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Lignite Utilization via Biotechnological Innovations

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FINAL REPORT

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Lignite Utilization via Biotechnological Innovations

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PART ONE:

Report on Activities and Accomplishments for Project Research
Conducted in the Environmental and Applied Microbiology Laboratories
Department of Biology, The University of Mississippi.

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Summary

Studies were made to identify optimal culture media for obtaining biosolubilized Mississippi lignite for applications to research on fuel gas production via fermentations of the solubilized coal. Both aerobic and anaerobic cultures of fungi and bacterial consortia were used to arrive at a system whereby evidence for methane generation from biosolubilized lignite was obtained for some preparations.

Project Component

A. Growth Media Characterization and Application to Microbial Lignite Solubilization

Objectives: to test several different formulations of mycological growth media for effects on aerobic lignite biosolubilization, and to define a minimal medium for producing solubilized products.

Summary

Known lignite-solubilizing fungal strains were tested against Mississippi Wilcox lignite. Enrichment media gave best results for all tested strains. One strain solubilized the coal in a defined minimal medium at acidic pH.

Methods and Results

The fungal isolates RWL-40 (a basidiomycete) and YML-1 (a Cunninghamella species), originally isolated from lignites (1) and later applied to several studies on coal biosolubilization (2-6), were chosen as test organisms. The diffusion zone assay (7) was used to evaluate relative degree of biosolubilization.

I. Solid Media (1.5% agar) (recipes, Table II)

SDA (Sabouraud Dextrose agar, Difco Laboratories)
Bristols + 4% Dextrose
Bristols + 4% Maltose
Czapek's

On SDA, the organisms RWL-40 and YML-1 showed (Table I) considerable solubilization of most of the coals tested. Of course, this varies with the type of coal and degree of weathering. But more often than not, the diffusion zones for RWL-40 average about 15-20 mm, and those for YML-1 average 20-25 mm.

A curious phenomenon occurs surrounding the piles of coal on RWL-40. Before the actual "dark" solubilization starts to take place, an orange pigment appears. This orange is apparent even against the yellowing background which the organism produces and which is barely evident on this agar, at least until very late in the incubation period (10 days). After this point, the yellow, which envelops the entire plate, is more evident, and the orange, which was more evident at first, has now been engulfed by the

TABLE I

Relative Degree of Lignite Solubilization by the Test Fungi

	Solid Medium		
	SDA	Bristol's + Bristol's + Czapek's 4% Dextrose 4% Maltose	
RWL-40	++++1 ++	(+?) ++	+++
YML-1	++++ ++	(+?) ++	+++

	Liquid Medium	
	SDB	Bristols
RWL-40	++++ +++	
YML-1	d—1—h+	+++

	Bristol		
	+ 0.001% Neo	+0.1% Neo	+ 1.0% Neo
RWL-40	++ +	+++	++++
YML-1	+++	+++	++++

i ++++ = optimum growth (on solid, includes covering surface, in liquid, includes thick mat and growth below surface)
+ = minimal growth (on solid, includes sparse growth on surface, in liquid, includes only floating patches of growth)

Table II

BRISTOL'S BROTH
(to prepare one liter)

10 ml of each of six stock solutions:

$NaNO_3$	(10<0g/400ml)
$CaCl_2$	(1.0g/400m1)
$MgSO4-7H_2O$	(3.0g/400ml)
K_2HPO4	(3.0g/400ml)
KH_2PO_4	(7.0g/400ml)
NaCl	(1.0g/400ml)

940 ml deionized H_2O
one drop 1.0% $FeCl3-6H_2O$ (1.lg/L)
2 ml Gaffron's microelements solution

(for solid medium, add 15g agar per L)

CZAPEK'S SOLUTION AGAR

Saccharose	(30g/L)
NaNOa	(2.0g/L)
K_2HPO_4	(1.0g/L·)
MgSO4	(0.5g/L)
KCI	(0.5g/L·)
Fe_2SO4	(0.01g/L)
Agar	(15g/L)

much darker brown solubilization diffusion zones which are present beneath each pile of coal sieve.

The orange pigment became a curiosity; to see whether or not it existed on another medium, and if it were actually a phase of the coal solubilization and required the presence of coal, another medium was chosen. Bristols + 4% Dextrose "BD" was the choice. It provided less background color (was not quite as amber as the SDA), and we were able to adjust the pH to 5.6 without difficulty.
Growth of RWL-40 on this medium was not as extensive or thick as on SDA, but it did grow reasonably well. And the yellow pigment characteristic to this organism was even more obvious on the virtually colorless medium. The orange was not present on organism controls or on controls using piles of glass beads instead of coal, only on the plates including piles of coal. These observations indicated that the orange pigment (which was more noticeable on this medium) was dependent upon something in the coal.
However, at the termination of this screening, there was no solubilization evident. The only coloration on any of the plates (RWL-40 or YML-1, et al) was the orange pigment, which by the final date had spread to become rather uniform across the entire plate (when viewed from bottom, of course). It was no longer localized around the piles of coal.

YML-1 grew only moderately well on BD, not nearly as well as on SDA. In fact, the culture did not have the height which is characteristic of itself on SDA. No solubilization or pigmentation occurred. Perhaps the absence of solubilization on BD medium indicates that something is present in SDA which induces the enzymatic reaction, or at least encourages it to occur.

Another point of difference between SDA and BD which might be worth noting is that for some coals, leaching occured on the SDA, but not on the BD. (Due to pH?)

Bristols + 4% Maltose agar "BM" was tried. The amount of growth on this medium was about equivalent to that of BD; it was not extensive, and the YML-1 did not attain its characteristic height. There was no real solubilization on this medium by either organism, although some very questionable diffusion might have been occuring with RWL-40 on one coal tested (RUS 2-1). This "diffusion" went nowhere, though, so it is doubtful that solubilization was happening. On BM, the RWL-40 once again produced a slight orangish pigment when the coal was present. But this pigment is not nearly so prominent on either of the Bristol agars as on the SDA or Czapek's. Is it a result of an enzymatic reaction, present only with requisite nutrients?

As a further medium differentiation screen, Czapek's agar was used. The organisms grew nicely on this, much better than on the Bristol agars, as might be expected, because the Czapek's has more nutrients available. (Growth not quite as good as on SDA). Leaching was evident on this agar, perhaps because of its pH of approx. 7.3. However, in addition to the leaching, there was actual solubilization also. Nice dark zones were present, indicating a great deal of biosolubilization going on. In fact, YML-1 showed a tremendous activity. After the first few days,

the leaching stopped spreading and became less evident. This may-have been because the solubilization "took over" and hid the leaching, or maybe the amount available for leaching had already been used.
As for the orange pigment of RWL-40, the color was much more evident on this medium (as on SDA) than on the Bristol's media. It was noted that this orange color was present throughout the entire mass of the agar, not only beneath where the organism had grown, but also where the organism had not covered the plate.

II. Liquid Media

SDB (Sabouraud Dextrose broth, Difco)
Bristols +4% Dextrose
Bristols +4% Dextrose + 0.001% Neopeptone
Bristols +4% Dextrose + 0.1% Neopeptone
Bristols +4% Dextrose + 1.0% Neopeptone

In SDB, the organisms grew well---------optimally, as far as compared to the other media explored (Table I). A considerable mat floated on the surface of the broth, and quite a bit of organism existed even below the surface.

The RWL-40, which produces a pigment in the agar on which it is placed, also produceed an intense yellow color in the SDB. This pigment is most evident in the organism controls, because in those systems with coal, solubilization causes the broth to turn brown, almost as soon as "good growth" is achieved.
Solubilization occurred also with YML-1, turning the broth brown (tea-colored).
The pH of SDB is initially 5.6 or so, but after 14 days of incubation, these organisms take the pH to alkaline range. RWL-40 ends up being around 7.1-7.3, and YML-1 around 7.6-7.9 (This is for controls; the systems with coal may rise even higher than this). The elevated pH may be responsible for some of the solubilization (alkaline conditions), or at least for some of the extent to which it is achieved.

To diminish the effects of alkaline solubilization, Bristols broth was chosen as medium. Four percent dextrose was added to the Bristols basal medium to provide a source of energy for the organisms (this is the same proportion as is found in SDB).
The growth of the organisms in this broth was not as full as in SDB, but did form a substantial mat on the surface (the main difference between the two broths is the thickness of the mat and the presence of growth below the mat----------Bristols + 4% Dex has very little if any organism extending below the mat).
Solubilization was difficult to evaluate in this medium, because the RWL-40 produced the characteristic yellow anyway, and this might only be a bit darker when coal was in the system, or it might turn orangish, reminiscent of the pigment observed on agar.
The YML-1 "solubilization" was scant and relatively unobservable by the naked eye, but could be detected by spectrophotometric methods.
RWL-40 produced the characteristic yellow pigment, very obvious in this colorless medium.

As for pH, while the broth was routinely brought to 5.6 (to equal SDB), it usually decreased (in control) to around 4.5-4.7 after

14 days of incubation. The pH of the organisms here were quite different. RWL-40 took the pH even lower, to around 4.2-4.5, whereas the YM1-1 took the pH higher, to around 5.9-6.2 (with coal in the system). Without coal in the system, the trends were still the same; RWL-40 controls decreased or remained close to the broth control, wheras YML-1 increased.

An add-back gradient assay was devised to reintroduce neopeptone (an organic nitrogen component of Sabourauds) to see if the presence of this protein was quantitively introducing enzymes which enhanced the biosolubilization.

The pH of these were all brought to 5.6, so the only difference between them was the concentration of neopeptone. These concentrations were 0.001%, 0.1%, and 1.0% neopeptone, which are 0.1%, 10.0%, and 100.0%, respectively, of the concentration of neopeptone found in SDB (lOg/L).

The organisms grew best in the 1.0% broth, as could have been predicted. The variations between the others could not be observed.

Czapeks's liquid medium was not tested due to time limitations at the end of the project period. We can only predict, from knowing what had occurred on the agar runs, that the Czapek's would have been a more hospitable environment for growth of the organisms (and probably would have fostered considerable solubilization because of its high pH), whereas the Bristol's + 3% Sucrose would most likely have provided enough for some growth, but certainly not optimal.

B. Fermentation of Solubilized Lignite

Objective: to obtain a microbial system that would anaerobically convert biosolubilized Mississippi lignite to fuel gases such as hydrogen and methane.

Summary

Biosolubilized Mississippi Wilcox lignite was incubated under anaerobic conditions in the presence of an unknown consortium of microorganisms derived from a wastewater treatment faciltiy. Gas production was monitored and analyzed by gas chromatography. Evidence of methane production occurred for some preparations. No hydrogen was detected. An unidentified heavier gas in some preparations indicated production of a two or three-carbon gas.

Methods and Results

(The following pages detail the experimentation and results for this component of the project.)

INITIAL FERMENTATION TESTING

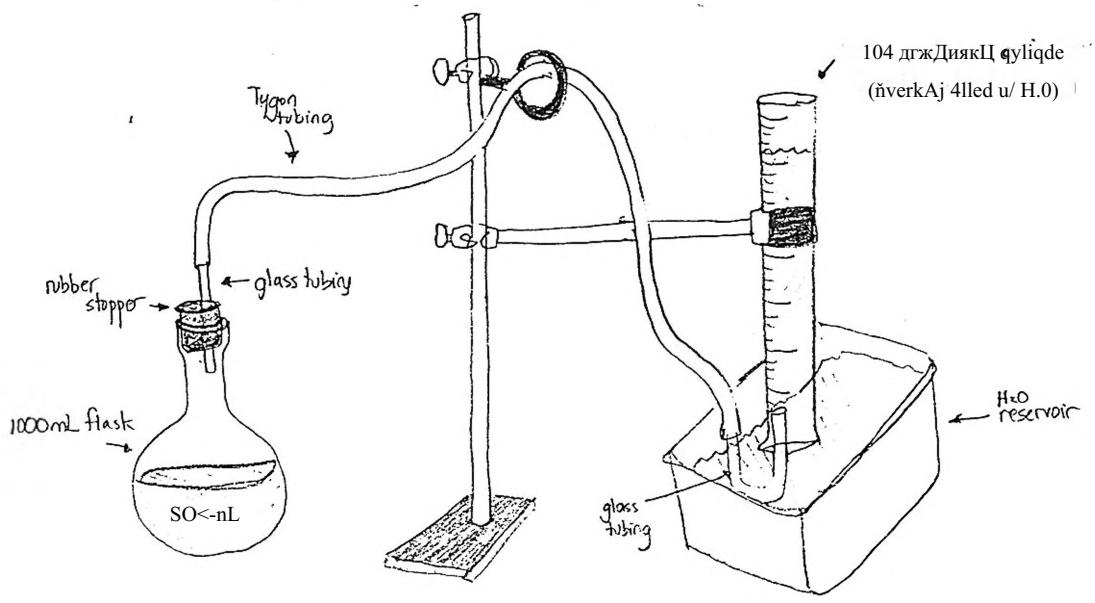
I. There were two original "sets" of cultures run in the primary assay dated September 1988-

A- SET 1: solubilized product + inoculum + NB
(NOTE: here and hereafter, "NB" is used to refer to Difco's Bacto Nutrient Broth)
solubilized product + inoculum + Bristol's
(recipe for Bristol's broth located in another section of this report)
solubilized product + NB
solubilized product + Bristol's

B. SET 2: coal sieve + inoculum + NB
coal sieve + inoculum + Bristol 's
coal sieve +NB
coal sieve +Bristol's

C. MUTUAL CONTROLS: inoculum + NB
inoculum + Bristol's
NB
Bristol 's

II . Those combinations listed above which contain the inoculum were arranged in an apparatus appearing as follows:



The "controls" (without inoculum) were arranged in simple 500ml flasks with cotton stoppers.

