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Biological Gasification of Coals

Final Report

March 1990

Work Performed Under Contract No.: DE-AC21-87MC23285

For
U.S. Department of Energy
Office of Fossil Energy
Morgantown Energy Technology Center
Morgantown, West Virginia

By
ARCTECH, Inc.
Alexandria, Virginia

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P.O. Box 880
Morgantown, West Virginia 26507-0880

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FOREWORD

This research project entitled "Biological Gasification of Coals" was performed under the sponsorship of Morgantown Energy Technology Center of the U.S. Department of Energy. ARCTECH is developing this novel approach for bioconversion of coals to methane under a joint agreement with Houston Lighting and Power Co. Support for basic research to develop this technology is also provided by the Electric Power Research Institute and Gas Research Institute.

ARCTECH acknowledges the continuous guidance and encouragement of Dr. Hsiao-Ya L. Lai, Technical Project Manager, of Morgantown Energy Technology Center.

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EXECUTIVE SUMMARY

This report describes the work performed on the project entitled "Biological Gasification of Coal" under DOE/METC Contract No. DE-AC21-87MC23285. The goal of this project was to develop a novel approach of utilizing microorganisms to convert low-rank coals into methane gas. Two approaches for biological conversion of coals to methane were evaluated. In the first approach, indirect bioconversion, coal is first depolymerized into low molecular weight compounds (chemical or aerobic biological treatment) followed by anaerobic conversion of these compounds to methane. The second approach, direct bioconversion, the coal was treated under anaerobic conditions to produce methane. This report presents the details of experimental procedures and the results obtained from these studies. Preliminary economic analysis of a potential biological gasification scheme was also performed to establish major cost components and recommendations for future research to develop an economical biological gasification process are presented.

Non-biological degradation of a number of coals was also evaluated. In some cases, a combination of chemical/thermal treatments was used to obtain maximum coal degradation. The chemicals used included different concentrations of nitric acid, hydrogen peroxide, sodium and potassium hydroxides, sodium and potassium carbonates, sodium bicarbonate and potassium phosphate. Several lignite subbituminous and bituminous samples were subjected to chemical/thermal degradation. Optical density (OD) measurements of liquid products at 450 nm using a spectrophotometer were used as a relative measurement of coal degradation. The wavelength selection was based on scans of control medium and medium after chemical/thermal (or biological) coal degradation had taken place. Absorption was good at this wavelength and appeared to eliminate some of the interferences observed at other wavelengths. Although single-ring aromatic compounds absorb at a lower wavelength, it was anticipated that products from coal degradation would include the humic acids and polyaromatic compounds cleaved from the coal structure rather than only the simplest aromatic compounds.

In preliminary studies, samples of Beulah lignite and Wyodak subbituminous were first treated with 0.5 to 8N HNO, and then treated with 1N NaOH followed by autoclaving. Both Wyodak and Beulah coals treated with 6 and 8N HNO, yielded

high concentrations of solubilized products. Whereas a bituminous treated with 8N HNO_3 was extremely resistant to chemical degradation and yielded an OD of 4 in comparison to 680 and 512 for Beulah and Wyodak samples respectively under similar treatment conditions. A combination of treatments using HNO_3, H_2O_2, NaOH-KOH and autoclaving did not soften Illinois #6 or Illinois #2 coal samples. The relative concentrations of degradation products for the bituminous were 100-250 times lower than the concentration of Beulah products when treated under similar conditions. These results indicate the recalcitrant nature of most bituminous samples to degradation.

Chemical depolymerization of both lignite and subbituminous was demonstrated when the coals were pretreated with 20% H_2O_2 followed by alkali (1N NaOH)/thermal treatments. Among the coals tested, a Beulah lignite was found to yield the highest concentration of water soluble products using this treatmen . Treatments with potassium hydroxide, sodium bicarbonate, sodium and potassium arbonates and potassium phosphate yielded significantly less chemical degradation products. Samples of bituminous were completely recalcitrant to all chemical/thermal degradation treatments evaluated in this study.

Experiments dealing with biological degradation of coal indicate that the use of microorganisms may be advantageous for degradation of coal in lieu of chemical depolymerization. Several aerobic and anaerobic microbial consortia have been found to degrade low-rank coals. Leonardite, a highly oxidized North Dakota lignite, was biologically degraded faster than many other lignite and/or subbituminous samples. The microbial cultures evaluated for biodepolymerizing activity were obtained from activated sludge, primary digestor sludge, horse manure-hay compost, and a consortium of bacteria and fungi isolated from coal-pile compost samples (CP1+2). The consortium, CP1+2, obtained from the ARCTECH culture collection depolymerized lignites very rapidly and appears to be an excellent candidate for use in future biological depolymerization of coal(s). This consortium depolymerized 40-70% of selected coals (MAF basis) within 48 hours. Repeated biodegradation using fresh cultures of CP1+2 for treatment of the residual coal resulted in additional depolymerization, although most of the coal substrate available for biological depolymerization had been depolymerized in the first treatment.

A yellow fungus, developed at ARCTECH, also degraded a North Dakota lignite very rapidly, particularly when the coal was added to 5-7 day pregrown cultures. In most cases, coals were pretreated with 20% H_2O_2 prior to bacterial degradation. However, the North Dakota lignite was biologically (aerobically) converted without any pretreatment. Interestingly, this culture did not depolymerize a Texas lignite, indicating biological substrate specificity. Microbial depolymerization of a North Dakota lignite using whole cells and the extracellular medium (cell-free) derived from this aerobic fungi was also compared. The whole cells and the culture supernatant (not autoclaved) showed higher depolymerization activity than the heat-killed (autoclaved cell-free) samples. The biodepolymerization activities obtained from whole cell and suspension samples was comparable, indicating that most of the biological products responsible for coal depolymerization are extra-cellular products and could be harvested easily.

Anaerobic treatment of a North Dakota lignite and a Texas lignite resulted in biodegradation of the coals to water soluble products. The rates of biodepolymerization, under this system, were not determined as these soluble products were rapidly converted to methane.

Both aerobic and anaerobic cultures were capable of growing with water soluble and/or acid precipitable products obtained from chemical/thermal degradation of coal. Microbial degradation products were also obtained and lyophilized. These coal degradation products were used as substrates for both aerobic and anaerobic bacteria. Several aerobic bacteria grew well in the presence of up to 5% of Beulah and Wyodak degradation products obtained from H_2O_2/NaOH/thermal treatments. However, the aqueous soluble products obtained after nitric acid pretreatment were toxic to microbial cultures, therefore, this method of chemical degradation of coal was abandoned. Microbially depolymerized coal products did not inhibit bacterial growth.

The biologically derived soluble coal products appeared to be preferred substrates for CH_4 production when compared to chemically derived soluble coal products as a biological substrate for methane production. Results from these experiments as well as THF solubilities determined for each of the different

products indicate that the products obtained from biological and chemical treatments are different.

Microbial cultures obtained from primary digestor sludge, leaf litter compost, chicken waste, ovine and bovine rumen fluids and horse manure-hay compost have demonstrated direct conversion of low-rank coals (i.e. untreated North Dakota lignite and Texas lignite) to methane. Studies evaluating adaptation of various anaerobic consortia to untreated Texas lignite and North Dakota lignite clearly show that adapted consortia can be used for direct conversion of coal carbon to CH_4. Total gas produced in reaction mixtures was measured using a syringe displacement method. The production of CH_4, H_2, and CO, were quantitated using gas chromatography.

Preliminary data analysis on methane production from an untreated Leonardite (North Dakota lignite), using a direct anaerobic conversion process, indicate that anaerobic bacteria converted up to 10% of the total coal carbon to methane during the initial testing period. The gas phase contained methane concentrations as high as 30 mole%. Although these anaerobic cultures were adapted for a short period of time, the culture may have potential for higher coal conversion after further adaptation. It is anticipated that culture enhancement and media manipulation will result in significantly increased coal carbon conversions.

Anaerobic bacteria derived from termite guts converted more than 68% of Texas lignite coal carbon to acetate and other short chain fatty acids presumed to be major precursors of methanogenesis. As it is estimated that more than 80% of the CH_4 formed in nature comes from acetate, these methanogenic precursors could be readily converted to CH_4 using known acetate-degrading and methane-forming bacteria such as *Methanothrix* sp. and *Methanosarcina* sp. Other acids and alcohols produced from coal or soluble coal products can also be converted to CH_4 using appropriate microbial consortia. These preliminary data clearly indicate that bacterial conversion of coal to CH_4 can taken place in both one stage or two stage culture systems.

Methane inhibition studies were conducted to identify the intermediary compounds produced from coal during biodegradation of the coal carbon to CH_4.

BESA (2-bromoethanesulfonic acid) and monensin inhibited methane production leading to the accumulation of various short chain acids and alcohols (intermediary metabolic products). Acetate and ethanol were the major compounds identified during the biodegradation of coal to methane. In addition, benzoic acid was identified as an intermediate in coal degradation during these studies. The short chain acids and alcohols were quantitated using gas chromatography. Bioconversion of these intermediates to methane was demonstrated using adapted cultures and, in some instances, the addition of known methanogenic microbial populations to ARCTECH's adapted cultures was required to enhance methane production. Addition of known H_2-CO_2 utilizing methanogens to reaction mixtures in which headspace gas composition showed large concentrations of CO_2 resulted in decreased CO_2 concentration and increased methane concentration.

The technical feasibility for bioconversion of untreated lignites (Leonardite and Texas lignite) to methane has been demonstrated. Preliminary data also indicate that efficient biological conversion of Beulah lignite and Wyodak subbituminous may be possible, although the best microbial consortia for conversion of these coals have not been identified. Direct conversion of Texas lignite by an adapted microbial consortia resulted in overall coal carbon conversions of greater than 70%. Direct conversion of coal carbon to methane ranged from 35% to as much as 50%.

The original concepts for biological coal conversion envisioned the use of a one-stage (direct bioconversion) or two-stage bioconversion process. Investigations into pretreatment of coals and subsequent carbon conversion to soluble coal products using aerobic biological systems resulted in relatively low recovery of parent carbon as soluble carbon products. Calculations showed that 100% conversion of these soluble coal-derived products to methane would result in overall parent coal carbon conversions of less than 50%. Based on these findings, the focus of the biogasification project became the direct anaerobic biomethanation of selected coals. Anaerobic consortia capable of directly biodegrading selected coals were identified and specific consortia/coal adaptations carried out. Consolidation of cultures and addition of methanogenic cultures to achieve maximum coal carbon conversions were subsequently performed.

Adaptation of cultures to specific coal(s) for bioconversion resulted in enhanced methane production. A two to three fold increase in methane production was observed following adaptation of a sewage sludge consortium with Leonardite and a termite consortium with Texas lignite. The addition of methanogens to cultures which methane precursors accumulated resulted in maximum conversion of coal carbon to methane. This manipulation increased methane production by an adapted culture by as much as 50%.

Continuous methane production in a bench-scale bioreactor was also evaluated. Bioreactor (rotating biological contactor) data indicate that accumulation of large quantities of biomass and conservation of this biomass will be of critical importance in a biogasification process. Production of a good biofilm on media discs within the rotating biological contactor appeared to result in high biomass concentration and conservation of the biomass over time. It is likely that the high biomass concentration in this bioreactor resulted in the production of methane in a reaction mixture containing 5% coal solids (w/v), the highest coal solids loading successfully used in these studies. It was anticipated that the use of up-flow fluidized bed reactors would also facilitate the accumulation and conservation of large concentrations of biomass while using long solids retention times and relatively short liquid retention times. Unfortunately, the up-flow fluidized bed experiments were not successful with respect to methane production.

Preliminary process design schematics and economic analyses for a biological biomethanation plant have been developed based on data accumulated during these laboratory and bench-scale studies. Based on these preliminary cost calculations using a best case scenario, the cost for bioconversion of lignite to methane falls in the range of $2.66 to $4.05/million Btu.

Major accomplishments of this project are summarized below:

- Bioproduction of soluble organic products from Leonardite occurred without pretreatment of the coal.
- Aerobic microorganisms depolymerized low-rank coals to water-soluble, acid-precipitable products of relatively low-molecular weight.

- The ARCTECH proprietary culture, CP1+2, was the most successful of the biological coal treatments.
- Coals pretreated with nitric acid were unsuitable for bioconversion to methane.
- Biological rather than chemical depolymerization products were preferred substrates for methane production.
- Short chain acids and alcohols were identified as intermediate products during bioconversion of coal to methane.
- Acetic acid and ethanol were the major compounds identified as intermediates when methane inhibitor studies were conducted using various coals and various microbial consortia.
- Direct bioconversion of Leonardite, Beulah lignite, Wyodak subbituminous and Texas lignite to methane has been demonstrated.
- Consortia derived from numerous anoxic environments have demonstrated biomethanation of low-rank coals.
- Coal/microbial cultures specificity with respect to bioconversion of coals to methane was conclusively demonstrated.
- Adaptation of selected consortia with specific low-rank coals has resulted in significantly enhanced methane production.
- Addition of specific methanogenic populations (acetate cleaving, H_2-CO_2 utilizing) results in increased methane production when intermediate products accumulate during bioconversion of coals.
- Overall coal carbon conversions (CH_4, CO_2, organic acids and alcohols) in excess of 70% have been demonstrated.
- Coal-carbon conversions to methane of up to 50% have been demonstrated.
- A rotating biological contactor was successfully used as a bioreactor for continuous production of methane over more than two months of operation.
- Methane concentration in the headspace gas of the RBC reached 83 mole%.
- Coal solids loading of 5% (w/v) was successfully used for biomethanation of Texas lignite in the RBC reactor.

The development of an economically acceptable, efficient process for conversion of coals to environmentally acceptable fuel forms would be a technological breakthrough for the United States in its quest for energy independence coupled with the maintenance of environmental quality and provision of jobs in the depressed coal mining areas of the country. Future research will address key areas to enhance the kinetics of methanogenesis and overall carbon conversion to methane from low-rank coals using microorganisms developed by ARCTECH using an innovative dual reactor system with above ground and underground

bioreactors. The focus of the future research should be characterization and optimization of the microbial consortia to achieve maximum methane production from two coals; enhancement of coal/bacterial interactions to achieve maximum methane production; and enhancement of the kinetics for each of the process steps (biomass production, substrate utilization, intermediate(s) production, carbon conversions and methane production). Data generated will provide the basis for future bioreactor design and construction.

The ultimate goal of the biogasification research is to develop an efficient, economically attractive biological process for conversion of low-rank coals to methane and to demonstrate this process at pilot-scale. Innovative concepts such as a dual reactor system to ensure greater process stability and control, ash removal to minimize wastes, a fixed film or fixed bed approach to fermentations to conserve biomass and allow for short liquid retention times, and the proposed use of above ground and underground reactors in process designs will be evaluated for commercial application as a coal gasification technology.

I. INTRODUCTION

Coal deposits within the United States are extensive. Significant portions of this fuel source are undesirable for combustion because of low energy content and/or contaminants found in varying concentrations in coals. The sulfur content of coals and, to a lesser extent, the nitrogen content have been linked to the acid rain problem prevalent in certain areas of the world. The use of such coal deposits as a feedstock for the production of a clean fuel has much appeal both as a native fuel supply and as the precursor for an environmentally acceptable fuel form. Interest in such coal conversions has been increasing in the fuel industry as a whole and in electric utilities in particular.

Although liquefaction processes can produce clean fuels from coal, these processes require extremes in pressure and temperature which render them unattractive from an economic standpoint. As an alternative, biological conversions of coal may have potential because of the mild and economically favorable process conditions generally associated with biological systems. Bioconversion of coal has been of interest for some time; however, only recently has biological depolymerization of coal by microorganisms been demonstrated. Many researchers (Cohen and Gabrielle, 1982; Scott et al., 1986a, b; Ward, 1985; Wyza et al., 1987; Yan, 1986) have isolated and studied microorganisms capable of degrading lignite coals to water soluble products.

Biological degradation of coal results in water soluble products of varying molecular weight under conditions of neutral or alkaline pH. In general, very slow degradation rates have been observed with natural samples of low-rank coals, the exception being a highly oxidized North Dakota II lignite (Scott et al., 1986b). Pretreatment of lignites and a subbituminous have resulted in increased biodepolymerization rates (Strandberg and Lewis, personal communication; Scott et al., 1986b, Wyza et al., 1987). Such pretreatments allow for more rapid generation of products for characterization and testing.

The major objective of this research project was to evaluate biological processes to demonstrate microbial conversion of low-rank coals to methane. ARCTECH has developed several microbial cultures which can degrade low-rank coals or depolymerized coal products (biological or chemical) to methane. Several

methanogenic precursors such as short chain acids and alcohols were also produced during bioconversion of coal to methane. The data presented in this report indicate that microbial cultures from natural sources can be developed for economical coal conversion to methane.

II. SELECTION OF COALS AND MICROORGANISMS

A. Selection and Preparation of Coals

Several coals of different ranks (lignite, subbituminous and bituminous) were selected for use in this research project. The list of the coals and their characteristics are reported in Tables 1 and 2. The coals were pulverized in a hammer mill to a nominal particle size of 100 mesh X 0. Pulverized coal was air dried before use in the experiments. Lignite and subbituminous samples were used in most of the experiments because these coals are more susceptible to microbial attack than bituminous. Several samples of bituminous were tested to compare the extent of depolymerization with other coals. The research was initiated to determine whether these coals, or water soluble coal-derived products, could be used as substrates for a biogasification process.

B. Coal Analyses

Leonardite, Beulah lignite and Wyodak subbituminous as well as their chemically and biologically depolymerized coal products were analyzed for ash, moisture, Btu, carbon, hydrogen, nitrogen, and total sulfur content under another project entitled "Biochemical Bond Breaking of Coal" (PETC, Contract No. DE-FG22-86PC90913). Significant changes occurred in the ash, nitrogen, and total sulfur contents after either chemical or chemical/biological treatments (Table 2). The ash and total sulfur contents of the soluble products were reduced compared to concentrations present in the parent coals. The nitrogen content of the coal products obtained from the CP1+2 treatment was increased except in the case of Wyodak. This increased nitrogen content was probably due to the presence of biologically produced proteins and/or ammonia.

C. Selection of Microorganisms

It was postulated that optimal biological degradation of coals to methane would require a multi-step process (Figure 1) involving a combination of aerobic and anaerobic bacteria. As a first step, several aerobic microorganisms were selected for use as biological agents for coal depolymerization. Microorganisms developed at ARCTECH were used to determine the rates of depolymerization of

Table 1. Type and Rank of Coal Used in Biogasification Project

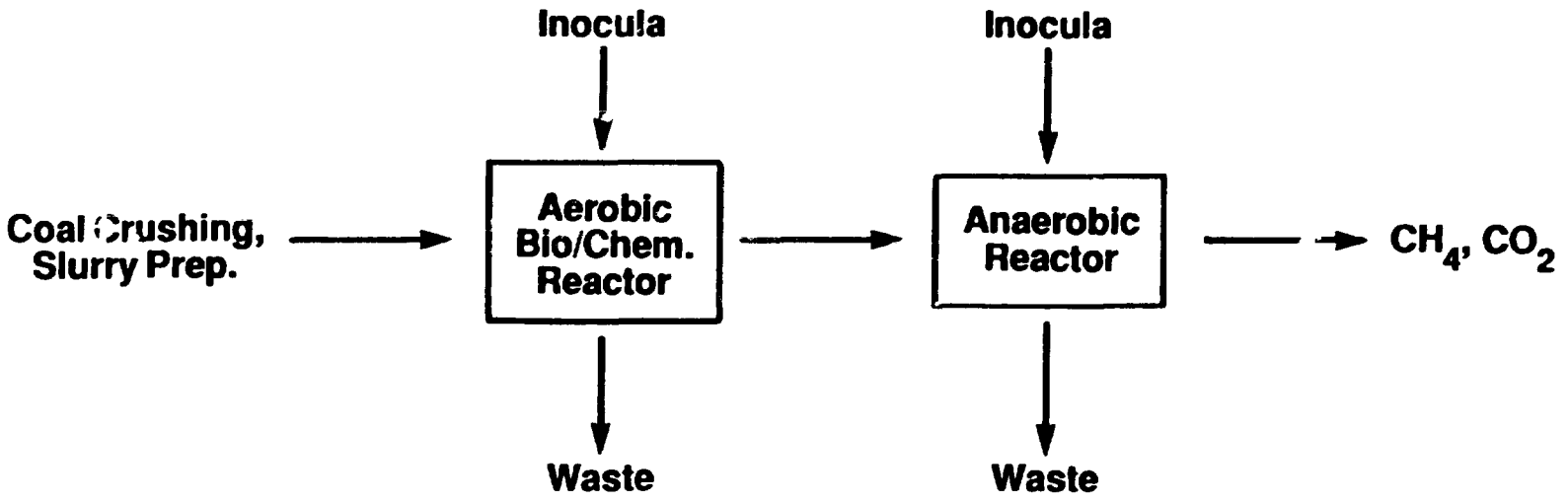
Coal	Rank
Beulah, North Dakota	Lignite
Leonardite, North Dakota	Lignite
San Miguel	Lignite
Spring Creek	Lignite
Texas (TX)	Lignite
AMAX fines	Subbituminous
Wyodak	Subbituminous
Ames	Bituminous
Illinois #2	Bituminous
Kentucky #9	Bituminous
Ohio	Bituminous
Peabody	Bituminous
Wellmore	Bituminous

Table 2. Characterization of Coals and Coal Depolymerization Products*

Coal & Rank	Sample	Analysis (% wt) Ash	Moisture	Btu	C	H	THF N	TS
Leonardite Lignite	Parent coal	22.1	11.0	9969	52.85	4.09	1.13	1.74
	CP1+2 depolymerized	4.4	4.0	8722	51.92	3.04	3.07	0.75
Beulah Lignite	Parent coal	11.93	15.67	9880	62.09	4.04	1.04	1.30
	CP1+2 depolymerized	1.35	4.12	9084	60.56	4.30	3.05	0.51
Wyodak Subbituminous	Parent coal	11.68	19.88	N.D.	63.13	4.68	1.01	1.25
	CP1+2 depolymerized	1.21	6.46	9248	57.74	3.57	0.63	0.59
Texas Lignite	Parent coal	11.07	21.02	10694	49.69	3.58	1.03	0.93

*Some analyses were performed under a separate research program entitled "Biochemical Bond Breaking of Coal" funded by PETC, Contract No. DE-FG22-86PC90913.

Scheme 1: Indirect Biogasification



Scheme 2: Direct Biogasification

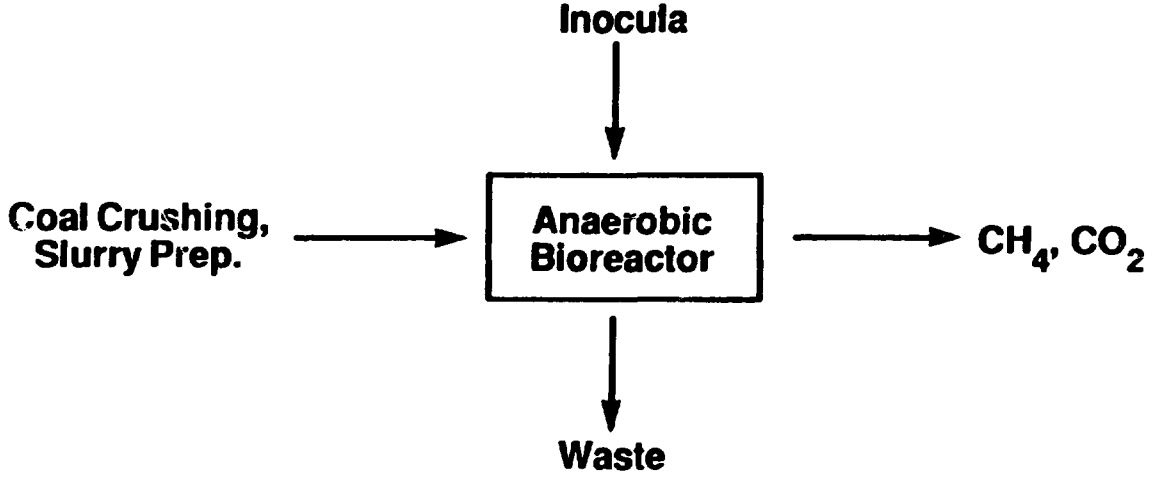


Fig.1 Bioconversion Flow Schemes

several low-rank coals. A mixed culture, CP1+2, and a mixture of yellow fungi were obtained from the ARCTECH culture collection for use in these experiments. Following aerobic depolymerization of coal, the water soluble products were collected and used as a feedstock for anaerobic bacterial cultures. The depolymerized products were used directly in the aqueous medium or were harvested and lyophilized for future use. The lyophilized products were reconstituted with water and tested as substrates for biogasification.

To determine whether direct anaerobic coal biodegradation to methane could be achieved, anaerobic cultures were incubated with untreated coal in later experiments. Anaerobic cultures are very versatile and are known to grow on a variety of substrates, including aromatic compounds and lignin derivatives. Therefore, direct conversion of coal or coal products to methane was studied using anaerobic bacteria obtained from natural sources. Samples from an anaerobic sewage digestor, Eastern shore mud samples, leaf litter compost, horse manure-hay compost, bovine and ovine rumen samples, and chicken waste were collected, carefully sealed and transported to the laboratory. The samples were then anaerobically inoculated into basal salts medium containing coal or coal depolymerized products. The details of the methods for preparing the anaerobic medium, inoculation, and transfer of cultures are described in Appendix 1 and Appendix 2.

III. RESULTS AND DISCUSSION

A. Coal Depolymerization

The major objective of this task was to break the coal matrix with conversion to water soluble products containing high molecular weight compounds. This process was demonstrated using both chemical and biological methods. The procedures used for chemical and biological depolymerization of coal are discussed separately.

1. Chemical Depolymerization

The primary objective of this subtask was to determine the extent of coal depolymerization using chemical processes. This was achieved by subjecting pulverized coal to high temperature and pressure and/or using a combination of chemical treatments. The best methods for the depolymerization of each of the coals were determined.

A variety of chemicals were tested to determine suitable agents for coal depolymerization. Chemicals were used to solubilize the coals to create water soluble products which were subsequently evaluated as substrates for further microbial conversions to methane or other chemicals. In some cases, the coals were subjected to chemical/thermal treatments to obtain maximum depolymerization. A list of various chemical treatments evaluated is presented in Appendix 3.

All chemical treatments were conducted using 10 ml chemical per gram of coal. The chemicals were added to the untreated, or pretreated coals, and incubated for 1 hour at room temperature. After 1 hour, the reaction mixture was autoclaved at 121°C (15 psi pressure) for 15 minutes. The reaction mixtures were then centrifuged for 10 minutes and both the residual coal and the supernatant were recovered for further experiments. The supernatants after centrifugation were read at OD_{450} to determine the extent of coal depolymerization. The optical density measurements of the supernatants at 450 nm using a spectrophotometer were used as a relative measurement of coal depolymerization by the chemical reagents. The wavelength determinations were

based on the scans of control medium and medium after chemical/thermal (or biological) coal degradation had taken place. Absorption was good at this wavelength and the use of this wavelength appeared to eliminate some of the interferences inherent to other wavelengths used to monitor aromatic compounds. Although mono-aromatics absorb at a lower wavelength, it was anticipated that the products from coal degradation would include the humic acids and polyaromatic compounds cleaved from the coal structure rather than only the simplest aromatic compounds. Thus, monitoring of the aqueous soluble products using this wavelength was used to obtain relative coal depolymerization data.

A variety of chemicals, including hydroxides, carbonates and bicarbonates, were used for pretreatment of the coals. Pretreatment of coals using sodium hydroxide, potassium hydroxide, a mixture of sodium and potassium hydroxides and hydrogen peroxide has been evaluated. Data on chemical depolymerization of several lignites (Tables 3 and 4) indicate that pretreatment with sodium hydroxide yielded good depolymerization of coal. Pretreatment of Beulah lignite with 20% H_2O_2 followed by thermal treatment resulted in the best OD_{450} readings, indicating the presence of more soluble compounds in the supernatant.

Several other concentrations of sodium and potassium carbonate were tested in order to find an optimum concentration of these chemicals for use in coal depolymerization. Samples of Beulah lignite, Wyodak subbituminous and bituminous (1 g each) were placed with 0.1 to 0.5 g of sodium or potassium carbonate in 10 ml water and allowed to react for 1 hour. The bituminous was pretreated with 8N HNO_3. The lignite and subbituminous samples were not pretreated. The coals were autoclaved at 121°C (15 psi) for 20 minutes. The reaction mixtures were then centrifuged at 12,000 rpm for 12 minutes and the OD_{450} of each supernatant was determined. Data from these studies show that the presence of 0.2 g sodium carbonate or 0.4 g potassium carbonate yielded the best results for Beulah and Wyodak coals (Table 5). Bituminous was highly resistant to chemical depolymerization but the presence of 0.4 g sodium or potassium carbonates yielded the best results. Bituminous was completely recalcitrant to chemical depolymerization unless acid pretreatment was performed.

