3 GEOCHEMISTRY

3.1 Re-Evaluation of Microbial Gas Generation under Expected Waste Isolation Pilot Plant Conditions[1]

Data Summary and Progress Report (February 1 – July 15, 2002)
July 18, 2002

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Abstract

Gas generation from the microbial degradation of the organic constituents of transuranic waste under conditions expected in the WIPP repository is being investigated at Brookhaven National Laboratory (BNL). This report summarizes progress from the period February 1 – July 15, 2002. During this period, we analyzed total gas and carbon dioxide (CO_2) production in initially aerobic and anaerobic humid samples at 3334 and 2945 days incubation (9.1 and 8.1 years), respectively. Carbon dioxide production in unamended inoculated and amended inoculated samples, initially aerobic and anaerobic, without bentonite, has leveled off, consistent with analyses over the past two years. Initially aerobic, unamended inoculated samples with bentonite continue to produce CO_2, from 244 ± 180 μmoles CO_2 g^{-1} cellulose at 3009 days to 300 ± 228 μmoles CO_2 g^{-1} cellulose at 3334 days (0.17 μmol CO_2 g^{-1} cellulose day^{-1} for the last 325 days); an increase in the rate of CO_2 production has been continuing in these samples since 804 days incubation. This treatment shows the stimulatory effect of bentonite on biodegradation of cellulose under humid conditions. Methane (CH_4) was analyzed in anaerobic humid samples and was below the minimum detectable amount (0.1 nmol ml^{-1}) in all treatments except those containing bentonite. Unamended inoculated samples with bentonite produced 25.5 ± 1.2 nmol CH_4 g^{-1} cellulose at 2653 days (7.3 years) incubation and amended inoculated samples produced 32.6 ± 9.3 nmol CH_4 g^{-1} cellulose. This finding verifies the presence of viable methanogenic bacteria in the humid samples. The extent of biodegradability of plastic and rubber materials, both unirradiated and irradiated, under WIPP-repository relevant conditions are being evaluated. The results for CH_4 analyses at 3070 days (8.4 years) incubation for polyethylene, polyvinylchloride, and neoprene and 2926 (8 years) for unleaded hypalon are summarized. Over a period of 2230 days incubation (6.1 years since CH_4 was last analyzed) the concentration of CH_4 in almost all samples did not increase but remained nearly equal to that measured at 840 days incubation. The exception to this was the unirradiated polyethylene, which increased from 2.14 ± 1.52 μmol $sample^{-1}$ at 840 days incubation to 2.50 ± 0.26 μmol

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sample^{-1} at 3070 days; however, this does not appear to be significant. Experiments performed at a relative humidity (RH) of 70% may no longer appropriately simulate WIPP disposal rooms post-closure due to the fact that magnesium oxide (MgO) is being emplaced. Experiments were initiated this period that employ radiolabelled carbon substrate (^{14}C) to examine microbial gas generation at 10, 40, 60, and 70% RH. The extreme sensitivity of this method for detecting CO_2 production will provide a means of assessing and quantifying microbial activity under the MgO-constrained water (H_2O) activity that simulates the current WIPP repository design.

BNL Project Objectives

1. Re-evaluation of the existing microbial gas data and development of appropriate technical approaches to improve the conservatism in the current gas generation model.
2. Re-examination and improvement of the experiment for cellulose degradation under humid conditions to derive a more realistic rate for humid microbial degradation.
3. Determination of the effect of MgO on the rate and extent of gas generation under humid conditions.
4. Clarification with scooping experiments of the factors that have caused a diminishing microbial gas generation rate with time in the ongoing experiments, including testing the effect of crystallinity on cellulose degradation under hypersaline conditions.
5. Determination of the rate and extent of methanogenesis by halophilic microorganisms. Due to the fact that methanogenesis is the terminal electron-accepting process in any system, it is important to understand the occurrence and rate of this process.

Progress Report

Long-term experiments designed to examine gas generation due to biodegradation of the organic fraction of transuranic wastes under WIPP repository-relevant conditions have been ongoing at BNL. Table A provides information about the status of these studies as of July 15, 2002.

Table A. Status of Microbial Gas Generation Experiments at BNL

Experiment	Start Date	Most Recent Analyses (Days/Years)	Data Reported (this report) (Days/Years)
Long-Term Inundated Cellulose	1/29/92	3462 / 9.5 aqueous metabolite analysis: 3561 / 9.9	(CO_2 analysis planned for FY02)
Initially Aerobic Humid Cellulose	4/7/93	3009 / 8.2	3334 / 9.1
Anaerobic Humid Cellulose	5/4/94	2616 / 7.2	2945 / 8.1 (methane at 2653 / 7.3)
Inundated PE, PVC, and Neoprene	3/9/93	2612 / 7.2	(methane at 3070 / 8.4) (CO_2 planned for FY02)
Inundated Hypalon	8/3/93	2464 / 6.8	(methane at 2926 / 8.0) (CO_2 analysis planned for FY02)

Research performed during this reporting period was conducted according to procedures specified by Francis et al. (2001) under contract AT-8739. Note that the SNL-approved BNL QA Program remained in effect during this reporting period, and is effectively implemented to date. During this period (February 1 – July 15, 2002) the following tasks were completed:

1. Total gas, CO_2, and CH_4 analysis of samples from the initially aerobic and anaerobic humid cellulose biodegradation experiment.
2. Methane analysis of samples from the plastic and rubber biodegradation experiment.
3. Experiments were started that will quantify gas generation due to microbial activity under conditions of MgO-constrained H_2O activity.
4. A planning meeting was held at Sandia Carlsbad on April 3, 2002, to discuss the status of ongoing studies and plans for this fiscal year. This meeting was attended by A.J. Francis and Jeff Gillow, BNL, and Yifeng Wang and Laurence Brush, SNL.
5. More complete details of the progress made during this reporting period are provided below.

QUALITY ASSURANCE PROGRAM

The program was approved by the SNL QA Team Lead on February 23, 2001 and remains in effect.

GAS ANALYSIS OF HUMID SAMPLES

Tables 1-4, Appendix A, provide data for total gas and CO_2 produced in initially aerobic humid cellulose samples incubated for 3334 days (9.1 years). All samples contain 1 g of cellulosic material. Table 5 provides data that has been corrected for CO_2 produced in the absence of cellulose due to metabolism of any dissolved organic carbon in the mixed inoculum. Table 6-9 provide data for total gas and CO_2 produced in anaerobic humid samples incubated for 2945 days (8.1 years); Table 10 provides corrected data for CO_2 production. All data are reported as gas produced g^{-1} cellulose and are the mean ± standard error of the mean of the analysis of triplicate samples. In all cases samples prepared to determine inoculum viability (succinate or glucose amended treatments) were not analyzed during this time period.

