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Microbial Conversion of High-Rank Coals to Methane

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ABSTRACT

It has been demonstrated recently that certain bacteria and fungi are capable of directly or indirectly converting low-rank coals into liquid and gaseous fuels. The Bureau of Mines has found preliminary evidence that indicates that microbial consortia are responsible for generating methane from bituminous coal. Building on this work, a study was undertaken to test selected anaerobic microbial consortia for their ability to degrade and produce methane from bituminous coals. Five consortia were collected from natural sources, including coal strip ponds, mine treatment areas, chemical waste deposits, and sewage sludge. Three coals were incubated into various media containing inocula from the consortia. At least three of the consortia showed an ability to produce methane from hard coals in the absence of yeast extract.

Index Entries: Methane; high-rank coals; consortia; anaerobic.

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INTRODUCTION

Microbial conversion of coal to methane offers a new environmentally friendly use of the country's abundant fossil fuel. The Bureau of Mines Advanced Mining Systems Program is exploring the application of biotechnology to new coal uses and alternative mining methods.

It has been demonstrated recently that certain bacteria and fungi may be used to convert low-rank coals directly or indirectly into liquid and gaseous fuels. The conversion of coal to these more convenient forms of energy could be a significant development for the mining industry because of the abundance of coal as a natural resource. Fungal species, such as *Polyporous* (*1,2*), *Phanerochaete* (*3*), *Paecilomyces* (*4*), *Cunninghamella* (*5*), and *Penicillium* (*6*), and bacterial genera, such as *Streptomyces* (*5,7*) and *Pseudomonas* (*8*), have been used to solubilize low-rank coals in producing a water-soluble mixture of highly polar compounds. This technology is based primarily on the success of microbially degrading lignin, and the chemical similarities between lignin and the low-rank coal lignite. Solubilized coal, in turn, has been used as a precursor for liquid fuels production (*9,10*). Small quantities of ethanol, acetate, and other low-molecular-weight products have been produced when utilizing inocula derived from natural sources, such as sewage sludge and animal rumens. Finally, methane has been produced from both presolubilized coal in combined aerobic and anaerobic steps, and directly from coal in a one-step anaerobic process (*11*). Sources of inocula for methane production included sewage sludge, animal waste, the animal rumen, and termite gut.

Each of the above studies has concentrated on the use of low-rank coals as microbial substrates. Although lower-rank coals are more amenable to microbial attack, there is at least preliminary evidence that shows that coals of higher rank can be microbially degraded, with subsequent conversion to methane. The Bureau of Mines (*12*) presented preliminary evidence that microbial consortia were responsible for generating methane from bituminous coal. The source of the microbial consortium was a Pittsburgh Seam A mine site abandoned about 75 yr ago, in which sewage dumping had occurred over a time period of 20–30 yr. After 48 d of incubation, the resulting gas phase from a coal mine sediment sample with 0.1% coal added contained 4.5% methane and 18.3% CO_2. The evidence of methanogenesis, with organisms possibly growing on volatilized coal, was postulated.

The purpose of this study was to build on the preliminary work of Volkwein in obtaining and testing microbial consortia able to degrade and produce methane from bituminous coals, such as Pittsburgh Seam A, Wyodak, and Hartshorne coals. The techniques used for selecting and utilizing the microbial consortia are presented, along with results obtained from consortia screening experiments. Special emphasis was given in the

experiments to the effects of pH and nutrients on coal conversion, especially in assuring that methane production occurred as a result of coal degradation alone and not as a result of conversion of nutrient sources.

MATERIALS AND METHODS

Coals

The following coals were obtained from the Penn State Coal Sample Bank and Data Base:

DECS-12	Pittsburgh Seam A coal collected from Green County, PA
DECS-5	Wyodak (also called Smith-Roland) collected from Campbell County, WY
PSOC-1521	Lower Hartshorne collected from Sebastian County, AR

Prior to use, the coals were reduced to a particle size of 60+ mesh using a Tumbler's Model A-R1 tumbler (Tru-Square Metal Products, Auburn, WA) and cylindrical burundum balls (13/16-in. nominal size) as the grinding medium. Since pretreatment of the coals by air oxidation, acid treatment, and so forth, would be cost-prohibitive in a large-scale operation, all experiments were carried out without coal pretreatment.

