EFFECTS OF CELL CONCENTRATION AND COAL PRETREATMENT ON DESULFURIZATION OF CIGALUGUR SUBBITUMINOUS COAL USING *THIOBACILLUS FERROOXIDANS*

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Abstract

The use of coal is forecast to increase substantially to generate energy through combustion. Coal contains chemical substances that might pollute environment if it is not properly burnt, such as sulfur and nitrogen. Sulfur removal can be carried out by chemical, physical, or biological methods. Removal of sulfur from coal by microbial action has many advantages over physical and chemical desulfurization methods, namely (i) low capital and operating cost, (ii) low energy requirements, and (iii) removal of finely dispersed sulfur. This paper reports an experimental study investigating the effects of microbial cell concentration and pretreatment of coal on sulfur removal from Indonesian subbituminous coal using *Thiobacillus ferrooxidans*. The experiments were conducted under conditions that promote performance of the microorganism, i.e. temperature of 28°C and pH of 2.5. The procedure includes blending coal with *Thiobacillus ferrooxidans* in a laboratory scale reactor. Coal particles were sampled periodically during biodesulfurization. Its sulfur content was then measured according to ASTM D2492-90 (for pyretic sulfur) and ASTM D4239-94 (for total sulfur). Experimental findings show that sterilized coals give higher sulfur removal (49.8% for pyretic sulfur and 39.5% for total sulfur) than those non-sterilized (30.2% for pyretic sulfur and 34% for total sulfur). Furthermore, observed cell concentration and percentage of sulfur removal (for pyretic and total sulfur) decrease with initial microbial cell concentration.

Keywords : coal, biodesulfurization, *Thiobacillus ferrooxidans*

Abstrak

Penggunaan batubara diramalkan akan meningkat secara pesat untuk membangkitkan energi melalui pembakaran. Batubara mengandung senyawa-senyawa yang dapat mencemari lingkungan apabila tidak dibakar secara tuntas, seperti sulfur dan nitrogen. Penghilangan sulfur dapat dilaksanakan secara kimiawi, fisik, maupun biologik. Penghilangan sulfur dari batubara melalui proses mikrobial menunjukkan banyak kelebihan dibandingkan dengan metode-metode desulfurisasi fisik dan kimiawi, yakni (i) biaya modal dan operasi yang rendah, (ii) kebutuhan energi yang rendah, dan (iii) kemampuan penghilangan sulfur yang terdispersi secara halus. Tulisan ini membahas kajian eksperimental mengenai pengaruh konsentrasi sel mikroba dan perlakuan awal batubara terhadap penghilangan sulfur dari batubara sub-bituminus Indonesia, oleh bakteri *Thiobacillus ferrooxidans*. Percobaan dilaksanakan dalam kondisi yang mendorong kinerja bakteri, yakni temperatur sebesar 28 °C dan pH sebesar 2,5. Prosedur percobaan mencakup pencampuran batubara dengan *Thiobacillus ferrooxidans* dalam reaktor berskala laboratorium. Partikel batubara dicuplik secara berkala selama proses biodesulfurisasi. Kandungan sulfurnya kemudian diukur dengan metode ASTM D2492-90 (untuk sulfur pirit) dan ASTM D4239-94 (untuk sulfur total). Hasil percobaan menunjukkan bahwa batubara yang disterilisasi memberikan penurunan kadar sulfur terbesar (49,8% untuk sulfur pirit dan 39,5% untuk sulfur total) daripada batubara yang tidak disterilisasi (30,2% untuk sulfur pirit dan 34% untuk sulfur total). Selain itu, konsentrasi sel dan persentase penghilangan sulfur (untuk sulfur pirit dan total) berkurang dengan konsentrasi sel mikroba awal.

Kata kunci: batubara, biodesulfurization, *Thiobacillus ferrooxidans*

1. Introduction

Indonesia has abundant coal resources. The Directorate of Coal, Ministry of Energy and Mineral Resources, has identified 38.8 billion MT of coal deposits. Major coal resource areas are Kalimantan and Sumatra, estimated at 21.1 billion MT and 17.8 billion MT, respectively. The Indonesian coal resources are provided in table below.

