



Biological treatment of wastewater with high concentrations of zinc and sulfate ions from zinc pyrithione synthesis

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Abstract: An enriched and domesticated bacteria consortium of sulfate-reducing bacteria (SRB) was used to treat wastewater from zinc pyrithione (ZPT) production, and the effects of different reaction parameters on sulfate reduction and zinc precipitation were evaluated. The single-factor experimental results showed that the removal rates of Zn^{2+} and SO_4^{2-} decreased with an increased ZPT concentration ranging from 3.0 to 5.0 mg/L. Zn^{2+} and SO_4^{2-} in wastewater were effectively removed under the conditions of 30–35 °C, pH 7–8 and an inoculum concentration of 10%–25%. The presence of Fe^0 in the SRB system enhanced Zn^{2+} and SO_4^{2-} removal and may increase the resistance of SRB to the toxicity of Zn^{2+} and ZPT in wastewater. A Box–Behnken design was used to evaluate the influence of the main operating parameters on the removal rate of SO_4^{2-} . The optimum parameter values were found to be pH 7.45, 33.61 °C and ZPT concentration of 0.62 mg/L, and the removal rate of SO_4^{2-} reached a maximum of 91.62% under these optimum conditions.

Key words: biological treatment; sulfate reduction; sulfate-reducing bacteria; wastewater; zinc pyrithione; zinc

1 Introduction

Zinc pyrithione (ZPT) is a broad-spectrum anti-bacterial agent and extensively used as the active antidandruff ingredient in hair care. ZPT is an extremely toxic substance whose EC_{50} value was found to be less than 1 mg/L for *Vibrio fischeri*, *Pseudomonas putida* and *Tetrahymena thermophila*, and some of the main microorganisms present in activated sludge [1]. In seawater, it was found that ZPT exposed to the sunlight degrades rapidly (half-life <2 min) into less toxic compounds, and so it is presumed to be environmentally neutral and non-persistent in the aquatic environment. However, other studies have reported that half of the initial ZPT amount remained after 48 h light exposure. Therefore, biological treatment processes for ZPT

wastewater could be adversely affected if ZPT production wastewater is directly discharged without proper pretreatment.

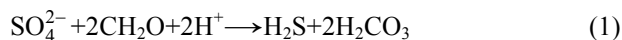
ZPT production wastewater contains high concentrations of Zn^{2+} and SO_4^{2-} , increasing the difficulties of ZPT production wastewater treatment. Generally, most common zinc salts at high concentrations can be separated effectively by physiochemical methods [2–4]. However, this implies prohibitively high operative cost and causes secondary pollution when applied on large scale. Bacterial sulfate reduction is considered to be a highly promising method for the removal of sulfate and heavy metal from environmental contamination due to low operating cost, high selectivity, complete removal and only small residual sludge. Anaerobic sulfate reducing bacteria (SRB) could oxidize various organic compounds by

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utilizing sulfate as an electron acceptor, resulting in alkalinity generation and metal cation (Me^{2+}) precipitation as sulphide, which is conducive to the sustainable development of the ZPT production industry.



Therefore, the immobilization removal of Zn^{2+} through microbial mediated reduction and precipitation has attracted a great deal of interest [5,6]. The tolerance concentration of SRB to Zn^{2+} depends on the type of SRB. AZABOU et al [7] found that zinc concentration more than 150 mg/L was lethal to SRB. ZHOU et al [8] reported that Zn^{2+} inhibited SRB when the concentration of Zn^{2+} was 300 mg/L, while the tolerance of SRB to Zn^{2+} could be enhanced to some extent by acclimatization. Although there are many reports on SRB and metal precipitation as mentioned above, literature on cost-effective processes for simultaneous removal of Zn^{2+} and SO_4^{2-} from wastewater in the presence of toxic ZPT is still not available. ZPT can be dead for SRB, which can lead to unstable biological treatment operation or even system failure. Most importantly, the concentration of zinc sulfate in ZPT production wastewater can reach 1000 mg/L that is harmful to SRB [9,10]. To address this problem, Fe^0 was introduced into the processing stream and found to be beneficial for forming an anaerobic reducing environment. A synergetic enhancement was also reported when Fe^0 was applied to the treatment of uranium-bearing wastewater [11]. Thus, it is reasonable to hypothesize that Fe^0 might promote Zn^{2+} removal as well [8,12].

The objective of this study was to investigate the effects of different experimental parameters on Zn^{2+} and SO_4^{2-} removal under different ZPT concentrations together with the growth dynamics of SRB. Batch experiments were performed and parameters including Zn^{2+} concentration, pH, inoculum concentration, temperature, and ZPT concentrations from 1.0 to 5.0 mg/L were examined. The reduction of Zn^{2+} and SO_4^{2-} over time in the combined system of SRB and Fe^0 (SRB+ Fe^0) was also investigated to study the feasibility of SRB+ Fe^0 in ZPT wastewater treatment. In addition, a Box–Behnken design was selected to determine the optimum conditions for ZPT production wastewater treatment and to illustrate the relationship between sulfate removal and three independent variables including temperature, pH, and initial ZPT concentration.

2 Experimental

2.1 Chemicals

ZPT, purity above 96%, was purchased from Shanghai DIBO Chemical Technology Co., Ltd. Zinc

standard solution (1.0 mg/mL) was supplied by Beijing PUXI Technology Co., Ltd. All other chemicals and reagents used in this study were of analytical grade.

2.2 Preparation of SRB for reduction

A mixed SRB consortium was isolated from landfill leachate collected from a landfill in Hunan Province, China. The landfill leachate was transferred immediately to a 250 mL serum bottle until it was completely filled, then the bottle was sealed tightly to prevent direct contact with atmospheric oxygen. After that, the sample was transported to a laboratory in a cool box. Once in the laboratory, the sample was removed from the cool box. The sample was purged with high purity nitrogen to minimize the exposure of SRB to oxygen.