Microbial Consortia Collection

Organisms capable of degrading bituminous coals are most likely to be found in environments rich in the particular coal. Lignin-degrading consortia are, in general, incapable of higher-ranked coal degradation without significant coal pretreatment (*13*). Organisms capable of metabolizing coal-derived products to produce methane might be found in a wide variety of environments, including soils, contaminated muds, and sludge. Environments having both types of organisms should hold the most potential for bituminous coal conversion to methane.

The locations shown in Table 1 were selected as having significant potential for harboring corsortia able to degrade high-rank coals to produce methane. A collection device was constructed in order to remove samples from natural areas while minimizing air introduction into the samples. The samples collected included muds, soils, sludge, and water.

Experimental Design

Studies were initially performed with 0.1% yeast extract (Difco, Detroit, MI) in the medium at initial pH levels of 4, 5, 6, 7, and 8. The

Table 1
Sources of Microbial Consortia

Inoculum source	Coal exposure
Strip pond, Stigler, OK	Hartshorne
PA mine treatment, Washington, PA	Pittsburgh Seam A
Oil/chemical waste El Dorado, AR	None
Wyodak strip, Wright, NY	Wyodak
Sewage treatment, Springdale, AR	None
Termite gut Fayetteville, AR	None

medium also contained per 100 mL of solution 1.0 mL of 5X Pfennig's minerals (*14*), 0.1 mL of Pfennig's trace minerals (*15*), 0.5 mL of B-vitamins solutions, 0.1 mL of a 0.1% solution of resazurin and distilled or deionized water, 0.1*M* KH_2PO_4, and 0.1*M* NaOH as appropriate to adjust the pH to the desired level. A 10% inoculum was used along with 0.1% coal.

Following the completion of the initial screening study, a secondary study was performed using the successful initial pH levels of 5 and 7. Yeast extract, a potential source of methane production, was gradually reduced and then eliminated from the medium by first performing studies with 0.05% yeast extract, followed by three successive transfers without yeast extract. The inoculum for each subsequent study was obtained from successful consortia in the previous study.

Experiments were run in duplicate in 26-mL serum stoppered tubes. Each tube contained 9 mL of medium, 1 mL of inoculum, and 16 mL of gas phase (N_2). Incubation was at 37°C with shaking at 150 rpm. Anaerobic techniques (*16–18*) were utilized in the experimental studies.

The tubes were sampled for gas composition every 2 wk and for liquid (pH and acetate) every 2 wk. Approximately 0.5 mL of sample was removed for each gas-phase analysis and 1.0 mL for each liquid phase analysis. The experimental duration was 12 wk in the initial and secondary studies, and 16 wk in the limited yeast extract studies. Although a significant fraction of the liquid culture volume was removed by sampling during experimentation, it should be remembered that the studies were performed only to demonstrate feasibility and culture potential. Controls without coal and without culture were also run.

Analytical Procedures

Acetate was determined as the free acid by gas–solid chromatography. A Hewlett-Packard 5890 Series II gas chromatograph equipped with a Porapak QS, 100/120 mesh, 150 cm × 3 mm PTFE column, and an FID detector was used for these tests. The oven temperature was 190°C, the detector and injector temperatures were both 240°C, and the carrier gas was nitrogen at a flow rate of 55 mL/min. An analytical procedure for the determination of the composition of the gas phase was developed for a Varian 3400 gas chromatograph with a TCD detector. A 180 cm × 3 mm PTFE column packed with Poropak Q, 100/120 mesh, allowed very accurate determination of methane and carbon dioxide. The oven temperature was maintained at 34°C, the detector and injector temperatures were both 150°C, and the carrier gas was helium at a flow rate of 30 mL/min.