Table 1. Indonesian Coal Resources

Company	Resources			Mineable Reserve
	Measured	Indicated	Total	
PTBA	1,902	4,657	6,559	2,804
Contractor	8,998	22,185	31,183	2,054
Others	584	442	1,026	504
Total	11,484	27,284	38,768	5,362

Source: Directorate of Coal

Coal as fossil fuel is expected to overcome the increasing of oil demand. However coal utilization can have negative impact to the environment due to air pollution. Sulfur in coal during combustion can be oxidized into sulfur dioxide which is the major source of SO_x pollution. When burned sulfur-bearing coal gives off sulfurous smoke, causing air pollution, corrosion of the metal, etc. The resulting residue or coke also retains some sulfur, called fixed sulfur. Furthermore, when coal mines are exposed to the atmosphere, pyrite is gradually oxidized. As a result, mine water becomes acidic, causing various environmental hazards, including severe damage to vegetation, soil erosion, and water pollution. Therefore, desulfurization process of coal is needed so that the various hazards arising from the sulfur compounds in coal can be avoided.

Coal desulfurization can be done by chemical, physical, or biological methods. Removal of sulfur from coal by microbial action has many advantages over physical and chemical methods. The advantages are low capital and operating cost, low energy requirements, and removal of finely dispersed sulfur. However, biological method (biodesulfurization) is rarely applied in commercial scale due to longer time in removing sulfur and lower organic sulfur removal. There are some of microorganisms suitable for removing sulfur compound in coal, such as *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Sulfolobus acidocaldarius*, and *Leptospirillum ferrooxidans*.

There are some studies that have been carried out in biodesulfurization process. These experiments conduct in different conditions.

Table 2. Researches on biodesulfurization

Authors	Experiment
Chandra and Mishra (1985)	Biodesulfurization of Assam coal using <i>Thiobacillus ferrooxidans</i> at pH 2 and T 28°C. Medium used are Peptone 10 g, beef extract 10 g, NaCl 5g, water 1000 mL and surfactant Tween-80 100 ppm
Chen and Skidmore (1981)	Biodesulfurization of Clarion 4-A (Ohio) coal using <i>Sulfolobus acidocaldarius</i> & <i>Sulfolobus brierleyi</i> at pH 2 and T 70°C. Medium used are MgSO ₄ 2H ₂ O 0.5 g/L; (NH ₄) ₂ SO ₄ 0.5 g/L; KH ₂ PO ₄ 0.5 g/L; yeast extract 0.02%(w/v)
Pandey et. al. (2003)	Biodesulfurization of Rajur coal using <i>Thiobacillus ferrooxidans</i> & <i>Thiobacillus thiooxidans</i> at pH 2 and T 32°C. Medium used are NH ₄ Cl 0.4 g/L; K ₂ HPO ₄ 0.4 g/L; Mg ₂ Cl 0.4 g/L
Siswanto and Nuella (2005)	Biodesulfurization of Indonesian subbituminous coal using <i>Thiobacillus ferrooxidans</i> at various pH (1.5, 2.0, 2.5) and T 35°C. Medium used is 9K medium and the surfactant is Tween-80 200 ppm

In this study, the aim is to investigate the effects of various cell concentration and coal pretreatment on percentages of pyretic sulfur removal and total sulfur removal on coal desulfurization. The microorganisms are *Thiobacillus ferrooxidans* and the type of coal that is used in this study is Cigalugur subbituminous coal. Moreover, the advantage of this study is to find the optimum operation condition in removing sulfur compound within coal by biological method, so that the coal that will be utilized is relatively clean.

The experimental procedure that is used in this research can be seen in the diagram below.

Figure 1. Experimental procedure

The effect of cell concentration (1x, 5x, and 10x dilution) and coal pretreatment (sterilization) on desulfurization of Cigalugur subbituminous coal using *Thiobacillus ferrooxidans* is investigated. The experiment is conducted under optimum conditions of the microorganism (28°C and pH values 2.5) with continuous supply of O₂ and CO₂. The total sulfur and pyretic sulfur content are determined using ASTM procedures and the cell concentrations are determined using spectrophotometer.

2. Fundamental

Coal is an organic rocklike (organic sediment) natural product that has undergone changes over several thousand years. Its resemblance to a rock is due to its physical nature and composition. Coal is formed from partially decomposed (and decomposing) plant debris that has collected in regions where waterlogged swampy conditions prevailed. In general, coal is composed mainly of carbon (50-98%), hydrogen (3-13%), oxygen, and smaller amounts of sulfur (organic and inorganic), nitrogen and other elements.

Coal can be classified into several major classification methods. One of the most common classifications is the general classification system of coal. In the general classification system of coal, coal can be divided into four major types: anthracite coal, bituminous coal, subbituminous coal, and lignite coal (Speight, 1994). They differ in their metamorphic range. The degree of metamorphic of coal decreases from anthracite to lignite.

