

PROJECT MANAGEMENT PLAN

A. Defined Effort Objectives

The objective of this project is to investigate the biotic and abiotic factors that influence microbial transformation of coal in order to assess the potential for stimulating microbial methane production *in situ*. Laboratory experiments and computer modeling will be performed. This is a single-phase project; funding will be sought for subsequent upscaling studies, as warranted by the results of this project.

Microcosm studies will be used to characterize the methane-generating potential of coals of different rank in response to nutrient additions, chemical pretreatments of the coal and/or environmental manipulations. By identifying the chemical constituents, natural microbial communities and the microbial metabolic products formed en route to methanogenesis, a greater understanding of the processes involved will be gained. Because microorganisms are responsible for catalyzing the transformation of organic matter to methane, we will take advantage of culture-independent phospholipid and molecular biology techniques to describe the salient microbial communities involved in coal conversion.

B. Tasks to be performed

Task 1.0. Project Management Plan (this document)

The Colorado School of Mines-University of Wyoming-USGS Coal Biogas consortium (hereafter referred to as CSM-CB) shall develop a Project Management Plan consisting of a work breakdown structure and supporting narrative that concisely addresses the overall project as set forth in the agreement. CSM-CB shall provide a concise summary of the objectives and approach for each Task and, where appropriate, for each subtask. CSM-CB shall provide schedules and planned expenditures for each Task including any necessary charts and tables, and all major milestones and decision points. CSM-CB shall identify key milestones that need to be met prior project proceeding to the next phase. This report will be submitted within 30 days of the Award. The RPSEA Contracts/Procurement Manager shall have 20 calendar days from receipt of the Project Management Plan to review and provide comments to CSM-CB. Within 15 calendar days after receipt of the RPSEA's comments, CSM-CB shall submit a final Project Management Plan to the RPSEA Contracts/Procurement Manager for review and approval.

Task 2.0. Technology Status Assessment

CSM-CB shall perform a Technology Status Assessment and submit a summary report describing the state-of-the-art of the proposed technology. The report will include both positive and negative aspects of each existing technology. The report will be no more than five typewritten pages in length. The report will not contain any proprietary or confidential data, as the report will be posted on the RPSEA website for public viewing. The report will be submitted within 30 days of the Award.

Task 3.0. Technology Transfer

CSM-CB shall designate 2.5% of the amount of the award for funding technology transfer activities. Throughout the project, CSM-CB shall work with RPSEA to develop and implement an effective Technology Transfer Program at both the project and program level.

Technology Transfer Plan:

- CSM-CB will make a minimum of two presentations in local professional organization meetings, one each in the Powder River Basin and the Denver area.
- CSM-CB will submit at least one paper for consideration for presentation at an SPE Annual Meeting.

- CSM-CB will coordinate with RPSEA to make a minimum of one presentation at a program level technology transfer activity. Forty percent (40%) of the total technology transfer budget will be set aside for participation and support of program level activities as directed by RPSEA.
- CSM-CB will prepare papers for publication. One of these papers should be in a producer-oriented trade journal.

Task 4.0. Sample Collection

Water, fresh drill cuttings and potentially some coal core samples will be collected with the cooperation of three partner CBM operating companies, Coleman Oil and Gas, Pinnacle Gas Resources and Pioneer Natural Resources. These contributors will supply samples from the Wyodak and Big George formations of the Powder River Basin (WY and MT), the Raton and Vermejo formations of the Raton Basin (southern CO), the Sand Wash Basin (northwestern CO) and the Green River Basin (WY). Coal ranks range from lignite through sub-bituminous to high volatile C, B and A. At least 4 of these samples will be collected across a gradient at different distances from the recharge zone of a coal seam, to enable characterization of differences in water and coal chemistry associated with typically higher rates of microbially generated methane near the recharge zone.