Initially Aerobic Treatments

Initially aerobic humid treatments with and without bentonite have ceased to produce gas as indicated by the decrease in total gas produced at 3334 days (Table 1 and 2). These samples have not produced any gas since the previous analysis period. The concentration of CO_2 in the samples is consistent with the previous analysis specifically in all of the control samples; e.g., the "No cellulose" control showed 2.89 ± 0.08 μmoles CO_2 $sample^{-1}$ at 3009 days and now shows 2.87 ± 0 μmoles CO_2 $sample^{-1}$. Carbon dioxide production in unamended inoculated and amended inoculated samples without bentonite has leveled off (Table 3 and 5), consistent with the analysis over the past two years. This observation is indicative of a limited capability for microbial growth on cellulose under initially aerobic humid conditions (RH = 70%). Unamended inoculated samples with bentonite continue to produce CO_2 (Table 4 and 5) from 244 μmoles CO_2 g^{-1} cellulose at 3009 days to 300 μmoles CO_2 g^{-1} cellulose at 3334 days (0.17 μmol CO_2 g^{-1} cellulose day^{-1} for the last 325 days); an increase in the rate of CO_2 production has been continuing in these samples since 804 days incubation (Table 5). This treatment shows the stimulatory effect of bentonite on biodegradation of cellulose under humid conditions. In addition, the treatment shows the viability of the microbial community over 9.1 years. Therefore the lack of gas production in samples without bentonite, which are relevant to the WIPP repository environment, is not due to a loss of microbial viability. Amended samples, however, have not produced CO_2 since 804 days. The unamended inoculated samples without bentonite showed 6.09 ± 2.41 μmol CO_2 g^{-1} cellulose at 3334 days and amended inoculated samples showed 0.48 ± 0.29 μmol CO_2 g^{-1} cellulose. Unamended inoculated samples with bentonite showed 300 ± 228 μmol CO_2 g^{-1} cellulose at 3334 days and amended inoculated samples showed 615 ± 250 μmol CO_2 g^{-1} cellulose. This is a decrease of 1.4, and 0.6, an increase of 56, and a decrease of 229 μmol, respectively, over 325 days since the last analysis. The changes in CO_2 production over this time period are not significant given the large variation (error) in CO_2 values between triplicate samples.

Anaerobic Treatments

Total gas produced in anaerobic humid samples is presented in Tables 6 and 7; the amended inoculated samples without bentonite showed an increase of 1.29 ml gas sample^{-1} (Table 6) and the unamended inoculated samples with bentonite showed an increase of 1.04 ml gas sample^{-1}; there was no increase in total gas in the other treatments at 2945 days. Carbon dioxide production has not increased at 2945 days in samples without bentonite and similar to initially aerobic samples continues to level off (Table 8). Samples with bentonite have also leveled in terms of CO_2 production (Table 9). After correcting for gas production in the absence of cellulose (Table 10), the unamended inoculated samples without bentonite showed 115 ± 20 μmol CO_2 g^{-1} cellulose at 2945 days and amended inoculated samples showed 21.9 ± 3.3 μmol CO_2 g^{-1} cellulose. Unamended inoculated samples with bentonite showed 541 ± 135 μmol CO_2 g^{-1} cellulose at 2945 days and amended inoculated samples showed 618 ± 125 μmol CO_2 g^{-1} cellulose. This is a decrease of 20, 4.9, 50, and 55 μmoles CO_2, respectively, over 329 days since the last analysis. It is important to note that samples that show a larger deviation from the mean generally show evidence of microbial activity (trending toward positive gas production). The larger spread in the data is indicative of microbial processes that may be occurring at slightly different rates in the active samples due to differences in overall microbial population or metabolic capability. Samples with a smaller variation in values between triplicate bottles have generally ceased to show additional microbial gas production.

ANALYSIS OF METHANE IN ANAEROBIC HUMID SAMPLES

Methanogenesis is a potential gas-consuming microbial process that may occur under repository conditions. In addition, methanogenic bacteria are extremely sensitive to changes in pH, Eh, the presence of oxygen (O_2), and have seldom been found to metabolize complex organic substrates under hypersaline conditions (Oren, 1999). The entire set of samples from the anaerobic humid cellulose biodegradation experiment was analyzed for the presence of methane at 2653 days (7.3 years) incubation. Methane was analyzed by gas chromatography using flame ionization detection and a set of National Institute of Standards and Technology-traceable calibration gases ranging in concentration from 5 to 100 ppm. Methane was detected in unamended inoculated samples with bentonite and amended inoculated samples with bentonite, but was below detectable (<0.1 nmol ml^{-1}) in all other samples. Table B summarizes the results of this analysis.

Table B. Methane Analysis of Anaerobic Humid Samples at 2653 Days Incubation

Sample	Methane (nmol g^{-1} cellulose)
Unamended inoculated + bentonite	25.5 ± 1.2
Amended inoculated + bentonite	32.6 ± 9.3

Gillow and Francis (2002) provided data for methane produced under inundated conditions; in these samples at 3462 days incubation (9.5 years), unamended inoculated sample with bentonite showed 4.51 ± 0.06 nmol CH_4 g^{-1} cellulose and amended inoculated samples with bentonite showed 3.41 ± 0.13 nmol CH_4 g^{-1} cellulose. Under humid conditions, and in the presence of bentonite, the production of methane appears more favorable; in unamended samples methane production was 6x greater than under inundated conditions and almost 10x greater for amended humid samples. This is further evidence of the stimulatory effect of bentonite on microbial activity and also verifies the presence of viable methanogenic bacteria, which should be capable of methanogenesis in all of the samples if conditions are favorable. In addition, the maintenance of strictly anaerobic conditions is verified by methanogenic bacterial activity which cannot occur even in the presence of trace O_2 (Ramakrishnan et al., 2000). The potential remains for the consumption of CO_2 by methanogenic bacteria and additional analyses will provide a suitable data set to compare CO_2 consumption with methane production.

ANALYSIS OF METHANE IN SAMPLES CONTAINING PLASTIC AND RUBBER MATERIALS

Plastic and rubber materials consist of long repeating single bonded carbon chains and are usually quite resistant to biodegradation. Irradiation causes the polymer to break down due to free radical formation, in addition there can be cross-linking of the polymer chain after free radical formation and reduction of the molecular mass of the polymer (Woods and Pikaev, 1994). The extent of biodegradability of plastic and rubber materials, both unirradiated and radiation-damaged, under WIPP-repository relevant conditions are being evaluated in studies initiated in 1993. Details of the experiment are provided elsewhere (Francis et al., 1997). Briefly, sheets of polyethylene (PE), polyvinylchloride (PVC), neoprene (NE), hypalon (HY) and leaded hypalon (LHE) were subject to electron-beam irradiation at Argonne National Laboratory's linear accelerator to create lower absorbed dose (700 Mrad, or 7 MGy) and high absorbed dose (4000-6000 Mrad (40-60 Mgy)) samples for study. Unirradiated and irradiated material was prepared to 2 cm^2 sub samples and these were added to serum bottles and inundated with mixed inoculum/G-Seep brine mixture. Samples were capped with butyl rubber stoppers and have been assessed for total gas, CO_2, H_2, N_2O, and CH_4 attributable to biodegradation of the polymer. Previously, at 7.2 years incubation, total gas and CO_2 was analyzed in these samples. Methane was last analyzed in samples containing PE, PVC, and HY at 840 days incubation, and in samples containing HY at 664 days incubation. Here results for CH_4 analyses at 3070 days (8.4 years) incubation for PE, PVC, and NE and 2926 (8 years) for HY are summarized in Table C (anaerobic treatments only).

