2.0 Gas Generation

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

Data Summary Report
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Abstract

Gas generation from the microbial degradation of the organic constituents of transuranic waste under conditions expected at the Waste Isolation Pilot Plant (WIPP) repository is under investigation at Brookhaven National Laboratory. The biodegradation of mixed cellulosics (various types of paper) and electron-beam irradiated plastic and rubber materials (polyethylene, polyvinylchloride, neoprene, hypalon, and leaded hypalon) is being examined. In addition, we are studying the effects of environmental variables such as starting atmosphere (air or nitrogen), water content (humid (~70% relative humidity) and brine inundated), and nutrient amendments (nitrogen, phosphate, yeast extract, and excess nitrate) on microbial gas generation. This report presents data obtained from samples incubated under humid conditions: i) initially aerobic (sample bottles sealed with air in the headspace) after 2553 days (7 years) of incubation at 30 ± 2°C; ii) anaerobic conditions incubated for 2156 days (6 years). In addition this report provides data from plastic (polyethylene and polyvinylchloride) and rubber (neoprene) materials (unirradiated and irradiated to simulate long-term radiation damage) incubated under brine-inundated conditions for 2612 days (7 years); and hypalon (unleaded and leaded, unirradiated and irradiated) incubated for 2464 days (6.75 years). Total gas production and carbon dioxide production are presented. Initially aerobic (sealed) humid samples did not show any marked increase in gas or CO_2 produced over the 4.75 year period since they were last analyzed; most notable was a decrease in CO_2 content in the inoculated samples in the absence of bentonite and an increase in unamended samples of 75 μmoles CO_2 g^{-1} cellulose in its presence. Loss of CO_2 may be due to gas consuming process such as methanogenesis; additional analysis is planned for this year to examine methane production. This trend is also observed under anaerobic humid conditions, with a decrease in CO_2 content in amended samples. An increase of 82 μmoles CO_2 g^{-1} cellulose was shown in unamended inoculated samples (no bentonite) during the 4.75 year period since the last analysis. Samples containing plastic and rubber materials did not show any significant increase in gas volume beyond control samples incubated without the polymer substrate. Carbon dioxide, a more sensitive analyte for microbial activity, increased somewhat in samples containing irradiated PE (10.9 μmoles CO_2 $sample^{-1}$ over 4.9 years since the last analysis); irradiated PVC (44.6 μmoles); irradiated neoprene (21.5 μmoles);

and unirradiated hypalon (unleaded, 11.2 μmoles, and leaded, 9.77 μmoles). Note that the amount of CO_2 generated over 4.9 years in samples containing plastic or rubber materials is not markedly higher than control samples. Additional analysis, including microscopy and spectroscopy, will be used to determine if material changes have occurred in the polymers due to microbial activity and biodeterioration.

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). A summary of these experiments for the period 1991 to 1996 was published in SAND96-2582 "Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions." The experiment to quantify gas generation due to cellulose biodegradation under inundated conditions was again analyzed in 1999, after 2718 days (7.4 years) incubation (4 years since the analyses reported in SAND96-2582). At that time total gas volume, carbon dioxide and methane were analyzed and this was reported in a memo dated September 23, 1999, a summary report for work conducted that year under TP-99-01 Rev. 0 (2/4/99). Table 1 provides information about the status of ongoing studies at BNL.

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

Experiment	Start Date	SAND96-2582 Data (Days)	Most Recent Analysis Date	Incubation Time of Recent Analysis (Days)
Long-Term Inundated Cellulose	1/29/92	1228	7/8/99 CH_4 7/28/99 CO_2	2718
Initially Aerobic Humid Cellulose	4/7/93	804	4/3/00 CO_2	2553
Anaerobic Humid Cellulose	5/4/94	415	3/29/00 CO_2	2156
Inundated PE, PVC, and Neoprene	3/9/93	840	5/3/00 CO_2	2612
Inundated Hypalon	8/3/93	664	5/2/00 CO_2	2464

This report presents data obtained during FY2000 from long-term studies designed to examine gas generation under: i) initially aerobic humid conditions with data reported here after 2553 days (7 years) of incubation at 30 ± 2°C; ii) anaerobic humid conditions incubated for 2156 days (6 years); iii) brine-inundated conditions with plastic and rubber materials (unirradiated and irradiated to simulate long-term radiation damage) incubated for 2612 days (7 years); and iv) brine-inundated conditions with hypalon (unleaded and

leaded, unirradiated and irradiated) incubated for 2464 days (6.75 years). Total gas production was analyzed as well as carbon dioxide in the headspace of 159 sample bottles.

