

LOUISIANA TECH UNIVERSITY

College of Engineering

JUN 18 1989

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MEMORANDUM

DOE/PC/88854--T1

DE89 015878

To: Mr. Elias George  
From: Brace H. Boyden *BB*  
Date: December —  
March 15, 1989  
Subject: Progress Report on DE-AC22-88PC88854  
*Quarterly Report*

**TITLE:** Application of Selected Microorganisms  
for Organic Sulfur Removal from Coal.

Please find enclosed a summary of the project activity for the period December 15, 1988 to March 15, 1989. If questions arise, please contact me at (318)257-2885.

**DISCLAIMER**

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## **DISCLAIMER**

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## 1 PROJECT PLANNING

This Task was completed as of 12/13/88.

## 2 COAL PROCUREMENT AND PREPARATION

### 2.1 Grinding, Sieving, and Storage

Ten pounds of Illinois #6 coal are ground, homogenized and stored under nitrogen. Arrangements are being made to procure quantities of Kentucky #11 and Pittsburgh #8 coals. Sulfur contents are such in the Illinois #6 coal (see below) that higher sulfur contents are desired.

### 2.2 Microbial Pyrite and Sulfate Removal

Treatment of the Illinois #6 coal for pyrite and sulfate removal is proceeding. Some delay was encountered due to inactive (i.e. old, lysed, few viable cells) cultures of Thiobacillus ferrooxidans and Thiobacillus thiooxidans. New cultures were obtained from Dr. Stevens at the University of Mississippi as well as from ATCC.

The first shipment of pyrite and sulfate free coal (approximately 200 grams) to Dr. Ward at U.M. Should occur within the next two weeks. Design and construction is currently underway to increase our treatment capacity to remove pyritic and sulfate sulfur. Immediate plans will utilize 20 liter containers anchored to a reciprocal shaker bed. Each container is filled with 15 liters of medium, 150 grams of coal, and 1.5 liters of inoculum of the aforementioned organisms. Plans for an even larger reactor system are being developed (see Attachment I) and should be realized within two weeks of this report. Desulfurization of the current coal batch (designated #1001) is being monitored using a Spectronic 20 spectrophotometer to analyze for sulfate release.

## 3 ANALYTICAL PROCEDURES FOR TOTAL ORGANIC SULFUR

### 3.1 Characterization

A typical proximate analyses of the Illinois #6 under nitrogen storage before pyrite and sulfate removal is as follows:

|                 |          |              |
|-----------------|----------|--------------|
| Total Sulfur:   | 3.39 wt% | +/- 0.05 wt% |
| Pyrite Sulfur:  | 1.59 wt% | +/- 0.04 wt% |
| Sulfate Sulfur: | 0.25 wt% | +/- 0.02 wt% |
| Organic Sulfur: | 1.55 wt% | +/- 0.05 wt% |
| Moisture:       | 8.98 wt% | +/- 0.09 wt% |
| Volatiles:      | 37.8 wt% | +/- 0.6 wt%  |
| Ash:            | 9.26 wt% | +/- 0.13 wt% |

These analyses could change slightly as initial tests were conducted on coal before it was completely homogenized. Caloric value of the coal, particle size distribution, and ultimate analyses will be determined in the near future.

### **3.2 Quality Assurance and Control**

All of the analytical procedures have been forwarded to Dr. Rowley for review. Attachments of two approved procedures (as examples) are included as Attachment II and III. A numbering scheme for each analytical procedure has been devised and these procedures are being kept in a central file.

### **3.3**

### **3.4 Microscopic Analysis**

#### **3.4.1 EDS-SEM**

Work in this area has been delayed to equipment breakdowns. However, most of the problems have been corrected and more activity on this Subtask should be forthcoming in the future.

## **4 ORGANIC SULFUR REMOVAL**

### **4.1 Desulfurization with OC7A**

#### **4.1.1 Louisiana Tech**

Formal work has ceased on this Subtask due to the inactivity displayed by the stored (since 1983), lyophilized culture. However, resampling of the area where the organism was originally isolated has yielded an organism with many of the same characteristics as was demonstrated by OC7A (e.g. production of colored products from DBT).

#### **4.1.2 University of Mississippi**

Work was also discontinued due to inactivity and contamination of stock OC7A culture.

### **4.2 Search for New Microorganisms**

#### **4.2.1 Louisiana Tech**

##### **4.2.1.1 Environmental Samples**

In addition to the environmental samples gathered from Oil City, LA (site of OC7A isolation), additional samples have been procured from a number of sources. A listing of the samples currently on hand is included in Attachment IV.

