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Removal of sulfur and ash from Indonesian coal by indigenous mixotrophic bacteria

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ABSTRACT

Coal is one of the alternative fuels which potentially provides most of the domestic energy needs. The steam-powered electric generator (PLTU) is a sector that dominates the utilization of domestic coal. Sulfur contained in the coal is an element impurity apart from other contaminants such as ash, soil, rocks, and minerals. The combustion of high sulfur from coal produces SO_2 which can interfere the human health, such as causing tightness in the respiratory tract, as well as the environment by causing acid rain and corrosion on plant equipment. Various efforts have been made by reducing the levels of sulfur to minimize the negative impact caused by coal combustion. The utilization of bacteria for bio desulfurization has been developed and widely studied as an alternative treatment to remove the sulfur and ash from the coal. Therefore, the purpose of this study was to remove the sulfur and ash from coal using various types of indigenous bacteria by the biomining method. The current study used coal from East Kalimantan of Indonesia with a total sulfur and ash content of 2.56 and 7.21%, respectively. The indigenous bacteria used in this study consisted of five bacterial isolates identified as *Citrobacter murliniae, Dietzia psychralcaliphila, Pseudomonas aeruginosa, Alcaligenes faecalis, Bacillus altitudinis*. The results showed that the bacterium *C. murliniae* was able to eliminate the sulfur by 19.61%, which was higher than the other bacterial isolates and remove ash from coal by 1.75%. The bacteria *D. psychralcaliphila, P. aeruginosa, A. faecalis, B. altitudinis* were capable of eliminating 16.49, 8.30, 3.61, 8.89% of total sulfur and 4.03, 4.56, 5.29, 4.21 of ash content in coal, respectively.

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1. Introduction

As an organic sedimentary rock that is used as fuel, coal was formed from plants that have undergone biochemical, chemical, and physical decomposition in oxygen-free conditions that take place at specific pressures and temperatures over a very long period. Coal is one of the sources of energy that is the focus of meeting the needs of energy and electricity in various countries, including Indonesia. Coal obtained from mining processes contains elements of impurities (impurity); this can occur during the formation of coal (coalification) or the mining process. Elements of the impurities found in coal include sulfur (pyritic sulfur, organic sulfur, and sulfate) and various minerals such as quartz, kaolin, clays, pyrite, and calcite obtained in coal ash (Sukandarrumidi, 2006).

Coal has been widely used as fuel in various industries, such as the cement industry, the pulp industry, the textile industry, steam power plants, and metallurgical industries. The need for coal for the steam power plant (PLTU) sector has increased in the last three years in 2017 by 88.4 million tons, in 2018 to 107.2 million tons, and in 2019 increased to 166 million tons (Kementerian PPN/Bapennas, 2019). When coal is burned to achieve industrial needs, besides producing industrial products for community needs, coal also produces other outputs. PLTU, as a producer of electrical energy also contributes to waste (residual combustion) and pollution to the environment. The primary emissions resulting from burning coal are sulfur dioxide (SO_2), nitrogen oxide (NOx), particulates, carbon dioxide (CO_2), heavy metals found in fly ash and bottom ash. The combustion process using coal, which has high sulfur content, can cause excessive SO_2 emissions, exceed the predetermined quality standards, and have a drawback on human health because it causes interference breathing. It is also harmful to the environment because it causes acid rain. Moreover, if high sulfur-containing coal is combusted, it will corrode and form a slag on the boiler pipes. In the metals casting industry, ash and sulfur from coal will form a slag, which is detrimental.

Sulfur is one of the coal quality parameters that should be taken concern. Sulfur contained in coal is an impurity element apart from other impurities such as ash, soil, rocks, minerals, and others. This element is found in both inorganic and organic forms (Huffman et al., 1991; Li et al., 2010). Inorganic sulfur is commonly found in the form of sulfide compounds (pyritic) and sometimes sulfates. Organic sulfur in coal is covalently bound into large and complex structures in coal molecules and distributed in coal substances (Constanti et al., 1994).