III. The proportions of the experiment were:

A. For SET 1:
10 ml of sol. product + 500 ml NB + 1 ml sludge
10 ml of sol. product + 500 ml Bristol's + 1 ml sludge

3 ml sol_ product + 150 ml NB
3 ml sol. product + 150 ml Bristol's

B. For SET 2:
0.6 g coal sieve + 500 ml NB + 1 ml sludge
0.6 g coal sieve + 500 ml Bristol's + 1 ml sludge
0.18 g coal sieve + 150 ml NB
0.18 g coal sieve + 150 ml Bristol's

C. For Controls:
1 ml sludge + 500 ml NB
1 ml sludge + 500 ml Bristol 's
200 ml NB
200 ml Bristol's

IU. Preparations:

A. For SET 1, the "solubilized product" was biosolubilized MS Wilcox (withdrawn from RWL—40 mat surfaces). The solubilized product was quite thick as liquids go, and did not exist in abundance. So to make the addition to each sample uniform and to provide enough for each , the solubilized product was diluted to about 1/20 full strength. This created enough to add the required amount for each sample in the experiment which required it.

B. For SET 2, the coal sieve was our standard sized MS Wilcox (35-60 mesh, .25-.50mm diam). This coal was introduced (in the amounts specified) into the broth before autoclaving either. Then both were autoclaved together. This high temp/high pressure solubilized the coal somewhat and created a dark brown solution in NB (instead of the golden color of the broth itself), and a slightly colored solution in Bristol's (instead of colorless).

C. The "sludge" used was obtained from the campus waste-water treatment plant, and is aerated, activated sludge.

D. The inverted cylinders filled with HsO were present to catch any gas and crudely quantitate it. If the fermentation was indeed working, then it is understood that the first gas to be forced into the cylinder would be the air initially present, containing Oa- But as the fermentation proceeded, presumably the gases being pushed off contained much CO2, and other gases such as CHU perhaps.

E. The entire flask/tubing apparatus was autoclaved (in pieces) before inoculation. So the procedure was aseptic from the beginning. Any active fermenters present in the assay must therefore have come directly from the sludge.

0. Data

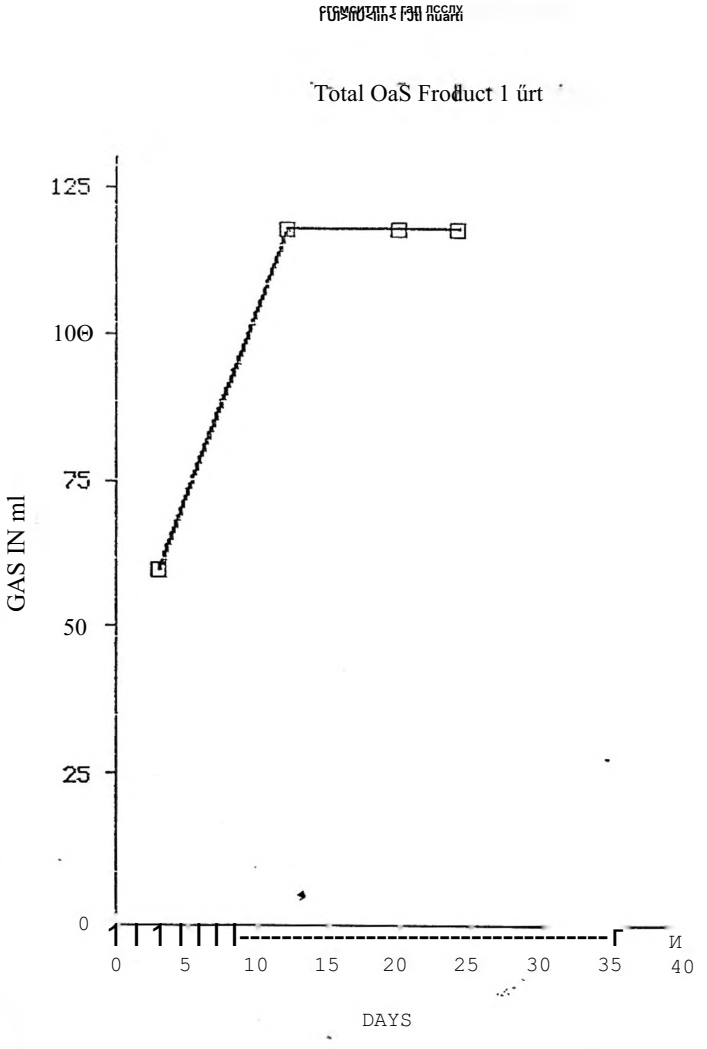
A. For NB, Coal sieve + inoculum (inoc. 09-17-88)

	DAY	GAS TOTAL	Δ GAS
09-20-88	3	47 ml	47 ml
09-29-88	12	126	79
10-07-88	20	146	20
10-11-88	24	174	28
10-18-88	31	212	38
10-24-88	37	250	38
11-01-88	45	261	11
11-03-88	47	270	9

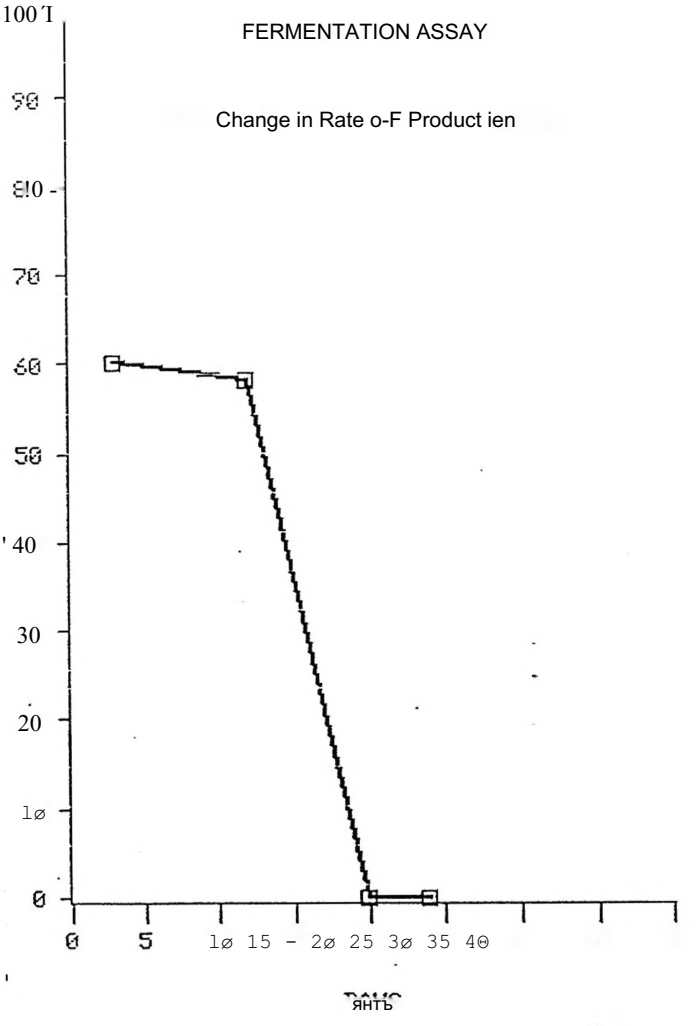
B. For NB, solubilized product + inoculum (09-17-88)

	DAY	GAS TOTAL	GAS
09-20-88	3	60 ml	60 ml
09-29-88	12	118	58
10-07-88	20	118	—
10-11-88	24	118	—

C. There was no notable gas production from any of the Bristol's samples, so none was recorded.'



NB, Solubilized product, and inoculum

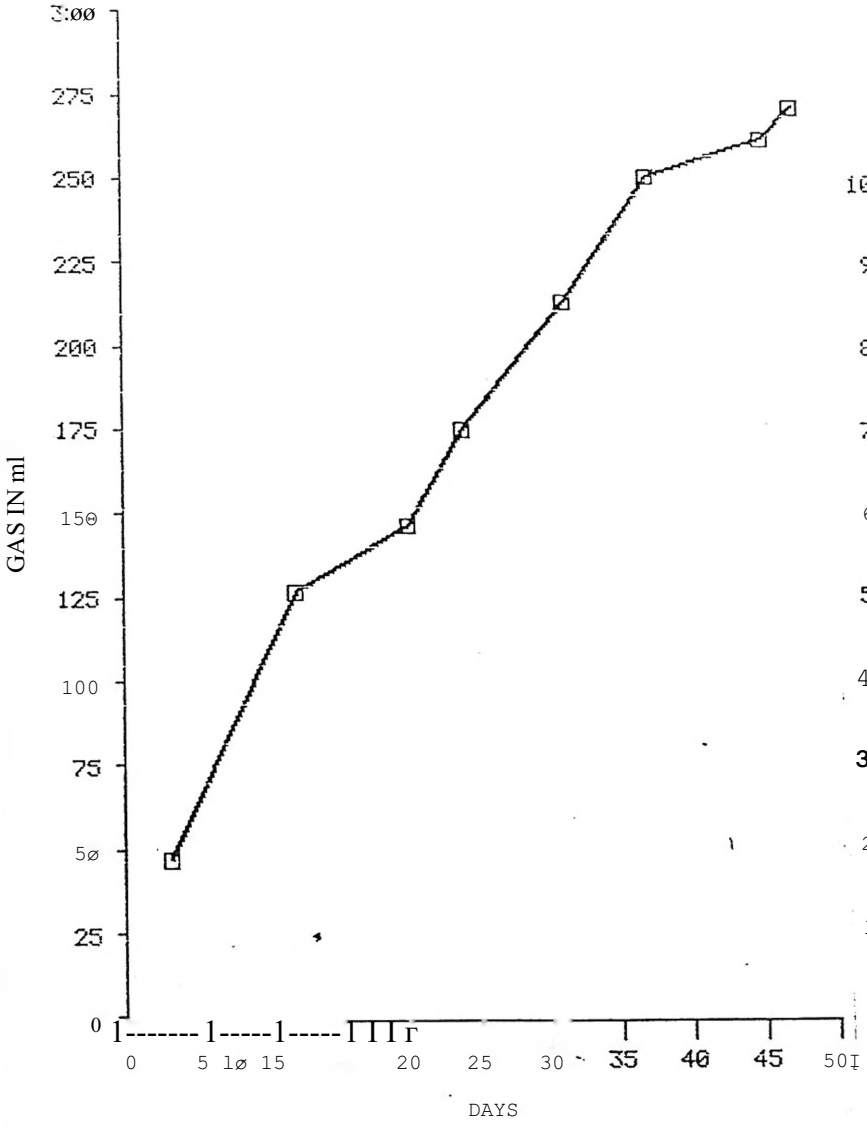


NB, Solubilized product, and inoculum

	DAY	GAS TOTAL	AGAS
09-20-88	3	60 ml	60 ml
09-29-88	12	118	58
10-07-88	20	118	—
10-11-88	24	118	—