Coal as fossil fuel contains sulfur, and the sulfur presents is released to the air when the fuels are combusted. Sulfur in coal is present in both inorganic and organic forms. The inorganic sulfur in coal consists predominantly of sulfides and sulfates. Pyrite is the main inorganic sulfur in coal. Particles of pyrite are randomly distributed as crystals throughout the coal, but are not bound to it.

Figure 2. Sulfur in coal

Coal also contains organic sulfur that is covalently bound into its large complex structure and is difficult to remove physically or chemically, in contrast to pyretic or inorganic sulfur. The structure of sulfur in coal is presented in figure 2.

Sulfur present in coal can be major source of air pollution when it is combusted. Treatment of coal is needed to remove sulfur compound prior to combustion. Sulfur removal can be done either by chemical, physical, or biological method. Furthermore, removal of sulfur in coal can be classified into several categories, which are pre-combustion, during combustion, post combustion, and conversion technologies that turn coal into gas or liquid that can be cleaned and used as fuel.

The inorganic sulfur can be a source of energy for the microorganisms. This is the reason why some microorganisms can live in the sulfur-rich coal, and also removing the sulfur on it. *Thiobacillus ferrooxidans* is one of the micro-organism that can oxidize pyrite and use it as its energy source. The mechanism of pyretic sulfur oxidation by microorganism can be divided into direct and indirect mechanism.

In direct mechanism, a physical contact exist between bacteria and sulfide mineral, and oxidation to sulfate takes place through several enzymatically catalyzed:

The overall reaction:

In the indirect mechanism, pyrite is oxidized by ferric iron to yield ferrous iron and elemental sulfur. The ferrous iron and elemental sulfur will then oxidized into ferric iron and sulfuric acid. And then the ferric iron can oxidize pyrite to produce more ferrous iron and elemental sulfur. In this mechanism, ferric iron is the leaching agent for bio-desulfurization. The reaction can be seen in the following equation.

There are several factors that have effects the biodesulfurization. The factors are as follows: extraction time, sterilization, temperature, pH, nutrient medium, particle size, inoculum size (cell concentration), surfactant, and coal concentration. The effect of sterilization and cell concentration will be discussed in this study.

3. Methodology

The experimental set up used in this study is based to the following methodology: temperature and agitation the process is controlled to achieve the optimum conditions. Medium used in the reactor is 9K medium. Inoculum's culture is *Thiobacillus ferrooxidans* that has been inoculated for 7 days in medium 9K without adaptation to obtain the optimum microorganisms activity with various numbers of cells. Coal used in this research is Cigalugur subbituminous coal.

The sulfur removal by *Thiobacillus ferrooxidans* is investigated by observing the decrease of pyrite and total sulfur in coal. Sulfur content is analyzed according to ASTM D2492-90 (for pyretic sulfur) and ASTM D4239-94 (for total sulfur). Observation of the effect of initial cell's number and its growth in the medium during the desulfurization is carried out in one experimental series using subbituminous coal. Observation of the effect of pretreatment of coal with the sterilization by autoclave before the desulfurization process is carried out in one experimental series using subbituminous coal.

The materials used in experimental are Cigalugur subbituminous coal, concentrated H_2SO_4 solution, 9K medium, demineralized water, CO_2 (gas), compressed air, Tween-80 as surfactant. Anionic surfactant belongs to polyoxyethylene derivatives of sorbitan fatty acid ester.

The proximate analysis for Cigalugur subbituminous coal that is used in the experiment presented in Table 3.

Table 3. Proximate analysis for Cigalugur coal

Parameters	Value (%)
Total moisture, (ar)	2.40
Moisture, (adb)	2.24
Ash content, (adb)	21.45
Volatile matter, (adb)	32.73
Fixed carbon, (adb)	43.58
Calorific value, (cal/g)	6092
Sulfur concentration, (adb)	8.04

Microorganism that used in this research is *Thiobacillus ferrooxidans* culture. Type of

medium used for inoculum development and in desulfurization reactor is 9K medium. The compositions are 0.5 g KH_2PO_4 , 3 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 20 g $FeSO_4 \cdot 7H_2O$, 0.1 g KCl, 0.01 g $Ca(NO_3)_2$, 1 L demineralized water

The medium except $FeSO_4 \cdot 7H_2O$ is sterilized in autoclave at $121^\circ C$ for 15 minutes. Every seven days, *Thiobacillus ferrooxidans* culture is removed to a new medium and then inoculated continuously. Inoculum's culture used for every run is 10% or 120 ml of the reactor working volume (1200 ml).