Table C. Analysis of Methane in Samples Containing Plastic and Rubber Materials. Nd = Not Detected.

Anaerobic Treatment	Incubation Time	
	T=840 days	T=3070 days
	Methane (μmol sample^{-1})	
Samples without polymer (no irradiation)		
Unamended	0.91 ± 0.14	0.99 ± 0.20
Amended	4.03 ± 0.17	3.65 ± 0.11
Polyethylene		
Unirradiated – Unamended	0.85	0.53
Amended	2.14 ± 1.52	2.50 ± 0.26
Lower-Dose- Unamended	1.01	0.72
Amended	4.13 ± 0.02	3.04 ± 0.11
High-Dose – Unamended	1.02	0.70
Amended	4.29 ± 0.13	1.73 ± 1.20
Polyvinylchloride		
Unirradiated – Unamended	1.27	1.00
Amended	4.88 ± 0.11	3.50 ± 0.37
Lower-Dose-Unamended	nd	nd
Amended	nd	0.004 ± 0.004
High-Dose – Unamended	nd	0.01
Amended	0.03 ± 0.02	0.04 ± 0.04
Neoprene		
Unirradiated – Unamended	0.03	0.02
Amended	4.03 ± 0.22	2.64 ± 0.34
Lower-Dose- Unamended	nd	0.01
Amended	3.87 ± 0.23	3.05 ± 0.14
High-Dose – Unamended	nd	2.79
Amended	4.91 ± 0.04	3.71 ± 0.01

Anaerobic Treatment		Incubation Time	
		T=840 days	T=3070 days
		Methane (μmol sample^{-1})	
Hypalon		*T=664 days*	*T=2926 days*
Unirradiated –	Unamended	nd	0.01
	Amended	0.02 ± 0.00	0.02 ± 0.01
Lower-Dose-	Unamended	nd	0.02
	Amended	0.01 ± 0.00	0.02 ± 0.01

Over a period of 2230 days incubation (6.1 years) the concentration of CH_4 in almost all samples containing polyethylene, polyvinylchloride, and neoprene did not increase but remained nearly equal to that measured at 840 days incubation. The exception to this was the unirradiated polyethylene, which increased from 2.14 ± 1.52 μmol sample^{-1} at 840 days incubation to 2.50 ± 0.26 μmol sample^{-1} at 3070 days; however, this does not appear to be statistically significant. In addition, samples containing hypalon did not show any increase in CH_4 over 2262 days incubation (6.2 years). The concentrations of methane detected at 664 and 840 days and at 2926 and 3070 days are extremely consistent indicating that no further methanogenesis has occurred in these samples. The CH_4 detected is most likely the result of metabolism of dissolved organic C in the mixed inoculum/inundation fluid; however, additional methane production due to biodegradation of the polymer is not evident. The inhibitory effect of irradiated PVC remained after 6.1 years indicating that the degradation products produced due to irradiation continue to be toxic to the microbial consortium in the samples. Additional analyses, including quantification of CO_2 in the samples since the last time period of this analysis (7.2 years), is planned. Characterization of the polymer using solid-state techniques (infrared spectroscopy), specifically in the case of PE, at this stage of incubation is also planned and will provide a more sensitive means of assessing the extent of biodegradation of the polymer.

MICROBIAL GAS GENERATION UNDER CONDITIONS OF MgO-CONSTRAINED WATER ACTIVITY

The two experiments in-progress at BNL are examining gas generation due to cellulose biodegradation under humid conditions. Samples were prepared to maintain a 70% RH environment. Most of the samples in these experiments received 2.0 to 2.5 ml of liquid (2.0 ml of liquid inoculum or 2.0 ml of liquid inoculum and 0.5 ml of a nutrient solution). Experiments performed at an RH of 70% may no longer appropriately simulate WIPP disposal rooms post-closure due to the fact that MgO is being emplaced. Microbial gas generation rates under MgO-constrained humid conditions may be much lower due to sequestering of water, which is necessary for microbial activity. An

experiment procedure has been prepared that tests microbial activity under more relevant MgO-constrained water activity conditions. In order to obtain relevant gas generation data rapidly and accurately, the following was used: (1) a "dry" inoculum; (2) ^{14}C-labeled substrate (for metabolism and growth); (3) extremely sensitive techniques for capturing and quantifying microbially produced CO_2 (alkaline trapping and $^{14}CO_2$ liquid scintillation counting). The experiment is described briefly.

Inoculum Preparation

The facultative anaerobe *Halomonas* sp. (WIPP-1A) was grown in the following media for 48 hours:

WDn Medium	($g\ L^{-1}$)
Sodium succinate	5
Potassium nitrate	1
Potassium phosphate monobasic	0.25
Yeast extract	0.25
Sodium chloride	200
pH	6.8-7.0
Filter sterilized	0.45 μm

The optical density (o.d.) of the culture at 48 hours was 0.8 (measured at 600 nm). The culture was centrifuged at 5,000 xg for 20 minutes, the supernatant decanted, and resuspended in sterile WDn medium diluted 1-4 and centrifuged again at 5,000 xg for 20 minutes. The supernatant was decanted and the cell pellet was resuspended in 20 ml of sterile WDn medium to prepare the inoculum. The o.d. of the inoculum was measured (1.0) and 1.0 ml was preserved with formalin (10% v/v) for direct counting by microscopy. The sample was split into two 10-ml aliquots and air-dried for 1-week followed by final drying in an oven at 30°C.

Sample Preparation

Glass serum bottles (60 ml) were acid washed (10% HCl), rinsed with DI water, autoclaved at 120°C, 20 psi, and air-dried at 80°C overnight. Once dried, 2 ml of WDn medium was added to each of 24 bottles. To six bottles, 40 ml of medium was added. Radiolabelled succinic acid, obtained from PerkinElmer Life Sciences, was added to each of the bottles. Exactly 5 μl of a 3.7 MBq ml^{-1} 1,4-^{14}C-succinic acid solution was pipetted into each bottle for a total of 1.1×10^6 dpm of ^{14}C in each. Sample bottles containing 2 ml of solution were placed in a desiccator along with a 20 ml sterile glass vial containing 10 ml of 0.5 N NaOH to trap, and quantify, any ^{14}C-CO_2 evolved during drying. Samples remained in the desiccator for seven days during which time ^{14}C-CO_2 was not evolved, followed by final drying in an oven at 30°C until dry at which time 12 bottles received air-dried inoculum (0.1 g inoculum $bottle^{-1}$). Tubes (13 x 120 mm, glass, sterile) of saturated salt solutions were added to the bottles in order to maintain the relative humidity at a desired level. An additional 12 bottles received the WDn nutrient

solution but not the ^{14}C substrate and were air-dried. These bottles were used to prepare triplicate samples for humidity testing during the course of the experiment to verify the humidity inside of the bottles using a portable digital humidity meter (Rotronic, Switzerland). The treatment matrix for these experiments is summarized in Table D.

