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Microbial desulfurization of three different coals from Indonesia, China and Korea in varying growth medium

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Abstract−Shake flask studies on microbial desulfurization of three different coal samples (Indonesian lignite, Chinese lignite and Korean anthracite) were performed to optimize the best suitable growth medium. Among the three different growth mediums (basal salt medium, basal salt medium supplemented with 9 g/L Fe and basal salt medium supplemented with 2.5% S^0) tested, the basal salt medium was found to be the best, considering process dynamics and economical factors. The extent of pyrite oxidation was highest with 95% in the experiments with Korean anthracite in basal salt medium supplemented with 9 g/L Fe, while the lowest pyrite oxidation of 70-71% was observed in the experiments with Indonesian and Chinese Lignite's in only basal salt medium. The microbial sulfur removal in the experiments with basal salt medium supplemented with 9 g/L Fe for all the three coal samples was between 94-97%, while the experiments on basal salt medium supplemented with 2.5% S^0 for all the coal samples were relatively much lower ranging between 27-48%. However, the overall study resulted with promising directions for further scaling up of microbial desulphurization in a best growth medium devoid of iron and sulfur supplement.

Key words: Biooxidation, Desulphurization, Coal, Redox Potential, Population Dynamics, Ferrous Iron

INTRODUCTION

Microbial desulfurization of coal could be an alternative technology to reduce SO_2 emissions from coal-fired power plants. Biological desulfurization of coal has an advantage over the conventional chemical and physical methods and would be much more efficient, fast and economically viable. Chemolithotrophic acidophiles, especially Fe and S oxidizers, are used to oxidize the FeS_2 present in the coal followed by S oxidation into soluble SO_4^{2-} ions in the aqueous phase [1]. Several studies regarding the feasibility of desulfurization of coal using micro-organisms have been carried out in past by several researchers [2-5]. Microbial method of coal desulfurization is well known for having certain advantages such as low capital and operating costs, higher energy efficiency compared to pyrometallurgical and hydrometallurgical processes possessing the potential to remove finely distributed sulfur compounds in the coal without hampering the coal quality [6]. However, the viability of microbial desulfurization method depends on several factors such as the group and location of the coal, type of microorganisms used in process, external surface properties, pH and temperature of the growth medium used and the sulfur content in the coal [7,8]. Among all the sulfide minerals, FeS_2 is an acid insoluble sulfide and mostly refractory by nature with relatively slow dissolution kinetics compared to others, whereas bioleaching of coal pyrite does not differ considerably from the pyrite of igneous origin, and hence could be influenced by the semiconductor properties of the mineral. Most of the research conducted using mesophilic microorganisms, moderate thermophiles or extreme thermophiles has proved to possess the potential of enhancing pyrite oxidation. However, mesophilic bioleaching is more attractive for commercial scale, as they have an advantage of low energy cost compared to the thermophilic bioleaching. Rossi [9] studied the technical and economical feasibility of a commercial scale biological depyritization of coal based on the results obtained from the pilot scale operation at the Porto Torres (Sardinia) factory of EniChem-Anic, in the framework project funded by the Commission of European Communities.

Microbial pyrite removal from hard coal using *Acidithiobacillius* species in a heap leaching process was tested to determine the seepage rate of the chemically defined growth medium and investigations on the modifications of the coal properties. A further detailed study on the scale up of the process by designing the plant dimensions and estimating the costs of pyrite removal based on existing plants was documented by Beier [10]. Investigations conducted on a packed bed column percolating microbial solution at pH 1.5 for desulfurization of coal for 42 days with proper monitoring of total iron concentration in the solution leaving the column indicated removal of 42% pyritic sulfur via pyrite oxidation. This was further scaled up to a pilot scale plant for the desulfurization of coal via heap bioleaching at a range of 20-30 tons [11]. Fabiańska et al. [12] reported biological desulphurization on bituminous coal together with density fractions by gas chromatography-mass spectrometry for organic matter alteration and atomic absorption spectroscopy to assess the concentration of trace elements. It was observed that the bio-

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logical desulfurization degraded the low-molecular weight fraction of coals relating density fractions, which means higher the mineral concentration larger is the biodegradation. A series of tests conducted on three different types of coal from India and Poland with high sulfur content with varying parameters like particle size, pH, pulp density and growth medium composition resulted in 92% sulfur removal from Rajasthan coal and 9.4% from Assam coal, India, while 63.1% from bituminous coal of Poland and the reason for high sulfur removal from lignite coal was stated due to high pyritic sulfur content in the coal making amenable for desulfurization due to pyrite oxidation by *Acidithiobacillus ferroxidans* [7].

