3.0 Microbial Degradation

Re-evaluation of Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Conditions[1]

Data Summary and Progress Report (February 1 – July 13, 2001)
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Abstract

Gas generation from the microbial degradation of the organic constituents of transuranic waste under conditions expected at the WIPP repository is being investigated at Brookhaven National Laboratory. The rates of gas production between 3.4 and 7.4 years from samples containing cellulose under anaerobic inundated conditions, reported previously, were in unamended uninoculated samples, 0.007 μmol CO_2 g^{-1} cellulose day^{-1}; unamended inoculated samples, 0.007; amended inoculated, 0.01; and amended, inoculated plus excess nitrate, 0.04. This report summarizes progress from the period February 1 – July 13, 2001. It includes total gas and carbon dioxide production data obtained from samples incubated under humid (~70% relative humidity) conditions analyzed after 3009 days (8 years) of incubation for initially aerobic samples and 2161 days (6 years) for anaerobic (N_2) samples. Initially aerobic humid samples did not show any marked increase in total gas or CO_2 production over the 1.25 year period since they were last analyzed and gas production has subsided. In samples incubated under anaerobic humid conditions CO_2 increased by 3.44 μmol g^{-1} cellulose over 460 days in the unamended uninoculated samples (0.007 μmol CO_2 g^{-1} cellulose day^{-1}); in the unamended inoculated samples CO_2 production has decreased and in the amended inoculated samples there was an increase of 16.9 μmoles CO_2 g^{-1} cellulose (0.04 μmol CO_2 g^{-1} cellulose day^{-1}). Analysis for CH_4 in anaerobic samples inundated with brine after 9.5 years showed continued methane production albeit at a very slow rate (2.5 pmol CH_4 g^{-1} cellulose d^{-1} in unamended inoculated samples). Work in-progress includes methane analysis of humid samples, gas analysis of inundated samples, initiation of microbial community analysis and species identification in inundated and humid samples and preparation of a protocol for the examination of microbial gas production under conditions of MgO-constrained water activity.

[1] This work is covered by BOE #1.3.05.04.01 and WBS #1.3.5.4.1. A more recent description of this work appears in Sandia National Laboratories WIPP/NTP Work Scope for FY01, December 14, 2000.

BNL Project Objectives

1. Re-evaluate the existing microbial gas data and develop appropriate technical approaches to reducing 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. Determine the effect of MgO on the rate and extent of gas generation under humid conditions.

4. Scoping experiments to test the effect of crystallinity on cellulose degradation under hypersaline conditions and to clarify the factors that caused a diminishing microbial gas generation rate with time in the previous experiments.

5. Determine the rate and extent of methanogenesis by halophilic microorganisms.

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 Brookhaven National Laboratory (BNL). Table 1 provides information about the status of these studies.

Table 1. Status of Microbial Gas Generation Experiments at BNL.

Experiment	Start Date	SAND96-2582 (Days/Years) (1996)	Most Recent Analysis Date	Incubation Time Most Recent Analysis (Days/Years)
Long-Term Inundated Cellulose	1/29/92	1228 / 3.4	**7/12/01 CH_4** 7/28/99 CO_2	**3462 / 9.5 (CH_4)** 2718 / 7.4 (CO_2)
Initially Aerobic Humid Cellulose	4/7/93	804 / 2.3	**7/9/01 CO_2**	**3009 / 8.2**
Anaerobic Humid Cellulose	5/4/94	415 / 1.1	**7/11/01 CO_2**	**2616 / 7.2**
Inundated PE, PVC, and Neoprene	3/9/93	840 / 2.3	5/3/00 CO_2	2612 / 7.2
Inundated Hypalon	8/3/93	664 / 1.8	5/2/00 CO_2	2464 / 6.8

Research performed during FY2001 has been conducted according to Sandia National Laboratories (SNL) Waste Isolation Pilot Plant Test Plan TP-99-01, effective 3/21/01,

under contract AT-8739. During this period (February 1 – July 13) the following was completed:

1. The quality assurance program for the project was approved by SNL February 23, 2001.
2. A quality assurance audit was performed on June 6-7, 2001, by SNL.
3. Methane (CH_4) was analyzed in select anaerobic samples from the long-term inundated cellulose biodegradation experiment (specifically those samples without bentonite).
4. Samples from the long-term inundated cellulose biodegradation experiment were prepared for microbiological characterization.
5. Specific emphasis during this period was placed on providing new data for total gas and CO_2 production under humid conditions (~70% relative humidity)
6. A protocol for the examination of gas production under conditions of MgO-constrained water activity was prepared and is undergoing review.