FERMENTATIOH ASSAY

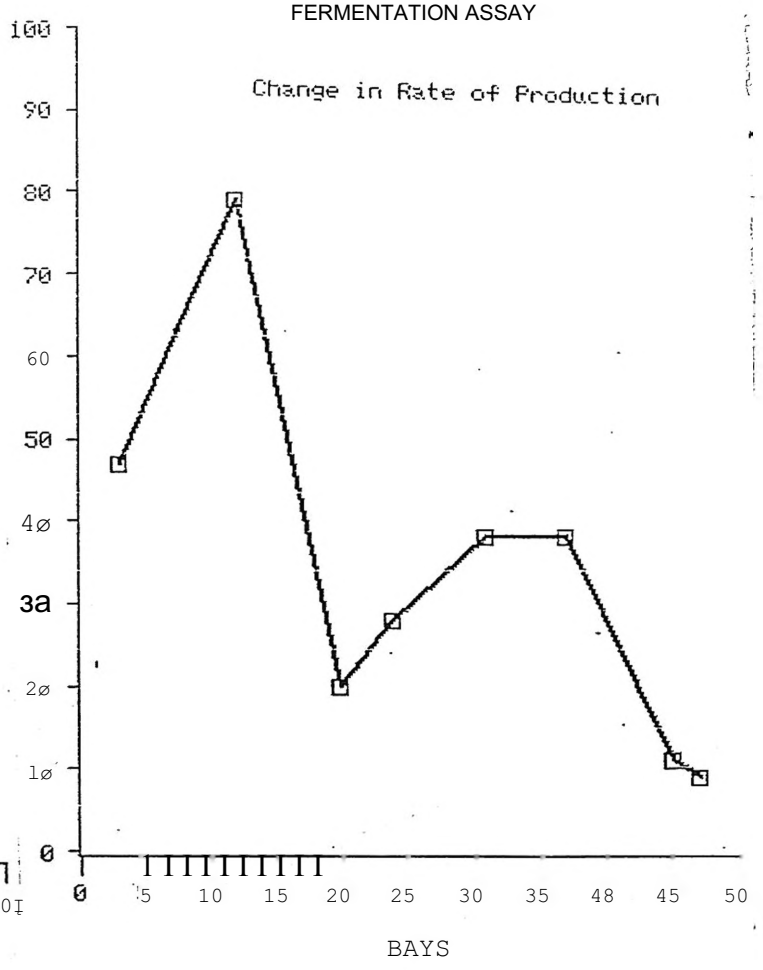
Total Gas Production



NB, Coal sieve, and inoculum

FERMENTATION ASSAY

Change in Rate of Production



NB, Coal sieve, and inoculum

	DAY	GAS TOTAL	A GAS
09-20-88	3	47 ml	47 ml
09-29-88	12	126	79
10-07-88	20	146	20
10-11-88	24	174	28
10-18-88	31	212	38
10-24-88	37	250	38
11-01-88	45	261	11
11-03-88	47	270	9

SMALLER SCALE FERMENTATION ASSAY

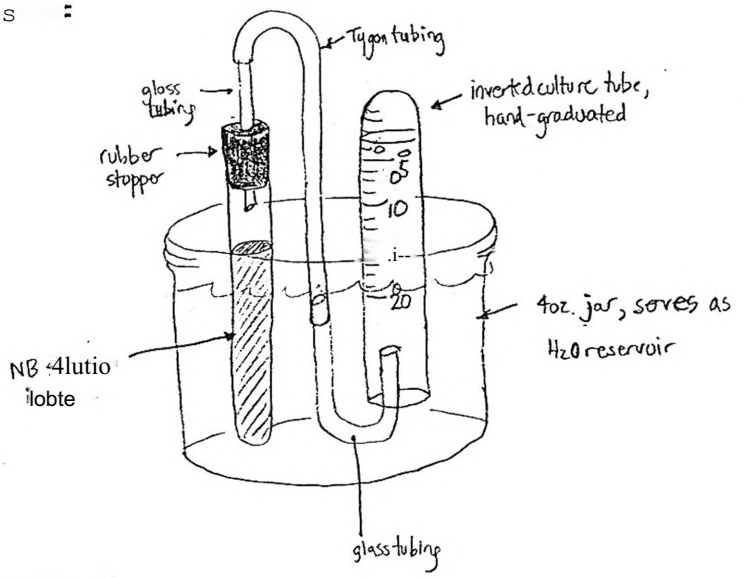
I. Materials used:

MB, inoculum + coal sieve (MS Wilcox)
(the broth and sieve autoclaved together)
NB, inoculum + solubilized product (MS Wilcox)
NB, inoculum only
NB, coal sieve only
NB, solubilized product only

II . Objective :

This was set up as an attempt to use spectrophotometric methods in hopes of quantifying or at least identifying the activity of the inoculum on the coal products. (We know from the larger-scale assay that fermentation occurs, and since the NB flasks of coal sieve + inoculum turned darker than those without inoculum, it could be concluded that some reaction is occuring there. Perhaps further solubilization.)

III. Apparatus :



IV . Preparat ion :

Note: where mentioned following, "set-up" is used to refer to the above apparatus.

3	set-ups	for NB, inoculum + coal	sieve
3	"	for NB, inoculum + sol.	product
3	"	for NB, inoculum	
6	"	for NB, coal sieve	
6	"	for NB, sol. product	
3	"	for NB only	

The quantities used in the above "set-ups" are as follows:

10 ml NB in each
0.1 ml inoculum (where used) (This was straight sludge.)
0.018 g coal sieve (where used)
I drop full strength solubilized product (where used)

The sieve and solubilized product are both MS Wilcox.
The proportions used for the coal sieve are the same as those for the large-scale assay.

The coal sieve and broth were autoclaved together, to provide some "solubilization" at the start.
To test the possible pH changes, pH was measured before and after autoclaving.

initial pH of NB = 6.70
after autoclaving, pH = 6-84
after autocl. with coal = 6.50

V. Assay begun -

Samples inoculated and incubation started on 10-28-88

By 11-16-88, still no change in color or gas formation evident in the tubes, so a "new" inoculum was introduced. I ml was taken from the already-ferment ing coal sieve + inoculum sample in the larger assay (still running in the incubator) and injected into each tube which called for an inoculum.

By 11-30-88, bubbles were present on the liquid surface of some of the samples (evidence of fermentation?), but no amount of gas was measurable yet.

By 02-03-89, there was supposedly some gas accumulated in the collecting tubes of several of the samples. (I say "supposedly" because the reservoirs had been allowed to run nearly dry over the break- But since they had been maintained since then by Anna, and this gas had been collected after that point, perhaps this was true accumulation of something produced by the system.)
At this point, two of the collection tubes were corked and stored for future analysis. One of the samples was from an "NB + coal sieve + inoculum" test, and one was from an "NB + sol. product + inoculum" test.

UI. Data

UU spectra were taken for the two units whose gas had been capped, along with one other "fermenting" unit. As a matter of comparison, spectra for the "control" counterparts of these were also taken (NB + coal sieve, and NB + sol .product). However, these "controls" themselves appeared to be housing organisms (they seemed a bit cloudier than when they were placed in the incubator fourteen weeks prior), so the data they provide may be skewed. (As it turned out, the control spectra were nearly

identical to all the others. In fact, all of the sample spectra closely resembled each other.)

Scans were run with H2O as baseline, and those samples which had been autoclaved with coal sieve had to be diluted.

The scans were as follows:

NBACCON.RTS = NB, autoclaved with coal sieve, "control" --- diluted 1=2

NBACINO5.RTS = NB, autoclaved with sieve, + inoculum (Set 5 in assay) --- diluted 1=2

NBACIN01.RTS = NB, autoclaved with sieve, + inoculum (Set 1 in assay) --- diluted 1=2

NBSPCON.RTS = NB, sol. product "control"

NBSPIN02.RTS = NB, sol. product, + inoculum (Set 2 in assay)

The spectra was also read for one of the larger samples - (inoculated on 11-16-88) from the larger assay, Just for comparison purposes.

All of these spectra have been saved on a floppy disk (dated 2-06-88). Since they look almost identical ,only one hard copy representative of them has been included. This representative spectrum is Figure 1 (following).

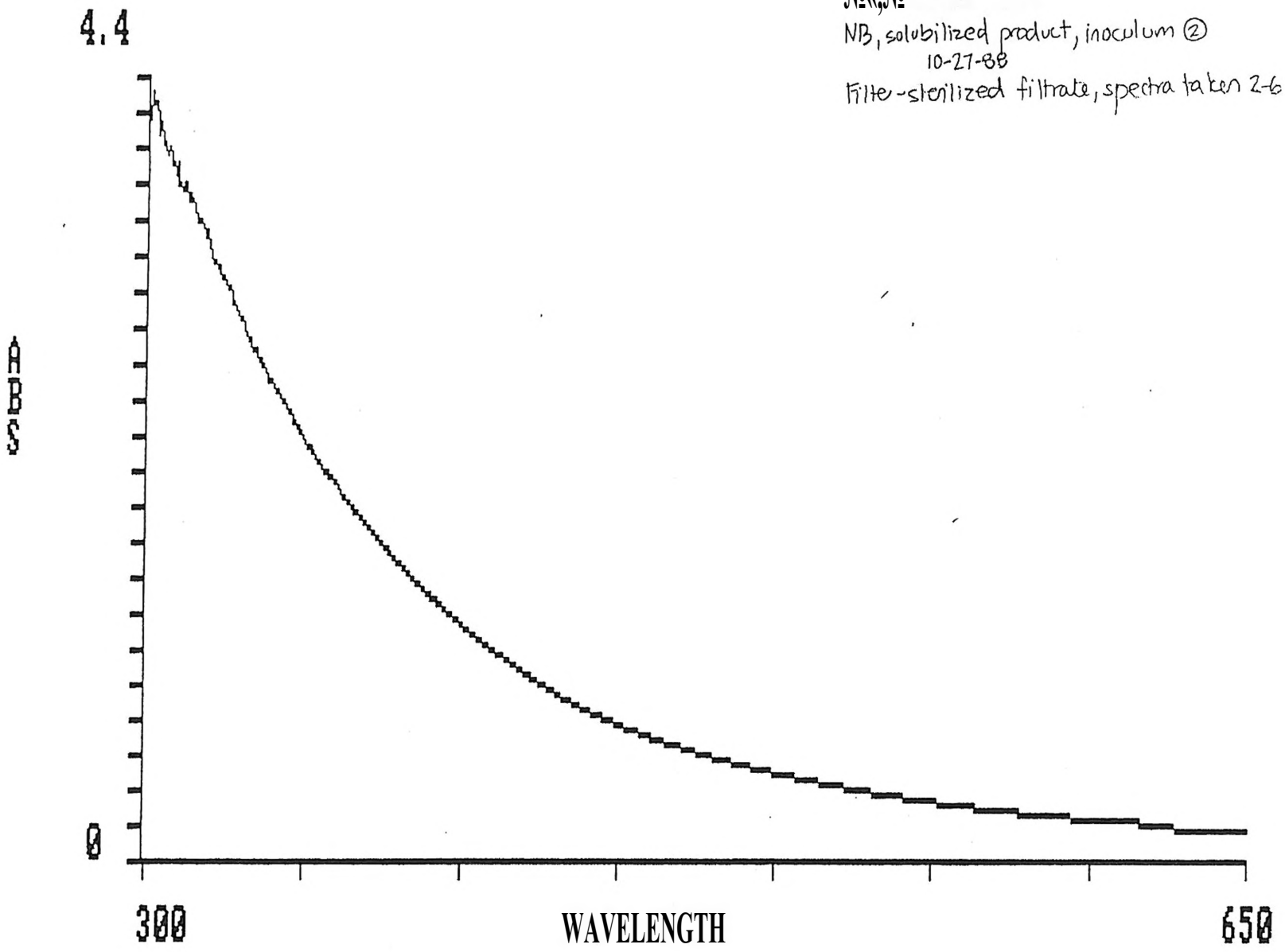


FIGURE 1. UV spectrum representative of the experimentals in small-scale fermentation assay. This experimental consisted of solubilized coal product + sludge inoculum in Nutrient Broth.

ASSAY OF GAS PRODUCTION BY FERMENTERS
IN A COAL-CONTAINING SYSTEM

I. Using cultures of activated sludge:

"Mother flask" (500 ml NB, 0.6g MS Wilcox autoclaved together, then inoculated with 1 ml activated sludge)
Date: 09-13-88

Several flasks from this parent, of same proportions (500 ml NB, 0.6g MS Wilcox) using an inoculum of 10 ml from the "mother flask"
Date: 11-16-88

Flask from one of these second generation cultures also, of same proportions , using an inoculum of 10 ml from one of these seconds.