Studies conducted by artificial neural networks procedures to remove sulfur from the coal resulted in 92% of pyritic sulfur removal using autotrophic *Acidithiobacillus ferrooxidans* and 36% of organic sulfur removal using heterotrophic *Rhodococcus* sp. [13]. Peeples and Kelly [14] investigated the bioenergetics of the metal/sulfur oxidizing potential using *Metallosphaera sedula* a thermoacidophile instead of generally used *Acidithiobacillus ferrooxidans*, a mesophile for inorganic sulfur removal from coal and compared the results with respect to leaching yields, metabolism of the microbe and enhancement of the leaching yield [14]. Microbial desulfurization studies conducted on the both untreated and treated (with aqueous NH_4OH and Na_2CO_3) coal sample obtained from Assam, India with 5.5% (wt%) of sulfur using mixed mesophiles dominated by *Acidithiobacillus ferroxidans* resulted in a higher desulfurization rate for pretreated coal (35-38 wt%) compared to untreated one (5-27 wt%) without removing the organic sulfur (major sulfur source) in the coal [15]. This study aimed to optimize the best growth medium among basal salt (BS) medium, basal salt (BS) medium supplemented with 9 g/L Fe and basal salt (BS) medium supplemented with 2.5% S^0 for the microbial desulfurization of three different coals from Indonesia, China and Korea.

MATERIAL AND METHODS

1. Samples

The mineral used for the bioleaching experiments consisted of coal samples collected from three different countries, Indonesia, China and Korea. The Chinese lignite came from Neimenggu Meng Tai, Indonesia from Sumatra Lahat and Korean anthracite from Hwasun. Both the Indonesia and Chinese lignite samples were lignite coal, while the Korean coal was anthracite type. The coal samples were wet ground by an attrition milling system [16]. The system consists of an attrition mill with a grinding chamber and an agitator, a vacuum pump, a cooling circulator and a drive motor [17]. The grinding chamber was stainless steel with an inner diameter of 450 mm with a height of 450 mm having a working volume of 17 L. The rotor in the instrument had a diameter of 80 mm with six arms with a dimension of 20 mm diameter and 200 mm length, each held apart from each other at 90°, and the rotor was positioned at a height of 40 mm from the bottom. The rotation speed was 1,000 rpm. The grinding time was 10 min for each batch. As the coal samples were in large lumps, an initial crushing was carried out before grinding in the attrition mill. The grinding in the attrition mill was carried out in wet condition at a pulp density of 5% (w/v). The grinding media used for the grinding of coal in attrition mill was stainless steel balls of 3 mm size each with a total weight of 15 kg. Both the Korean anthracite coal and Chinese lignite coal did not have any problem with filtration, but the Indonesian lignite was difficult to filter after long time filtration, which could be due to over-grinding seen from the particle size analysis conducted on the Mastersizer 2000 particle size analyzer. The Indonesia lignite coal was ground too fine, probably due to its nature and property with the same grinding time for all the samples. All the coal samples were ground to a particle size of <100 μm and all the ground samples showed 80% passing below 100 μm. After filtration the filter cake was dried in a hot air oven for 56 hours to ensure complete drying and thereafter ground by mortar and pestle and divided with zones divider prior to its use in the bioleaching experiment. The chemical composition was analyzed by LECO analyzer and induced coupled plasma spectroscopy (ICP) for the three different coal samples as shown in Table 1.

2. Microorganisms

The microbial culture used for all the bioleaching experiments was isolated from an acidic mine drainage (AMD) region of the Dalsung mine area in South Korea, and grown in a shake flask on a basal salt (BS) medium supplemented with 4.5 g/L Fe and 2.5%

Table 1. Chemical analysis of the three different coal samples

Analysis	Unit	Indonesian lignite (IL)	Chinese lignite (CL)	Korean anthracite (KA)
Total moisture	wt%	24.7	28.1	10.6
Total iron	%	1.08	0.80	2.62
Total pyritic sulfur	%	0.23	0.20	0.23
Proximate analysis (air dry basis)				
Inherent moisture	wt%	13.6	12.9	4.5
Ash	wt%	0.9	13.91	36.5
Volatile matter	wt%	43.5	35.5	5.0
Fixed carbon	wt%	42.1	37.7	54.0
Total sulfur (air dry basis)	%	0.45	0.26	0.2
Higher heating value				
Gross caloric value (air dry basis)	kcal kg^{-1}	5870	4710	4360
Gross caloric value (dry basis)	kcal kg^{-1}	6790	5410	4560
Hardgrove grindability index	HGI	63.9	48.4	106.5

S^0 at pH 1.5 and temperature of 35 °C. The basal salt (BS) growth medium was comprised of $(NH_4)_2SO_4$, 3.0 g/L; KCl, 0.1 g/L; K_2HPO_4, 0.5 g/L; $MgSO_4*7H_2O$, 0.5 g/L; $Ca(NO_3)_2*4H_2O$, 0.01 g/L, supplemented with $FeSO_4*7H_2O$, 44.8 g/L and 2.5% S^0 [18]. After several sub-culturings in the Fe and S supplemented basal salt (BS) medium, the biomass was collected and subjected to 16s r RNA sequencing. For this purpose, a total of 10 sets of biomass samples were analyzed, with 99% of identities and was found to be *Acidithiobacillus ferrooxidans* (ATCC 23270 type). However, the *Acidithiobacillus ferrooxidans* is well known for its potential to oxidize both Fe and S [19]; therefore, this microbial culture was transferred to a batch reactor at a working volume of 1 L in Fe and S supplemented basal salt (BS) medium to revive both its Fe and S oxidation potential prior to its use in bioleaching experiments. The batch culture reactor was maintained at a pH level of 1.45±0.05 and a redox potential of 680 mV vs. Ag/AgCl. The viable planktonic microbial cell population in the full grown batch culture reactor was 2.8×10^9 cells/mL.

