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NOVEL MICROORGANISM FOR SELECTIVE SEPARATION OF COAL FROM ASH AND PYRITE

DOE Grant No: DE-FG22-93PC93215

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SUMMARY

This report summarizes the progress made during the second quarter of the research project entitled "A Novel Microorganism for Selective Separation of Coal from Ash and Pyrite", DOE Grant No: DE-FG22-93PC93215.

The objective of this project is to study the effectiveness of a novel hydrophobic microorganism, *Mycobacterium phlei* (*M.phlei*), for the selective flocculation of coal from pyrite and ash forming minerals. During the reporting period, the hydrophobicity of different coal samples was studied both in the presence and absence of *M. phlei* cells. In the absence of *M. phlei*, Il No.6 and Pa No.8 exhibited higher contact angles as compared to Ky No.9 coal. All the coal samples exhibited a maximum in contact angle around pH 5-7, which roughly coincides with the iso-electric point (iep) of different coals studied in this investigation. In the presence of *M. phlei*, maximum contact angle shifted to lower pH range of 2-3 which coincides with the iep of the *M. phlei*. These measurements reinforce the notion that good flocculation of coal with *M. phlei* can be achieved around pH 2-3.

The amount of soluble fraction released during rupturing of *M. phlei* cells was studied as a function of sonication time. The rupturing experiments showed that the whole cells (unruptured cells) contain nearly 40% by weight of soluble fractions. Also, during the reporting period, the fabrication of the counter-current flocculation device was completed.

INTRODUCTION

The increasing demand for clean coal requires the removal of ash and pyrite from run-of-mine coal. In order to produce compliance quality coal, inorganic minerals such as silica, clay and pyrite have to be removed by ultra-fine grinding followed by physico-chemical separation processes. Considering the tonnage of coal processed in the United States, it is obvious that improved physical coal cleaning techniques will be attractive in terms of economics and process feasibility. The selective separation of pyrite and ash-forming minerals can be accomplished by flotation, agglomeration and selective flocculation. The methods currently used for selective flocculation of coals include addition of natural or synthetic polymeric flocculants along with precise pH control. In some cases, these flocculants are nonselective or work imperfectly.

It is known that many highly charged planktonic algae and bacteria will adhere to certain solid surface if the charge or hydrophobic interaction between the organism and the solids are favorable for adhesion. The resultant microorganism-mineral entities if formed can flocculate and can be separated. In addition, many living organism produce extracellular biopolymers that can also cause flocculation. The microorganism, *M. phlei*, has the properties of being both highly charged and highly hydrophobic. The aim of the present investigation is to study the effectiveness of *M. phlei* and biopolymers derived from the organism for selective flocculation of fine coal from ash and pyrite.

EXPERIMENTAL PROCEDURES

1. Culturing of *Mycobacterium phlei*

The freeze-dried culture of microorganism, *M. phlei*, was first transferred to a rehydration medium (supplied by Carolina Biological Supply Company) and incubated at

35°C for 48 hours. *M. phlei* was grown in a culture medium consisting of the following : 10 g/l D-(+)-glucose, 1 g/l beef extract, 1g/l yeast extract, and 2 g/l enzymatic hydrolysate casein (1-2). The above materials were supplied by Sigma Chemical Company, St. Louis, MO. The culture medium was sterilized at 121°C for 25 minutes in a Spectroline Model 750 autoclave. The sterilized culture medium was cooled and the incubated bacteria was inoculated into the medium. Culturing was carried out in 250 ml flasks continuously shaken at 150 rpm at 35°C in a G24 Environmental incubator shaker manufactured by New Brunswick Scientific Co. Inc., NJ. After about 30 hours of culturing, *M. phlei* was harvested by centrifugation, washed twice in distilled water and then resuspended in distilled water. Sterilized glassware and distilled water were used throughout the experiments.

