. In this experiment, biologically activated lignite was used as SRB carbon source to explore the optimal ratio of immobilized particle composition of mineral carbon source in the treatment of AMD by immobilization technology, and the reaction kinetic process and adsorption capacity of immobilized particles to SO_4^{2-} and Zn^{2+} were analyzed, so as to provide a strong basis for selecting minerals as carbon source for SRB treatment of acid mine wastewater.

2 Experimental Methods

2.1 Single Factor Experiment and Orthogonal Experiment

According to the preparation method of immobilized particles⁵, 6 groups of Rhodopseudomonas spheroides immobilized particles with different mass fractions (0%, 10%, 20%, 30%, 40%, 50%), 4 groups of lignite

immobilized particles with different mass fractions (1%, 3%, 5%, 7%), 3 groups of immobilized particles with different SRB bacterial dosage (10%, 30%, 50%) and 3 groups of immobilized particles with different lignite particle sizes (80 mesh, 100 mesh 200 mesh) were prepared successively, and activated in an improved Starkey medium solution without organic components for 12 hours. Different immobilized particles were added to 300 ml of wastewater according to the solid-liquid ratio of 1:10, and placed in a constant temperature shaking table at 30°C and 150 r/min for continuous oscillation for single factor test⁶. Among them, the initial concentrations of SO_4^{2-} , Cu^{2+} , Zn^{2+} , Ca^{2+} and Mg^{2+} in the wastewater are 816 mg/L, 10 mg/L, 20 mg/L, 100 mg/L and 50 mg/L respectively, and the initial pH value is 4.0. Based on the single factor test results, $L_9(3^4)$ orthogonal test is carried out for the above four factors. Table 1 is the specific test scheme. The removal rate of SO_4^{2-} , Cu^{2+} , Zn^{2+} in wastewater, the effect of pH improvement, the release of COD and the change of ORP value were analyzed by single factor real test and orthogonal test. Among them, the concentration of SO_4^{2-} is determined by V-1600PC visible spectrophotometer (HJ/T 342-2007), the concentration of Cu^{2+} and Zn^{2+} is determined by Z-2000 flame atomic spectrophotometer (GB/T 7475-2015), the pH of the solution is determined by PHS-3C pH meter (GB/T 6920-2015), the content of COD is determined by ET99730 COD tester (HJ/T 399-2007), and the ORP value is determined by calomel electrode (SL 94-1994).

2.2 Characteristic Experiment

Conduct SO_4^{2-} and Zn^{2+} characteristic test on the immobilized particles with the best composition ratio: Configure the simulated wastewater containing only 816 mg/L SO_4^{2-} concentration, adjust the pH of the wastewater to 4, put it in the wastewater according to the solid-liquid ratio of 1:10, and continuously vibrate in a shaking table at 30°C and 150 r/min. At 6 h, 12 h, 24 h, 48 h, 72 h, 96 h and 120 h after the start of the test, the concentration of residual SO_4^{2-} and the changes of pH, COD and ORP values shall be measured until the residual concentration of SO_4^{2-} is stable; Prepare 20 mg/L of simulated wastewater containing only Zn^{2+} , adjust the pH value of the solution to 4.0, take 30 g of immobilized particles in 300 ml of wastewater according to the ratio of solid-liquid ratio of 1:10, and shake continuously in a shaking table at 30°C and 150 r/min. At 3 h, 6 h, 9 h, 12 h, 24 h, 48 h, 72 h, 96 h and 120 h after the start of the test, the concentration of residual Zn^{2+} , the changes of pH, COD and ORP values shall be measured until the concentration of each ion in the system is stable.

3 Experimental Results and Discussion

3.1 Results of Single Factor Experiment

According to Fig. 1, when the dosage of *Rhodopseudomonas spheroides* is 30%, the removal effect of SO_4^{2-} is the best; When the dosage of *Rhodopseudomonas spheroides* is 20%, the removal effect of Cu^{2+} and Zn^{2+} is the best; When the dosage of *Rhodopseudomonas spheroides* is 50%, the effect of pH enhancement is the best; When the dosage of *Rhodopseudomonas spheroides* is 30%, it has the best effect on stimulating SRB activity.

According to Fig. 2, when the lignite dosage is 5%, the SO_4^{2-} removal effect is the best; When the dosage of lignite is 5%, the removal effect of Cu^{2+} is the best; When the dosage of lignite is 1%, the removal effect of Zn^{2+} is the best; When the dosage of lignite is 5%, it has the best effect on increasing the pH of the solution; When the dosage of lignite is 5%, it has the best effect on stimulating SRB activity.

According to Fig. 3, when the SRB dosage is 30%, the SO_4^{2-} removal effect is the best; When SRB dosage is 10%, the removal effect of Cu^{2+} is the best; When SRB dosage is 30%, the removal effect of Zn^{2+} is the best; When SRB dosage is 10%, it has the best effect on increasing the pH of the solution; When the dosage of SRB is 30%, it has the best effect on stimulating SRB activity.