#### 4.2.1.2 Screening for 4S Pathway Isolates

Several samples exhibited colored compounds of the "Kodama" type (refer to Attachment IV): Samples 1, 2, 3, 4, 11, & 12. It is believed that the lack of observed 4S fluorescence may be due to high DBT concentrations (10 % w/v) applied to the plates. In the future, lower concentrations of DBT solutions will be used to spray the plates (e.g. 0.1 % (w/v) as suggested by Dr. Ward).

Other steps in our screening procedure will remain essentially the same. The samples are streaked on 21C agar plates, sprayed with DBT solution after growth is observed, and monitored every 15 minutes for fluorescence. Our plating medium (21C) is consistent with that used by Dr. Ward at UM.

#### 4.2.2 University of Mississippi

##### 4.2.2.1 Personnel

Beginning on January 1, 1989, Dr. Carol J. Stevens ("CJ") joined the project as a Postdoctoral Research Associate. Dr. Stevens received her Ph.D. in Microbiology in December, 1988 from Ohio State University, where she studied the physiology of Thiobacillus ferrooxidans. Also on January 1, Mr. Mannish Petrekh, and on January 15, Mrs. Kathleen Odum began work on the project as research assistants.

##### 4.2.2.2 Apparatus

During the last of January and first of February, two waterbath (culture) shakers and a Hewlett Packard Model 5890A gas chromatograph with Model 3396A integrator were installed.

##### 4.2.2.3 Screening for 4S Bacteria

###### 4.2.2.3.1 Methods

Bacteria that were potentially positive for the 4S pathway were screened by two methods. The first method was developed at LeHigh University by Dr. Steven Krawiec and his staff. The other method was developed by INEL by Tom Ward and Diane Key. The Krawiec method involved growing bacteria on solid 21C agar, spraying the plates with 3% DBT (w/v) in ethyl ether, allowing the plates to dry, and observing the plates under a 254 nm light source with the intention of observing the production of the fluorescent compound 2,2'-dihydroxybiphenyl (Krawiec's staff claimed the results should be positive within 2 hours). The TTC assay involved growing bacteria on TTC agar that had first been sprayed with 3% (w/v) DBT in ethyl ether. Those bacteria able to utilize the DBT produced an excess of electrons that were used to reduce the TTC dye to a red color and therefore produced red colonies. Bacteria unable to utilize DBT could not reduce the dye

and produced white colonies. The red colonies were then transferred to TTC agar without DBT and allowed to grow. The lack of excessive carbon source and, hence, electrons resulted in white colonies; red colonies were regarded as negative because the bacteria were not limited by the yeast extract.

#### 4.2.2.3.2 Sample Sources

Bacteria to be screened were isolated from the following sources:

- (a) the soil from a coal yard in Memphis, TN,
- (b) the soil contaminated with oil from an oil company in Oxford, MS,
- (c) a natural crude-oil seepage site at Beaver Dam Creek, MS (these samples were obtained by a state agency in 1985 and have been stored; plans to obtain fresh samples have been hampered by flooding in the area,
- (d) airborne bacteria that contaminated medium containing DBT and,
- (e) cultures procured from Krawiec's lab (A4, B24, C1, C2, C18, D20, E1, F14, & F23). These cultures were used as positive controls due to our inability to reproduce the positive results obtained by the "Krawiec" method.

Numerous unsuccessful attempts were made to obtain DBT-dependent fluorescence (of presumed 4S products) from microbial isolates obtained from our sample sources. Techniques were double checked to insure conformity with those utilized by Dr. Steve Krawiec and his colleague, Diane Dutt at LeHigh University. Nine of Dr. Krawiec's isolates were tested unsuccessfully for DBT fluorescence, using a 3% diethyl ether solution of DBT as described by the LeHigh team. During a series of exploratory tests in our lab, Dr. Stevens discovered that vivid DBT-dependent fluorescence occurred when a 0.1% solution of DBT in ether was sprayed onto developed colonies of the LeHigh isolate strain C-18. This finding confirmed reports by Dr. Krawiec. We note here that a very light coating of DBT gave the best results, e.g., the amount of DBT crystals deposited on the agar surface produced a barely detectable "sheen" when viewed at an angle under room fluorescent lighting. Table I presents our data on presumptive 4S and "Kodama" activity of the LeHigh cultures.