Materials and Methods

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.

Inoculum

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.

Control Samples

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.

Humidity Maintenance

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.

Atmosphere

Initially aerobic (sealed) samples were sealed with butyl rubber stoppers and aluminum crimp seals in an air atmosphere. Anaerobic samples were prepared in a N_2-filled glove box, and all components (mixed inoculum, nutrient solutions, and sterile brine) were flushed with N_2 before they were added to the sample.

Microbial Activity Measurements under Humid Conditions

In addition to the above treatments, 1% succinate or glucose was added with the nutrient amendment to certain samples to determine microbial gas generation under humid conditions in the presence of a readily metabolizable source of carbon. The ability of specific microorganisms (i.e., denitrifiers) to grow under such low-moisture conditions was examined. We point out that WIPP halophiles can function under low-moisture conditions because they can grow in highly concentrated brine, which has a low water activity.

Two of the inoculated, succinate-amended treatments (one with bentonite, the other without bentonite) were incubated with 0.1 atm of acetylene to examine N_2O production from denitrification.

Incubation

Seventy-two samples were incubated at 30 ± 2°C. In this study, we attempt to determine the rate and extent of gas production due to biodegradation of unirradiated and electron-beam irradiated plastic and rubber materials under conditions relevant to the WIPP repository. In the case of irradiated materials, these were accelerated tests because the entire structure of the polymer was altered as opposed to the effects of alpha-irradiation, which alter only the surface of the polymer. These samples, therefore, represented "overtest" conditions in terms of overall radiation dose. The influence of adding nutrients (nitrogen, phosphorus, and yeast extract) on the extent of biodegradation also was determined.

E-Beam Irradiated Plastic and Rubber Materials

The plastics examined were polyethylene and polyvinylchloride; the rubber materials were neoprene and hypalon (leaded and unleaded). These materials were exposed to electron-beam irradiation at the linear accelerator (LINAC) at Argonne National Laboratory by Dr. D. Reed, Chemical Technology Division. The polymer samples received an absorbed dose of either 500-700 Mrad (low-dose) or 4000-6000 Mrad (high dose), see Table 2. Tests with unleaded and leaded hypalon did not include a high-dose irradiation because it caused extensive degradation (melting) of the leaded sample.

Table 2. Irradiation conditions and material characteristics.

Irradiation Conditions (samples irradiated in air):

Polymer	Density (g\cm^3)	Thickness (mm)	Absorbed Dose (Low) Mrad	Absorbed Dose (High) Mrad
Polyethylene	0.92	0.28	500	4,140
Polyvinylchloride	1.30	0.28	700	5,850
Neoprene	1.23	0.46	660	5,535
Unleaded Hypalon	NA	NA	NA	NA
Leaded Hypalon	NA	NA	NA	NA

NA - not available

Material Characteristics:

Polymer	Unirradiated	Low-Dose	High-Dose
Polyethylene	clear	light yellow	darker yellow/brittle weight loss
Polyvinylchloride	clear	dark brown/sticky liquid droplets weight loss	Black/sticky weight loss
Neoprene	black	loss of flexibility weight loss	brittle weight loss
Unleaded Hypalon	dull white	brown discoloration	NA
Leaded Hypalon	dull white	brown discoloration	NA

NA = Not applicable

Triplicate samples of unirradiated and low-dose irradiated polymers and duplicate samples of the material that received high doses of electron-beam irradiation were tested. Each polymer was cut into 2 cm^2 pieces, the weights were recorded, and the pieces placed in acid-washed sterilized (autoclaved) 70 ml glass serum bottles. Mean weights (22 samples for each polymer) were as follows: Polyethylene (86.1 mg), Polyvinylchloride (134.6 mg), Neoprene (257.5 mg).

