



Biodesulfurization of coal with *Acidithiobacillus caldus* and analysis of the interfacial interaction between cells and pyrite

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ABSTRACT

In the present work, the acidophilic and thermophilic strain *Acidithiobacillus caldus* was used for biodesulfurizing coal and bioleaching coal pyrite. The surface characteristics of cells and minerals and the mineral transformation during the coal pyrite leaching process were investigated combined with zeta-potential, FT-IR spectroscopy, scan electronic microscopy and X-ray absorption near edge structure spectroscopy (XANES). The results showed that the coal pyritic desulfurization with *A. caldus* was about 47% and the total desulfurization was 19%. After processed by cells, there was clear corrosion on the pyrite surface. Coal pyrite and elemental sulfur-grown cells had more hydrophilic functional groups than thiosulfate-grown cells. During the coal pyrite leaching course, the elemental sulfur was the main sulfur speciation but no other secondary mineral components were detected.

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1. Introduction

Coal is the most abundant fossil fuel in the world. However, the combustion of coal containing high sulfur causes serious environmental damage such as acid rain because of emission of sulfur dioxide [1]. Sulfur is present in coal mainly in three forms: pyritic, organic and sulfate sulfur. The organic sulfur is the integral part of the coal matrix, whereas pyritic sulfur is present in coal as mineral matter. There are several physical–chemical methods employed to eliminate the organic or inorganic sulfur fractions of coal by floatation, oxidation and reduction with chemicals [2]. Nevertheless, these physical methods cannot remove the pyrite when these sulfides are finely dispersed in coal matrix. Furthermore, although the chemical methods can remove organic and inorganic sulfur from coal, which will also produce hazardous secondary products and lead to loss of partial combustible matter [3,4].

Biodesulfurization offers a clean alternative method to remove sulfur from coals, in which the microbes can catalyze the biochemical reaction in an aqueous medium resulting in the oxidation and dissolving of the sulfur content into sulfate [4,5]. Among of them, the mesophilic microbes, mainly *A. ferrooxidans* and *A. thiooxidans* have been frequently applied to remove the pyrite from coals [4,6,7]. However, in the last decade, limited literatures reported that the thermophilic microbes were used to eliminate the sulfur from coal [8,9]. Recently, *A. caldus* has

been reported as the dominant sulfur-oxidizing thermophilic bacterium accounting for the relatively high percentage in bioleaching pyrite at 45 °C [10,11]. To our knowledge, it has never been used in coal's biodesulfurization process.

It is well known that the attachment of cells on the surface of mineral is reported to be involved in the oxidation of sulfide minerals, which depends not only on the interfacial process between the cells and mineral surface but also the biochemical properties of the cells. Furthermore, it has been reported that the surface properties of bacterial cells are significantly influenced by the growth conditions [12–14]. Hence, a better understanding of the surface properties of functional bacteria under different conditions is helpful to illustrate the interfacial interaction between cells and mineral surface [15,16].

The bio-oxidation of pyrite has been widely investigated and a possible mechanism has been proposed: the stepwise oxidation and the formation of surface-bound thiosulfate, and then oxidized via tetrathionate and other polythionates, finally to sulfate [17–19]. Many investigators agreed that the precipitation of mineral phases during the pyrite oxidation process may affect the efficiency of sulfur removal [20,21]. Besides the reported jarosite, the sulfur-rich layers may also deposit onto the surface of pyrite, thus preventing the contact of the microorganisms and hindering the leaching process [21–23].

The object of this study is to investigate the coal's biodesulfurization and the coal pyrite's bioleaching by thermophilic *A. caldus* cells and the interfacial interaction between cells and coal pyrite surface with the approaches of zeta-potential, FT-IR spectroscopy, SEM and sulfur K-edge XANES.

