

MICROBIAL DESULFURIZATION OF MALAYSIAN COAL IN BATCH PROCESS USING MIXED CULTURE

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Abstract Biodesulfurization of coal has been carried out in batch culture of single strain and mixed culture of sulfur oxidizing bacteria. The pure cultures of *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* utilized inorganic sulfur content of Malaysian coal. The chemolithotroph bacteria were able to grow on coal and metabolize the coal's sulfur content. The batch cultures of coal with 3-5 percent sulfur were used for growing microorganisms. A few species of bacteria and fungi had potential to utilize coal complex media, which were isolated from coal rice hulls and pharmaceutical wastewater. *Asperigillus niger* and *Thiobacillus* species isolated from coal, fungi from rice hulls and *Saccharomyces* species from wastewater, all microorganisms capable to grow on coal. The main concern was the sulfur oxidizing bacteria were screened out from all non-sulfur reducing bacteria. In the Batch cultures, the biooxidation coal's sulfur was affected by several parameters, such as pH, nutrients, agitation rate, growth stimulants and percentage coal. The trace amount of magnesium and cobalt ions, 200 rpm agitation rate with initial pH of 2.6 for mixed culture of *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* showed maximum growth rate and optimum batch operating conditions. The highest sulfur oxidation was obtained at the optimal *Thiobacillus* growth rate. More than 75 percent of sulfur was removed from coal. The biooxidation of coal, the kinetic model followed by Monod rate equation with specific growth rate, $\mu_{max} = 4.8 \text{ d}^{-1}$ and rate constant, $K_s = 16.7 \text{ g/L}$.

Key Words Biodesulfurization, Microbial Oxidation of Coal, Sulfur Removal, *Thiobacillus Thiooxidans*, *Thiobacillus Ferrooxidans*

چکیده سولفور زدایی ذغال سنگ به روش بیولوژیکی در فرآیند ناپیوسته با گونه ای منفرد و کشت مرکب به کمک باکتریهای اکسید کننده سولفور انجام شده است. کشتهای خالص *Thiobacillus thiooxidans* و *Thiobacillus ferrooxidans* از محتوای سولفور معدنی بهره می گیرند. باکتریها کیمولیتوتروف قادر اند بر روی ذغال سنگ رشد نموده و سولفور ذغال سنگ را در متابولیسم خود شرکت دهند. کشت ناپیوسته با سه یا پنج درصد سولفور برای رشد باکتریها به کار رفته است. چند گونه از باکتریهای جدا شده از پوسته برنج، فاضلاب کارخانه های داروسازی پتانسیل آن را دارند تا از محیط کشت پیچیده استفاده کنند. گونه های تیوباسیلوس و *Asperigillus niger* جدا شده ذغال سنگ و گونه های جدا شده از پوسته برنج و گونه *Saccharomyces* جدا شده از فاضلاب صنعتی قادر به کشت بر روی ذغال سنگ می باشند. نکته اساسی در کاوش باکتریها قادر به اکسیداسیون سولفور اند از باکتریهای که قدرت کاهش سولفور را ندارند متمایز می گردند. کشت ناپیوسته بیواکسیداسیون سولفور ذغال تحت تاثیر چندین عوامل از جمله pH با شدت همزن دوپست دور در دقیقه و محیط کشت *Thiobacillus thiooxidans* و *Thiobacillus ferrooxidans* با اولیه pH ۲/۶ حداکثر رشد در شرایط بهینه به دست آوردند. بالاترین نرخ اکسیداسیون سولفور با بهینه نرخ رشد تیوباسیلوس به دست آمده است. در این شرایط بیش از هفتاد و پنج درصد سولفور از ذغال سنگ حذف شده است. بیواکسیداسیون ذغال سنگ مدل سینتیکی منود Monod را پیروی می کند که نرخ رشد ویژه آن $\mu_{max} = 4.8 \text{ d}^{-1}$ و ضریب ثابت واکنش $k_s = 16.7 \frac{\text{g}}{\text{L}}$ می باشند.

1. INTRODUCTION

Coal is considered as energy resources for power

generation and industrial use. In steel industry coal is used as coke. Direct combustion of untreated coal creates air pollution and causes acid rain.

Production of clean coal via physical and chemical treatments required complicated processes. The conventional methods need extensive improvement. Recent development in biodesulfurization of coal has many advantages over chemical oxidation process. It requires low capital and operating costs as microbial desulfurization process operates at relatively low temperature [1,2].