To a 250 mL serum bottle, 25 mL of landfill leachate was added followed by 225 mL of sterilized Postgate's B medium [13] (autoclaved at 121 °C and 2.026×10^5 Pa for 20 min) at pH 7.5. After purging with pure nitrogen for 10 min, the bottle was capped with a tertbutyl rubber stopper and crimp-sealed, then incubated for at 35 °C 4 d in an incubator. Enriched SRB culture was obtained by repeating the process mentioned above three times. Postgate's B medium contained 3.5 g/L sodium lactate, 1.0 g/L yeast extract, 2.0 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L Na_2SO_4 , 0.5 g/L K_2HPO_4 , 1.0 g/L NH_4Cl , 0.5 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/L ascorbic acid, and 0.1 g/L of thioglycolic acid.

The SRB was acclimated to Zn^{2+} tolerance in the following manner. After enrichment, 25 mL SRB was inoculated into 250 mL serum bottle containing 225 mL sterilized Postgate's B medium at pH 7.5 and amended with approximately 50 mg/L Zn^{2+} . The headspace of serum bottle was filled with pure N_2 before being capped and crimp-sealed. The bottle was maintained in an incubator at 35 °C for 4 d. This procedure was repeated until the medium turned black within 2 d. The zinc concentration in the Postgate's B medium was increased at a step of 50 mg/L from 100 to 350 mg/L. Once the acclimated SRBs were all prepared, they were streaked on agar slants spiked with Zn^{2+} , incubated at 35 °C for 4 d and stored at 4 °C for use.

2.3 Analytical methods

All glassware and plasticware used were thoroughly cleaned by soaking in 10% nitric acid for 48 h followed by ultrasonic cleaning for 30 min, rinsed several times with ultrapure water and finally oven dried. Before the measurement of soluble Zn and sulfate, samples were centrifuged at 4000 r/min for 10 min. The supernatant was analyzed for Zn^{2+} by atomic absorption spectroscopy (Perkin Elmer Analyst 700). 5.0 mL of the supernatant was transferred to 50 mL volumetric flasks and diluted with 0.1 mol/L HNO_3 to dissolve Zn particles. Diluted

samples were then filtered through a sterile nitrocellulose filter (0.45 mm) to remove biomass and other particles before analysis.

Barium chromate spectrophotometry was used for determination of sulfate by visible spectrophotometer (Shanghai No. 3 Analytical Instruments Company). Sulfate was measured immediately after centrifugation mentioned above. Each experiment was carried out in triplicate under identical condition.

The growth curves of SRB under different concentrations of ZPT were studied using nephelometry by visible range spectrophotometer. ZPT was added into the modified Postgate's B medium according to the concentrations of 0, 1.0, 3.0 and 5.0 mg/L, respectively. 1.0 mL of acclimated SRB and 9.0 mL of sterilized Postgate's B medium containing ZPT (pH 7.5) were transferred into 10 mL sterile centrifuge tube. The centrifuge tubes were put into an incubator at 35 °C for 50 h, and the peak absorbance at 600 nm ($O_{D_{600}}$) was monitored every 2 h. The SRB concentration showed a linear relationship with $O_{D_{600}}$ as follows:

$$c=4.6 \times 10^7 O_{D_{600}}, R^2=0.990 \quad (3)$$

where c is the SRB concentration (cfu/mL).

2.4 Batch studies

All experiments for Zn^{2+} removal and SO_4^{2-} reduction were performed in duplicate 250 mL serum bottles, capped with tertbutyl rubber stopper and crimp-sealed. 25 mL SRB, a given quantity of $ZnSO_4$ and

225 mL sterile Postgate's B medium at pH 7.0 were added into 250 mL serum bottle. In order to estimate and assess the potential toxicity that ZPT could cause on SRB, ZPT was added into the bottles at a concentration of 0, 1.0, 3.0 and 5.0 mg/L, respectively. The bottles were put into an incubator at 30 °C for 7 d. The residual concentration of Zn^{2+} was measured every day at various initial concentrations of 50, 100, 150, 200, 250, 300 and 350 mg/L. The solution pH was adjusted to the desired values (5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0) by 2 mol/L NaOH and HCl to evaluate the effect of pH on precipitation of Zn^{2+} and degradation of SO_4^{2-} . The influence of temperature on the removal of Zn^{2+} and SO_4^{2-} was examined at 15, 20, 25, 30, 35 and 40 °C. The effect of Fe^0 on removal of Zn^{2+} and SO_4^{2-} was investigated by measuring the residual Zn^{2+} and SO_4^{2-} each day for 7 d.

2.5 Experimental design

The sulfate removal rate was optimized by response surface methodology (RSM) using a Box–Behnken design (BBD). The statistical software Design Expert 8.0.6 was used for the analysis. Three independent parameters, pH, X_1 (5.5–9), temperature, X_2 (15–40 °C) and ZPT concentration, X_3 (1–5 mg/L) were confirmed to optimize the sulfate removal. The coded and uncoded levels of these variables were presented in Table 1. With statistical analysis of the gained experimental data, a quadratic equation (Eq. (4)) was attained as an empirical model for the optimization process.

