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# NOVEL MICROORGANISM FOR SELECTIVE SEPARATION OF COAL FROM ASH AND PYRITE

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First Quarterly Technical Progress Report  
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## SUMMARY

This report summarizes the progress made during the first 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, three different coal samples : Illinois No. 6 coal, Kentucky No. 9 coal and Pittsburgh No. 8 coal, were collected to be used in the investigation. The microorganism, *M. phlei*, was obtained as freeze-dried cultures and the growth characteristics of the bacteria were studied. Scanning electron microphotographs revealed that *M. phlei* cells are coccal in shape and are approximately 1  $\mu\text{m}$  in diameter. Electrokinetic measurements showed that the Illinois No. 6 and Pittsburgh No. 8 coal samples had an isoelectric point (IEP) around pH 6 whereas *M. phlei* had an IEP around pH 1.5. Electrokinetic measurements of the ruptured microorganisms exhibited an increase in IEP. The increase in IEP of the ruptured cells was due to the release of fatty acids and polar groups from the cell membrane.

## 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 ultrafine 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 well 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 conducive for adhesion. The resultant microorganism-mineral entities if formed can flocculate readily. In addition, many living organisms produce extracellular biopolymers that can cause flocculation. The microorganism, *M. phlei*, is a remarkable one. It has the properties of being both highly negatively charged and highly hydrophobic. The aim of the present investigation is to study the effectiveness of *M. phlei* and biopolymers derived from the ruptured organism for selective flocculation of fine coal from ash and pyrite.

## EXPERIMENTAL PROCEDURE AND ANALYSIS

### 1. Acquisition of Samples

Three different coal samples were acquired to be used in this investigation. These coal samples are : (1) Illinois No. 6 coal, (2) Kentucky No. 9 coal, and (3) Pittsburgh No. 8 coal. The first two coal samples are high-sulfur coals and the third one is a low-sulfur coal. The proximate and sulfur analyses of these coal samples are given in Table 1.

Table 1. Proximate and sulfur analyses of the three coal samples

Composition	Pittsburgh No. 8 (%)	Illinois No. 6 (%)	Kentucky No. 9 (%)
Moisture	1.93	9.47	8.27
Volatile Matter	37.93	31.13	28.20
Fixed Carbon	54.92	42.70	40.06
Ash	5.22	16.70	23.47
Total Sulfur	1.12	4.34	4.06
Sulfatic Sulfur	0.08	0.34	0.14
Pyritic Sulfur	0.56	2.34	2.10
Organic Sulfur	0.48	1.66	1.82

Table 2. Composition of the soluble fraction released during rupturing of *M. phlei* cells

Name	(%)
14 - Carbon Chain Length Fatty Acid	4.01
16 - Carbon Chain Length Fatty Acid	13.61
18 - Carbon Chain Length Fatty Acid	43.53
20 - Carbon Chain Length Fatty Acid	38.85

The microorganism, *M. phlei*, was obtained from Carolina Biological Supply Company, Burlington, North Carolina. The microorganism was in the form of a freeze-dried culture containing lyophilized pure strains of viable bacteria and was stored in a refrigerator at 4°C.

## **2. Culturing of *Mycobacterium phlei***

The freeze-dried culture of the 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, 1 g/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, the *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.

## **3. Identification and Characterization of *M. phlei***

Identification of the bacterial culture was performed at Analytical Services, Inc., Essex Jct., VT, by using a fatty acid analysis. Each fatty acid profile is compared with the Microbial Identification System database and the microorganisms are identified. The fatty acid analysis indicated that the strain contained *Mycobacterium phlei* and *Staphylococcus species*.

Scanning Electron Microscopy (SEM) studies were carried out using a JSM-840A scanning electron microscope. *M. phlei* samples for SEM studies were prepared by centrifugation followed by drying in petri dish at 45°C.