RESULTS AND DISCUSSION

In these preliminary studies, efforts were made to maintain a constant inoculum size in each experiment. In addition to using a constant inoculum volume, qualitative examinations of the inocula under the microscope showed similar cell counts. In general, methane production from the two duplicate tubes was similar, reaching a constant value with time. Methane production maxima for the duplicate set of tubes is herein reported.

Tables 2 and 3 show maximum methane production volumes at pH 5 and 7 during the 12-wk initial and secondary screening studies, performed with 0.1% yeast extract present in the medium. Acetate production data, although not shown, also signaled the onset of methanogenesis. As is noted, significant volumes of methane were produced by five of the six inocula, although the controls without coal also produced significant amounts of methane. The controls without inocula, but with coal, produced no methane in any of the studies.

Although the results of Tables 2 and 3 are encouraging, it is clear that the presence of yeast extract in the medium was also causing methane production. Thus, an experimental program was undertaken to eliminate yeast extract from the medium. Other potential carbon sources in the medium besides yeast extract (such as B-vitamins) can yield only negligible amounts of methane, even if completely converted.

Tables 4–6 show the maximum methane production volumes at pH 5 and 7 for the 16-wk studies performed without yeast extract in the medium. The inoculum for the studies of Table 4 was a 10% inoculum from successful cultures with 0.05% yeast extract under the same experimental conditions. The inoculum for the studies of Table 5 was a 10% inoculum from

Table 2
Maximum Methane Production from Initial Screening Studies
(12-wk Study)

	Maximum reported production, vol %						
	pH 5			pH 7			
Inoculum source	P	H	W	P	H	W	Control, no coal
Strip Pond Stigler, OK	10.38	12.36	11.45	12.18	11.80	12.53	10.93
PA Mine Treatment Washington, PA	24.39	22.45	24.01	21.13	21.10	20.56	19.63
Oil/Chemical Waste El Dorado, AR	19.16	19.46	20.54	19.26	16.47	19.47	17.05
Wyodak Strip Wright, NY	11.64	12.02	10.64	6.60	6.71	6.97	6.88
Sewage Treatment Springdale, AR	16.50	16.43	15.66	15.92	16.13	13.55	16.76
Termite gut Fayetteville, AR	0.19	0.20	0.32	0.80	0.63	0.84	0.73

P —Pittsburgh Seam A.
H —Hartshorne.
W—Wyodak.

Table 3
Maximum Methane Production from Secondary Screening Studies
(12-wk Study)

	Maximum reported production, vol %							
	pH 5				pH 7			
Inoculum source	P	H	W	Control, no coal	P	H	W	Control, no coal
Strip Pond Stigler, OK	8.76	11.81	9.74	9.07	8.05	7.60	7.55	5.55
PA Mine Treatment Washington, PA	20.46	17.04	25.47	19.85	16.79	18.02	16.63	16.99
Oil/Chemical Waste El Dorado, AR	23.13	21.85	24.80	16.58	15.80	15.37	17.62	15.71
Wyodak Strip Wright, NY	12.17	11.03	15.28	5.65	1.43	1.28	4.72	1.55
Sewage Treatment Springdale, AR	14.85	14.36	15.27	13.83	6.63	13.74	14.75	6.80

P —Pittsburgh Seam A.
H —Hartshorne.
W—Wyodak.

Table 4
Maximum Methane Production from Initial Studies Without Yeast Extract
(8-wk Study)

	Maximum reported production, vol %							
	pH 5				pH 7			
Inoculum source	P	H	W	Control, no coal	P	H	W	Control, no coal
Strip Pond Stigler, OK	0.04	0.08	0.09	0	0	0.13	0.09	0
PA Mine Treatment Washington, PA	2.47	3.44	0.42	2.05	2.31	1.37	2.74	2.24
Oil/Chemical Waste El Dorado, AR	2.65	5.33	5.49	3.13	3.33	3.41	2.48	1.30
Wyodak Strip Wright, NY	0.05	0.14	0.52	0.06	0.08	0	0.12	0
Sewage Treatment	3.30	2.78	1.39	2.80	2.85	0.67	3.37	1.50

P —Pittsburgh Seam A.
H —Hartshorne.
W—Wyodak.

