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Bench-scale microbial remediation of the model acid mine drainage: Effects of nutrients and microbes on the source bioremediation

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ABSTRACT

In this work, a bench-scale microbial remediation system was established by supplemented nutrients, sulfate reducing bacteria and iron reducing bacteria to inhibit key factors (iron oxidizing bacteria and oxidation reduction potential) during the formation of acid mine drainage. The results indicated sodium lactate could inhibit the growth of *A. ferrooxidans* and their ability of oxidizing ferrous ion, could alleviate but not prevent the pyrite oxidizing process. Organic nutrients prevented pyrite oxidation effectively by the adjustment of microbial community. Sulfate reducing bacteria adapted to the remediate environment and improved physicochemical index of group 1. Sulfate reducing bacteria and nutrients are the key factor for bioremediation.

1. Introduction

Due to the characteristics of large mount, low pH, high concentration and various of heavy metals, long duration and serious pollution (Valente et al., 2013), acid mine drainage (AMD) has been an increasingly serious issue in most mines (Akcil and Koldas, 2006). In the condition of water, oxygen and iron oxidizing bacteria existed, sulfides, especially the pyrite oxidation, was the main source of AMD (Johnson and Hallberg, 2005): (Equation (1))

$$4FeS_2 + 15O_2 + 14H_2O = 4Fe(OH)_3 + 8SO_4{}^{2-} + 16H^+ \quad (1)$$

Intermediate reaction was the oxidation of ferrous ion (Equation (2)) and other metals (Equation (3))

$$4Fe^{2+} + O_2 + 4H^+ = 4Fe^{3+} + 2H_2O \quad (2)$$

$$MeS + 2Fe^{3+} = Me^{2+} + 2Fe^{2+} \quad (3)$$

Pyrite oxidation follows the electrochemistry mechanism, and the critical potential (Yeqing et al., 2000) is 600 mV (vs. selective hydrogen electrode (FSHE)). With the oxidation reduction potential (ORP) increasing, pyrite oxidizes more violently in above-critical potential environment. The under-critical potential environment is helpful to prevent pyrite oxidation and AMD formation. However, ORP of the environment is controlled by the ratio of $[Fe^{3+}]$ and $[Fe^{2+}]$, Equation (4):

$$E = 0.771 + 0.0591 \lg \frac{[Fe^{3+}]}{[Fe^{2+}]} \quad (4)$$

Without biological factors, the oxidation process of ferrous ion is the controlling step of AMD formation. But iron oxidizing bacteria, especially the *Acidthiobacillus ferrooxidans* can oxidize ferrous iron to ferric iron and elevate the ORP, this process can accelerate pyrite oxidation process by 10^6 times, and facilitates the formation of AMD (Singer and Stumm, 1970).

Recently, a large number of researches were focusing on the microbial remediation of AMD; especially the remediation by sulfate-reducing bacteria (SRB) and iron reducing bacteria (IRB) was attracting increasing attention. SRB have been proved efficient in the treatment of AMD (Liu et al., 2013), promoting the pH and recovering heavy metals (Martins et al., 2009). In the SRB remediation process, sulfate is reduced to sulfide, then the dissolved metals ion are precipitated as metal sulfides and the concentration of heavy metal ions in solution will decrease (Barbosa et al., 2014; Lee et al., 2014). At the same time, the hydrogen ion is consumed, pH will increase. IRB could reduce Fe (III) to ferrous ion (Equation (5)), which contributes to consume acid and maintain low ORP level (Johnson and Hallberg, 2005). Therefore, the use of SRB for AMD remediation would become an attractive biotechnology in the

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future.

$$Fe(OH)_3 + e + 3H^+ \rightarrow Fe^{2+} + 3H_2O \quad (5)$$

The key to control AMD at source lies in inhibiting multiple factors of the pyrite oxidation process. Some infeasible methods such as alkaline lime neutralization, pyrite-coating and bactericide are due to only focus on single factor of the AMD formation (Egiebor and Oni, 2007). In order to prevent the oxidation of pyrite and remediation AMD, source control of AMD can be realized by two key points: inhibiting of *A. ferrooxidans* and promoting of SRB and IRB.

A. ferrooxidans is autotrophic bacteria, which can manufacture complex organic compounds from simple inorganic sources such as carbon dioxide, water, and nitrates (Yuan et al., 2012). However, *A. ferrooxidans* can't utilize organic substances, even could be inhibited or killed by some organic substance (Zhang et al., 2006). On the contrary, heterotrophic SRB and IRB entirely dependent on complex organic substances for growth, the growth of SRB and IRB could be promoted by organic substance. According to the difference of nutritional characteristics between *A. ferrooxidans* and heterotrophic reducing bacteria, the microbial community structure might be adjusted to inhibit AMD produce and promote bioremediation.

In this work, a bioremediation system was investigated, which combined adjustment of microbial community structure with maintaining low ORP level by introducing remediate bacteria SRB, IRB and organic nutrients. This study aims to research the effects of nutrients on the microbial community, investigate the correlation of nutrients, microbial community and AMD remediation.

