Bioremediation of heavy metals pollution from acidic coal gangue with sulfate-reducing bacteria

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Abstract. Acid mine drainage (AMD) generated by acidic coal gangue has caused coal mine area environmental degradation. Sulfate-reducing bacteria (SRB) was used to inhibit the oxidation of metal sulfide minerals from acidic coal gangue in Kaili city, Guizhou province. Four experimental groups (named as CK, R-M, R-S, R-SCa) were carried out, the results showed that SRB can effectively raise the pH (2.3 in CK, 7.8 in R-SCa) and inhibit heavy metals leachability. The low initial pH of the system can retard the start-up of sulfate reduction process. The leaching toxicity test indicated that the leachate of heavy metals in coal gangue after bioremediation was significantly reduced. High-throughput sequencing analysis showed the diversity of SRB genera in R-SCa (e.g., Desulfotomaculum and Desulfobulbus was detected) was enhanced in comparation to R-M and R-S. The results demonstrated that SRB significantly inhibit acidic coal gangue contamination and the biostimulation is also a potential solution due to indigenous SRB existence.

1. Introduction

As China main energy and industrial raw materials, coal plays a vital role in China economic development [1]. Although Chinese government continues to optimize the domestic energy structure, the proportion of coal in total energy consumption still in a non-negligible position[1].

The intensive demand for coal leading to the accumulation of coal gangue is generated by coal extraction and process. In China, the total accumulation of coal gangue has exceeded 5 billion tons, and the data continues to grow with a 300 to 350 million tons per year. It was estimated that coal gangue production will reach 790 million tons in 2020, making coal gangue became a huge environmental concern in China[3].

Pyrite (FeS₂) in coal gangue is main sources to form Acid Mine Drainage (AMD) with low pH and rich in metalloid through chemical and biological oxidation, which can cause severe pollution in mine area for centuries. The heavy metals in coal gangue can be dissolved due to the change of pH value, thus acidity removal is critical in the treatment of AMD[3].

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Sulfate-reducing bacteria (SRB) have attracted widespread attention in the treatment of acid mine wastewater from sulfide mines area, because they can raise the pH of the system through oxidation of organic matters (as electron donor) and reduction of $\text{SO}_4^{2-}$ (as electron acceptor) (Equation 1), reduce the environmental redox potential, precipitate heavy metal ions by sulphide (Equation 2), without secondary pollution, low operating costs, and recover monomeric sulfur or sulfide[3]. In this study, the control of acid production on coal gangue was investigated from the analysis of leaching water samples, post-remediation coal gangue stability and microbial community structure in each experimental groups.

$$2\text{CH}_2\text{O} + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{S}$$ (1)

$$\text{Me}^{2+} + \text{H}_2\text{S} \rightarrow \text{MeS} \downarrow + 2\text{H}^+$$ (2)

2. Materials and Method

2.1. Characterization of coal gangue
Experimental samples of coal gangue were collected from Yudong River basin, Kaili City, Guizhou province, China. Once arriving lab, all samples were naturally air dried and crushed into powder(< 2mm). Then XRF analysis was performed to identify the chemical composition of coal gangue. Table 1 showed the physical and chemical properties of coal gangue.

| Parameters | pH | Moisture content | SiO$_2$(ω%) | Al$_2$O$_3$(ω%) | Fe$_2$O$_3$(ω%) | SO$_3$(ω%) | K$_2$O(ω%) | MgO(ω%) |
|------------|----|-----------------|-------------|----------------|----------------|-------------|------------|----------|
| Value      | 2.3| 16.4%           | 66.28       | 19.54          | 4.69           | 4.15        | 2.47       | 1.16     |

2.2. SRB cultures Preparing
The SRB consortium came from National Engineering Laboratory of Biohydrometallurgy. The consortium (10% v/v) was inoculated into 200 mL conical flask containing medium (Table 2). The flask was incubated at 30℃ in Electro-thermal incubator for 3 days to obtain the SRB cultures.

| Composition   | Glucose | NH$_4$Cl | Na$_2$SO$_4$ | MgCl$_2$ | K$_2$HPO$_4$ | CaCl$_2$ |
|---------------|---------|----------|--------------|----------|--------------|----------|
| Concentration | 2       | 1.5      | 0.8          | 2        | 0.5          | 0.2      |

2.3. Experimental set up
In order to study the inhibition effect of SRB on acidic coal gangue, four experimental groups were carried out, the details and information was shown in Table 3.

| Groups | Experimental conditions |
|--------|-------------------------|
| CK     | 100g coal gangue+250ml deionized water |
| R-M    | 100g coal gangue+250ml medium |
| R-S    | 100g coal gangue+225ml medium+25ml SRB cultures |
| R-SCa  | 100g coal gangue+1g CaCO$_3$+225ml medium+25ml SRB cultures |