Table 1 Box–Behnken design matrix

Run	Code value			Actual value			SO_4^{2-} removal rate/%		
	X_1	X_2	X_3	X_1	$X_2/^\circ C$	$X_3/(mg \cdot L^{-1})$	Y_{exp}	Y_{pred}	Residual
1	0	0	0	7.25	27.5	2.5	2.53	2.54	−0.01
2	1	−1	0	9	15	2.5	7.87	7.83	0.037
3	0	0	0	7.25	27.5	2.5	25.87	25.98	−0.11
4	1	0	1	9	27.5	5	46.23	46.29	−0.063
5	−1	0	−1	5.5	27.5	0	32.87	33.18	−0.31
6	0	0	−1	7.25	27.5	0.05	47.87	48.22	−0.35
7	0	−1	−1	7.25	15	0	20.46	20.03	0.43
8	0	0	0	7.25	27.5	2.5	30.97	30.59	0.38
9	0	0	0	7.25	27.5	2.5	26.57	26.14	0.43
10	0	−1	1	7.25	15	5	85.79	85.26	0.53
11	0	1	1	7.25	40	5	38.47	38.92	−0.45
12	−1	−1	0	5.5	15	2.5	41.35	41.70	−0.35
13	0	1	−1	7.25	40	0	85.12	85.19	−0.073
14	1	1	0	9	40	2.5	85.28	85.19	0.087
15	1	0	−1	9	27.5	0	80.50	80.80	−0.3
16	−1	0	1	5.5	27.5	5	85.17	85.19	−0.023
17	−1	1	0	5.5	40	2.5	85.36	85.19	0.17

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (4)$$

where Y is the response, β_0 , β_i , β_{ii} , and β_{ij} are coefficients of the intercept, linear, square and interaction effects, respectively. The optimum response (Y_{opt}) and the corresponding process parameters were also determined.

3 Results and discussion

3.1 Effect of ZPT concentration on growth of SRB

As an antimicrobial agent, ZPT is active against a range of micro-organisms. The growth characteristics of SRB under ZPT concentrations of 0, 1.0, 3.0 and 5.0 mg/L in 50 h batch tests were investigated (Fig. 1). All the curves illustrate the characteristic growth phase in SRB batch culture. From Fig. 1, it can be seen that the concentration of SRB decreased sharply within 8 h after inoculation for all tests. The SRB biomass reached a minimum at 8 h. After a period of acclimatization, SRB entered logarithmic growth phase. Although the logarithmic growth phase of SRB under different concentrations of ZPT was similar, its concentration decreased with the increase of ZPT concentrations at the end of logarithmic growth phase. In culture medium with ZPT, SRB showed poor growth and entered into aging phase much earlier as compared to SRB in medium without ZPT. In addition, the concentration of SRB decreased rapidly during aging phase with ZPT, while the concentration of SRB showed only a slight drop in the absence of ZPT. Moreover, the concentration of SRB with ZPT was significantly lower than that without ZPT when the trials ended.

During the aging phase, there was no increase in the concentration of cells for the ZPT-free group and growth was limited by insufficient nutrients and the accumulation of cellular metabolism by-products [14]. Possibly, the nutrient limitations and the accumulation of high concentrations of sulfide produced during the

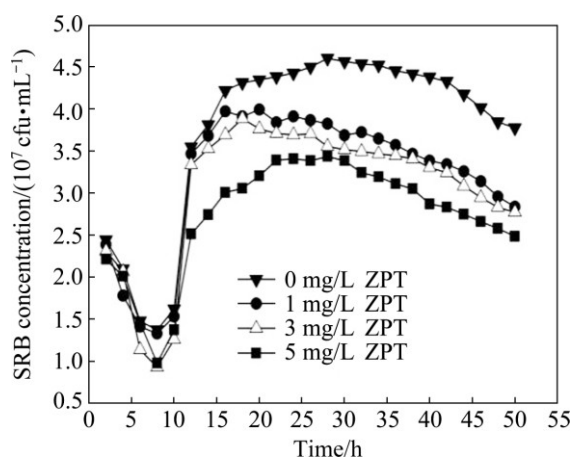


Fig. 1 Growth curves for SRB grown under four ZPT concentration levels within 50 h

exponential phase hindered the growth of SRB. It was suggested that high sulfide concentration has a reversible and direct toxicity effect on SRB. The inhibition may be the result of an intrinsic toxicity of H_2S to living systems or it may be a result of indirect toxicity generated by rendering the iron insoluble as iron sulfide [15]. Iron is needed as an essential cofactor to various cytochromes involved in cellular respiration (e.g., cytochrome-C). In addition to the negative effects of insufficient nutrients and H_2S , ZPT inhibited the growth of SRB in the ZPT-containing batch experiments. ZPT inhibited the growth and metabolism of SRB, and the extent of inhibition increased with increasing ZPT concentration, with the toxicity especially strong when the ZPT concentration was 5.0 mg/L. Similarly, ZPT was shown to inhibit yeast growth at concentrations lower than 1 mg/L through copper influx and inactivation of iron-sulfur proteins. Perhaps, this was because ZPT was toxic for SRB.

3.2 Effects of initial Zn^{2+} concentration on removal of Zn^{2+}

Although Zn^{2+} in trace concentration is beneficial to the growth of SRB, it inhibits its growth at high concentrations by interfering with nucleic acids and active sites of enzymes [16,17]. The effects of the initial concentration of Zn^{2+} on the removal of Zn^{2+} are presented in Fig. 2. From Fig. 2, it can be seen that the removal rate of Zn^{2+} gradually decreased with an increased concentration of Zn^{2+} , and exceeded 85.0% when the initial Zn^{2+} concentration was lower than 250 mg/L. When the initial Zn^{2+} concentration was higher than 250 mg/L, a much more dramatic drop of Zn^{2+} removal rate was observed and displayed a severe toxic effect on the growth of SRB. The results showed that 250 mg/L was the maximum tolerable concentration of Zn^{2+} for SRB, higher than 150 mg/L obtained in an earlier study [18]. It should be noted that not only high