The sulfurs in coal are categorized into organic and inorganic sulfurs. *Rhodococcus rhodochrous* is able to utilize organic sulfur [3]. *Thiobacillus* species generally have the potential to oxidize inorganic sulfur. *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* are the most common microorganisms grown on coal for sulfur removal [4,5]. It is believed that the acidophilic bacteria oxidize pyrite directly and gain energy for metabolizing other nutrients [6]. The mixed culture was the most effective method of bioleaching used on Hungarian coal.

The microorganisms were grown on coal as they utilized sulfur for energy source [7]. *Thiobacillus ferrooxidans* has a great potential to oxidize sulfur compounds in coal [8]. Chemolithotroph bacteria are able to oxidize and to reduce the sulfide ions, which are considered as impurities in the coal. The chemolithotroph microorganisms are also used for ore's bioleaching; the pyrite is converted to sulfate ions. The biooxidation process is based on microbial activities to oxidize pyrite at effective pH. It was reported that acid pH has shown high microbial growth rate [8,9].

The objective of this research was to find a desire media for microbial optimal growth and kinetic rate model for efficient biodesulfurization of coal in aqueous phase. The effective operating parameters in batch culture experiments were carried out with coal for biooxidation of inorganic sulfur.

2. MATERIALS AND METHODS

The coal, Banjar Masin Haji Ali-Aliansar, a sub bituminous type, Indonesian coal was obtained in small chunks. The coal was grinded and sieved to reduce to a suitable size of 200 meshes. The fine coal was dried in an oven, Thelco Laboratory oven model 160M at temperature of about 80 °C for 3 hours, stored in desiccators to avoid any moisture adsorption. The proximate analysis and ultimate

analysis were carried out by the standard methods using Thermo-gravimetric Analyzer, Perkin Elmer, TGA-7 and Elemental Analyzer, Perkin Elmer model 2400 series II respectively. The sulfur content of the untreated coal was measured, 3.8 percent.

The microorganisms *Thiobacillus ferrooxidans* ATCC 19859, *Thiobacillus thiooxidans* ATCC 15494 were obtained from American Type Culture Collection, Virginia, and USA. Also chemolithotroph bacteria were isolated from coalmine, fungi from rice hull and *Saccharomyces* species from pharmaceutical wastewater. The bacterial media was determined that best permits the fast growth of *Thiobacillus* species.

The bacteria were grown on coal media with the specified cultures were adapted on coal. The microorganisms were initially acclimated to grow on coal samples to reduce the lag phase, which was usually occurred for the first 2 days of incubation period. All of the prepared media were autoclaved at 121°C for 30 minutes for pure culture inoculation. Microbial Optical Density was used to monitor growth of microorganisms on coal based on light absorbance using Spectrophotometer (UV/VIS) Hitachi, model U 2200. The inoculation of seed culture to coal media was done under sterilized condition using a laminar flow, workstation, Gelman Class 100 series.

In the process of coal oxidation sulfate ions were liberated, which were determined by turbidity created with interaction of barium chloride. The turbidity was measured by spectrophotometer at wavelength of 420 nm. A buffer solution with addition of barium chloride may react with liberated sulfate ions. Standard method was developed using sodium sulfate solution [10]. A linear calibration curve was obtained for the range 0 - 45 mg/l with 5 mg/l increments.

Simple and complex media were prepared for microbial growth. The simple medium was used for seed culture, consisted of glucose or coal as carbon source, 1.0 g, yeast extract and peptone, 0.4 g in 100 ml distilled water. The complex medium for *Thiobacillus ferrooxidans* consisted of trace amount of metals and minerals ions such as magnesium, manganese, cobalt, zinc and copper ions with nitrogen source of ammonium sulfate. The medium for *Thiobacillus thiooxidans* consisted of ammonium sulfate, (0.2 g/l), magnesium sulfate, (0.5 g/l) and calcium chloride (0.25 g/l). Phosphates buffer was used for the desired pH. The initial pH

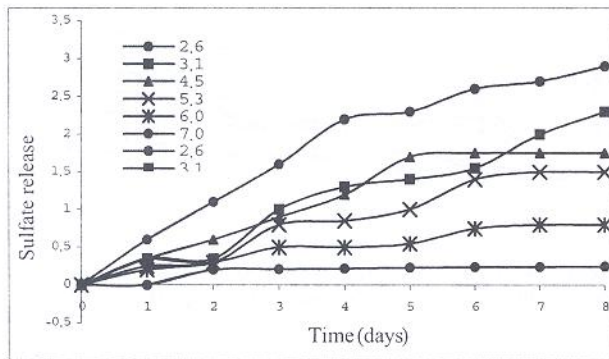


Figure 1. Effect of PH on sulfate released by *T. ferrooxidans* using phosphate buffer.

settings for the culture media in presence of coal, was contributed by use of phosphate buffer. The pH range of 2.6, 3.1, 4.5, 5.3, 6.0 and 7.0 were used for pH experiments. The compositions of the phosphate buffer used for initial pH setting were obtained from the literature [11]. Addition of 2percent agar was used if a solidified media for stock culture was required. Trace metals and minerals standard solution of 1 g/l of each was prepared. Based on required ions in the coal media, a defined volume of the standard solution was used.