2.4. Analysis Methods
The leaching liquid were taken out periodically, pH was measured by pH meter (Thermo Scientific Orion 3-Star, Germany), Eh was measured by Eh meter (pH/ORP controller PC-350, China), the concentration of heavy metals were determined by ICP-OES (Agilent 725, USA). High-throughput sequencing analysis of the 16S rRNA gene was performed for determining bacterial diversity and community structure at different experimental groups. At the end of the experiment, the coal gangue was conducted to leaching toxicity extraction procedure (HJ/T299-2007) to verify the stability of remediation.
3. Result and discussion

3.1. pH
The pH is most notable environmental factors contribute to microorganism growth and heavy metals leachability. Figure 1 showed the pH variation in different experimental groups. The pH of R-M, R-S and R-SCa present an upward trend, but R-SCa reached equilibrium earlier than R-M and R-S, because of higher initial pH value neutralized by CaCO₃. For CK group, pH value remained stable throughout the experiment. The final pH values of each group were 2.10, 4.42, 6.24 and 7.90, respectively.

![Figure 1. pH variation in different experimental groups.](image1)

3.2. Eh
Figure 2 showed Eh change in different experimental groups. The redox potential variations in CK group were not significant, and the changes in the R-M and R-S groups were almost identical, both remaining stable in the early stages and decreasing significantly around 30 days. This was perfectly synchronized with the pH changes in Figure 1. At 50 days, the redox potential of two groups were 139mV and -79.33mV, respectively. The R-SCa group had lower potentials value than others from 0 to 16 days, and the decrease trend was obvious. However, the redox potential was increased after 16 days, which may be due to the heavy metal concentrations were already close to zero in the system. Thus the hydrogen sulfide produced by SRB can’t be consumed, leading to its accumulation in the system and inhibiting the growth of sulfate-reducing bacteria.

![Figure 2. Eh variation in different experimental groups.](image2)
3.3. Heavy metals
As shown in Figure 3, in R-SCa group, leachability of heavy metals was minimal and all heavy metals were undetectable in aqueous solution through precipitated as metallic sulfides by SRB (black precipitation formation). The R-M group showed the same behavior of R-S group, heavy metal content in water first increased and then decreased. But the heavy metal removal rate was higher in R-S than in R-M (Fe: 68.13%, 81.73; Mn: 7.23%, 44.13%; Ni: 57%, 98.04%; Zn: 72.37%, 100%). For CK group, the highest leaching concentration of Fe was 818.103 mg/L, which is higher than R-M and R-S. While Mn, Ni and Zn were similar to other groups. In addition to a slow decrease in Fe concentration may probably due to absorption, Mn, Ni, Zn did not show a decreasing trend.

3.4. Microbial community analysis
The relative abundance of SRB at genus level were shown in Figure 5. The results indicated that R-SCa group have the richest typical SRB, containing six genera, Desulfosporosinus, Desulfovibrio, Desulfotomaculum, Desulfobulbus, Desulfitobacterium, and Desulfurella, the relative abundance was 0.17%, 2.88%, 0.28%, 0.14%, 0.1% and 0.01%, respectively. In contrast, R-M and R-S contain only Desulfosporosinus and Desulfovibrio, and the Desulfosporosinus abundance is significantly higher than that of Desulfovibrio, which is also in contrast to the results of R-SCa. According to these data, we can infer that Desulfosporosinus was the indigenous bacteria in coal gangue, because it had higher abundance in R-M than experimental groups with exogenous bacteria and wasn’t detected in SRB cultures.
3.5. Toxicity characteristic leaching procedure
According to the figure 4, no relevant ion concentration was detected, except for Fe, which reached 72.44 mg/L in CK group, 1.485 mg/L in R-M, 1.578 mg/L in R-S, and 0.974 mg/L in R-SCa. The results provide a good indication that SRB can effectively improve the stability of heavy metals in coal gangue and reduce the pollution potential of coal gangue.

4. Conclusions
Sulfate-reducing bacteria (SRB) have great potential in environmental remediation. This study confirmed that inoculate SRB can effectively inhibit the acidity and heavy metal pollution of coal gangue. In R-SCa group, pH value was rapidly increased to neutral within 16days, and ultimately, all metallic ions in leaching liquid were lower than detection limit. The leaching toxicity extraction procedure of coal gangue after remediation further proves the feasibility of this method. The microbial community structure reveals the introduction of exogenous SRB can effectively increase the diversity of SRB in the system. The existence of indigenous SRB in coal gangue also be proved, so biostimulation can be a promising alternative for bioremediation of acidic coal gangue.

Acknowledgments
This research was supported by the National Natural Science Foundation of China [grant numbers U1402234, 41573074, and 51974279], the National Key Research and Development Program of China [grant numbers 2018YFC18018, 2018YFC18027 and 2019YFC1805903], KeJunPing [2018]
No. 159, the Guangxi Scientific Research and Technology Development Plan [grants number GuikeAB16380287 and GuikeAB17129025].

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