Table 3. Chemical Depolymerization of Coals

Sample	Treatment	OD_{430}
San Miguel	1N NaOH	310
	2N NaOH	260
	20% H_2O_2	234
	15% H_2O_2	86
Spring Creek	1N NaOH	145
	2N NaOH	260
	20% H_2O_2	198
Beulah	1N NaOH	192
	2N NaOH	150
	20% H_2O_2	264

Additional testing of the Beulah lignite was performed and data from these experiments are presented in Table 4.

Table 4. Chemical Pretreatment of Beulah Lignite

Treatment	OD_{430}
1N NaOH	120, 196
1N NaOH, $NaHCO_3$, ethanol	28, 17
1N NaOH/KOH	82, 200
1N NaOH/KOH, $NaHCO_3$, Etoh	70, 76
1N KOH	80, 2
1N KOH, $NaHCO_3$, Etoh	9, 44
15% H_2O_2	100
30% H_2O_2	17

Table 5. Chemical Depolymerization of Three Coals with Different Concentrations of Carbonates Followed by Heat Treatment

Carbonates, g/l	g Coal	OD_{∞} Beulah lignite	Wyodak subbituminous	Wellmore* bituminous
Na_2CO_3	0.1	78	26	---
	0.2	178	54	7.5
	0.4	136	46	9.5
	0.5	120	42	8.0
K_2CO_3	0.1	32	10	---
	0.2	96	32	7
	0.4	144	44	9
	0.5	156	42	8.5

* Previously treated with 8N HNO_3. Untreated Wellmore coal did not yield any depolymerized products.

In another experiment, Beulah lignite and Wyodak subbituminous were first treated with 0.5 to 8N HNO_3 and then treated with 1N sodium hydroxide. After acid pretreatment the coals were washed with distilled water several times until a neutral pH was obtained and were then resuspended in 10 ml of 1N sodium hydroxide. After a 1 hour incubation at room temperature, the samples were autoclaved at 121°C, 15 psi for 20 minutes, centrifuged and the supernatant OD_{400} determined. Beulah lignite treated with 8N HNO_3 and Wyodak subbituminous treated with 6N HNO_3 yielded higher concentrations of depolymerized products (Table 6). The bituminous sample treated with 8N HNO_3 was extremely resistant to chemical depolymerization and yielded an OD_{400} of 4 in comparison to OD_{400} readings of 680 and 512 for Beulah and Wyodak samples respectively, under similar conditions.

Pretreatment with sodium carbonate resulted in good depolymerization of lignite coals. Tests were performed to evaluate this pretreatment followed by thermal treatment. Data from these experiments indicate that the presence of both sodium and potassium carbonate could depolymerize Beulah lignite but had little effect on subbituminous or bituminous (Table 7) samples. Several other bituminous samples were treated with 1N HNO_3 and then treated with 1N sodium or potassium hydroxide. Methods similar to those described above were followed. The results (Table 8) indicate that all four of the bituminous samples were resistant to chemical depolymerization. Chemical treatment of the Ames coal yielded the best results with an OD_{400} of 10.75 and 11.5 following treatment with sodium and potassium hydroxides, respectively.

In another experiment, the coals were first pretreated with 20% H_2O_2 and then with 1N sodium or potassium hydroxide, followed by heat treatment. This method increased coal depolymerization slightly. Chemical treatment of the Ames coal yielded the best results (Table 9). Approximately a three fold increase in OD_{400} was observed following treatment of the coal with H_2O_2, hydroxide and heat compared to depolymerization of the coal using the HNO_3 method.

The results from these studies clearly indicate that H_2O_2 pretreatment rather than nitric acid is a preferred chemical pretreatment for coals. Coals pretreated with 20% H_2O_2 and subjected to depolymerization using 1N NaOH yielded the maximum depolymerized products. These products were found to be suitable substrates for microbial conversion to methane.

Table 6. Depolymerization of Coal Pretreated with Different Concentrations of Nitric Acid Followed by 1N Sodium Hydroxide/Thermal Treatment

Nitric Acid Conc. (N)	OD_{450} Beulah lignite	OD_{450} Wyodak subbituminous
0.5	112	---
1	144	---
2	140	98
4	320	272
6	640	580
8*	680	512

* Similar treatments with Wellmore bituminous yielded an OD_{450} of 4.

Table 7. Depolymerization of Coals in the Presence of Sodium and Potassium Carbonate

Coals	OD_{450} Na_2CO_3 1 g	Na_2CO_3 1.5 g	K_2CO_3 1 g	K_2CO_3 1.5 g
Beulah lignite	62	58	88	46
	.5 g	1 g	.5 g	1 g
Beulah lignite	108	61	136	80
Wyodak subbituminous	37	22	48	24
Illinois #2	0.9	0.42	0.75	0.65

Table 8. Depolymerization of Coals Pretreated with 1N HNO, Followed by 1N Sodium or Potassium Hydroxide/Thermal Treatment

	OD_{450}	
	NaOH	KOH
Kentucky #9	0.8	0.7
Ohio #8	0.8	0.72
AMAX, Illinois #6	1.82	1.2
AMES	10.82	11.5

Table 9. Depolymerization of Coals Pretreated with Hydrogen Peroxide Followed by 1N Sodium or Potassium Hydroxide/Thermal Treatment

	OD_{450}	
	NaOH	KOH
Kentucky #9	4.3	5.5
Ohio #8	3.3	2.5
AMAX, Illinois #6	6.5	5.5
AMES	31.0	33.0

2. Biological Depolymerization

The use of microorganisms offers advantages for the depolymerization of coal over chemical depolymerization methods. The major advantage, as determined by our research, is that the biological depolymerization products do not create any inhibition and/or toxicity problems and could serve as substrates for both aerobic and anaerobic bacteria. Another advantage is that some of the coals can be depolymerized and converted to valuable products using a single stage reactor system under anaerobic environments, whereas, separate reactors are required for chemical depolymerization and subsequent biological conversion to methane.

Pretreatment of most of the coals, except a North Dakota lignite, was required before their biological depolymerization. Nitric acid (1-8N) and hydrogen peroxide (H_2O_2 at 10-20%) were used as the chemical reagents. A few drops of ethanol were added to evenly wet the coal and the mixture was gently agitated using magnetic stirrers, or by occasional swirling. All of the procedures were carried out in a chemical fume hood. An exothermal reaction which occurs during this process usually varies in intensity with different coals and the concentration of chemicals used. Generally coals were reacted for 1 hour when nitric acid was used as the pretreatment chemical. However, the time of reaction ranged from 1 to 2 hours when H_2O_2 was used. When the reaction was over, the reaction mixtures were centrifuged at 3000 rpm for 5 minutes. The residual coals were washed several times with distilled water, or until a near neutral pH was obtained. The coals were air dried, or dried in an oven at 80°C, and stored at room temperature for future experimental use.

Although nitric acid treatment removes much of the pyritic sulfur and may leach metals from the coals, it has many disadvantages. It is believed that the treatment adds nitrogen to the coal and results in products which are toxic to bacterial growth. The recovery of coal after nitric acid treatment was more than 95% by weight. No attempt was made to measure the soluble products obtained after nitric acid treatments as these products were unsuitable for bacterial growth. The coals treated with nitric acid were washed thoroughly (using hot water or dilute mineral salts solution buffered at pH 7.0), air dried, and stored for use in future experiments.

Chemical softening using H_2O_2 was the best pretreatment for most coals used in this project. Coals pretreated with H_2O_2 were also found to yield more depolymerization products when treated with alkali or carbonates. Experiments with different concentrations of hydrogen peroxide and solids loadings indicated that 20% hydrogen peroxide and 30% coal solids loading were suitable conditions for coal pretreatment. An experiment was conducted to determine the percent recovery of coal after 20% hydrogen peroxide treatment at 30% solids loading using different coals. One hundred grams of Leonardite, Amax, Beulah lignite and Wyodak subbituminous samples were weighed and treated with 20% hydrogen peroxide. The reaction times varied between 1 and 2 hours, depending on the coal. After the reaction was complete, the contents of each flask were centrifuged at 5000 rpm for 10 minutes. The residual coal was carefully collected, dried in an oven and weighed. Results showed that Leonardite was the most reactive of the coals treated (Table 10) and that the Wyodak subbituminous was the least reactive. Only 46% of the Leonardite coal was recovered following the hydrogen peroxide treatment, indicating that more than 50% of the coal was depolymerized to water soluble products and CO_2 during H_2O_2 treatment. The total mass recoveries of Amax, Beulah and Wyodak coals were 53, 55 and 60% respectively.

From this data, it appears that a large portion of low-rank coals is depolymerized or lost as CO_2, during the pretreatment process. Thus, chemical pretreatment of coals, unless absolutely necessary, should be avoided.

Aerobic and anaerobic bacterial cultures were grown with various coals to determine coal depolymerization activities. Total coal recovery and % coal depolymerization were used as the criteria to calculate the extent of coal depolymerization. A mixed culture, CP1+2, developed earlier at ARCTECH, was used to solubilize most of the lignites and one subbituminous sample. Previous studies with CP1+2 at ARCTECH indicated that the coal biodepolymerization is dependent on culture medium, culture age, pH, coal particle size and coal pretreatment (Biochemical Bond Breaking of Coal Project, PETC, Contract No. DE-FG22-86PC90913).

A yellow fungal culture, isolated and developed under this research contract, also showed depolymerization activity with several lignites. The

Table 10. Recovery of Coals after Chemical Pretreatment*

Coals	Before Treatment (g)	After Treatment (g)
Leonardite	100	46.0
Amax	100	52.7
Beulah lignite	100	54.8
Wyodak subbituminous	100	60.1

* Coals pretreated with 20% hydrogen peroxide at 30% solids loading.

yellow fungal culture was grown in Yeast Malt Broth (YMB). Experiments were also conducted using the yellow fungal culture to determine the parameters influencing coal depolymerization activity.

Other cultures, derived from activated sludge and a primary digestor, were also evaluated for coal depolymerization. Anaerobic cultures obtained from various sources were grown in the presence of lignites but anaerobic coal depolymerization activity could not be calculated, as the depolymerized products were rapidly metabolized to other products, such as alcohols, acids and methane.

To evaluate bacterial depolymerization of a variety of coals, CP1+2 was grown in Trypticase Soy Broth (TSB) at pH 8.0, for 7-10 days. Experiments conducted under another ARCTECH project, Biochemical Bond Breaking of Coal, indicated that a 7-10 day old culture exhibits maximum depolymerization of coal activity. Before using the culture in the experiments, an aliquot of 10 ml of culture was taken in triplicate, placed in preweighed aluminum boats and dried in an oven at 80°C overnight. The dry weight of the bacterial cells was recorded until a constant cell weight was obtained. Total dry cell weight added to the coals was calculated based on 0.014 g dry biomass per ml of culture. Total coal recovery and total amount of depolymerized coal products were determined.

a. CP1+2 Biodepolymerization of Coal

A 20% hydrogen peroxide pretreated coal (100 g) was weighed into 2 liter pre-sterilized Erlenmeyer flasks in triplicate. Leonardite was not pretreated with hydrogen peroxide before biological treatment. Two hundred ml of bacterial culture (2.82 g dry cell weight) were added to the coal and the mixtures were incubated on a shaker table at room temperature for 48 hours. Aliquots of samples collected at different time periods were centrifuged and OD_{∞} of the supernatant was determined using a spectrophotometer for measurement of coal biodegradation. After 48 hours, the contents of the flasks were centrifuged at 10,000 rpm for 15 minutes. The coal and the supernatant were carefully collected. The coal containing most of the bacterial biomass was dried in an oven. The dry weight of the coal was measured until a constant weight was obtained. The cell dry weight was deducted from the total residual coal dry weight to calculate the percent recovery. The supernatant, containing coal

depolymerization products, was frozen in a preweighed container and lyophilized. After lyophilization, the containers were weighed to determine product recovery from the coal. Maximum depolymerization occurred with Amax fine coal. More than 68% of the Amax coal was depolymerized by CP1+2. The corresponding product recoveries for Wyodak subbituminous, Leonardite and Beulah lignite were 43, 47, and 35% respectively. However, it should be mentioned that Leonardite did not receive any pretreatment, therefore a yield of more than 45% as product can be considered to be good depolymerization. The recovery of product and residual coal was nearly 100%, indicating that methods established for product recovery were good. These products were used for growth of anaerobic bacteria for conversion to methane. The experiments with other coals were performed using methods similar to those described above.

The residual (unreacted) coal recovered after the first biological depolymerization was treated again with CP1+2 to determine whether more depolymerized products can be derived from this coal using CP1+2. Residual coal and product recoveries were calculated at the end of the experiments. Aliquots of each reaction mixture were taken at different time intervals and OD_{450} was determined after centrifugation to separate particulate coal and bacterial cells from the product containing aqueous fraction. Little additional water soluble products were obtained during this second biological treatment (Figure 2) indicating that CP1+2 was not capable of further depolymerization of this coal under these conditions.

Several lignite and subbituminous samples were used to monitor the biological depolymerization of coals using CP1+2. The mixed culture depolymerized Leonardite and Beulah lignites by 41 and 30% on a MAF (moisture-ash free) basis. The maximum depolymerization was observed when Amax fine coal was used. These data were compared to the % depolymerization using chemical-thermal treatment (Table 11). Results from this experiment indicate that higher rank coals were more amenable to biological treatment with decreased depolymerization activity when coals were treated using chemical processes.

In another experiment, a pretreated (nitric acid or 10-30% H_2O_2) lignite was used to monitor biodegradation of the coal using a mixed CP1+2 culture. Following the pretreatment, each coal was washed several times and the

Fig.2 BIOCONVERSION OF BEULAH LIGNITE COAL USING CP1+2

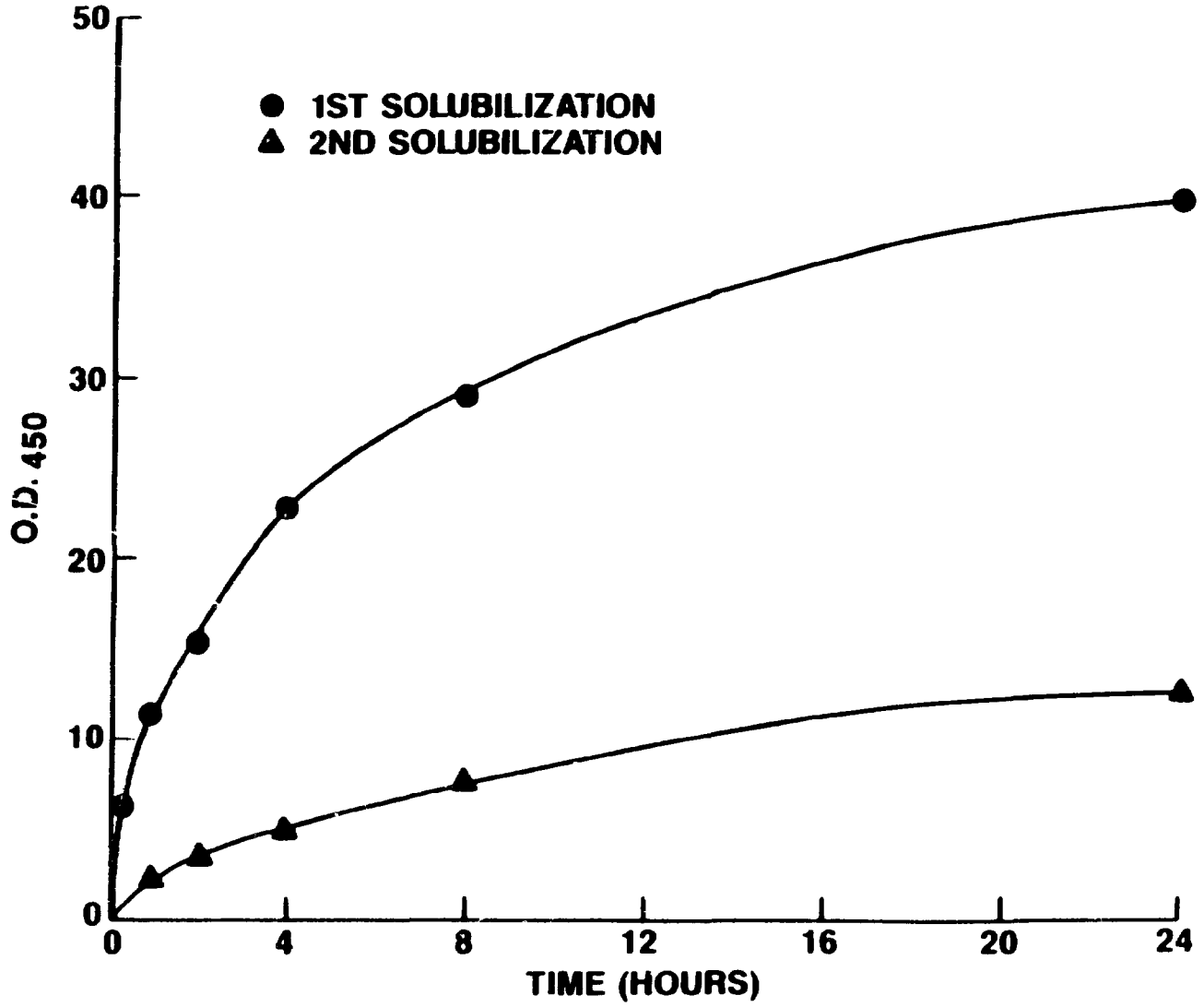


Table 11. Depolymerization of Coals Using Chemical and Biological Treatments

Coals	% Depolymerization (MAF)	
	Chemical	Biological **
Leonardite	57.8	41.0
Beulah'	43.3	30.0
Wyodak'	30.0	39.0
Amax fines'	25.9	60.0

L, Lignite; S, Subbituminous

* Coals treated with 20% H_2O_2 - NaOH/Thermal.
** Coals treated with 20% H_2O_2 - CP1+2 Culture.

Leonardite coal was not treated.

residual coal (softened product) was incubated with a fully grown culture of CP1+2 for evaluation of biological degradation of the coal. After a 12 hour incubation period, 1 ml of the coal/microbial culture mixture was removed, centrifuged to separate the particulates from the aqueous phase, and OD_{∞} of the supernatant fraction was determined. Microbial treatment of the coal softened with nitric acid before biological degradation, resulted in a higher yield of liquid products than the coal softened with hydrogen peroxide (Table 12). Biological treatment of 10% H_2O_2 pretreated coal appeared to yield more biological degradation products than treatment using 30% hydrogen peroxide.

Coal depolymerization using CP1+2 appears to be a very effective means of obtaining water soluble products. These products can be further bioconverted to methane precursors or methane under anaerobic conditions.

b. Depolymerization of Coal Pretreated with Acetic Acid by CP1+2

In this experiment, Beulah lignite was treated with various concentrations of acetic acid followed by treatment with 10% hydrogen peroxide. After several water washings, the residual coal was incubated with the mixed culture, CP1+2, with agitation at room temperature. At the end of a one hour incubation, 1 ml reaction mixture was removed, centrifuged to remove particulates and the OD_{∞} of the supernatants was determined. Results indicate that biodepolymerization of coal pretreated with 5% acetic acid gave good product formation (Table 13) and the product yield was approximately four times as great as that determined when the coal was not pretreated with acetic acid. It appears that pretreatment with acetic acid followed by H_2O_2 treatment creates a Beulah lignite more readily convertible to depolymerized products using CP1+2 microbial cultures.

c. Depolymerization of Coals Pretreated with H_2O_2 by CP1+2 and Its Subcultures

Two coals, Texas and Beulah lignites, were pulverized and ground to a D_{∞} of 200 mesh. The ground coal samples (750 grams of each) were pretreated with 20% H_2O_2 (30% solids loading). After pretreatment, the solids

Table 12. Microbial Degradation of a Lignite Pretreated with HNO_3 and H_2O_2

	OD_{450}	pH
1N HNO_3	184	7.0
4N HNO_3	231	7.0
10% H_2O_2	96	8.0
30% H_2O_2	11	8.0

Table 13. Microbial Degradation of a Beulah Lignite Pretreated with Various Concentrations of Acetic Acid Followed by 10% H_2O_2

% Acetic Acid	OD_{450}
0	2.6
2.5	7.6
5.0	9.8
10.0	10.3

were collected and washed free of H_2O_2 by means of pressure filtration. ARCTECH's mixed culture, CP1+2, capable of degrading several low-rank coals to water soluble products and two subcultures derived from this consortium were inoculated into 100 ml of trypticase soy broth (TSB) and monitored daily over a two week period for depolymerization capability and culture pH.

The relative depolymerization capability was evaluated by determining the presence of aromatic compounds as measured by the samples' optical density at 450 nm. To measure the O.D., 0.05 grams of coal were added to 1 ml of each culture and incubated overnight at room temperature. The samples were then centrifuged, and the supernatant diluted. The OD_{450} of each diluted supernatant was measured. Results showed no significant differences in depolymerization between the CP1+2 consortium and its two subcultures. All three cultures achieved a maximal pH of 9.3 on day 12 and maximal OD_{450} of 25 on day 11 (Figure 3).

Ultimate analyses were performed on raw Beulah lignite, the hydrogen peroxide pretreated Beulah product and on Beulah lignite which was first pretreated with hydrogen peroxide, biodepolymerized with CP1+2 and then acid-precipitated to separate the coal product from any media residual. The results are shown in Tables 14 and 15. Each step in coal pretreatment removes more carbon, thus reducing the amount of methane that could be produced from the coal. The oxygen content, calculated by difference, increases with each successive step. These results indicate that an optimized biogasification process must be a direct bioconversion using untreated coal; however, the use of soluble coal products provides a useful modelling technique for these studies.

d. Fungal Biomass

Bioconversion of a North Dakota lignite using fungi was investigated. A mixture of yellow fungi isolated at ARCTECH laboratories was grown in malt extract, yeast malt (YM) broth and TSB for one week. An aliquot of 5 ml from each of these cultures was then inoculated into 100 ml of identical medium containing 1% untreated North Dakota lignite. The pH of the medium was not adjusted and the flasks were incubated under shaking conditions at room temperature. After five days of incubation, 1 ml of the culture medium was

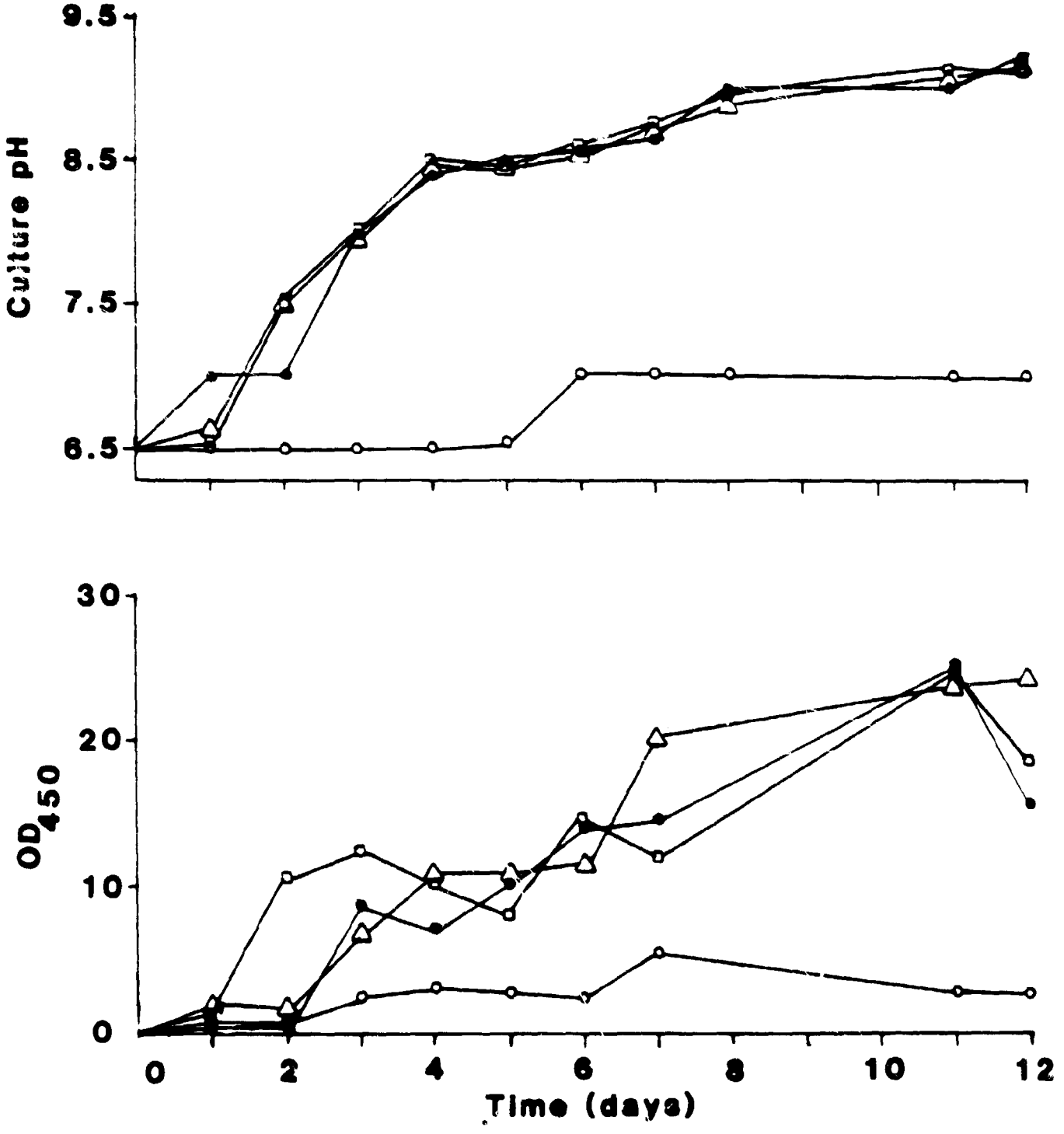


Figure 3. Culture pH and solubilization capability of CP1+2 consortium and two CP1+2-derived subcultures versus time.

Legend: ○ TSB control
□ CP1+2
△ MY (CP1+2 subculture
● MY+1 (CP1+2 subculture)

Table 14. Effects of Pretreatment Steps on Constituents of Beulah Lignite

% Dry Basis	Untreated	H_2O_2 Treated	CP1+2 Treated
Carbon	62.95	57.85	45.06
Hydrogen	3.98	3.57	4.13
Nitrogen	0.99	0.92	2.97
Sulfur	0.55	0.67	0.54
Ash	9.64	9.82	4.57
Oxygen (by difference)	21.89	27.17	42.73
Total	100.00	100.00	100.00

Table 15. Loss of Coal During Pretreatment Steps

	Texas Lignite (g)	Lab Beulah (g)	Premium Beulah (g)
Parent Coal	723	750	115
H_2O_2	497	317	83
CP1+2 Treated	300	192	37
Percentage Lost	59%	74%	68%

removed, centrifuged and the OD_{450} of the supernatant was determined. The results indicated that biodegradation of coal occurred only when the culture was grown in YM (a medium specific for fungal growth) broth (Table 16). Although the fungus grew in both malt extract broth and TSB medium, the coal was not degraded by the fungi in these medium as indicated by the OD_{450}. The YM medium maintained an approximate pH of 6 during growth of the fungus. Results from previous experiments have shown that chemical degradation of low-rank coals is minimal at this low pH, thus the conversion of coal may be completely attributed to the activity of the fungi.

Two untreated coals, a Texas (TX) lignite and a North Dakota (ND) lignite, were used in fungal biodegradation. A mixture of yellow fungi isolated at ARCTECH was grown in 100 ml yeast malt medium under static conditions at 30°C. After 7 days of growth, 1 gram of Texas lignite or North Dakota lignite was added to a pregrown culture for incubation at room temperature with agitation. At specific intervals, 1 ml of the culture medium was removed, centrifuged and the OD_{450} of the supernatant was determined. The results indicated that the yellow fungus produced water soluble compounds from North Dakota lignite coal (Table 17); however, the Texas lignite was resistant to biodegradation by this fungi.

After 5 days of incubation, when the maximum depolymerization had occurred, an additional amount of coal (1 g per 100 ml) was added to each of these cultures to determine whether the depolymerization activities were lost or if the substrate availability was limiting. The results (Table 18) indicate that the cultures were capable of enzymatic (depolymerization) activities when additional substrate was added. As expected, after another 4 days of incubation, the concentration of microbial product(s) from North Dakota lignite had doubled. Texas lignite did not yield any products during either of the reaction periods monitored. The pH of the medium remained within a 6.5 to 7.0 range and experience from previous experiments suggests that pH did not play a significant role in depolymerization of North Dakota lignite.