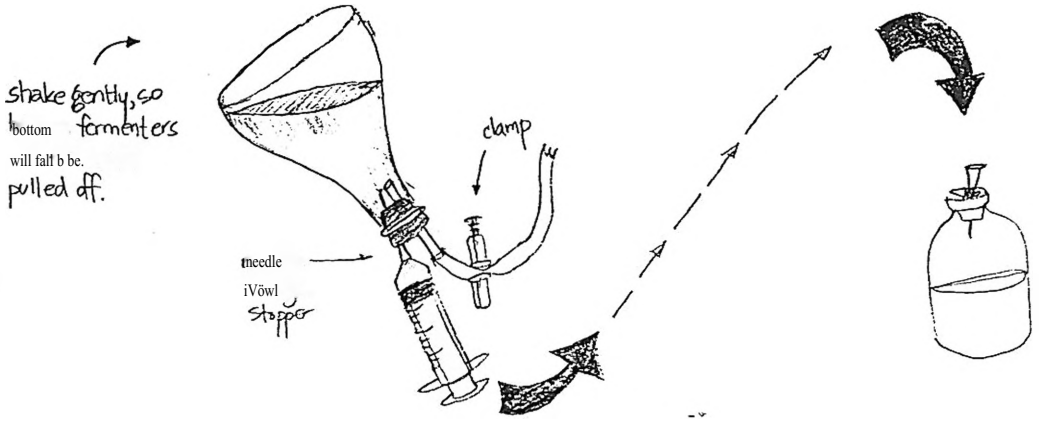
The most active of these cultures from 11-16-88 (determined "active" by amount of bubbles at surface—evidence of the fermentation underway) was used as inoculum for the assay described herein.

II . Small culture bottles, crimp-sealed, were employed for a small-seaie gas collection assay.

The cultures would be allowed to incubate for about one month, then the gas in the headspace would be sampled and assessed on a gas chromatograph.

III . Set-up as follows:

A. Inoculum withdrawn thus:



B. Two general types of samples used.

1. 30 ml of liquid (and cells) removed directly from the large flask and injected into small serum vial (which is sterile and has been purged with N2)
2. 30 ml of Bristol's broth (no sugar added) autoclaved together with 0.04g MS Wilcox in small

serum vial (purged with N2) into which was injected 1 ml (in three bottles) or 2 ml (in two bottles) of inoculum from the large flask

C. Three types of control were prepared.
1. 30 ml of Bristol's broth autoclaved together with 0.04g MS Wilcox in small serum vial (purged w/ N2)
2. 30 ml of Bristol's broth (purged with Ns)
3. Bottle purged with Ns only

D. The proportions of 0.04g coal / 30 ml approximate those used in the larger original assay, which used 0.18g / 150 ml (or 0.6g / 500 ml).

E. Inoculation, experiment underway 05-10-8?

IU. Chromatographic analysis

A. HP5790A Series GC was used
60 C
Detector Sensitivity = 3 I
Polarities A & B both used, respectively
("Polarity A" column used Supelco Chromosorb 102, 60/80 mesh, Lot# 369411
"Polarity B" column used Supelco Molecular sieve 13X, 60/80 mesh, Lot# 309)

B. References were run for comparison
(Нг, C0г, 0¿, N2, CO, House Gas/CH.4)
Each of these was crudely prepared by flushing a bottle with cylinder gas (or in the case of "house gas", with gas from the natural gas spout in the lab) and sealing it. Although the chromatographs of these may evidence some bit of impurity as a result, for the most part the references are dependable.
An injection of air was also run

C. "A" Polarity=most sensitive to CO2 and H2
On this polarity, results on references (and on the experimental samples) were a little vague and uncertain.
These were the best "guesstimates" we could decipher as far as standards go:

Нг	0.37-0.41	
0г	0.40-0.43	
Иг	0.42	(?)
CO2	0.81	
CHA	0.53	(?)

Since N2 and CHл are not theoretically designed to be detected by this column, the (?) indicates that these were the retention peaks evident, but whether they are the correct ones or not could be debatable.

"B" Polarity----- most sensitive to Ns, Hs, and CO
On this polarity, the standards had more accurate (or more dependable, as the case may be) retention times, as follows:

Hs	0.47-0.50
Os	0 .09-0.70
Ns	0.82-0.85
ChU	1.44-1.45

D. Standard injection volume was 25 microliters, but in some cases, less was used. This is noted on the chromatographs where applicable.

E. Chromatographs follow:

On "A" Polarity_____

air
COs
Os
Hs
House Gas (CHл)

Control #1
Exp #2
Exp #3
Exp #4
, Exp #5
Exp #6
Exp #7
Exp #8
Exp #9
Parent Flask

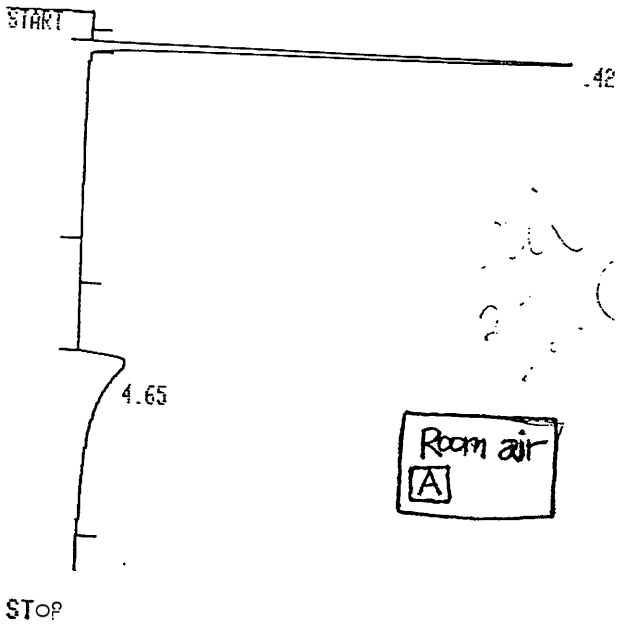
On "B" Polarity __

air
Ns
Os
Hs
House Gas (CH^)

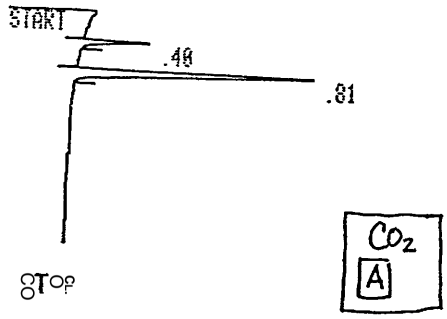
Control
Control #1
Exp $2
Exp #3
Exp #4
Exp 115
Exp #6
Exp #7
Exp #8
Parent Flask

A – STANDARD REFS

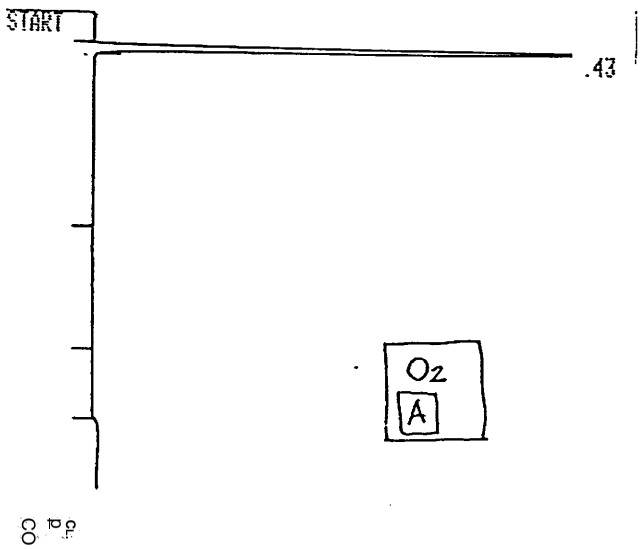
AIR



CO_2



O_2



TIME IN MINUTES

m

A - STANDARD **REFE**

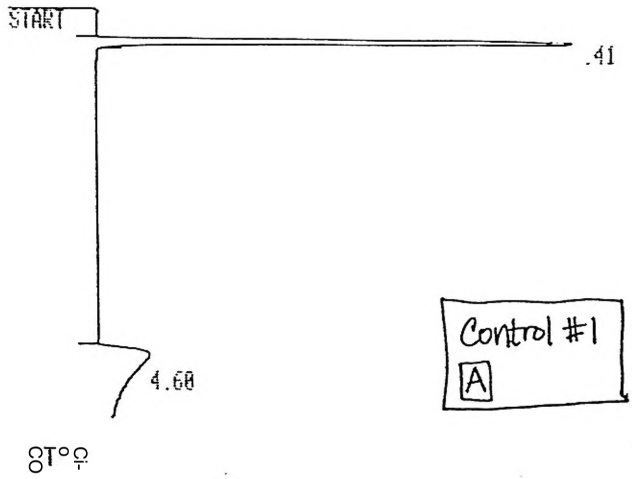
TIME IN MINUTES

H_2

CH_4

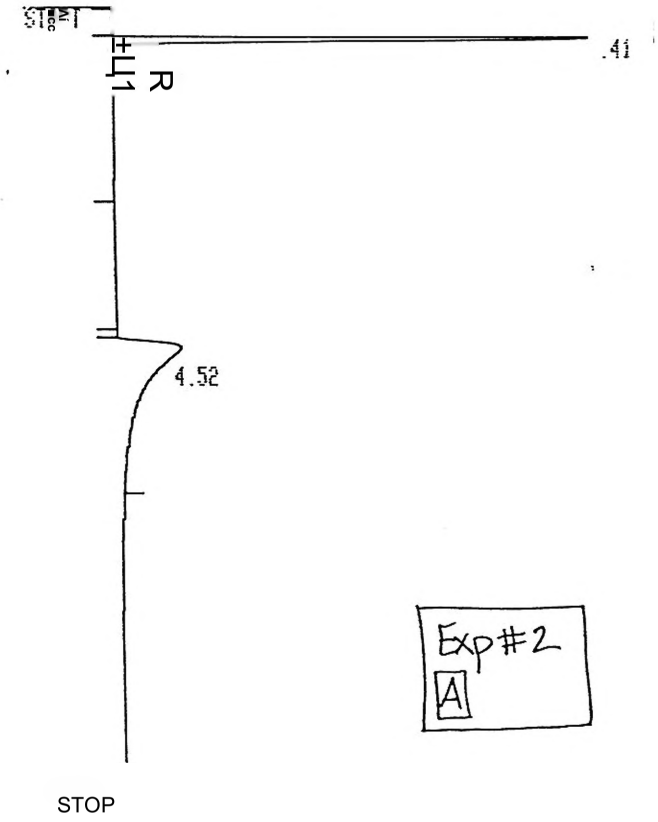
A

CONTROL #1



Control #1
A

EXP #2

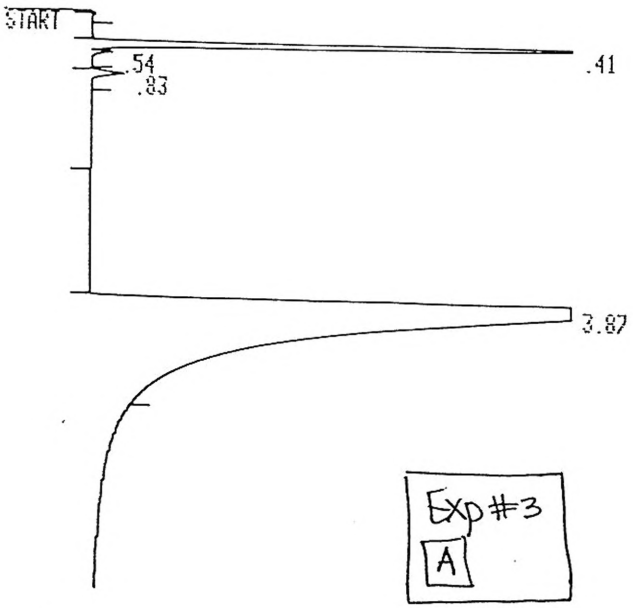


Exp #2
A

TIME IN MINUTES

A

EXP #3



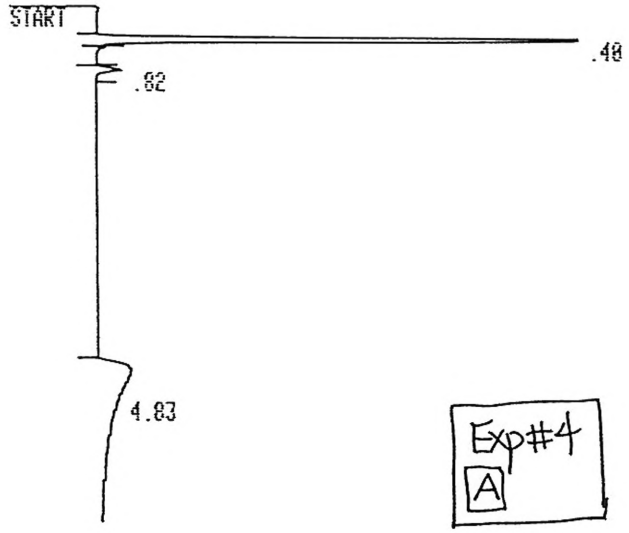
0.41 – N_2 (?) (some O_2?)