The equipment that used in the research is given in the figure 3 below.

Figure 3. Experimental equipment

The main equipment is a five-neck flask reactor. It is connected with air and CO_2 regulator, venturimeter, thermometer, air filter, magnetic stirrer, and heater. Two sets of equipment are used for this experiment.

Pyretic sulfur content in coal analysis is measured using ASTM D2492-90 Standard. Coal particle is suspended in dilute HNO_3 to extract ferrous, which is determined by AAS (Atomic Adsorption Spectrophotometer). Total Sulfur Content in Coal Analysis is measured using ASTM D4239-94 Standard, which is a high-temperature combustion method with acid-base titration detection procedures. Number of cell in solution is measured by analysis using spectrophotometer after solution is diluted.

4. Results and Discussions

Sterilization process is one of several methods of coal pretreatment that has effects on coal biodesulfurization. The non-sterilized coal is reported as the most advantageous for removal of sulfur from coal. It is appeared that coal itself served as a desulfurizing agent. In other words, raw coal contained microbes that are capable of removing pyretic sulfur from coal (Chandra and Mishra, 1985). The result of this study is given in Table 4 and Figure 4.

Table 4. Quantitative analysis of sulfur removal on sterilized and non-sterilized coal Run I (Sterilized coal)

Run I (Sterilized coal)		
Hour	%Total sulfur removal	%Pyritic sulfur removal
1	20.36	4.74
3	29.05	10.08
6	27.47	27.08
24	39.53	49.80

Run II (Non-sterilized coal)		
Hour	%Total sulfur removal	%Pyritic sulfur removal
6	22.30	13.83
24	34.04	30.24

(a)

(b)

Figure 4. Quantitative analysis of sulfur removal on Cigalugur coal
(a) Sterilized coal (b) Non-sterilized coal

Figure 5 shows that sterilized coal gives higher percentages in both total sulfur and pyritic sulfur removal. This result differs with Chandra and Mishra (1985) who found that biodesulfurization of non-sterilized coal using *Thiobacillus ferrooxidans* gives higher percentage of sulfur removal than the sterilized coal. This difference probably caused by the characteristic of inherent bacteria in the coal sample and the *Thiobacillus ferrooxidans* itself. There might be attributed to the characteristics of the inherent bacteria that are not very good

compatible with *Thiobacillus ferrooxidans*. In other words, the present of these bacteria cause competition for nutrition with *Thiobacillus ferrooxidans*. Another reason is the inherent bacteria in the coal form metabolite products that cause inhibition for *Thiobacillus ferrooxidans*. If this phenomenon happens, it would cause the lower percentage of sulfur removal in the non-sterilized coal than that in the sterilized coal.

Figure 5. Sulfur removals on sterilized and non-sterilized coal after 24 hours biodesulfurization

Gokcay and Yurteri (1990) reported that sulfur removal in flasks inoculated with concentrated cells (>108 cells/mL) is higher than in flasks inoculated with the less concentrated inoculums (108 cells/mL). Consequently, it can be concluded that increasing of cell concentration has a dual effect: increasing the observed cell concentration plus specific rates of desulfurization.

Table 5. Quantitative analysis of sulfur removal on various initial cell concentration

Run II (No dilution)		
Hour	%Total sulfur removal	%Pyritic sulfur removal
6	22.30	13.83
24	34.04	30.24

Run III (5 times dilution)		
Hour	%Total sulfur removal	%Pyritic sulfur removal
1	11.48	7.98
3	12.14	14.40
6	12.93	20.50
24	6.46	20.34

Run IV (10 times dilution)		
Hour	%Total sulfur removal	%Pyritic sulfur removal
1	13.72	18.58
3	20.18	17.08
6	15.44	17.39
24	6.60	17.39

This phenomenon also occurs during the biodesulfurization in this study. The investigation shows that the observed cell concentration and the percentage of sulfur removal (for pyretic and total sulfur) decrease with the decreasing number of initial cell concentration. To have different initial cell concentration that used in the experiment, the cell suspension is diluted for 5 and 10 times which means the dilution has smaller number of cell concentration. The effect of initial cell concentration on biodesulfurization using *Thiobacillus ferrooxidans* can be seen in table 5.

Figures 6 to 8 show the effect of initial cell concentration on sulfur removal for 1, 5, and 10 times dilution respectively.