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2. Material and method

2.1. Coal sample and bacterial strains' culture conditions

The coal sample used in the experiment was collected from Indonesia area named MTWL-7 and the total sulfur of it was 2.15%, which was sterilized at 120 °C for 20 min. The pyrite powders used in the experiments were picked up from the coal samples and the component was analyzed with XRD (data not shown). *A. caldus* derived from the acid hot spring of Yun-nan province, south east of China was cultivated in the Starkey basal salt medium [24] supplemented with sulfur powder, pyrite, coal and thiosulfate, and the initial pH of culture were 2.5, 2.5, 2.5 and 4.8, respectively. The culture of *A. caldus* was grown in 250 mL flasks containing 100 mL medium and incubated at 40 °C with 170 rpm shaking. Bacterial cells were cultured until late exponential phase and harvested by the following procedure: the cultures were filtered three times through filter paper (pore size 7–10 μm) to remove the suspended solid particles and then the filtrates were centrifuged at 10,000 rpm for 15 min, and the cell pellets were washed thrice in diluted sulfuric acid (0.1 mol.L⁻¹) in order to remove any trapped ions, finally all the samples were freeze-dried and stored at -20 °C for zeta-potentials and the FT-IR test.

2.2. Coal biodesulfurization experiment

The biodesulfurization processes were carried out in flasks with a volume capacity of 100 mL in 250 mL of basic salt medium, 10% w/v pulp density, particle size of -65 Tyler mesh, the initial cell concentration was 1.0 × 10⁶ cells.mL⁻¹, processing time of 30 d. Every four days, the pH values, and concentration of iron of biodesulfurization system were determined. At the end of the experiments, the coal samples were prepared with the method mentioned in the literature [25], then the total sulfur and the relative sulfur contents in the coal samples were measured [26].

2.3. Pyrite bioleaching experiment

For leaching pyrite, *A. caldus* cells were inoculated into 250 mL flasks containing 100 mL sterilized culture medium and 3 g pyrite (the initial cell concentration was 1.0 × 10⁶ cells.mL⁻¹, the particle size of pyrite was -200 Tyler mesh), and were then incubated at 40 °C with 170 rpm shaking, processing time of 40 d. Every four days, the pH values, and concentration of iron of bioleaching system were determined. On the other hand, a 10 mL sample was taken from the flasks and centrifuged at 4000 rpm for 10 min. The solid fractions were prepared with the same process as in our previous report [21] and analyzed by FT-IR and SEM and XANES in order to establish mineralogical transformations of pyrite. Triplicate leaching experiments were performed under identical conditions. Parallel experiments (without cells; but the same mixing culture medium and pyrite) were prepared as sterile control.

2.4. Sample analysis

Cells' concentration grown on pyrite containing media was monitored by using a counting chamber; the total iron ions were measured with atomic absorption spectrometer (WFX-110) and the ferrous concentration was determined with the method of potassium dichromate titration.

Table 1
The total sulfur and relative sulfur contents in coal samples.

Sulfur forms (%)	Original coal (%)	Residual coal (%)
Total sulfur	2.15	1.75
Pyrite	1.34	0.71
Sulfate	0.25	0.41
Organic sulfur	0.56	0.63

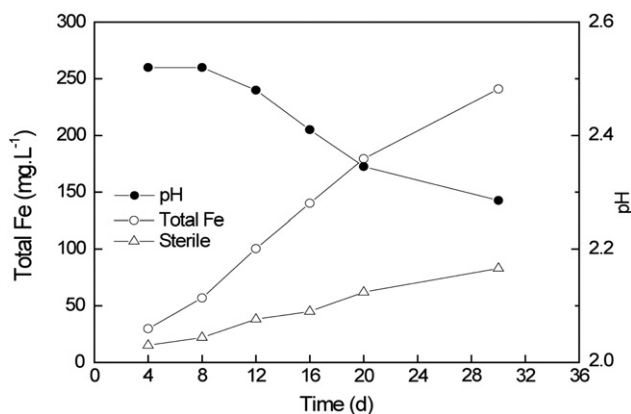


Fig. 1. Growth characteristics of coal-grown *A. caldus*.