#### 4.2.2.3.3 Experimental Procedures

A "Chromist" (Gelman Cat. No. 15901) sprayer was used to deliver one short burst of DBT solution directly onto the agar surface (in a fume hood with 100 ft/min velocity air flow). The culture dish lid was replaced after several minutes of drying. Preparations were monitored at room temperature (ca. 25 Celsius) from time "zero" at 15 minute intervals up to 2 hours, or after incubation at 30 Celsius overnight. Fluorescence was monitored under a Spectroline Model ENF-260C, 254 nm UV lamp (about 430  $\mu\text{W}/\text{cm}^2$  at 15 cm), with plates held at an angle at 5-6 cm from the lamp fitted to a Spectroline Model CM-10 viewing cabinet. Subsequent tests using 0.1% DBT on the other LeHigh isolates resulted in detectable fluorescence by five of the nine tested. Some of the LeHigh isolates produced colored "Kodama"-type products, as did several of our own source sample isolates sprayed with 3% DBT. We cannot now explain why the LeHigh isolates did not exhibit 4S-type fluorescence when sprayed with 3% DBT. It is likely that the LeHigh team applied considerable less of the 3% solution than we applied using the commercial pressurized spray applicator. Yet fluorescence was still not observed for three of the LeHigh strains.

#### 4.2.2.3.4 Media

Cultures were maintained and tested with medium 21C<sup>1</sup> as described below.

Stock Solutions: (autoclaved) 50g NH<sub>4</sub>Cl/500 ml water; 69.25 g KH<sub>2</sub>PO<sub>4</sub>/500 ml water; 50.25 g Na<sub>2</sub>HPO<sub>4</sub>/500 ml water; (filter-sterilized) 30 mg glucose/100 ml water. Vitamin stock (filter sterilized): 0.5 mg biotin, 50 mg nicotinic acid, and 25 mg thiamine-HCl/50 ml water and Hütner's mineral base (filter-sterilized).

Recipe for 1 liter Preparation:

1. 10 ml NH<sub>4</sub>Cl, 20 ml KH<sub>2</sub>PO<sub>4</sub>, and 20 ml Na<sub>2</sub>HPO<sub>4</sub> stocks in 920 ml distilled water, autoclaved and cooled.
2. To the solution from no. 1, the following filter-sterilized solutions are added aseptically to the solution: 20 ml of trace metals (Hütner's vitamin-free trace metal mix), 1 ml of vitamin mix, and 10 ml glucose. If solid medium is required, 15 g of agar is added prior to autoclaving. In certain circumstances, the glucose and vitamin mixture are replaced by yeast extract.

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<sup>1</sup> Guirard, B.V., & E.E. Snell. Biochemical Factors in Growth. "Manual of Methods for General Bacteriology," American Society for Microbiology, Washington, D.C., 1981.

TTC Agar: This medium contained the same basal salts as the 21C medium (10 ml NH<sub>4</sub>Cl, 20 ml each KH<sub>2</sub>HPO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub>) as well as 0.25 g yeast extract, 0.025 g triphenyltetrazolium chloride (TTC), 15 g agar, and 930 ml distilled water. The medium was autoclaved for 15 minutes at 121 Celsius and 15 psig. When cool, 20 ml of Hutner's trace metal mix was added.<sup>2</sup>

#### 4.2.2.4 Analysis of DBT Degradation

##### 4.2.2.4.1 Gas Chromatography

DBT (5% w/v) in acetone was filter sterilized and 1 ml aliquots dispensed into sterile 125 ml Erlenmeyer flasks. After drying overnight, 50 ml of the 21C medium were added to each flask. The flasks were inoculated with 10 ml of culture grown overnight in 21C medium. The flasks were then incubated at 30 Celsius, 125 rpm for 48 h. The DBT and hopefully its by-products were extracted by the following method. After prerinising all glassware with chloroform, 0.5 ml of 0.1% thianthrene was added to a 50 ml centrifuge (1/flask). The culture was added to the centrifuge tube, 9 ml of chloroform added to the flask and subsequently transferred to the centrifuge tube. The tubes were inverted 50 times, centrifuged, and the organic layer saved. The tubes were extracted twice more with 8 ml of chloroform each time for a final total volume of 25 ml. One microliter of the extract was then injected into the gas chromatograph (FID detector, 15 ml/min, He as the carrier gas, 10 meter, HP-5 column). Standard chromatograms have been prepared and preliminary trials have begun on assays for DBT bioconversions by the LeHigh cultures as well as some of the UM isolates.