3. Analytical and Instrumentation Techniques

The analytical techniques used in the present study were followed by the estimation of the residual Fe^{2+} ion concentration, redox potential, planktonic viable cell count and chemical analysis. The residual Fe^{2+} concentration in the bioleaching solution was determined by a titrimetric method using cerium sulfate with 1,10-phenanthroline indicator [20]. Redox potential was measured with a platinum electrode against the Ag, AgCl reference electrode, while the pH was measured by Orion portable pH meter. Three point calibration of pH meter was carried out once every day on the standard buffers of pH 1.68, 4.0 and 7.0, and the slope obtained ranged from 95 to 105 ensuring good condition of the pH electrodes. Microbial population dynamics were studied by regular cell count of the viable planktonic cells using an improved Neubauer Haemocytometer under a phase-contrast microscope (Olympus Model No BX51TF). Total iron and sulfur content of the three different coal samples together with the bioleaching residues and bioleach liquor obtained from different experiments was determined by induced coupled plasma-mass spectrometer (ICP-MS).

4. Bioleaching Experiment

Batch bioleaching experiments were conducted in 250 ml Erlenmeyer flasks at a working volume of 100 ml in a shaker-incubator at a rotation speed of 180 rpm for homogeneous mixing of the bioleaching pulp. All the bioleaching experiments were conducted at pulp density of 5% with three different types of coal samples: Indonesian Lignite (IL), Chinese Lignite (CL) and Korean Anthracite (KA). The bioleaching medium consisted of three different composition: basal salt (BS) medium, basal salt (BS) medium supplemented with 9 g/L Fe (i.e., 44.7 g/L of $FeSO_4\cdot7H_2O$) and basal salt (BS) medium supplemented with 2.5% sulfur (S^0). The aim was to investigate the desulfurization potential of the mixed culture of iron and sulfur oxidizers in the various growth media with sulfur source, iron source and without iron or sulfur source during bioleaching of three different types of coal. The leaching solution for all the experiments consisted of 90 ml of growth medium and 10 ml of the inoculum adding up to 100 ml of working volume. The inoculum for all the experiments was taken from the active and fresh mixed microbial culture grown in shake flask prior to the experiments. Experiments were followed by regular measurements of several parameters like pH and redox potential. All the experiments were conducted under pH controlled condition with a pH of 1.5 by regular addition of 2 M/5 M H_2SO_4 and slaked lime ($Ca(OH)_2$) based on its requirement. Samples were drawn at various intervals from the bioleaching pulp to determine the Fe^{2+} ion concentration and viable planktonic microbial cell count. Experiments continued until a stable redox potential, pH and Fe^{2+} concentration was observed; thereafter, all the flasks were harvested and filtered followed by a thorough washing of the residues with acidified deionized water at pH 1.5 to avoid iron precipitation. The bioleach liquor obtained was also analyzed accounting to the amount of wash water added. The desulfurization percentage was calculated based on both residue analysis and leach liquor analysis, resulting in a good mass balance. Pyrite oxidation percentage was calculated based on the feed and residue analysis as per the formulae given below.

$$\text{Pyrite oxidation}\% = \left(1-\frac{\text{Fe(r)}}{\text{Fe(f)}}\right)\times 100$$

Where, Fe(r) is the iron content in residue and Fe(f) is the iron content in the feed. All experiments were conducted in triplicates with an average deviation was ±5%.

RESULTS AND DISCUSSION

1. pH Evolution with Acid and Base Consumption during the Microbial Desulfurization

The bioleaching experiment started with three different coals--Indonesian Lignite (IL), Chinese Lignite (CL) and Korean Anthracite (KA)--in three different growth media such as basal salt (BS) medium, basal salt (BS) medium supplemented with 9 g/L Fe and basal salt (BS) medium supplemented with 2.5% S^0 as discussed above. As discussed earlier about the addition of 10% (v/v) of the inoculum (microbial culture) to the growth medium, which was rich in ferric iron (0.09 g/L) responsible for the initial attack on the pyrite in the coal. The Fe^{3+} iron attack on the pyrite led to the oxidation of the pyrite producing $S_2O_3^{2-}$ and Fe^{2+} ion as intermediate products (Eq. (1)). Thereafter, the Fe^{2+} ion released from FeS_2 oxidation is further oxidized to Fe^{3+} ion by the Fe-oxidizing microorganisms (Eq. (2)), while $S_2O_3^{2-}$ is oxidized by Fe^{3+} ions to produce SO_4^{2-} ion (Eq. (3)) [21].

$$FeS_2+6Fe^{3+}+3H_2O\rightarrow 7Fe^{2+}+S_2O_3^{2-}+6H^+ \quad (1)$$

$$Fe^{2+}+1/4O_2+H^+\rightarrow Fe^{3+}+1/2H_2O \quad (2)$$

$$S_2O_3^{2-}+8Fe^{3+}+5H_2O\rightarrow 2SO_4^{2-}+8Fe^{2+}+10H^+ \quad (3)$$

The $S_2O_3^{2-}$ produced can be oxidized alternatively by S-oxidizing microorganisms into SO_4^{2-} ion. The overall reaction based on the primary oxidant is given in Eq. (4).