#### **4. 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 subjected to ultrasonic treatment. Sonication was carried out for about 18 minutes at 20,000 Hz. After sonication, the ruptured cells were separated from the supernatant by centrifugation and used for electrokinetic studies. The supernatant was sent for analysis to Analytical Services, Inc., Essex Jct., VT.

#### **5. Electrokinetic Measurements**

Electrokinetic studies were carried out using a Lazer Zee Meter, Model 501, manufactured by Pen Kem, Inc., NY. The Lazer Zee Meter is a highly sophisticated instrument and is composed of an electrophoresis chamber, laser ultra-microscope, prism/galvanometer assembly, and readout display as shown in Figure 1.

Coal samples of -400 mesh were used for measurement of zeta potential. The sample was first conditioned in  $1 \times 10^{-2}$  M  $\text{NaNO}_3$  for 3 minutes. After pH adjustments were made, the suspension was further conditioned for 3 minutes. The suspension was then transferred to the electrophoresis cell and measurements were made. The readings reported here are the average of 10 readings. pH adjustments were done using either  $\text{HNO}_3$  or  $\text{NaOH}$  and pH measurements were done using a Accumet Model 5 pH meter.

In the case of *M. phlei*, both whole cells and ruptured cells, were harvested by centrifugation, washed twice in distilled water and then resuspended in distilled water. The suspension was then used for electrokinetic measurements.

## EXPERIMENTAL RESULTS

### 1. Growth Characteristics of *M. phlei*

The growth kinetics of *M. phlei* were studied by determining the mass of the cells produced as a function of time using the culture medium described earlier. The growth curve for *M. phlei* is presented in Figure 2. The cell growth can be divided into three distinct stages viz. lag phase or incubation period, exponential growth phase and stationary phase. The lag phase represents the time when the freeze-dried cultures are incubated in the rehydration medium. Once the incubated microorganisms are inoculated in the culture medium, exponential growth occurs. After sometime, the growth of the cells reaches a stationary phase. The cells are harvested at the end of the exponential growth period to be used in the flocculation experiments.

### 2. Identification and Characterization of *M. phlei*

Identification of the microbial culture grown was performed by fatty acid analysis. The microorganism was identified as *M. phlei*. However, the culture was slightly contaminated with the species, *Staphylococcus hominis* and *Staphylococcus epidermis*. The results of SEM studies are shown in Figure 3. It can be seen that *M. phlei* cells are coccal or spherical in shape with a diameter of about 1  $\mu\text{m}$ . The shape of the cell, however, can be altered depending on the culture medium and conditions. The shape of *M. phlei* cells after rupturing will be studied by using SEM.

### 3. Electrokinetic Measurements

The results of electrokinetic measurements of the Pittsburgh No. 8 and Illinois No. 6 coals are shown in Figure 4. Pittsburgh No. 8 coal has an isoelectric point (IEP) at around pH 6.3 while Illinois No. 6 coal has an IEP around pH 5.9. The coals are positively charged at pH values less than the  $\text{pH}_{\text{IEP}}$  and become negatively charged at pH values higher than that.

However, in the case of *M. phlei* whole cells (as shown in Figure 4), the electrokinetic behavior is different. *M. phlei* has a IEP at around pH 1.5 and becomes negatively charged at pH values greater than  $pH_{IEP}$ . No visible movement could be observed below pH value of 1.5 and hence measurements could not be made. The negative charge imparted to *M. phlei* may be attributed to the presence of large number of polar groups i.e., carboxyl-, hydroxyl-, amino-, and phosphate-groups, in the cell wall and is shown in Figure 5 (3). In the pH range where, both *M. phlei* and coal possess opposite charges, favorable adhesion of *M. phlei* on coal can be anticipated due to strong coulombic interaction.