According to Fig. 4, when the particle size of lignite is 100 mesh, the removal effect of SO_4^{2-} is the best; When the particle size of lignite is 80 mesh, the removal effect of Cu^{2+} is the best; When the particle size of lignite is 200

mesh, the removal effect of Zn^{2+} is the best; When the particle size of lignite is 200 mesh, it has the best effect on increasing the pH of the solution; Lignite with particle size of 200 mesh has the best effect on stimulating SRB activity.

3.2 Analysis of The Results of Orthogonal Experiment

The orthogonal test was carried out on the basis of the optimal dosage of each factor of immobilized particles obtained from the previous single factor test (the dosage of *Rhodopseudomonas spheroides* was 30%; the dosage of lignite was 5%; the dosage of SRB bacteria was 30%; and the particle size of lignite was 200 mesh).

The range of a factor in the orthogonal test results is positively correlated with the effect of affecting the test results. The greater the range, the greater the influence of the influencing factor on the test results⁷⁻⁹. According to Table 1, the order of influence of various factors on SO_4^{2-} removal effect is: $A > D > C > B$. The analysis of variance is mainly carried out with item B, as shown in Table 2 and Table 3. The P value of the four factors and their influence in this experiment is very low ($P < 0.05$), indicating that the four factors have no significant correlation with the removal effect of SO_4^{2-} . According to the mean value of four factors¹⁰, when the combination type of immobilized particles is $A_1B_1C_1D_3$, the removal effect of SO_4^{2-} is the best.

According to Table 1, the order of influence of various factors on Cu^{2+} removal effect is: $A > D > C > B$. The analysis of variance based on item B is shown in Table 2 and Table 3. The results show that the removal effect of factor A on Cu^{2+} is significant, and factors B, C and D are not significant. According to the mean value of the four factors, when the combination type of immobilized particles is $A_1B_2C_2D_3$, the removal effect of Cu^{2+} is the best.

According to Table 1, the order of influence of various factors on Zn^{2+} removal effect is: $D > A > B > C$. The analysis of variance based on item C is shown in Table 2 and Table 3. The results show that the removal effect of factors A, B and C on Zn^{2+} is not significant, and factor D is significant. According to the mean value of the four factors, when the removal effect of Zn^{2+} is the best, the optimal type of immobilized particle combination is $A_1B_3C_1D_3$.

According to Table 1, the order of the influence of various factors on the pH enhancement effect is: $A = C > D > B$. The analysis of variance based on item B is shown in Table 2 and Table 3. The results show that the four factors have no significant correlation with the effect of pH improvement. According to the mean value of the four factors, the optimal combination type of immobilized particles with the best effect on pH improvement is $A_3B_3C_3D_3$.

According to Table 1, the order of influence of various factors on COD release is: $A > D > B > C$. The analysis of variance based on item C is shown in Table 2 and Table 3. The results show that factors A, B and D show significant differences in COD release, while factor C shows no significant differences. According to the mean value of the four factors, the optimal combination type of immobilized particles affecting cod release is $A_3B_1C_2D_3$.

According to Table 1, the order of influence of various factors on ORP value change is: $A > D > B > C$. The analysis of variance is mainly carried out with item C, as shown in Table 2 and Table 3. The experimental results show that factors A and D show a significant level on the change of ORP value, while factors B and C show a non significant level. According to the mean value of the four factors, the best combination type of immobilized particles affecting the change of ORP value is $A_1B_1C_1D_1$.

3.3 Analysis of Characteristic Test Results

3.3.1 Physical Properties of Particles

The immobilized particles were prepared according to the best composition ratio obtained by orthogonal test. The particle morphology before and after AMD treatment is shown in Supplementary Figure S1. Before the treatment of AMD with immobilized particles, the appearance was spherical with smooth surface, hard texture, no

obvious agglomeration, and the inner black core was faint under the white coat; After AMD treatment, the appearance of the immobilized particles presents a full spherical shape, but the texture becomes softer and more moist, and the black core of the particles is clearly visible¹¹.

3.3.2 Kinetics of SO₄²⁻ Reduction

As the reaction continues, the overall change trend of SO₄²⁻ removal rate is gradually increased at first and then stabilized, and the SO₄²⁻ concentration in the system gradually decreases to a stable level. When the reaction lasted for 1 day, the reduction rate of SO₄²⁻ was 12.283 mg/(L·h); The average reduction rate of SO₄²⁻ in 1-4 days was 5.839 mg/(L·h); When the reaction reaches 5d, the reduction rate of SO₄²⁻ is only 0.840 mg/(L·h)(Supplementary Figure S2(a)). SRB is a microorganism that uses sulfur oxide such as sulfate as electron receptor to obtain energy supply¹². The test results show that SRB immobilized particles have strong adaptability to the environment, and SRB can quickly enter the state of high growth rate and vigorous metabolism¹³. The fundamental reason is that at the initial stage of the reaction, Rhodopseudomonas spheroides degraded lignite into organic matter that can be utilized by SRB¹⁴⁻¹⁵. The rich organic matter in the system promoted the growth of SRB, enhanced microbial SRB activity and promoted the removal of SO₄²⁻⁵; Lignite can also adsorb heavy metal ions and H⁺ in the system, reduce environmental load and reduce the stimulation of metal ions and peracid conditions on SRB. According to the pH change curve of the solution, the environment of the system in the early stage of the reaction is too acidic. When the pH is between 7-8, the SRB activity is the best, and both acid and alkali will inhibit the SRB activity. Therefore, the growth and activity of SRB in the early stage of the reaction are inhibited, which is consistent with the results of this test; When the pH value gradually increases to 7-8, the SRB activity is the best. However, with the continuous occurrence of the reaction, the nutrients in the system are gradually consumed, and the SO₄²⁻ content is gradually reduced due to the reduction, so that the SRB growth rate and activity are slowed down in the later stage of the reaction, and the removal efficiency of SO₄²⁻ is reduced⁶, and the sediment produced by the early reaction is attached to the particle surface, It blocks the internal channel of the particles and affects the ion exchange reaction between the particles and the outside¹⁶, so the SRB activity is inhibited and the removal effect of SO₄²⁻ is reduced.