Figure 6. Sulfur removal on subbituminous coal during desulfurization with no dilution (6.46 mg cells/L)

Figure 7. Sulfur removal on subbituminous coal during desulfurization with 5 times dilution (4.25 mg cells/L)

Figure 9 shows the effect of initial cell concentration on sulfur removal in coal. Large initial cell concentration gives higher microbial activities on biodesulfurization. Therefore, the percentages of sulfur removal decrease with the decreasing of initial cell concentration.

Figure 8. Sulfur removal on subbituminous coal during desulfurization with 10 times dilution (3.14 mg cells/L)

Figure 9. Sulfur removal on subbituminous coal for various initial cell concentration after 24 hours biodesulfurization

Figure 10 shows the concentration of *Thiobacillus ferrooxidans* during the biodesulfurization. The bacteria undergo logarithmic phase for the first 3 hours, which its growth enhance rapidly. After the logarithmic phase, the growth of the bacteria declines. At the end of the biodesulfurization, the growth of bacteria decreases progressively and is nearly constant. Further prolongation is possible because the growth is still continued although the rate is very slow.

Figure 10. Cell concentration during biodesulfurization

The total sulfur and pyretic sulfur content decrease progressively with the length of time during biodesulfurization. However, it is appeared that the bacteria have a limited effect on removing sulfur. Hoffmann *et. al.* (1981) and Juszczak *et. al.* (1995) reported that in biodesulfurization using *Thiobacillus ferrooxidans*, the appreciable quantities of pyretic are oxidized in 3 to 4 days. The action of microorganism is limited with time because the microorganism can be inhibited by its metabolite product. However, it finds out that the results in this study are differing with the results from the previous studies (Hoffmann *et. al.*, 1981; Juszczak *et. al.*, 1995).

In this study, the effective elimination of the total amount of sulfur and pyretic sulfur is reached within 24 hours. The results probably are caused by differences of the coal characteristic that are used in the literature and in the experiment. Further prolongation of the biodesulfurization time to 3 days resulted in a decrease of the sulfur removal percentages. The decreasing total sulfur and pyretic sulfur removal percentages probably are caused by sulfate ion adsorption, which is the product of biodesulfurization, on the coal surface. The adsorbed sulfates and the increasing amount of protein formed a film on the surface of coal thus becoming difficult to wash out (Juszczak *et. al.*, 1995). The adsorbed sulfate ions can cause increasing of sulfur content in analysis, and causing an error. The result is presented in table 6 and figure 11.

Table 6. Quantitative analysis of sulfur removal on subbituminous coal during 72 hours biodesulfurization

Run V (72 hours biodesulfurization)		
Hour	%Total sulfur removal	%Pyritic sulfur removal
20	15.57	24.31
72	9.10	7.31

Figure 11. Sulfur removal during 72 hours biodesulfurization

The effect of biofilm that formed in coal surfaces is investigated by NaOH treatment for coal sample before it is analyzed. The coal sample is mixed with NaOH solution (0.3 N) for an hour in order to break the biofilm in its surfaces. Figure 12 and table 7 show the effect of NaOH treatment on the sulfur removal. The total sulfur and pyretic sulfur removal for treated coal sample is higher than untreated coal samples. The increasing is probably caused by the characteristic of NaOH (alkali), which is the opposite of the biofilm characteristic (acid), therefore breaks the biofilm by neutralizing it. However, the NaOH treatment that is carried out in this study cannot be concluded as the most effective treatment in removing the biofilm. Further studies should be carried out to find the most effective treatment for removing the biofilm.

Table 7. Quantitative analysis of sulfur removal during 72 hours biodesulfurization after NaOH treatment

Run V (72 hours biodesulfurization after NaOH treatment)		
Hour	%Total sulfur removal	%Pyritic sulfur removal
20	15.57	24.31
53	10.29	22.92

Figure 12. Total and pyretic sulfur removal during 72 hours biodesulfurization after NaOH treatment

5. Conclusions

It is concluded that the sterilized coals give higher sulfur removal (49.8% for pyretic sulfur and 39.5% for total sulfur) than non-sterilized coals (30.2% for pyretic sulfur and 34% for total sulfur). The observed cell concentration and the percentage of sulfur removal (for pyretic and total sulfur) decrease with the decreasing in initial cell concentration.

Total sulfur and pyretic sulfur content in coal affect the biodesulfurization process of coal. High total and pyretic sulfur content are simply effective for short time biodesulfurization.

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