Task 5.0. Coal Pretreatment

Biogenic methane production from coal requires the conversion of the solid coal matrix into soluble constituents. In addition to studying methane production resulting from naturally occurring solubilization processes, various chemical coal pretreatments designed to enhance coal solubilization will be examined. Treatment agents including acids, bases, oxidants, solvents, and enzymes may increase the extent and rate of coal solubilization and depolymerization, resulting in enhanced methane production. Fractional and full-factorial bench-scale studies will be used to evaluate treatment effectiveness and assess the feasibility of reagent coupling. Variables including the types of coal and reagents, reagent-to-coal ratio, residence time, temperature, and pressure will be evaluated using stainless steel micro-reactors. The rate of solubilization is also important and will be examined in separate time-series experiments. Chemical constituents in the most promising factorial experiments will be identified by a combination of GC-MS and LC-ESI-MS (Task 6). In addition, selected samples of treated coal and associated water will be examined using the microbial techniques described in Task 7.

Task 6.0. Chemical Characterization of Different Coals, Pre-treated Coals and Associated Waters

Organic matter will be extracted from the various coal samples to determine the amount of bioavailable carbon and reveal the identity of putative metabolites that may represent a parent substrate or chemical intermediate in the microbial food chain. The organic matter extracted by the coal pre-treatments (Task 5.0) and from untreated coals will be analyzed. To this end, solvents will be selected that when used separately will allow extraction of either polar or non-polar organic matter (e.g. water and chloroform, respectively) from the untreated coals. Additionally, water samples collected from corresponding coal zones will be analyzed to establish the quantity and character of organic matter present in the coal formations before additional biological or chemical treatment and provide a baseline for Tasks 5.0 and 7.0.

A broad-spectrum analysis (BSA) approach will be used to examine the above samples. Solid-phase micro-extraction (SPME) and solvent extraction will be used to simultaneously extract compounds of a wide range of properties and subsequently analyze them by GC-MS and/or Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS). Identified substrates from the water samples and coal extractions will be tested for bioavailability with consortia in Task 7.0.

Task 7.0. Microbial Enrichment and Characterization

This task consists of several integrated efforts designed to better understand the metabolic pathways of microbial consortia involved in methane generation from coal. The effect of nutrients, environmental conditions and pre-treatments on methane generation will also be assessed. Microbes associated with native coal samples and produced waters will be identified by a variety of methods, microbial cultures that

generate methane from coal will be enriched and characterized and coal biotransformation intermediates will be identified.

Subtask 7.1. Microbial Enrichment. Untreated and pretreated coal and water samples will be incubated under a variety of conditions to stimulate methane generation by associated microbes. Microcosms containing slurries of ground coal samples and liquid media will be prepared for laboratory incubations. The incubation parameters to be tested include coal type, pH, temperature, nutrient and salt addition, carbon amendments, and hydrogen concentrations. Appropriate controls will be run in parallel to ensure that methane production is biogenic and due to coal conversion; these controls will include autoclaved coal, autoclaved coal plus amendments, and amendments with no coal. The rate and extent of methane production will be quantified in these slurries and normalized to the mass and surface area of coal used in the incubation. The microcosms will also be periodically sampled and analyzed for parameters such as hydrogen concentration and production, volatile fatty acid concentrations (especially acetic acid, butyric acid and propionic acid) and dissolved organic carbon. In addition, the dissolved organic constituents will be periodically characterized using both GC-MS and LC-MS analyses on select microcosms. Upon confirmation of a culture's ability to produce methane from coal, sub-cultures will be made from selected replicates by transferring 10% of the culture to fresh bottles.

Subtask 7.2. Microbial characterization. The microbes associated with coal will be characterized by physiological and genetic methods such as phospholipid analysis and DNA sequencing. Microbial communities will be characterized in selected native coals and associated waters (at least one sample per site), as well as in those enrichment cultures that produce significant methane from coal. Using GC-MS techniques, we will measure the abundance of sulfate-reducing bacteria and methanogenic archaea in groundwaters associated with CBM deposits from structural analysis of microbial membrane phospholipid fatty acids (PLFAs) and phosphoether lipids (PELs), respectively. We will conduct similar analyses of selected (high methane production) microcosms. In particular, the methanogenic communities in the microcosms will be characterized from the composition and $\delta^{13}\text{C}$ of the PLFAs and PELs. Similarly, DNA will be extracted from coal, formation water samples and selected high-methane microcosms. Extracted DNA of eukaryotes, bacteria, and archaea will be amplified, cloned and sequenced. The DNA of different microbes can be quantified, allowing for determination of relative abundance of different microorganisms within the microbial community.