0.54 – CH_4

0.83 – CO_2

3.87 – ?

EXP #4



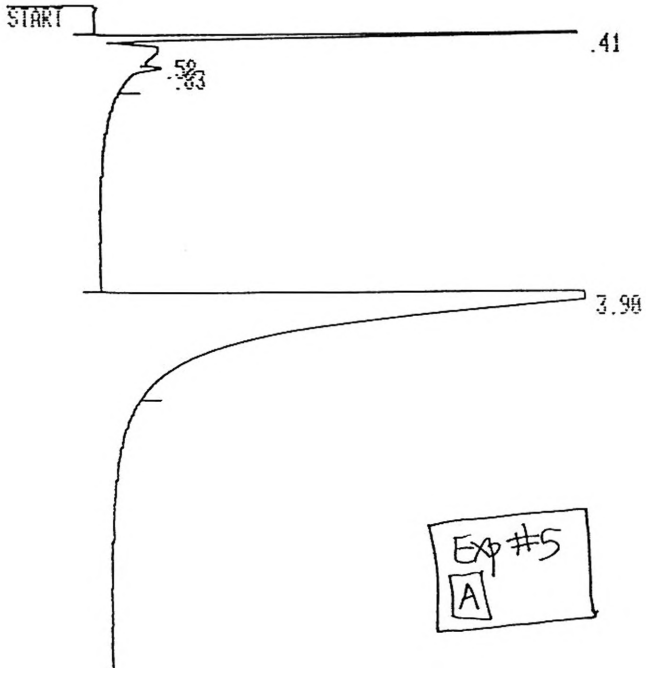
0.40 – N_2 (?) (some O_2?)

0.82 – CO_2

4.83 – ?

TIME IN MINUTES

EXP #5

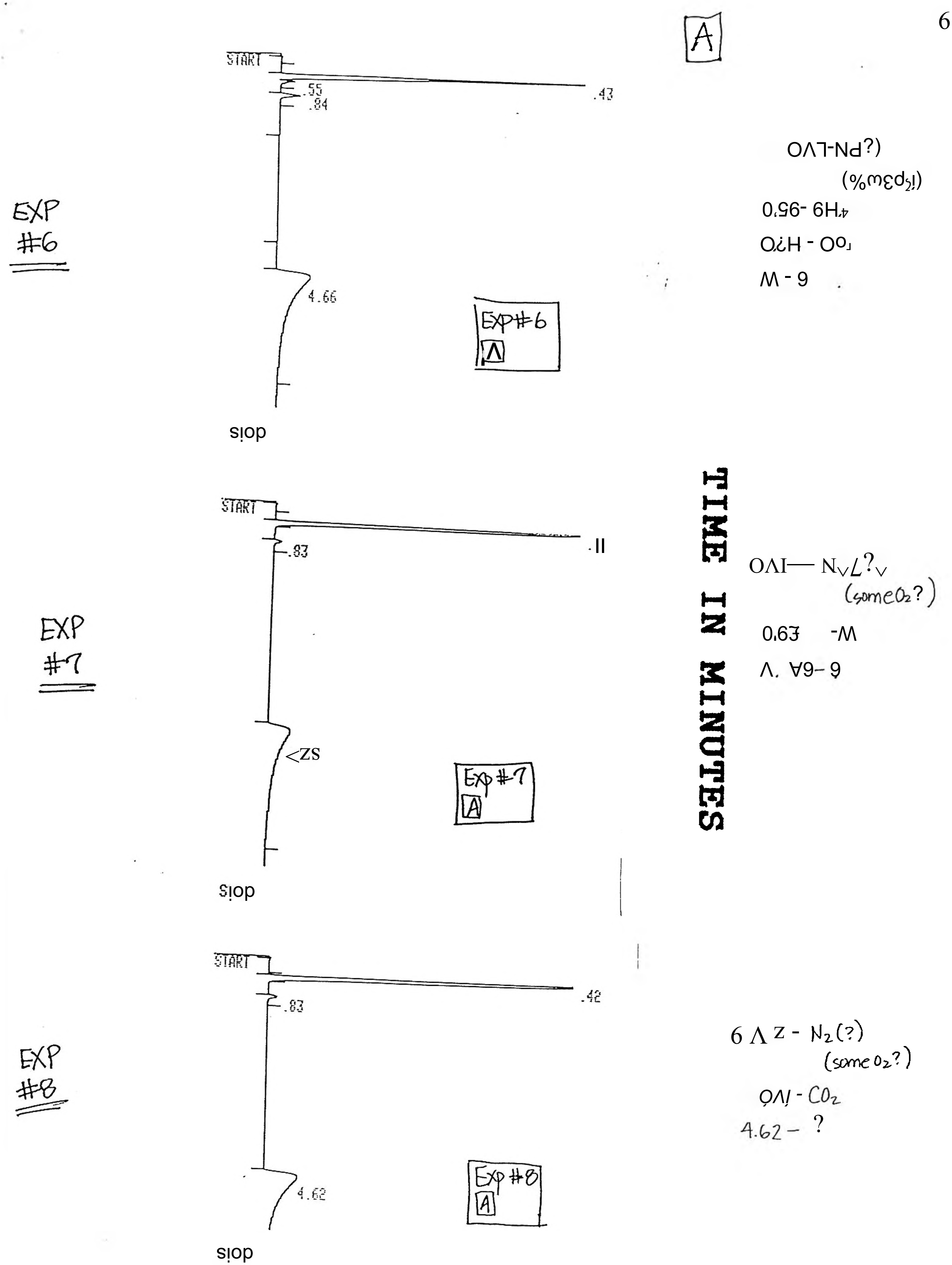


0.41 – N_2 (?) (some O_2?)

0.58 – CH_4

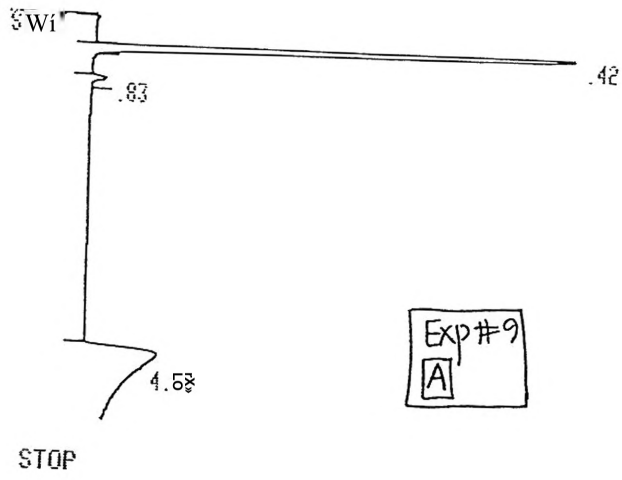
0.83 – CO_2

3.90 – ?


A
EXP #6
START
.43
.55
.84
4.66
Exp #6
EXP #7
.83
Exp #7
A
EXP #8
.42
.83
4.62
Exp #8
A
TIME IN MINUTES
(some O2?)
N2(?)
(some O2?)
CO2
4.62 – ?

A

EXP #9



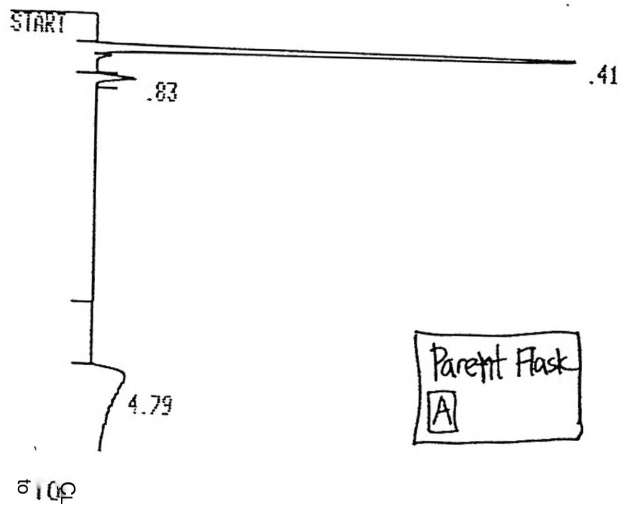
0.42 – N_2 (g)
(some O_2)

0.83 – $S_2O_3^{2-}$

4.68 – ?

TIME IN MINUTES

Parent Flask



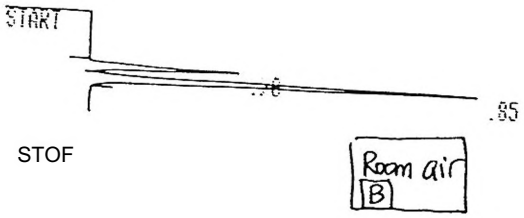
0.41 – N_2 (g)
(some O_2 (g))

0.83 – $S_2O_3^{2-}$

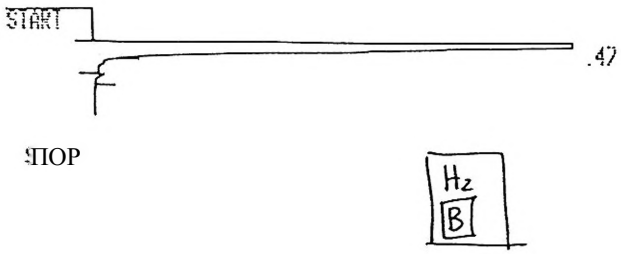
4.79 – ?

B + STANDARD REFS"

