Characterization of sulfate reducing bacteria isolated from urban soil

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Abstract. Sulfate reducing bacteria (SRB) was isolated from urban soil and applied for the remediation of heavy metals pollution from acid mine drainage. The morphology and physiological characteristics (e.g. pH and heavy metals tolerance) of SRB was investigated. The SRB was gram-negative bacteria, long rod with slight curve, cell size 0.5× (1.5-2.0) μm. The pH of medium had significant effect on SRB growth and the efficiency of sulfate reduction, and it showed that the suitable pH range was 5-9 and SRB could not survive at pH less than 4. The maximum tolerance of Fe (II), Zn (II), Cd (II), and Cu (II) under acidic condition (pH 5.0) was about 600 mg/L, 150 mg/L, 25 mg/L and 25 mg/L, respectively. The result indicated that SRB isolated in this study could be used for the bioremediation of acid mine drainage (pH>4) within the heavy metals concentrations tolerance.

1. Introduction

Acid mine drainage (AMD) can be formed by the oxidation of sulfide minerals when they are exposed to water and air. AMD is one of the most serious environmental issue to the mining area because of its strong acidity and high concentrations of heavy metals. Microbial remdiation by sulfate reducing bacteria (SRB) has been considered as one interesting biological process. SRB can reduce sulfate to hydrogen sulfide when supplied with reliable organic carbon, and metal sulfide precipitation can be formed during the remediation process. However, one reported problem associated with sulfate reducing process is the negative effect of metal toxicity which can inhibit SRB growth and affect AMD remediation. SRB growth will be completely inhibited if heavy metal ions concentrations reach a certain extent. In this study, one strain of SRB was isolated from urban soil and physiological characteristics of SRB were studied. The effect of pH and heavy metal to SRB was also investigated. The study will be helpful for SRB used for the remediation of heavy metals pollution from acid mine drainage.

2. Materials and Methods

2.1. Isolation of SRB

In this experiment, one strain of sulfate-reducing bacteria was isolated from Jinan urban soil. A modified Postgate growth medium was used in all experiments. The medium was composed of: KH_2PO_4 0.5 g/L; NH_4Cl 1.0 g/L; Na_2SO_4 4.5 g/L; $CaCl_2{\cdot}6H_2O$ 0.06 g/L; $MgSO_4{\cdot}7H_2O$ 0.06 g/L; sodium lactate 6 g/L; yeast extract 1 g/L; $FeSO_4{\cdot}7H_2O$ 0.5 g/L; sodium citrate$\cdot 6H_2O$ 0.3 g/L. The pH of

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the medium was not adjusted at about 5.5. The experiment was conducted in anaerobic conditions at 30°C in a biochemical incubator for 7 days. The soil soaking solution was added into a stoppered flask filled with Postgate liquid culture, sealed until the culture medium became dark black, and lead acetate test paper turned black, and strong smell could be smelled. It indicated that SRB growth was good. Enrichment and isolation described above were repeated 3-5 times.

Spread plate method was used for isolation of SRB, and the plate was placed in the biochemical incubator for about 5-7 days for black colonies growth, and then pick well-growing single colony, inoculated in liquid medium for enrichment. The isolation described above was repeated 3-5 times. Finally the well-grown sulfate-reducing bacteria were isolated.

2.2. Physiological Characteristics of SRB

The morphology of the SRB strain was observed by scanning electron microscopy (SEM, FEI Quanta FEG250). Test of SRB growth curve: Postgate medium with 5% inoculation amount with the initial pH of 5.0 and 7.0, was placed in a biochemical incubator at 30°C for 7 days. The concentration of sulfate was collected and the growth of bacteria was measured periodically. Test of different carbon sources for SRB: sodium lactate, yeast extract, sodium acetate, ethanol or sodium citrate as a single carbon source was used as the potential carbon source. Considering acid mine drainage remediation during sulfate reducing process, the acidic environment and heavy metals in acid mine drainage have inhibitory effect on SRB activity, so the effect of pH and heavy metal ions on SRB growth will be analyzed.