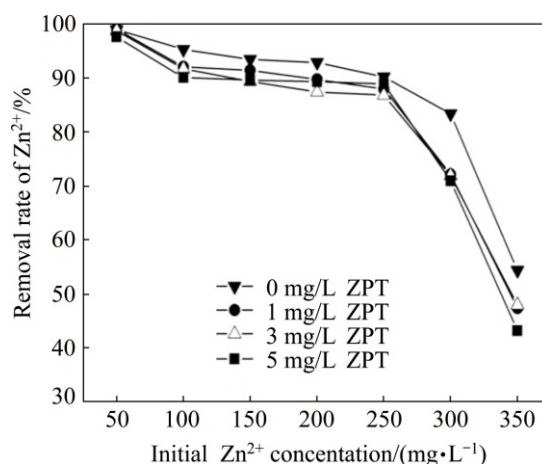


Fig. 2 Effect of initial Zn^{2+} concentration on Zn^{2+} removal rate by SRB consortium at four different concentrations of ZPT

concentration of heavy metal but insoluble metal sulfides can inhibit the SRB activity as well [19]. Metal sulfides concentrated near the bacteria cells or deposited on the surface of the SRB hindered further metabolism by preventing the contact between the necessary enzymes and reactants. The toxicity of the combination of ZPT and Zn^{2+} on SRB was marginally higher than that of Zn^{2+} only.

3.3 Effect of pH on removal of Zn^{2+} and SO_4^{2-}

The optimum pH for growth of SRB is pH 5.5 to 9 [20]. pH plays an important role in the biogenic sulfide production which in turn affects the removal of Zn^{2+} and SO_4^{2-} [8]. The results are presented in Fig. 3. There were clear differences between the removal of Zn^{2+} and SO_4^{2-} . When the concentration of ZPT was greater than 1.0 mg/L and the pH was lower than 7.0 or higher than 8.0, the removal rate of SO_4^{2-} decreased substantially while Zn^{2+} could be efficiently removed at pH 6.5–9.0. This occurred because the solubility of Zn^{2+} is pH-dependent, while at higher pH zinc is less soluble in water. In addition, the bioavailability of heavy metals is critically dependent on its speciation, and it is generally assumed that free metal ions are most toxic to

microorganisms [21]. Therefore, the effects mentioned above facilitate the removal of Zn^{2+} while in contrast the removal rate of SO_4^{2-} is low in alkaline conditions.

The removal rate of SO_4^{2-} is an important indicator for the activity of SRB. From Fig. 3(b) both acidic and basic conditions pose a significant barrier for the growth of SRB. In addition, when the culture was not given as ZPT, the removal rates of Zn^{2+} and SO_4^{2-} are higher than those cultured with ZPT addition, especially for SO_4^{2-} . These results might be attributed to the inhibition effect of ZPT on SRB being enhanced in acidic and alkaline conditions. The removal rates of Zn^{2+} and SO_4^{2-} are the highest in the pH range from 7.0 to 8.0, with the highest at pH 7.5. In this range, ZPT had little effect on the SRB.

3.4 Effect of temperature on removal of Zn^{2+} and SO_4^{2-}

The effects of temperature on removal rates of Zn^{2+} and SO_4^{2-} are presented in Fig. 4. From Fig. 4, it can be seen that the removal rates of Zn^{2+} and SO_4^{2-} increased with increasing temperature until 35 °C. The removal rates of Zn^{2+} and SO_4^{2-} with ZPT were lower than that of

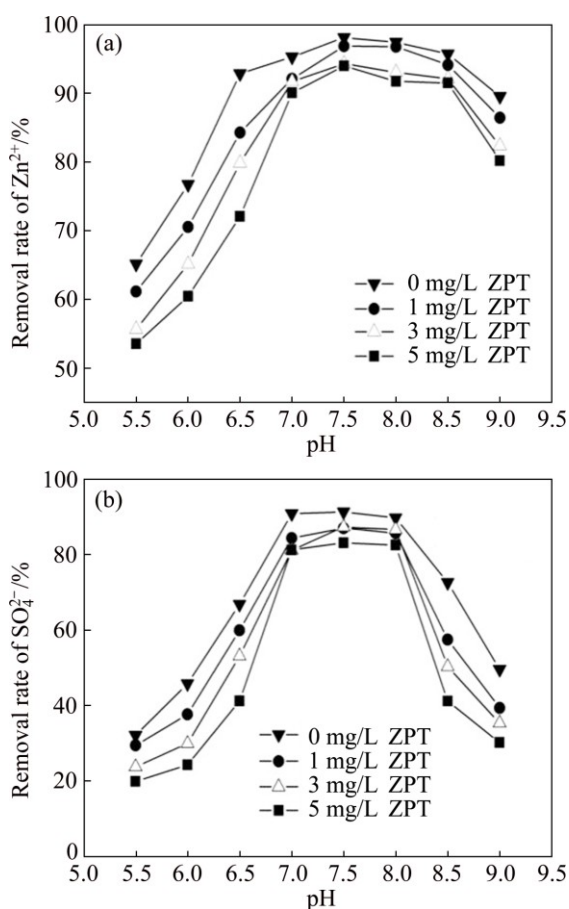


Fig. 3 Effect of pH on Zn^{2+} (a) and SO_4^{2-} (b) removal rates by SRB consortium at four different concentrations of ZPT and initial Zn^{2+} concentration of 100 mg/L