$$FeS_2+7/2O_2+H_2O\rightarrow Fe^{2+}+2SO_4^{2-}+2H^+ \quad (4)$$

From the above equations it is seen that oxidation of pyrite is an acid producing process followed by a further acid production due to the hydrolysis of ferric iron. As the microorganisms used in this bioleaching processes are chemolithotrophic and acidophilic having an affinity towards low pH and thrive very well at pH 1.5, addition of acid/base as per the increase/decrease in pH below/above 1.5 is required to maintain a luxuriant condition for the microor-

Table 2. Acid and Base consumption during biooxidation of coal

Experimental conditions	Conc. H_2SO_4 required ($kg \cdot ton^{-1}$)	$Ca(OH)_2$ required ($kg \cdot ton^{-1}$)
IL (BS+9 g/L Fe)	377	131
IL (BS+2.5% S)	304	712
IL (BS)	299	43
CL (BS+9 g/L Fe)	299	36
CL (BS+2.5% S)	94	769
CL (BS)	144	28
KA (BS+9 g/L Fe)	261	60
KA (BS+2.5% S)	160	524
KA (BS)	199	44

ganisms. However, all the bioleaching experiments had a higher consumption of acid in the initial 2-3 days due to the presence of acid consuming gangue minerals in coal. One of the plots showing the pH evolution followed by acid/base addition in the bioleaching experiment with Indonesian Lignite (IL) in basal salt (BS) medium supplemented with 9 g/L Fe. All the other pH evolution studies carried out for all the experiments were used to follow the pH regulation and maintenance during the complete process as well as for the calculation of the amount of the acid and base required for each experiment (Table 2).

The total amount of concentrated sulfuric acid required for each experiment carried out in basal salt (BS) medium supplemented with 9 g/L Fe on three different coal samples ranged between 261-377 kg/ton coal, which was comparatively higher than the other experiments with basal salt (BS) medium only and basal salt (BS) medium supplemented with 2.5% S^0 and was due to acid consuming Fe^{2+} ion oxidation (Eq. (2)). Apart from that, the acid consumption for the Indonesian lignite (299-377 kg/ton) compared to Chinese lignite (94-299 kg/ton) and Korean anthracite (160-261 kg/ton) was found to be relatively higher probably due to the presence of high content acid consuming gangue minerals in the Indonesian lignite. A lower amount of acid consumption was observed in experiments with basal salt (BS) medium supplemented with 2.5% S^0 with all the coal samples due to high content of acid produced via sulfur oxidation by the sulfur oxidizing microorganisms as per Eq. (5) [22-24].

$$S^0+3/2O_2+H_2O \rightarrow SO_4^{2-}+2H^+ \quad \text{(Sulfur oxidizing microorganism)} \quad (5)$$

Among all the experiments with basal salt (BS) medium supplemented with 2.5% S^0 with all three coal samples, Chinese lignite experiment with basal salt (BS) medium supplemented with 2.5% S^0 had the lowest (94 kg/ton) conc. sulfuric acid per ton of coal (Table 2). The reason for the lowest amount of acid consumption may be due to the luxuriant condition for the sulfur oxidizers to thrive well and conduct the oxidation process quickly, producing a large amount of acid partially fulfilling the acid requirement during the process. The luxuriant growth of the sulfur oxidizers in Chinese lignite may also be due to its property favoring the microbial growth.

Generally, all the tank biooxidation full scale operation existing today uses lime/limestone as a neutralizing agent to control the pH at desired level of 1.5 to avoid very low pH resulting from the pyrite oxidation and provide luxuriant growth condition for the microorganisms. Most lab scale experiments use slaked lime, where the chemistry underlying the neutralization process of the acid produced during to pyrite oxidation by slaked lime is represented in the equation [25]:

$$Ca(OH)_2+H_2SO_4 \rightarrow CaSO_4+2H_2O \quad (6)$$

The amount of slaked lime ($Ca(OH)_2$) required in all the experiments was calculated based on the additions carried out during complete course of the experiment. However, it was observed that the highest amount of slaked lime was required for each experiment conducted with 2.5% S^0 on all the three different coal samples ranging from 524-769 kg/ton coal, while the highest amount of slaked lime (769 kg/ton) was required in case of the experiment with Chinese lignite (CL) (Table 2). The highest slaked lime consumption is in correlation with the lowest acid consumed in the same experiment as stated before. The slaked lime required for the experiments with basal salt (BS) medium only for all three different coals was low among all the experiments ranging between 28-44 kg/ton coal, whereas the experiment with Chinese lignite (CL) in basal salt (BS) medium had the lowest with 28 kg/ton coal. Overall, in any biooxidation plant the neutralization cost is one the biggest cost [26], as huge amount of acid is produced due to pyrite biooxidation, while the pH has to be controlled at 1.5 to allow the microorganisms to carry out the biooxidation process comfortably. Therefore, the low slaked lime consumption mostly with the experiments with basal salt (BS) medium only without supplementing Fe/S source has a two-fold advantage: one is low consumption of slaked lime saving neutralization cost and the other is no addition of Fe or S^0 to the growth medium also saving growth medium cost and resulting in an overall cheaper operation cost.