When the whole cells were ruptured by sonication, the electrokinetic behavior of the ruptured cells changed and is shown in Figure 6. The isoelectric point of the ruptured cells of *M. phlei* is close to pH 7. This indicates that with the rupture of *M. phlei* whole cells, the polar functional groups present in the cell wall are released thus making the surface more positively charged. Analysis of the extracellular fraction released during sonication showed that fatty acids of different carbon chain lengths were released and the results are tabulated in Table 2. The exact nature of the functional groups attached to these fatty acid chains will be studied by FTIR analysis during the next quarter.

## **FUTURE WORK**

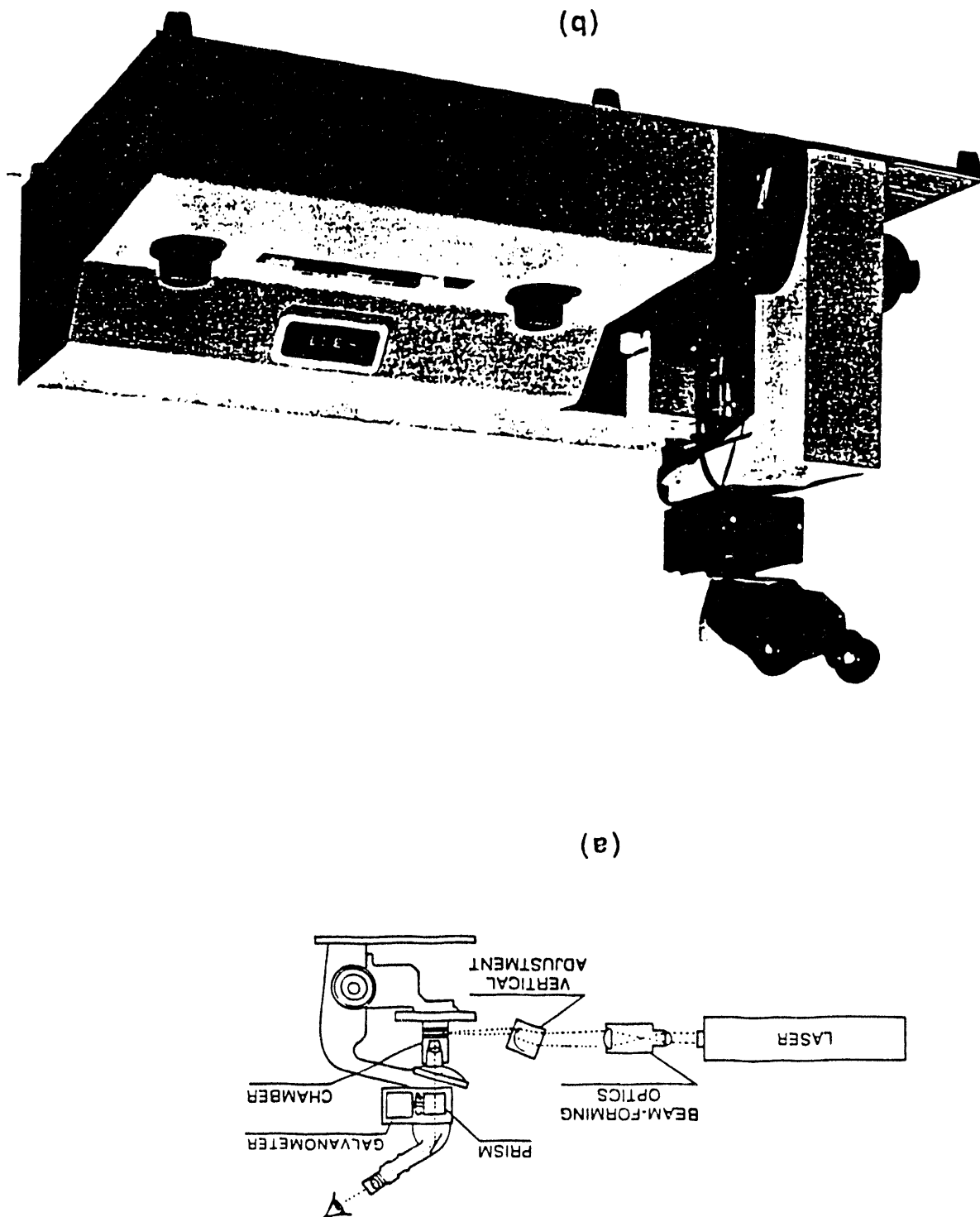
During the next quarter, the hydrophobicity of the different coals as a function of *M. phlei* concentration and solution pH will be studied. In addition, the electrokinetics behavior and hydrophobicity of ore pyrite and coal-pyrite will be studied. Fabrication of a counter-current flocculation device is under way and it will be completed for testing during the next quarter.