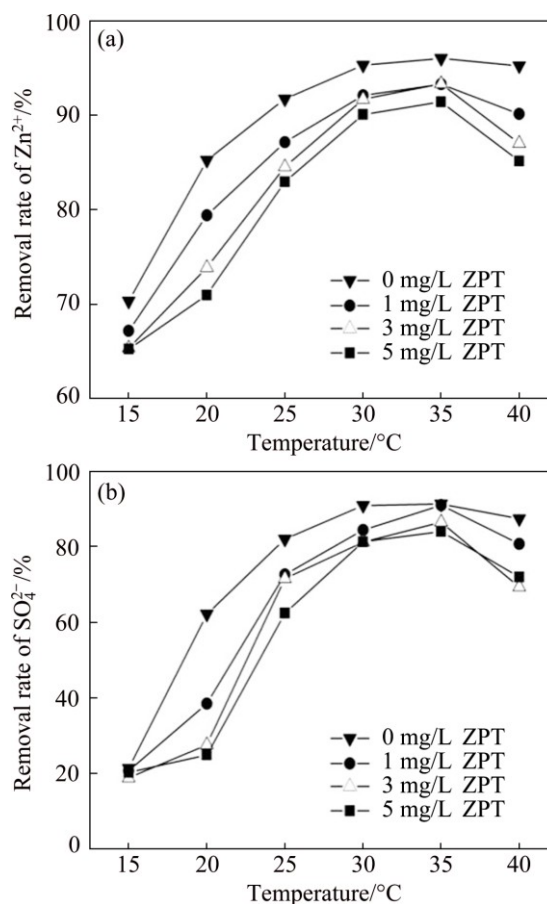


Fig. 4 Effect of temperature on Zn^{2+} (a) and SO_4^{2-} (b) removal rates by SRB consortium at four different concentrations of ZPT and initial Zn^{2+} concentration of 100 mg/L

the ZPT-free group and decreased with an increase of ZPT concentration within the temperature range of 20–40 °C. The removal rates of Zn^{2+} and SO_4^{2-} in all of the trials reached a maximum value at 35 °C.

As an important factor in biological and chemical reduction [8], temperature may provide insight into the mechanism of the interaction between SRB and ZPT, which could provide new significant information to promote technological advance in practical application. SO_4^{2-} reduction is highly sensitive to temperature change with effective reduction occurring at 30–40 °C, while Zn^{2+} reduction has a much wider temperature range (20–40 °C). As 35 °C is the most suitable temperature for SRB reduction reaction, a temperature increase or decrease suppresses these reactions [8]. When ambient temperatures were as low as 15 °C, the activity of sulfate reducing bacteria was significantly inhibited. It may be caused by the decrease in enzyme activity related to sulfate reduction and the extension of the acclimation period [22]. The experiment results also show that the toxicity of the ZPT on SRB is reduced within the favorable temperature range of 30–35 °C.

3.5 Effect of inoculum concentration on removal of Zn^{2+} and SO_4^{2-}

Regarding the effect of inoculum concentration, the common assumption is that excessive cell density leads to a decrease in the substrate/microorganism (S/M) ratio, causing a shortage of substrate to support microbial metabolism. However, low inoculum concentration may result in the death of bacteria. Therefore, the suitable inoculum concentration could shorten the time of the acclimatization period, which in turn affects the removal of heavy metals [23].

The effect of inoculum concentration on the removal rates of Zn^{2+} and SO_4^{2-} is presented in Fig. 5. When the inoculum concentration was 5%, the removal rates of Zn^{2+} and SO_4^{2-} were much more sensitive to the addition of ZPT. In contrast, the removal rates of Zn^{2+} and SO_4^{2-} significantly increased for SRB exposed to ZPT when the inoculum concentration increased from 5% to 10%. As seen from Fig. 5(b), when the inoculum concentration was 10%, the removal rate of SO_4^{2-} was between 81.2%–90.8% with and without ZPT. In comparison, at a 5% inoculation level, the range of SO_4^{2-} removal rate was much lower between 30%–59% at ZPT concentrations of 1.0, 3.0 and 5.0 mg/L. Inoculum concentrations higher than 10% showed little improvement in the removal rates of Zn^{2+} and SO_4^{2-} .

The reasons might be the high toxicity of ZPT to SRB. When the SRB inoculum concentration was low at 5%, ZPT inhibited SRB activity and inactivated most of the bacteria, making it difficult for SRB to multiply rapidly. Low ZPT concentrations (1 mg/L) showed low

toxicity to SRB, while strong inhibition was observed at high concentrations of ZPT. As seen in Fig. 5(b), 3.0–5.0 mg/L ZPT significantly inhibited the growth of SRB at an inoculum concentration of 5%. However, 3.0–5.0 mg/L ZPT was tolerable to SRB when the inoculum concentration was more than 10%.

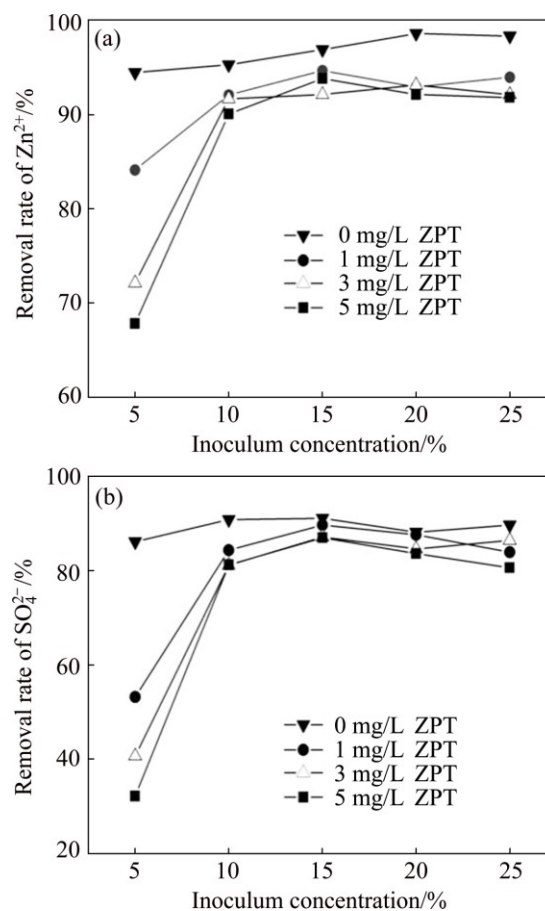


Fig. 5 Effect of inoculum concentration on Zn^{2+} (a) and SO_4^{2-} (b) removal rates by SRB consortium at four different concentrations of ZPT and initial Zn^{2+} concentration of 100 mg/L