2. Redox Potential Trend during the Microbial Desulfurization

The redox potential in all the experiments was measured on regular basis to follow growth of the microorganism and the bioleaching profile with time. The redox potential states the ratio of ferric to ferrous concentration in the leaching solution, which means more ferric iron in the solution compared to the ferrous iron, the higher would be the redox potential value and vice versa. As discussed earlier, during the initial days of the experiment Fe^{3+} ion coming from the inoculum was consumed for FeS_2 oxidation releasing Fe^{2+} ion into solution resulting in increase of the Fe^{2+} ion in the solution and fall in the redox potential, which can be clearly seen from Fig. 1. During the initial days of the experiment the fall in redox potential shows the lag phase for the microbial growth in the bioleaching experiments, and once the Fe^{2+} ion in the solution starts to oxidize to Fe^{3+} ion the redox potential starts to increase, which will be discussed in the later part considering the Fe^{2+} ion profile. All the experiments started with an initial redox potential value ranging between 379-502 mV on the first day and followed by a decrease 409-445 mV and later started to increase showing the active iron oxidation. The highest redox potential value achieved in the experiments for the Indonesian lignite, Chinese lignite and Korean anthracite ranged between 616-652 mV, 606-651 mV and 446-643 mV stating a good activity of the iron oxidation except the one experiment with Korean anthracite in 9 g/L Fe (Fig. 1). The very low redox potential observed in the experiment with Korean anthracite in basal salt (BS) medium supplemented with 9 g/L Fe could be due to the presence of some

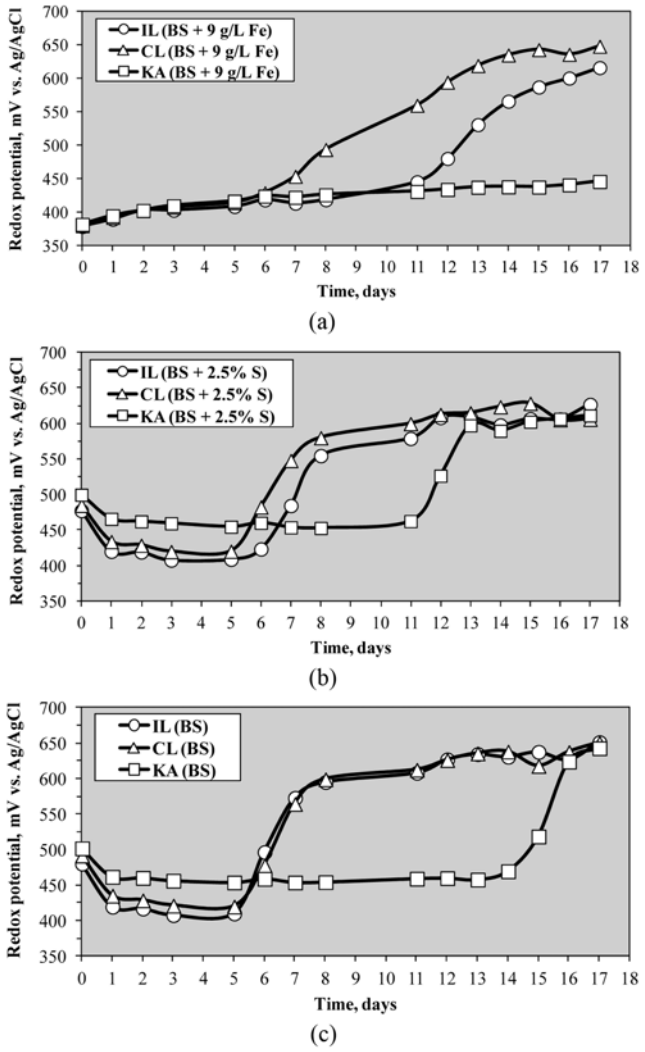

Fig. 1. Redox potential profile during biooxidation of coal in different experimental conditions.
(a) Redox potential trend of the bioleaching experiment in BS medium supplemented with 9 g/L Fe
(b) Redox potential trend of the bioleaching experiment in BS medium supplemented with 2.5% S^0
(c) Redox potential trend in the bioleaching experiment in BS medium