To determine the optimal coal concentration for maximum biodepolymerization, various concentrations of Leonardite were separately added to 100 ml of culture (seven days old) of the yellow fungus. The contents were

Table 16. Medium Effects on Degradation of a Lignite by Aerobic Fungi

	Malt Extract[1]	YM Broth[2]	TSB[3]
Medium Control	0.5	2.0	2.0
Fungal Cultures	0.5	9.4	2.5

[1] pH 4.5 - For control and culture

[2] pH 6.0

[3] pH 6.5

Table 17. Microbial Degradation of North Dakota and Texas Lignites Using a Mixture of Aerobic Fungi (Yellow Fungus)

Coal	pH	OD_{450} 0 d	5 d	10 d
TX lignite	6.5	0.05	0.1	0.2
ND lignite	6.5	0.50	22.0	24.0
Control TX lignite	6.5	0	0.1	0.2
Control ND lignite	6.5	0	1.0	2.3

Table 18. Microbial Degradation of Lignite Following a Second Coal Addition Using a Mixed Aerobic Fungal Culture (Yellow Fungus)

Coal	pH	OD_{450} 0 d	4 d	8 d
TX lignite	7.5	0.1*	0.4	0.5
ND lignite	7.5	22.0*	54.0	63.0
Control TX lignite	6.5	0	0.05	0.2
Control ND lignite	6.5	0	1.2	2.0

* OD_{450} of 5 day fungal lignite depolymerization experiment. Coal addition to the appropriate flask for subsequent microbial degradation of the coal.

gently shaken at room temperature. The fungus was grown in YM medium as described earlier. Aliquots were taken periodically, centrifuged and the OD_{430} of the supernatant was determined. The results (Table 19) indicate that good microbial depolymerization was obtained when the concentration of coal ranged from 1-4%. The concentration of microbial degradation products from the reaction mixture containing 5% coal was significantly lower than the quantity of products obtained at 1-4% coal concentration based on grams of coal treated. At zero day, however, samples containing 4% coal showed more depolymerization, perhaps as the result of slightly higher pH than the other samples. Maximum depolymerization based on the gram weight of coal in the reaction mixture was obtained when coal was treated at the 1% level.

Based on these results, biodepolymerization of these coals may be attributed to the activity of the fungi. It appears that the enzyme(s) responsible for the coal depolymerization activity using the yellow fungal culture may be constitutive; however, they are produced only when the fungi are grown in YM broth.

e. Culture Combinations

To investigate the potential for enhancing the biodepolymerization activity, the yellow fungal culture and CP1+2 cultures were grown together in YM broth under static conditions at 30°C. After 7 days of growth, both Texas lignite and North Dakota lignite (1g per 100 ml) were added to separate flasks which were incubated at room temperature under stirred conditions. An aliquot of 1 ml of culture medium was removed at specific intervals, centrifuged and the OD_{430} of the supernatant was determined. Neither the Texas lignite nor the North Dakota lignite was rapidly biodegraded (Table 20). After several days, the North Dakota lignite was degraded to water soluble products as indicated by the increase in the OD_{430}. As reported earlier, both CP1+2 and the yellow fungus as separate cultures degrade North Dakota lignite very rapidly. The anticipated synergistic effect of their enzyme systems was not demonstrated as evidenced by the decreased activity when the combined culture was used to solubilize coals.

Table 19. Microbial Degradation of an Untreated Leonardite Incubated with a Yellow Fungus

	OD_{450}		
Coal Concentration (%)	0 d	7 d	12 d
1	2.2	21.6	33.0
2	3.8	26.4	42.0
4	7.4	24.8	38.0
5	3.7	17.2	32.0

Table 20. Microbial Degradation of North Dakota and Texas Lignites Using a Pregrown Culture of CP1+2 and Yellow Fungus in a YM Broth

		OD_{450}		
Coal	pH	0 d	7 d	11 d
TX lignite	7.0	0.4	0.4	0.4
ND lignite	7.0	0.8	9.3	19.4
Control TX lignite	6.5	0	0.2	0.4
Control ND lignite	6.5	0	1.8	2.6

f. Fungal Enzyme

Depolymerization of coal using the extracellular enzyme (and/or coal solubilizing factor) from the yellow fungal culture was studied. An experiment was conducted using the yellow fungus grown in YM broth for one week at 30°C. The contents of one flask containing 100 ml culture broth, were aseptically divided into three equal parts. Two parts were centrifuged at 12,000 rpm for 15 minutes to remove the whole cells. The supernatants (cell-free medium) were collected separately and one portion was autoclaved at 121°C for 15 minutes. An untreated, water washed-Leonardite coal was weighed and added at 1% (w/v) level to each of the three samples (whole culture-not centrifuged, supernatant-boiled and supernatant-not boiled) contained in 100 ml Erlenmeyer flasks. The flasks were then incubated on a shaker table at room temperature. Aliquots of reaction mixture were removed at appropriate time intervals, centrifuged, and the OD_{430} of the supernatant was determined. The results indicated that the whole cells and the supernatant which was not autoclaved showed higher depolymerization activity than the heat killed (boiled) sample (Table 21). Initially, the whole cells showed higher activity than the cell-free extract but at the end of seven days both these samples showed identical depolymerization as indicated by OD_{430} readings. The heat killed sample showed increasing degradation activity with time. Microbes present in the coal itself could account for this delayed activity. These results indicate that the biologically produced factor responsible for coal depolymerization is primarily extracellular and could be harvested easily. The initial rate of extracellular depolymerization activity appears to be much slower than that of the whole cell reaction mixture.

g. Coal Pretreatment with Chlorine Bleach

Chlorine bleach (5.25% sodium hypochlorite) was evaluated as a possible substitute for 20% hydrogen peroxide pretreatment. Bleach would provide an economical means of pretreatment, can be stored at room temperature and is readily available. Bleach concentrations of 5, 10, 50 and 100% were compared to 20% hydrogen peroxide and a distilled water control as coal oxidants. Visual observation and heat generation indicated that oxidation reactions occurred with a bleach concentration as low as 50%.

Table 21. Microbial Degradation of an Untreated Leonardite Incubated with a Yellow Fungus

Sample	OD_{450} 0 d	3 d	7 d
Whole Culture	0.4	8.8	20.0
Supernatant - Not Boiled	0.4	6.4	20.0
Supernatant - Boiled	0.5	4.8	15.0

The bleach pretreated Beulah lignite samples were washed twice with 250 ml of deionized water and centrifuged. They were oven-dried for several hours and then stored at room temperature. The bleach pretreated coals were subjected to CP1+2 treatment and NaOH/heat treatment to compare chemical depolymerization to biodepolymerization. The CP1+2 treatment was carried out as follows: 0.5 grams of each pretreated coal or the untreated coal was added to 10 ml of CP1+2 culture in a 50 ml screw cap tube. The tubes were incubated overnight on a shaker, centrifuged and the OD_{450} determined. For chemical depolymerization, 0.5 grams of each coal was added to 5 ml of 2N NaOH and allowed to stand one hour at room temperature followed by autoclaving for 15 minutes. The results are presented in Table 22. Clearly, the optimal depolymerization (chemical or biological) was achieved with the hydrogen peroxide pretreated coal. Pretreatment with 100% bleach increased subsequent chemical depolymerization of the coal. Pretreatment with 50% bleach resulted in relatively low CP1+2 depolymerization activity. Residual chlorine in the bleach pretreated coal may be inhibiting depolymerization by CP1+2. It may be possible to increase depolymerization by CP1+2 if the chlorine residual could be decreased.

h. Other Bioconversions

The activated sludge and primary digestor cultures, grown with a North Dakota lignite, depolymerized the coal to water soluble products very slowly. The slow biological degradation required that investigation of these cultures be discontinued as it was evident that CP1+2 was the best aerobic consortium for degrading coal rapidly with a high yield of depolymerized products.

3. Other Parameters

a. Substrate Limitations

Different concentrations of coal were incubated with CP1+2 in order to determine the optimum coal concentration for biodepolymerization. In these experiments, CP1+2 was grown in the presence of 1% untreated lignite for five days before the contents of the culture flask were divided. One gram of

Table 22. Chemical and Biological Depolymerization of Bleach and Hydrogen Peroxide Pretreated Coals

	CD_{450}	
Pretreatment	CP1+2	NaOH/Heat
None	0.6	236
20% H_2O_2	69.0	720
100% Bleach	0.5	400
50% Bleach	11.0	184
10% Bleach	0.8	216
5% Bleach	0.6	240

coal was added to flask #1 and fresh CP1+2 culture (5 ml) was added to flask #2 Both flasks were incubated with agitation at ambient temperature for two days. At the end of the second day, aliquots were removed from each flask, centrifuged to remove particulates, and the OD_{450} of each supernatant determined. The addition of fresh microbial culture resulted in an increase in the OD_{450} from 9.6 to 11. The flask which received fresh coal (no additional microorganisms), was found to have an OD_{450} of 20.0. These results indicate that the microorganisms are capable of degrading additional coal and perhaps were limited by nutrient availability in the raw coal. The readily available nutrients would be degraded and the bonds most easily cleaved in the coal would be broken during the first 5 day incubation. The increase in OD_{450} over five days reached 9.6 with a comparable increase in OD_{450} to 20 (an increase of 10.4 OD_{450} units) when a second gram of coal was added to the flask.

b. Toxicity Assessments and Further Biological Degradation of Depolymerized Products

The depolymerized coal products (chemical and biological) were tested for their ability to support growth of microbial cultures. For these experiments, Beulah lignite was treated with various chemicals and the soluble products were used to evaluate the bacterial growth and utilization of these compounds. Coal products from lignite pretreated with HNO_3 followed by NaOH/thermal treatment were also evaluated as a substrate for growth of bacterial cultures. Results showed that the chemically produced coal products were highly toxic to the biological cultures. These studies indicated that the use of nitric acid for enhancing the alkali degradation of this coal would not be useful in this project.

The coal depolymerized products at different concentrations were added to a minimal basal salts medium (1 XE) containing (grams per liter), $MgSO_4$ 0.2, citric acid 0.2, K_2HPO_4 5.0 and $NH_4H_2PO_4$ 1.9. TSB in various concentrations (up to 0.5%) was added to this medium. The flasks contained 0.5 to 5% of the depolymerized products in 100 ml medium and an ARCTECH mixed culture, CP1+2 was inoculated at 3% (v/v). Bacterial growth was monitored over time at 520 nm using a spectrophotometer. The results obtained from the experiment with Beulah lignite extract added to TSB medium are shown in Table 23.

Table 23. Growth of Bacterial Cultures on Depolymerized Products of Beulah Lignite

Primary Treatments	Depolymerized Product Added 1%	2%	5%
8N HNO_3 - 1N NaOH - Heat	+	-	-
20% H_2O_2 - 1N NaOH - Heat	+++	++	+
1N NaOH - Heat	+++	++	++
1N KOH - Heat	+++	++	+

+++ no inhibition of bacterial growth

- complete inhibition of bacterial growth

TSB medium

No bacterial growth occurred in medium without TSB. A minimum of 5 ml of TSB per 100 ml 1 XE medium was required to initiate bacterial growth in the presence of up to 2% Beulah depolymerized products. When 0.2% glucose was added to 1 XE medium, bacterial growth occurred in medium containing up to 5% depolymerized coal products. When the coal was pretreated with 8N HNO_3, the depolymerized product inhibited bacterial growth even in the TSB medium, indicating that these products are toxic to bacterial cultures. Products obtained following pretreatment of the coal with 20% H_2O_2, 1N NaOH or KOH were not inhibitory to bacterial cultures which cultures tolerated up to 5% depolymerized products when grown in TSB medium. When 5 ml TSB was added to 1 XE medium, the maximum depolymerized coal concentration for a good biological growth was about 2% (v/v). Although the toxicity experiment was conducted with only one bacterial culture, it is likely that at the 2% addition level, inhibition of bacterial growth will not occur. Tolerance to elevated product concentration may be developed by adaptation of bacteria through several transfers with a gradual increase in the product concentration.

Chemically depolymerized products from different coals were tested to determine whether CP1+2 could degrade these products to smaller molecules. The mixed bacterial culture was inoculated into 100 ml 1 XE medium containing 5 ml TSB and 0.4-0.5% (w/v) concentrations of lyophilized depolymerized coal products. The flasks were then incubated aerobically on a shaking platform at room temperature. The disappearance of compounds absorbing at 450 nm was monitored spectrophotometrically over time. A 1 ml aliquot of each culture was removed, centrifuged at 15,000 rpm for 5 minutes and the OD_{450} of the supernatant was determined. The results showed that compounds absorbing at 450 nm disappeared during incubation with the microbial cultures (Table 24), although at a slower rate. Products from these aerobic degradations were later used as substrates for anaerobic bacteria which converted these compounds to methane.

In another experiment, the depolymerized products obtained from various coals were incubated with CP1+2 to determine whether this consortium could convert these products to simple compounds suitable for anaerobic fermentations. In this experiment, 0.5 to 1% (v/v) of various coal depolymerized products was added to a TSB medium containing CP1+2 microbial culture grown under

Table 24. Microbial Breakdown of Chemically Depolymerized Coal Products in the Presence of TSB

Depolymerized Products	Products % Added	$OD_{...}$ 0 day	21 days
Beulah	0.4	1.2	0.8
Wyodak	0.4	0.5	0.2
Spring Creek	0.4	0.3	0.2
North Dakota	0.5	0.8	0.5

agitated conditions. The dark brown color, characteristic of the water soluble coal products, disappeared with time. A 5 ml aliquot of culture was removed after 5 days incubation, centrifuged to remove bacterial cells and the OD of each supernatant was determined. Supernatant O.D. decreased when CP1+2 was grown with Beulah, Wyodak and San Miguel products (Table 25). This indicates that CP1+2 most likely transformed the dark brown products to other compounds which may be suitable for anaerobic fermentations.

B. Anaerobic Bioconversion of Coal or Coal Products to Methane

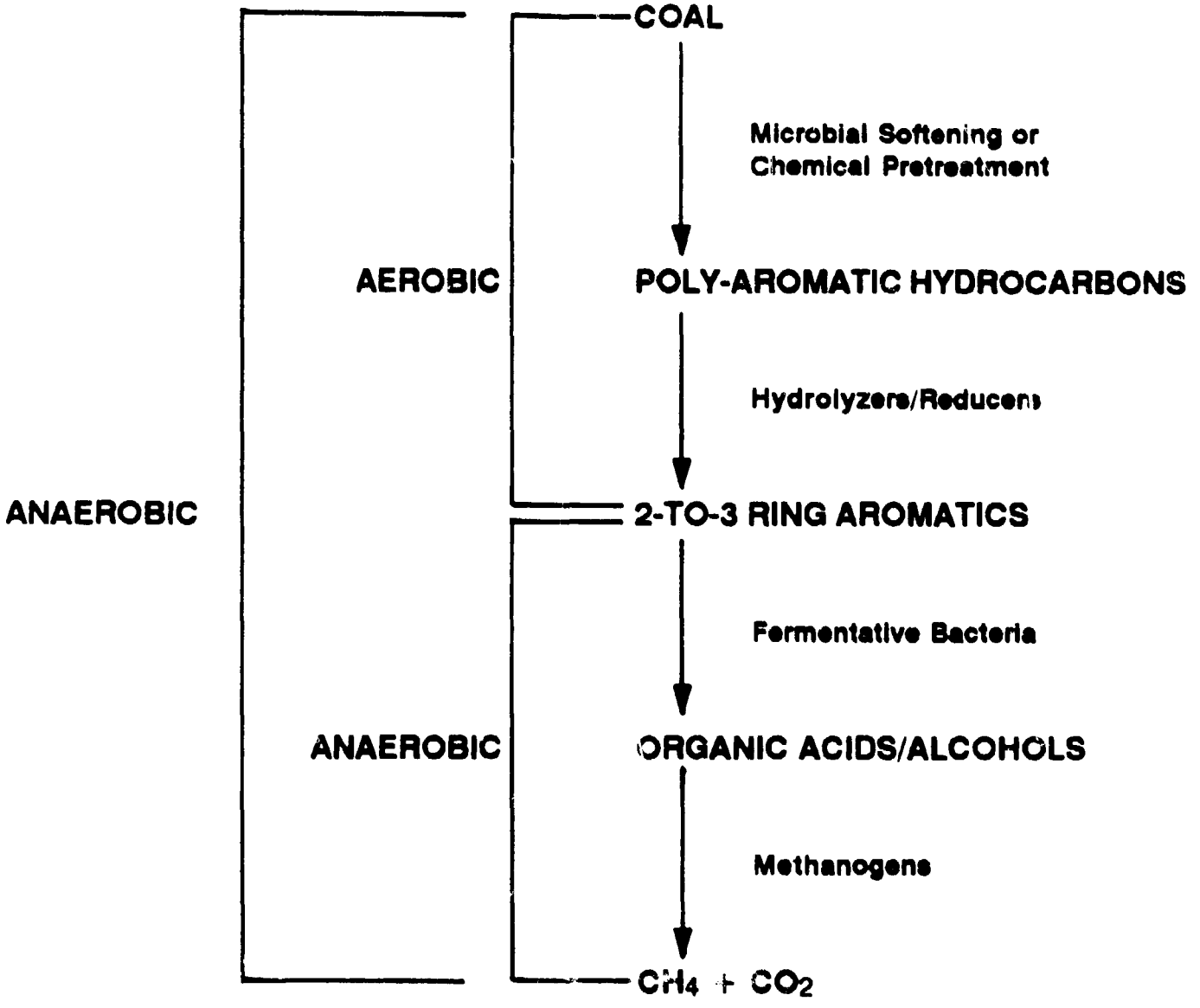
Anaerobic bioconversion of coal or coal depolymerized products to methane could offer many advantages over chemical conversion processes. Bioconversion processes are generally more economical than chemical processes. Theoretically, this conversion could involve a multi-stage process requiring: 1) the involvement of both aerobic and anaerobic bacteria, 2) a two-stage anaerobic process; or ideally, 3) a one-stage anaerobic process for direct conversion of coal to methane (Figure 4). During any of these bacterial fermentations, the bacteria transform coal into methane precursors. These precursors to methane formation must be identified in order to manipulate the methanogens as well as other acetogenic bacteria in the mixed population responsible for the bioconversion of coal to methane.

Two approaches to biogasification, direct methanation and a two-stage aerobic/anaerobic methanation, were proposed because coal is a very complex, heterogeneous, polymeric material which is not soluble in aqueous medium. It was anticipated that direct bioconversion of coal to methane would be difficult because the degradation must proceed through multiple steps, i.e. coal modification, coal depolymerization to create aqueous soluble products of high molecular weight, degradation of high molecular weight compounds to low molecular weight materials which can serve as precursors to methanogenesis, and finally methane production. It is obvious that such a wide variety of degradative steps would require numerous microbial populations. Because microorganisms are generally more efficient in degrading water soluble, low molecular weight compounds, an aerobic coal treatment was proposed to achieve the preliminary coal modification and depolymerization steps and provide substrates for anaerobic

Table 25. Microbial Breakdown of Coal Depolymerized Products Using Cultures of CP1+2

		OD_{450}	
Coal Product	% Added	+ CP1+2	- CP1+2
Spring Creek	0.5	0.6	0.6
Beulah	0.5	1.4	1.6
Wyodak	0.5	0.7	0.8
San Miguel	0.5	1.3	1.4
Peabody	1.0	0.3	0.2

Fig. 4 CONCEPTS FOR BIOCONVERSION OF COALS



fermentation to methane. Evaluations of both the direct and indirect biogasification approaches were performed.

Bacterial cultures were grown directly on coal or coal depolymerization products and methane formation was monitored. When methane production was demonstrated, the identification of methane precursors was undertaken using various analytical techniques. This approach allowed ARCTECH to adapt anaerobic bacterial cultures for direct bio-methanation of coal before identification of the products was undertaken. Experiments were conducted to demonstrate methane production by different bacterial enrichment cultures followed by experiments in which methane formation was blocked using methane inhibitors (BESA and monensin). The use of methane inhibitors forced the accumulation of methane precursors to facilitate detection and identification of these methane pathway intermediates.

1. Coal

Biological degradation of coal to methane was studied using untreated coals (North Dakota and Texas lignites) or coal products obtained via chemical and/or biological coal depolymerization. Three coals, North Dakota lignite, Beulah lignite and Wyodak subbituminous were treated to obtain biological and chemical depolymerized coal products. Biological treatment of coals at 1% (w/v) and treatment of the depolymerized products 1% (v/v) were performed, except as noted, during all the experiments.

2. Inoculum

The anaerobic samples were collected from primary sewage sludge digestor, Eastern shore mud, bovine and ovine rumen, horse manure-hay compost, leaf litter compost and chicken wastes. The samples were collected and transported to the laboratory using precautions to maintain an anaerobic environment until they were used for inoculation.

3. Preparation of Anaerobic Medium

The methods used for the medium preparation and microbial inoculation conformed to Hungate anaerobic procedures modified by Bryant (1972) and Balch and Wolfe (1976). For the preparation of the medium, mineral salts and other constituents in appropriate amounts were placed in a round bottomed flask and were boiled 3-5 minutes under a nitrogen-carbon dioxide (80:20) atmosphere. More details of this method and the formulations for the basal salts medium used to grow the bacterial cultures are described in Appendix 1. Yeast extract (0.2%) (to enhance microbial replication) and sodium benzoate (0.05%) were added to serve as growth supplements. Benzoate was provided in the culture medium to confirm that methanogens were present in the culture. Sodium bicarbonate (0.35 g/100 ml) was added when the medium was cooled to about 55°C. Addition of sodium bicarbonate enhances the buffering capacity of the medium. Medium, in appropriate amounts, was then pipetted anaerobically into serum stoppered tubes (18 x 150 mm) or serum bottles (ca. 120 or 150 ml) capped with black butyl rubber stoppers. The tubes and the bottles were then crimp sealed and autoclaved at 121°C for 15 minutes. Sodium sulfide (2.5%) was used to reduce the medium prior to use.

4. Establishment of Enrichment Cultures

Microbial consortia for use in anaerobic fermentation of coal carbon (raw coal and coal products) were developed from numerous anoxic environments. A list of the microbial consortia evaluated in these studies is presented in Table 26.

Coals used as carbon substrates for anaerobic fermentation included a highly oxidized North Dakota lignite (Leonardite), Beulah lignite, Texas lignite and Wyodak subbituminous. Products were obtained from each of these coals by pretreatment using 20% H_2O_2 followed by incubation with an ARCTECH proprietary culture, CP1+2, capable of depolymerizing the oxidized coals into water soluble products. These products were used as substrates for bioconversion in liquid form or as dry coal-like particulates following lyophilization. The use of the lyophilized products allows for more precise calculation of carbon conversion based on grams of product and percent carbon in the specific product.

Table 26. Source of Anaerobic Microbial Consortia

Horse manure/Hay compost
Chicken Waste
Sewage Sludge
Eastern Shore Mud
Coal Slurry Pit
Coal Tar Pit
Coal Settling Pond
Coal Pile Run-off (waste-water)
Leaf Litter
Ovine Rumen
Bovine Rumen
Termite Guts

Total gas production from reaction mixtures is measured using a syringe displacement method described in Appendix 4. The concentration of methane in head space gases is determined using gas chromatography. Quantitation of intermediates present in the aqueous phase of reaction mixtures is performed using gas chromatography (Appendix 5).

Identification of intermediates formed during bioconversion of coals and coal products was of interest in this project as a means of further elucidating the microbial pathways used for biodegradation of the coal carbon. Initial biomodification of the coal and subsequent creation of soluble products did not result in products which could be readily identified. To force the accumulation of high molecular weight metabolic intermediates would require the use of inhibitors to block specific steps in the biodegradative pathway. No such inhibitors are currently available; however, 2-bromoethanesulfonic acid (BESA) and monensin are widely used inhibitors to block methane formation resulting in the accumulation of low molecular weight methane precursors such as short chain organic acids and/or alcohols. Data obtained in these studies on accumulation and identification of intermediate products has added to the knowledge on biochemical pathways for bioconversion of coal or coal products to methane. Surprisingly, in some cases, the addition of these inhibitors did not lead to methane inhibition. ARCTECH believes that the cultures which exhibited methane production in the presence of the inhibitors may belong to a special group of microorganisms and may be functioning via a different metabolic pathway.

All preliminary screening studies were performed using Balch tubes (10 ml medium). In later studies these cultures were transferred to serum bottles containing 20-75 ml medium. The cultures were visually observed for bacterial growth and total gas and methane production were monitored. Total gas was measured using the syringe displacement method. Total gas evolved was measured at least once every 5 days, shorter intervals for sampling were used when gas evolution was higher. The procedure for gas phase analysis is described in Appendix 6.

The bacterial cultures were transferred to fresh medium every 28-45 days depending on the maximum gas and methane production. Some cultures were not transferred even after 60 days because the cultures were slow to adapt to

certain coals or coal depolymerization products. A long adaptation period is not surprising because coal contains high molecular weight polyaromatic structures.

5. Methane Production

Bacterial screening to evaluate microbial consortia derived from different anoxic environmental sources used four coals (three lignites and one subbituminous) as substrates for anaerobic fermentations. Total gas production as well as methane production was monitored over time using gas chromatography. Calculation of total quantities (cc) of methane produced by each reaction (10 ml reaction mixture; 0.1 g coal or coal products) were determined from the gas chromatographic data (mole% CH_4 and total gas) and multiplied by 10 to obtain total methane produced per gram of coal carbon. Total carbon analysis of each coal was performed at the University of Kentucky and is presented in Table 2. Total carbon in each coal allowed for subsequent conversion of the data to methane production per gram of coal carbon.