Table 5
Maximum Methane Production from a Second Experiment
Performed Without Yeast Extract
(8-wk Study)

	Maximum reported production, vol %							
	pH 5				pH 7			
Inoculum source	P	H	W	Control, no coal	P	H	W	Control, no coal
Strip Pond Stigler, OK	0	0	0	0	0	0	0	0
PA Mine Treatment Washington, PA	2.93	1.77	0.52	0.26	2.16	2.19	2.32	1.92
Oil/Chemical Waste El Dorado, AR	3.19	2.14	2.48	0	3.66	2.69	6.87	3.09
Wyodak Strip Wright, NY	0	0	1.06	0	0.54	0	0.10	0.56
Sewage Treatment	1.22	0.11	1.23	0.46	0.22	0	1.68	2.93

P —Pittsburgh Seam A.
H —Hartshorne.
W—Wyodak.

Table 6
Maximum Methane Production from a Third Experiment Performed Without Yeast Extract
(8-wk Study)

	Maximum reported production, vol %							
	pH 5				pH 7			
Inoculum source	P	H	W	Control, no coal	P	H	W	Control, no coal
PA Mine Treatment Washington, PA	3.79	2.98	0.25	0.78	4.81	4.96	3.20	3.93
Oil/Chemical Waste El Dorado, AR	1.70	3.17	5.25	0	3.40	3.30	6.11	2.96
Sewage Treatment Springdale, AR	2.53	1.95	2.39	0	0	0	1.72	0.11

P —Pittsburgh Seam A.
H —Hartshorne.
W—Wyodak.

successful cultures from Table 4, and the inoculum for the studies of Table 6 was a 10% inoculum from the successful cultures of Table 5. Thus, the tubes of Table 6 contained at most 0.005% yeast extract, assuming that none of the yeast extract present in the 0.05% yeast extract tubes had been utilized in the experimental study prior to transfer.

As is noted in Tables 4–6, the elimination of yeast extract greatly reduced methane production. However, a significant amount of methane was produced even after three successive transfers, indicating that coal components were being utilized for methane production. After three transfers, Inoculum 3 (oil/chemical waste from El Dorado, AR) showed over 5% methane from Wyodak coal at pH 5, whereas the control produced no methane. Similarly, Inoculum 5 produced over 2% methane from coal, but only at pH 5. It is unclear why the control tubes containing no coal produced methane in some cases. It is possible that the cells could be digesting themselves to produce some methane. This observation requires further study.

It is interesting to trace the evolution of the cultures producing methane as yeast extract is removed from the medium. Table 7 shows the maximum methane production volumes for the three best inocula using Pittsburgh Seam A coal at pH 5. The methane production volumes shown have been corrected by subtracting the methane produced in the controls. As is noted, after the removal of yeast extract, methane production was fairly steady for each of the inocula, even for three transfers. It is quite possible that at least some fraction of the coal is being converted by the consortia.

Table 7
Maximum Methane Production from Pittsburgh Seam A Coal Using Various Inocula with and Without Yeast Extract Present (pH 5)

	Maximum reported production, vol %				
	0.1% yeast extract		No yeast extract		
Inoculum source	Initial	Secondary	1st Transfer	2nd Transfer	3rd Transfer
PA Mine Treatment Washington, PA	5.76	3.46	0.42	2.67	3.01
Oil/Chemical Waste El Dorado, AR	2.11	7.42	0	3.19	1.70
Sewage Treatment Springdale, AR	0	8.05	0.50	0.76	2.53

CONCLUSIONS

Based on the results of this consortia screening study, at least three consortia have been isolated from natural sources that are capable of anaerobically converting at least portions of higher-rank coals to methane. These results are particularly impressive, since three transfers of these consortia have been made into media devoid of yeast extract. The most promising consortia were obtained from Pennsylvania mine treatment of Washington, PA; the oil/chemical waste sample from El Dorado, AR; and the sewage treatment sample from Springdale, AR. The consortia were able to utilize Pittsburgh Seam A, Hartshorne, and Wyodak coals equally well at pH 5 and 7. Additional studies will be performed in an effort to develop the technology further.

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