Microorganisms were grown on coal in 200 ml media for the duration of 1-2 weeks. Throughout the incubation time, samples were taken for analysis. The experiments were carried out to optimize the culture media and to find any microbial growth stimulants for maximum sulfur removal from coal. Different parameters, such as initial pH setting, and agitation rate of 100 to 300 rpm were examined.

Batch experiments of coal desulfurization were carried out in Biostat B, B. Braun fermentor with 750 ml of working volume using mixed culture of *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*. The optimum operating conditions and defined media compositions were implemented to obtain kinetic data. The media pH was controlled at 2.6. The agitation rate was fixed at 200 rpm. The coal pulp density of was 3 percent in the media. The temperature was also controlled at 35 °C, for maximum microbial cell cultivation.

3. RESULTS AND DISCUSSION

In early stages of microbial screening, experiments were

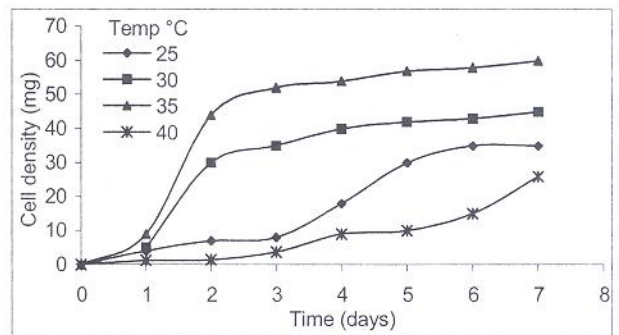


Figure 2. Effect of temperature on cell density of *Thiobacillus ferrooxidans*, with phosphate buffer at pH 2.6.

carried out with several sulfur oxidizing microbial cultures to determine an effect coal bacteria based on maximum cell density and growth yield. All the bacteria were isolated from coalmine, fungi from rice hull and *Saccharomyces* species from pharmaceutical wastewater were able to grow on coal media. All fungi and bacteria even had the capability to reduce sulfur but to justify the microbial metabolites were too complicated, therefore focusing on sulfur oxidizing bacteria yielded meaningful results. The most effective sulfur oxidizing bacteria identified were *Thiobacillus* species. The cell density and growth curve for *T. ferrooxidans* and *T. thiooxidans* grown on coal were linear for incubation period of 24 hours. Culture studies have been conducted to obtain the optimum conditions for coal desulfurization. The influential factors on *Thiobacillus* cultures were determined. The optimum conditions for batch experiments were carried out. Further research is required for large-scale bioreactor operation. The parameters were initial pH, temperature, agitation, percentage of coal and trace minerals and metals. Finally the mixed cultures for reaction rate model were carried out in coal desulfurization process.

The effects of pH on oxidizing coal's sulfur with variation of media's initial pH were studied. The coal desulfurization was conducted using *T. ferrooxidans*, phosphate buffer was used to maintain the pH of media in a stable condition throughout the experiments. The optimum media pH for coal oxidation was in the range of 2.6 to 4.5. Figure 1 presents the sulfate released from coal oxidation using *T. ferrooxidans* with different initial pH. Based on comparison studies the amount of sulfate ions liberated was maximized at pH of 2.6, concentration

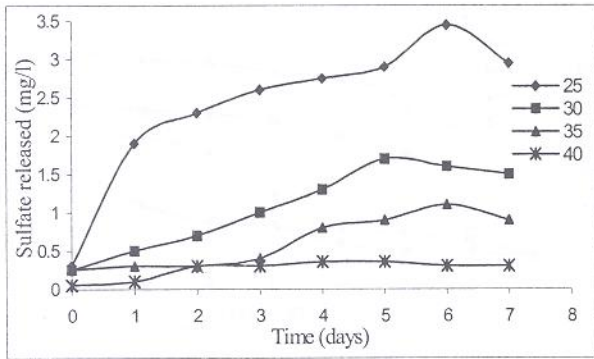


Figure 3. Sulfate released with *Thiobacillus ferrooxidans*, with phosphate buffer at pH 2.6.

of 2.9 mg/l. The cell density at pH of 2.6 was 50 mg/l. Therefore, phosphate buffer directly enhanced the growth rate of *T. ferrooxidans* and has further increased the oxidation ability of bacteria on coal.