Besides being constrained by the sulfur content, coal ash will also become a serious obstacle if it contains alkali metals, such as sodium (Na) and potassium (K). Alkali metals show the strongest sublimation tendency among the main minerals in coal (Sugawara et al., 2002). The high sodium content in coal causes slagging and fouling on the boiler walls, so that boiler operation is hazardous and not economical (Bryers, 1996; Zheng and Xu, 2003; van Alphen, 2007).

Several efforts have been performed to remove coal impurities, especially the problem of sulfur and ash (Demirbas and Balat, 2004). Various desulfurization techniques are used to reduce the adverse environmental impacts caused by the coal combustion process. Based on the process of desulfurization, it consists of physical desulfurization methods, chemical methods, and biological methods. Coal desulfurization using physical methods has a disadvantage because it is unable to remove organic sulfur in coal. The chemical desulfurization method produces better results than physical methods, but it generally requires high energy consumption. It can also create hazardous substances and affect the structural integrity of coal (Aytar et al., 2008; Jiang et al., 2009). Because of the shortcomings of physical and chemical methods, the increased impulse is currently given to biological processes due to many advantages compared to conventional physical and chemical processes. The biological process is carried out in easy conditions and not dangerous. It can reduce organic sulfur, and the coal structure is not affected (Monticello, 2000). This process changes the oxidative form of sulfur, which is reduced to soluble and easily washable compounds (Larsson et al., 1990; Cardona and Márquez, 2009). The use of bacteria for biodesulfurization has been developed and widely studied as an alternative treatment to overcome sulfur and ash content in coal (Cara et al., 2005). The utilization of bacteria is considered effective to be used in the desulfurization of inorganic and organic sulfur in coal. Various microorganisms have been found capable of desulfurization, each of which has its significance based on the environmental conditions and the origin of coal throughout the world. Bacteria are generally isolated from soil, and mine acid water that is located at coal mining locations. The most widely used microorganisms in the desulfurization process until now are meso-acidophilic bacteria, namely *Acidithiobacillus ferrooxidans* (Ghosh et al., 2015).

The use of bacteria in coal desulfurization is one example of the role of the use of biotechnology in mining or known as biomining (herein involving two processes: bio-oxidation and bioleaching). Potential applications of biotechnology for mining and bioprocessing have been carried out in many countries. However, little or no study has been reported on coal beneficiation using biomining technology in Indonesia. Therefore, this study investigated the biomining method employed in coal bio beneficiation, also referred to as clean coal technology, by using five indigenous mixotrophic bacteria; five bacteria were chosen to find out whether these bacteria can oxidize sulfur and iron. The characteristics of the pre- and post-microbial processes of coal are compared based on total proximate and sulfur analysis. From the results of this study, the alternative clean coal technology that is more effective and environmentally friendly can be achieved.

Table 1. Characteristics of the initial coal sample

Proximate analysis (%)	Ash	7.21
	Volatile matter	46.38
	Fixed carbon	46.41
Sulfur distribution (%)	Total sulfur (TS)	2.56
	Organic sulfur (OS)	1.31
	Pyritic sulfur (PS)	1.17
	Sulfate sulfur (SS)	0.08
Gross calorific value		5,582.57 cal/g

2. Materials and methods

2.1. Coal samples

A sub-bituminous coal sample was collected from East Kalimantan, Indonesia. Initial coal was characterized by total sulfur and ash content of 2.56% and 7.21%, respectively. The coal samples were crushed in roll crusher, further ground in a tumbling mill, and separated into various particle size fractions by a sieving machine to obtain the grain size of -200 +325 mesh (-74+44 μm).

2.2. Bacteria and bacterial growth medium

The indigenous bacteria used in this study consisted of five bacterial isolates, identified as *Citrobacter murliniae* (isolated from the water sample of the Domas Crater, Tangkuban Perahu, West Java), *Dietzia psychralcaliphila* (isolated from the culture containing crude oil), *Pseudomonas aeruginosa* (isolated from the seawater contaminated with crude oil), *Alcaligenes faecalis* (isolated from the soil contaminated with chromium (Cr)), *Bacillus altitudinis* (isolated from a mixture of crude oil samples and water sample). All the bacterial isolates were obtained from the Laboratory of Geomicrobiology, Biomining & Biocorrosion (GBBL), Institut Teknologi Bandung.