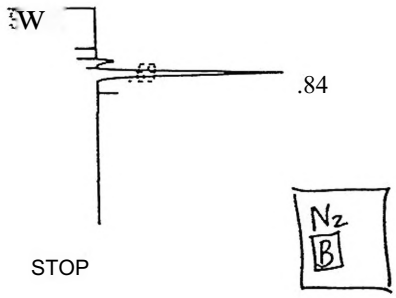
air



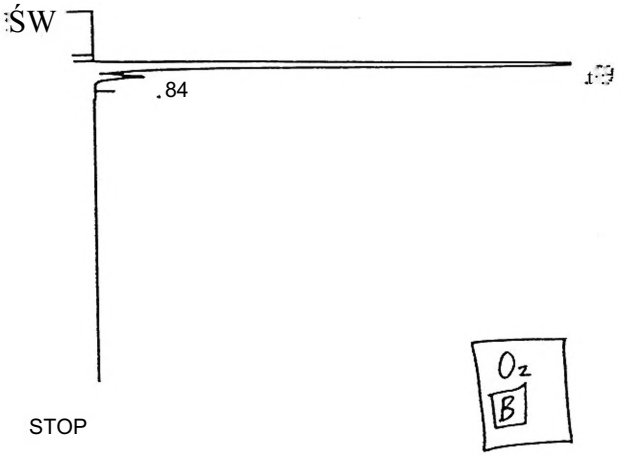
H_2



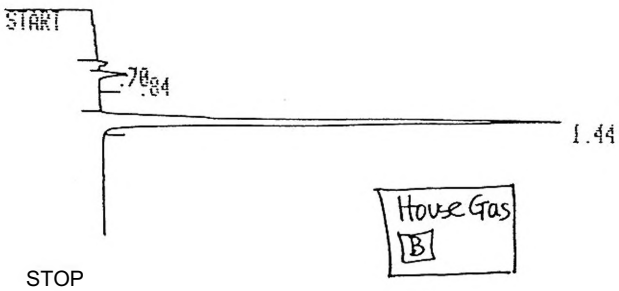
N_2



O_2



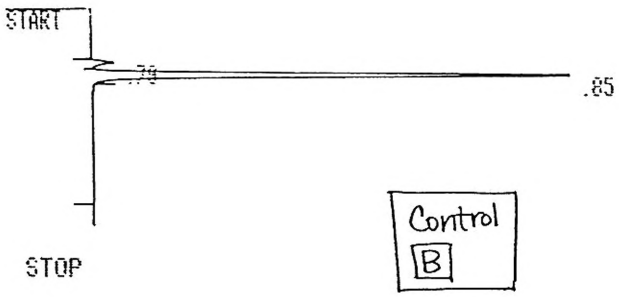
CH_4



TIME IN MI §Ł
W
СП

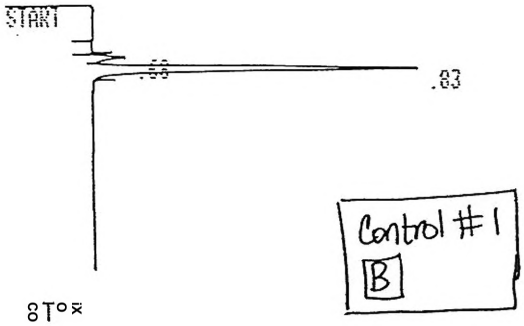
B

Control



0.70 - O_2
0.85 - N_2

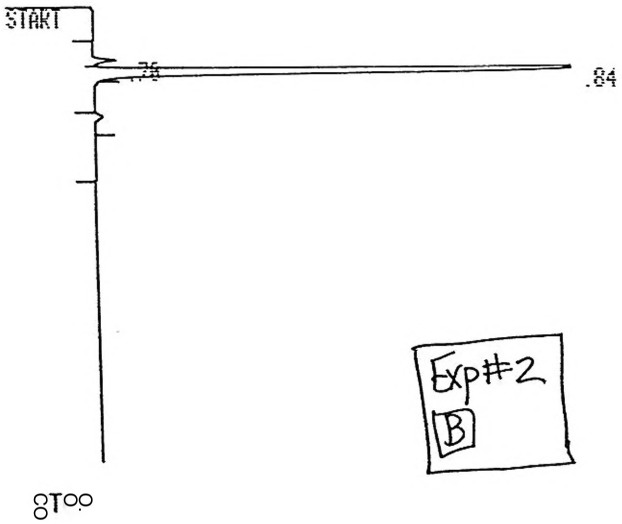
Control #1



0.68 - O_2
0.83 - N_2

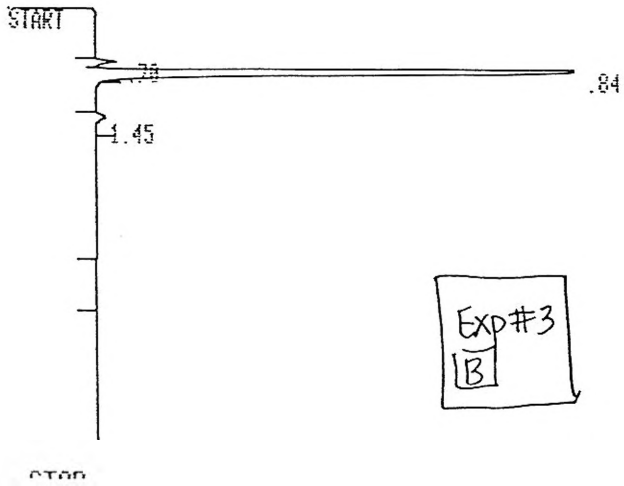
TIME IN MINUTES

EXP #2



0.70 - O_2
0.84 - N_2
(1.45) - CH_4

EXP #3

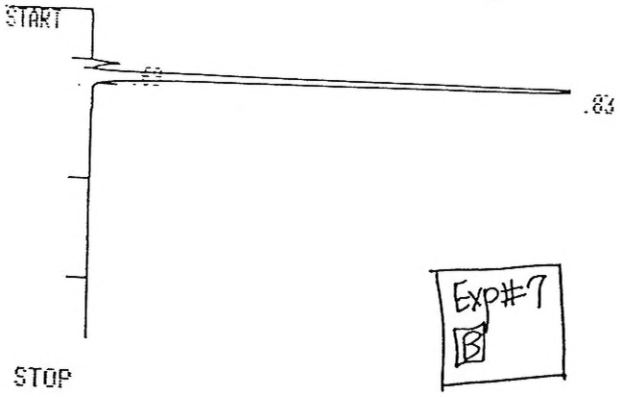


0.7
0.8
1.4

EXP #4
dois
START
.84
EXP #5
dois
START
.84
EXP #6
STOP
1.45
.84
IMS

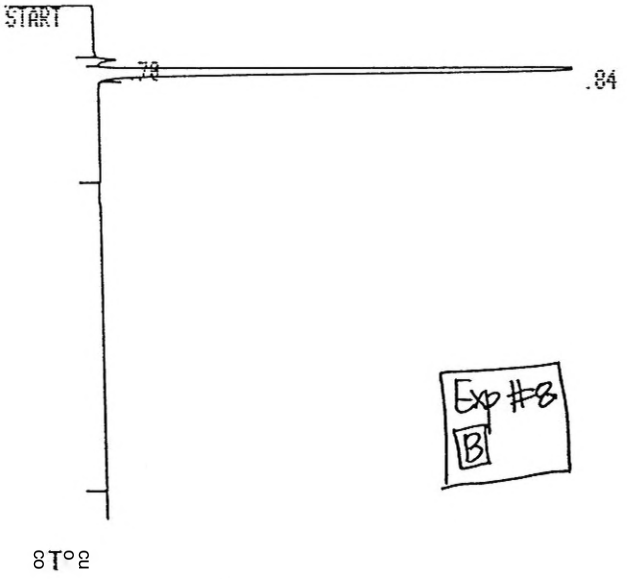
B

EXP #7



0.69 — O_2
0.82 — N_2

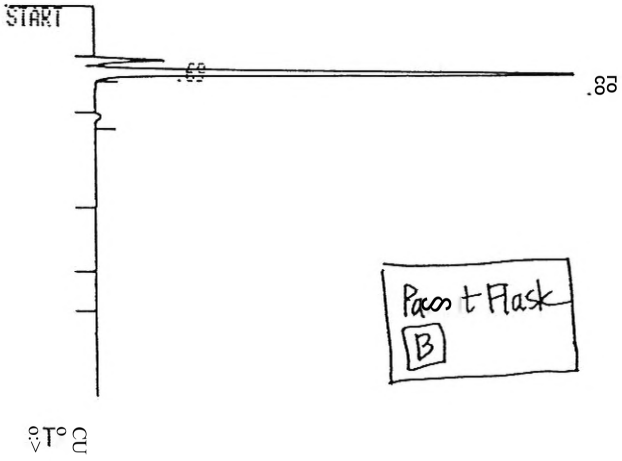
EXP #8



0.70 - O_2
0.84 — N_2

TIME IN MINUTES

Parent Flask



0.69 — O_2
0.83 — N_2
(1.45) — CH_4

V. Interpretation and Compilation

A. All of the bottles tested contained some amount of Ns, as would be anticipated, since the headspaces were initially purged with Ns-

Ns on "A" = most probably -42, since that is where the major peak occurs (air is 79% Ns)

Ns on "B" = .84

B. In addition, most of the bottles apparently contained some Os- This is difficult to tell when employing the "A" polarity column, but becomes more obvious with "B". The origin of this Os is uncertain. It could be left as a result of incomplete purging with the Ns, or it could be some product of the reaction occur ing within the system. (Perhaps Os is released from the breakdown of the structure of the coal—at the COOH locations, for instance.)

Os on "A" = .43

0s on "B" = .69

C. The "A" polarity is sensitive to COs moreso than the "B", so we can see what is probably COs appearing on some "A" graphs. (COs cannot be identified on "B" .)
The COs is present in all of the experimentals, but not in the control.

COs on "A" = .81

D. CH4 can supposedly be picked up some on both polarities, but seems to be more apparent on "B".
CHA is not present in the controls, nor is it found in the "broth/coal + inoculum" samples. It is only in the parent flask graph and some samples drawn directly from the parent.

CHд on "A" = .53 (Exp #3, #5, #6 show this)

C1U on "B" = 1.44 (Exp #2, #3, #4, #5, #6, and parent show this)

E. The peak at or around 4.50-4.85 on the "A" polarity runs is as yet unidentified. But it can be seen in the chromatograph of room air also. With such a high retention time, the gas must be relatively larger. It was suggested that the lab we were conducting the assays in was full of fumes remaining from the acetogen research taking place there. So it is slightly possible that this mystery peak may be something such as acetylene.

Additional high retention-time mystery peaks appeared between 3.8 and 4.0 on a few of the samples (instead of at the 4.5 mark).

By way of explanation, the reason for the "broth/coal + inoculum" was to deprive the culture of any additional carbon source other than the coal itself, to see if it would utilize the coal. These sample bottles prepared in this manner showed no obvious additional growth (still relatively clear except for the slight population introduced with the 1-2ml inoculum). Also, these did not produce the Cl-U found in the directly transferred samples. They did, however, possess some quantity of CO2 (if the peaks can be truly interpreted as such))

As for the chromatographs, the following can also be provided for the graphs present:

Area %
Area Type
Area Height
Total Area

Some additional settings on the chromatograph can be provided also.

References

1. Ward, H.B. Isolation and application of coal-solubilizing microorganisms. In Press for: Wise, D.L. ed. Bioprocessing and Biotreatment of Coal" Marcel Dekker, Inc., New York.

2. Quigley, D.R., B. Ward, D.L. Crawford, H.J. Hatcher, and P.R.Dugan. 1989. Evidence that microbially produced alkaline materials are involved in coal biosolubilization. Appi. Blochem. Biotech. Vol. 20/21, pp. 753-763.

3. Quigley, D.R., C.R. Breckenridge, P.R. Dugan, and B. Ward. 1989. Effects of multivalent cations found in coal on alkali or biosolubility. In Press: Energy and Fuels (accepted with revisions).

4. Ward, B.. D. R. Quigley, and P. R. Dugan. 1988. Relationships between natural weathering and lignite biosolubility or alkali solubility. Preprint and In Press: Proceedings: Institute of Gas Technology Symposium on Gas, Oil, and Coal Biotechnology, Dec. 5-7, 1988, New Orleans, LA.

5. Ward, B., and A. Sanders. Solubilization of coals by fungi. In Press: Proceedings of the Electric Power Research Institute Symposium on Biological Processing of Coal and Coal-derived Substances, Palo Alto, CA, May 16-17, 1989.

6. Yeh, G.J.C., B. Ward, D.R. Quigley, D.L. Crawford, and H.L.C. Meuzelaar. 1988. Numerical Comparisons between the pyrolysis mass spectra of twelve U.S. coals and their relative solubility in microbial cultures or alkaline buffer. American Chemical Society preprint, Vol. 33, No. 4, pp. 612-622.

7. Ward, B. 1988. Biodegradation and bioconversion of coals by fungi. Fossil, U.S. Department of Energy Document DOE/PC/80913-8 (DE88006665). 33 p. Available from NTIS Energy Distribution Center, Oak Ridge, TN.

FINAL REPORT

MISSISSIPPI MINERAL RESOURCES INSTITUTE
PROJECT NUMBER 89-11F
BOM No. G1184128

Lignite Utilization Via Biotechnological Innovations

PART TWO

Report on activities and accomplishments for Project Research conducted in the Biochemical Engineering Laboratories, Chemical Engineering Department, the University of Mississippi.

Robert J. Fisher, Ph.D., Principle Investigator

Kao-Sheng Lay, Graduate Research Assistant

SUMMARY - PART IT

This phase of the project was divided into two distinct paths: (1) Characterization of the raw materials and biosolubilization products, and (2) understanding of the bioprocesses via reaction and transport studies. The accomplishments in each area are summarized as follows:

(1) Characterization Studies

Analytical pyrolysis, with GC/MS interfacing, was used to characterize the raw materials and biosolubilization products. Only partial success was obtained, identifying a limited number of species. The destructive nature of this technique was the major shortcoming. Additional analytical instruments are being evaluated, such as Supercritical Fluid-High Performance Liquid Chromatography (SCF/HPLC). This system is new and the protocols are not well established, therefore, only limited results are available. First indications are that it will not be as effective as first anticipated. The technique is still in the "art" phase and not a proven scientific instrument for these applications. The major limitation to date seems to be column contamination. Effort is continuing to be expended to resolve the difficulties encountered in these characterization studies.