2.3. Effect of pH on SRB growth

SRB is very sensitive to the pH of the growing environment, which directly affects SRB growth and the reduction efficiency of sulfate. In the Postgate medium with 5% bacterial solution was inoculated with the initial pH of 2, 4, 5, 6, 7, 8, 9, and 10 in the biochemical incubator at 30 °C for 4 days. The concentration of sulfate and SRB growth were measured periodically.

2.4. Effect of heavy metals on SRB activity

The effect of heavy metals on SRB activity was studied by the following batch test. Modified Postgate medium described above was used as control medium for SRB's tolerance toward different concentrations of heavy metal ions. The experiments were conducted in anaerobic conditions at 30°C in a biochemical incubator for 14 days, using 150 mL Erlenmeyer flask with stopper containing 100 mL of growth medium with 5 mL of SRB inoculum of log phase cells (pH around 5.5, not adjusted). The pH, Eh and sulfate concentration of solution were measured to study the tolerance of SRB toward heavy metals. The effects of pH and temperature on SRB activity was also studied by similar batch tests.

2.5. Analytical methods

The pH and Eh of samples were measured immediately after collection. Sulfate concentration was determined using the turbidimetric method. OD_{600} was measured by UV-VIS spectrophotometer (Shimadzu). The samples were filtered and acidified with one drop of nitric acid and atomic absorption spectroscopy (AAS) (AA-7000 model spectrometer, Shimadzu) was used for the measurement of the concentrations of Fe, Zn, Cu and Cd.

3. Results and Discussions

3.1. The morphology of the SRB

The soil soaking solution was added to the liquid medium and sealed for 7 days for SRB enrichment. The SRB were grown in large quantities (Fig.1). Gram staining indicated that the strain was gram-negative. It was found that the isolated SRB strain had a slightly curved rod shape with a size of about

0.5 × (1.5-2) μm by scanning electron microscopy. The identified SRB strain belongs to *Desulfovibrio*, and it can be used sodium lactate, ethanol, yeast extract and sodium citrate as the electron donor.

3.2. Growth curve

The growth curve of isolated SRB strain is shown in Fig.2. The strain growth was in lag phase in16h without H_2S production and lead acetate test paper did not turn black. Sulfate concentration did not significantly decrease. In 16-48 h the strain shifted from lag phase to the logarithmic growth phase, and the concentration of sulfate decreased sharply from 3372mg/L to 964mg/L (at pH 7.0), and a large amount of H_2S was generated. After 48-72h, the OD_{600} reached the maximum (0.66) and entered the stable period, and the Eh remained -380 ~ -400 mV. After 72 hours, OD_{600} showed a downward trend (0.46-0.53), indicating the strain entered the decay period. At pH 5.0, the lag phase of SRB strain was significantly increased to 24 h, and the reduction rate of sulfate were decreased compared with the condition at pH 7.0.