Subtask 7.3. Identification of metabolic intermediates and capabilities of the microbial community. The rate-limiting metabolic process(es) will be determined by inhibiting specific processes and measuring the production rates of intermediates and also by adding different known intermediates and measuring the removal rates. The first test will be to inhibit methanogenesis from coal conversion using methanogenic-specific inhibitors such as bromoethanesulfonic acid. The accumulation rate of the expected intermediates hydrogen and acetic acid will be determined, providing an indication of their production rate by other organisms; the accumulation rate of other constituents, such as phenolic compounds and long-chain fatty acids, will also be determined. To stop acetic acid metabolism by all organisms including sulfate-reducing organisms, fluoroacetic acid, a potent inhibitor of acetate metabolism, may be used. Additional studies using non-methanogenic inhibitors including antibiotics, will allow examination of the inherent methanogenic activity as well as fermentative processes that may be required to initiate degradation of the parent coal constituent. Selected intermediate compounds, determined from the inhibition studies, will then be added to inhibited and uninhibited cultures and their rates of removal measured. Hydrogen, acetic acid, methane, biomass concentration and other constituents will be measured using established methods.

Task 8.0.

The standard model for anaerobic processes (Anaerobic Digestion Model 1 (ADM1)) considers a number of different metabolically active groups but was developed primarily for wastewater and sludge treatment. The approach used in ADM1 can be readily used to model other anaerobic systems (Bagley 1998) but the microbial populations and the metabolic pathways must be defined appropriately. The model developed for this work will examine hydrolysis and fermentation separately and include hydrogen production, hydrogen consumption and hydrogen feedback inhibition for intermediate degradation.

Parameter values will be estimated using an inverse modeling approach appropriate to upscaling. The data generated in Tasks 5, 6 and 7 will be used to develop a coupled microbial kinetic model that tracks C and N compounds from coal through intermediates to methane. Collection of data required for modeling will include changes in reactant concentrations that include: coal mass, bulk coal chemical composition, added nitrogen and phosphorus and changes in product concentrations including soluble carbon products, methane, carbon dioxide. Additionally, data on the changes in community structure and active biomass concentration will be collected in Task 7.

C. Labor Distribution

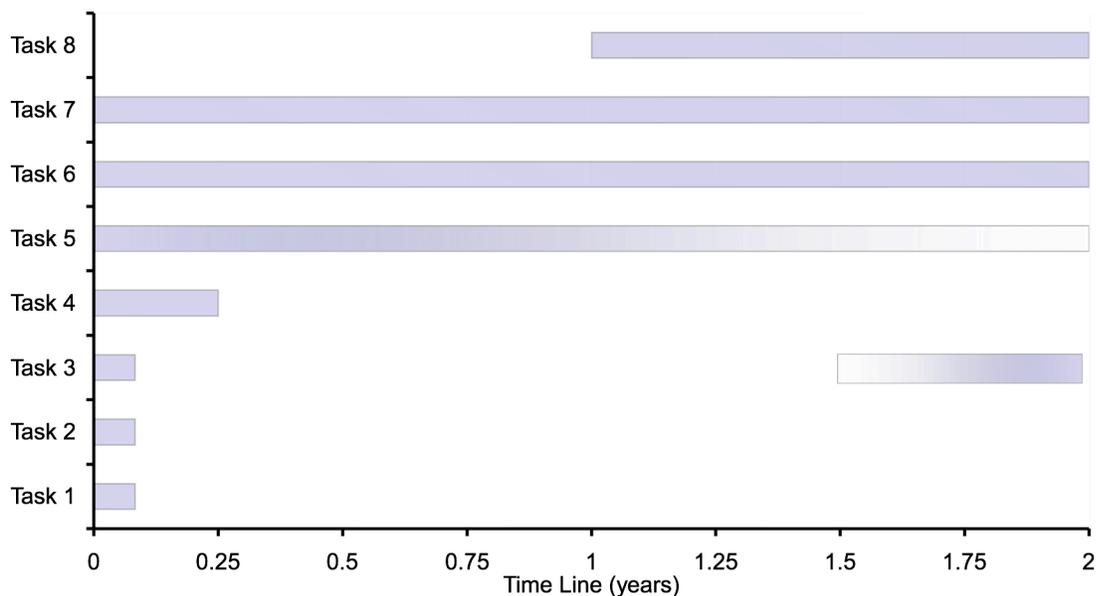
Participants are anticipated to expend efforts on the different tasks as outlined in the table below.