Table D. Treatment Matrix For C-14 Humid Study

Treatment	Relative Humidity				
	Inundated	70 % (G-Seep)	60% (NaBr)	40% (NaI)	10% (LiI)
	Number of Sample Bottles				
Inoculated	3	3	3	3	3
Uninoculated	3	3	3	3	3
Humidity test	3	3	3	3	3

Samples were capped with thick butyl rubber stoppers (Bellco, NJ) affixed with 1-ml sterile glass tubes by running a sterile stainless steel (Nichrome V) wire through the stopper and wrapping the tube to hang it within the bottle. The samples were placed into an incubator set at 30 ± 2°C and alarmed for temperature excursions. The inundated samples serve as a test for inoculum viability and it is expected that CO_2 production will be detected in these samples after a short amount of time relative to humid samples. At approximately 2 months 1.0 ml of sterile 0.5 N NaOH will be added to the hanging-tube within the bottles by using a sterile needle and syringe. The NaOH will be left in the bottle for 1 hour (reaction time is currently being optimized) in order to capture the ^{14}C-CO_2 that is produced in the bottle. The NaOH will then be withdrawn and added to 10 ml of Ultima Gold XR (Packard Instrument Corp.) scintillation fluid for analysis by liquid scintillation counting (LSC). The LSC has a minimum detectable amount of 20 dpm ^{14}C, which will correlate with metabolism of 7.2×10^{-14} mol of ^{14}C-succinate (1.74×10^{-6} mol of ^{14}C-succinate will be present in each bottle). The extreme sensitivity of this method to succinate metabolism and CO_2 production will provide a means of assessing and quantifying microbial activity under the MgO-constrained water activity that simulates the current WIPP repository design. Results of the first round of analyses of these samples will be provided prior to the end of FY'02.

ONGOING WORK

- A draft manuscript, planned for submittal to a peer-reviewed journal, has been prepared that details gas production due to cellulose biodegradation under hypersaline conditions; this is currently undergoing review and revision at SNL and BNL.
- The experiment to examine microbial growth and gas production under conditions of MgO-constrained water activity is underway and analyses continue.

FUTURE WORK

- Total gas, CO_2, and CH_4 will be analyzed in samples prepared to examine gas generation due biodegradation of cellulose under inundated conditions.
- Total gas and CO_2 will be analyzed in samples containing plastic and rubber materials.
- Material characterization techniques including infrared and x-ray spectroscopy will be used to assess the extent of biopolymer degradation due to microbial activity in samples containing cellulose and plastic and rubber materials thus providing an assessment of polymer crystallinity and its relation to the extent and rate of gas generation
- A manuscript concerned with methanogenesis under hypersaline conditions will be prepared for submittal to a peer-reviewed journal.
- A manuscript detailing the effect of bentonite on microbial activity will be prepared for submittal to a peer-reviewed journal.

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Brookhaven National Laboratory
WIPP Gas Generation Project

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Appendix

Data Tables

Total Gas and CO_2 Produced in Samples of the Initially Aerobic and Anaerobic Humid Cellulose Biodegradation Experiment

Tables 1-10

Table 1. Total Volume of Gas Produced in Initially Aerobic Humid Treatments (without bentonite)

Treatments (without bentonite)	Volume of Gas Produced (ml/sample)								
	Incubation Time (Days)								
	6	120	317	399	593	804	2553	3009	3334
Control									
Empty bottle	7.15	-0.22	0.28	1.08	1.19	2.51	0.73	3.37	1.24
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52	0.32	-0.89	1.88	-1.18
No cellulose (salt/ inoculum/ tube+brine)	6.23 ± 0.09	-2.36 ± 0.04	-0.21 ± 0.07	0.73 ± 0.07	0.23 ± 0.04	3.01 ± 0.22	-0.48 ± 0.87	0.20 ± 0.02	-0.62 ± 0.05
Carbon Source: Cellulose Only									
Unamended uninoculated	6.87 ± 0.11	-0.03 ± 1.85	-0.41 ± 0.09	-0.20 ± 0.14	0.12 ± 0.03	1.10 ± 0.17	0.77 ± 0.16	3.84 0.38	-0.73 ± 0.12
Unamended inoculated	7.50 ± 0.33	-0.31 ± 1.62	0.19 ± 0.33	-0.61 ± 0.25	0.31 ± 0.05	1.29 ± 0.25	1.15 ± 0.39	2.91 ± 0.49	-0.96 ± 0.14
Amended uninoculated	6.98 ± 0.18	-0.03 ± 1.68	-0.23 ± 0.10	-0.29 ± 0.13	0.20 ± 0.10	0.50 ± 0.21	1.26 ± 0.24	2.12 ± 0.36	-0.73 ± 0.07
Amended inoculated	7.39 ± 0.11	-0.21 ± 1.57	-0.02 ± 0.18	-0.39 ± 0.07	0.13 ± 0.17	0.77 ± 0.18	0.91 ± 0.12	1.33 ± 0.27	-0.46 ± 0.40
Carbon Source: Cellulose + Glucose									
Amended uninoculated	6.45 ± 0.11	-2.08	0.75 ± 0.00	-0.06 ± 0.21	0.02 ± 0.14	0.13 ± 0.28	1.05 ± 0.22	1.10 ± 0.77	NA
Amended inoculated	7.03 ± 0.07	-1.92 ± 0.11	0.79 ± 0.33	0.35 ± 0.23	0.15 ± 0.04	0.50 ± 0.22	1.15 ± 0.00	1.31 ± 0.40	NA
Amended uninoculated (RG salt)	NA	3.12	1.99 ± 1.90	-0.80 ± 0.11	-0.34 ± 0.33	0.18 ± 0.40	2.87 ± 0.99	2.09 ± 0.29	NA
Carbon Source: Cellulose + Succinate									
Amended uninoculated (w/ acetylene)	19.5	NA	0.64	-0.10	1.66	-0.10	1.98	1.05	NA
Amended uninoculated (w/o acetylene)	5.15	-2.08	0.98	-0.37	-0.08	0.72	0.74	0.22	NA
Amended inoculated (w/ acetylene)	12.9	NA	1.17	0.35	-0.34	-0.10	NA	NA	NA
Amended inoculated (w/o acetylene)	5.88	-2.29	1.27	0.05	0.17	0.72	2.18	1.25	NA

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed
3334 day data from notebook JG121101

Table 2. Total Volume of Gas Produced in Initially Aerobic Humid Treatments (with bentonite)