Preliminary data indicated that methane formation occurred when coal or coal products were inoculated with anaerobic microorganisms collected from various sources. Data on methane produced from an untreated North Dakota coal and from the depolymerized products of a Beulah lignite are presented in Figure 5. Biological production of methane was demonstrated when an untreated North Dakota or the depolymerized products of a chemically treated Beulah lignite were inoculated with horse manure-hay microbial cultures. Methane formation from the samples containing coal or coal products as substrates was slightly higher than from the control samples (Figure 5). Methane in control samples is generated from benzoate and/or yeast extract in the medium.

a. Sewage Sludge/Horse Manure

Methane production from both Leonardite and depolymerized Beulah products was monitored using sewage sludge and horse manure (HM) compost as inocula. The production of methane from Leonardite and Beulah depolymerized products inoculated with sewage sludge and horse manure samples is reported in Table 27. Methane was produced at higher concentrations from both Beulah

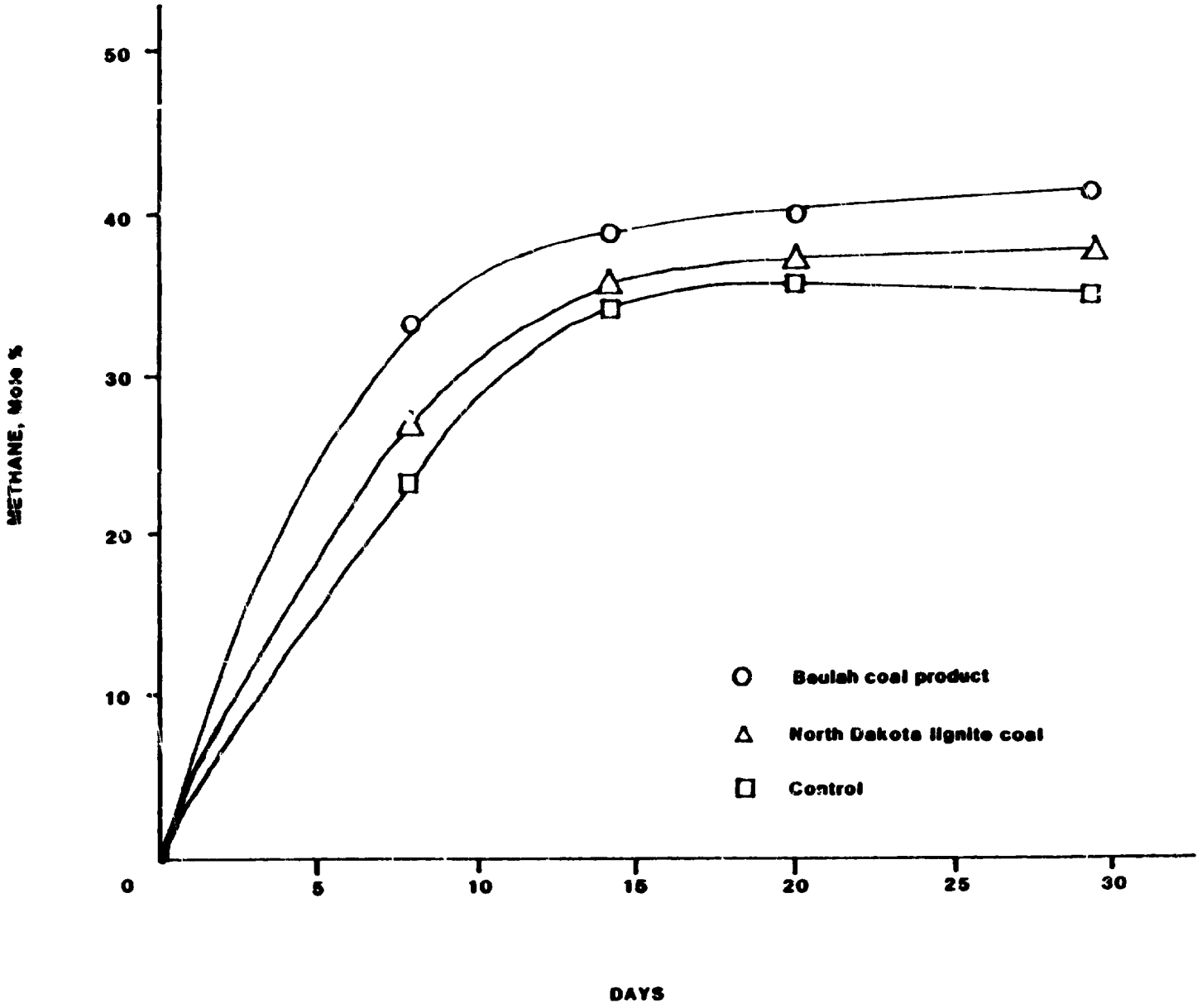


Figure 5. Production of methane from a ND Coal and Beulah Coal Product Inoculated with Horse Manure-Hay Organisms

Table 27. Production of Methane from Coal or Coal Products Inoculated with Primary Digestor Sludge and Horse Manure-Hay Compost Samples

	Mole%, 42 days			
	8 d		42 d	
Samples	CH_4	CO_2	CH_4	CO_2
Sludge inoculum:				
Control	7.3	24.5	36.0	23.8
Leonardite	7.8	32.4	39.3	34.2
Beulah product	4.3	18.0	42.2	26.4
Horse Manure-Hay Compost:				
Control	22.6	30.1	34.5	32.6
Leonardite	26.5	33.9	38.0	36.5
Beulah product	32.9	29.6	41.3	31.8

depolymerized products and Leonardite coal by two microbial consortia containing sludge and horse manure organisms. Initial results indicated that HM microbial cultures adapted faster than the sludge cultures. However, at the end of 42 days, sludge cultures did as well as the HM samples and produced almost equivalent amount of methane from these samples. In both cases, more methane was produced by the microbially inoculated samples containing coal products. Production of methane from the control samples was due to the provision of benzoate which was used as a growth supplement for the consortium.

b. Chicken Waste

Chicken waste samples were collected to evaluate this source of inoculum for bioconversion of coal or coal products to methane. Chicken waste organisms were first grown anaerobically with benzoate for two weeks and then transferred to the medium containing small amounts (0.02%) of benzoate and coal or coal depolymerization products. Methane was produced from Beulah coal products and from untreated Leonardite coal within seven days after the transfer (Table 28). The formation of methane was greater in the samples containing coal or coal products than in the control samples containing no coal or coal products. Similar results were observed at the end of 20 days of incubation. The mole% methane produced from Leonardite and Beulah products was essentially the same. Very little methane was produced from Texas lignite, even after 20 days of incubation. Visual observations during the experiments indicated a change in the color of the reaction medium usually associated with coal depolymerization. These cultures were incubated further to allow complete bacterial adaptation to coal or coal depolymerization products.

c. Leaf Litter

Similar experiments with Leonardite coal and Beulah coal products were conducted using leaf-litter samples as inoculum. These organisms required a longer adaptation period before coal or coal depolymerization products were converted to methane. Very little difference in methane production between samples with and without coal or coal products was observed after 30 days of incubation (Table 29). At 60 days of incubation, methane production from Beulah coal products was 10% higher than the control samples, indicating that the

Table 28. Production of Methane from Untreated Leonardite and TX Lignite and Chemically Depolymerized Beulah Products Inoculated with Chicken Waste Microorganisms

	Methane, Mole%	
Sample	7 d	20 d
Control (no coal)	3.2	7.3
Leonardite	5.9	32.9
Beulah product	19.3	31.0
TX Lignite	4.0	7.0

Table 29. Production of Methane from Untreated Leonardite and Chemically Depolymerized Beulah Products Inoculated with Leaf-Litter Samples

	Methane, Mole%		
Sample	30 d	45 d	60 d
Control (no coal)	22.1	23.7	25.1
Leonardite	23.0	25.6	25.5
Beulah product	27.5	33.1	33.7

products obtained after H_2O_2-NaOH pretreatment of Beulah coal contained compounds that are converted to methane by anaerobic bacteria. The leaf-litter microorganisms were unable to degrade Leonardite coal to methane. The control samples (no coal) produced methane from benzoate confirming that methanogens were present in the cultures. Longer adaptation periods might be required for bioconversion of certain coals.

d. Eastern Shore Mud

An anaerobic consortium derived from an Eastern shore mud sample was tested for methane production from the four coals. Control reactions containing no coal carbon showed very little methane production (Table 30). Leonardite and Texas lignite appeared to be good substrates for anaerobic conversion by this consortium. Beulah lignite and Wyodak subbituminous were less readily converted with little methane produced after 45 days of incubation. Transfer of this consortium and subsequent testing with Texas lignite, Beulah lignite and Leonardite were carried out. Experiments included addition of BESA and/or monensin as methane inhibitors as well as reactions in the absence of inhibitors. Methane production was only observed when Texas lignite was supplied as the carbon substrate, no methane was produced in reactions containing Leonardite or Beulah lignite as substrates over a 15 day incubation. The addition of monensin to the Texas lignite reaction mixture resulted in complete inhibition of methane; however, BESA addition resulted in a 30% reduction in methane production.

Additional inocula for the Eastern Shore mud consortium was prepared and used in experiments for production of methane from the four coals, three lignites and one subbituminous, with and without BESA. No methane was obtained from samples containing no coal carbon. In these experiments, conversion of Beulah coal was best followed by Wyodak, Texas lignite and Leonardite (Table 31).

Further investigation used Eastern Shore mud samples with coal as the carbon source in the presence and absence of BESA, a methane inhibitor. Methane production and organic acid accumulation were monitored. Control reactions (no coal) showed little methane production with accumulation of acetate

Table 30. Production of Methane from Various Inoculated with Eastern Shore Mud Sample Microorganisms

Coals	Total Methane (cc) produced per Gram of Coal or per ml of Coal Products	
	30 days	45 days
Control, No Coal	4.8	5.33
Leonardite, lignite	68.2	112.94
Texas lignite	57.0	117.95
Beulah lignite	13.05	15.69
Wyodak subbituminous	6.48	11.65

Table 31. Production of Methane from Coals Inoculated with Eastern Shore Mud Samples

Coals	cc Methane Produced per gram of Coal, 24 days Incubation
Control (no coal)	0.58
Beulah	62.15
Leonardite	18.69
TX lignite	28.79
Wyodak	31.86
Control + BESA (no coal)	0.07
Beulah + BESA	256.54
TX lignite + BESA	31.62
Wyodak + BESA	345.77
Leonardite + BESA	ND

ND: Not determined

both with and without BESA. After 24 days of incubation, bioconversion of the raw Wyodak subbituminous to methane was better than conversion of Texas, Beulah or Leonardite (Table 32). A two-fold increase in methane production from Wyodak coal was observed when BESA was present in the reaction mixture. Acetate accumulations were increased when BESA was present in reaction mixtures for all coals tested. These results were notable because less methane was produced from Leonardite, a highly oxidized lignite, than from Wyodak subbituminous. Methane production from Beulah lignite and from Wyodak subbituminous in the presence of BESA was surprising. The reasons for this phenomenon are not known. These results have been reproduced in several experiments with the Eastern Shore mud culture and should be investigated further.

e. Coal-Associated Anaerobic Cultures

Anaerobic microbial cultures were obtained from a coal slurry pit, a coal tar pit and a coal pile run-off waste water reservoir. These samples were incubated anaerobically with various coals and/or coal products. Gas samples and aqueous samples were taken and monitored for methane production and accumulation of methane precursors respectively.

Bioconversion of coal carbon in Texas lignite, Leonardite, Wyodak product and Beulah product by the coal slurry/wastewater pit consortium was evaluated. Control reactions (no coal carbon) produced only small amounts of methane (Table 33). Direct conversion of coal (Texas lignite and Leonardite, no pretreatment) showed the greatest methane production. Conversion of the Wyodak and Beulah products resulted in the production of about 20% of the methane produced from the lignites.

Bioconversion of four coals, three lignites and a subbituminous, by inoculum derived from a coal pit were also tested. At neutral pH, good bioconversion of raw Texas lignite and Leonardite was demonstrated (Table 34). Bioconversion of Leonardite and Beulah at pH 5 and 6 in the presence of BESA or monensin (methane inhibitors) was also investigated. Monensin did not result in good inhibition with Leonardite under either of the lower pH regimes. Inhibition of methane production by BESA was more complete at pH 5 (95% inhibition) than at pH 6, where 63% inhibition was noted. In the case of Beulah

Table 32. Production of Methane and Acetate from Coals Inoculated with Eastern Shore Mud Samples

Coals	Acetate* µg/ml	cc Methane/g Coal*
Control (no coal)	185.1	0.2
Control + BESA	302.3	0.1
Beulah	145.6	15.1
Beulah + BESA	647.4	27.5
TX lignite	30.8	22.4
TX lignite + BESA	281.8	28.9
Wyodak	171.6	109.4
Wyodak + BESA	213.3	216.0
Leonardite	111.2	8.7
Leonardite + BESA	ND	ND

ND - Not determined
*After transfer, 24 days incubation

Table 33. Production of Methane from Coals or Coal Products Inoculated with Coal-Slurry Wastewater Pit Samples

Coals		CH_4 Produced (cc per gram of coal or per ml of coal products) 8 days	18 days
Control (No Coal)	+ Benzoate	2.6	3.4
	- Benzoate	1.8	2.5
Texas lignite	+ Benzoate	54.2	53.6
	- Benzoate	49.7	49.3
Leonardite	+ Benzoate	41.6	51.4
	- Benzoate	42.2	78.6
Wyodak Product	+ Benzoate	8.50	11.3
	- Benzoate	7.4	12.8
Beulah Product	+ Benzoate	2.4	3.3
	- Benzoate	7.8	10.7

Table 34. The Effect of pH on the Production of Methane from Various Coals*

pH	Coal	cc Methane Produced per gram of coal, 45 d incubation
7.0	Control, no coal	3.61
	TX lignite	90.79
	Wyodak	14.53
	Leonardite	80.91
	Beulah lignite	10.98
6.0	Control, no coal	6.01
	Leonardite + monensin	67.77
	Leonardite + BESA	29.40
	Beulah + monensin	19.10
	Beulah + BESA	0.91
5.0	Control, no coal	6.29
	Leonardite + monensin	75.89
	Leonardite + BESA	3.62
	Beulah + monensin	24.88
	Beulah + BESA	1.67

* Inoculum was collected from a coal pit.
All coals used at 0.5% solids loadings.

lignite, the presence of monensin resulted in increased methane production (compared to bioconversion in the absence of monensin) with good methane inhibition in the presence of BESA.

Direct production of methane from four coals using the four coal-associated inocula was monitored over a three month incubation period. Control reactions (no coal carbon) resulted in little methane production. These data (Table 35) conclusively illustrate that microbial/coal substrate specificity is a factor which must be addressed for successful bioconversion of coal. The settling pond and the coal pit inocula were most successful in bioconversion of Wyodak subbituminous, with production of 285 and 319 cc methane per gram of coal respectively. The coal pit inoculum was also successful in bioconversion of Beulah lignite . The coal waste-water sample was only successful in significant bioconversion of Wyodak subbituminous with production of 56 cc methane per gram of coal. The coal slurry inoculum was successful in bioconversion of Beulah lignite (155 cc methane per gram of coal) and Wyodak subbituminous (134.5 cc methane per gram of coal). Maximum bioconversion of Leonardite was achieved (59 cc/g coal) by the coal settling pond and coal pit inocula. Texas lignite was converted by the coal pit inocula (60 cc methane/g coal) and by the coal settling pond inocula (38 cc methane/g coal).

Aqueous samples from reactions described above were also taken and monitored for accumulation of methane intermediates. No alcohols were detected during these bioconversions. Acetic acid was the major product although other intermediates were detected in very low concentrations. Little acetic acid was detected in the control reactions (no coal carbon) or in reaction mixtures containing Leonardite and Texas lignite (Table 36). Acetic acid accumulations of up to 87 mg/g of coal were observed in Beulah lignite reaction mixtures, although only the coal settling pond inoculum with Wyodak showed significant acetic acid accumulation. The accumulation of acetic acid in Beulah and Wyodak reaction mixtures with the coal settling pond inocula indicates that the concentration of the desired methanogens in this consortium may be too low for maximum bioconversion of the coal carbon to methane. As the control samples did not produce methane or accumulate acetate, these products resulted from bioconversion of the coals.

Table 35. Direct Production of Methane from Coals Inoculated with Four Coal-Associated Consortia

Inocula	cc Methane Produced/g of Coal*				
	Control**	Leonardite	Beulah Lignite	Texas Lignite	Wyodak Subbituminous
Coal Settling Pond	2.1	59.3	157.9	37.9	284.5
Coal Pit	2.5	59.0	280.7	60.1	318.7
Waste Water	0.3	8.0	22.0	5.1	55.5
Coal Slurry	1.2	25.4	155.0	22.4	134.5

*Methane produced during three month incubation period.
**No coal added. Samples did not contain any other carbon sources.

Table 36. Production of Acetate as Intermediate During Conversion of Coals to Methane in Samples Inoculated with Four Coal-Associated Consortia

Inocula	mg Acetate Accumulated/g of Coal*				
	Control**	Leonardite	Beulah Lignite	Texas Lignite	Wyodak Subbituminous
Coal Settling Pond	0.6	1.4	86.9	2.1	75.7
Coal Pit	0.03	0.7	6.4	1.8	6.3
Waste Water	0.3	2.0	79.3	0	0
Coal Slurry	0.1	1.1	52.6	1.0	12.5

*Methane acetate accumulated during three month incubation period
**No coal added. Samples did not contain any other carbon sources.

f. Ovine and Bovine Rumen Consortia

Samples were obtained from ovine (sheep) and bovine (cow) rumen and cultured for use as inocula to evaluate bioconversion of coal carbon by these naturally occurring microorganisms. During a two month incubation period, little methane was produced by the control reactions (no coal carbon). The ovine rumen consortium demonstrated excellent bioconversion of pretreated Wyodak subbituminous (production of 1136 cc methane/g of coal carbon) and Beulah lignite (875 cc methane/g coal carbon) (Table 37). The bovine rumen consortium also gave good conversion of both pretreated Beulah and Wyodak coals, although conversion was not as good as that achieved by the ovine rumen consortium. Bioconversion of untreated Leonardite and Texas lignite was observed in both ovine and bovine reaction mixtures. Although good conversion of soluble coal products to methane was demonstrated, direct bioconversion of coals had been determined to be the best approach for efficient conversion of parent coal carbon. Thus, direct conversion of coals by rumen cultures was the focus of subsequent studies.

Analysis of aqueous samples from the reaction mixtures described above, showed that acetic acid accumulated in all reaction mixtures except for the controls. Based on methane and acetic acid data presented, it appears that the ovine consortium contained an appropriate mixture of acetogens and methanogens, with a high acetic acid accumulation found only in the Texas lignite reaction mixture. High acetic acid concentrations were observed in three of the four bovine consortium reactions, indicating that methanogens capable of acetate cleavage may not be present in sufficient numbers to effect maximum coal bioconversion to methane. It is possible that the addition of *Methanothrix* sp., a methanogen which preferentially converts acetate to methane, could enhance the conversion of these coals to methane and preclude the accumulation of acetate.

Further adaptation of these cultures was carried out and subsequent experiments monitoring methane production and acetic acid in reaction mixtures containing four coals. After 40 days of incubation, the ovine rumen cultures had produced significant amounts of methane from both Beulah and Wyodak coals (Table 38). Much less methane was produced from Leonardite and Texas lignites. Similar results were obtained with these four coals incubated with

Table 37. Direct Production of Methane from Coals with Ovine and Bovine Rumen Inocula

	cc Methane Produced/g of Coal*				
Inocula	Control**	Leonardite	Beulah Lignite	Texas Lignite	Wyodak Subbitum.
Ovine Rumen	4.7	171.5	875.4	126.8	1136.2
Bovine Rumen	7.0	131.6	766.3	143.9	613.3

*Maximum methane production during two month incubation period.
**Controls contained no coal. Samples contain 0.02% sodium benzoate to facilitate culture adaptation.

Table 38. Direct Biological Production of Methane and Acetic Acid from Coals using Two Bacterial Consortia*

Coals	Ovine Rumen		Bovine Rumen	
	CH4 (cc/g coal)	acetic acid (mg/L)	CH4 (cc/g coal)	acetic acid (mg/L)
Leonardite	171.5	8.1	131.6	59.6
Beulah lignite	875.4	11.2	766.3	49.8
Wyodak Subbit.	1136.2	51.1	613.3	222.1
TX lignite	126.8	25.5	143.9	469.0

* Benzoate medium, controls subtracted; 40 days incubation.

bovine rumen consortium. Acetic acid was detected only at low levels in each of the bovine/ovine rumen reaction mixtures. Higher concentrations of acetic acid were detected when bovine rumen cultures were used for conversion of Wyodak and Texas lignite. The accumulation of acetic acid from conversion of Texas lignite by the bovine rumen consortium is significant since methane production was quite low in this system.

The adapted ovine rumen consortium was grown with untreated and pretreated coals (Texas lignite, Beulah lignite and Wyodak subbituminous). These cultures produced large concentrations of acetate which accumulated in the culture medium during coal bioconversion. A mixed population of methanogenic bacteria was added to the adapted ovine rumen consortium grown with untreated coals and pretreated coals (Texas lignite, Beulah lignite, Wyodak subbituminous). Coal/microbial culture specificity was demonstrated when the ovine rumen consortium was incubated with the four coals. This culture produced methane from Texas lignite and Wyodak subbituminous but failed to produce significant amounts of methane from Leonardite or Beulah lignite (Table 39). Maximum methane produced was 39 cc/g of Wyodak coal within 50 days of incubation. Methane (35 cc/g coal) was produced from Texas lignite within 25 days of incubation.

Neither of these two consortia have adapted sufficiently for rapid conversion of coal carbon to methane in the time-frame of this research project. The consortia have been stored for future study as time permits.

g. Bioconversion of Coal-derived Products

Anaerobic conversion of biological and chemical depolymerized products of Leonardite, Beulah and Wyodak coals was compared. The data on methane production from these experiments show that biological methane production was higher when biodepolymerized coal products were the substrates as compared to methane from chemically (20% H_2O_2 or NaOH/heat) depolymerized coal products (Table 40). The experimental data indicate that the products formed during biological and chemical treatments are different. These data also clearly demonstrate the relative preference of the anaerobic bacteria for biologically derived coal depolymerized products over chemically derived products for methane production.

Table 39. Production of Methane from Coals Using a Bacterial Consortium Derived from an Ovine Rumen

Coals	Methane produced, cc/ g Coal	
	25 d	50 d
Control (No coal)	0.1	0.1
Texas lignite	34.8	33.7
Wyodak subbituminous	22.9	39.1
Beulah lignite	12.5	19.1
Leonardite	6.1	4.5

Note: Coal concentration used: 0.1%; static conditions; 37° C.
Methane production from the control samples were deducted before cc/g of coal was calculated.

Table 40. Production of Methane from Depolymerized of Coal Products Using Anaerobic Bacteria

	Methane** Mole% from Coal Depolymerized Products	
Coal*	Biological	Chemical
Leonardite	20.4	4.5
Beulah	24.0	13.9
Wyodak	13.6	2.7

* Coal products used at 1% v/v.
** Methane from control (no coal) samples was subtracted.

6. Identification of Intermediates during Coal Biodegradation to Methane

The mixed anaerobic cultures developed at ARCTECH degraded both coal and coal depolymerization products to methane. The bacterial cultures likely metabolize the parent substrates through various steps leading to methane as the final product. It is believed that at least two groups of bacterial cultures, acetogens and methanogens, are involved in biodegradation of coal to methane, similar to the anaerobic degradation of most aromatic compounds. The role of these two groups of bacteria are interrelated and one is dependent on the other for growth and survival. Acetogens, containing several species of microflora, degrade coal to methane precursors which serve as substrates for methanogens. Most of the methanogenic precursors are transitory and usually do not accumulate in the medium unless methanogenesis is blocked or the methanogenic population is inactive.

Our experiments indicated that methanogens were present and active in producing methane from coals when the active acetogenic population is present. Therefore, experiments were initiated to block or inhibit methane formation. Several experiments were conducted as described previously with the exception that methane inhibitors were added to the culture. Two inhibitors, BESA (2-bromoethanesulfonic acid) and monensin, were used in preliminary studies. BESA is an analogue of methyl-CoM and competitively inhibits methane production in the samples, whereas monensin serves as a selective inhibitor at specific biochemical steps in the production of methane. Liquid samples were analyzed for the accumulation of alcohols or short chain fatty acids. The gas phase was also analyzed for the accumulation of H_2 and formation of methane.

The results of these studies are reported in Table 41. BESA completely inhibited methane production from Leonardite and Beulah coal products inoculated with sludge cultures and from Leonardite inoculated with HM samples. Interestingly, BESA failed to inhibit methane production from Beulah coal inoculated with HM samples. In fact, BESA enhanced the methane production (up to 10%) in comparison to control samples. On the other hand, monensin inhibited methane production only from Beulah samples inoculated with sludge cultures. In all other cases, methane production was equal to or greater than the control samples. The liquid samples were analyzed to determine if any other products

Table 41. Production of Methane from Leonardite or Beulah Products Inoculated with Primary Sludge Digestor and Horse Manure-Hay Compost Organisms in the Presence of Methane Inhibitors

	Mole%, 42 days	
Samples	Methane	Carbon Dioxide
Sludge inoculum:		
Control (no inhibitor)	36.0	23.8
Leonardite + BESA	3.0	30.6
+ Monensin	27.7	26.3
no inhibitor	39.3	34.2
Beulah + BESA	2.7	20.1
+ Monensin	11.3	17.4
no inhibitor	42.2	26.4
Horse Manure-Hay Compost Samples:		
Control (no inhibitor)	34.5	32.6
Leonardite + BESA	5.3	34.8
+ Monensin	35.2	32.1
no inhibitor	38.0	36.5
Beulah + BESA	44.7	12.7
+ Monensin	43.4	21.0
no inhibitor	41.3	31.8

(such as alcohols, ketones, and volatile fatty acids) were being produced by virtue of methane inhibition. The details analytical procedures for volatile fatty acids and alcohols are described in the Appendix 5.

Analysis of the liquid phase during bacterial fermentation showed that several short chain alcohols including methanol, ethanol, propanol and butanol were present. In some cases, acetone was produced in small quantities. Benzoic acid was also identified as an intermediate in the coal degradation pathway. Alcohols were produced when either coal or coal depolymerized products were present in the reaction medium (Figures 6 and 8). No alcohols were produced from control cultures grown without coal (Figures 7 and 9). A chromatogram showing mixtures of alcohols and their retention times is presented in Figure 10.

Preliminary results obtained from quantitative measurements of alcohols produced during bacterial metabolism indicate that the alcohols are being produced at levels of 1000 - 2300 ppm (Table 42). Short chain alcohols were produced from Leonardite and Beulah lignite products when methane inhibitors were added to the culture. Ethanol was the predominant alcohol produced during the bacterial degradation of coal or coal depolymerized products. The concentration of ethanol ranged between 800 - 2300 ppm. Methanol accumulated to some extent when monensin was used as an inhibitor. Lower pH favored the production of alcohols. No alcohols were detected at pH 7.0 in any culture or at pH 6.0 when sewage sludge was used as inoculum. The control samples without coal did not produce alcohols.

These microbial consortia not only produced alcohols, but also produced several short chain acids which accumulated when methane inhibitors were added to the cultures. Acetic acid was the principal acid produced, with accumulation up to 1300 ppm. Small quantities of propionic, butyric and valeric acid were also produced. The results obtained using three coals indicated that short chain acids can be produced from coals or coal depolymerization products using anaerobic bacteria. In most cases more acetic acid was produced when BESA was used as an inhibitor (Table 43). The control samples produced little or no acids. The chromatograms showing production of short chain acids from Leonardite and Beulah coal depolymerization products are also reported in Figures 11 and 12. Both of these coals were treated with CP1+2 to obtain biodepolymerized

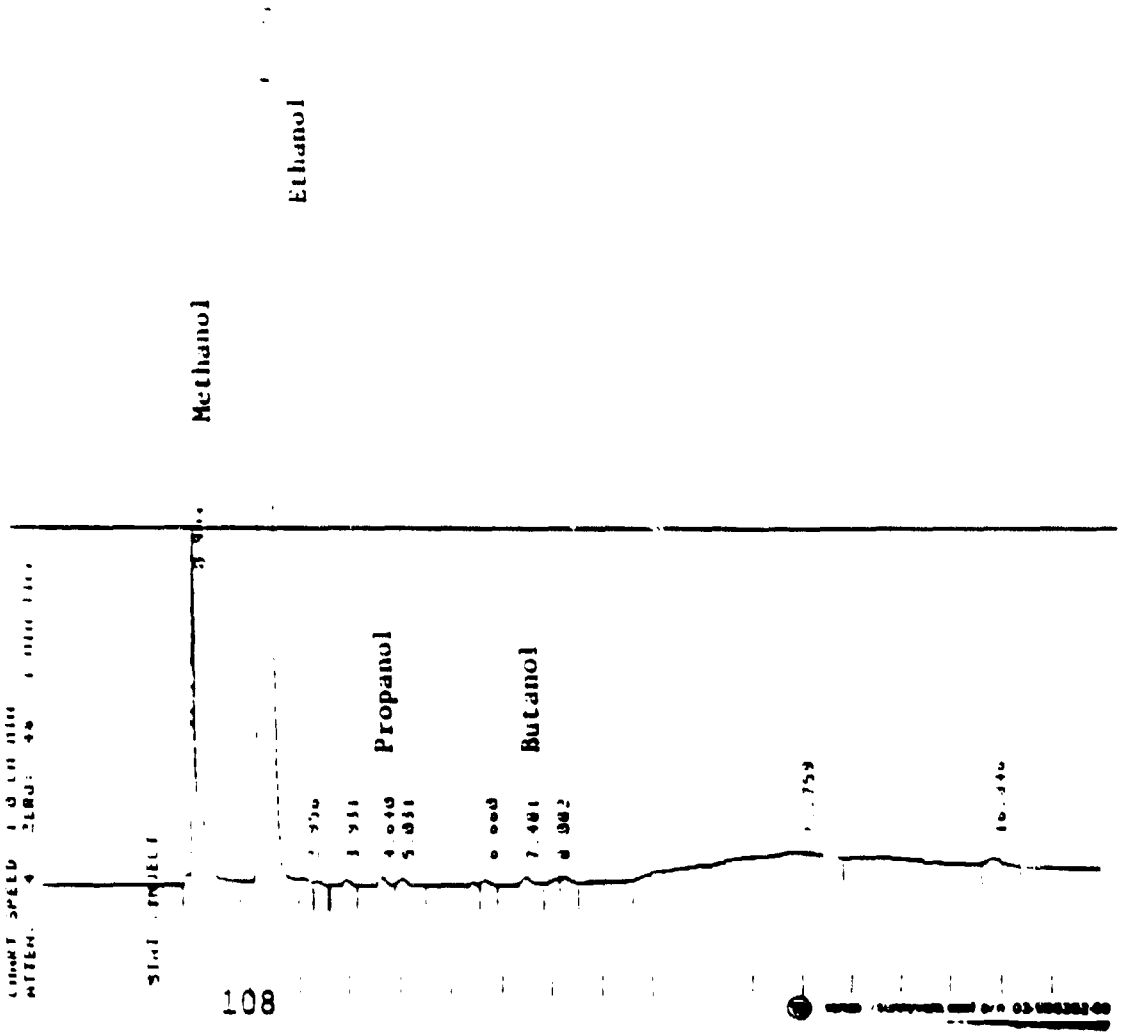


Figure 6. Chromatogram Showing Biological Production of Alcohols from Untreated Leonardite (1% w/v) Using a Mixed Anaerobic Bacterial Culture (SB1) Developed at ARCTECH

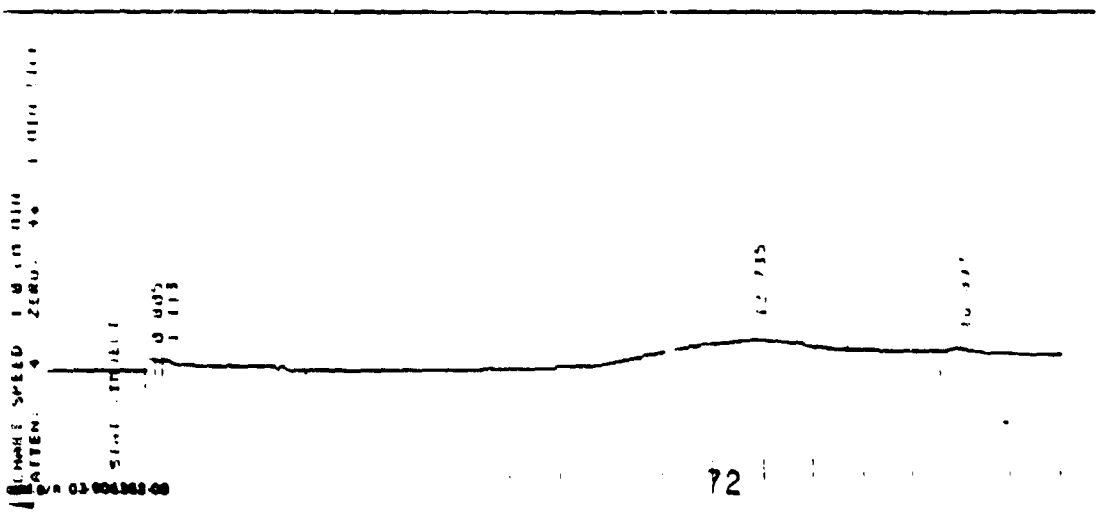


Figure 7. Chromatogram Showing No Alcohol Production from Control Cultures of SB1 Grown without Leonardite Products