Effect of temperature was also determined based on optimum microbial growth with the highest sulfate release in bio-oxidation. Therefore, range of temperature of 25° C to 40° C has been experimented on coal desulfurization with *T. ferrooxidans*. The highest cell density was obtained at 35° C, illustrated in Figure 2. At the optimal

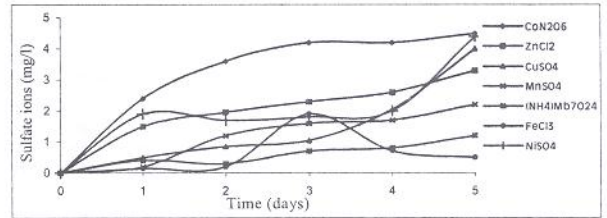


Figure 4. Effect of trace metals on sulfate released by *Thiobacillus ferrooxidans*, without controlling pH.

temperature of 35° C, maximum cell density was achieved: concentration of 33 mg/l without controlling pH. At initial pH of 2.6 and temperature of 35° C were the optimum conditions for sulfur oxidizing bacteria in coal media. The microbial cell density was improved by 82 percent using phosphate buffer. The cell density was increased to 60 mg/l for 7 days of incubation. The concentration of sulfate released was also increased and reached to 3.35 mg/l. Figure 3 shown the sulfate ions concentration profile with respect to time at various temperatures. At optimum temperature of 35° C, maximum concentration of sulfate ions was detected.

Trace metals had stimulated the sulfur oxidizing bacterial growth and enhanced the oxidation process,

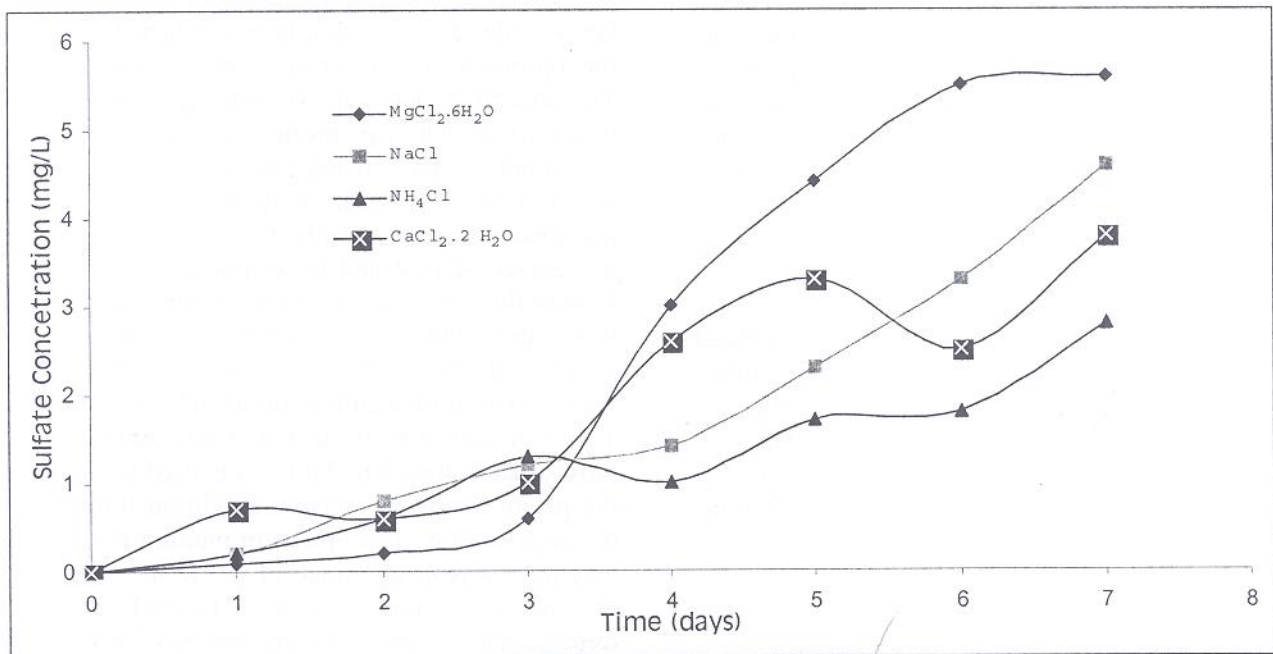


Figure 5. Effect of trace minerals on minerals on liberation of sulfate ions.