Task	Labor Category	Labor Hours
Task 1: Project Management Plan	PI, PI1-7*	PI=20, PI1=8, PI3-7=2 ea
Task 2: Technology Status	PI, PI1-7	PI=8, PI1=20, PI3-7=2 ea
Task 3: Technology Transfer	PI, PI1-7, GS	PI=20, PI1=40, PI3-7=16 ea, GS=160
Task 4: Sample Collection	PI1,6, GS	PI1=24, GS=160
Task 5: Coal Pre-Treatment	PI, PI1, PI6, GS	PI=20, PI1=40, PI6=182, GS=1150
Task 6: Chemical Characterization	PI, PI1,4,7, GS	PI=20, PI1=40, PI4=320, PI7=91, GS=2100
Task 7.1: Microbial Enrichment	PI, PI1,4,, GS	PI=62, PI1=320, PI4=1350, GS=900
Task 7.2: Microbial Characterization	PI, PI1,2, GS	PI=62, PI1=380, PI2=182, GS=2100
Task 7.3: Metabolic Intermediates...	PI, PI1,5,7, GS	PI=20, PI1=40, PI5=182, PI7=91, GS=2150
Task 8.0 Modeling	PI1,3, GS	PI1=160, PI3=182, GS=400
Reports:	PI, PI1-7, GS	PI=30, PI1=80, PI2-7=30
* PI= Munakata-Marr, PI1=Landkamer, PI2=Mandernack, PI3=Figueroa, PI4=Harris, PI5=Bagley, PI6=Urynowicz, PI7=Basile, GS=Graduate Students		

D. Schedule

The first step of the project involves collecting samples of coal and water upon which the rest of the project is dependent. Task 4 will be mostly completed within the first three months of the project. Once the first samples are collected, Tasks 5,6 and 7 will begin immediately and progress simultaneously. Task 5 (Coal Pretreatment) will begin by evaluating different coal pre-treatment methods on the first samples collected and will proceed as new samples are acquired. Task 6 will begin by evaluating the soluble organic present in untreated coals and associated water samples; extracts resulting from the various treatments in Task 5 will also be analyzed in Task 6. Task 7.1 will use untreated coal and water samples to start microcosm experiments to evaluate the effect of nutrient additions and changes in environmental conditions (e.g., temperature and pH); extracts and the associated treated coals from Task 5 will also be used to start microcosm experiments in Task 7.1. Task 7.2 (Microbial Characterization) will be started using untreated and treated coal and water samples, as well as existing coal-to-methane enrichment cultures, to identify the microbial constituents. Microcosms that produce significant quantities of methane in Task 7.1 will be re-characterized in Task 7.2 to assess any changes. Task 7.3 will be initiated by examining the initial results of Task 6 for potential metabolites. Once microcosms that produce significant methane are identified in Task 7.1, Task 7.3 will begin to examine the metabolic processes occurring in these microcosms to gain an understanding of the rate limiting steps. This knowledge will feed back to Task 7.1 where methods to overcome these rate-limiting steps will be developed. Task 5, 6 and 7 are closely interrelated and will be carefully managed by having weekly meetings to communicate and

discuss results. As an understanding of the kinetics of methanogenesis in the microcosms is gained, this information will be incorporated in a mathematical model in Task 8. Task 5 is expected to be largely finished by the end of year one, although work on Task 5 will continue at a slower pace in year two. Tasks 6 and 7 identify specific metabolites solubilized in Task 5 that result in enhanced methanogenesis. Once the identity of these key metabolites is discerned, the pre-treatments will be fine-tuned to target these metabolites. These feedback mechanisms will be used to maximize the coal to methane conversion rates over the entire project period. The timeline for the project tasks is depicted in the figure below.



E. Expected Expenditures

Details for Colorado School of Mines supply costs are provided in the table below. The indirect cost rate for Colorado School of Mines is 46.35% on total direct costs except for tuition and fees and permanent equipment.