The adsorption kinetic process of SO₄²⁻ by immobilized particles was analyzed by zero order kinetic model and first-order kinetic model respectively, and See equations (1) and (2) for the expression:

$$C_t = C_0 - k_0 t \quad (1)$$

$$\ln C_t = \ln C_0 - k_1 \cdot t \quad (2)$$

In the formula: C_0 ——Initial concentration of SO₄²⁻, mg/L;

C_t ——The concentration of SO₄²⁻ at t , mg/L;

k_0 ——Zero order reaction rate constant, mg/L⁻¹·h⁻¹;

k_1 ——First order reaction rate constant, h⁻¹.

According to the fitting results(Fig. 5(a, b)), we can know the SO₄²⁻ reduction kinetic parameters. According to the SO₄²⁻ reduction kinetic fitting parameters, for the linear correlation coefficient R^2 , the first-order reduction kinetic model is greater than the zero order reduction kinetic model, indicating that the first-order reduction kinetic model can more accurately describe the SO₄²⁻ reduction reaction process than the zero order reduction kinetic

model, The linear correlation coefficient of the first-order reduction kinetic model was $R^2=0.98821$. It shows that the reduction process of SO_4^{2-} is mainly electron receptor¹⁷.

According to Fig. 5(c), with the progress of the reaction, the pH first increased and then stabilized, and finally showed weak alkalinity, pH=8.1. The initial pH of the system is 4.0. The peracid reaction conditions are not conducive to the growth and metabolism of SRB, and the SRB activity is low. At this time, the pH rise of the solution is mainly due to the rich ionizable groups ($\text{RCOOH} \rightleftharpoons \text{RCOO}^- + \text{H}^+$) in the lignite inside the immobilized particles, which has a good two-way pH regulation ability¹⁸. Therefore, the high concentration of H^+ in the system before the reaction will inhibit the dissociation of active groups, the dissociation equilibrium moves reversely, and the pH value of the solution gradually increases. At this time, the adsorption is mainly physical adsorption, mainly ion exchange¹⁹; However, the ability of lignite to adjust pH is limited, so when the pH value rises to a certain extent and the physiological activity of SRB is no longer inhibited, SRB begins to actively carry out SO_4^{2-} reduction reaction. At this time, the root cause of the decrease of H^+ concentration in the system is the biochemical reaction of SRB. The H^+ in the solution provides electrons for SRB to reduce SO_4^{2-} and improves the pH of the solution to a great extent. Until the later stage of the reaction, although the pH of the system is very suitable for the growth and metabolism of SRB, the concentration of SO_4^{2-} in the system is low and the content of electron receptor is insufficient, resulting in the ability of SRB to reduce SO_4^{2-} was reduced and the demand for H^+ , so the pH value of the system finally tends to be stable.

The overall change trend of COD release is that it increases gradually with the reaction, decreases after a period of reaction, and then increases gradually. The final release of COD is 700 mg/L. The above changes in COD release are related to the leakage of organic matter released from lignite and SRB biological metabolites in immobilized particles¹⁶. At the initial stage of the reaction, *Rhodopseudomonas spheroides* degraded the lignite, so that the lignite was degraded into organic matter, and a large amount of organic matter leaked out. At the initial stage, SRB was not enough to make use of such rich organic matter, so the organic matter in the solution gradually accumulated and the COD content increased. With the progress of the reaction, the activity of SRB and the ability to use organic matter are enhanced. The lignite degraded into organic matter provides carbon source for the growth of SRB, promotes the growth and metabolism of SRB, and the organic matter in the solution is gradually consumed, so the organic matter content shows a downward trend. When the reaction proceeds to the later stage, more sediments are attached to the lignite surface, which makes the lignite degradation process more difficult, the organic carbon source available for SRB growth and metabolism in the system is reduced, the SRB activity and the ability to utilize organic matter are limited, and the COD in the system begins to accumulate again.