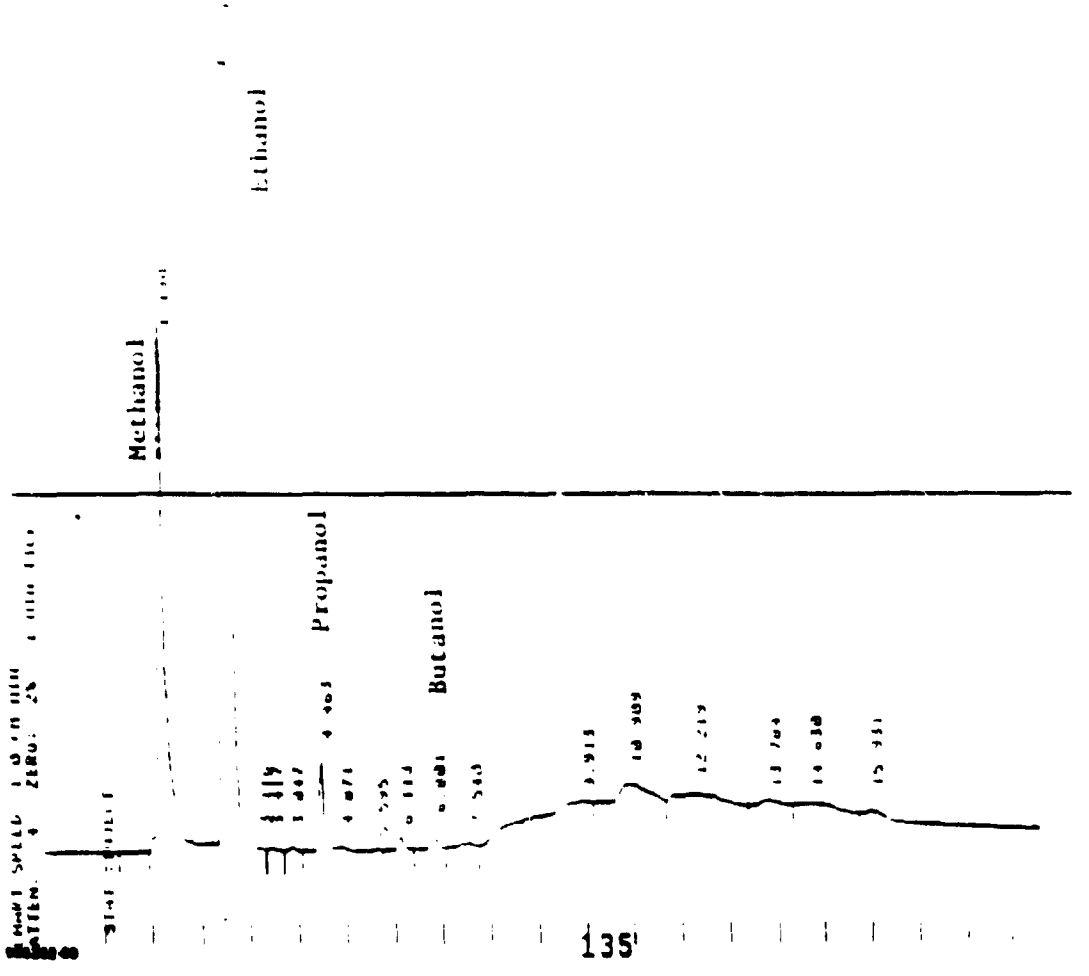


Figure 8. Chromatogram Showing Biological Production of Alcohols from Depolymerization Products of Beulah Lignite (1% v/v) using a Mixed Anaerobic Bacterial Culture (SB1) Developed at ARCTECH

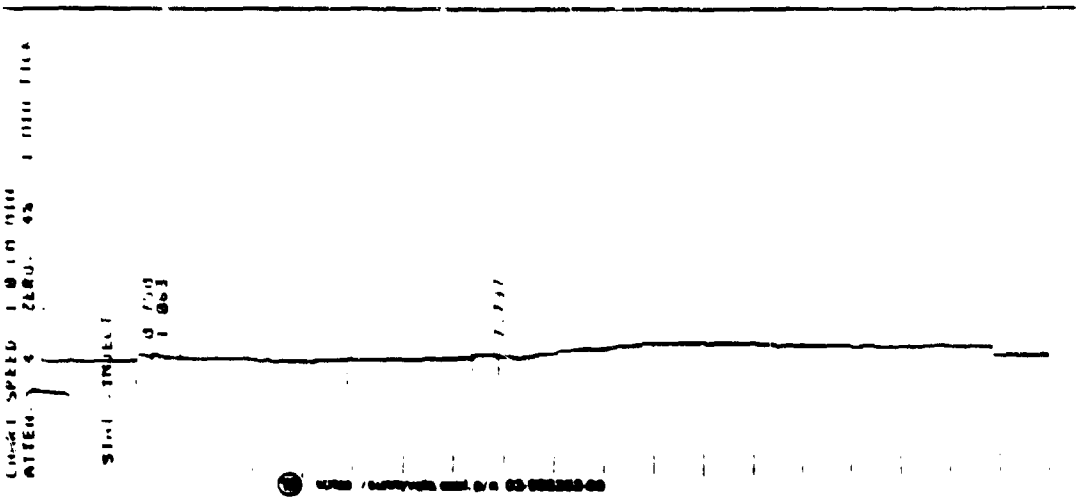


Figure 9. Chromatogram Showing No Alcohol Production from Control Cultures of SB1 Grown without Beulah Products

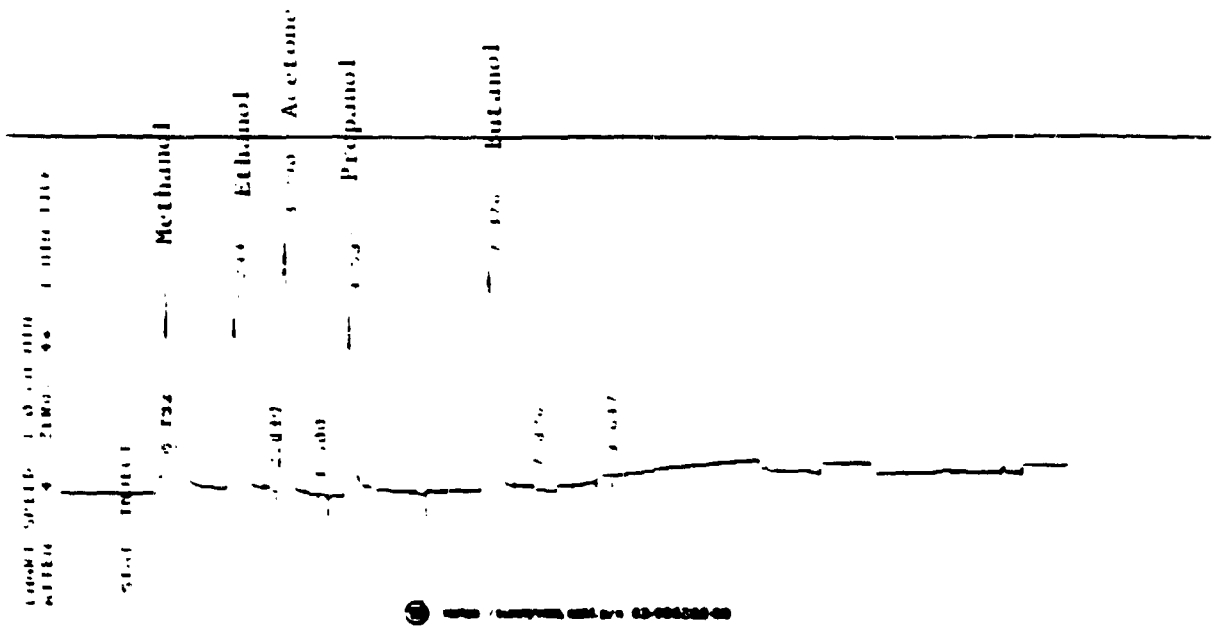


Figure 10. Chromatogram Showing a Mixture of Alcohols (40 ppm) and Their Retention Times

Table 42. Production of Alcohols from Untreated Leonardite and Depolymerized Beulah Products Using Sewage Sludge and Chicken Waste Microorganisms

				Alcohols Produced, ppm			
Inoculum	pH	Coal	Inhibitors	Methanol	Ethanol	Propanol	Butanol
SS	5.0	L	BESA	7.2	2288.2	22.3	18.6
SS	5.0	L	Monensin	275.3	2053.3	3.9	1.9
SS	5.0	L	Control	0	10.9	25.6	0
CW	6.0	L	BESA	7.06	1335.9	173.5	38.3
CW	6.0	L	Monensin	315.9	1975.5	1.7	0
CW	6.0	L	Control	0	16.2	19.9	1.7
CW	6.0	B	BESA	13.5	756.5	202.9	88.9
CW	6.0	B	Monensin	129.2	911.4	10.1	8.5
CW	6.0	B	Control	29.1	4.9	4.5	6.2

SS - Sewage Sludge
CW - Chicken Waste
L - Leonardite
B - Beulah lignite
BESA - 2-bromoethanesulfonic acid

Table 43. Production of Short Chain Acids from Coals and Depolymerized Coal Products Using Sewage Sludge and Chicken Waste Microorganisms

			Acids Produced, ppm			
Inoculum	Coal	Inhibitors	Acetic	Propionic	Butyric	Valeric
SS	L	BESA	925.8	70.9	28.8	280.7
SS	L	Monensin	626.5	77.7	34.1	266.1
SS	L	Control	34.7	0	0	0
CW	L	BESA	1311.5	306.5	80.7	501.0
CW	L	Monensin	618.9	92.6	83.8	436.5
CW	L	Control	4.5	0	0	0
CW	B	BESA	932.4	233.9	62.5	0
CW	B	Monensin	805.0	261.8	57.9	0
CW	B	Control	99.6	118.7	0	0
CW	TXL	BESA	385.7	158.4	34.7	0

SS - Sewage Sludge
CW - Chicken Waste
L - Leonardite
B - Beulah lignite
TXL - Texas lignite
BESA - 2-bromoethanesulfonic acid

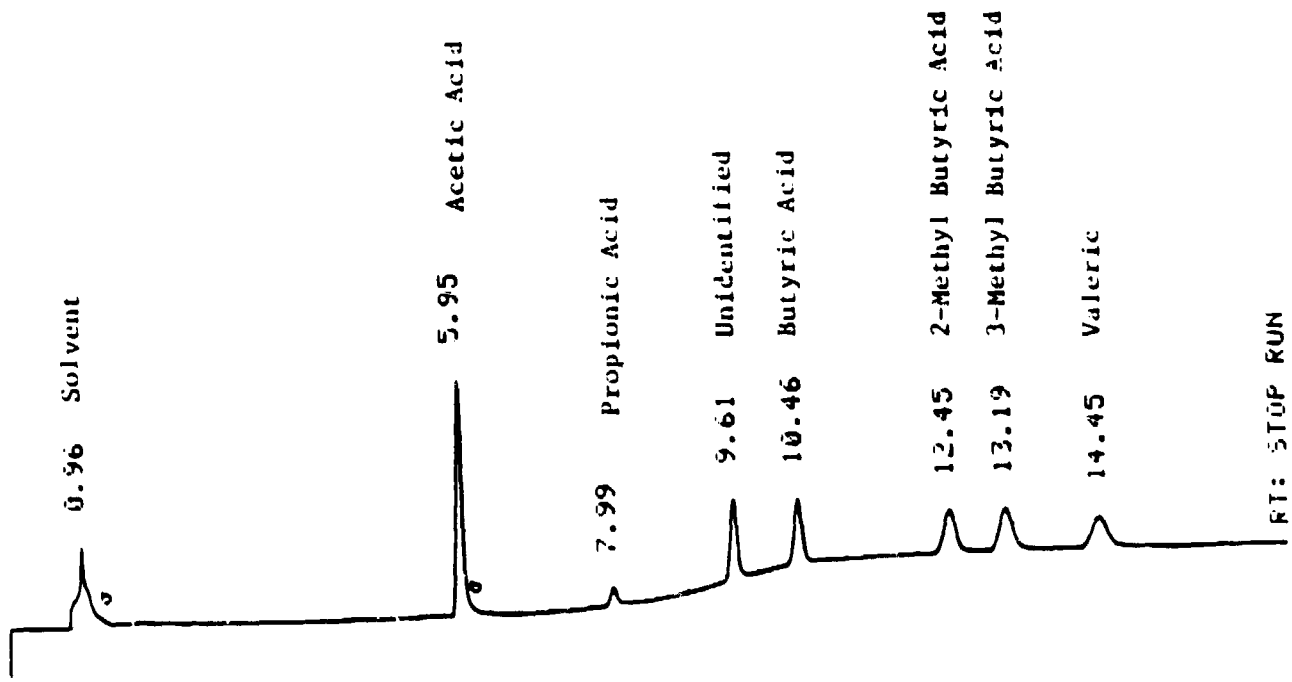


Figure 11. Chromatograph Showing the Production of Short Chain Acids from CP1÷2 Depolymerized Products of a Leonardite using a Mixed Anaerobic Culture Developed at ARCTECH

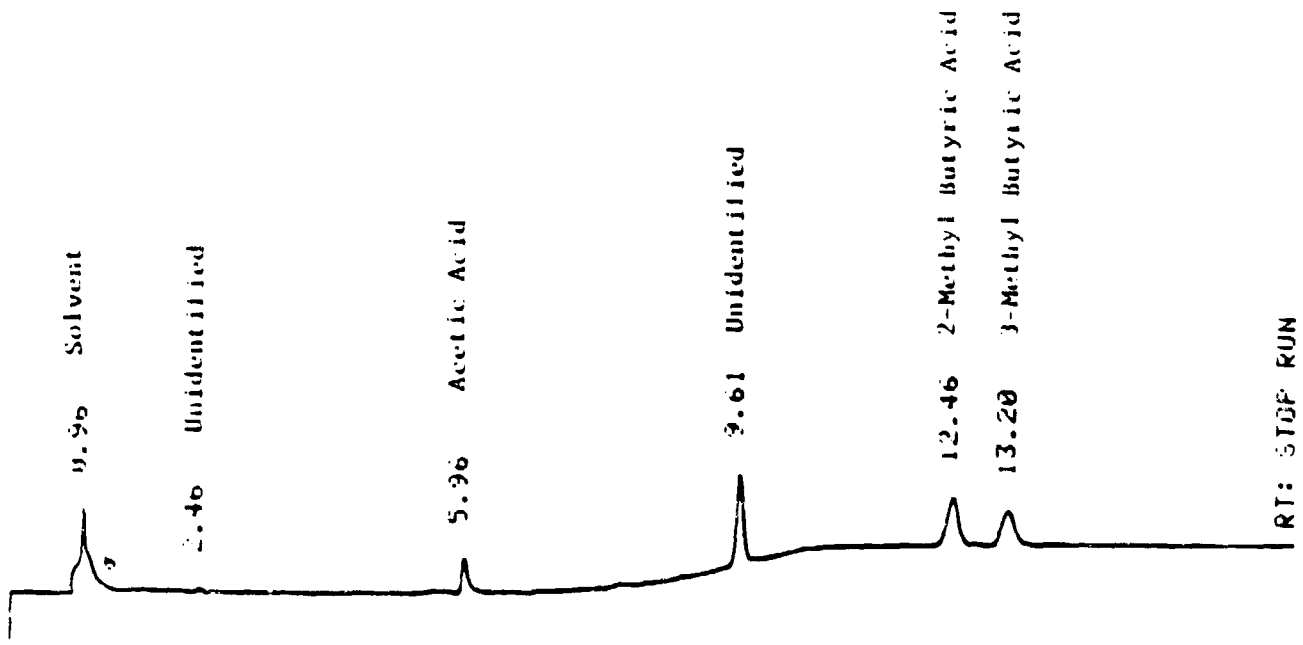


Figure 12. Chromatogram Showing the Production of Short Chain Acids from CP1+2 Depolymerized Products of a Beulah Lignite using a Mixed Anaerobic Culture Developed at ARCTECH

products, and the anaerobic bacteria were then adapted using these materials. The data indicated that more acidic compounds were produced from Leonardite coal products (Figure 12) than from Beulah products (Figure 11). Short chain fatty acids were also produced when Leonardite coal was used directly for anaerobic conversion to methane.

The formation of different types of acids from these coals, and biodepolymerization products indicated that these feedstocks are different. The formation of various methanogenic precursors from different coals is, perhaps, most important in determining the bioreactivity of the coals for use as a biological substrate for methane production.

These results suggest that short chain alcohols and acids are produced during coal biodegradation to methane. Because the culture systems contain mixed bacterial populations, these products are being used as methane precursors and do not accumulate unless methanogenesis is blocked.

7. Culture Adaptation

Early experiments were conducted in the presence of 0.02% benzoate. Control tubes were included in all the experiments and methane production from controls was subtracted from methane production in the presence of coal or coal products. In the course of adaptation, benzoate concentration was decreased and finally excluded from the medium so that the cultures are grown only with coal or depolymerized coal products as the sole carbon sources. This process of bacterial adaptation is the key to coal bioconversion to methane. When the bacterial cultures are well acclimated, experiments dealing with carbon mass balance, and the rate of methane production per unit of carbon utilized can be conducted.

Adaptation experiments using sewage sludge cultures, horse manure-hay compost cultures incubated with 1) Leonardite (untreated), 2) Texas lignite (untreated), 3) Beulah products (H_2O_2-NaOH/heat treated), 4) Beulah (untreated), 5) Wyodak product (H_2O_2-NaOH-acid precipitated) and 6) aqueous soluble coal products (no acid precipitation) were also conducted. The preliminary data indicated that the coal or coal biodepolymerization product(s) were converted

to methane by anaerobic bacterial consortia. The active cultures were transferred to fresh anaerobic medium containing coal (with and without benzoate) for further adaptation.

Anaerobic conversion of Texas lignite was also evaluated. Preliminary experiments with this coal indicated that chicken waste organisms adapted well to this coal. Methane production from coal incubated with adapted chicken waste and sewage sludge cultures is reported in Table 44. Methane was produced from both of the untreated coals by the chicken waste and sewage sludge adapted cultures. Maximum methane production occurred between 35 and 45 days. The production of methane was enhanced when the cultures were adapted for a longer time, indicating that bacterial acclimation had occurred and may continue with additional adaptation to specific coal(s).

Although adaptation of microbial consortia for conversion of coal to methane requires extended incubation periods, the resultant increase in methane production is significant (Figure 13). Adaptation of the sewage sludge consortium with Leonardite resulted in at least a two-fold increase in methane production. A similar increase in methane produced from Texas lignite by a termite-derived culture was also observed. Based on these data, the time required for specific microbial culture/coal adaptation is time well spent in the quest for maximum coal carbon conversion to methane.

An additional manipulation of the consortium was also evaluated. In some instances key methanogens producing methane from intermediary compounds such as acetate and propionate were lost during culture transfer. This phenomenon is not unusual because of the lower biomass of methanogens in the culture medium. The addition of methanogens such as *Methanosarcina* sp., which metabolize acetate, H_2 and CO_2, or *Methanothrix* sp., which uses acetate as the carbon source, will be used to achieve the optimal proportion of acetogens and methanogens in the culture system.

Addition of these methanogens to two consortia derived from termites resulted in a two- to three-fold increase in methane production from Texas lignite (Figure 14). These results confirm that the ratio of acetogens to active

Table 44. Direct Production of Methane from Coals by Anaerobic Bacteria

Coals	Inoculum Used	Methane Produced,** Mole%	
		2 Mos. Adaptation	6 Mos. Adaptation
Leonardite	Chicken waste	7.8	25.6
	Sewage sludge	11.4	19.3
Texas lignite	Chicken waste	9.1	----
	Sewage sludge	6.4	----

* 1% (w/v) coals were used.
** Methane from control samples (no coal) was substracted.

Fig.13

Effect of culture adaptation on methane production from coals

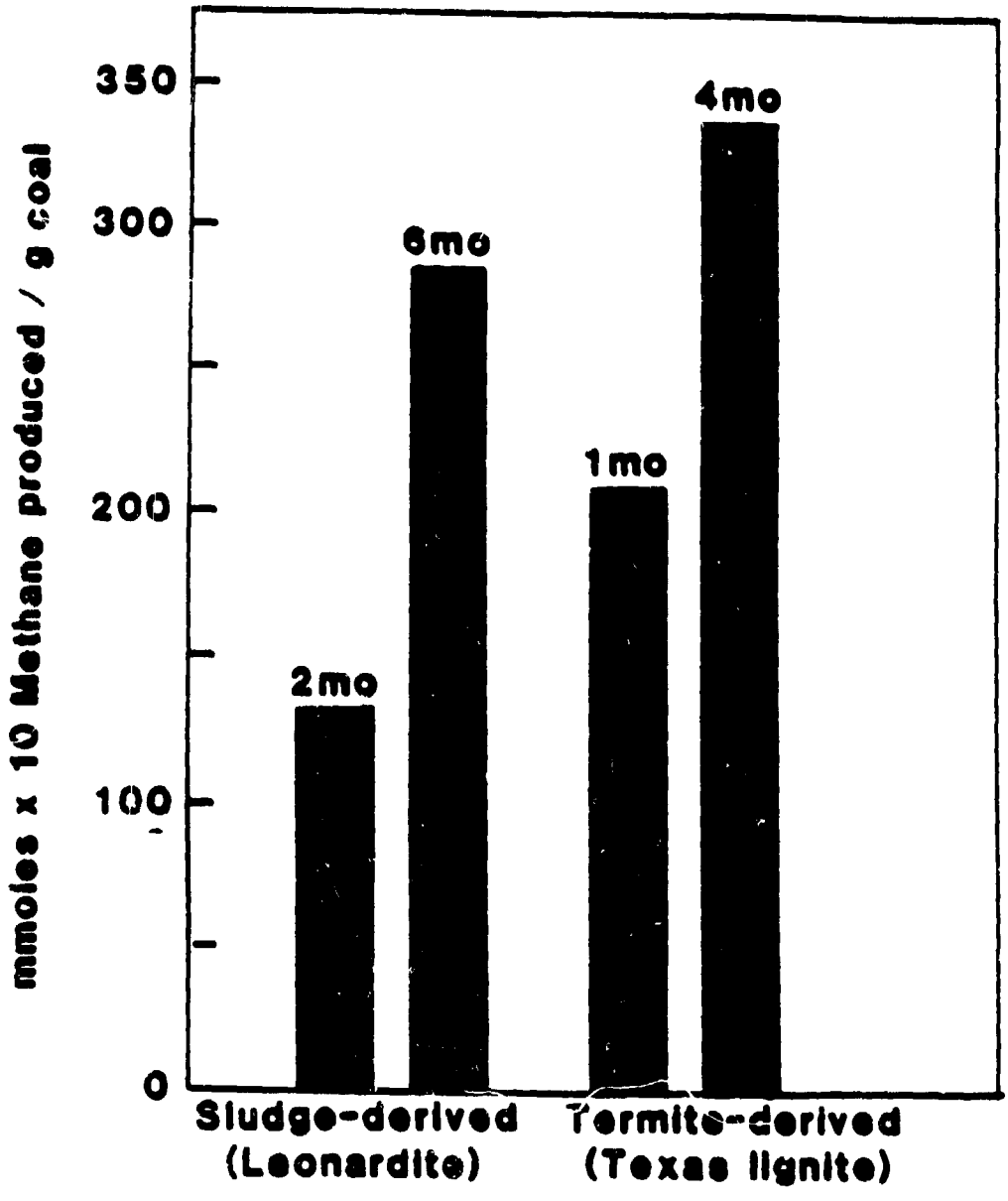
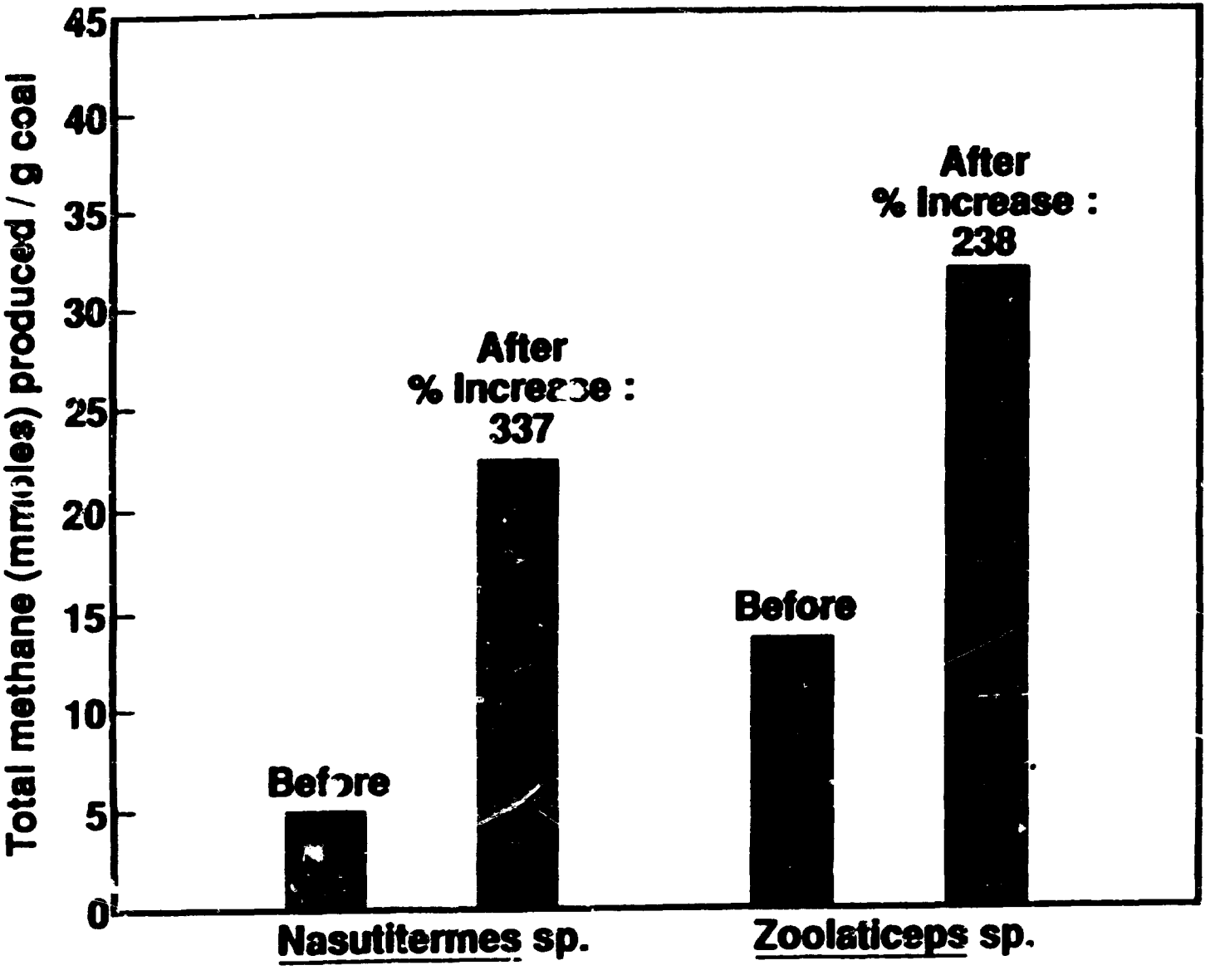


Fig.14 **ENHANCED METHANE PRODUCTION BY METHANOGEN ADDITIVES***



*15 day incubation

methanogens in a consortium is extremely critical for efficient conversion of coal carbon to methane.

8. Culture Development

Enriched bacterial cultures obtained from different natural anoxic environments were monitored for coal-substrate specificity. Each of the four cultures was incubated with untreated Texas lignite and methane production was monitored. Control mixtures resulted in negligible methane production in the absence of coal, irrespective of the source of inoculum. Methane production was demonstrated when coal was present in the reaction mixture. The sewage sludge consortium produced the most methane, followed by horse manure/hay compost, chicken waste and leaf litter consortia. Additional adaptation of the cultures was carried out and subsequent testing of the cultures with untreated coals was carried out. In spite of regular transfers some cultures have failed to produce methane in significant quantity during the adaptation period. Specifically, the chicken waste consortium seems to accumulate large amounts of acetic acid and other intermediate products but does not reproducibly produce methane. This result is not totally unexpected as the major components of chicken waste itself are organic acids, indicating that these products are the preferred end products for the naturally occurring chicken waste microorganisms. Testing of these cultures will be discontinued in order to focus attention on cultures which seem better suited for use in the bioreactor studies.

a. Consolidated Cultures and Termite-Derived Culture

The best cultures identified during the first part of this project were transferred to fresh medium with and without coal, benzoate was removed from the medium to force cultures to grow only on coal or coal products and inoculation of cultures into bottles containing 40-60 ml medium was carried. Total gas and methane production were used as an indication of culture activity with analysis of liquid samples for short chain fatty acids.