(2) Transport and Reaction Studies

The solubilization process has been identified as extracellular. Previous investigators also showed that the process is more efficient when whole cells are present versus a "cell-free" extracellular extract. This indicates that a multistep reaction pathway utilizing (a) enzymes produced "on demand" is in operation and/or (b) possible sites on/or within the cell membrane. Thus, the need to study transport processes in conjunction with reaction kinetics. Consequently, we have developed (and published) various models for diffusion with immobilization in membranes. The immobilization can be physi- and/or chemiadsorption with or without reaction at these sites. Analysis of the proposed reactions responsible for the solubilization has showed that the rate is primarily dependent upon (1) temperature, (2) pH, (3) moisture content of the lignite/coal and (4) its oxidation state (i.e. composition and degree of weathering). The carbon-oxygen bonds appear to be the attack sites by the microorganisms (such as Polyporus versicolor, a basideomycete fungi) in this coal solubilization process. The rate is highest, apparently, at the ether linkages. Naturally weathered coals are highest in these types of bonds, consistent with the observations that these coals are easiest to solubilize. Countering this enhancement due to favorable bond formation however, is the extent of pyrite oxidation. This leads to the formation of H_2SO_4 during solubilization which has an inhibiting affect. Our theories and the proposed reaction mechanisms account for all these observations. Thus, successful processes can be developed

once validification experimentation is designed, conducted, and analyzed providing the appropriate data base.

PROJECT COMPONENT

A. Characterization Studies

I. Pyrolysis - Mass Spectrometry

Biosolubilization products in addition to the raw materials themselves and the post-solubilized solids have been/are being characterized by pyrolysis with GC/MS interfacing. Metcalf et al. (1987) characterized U.S. lignites and the micro-organism itself in addition to liquid products were studied by many investigators. For example, analytical pyrolysis, either in (PGC), (P-GC/MS) or (P-MS) systems has been reported for drugs in biological fluids (Sano et al. (1980)), in metabolic studies (Roy, 1978) and in taxonomy for microorganisms (Quinn, 1979), (Blomquist, et al. (1979)), (Fisher (1989a)). The usefulness of the pyrolysis techniques are limited for a number of reasons. First, our unit needs updating, particularly with cryofocusing capabilities, and improvements in the handling of liquid samples. Secondly, the inherent limitations of a destructive technique, allowing only certain components to be successfully identified, makes complete identification of all species an unrealistic goal. Gaseous components are handled successfully with GC techniques. Analysis of the results from short and long term fermentation experiments with the production of fuel gases was discussed in Part I. Thus, our major problem is still with the liquid products. We have selected key components (as discussed in Section B - Transport and Reaction Studies) from various stages of the solubilization process, and are currently developing the separation/identification techniques.

II. Alternative Techniques For Liquid Product Analysis

Alternative analytical instrumentation is being evaluated. Presently, our new Supercritical Fluid-High Performance Liquid Chromatography (SCF/HPLC) unit has not proven itself effective, for general application. Success has been reported by Fisher (1989b) and Fisher and Tang (1989) for applications in Food Biochemistry such as the isolation of Vitamin E and other tocopherals. Column contamination while in the Supercritical extraction mode has been the main difficulty. Work continues in this phase, without a great deal of success, however.

B. Transport And Reaction Studies

I. Transport Studies

The solubilization process has been identified as extracellular (see documentation in next section, i.e. B-II -

Reaction Studies). Campbell, et al (1988) verified, using coal-related model compounds, that the biodegradation process is more efficient when whole cells are present versus a "cell free" extracellular extract obtained during the growth of C. Versicolor. Their results confirmed the observation of many investigation (see next section) using various coals. These results indicate that a multistep reaction pathway is operational utilizing (a) enzymes produced "on demand" by the cells and/or (b) possible sites on/or within the cell wall/membrane structure. Hence, Transport processes play a major role and must be dealt with in concert with reaction kinetics studies. Various models have been developed, (Fisher, R.J. (1989c)), for studying diffusion with immobilization in membranes. The immobilization can be by physi- and/or chemi-adsorption with or without reaction at these sites.

The diffusion problems are from the general class relating diffusion of components into a medium which will immobilize a portion of them and thus remove them from further participation in the diffusion process. They are best represented as moving boundary problems. The three cases discussed are (1) concentration dependent diffusivity with rapid immobilization in simple media, (2) finite absorption rate with constant diffusivity in simple media, and (3) diffusion anomalies in polymers, i.e. complex media. The complex nature of the media can arise from changes in physical properties with concentration of diffusing molecules, time dependent internal stresses, and changes in geometry with time, most likely anisotropic. The systems studied are all characterized by immobilization processes that are irreversible and rates that are either instantaneous or comparable to diffusion. The fact that only a finite amount of material can be immobilized is also accounted for i.e. solubility limits are incorporated. Approximate solutions are presented. Coupling these results with the ideas/results of the following section is our next objective.

II. Reaction Studies

The emphasis of our work has been to develop an understanding of the mechanism of microbial solubilization of low-rank coals such as lignite, with particular interest in the effects due to the weathering phenomena (Fisher and Lay, 1989).

Changes in the functional groups of the low-rank coal after natural weathering or low-temperature oxidation were investigated. A substantial literature survey was conducted related to coal weathering, lignin degradation and coal solubilization by microorganisms. The objective was to elucidate the mechanism of enhanced fungal degradation observed with weathered samples.

Natural weathering can significantly affect the coal structure; of particular concern is an increase in certain oxygen-containing groups. Carbonylation of the aliphatic

structure can expose the C-C bond, making it more susceptible to enzyme cleavage. The increase of the carboxylic acid content in the fungal degraded liquid products is believed to be a result of the oxidative cleavage of aromatic rings by fungi leading to the formation of the aliphatic acid groups. Ether linkages can also be formed during natural weathering of a low-temperature oxidation process due either to the condensation of hydroxyl groups or the thermal decomposition of the carbonyl groups. Ether groups interconnecting the aromatic rings lead to the observed increase in molecular size and the loss of the plastic properties of low-rank coals. Enzymes from white-rot fungi most probably split the C-O bond, yielding a coal liquefaction product via an extracellular process.

(a) Background/Literature Review

Bioconversion of coal has been studied for many years, and has increased in potential because of its low cost. Since the beginning of the century, investigators have been able to isolate some microorganisms from coals, and some were grown on low rank coal as its sole substrate. A comprehensive review is given by Fisher and Lay (1989) and will not be duplicated here. Some of the Key research groups are: Rogoff, et al. (1962) providing a compilation of microbial species that were often associated with coal or related to coal studies, and identified certain variables that enhance growth rates; Koburger (1964) reports some factors that apparently influence the rate and extent of growth; Kucher, et al. (1977) used coal substrates containing aqueous extracts, obtained after partial oxidation of the coal, by hydrogen peroxide or nitric acid, for the cultivation of yeast.

Coal is considered to have been formed from peat deposits produced in swamps through the accumulation of plant substrate. Thus, it contains distinctive organic chemical structures such as cellulose and lignin (Davidson, 1982). These vegetable plant substances were subjected to various physio-chemical processes and hence converted into various coals. Obviously, low-ranked coal originates from fossilized plant material and has a structure similar to cellulose and lignin.

The analysis studied by Buravas et al. (1970) indicated that 35-70% lignin-like compound was contained in Tertiary lignite. They also mentioned that the infra-red spectra of early lignites and lignins? are similar. The early lignites contain phenolpropane type structures while the later (older) lignites and coals may contain material formed by the condensation of such substrate. It is not surprising, therefore, that it has been demonstrated that some wood-rot fungi degrade low-ranked coal.

Scott et al. (1986) collected (and cultured) seven strains of fungi (Polyporus versicolor ACTT12679, Poria monticola ATCC 11538, Pénicillium waksmanii ML-20, Candida sp. ML-13,

Asperguillus sp., Sporothrix sp. and Paecilomyces sp.), and used various types of coals as test substrates. Based on the solubilization experiments they concluded that Candida sp., P. waksmanii and Sporothrix sp. were the more active fungi for the degradation of low-ranked coals. Interestingly, they observed that the tested North Dakota II lignite (naturally oxidized lignite), which had the highest oxygen content (ultimate analysis 41.8%) among the coals, was the best substrate for microbial solubilization. That is, it had the highest solubilization rate and ultimate degree of solubilization. Thus, it is reasonable to consider oxygen content an important factor in coal biodegradation. Further, they observed that low-ranked coals with differing oxidation states give different solubilization rates. For example, the solubilization rate, with Candida sp. on naturally weathered Mississippi lignite was more effective than on unoxidized samples from the same coal seam.

Strandberg and Lewis (1987) reported that two strains of lignin-degrading bacteria (Streptomyces viridosporous T7A and S. setonii 75VÍ2) have the ability to solubilize low-rank coals dispersed on the agar culture surface. The S. viridosporous T7A did not produce any cell-free activity, while extracellular compounds with coal-solubilizing activity were produced. The results indicated that the extracellular components are not enzymes and could be basic polypeptides or polyamines.

(b) Objectives and Approach

The characteristics of the solubilization of coal by lignin-degrading bacteria appears to be different than those of lignin-degrading fungi. The observations from the previously mentioned studies suggest that the oxidation state of low-ranked coal plays an important factor on coal degradation by microorganisms. New functional groups or substances are probably produced after oxidation (naturally weathered or chemical oxidation) and these may provide either active sites for microbial attack or nutrients for growth. Among the coal samples for biosolubilization, naturally weathered low-ranked coals are effective substrates for fungal growth. Studies of natural weathering effects on coal will provide us some valuable information to identify the factors responsible for the increase in bioliquefaction rate. Furthermore, since the low-ranked coal structure is similar to lignin structure, the studies of degradation mechanisms on lignin by wood-rot fungi will also provide some important information about coal solubilization by the same organisms. The objective of this research is to collect and theoretically analyze the data from literature on coal bioliquefaction, lignin biodegradation and coal weathering, and to present further evidence to support proposed degradation mechanisms of weathered low-ranked coal by wood-rot fungi.

(c) Effects Of Weathering On Coal Properties

To study the reaction of low-rank coal, several studies have been made to elucidate its organic structure. Since coal is not a well-defined structure, investigators agree that it is too complex and heterogeneous a material to be usefully represented by a single, average structure. Various hypothetical models have been proposed for its structure to explain the nature, distribution and reactivity of the oxygen containing groups in low rank coals.

Low-ranked coals generally have higher moisture content and are more strongly hydrophilic in character than high-rank coals. This is due to the gradual elimination of polar groups (e.g. OH and COOH groups) during coalification. Table 2-1 shows the relationship between the functional groups and coal rank.

TABLE 2-1. Oxygen-containing groups in different coal structure (modified from Van Krevelen, 1962)

Coal Type	C	O(COOH)	O(OCH3)	O(OH)	O(C=O)	O(n)	O(S)
peat	60.0	5.1	0.9	12.3	11.6		29.9
leonardite (1) **	63.9	17.4	0.5	4.8	4.6	1.5*	28.4
brown coal	65.5	8.0	1.1	7.2	6.5		22.7
lignite	69.6	3.9	—	12.5	7.2		23.6
	71.7	5.1	0.4	7.8	9.3		22.6
(2) **	73.8	9.2	0.5	4.8	4.3	1.1*	19.6
(3) **	75.0	5.0	(12. hetero)		3.0		25.0
	75.9	1.6	0.3	7.5	7.4		16.8
hard coal	79.5	0.3	0	6.1	4.9		11.3
	80.2	1.0	0	8.3	3.1		12.4
	85.5	0	0	5.6	1.6		7.2
	87.0	0	0	3.1	1.7		4.8
	88.6	0	0	1.9	1.8		3.7
	90.3	0	0	0.5	2.0		2.5
	90.9	0	0	0.6	1.9		2.5

* Is assigned to indicate the total content of oxygen.
** (1) and (2) from Fowkes and Frost (1960), (3) from Davidson (19).

The above Table shows that oxygen-containing groups are more abundantly distributed in the low-rank coal structure versus high-rank coal. The principle groups are carboxylic and phenolic. Generally, low-rank coal aromatic structure consists of one-, two-, or three-ring fused systems, mostly one- or two-rings. Small aromatic clusters, high

concentration of oxygen-containing groups as well as high internal porosity (Sondreal et al., 1984) are unique features of low-rank coals; each of these features contributing to activity.

Since weathering can significantly enhance the rate of coal solubilization by microorganisms, it can be reasoned that the increase in oxidation state can positively affect coal biodegradation.

The conclusions from various studies of weathering of low-rank coal, are limited since few studies report the properties of low-rank coal. Furthermore, the chemical structural change induced by weathering was studied at different temperature and coal rank by the various researchers. Fortunately, Gethner (1987) has reported that coals (excluding anthracites) of various ranks appear to have the same oxidation mechanism. Thus, studying the oxidation mechanisms of different coal ranks can be useful in elucidating the oxidation reactions for low-rank coal. An object of our work is to draw a general conclusion about oxidation mechanisms of low-rank coal structure based on examinations of different coal ranks and oxidation temperature; and then to predict new oxidized compositions in low-rank coal structure.