##### 4.2.2.4.2 Sulfate Assay

The release of sulfate from DBT by microorganisms can potentially be measured using the ASTM turbidimetric method of sulfate analysis. So far, we are still trying to determine the reproducibility and detection limits for using this assay. Cultures are grown in 100 ml of 21C media in 250 ml Erlenmeyer flasks, incubated at 30 Celsius, 125 rpm, for 48 h. Samples are centrifuged and filtered to reduce turbidity. Then 50 ml of the sample (or a diluted sample) is mixed with 10 ml of glycerin (diluted 1:1 with water) and 5 ml of NaCl solution (240 g NaCl, 20 ml conc. HCl in 1 liter of water). The spectrophotometer is zeroed with the sample and 0.3 g of BaCl<sub>2</sub>·2H<sub>2</sub>O crystals are added and the sample stirred for 1 minute, allowed to be static for 4 minutes, and stirred for 15 seconds to read the absorbance at 390 nm. The

2 The TTC assay indicated that several of the screened bacteria (SOC-3, S2, S4, S8, SOM1-17-1, SOM1-17-3a, SOM1-17-3b, SOM1-17-4, SOM1-17-5, SOM1-17-6a, SOM1-17-6b, Krawiec's cultures) were capable of utilizing DBT. However this does not indicate if usage is via the "Kodama" or 4S pathways. These bacteria can be further screened by the 0.1% DBT fluorescence assay, determining the products of DBT degradation via GC, and possibly by SO<sub>4</sub><sup>2-</sup> release.

amount of sulfate present is determined by comparison to a calibration curve. The amount of sulfate used or released by the bacteria is determined by comparing the inoculated cultures to an uninoculated control. Thus far, the calibration curve is reproducible and it appears that the detection limits for sulfate used by pure cultures in 21C media may be within a useful range, provided significant amounts of sulfate are released by oxidation of DBT.

#### 4.2.2.4.3 UV Spectrophotometry

Attempts were made to determine if the degradation of DBT to 2,2'-dihydroxybiphenyl (o,o'biphenol) could be followed using UV spectrophotometry. DBT was dissolved in t-butanol (0.5% to 0.0005% w/v) and scanned from 350 to 250 nm. Similar scans were conducted on biphenol solutions in t-butanol. It was determined that although there was overlap of the two spectra, the DBT and biphenol spectra were dissimilar enough that decreases in the DBT concentration could be monitored. Increases in biphenol would have to be greater than about 10% to be detected. Following this premise, it should be possible to grow cultures in 21C media with DBT, extract the DBT, and determine decreases when inoculated media are compared to uninoculated controls.

Table I

Presumptive 4S or "Kodama" Activity of Nine LeHigh  
 University Cultures (Dr. Krawiec) as Tested at  
 University of Mississippi

