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ANAEROBIC BIOPROCESSING OF LOW-RANK COALS
CONTRACT No. DE-AC22-90PC90051

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PROGRESS REPORT

January 1, March 31, 1992

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QUARTERLY PROGRESS REPORT

Anaerobic Processing of Low-rank Coals

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INTRODUCTION

The overall goal of this project is to find biological methods to remove carboxylic functionalities from low-rank coals and to assess the properties of the modified coal towards coal liquefaction. The main objectives for this quarter were : (i) continuation of microbial consortia development and maintenance, (ii) crude enzyme study using best decarboxylating organisms, (iii) decarboxylation of lignite, demineralized Wyodak coal and model polymers, and (iv) characterization of biotreated coals.

QUARTERLY PROGRESS SUMMARY

- Two batch fermentor systems containing 20 g of coal were completed and two other fermentors are under progress.
- 5% H₂ gas in the fermentor gas phase during bioprocessing increased the H/C content of coal, but overall CO₂ production was low in comparison to 100% N₂ gas atmosphere.
- Currently four adapted microbial consortia with decarboxylating capability are being subcultured/maintained.
- Decarboxylation experiments using lignite, demineralized coal and model polymers were initiated.
- Preliminary crude enzyme study showed that the decarboxylating organisms may have extracellular enzyme activity.

PROGRESS REPORT

I. Continue Coal Decarboxylation and Development of Microbial Consortia

1. Batch Fermentor Systems

Wyodak subbituminous coal was decarboxylated in the batch configuration system where CO₂ produced from the fermentor was continuously trapped in a concentrated alkali solution.

Parameters to operate different batch systems are given in Table 1. The trapped CO₂ in the alkali solution was released by acidifying the solution in a sealed tube and the evolved CO₂ in the gas phase was quantified using gas chromatograph. The profiles of CO₂ production in the

Table 1. Summarized culture conditions for batch fermentor systems (#10 - #14)

Batch No.	Gas phase	Coal particle size	Carbon and Nitrogen sources	Time (days)
#10	N ₂	100 mesh	0.2% Na.Succinate + 0.2% Yeast ext.	37
#11	N ₂ +H ₂ (95:5)	100	0.2% Na.Succinate + 0.2% Yeast ext.	37
#13	N ₂	100	0.4% Na.Lactate + 0.2% NH ₄ Cl	56
#14	N ₂	20	0.4% Na.Lactate + 0.2% NH ₄ Cl	56

batch fermentor system (#10, #11, #13 and #14) are shown in Figures 1 - 4, respectively. Biotreatment of coal under H₂ (5%) gas phase (Figure 2) significantly decreased total CO₂ production from coal in comparison to N₂ atmosphere (Figure 1). These results indicate that H₂, to some extent, may inhibit some organisms in the microbial consortium. When the biotreated coals were analyzed for the CHN content (Table 2), the H/C ratio of the biotreated coal under N₂ (fermentor #10) and under H₂ (fermentor #11) was found to be increased by 4.3% and 4.9%, respectively. These results confirm our previous observation (quarterly report, December, 1991), that bioprocessing of coal under H₂ gas phase may result in a coal product with slightly higher H/C ratio.

Table 2. Elemental analysis of biotreated coals

Coal	% C	% H	% N
Control	66.88 ± 0.30	4.50 ± 0.09	0.94 ± 0.00
Fermentor #10	66.90 ± 0.11	4.69 ± 0.06	1.07 ± 0.01
Fermentor #11	66.61 ± 0.35	4.70 ± 0.06	1.11 ± 0.07

In another experiment, the newly developed consortium LC4 was inoculated in batch #13 and #14 systems containing coals of different particle sizes (100 mesh vs. 20 mesh). The consortium LC was developed by successive enrichment of rumen inoculum on sodium lactate as carbon source and NH_4Cl as nitrogen source. 100 mesh coal contained in #13 fermentor from Argonne Premium Coal Sample Program, whereas 20 mesh Wyodak coal contained in #14 fermentor was obtained from Penn State Coal Bank Program. Figures 3 and 4 exhibits the differences in CO_2 production from these coals. Total CO_2 production on day 42 was about 25% higher in batch #14 with 20 mesh coal (39.5 mmoles) than in fermentor #13 with 100 mesh coal (31.4 mmoles). However, CO_2 production is still increasing on day 42 in batch #13, but it has slowed down in batch #14. These batches will be terminated on day 56 and characterization of biotreated coals will be followed.