More complete details of the progress to date is provided below.

1. Quality Assurance Program: In order to meet the requirements of the DOE Carlsbad Field Office (CBFO) Quality Assurance Program Description (QAPD) Revision 3, the BNL staff worked closely with SNL to revise the QA program. It was decided that all experimental work performed at BNL under contract AT-8739 will be conducted in accordance with current SNL Nuclear Waste Management Program Procedures (NPs) and SPs, with the following exceptions: BNL Standards Based Management System (SBMS) procedures will be followed for calibration of measuring and test equipment and for purchasing items. This program was approved by Greg Miller, SNL QA Team Lead on February 23, 2001.

2. Quality Assurance Audit: An audit was performed on June 6-7, 2001, to verify effective implementation and compliance with QA requirements. Greg Miller, SNL QA Team Lead and Dr. Yifeng Wang of SNL visited BNL to perform the audit. There were no deficiencies that needed remedy after audit close-out.

3. Methane Analysis: Table 2 provides the latest methane data for the inundated cellulose samples incubated for 3462 days. Methane was analyzed by gas chromatography using flame ionization detection. The minimum detectable quantity is 0.2 nmol CH_4 g^{-1} cellulose dry wt. Methane was first detected in small quantities in most anaerobic samples except those with excess nitrate (Table 2, 2718 days). The lack of methane production in amended samples that contain nitrogen-compounds was consistent with information in the literature related to the inhibitory effect of nitrate on methanogenic activity (Kluber 1998). At 3462 days (9.5 years) methane was still detected in greatest quantity in samples that were not amended with any nitrogen-containing compounds at all (NH_4NO_3, KNO_3) specifically the unamended/inoculated samples. However, for the first time, methane has been detected in samples that initially contained excess nitrate. It is likely at this point that nitrate has been completely converted to N_2 gas by denitrifying bacteria; this will be confirmed by nitrate, nitrite, and nitrous oxide analysis. The rate of methane production is very slow, on the order of 2.5 pmol CH_4 g^{-1} cellulose d^{-1} in unamended inoculated samples. This may be due to the extreme difficulty methanogens have in metabolizing acetate, CO_2, and H_2 (all confirmed to be present in the samples) under hypersaline conditions due to bioenergetic constraints (Oren 1999). Further analyses will include an attempt at subculturing the methanogens in these samples and molecular biological detection of methanogens (see 4. below).

Table 2. Methane analysis of inundated cellulose samples.

Sample	Incubation Time (d) 1228	2718	3462
	----------(nmol g^{-1} cellulose)----------		
Anaerobic			
Unamended	nd	3.92 ± 0.27	4.40 ± 0.28
Unamended/Inoculated	nd	4.03 ± 1.38	5.89 ± 1.30
Amended/Inoculated	nd	0.85 ± 0.7	2.74 ± 0.90
Amended/Inoc. + Exc. Nitrate	nd	nd	2.57 ± 0.79
Anaerobic + Bentonite			
Unamended	nd	3.84 ± 0.40	na
Unamended/Inoculated	nd	3.52 ± 0.20	na
Amended/Inoculated	nd	1.12 ± 0.03	na
Amended/Inoc. + Exc. Nitrate*	nd	nd	na
Initially Aerobic			
Unamended	nd	1.25 ± 0.29	na
Unamended/Inoculated	nd	1.10 ± 0.13	na

nd = not detected; methane was not detected in initially aerobic samples with nutrient amendments and excess nitrate nor in initially aerobic samples with bentonite.
na = not analyzed; analysis is planned for FY'01.