0.1% DBT

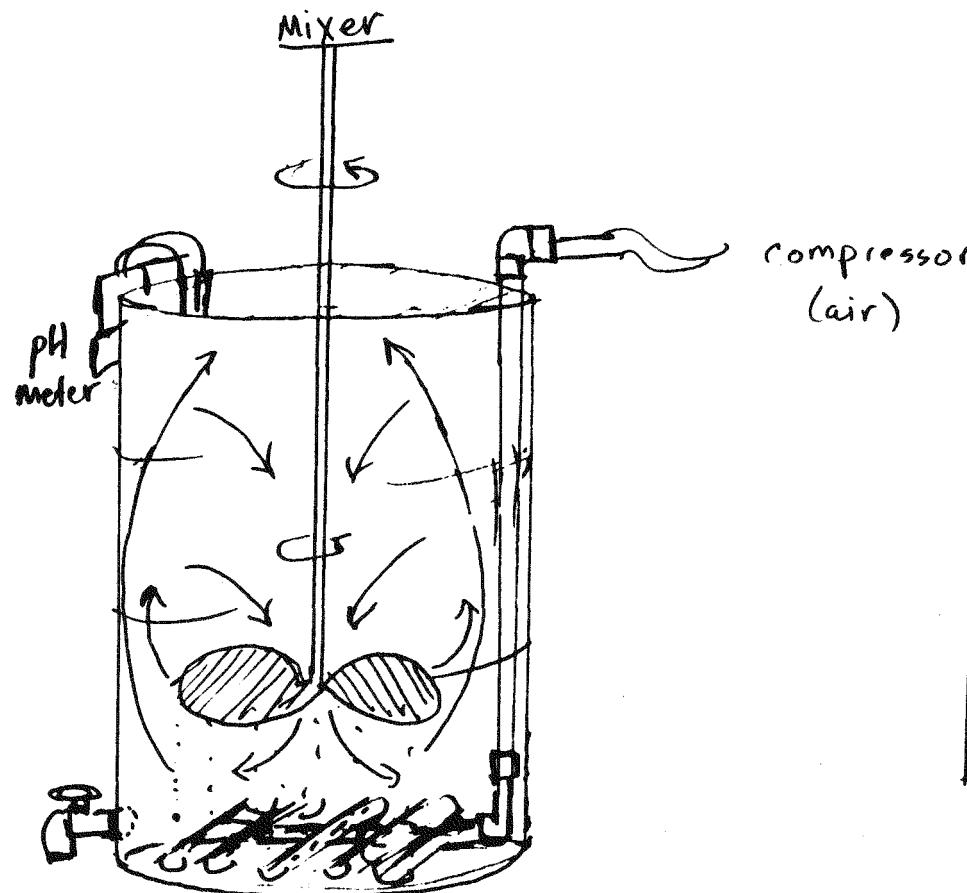
3.0% DBT

| Strain           |      | Time, Floursce | Orange  |
|------------------|------|----------------|---------|----------------|---------|----------------|---------|----------------|---------|
| ID               |      | hr.            | Prodts. | hr.            | Prodts. | hr.            | Prodts. | hr.            | Prodts. |
| A4               | None | -              | Yes     | 18             |         | None           | -       | Yes            | 18      |
| B24              | None | -              | Yes     | 18             |         | None           | -       | None           | -       |
| C1               | Yes  | 0.25           | None    | -              |         | None           | -       | Yes            | 18      |
| C2               | Yes  | 0.25           | Yes     | 18             |         | None           | -       | Yes            | 18      |
| C18 <sup>3</sup> | Yes  | 0.25           | Yes     | 18             |         | None           | -       | Yes            | 0.5     |
| D20              | Yes  | 18             | Yes     | 18             |         | None           | -       | None           | -       |
| E1               | Yes  | 18             | Yes     | 18             |         | None           | -       | Yes            | 6       |
| F14              | None | -              | Yes     | 18             |         | None           | -       | None           | -       |
| F23              | Yes  | 2              | None    | -              |         | None           | -       | None           | -       |

<sup>3</sup> Stain which gave the most vivid fluorescence.

.. Bio-Reactor  
with side arm. aeration

~~★ BEST~~



55 gal  
polypropylene  
tank

Chem o Stat

Reactor

for the aeration/mixing  
and suspension  
of coal slurry -

[De-pyritization]

Constant Temp

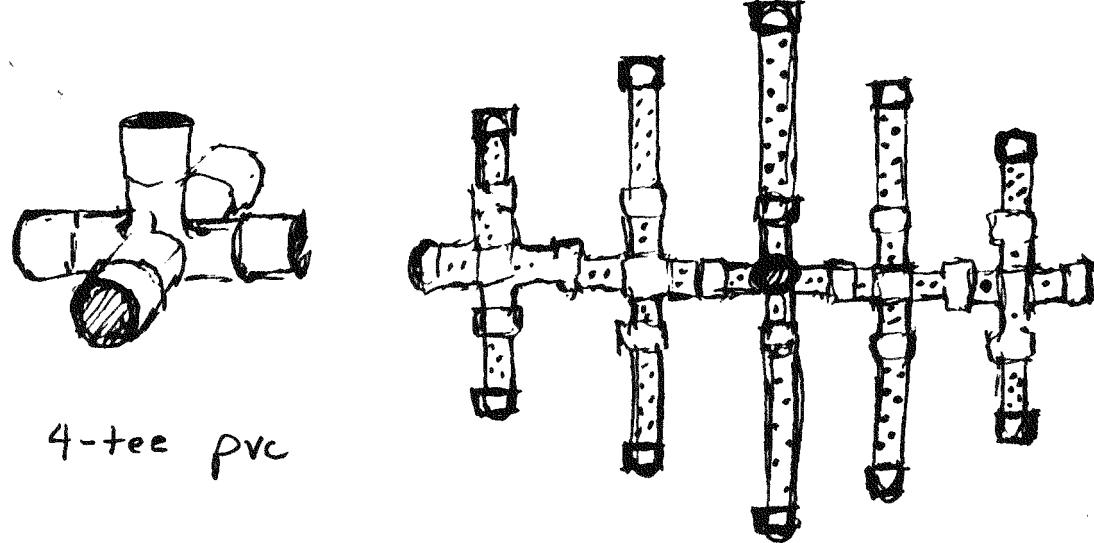
Also continual pH  
monitoring

- \* Also the side arm can be perforated, but the compressor would have to provide enough pressure to produce even aeration.