Treatments (with bentonite)	Volume of Gas Produced (ml/sample)								
	Incubation Time (Days)								
	6	120	317	399	593	804	2553	3009	3334
Control									
Empty bottle	7.15	-0.22	0.28	1.08	1.19	2.51	0.73	3.37	1.24
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52	0.32	-0.89	1.88	-1.18
No cellulose (salt/ inoculum/ tube+brine)	7.25 ± 0.03	-2.42 ± 0.08	-0.42 ± 0.07	0.52 ± 0.18	0.33 ± 0.04	1.68 ± 0.95	1.47 ± 0.51	1.11 ± 0.48	-0.80 ± 0.14
Carbon Source: Cellulose Only									
Unamended uninoculated	5.67 ± 0.00	1.03 ± 1.41	-0.62 ± 0.17	-0.39 ± 0.15	0.31 ± 0.05	-0.01 ± 0.10	1.38 ± 0.25	4.67 ± 0.34	2.21 ± 0.16
Unamended inoculated	6.35 ± 0.48	-0.59 ± 1.52	0.11 ± 0.13	-0.40 ± 0.08	0.06 ± 0.12	0.02 ± 0.32	1.05 ± 0.30	2.39 ± 0.69	0.76 ± 0.15
Amended uninoculated	6.09 ± 0.00	0.08 ± 1.85	0.01 ± 0.13	-0.15 ± 0.13	0.11 ± 0.05	0.19 ± 0.27	2.05 ± 0.99	1.36 ± 0.29	-0.46 ± 0.03
Amended inoculated	7.81 ± 0.26	0.78 ± 1.56	0.35 ± 0.31	0.02 ± 0.24	0.11 ± 0.14	0.51 ± 0.19	1.15 ± 0.18	0.43 ± 0.48	0.02 ± 0.00
Carbon Source: Cellulose + Glucose									
Amended uninoculated	6.35 ± 0.04	-1.98	-1.45 ± 0.29	-0.09 ± 0.25	0.07 ± 0.07	1.03 ± 0.76	1.41 ± 0.40	3.38 ± 0.76	NA
Amended inoculated	7.29 ± 0.11	-1.45 ± 0.07	-0.42 ± 0.07	0.23 ± 0.11	0.20 ± 0.04	1.28 ± 0.83	1.20 ± 0.04	NA	NA
Amended uninoculated (RG salt)	NA	2.60	1.78 ± 1.57	-0.82 ± 0.21	0.13 ± 0.04	1.59 ± 0.76	1.26 ± 0.37	4.06 ± 0.22	NA
Carbon Source: Cellulose + Succinate									
Amended uninoculated (w/ acetylene)	18.7	NA	0.74	-0.15	0.07	-0.63	1.46	2.18	NA
Amended uninoculated (w/o acetylene)	5.56	-1.98	1.71	-0.76	0.27	-0.33	0.84	2.30	NA
Amended inoculated (w/ acetylene)	18.0	NA	2.00	0.05	0.10	0.55	NA	NA	NA
Amended inoculated (w/o acetylene)	6.82	-2.29	2.30	0.67	-0.11	1.16	0.74	-0.19	NA

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed
3334 day data from notebook JG121101

Table 3. Production of Carbon Dioxide in Initially Aerobic Humid Treatments (without bentonite)

Treatments (without bentonite)	Carbon Dioxide (µmoles/sample) Incubation Time (Days)								
	6	120	317	399	593	804	2553	3009	3334
Control									
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71	2.68	2.94	3.07
Blank (tube+brine only)	4.18	4.64	4.54	4.63	3.00	2.76	2.74	3.50	3.48
No cellulose (salt / inoculum/ tube+brine)	7.93 ± 0.19	14.0 ± 0.1	10.7 ± 0.3	9.21 ± 0.06	6.28 ± 0.22	3.61 ± 0.18	3.55 ± 0.2	2.89 ± 0.08	2.87 ± 0
Carbon Source: Cellulose Only									
Unamended uninoculated	7.45 ± 0.21	10.7 ± 0.2	12.2 ± 0.7	12.2 ± 0.9	11.2 ± 1.5	8.96 ± 1.82	8.73 ± 2.43	7.40 ± 1.66	5.99 ± 1.14
Unamended inoculated	11.7 ± 0.1	56.0 ± 4.4	72.6 ± 11.4	65.5 ± 11.5	45.3 ± 8.1	27.6 ± 5.3	12 ± 3.25	10.4 ± 2.68	8.96 ± 2.41
Amended uninoculated	14.0 ± 1.1	28.1 ± 0.8	24.1 ± 1.8	22.9 ± 2.6	17.4 ± 3.1	12.2 ± 2.7	6.08 ± 1.78	6.23 ± 1.88	5.94 ± 1.88
Amended inoculated	35.9 ± 1.3	42.4 ± 1.5	31.1 ± 2.4	24.8 ± 2.9	14.7 ± 2.4	8.21 ± 1.75	4.48 ± 1.09	3.96 ± 0.56	3.35 ± 0.29
Carbon Source: Cellulose + Glucose									
Amended uninoculated	12.7 ± 0.4	32.7	39.7 ± 0.6	38.6 ± 1.2	35.0 ± 3.07	26.5 ± 4.5	29.83 ± 5.84	28.4 ± 10	NA
Amended inoculated	28.3 ± 1.6	183 ± 98	236 ± 140	166 ± 96	79.8 ± 39.8	28.2 ± 9.0	9.1 ± 1.46	8.41 ± 2.77	NA
Amended uninoculated (RG salt)	NA	36.0	44.8 ± 0.1	46.5 ± 0.1	47.4 ± 2.6	39.4 ± 5.6	56.81 ± 3.99	61.0 ± 5.8	NA
Carbon Source: Cellulose + Succinate									
Amended uninoculated (w/ acetylene)	15.1	NA	28.8	27.7	21.0	16.8	22.12	NA	NA
Amended uninoculated (w/o acetylene)	15.7	26.0	22.7	19.7	14.4	7.06	4.75	3.25	NA
Amended inoculated (w/ acetylene)	14.5	NA	1384	1450	1470	1270	NA	NA	NA
Amended inoculated (w/o acetylene)	15.8	42.4	40.0	38.2	29.5	23.8	16.86	11.3	NA

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed
3334 day data from notebook JG121101

Table 4. Production of Carbon Dioxide in Initially Aerobic Humid Treatments (with bentonite)

Treatments (with bentonite)	Carbon Dioxide (µmoles/sample) Incubation Time (Days)								
	6	120	317	399	593	804	2553	3009	3334
Control									
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71	2.68	2.94	3.07
Blank (tube+brine only)	4.18	4.64	4.54	4.63	3.00	2.76	2.74	3.50	3.48
No cellulose (salt / inoculum/ tube+brine)	34.2 ± 0.8	164 ± 1	168 ± 8	144 ± 4	89.1 ± 0.8	42.3 ± 3.0	16.13 ± 4.52	13.6 ± 4	10.6 ± 2.5
Carbon Source: Cellulose Only									
Unamended uninoculated	9.15 ± 0.58	12.1 ± 0.6	13.2 ± 0.6	13.1 ± 0.3	11.0 ± 0.5	9.82 ± 0.15	9.98 ± 1.15	10.5 ± 0.3	10.2 ± 0.3
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61	184 ± 76	233 ± 152	258 ± 180	311 ± 228
Amended uninoculated	15.2 ± 0.9	52.2 ± 1.8	49.9 ± 1.1	45.1 ± 2.4	33.2 ± 4.2	23.1 ± 5.5	22.1 ± 6.29	15.1 ± 6.9	12.0 ± 6.0
Amended inoculated	53.7 ± 2.4	1030 ± 80	1620 ± 30	1600 ± 40	1520 ± 40	1469.8 ± 40	1059 ± 207	858 ± 219	626 ± 250
Carbon Source: Cellulose + Glucose									
Amended uninoculated	14.8 ± 0.5	46.3	590 ± 364	625 ± 394	694 ± 438	631 ± 401	53.8 ± 26.3	50.5 ± 27.5	NA
Amended inoculated	44.9 ± 2.6	1590 ± 40	1240 ± 20	1250 ± 160	1240 ± 240	816 ± 355	964 ± 230	n/a ±	NA
Amended uninoculated (RG salt)	NA	39.5	50.9 ± 1.3	54.6 ± 2.4	55.7 ± 6.7	45.7 ± 8.6	82.0 ± 37.0	90.7 ± 45.3	NA
Carbon Source: Cellulose + Succinate									
Amended uninoculated (w/ acetylene)	22.9	NA	50.0	50.8	46.1	38.9	27.8	27.7	NA
Amended uninoculated (w/o acetylene)	21.7	47.7	50.4	46.8	43.6	37.3	34.0	30.3	NA
Amended inoculated (w/ acetylene)	38.5	NA	1430	1470	1540	1460	NA	NA	NA
Amended inoculated (w/o acetylene)	52.8	1130	1460	1500	1520	1400	631	320	NA