2. Maintenance of Microbial Consortia

Currently, four adapted microbial consortia are being maintained in anaerobic vials for coal decarboxylation experiments. These consortia are being subcultured in different media as shown in Table 3 and have shown distinct coal decarboxylation profiles. Each consortium is subcultured at every 6 week interval. Old microbial consortia have been preserved at 4 °C. Typical profiles of gas production from four microbial consortia in the presence and absence of coal are presented in Figure 5. Consortium LC exhibited highest net gas production from coal followed by consortia RW and RN. However, consortium RN was not used further due to limited total gas production. Consortia LC and L are being subcultured in the presence and absence of coal, respectively, to examine if the coal decarboxylation ability of the microbial consortia will be lost. Highest net gas production from coal was usually observed between 3 and 4 weeks of biotreatment period.

Table 3. Current status of adapted microbial consortia

Consortium	Original inoculum	Carbon and Nitrogen Source	# of Subculture Made
RW	Rumen(R2) + Waste digester(#1B)	0.4% Sodium succinate + 0.2% Yeast Extract	9
#34	From #3 and #4 batch fermentors	Same as RW	5
L and LC*	Rumen 991	0.4% Sodium lactate + 0.2% NH_4Cl	5
RWNH	RW7-5C	0.4% Sodium succinate + 0.05% Yeast Extract + 0.2% NH_4Cl	4

*Consortia L and LC are maintained in the absence and presence of coal, respectively.

II. Decarboxylation of Lignite and Penn State Coal

1. Beulah Lignite

One of the important questions to ask is whether these microbial consortia developed with Wyodak coal will be equally effective in removing oxygen from other coals. For this purpose, Beulah lignite was obtained from Argonne Premium Coal Sample Program. Microbial consortium RW9, which was developed and adapted on Wyodak subbituminous coal was examined for its ability to remove oxygen from lignite. The experiment was conducted in anaerobic serum vials. The results at the end of 4 weeks have shown that the consortium RW9 was effective in removing oxygen as CO₂ from lignite. The biotreated lignite coal will be characterized to determine using FT-IR and titration methods if the CO₂ produced was from carboxyl groups.

2. Penn State Wyodak Coal (20 mesh)

We have used a 100 mesh Wyodak coal (Argonne Premium Coal Sample Program) to develop the microbial consortia and to examine their decarboxylation potential. It may, however, be important to examine the utility of these microbial consortia on coals with larger particle size. For this purpose a 20 mesh Wyodak coal was obtained from Penn State Coal Bank. Penn State Wyodak coal was examined for coal decarboxylation using microbial consortia #34 and LC in anaerobic vials (Figure 6). The gas production profiles from Penn State Wyodak coal was almost identical to that obtained from Wyodak coal (100 mesh; Argonne Coal Sample Program) when inoculated with the same microbial consortium. This suggests that our adapted microbial consortia can decarboxylate Wyodak coals even if the particle size is larger than 100 mesh.

III. Decarboxylation of Modified Coal and Polymers

1. Demineralized Wyodak Coal

A small amount of demineralized (low calcium) Wyodak subbituminous coal was obtained from Dr. Michael Serio (Advanced Fuel Research, East Hartford, CT) to examine the efficiency of microbial consortium for removal of carboxyl groups which are no longer held by calcium cations. The vials were inoculated separately with microbial consortia RW9 and LC5. The experiment is under progress.

2. Model Polymers

In order to better understand coal decarboxylation mechanism(s), it is necessary to use model compounds with carboxyl groups which have physicochemical properties similar to coal. Two model polymers, poly (acrylic acid) and poly (methyl methacrylate), were procured to examine the decarboxylation ability of the microbial consortia. Preliminary results indicate that CO₂ produced from each polymer varied depending on microbial consortium, indicating that the microbial consortium may have different decarboxylase specificity for different polymers. Currently, poly (methyl methacrylate) is being partially hydrolyzed to provide different amounts of carboxyl groups. On termination of decarboxylation, biotreated polymers

will be analyzed to determine the removal of carboxyl groups using FT-IR method.

Poly (methyl methacrylate)

Polyacrylic acid

IV. Crude Enzyme Study with Decarboxylating Organisms

1. Cultures Grown in the Absence of Coal:

Two selected cultures, Veillonella alcalescens and culture #8, were grown in the absence of coal in their respective media for crude decarboxylase study. Cell-free broth of each culture and washed cells were prepared anaerobically to conduct the enzyme study. Data presented in Table 4 indicate that culture #8 cell-free broth showed higher CO₂ production, while V. alcalescens did not produce higher gas from coal than control. On the other hand, washed cells exhibited very low enzyme activity with little increase in CO₂ in the presence of coal. These preliminary results are not conclusive, but adapted culture, i.e., culture #8, seems to have extracellular coal decarboxylase activity.