Bacterial growth as the biotic experiments was undertaken in duplicate in 300 mL Erlenmeyer flasks containing the SKC-broth medium. All flasks and their content were then autoclaved at 121°C for 15 min, and subsequently inoculated by 10% v/v of each bacterial strain. The bacterial cultures were then incubated in a rotary shaker at a speed of 180 rpm at room temperature under aerobic conditions. Every 4 hours, sampling was carried out by measuring the OD (optical density) value of 1 ml culture using a UV-Vis Spectrophotometer with a wavelength of 600 nm. The sampling activity was carried out until the stationary phase of the bacterial growth. Also, oxidation-reduction potential (Eh) and pH measurements were conducted to determine the bacterial culture conditions during bacterial growth and the abiotic control experiment without bacteria was also carried out, which was identical to the biotic experiments.

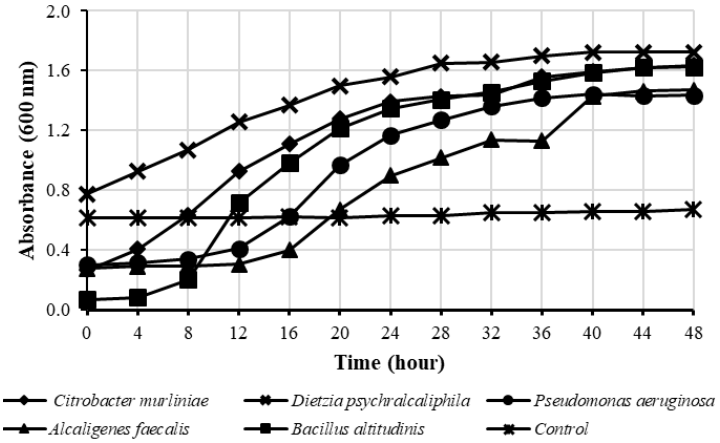

Fig. 1. The growth curve in optical density for five bacterial isolates. Control was optical density (OD) of the medium without bacteria.

2.3. Biomining experiment

Batch biomining experiments were carried out in duplicate in 500 ml Erlenmeyer flasks containing 300 ml of SKC-broth medium supplemented with 10% v/v bacterial inoculum of each bacterial strain, 25% w/v coal (25% pulp density) with a particle size of -200+325 mesh (-74+44 μm). The biomining experiments were conducted for ten days by agitating the cultures with a rotating speed of 180 rpm at room temperature. During the experiments,

oxidation-reduction potential (Eh) and pH in the culture medium were also measured periodically. After ten days, the coal samples were separated from the culture medium using a centrifuge, then dried at 40^0C for about 10 minutes and left for another two days at room temperature. Eventually, the dried coal samples were prepared for the analysis of their sulfur and ash contents.

2.4. Data analysis

The pH and redox potential (Eh) of the culture medium and the suspension were monitored with pH meter (Lutron pH-201) and ORP meter (Lutron ORP-203), respectively. The Eh value was recalculated with a standard hydrogen electrode (SHE) according to the following equation: Eh vs SHE (mV) = Eh+197 (Syarif et al., 2019). Proximate analysis of coal was performed to determine the main characteristics of coal (i.e., moisture, volatile matter, fixed carbon, and ash content) before and after biomining treatment following standard methods (ASTM D3173 for moisture content, ASTM D3174 for ash content and ASTM D3175 for the volatile matter). To determine the quality of coal before and after biomining treatment, sulfur contents in coals were measured according to ASTM D3177 (for total sulfur) and ASTM D2492 (for sulfur forms). On the other hand, the calorific value of the initial coal used in this study was also measured based on ASTM D2015.

3. Results and discussion

3.1. Mineralogical and chemical characteristics of coal

Table 1 shows the characteristics of the initial coal used in this study. Coal samples from East Kalimantan of Indonesia are known to have a moderate ash content of <10%. The coal used is classified as sub-bituminous coal, which has a moderate calorific value of 5,582.57 cal/g. However, the presence of a higher amount of sulfur, especially organic sulfur, is not beneficial for its applications. Total sulfur contained in coal samples was 2.56% (consisting of 1.31% organic sulfur, 1.17% pyritic sulfur, 0.08% sulfate sulfur). Based on the mineralogical analysis, the coal contains illite ($K_{0.65}Al_{2.0}[Al_{0.65}Si_{3.35}O_{10}](OH)_2$), quartz ($SiO_2$), pyrite ($FeS_2$), and a small amount of manganosite (MnO) (data not shown).