4. Microbiological Characterization: In order to address Objective 1, 4, and 5, we need to understand the composition (identity and microbial community structure) of microorganisms in samples that show CO_2 and methane production. The predominant microorganisms in samples from the BNL-WIPP gas generation experiments, and differences in community structure which may help to explain difference in gas generation rates, will be assessed by DNA analysis and phospholipid fatty acid (PLFA) analysis (Pancost et al., 2001; Petsch et al., 2001; Lehman et al., 2001). For the DNA analysis, polymerase chain reaction (PCR) of bacterial and archaeal 16S rRNA gene fragments will be performed. The PCR products are then run through denaturing gradient gel electrophoresis (DGGE) to separate fragments according to their melting properties. Ethidium bromide stains the fragment bands and these are excised and re-amplified by PCR. Specific emphasis will be placed on searching for methanogens at this stage. The PCR product is sequenced using an automated sequencer and the sequences are identified using the BLASTN facility of the National Center for Biotechnology Information (NCBI) or the Ribosomal Database Project (RDP). Four sub-samples will be taken from anaerobic inundated samples: (i) unamended uninoculated, (ii) unamended inoculated, (iii) amended inoculated, and (iv) amended and inoculated and excess nitrate. In addition, PLFA analysis will be used to identify the bacteria and archaea in these samples. A "QA mix" has been prepared containing known halophilic isolates to validate the analysis. A procedure for this analysis has been prepared and a commercial service identified that is capable of performing the DGGE and PLFA analyses. A site visit was made in June to discuss sample handling, analysis, and to review the quality assurance program. Upon successful analysis of inundated samples we will examine humid samples.

5. Microbial Gas Generation Under Humid Conditions:

Background - Composition of Samples

Samples were prepared in 160 ml glass serum bottles, with 1 g of mixed cellulosics (0.25 g each of Whatman® #1 filter paper, brown paper towel, white paper towel, and Kimwipes®) mixed with (i) 5.00 g of reagent-grade NaCl (Aldrich), (ii) 5.00 g of crushed WIPP muck pile salt from the WIPP underground workings (100% E140, N635 salt), and (iii) a mixture of 3.50 g WIPP muck pile-salt and 1.50 g bentonite MX-80

(70% salt/30% bentonite). Samples were prepared with and without added nutrients. The nutrients added (amended samples) consisted of a 0.50 ml solution containing nitrogen (ammonium nitrate, 0.1% w/v), phosphorus (potassium phosphate, 0.1% w/v), and yeast extract (0.05% w/v). Unamended samples received 0.50 ml of a filtered, sterilized reagent-grade salt solution (20% w/v). All samples were prepared in triplicate. A mixed inoculum was prepared as described in SAND96-2582 and 2.0 ml was pipetted onto the cellulose with a calibrated pipette. The uninoculated samples (controls) received 2.0 ml of filter sterilized (0.2µm, Millipore Corp.) reagent-grade NaCl (Aldrich) solution (20% w/v deionized H_2O) to duplicate the moisture content of the inoculated samples. To examine the viability and potential gas-producing activity of the mixed inoculum, as well as elucidate the nutrient conditions in the mixed inoculum, 20 ml aliquots were prepared in duplicate with the following additions: i) no nutrients; ii) nutrients; iii) glucose + nutrients; and iv) succinate + nutrients. Because WIPP crushed salt contains viable bacteria adding it to the samples provided an additional, but integral, source of inoculum. Samples containing WIPP salt but without inoculum are not true "abiotic" controls. Therefore, reagent-grade NaCl was added to specific uninoculated samples to serve as abiotic controls. In order to maintain the desired relative humidity of approximately 70-74%, 3 ml of G-Seep brine (a_W (water activity of the brine) = 0.73) in an unsealed 5 ml glass tube (1.0 x 7.5 cm) was placed inside the 160 ml serum bottle containing 1 g of mixed cellulose. Upon sealing the sample bottles, the relative humidity was measured using a Hygroskop GT™ (Rotronic, Zurich) portable humidity meter, the probe of which was fitted with a rubber seal to allow measurements to be taken inside of an uncapped serum bottle. The meter was calibrated before use with a standard solution (80% relative humidity) according to the manufacturer's specifications. The relative humidity in the sample bottles (72%) was verified using this method. Samples were sealed with butyl rubber stoppers and aluminum crimp seals in an air atmosphere (samples defined as "initially aerobic") or in a N_2-filled glove box (anaerobic samples). Seventy-two samples were incubated at 30 ± 2°C.