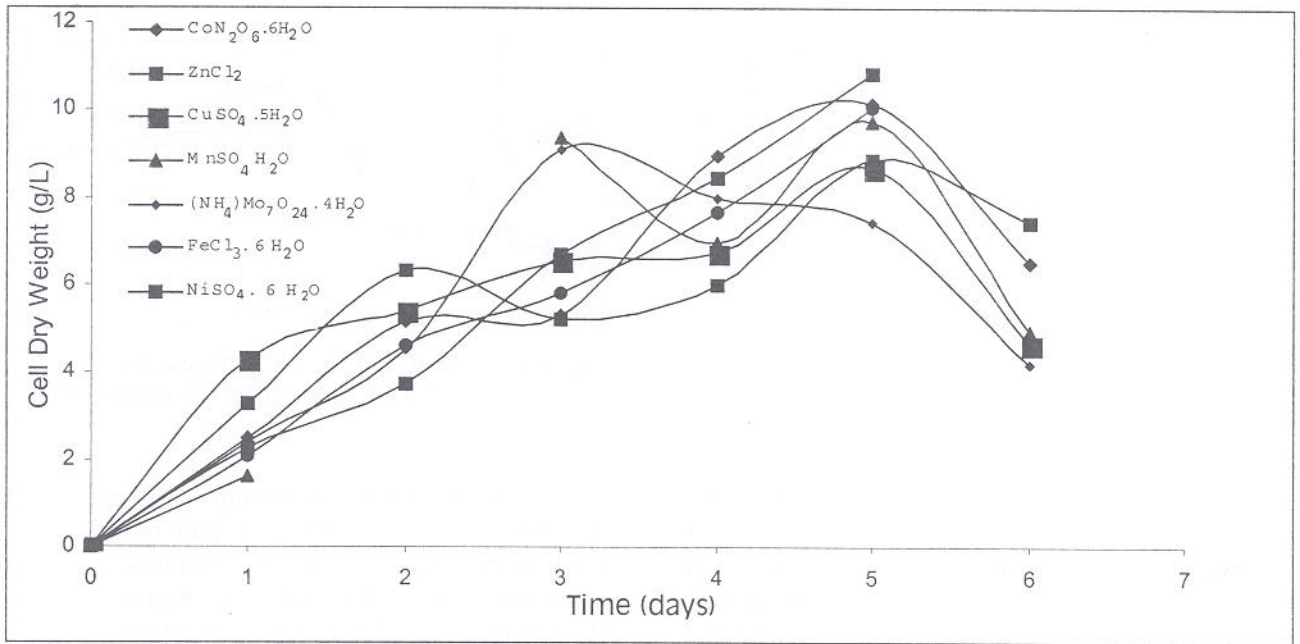


Figure 6. Effect of trace metals on Thiobacillus ferrooxidans growth in the coal media.