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed
3334 day data from notebook JG121101

Table 5. Summary of Carbon Dioxide Production per gram Cellulose in Initially Aerobic Humid Treatments (including corrected data)

Treatments ***without bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 6	120	317	399	593	804	2553	3009	3334
Control									
No cellulose (salt/ inoculum/ tube+brine)	7.93 ± 0.19	14.0 ± 0.1	10.7 ± 0.3	9.21 ± 0.06	6.38 ± 0.22	3.61 ± 0.18	3.55 ± 0.2	2.89 ± 0.08	2.87 ± 0
Carbon Source: Cellulose									
Unamended inoculated	11.7 ± 0.1	56.0 ± 4.4	72.6 ± 11.4	65.5 ± 11.5	45.3 ± 8.1	27.6 ± 5.3	12.0 ± 3.25	10.4 ± 2.68	8.96 ± 2.41
Amended Inoculated	35.9 ± 1.3	42.4 ± 1.5	31.1 ± 2.4	24.8 ± 2.9	14.7 ± 2.4	8.21 ± 1.75	4.48 ± 1.09	3.96 ± 0.56	3.35 ± 0.29
*Unamended inoculated (corrected)**	3.77 ± 0.22	42.1 ± 4.4	62.0 ± 11.4	56.3 ± 11.5	38.9 ± 8.1	24.0 ± 5.3	8.45 ± 3.26	7.51 ± 2.68	6.09 ± 2.41
*Amended inoculated (corrected)**	28.0 ± 1.3	28.5 ± 1.5	20.5 ± 2.4	15.6 ± 2.9	8.32 ± 2.41	4.60 ± 1.76	0.93 ± 1.11	1.07 ± 0.57	0.48 ± 0.29

Treatments ***with bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 6	120	317	399	593	804	2553	3009	3334
Control									
No cellulose (salt/ inoculum/ tube+brine)	34.2 ± 0.8	164 ± 1	168 ± 8	144 ± 4	89.1 ± 0.8	42.3 ± 3	16.13 ± 4.52	13.6 ± 4.0	10.6 ± 2.5
Carbon Source: Cellulose									
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	288 ± 44	219 ± 61	184 ± 76	233 ± 152	258 ± 180	311 ± 228
Amended inoculated	53.7 ± 2.4	1033 ± 76	1623 ± 26	1600 ± 44	1520 ± 40	1470 ± 40	1059 ± 207	858 ± 219	626 ± 250
*Unamended inoculated (corrected)**	-13.5 ± 0.8	8.00 ± 5.41	105 ± 26	124 ± 44	130 ± 61	142 ± 76	217 ± 152	244 ± 180	300 ± 228
*Amended inoculated (corrected)**	19.5 ± 2.5	869 ± 76	1455 ± 28	1456 ± 44	1431 ± 40	1428 ± 40	1043 ± 207	844 ± 219	615 ± 250

** These samples have been corrected with the appropriate control for gas production in the absence of cellulose*

Table 6. Total Volume of Gas Produced in Anaerobic Humid Treatments (without bentonite)

Treatments (without bentonite)	Total Volume of Gas Produced (ml/sample)													
	Days													
	6	*100*	gas produced* (94 d)	*140*	gas produced (40d)	*415*	gas produced (275 d)	*2156*	gas produced (1741 d)	*2616*	gas produced (460 d)	*2945*	gas produced (329 d)
Control													
Empty bottle	7.98 ± 0.59	4.62 ± 0.54	-3.38	3.61 ± 0.66	-1.01	2.01 ± 1.04	-1.60	0.72	-1.29	0.29	-0.43	2.51 ± 0.46	2.22
Blank (tube+brine only)	6.85 ± 0.38	3.81 ± 0.34	-3.04	2.80 ± 0.27	-1.01	0.37 ± 1.02	-2.43	-0.89	-1.26	n/a		-0.85 ± 0.11	
No cellulose (salt/ inoculum/ tube+brine)	6.49 ± 0.04	3.07 ± 0.07	-3.42	1.56 ± 0.63	-1.51	2.76 ± 0.88	1.20	5.53	2.77	2.33	-3.20	-0.57 ± 0.93	-2.90
Carbon Source: Cellulose Only													
Unamended uninoculated	7.33 ± 0.80	1.59 ± 1.25	-5.74	0.01 ± 1.07	-1.58	-2.26 ± 0.17	-2.27	0.09 ± 0.18	2.35	2.51 ± 0.59	2.42	-0.64 ± 0.73	-3.15
Unamended inoculated	9.49 ± 0.45	2.40 ± 1.23	-7.09	1.17 ± 1.39	-1.23	-0.28 ± 1.23	-1.45	2.00 ± 1.02	2.28	1.42 ± 0.58	-0.58	-0.50 ± 0.31	-1.92
Amended uninoculated	7.50 ± 0.13	0.93 ± 1.25	-6.57	-0.92 ± 1.12	-1.85	-1.87 ± 0.24	-0.95	1.70 ± 1.05	3.57	1.86 ± 1.01	0.16	-0.57 ± 0.74	-2.43
Amended inoculated	7.64 ± 0.37	0.89 ± 0.69	-6.75	-0.54 ± 1.03	-1.43	-1.07 ± 1.15	-0.53	0.43 ± 0.00	1.50	0.19 ± 0.15	-0.24	1.48 ± 1.14	1.29
Amended inoculated (w/ acetylene)	20.4 ± 0.1	16.6 ± 0.8	-3.87	14.85 ± 0.48	-1.81	7.15 ± 5.15	-7.80	0.32 ± 0.08	-6.83	0.25 ± 0.23	-0.07	NA	
Carbon Source: Cellulose + Glucose													
Amended uninoculated	6.55 ± 0.63	3.82 ± 0.73	-2.73	2.07 ± 0.86	-1.75	-0.51 ± 0.44	-2.58	2.50 ± 0.62	3.01	1.57 ± 0.62	-0.93	NA	
Amended inoculated	7.18 ± 0.04	4.83 ± 0.11	-2.35	1.77 ± 1.10	-3.06	0.68 ± 1.90	-1.09	3.27 ± 1.74	2.59	2.34 ± 1.89	-0.93	NA	
Amended uninoculated (RG salt)	6.60 ± 0.00	2.35 ± 1.90	-4.25	0.18 ± 2.28	-2.17	0.09 ± 1.46	-0.09	3.83 ± 0.51	3.74	1.27 ± 0.15	-2.56	NA	
Carbon Source: Cellulose + Succinate													
Amended uninoculated (w/ acetylene)	18.9 ± 0.1	10.8 ± 4.1	-8.11	3.66 ± 1.90	-7.15	8.11 ± 5.24	4.45	NA	NA	1.60		NA	
Amended uninoculated (w/o acetylene)	8.30 ± 0.19	4.50 ± 0.29	-1.80	4.21 ± 0.37	-0.29	2.49 ± 1.80	-1.72	8.69	6.20	NA		NA	
Amended inoculated (w/ acetylene)	18.7 ± 0.1	7.27 ± 6.63	-11.46	6.83 ± 6.43	-0.44	6.46 ± 4.32	-0.37	5.70 ± 3.19	-0.78	3.25	-2.45	NA	
Amended inoculated (w/o acetylene)	5.67 ± 0.04	1.70 ± 1.72	-3.97	0.67 ± 1.71	-1.03	2.48 ± 1.61	1.79	7.05	4.59	NA		NA	