3.2. Bacterial growth

The turbidimetric method was used to measure bacterial growth based on turbidity; microbes in a liquid material can be detected based on turbidity. The growth of bacterial cells in a liquid medium will increase the turbidity of the media, which will affect the amount of light that can be transmitted through the medium (absorption) (Agustiyani et al., 2004). The growth curve provides an overview of the overall bacterial growth cycle, which includes the lag, exponential, stationary, and death phases (Madigan et al., 1997). The bacterial growth curve of five bacterial isolates is shown in Fig. 1. Overall, bacterial growth fluctuated from 0 to 16 hours, then increased significantly up to 30 hours. Subsequently, the bacterial growth plateaued up to 48 hours, indicating the stationary phase. The bacteria *C. murliniae* and *D. psychralcaliphila* had the same growth time, which had the lag phase less than 4 hours. The exponential phase started from 0 to 36 hours, and the stationary phase occurred from 36 to 48 hours. The lag phase of the bacterium *P. aeruginosa* occurred at 0 to 4 hours; the exponential phase occurred at 4 to 44 hours, and the stationary phase started from 44 to 48 hours. The bacteria *A. faecalis* and *B. altitudinis* demonstrated a similar growth curve pattern, which had the lag phase at 0 to 12 hours; the exponential phase occurred at 12 to 40 hours, and the stationary phase occurred from 40 to 48 hours.

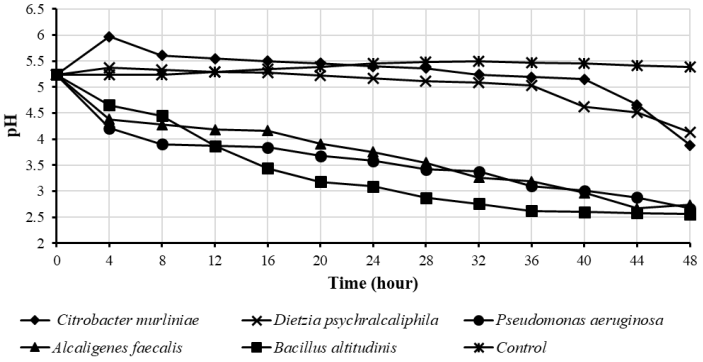

Fig. 2. The pH value of the solution during bacterial growth. Control was the pH of the medium without bacteria.

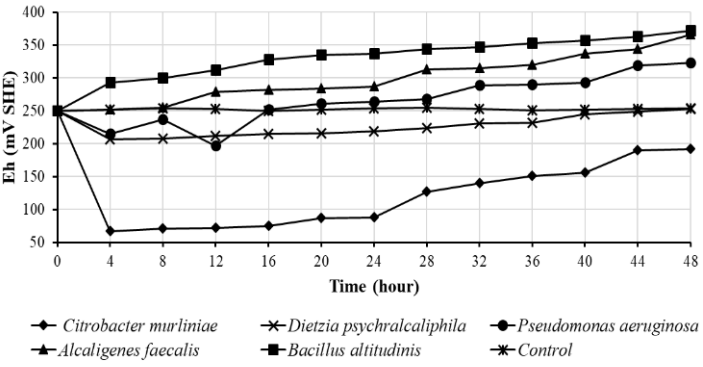

Fig. 3. Redox potential (Eh) changes over a 48-hour bacterial growth of five bacteria. Control was Eh of the medium without bacteria