## REFERENCES

1. Pratt, D., 1952, "Nutrition of *Mycobacterium phlei*: Requirement for rapid growth," *Journal of Bacteriology*, Vol. 16, pp. 651-657.
2. Guirrad, B. M., and Snell, E. E., *Biochemical Factors in Growth, Manual of Methods for General Bacteriology*, P. Gerhardt, (ed.), American Society of Microbiology, pp. 79-111.
3. Smith, R. W., Misra, M., and Chen, S., 1993, "Adsorption of a hydrophobic bacterium onto hematite: implications in the froth flotation of the mineral," *Journal of Industrial Microbiology*, Vol. 11, pp. 63-67.

Figure 1. The Pen Kem Model 501 Lazer Zee Meter (a) Schematic and (b) Pictorial View



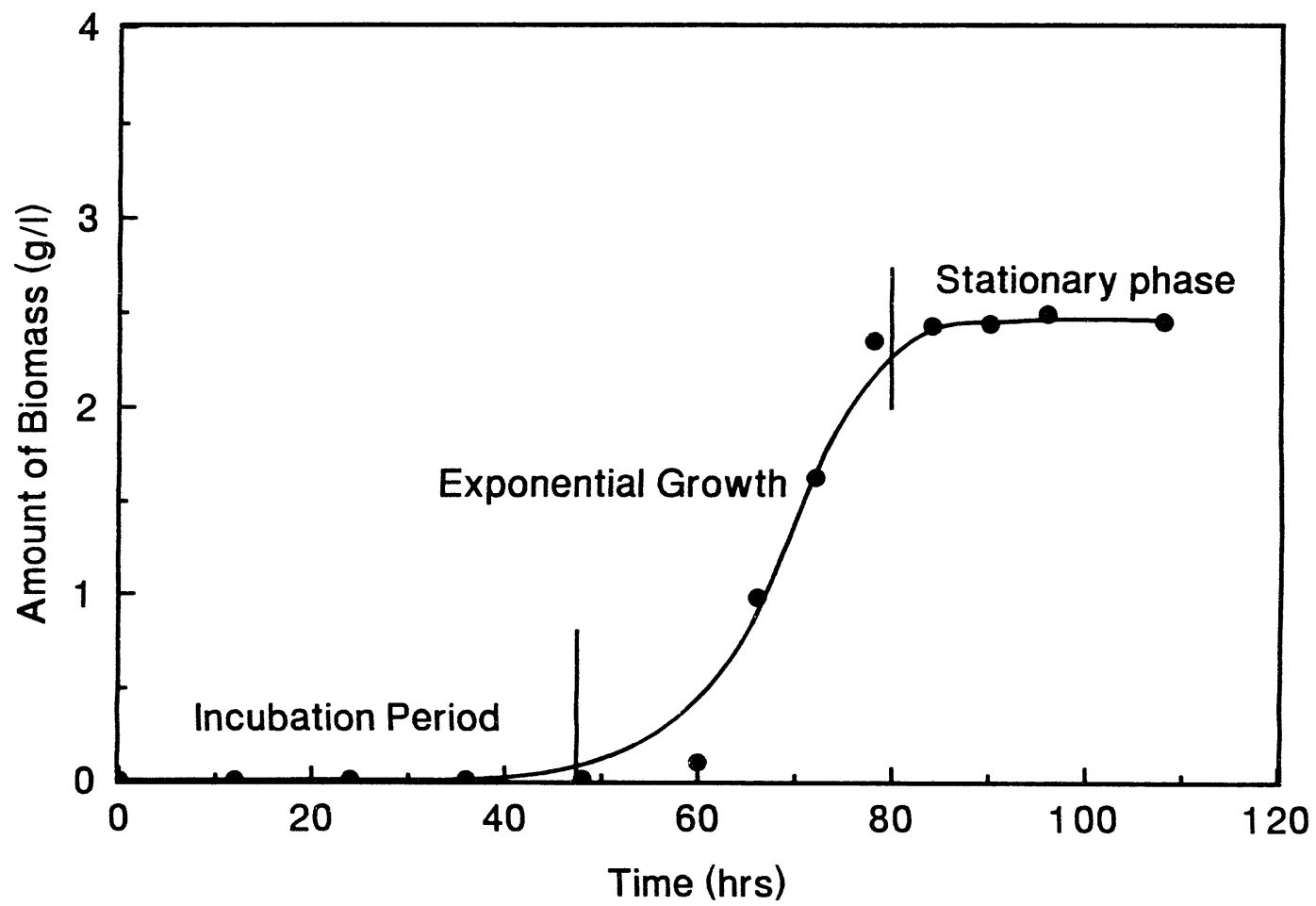