The overall change trend of ORP value is: It gradually decreases and then tends to be stable, and the final ORP value of the system was -136 mV. The lower the ORP value, the better the bacterial activity in the system. The test results showed that the SRB activity in the reaction system gradually increased until the SRB activity remained unchanged. At the initial stage of the reaction, lignite is both a carbon source and an electron donor of SRB²⁰. Lignite degraded by *Rhodopseudomonas spheroides* provides nutrients for SRB growth and metabolism and enhances SRB activity; Lignite can also be used as an electron donor of SRB to further enhance SRB activity, so the ORP value decreases. In the later stage of the reaction, due to the limitation of the content of carbon source and terminal electron receptor, the proliferation activity of SRB is inhibited and the activity is low. Therefore, the ORP value tends to be stable, and the gradual adsorption saturation of lignite to H^+ is also one of the reasons.

3.3.3 Adsorption Capacity of Zn^{2+} and Analysis of Fitting Results of Isotherm Adsorption Equation

(1) Adsorption capacity and isotherm adsorption equation of Zn^{2+}

$$q_e = \frac{(C_0 - C_e) \cdot V}{M} \quad (3)$$

In the formula: q_e ——Equilibrium adsorption capacity of Zn^{2+} on particles, mg/g;

C_0 、 C_e ——Initial and equilibrium concentrations of Zn^{2+} in solution, mg/L;

V ——Volume of Zn^{2+} Solution, L;

M ——Dosage of particles, g.

See equation (3) for adsorption equation, and the adsorption capacity of Zn^{2+} by immobilized particles gradually increases with the increase of equilibrium concentration (Supplementary Figure S2(b)), and the adsorption rate of Zn^{2+} by particles in the early stage is fast, and then the rate gradually slows down. When the equilibrium concentration is 8.6175 mg/L, the equilibrium adsorption capacity of Zn^{2+} by particles is 0.759 mg/g. Langmuir and Freundlich²¹ isothermal adsorption equations are used to fit the isothermal adsorption process of Zn^{2+} respectively. See equations (4) and (5) for isothermal adsorption equation:

$$q_e = \frac{b \cdot q_{\max} \cdot C_e}{1 + b \cdot C_e} \quad (4)$$

$$q_e = k \cdot C_e^{\frac{1}{n}} \quad (5)$$

In the formula: b ——Constant, which is related to the enthalpy of adsorption reaction, expressed as reciprocal of concentration;

k ——Freundlich adsorption coefficient;

n ——Constant, which indicates the relative size of surface heterogeneity and adsorption strength of adsorbent, is usually greater than 1;

$q_{\max}$ ——Maximum theoretical adsorption capacity of Zn^{2+} on particles, mg/g.

(2) Analysis of fitting results

According to the fitting results (Fig. 5(d, e)), the isotherms of the two isothermal adsorption types are regular, non negative and concave to the concentration axis, and the adsorption reaches equilibrium at high concentration¹⁷. For the linear correlation coefficient R^2 , both Freundlich isothermal adsorption equation and Langmuir isothermal adsorption equation are greater than 0.95, indicating that the adsorption of Zn^{2+} on the particle surface is mainly chemical adsorption, and for the adsorption correlation coefficient, Freundlich isothermal adsorption equation is greater than Langmuir isothermal adsorption equation. Therefore, Freundlich isothermal adsorption equation can more accurately describe the adsorption process of Zn^{2+} in AMD wastewater than Langmuir isothermal adsorption equation. The detailed analysis of the parameters of Freundlich isothermal adsorption equation shows that Freundlich isothermal adsorption equation is used to describe the heterogeneity of adsorbent surface and the adsorption strength of adsorbent $1/n=0.41701$, $0.1 < 1/n < 0.5$. The results show that the immobilized particles have strong heterogeneity²² and the Zn^{2+} adsorption process on lignite surface is easy to occur²³⁻²⁴.

3.3.4 Adsorption Kinetics of Zn^{2+}

Langergren equation (pseudo first-order kinetic model) and McKay equation (pseudo second-order kinetic model) are used to study the adsorption kinetic process of Zn^{2+} by immobilized particles. See equations (6) and (7) for the expression:

$$\ln(q_e - q_t) = \ln q_t - k_1 \cdot t \quad (6)$$

$$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e} \quad (7)$$

In the formula: q_t ——The adsorption capacity at the time of adsorption time t , mg/g;

t ——Adsorption time, min;

k_1 ——Rate constant of pseudo first order kinetic reaction, min^{-1} ;

k_2 ——Pseudo second order kinetic rate constant, $\text{g}/(\text{mg} \cdot \text{min})$;

h ——Initial adsorption rate, $h = k_2 \cdot q_e^2$, $\text{mg}/(\text{g} \cdot \text{min})$.

According to Fig. 5(f, g), the adsorption capacity of Zn^{2+} by immobilized particles gradually increases with the progress of the reaction until it is stable. When the adsorption reaches equilibrium, the equilibrium adsorption capacity of Zn^{2+} by immobilized particles is 0.2 mg/g. The kinetic model is fitted based on the adsorption curve model, and the primary and secondary kinetic fitting diagrams of $\ln(q_e/(q_e-q_t)) \sim t$ and $t/q_t \sim t$ are drawn respectively (Fig. 5(h, i)).