Mixed Inoculum/Inundation Fluid

Every sample bottle containing plastic or rubber was filled with 50 ml of a mixture consisting of 56% G-Seep Brine #10 (collected 12/13/89-1/10/90), 27% WIPP muck pile salt slurry, and 17% surficial lake brine/sediment slurry. The salt slurry and brine/sediment slurry were prepared as previously described. The inundation fluid differed from that added to the sample bottles containing cellulose; the sample bottles containing plastic or rubber material were inundated with fluid comprised of 100% mixed inoculum. The mixed inoculum was used without dilution to increase the proportion of potential plastic/rubber degrading microorganisms in the experiment. This was done to provide an additional "overtest" because we expected at the outset that biodegradation

rates potentially would be very low, especially if the same concentration of mixed inoculum (3.8% v/v) was used as in the cellulose experiment.

Sample Treatments

Samples were incubated either unamended (without added nutrients) or amended (with nutrients). Table 3 lists the composition of the nutrient addition. The pH of the nutrient solution was adjusted to 7.0 with NaOH and 2.50 ml of the filter-sterilized concentrated stock solution was added to the appropriate samples using a calibrated continuously adjustable pipette (Pipetteman™, Rainin Corp.).

Table 3. Composition of the nutrient amendment.

Nutrient	Final concentration (g/L)	Final concentration (w/v %)
NH_4NO_3	0.5	0.1
K_2HPO_4	0.5	0.1
Yeast extract	0.25	0.05

Unirradiated, low and high dose electron beam or alpha-irradiated polymers were treated as follows:

i) Polymer + no nutrients (unamended) + mixed inoculum (one sample each);

ii) Polymer + nutrients (amended) + mixed inoculum (triplicate);

iii) No polymer + nutrients (control) + mixed inoculum (triplicate); and

iv) No polymer + no nutrients (control) + mixed inoculum (triplicate).

One set of each treatment detailed above was prepared for each material for aerobic and anaerobic incubations, giving a total of 87 bottles. The final aqueous sample volume of the unamended treatments was 50 ml, and 52.5 ml for the amended treatments; the headspace volume was 20 ml, and 17.5 ml, respectively.

Incubation

Samples were incubated under initially aerobic and anaerobic conditions in serum bottles fitted with butyl rubber stoppers and sealed with aluminum crimps. Anaerobic samples were prepared in a glove box and incubated under a N_2 atmosphere, whereas aerobic samples were prepared on the lab bench. We expected that the aerobic samples would eventually become anaerobic due to consumption of oxygen by aerobic microorganisms in the sealed bottle. All samples were incubated unshaken (static) at 30 ± 2°C.

Gas Analysis

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

The appendix provides the following tables of data: 1-4, total gas and CO_2 produced in aerobic humid experiments; Table 5 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 6-9 provide total gas and CO_2 produced in anaerobic humid experiments; Table 10 provides a summary of CO_2 production; 11-15, total gas produced in samples containing plastic and rubber materials; and 16-20, CO_2 produced in samples containing plastic and rubber materials. 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.

Summary

Initially Aerobic Humid Samples (Tables 1-5)

Initially aerobic (sealed) humid samples did not show any marked increase in gas or CO_2 production over the 4.75 year period since they were last analyzed (Tables 1-4); most notable was a decrease in CO_2 content in unamended and amended samples in the absence of bentonite (Table 5) and an increase in unamended samples of 75 μmoles CO_2 g^{-1} cellulose in its presence (Table 5, these studies show a stimulatory effect of bentonite on microbial gas generation under humid conditions). Loss of CO_2 may be due to a gas

consuming process such as methanogenesis; additional analysis planned for this year will examine methane production.

Anaerobic Humid Samples (Table 6-10)

A decrease in CO_2 content was observed in specific samples (amended, uninoculated and inoculated, Table 10). An increase of 82 μmoles CO_2 g^{-1} cellulose was shown in unamended inoculated samples (no bentonite) during the 4.75 year period since the last analysis (Table 10); while the same samples with bentonite only showed an increase of 7 μmoles (unamended inoculated samples with bentonite but without cellulose produced 42.2 μmoles CO_2 $sample^{-1}$, this value is used to correct for gas production in the absence of cellulose). Amended samples without bentonite showed a decrease in CO_2; those with bentonite showed an increase of 70 μmoles CO_2 g^{-1} cellulose (Table 10).