Total sulfur in coals was determined by using sulfur analyzer (CTS-3000). For the SEM observation, a 100 μL culture solution (containing mineral powder) was fixed with formaldehyde (final conc. 2.5% V/V). The fixed samples were then dehydrated with ethanol, conductive coated, and then finally introduced into the chamber of the SEM (JEOL JSM-6360 LV) for mineral surface analysis. The zeta-potential measurements of cells were determined by a Zeta-Potential & Size Distribution Analyzer (Delsa 440SX) at a specified pH value. The cells' zeta-potential measurements were conducted on bacterial suspensions of 1.0 × 10⁸ cells.mL⁻¹ with ionic strength of 10⁻³ mol L⁻¹ KCl. FT-IR spectra of modified and original pyrite and prepared cells were obtained using a Fourier transform spectrometer (Nicolet 380, Thermo Nicolet) with diffuse reflectance attachment. X-ray absorption spectra were performed at the same conditions as in our previous studies [27]. All the data were calculated with the same program as in our previous report [27]. A series of typical sulfur compounds (zinc sulfate, thiosulfate, potassium tetrathionate, pyrite, jarosite and elemental sulfur) were selected as the sulfur references. The sulfur K-edge XANES spectra of the reference compounds were used to deduce the chemical speciation transformation of pyrite modified by *A. caldus*.

3. Results and discussion

3.1. Coal biodesulfurization process

Table 1 and Fig. 1 show the analysis of coal samples before and after processing with cells and the biodesulfurization characteristics of coal. There are small distinctions of total sulfur between original and processed coal samples, 2.15% versus 1.75%. However, the pyritic

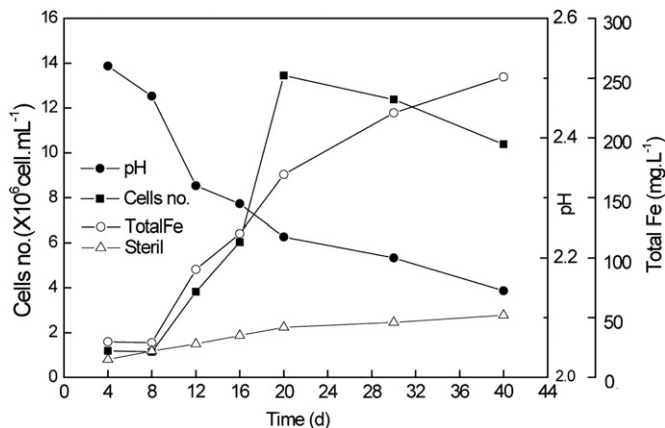


Fig. 2. Growth characteristics of coal pyrite-grown *A. caldus*.

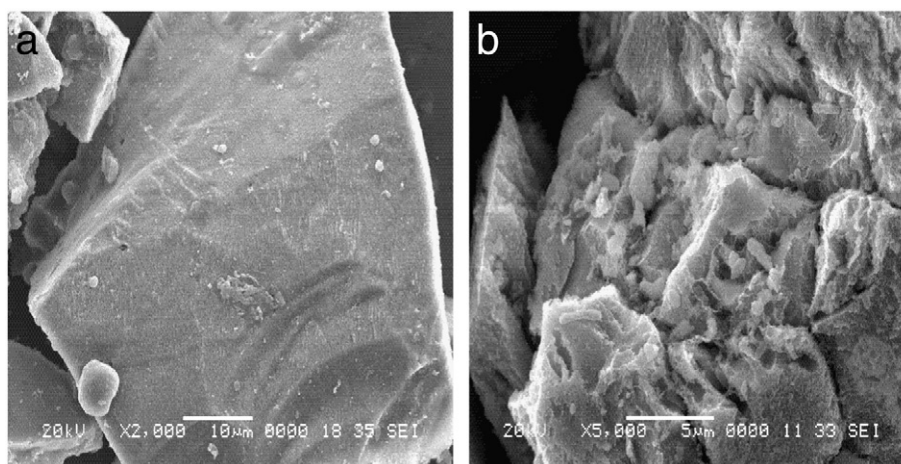


Fig. 3. SEM micrographs of pyrite before (a) and after (b) modified by cells.

sulfur was reduced from 1.34% to 0.71% and the pyritic desulfurization was about 47% (with a total desulfurization of 19%). As shown in Fig. 1, the total iron concentration was up to 241 mg L^{-1} . Based on these results, it is remarkable that the cells removed majority pyrite from the coal sample.