Figure 1. The isolated SRB in liquid medium

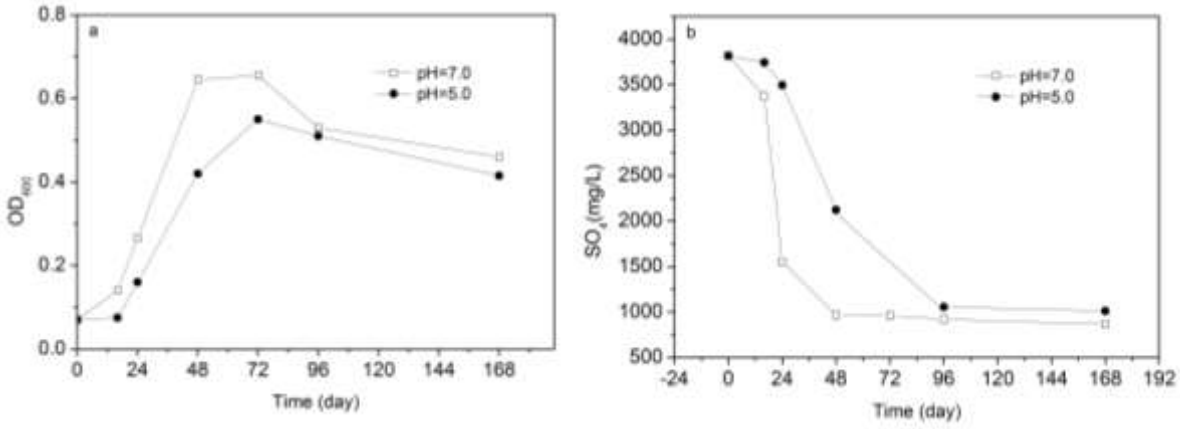

Figure 2. Growth curves of SRB

3.3. Effect of pH

Solution pH has an important effect on the growth of SRB. Although eosinophilic SRB strains in extreme acidic wastewater were isolated by some scholars, most of SRBs have a suitable pH range of 5-8. The effect of pH on the growth of SRB strains isolated from this experiment is shown in Fig.3.

The results showed that the optimum growth pH of the isolated SRB was 7, and the SRB could grow well at pH 5-9, and the concentration of sulfate decreased obviously. When the pH was less than 4 and more than 10, the growth of the strain was inhibited, and no H_2S was produced, and the sulfate concentration did not decrease obviously. It was found that although the isolated SRB was able to grow under the conditions of pH 5-9, lag phase was significantly different. The lag phase was only 16 hours at pH 6-8, and it was 24 hours pH 5 or 9. Therefore, the provision of appropriate pH is an important prerequisitesto improve the SRB growth activity and effective remediation of acid mine drainage.

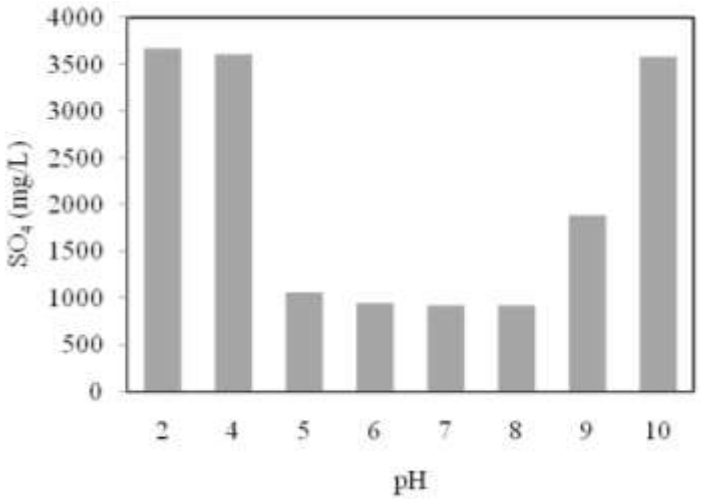

Figure 3. Effect of pH on sulphate reduction by SRB

3.4. Heavy metals tolerance

The effects of different concentrations of Fe (II), Zn (II), Cd (II) and Cu (II) on SRB growth are shown in Fig.4. It showed that the removal of sulfate was generally decreasing with the increase of heavy metal ion concentration. The maximum tolerable concentrations of SRB to Fe (II), Zn (II), Cd (II) and Cu (II) were 600 mg/L, 150 mg/L, 25 mg/L and 25 mg/L. In tolerable concentrations, SRB growth was good which was evidenced that sulfate concentration decreased significantly, the solution pH increased to 6 and the Eh decreased to below -100 mV.

When the concentration of Fe(II) was 800 mg/L, the solution Eh was postive (91 mV) and pH was 4.98 (slightly lower than the initial pH 5.0), indicating that the SRB strain was completely inhibited and could not tolerate Fe (II) at 800mg/L. It was found that the lag phase of SRB growth was prolonged with the increase of heavy metal concentration. The lag phase of SRB growth was 1 day at Fe 100 mg/L, lag phase was 4 days with Fe 600 mg/L, and SRB will stop growing at Fe 800 mg/L. The lag phase of SRB growth was 3 days at Zn 25 and 50 mg/L, and SRB displayed a prolonged lag phase of 4 days at Zn 100 mg/L. SRB will stop growth at Zn 150 mg/L. It indicated that heavy metal ions had a significant impact on the SRB growth.