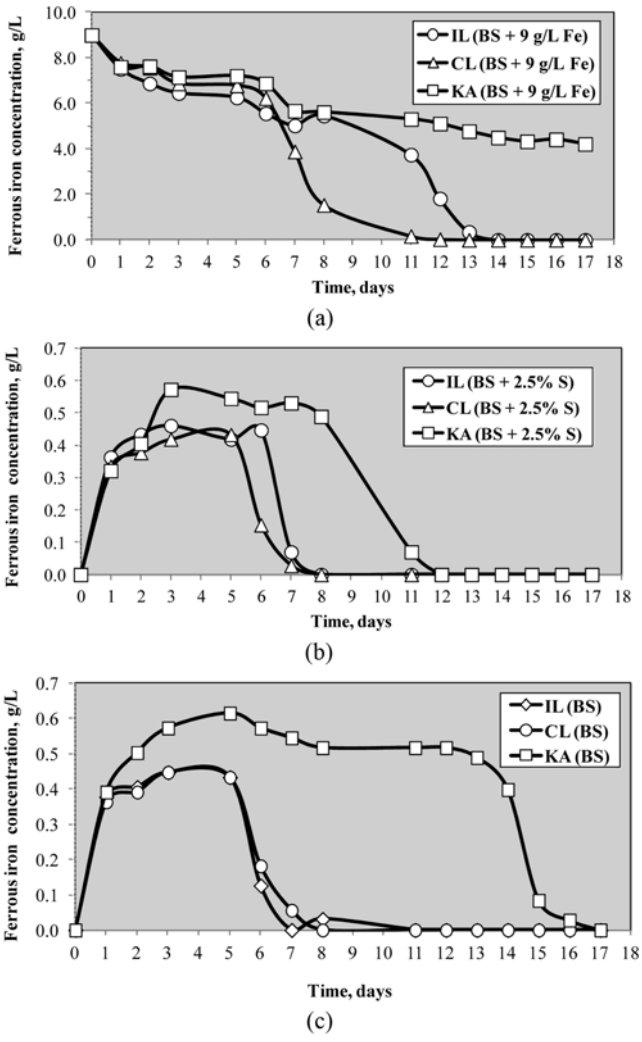

Fig. 2. Ferrous ion concentration profile during biooxidation of coal in different experimental conditions.
(a) Fe^{2+} concentration with time in experiment with BS medium supplemented with 9 g/L Fe
(b) Fe^{2+} concentration with time in experiment with BS medium supplemented with 2.5% S^0
(c) Fe^{2+} concentration with time in experiment with BS medium

toxic elements like Hg usually present in Korean anthracite [27]. This low redox potential also matched the ferrous iron profile and could be explained that the low redox was only due to incomplete iron oxidation in the solution. However, other experiments with Korean anthracite also had a similar problem as the lag phase was prolonged by 11-14 days in two other experiments with Korean anthracite (Fig. 1). It seems that iron oxidizers were much easily affected by the toxic influence of the Korean anthracite, while the sulfur oxidizers and Fe- and S-oxidizers like *Acidithiobacillus ferrooxidans* might have tolerated the toxic effect and switched off to S-oxidation instead of Fe-oxidation, and later adapted to the toxicity before it could oxidize ferrous iron in the solution in the other two experiments. On the contrary, the dominating species of iron oxidizers in the experiment with basal salt (BS) medium supplemented by 9 g/L Fe could not execute the iron oxidation function with the toxic influence of the Hg and needs to be further investigated in future studies. However, it was also observed in all the three coals the experiment with basal salt (BS) medium was found to be promising with very good biooxidation rate.

3. Ferrous Iron Trend during the Microbial Desulfurization Process

The ferrous iron content in the bioleaching solution measured on regular basis for all the experiments supported the results obtained from the redox potential profile and acid consumption during the microbial desulfurization process. As per Fig. 2, it was observed that all the ferrous iron oxidized to ferric resulting to 0 g/L of ferrous iron towards the end of the experiments except the one with Korean anthracite in basal salt (BS) medium supplemented by 9 g/L Fe, which stopped at 4.2 g/L starting from the initial value of 9 g/L. The most important results obtained from these experiments were the experiments where no Fe was supplemented to the growth

medium with no initial ferrous iron keeping on increasing with time until all the pyrite was oxidized (Fig. 2). The ferric iron in the inoculum as discussed earlier was responsible for the oxidation of the pyrite mineral in the coal releasing ferrous iron into solution and was observed from the increasing trend from 0 to 0.46 g/L, 0.45 g/L and 0.66 g/L of Fe^{2+} in the experiments with Indonesian lignite, Chinese lignite and Korean anthracite, respectively (Fig. 2). The release of Fe^{2+} ion into the solution undoubtedly came from the pyrite oxidation releasing Fe^{2+} and $S_2O_3^{2-}$ into solution (Eq. (1)), where the Fe^{2+} was oxidized to Fe^{3+} by the Fe-oxidizers (Eq. (2)) and $S_2O_3^{2-}$ to SO_4^{2-} either by Fe^{3+} or by the S-oxidizers (Eq. (3) and (5)). The decrease in pH due to acid production by sulfur oxidation is also a confirmation of the formation of sulfate ions as discussed earlier. A remarkable prolongation of the ferrous iron oxidation was observed in concurrence with the redox potential profile in case of the Korean anthracite experiments, possibly due to the toxic influence of expected elements like Hg, but this needs to be investigated in further studies.