Samples Containing Plastic and Rubber Materials (Tables 11-20)

Samples containing plastic and rubber materials did not show any significant increase in gas volume beyond that produced by control samples incubated without the polymer substrate. Carbon dioxide, a more sensitive analyte for microbial activity, increased somewhat in samples containing irradiated PE (10.9 μmoles CO_2 sample over 4.9 years since the last analysis); irradiated PVC (44.6 μmoles); irradiated neoprene (21.5 μmoles); and unirradiated hypalon (unleaded, 11.2 μmoles, and leaded, 9.77 μmoles). Note that the amount of CO_2 generated over 4.9 years in samples containing plastic or rubber materials is not markedly higher than control samples. Additional analysis, including microscopy and spectroscopy, will be used to determine if material changes have occurred in the polymers due to microbial activity.

Future Work

Further analysis of the data presented here will entail correcting the gas generation data from samples containing plastic and rubber materials using the control samples. In addition, gas production on a per-gram polymer basis will be determined. During the second quarter of FY2001 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 containing plastic/rubber materials will also be analyzed for methane production. Material characterization techniques including infrared and x-ray spectroscopy will be used to assess the extent of polymer degradation due to microbial activity.

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
Control							
Empty bottle	7.15	-0.22	0.28	1.08	1.19	2.51	0.73
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52	0.32	-0.69
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
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
Unamended inoculated	7.50 ± 0.33	-0.31 ± 1.82	0.19 ± 0.33	-0.61 ± 0.25	0.31 ± 0.05	1.29 ± 0.25	1.15 ± 0.39
Amended uninoculated	6.98 ± 0.18	-0.03 ± 1.88	-0.23 ± 0.10	-0.29 ± 0.13	0.20 ± 0.10	0.50 ± 0.21	1.26 ± 0.24
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
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
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
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
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	19.5	NA	0.64	-0.10	1.68	-0.10	1.98
Amended uninoculated (w/o acetylene)	5.15	-2.08	0.98	-0.37	-0.08	0.72	0.74
Amended inoculated (w/ acetylene)	12.9	NA	1.17	0.35	-0.34	-0.10	n/a
Amended inoculated (w/o acetylene)	5.88	-2.29	1.27	0.05	0.17	0.72	2.18

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

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)						
	8	120	317	399	593	804	2553
Control							
Empty bottle	7.15	-0.22	0.28	1.08	1.19	2.51	0.73
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52	0.32	-0.89
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
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
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
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
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
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 ± 0.78	1.41 ± 0.40
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
Amended uninoculated (RG salt)	NA	2.80	1.78 ± 1.57	-0.82 ± 0.21	0.13 ± 0.04	1.59 ± 0.76	1.26 ± 0.37
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	18.7	NA	0.74	-0.15	0.07	-0.63	1.48
Amended uninoculated (w/o acetylene)	5.56	-1.98	1.71	-0.78	0.27	-0.33	0.84
Amended inoculated (w/ acetylene)	18.0	NA	2.00	0.05	0.10	0.55	n/a
Amended inoculated (w/o acetylene)	6.82	-2.29	2.30	0.67	-0.11	1.16	0.74

RG salt = reagent grade NaCl was used in this treatment in place of WIPP salt

NA=not analyzed

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
Control							
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71	2.68
Blank (tube+brine only)	4.18	4.64	4.54	4.83	3.00	2.76	2.74
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
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	8.98 ± 1.82	8.73 ± 2.43
Unamended inoculated	11.7 ± 0.1	58.0 ± 4.4	72.8 ± 11.4	65.5 ± 11.5	45.3 ± 8.1	27.8 ± 5.3	12 ± 3.25
Amended uninoculated	14.0 ± 1.1	28.1 ± 0.6	24.1 ± 1.8	22.9 ± 2.6	17.4 ± 3.1	12.2 ± 2.7	6.08 ± 1.78
Amended inoculated	35.9 ± 1.3	42.4 ± 1.5	31.1 ± 2.4	24.6 ± 2.9	14.7 ± 2.4	8.21 ± 1.75	4.48 ± 1.09
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
Amended inoculated	28.3 ± 1.6	163 ± 98	236 ± 140	166 ± 96	79.8 ± 39.8	26.2 ± 9.0	9.1 ± 1.46
Amended uninoculated (RG salt)	NA	36.0	44.8 ± 0.1	46.5 ± 0.1	47.4 ± 2.8	39.4 ± 5.6	56.81 ± 3.99
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	15.1	NA	28.8	27.7	21.0	16.8	22.12
Amended uninoculated (w/o acetylene)	15.7	26.0	22.7	19.7	14.4	7.06	4.75
Amended inoculated (w/ acetylene)	14.5	NA	1384	1450	1470	1270	n/a
Amended inoculated (w/o acetylene)	15.8	42.4	40.0	38.2	29.5	23.6	16.86