3.6 Removal of Zn^{2+} and SO_4^{2-} in systems using SRB+ Fe^0

The removal rates of Zn^{2+} and SO_4^{2-} in different systems were investigated over time and the results are shown in Fig. 6. In SRB system, the residual Zn^{2+} concentration showed a consistent pattern of the first up and then down fluctuation. This fluctuation increased with an increased ZPT concentration. However, there was no evident fluctuation of Zn^{2+} removal in SRB+ Fe^0 system. SO_4^{2-} removal rates in SRB+ Fe^0 system and SRB system without ZPT reached more than 90% even on the 5th day after inoculation, and the removal rates on the 7th day could reach 91.9% and 90.8%, respectively. In contrast, SO_4^{2-} removal rate with ZPT was stably increasing until the end of experiment, correspondingly arriving at 84.4%, 81.2%

and 81.2% under the ZPT concentrations of 0, 1.0, 3.0 and 5.0 mg/L. From Fig. 6(b), it can be seen that the consumption rate of SO_4^{2-} in this study decreased as the ZPT concentration increased. Comparing three concentrations of ZPT, the final removal rates of SO_4^{2-} had no significant difference on the 7th day, which were much lower than that of the system without ZPT.

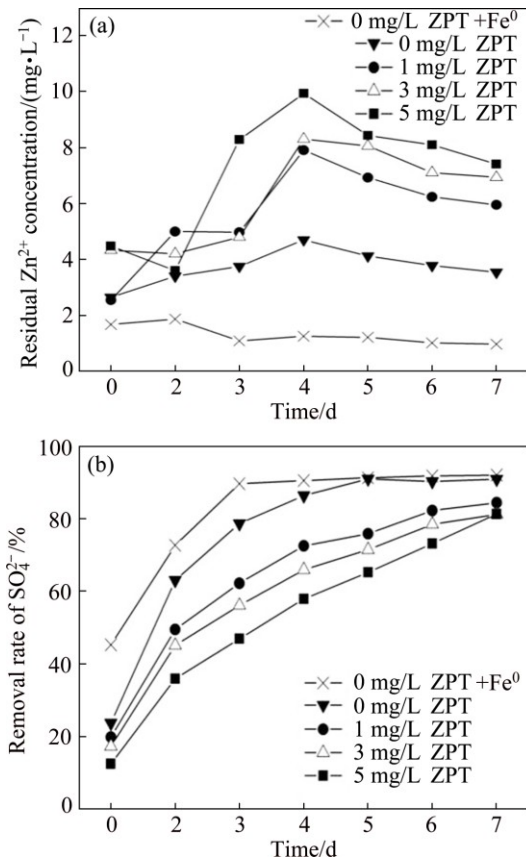


Fig. 6 Variations of residual Zn^{2+} concentration (a) and SO_4^{2-} removal rate (b) with time under four different concentrations of ZPT in different systems at initial Zn^{2+} concentration of 100 mg/L

The mechanisms of zinc removal in SRB system were sulfide precipitation and integral components of biosorption [24]. Biodegradation was the major mechanism of Zn^{2+} removal on the first day since the removal rate of SO_4^{2-} on the first day was very low and the residual Zn^{2+} concentration increased until the 4th day. The reason for the decrease in removal rate of Zn^{2+} within 4 d was that the activity of SRB was constrained seriously by ZPT and ZnS , which weakened the effect of biosorption. The mechanisms of Zn^{2+} removal in SRB+ Fe^0 system were rather complex, including sulfide precipitation, biosorption and reductive precipitation [25].

3.7 Optimization by Box–Behnken design

The Box–Behnken design was applied in this study

and 17-experimental runs were conducted at orders randomly for the optimization of sulfate removal. The effects of key parameters, pH (X_1), temperature (X_2) and ZPT concentration (X_3) on sulfate production were evaluated. According to the RSM results in regard to the response variables of sulfate removal rate, which were acquired from 17 groups of experiments with the help of Design-Expert software, regression analysis of data from Table 2 resulted in the following quadratic expression (see Eq. (5)):

$$Y_{\text{SO}_4^{2-}} = 85.19 + 6.40X_1 + 15.48X_2 - 7.69X_3 + 3.75X_1X_2 - 1.12X_2X_3 - 14.08X_1X_3 - 39.77X_1^2 - 24.76X_2^2 - 12.42X_3^2 \quad (5)$$