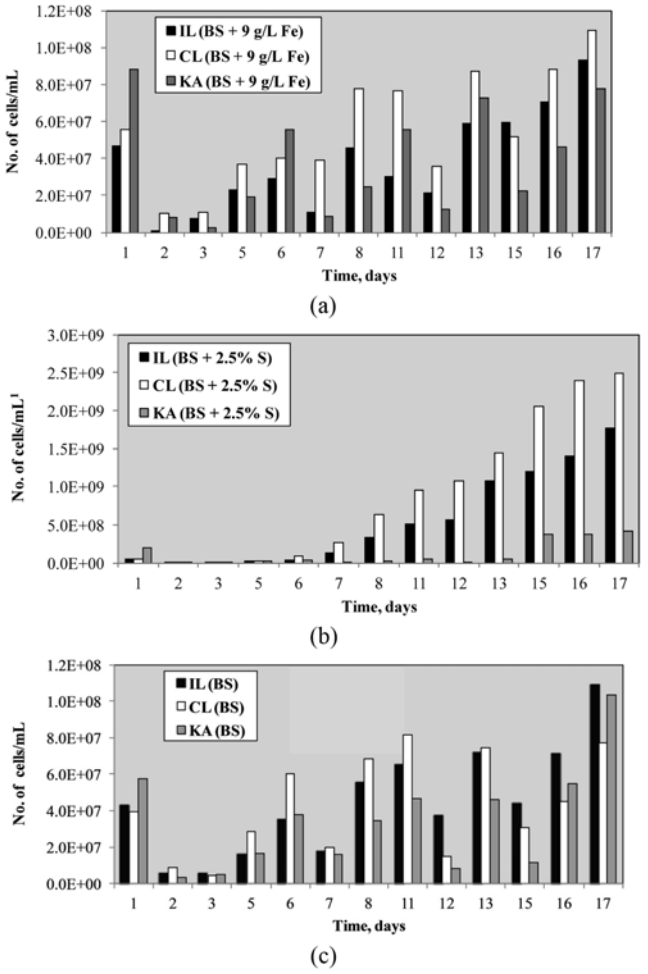

Fig. 3. Microbial cell (planktonic) population dynamics during bio-oxidation of coal in different experimental conditions.
(a) Planktonic microbial cell population dynamics during the experiment with BS medium supplemented with 9 g/L Fe
(b) Planktonic microbial cell population dynamics during the experiment with BS medium supplemented with 2.5% S^0
(c) Planktonic microbial cell population dynamics during the experiment with BS medium

However, the iron oxidation was much faster in the experiments conducted with Indonesia and Chinese lignite, proving it as an excellent material for microbial desulfurization.

4. Microbial Viable Planktonic Cell Population Dynamics

The microbial population dynamics study conducted by counting the viable planktonic cells actively involved in the desulfurization process revealed information supporting the redox potential and ferrous oxidation profile as discussed earlier (Fig. 3). The number of viable planktonic cells in the inoculums was 2.87×10^8 cells/mL, and after one day the population ranged between 3×10^7 to 8×10^7 cells/mL and was expected due to 10 times dilution of the inoculum bioleaching solution, which was taken added as 10% (v/v) as discussed in earlier sections. The important fact observed in all the experiments is that the viable planktonic cell count suddenly fell down to a range of 1.0×10^6 to 1.1×10^7 cells/mL on the 2nd and 3rd day of the experiment due the shock encountered by the microorganisms unable to withstand sudden changes redox potential (Figs. 1 and 3). Similar observations were made by Gahan et al. [28] in a study with pyrite concentrate, where it was observed that iron oxidizing microorganisms like *Leptospirillum* species thriving in the inoculum suddenly disappeared in the bioleaching solution and recovered back when the redox potential reached 650-700 mV. The decrease in viable planktonic cells in all the experiments conducted on the coal samples might have encountered a similar problem as observed by Gahan et al. [28] as the iron oxidizers present in the inoculum could have disappeared due to fall in redox potential from 650 mV to a range of 380-450 mV (Fig. 3). The other viable organisms in the system could be few sulfur oxidizers and Fe and S-oxidizers, which seem to be very robust organisms not much influenced by the drop in redox potential as demonstrated by Gahan et al. [28]. As per the lag phase discussed with regards to the redox potential profile in an earlier section, the microbial cell count also resulted with a similar lag phase by a slowly increasing population with time. However, an interesting correlation has been observed between the pH evolution, pyrite oxidation, ferrous oxidation and redox potential profile with the microbial population dynamics. As a toxic effect which was observed in the case of Korean anthracite discussed in earlier section could also be seen with a lower cell count of the viable planktonic cells compared to other two experiments with Chinese lignite and Indonesian lignite. Highest population of the microorganisms was observed in the experiments with basal salt (BS) medium supplemented with 2.5% S^0 and supports the reason for high acid production due to sulfur oxidation resulting in ample energy source for its replication.