Six inoculum sources -- horse manure compost, chicken waste, two sewage sludge samples and two leaf litter samples -- which showed significant bioconversion of Beulah coal product were combined in 150 ml serum bottles with

75 ml of medium and monitored for methane and for organic acid and alcohol production. The medium, pH 7.2, contained pretreated Beulah coal as the only source of carbon other than a small amendment of yeast extract. Within 19 days, 33.8 cc of total gas were produced, 28 mole% as methane. Calculations showed that bioconversion of the pretreated Beulah lignite resulted in production of 1556 cc methane per gram of coal carbon. This result indicated that the consolidated consortium was capable of rapid and efficient coal carbon conversion. This consolidated consortium (BC-1) was further adapted in anticipation that it could serve as an ideal inoculum for the bioflo reactor studies. The consortium was transferred into multiple 150 ml serum bottles with fresh medium containing Beulah lignite as the carbon substrate. During the adaptation/incubation period, samples of the headspace were removed to monitor gas production and aqueous samples were analyzed for methane precursors.

The consolidated culture was monitored for methane production from various coal derived products and raw coals. Inoculation of a reaction mixture with BC-1 for fermentation of pretreated Beulah coal was carried out at 37°C under static conditions. Small amounts of methane were produced in control samples (no coal carbon). The control methane was subtracted from methane production in the experimental reaction mixtures to allow for calculation of cc methane/gram of coal carbon. Within 20 days of incubation, the coal reaction mixtures produced 291 cc methane per gram of Beulah coal product. This represents at least a 53% coal carbon conversion to methane. The methane produced in control samples is likely derived from carry-over coal and intermediate products present in the inocula and from media constituents, in particular the yeast extract.

Preliminary data obtained using the BC-1 culture in biogasification of Texas lignite, Beulah lignite and Beulah coal-derived products indicate that methane was produced during the first five days of incubation. Controls containing no coal showed very little methane in the headspace gas. Methane concentrations from Texas lignite provided as a substrate at 0.1% and 0.5% solids were essentially the same. Methane concentrations in the headspace of the Beulah lignite (0.1% w/v) reaction mixture were similar to methane production from Texas lignite, with somewhat more methane being produced from Beulah lignite at 0.5% solids loading. The most dramatic difference in

biogasification was noted when Beulah coal-derived products were used as the substrate. When the Beulah products were provided at 0.1% solids, significantly more methane was produced from these products than from the raw coals. However, when the coal products were provided at 0.5% solids, methane production was similar to that observed with the raw coals.

A different consolidated culture (KC-1) created by combining the chicken waste consortium, the horse manure compost consortium, and two sewage sludge consortia was also tested. This culture produced methane from a mixture of Beulah lignite coal products and Texas lignite and was used to test the simulated underground stirred tank reactor. An aliquot of the KC-1 consortium was used to inoculate this reactor vessel (total volume 1500 ml). Methane was produced by this consortium in the stirred tank reactor (11 mole% in headspace gas). A leak in the bioreactor inactivated the consortium and resulted in shutdown of the system and subsequent modification of the reactor.

The two consolidated cultures, KC-1 and BC-1, were used in experiments to monitor methane production from various raw coals and the Beulah coal product. BC-1 was inoculated into a basal salts medium with and without coal or Beulah coal products and incubated at 37°C under static conditions. The gas phase was sampled and analyzed for methane. The aqueous phase was analyzed for short-chain fatty acids and alcohols. Small amounts of methane were produced in samples without coal or coal products (controls) (Table 45). Within 6 days of incubation, this culture produced up to 61 cc methane per gram of Beulah product. This represents at least a 10% coal carbon conversion to methane. Methane was also produced from raw Texas lignite and Beulah lignite, but in small quantities. The liquid samples were analyzed for the presence of methane precursors. Acetic and propionic acids were the major acids accumulated during this short incubation period. Up to 133 mg of acetic acid and 43 mg of propionic acid per gram of coal accumulated in the medium. Small amounts of acetic and propionic acid were detected in samples without coal (medium only).

The KC-1 consortium was used in biogasification of raw Texas lignite, Beulah lignite and a premium Beulah lignite. Preliminary data indicated that methane was produced within 7 days of incubation. This experiment was conducted using 500 ml bottles containing 225 ml anaerobic medium and 5 ml of

Table 45. Production of Methane and Short Chain Fatty Acids from Different Coals Inoculated with a Consolidated Culture (BC-1)

	6 Day Incubation Acids, mg/g of Coal		
Coals	Methane cc/g Coal	Acetic	Propioinic
Control, No coal	0.2	1.7	0.7
Texas lignite, 0.1%	16.9	131.8	42.7
Beulah lignite, 0.1%	17.7	115.7	37.3
Beulah - product, 0.1%	60.7	132.9	36.4

inoculum (rather than the usual 10% inoculum). The use of small amounts of inocula should minimize the carry-over of unreacted coal and other short chain fatty acid products accumulated during culture build-up. Methane production from Beulah lignite was higher than methane obtained from Texas lignite and premium Beulah lignite samples (Table 46). Methane production continued and within 12 days of incubation up to 77.5 cc methane was produced per gram of Beulah lignite.

Preliminary studies with KC-1 indicated that thi culture produced up to 129 cc of methane/g of Texas and Beulah lignites within 26 days of incubation. The other consortium, BC-1, also produced methane from raw (untreated) Texas and Beulah lignites; however, upon transfer, neither of the consolidated cultures produced significant amounts of methane from any of the coals tested. The cultures ceased producing methane after a few days of incubation. The results from the BC-1 consolidated culture at 4 days incubation are presented in Table 47. This culture produced methane from untreated Texas and Beulah lignites as well as from Beulah coal products. Acetate (up to 80 mg/g of coal) was also noted when cultures were grown in the presence of Texas and Beulah lignites but not when Beulah coal products were fermented. A stock culture of each consortium was defrosted and inoculated into anaerobic medium for build-up of biomass and subsequent use in comparative biomethanation experiments.

Biogasification of Texas lignite (0.2% w/v) by BC-1 and KC-1 was compared to biogasification using a *Zoogermopsis* sp. derived culture. The basal salts medium for the termite-derived culture was somewhat different than the anaerobic medium used for the consolidated culture. The termite culture medium contained low-sulfate minerals and 0.1% each of Trypticase soy broth (TSB) and yeast extract. Cultures (5 ml/20 ml medium) were inoculated in duplicate. Control bottles contained no coal but were inoculated with equal amounts of seed culture. Methane production by the termite culture reached 73 cc methane/g of coal within 18 days of incubation. Methane production increased with further incubation and reached 272 cc/g of coal within 45 days of incubation. After 45 days of incubation this consortium was supplemented with a 0.5 ml inoculum of a methanogenic culture containing predominantly *Methanothrix* sp. and *Methanosarcina* sp. The cultures were incubated further and the production of

Table 46. Production of Methane from Different Coals Inoculated with a Consolidated Culture (KC-1)

Coals	Methane, cc/g of Coal	
	Day 7	Day 12
Control, No coal*	7.3	28.4
Texas lignite**, 0.1%	16.3	68.9
Beulah lignite**, 0.1%	51.5	77.5
Premium Beulah lignite**, 0.1%	12.9	41.8

*Large volume of media might have contributed higher methane production in this sample.
**Control methane was deducted before calculating methane per gram of coal.

Table 47. Production of Methane and Acetate from Different Coals Using ARCTECH's Consolidated Bacterial Consortium (BC-1)

	Incubation, 4 days	
	Methane cc/g Coal	Acetate mg/g Coal
Texas lignite	52.3	75.8
Beulah lignite	31.0	80.3
Beulah lignite	9.4	14.9
Control (No coal)	1.4	1.5

Note: Coal concentration used: 0.1% static conditions; 37°C. Methane and acetate produced from the control samples were deducted before cc or mg/g of coal was calculated.

methane and CO_2 was monitored 15 days after the cultures were supplemented with the methanogenic populations. CO_2 concentrations decreased from 410 to 53.8 cc/g of coal while methane increased from 272 to 439 cc methane/gram of coal (Table 48). Methane obtained from controls (no coal plus inoculum) was subtracted from experimental methane production before calculating methane production per gram of coal even though control methane was minimal. Initial headspace CO_2 and CO_2 produced by controls were also deducted before calculating CO_2 production. Total carbon conversion in this experiment was calculated to be greater than 71%.

The BC-1 consortium was grown with untreated Texas lignite and Beulah lignite. Results indicated that methane was produced from both coals by this consortium although the Texas lignite at 0.1% solids was found to be the best substrate for methane production. Within 60 days of incubation, the BC-1 consortium produced up to 111 cc methane per gram of Texas lignite. The methane production from Beulah lignite (0.1% w/v) was 45.3 cc/g of coal.

9. Bioreactor Studies

Three bioreactor designs were considered as viable candidates for application in a demonstration of continuous methane production. Three configurations identified were: 1) an anaerobic batch reactor simulating an underground stirred tank biogasification process, 2) a rotating biological contactor (RBC) and 3) up-flow fluidized-bed reactors.

a. Simulated Underground Reactor

The anaerobic batch reactor simulating the use of an underground cavern as a bioreactor is a two liter glass jar fitted with temperature control via a heating tape, gas inlet and gas outlet ports (Figure 15). Nitrogen is bubbled into the medium to provide agitation of the reaction mixture and to provide anaerobic conditions. Anaerobic basal salts medium used in this reactor was similar to medium used in the bacterial enrichment studies. Initially 400 ml of the medium was inoculated with 100 ml of culture. A coal concentration of 0.2% (w/v) was used. The medium-coal mixture was autoclaved before inoculation. Resazurin was used as an oxygen

Table 48. Production of Methane and Carbon Dioxide from Texas Lignite by *Zootermopsis* Termite-Derived Bacterial Consortium Supplemented with Methanogenic Bacterial Cultures

	cc Gas Produced/g of Coal*			
	Before Methanogen Addn		After Methanogen Addn	
	Methane	CO_2	Methane	CO_2
Control, No Coal	5.5	9.9	1.3	16.2
TX Lignite (0.1%)	272.2	410.0	439.0	53.8

*Methane and CO_2 produced from the control samples were deducted before cc gas/g coal was calculated.
Static incubation, 37°C.

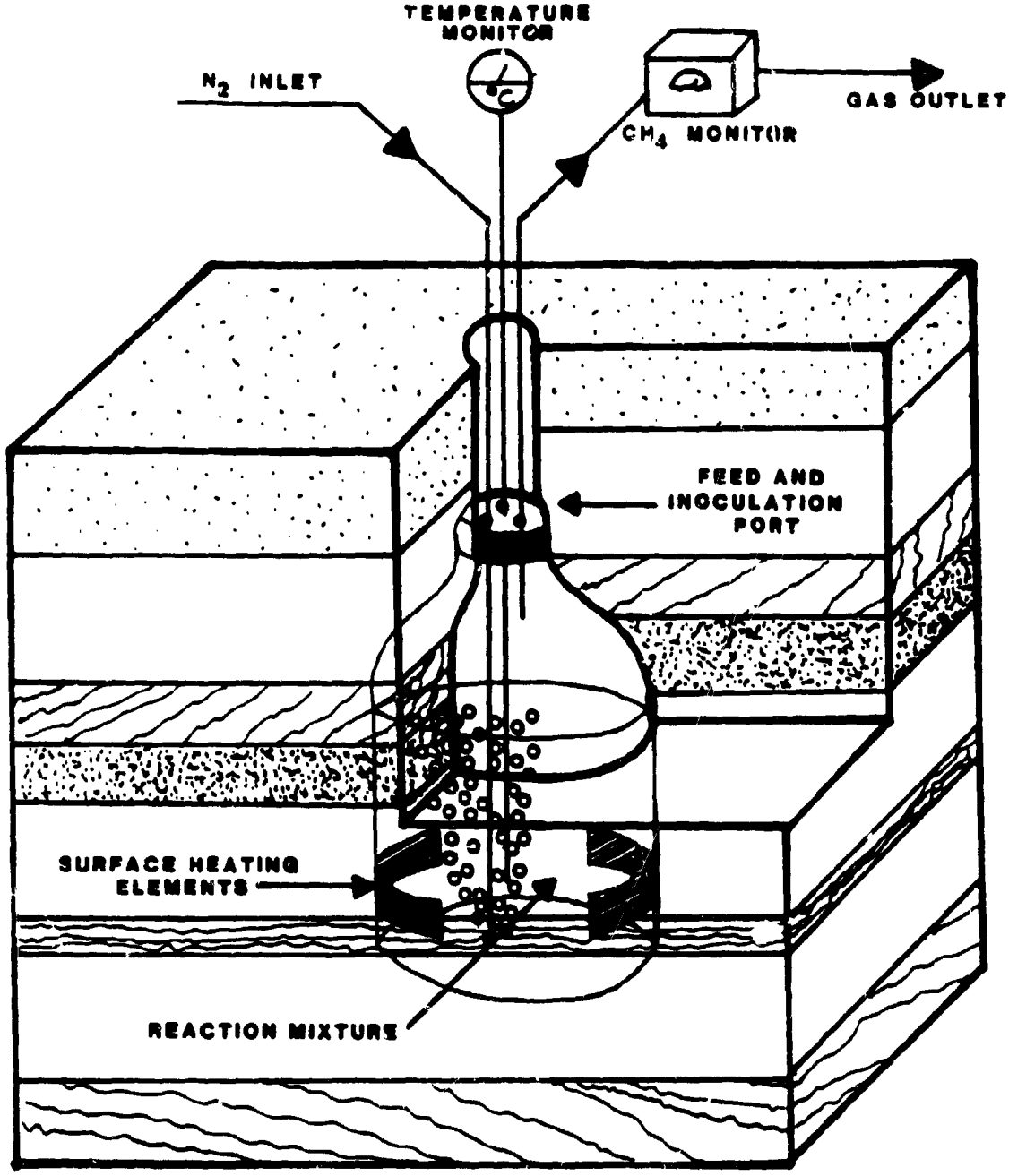


Fig. 15

SIMULATED UNDERGROUND BIOGASIFICATION REACTOR

indicator and sodium sulfide was used to reduce the medium. The temperature of the reactor was maintained at 37°C and additional agitation was provided using a magnetic stirrer. Gas flow in and out was measured to monitor bacterial activity in the fermentative reaction mixture. Liquid samples were taken periodically to monitor for intermediate products.

The consolidated culture, BC-1, obtained by mixing the cultures demonstrating the best biogasification of lignite coal during the culture development studies, was used as the inoculum for the simulated underground reactor. The reactor contained 400 ml medium and 100 ml culture for the initial incubation period in which biomass accumulation was the objective. Once biomass had accumulated, additional medium was added to the reactor to make a total working volume of 1500 ml. Texas lignite was used as the coal substrate at 0.2% (w/v).

The bioreactor was not airtight as shown by oxidation of the medium in the reactor. The presence of oxygen resulted in the inactivation of the anaerobic microorganisms in the reactor. The bioreactor was modified and tested for maintenance of anaerobic conditions using reduced medium containing resazurin as an indicator for oxidation. Upon demonstration of sustained anaerobic conditions in the bioreactor, the BC-1 consolidated culture was inoculated into 400 ml medium for production of additional biomass. A mixture of Texas lignite (0.2% w/v) and Beulah coal (0.1% w/v) derived product was used as the substrate in a total working volume of 1500 ml in the reactor. Methane production reached 11 mole% in the headspace gas but did not increase significantly thereafter.

b. Semi-continuous Flask Reactor

A semi-continuous reactor was set-up using a 500 ml side-arm flask closed with a black rubber stopper (Figure 16). This reactor is provided with medium inlet, outlet and a gas sampling ports. Semi-continuous feed of untreated Texas lignite at 0.5% solids concentrations is provided through the medium inlet. The KC-1 consolidated culture and two termite-derived cultures (*Zootermopsis* and *Nasutitermes* sp.) were used as inocula. The reactor was fed with 0.5% coal slurry prepared in anaerobic medium at a rate of 25 ml/day (30 day

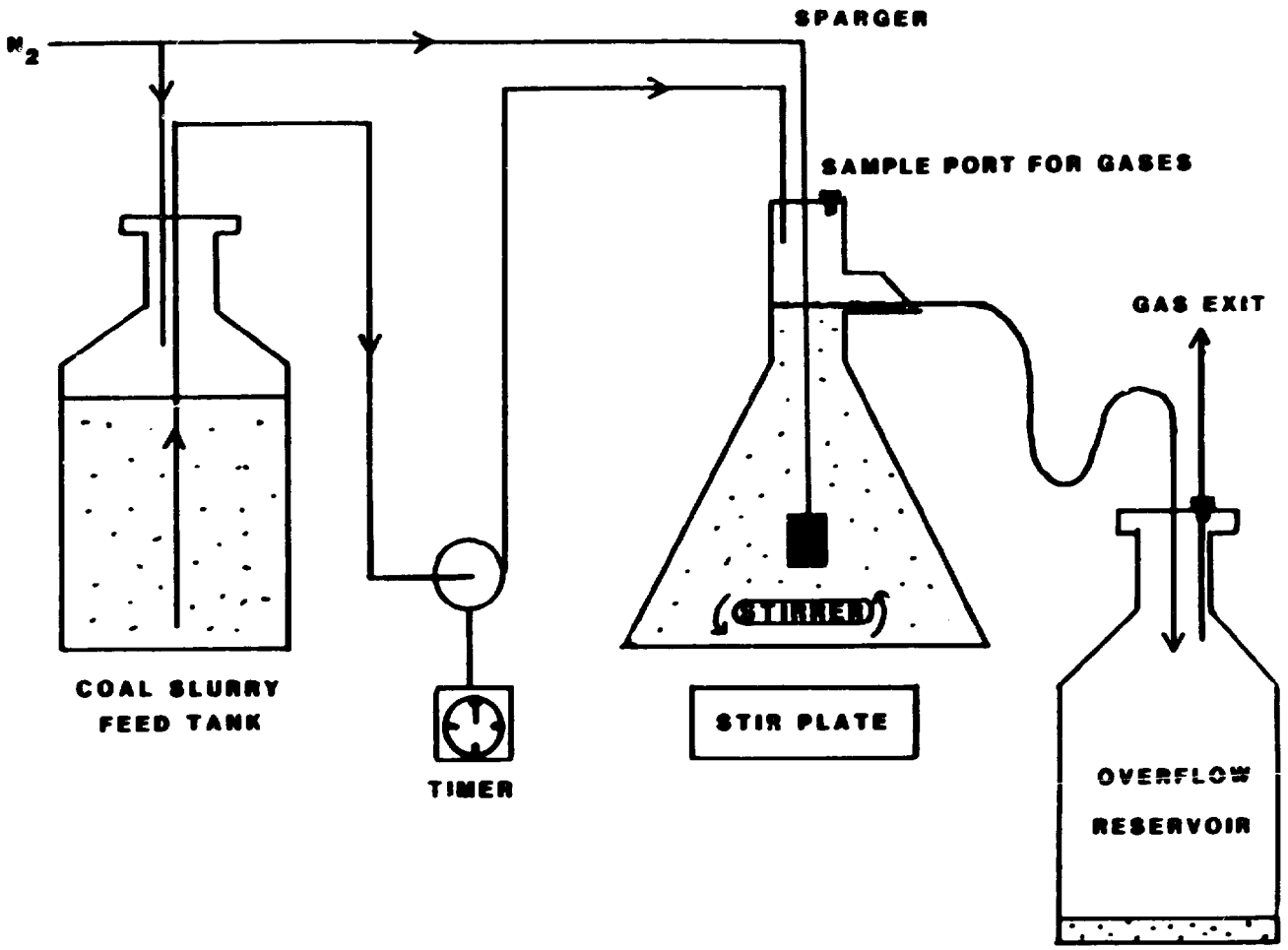


Fig. 16 SCHEMATIC DIAGRAM OF A SEMI-CONTINUOUS BIOREACTOR

retention time). Methane was produced in this reactor when the retention time was between 25 and 30 days. When the feed rate was changed to achieve a 20 day retention time, methane production was decreased somewhat. Following an adjustment to the feeding rate which resulted in a retention time of 16 days, methane production was no longer observed. This brief study indicated that continuous production of methane can be achieved in a semi-continuous system when the retention time is adjusted to conserve the biomass within the system for methanogenesis.

c. Up-flow Fluidized Bed

Five up-flow fluidized column reactors were constructed and are being tested for another project (EPRI). These reactors are constructed from plexiglass columns which are 2' high with a 3" I.D. and an approximately liquid volume of 2.65 liters (Figure 17). The reactor is provided with a port for medium recirculation and a second port which serves as a gas outlet. Additional sample ports can be provided on the plexi-glass column itself as required. Anaerobic medium with coal solids loadings of 1-10% (w/v, dry weight) Texas lignite (-325 mesh) was inoculated with anaerobic sewage sludge and ARCTECH's compost culture at the 10% level (v/v). The slurry is recirculated through each reactor using a Masterflex pump. Methane production and fluidization of the coal were monitored. Minimal methane production was observed in the control column reactor without coal over seven days of incubation. At 1% and 2% solids loadings, the methane concentration in the headspace reached more than 7 mole% in 4 days but decreased steadily thereafter. The concentration of methane in the headspace of the 5% solids reactor reached 2.3 mole% by day 4 and insignificant methane production was observed when solids were provided at 10%. Additional investigations are needed to further evaluate the potential of up-flow fluidized bed reactors. The major advantage of such a reactor system is the conservation of biomass in the fluidized zone of the reactor and the provision of relatively particulate-free substrates via an acetogenic reactor into a methanogenic reactor.

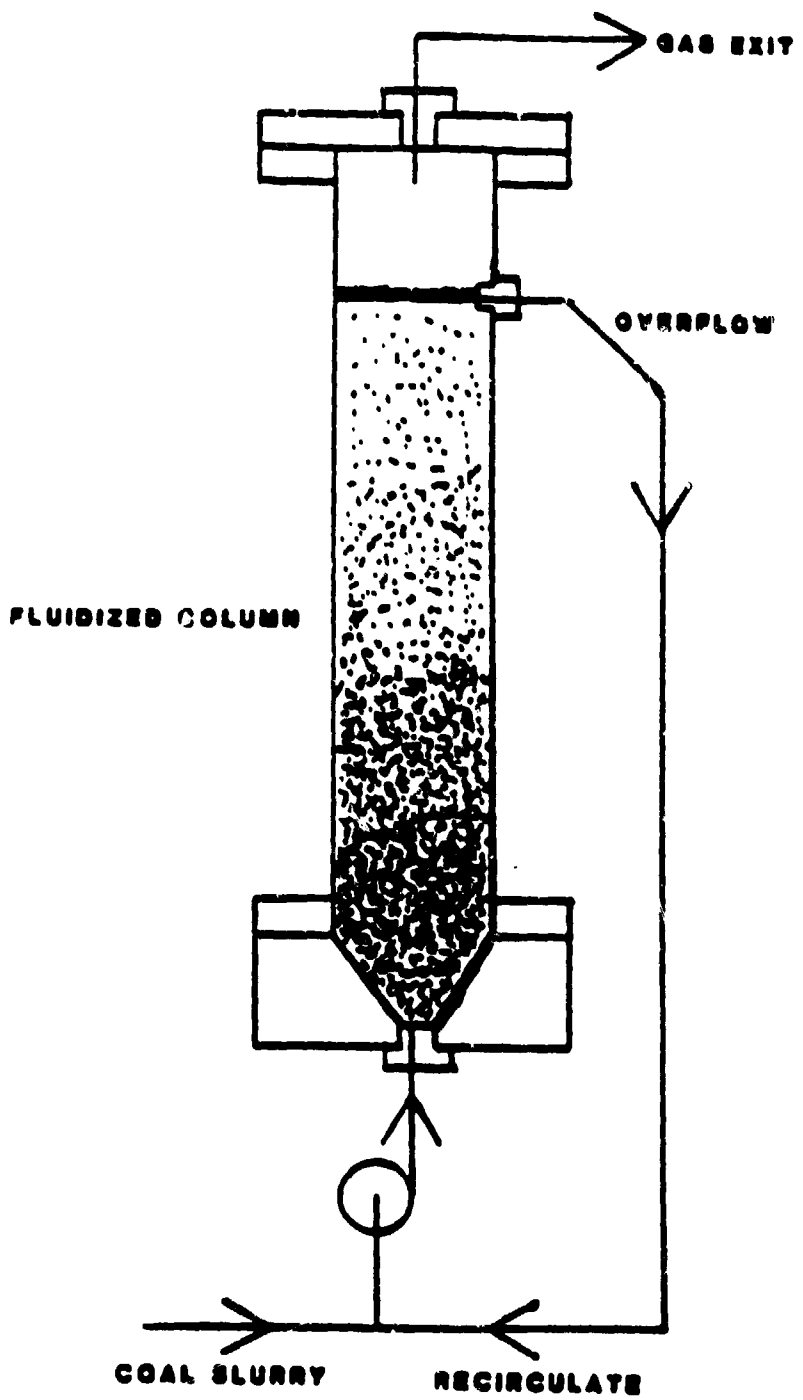


Figure 17 Schematic diagram of fluidized column reactor

d. Rotating Biological Contractor

A bench-scale rotating biological contactor type reactor (total volume 7.5 liters) was constructed at ARCTECH and developed for use under anaerobic conditions. This reactor (Figure 18) is equipped with sample ports, media feed inlets and overflow outlets. A system has been devised to measure total gas production. Preliminary tests evaluated the mixing potential for this reactor configuration. Good mixing of the coal at 5% solids was demonstrated. Sewage sludge and ARCTECH's coal compost culture were used as inocula. A 5% (w/v) coal slurry prepared in anaerobic medium was used as substrate. Development of the desired biofilm on the medium used for the plates in the reactor was monitored. Visual observation of the reactor biofilm formation indicated that good biofilm formation occurred and was quite stable within the rotating system.

During operation of the bioreactor, nitrogen was provided to maintain anaerobic conditions. Initially, acetate and benzoate at 0.1% (w/v) were provided as a feed supplement for acetogens and methanogens to allow for the most rapid biofilm production. The addition of these substrates initiated methane production (up to 4.4 mole%) in the reactor within four days of operation.

A second bench-scale rotating biological contactor reactor was constructed at ARCTECH to address problems such as leakage and breakage of the shaft during continuous operation. Mixing of a 5% coal water slurry in this reactor was monitored and results indicate that coal settling is minimal with more than 90% of the particulate coal being suspended in the liquid due to the agitation provided by the constantly rotating media discs. This reactor is a modification of the previous reactor designed to withstand the stress and shear imposed on the shaft during constant rotation. The reactor was fed with 5% coal slurry (Texas lignite, D_m of -325 mesh) and inoculated with a mixture of ARCTECH's coal compost and sewage digestor sludge at 10% (v/v). Neither benzoate nor acetate was added to the reaction mixture. Good biomass buildup was observed on the rotating discs and suspension of more than 90% of the particulate coal in the system was confirmed. Methane production was monitored on a regular basis and data indicated that methane production increased with time (Figure 19).