Analyses - Total Gas and Carbon Dioxide

The composition of the headspace gas of each sample was determined over time and compared to the baseline composition at time zero (t=0). For each sampling, the serum bottle fitted with a butyl rubber septum was pierced with a sterile 22-gauge needle (Becton Dickenson) attached to a digital pressure gauge (-5.00 to 35.00 psi (calibrated to NIST by the manufacturer (Wallace and Tiernan): 0.00 to 35.00 psi), to measure the headspace gas pressure to calculate total gas production. At the same time, the room temperature was recorded with a thermometer calibrated to NIST (Princo Instruments).

Immediately after this, a gas-tight syringe (Pressure-Lok™, Precision Instrument Corp.) fitted with a stainless-steel side-port needle was used to remove 0.3 ml of headspace gas to determine the various gases quantitatively by gas chromatography (GC). All analyses were performed according to written procedures prepared as part of the BNL Quality Assurance Program (QAP).

Carbon dioxide was analyzed using a Varian 3400 gas chromatograph according to methods detailed in SAND96-2582. Gas production was assessed by examining the increase in total gas volume over time, in addition CO_2 is quantitated as an indicator of microbial activity. The values were measured against the baseline (t=0), or against control values. For these experiments we prepared the following control samples: i) unamended, uninoculated samples; ii) and samples without organic substrate (cellulose or plastic/rubber material). The gas data in this report are cumulative from t=0.

Results

During this period, total gas and CO_2 was analyzed in all of the humid treatments (initially aerobic at 3009 days (8.2 years) incubation and anaerobic at 2616 days (7.2 years) incubation). The following tables of data are provided at the end of this report: 3-6, total gas and CO_2 produced in aerobic humid experiments; Table 7 presents a summary of CO_2 production on a per-gram cellulose basis with corrections made in the data for CO_2 produced in control samples; Tables 8-11 provide total gas and CO_2 produced in anaerobic humid experiments; Table 12 provides a summary of CO_2 production. Data are the mean of triplicate samples with the standard error reported except where single

samples were analyzed due to either holding the replicate in reserve or prior destructive testing of the replicate samples.

Initially Aerobic Humid Samples (Tables 3-7)

Initially aerobic (sealed) humid samples did not show any marked increase in gas or CO_2 production over the 1.25 year period since they were last analyzed (Tables 3-6); most notable was a continued decrease or leveling-off in CO_2 content in unamended and amended samples in the absence of bentonite (Table 7) and an increase in unamended inoculated samples of 27 µmoles CO_2 g^{-1} cellulose in its presence (0.06 µmol CO_2 g^{-1} cellulose day^{-1}, Table 7). These studies continue to show a stimulatory effect of bentonite on microbial gas generation under humid conditions; while this is not directly applicable to the WIPP repository it does provide a consistent companion data set to help explain trends in more relevant samples. Loss of CO_2 may be due to a gas consuming process such as methanogenesis (corrections are made for loss of gas due to sampling); additional analysis planned for this year will examine methane production.

Anaerobic Humid Samples (Table 8-12)