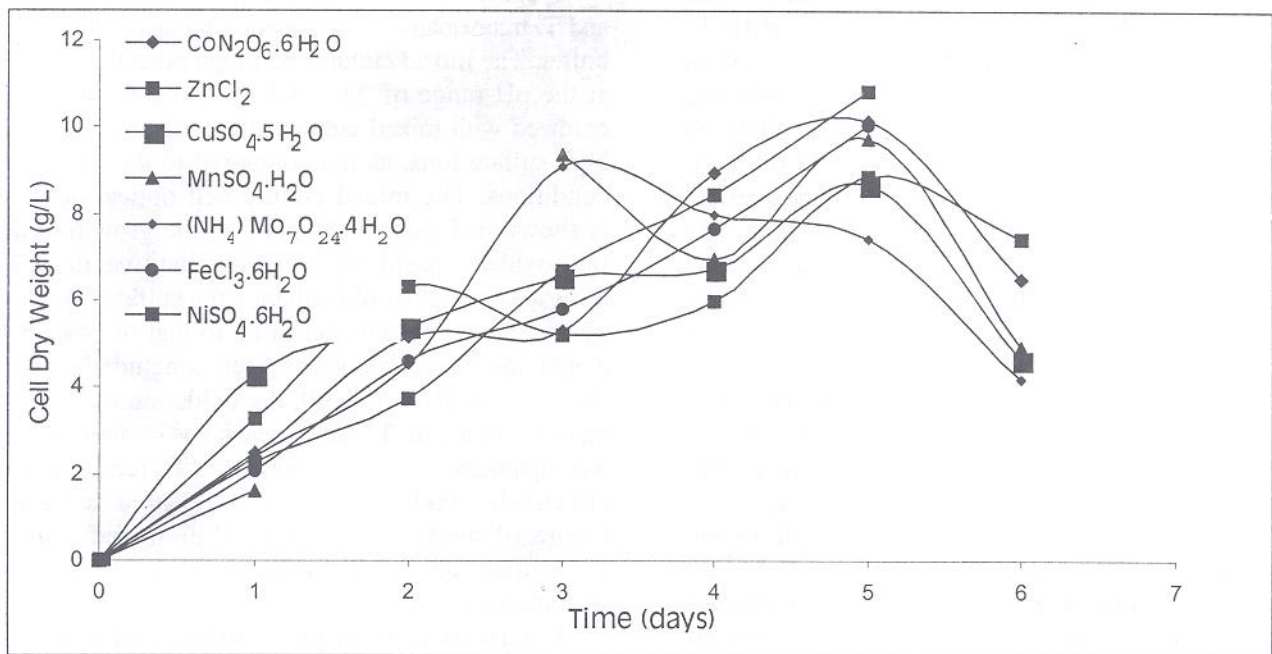


Figure 7. Bacterial growth with Thiobacillus ferrooxidans at different agitation rate and at optimum conditions.

shown in Figure 4. The cell growth had reached to a maximum level with nickel ions.

The cell density of *T. ferrooxidans* was 54.0 mg/l. Manganese ions shown fast cell growth. Bacteria also showed high growth with other

stimulators like cobalt, zinc, copper, ferric and nickel. Cobalt ions stimulated the oxidation process of coal by releasing sulfate in to a maximum sulfate concentration of 4.3 mg /l. The rate of sulfate released was 45 percent higher for

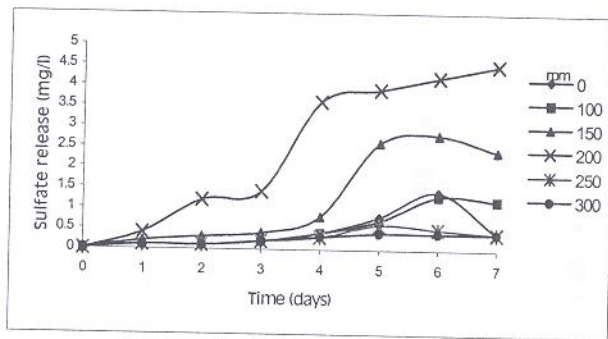


Figure 8. Sulfate release with *Thiobacillus ferrooxidans* at different agitation rate and at optimum conditions.

cobalt than that of zinc. Therefore; the significant stimulator for the oxidation of coal by *T. ferrooxidans* was cobalt ions at 0.016 v/v. The cobalt ions were considered as effective growth stimulants for *T. ferrooxidans* in the 3 percent coal media.

Trace amount of magnesium chloride, sodium chloride, ammonium chloride, and calcium chloride solutions were tested on coal media with *T. ferrooxidans* at concentration of 0.016 v/v. With magnesium and sodium ions the cell growth was tremendously increased. The growth was observed and the cell density was at the highest of 69 mg/l. Figure 5, shows that Mg^{+2} ion had maximum effect compared to other ions on the liberation of sulfate ions from pyrite. This stage was required for the biodesulfurization of coal. The Mg^{+2} showed maximum sulfate concentration of 5.8 mg/l after six days of incubation with coal in the broth media.

Effect of trace metals on cell optical density is presented in Figure 6, which is shown as cell dry weight. The cobalt and nickel ions are the growth stimulants for the biodesulfurization process.

The bacteria growth was proportional to the coal density. When coal density increased, the cell growth also increased. A 0.5 -10 percent of coal density percentage was used. The optimum coal concentration for, maximum sulfur reduction was 3 percent of coal.

Agitation rate in coal broth was conducted in the range of 100 to 300 rpm. Figure 7, shows at 200 rpm the cell growth was maximized, 135 mg/l. At high agitation rate of 250 and 300 rpm the cell growth was gradually decreased with cell density of only 31 mg/l that was due to high shear rate generated in coal media. The optimum agitation rate

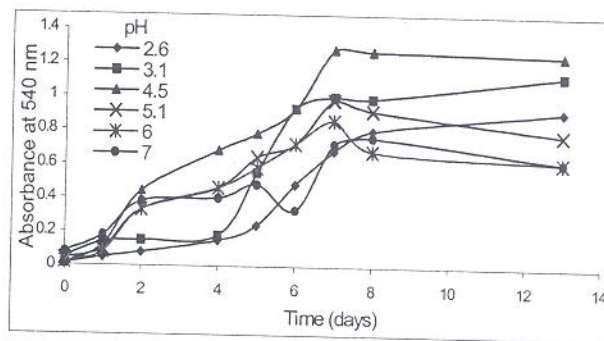


Figure 9. Growth of mixed cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* at pH 2.6 with phosphate buffer.

was at 200 rpm when maximum cell density was achieved. Also it is shown at 200 rpm, the coal oxidation process by sulfur bacteria was successful, with maximum sulfate released. Figure 8 shown the sulfate released with agitation rate also follows the microbial growth trend with maximum sulfate concentration of 4.85 mg/l was obtained at 200 rpm.