Figure 2. Growth Curve for *Mycobacterium phlei*

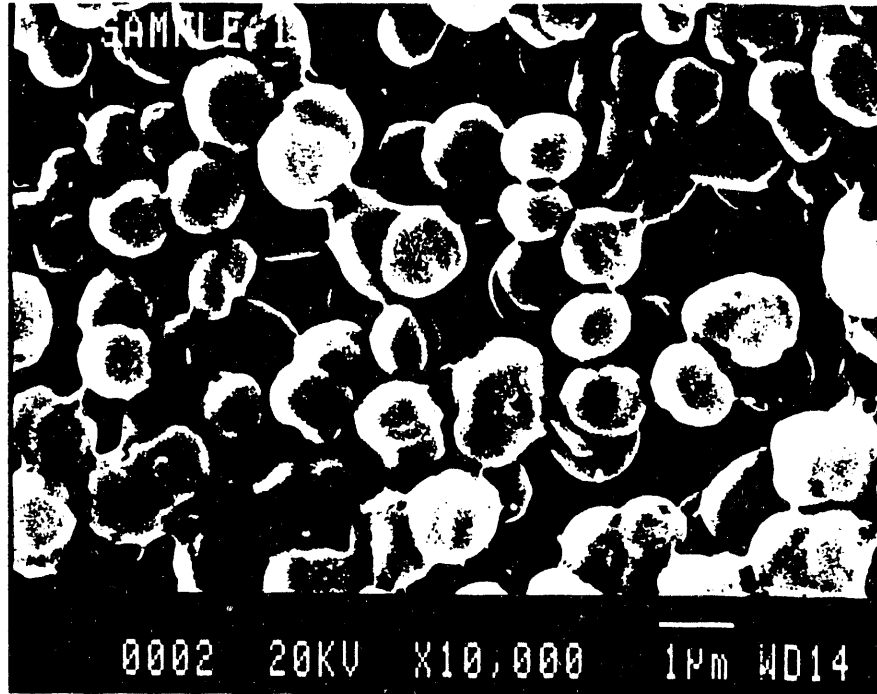


Figure 3. Scanning Electron Microphotograph of *Mycobacterium phlei*

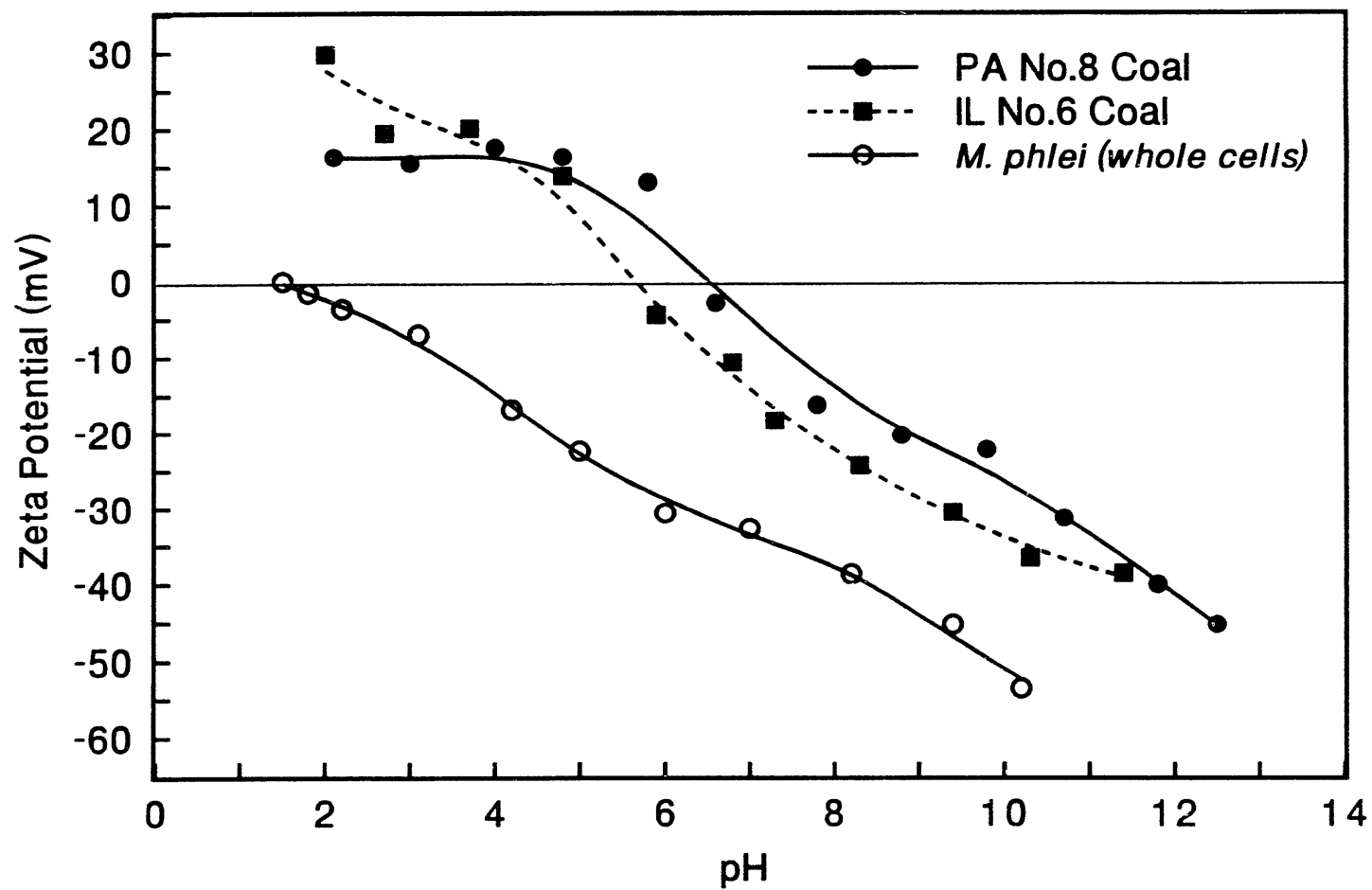


Figure 4. The Zeta Potential of Pittsburgh No.8 Coal, Illinois No.6 Coal and *Mycobacterium phlei* whole cells.