Colorado School of Mines Supply Costs				
Description	Unit Cost	Quantity	Total Direct Cost	Cost-Shared Amount
Capital Equipment				
<i>Autosampler for gas chromatograph (GC-MS)</i>				
Cooling/Heating plate	\$370	1	\$370	
Cooling/Heating tray	513	1	513	
Autosampler	10,117	1	10,117	\$5,500
		sub-total	\$11,000	\$5,500
<i>Upgrade to current GC-FID, including a computer & GC software</i>				
Upgrade from CVP4.1 or CVP4.2 System to Class VP 7.4 SP1	\$2100	1	\$2100	
SS420x Instrument Interface for Class-VP (4.3 and 7.x only)	2700	1	2700	
Upgrade GC Control, First Option (Class VP 7.4 SP1)	400	1	400	

Enhanced Data Station (Win XP) w/ 19" LCD	2800	1	2800	\$1,000
		sub-total	\$8,000	\$1,000
Materials and Supplies				
<i>Compressed gases for GC operation</i>				
UHP He LW800	\$72.30	18	\$1,301.40	
UHP H ₂ LW500	83.40	18	1,501.20	
UHP N ₂ LW411P	75.60	18	1,360.80	
UHP Air LW700	46.48	18	836.64	
		sub-total	\$5,000.04	
<i>Reagents and solvents for GC-MS analysis</i>				
HPLC grade Chloroform	\$26.36	40	\$1,054.40	
HPLC grade Methanol	12.25	40	490.00	
HPLC grade Acetone	13.82	40	552.80	
HPLC grade Ethyl acetate	19.13	40	765.20	
HPLC grade 95% <i>n</i> -hexane	43.78	30	1,313.40	
Toluene	14.56	10	145.60	
HPLC grade H ₂ O	10.69	40	427.60	
Dimethyl disulfide	33.50	10	335.00	
Tetrahydrofuran	31.76	13	412.88	
		sub-total	\$5496.88	
<i>Reagents for DNA analysis</i>				
Extraction	402	1	\$402	
Amplification	113	25	2,825	
Cloning	572	6	3,432	
Primers, probes, etc.	64	10	640	
		sub-total	\$7,299	
GC column	500	1	500	
<i>Plastic- and glass- ware (serum bottles, stoppers, etc.)</i>				
	120	20	2,400	
<i>Disposables and consumables (gloves, paper towels, pipet tips, etc.)</i>				
	110	20	2,200	
Outside Services				
DNA sequencing (incl. some cloning)	12	1000	12,000	
GC-irMS ¹³ C lipid analysis	300	20	6,000	
HPLC-MS intact phosphoether lipid analysis	100	24	2,400	

Details for University of Wyoming supply costs are provided in the table below. The indirect cost rate for University of Wyoming is 43% on total direct costs except for tuition and fees.

University of Wyoming Supply Costs¹

Description	Unit Cost	Quantity	Total Direct Cost	RPSEA ²
Materials and Supplies				
<i>Culture Growth and Treatment</i>				
Culture serum bottles (20 mL)	\$1.50	500	750	750
Culture serum bottles (125 mL)	2.00	500	1,000	1,000
Serum bottle septa	1.00	3,000	3,000	3,000
Medium chemicals (per L of medium)	0.50	10,000 L	5,000	5,000
Reagent chemicals (coal treatment, per treatment)	0.25	5,000 treatments	1,250	1,250
<i>Culture Sampling and Analysis</i>				
Automatic pipettors	\$200.00	6	1,200	1,200
Reagent chemicals (per measurement)	0.25	10,000	2,500	2,500
Gas-tight syringe, 50 µL	125.00	12	1,500	1,500
Gas-tight syringe, 100 µL	125.00	12	1,500	1,500
Gas-tight syringe, 1 mL	125.00	12	1,500	1,500
Gas-tight syringe, 5 mL	125.00	12	1,500	1,500