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed
*net gas produced between two time periods (duration between analyses given in parentheses).
2945 day data from notebook JG121101

Table 7. Total Volume of Gas Produced in Anaerobic Humid Treatments (with bentonite)

Treatments (with bentonite)	Total Volume of Gas Produced (ml/sample)														
	Days														
	6	100	gas produced* (94 d)	140	gas produced (40 d)	415	gas produced (275 d)	2156	gas produced (1741 d)	2616	gas produced (460 d)	2945		gas produced (329 d)
Control														
Empty bottle	7.98 ± 0.59	4.62 ± 0.54	-3.36	3.61 ± 0.66	-1.01	2.01 ± 1.04	-1.60	0.72	-1.29	0.29	-0.43	2.51	± 0.46	2.22
Blank (tube+brine only)	6.85 ± 0.38	3.81 ± 0.34	-3.04	2.80 ± 0.27	-1.01	0.37 ± 1.02	-2.43	-0.89	-1.26	n/a		-0.85	± 0.11	
No cellulose (salt/ inoculum/ tube+brine)	6.18 ± 0.19	4.60 ± 0.37	-1.58	0.87 ± 1.85	-3.73	1.93 ± 0.37	1.06	-1.79	-3.72	0.78	2.57	-0.83	± 0.11	-1.61
Carbon Source: Cellulose Only														
Unamended uninoculated	7.22 ± 0.25	2.91 ± 0.90	-4.31	1.40 ± 1.22	-1.51	-0.65 ± 1.05	-2.05	0.98 ± 0.52	1.63	-1.04 ± 0.28	-2.02	0.00	± 0.79	1.04
Unamended inoculated	6.63 ± 0.03	6.36 ± 1.22	-0.27	5.86 ± 3.11	-0.50	11.22 ± 5.42	5.36	6.37 ± 2.06	-4.85	-0.59 ± 0.62	-6.96	-3.09	± 0.50	-2.50
Amended uninoculated	6.18 ± 0.08	3.72 ± 0.51	-2.46	1.57 ± 1.11	-2.15	-0.79 ± 1.06	-2.36	1.05 ± 0.47	1.84	2.92 ± 0.56	1.87	-1.24	± 0.63	-4.16
Amended inoculated	6.81 ± 0.12	10.4 ± 1.7	3.59	15.31 ± 1.70	4.91	8.60 ± 2.97	-6.71	2.58 ± 1.49	-6.02	1.52 ± 0.20	-1.06	-2.19	± 1.18	-3.71
Amended inoculated (w/ acetylene)	18.2 0.3	17.2 ± 0.3	-1.02	15.54 ± 0.74	-1.61	7.32 ± 5.11	-8.22	8.16 ± 4.20	0.84	6.22 ± 2.44	-1.94	NA		
Carbon Source: Cellulose + Glucose														
Amended uninoculated	7.18 ± 0.04	3.18 ± 1.10	-4.00	-0.39 ± 0.77	-3.57	-1.91 ± 0.00	-1.52	0.19	2.10	-0.43	-0.62	NA		
Amended inoculated	6.97 ± 0.11	9.79 ± 3.73	2.82	7.87 ± 4.78	-1.92	7.46 ± 6.62	-0.41	7.73 ± 4.82	0.27	7.73 ± 4.53	0.00	NA		
Amended uninoculated (RG salt)	7.18 ± 0.14	5.51 ± 0.04	-1.67	3.27 ± 0.29	-2.24	2.43 ± 0.95	-0.84	6.23 ± 1.15	3.80	5.01 ± 0.94	-1.22	NA		
Carbon Source: Cellulose + Succinate														
Amended uninoculated (w/ acetylene)	19.9 ± 0.4	8.36 ± 2.14	-11.52	4.75 ± 3.05	-3.61	-1.54 ± 0.03	-6.29	2.34 ± 0.62	3.88	1.51 ± 0.10	-0.83	NA		
Amended uninoculated (w/o acetylene)	7.91 ± 0.48	4.26 ± 1.10	-3.65	3.20 ± 1.03	-1.06	3.86 ± 0.24	0.66	3.37 ± 2.03	-0.49	2.86 ± 1.60	-0.51	NA		
Amended inoculated (w/ acetylene)	19.6 ± 0.1	16.7 ± 0.5	-2.89	8.59 ± 4.01	-8.12	5.36 ± 5.00	-3.23	10.04	4.68	1.46	-8.58	NA		
Amended inoculated (w/o acetylene)	6.76 ± 0.18	10.2 ± 0.3	3.42	10.41 ± 1.22	0.23	3.84 ± 1.94	-6.57	-0.53	-4.37	0.50	1.03	NA		

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed
*net gas produced between two time periods (duration between analyses given in parentheses).
2945 day data from notebook JG121101

Table 8. Production of Carbon Dioxide in Anaerobic Humid Samples (without bentonite)