The CO_2 content of the unamended inoculated samples has leveled-off to 135 µmol g^{-1} cellulose, this marks the end of gas generation at a rate of 0.05 µmol g^{-1} cellulose day^{-1} over 1741 days (between 1 and 6 years incubation) to a loss of 0.04 µmol g^{-1} cellulose day^{-1} for the last 1.26 years incubation. The amount of total gas produced by unamended inoculated samples has declined as well; however, unamended uninoculated samples showed an increase in gas volume and CO_2 concentration (0.007 µmol CO_2 g^{-1} cellulose day^{-1}, Table 8). Amended samples without bentonite showed a decrease in CO_2 at 6 years incubation (18.2 ± 1 CO_2 g^{-1} cellulose) but this loss was made up over the past 1.26 years to a total of 26.8 ± 0.8 µmol CO_2 g^{-1} cellulose (0.04 µmol CO_2 g^{-1} cellulose day^{-1}). The samples with bentonite continue to produce gas at a rate of 0.44 and 0.46 µmol CO_2 g^{-1} cellulose day^{-1} in unamended and amended samples respectively (Table 12), even with a loss in total gas volume (Table 9). Samples designed to test the viability and activity of the mixed inoculum continue to produce CO_2 (Tables 10,11).

We effectively recovered microorganisms from the cellulose in one of the succinate amended anaerobic samples. The bacterial cells were extremely small (<1 μm) and morphologically homogenous indicating that the addition of succinate may have stimulated the growth of a monoculture. The procedure for this recovery will be used to examine the microbial population in the more relevant anaerobic samples.

6. Microbial Gas Generation Under Conditions of MgO-Constrained Water Activity: The two experiments in-progress at BNL to examine gas generation due to cellulose biodegradation under humid conditions were prepared to maintain a 70% relative humidity 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. A procedure has been prepared and is undergoing review that tests microbial activity under more relevant MgO-constrained water activity conditions (with activity bounded at the high end by the absence of MgO and at the low-end by its presence). In order to obtain relevant gas generation data rapidly and accurately, the following will be used: i) a "dry" inoculum, ii) ^{14}C-labelled substrate (for metabolism and growth) and, iii) extremely sensitive techniques for capturing and quantifying microbially produced CO_2 (alkaline trapping and $^{14}CO_2$ liquid scintillation counting).

The dry inoculum was prepared by growing a *Halomonas* sp. (isolated from the WIPP environment) to mid-log phase, harvesting and washing the cells, and resuspending in brine (20% w/v NaCl) followed by drying in a dessicator over $CaSO_4$ for 1-week. The dried material (salt crystals laden with biomass) was resuspended in aqueous growth medium and incubated at 30 ± 2°C. After 5 days the bacterium was revived, analyzed by epifluorescence microscopy to confirm the presence of cells and morphology, thus demonstrating the viability of the proposed "dry" inoculum for these experiments. Upon completion of review these experiments will be started this FY.

Future Work

The experiment to examine microbial growth and gas production under conditions of MgO-constrained water activity will be started during the last quarter of FY2001. At this time select samples from the long-term inundated cellulose biodegradation experiment will again be analyzed for total gas, CO_2, and most importantly methane. These samples will be studied for the presence of methanogenic bacteria. Select samples from the humid studies and samples will also be analyzed for methane production. 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. A manuscript is in preparation that details gas production due to cellulose biodegradation under hypersaline conditions, and a manuscript concerned with methanogenesis under these conditions will be prepared.

Summary

- Samples prepared to examine biodegradation of cellulose under anaerobic inundated conditions showed the following gas production rates between 3.4 and 7.4 years incubation (reported 9/23/99): in unamended uninoculated samples, 0.007 µmol CO_2 g^{-1} cellulose day^{-1}; unamended inoculated samples, 0.007; amended inoculated, 0.01; and amended, inoculated plus excess nitrate, 0.04. Over this same time period, enhanced gas production due to the presence of bentonite was observed in inundated samples, specifically initially aerobic samples: unamended inoculated samples produced gas at a rate of 0.03 µmol CO_2 g^{-1} cellulose day^{-1}; and amended inoculated samples containing excess nitrate gained 0.05 µmol CO_2 g^{-1} cellulose day^{-1}.

- *Initially aerobic (sealed) humid samples did not show any marked increase in gas or* CO_2 production over the 1.25 year period since they were last analyzed, most notable was a continued decrease or leveling-off in CO_2 content in unamended and amended samples.

- Addition of bentonite stimulated gas production in initially aerobic humid samples. In unamended inoculated samples CO_2 increased to 27 µmoles CO_2 g^{-1} cellulose between 7 and 8 years at a rate of 0.06 µmol CO_2 g^{-1} cellulose day^{-1}. Amended inoculated samples showed a loss of CO_2.