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

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
Control							
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71	2.68
Blank (tube+brine only)	4.18	4.84	4.54	4.63	3.00	2.76	2.74
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
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	9.62 ± 0.15	9.98 ± 1.15
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61	164 ± 76	233 ± 152
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 ± 6.28
Amended inoculated	53.7 ± 2.4	1030 ± 80	1620 ± 30	1600 ± 40	1520 ± 40	1469.8 ± 40	1059 ± 207
Carbon Source: Cellulose + Glucose							
Amended uninoculated	14.8 ± 0.5	46.3	590 ± 364	625 ± 394	694 ± 438	631 ± 401	53.8 ± 26.3
Amended inoculated	44.9 ± 2.6	1590 ± 40	1240 ± 20	1250 ± 160	1240 ± 240	816 ± 355	964 ± 230
Amended uninoculated (RG salt)	NA	39.5	50.9 ± 1.3	54.6 ± 2.4	55.7 ± 6.7	45.7 ± 8.6	82 ± 37
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	22.9	NA	50.0	50.8	46.1	38.9	27.8
Amended uninoculated (w/o acetylene)	21.7	47.7	50.4	46.8	43.6	37.3	34
Amended inoculated (w/ acetylene)	38.5	NA	1430	1470	1540	1460	n/a
Amended inoculated (w/o acetylene)	52.8	1130	1460	1500	1520	1400	631

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

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
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
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
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
*Unamended Inoculated (corrected)**	3.77 ± 0.03	42.1 ± 3.3	62.0 ± 9.6	56.3 ± 9.9	38.9 ± 7.0	24.0 ± 4.6	8.45 ± 2.29
*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

Treatments *with bentonite*	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 6	120	317	399	593	804	2553
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
Carbon Source: Cellulose							
Unamended Inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61	184 ± 76	233 ± 152
Amended Inoculated	53.7 ± 2.4	1033 ± 76	1623 ± 26	1600 ± 44	1520 ± 40	1470 ± 40	1059 ± 207
*Unamended Inoculated (corrected)**	-13.5 ± 0.0	6 ± 0	105 ± 9.6	124 ± 20.4	130 ± 38.2	142 ± 58.5	217 ± 141
*Amended Inoculated (corrected)**	19.5 ± 0.9	869 ± 63.9	1455 ± 23.7	1456 ± 40.0	1431 ± 37.7	1428 ± 38.8	1043 ± 204

** 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								
	8	100	gas produced* (94 d)	140	gas produced (40d)	415	gas produced (275 d)	2156	gas produced (1741 d)
Control									
Empty bottle	7.98 ± 0.59	4.62 ± 0.54	-3.38	3.81 ± 0.86	-1.01	2.01 ± 1.04	-1.60	0.72	-1.29
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
No cellulose (salt/ inoculum/ tube+brine)	6.49 ± 0.04	3.07 ± 0.07	-3.42	1.56 ± 0.83	-1.51	2.76 ± 0.88	1.20	5.53	2.77
Carbon Source: Cellulose Only									
Unamended uninoculated	7.33 ± 0.80	1.59 ± 1.25	-5.74	0.01 ± 1.07	-1.58	-2.28 ± 0.17	-2.27	0.09 ± 0.16	2.35
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
Amended uninoculated	7.50 ± 0.13	0.93 ± 1.25	-6.57	-0.92 ± 1.12	-1.85	-1.67 ± 0.24	-0.95	1.70 ± 1.05	3.57
Amended inoculated	7.64 ± 0.37	0.89 ± 0.89	-6.75	-0.54 ± 1.03	-1.43	-1.07 ± 1.15	-0.53	0.43 ± 0.00	1.50
Amended inoculated (w/ acetylene)	20.4 ± 0.1	16.6 ± 0.6	-3.67	14.95 ± 0.48	-1.81	7.15 ± 5.15	-7.80	0.32 ± 0.08	-6.83
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 ± 0.82	3.01
Amended inoculated	7.18 ± 0.04	4.63 ± 0.11	-2.35	1.77 ± 1.10	-3.06	0.68 ± 1.90	-1.09	3.27 ± 1.74	2.59
Amended uninoculated (RG salt)	8.60 ± 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
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
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	8.20
Amended inoculated (w/ acetylene)	18.7 ± 0.1	7.27 ± 8.63	-11.46	8.83 ± 8.43	-0.44	8.46 ± 4.32	-0.37	5.70 ± 3.19	-0.76
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