Treatments (without bentonite)	µmoles CO_2/Sample						
	Days						
	6	100	140	415	2156	2616	2945
Control							
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00	4.13	1.84	1.80 ± 0.09
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14	2.39	2.37 ± 0.04
Salt / inoculum/ tube+brine (no cellulose)	3.60 ± 0.01	5.90 ± 0.11	7.63 ± 1.08	16.4 ± 0.6	8.35	6.81	5.38 ± 1.97
Carbon Source: Cellulose Only							
Unamended uninoculated	4.07 ± 0.09	5.44 ± 0.10	6.22 ± 0.82	8.05 ± 0.18	15.8 ± 0.46	17.7 ± 0.3	16.5 ± 0.8
Unamended inoculated	11.3 ± 0.12	25.9 ± 3.8	36.1 ± 7.0	89.0 ± 24.4	163 ± 36	142 ± 28	120 ± 20
Amended uninoculated	3.34 ± 0.22	34.3 ± 1.44	39.8 ± 0.9	32.3 ± 1.5	13.5 ± 2.76	31.2 ± 7.0	25.1 ± 8.0
Amended inoculated	16.9 ± 1.15	36.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9	18.2 ± 1	33.6 ± 1.0	27.3 ± 2.7
Amended inoculated (w/ acetylene)	13.7 ± 1.3	38.5 ± 2.2	42.7 ± 2.5	61.0 ± 16.9	47.3 ± 17	76.5 ± 27.0	n/a
Carbon Source: Cellulose + Glucose							
Amended uninoculated	3.34 ± 0.27	23.5 ± 1.6	31.3 ± 0.0	38.6 ± 2.1	42.9 ± 5.2	54.9 ± 8.9	NA
Amended inoculated	17.7 ± 0.47	39.8 ± 0.2	42.2 ± 0.9	41.8 ± 4.2	52.8 ± 10.8	58.9 ± 12.2	NA
Amended uninoculated (RG salt)	4.07 ± 0.37	19.8 ± 2.4	28.9 ± 0.6	26.3 ± 2.9	47.8 ± 12.3	48.2 ± 19.7	NA
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	3.21 ± 0.04	22.6 ± 0.8	29.4 ± 2.5	28.8 ± 3.0	NA	33.8 ± 7.2	NA
Amended uninoculated (w/o acetylene)	3.19 ± 0.18	21.4 ± 0.2	27.9 ± 0.5	34.1 ± 2.5	984	NA	NA
Amended inoculated (w/ acetylene)	13.5 ± 0.7	78.1 ± 33.4	123 ± 63	308 ± 175	99.8	133 ± 79	NA
Amended inoculated (w/o acetylene)	14.8 ± 0.2	60.5 ± 16.0	106 ± 21	328 ± 78	1034	NA	NA

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
n/a =not analyzed
2945 day data from notebook JG121101

Table 9. Production of Carbon Dioxide in Anaerobic Humid Samples (with bentonite)

Treatments (with bentonite)	µmoles CO_2/Sample						
	Days						
	6	100	140	415	2156	2616	2945
Control							
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00	4.13	1.84	1.80 ± 0.09
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14	2.39	2.37 ± 0.04
Salt / inoculum/ tube+brine (no cellulose)	14.2 ± 0.51	36.6 ± 6.1	39.8 ± 5.5	51.6 ± 3.4	93.8	59.21 ± 14.1	63.9 ± 11.8
Carbon Source: Cellulose Only							
Unamended uninoculated	5.04 ± 0.15	12.1 ± 3.2	14.4 ± 3.6	26.5 ± 8.9	37.6 ± 19.1	70.5 ± 36.4	80.7 ± 40.6
Unamended inoculated	20.3 ± 0.2	93.7 ± 2.6	186 ± 6	434 ± 39	483 ± 133	650 ± 175	605 ± 134
Amended uninoculated	6.65 ± 0.80	39.2 ± 1.5	45.5 ± 1.5	49.6 ± 1.6	41.7 ± 3.2	70.3 ± 4.3	67.1 ± 10.1
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7	732 ± 47	682 ± 124.0
Amended inoculated (w/ acetylene)	26.8 ± 0.7	94.0 ± 18.6	123 ± 30	251 ± 92	558 ± 270	609 ± 273	NA
Carbon Source: Cellulose + Glucose							
Amended uninoculated	6.71 ± 0.12	44.5 ± 0.2	53.1 ± 0.4	64.3 ± 1.0	177	201 ± 4	NA
Amended inoculated	31.4 ± 0.7	396 ± 13	487 ± 1	584 ± 28	754 ± 94	641 ± 16	NA
Amended uninoculated (RG salt)	5.26 ± 0.45	45.9 ± 0.7	55.1 ± 1.4	74.9 ± 2.2	178 ± 3	209 ± 1	NA
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	5.77 ± 0.60	0.00 ± 0.00	41.5 ± 3.1	36.7 ± 0.9	48.5 ± 0.5	75.0 ± 6.3	NA
Amended uninoculated (w/o acetylene)	8.58 ± 0.74	44.9 ± 1.6	51.5 ± 1.0	54.0 ± 2.0	79.4 ± 3.4	44.8 ± 0.6	NA
Amended inoculated (w/ acetylene)	27.7 ± 0.27	70.3 ± 2.7	114 ± 0	324 ± 30	447	568	NA
Amended inoculated (w/o acetylene)	28.0 ± 0.82	237 ± 2	317 ± 6	516 ± 0	1356	944 ± 110	NA

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed
2945 day data from notebook JG121101

Table 10. Summary of Carbon Dioxide Production per gram Cellulose in Anaerobic Humid Samples

Treatments ***without bentonite***	*Carbon dioxide (µmoles/ gram cellulose)* Days						
	6	100	140	415	2156	2616	2945
Control							
No cellulose (salt/ inoculum/ tube+brine)	3.60 ± 0.01	5.9 ± 0.1	7.64 ± 1.08	16.4 ± 0.6	8.35	6.81	5.38 ± 1.97
Carbon Source: Cellulose							
Unamended inoculated	11.3 ± 0.1	25.9 ± 3.8	36.1 ± 7	89 ± 24.4	163 ± 36	142 ± 28	120 ± 20
Amended inoculated	16.9 ± 1.2	36.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9	18.2 ± 1.0	33.6 ± 1.0	27.3 ± 2.7
*Unamended inoculated (corrected)**	7.70 ± 0.12	20.0 ± 3.8	28.5 ± 7.1	72.6 ± 24.4	155 ± 36	135 ± 28	115 ± 20
*Amended inoculated (corrected)**	13.3 ± 1.2	30.5 ± 0.8	32.8 ± 1.3	18.3 ± 1.1	9.9 ± 1.0	26.8 ± 1.0	21.9 ± 3.3

Treatments ***with bentonite***	*Carbon dioxide (µmoles/ gram cellulose)* Days						
	6	100	140	415	2156	2616	2945
Control							
No cellulose (salt/ inoculum/ tube+brine)	14.2 ± 0.5	36.6 ± 6.1	39.6 ± 5.5	51.6 ± 3.4	93.6	59.2 ± 14.1	63.9 ± 11.8
Carbon Source: Cellulose							
Unamended inoculated	20.3 ± 0.2	94 ± 3	186 ± 6	434 ± 39	483 ± 133	650 ± 175	605 ± 134
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7	732 ± 47	682 ± 124
*Unamended inoculated (corrected)**	6.10 ± 0.55	57.1 ± 6.6	146 ± 8	382 ± 39	389 ± 133	591 ± 176	541 ± 135
*Amended inoculated (corrected)**	18.0 ± 1.2	213 ± 31	433 ± 26	390 ± 152	460 ± 36	673 ± 49	618 ± 125

** These samples have been corrected with the appropriate control for gas production in the absence of cellulose*