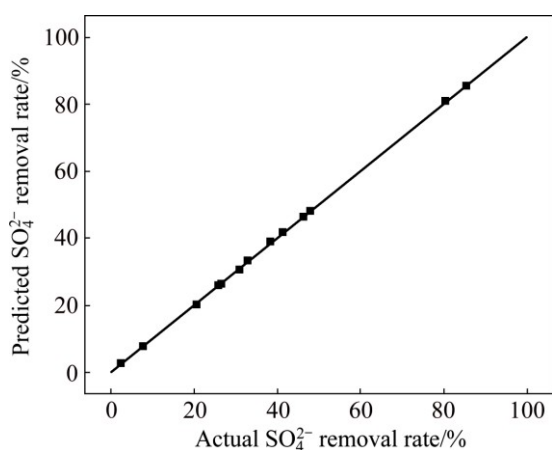
The results of analysis of variance (ANOVA) are listed in Table 2. Statistical testing was carried out by the calculated Fischer values (F -test) and probability values (p -value). The corresponding parameter is more significant if its p -value is smaller than 0.05 at 95% confidence level [26]. Obviously, as shown in Table 2, the model F -value of 7339.99 and values of “ p -value> F ” less than 0.0001 indicated that the model was significant. There was only a 0.0089% chance that a large “Model F -value” could occur due to noise. The “lack of Fit F -value” of 31.11 implied that the lack of fit was significant. In this study, the independent variables of the quadratic model pH X_1 , temperature X_2 and ZPT concentration X_3 , the interaction between pH and temperature (X_1X_2), the interaction between pH and ZPT concentration (X_1X_3) and the interaction between temperature and ZPT concentration (X_2X_3) were quite significant because the p -value was less than 0.05. Judging by the F -values of the items in the regression model, the temperature (X_2) had the highest F -value (9000.28) with the lowest p -value (<0.0001) among other parameters, so the degree of importance of the three parameters on SO_4^{2-} removal rate was: temperature X_2 >ZPT concentration X_3 >pH X_1 . The quite high R^2 values of 0.9999 for SO_4^{2-} removal rate indicated that the predicted polynomial model was reasonably well fitted with the data. The predicted R^2 (Pred) values of 0.9998 for sulfate removal rate were in reasonable agreement with the adjusted R^2 (Adj) values of 0.9987 for SO_4^{2-} removal rate. The comparisons between experimental and predicted values of SO_4^{2-} removal rate were exhibited graphically with 45 °C-lines in Fig. 7. Very little deviation was discovered between points that represented experimental values and the regression line that represented predicted values, indicated a satisfactory degree of precision and reliability of the experimental values.

Figure 8(a) clearly represented the effects of pH (X_1) and temperature (X_2) on the sulfate removal rate, when the ZPT concentration (X_3) was fixed at 2.5 mg/L. The three-dimensional response surface plot suggests

Table 2 Analysis of variance (ANOVA) for sulfate removal rate

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value>F
Model	14061.55	9	1562.39	7339.99	<0.0001
pH, X_1	327.81	1	327.81	1540.01	<0.0001
Temperature, X_2	1915.81	1	1915.81	9000.28	<0.0001
ZPT concentration, X_3	519.4	1	519.4	2440.1	<0.0001
X_1X_2	56.4	1	56.4	264.96	<0.0001
X_1X_3	5.04	1	5.04	23.68	0.0018
X_2X_3	793.55	1	793.55	3728.02	<0.0001
X_1^2	6630.2	1	6630.2	31148.11	<0.0001
X_2^2	2571.22	1	2571.22	12079.36	<0.0001
X_3^2	642.04	1	642.04	3016.25	<0.0001
Residual	1.49	7	0.21		
Lack of fit	1.45	4	0.36	31.11	0.0089
Pure error	0.035	3	0.012		
Cor. tal	14063.04	16			

$R^2=0.9999$; R^2 (Adj)=0.9998; R^2 (Pred)=0.9987

**Fig. 7** Comparison of experimental SO_4^{2-} removal rate with calculated one via Box–Behnken design (BBD) resulted equation

that the interaction effect of pH and temperature significantly influenced sulfide production (p -value <0.0001). At the lower pH, sulfate removal rate decreased gradually at the lower or higher of the temperature. In addition, when a high level of temperature was applied (40 °C), the removal rate of SO_4^{2-} was found to increase and then decline by increasing pH. Temperature and pH were key factors influencing the efficiency of sulfide production, which could affect the activity of the enzyme in the reaction of sulfate reduction [22]. However, the SO_4^{2-} removal rate was more sensitive to temperature. Figure 8(b) represents response surface plot of two variables pH (X_1) and ZPT concentration (X_3) while temperature (X_2) was kept constant at 27.5 °C. With pH at low levels, SO_4^{2-} removal rate was higher with the decrease of the ZPT concentration owing to the increase

of toxicity of ZPT in high ZPT concentration. On the other hand, with pH at high levels, the SO_4^{2-} removal rate was found to increase first followed by a slight decline with the increasing pH. That was probably because enzyme activity was suppressed under acid–base environment. The result is consistent with AHMADI et al [27], who suggested that there was a strong correlation between the SO_4^{2-} removal rate and the pH. The lower the SO_4^{2-} removal rate was, the lower the observed solution pH was. PIKUTA et al [28] also reported that the reduction of sulfate was usually suppressed at pH values lower than 6 or higher than 9. When the pH value was below 6, the production of the undissociated form (H_2S) was a strong inhibitor of SRB [29].

Figure 8(c) illustrated the effect of temperature (X_2) and ZPT concentration (X_3) on sulfate removal rate at pH value of 7.25. SO_4^{2-} removal rate was sensitive to changes in temperature and ZPT concentration, which has the greatest significant interaction on sulfide production (F -value=3728.02, p -value <0.0001). It was obvious that the SO_4^{2-} removal rate decreased rapidly with the increase of ZPT concentration under low temperature condition, while the SO_4^{2-} removal rate decreased slightly under high temperature condition. It may be because the toxicity of ZPT to SRB was weakened under the high temperature condition. Most importantly, all these results are consistent with the single factor experiment.