- The CO_2 content of the anaerobic humid unamended uninoculated samples showed an increase in gas volume and CO_2 concentration (0.007 µmol CO_2 g^{-1} cellulose day^{-1}). The CO_2 production in unamended inoculated samples has leveled-off to 135 µmol g^{-1} cellulose, with a loss of 0.04 µmol g^{-1} cellulose day^{-1} for the last 1.26

years incubation. The amended inoculated samples showed an increase to a total of 26.8 ± 0.8 µmol CO_2 g^{-1} cellulose (0.04 µmol CO_2 g^{-1} cellulose day^{-1}).

- The anaerobic humid samples with bentonite continue to produce gas at a rate of 0.44 and 0.46 µmol CO_2 g^{-1} cellulose day^{-1} in unamended inoculated and amended inoculated samples respectively.

- A procedure has been prepared and is undergoing review that tests microbial activity under more relevant MgO-constrained water activity conditions (with activity bounded at the high end by the absence of MgO and at the low-end by its presence). In order to obtain relevant gas generation data rapidly and accurately, the following will be used in experiments: i) a "dry" inoculum, ii) ^{14}C-labelled substrate (for metabolism and growth) and, iii) alkaline trapping and $^{14}CO_2$ liquid scintillation counting, an extremely sensitive technique for capturing and quantifying microbially produced CO_2.

- A procedure for microbial community and species identification in inundated and humid samples has been prepared and a commercial service identified that is capable of performing the DNA-based and biomarker phospholipid fatty acid analyses. A site visit was made in June to discuss sample handling, analysis, and to review the quality assurance program.

- We effectively recovered microorganisms from the cellulose in one of the succinate amended anaerobic humid samples. The bacterial cells were extremely small (<1 µm) and morphologically homogenous indicating that the addition of succinate may have stimulated the growth of a monoculture. The procedure for this recovery will be used to examine the microbial population in the more relevant anaerobic samples.

References

Kluber, H. D., and R. Conrad (1998). "Effects of nitrate, nitrite, NO and N_2O on methanogenesis and other redox processes in anoxic rice field soil." FEMS Microbiology Ecology **25**: 301-318.

Lehman, R. M., F. F. Roberto, D. Earley, D. F. Bruhn, S. E. Brink, S. P. O'Connell, M.E. Delwiche, and F. S. Colwell. 2001. "Attached and unattached bacterial communities in a 120-meter corehole in an acidic, crystalline rock aquifer." Appl. Environ. Microbiol 67:2095-2106.

Oren, A. (1999). "Bioenergetic aspects of halophilism." Microbiology and Molecular Biology Reviews **63**(2): 334-348.

Pancost, R.D., E.C. Hopmans, J.S. Sinninghe Damst, and the Medinaut Shipboard Scientific Party. 2001. "Archaeal lipids in Mediterranean cold s eeps: molecular proxies for anaerobic methane oxidation." Geochimica et Cosmochimica Acta, Vol. 65, No. 10, pp. 1611–1627.

Petsch, S.T., T. I. Eglinton, K. J. Edwards. 2001. "^{14}C-Dead living biomass: evidence for microbial assimilation of ancient organic carbon during shale weathering." Science, Vol. 292, Issue 5519, 1127-1131.

Table 5. 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
Control								
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71	2.68	2.94
Blank (tube+brine only)	4.18	4.64	4.54	4.63	3.00	2.76	2.74	3.50
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
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
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
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
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
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
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
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
Carbon Source: Cellulose + Succinate								
Amended uninoculated (w/ acetylene)	15.1	NA	28.8	27.7	21.0	16.8	22.12	NA
Amended uninoculated (w/o acetylene)	15.7	26.0	22.7	19.7	14.4	7.06	4.75	3.25
Amended inoculated (w/ acetylene)	14.5	NA	1384	1450	1470	1270	NA	NA
Amended inoculated (w/o acetylene)	15.8	42.4	40.0	38.2	29.5	23.6	16.86	11.3