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 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)
Control									
Empty bottle	7.98 ± 0.59	4.62 ± 0.54	-3.36	3.81 ± 0.66	-1.01	2.01 ± 1.04	-1.60	0.72	-1.29
Blank (tube+brine only)	6.85 ± 0.36	3.81 ± 0.34	-3.04	2.80 ± 0.27	-1.01	0.37 ± 1.02	-2.43	-0.89	-1.28
No cellulose (salt/ inoculum/ tube+brine)	8.16 ± 0.19	4.60 ± 0.37	-1.58	0.87 ± 1.85	-3.73	1.93 ± 0.37	1.06	-1.79	-3.72
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	1.83
Unamended inoculated	8.63 ± 0.03	6.36 ± 1.22	-0.27	5.68 ± 3.11	-0.50	11.22 ± 5.42	5.36	6.37 ± 2.08	-4.85
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
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
Amended inoculated (w/ acetylene)	16.2 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
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
Amended inoculated	8.97 ± 0.11	9.79 ± 3.73	2.62	7.67 ± 4.78	-1.92	7.48 ± 6.62	-0.41	7.73 ± 4.82	0.27
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	3.60
Carbon Source: Cellulose + Succinate									
Amended uninoculated (w/ acetylene)	19.9 ± 0.4	6.36 ± 2.14	-11.52	4.75 ± 3.05	-3.81	-1.54 ± 0.03	-6.29	2.34 ± 0.62	3.88
Amended uninoculated (w/o acetylene)	7.91 ± 0.48	4.28 ± 1.10	-3.85	3.20 ± 1.03	-1.08	3.86 ± 0.24	0.66	3.37 ± 2.03	-0.49
Amended inoculated (w/ acetylene)	19.8 ± 0.1	16.7 ± 0.5	-2.69	6.59 ± 4.01	-6.12	5.36 ± 5.00	-3.23	10.04	4.68
Amended inoculated (w/o acetylene)	6.76 ± 0.16	10.2 ± 0.3	3.42	10.41 ± 1.22	0.23	3.84 ± 1.94	-6.57	-0.53	-4.37

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

Treatments (without bentonite)	µmoles CO_2/Sample				
	Days				
	6	100	140	415	2156
Control					
Empty bottle	0.00 ± 0.00	0.68 ± 0.46	1.34 ± 0.95	0.00 ± 0.00	4.13
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14
Salt / inoculum/ tube+brine (no cellulose)	3.60 ± 0.01	5.90 ± 0.11	7.63 ± 1.08	16.4 ± 0.6	8.35
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
Unamended inoculated	11.3 ± 0.12	25.9 ± 3.8	36.1 ± 7.0	89.0 ± 24.4	163 ± 36
Amended uninoculated	3.34 ± 0.22	34.3 ± 1.44	39.8 ± 0.9	32.3 ± 1.5	13.5 ± 2.76
Amended inoculated	16.9 ± 1.15	36.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9	18.2 ± 1
Amended inoculated (w/ acetylene)	13.7 ± 1.3	38.5 ± 2.2	42.7 ± 2.5	61.0 ± 16.9	47.3 ± 17
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
Amended inoculated	17.7 ± 0.47	39.8 ± 0.2	42.2 ± 0.9	41.8 ± 4.2	52.8 ± 10.8
Amended uninoculated (RG salt)	4.07 ± 0.37	19.8 ± 2.4	28.9 ± 0.6	26.3 ± 2.9	47.8 ± 12.3
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
Amended uninoculated (w/o acetylene)	3.19 ± 0.18	21.4 ± 0.2	27.9 ± 0.5	34.1 ± 2.5	984
Amended inoculated (w/ acetylene)	13.5 ± 0.7	78.1 ± 33.4	123 ± 63	308 ± 175	99.8
Amended inoculated (w/o acetylene)	14.8 ± 0.2	60.5 ± 16.0	106 ± 21	328 ± 78	1034