In this experiment, in the reaction stage with fast adsorption rate of Zn^{2+} , chemical precipitation and biological adsorption exist at the same time, and the first process is chemical precipitation; In the later stage, the adsorption reaction of Zn^{2+} is mainly biosorption. Chemical precipitation works through lignite in the particle. Lignite adsorbs a large number of positively charged metal ions in the solution by virtue of its developed specific surface area and rich oxygen-containing functional groups; SO_4^{2-} is reduced to sulfide by SRB and combined with the remaining Zn^{2+} not adsorbed by lignite in the solution to form precipitation, which is removed.

The fitting results show that the pseudo second-order kinetic regression coefficient is greater than the pseudo first-order kinetic regression coefficient, and the pseudo second-order kinetic regression coefficient $R^2=0.99992$, and the correlation is very high. In this case, the pseudo second-order kinetics can more accurately describe the adsorption behavior of immobilized particles on Zn^{2+} ²⁵. It shows that the adsorption process of Zn^{2+} by immobilized particles is dominated by chemical adsorption, and the influence of chemical adsorption is higher than that of mass transfer step control²⁶⁻²⁸.

The pH value of the solution increased first and then tended to be stable as a whole (Fig. 5(j)). Lignite in the particle contains rich active groups. The dissociation of active groups ($\text{RCOOH} \rightleftharpoons \text{RCOO}^- + \text{H}^+$) makes the lignite surface negatively charged. At the initial stage of the reaction, the pH of the solution is 4, indicating that there is a large amount of H^+ in the solution, and the negatively charged lignite on the surface is very easy to attract positively charged H^+ , resulting in the decrease of H^+ content in the solution, so the pH value of the solution increases; Moreover, as the pH of the solution gradually approaches neutral, the SRB activity in the system also increases. In addition to the adsorption of lignite on H^+ , the process of SRB reducing SO_4^{2-} has also become a way to consume H^+ , and the pH value of the solution gradually increases with the decrease of H^+ concentration. As shown in the test results, the pH of the solution can be increased rapidly when the two reactions act at the same time. However, with the continuous occurrence of the reaction, the spare sites on the lignite surface are gradually occupied by metal ions and H^+ , resulting in the reduction of its adsorption capacity. Moreover, the precipitates produced by the early reaction adhere to the lignite surface, blocking the internal channels of lignite and further affecting the adsorption capacity of lignite. Therefore, the pH value of the later system basically does not change. In addition, in the early stage of the reaction, the pH of the solution has been raised to neutral, the H^+ concentration in the solution is low, and the ability to provide electrons for SRB is weak. Therefore, the H^+ concentration in the

system basically does not change significantly, and the pH of the solution tends to be stable.

The overall change of COD shows a trend of first increasing, then decreasing, and then increasing. Finally, the cumulative release of COD is 1090 mg/L. The organic matter released from lignite and the leakage of SRB biological metabolites in immobilized particles are the reasons affecting the change of COD release. The gradual increase of COD content in the initial stage of the reaction is due to the degradation of Lignite by *Rhodopseudomonas spheroides*, which releases a large amount of organic matter. At the initial stage, SRB activity is low and all organic matter can not be used quickly, resulting in the overflow of some organic matter, the increase of organic matter content in the solution and the gradual increase of COD. As the reaction proceeds, SRB begins to adapt to the current living environment and uses the degraded lignite as the carbon source for its own proliferation. Sufficient carbon source in the system can accelerate the growth and metabolism of SRB and enhance the activity of SRB, while organic matter can be utilized by SRB, so the release of COD in the solution is reduced. In the later stage of the reaction, the growth, metabolism and activity of SRB are affected by the content of carbon source and terminal electron receptor, resulting in the limited utilization of COD by SRB. Therefore, COD accumulates again and the release increases.

The ORP value first decreased and then stabilized with the increase of reaction time. The final ORP value of the system was -106 mV. The results showed that the SRB activity in the reaction system increased gradually and remained unchanged. Combined with the pH change diagram, it can be seen that due to the dissociation of active groups, the lignite surface inside the particles is negatively charged, and has obvious adsorption on positively charged heavy metal ions and H^+ , so it reduces the toxic effect of acid and heavy metal ions on SRB in AMD, enhances SRB activity, and the ORP value of the system decreases; Moreover, under the degradation of *Rhodopseudomonas spheroides*, lignite also acts as a carbon source for SRB proliferation, provides nutrients for SRB growth and metabolism, further enhances SRB activity and reduces ORP of the system. At the later stage of the reaction, the solution environment has been neutral and lignite has basically reached adsorption saturation, and the change of H^+ concentration in the solution is not obvious, indicating that there are less electrons in the system for SRB reduction of SO_4^{2-} process. At this time, SRB activity is low, and ORP value basically does not change. The change trend of ORP value in the experimental results is consistent with the change law of pH.

4 Conclusion

(1) The single factor experiment shows that when the dosage of *Rhodopseudomonas spheroides* is 30%, the dosage of lignite is 5%, the dosage of SRB is 30% and the particle size of lignite is 200 mesh, the biological activated lignite fixed SRB particles has the best effect on AMD treatment.