Syringe filters	3.00	1,000	3,000	3,000
<i>GC and TOC Operation</i>				
UHP H ₂	\$100.00	12	1,200	1,200
UHP N ₂ (also culture sparging)	100.00	36	3,600	3,600
UHP Air	100.00	12	1,200	1,200
GC Columns	600.00	2	1,200	1,200
Headspace sampling vials	1.00	1,000	1,000	1,000
<i>GC-MS Analyses</i>				
GC-MS Column (30 m) nonpolar	\$500.00	2	1,000	1,000
GC-MS Column (30 m) intermediate polarity	500.00	2	1,000	1,000
GC-MS Column(60m) non-polar(DB-5)	880.00	1	880	880
GC-MS Column (60 m) intermediate polarity (DB-35)	860.00	1	860	860
GC Injector Septa (box of 250)	300.00	2	600	600
GC Column graphite ferrules(box of 10)	35.00	20	700	700
GC injector liners (box of 25)	270.00	2	540	540
GC in-lin gas purification	350.00	1	350	350
SPE 24 position vacuum manifold set	835.00	1	835	835
SPE tubes (box of 50 tubes)	285.00	6	1,710	1,710
GC-MS Syringes (autosampler)	125.00	12	1,500	1,500
GC-MS syringes (manual)	110.00	5	550	550
GC autosampler vials (box of 200)	55.00	20	1,100	1,100
Vial crimp seals (box of 100)	30.00	10	1,230	1,230
Pressure pump oil for GC-MS	80.00	1	80	80
Vacuum pump exhaust filter	25.00	12	300	300
UHP He (carrier gas)	100.00	24	2,400	2,400
<i>LC-MS Analyses</i>				
LC-MS syringes (gas-tight)	80.00	10	800	800
HPLC Columns (polar and C18)	650.00	2	1,300	1,300
LC-MS Fittings (Upchurch)	100.00	40	4,000	4,000
LCQ (ESI-MS) heated capillary interface	985.00	2	1,970	1,970
Ionization filament repair (MS)	175.00	3	525	525
MS Ion Volume cleaning supplies	70.00	1	70	70
MS Electron Multiplier (2-yr lifetime)	850.00	1	850	850
ESI-MS Vacuum oil	80.00	1	80	80
UHP N ₂ (for ESI)	100.00	3	300	300
<i>General Chromatography Supplies</i>				
Fused silica capillary (75 µm id x 10m)	130.00	2	260	260
Fused silica capillary (125 µm id x 10m)	130.00	2	260	260
Swagelok 1/8, ¼ fittings	450.00	1	450	450
Screw top vials-glass (box of 100)	40.00	40	1,600	1,600
Screw cap PTFE liner (box of 100)	20.00	35	700	700
Standard compounds, 100 mg average sample size	100.00	20	2,000	2,000
Standard compounds, 50 mg average sample size	250.00	10	2,500	2,500
GC and HPLC solvents	700.00	1	700	700
<i>Consumables</i> (gloves, paper towels, etc.)	4.00	900	3,600	3,600
<i>Pipette supplies</i> (tips)	0.05	10,000	500	500
Total Supplies			70,000	70,000
Indirect Costs (43%)			30,100	30,100
Total			100,100	100,100

¹Over the two year project life.

²There is no cost share for supplies.

Details for USGS supply costs are included in the table below. The indirect cost rate for USGS is 50%. The requested funds for supplies are relatively small because they will be supplemented by existing

funds.

U.S. Geological Survey Supply Costs			
Description	Unit Cost	Quantity	Total Direct Cost
Supplies			
Chloroform	295.16	2 (case)	590.32
borosilicate soxhlet thimbles	200.00	2 (case)	400.00
gas purifying converter tube	118.00	2	236.00
glass serum bottles	264.70	4 (case)	1058.80
Travel to 1 meeting (domestic)	2000.00	1	2000.00
Overhead	2142.56		2142.56
Total			6428

E. Milestones

Key milestones of the project include sample collection upon which the rest of the project is dependent. However, Dr. Harris already has some samples of Powder River Basin coal and enrichment cultures based on these samples are currently being cultivated; samples from these coals and microcosms will be used to initiate the project. The most important milestone of the project will be the identification of nutrients, environmental conditions and coal pre-treatments that enhance the biological conversion of coal to methane (Task 5). Although microbial activity is notoriously difficult to predict, this milestone is expected to be reached within the first year of the project. Once this is accomplished, the milestones of metabolite identification and identification of microorganisms responsible for methanogenesis, including those involved in hydrolyzing the coal and converting these organic precursors into metabolites used by the methanogens, can be achieved. Identifying the rate limiting steps of methanogenesis from coal is an important milestone that should be reached during the final year of the project.