The chemical properties and structure of most coals are extremely sensitive to natural weathering, with oxidation believed to be the principle cause of these changes (Gethner, 1987). Oxidation alters properties of the coal and increases some active sites available for microbial solubilization. Fisher and Lay (1989) review the literature for the studies of the oxidation of coal examined from natural weathering, to low and/or high temperature induced oxidation. The coal ranked from brown coal, lignites to bituminous coal. Focus is on the determination of functional group changes. Through a critical review of this literature we developed general conclusions, as summarized and discussed in later sections, possible reaction mechanisms are thus proposed for low-rank coal oxidation.

(d) Low-Temperature Oxidation Reaction

The oxidation state of coal is dependent on many factors, generally including coal rank, temperature, time and particle size. Low rank-coals have a high porosity and high concentration of functional groups which contribute to reactivity. They, therefore, oxidize significantly faster than high-rank coal (Wu et al., 1987). The principle variations are due to the different relative proportions of the reactive species (e.g. a-carbon) in the different coals. Fortunately, this statement strongly supports the premise of this section.

Gethner (1987) has proposed an overall reaction network of low-temperature oxidation which consists of three chemical reactions. Two of the reactions are oxidation reactions

while the third is a thermal decomposition reaction. These temperature-dependent reactions are briefly discussed as follows:

1. When the temperature is near and slightly above ambient, the first oxidation reaction will take place, (free-radical reaction). The investigation (Dack et al., 1983, 84) of low-rank coal (brown coal) by electron spin resonance revealed that at least two types of free radicals are present in the coal structure, i.e. organic free radicals and heteroatom free radicals. Retcofsky et al. (1968) reported that some element other than carbon or hydrogen, plays a part in the free radical species present in the low-rank coal, and oxygen-containing radicals, phenoxy radicals or those of the semiquinone type appear to be most probable. Aliphatic groups, especially a-carbons, are likely to be the active sites, forming free-radicals during exposure. These organic free radicals are stable to oxygen. Since the moisture in the coal structure is likely to be firmly bonded in the internal charge sites and prevents oxygen addition, a drying process can lead to a significant increase in free radical concentration. The formation of hydroperoxides may occur through the direct addition of oxygen on non-radical sites, or through the abstraction of a hydrogen by peroxy radical.

Low-rank coals have abundant oxygen-containing functional groups, (i.e. carboxylic acid, phenols or semiquinone structure). These functional groups contribute to the formation of the heteroatom free radicals. Organic free radicals always occur in aliphatic groups and form unstable radicals with oxygen, and lead to the significant loss of aliphatic structure in coal oxidation.

2. At temperatures between 25 °C to 100°C, the reaction is suspected to be a thermal decomposition reaction (Gethner (1987)). The thermolysis reaction is competitive with the low-temperature oxidation, since both of the reactions may involve reactions with the same species or proceed through a common intermediate. When the coal is exposed to oxygen at room temperature, the relative contributions of thermal decomposition and lower-temperature radical reactions will depend on the experimental conditions (e.g. moisture, time and temperature). Low heating will increase the contribution of thermal decomposition, leading to the loss of ketonie and carboxylic functional groups (decarbonylation and/or decarboxylation) in coal. The moisture in coal, which will move to active binding sites within the coal, may appear to become firmly attached to the sites and prevent oxygen addition or attack on the organic portion of the coal, consequently retarding the low-temperature oxidation. The principle chemical change that occurs during the thermal decomposition reaction is the conversion of carbonyl functional groups (probably most are aromatic carboxylic groups), and the subsequent formation of the various ether species (benzylic ethers are predominant). Referring to the results reported earlier, investigators have discovered that the significant difference between the naturally weathered coal and the high temperature oxidized or unoxidized coal is the ether linkage

formation. This result strongly supports Gethner's observation, although most investigators suggested that the formation of ethers is due to the condensation of hydroxyl or quinonic groups. Although ether groups are clearly present in most low-temperature or naturally oxidized coal, the mechanism is still not well known. Gethner (1987) reported that ether (benzyl ether) can be produced through the decarboxylation of an aromatic carboxylic acid or the decarbonylation of ketonie groups, but that the reactions are not obvious. Some investigators (Painter et al., 1980) believed that an ester (phenyl ester) can be formed in a similar manner, while others suggested that condensation reactions between adjacent hydroxyl groups led to ether cross-linkages and production of water. Some carboxylic groups can decompose into carbon radical and carbon dioxide.

3. Another oxidation can be observed when temperature is near 100°C. This principle oxidation reaction involves oxygen addition to a aliphatic carbon chain to produce thermally unstable hydroperoxides which subsequently decompose to form a variety of ketones, aldehydes and ester species, finally resulting in a net increase of carbonyl functional groups.

The systematic studies of three separate reactions of low-temperature oxidation (proposed by Gethner, 1987) will provide a better understanding about the reaction mechanisms of natural weathering, and also provide an insight into the formation of oxygen-containing functional groups upon oxidation.

(e) Aliphatic And Aromatic Structure

Aliphatic groups are more easily oxidized than aromatic groups in coal structure, resulting in the formation of hydroperoxides and subsequent decomposition to stable oxygen-containing groups. Aliphatic CH_2 groups are very susceptible to oxygen attach, and the initial oxidation is believed to occur at the α-carbon. Since investigators (Fuller et al., 1982) have reported that naturally weathered coal was oxidized in the same manner as under rather mild oxidation (about 150 °C), the content of aliphatic CH_3 groups (less reactive) in the outcrop coal is nearly the same as those in unoxidized coal (Teo et al., 1982). During weathering, aliphatic moieties seem to be oxidized and break into oxygen-containing groups or short chain aliphatic hydrocarbon. Although Jakab et al. (1988) have reported that benzene carboxylic acids could be formed upon oxidation (80-100°C), many investigators believed that aromatic groups are not easily affected by natural weathering, even when the oxidation temperature is high (200°C) (Kister et al, 1988).

(f) Oxygen-Containing Groups

Various oxygen-containing groups could be formed within the coal structure after natural weathering, and after the chemical/physical properties of the oxidized coal. Those functional groups include carboxylic groups, ketones, aldehydes, quinones, hydroxyl groups, esters and ethers, but the amount of each group is apparently different in different naturally weathered coal. The variables during natural oxidation (moisture content, temperature history and exposure time) as well as the coal properties will affect the formation of the oxygen-containing groups. Several important oxygen-containing groups formed upon natural weathering, are discussed as follows:

1. Carbonyl Group. Carbonyl functional groups are one of the predominant products during the natural weathering and/or temperature-induced oxidation, and are produced mostly through the oxidation of the aliphatic groups. The presence of carbonyl groups in naturally weathered coal suggests that the high-temperature oxidation reaction could take place under "natural oxidation" conditions. The carbonyl groups formed under natural oxidation include ketones (aryl-alkyl ketones), esters (aryl esters), aldehydes, carboxylates and carboxylic groups (aliphatic carboxyls).

2. Hydroxyl Group. Low-rank coals have higher hydroxyl group contents than high-rank coal, and contain about 1/2 total oxygen content in the low-rank coal.

The change in the amount of hydroxyl groups present under low-temperature or natural oxidation reported by many investigators are not consistent: (1) Some investigators reported that phenolic OH groups are not chemically altered at ambient-temperature oxidation (Liotta et al., 1983) or are not significantly formed under 350°C oxidation (Fuller et al., 1982); (2) other investigators (Albers et al., 1974) reported that hydroxyl groups are slightly increased during low-temperature oxidation, increase with increasing temperature and rank. (3) However, most of the studies reported an apparent loss of hydroxyl groups in the oxidation process. It is postulated that hydroxyl groups may undergo further oxidation to form quinones or undergo condensation reaction to produce ether linkages under low-temperature/natural oxidation resulting in the decrease in swelling properties in coal structure (Ignasiak et al., 1972; Jakab et al., 1988; Cronauer et al., 1983).

3. Ether Linkage. Ether linkages are the most specific functional groups formed during low-temperature or natural weathering. Perry et al. (1983), using XPS, have shown that the major oxygen-containing groups formed on the surface of an oxidized coal are singly-bonded C-O species (i.e. ether or hydroxyl groups).

Interestingly, Liotta et al. (1983) reported that all the added oxygen under low-temperature oxidation (about 20°C) eventually formed ether groups (which lead to the destruction of the plastic properties of the coal), and that no carbonyl groups were produced under these conditions.

Fuller et al. (1982) reported that the strongest difference in spectrum (IR 12 00 cm^{-1}) is between the outcrop coal and unoxidized coal from the same coal seam. This indicates the formation of aryl ether linkages during natural weathering.

Upon comparison of naturally weathered (20 and 60 °C) coal and high-temperature-induced (100 and 2 00°C) coal by Kister et al. (1988) , significantly different IR absorption spectrum (1200-1300 cm^{-1}) were obtained. The difference in spectrum is attributed to the formation of ethers, and indicates that different conditions lead to different mechanisms. We conclude that the ether formation mechanisms are as follows:

(1) Ether groups are produced from the combination of an alkoxy-radical (due to the cleavage of hydroperoxide under 2 0-60°C) with a carbon radical, and hydroxyl groups are not affected or involved in the reaction.

(2) The formation of ethers is due to decarbonylation, from the thermal decomposition reaction. The reaction mechanism is still not obvious, although the carbonyl groups of aldehydes could react with alcohols to yield acetals (an ether) in the presence of anhydrous acids.

(3) The condensation of hydroxyl groups is responsible for the ether formation. The hydroxyl groups involved in the reaction are inherent in the coal structure or are produced during the peroxide-radical rearrangement.

Most of the investigators believed that the formation of ether cross-linking is an important reaction that increases the size of coal molecules, leading to the destruction of plasticity, swelling property and the loss of the fluidity.

Finally, the results indicate that the ether groups formed in naturally weathered coal are primary aryl ether, including monoaryl ethers and benzylic ethers.

C. Conclusions

1. Weathering can significantly alter the low-rank coal, and make the coal structure more susceptible to degradation by microorganisms. Since the oxygen-containing groups are increased in the oxidized coal, the alternation of the functional groups should be responsible for the enhancement of coal biosolubilization.

2. Weathering is a oxygen-induced reaction, and increases oxygen-containing groups in the coal structure. These oxygen-containing groups include carboxylates, ketones, aldehydes, quinones, esters and ether linkage.

3. Natural weathering can effectively oxidize the aliphatic structure, carbon oxidation is believed to be the initial reaction during weathering.

4. Carbonylation of aliphatic structure caused by weathering can open up the coal structure, reduce its steric complexity making it more accessible to enzymatic attack.

5. The cleavage of oxidized aliphatic C-C bonds which connect the aromatic structure, is an important reaction for coal liquefaction by white-rot fungi. Other carbonyl groups formed are easily degraded by the fungi.

6. The increase of carboxylic acid contents in the biodegraded product of low-rank coal is due to the cleavage of aromatic rings to aliphatic acid fragments by fungi.

7. Natural weathering or low-temperature oxidation, especially at low heating and high moisture content, favors the condensation reaction of phenols or quinones and thermal decomposition reaction of carbonyl or carboxylic groups, leading to the formation of ether groups or some ester groups.

8. The splitting of ether linkages is an important reaction for lignin degradation by enzymes from wood-rot fungi, certain enzymes are believed to be responsible for the ether bond cleavage. Therefore, ether linkages which increase the cross-link density of the oxidized low-rank coal will also be cleaved easily by the enzymes during the coal degradation. The ester groups will react in the similar manner as ether linkages.

9. The fungal degradation of the oxidized low-rank coal can be considered as a further oxidation reaction.

10. Enzymes from white-rot fungi seem to cleave the bonds which are of less steric complexity, e.g. ether or ester C-O bonds and the C-C bonds near the carbonyl groups.

D. Recommendation

Because of the variation of the chemistry of the low-rank coal structure and the complexity of the enzyme systems during the coal degradation by microorganisms, we recommend use of simple aryl-alkyl compounds for modelling of the low-rank coal structure to simplify and further study the mechanisms of coal biodegradation related to the weathering effects.

Increase in degradation rate of model compounds by enzymes can be detected by a-carbon oxidation of the aryl-alkyl compounds to aryl-alkyl ketones. This technique needs to be substantiated.

Hydroxylation of aromatic rings in the model compounds is also recommended to test the enhancement of aromatic ring cleavage into aliphatic materials during degradation by white-rot fungi, in order to reduce the polycondensed aromatic concentration in digestion products.

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