2. Culture Grown in the Presence of Coal

To examine the localization of enzyme(s) responsible for decarboxylation of coal, the culture #8 was grown in the presence of 5% Wyodak coal. Using this culture, three preparations were made to examine the possible nature of the enzyme, i.e., extracellular, cell-bound, and membrane bound or intracellular. The three preparations were: cell-free broth (I), cell-free broth (II) and washed cells. The cell-free broth (I) was obtained by centrifuging the culture broth containing coal followed by filtration of the supernatant through a 0.22 μ millipore filter. Cell-free broth (II) was obtained by suspending the pellet containing cells and coal in phosphate buffer (pH 7) and shaking it for 5 min to release cell bound enzymes(s). The mixture was centrifuged and the supernatant passed through the 0.22 μ millipore filter to obtain a cell-free broth (II) preparation. The remaining cell pellet was considered as washed cells preparation.

Table 4. Decarboxylase activities in cell-free broth and washed cells prepared from V. alcalescens and culture #8.

Organism	Enzyme Mixture	% CO ₂ produced on 16 hr	
		<u>Total</u>	<u>Net*</u>
<u>V. alcalescens</u>	Control coal	0.11	
	Cell-free broth	2.49	
	Cell-free broth + coal	2.10	-0.50
	Washed cells	0.07	
	Washed cells + coal	0.23	0.05
Culture #8	Control coal	0.10	
	Cell-free broth	3.39	
	Cell-free broth + coal	4.54	1.04
	Washed cells	0.0	
	Washed cells + coal	0.11	0.01

* Net CO₂ was calculated by subtracting control.

Coal decarboxylation was examined in the presence of these three enzyme preparations in anaerobic tubes. After an incubation for 16 hr, the gas phase analysis in comparison to control (no coal) showed net CO₂ production only in tubes containing cell-free broth (I). This observation indicates that the enzyme responsible for production of CO₂ from coal may be extracellular in nature. To confirm this observation further, this experiment will be repeated. Also, a large amount of cell biomass will be produced to examine the presence of intracellular decarboxylase, if any.

Table 5. Decarboxylase activities in cell-free broth (I), cell-free broth (II) and washed cells prepared from culture #8

Enzyme Mixture	%CO ₂ produced on 16 hr	
	<u>Total</u>	<u>Net*</u>
Control coal	0.33	
Cell-free broth (I)	1.98	
Cell-free broth (I) + coal	2.86	+0.54
Cell-free broth (II)	0.51	
Cell-free broth (II) + coal	0.70	-0.14
Washed cells	0.14	
Washed cells + coal	0.29	-0.18

*Net CO₂ was calculated by subtracting control.

SIGNIFICANT ACHIEVEMENTS

1. A slight increase in H/C ratio due to biotreatment of coal under H₂ gas phase confirmed.
2. A 4.3%-4.9% increase in H/C content of biotreated coal as a result of removal of oxygen from coal demonstrated.
3. Efficiency of the microbial consortia to effectively remove oxygen from Wyodak coal of particle size larger than 100 mesh demonstrated.
4. The nature of the decarboxylase present in the microbial consortia decarboxylating coal may be extracellular in nature.
5. The effectiveness in removing oxygen from lignite by microbial consortium developed on Wyodak coal observed.

OBJECTIVES FOR NEXT QUARTER

1. Complete evaluation of known cultures for their decarboxylation potential.
2. Complete selection of a culture with a high decarboxylating ability.
3. Complete evaluation of crude enzyme from best culture for coal decarboxylation.
4. Demonstrate decarboxylating ability of developed microbial consortium through use of modified coals and polymers.
5. Continue characterization of biotreated coal.

Figure 1. Profiles of carbon dioxide production from #10 batch fermentor system under nitrogen gas phase

Figure 2. Profiles of carbon dioxide production from #11 batch fermentor system under 5% hydrogen gas phase

Figure 3. Profiles of carbon dioxide production from #13 batch fermentor system containing 100 mesh Wyodak coal

Figure 4. Profiles of carbon dioxide production from #14 batch fermentor system containing 20 mesh Wyodak coal

Figure 5. Gas production profiles from Wyodak coal by four adapted microbial consortia in vials

—◆— (-) coal
 —□— (+) coal
 - - -▲- - - Net gas

Figure 6. Gas production profiles from 20 mesh coal by two adapted microbial consortia in vials

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