2. Rupturing of *M. phlei* Cells

Rupturing of *M. phlei* cells was performed using a Branson 184V ultrasonic probe. *M. phlei* was harvested by centrifugation, washed twice in distilled water and resuspended in distilled water. This suspension was then sonicated at 20,000 Hz. After sonication, the ruptured cells were separated from the supernatant by centrifugation. The ruptured cells were then used in the experiments.

3. Contact Angle Measurements

The hydrophobic/hydrophilic balance at the coal surfaces was measured by using a Ramé-hart contact angle goniometer Model A-100. The equipment, shown in Figure 1, consists of a goniometer with two rotatable cross hairs for measuring the contact angle, a specimen stage and a variable intensity illuminator. All of these are mounted on a optical bench.

Sample Preparation : Samples of approximately 1 x 1 x 0.5 cm size were selected

and then molded with epoxy resin. Freshly polished surfaces were prepared by polishing the surface first with 1000 and 4000 grit SiC polishing paper and then with 0.05 μm alumina suspension. The samples were thoroughly washed with distilled water to remove any adhering alumina and then used for measuring contact angle. Latex gloves were used throughout the experiments in order to avoid contamination of the surfaces.

Technique of Measuring Contact Angle: Contact angles of different coal samples were measured using the captive bubble technique. This technique involves immersing the freshly polished sample into an optical glass cell containing water and then attaching an air bubble to the surface. The angle (θ) formed between the air bubble and the solid surface is measured through the liquid medium and is known as the contact angle. This is illustrated in Figure 2. Depending on the hydrophobicity of the surface, the contact angle varies. The greater the contact angle, the greater the hydrophobicity of the surface. The contact angles on both sides of the air bubble were measured. An average of at least 4 measurements is reported here. The standard deviation in measuring single contact angle was about $\pm 2^\circ$. All measurements were carried out at $25 \pm 2^\circ \text{C}$.

Method of Coating Coal Samples With *M. phlei* : *M. phlei* cells, both whole and ruptured, were harvested by centrifugation and then dried in a Fisher Isotemp Vacuum Oven Model 281A at 45°C . Then suspensions of different concentrations and at different pH were prepared. A few drops of the suspension were placed on the surface of freshly prepared coal samples and dried at 45°C . The coal samples were then rinsed slowly for a few minutes in distilled water to remove any weakly adhered cells from the surface. The samples were again dried and then transferred to the quartz cell containing distilled water. The ionic strength of the water was maintained constant at 0.01 M NaNO_3 . The pH of the water was adjusted such that it was the same as that at which the *M. phlei* suspension was conditioned. The pH was adjusted by NaOH or HNO_3 .

EXPERIMENTAL RESULTS

1. Rupturing of *M. phlei* cells

It was shown in the last report that rupturing of *M. phlei* cells releases the soluble fraction consisting of straight chain fatty acids. During this quarter, the amount of soluble fraction released was studied as a function of sonication time and the results are shown in Figure 3. It can be seen that the amount of soluble fraction released increases with sonication time and after about 20 minutes no appreciable change is noticed. This graph indicates that nearly 40% of the cell is made up soluble fraction. Based on these results, the sonication time of rupturing *M. phlei* cells for future experiments was fixed at 20 minutes. However, the exact functional groups associated with these straight chain fatty acid compounds is not known and studies are in progress to identify the soluble fractions.

2. Hydrophobic / Hydrophilic Balance

The hydrophobic/hydrophilic balance at the surface of three different coal samples [Illinois No.6 (Il No.6), Kentucky No.9 (Ky No.9) and Pittsburgh No.8 (Pa No.8)] was studied using the captive bubble technique. Figure 4 shows the intrinsic hydrophobicity of the three coals as a function of pH. Both Il No.6 and Pa No.8 are more hydrophobic than Ky No.9. Figure 5 shows the variation of contact angle of the coals as a function of fixed carbon content at pH 7.2. As the carbon content increases, the contact angle increases. which suggests that the hydrophobic/hydrophilic balance is controlled by the amount of carbon. All the three coal samples show a variation in contact angle as a function of pH exhibiting a maximum at around neutral pH. The maximum in contact angle is close to the iso-electric point for all the coals as shown in Figure 6.