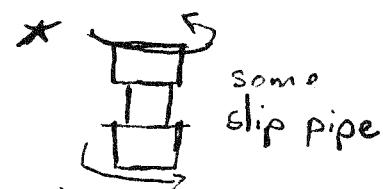


compressor

Most Feasible - stationary aeration  
w/ mixer



pipe  
out of page



(This will be circular)

perforated pvc pipe — fits  
in bottom of drum

~ If find — possibility  
of connecting to  
mixer and using for  
both mixing and air

QC/QA PROGRAM  
LOUISIANA TECH UNIVERSITY

DOE COAL BIODESULFURIZATION TECHNIQUES:  
APPLICATION OF SELECTED MICROORGANISMS FOR ORGANIC  
SULFUR REMOVAL FROM COAL

DE-AC22-88PC8854

TECHNICAL PROCEDURE REVIEW FORM

PROCEDURE DESIGNATION TP-001, Rev.0: Procedure for Adiabatic  
Calorimeter

REVIEW DATE November 29, 1988

REVIEWER John C. Rowley

COMMENTS \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

DISPOSITION

APPROVED ✓

DISAPPROVED \_\_\_\_\_

APPROVED CONDITIONALLY \_\_\_\_\_

CONDITIONS TO BE MET FOR APPROVAL \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Signature of reviewer John C. Rowley  
Date 11/29/88

Note: See typo and suggested number / designator system.  
\_\_\_\_\_  
\_\_\_\_\_

PROCEDURE FOR ADIABATIC COLORIMETER

Read and understand ASTM Procedure D 2015-77.

Turn on water supply under counter.

Turn on water heater, water cooler, distilled water tank, master controller, and calorimeter.

Set buret stopcock to direct water through buret. Be sure hot and cold water lights cycle even when the tank temperature stabilizes. (5 seconds/cycle see page 6 of Manual No. 156).

Loading Bomb

Place preignited stainless steel crucible on balance and tare weight.

Place sample pellet in crucible and note weight.

Install 10 cm of firing wire on the bomb head electrodes as shown on page 6 of operations manual.

Place crucible in ring with the wire in contact with sample.

Pipet 1 ml of distilled water into bottom of bomb.

Put top on bomb, hand tighten ring and close valve.

Attach oxygen line to bomb and pressurize to 20 atm.

Release pressure in line with toggle and remove line.

Place bomb in corresponding bucket and fill bucket with 2000 ml of distilled water from buret.

Open calorimeter by raising thermometer (pull black ball) and releasing (pull black ball and lift) cover and slide to right.

Place filled bucket in opening and attach wires to electrodes. Close cover and lower thermometers.

Standardization

Load bomb with benzoic acid pellet and place in calorimeter. Set toggle switches on Master Controller to Ref., Stand., and BTU/lb.

Press reset, then start and respond to lights.

CAL. ID. requires a number for the bomb and bucket combination--1 or 2.

SAMPLE ID is a number for this run.

Attachment II (Continued)

SAMPLE WT. is the weight in grams of benzoic acid.

Master Controller will now conduct the test and give a preliminary report.

After the report is printed, remove the bomb and slowly release the pressure. Open the bomb and rinse the inside with methyl red solution into a beaker.

Titrate this solution till the red color disappears.

Multiply the ml. used by 10 to get acid correction.

Measure the length of unburned wire on the card. This is the wire correction.

Press Sample ID, its numeral, and Enter.

Enter the acid and wire correction and receive the final report which contains the energy equivalent.

Average ten energy equivalent runs for each bomb and bucket combination. The standard deviation of each series must not be greater than 6.5 BTU/°C (see ASTM D2015 TABLE XI).

Check standardization at least once a month if the new standard value exceeds old value by  $\pm$  6 BTU/°C, see ASTM D2015 Sec. 9.

Input the average of the energy equivalent for each bomb and bucket combination by pressing \*, 0, Enter; the number you selected for the bomb and bucket combination, Enter; and the corresponding energy equivalent.

Running a Sample

Use the pellet press to make a coal pellet from 1 gram of coal, and load the bomb as previously described.

Set the toggle switches to Ref., Det. and BTU/lb.

Press reset and start.

Respond to lights - CAL. ID and SAMPLE WEIGHT.

After preliminary report is printed, open bomb, titrate content and measure wire.

Press SAMPLE ID, enter ID NO. and respond to lights with fuse correction, % sulfur of sample, and acid correction. Final report will then print.

When finished for the day, turn off heater, bucket filling system, calorimeter and water supply.

Leave Master Controller on but set 3-position switch to Man. and press start to go to standby status.

Attachment II (Continued)

Duplicate results should not be considered suspect unless they differ by more than 50 BTU/lb, dry basis.