Table 2. The evaluated quality of initial and bacteria-treated coal samples

Analysis, %db	Initial coal	After treated with the bacterium				
		C. *murliniae*	*D. psychralcaliphila*	*P. aeruginosa*	*A. faecalis*	*B. altitudinis*
Ash	7.21 (0.02)	7.09 (0.34)	6.92 (0.02)	6.88 (0.50)	6.83 (0.45)	6.91 (0.32)
Volatile matter	46.38 (0.07)	50.35 (1.28)	46.19 (0.57)	40.27 (0.27)	37.87 (0.36)	40.71 (0.21)
Fixed carbon	46.41 (0.01)	43.69 (0.50)	46.89 (0.60)	52.84 (0.97)	55.30 (0.08)	52.38 (0.04)
Total sulfur	2.56 (0.05)	2.06 (0.00)	2.14 (0.02)	2.35 (0.05)	2.47 (0.02)	2.33 (0.00)

The numbers in parentheses are standard deviation based on duplicate experiments

One crucial factor that affects bacterial growth is pH. If the environmental pH is not suitable for enzyme activity optimally, the bacteria will not metabolize properly. As a result, bacteria cannot grow optimally. During the growth phase, the pH can change, rise, or fall, depending on the composition of the medium used, where no buffer was added to the culture medium to adjust the initial pH of the solution. The pH changes during bacterial growth are shown in Fig. 2. In this study, no buffer was added to the culture medium to adjust the initial pH of the solution. During bacterial growth, the pH of the culture medium decreased within 48 hours, with the average pH values of ~ 3.2, indicating that by using the SKC-broth medium, the bacteria were able to produce organic acids without the addition of any buffers (e.g., sulfuric acid or hydrochloric acid). Organic acids produced by bacteria were expected to be able to dissolve sulfur contained in coal in the biomining experiment

described later. Moreover, the molasses contained in the medium was a carbon source of bacterial growth.

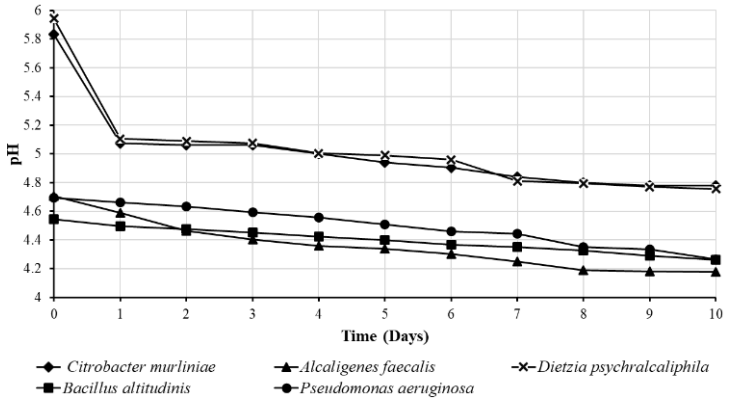

Fig. 4. pH changes over a 10-day biomining process by five bacterial isolates. Data reported are the averages obtained from batch cultures run in duplicate

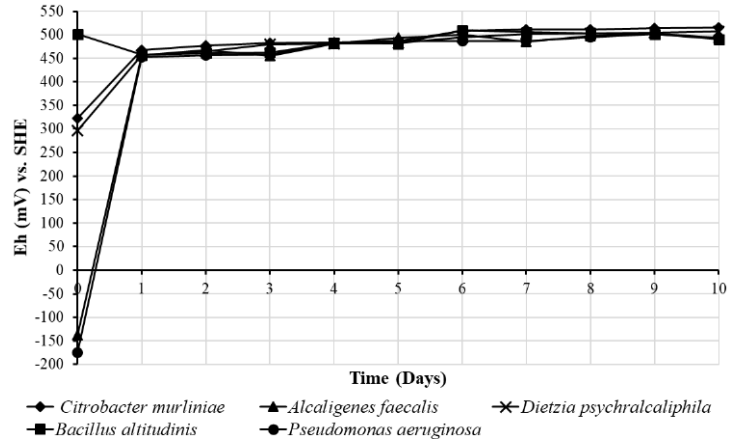

Fig. 5. Redox potential (Eh) changes over a 10-day biomining process by five bacterial isolates. Data reported are the averages obtained from batch cultures run in duplicate

Furthermore, another intrinsic factor affecting the growth of microorganisms is oxidation-reduction potential (redox). Microbes have a specific sensitivity to the oxidation-reduction potential (redox) of the growth medium. The redox potential (E_h) changes during bacterial growth are shown in Fig. 3. The potential redox value (Eh) increased during bacterial growth, and the Eh values reached ~300 mV after 48 hours of incubation. The increased redox potential value (E_h) causes the environment to become more oxidative. A low Eh value below 300 mV (vs SHE) indicates a low oxidizing medium (Cardona and Marquez, 2009).