The variation of contact angle with the addition of whole and ruptured cells of *M.*

phlei was carried out as a function of pH. The results are shown in Figs. 7-9. As can be seen, conditioning coal samples with whole cells (180 ppm, without rupturing) increased contact angle significantly. For example, conditioning Il No.6 coal with whole cells (180 ppm) increases contact angle from 55 to 67° at pH 3.0. It is striking to note that maximum contact angle of 65° was measured at pH 3.0 with *M. phlei*. On the contrary, maximum contact angle was measured at pH 6.5 without the addition of *M. phlei*. Although, at pH 6.5 the coal showed an increase in contact angle with addition of *M. phlei*, however, a drastic shift of maximum contact angle to lower pH of 3.0 is interesting. This maximum contact angle at pH 3.0 corresponds to the iso-electric point of *M. phlei*. It is quite possible that at iso-electric point the adhesion of microorganism is favorable thus exhibiting a high contact angle. These measurements indicate that the flocculation of fine coal due to hydrophobic interaction will be favorable at pH 2-3. It is planned to run flocculation experiments with *M. phlei* in the pH range of 2-3.

Similarly, the effect of ruptured cells of *M. phlei* on the hydrophobicity of the coals was studied as a function of pH and the results are shown in Figs. 7-9. The effect on hydrophobicity was different than the whole cells. Although, an increase in contact angle was observed, it was not as significant as in the case of whole cells. The cell wall of *M. phlei* contains various fatty acid chains and functional groups which contribute to the surface charge and hydrophobicity of the microorganism (3). However, upon rupturing, these biopolymers are released thus making the cell wall less hydrophobic. Hence lower contact angles are observed with ruptured cells when compared to whole cells of *M. phlei*.

The effect of concentration of *M. phlei*, both whole and ruptured cells, on the contact angle was studied and the results are shown in Figs. 10-12. It can be seen that contact angle increases with increasing concentration of *M. phlei* cells before reaching a maximum value. The concentration of ruptured cells required to achieve maximum contact angle is almost twice than that of whole cells.

FUTURE STUDIES

Future studies will include studying the adhesion of *M. phlei* cells on coal and pyrite surfaces by Scanning Electron Microscopy and the effect of *M. phlei* on hydrophobicity of coal and ore pyrite will be investigated. Also, flocculation experiments will be conducted using the counter-current flocculation device.

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Figure 1. Schematic diagram of the contact angle goniometer apparatus

Figure 2. The captive bubble technique of measuring contact angles

Figure 3. The amount of soluble fraction released during rupturing of *M. phlei* cells as a function of sonication time.

Figure 4. The variation of contact angle of different coal samples as a function of pH in deionized water

Figure 5. The variation of contact angle of different coals as a function of carbon content.

Figure 6. Zeta potential of different coal samples as a function of pH
(Studies using Ky No.9 are in progress)

Figure 7. The effect of *M. phlei* cells (whole and broken cells) on the contact angle of Illinois No. 6 coal as a function of pH.

Figure 8. The effect of *M. phlei* cells (whole and ruptured cells) on the contact angle of Pittsburgh No. 8 coal as a function of pH.

Figure 9. The effect of *M. phlei* cells (whole and ruptured cells) on the contact angle of Kentucky No. 9 coal as a function of pH.

Figure 10. The variation of contact angle as a function of *M. phlei* concentration for Illinois No. 6 coal.

Figure 11. The variation of contact angle as a function of *M. phlei* concentration for Pittsburgh No. 8 coal.

Figure 12. The variation of contact angle as a function of *M. phlei* concentration for Kentucky No. 9 coal.

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