(2) Based on the single factor experiment of the ratio of immobilized particles in the early stage, based on the orthogonal experiment, combined with the changes of SO_4^{2-} , Cu^{2+} , Zn^{2+} removal rate, pH improvement effect, COD release and ORP value in the solution, it is finally determined that the best combination of biologically activated lignite immobilized SRB particles is $A_1B_1C_1D_3$. That is, the dosage of *Rhodopseudomonas spheroides* in the immobilized particles is 10%, and the dosage of lignite is 3%, SRB dosage is 10% and lignite particle size is 200 mesh. Under this ratio, the removal rates of SO_4^{2-} , Zn^{2+} and Cu^{2+} by immobilized particles were 83.21%, 99.59% and 99.93%, the pH was raised to 7.43, the COD release was 523mg/L and the ORP value was -134mV.

(3) The reduction process of SO_4^{2-} by immobilized particles conforms to the pseudo first-order kinetics, and the fitting equation is: $y = -0.02019 \cdot x + 6.73245$, $R^2 = 0.98821$. The main factor affecting the reduction process is electron receptor.

(4) The isothermal adsorption of Zn^{2+} by immobilized particles is more in line with Freundlich isothermal

adsorption equation, and the fitting equation is: $q_e = 0.32156 \cdot C_e^{0.41701}$, $R^2 = 0.96532$. When the equilibrium concentration is 8.6175 mg/L, the equilibrium adsorption capacity can reach 0.759 mg/g. The results show that the lignite in the immobilized particles can easily adsorb Zn^{2+} , and the surface of the adsorbent is uneven; The adsorption kinetics of Zn^{2+} by lignite inside the immobilized particles is more in line with the pseudo second-order kinetic model, and the fitting equation is: $y = 5.07431 \cdot x + 0.14878$, $R^2 = 0.99992$. It shows that the adsorption of Zn^{2+} by lignite is mainly chemical adsorption.

Table1 L₉(3⁴) orthogonal test and result

Serial number	Rhodopseudomonas spheroides A/%	Lignite content B/%	SRB C/%	Particle size of lignite D/mesh	SO ₄ ²⁻ Test result	Cu ²⁺ Test result	Zn ²⁺ Test result	pH Test result	COD Test result	ORP Test result
1	10	3	10	80	80.96	99.18	96.77	7.13	499	-134
2	10	5	30	100	77.70	99.93	95.58	7.28	482	-129
3	10	7	50	200	82.28	99.18	99.59	7.42	516	-123
4	30	3	30	200	75.17	98.81	98.28	7.31	511	-119
5	30	5	50	80	71.77	96.57	95.66	7.23	479	-124
6	30	7	10	100	72.21	95.59	96.58	7.23	485	-120
7	50	3	50	100	81.49	93.05	94.67	7.43	516	-119
8	50	5	10	200	83.21	95.59	97.12	7.35	521	-114
9	50	7	30	80	75.21	94.74	95.00	7.36	523	-116

Table2 Intuitive analysis table

Serial number	Rhodopseudomonas Spheroides A	Lignite content B	SRB C	Particle size of lignite D
SO ₄ ²⁻ Mean value 1	80.311	79.207	78.793	75.980
SO ₄ ²⁻ Mean value 2	73.050	77.560	76.027	77.133
SO ₄ ²⁻ Mean value 3	79.970	76.567	78.513	80.220
SO ₄ ²⁻ Range	7.263	2.640	2.766	4.240
Cu ²⁺ Mean value 1	99.430	97.013	96.787	96.830
Cu ²⁺ Mean value 2	96.990	97.363	97.827	96.190
Cu ²⁺ Mean value 3	94.460	96.503	96.267	97.860
Cu ²⁺ Range	4.970	0.860	1.560	1.670
Zn ²⁺ Mean value 1	97.313	96.573	96.823	95.810
Zn ²⁺ Mean value 2	96.840	96.120	96.287	95.610
Zn ²⁺ Mean value 3	95.597	97.057	96.640	98.330
Zn ²⁺ Range	1.716	0.937	0.536	2.270
pH Mean value 1	7.277	7.290	7.237	7.240
pH Mean value 2	7.257	7.287	7.317	7.313
pH Mean value 3	7.380	7.337	7.360	7.360
pH Range	0.123	0.05	0.123	0.120
COD Mean value 1	499.000	508.667	501.667	500.333
COD Mean value 2	491.667	494.000	505.333	494.333
COD Mean value 3	520.000	508.000	503.667	516.000
COD Range	28.333	14.667	3.666	21.667
ORP Mean value 1	-128.667	-124.000	-122.667	-124.667
ORP Mean value 2	-121.000	-122.333	-121.333	-122.667
ORP Mean value 3	-116.333	-119.667	-122.000	-118.667
ORP Range	12.334	4.333	1.334	6.000