Effect of pH on mixed culture of *T. ferrooxidans* and *T. thiooxidans* were examined by using phosphate buffer. The mixed cultures had high potential growth at the pH range of 2.6 - 4.5. At pH 2.6, coal was oxidized with mixed culture effectively by releasing high sulfate ions, as it's compared to the other pH conditions. The mixed culture cell optical density is shown in Figure 9. At pH 2.6, the growth of *T. ferrooxidans* could be more competitive than *T. thiooxidans* that would enhance the sulfate released by oxidation process compared to that of other pH conditions. Therefore, it has been concluded that in the range of pH 2.6 to 3.0, the oxidation of coal by mixed culture of *T. ferrooxidans* and *T. thiooxidans* was optimum whereby at pH 2.6, *T. ferrooxidans* effectively oxidized coal and released sulfates. Figure 10 shows the effect of pH on mixed culture for sulfate ions concentration released by bio-oxidation of coal.

T. ferrooxidans as pure culture and a mixed culture of *T. ferrooxidans* and *T. thiooxidans* were experimented in a bioreactor for desulfurization of coal. At the optimum conditions, pH 2.6, media temperature of 35 °C, 200 rpm, 2 percent inoculum with 20 g coal as substrate in the bioreactor with working volume of 750 ml. The bacterial cell density was nearly at 224 mg/l with an increased of sulfate ions released, an increase up to nearly 23 mg/l of sulfate ions. The oxidation ability increased

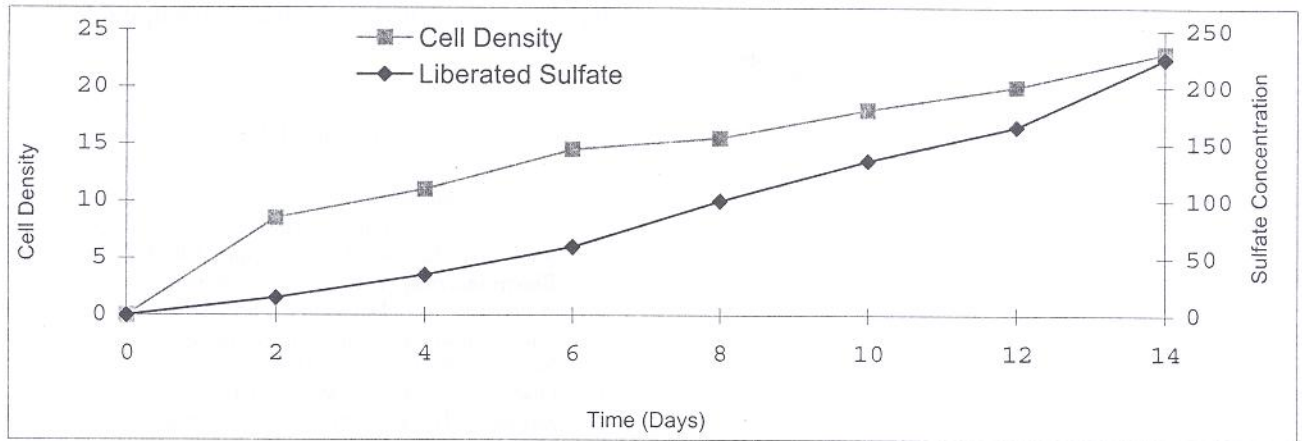


Figure 10. Sulfate released by Thiobacillus ferrooxidans & T. thiooxidans at different pH with optimum conditions.