3.2. Pyrite bioleaching performance of *A. caldus*

As shown in Fig. 2, the changes of cell concentration, pH value and total iron concentration are presented, respectively. It shows that the *A. caldus* has promoted the leaching process of pyrite, with a final total iron concentration of 251 mg L^{-1} , compared with that of 52 mg L^{-1} in the sterile control. During the process, the pH decreased continuously to a value below 2.1. Cell numbers decreased sharply after 20 d, which might be caused by the low pH at which the bacteria could not thrive.

3.3. SEM analysis of pyrite

The SEM graphs collected from the bioleached particles are shown in Fig. 3a and b. In Fig. 3a, the micrograph shows that the pyrite surface was smooth before leaching but became rough (Fig. 3b) after 40 d leaching and have holes where cells were clearly attached. Previous studies reported that the cells' attachment on the mineral surface may occur via diffusion, convection and/or chemotaxis [16]. Moreover, the attachment

to the metal sulfide does not occur randomly and the mesophilic cells, such as *A. ferrooxidans*, are preferentially attached to sites with visible surface imperfections [17]. The present results indicated that the *A. caldus* might have the same attachment mode as the mesophilic microbes.

3.4. Surface characteristics of cells and pyrite

The zeta-potentials of *A. caldus* cells grown on sulfur, thiosulfate and pyrite and the pyrite before and after modified with cells as a function of pH are shown in Fig. 4. The *A. caldus* grown on thiosulfate showed an isoelectric point of pH 2.5. However, the higher isoelectric point values were observed in the sulfur and pyrite-grown cells. Two solid substrate-grown cells exhibited isoelectric point at 2.6 and 2.8, respectively. The maximum negative charge occurred at the point of about pH 6.3, and the maximum were -22 , -33 , -50 mV for thiosulfate, sulfur and pyrite, respectively. These results show that the growth conditions significantly influence the net surface charge. It has been reported that the isoelectric point is a parameter indicating the presence of functional groups, which are the components of cell surface polymers and decide the surface charge of cells [18]. According to the previous reports, thiosulfate-grown *A. caldus* cells which presented the isoelectric point at pH 2.5 might indicate existing significant amounts of glucuronic acids or other polysaccharides containing negatively charged phosphate and/or carboxyl groups [13]. However, the isoelectric point at pH 2.6–2.8 might be due to the presence of a $-\text{NH}_3$ group on the surface, which might mean

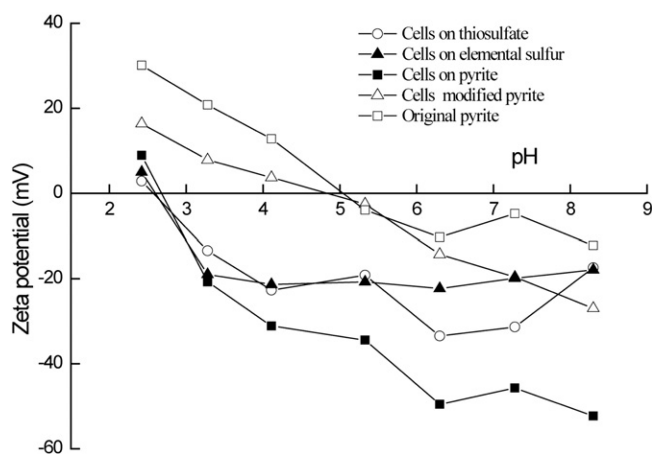


Fig. 4. Zeta-potentials of *A. caldus* grown on different substrates and the original pyrite and cell modified pyrite.