Table3 Variance analysis

Source of variance	Rhodopseudomonas spheroides /A	Lignite content /B	SRB /C	Particle size of lignite /D	Error /E	Sum
SO ₄ ²⁻ Sum of squares of deviations	100.760	10.668	13.916	28.835	10.670	164.849
SO ₄ ²⁻ Free Degree	2	2	2	2	2	8
SO ₄ ²⁻ Mean Square	50.38	5.334	6.958	14.4175	5.335	
SO ₄ ²⁻ F value	9.445	1.000	1.304	2.703		
SO ₄ ²⁻ P value	<0.05	<0.05	<0.05	<0.05		
SO ₄ ²⁻ Significant	⊙	⊙	⊙	⊙		
Cu ²⁺ Sum of squares of deviations	37.055	1.122	3.786	4.259	1.12	47.342
Cu ²⁺ Free Degree	2	2	2	2	2	8
Cu ²⁺ Mean Square	18.5275	0.561	1.893	2.1295	0.56	
Cu ²⁺ F value	33.026	1.000	3.374	3.796		
Cu ²⁺ P value	>0.05	<0.05	<0.05	<0.05		
Cu ²⁺ Significant	*	⊙	⊙	⊙		
Zn ²⁺ Sum of squares of deviations	4.717	1.316	0.446	13.789	0.45	20.718
Zn ²⁺ Free Degree	2	2	2	2	2	8
Zn ²⁺ Mean Square	2.3585	0.658	0.223	6.8945	0.225	
Zn ²⁺ F value	10.576	2.951	1.000	30.917		
Zn ²⁺ P value	<0.05	<0.05	<0.05	>0.05		
Zn ²⁺ Significant	⊙	⊙	⊙	*		
pH Sum of squares of deviations	0.026	0.005	0.023	0.022	0.01	0.086
pH Free Degree	2	2	2	2	2	8
pH Mean Square	0.013	0.0025	0.0115	0.011	0.005	
pH F value	5.200	1.000	4.600	4.400		
pH P value	<0.05	<0.05	<0.05	<0.05		
pH Significant	⊙	⊙	⊙	⊙		
COD Sum of squares of deviations	1297.556	411.556	20.222	750.889	20.22	2500.443
COD Free Degree	2	2	2	2	2	8
COD Mean Square	648.778	205.778	10.111	375.4445	10.11	
COD F value	64.166	20.352	1.000	37.132		
COD P value	>0.05	>0.05	<0.05	>0.05		
COD Significant	*	*	⊙	*		
ORP Sum of squares of deviations	232.667	28.667	2.667	56.000	2.67	322.671
ORP Free Degree	2	2	2	2	2	8
ORP Mean Square	116.3335	14.3335	1.3335	28	1.335	
ORP F value	87.239	10.749	1.000	20.997		
ORP P value	>0.05	<0.05	<0.05	>0.05		
ORP Significant	*	⊙	⊙	*		

Note: $F_{0.05}(2,2) = 19$, $F_{0.01}(2,2) = 99$; $0.05 \leq P \leq 0.01$, significant; $\odot$ $P < 0.05$ was not significant.

Reference

- 1 Sun, K., Hwang, Hea, E. & Jho. Corrigendum to "Heavy metal and sulfate removal from sulfate-rich synthetic mine drainages using sulfate reducing bacteria" [Sci. Total Environ. 635 (2018) 1308-1316]. *The Science of the total environment*, doi:10.1016/j.scitotenv.2018.10.142 (2018).
- 2 Watanabe, M., Higashioka, Y., Kojima, H. & Fukui, M. Proposal of Desulfosarcina ovata subsp. sediminis subsp. nov., a novel toluene-degrading sulfate-reducing bacterium isolated from tidal flat sediment of Tokyo Bay. *Systematic and Applied Microbiology* **43**, 126109, doi:10.1016/j.syapm.2020.126109 (2020).
- 3 Tran, T. T. T., Kannoopatti, K., Padovan, A. & Thennadil, S. Sulphate-Reducing Bacteria's Response to Extreme pH Environments and the Effect of Their Activities on Microbial Corrosion. *Applied Sciences* **11**, 2201, doi:10.3390/app11052201 (2021).
- 4 Zhang, Q. *Preparation of Coal-based Adsorbent and the removal of Heavy Metals from Waste Water*, Wuhan University of Science and Technology.
- 5 An, W., Wang, L. & Di, J. Dynamic Experimental Study on the Iron Cooperated with SRB Sludge Immobilized Particles to Treat AMD. *Non-Metallic Mines* (2017).
- 6 Junzhen, D. I. *et al.* Best conditions of modification about corncob as SRB fixed carbon source. *Chinese Journal of Environmental Engineering* (2017).
- 7 Zhu, Z. *Study on Acid Waste water of SRB sludge particles*, Fuxin: Liaoning University of Engineering and Technology, (2015).
- 8 Chen, Y. The extraction solvent of tangerine total flavonoids was screened by orthogonal analysis. *Everybody Health (Academic)* **6**, 4-6, doi:CNKI:SUN:JKXS.0.2012-12-003 (2012).
- 9 Kiran, M. G., Pakshirajan, K. & Das, G. Metallic wastewater treatment by sulfate reduction using anaerobic rotating biological contactor reactor under high metal loading conditions. *Academic abstracts of Chinese colleges and universities · environmental science and Engineering* **012**, 111-121, doi:10.1007/s11783-018-1073-4 (2018).
- 10 Zhu, Y. Language-based orthogonal experimental extreme analysis—takes the orthogonal table of L-9(3~4) without interaction as an example. *Beverage Industry*, 31-34, doi:CNKI:SUN:RYLG.0.2011-02-021 (2011).
- 11 Lin, Y. *et al.* Preparation and characterization of magnetic Fe₃O₄-chitosan nanoparticles for cellulase immobilization. *Cellulose*, doi:10.1007/s10570-017-1520-6 (2017).
- 12 Dongmei, Z., Shiwei, Z., Huanchi, M. & Huiying, L. Simulation of Methylmercury Content and SRB Methylation in Phragmites australis Soil Under Different Salinity Conditions. *Water, Air, and Soil Pollution* **231**, 18.11-18.18, doi:10.1007/s11270-019-4382-8 (2020).
- 13 Yu, H. *et al.* Transcriptome Analysis of the Acid Stress Response of Desulfovibrio vulgaris ATCC 7757. *Current Microbiology*, doi:10.1007/s00284-020-02051-x (2020).
- 14 Pantoja-Guerra, M. & Valero-Valero, N. Design of a bio-conditioner prototype for the treatment of degraded soils: biomass production and lignite formulation for Microbacterium sp. CSB3. *Chemical and Biological Technologies in Agriculture* **7**, 3, doi:10.1186/s40538-019-0167-y (2020).
- 15 Nelson, V., Liliana, G., Manuel, P. & Ramiro, R. Production of humic substances through coal-solubilizing bacteria. *Brazilian Journal of Microbiology* **45**, doi:10.1590/S1517-83822014000300021 (2014).
- 16 Dong, Y. *et al.* Dynamic Experimental Study on Treatment of Acid Mine Drainage by Bacteria Supported in Natural Minerals. *Energies* **13**, doi:10.3390/en13020439 (2020).
- 17 B, D. M. A. & A, S. C. Single, binary, and multicomponent sorption of iron and manganese on lignite - ScienceDirect. *Journal of Colloid and Interface Science* **299**, 76-87, doi:10.1016/j.jcis.2006.02.010 (2006).
- 18 B, D. M. A. & A, S. C. Single, binary, and multicomponent sorption of iron and manganese on lignite - ScienceDirect. *Journal of Colloid and Interface Science* **299**, 76-87, doi:10.1016/j.jcis.2006.02.010 (2006).