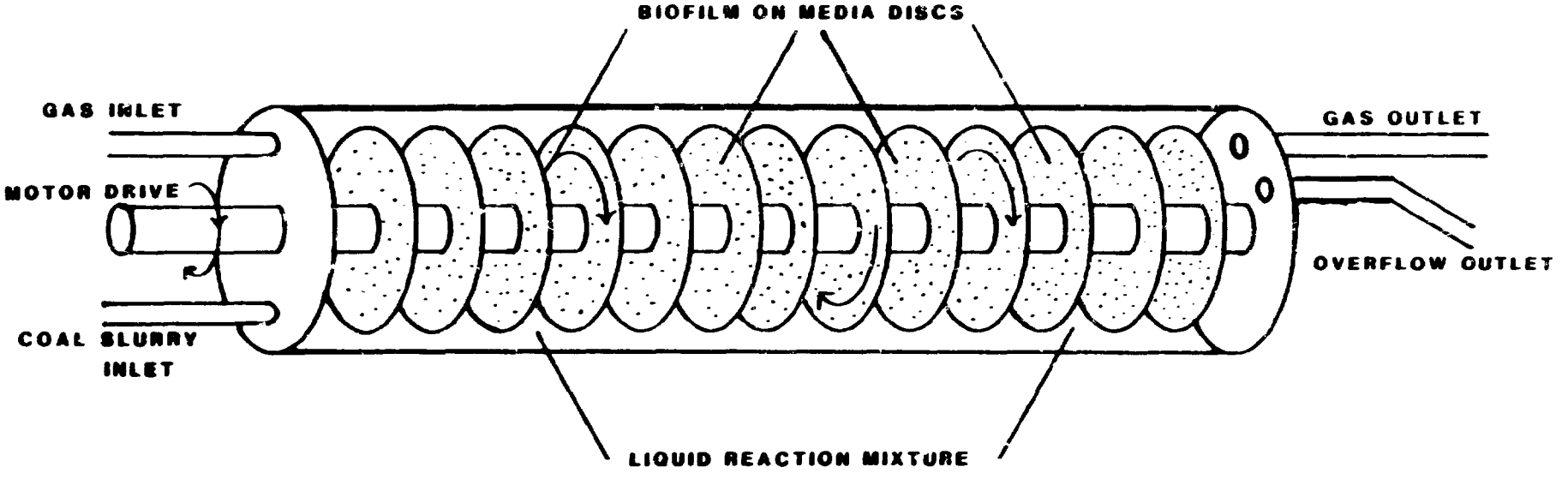
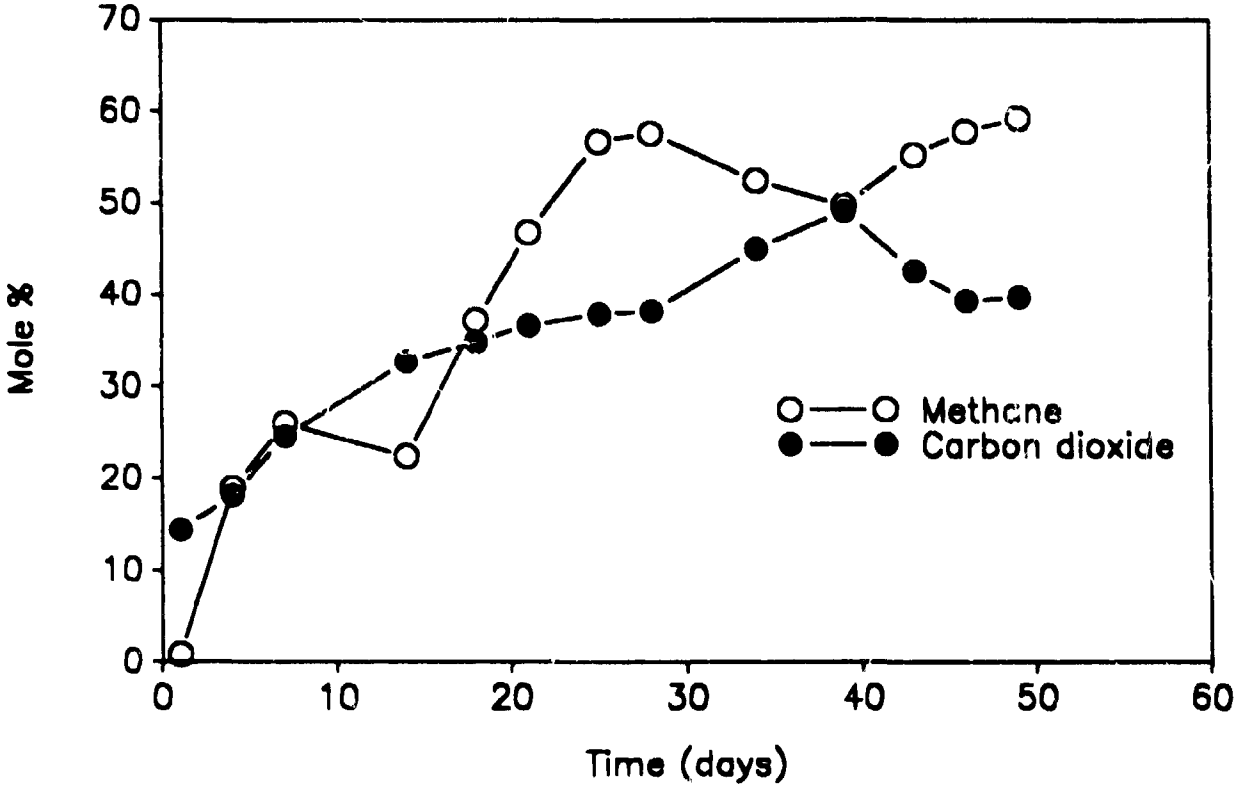


Fig.18 RBC BIOREACTOR DESIGN FOR CONVERSION OF COAL TO METHANE

Figure 19. Production of methane and carbon dioxide from Texas lignite with sewage sludge culture grown in a Biodisc reactor.



Within twenty days of operation, methane concentrations found in the headspace gas has reached 43.6 mole% with a CO_2 concentration of 36.7%.

Methane production increased steadily up to 26 days of incubation, followed by a slight decrease over the next 4 day period. The decrease in methane production corresponds to an environmental change brought about by the addition of heat to maintain the reactor temperature at or near 35°C (rather than at ambient temperature). After the four day period in which no increasing methane production occurred, an increase in methane production was observed. The CO_2 concentration peaked at 44.9 mole% at day 35 and decreased steadily (Table 49). By day 49 of operation, total gas produced reached 1200 cc per day with a methane concentration of 59.2 mole% and CO_2 concentration of 39.7 mole%. Methane production has continued over more than two months of continuous operation. A maximum methane concentration of more than 80 mole% in the headspace gas was reached after 80 days of operation.

This reactor, the rotating biological contactor, appears to create good mixing of particulate coal at 5% solids and to result in the production of adequate biofilm to achieve good conversion of Texas lignite. This reactor is the first demonstration that increased solids (up to 5% w/v) can be successfully used in continuous production of methane. It is believed that scale-up of the volume, coupled with build-up and conservation of biomass via the biofilm on the disc media are the key factors for the success in bioconversion of coal carbon to methane in this reactor. This reactor is in operation and will be monitored for methane, CO_2, total gas, organic acids and alcohols until methane production peaks. Upon observation of decreasing methane production, 50% of the reactor contents will be removed for analysis of the residual coal and the aqueous phase. Continuous feed of coal slurry in anaerobic medium will be initiated at a flow rate designed to result in a 25 day retention time. Headspace gases and the aqueous phase will be monitored on a regular basis.

Table 49. Production of Total Gas, Methane and Carbon Dioxide from the Bio-disc Reactor Fed with 5% Texas Lignite Slurry and Inoculated with Sewage Digestor Culture

Days	Total gas (cc/d)	Methane (mole%)	Carbon dioxide (mole%)
7	n.d.	26.0	24.6
14	n.d.	22.4	32.7
21	n.d.	46.8	36.7
28	n.d.	57.6	38.2
35	n.d.	53.2	44.9
41	425	52.7	43.9
49	1200	59.2	39.7

n.d.: Not determined

IV. PROCESS DESIGN AND PRELIMINARY ECONOMIC EVALUATION FOR A LIGNITE BIOGASIFICATION PROCESS

A. Introduction

Sufficient laboratory data on the biological conversion of low-rank coals to methane has now been obtained to allow a preliminary process design and cost evaluation. These preliminary estimates will undoubtedly change as process conditions are more precisely defined by further research.

The process design is based on current state-of-the-art technology for high-rate anaerobic bioconversion of soluble organic substrates. ARCTECH has proposed a two-stage process for bioconversion of coal to methane. In the stage-one reactor, pulverized coal is solubilized and converted to methane precursors by hydrocarbon degraders and/or hydrolyzers. Products (short chain acids and alcohols, CO_2, H_2) from the stage-one reactor serve as substrates for methanogenesis in the stage-two reactor.

The proposed bioreactors are up-flow type which combine the desirable characteristics of both sludge blanket and fluidized bed reactor. Biomass retention and conservation in the reactors is achieved by microbial attachment to fluidized coal particles, and hydraulic design which ensures minimal washout of solids. These conditions allow for shorter hydraulic residence time coupled with longer solids residence time. This means that in the stage-one solubilization/acid forming reactor, delivery of solubilized products to the stage-two methanogenic reactor is more rapid. In the stage-two reactor, maximum biomass concentration is maintained to enhance rates of methanogenesis.

When utilizing laboratory data in a full-scale plant costing exercise, ARCTECH has made certain assumptions. Productivity of bioreactors is dependent upon the biomass concentration within the reactor. Most of ARCTECH's work to date has involved batch-mode screening and enrichment experiments which are limited by biomass concentration, although it is known that biomass concentrations of at least 3% w/v can be achieved in continuously fed up-flow reactors. ARCTECH has achieved great improvements in coal carbon conversion to methane in its Biodisc reactor, which retains more biomass than batch reactors.

Based on these assumptions ARCTECH has designed a full-scale bioreactor at 80% carbon conversion and 75% (v/v) CH_4 in the biogas.

State-of-the art technology for coal grinding, waste handling and biogas purification are also included. Biogas purification is necessary to separate CO_2 from CH_4 so that pipeline quality (> 94%) methane is produced. CO_2 recovered from the biogasification process could be sold as a byproduct.

A design basis of 100 Te hr^{-1} coal (Texas lignite) feed has been chosen to provide sufficient energy to feed a 300 MW gas-fired power plant. The biogasification plant is situated at the mine. Run-of-mine lignite is assumed to be available at the battery limits. Products are pipeline quality methane and carbon dioxide.

PLANT SIZE:	100 Te hr^{-1} lignite
OPERATION:	330 days per year
METHANE PRODUCTION:	768 Te d^{-1} (38 MSCFD)
	(to feed 300 MW power plant)
CARBON DIOXIDE PRODUCTION:	748 Te d^{-1} (13.5 MSCFD)

For costing purposes the plant has been divided into plant areas I through IV, i.e. Coal Grinding, Bioreactors, Gas Purification and Waste Handling. Since bioconversion is the subject of ARCTECH's research for METC, and the bioreactors are the major cost component of the overall process, plant area II (Bioreactors) has been the focus of the cost sensitivity analysis. Two types of bioreactor scenarios have been considered - above ground and below ground. Base cases and best scenario (increased productivity) case have been developed for each of the bioreactor systems.

Equipment and ancillary project costs have been derived from extrapolation of previous cost calculations: 1) anaerobic wastewater treatment plant designs, 2) HL&P projections for a Texas lignite bioconversion process, 3) engineering judgement and 4) generally acceptable scaling and cost factors

B. Process Description

1. Plant Area I: Coal Grinding

A conveyor will transport the ROM lignite from the coal mine to the processing plant where it is initially crushed to 1 1/2" x 0 and stored in a surge silo. The crushed coal is then passed through two pulverizers that sequentially grind it to D_{90} of 100 mesh. It is then dropped into a slurry tank where water is added to prepare the correct lignite concentration (5% w/v in the base case) for feed to the bioreactors. It is anticipated that approximately 90% of this makeup water can be recycled from the biogasification process. Nutrient solution is also added to the slurry tank to satisfy the growth requirements of the microorganisms in the bioreactor.

2. Plant Area II: Bioreactors

The bioconversion process proceeds optimally as a two-stage process. The first stage is a coal solubilization/volatile acid formation reactor operating at a lower pH than the second stage methane forming reactor. Each reactor operates in an up-flow mode to maximize coal solids and biomass retention to result in long solids retention time and short liquid retention time. Each reactor also operates in a recycle mode (approximately 5:1 recycle ratio) which alleviates substrate inhibition effects, allows mixing of the coal/biomass sludge bed, and also allows the external recycle line to be heated to maintain optimum reaction temperature. The recycle mode also facilitates start-up and allows for simple process control.

The bioreactors can either be above-ground or below-ground. The above-ground bioreactors will contain a small amount of plastic packing (approximately 10% of reactor volume) near the top of the reactor. The use of this "barrier" could increase biomass retention, decrease particulate carryover to the phase-two bioreactor and facilitate gas release. The stage-two below-ground bioreactors will be 50% filled with crushed rock to serve as growth support for microbial biomass. There will be no heating requirement for the below ground bioreactors since underground temperatures will be close to optimum.

3. Plant Area III: Gas Purification

Gas produced in the first stage reactor (carbon dioxide and H_2) is directed to the second stage reactor to allow conversion of the carbon dioxide and H_2 to methane by specific methanogenic bacteria. Gas produced by the second stage reactor will be approximately 75% (v/v) methane and 25% (v/v) carbon dioxide. This gas is purified to 97% (v/v) methane by selective solvent absorption of the carbon dioxide. Subsequently carbon dioxide can be stripped from the solvent. Each gas stream (CH_4 and CO_2) is then compressed for pipeline delivery.

4. Plant Area IV: Waste Handling

Unconverted coal solids and ash are removed from the bottom of the reactors (mainly stage-one reactor) and can be directly disposed in landfill, or alternatively, be dewatered and sent to a steam generating boiler for energy recovery. In this study, the solids disposal option has been chosen.

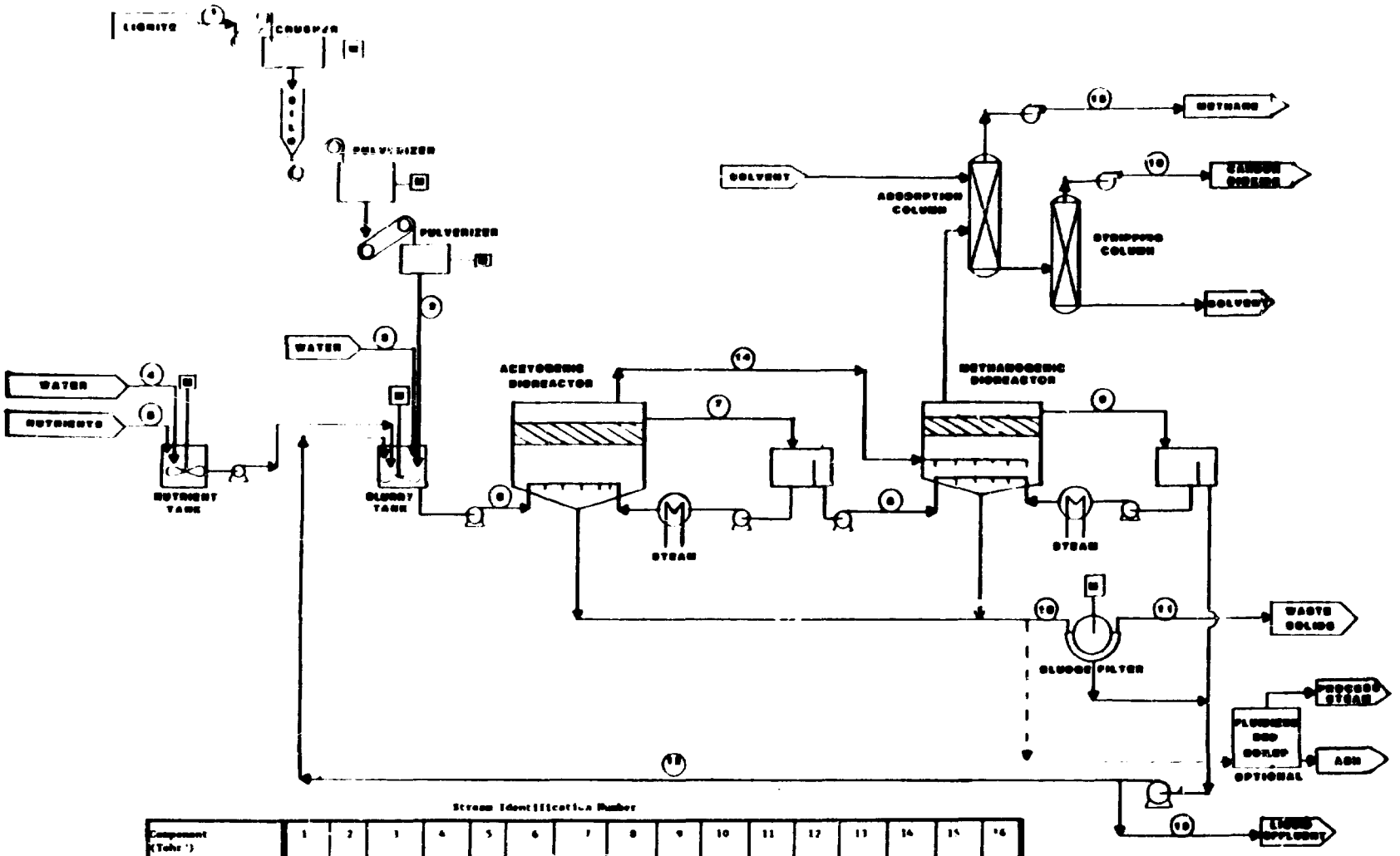
Ninety percent (90%) of the aqueous overflow from the second stage reactor is recycled to the slurry tank at the front end of the process. The other 10% is bled off and discharged to a municipal wastewater treatment plant as a low strength biodegradable effluent. This discharge will remove recalcitrant compounds from the process stream and decrease the potential for build-up of inhibitory materials.

C. Process Costing and Cost Sensitivity Analysis

A flow sheet and mass balance depicting base case conditions for the above-ground bioreactor system are shown in Figure 20. Capital and operating costs are shown in Tables 50 through 53 for the following four cases:

Case A Above-Ground Reactor - Base Case
Case B Below-Ground Reactor - Base Case
Case C Above-Ground Reactor - Increased Productivity
Case D Below-Ground Reactor - Increased Productivity

Each case is discussed separately.



Stream Identification Number

Component (Tons)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Insoluble Carbon	67	67				67				15.9	15.9					
Soluble Carbon							168.1	24.7	17	< 0.1	< 0.1	1.8	< 0.1			
CH_4																32.0
CO_2						Sat'd	Sat'd	Sat'd	Sat'd	Sat'd	Sat'd	Sat'd	Sat'd	31.2	31.2	
Ash	18	18				26	68	8	48	17.6	17.3	7.2	0.7			
Other volatile elements	5	5			2	7	21.0	3.5		7	7					
Water	30	30	193.5	8		2031.5	12000	2031.5	12000	96.5	26.8	1000	172.6	Sat'd		
Total (Tons)	100	100	193.5	8	2	2111.5	12217.1	2067.7	12065	135	67	1009	173.3	31.2	31.2	32.0

Figure 20. Process Flow Sheet and Mass Balance

Table 5A.

COSTING OF LIGNITE BIOGASIFICATION PROCESS
CASE A ABOVE GROUND BASE CASE
BIOREACTOR CONDITIONS : 6 DAY TOTAL RESIDENCE TIME

CAPITAL COSTS

PLANT AREA COSTS $MM	I COAL GRINDING	II BIOREACTORS	III WASTE HANDLING	IV GAS SEPARATION/ COMPRESSION	TOTAL CAPITAL COSTS $MM
CAPITAL COSTS					
1-Capital Equipment	1.00	22.60	0.50	2.05	26.15
2-Electrical, Controls, Instruments	0.20	4.52	0.10	0.41	5.23
3-Foundations Steel	0.15	3.39	0.08	0.31	3.92
4-Piping Installation Construction	0.27	6.10	0.14	0.55	7.06
5-General Facilities, Buildings	0.16	3.66	0.08	0.33	4.24
6-Design Project Management Start up	0.62	14.10	0.31	1.28	16.31
BATTERY LIMITS PROJECT COST $MM	2.41	54.37	1.20	4.93	62.91
7-Utilities	1.48	10.87	0.24	0.99	12.58
8-Working Capital	0.24	5.44	0.12	0.49	6.29
9-Contingency	0.48	10.87	0.24	0.99	12.58
PROJECT COST $MM	3.61	81.55	1.80	7.40	94.36

*BY
1- Delivered
2- 20% of 1
3- 15% of 1
4- 5.0% of 1+2+3
5- 11% of 1+2+3+4
6- 25% of 1+2+3+4+5
7- 20% of Battery Limit Cost
8- 5% of Battery Limit Cost
9- 20% of Battery Limit Cost

Table 50. (Cont.)

OPERATING COSTS
CASE A

TOTAL PROJECT CAPITAL COST $MM=					94.26
OPERATING COSTS	I	II	III	IV	TOTAL ANNUAL OPERATING COST $MM
1- Amortization					4.72
2- Cost of Capital					14.15
3- Fixed Cost O/M					1.89
4- Maintenance					4.72
5- Labor					2.00
6- Chemicals					0.25
7- Nutrients		63.40			63.40
8- Steam					15.84
9- Equipment Power	900kW	1000kW	300kW	300kW	1.19
10- Lignite					7.92
11- Process Water					0.50
12- Misc. Consumables					0.25
13- Ash Disposal			1.18		1.18
14- Sludge Disposal			1.05		1.05
15- Wastewater Disposal			3.17		3.17
TOTAL ANNUAL OPERATING COSTS $MM					122.23
REVENUES					
1-Carbon Dioxide					7.40
NET REVENUES REQUIRED FROM METHANE SALE					114.83
EQUIVALENT PRICE PER MM BTU=					9.68

KEY
1- 5% of Total Project Capital Costs
2- 15% of Total Project Capital Costs
3- 2% of Total Project Capital Costs
4- 5% of Total Project Capital Costs

Table 51.

COSTING OF LIGNITE BIOGASIFICATION PROCESS
CASE B BELOW GROUND BASE CASE
BIOREACTOR CONDITIONS : 6 DAY TOTAL RESIDENCE TIME

CAPITAL COSTS

PLANT AREA COSTS $MM	I COAL GRINDING	II BIOREACTORS	III WASTE HANDLING	IV GAS SEPARATION/ COMPRESSION	TOTAL CAPITAL COSTS $MM
CAPITAL COSTS					
1-Capital Equipment	1.00	14.40	0.50	2.05	17.95
2-Electrical Controls Instruments	0.20	2.88	0.10	0.41	3.59
3-Foundations/Steel	0.15	2.16	0.08	0.31	2.69
4-Piping Installation Construction	0.27	3.89	0.14	0.55	4.85
5-General Facilities/ Buildings	0.16	2.33	0.08	0.33	2.91
6-Design Project Management Start Up	0.62	8.98	0.31	1.28	11.20
BATTERY LIMITS PROJECT COST $MM	2.41	34.64	1.20	4.93	43.18
7-Offsites	0.48	6.93	0.24	0.99	8.64
8-Working Capital	0.24	3.46	0.12	0.49	4.32
9-Contingency	0.48	6.93	0.24	0.99	8.64
PROJECT COST $MM	3.61	51.96	1.80	7.40	64.77

KEY
1- Delivered
2- 2.5 of (1)
3- 15% of (1)
4- 20% of (1)+(2)+(3)
5- 10% of (1)+(2)+(3)+(4)
6- 25% of (1)+(2)+(3)+(4)+(5)
7- 20% of Battery Limit Cost
8- 10% of Battery Limit Cost
9- 20% of Battery Limit Cost

Table 51. (Cont.)

OPERATING COSTS
CASE B

TOTAL PROJECT CAPITAL COST $MM=					54.77
OPERATING COSTS	I	II	III	IV	TOTAL ANNUAL OPERATING COST $MM
1- Amortization					3.24
2- Cost of Capital					9.72
3- Fixed Cost O/M					1.30
4- Maintenance					3.24
5- Labor					2.00
6- Chemicals					0.25
7- Nutrients		63.40			63.40
8- Steam					0.00
9- Equipment Power	800KW	1000KW	200KW	300KW	1.19
10- Lignite					7.92
11- Process Water					0.50
12- Misc. Consumables					0.25
13- Ash Disposal			1.18		1.18
14- Sludge Disposal			1.05		1.05
15- Wastewater Disposal			3.17		3.17
TOTAL ANNUAL OPERATING COSTS $MM					98.40
REVENUES					
1-Carbon Dioxide					7.40
NET REVENUES REQUIRED FROM METHANE SALE					91.00
EQUIVALENT PRICE PER MM BTU=					7.67

KEY
1- 5% of Total Project Capital Costs
2- 15% of Total Project Capital Costs
3- 2% of Total Project Capital Costs
4- 5% of Total Project Capital Costs

Table 52.

COSTING OF LIGNITE BIOGASIFICATION PROCESS
CASE 1 ABOVE GROUND
BIOREACTOR CONDITIONS: 1 DAY RESIDENCE TIME
LOWER NUTRIENT COSTS $1 MM
HIGHER LIGNITE LOADING 25%

CAPITAL COSTS

PLANT AREA COSTS $MM	I COAL SPACING	II BIOREACTORS	III WASTE HANDLING	IV GAS SEPARATION/ COMPRESSION	TOTAL CAPITAL COSTS $MM
CAPITAL COSTS					
1-Capital Equipment	1.00	5.10	0.50	2.05	8.65
2-Electrical Controls/ Instruments	0.20	0.82	0.10	0.41	1.33
3-Foundations Steel	0.15	0.47	0.08	0.31	1.00
4-Piping Installation Construction	0.27	0.84	0.14	0.55	1.80
5-General Facilities/ Buildings	0.16	0.50	0.08	0.33	1.08
6-Design Project Management Start Up	1.62	1.90	0.31	1.28	4.15
BATTERY LIMITS PROJECT COST $MM	2.41	7.46	1.20	4.93	16.00
7-Offsites	0.48	1.49	0.24	0.99	3.20
8-Working Capital	0.24	0.75	0.12	0.49	1.60
9-Contingency	0.48	1.49	0.24	0.99	3.20
PROJECT COST $MM	3.61	11.19	1.80	7.40	24.00

*EX
1- Delivered
2- 20% of (1)
3- 15% of (1)
4- 30% of (1+2+3)
5- 10% of (1 + 2+3+4)
6- 25% of (1+2+3+4+5)
7- 20% of Battery Limit Cost
8- 10% of Battery Limit Cost
9- 20% of Battery Limit Cost

Table 52. (Cont.)

OPERATING COSTS
CASE C

TOTAL PROJECT CAPITAL COSTS $MM =					24.00
OPERATING COSTS	I	II	III	IV	TOTAL ANNUAL OPERATING COST $MM
1- Amortization					1.20
2- Cost of Capital					3.60
3- Fixed Cost O/H					0.48
4- Maintenance					1.20
5- Labor					2.00
6- Chemicals					0.25
7- Nutrients		15.85			15.85
8- Steam					15.84
9- Equipment Power	900kW	1000kW	300kW	200kW	1.19
10- Lignite					7.92
11- Process Water					0.25
12- Misc. Consumables					0.25
13- Ash Disposal			1.18		1.18
14- Sludge Disposal			1.05		1.05
15- Wastewater Disposal			3.17		3.17
TOTAL ANNUAL OPERATING COSTS $MM					55.43
REVENUES					
1-Carbon Dioxide					7.40
NET REVENUES REQUIRED FROM METHANE SALE					48.03
EQUIVALENT PRICE PER MM BTU=					4.05

KEY
1- 5% of Total Project Capital Costs
2- 15% of Total Project Capital Costs
3- 2% of Total Project Capital Costs
4- 5% of Total Project Capital Costs

Table 53.

COSTING OF LIGNITE BIOGASIFICATION PROCESS
CASE D BELOW GROUND
BIOREACTOR CONDITIONS : [illegible] DAY TOTAL RESIDENCE TIME
LOWER NUTRIENT COST $[illegible]/KG
HIGHER LIGNITE LOADING 25%

CAPITAL COSTS

PLANT AREA COSTS $MM	I COAL GRINDING	II BIOREACTORS	III WASTE HANDLING	IV GAS SEPARATION, COMPRESSION	TOTAL CAPITAL COSTS $MM
CAPITAL COSTS					
1-Capital Equipment	[illegible]	2.50	[illegible]	2.05	[illegible]
2-Electrical, Controls Instruments	[illegible]	1.50	[illegible]	1.41	[illegible]
3-Foundations Steel	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4-Piping Installation Construction	[illegible]	[illegible]	0.14	0.55	[illegible]
5-General Facilities Buildings	[illegible]	0.41	[illegible]	[illegible]	[illegible]
6-Design Project Management Start up	[illegible]	[illegible]	[illegible]	[illegible]	3.77
BATTERY LIMITS PROJECT COST $MM	2.41	6.01	[illegible]	[illegible]	14.55
7-Offsites	0.48	1.20	0.24	[illegible]	2.91
8-Working Capital	0.24	0.60	0.12	0.49	1.46
9-Contingency	0.48	1.20	0.24	0.99	2.91
PROJECT COST $MM	3.61	[illegible]	[illegible]	7.40	[illegible]

KEY
1- Delivered
2- [illegible]% of 1
3- [illegible]% of 1
4- [illegible]% of (1 + 2 + 3)
5- [illegible]% of (1 + 2 + 3 + 4)
6- [illegible]% of (1 + 2 + 3 + 4 + 5)
7- [illegible]% of Battery Limit Cost
8- [illegible]% of Battery Limit Cost
9- [illegible]% of Battery Limit Cost

Table 53. (Cont.)