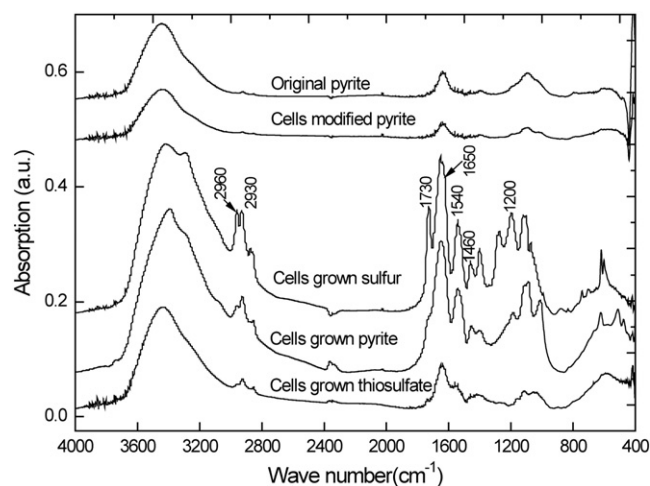


Fig. 5. FT-IR spectra of *A. caldus* grown on different substrates and the pyrite before and after modified by cells.

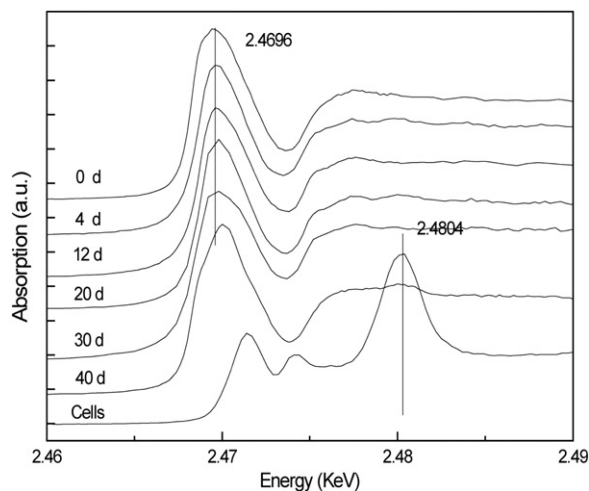


Fig. 6. Sulfur K-edge spectra of pyrite leaching with *A. caldus* and the cells grown on pyrite.

more proteins on the surface of solid substrate-grown cells [28]. On the other hand, the isoelectric point of residual pyrite impacted by cells presented a subtle variation compared to the original pyrite (reduced from 5.1 to 4.8). The changes might be caused by the cells' surface polymers.

The FT-IR spectra of *A. caldus* grown on different culture media and the pyrite modified before and after cells are shown in Fig. 5. Although the shape of FT-IR spectra of original and residual pyrite is very similar, the cells grown on different culture media present different absorption features. The bands were assigned according to previous reports [13,29]. Compared with culturing in thiosulfate, the cells growing in solid substrate exhibited a gradual absorption bands at 2960, 2930 and 2870 cm^{-1} , which were characterized as asymmetric $-\text{CH}_3$ stretching, asymmetric $-\text{CH}_2$ stretching and symmetric $-\text{CH}_2$ stretching, respectively. The very intense bands between 1730 and 1650 cm^{-1} are related to the $-\text{C}=\text{O}$ group, whereas another intense sharp band at 1540 cm^{-1} indicates the presence of an $-\text{NH}_3$ group. The bands at 1460 and 1400 cm^{-1} indicate the presence of $-\text{CH}_3$ and $-\text{CH}_2$ groups. The band at 1280 cm^{-1} is due to $-\text{CH}_3$ wagging modes. The band at 1100 cm^{-1} is assigned to $-\text{CH}_3$ wagging modes.

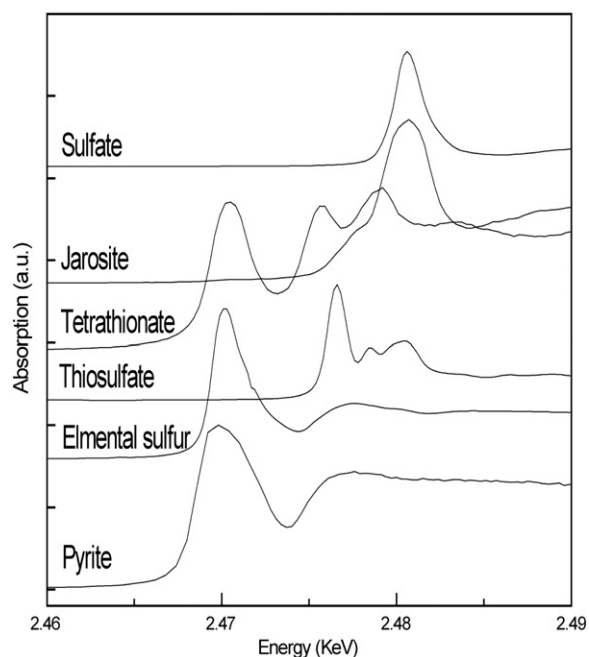


Fig. 7. Sulfur K-edge of model compounds.