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed

Table 6. 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
Control								
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71	2.68	2.94
Blank (*tube+brine only*)	4.18	4.64	4.54	4.63	3.00	2.76	2.74	3.50
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 ± 4.0
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
Unamended Inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61	184 ± 76	233 ± 152	258 ± 180
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
Amended inoculated	53.7 ± 2.4	1030 ± 80	1620 ± 30	1600 ± 40	1520 ± 40	1469.8 ± 40	1059 ± 207	858 ± 219
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
Amended inoculated	44.9 ± 2.6	1590 ± 40	1240 ± 20	1250 ± 160	1240 ± 240	816 ± 355	964 ± 230	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
Carbon Source: Cellulose + Succinate								
Amended uninoculated (w/ acetylene)	22.9	NA	50.0	50.8	46.1	38.9	27.8	27.7
Amended uninoculated (w/o acetylene)	21.7	47.7	50.4	46.8	43.6	37.3	34.0	30.3
Amended inoculated (w/ acetylene)	38.5	NA	1430	1470	1540	1460	NA	NA
Amended inoculated (w/o acetylene)	52.8	1130	1460	1500	1520	1400	631	320

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed

Table 7. 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
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
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
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
*Unamended inoculated (corrected)**	3.77 ± 0.03	42.1 ± 3.3	62.0 ± 9.8	56.3 ± 9.9	38.9 ± 7.0	24.0 ± 4.6	8.45 ± 2.29	7.51 ± 1.94
*Amended inoculated (corrected)**	28.0 ± 1.0	28.5 ± 1.0	20.5 ± 1.6	15.6 ± 1.8	8.32 ± 1.4	4.60 ± 0.98	0.93 ± 0.23	1.07 ± 0.15

Treatments ***with bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 6	120	317	399	593	804	2553	3009
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
Carbon Source: Cellulose								
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61	184 ± 76	233 ± 152	258 ± 180
Amended inoculated	53.7 ± 2.4	1033 ± 76	1623 ± 26	1600 ± 44	1520 ± 40	1470 ± 40	1059 ± 207	858 ± 219
*Unamended inoculated (corrected)**	-13.5 ± 0.0	8 ± 0	105 ± 9.6	124 ± 20.4	130 ± 36.2	142 ± 58.5	217 ± 141	244 ± 171
*Amended inoculated (corrected)**	19.5 ± 0.9	869 ± 63.9	1455 ± 23.7	1456 ± 40.0	1431 ± 37.7	1428 ± 38.8	1043 ± 204	844 ± 216

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

Table 8. 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)*
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*
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*	0.02	*0.91*
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*
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*
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 ± 0.56	*-0.58*
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*
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*
Amended inoculated (w/ acetylene)	20.4 ± 0.1	16.6 ± 0.6	*-3.87*	14.95 ± 0.48	*-1.61*	7.15 ± 5.15	*-7.80*	0.32 ± 0.08	*-6.83*	0.25 ± 0.23	*-0.07*
Carbon Source: Cellulose + Glucose											
Amended uninoculated	6.55 ± 0.63	3.82 ± 0.73	*-2.73*	2.07 ± 0.66	*-1.75*	-0.51 ± 0.44	*-2.58*	2.50 ± 0.62	*3.01*	1.57 ± 0.62	*-0.93*
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*
Amended uninoculated (RG salt)	6.60 ± 0.00	2.35 ± 1.90	*-4.25*	0.18 ± 2.28	*-2.17*	0.09 ± 1.48	*-0.09*	3.83 ± 0.51	*3.74*	1.27 ± 0.15	*-2.56*
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	
Amended uninoculated (w/o acetylene)	6.30 ± 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	
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	*-0.76*	3.25	*-2.45*
Amended inoculated (w/o acetylene)	5.67 ± 0.04	1.70 ± 1.72	*-3.97*	0.67 ± 1.71	*-1.03*	2.46 ± 1.61	*1.79*	7.05	*4.59*	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).

Table 9. 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)*
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*
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*	0.02	*0.91*
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*
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*
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*
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*
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*
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*
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*
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*
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*
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*
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*
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*
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*

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).