QC/QA PROGRAM  
LOUISIANA TECH UNIVERSITY

DOE COAL BIODESULFURIZATION TECHNIQUES:  
APPLICATION OF SELECTED MICROORGANISMS FOR ORGANIC  
SULFUR REMOVAL FROM COAL

DE-AC22-88PC8854

TECHNICAL PROCEDURE REVIEW FORM

PROCEDURE DESIGNATION TP-002, Rev.0: Microbial Pyrite and Sulfate

Removal

REVIEW DATE November 29, 1988

REVIEWER John C. Rowley

COMMENTS \_\_\_\_\_

DISPOSITION

APPROVED ✓

DISAPPROVED \_\_\_\_\_

APPROVED CONDITIONALLY \_\_\_\_\_

CONDITIONS TO BE MET FOR APPROVAL \_\_\_\_\_

Signature of reviewer John C. Rowley  
Date 11/29/88

Note: See type and suggested number  
for identifier.

11-002, REV.0  
Nov. 11, '88  
Page 1071

## MICROBIAL PYRITE AND SULFATE REMOVAL

Samples of acid mine water and pond mud from Madisonville, Kentucky, were set up in shake flasks of ISP medium to screen for iron-oxidizing and inorganic sulfur utilizers. Incubation was at room temperature on a reciprocal shaker at 100 shakings/min until the medium showed an orange-brown color. Plates of ISP agar were streaked from the flasks showing the presence of iron-oxidizing and inorganic sulfur utilizers to determine the bacterial types. Plates of ISP agar were also streaked directly from the acid mine water samples and the pond <sup>mud</sup> used to detect inorganic sulfur utilizers.

METHOD II  
PROCESSING TREATED COAL SAMPLES --  
WATER WASH SHAKE + CENTRIFUGATION METHOD

1. Shake samples vigorously as in #1, Method I.
2. Centrifuge the coal-water suspension at 1000 X g for 5 min. (500 X may also work.)
3. Aspirate or decant the supernatant.
4. Resuspend the pellet in water and filter as in #7, Method I.
5. Transfer sample as in #8, Method I for drying.
6. Transfer sample for analysis as in #9, Method I.

**ENVIRONMENTAL SAMPLE INVENTORY**  
**Louisiana Tech**

| <u>Sample Number</u> | <u>Type</u>                | <u>Site Taken From</u>   |
|----------------------|----------------------------|--|
| 1                    | Soil                       | Highway 1 at 1/2 mile south of Vivian at a tank battery storage area (Oil City)      |
| 2                    | Soil                       | 1/2 mile north of highway 2 on highway 1 at new tank battery storage area (Oil City) |
| 3                    | Soil                       | Same as above but from old battery storage area (Oil City)                           |
| 4                    | Soil                       | Sludge pit on highway 2, NE of Vivian, 1 mile before Caddo Lake (Oil City)           |
| 5                    | Liquid                     | Oil pit on the same site as samples 2 and 3 (Oil City)                               |
| 6                    | Liquid                     | Same site as sample 1  |
| 7                    | Liquid                     | Same site as sample 2  |
| 8                    | Liquid                     | Same site as sample 3  |
| 9                    | Liquid                     | Same site as sample 4  |
| 10                   | Liquid                     | Same site as sample 5  |
| 11                   | Weathered lignite          | Dolet Hill in Mansfield, La.   |
| 12                   | Weathered lignite and soil | Dolet Hill in Mansfield, La.   |
| 13                   | Pristine lignite           | Dolet Hill mine, F-2 range, Mansfield, La.   |
| 14                   | Soil                       | Oil seepage in California (California State University, Northside)                   |
| 15                   | Soil                       | From 2.6 pH pond, Kentucky   |

## Attachment IV (Continued)

| <u>Sample Number</u> | <u>Type</u>                   | <u>Site Taken From</u>   |
|----------------------|-------------------------------|--|
| 16                   | Weathered coal<br>--- Andelex | Near pond in sample 15   |
| 17                   | Liquid                        | pH 2.6 pond, Kentucky  |
| 18                   | Liquid                        | pH 2.34 pond, Kentucky   |
| 19                   | Liquid                        | 1/2 mile south of I-70 on Indiana Hwy. 42                            |
| 20                   | Liquid                        | Kentucky Hwy. 813 Exit 37  |
| 21                   | Liquid                        | Active slurry pond in Kentucky                                       |
| 22                   | Liquid                        | Runoff of Peabody strip mine slurry                                  |
| 23                   | Liquid                        | 1/2 mile south of I-70 on Indiana Hwy. 42                            |
| 24                   | Soil                          | Hot Sulfur Springs Colorado  |
| 25                   | Soil                          | Hot Sulfur Springs Colorado  |
| 26                   | Soil                          | Hot Sulfur Springs Colorado  |
| 27                   | Soil                          | Hot Sulfur Springs Colorado  |
| 28                   | Soil                          | Hot Sulfur Springs Colorado  |
| 29                   | Soil                          | Hot Sulfur Springs Colorado  |
| 30                   | Soil                          | Pressboard Plant<br>(Dr. Boyden)                                     |
| 31                   | Soil                          | Chevron Oil Refinery, Port Arthur, Texas -- West Lagoon Conduit Bank |
| 32                   | Soil                          | Chevron Oil Refinery, Port Arthur, Texas -- #6 Tank hold             |