3.3. Biomining experiment

During the biomining experiment, potential redox value (E_h), pH, and total sulfur and ash decreased for ten days. During biomining processes, the bacteria obtained their energy source from the sulfide mineral oxidation process. The pH changes during the biomining experiment are shown in Fig. 4. Based on the measured pH value, the average pH of the solution began to decline from the first day and continued to decrease up to day 10 of incubation time. The pH changes during the biomining experiment are shown in Fig. 4. The average pH value of all the bacterial suspensions was ~5.2 at the onset of the experiments and subsequently decreased gradually to pH ~4.5 at the end of the biomining experiments. The initial pH value of the suspension differed from each other due to different bacterial inoculum and coal addition, and no pH adjustment was performed at the onset of the experiments. Changes in pH in the coal biodesulfurization process were studied previously by Liu et al. (2017) on different types of coal and bacteria, exhibiting that the pH of the optimum solution at the end of the coal biodesulfurization process was achieved at 4 and 5. However, the pH was adjusted at the beginning of the coal biodesulfurization process. Unlike the case in this study, pH adjustment was not carried out. The decreased pH is influenced by bacterial activity; the coal sample itself contains some humid acids; therefore, when coal samples dissolve in culture media, the pH value of the system decreases. Bacteria adsorbed on the surface of coal particles can constantly metabolize sulfur pyrite effectively, and large amounts of H^+ are produced. Bacteria oxidize sulfide minerals, such as pyrite during the biomining process, so that the pH value of a declining suspension ORP value increases along with bacterial growth (Chaerun and Tazaki, 2003). Bacteria produce ferric ions during the oxidation of pyrite, which is then reduced to ferrous ions. Ferrous ions can be oxidized again by bacteria in the biomining process, and the Fe cycle will continue. Fe ions play an essential role in the oxidation of pyrite. Hence, changes in pH and ORP suspension can be used to evaluate coal biomining processes (herein bio-oxidation) (Boxall et al., 2017).

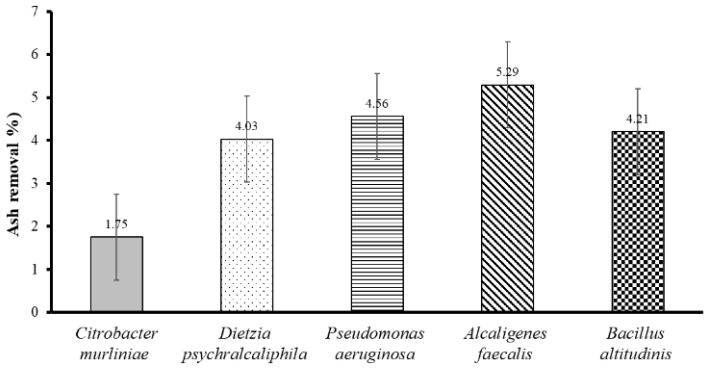

Fig. 6. Ash removal (%) from coal after 10 days of biomining process by five bacterial isolates. Data reported are the averages obtained from batch cultures run in duplicate

Correspondingly, the redox potential (Eh) changes during the biomining experiments are shown in Fig. 5. The Eh increased up to the last day of the biomining experiments. The Eh value was initially low, then increased gradually and began to be constant on the 7th day. An increase in the Eh value caused the environment to become more oxidative, thus supporting the dissolution of sulfide minerals contained in coal (herein pyrite). At the end of the biomining process, the average Eh value reached ~500 mV. Changes in the Eh values had been studied previously by Ye et al. (2018), reporting that the Eh value at the end of the bio-desulfurization process of coal reached 511 mV.