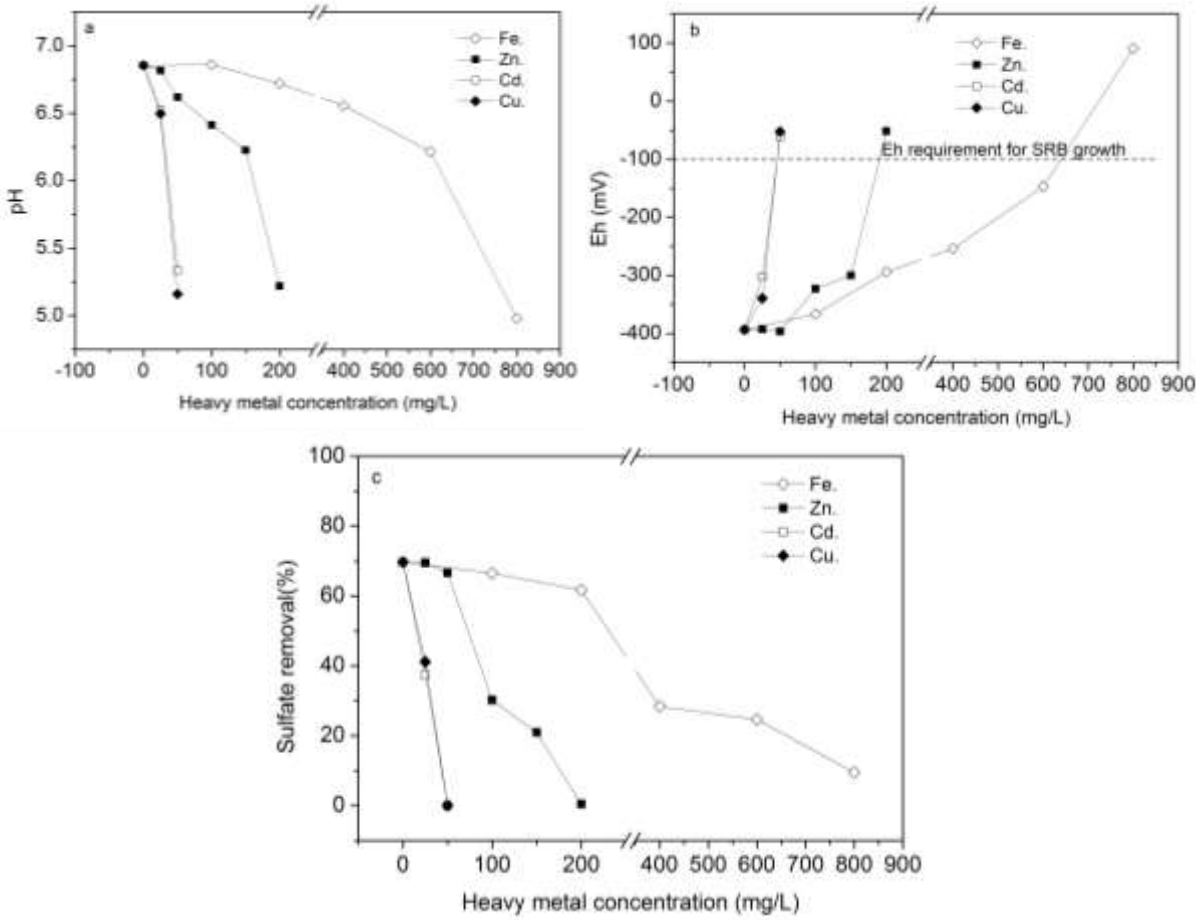

Fig. 4 The tolerance of heavy metals, (a) pH variation, (b) Eh variation, (c)Sulphate reduction varitation

4. Conclusion

The SRB isolated from soil could not survive at pH less than 4, and the suitable pH range was 5-9. The maximum tolerance of Fe (II), Zn (II), Cd (II), and Cu (II) under acidic condition (pH 5.0) was about 600 mg/L, 150 mg/L, 25 mg/L and 25 mg/L, respectively. The result indicated that SRB isolated in this study may be used for the bioremediation of acid mine drainage (pH >4) within the heavy metals concentrations tolerance.

Acknowledgments

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