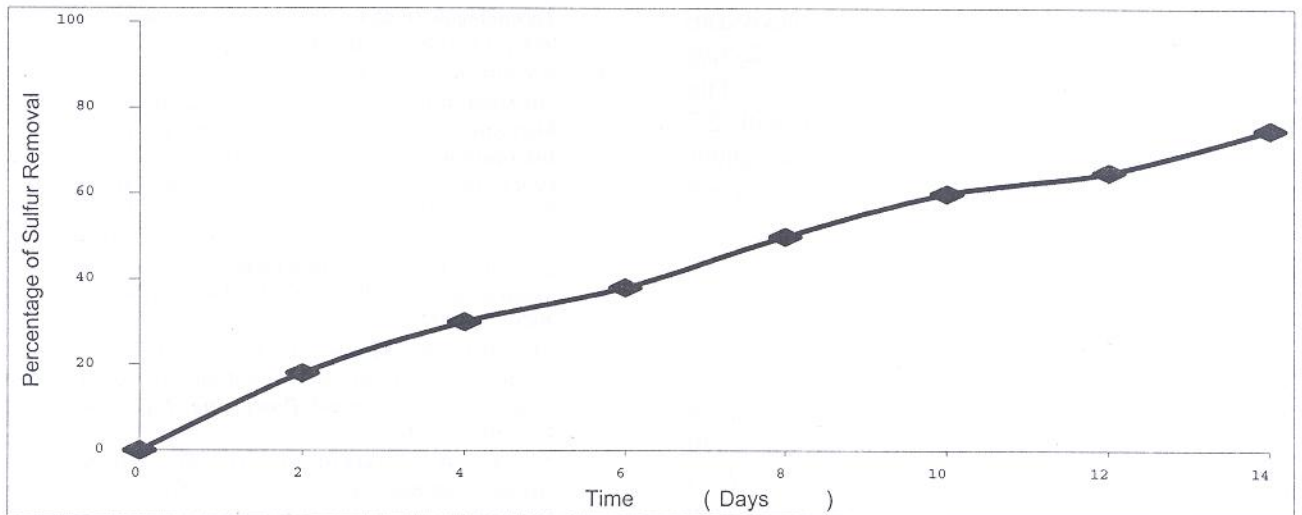


Figure 11. Sulfur removal from coal using mixed culture of by Thiobacillus ferrooxidans & T. thiooxidans.

approximately 4 times from the normal oxidation of coal with optimum conditions. The presence of magnesium and cobalt ions each at 0.016 v/v was believed to enhance the oxidation of coal by T. ferrooxidans. The bacteria had actively oxidized coal and released sulfate.

Figure 11, shows the percentage of sulfur reduction of coal in the bioreactor. The trend of sulfur removal was based on total sulfur presence in coal shown to be almost linear for incubation period of 14 days. The coal sulfur was reduced nearly more than 75 percent of the initial sulfur analyzed in the coal.

Based on experimental results obtained in coal

biodesulfurization using mixed culture in the bioreactor, the kinetic model, the Monod type rate equation shown, as following was quite suitable to represent bio-oxidation of sulfur. The obtained data shown in Figure 12 is linear, illustrated in Lineweaver-Burk plot with best fit. The specific growth rate (μ_{max}) and saturation constant (K_s) were 4.78 .d⁻¹ and 16.7 g/l respectively.

$$\mu = \mu_{max} S / (K_s + S)$$

$$-r_s = \frac{4.78 S}{16.7 + S}$$

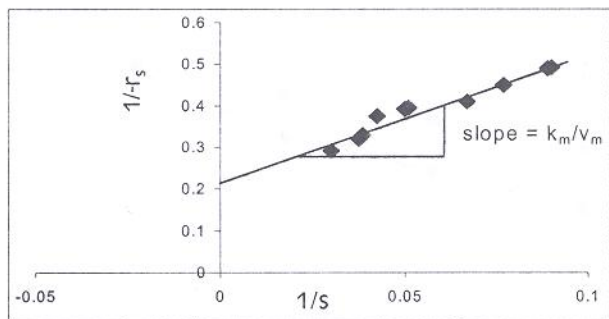


Figure 12. Kinetic model for coal desulfurization using mixed culture of *T. ferrooxidans* and *T. thiooxidans* at optimum conditions, 35 °C, 200 rpm and pH 2.6.

4. CONCLUSION

Results obtained for mixed culture of *Thiobacillus ferrooxidans* ATCC 19859 and *Thiobacillus thiooxidans* ATCC 15494, for their high growth rate and effective coal desulfurization at pH 2.6-4.5 and 35°C. The experimental results obtained shown that with 2.7 percent of coal presence in the mixed microbial culture, 75 percent of sulfur was removed and maximum cell growth was obtained.

5. ACKNOWLEDGEMENT

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6. NOMENCLATURE

μ	=	Specific Growth Rate, d^{-1}
μ_{max}	=	Maximum Specific Growth, d^{-1}
S	=	Substrate, Sulfur Concentration, g/l
K_S	=	Saturation Constant/ Half Velocity Constant, g/l

$-r_s$ = Rate of Sulfur utilized, g/l.d

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