Response optimization technique contributed to identifying a production of a combination of input variables, which collectively optimized a single response or a set of responses [30]. It is useful to note that the goal

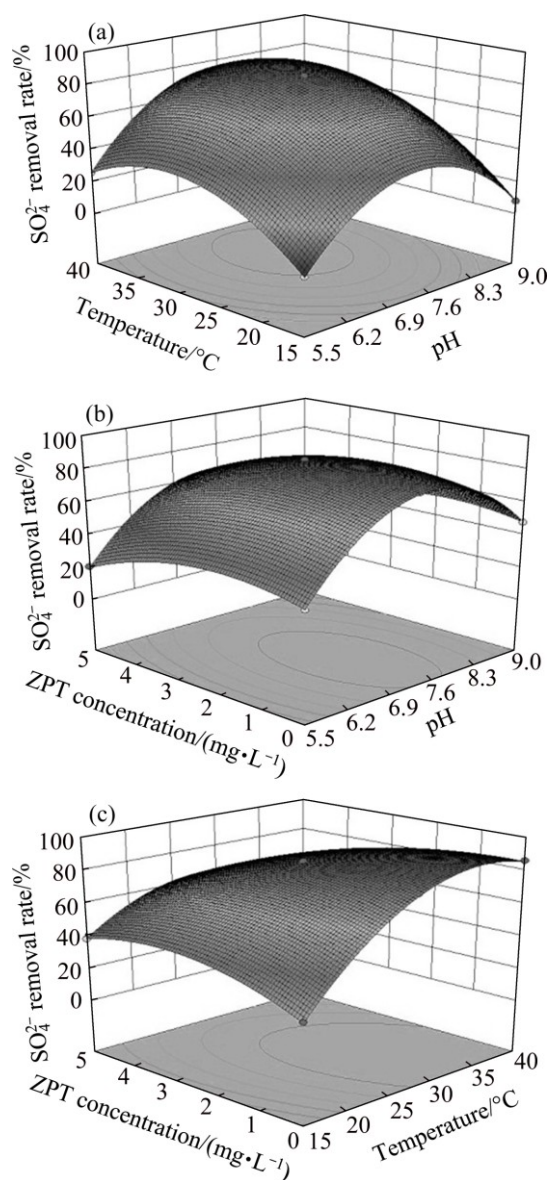


Fig. 8 3D surface plots of SO_4^{2-} removal rate as function of pH and temperature (a), pH and ZPT concentration (b) and temperature and ZPT concentration (c)

of optimization is to find a good set of experimental conditions. The optimum conditions proposed by the model were initial pH 7.45, temperature 33.61 °C, and ZPT concentration 0.62 mg/L, at which maximum SO_4^{2-} removal rate of 91.62% was achieved. Similar results regarding to pH and Cr(VI) removal rate can be found in the work of AHMADI et al [27], where pH 7.5 was enough to reach the maximal Cr(VI) removal rate. In order to confirm the reliability and accuracy of the predicted value, the predicted conditions were validated by conducting an experiment twice for the reproducibility of the data [26]. As shown in Table 3, the SO_4^{2-} removal rate of 91.37% (average of three replicates) was obtained by using optimized conditions,

which was nearly 0.25% lower than the predicted value. The results were economically and technically feasible. It also confirmed that RSM was a powerful tool for optimizing the operational conditions of biodegradation experiment with great accuracy.

Table 3 Process parameters for maximum SO_4^{2-} removal rate

Parameter	Value
SO_4^{2-} removal rate/%	91.62
X_1 (pH)	7.45
X_2 (temperature)/°C	33.61
X_3 (ZPT concentration)/(mg·L ⁻¹)	0.62

4 Conclusions

1) Sulfate reduction by SRB and consequent precipitation showed the potential to be cost-effective for simultaneous removal of high concentrations of Zn^{2+} and SO_4^{2-} from wastewater of ZPT production. High removal rates for both Zn^{2+} and SO_4^{2-} in ZPT production wastewater reached under conditions of 30–35 °C, pH 7.0–8.0 and inoculum concentration of 10%–25%.

2) ZPT inhibited the growth of SRB, resulting in the decrease of removal rates of Zn^{2+} and SO_4^{2-} . The inhibition by ZPT was enhanced with an increased ZPT concentration. In addition, the presence of Fe^0 could not only enhance Zn^{2+} and SO_4^{2-} removal rates, but also increase the resistance of SRB to ZPT toxicity.

3) The effects of variables using RSM, optimal operating conditions were found to be: pH 7.45, 33.61 °C, and ZPT concentration of 0.62 mg/L. Under these conditions, the removal rate of SO_4^{2-} was found to be 91.62%, which was consistent with the overlay plot results. Therefore, RSM could be effectively adopted to optimize the operating multifactor in complex biodegradation process.

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生物法处理吡啉硫酮锌生产废水中 高浓度锌离子和硫酸根离子

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摘要: 采用富集和驯化后的硫酸盐还原菌处理吡啉硫酮锌生产废水, 并考察不同实验参数对硫酸盐降解和锌沉淀的影响。单因素实验结果表明: 当吡啉硫酮锌浓度为 3.0~5.0 mg/L 时, 锌离子和硫酸根离子的去除率呈明显下降趋势。当温度为 30~35 °C、pH 为 7~8、接种量为 10%~25% 时, 锌离子和硫酸根离子都能得到有效去除。零价铁不仅能提高硫酸盐还原菌对硫酸根离子和锌离子的去除效果, 还能加强硫酸盐还原菌对锌离子和吡啉硫酮锌毒性的抗性。此外, 采用 Box-Behnken 法研究主要实验参数对硫酸盐去除率的影响。得到最佳实验条件如下: pH 7.45、温度 33.61 °C、吡啉硫酮锌浓度 0.62 mg/L。在最佳实验条件下硫酸根离子的去除率能达到最大值 91.62%。
关键词: 生物处理; 硫酸盐还原; 硫酸盐还原菌; 废水; 吡啉硫酮锌; 锌

(Edited by Wei-ping CHEN)