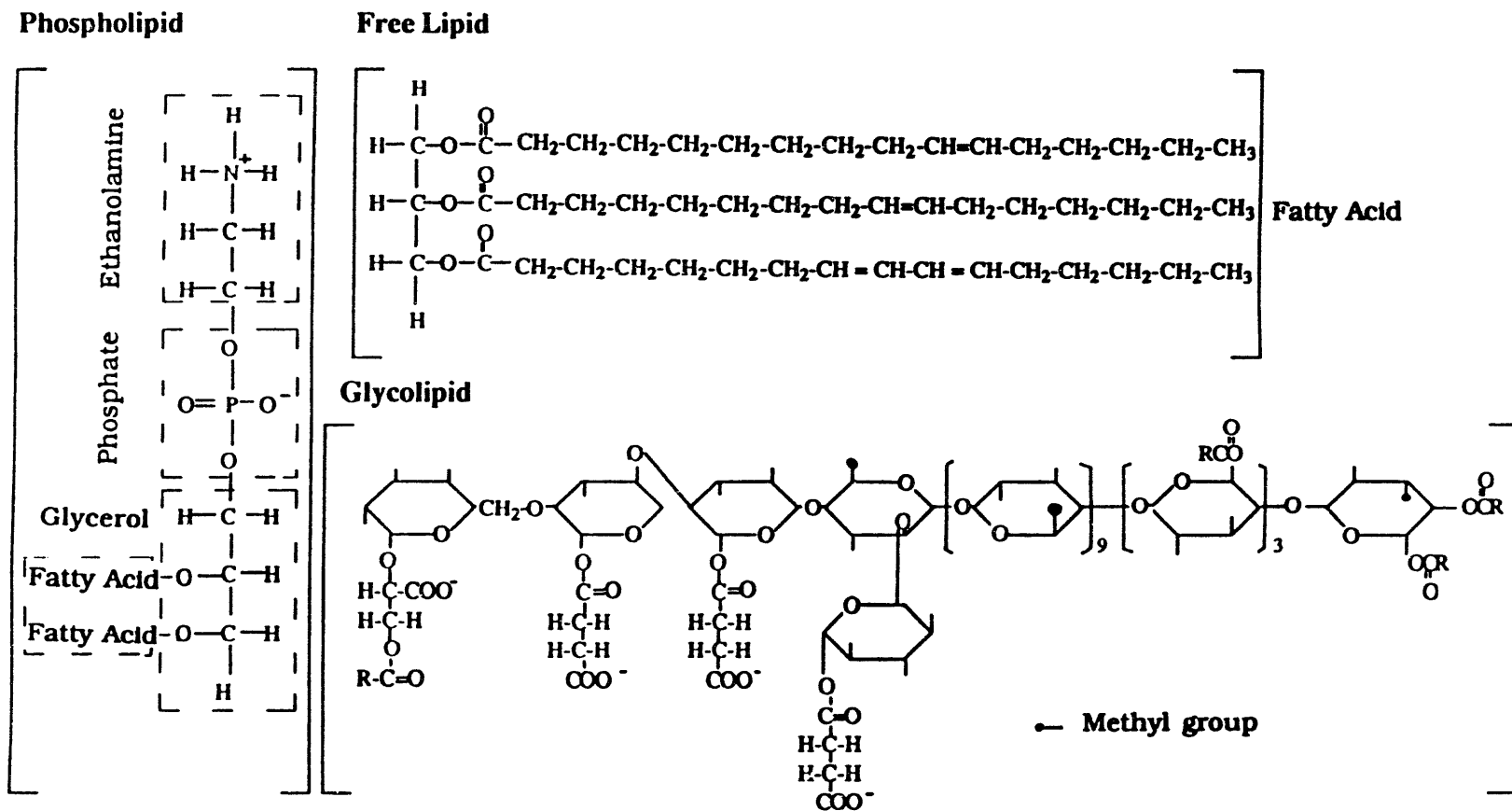


Figure 5. Average cell surface composition of *Mycobacterium phlei*

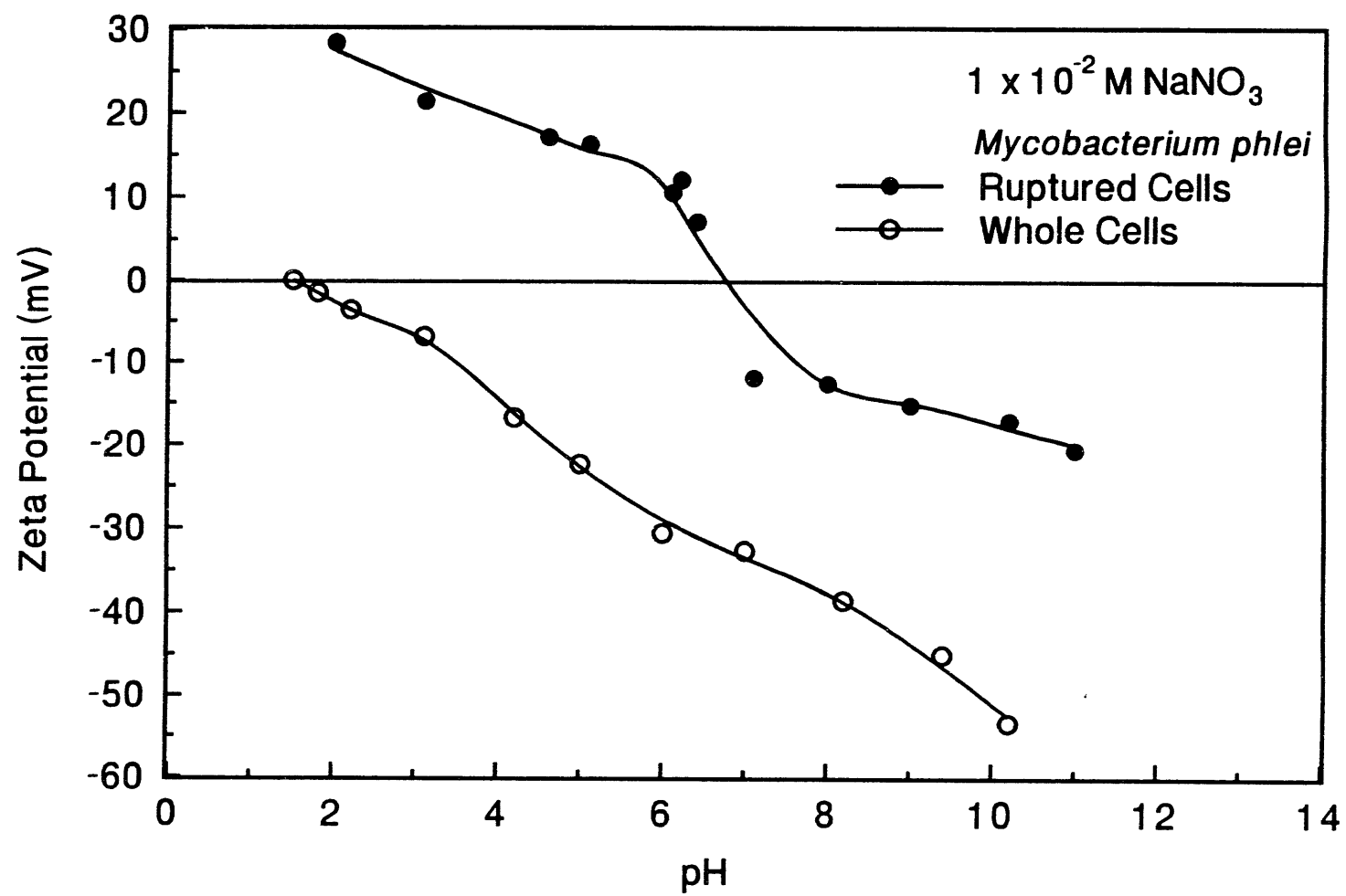


Figure 6. The Zeta Potential of Whole Cells and Ruptured Cells of *Mycobacterium phlei*

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