OPERATING COSTS
CASE D

TOTAL PROJECT CAPITAL COSTS $MM =					21.83
OPERATING COSTS	I	II	III	IV	TOTAL ANNUAL OPERATING COST $MM
1- Amortization					1.09
2- Cost of Capital					3.27
3- Fixed Cost O/M					0.44
4- Maintenance					1.09
5- Labor					2.00
6- Chemicals					0.25
7- Nutrients		15.85			15.85
8- Steam					0.00
9- Equipment Power	9.0kW	1300kW	200kW	300kW	1.19
10- Lignite					7.92
11- Process Water					0.25
12- Misc. Consumables					0.25
13- Ash Disposal			1.18		1.18
14- Sludge Disposal			1.05		1.05
15- Wastewater Disposal			3.17		3.17
TOTAL ANNUAL OPERATING COSTS $MM					39.00
REVENUES					
1-Carbon Dioxide					7.40
NET REVENUES REQUIRED FROM METHANE SALE					31.60
EQUIVALENT PRICE PER MM BTU=					2.66

REV
1- 5% of Total Project Capital Costs
2- 15% of Total Project Capital Costs
3- 2% of Total Project Capital Costs
4- 5% of Total Project Capital Costs

1. CASE A - ABOVE GROUND REACTOR BASE CASE

FEED COMPOSITION:	52% volatile solids
(TEXAS LIGNITE):	Comprising 47% C
	5% other elements
	18% Ash
	30% Water
LIGNITE COST	$10/Te
FEED PREPARATION	Lignite ground to D_{90} of 100 mesh
BIOREACTOR FEED	5% w/v lignite (as received)
	(2000 $m^3 hr^{-1}$ @ 2.35% w/v C)
BIOREACTOR RESIDENCE TIME	Stage I 4 days
	Stage II 2 days
CARBON MASS BALANCE IN BIOREACTOR	65% ———> CH_4
	22.5% ———> CO_2
	12.5 ———> Biomass
CARBON CONVERSION IN BIOREACTOR	80%
NUTRIENT LOADING	0.1% w/v @ $4/kg
1 Te TEXAS LIGNITE	———> 320 kg CH_4
	(@ 80% C conversion and 312 kg CO_2)
100 $Tehr^{-1}$ TEXAS LIGNITE	———> 1.58 MSCFH CH_4
	(@ 80% C conversion and 0.56 MSCFH CO_2)
BIOGAS CALORIFIC VALUE	———> 700 Btu/SCF
ANNUAL ENERGY PRODUCTION	———> 11.86 x 10^{9} Btu

Lignite composition and loading rate are the same as presently utilized in laboratory experiments. Residence times (4 d in stage-one and 2 d in stage-two reactors), carbon mass balance and carbon conversion efficiencies are with 3% (w/v) biomass maintained in each reactor (see discussion in Section IV.A).

Nutrient supplementation merits special attention since annualized operating costs and the ultimate cost of producing methane are extremely sensitive to nutrient cost (Table 50). In this base case nutrient costs represent yeast-extract at the concentration presently used in the laboratory.

This concentration has not yet been minimized and could be significantly reduced in the future. Yeast extract contains nitrogen and phosphorus in high concentrations as well as growth factors, all of which are required and used by growing biomass. Other components of the media used in laboratory experiments provide the micro-environment but are not depleted to a significant extent. Therefore, when 90% of the liquid overflow from the second stage bioreactor is recycled to the front end of the process, make up requirements are minimal. A total project capital cost of $94.36 mm is projected for this above ground base case resulting in a methane cost of $9.68 per mm Btu.

2. CASE B - BELOW GROUND BIOREACTOR BASE CASE

The cost basis for Case B is the same as in Case A except that an underground reactor which has no steam requirement is utilized. Underground bioreactor costs are based on excavation of undisturbed rock strata (no previous mining operations) to form underground caverns. The lower cost of an underground reactor significantly reduces overall project capital costs from $94.36 mm to $64.77 mm. Annualized operating costs are further reduced by the elimination of the process heating requirement. The resulting cost of methane production is $7.67 per mm Btu.

3. CASE C - ABOVE GROUND BIOREACTOR, SHORTER RESIDENCE TIME, LOWER NUTRIENT COST, HIGHER LIGNITE LOADING

As in Case A except:

BIOREACTOR RESIDENCE TIME	Stage I	1.3 days
	Stage II	0.7 day
NUTRIENT COST	$1/kg	
LIGNITE LOADING	25% w/v (as received)	

The major difference between costs for Case C and base Cases A and B is the reduced nutrient cost. This cost reduction is achievable with additional research to define a suitable low-cost medium for the biogasification process. The cost reduction could be obtained by retaining yeast extract

supplementation for provision of growth factors and substituting low cost mono- or di-ammonium phosphate for the nitrogen and phosphorous requirements.

Results from ARCTECH studies indicate that both higher lignite loadings and lower residence times may be feasible. In laboratory experiments to date, significant progress has been made in increasing lignite loading rates (0.01 to 0.1% to 5% w/v) and rates of coal carbon conversion to methane (up to 50%). ARCTECH predicts that further progress in this direction can be made.

The result of these changes is reduction in capital costs to $24.00 mm (since bioreactors are modular units at this scale of operation and the cost reduction for bioreactors is in direct proprotion to the reduction in total reactor volume requirement). Reduced capital costs bring down annualized operating costs but the greatest reduction in operating cost is brought about by the reduced nutrient cost (Table 52). Consequently the cost of methane production is reduced to $4.05 per mm Btu.

4. CASE D - BELOW GROUND BIOREACTOR, SHORTER RESIDENCE TIME, LOWER NUTRIENT COST, HIGHER LIGNITE LOADING

As in Case B except:

BIOREACTOR RESIDENCE TIME	Stage I	1.3 days
	Stage II	0.7 days
NUTRIENT COST	$1/kg	
LIGNITE LOADING	25% w/v (as received)	

The assumptions in Case C, above ground reactor, when applied to Case D, underground reactor, result in a total project capital cost of $21.83 mm and methane production costs of $2.66 per mm Btu.

These preliminary cost sensitivity analyses indicate that key areas to be developed for successful commercialization of a process for bioconversion of low-rank coals to methane are: 1) enhanced kinetics of methane production, 2) reduced bioreactor costs, and 3) lower nutrient costs.

V. CONCLUSIONS AND MAJOR ACCOMPLISHMENTS

The technical feasibility for biological conversion of untreated lignites (Leonardite and Texas lignite) and coal-derived products to methane has been demonstrated. Preliminary data also indicate that efficient biological conversion of Beulah lignite and Wyodak subbituminous may be possible, although the best microbial consortia have not been identified. The production of short chain organic acids and alcohols during degradation of coal to methane was confirmed and an intermediate not previously reported, benzoic acid, was identified. Bioconversion of these intermediates to methane was demonstrated using adapted cultures and, in some instances, the addition of known methanogenic microbial populations to ARCTECH's adapted cultures. Addition of known H_2-CO_2 utilizing methanogens to reaction mixtures (where CO_2 concentrations were high) resulted in decreased CO_2 concentration and increased methane production. Direct conversion of selected low-rank coals by adapted microorganisms resulted in overall coal carbon conversions of greater than 70%. Direct conversion of coal carbon to methane ranged from 35% to as much as 50%.

The original concepts for coal conversion envisioned the use of a one-stage (direct bioconversion) or two-stage bioconversion process (Figure 1). Experiments investigating the pretreatment of coals and subsequent carbon conversion to aqueous soluble products using an aerobic biological system resulted in relatively low recovery of parent carbon in soluble carbon products. The soluble products created had increased oxygen and nitrogen contents. Data indicated that 100% conversion of these soluble coal-derived products to methane would result in overall parent coal carbon conversions of less than 50%. Based on these findings, the primary focus of the project became the direct anaerobic biomethanation of selected coals. The approach used was a) the identification of anaerobic consortia capable of directly biodegrading selected coals, b) adaptation of selected cultures, c) consolidation of cultures to achieve maximum coal carbon conversion, d) addition of known methanogenic populations to consortia in which methane precursors accumulated in the culture medium, and e) evaluation of continuous methane production in a bench-scale bioreactor.

Adaptation of cultures to specific coal(s) for bioconversion was found to result in enhanced methane production (Figure 13). The addition of methanogens to cultures in which methane precursors (organic acids and alcohols) accumulated resulted in maximum conversion of coal carbon to methane (Figure 14). Bioreactor data indicate that accumulation of large quantities of biomass and conservation of this biomass will be of critical importance in a biogasification process. Information from other anaerobic fermentations in which more easily accessible substrates are bioconverted to methane indicates that biomass concentrations of 5g/L are necessary to achieve maximum methane production. The rotating biological contactor reactor appeared to achieve maximum biomass accumulation and conservation by the production of biofilm on media discs within the reactor. It is likely that the high biomass concentration in this bioreactor resulted in the production of methane in the presence of 5% coal solids (w/v), the highest coal solids loading successfully used in these studies. It was anticipated that the use of up-flow fluidized bed reactors could also facilitate the accumulation and conservation of large concentrations of biomass while using long solids retention times and relatively short liquid retention times. Unfortunately, the up-flow fluidized bed experiments were less successful with respect to methane production.

Preliminary process design schematics and economic analyses for a biological biomethanation plant have been developed based on data accumulated during these laboratory and bench-scale studies. Based on these preliminary cost calculations using a best case scenario, the cost for bioconversion of lignite to methane falls in the range of $2.66 to $4.05/million Btu.

Major accomplishments of this project are summarized below:

- Bioproduction of soluble organic products from Leonardite occurred without pretreatment of the coal.
- Aerobic microorganisms depolymerized low-rank coals to water-soluble, acid-precipitable products of relatively low-molecular weight.
- The ARCT'CH proprietary culture, CP1+2, was the most successful of the biological coal treatments.
- Coals pretreated with nitric acid were unsuitable for bioconversion to methane.

- Biological rather than chemical depolymerization products were preferred substrates for methane production.
- Short chain acids and alcohols were identified as intermediate products during bioconversion of coal to methane.
- Acetic acid and ethanol were the major compounds identified as intermediates when methane inhibitor studies were conducted using various coals and various microbial consortia.
- Direct bioconversion of Leonardite, Beulah lignite, Wyodak subbituminous and Texas lignite to methane has been demonstrated.
- Consortia derived from numerous anoxic environments have demonstrated biomethanation of low-rank coals.
- Coal/microbial cultures specificity with respect to bioconversion of coals to methane was conclusively demonstrated.
- Adaptation of selected consortia with specific low-rank coals has resulted in significantly enhanced methane production.
- Addition of specific methanogenic populations (acetate cleaving, H_2-CO_2 utilizing) results in increased methane production when intermediate products accumulate during bioconversion of coals.
- Overall coal carbon conversions (CH_4, CO_2, organic acids and alcohols) in excess of 70% have been demonstrated.
- Coal-carbon conversions to methane of up to 50% have been demonstrated.
- A rotating biological contactor was successfully used as a bioreactor for continuous production of methane over more than two months of operation.
- Methane concentration in the headspace gas of the RBC reached 83 mole%.
- Coal solids loading of 5% (w/v) was successfully used for biomethanation of Texas lignite in the RBC reactor.

The development of an economically acceptable, efficient process for conversion of coals to environmentally acceptable fuel forms would be a technological breakthrough for the United States in its quest for energy independence coupled with the maintenance of environmental quality and provision of jobs in the depressed coal mining areas of the country. Future research will address key areas to enhance the kinetics of methanogenesis and overall carbon conversion to methane from low-rank coals using microorganisms developed by ARCTECH using an innovative dual reactor system with above ground and underground bioreactors. Future research should focus on characterization and optimization of the microbial consortia to achieve maximum methane production from two coals; enhancement of coal/bacterial interactions to achieve maximum methane production;

and enhancement of the kinetics for each of the process steps (biomass production, substrate utilization, intermediate(s) production, carbon conversions and methane production). Data generated will provide the basis for future bioreactor design and construction.

The ultimate goal of the biogasification research is to develop an efficient, economically attractive biological process for conversion of low-rank coals to methane and to demonstrate this process at pilot-scale. Innovative concepts such as a dual reactor system to ensure greater process stability and control, ash removal to minimize wastes, a fixed film or fixed bed approach to fermentations to conserve biomass and allow for short liquid retention times, and the proposed use of above ground and underground reactors in process designs will be evaluated for commercial application as a coal gasification technology.

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APPENDIX 1. ANAEROBIC MEDIA PREPARATION

Basal Salts Medium (McInerney et al., 1979)

This medium is routinely used for enrichment of anaerobic consortia capable of converting coal to methane. The basal medium contains the following constituents at the final concentrations indicated in percent:

Pfennig's Mineral Solution	5.0	
Pfennig's Metal Solution	0.1	
Yeast Extract	0.1	
Resazurin, 0.1% Stock Solution	0.1	
B-vitamin Solution	0.5	
Sodium selenite, 0.1% solution	0.1	(added after
Sodium Bicarbonate	0.35	cooling)

The medium is prepared in a round-bottom flask under an 80% nitrogen : 20% carbon dioxide gas phase. It is heated to boiling and then allowed to cool to room temperature. The sodium bicarbonate is added and the pH is checked to ascertain that it is between 7.0 and 7.5. If not, it is adjusted with sodium hydroxide or hydrochloric acid. The medium is dispensed into vessels which have been flushed with the gas phase and contain preweighed amounts of raw coal, if necessary. The vessels are fitted with black butyl rubber stoppers, sealed with aluminum crimp seals and autoclaved at 121°C for 20 minutes. The medium is reduced with $Na_2S \cdot 9H_2O$ (0.05% w/v final concentration) immediately prior to use. The method is based on that of Hungate (1969) as modified by Bryant (1972).

Pfennig's Mineral Solution Composition (g/L)

KH_2PO_4	10.0
$MgCl_2 \cdot 6H_2O$	6.6
NaCl	8.0
NH_4Cl	8.0
$CaCl_2 \cdot 2H_2O$	1.0

Pfennig's Metal Solution Composition (g/L)

$ZnSO_4 \cdot 7H_2O$	0.10
$MnCl_2 \cdot 4H_2O$	0.03
H_3BO_3	0.30
$CoCl_2 \cdot 6H_2O$	0.20
$CaCl_2 \cdot 2H_2O$	0.01
$NiCl_2 \cdot 6H_2O$	0.02
$Na_2MoO_4 \cdot 2H_2O$	0.03
$FeCl_2 \cdot 4H_2O$	1.50

B-Vitamin Solution Composition (mg/100 ml)

Nicotinic Acid	2.0
Cyanocobalamin	2.0
Thiamine	1.0
p-aminobenzoic acid	1.0
Pyrodoxine	5.0
Pantothenic Acid	0.5

APPENDIX 2. INOCULATION AND TRANSFER OF CULTURES

Prior to inoculation, any necessary supplements such as inhibitors or biosolubilized coal products are added to the media which was previously dispensed, autoclaved and reduced. The stoppers are sterilized with alcohol and flaming. Inoculation is carried out by introducing the inoculum source by means of a needle and syringe through the rubber stopper. Alternatively, the stoppers and seals may be removed and inoculation accomplished in the anaerobic chamber with a pipet. Usually, inoculum is added to a final concentration of 10%. Equivalent volumes of headspace gas are removed in order to maintain ambient pressure in the vessels.

All experiments are performed in duplicate tubes -- one for gas measurement and analysis and one for liquid sampling for acid/alcohol analysis. Generally, these analyses are performed biweekly (or as necessary) and the cultures are transferred into fresh media and coal after three samplings (approximately six weeks post-inoculation).

The cultures are transferred into vessels containing the same amount of coal initially added to the original culture, but only half the volume of media. The transfer is performed by adding an equal volume of the ongoing culture, resulting in a 50% transfer rate. Some experiments require a smaller carryover, so less volume from the original culture is transferred, and the volume of media in the new vessel is increased proportionally.

All culture manipulations are carried out using strict anaerobic techniques, either in the anaerobic chamber or using the method of Hungate (1969) as modified by Bryant (1972).

Appendix 3. List of Chemical Treatments Used to Pretreat and/or Solubilize Coals

1. Nitric acid (HNO_3) 1-8N
2. Hydrogen peroxide (H_2O_2) 5-20%
3. Sodium hydroxide (NaOH) 1N
4. Potassium hydroxide (KOH) 1N
5. Sodium carbonate (Na_2CO_3) 0.1 to 2 g
6. Potassium carbonate (K_2CO_3) 0.1 to 2 g
7. Acetic acid (CH_3COOH) up to 10%

APPENDIX 4. GAS SAMPLING AND VOLUME DETERMINATIONS

Periodically, the cultures are monitored for methane production. The initial step in the process is to measure the amount of excess gas formed from the coal substrate by the cultures. The total volume of the serum tubes or bottles is known and the headspace volume is determined from the difference between total volume and the volume of the media.

The additional gas in the headspace is measured by means of a glass syringe, equipped with a one-way stopcock with a swage lock fitting to accomodate a needle. The stoppers on the cultures are sterilized by alcohol and flaming. The stopcock valve is closed and the needle inserted through the stopper. The valve is then opened and the plunger of the syringe is allowed to displace the excess gas in the culture headspace. The gas volume is determined by the volume indicated by the syringe markings.

Similarly, the pressure in the cultures could be measured by means of a pressure transducer. This method would provide greater accuracy and convenience. The transducer could be attached to an integrator which can be programmed to translate the electrical current production into meaningful pressure units.

APPENDIX 5. ANALYSIS OF LIQUID SAMPLES FOR VOLATILE ACIDS AND ALCOHOLS

1. Scope

1.1 This method describes the determination of chemical composition of liquid samples containing the following components: acetone, methanol, ethanol, propanol, butanol, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid.

1.2 The method should allow for determination of components in the range of 10 to 1500 ppm.

2. Applicable Documents

2.1 ASTM Standards - E 260 Recommended Practice for General Gas Chromatography Procedures

2.2 U.S. Environmental Protection Agency - 40 CFR Part 792, Toxic Substances Control Act; Good Laboratory Practice Standards (8/89)

3. Terminology

3.1 VA - volatile acids (C1-C5); acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid.

3.2 Alcohols - methanol, ethanol, propanol, and butanol.

4. Summary of Method

4.1 Components in a liquid sample of culture medium are physically separated by gas chromatography and compared to corresponding components of a reference standard separated under identical operating conditions, using a reference standard mixture of known composition. The composition of the liquid sample is calculated by comparison of the area response of each component with the corresponding value of that component in the reference standard.

5. Significance and Use

5.1 The information about the chemical composition of the liquid medium can be used to determine the extent of coal- conversion to VA's and alcohols.

6. Apparatus

6.1 Hewlett Packard 5880A - capillary column gas chromatograph with Hewlett Packard 7672A automatic sampler.

6.1.1 Detector - The detector is a flame ionization type and is operated at 300°C.

6.1.2 Column - Bonded phase, wide bore, Nukol fused silica capillary column, 15 m, 0.53 mm ID, 0.50 um film thickness (Supelco #2-5326). The life and resolution of the Nukol column will be greatly enhanced with frequent replacement of the pre- column section.

6.1.3 Pre-column - Non-bonded phase, wide bore, SP-1000 borosilicate glass column, 0.75 m, 0.75 mm ID, 1 um film thickness (Supelco #2-3757).

6.1.4 Oven temperature control - This method utilizes a multi-level oven temperature program. The pre-injection equilibration time is 1.5 min at 48°C.

Program	Rate (°C/min)	Final Temperature (°C)	Final Time (min)
Initial	N/A	48	0.70
Rate #1	15	120	0.20
Rate #2	10	155	0.50
Rate #3	5	200	20.00

6.1.5 Gases - gases are obtained from a commercial gas distributor and stored outside the building in the cylinder shed.

Gas	Function	Regulator Pressure (PSI)	Flow Rate (ml/min)
Helium[a]	Carrier gas[a]	110	5 column 25 split vent
Nitrogen[a]	Make-up gas	50	30
Hydrogen	Flame	50	35
Air	Flame	55	200 igniter off 30 igniter on
Nitrogen	Autosampler	70	N/A

[a] Passed through a heated carrier gas purifier (Supelco #2-3000).

[b] The carrier gas is split (1:5) using an ALLTECH 0993 split/splitless injector.

6.1.6 Injector temperature - The injector temperature is 205°C. The septa [11 mm Thermogreen LB-2 (Supelco #2-0654)] should be replaced every 120-150 injections. Also, the injector lining should be cleaned periodically.

6.2 Electronic Integrator (Hewlett Packard 5880A series GC terminal)

6.2.1 Attenuation - 2[a]

6.2.2 BASIC program - The analysis is automated and controlled by a 45-line BASIC program.

6.2.3 Calibration table - The calibration table is used to calculate the amount of each sample component. For each set of analyses, the calibration table is recalibrated following analysis of five external standards.

6.3 Autosampler - 2 ul injection volume, 5 flushes with distilled water, 3 purges with the sample prior to injection.

7. Reference Standards

7.1 Calibration Standards - Calibration standards are used at the beginning of each set of analyses. Concentrated stock solutions are prepared as needed or at 2 month intervals and stored at 4°C. Working stocks are prepared as needed or at 2 week intervals from the concentrated stocks and stored at 4°C. Each working stock is assigned a lot number and subjected to quality assurance. Concentrated stocks and working stocks are prepared in the following manner:

7.1.1 Concentrated stocks - Acids and alcohols are not mixed in preparing the concentrated stock standards due to ester formation over time.

CONCENTRATED STOCK 1B - ALCOHOLS

0.5135 g Methanol (Burdick & Jackson 23C)
0.6032 g Ethanol (Polysciences, Inc. 16020)
0.5097 g Propanol (Burdick & Jackson 322)
0.5020 g n-Butanol (Sigma Chemical Co. BT-105)

CONCENTRATED STOCK 2B - ACIDS

0.6045 g Acetic acid (Fisher Scientific A-38)
0.4990 g Propionic acid (Mallinckrodt, Inc. 7179)
0.5024 g n-Butyric acid (Sigma Chemical Co. B-2503)
0.5027 g Isobutyric acid (Sigma Chemical Co. I-1754)

CONCENTRATED STOCK 3 - ACETONE & VALERIC ACIDS

0.1310 g Acetone (Burdick & Jackson 010-4)
0.1260 g Isovaleric acid (Aldrich Chemical Co. 12,954-2)
0.1240 g Valeric acid (Aldrich Chemical Co. 24,037-0)

CONCENTRATED STOCK 4 - 2-METHYL-BUTYRIC ACID

0.1507 g 2-methyl-butyric acid (Sigma Chem. Co. M-0516)

7.1.2 - Working standards - All dilutions are made using 0.05 M phosphoric acid to a final volume of 25 ml.

Standard I (400 & 480 ppm) = 1 ml STOCK 1B + 1 ml STOCK 2B
Standard II (200 & 240 ppm) = 0.5 ml STOCK 1B + 0.5 ml STOCK 2B
Standard III (80 & 96 ppm) = 5 ml Std I + 5 ml Std VI
Standard IV (30 & 36 ppm) = 1.875 ml Std I + 2.5 ml Std VI
Standard V (12 & 14 ppm) = 1.25 ml Std II + 1.25 ml Std VI
Standard VI (500 ppm) = 2.5 ml STOCK 3 + 2.0 ml STOCK 4

7.2 Internal Standards - Standard solutions containing VA's and alcohols are diluted 1:2 and 1:4 using 0.05 M phosphoric acid and prepared as described in section 9.

Component	Concentration (ul/l) Full	1:2	1:4
Alcohol Standard (Supelco #4-8806)			
Ethanol	783	391.5	195.75
Propanol	301	150.5	75.25
n-Butanol	74	37.0	18.50
Isobutanol	37	18.5	9.25
Isopentyl alcohol	44	22.0	11.00
Pentanol	44	22.0	11.00
Volatile Acid Standard (Supelco #4-8975)			
Formic acid	460	230.0	115.00
Acetic Acid	601	300.5	150.25
Propionic Acid	741	370.5	185.25
Isobutyric Acid	881	440.5	220.25
n-Butyric Acid	881	440.5	220.25
Isovaleric Acid	1021	510.5	255.25
n-Valeric Acid	1021	510.5	255.25
Isocaproic	1162	581.0	290.50
n-Caproic	1162	581.0	290.25
Heptanoic	1302	651.0	325.50

8. Hazards and Precautions

8.1 If the operator follows GLP's while using this method there should not be any exposure to hazards.

9. Preparation of Samples

9.1 Sample Removal - One ml of liquid sample is aseptically removed from the culture bottle using a sterile 1 cc tuberculin syringe and 20 gauge needle previously flushed three times with 80% N_2:20% CO_2. Samples are placed in 13x100 screw cap borosilicate glass tubes. Samples may be frozen at -20°C for a period no longer than 3 months.

9.2 Sample Dilution - Liquid samples are diluted using 1 ml of 0.05 M phosphoric acid. The diluent is made using 5.76 g 85% H_3PO_4 (Fisher Scientific, Fair Lawn, NJ) in 100 ml Type I (18 M ohm) reagent grade water (Nanopure)

9.3 Centrifugation - Samples are mixed by vortexing, placed in 1.5 ml microfuge tubes, and centrifuged at 13,000 rpm for 5 min using a Sorvall Microspin 24s centrifuge. The supernatant is removed, placed in sample vials (Cat. #1100, 12x32 mm, clear borosilicate glass, Type I, Class "A", Sunbrokers, Inc.), and crimp sealed (Cat. #1150, Sunbrokers, Inc.). This procedure removes particulates and biomass allowing direct aqueous injection into the gas chromatograph.

10. Preparation of Apparatus

10.1 Gas Chromatograph - Prior to starting gas chromato- graph, check the helium for proper pressure and supply (see section 6.1.6).

10.1.1 Flame - To ignite the flame, open the hydrogen valve on the front panel of the gas chromatograph. Push the igniter switch to "auto" and slowly open the air valve at the front of the gas chromatograph. Check the flame for ignition by monitoring the baseline. The zero should not read <1.0 and would optimally be between 4 and 15. You may also use a mirror or beaker of water to see water vapor from the flame.

10.1.2 Autosample tray - Samples are loaded on the sample tray as noted in the sample table.

10.2 Electronic Integrator - Load BASIC program, modify sample table, modify run table, start program.

11. Calibration and Standardization

11.1 Calibration - Lab-prepared standards are used for a 5- point calibration with recalibration prior to each set of sample analyses.

11.2 Internal Standardization - The use of internal standards allow for the determination of run accuracy. Standards are obtained from Supelco for VA and alcohols. Samples are prepared as described in section 9 for liquid sample analysis.

12. Procedure

12.1 Sample Volume - Analysis will be performed by direct aqueous injection after the sample was centrifuged to remove particulates and biomass. A sample volume of 2 ul is injected by the autosampler equipped with a Hamilton 701N, 10 ul syringe (Supelco #5-50779).

12.2 Chromatogram - The following is a sample chromatogram using a chart speed of 1.00 cm/min and 3% offset.

APPENDIX 6. ANALYSIS OF GAS SAMPLES FOR PERMANENT GASES

1. Scope

1.1 This method describes the determination of chemical composition of gas samples containing the following components: hydrogen, nitrogen, carbon monoxide, methane, and carbon dioxide.

2. Applicable Documents

2.1 ASTM Standards - E 260 Recommended Practice for General Gas Chromatography Procedures

2.2 U.S. Environmental Protection Agency - 40 CFR Part 792, Toxic Substances Control Act; Good Laboratory Practice Standards (8/89)

3. Summary of Method

3.1 Components in a gas sample are physically separated by gas chromatography and compared to corresponding components of a reference standard separated under identical operating conditions, using a reference standard mixture of known composition. The composition of the gas sample is calculated on a percentage of total area basis by comparison of the area response of each component.

4. Significance and Use

4.1 The information about the chemical composition of the gas sample can be used to determine the extent of coal- conversion to methane and carbon dioxide.

5. Apparatus

5.1 Varian 3700 gas chromatograph with 1/8" on-column injection.

5.1.1 Detector - The detector is a thermal conductivity type and is operated with a filament temperature of 280°C.

5.1.2 Column - 10' x 1/8" OD stainless steel column packed with 100/120 mesh Carbosieve S-II (Supelco # 1-2579).

5.1.3 Oven temperature control - The oven is maintained isothermally at 210°C.

5.1.4 Gases - Helium is the carrier gas (6 ml/min, 110 psi at regulator) and is passed through a heated carrier gas purifier (Supelco #2-3800).

5.1.5 Injector temperature - The injector temperature is 220°C. The septa [11 mm Thermogreen LB-2 (Supelco #2-0654)] should be replaced every 30 injections.

5.1.6 - TCD controller - Sensitivity 4 x 0.5 mvfs (0.. mV, attenuation 4, output +)

5.2 Electronic Integrator (Hewlett Packard 3396A)

5.2.1 Attenuation - 2'

5.2.2 Calibration table - The calibration table is used to calculate the amount each sample component. For each set of analyses, the calibration table is recalibrated following analysis of an external standard.

6. Reference Standards

6.1 Calibration Standards - The calibration standard is used at the beginning of each set of analyses. The calibration standard gas mixture is obtained from Air Products and contains the following:

4.9% Hydrogen
24.9% Nitrogen
10.2% Carbon monoxide
30.7% Methane
29.3% Carbon dioxide

7. Hazards and Precautions

7.1 If the operator follows GLP's while using this method there should not be any exposure to hazards.

8. Preparation of Samples

8.1 Gas Removal - Prior to GC analysis the total amount of gas produced is measured with a sterile syringe.

9. Preparation of Apparatus

9.1 Gas Chromatograph - Prior to the starting gas chromatograph, check the helium gas for proper pressure and supply (see section 5.1.4).

9.2 Electronic Integrator

10. Calibration and Standardization

10.1 Calibration - After analysis of the standard, recalibrate the amount/area for each component and correct the calibration table.

11. Procedure

11.1 Removal of Sample - Wet the surface of the rubber butyl stopper with methanol to prevent contamination of the sample. Allow the methanol to evaporate before puncturing the stopper, then remove 200 ul.

11.2 Sample Volume - Analysis will be performed by direct gaseous injection of 125 ul using a Dynatech 010031 Pressure-Lok series "A" (0-0.25 cc) gas syringe (Fisher Scientific #11-124B).

11.3 Chromatogram - The following is a sample chromatogram using a chart speed of 1 cm/min and 5% offset.