- 19 Hao, Y., Fan, F. & Guo, C. Thermodynamic mechanism of copper adsorption in lignite. *Material Protection* **07**, 85-88, doi:CNKI:SUN:CLBH.0.2017-07-023 (2017).
- 20 Detmers, J., Schulte, U., Strauss, H. & Kuever, J. Sulfate Reduction at a Lignite Seam: Microbial Abundance and Activity. *Microb Ecol* **42**, 238-247, doi:10.1007/s00248-001-1014-8 (2001).
- 21 Xue, L. *Experimental study on the repair of acid wastewater*, Liaoning University of Engineering and Technology, (2019).
- 22 Xin, F., Xu, H., Tang, D. & Cao, L. Properties of lignite and key factors determining the methane adsorption capacity of lignite: New insights into the effects of interlayer spacing on adsorption capacity. *Fuel Processing Technology* **196**, 106181, doi:10.1016/j.fuproc.2019.106181 (2019).
- 23 Chethan, P. D. & Vishalakshi, B. Adsorption Efficiency of Cr(VI) by Ethylene-1,2- Diamine-6-Deoxychitosan. *Separation Science and Technology* **50**, 1158-1165, doi:10.1080/01496395.2014.967405 (2015).
- 24 Panadare, D. C., Lade, V. G. & Rathod, V. K. Adsorptive removal of copper(II) from aqueous solution onto the waste sweet lime peels (SLP): equilibrium, kinetics and thermodynamics studies. *Desalination & Water Treatment* **52**, 7822-7837, doi:10.1080/19443994.2013.831789 (2014).
- 25 Mhca, B. *et al.* Preparation, characterization and adsorption kinetics of methylene blue dye in reduced-graphene oxide supported nanoadsorbents - ScienceDirect. *Journal of Molecular Liquids* **309**, doi:10.1016/j.molliq.2020.113171.
- 26 Agarry, S. E., Ogunleye, O. O. & Aworanti, O. A. Biosorption equilibrium, kinetic and thermodynamic modelling of naphthalene removal from aqueous solution onto modified spent tea leaves. *Environmental Technology* **34**, 825-839, doi:10.1080/09593330.2012.720616 (2013).
- 27 Bao, X. *Absorption and mechanism of modified lignite*, Henan: Henan Agricultural University.
- 28 Vimonses, V., Lei, S., Jin, B., Chow, C. & Saint, C. Kinetic study and equilibrium isotherm analysis of Congo Red adsorption by clay materials. *Chem.eng.j* **148**, 354-364, doi:10.1016/j.cej.2008.09.009 (2010).

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1 School of Civil Engineering, Liaoning University of Engineering and Technology, Fuxin, Liaoning Province 123000, China

* corresponding author: dijunzhen@126.com

Figures

Figure 1

Effects of different dosages of Rhodopseudomonas Spheroides on various indexes

Figure 2

Effect of different dosage of lignite on various indexes

Figure 3

Effects of SRB dosage on various indexes

Figure 4

Effect of different particle size of lignite on various indexes

Figure 5

Fitting curves

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