5. Extent of Pyrite Oxidation and Sulfur Removal

The pyrite oxidation percentage for the experiment on three different coal samples with basal salt (BS) medium, basal salt (BS) medium supplemented with 9 g/L Fe and basal salt (BS) medium supplemented with 2.5% S^0 ranged between 71-81%, 81-95% and 70-80%, respectively (Fig. 4). The extent of pyrite oxidation was lowest with 70-71% in the experiments with Indonesian lignite and Chinese lignite in basal salt (BS) medium only, while the highest pyrite oxidation of 95% was observed in the experiment with Korean anthracite in basal salt (BS) medium supplemented with 9 g/L Fe (Fig. 4). The sulfur removal percentage calculated was found to be very low for experiments with basal salt (BS) medium supplemented with 2.5% S^0 for all the coals ranging from 27% to 48%, showing

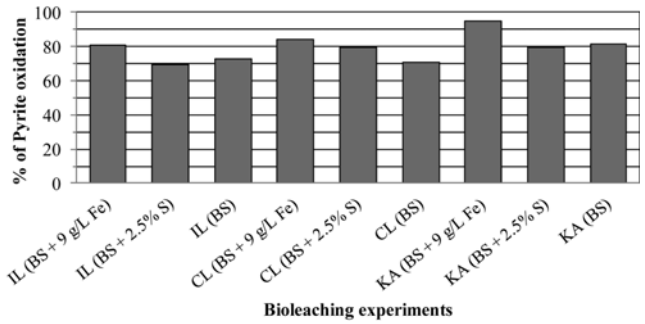

Fig. 4. Histogram showing the percentage of pyrite oxidation in different bioleaching experiments.

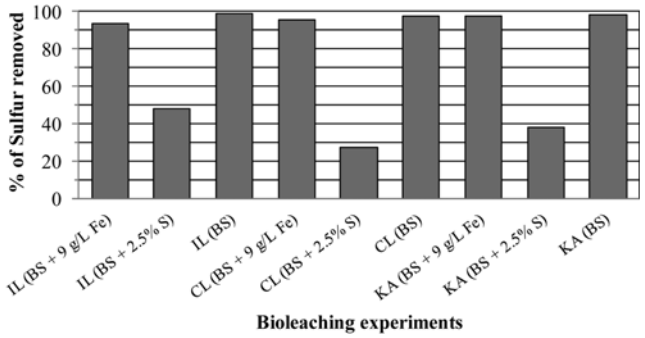

Fig. 5. Histogram showing the percentage of sulfur removal in different bioleaching experiments.

the importance of iron oxidizers in the microbial desulfurization process (Fig. 5). The sulfur removal percentage for the experiments with basal salt (BS) medium supplemented with 9 g/L Fe for all the three coals ranged between 94-97%, showing good desulfurization despite of weak growth in case of Korean anthracite (Fig. 5). However, it is inevitable that the microbes having the potential to oxidize both Fe and S have shown their prominence with a successful removal of sulfur compared to Fe-oxidizers and S-oxidizers. The sulfur removal percentage for the experiments with basal salt (BS) medium for all the three different coal samples ranged between 97-99% with Indonesian lignite being the highest with 99% followed by Korean anthracite as 98% and Chinese lignite as 97% (Fig. 5). Even though the sulfur removal percentages in the experiments with basal salt (BS) medium supplemented with 9 g/L Fe and basal salt (BS) medium only were similar, but the cost of the medium without Fe would be economical for the operation cost. Therefore, the experiments with basal salt (BS) medium only without Fe/S supplement stands to be a very economical and environmentally friendly process to desulfurize all three different coals. However, it should also be noted that the pulp density (w/v) used in this present study was 5%, which was comparatively half or one-fourth of the ratio used in a full scale operation in continuous stirred tank biooxidation. The reason for considering such a low pulp density was to avoid solid settlement in the bottom of the shake-flask due to insufficient mixing by an incubator orbital shaker. This pulp density can be further increased when scaled up in a bioreactor as the conditions for homogeneous mixing of solids even at higher pulp density is easier compared to shake-flask studies until a certain limit.

CONCLUSIONS

Our comparative study of the desulfurization of three different coal samples obtained from Indonesia, China and Korea in three different growth media--basal salt (BS) medium, basal salt (BS) medium supplemented with 9 g/L Fe and basal salt (BS) medium supplemented with 2.5% S^0--resulted in very promising findings. It was observed that the basal salt (BS) medium was the best among all the different media tested in all three types of coal samples. The growth parameters considered for the assessment of the successful microbial sulfur removal stated that the basal salt (BS) medium without additional iron or sulfur supplement was the best growth medium among all. This was concluded as economical compared with other growth media as additional iron and sulfur supplement did not have any benefit to the process. Some toxic effect of Korean anthracite encountered by the microorganism depicted in the results obtained of microbial activity needs to be further investigated in future studies. The extent of pyrite oxidation and percentage of sulfur removal were promising, suggesting its feasibility to operate and scale up for future. Considering the limitations of the shake-flask studies with regard to the low S/L ratio used in this present study, further studies in batch and continuous stirred tank reactors would help to evaluate the observed performances of *Acithiobacillus* species together with mixed cultures in particular at high S/L ratios maintaining optimum bioleaching. As it has been well known that biomining of sulfide minerals has proven to be environmentally friendly compared to high temperature pyrometallurgical processes, where toxic gases such as sulfur dioxide and mercury emissions are the major problems encountered together with energy cost and retention of coal properties. Similarly, physical separation of sulfur from pyritic coal leads to dumping of fines rich in pyrite leading to acid mine drainage, which is in fact one of the major problems faced by the mining companies at the mining sites. To overcome such problems with regards to environmental issues, biohydrometallurgical application for desulfurization of coal would provide technical, economical and environmental advantages.

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