## Attachment IV (Continued)

| <u>Sample Number</u> | <u>Type</u> | <u>Site Taken From</u>  |
|----------------------|-------------|---|
| 33                   | Soil        | Chevron Oil Refinery, Port Arthur, Texas -- 47PH Manifold Conduit |
| 34                   | _____       | _____   |
| 35                   | _____       | _____   |
| 36                   | _____       | _____   |
| 37                   | _____       | _____   |
| 38                   | _____       | _____   |
| 39                   | _____       | _____   |
| 40                   | _____       | _____   |
| 41                   | _____       | _____   |
| 42                   | _____       | _____   |
| 43                   | _____       | _____   |
| 44                   | _____       | _____   |
| 45                   | _____       | _____   |
| 46                   | _____       | _____   |
| 47                   | _____       | _____   |
| 48                   | _____       | _____   |

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111-84)

U.S. DEPARTMENT OF ENERGY  
MILESTONE SCHEDULE  PLAN  STATUS REPORT

FORM APPROVED  
OMB NO 1901-1400

| 1. TITLE  |                       | Biodesulfurization Techniques: Application of Selected Microorganisms for Organic Sulfur Removal from Coals  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    | 2. REPORTING PERIOD |    | 3. IDENTIFICATION NUMBER |         |       |    |
|---|-----------------------|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----|-----|----|-------|----|---------------------|----|--------------------------|---------|-------|----|
|   |                       |  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    | 12/15/88 - 3/15/89  |    | DE-AC22-88PC88854        |         |       |    |
| 4. PARTICIPANT NAME AND ADDRESS                         |                       | Dr. Joseph B. Fernandes<br>Louisiana Tech University and Dr. Brace H. Boyden<br>Ruston, LA 71272-0046 Louisiana Tech University<br>Ruston, LA 71272-0046 |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    | 5. START DATE       |    | August 1, 1988           |         |       |    |
|   |                       |  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    | 6. COMPLETION DATE  |    | January 31, 1991         |         |       |    |
| 7. ELEMENT CODE   | 8. REPORT-ING ELEMENT | 9. DURATION  |     |     |     |     |     |     |     |     |     |     |     | FY 90 |     |     |    | FY 91 |    |                     |    | 10. PER CENT COMPLETE    | a. Plan | b. Ac |    |
|   |                       | FY 88  | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul   | Aug | Sep | Q1 | Q2    | Q3 | Q4                  | Q1 |                          |         |       | Q2 |
| Task 1  | P. P.                 | xxx  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          | 100     | 100   |    |
| Task 2  |                       |  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         |       |    |
| Subtask 2-1   | G. S.<br>& S.         | XXXXXXXXXXXX   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 100   | 95 |
| Subtask 2-2   | M. P. &<br>S. R.      | XXXXXXXXXXXXXXXXXXXX   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 67    | 15 |
| Task 3  |                       |  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         |       |    |
| Subtask 3-1   | Character-<br>ization | XXXXXXXXXXXXXXXXXXXXXXXX   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 25    | 25 |
| Subtask 3-2   | Q. A./<br>Q. C.       | XXXXXXX  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 100   | 95 |
| Subtask 3-3   | N. A.<br>Proc.        |  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 0     |    |
| Subtask 3-4   | Micro.<br>Analysis    | XXXXXXXXXXXXXXXXXXXX   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 36    | 15 |
| Task 4  |                       |  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         |       |    |
| Subtask 4-1   | D. with<br>OC7-A      | xxxx (Work has ceased)   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 17    | 17 |
| Subtask 4-2   | S. for<br>N. M.       | XXXXXXXXXXXXXXXXXXXX   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 25    | 40 |
| Subtask 4-3   | P.-M.<br>Tech.        | XXXXXXXXXXXX   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 22    | 5  |
| Task 5  | P. M & R.             | XXXXXXXXXXXXXXXXXXXX   |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         | 27    | 27 |
| 11. SIGNATURE OF PARTICIPANT'S PROJECT MANAGER AND DATE |                       | D. J. Fernandes  |     |     |     |     |     |     |     |     |     |     |     |       |     |     |    |       |    |                     |    |                          |         |       |    |