2. Materials and methods

2.1. Pyrite, bacteria and medium

The bioremediation system was constituted with pyrite, microorganism and medium. High purity (>99.5%) and low granularity (<74 μm) pyrite was used to simulate the easily oxidized sulfide in waste rock pile and tailing pond. Anaerobic microbes containing *Desulfosporosinus, Desulfitobacterium* and *Desulfotomaculum* were used as sulfate reducing bacteria (SRB), *Alicyclobacillus* was used as iron reducing bacteria (IRB), and *A. ferrooxidans* was added to the bioremediation system to simulate the mineral environment. *Desulfosporosinus, Desulfitobacterium*, *Desulfotomaculum, Alicyclobacillus* and *A. ferrooxidans* were isolated from mine tailings in our Lab. Using 9K medium with different concentration of sodium lactate to research the inhibitory effect of organic carbon source on *A. ferrooxidans*, the detailed composition of 9 K medium was: $(NH_4)_2SO_4$ 3.0 g/L, KCl 0.1 g/L, K_2HPO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $Ca(NO_3)_2$ 0.01 g/L, $FeSO_4$ 44.3 g/L, and pH was adjusted to 1.7 by H_2SO_4 (50%, v/v) (Li et al., 2016). The bioremediation medium was consisted of modified 0 K salt (Li et al., 2016), carbon and nitrogen source, and the detailed composition was KCl 0.1 g/L, K_2HPO_4 0.5 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $Ca(NO_3)_2$ 0.01 g/L, Yeast extract 0.1 g/L, sodium lactate 1.0 g/L, and pH was adjusted to 7 with NaOH (2%, w/w) and H_2SO_4 (5%, v/v).

2.2. The inhibitory experiment of sodium lacatae on A. ferrooxidans

This research was conducted by supplementing different concentration of sodium lactate in 9K medium, the concentration of sodium lactate were 0 g/L, 0.1 g/L, 0.3 g/L, 0.6 g/L, 1.0 g/L, 3.0 g/L, 6.0 g/L, respectively. The medium was treated by high temperature sterilization, 121 °C, 30 min. Pyrite was treated by ultraviolet sterilization for 10 min. Pyrite was supplemented in accordance with the proportion of 0.5%. In the condition of 33 °C, 150 r/min, *A. ferrooxidans* were cultured in the medium.

In order to evaluate the growth of *A. ferrooxidans* and its ability of oxidizing ferrous ion, the concentration of *A. ferrooxidans* was determined using real-time PCR (Zammit et al., 2008), and real-time PCR was performed according to the manufacturer's instructions (SYBR Primix Ex Taq, RR420, TAKAKA) with Rotor-Gene 6000 (Corbett Research), all tests were conducted in triplicate. The ORP were determined with ORP meter.

2.3. The bioremediation experiment with reducing bacteria and nutrients

To simulate the site remediation of waste rock pile and tailing pond, the bioremediation system was constructed by adding pyrite, microorganism and the medium in the conical flasks. The experimental groups were listed in Table 1.

According to settings in Table 1 and 1g pyrite was supplemented into 300 mL conical flasks with 200 mL modified 0 K medium, the final concentration of sodium lactate and yeast extract in which were 1.0 g/L and 1.0 g/L respectively. The reducing microbes and iron oxidizing microbes were inoculated with about 4×10^8 respectively. Oxygen limitation in the system was reached by sealing each conical flask with rubber plugs. Conical flasks were put into 33 °C incubator for 28 days. The oxidation of pyrite was investigated every 7 days by analyzing pH, ORP, concentration of total soluble Fe and sulfate.

pH and ORP were detected by pH meter and ORP meter, respectively. The concentration of total soluble Fe was detected by o-phenanthroline spectrophotometric method (Tamura et al., 1974). The concentration of sulfate ion was analyzed by barium sulfate turbidity (Tabatabai, 1974). The microbial community structure was revealed by establishing 16S rDNA clone library (Chen et al., 2009; Liang et al., 2014; Pinar et al., 2013; Zhang et al., 2016). The sequencing data of clone library can be obtained from: https://dx.doi.org/10.6084/m9.figshare.4047252.v1.

3. Results & discussion

3.1. The inhibitory effect of sodium lacatae on A. ferrooxidans

The concentration of bacteria and the ORP change of the

Table 1
Experimental groups for investigating the effect of the bioremediation system.

Numbering	Carbon Source	Nitrogen Source	modified 0K salt	Reducing Bacteria		Iron Oxidizing Bacteria
	Sodium Lactate	Yeast Extract	Modified 9K	SRB	IRB	*A.ferrooxidans*
Group 1	✓	✓	✓	✓	✓	✓
Group 2	✓	✓	✓	–	–	✓
Group 3	✓	✓	✓	–	–	–
Group 4	–	–	✓	–	–	–

Group 1 was the remediate group, as well as the bioremediation system, group 2 was the none-remediate bacteria group, group 3 was the none-bacteria group and group 4 was the blank group without bacteria and organic. Setting parallel experiments for each group decreased the accidental error.

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