3.4. Sulfur and ash removal from coal

Removal of sulfur and ash from coal before and after biomining treatment is shown in Table 2, Fig. 6, and Fig. 7. Based on Table 2, the ash content in coal can be reduced through biomining treatment with bacteria. The bacteria *C. murliniae*, *D. psychralcaliphila*, *P. aeruginosa*, *A. faecalis*, and *B. altitudinis* can reduce ash content of 1.75%, 4.03%, 4.56%, 5.29%, and 4.21%, respectively. However, some researchers have reported that the reduction in ash content may be mainly attributed to the dissolution of minerals (Cardona and Márquez, 2009). The decrease in ash content can be considered to have a positive environmental impact, related to heavy metals existing in the bottom ash and fly ash after combustion of untreated coals. Analysis of volatile matter showed that the volatile matter after biomining treatment with *C. murliniae* increased, while that with four other bacteria decreased. This increased volatile matter

content might be due to low volatile matters converted into the high volatile matters in the biomining process. The fixed carbon content in coal after the biomining treatment for the bacterium *C. murliniae* decreased, while that for four other bacteria increased.

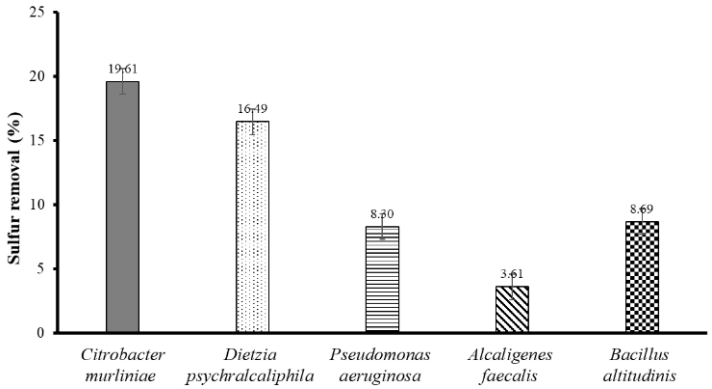

Fig. 7. Sulfur removal (%) from coal after 10 days of biomining process by five bacterial isolates. Data reported are the averages obtained from batch cultures run in duplicate.

Based on the results shown in Fig. 6 and 7, the bacterium *C. murliniae* can produce biosurfactants and oxidize sulfur (Wahyuningsih et al., 2017). For these reasons, *C. murliniae* was able to eliminate the sulfur by 19.61% (from 2.56% to 2.06%), which was higher than the other bacterial isolates and remove ash from coal by 1.75% (from 7.21% to 7.09%). Although the sulfur removal by *C. murliniae* was high, the ash removal was low due to the bacterial ability to break down sulfide minerals through oxidative reaction but not oxide minerals contained in the coal. Subsequently, the bacterium *D. psychralcaliphila* was able to eliminate 16.49% of sulfur (from 2.56% to 2.14%), and 4.03% of ash (from 7.21% to 6.92%). *D. psychralcaliphila* had a higher ability to remove both sulfur and ash than the other bacteria. The bacterium *P. aeruginosa* was capable of eliminating 8.3% of sulfur (from 2.56% to 2.35%) and 4.56% of ash (from 7.21% to 6.88%). The bacterium *B. altitudinis* demonstrated its ability to eliminate 8.69% of sulfur (from 2.56% to 2.34%) and 4.21% of ash (from 7.21% to 6.91%). Both the bacteria *P. aeruginosa* and *B. altitudinis* showed a moderate ability in the removal of both sulfur and ash. Eventually, the bacterium *A. faecalis* exhibited its capability to remove 3.61% sulfur (from 2.56% to 2.47%) and 5.29% ash (from 7.21 to 6.83%) from coal. The bacterium *A. faecalis* demonstrated the highest capacity in removing ash but the lowest ability to eliminate sulfur compared with other bacteria.

4. Conclusion

Based on the results of this study, of the five bacterial isolates tested, the highest removal of sulfur was achieved by the bacterium *Citrobacter murliniae* (19.61%), and the ash could best be removed from the coal using *Alcaligenes faecalis* (5.29%). Hence, both bacteria can be recommended to be used as a consortium to achieve the best removal of sulfur and ash from the coal. Thus, biomining technology can be an alternative to reduce the high sulfur and ash content of coal because the technology is environmentally friendly and does not change the properties of coal.

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Conflict of interest

The authors declare there is no conflict of interest in this study.

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