Table 10. Production of Carbon Dioxide in Anaerobic Humid Samples (without bentonite)

Treatments (without bentonite)	µmoles CO_2/Sample					
	Days					
	6	100	140	415	2156	2616
Control						
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00	4.13	1.84
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14	2.39
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
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
Unamended inoculated	11.3 ± 0.12	25.9 ± 3.8	36.1 ± 7.0	89.0 ± 24.4	163 ± 36	142 ± 28
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
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
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
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
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
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
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	3.21 ± 0.04	22.5 ± 0.8	29.4 ± 2.5	28.8 ± 3.0	NA	33.8 ± 7.2
Amended uninoculated (w/o acetylene)	3.19 ± 0.18	21.4 ± 0.2	27.9 ± 0.5	34.1 ± 2.5	984	NA
Amended inoculated (w/ acetylene)	13.5 ± 0.7	78.1 ± 33.4	123 ± 63	308 ± 175	99.8	133 ± 79
Amended inoculated (w/o acetylene)	14.8 ± 0.2	60.5 ± 16.0	106 ± 21	328 ± 78	1034	NA

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=not analyzed

Table 11. Production of Carbon Dioxide in Anaerobic Humid Samples (with bentonite)

Treatments (with bentonite)	µmoles CO_2/Sample					
	Days					
	6	100	140	415	2156	2616
Control						
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00	4.13	1.84
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14	2.39
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
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
Unamended inoculated	20.3 ± 0.2	93.7 ± 2.6	186 ± 6	434 ± 39	483 ± 133	650 ± 175
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
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7	732 ± 47
Amended inoculated (w/ acetylene)	26.8 ± 0.7	94.0 ± 18.6	123 ± 30	251 ± 92	558 ± 270	609 ± 273
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
Amended inoculated	31.4 ± 0.7	396 ± 13	487 ± 1	584 ± 28	754 ± 94	641 ± 16
Amended uninoculated (RG salt)	5.28 ± 0.45	45.9 ± 0.7	55.1 ± 1.4	74.9 ± 2.2	178 ± 3	209 ± 1
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
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
Amended inoculated (w/ acetylene)	27.7 ± 0.27	70.3 ± 2.7	114 ± 0	324 ± 30	447	568
Amended inoculated (w/o acetylene)	28.0 ± 0.82	237 ± 2	317 ± 6	516 ± 0	1356	944 ± 110

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt
NA=*not analyzed*

Table 12. 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
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
Carbon Source: Cellulose						
Unamended inoculated	11.3 ± 0.1	25.9 ± 3.8	36.1 ± 7.0	89 ± 24.4	163 ± 36	142 ± 28
Amended inoculated	16.9 ± 1.2	36.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9	18.2 ± 1	33.6 ± 1.0
*Unamended inoculated (corrected)**	7.70 ± 0.08	20.0 ± 2.9	28.5 ± 5.5	72.6 ± 19.9	154.7 ± 34.2	135 ± 27
*Amended inoculated (corrected)**	13.3 ± 0.9	30.5 ± 0.7	32.8 ± 0.6	18.3 ± 0.5	9.9 ± 0.5	26.8 ± 0.8

Treatments ***with bentonite***	Carbon dioxide (µmoles/ gram cellulose) Days					
	6	100	140	415	2156	2616
Control						
No cellulose (salt/ inoculum/ tube+brine)	14.2 ± 0.5	36.6 ± 6.1	39.8 ± 5.5	51.6 ± 3.4	93.8	59.2 ± 14.1
Carbon Source: Cellulose						
Unamended inoculated	20.3 ± 0.2	94 ± 3	186 ± 6	434 ± 39	483 ± 133	650 ± 175
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7	732 ± 47
*Unamended inoculated (corrected)**	6.10 ± 0.06	57.1 ± 1.6	146 ± 5	382 ± 34	389 ± 107	591 ± 159
*Amended inoculated (corrected)**	18.0 ± 0.6	213 ± 26	433 ± 23	390 ± 134	460 ± 30	673 ± 43

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