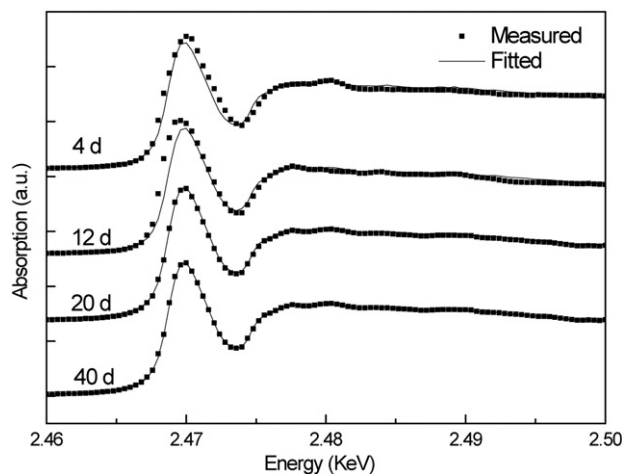


Fig. 8. Sulfur K-edge measured and fitted spectra of pyrite leaching with cells.

Based on the foregoing analysis, it can be concluded that elemental sulfur and pyrite-grown cells secreted more extracellular proteins than that of thiosulfate grown cells. The results of FT-IR spectra are consistent with the results obtained from zeta-potential experiment.

3.5. Sulfur speciation analysis of pyrite's bioleaching

As an advanced technique for sulfur speciation analysis, sulfur K-edge XANES has been widely applied to geochemical sulfur forms in coals [30,31]. The sulfur K-edge XANES spectra of pyrite were analyzed at different intervals during the leaching process, and the results are shown in Fig. 6. The sulfur K-edge XANES spectra of pyrite bioleached after 4 d and 12 d show almost similar absorption features to the original pyrite, in both edge position and intensity (the maximum absorption peak at 2.4696 keV). After 20 d of bioleaching pyrite, there appears a subtle excursion of the maximum peak, especially after 40 d processing, the absorption spectra of pyrite sample present a strong resonance peak at 2.470 keV. In comparison, the sulfur K-edge XANES spectrum of pyrite in the control shows similar absorption features in the near edge as well as post edge (data not shown). It has been reported that the formation of jarosite or sulfur-rich layer on the mineral surface significantly hinders the bioleaching of pyrite [21,23]. To obtain the detailed information of the sulfur speciation during the bioleached pyrite, the absorption spectra of model compounds were recorded and represented in Fig. 7. As shown in Fig. 7, the model compounds with different functional groups show distinct spectra. Fig. 8 shows the fitted curve of the bioleached pyrite residual after being processed by cells. The results suggested that elemental sulfur appeared after 20 d leaching, and increased with the leaching time. The fitted results indicate that the spectra of pyrite after 20 d and 40 d were overlapped with 98.1% pyrite 1.1% elemental sulfur and 88.3% pyrite and 12.6% elemental sulfur, respectively. In the present work, it is notable that the elemental sulfur was detectable until day 12, but no other secondary mineral components were detected.

4. Conclusion

Acidophilic and thermophilic strain *A. caldus* was firstly used for biodesulfurization of coals. The final results showed it achieved pyritic desulfurization and total desulfurization was about 47% and 19%, respectively. After processed for 40 d, the cells attached to the surface of pyrite and there was clear corrosion on the mineral surface. Moreover, the cells grown on different sulfur containing substrates presented distinct surface characteristics. In contrast, the cells grown on pyrite and elemental sulfur had more hydrophilic functional

groups than thiosulfate. The elemental sulfur was the main sulfur speciation occurring in the coal pyrite bioleaching but no other secondary mineral components were detected.

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