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

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
Control					
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00	4.13
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14
Salt / inoculum/ tube+brine (no cellulose)	14.2 ± 0.51	36.6 ± 6.1	39.8 ± 5.5	51.6 ± 3.4	93.8
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
Unamended inoculated	20.3 ± 0.2	93.7 ± 2.6	186 ± 6	434 ± 39	483 ± 133
Amended uninoculated	6.65 ± 0.80	39.2 ± 1.5	45.5 ± 1.5	49.6 ± 1.6	41.7 ± 3.2
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7
Amended inoculeted (w/ acetylene)	26.6 ± 0.7	94.0 ± 18.6	123 ± 30	251 ± 92	558 ± 270
Carbon Source: Cellulose + Glucose					
Amended uninoculated	6.71 ± 0.12	44.5 ± 0.2	53.1 ± 0.4	64.3 ± 1.0	177
Amended inoculated	31.4 ± 0.7	396 ± 13	487 ± 1	584 ± 28	754 ± 94
Amended uninoculated (RG salt)	5.28 ± 0.45	45.9 ± 0.7	55.1 ± 1.4	74.9 ± 2.2	178 ± 3
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
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
Amended inoculated (w/ acetylene)	27.7 ± 0.27	70.3 ± 2.7	114 ± 0	324 ± 30	447
Amended inoculated (w/o acetylene)	26.0 ± 0.82	237 ± 2	317 ± 6	516 ± 0	1356

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

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
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
Carbon Source: Cellulose					
Unamended Inoculated	11.3 ± 0.1	25.9 ± 3.6	36.1 ± 7	89 ± 24.4	163 ± 36
Amended Inoculated	16.9 ± 1.2	38.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9	18.2 ± 1
*Unamended inoculated (corrected)**	7.70 ± 0.08	20.0 ± 2.9	28.5 ± 5.5	72.6 ± 19.9	154.7 ± 34.2
*Amended inoculated (corrected)**	13.3 ± 0.9	30.5 ± 0.7	32.8 ± 0.6	18.3 ± 0.5	9.9 ± 0.5

Treatments *with bentonite*	Carbon dioxide (µmoles/ gram cellulose) Days				
	6	100	140	415	2156
Control					
No cellulose (salt/ inoculum/ tube+brine)	14.2 ± 0.5	38.8 ± 6.1	39.8 ± 5.5	51.8 ± 3.4	93.8
Carbon Source: Cellulose					
Unamended Inoculated	20.3 ± 0.2	94 ± 3	186 ± 6	434 ± 39	483 ± 133
Amended Inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7
*Unamended Inoculated (corrected)**	6.10 ± 0.08	57.1 ± 1.6	146 ± 5	382 ± 34	389 ± 107
*Amended Inoculated (corrected)**	16.0 ± 0.6	213 ± 26	433 ± 23	390 ± 134	460 ± 30

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

Table 11. Total Volume of Gas Produced in Samples Containing Polyethylene.

Sample	Milliliters of Gas Produced/Sample Days 0	30	189	334	488	840	2612
No Plastic or Rubber							
Aerobic							
Unamended	0.93	0.97 ± 0.13	-1.09 ± 0.63	0.45 ± 0.50	0.78 ± 0.52	1.70 ± 0.35	3.29 ± 0.37
Amended	0.65	1.74 ± 0.17	1.56 ± 0.03	0.90 ± 0.48	1.73 ± 0.57	2.69 ± 0.59	2.86 ± 0.49
Anaerobic							
Unamended	1.07	1.17 ± 0.05	0.98 ± 0.08	0.66 ± 0.37	1.59 ± 0.42	2.48 ± 0.34	2.31 ± 0.4
Amended	0.93	4.96 ± 0.24	3.13 ± 1.19	3.13 ± 1.15	3.66 ± 0.98	4.24 ± 0.82	5.27
Polyethylene - Aerobic							
Unamended							
Unirradiated	1.06	1.50	-1.97	2.47	2.42	3.46	4.53
Irradiated (Low-Dose)	1.17	1.56	-2.37	1.30	1.61	2.51	3.33
Irradiated (High-Dose)	1.02	1.25	-2.32	2.19	1.33	3.02	4.39
Amended							
Unirradiated	1.06	1.73 ± 0.05	1.55 ± 0.34	1.78 ± 0.49	1.87 ± 0.44	2.70 ± 0.25	3.84 ± 0.42
Irradiated (Low-Dose)	0.95	2.09 ± 0.09	0.98 ± 0.32	1.54 ± 0.41	1.55 ± 0.36	2.49 ± 0.38	2.85 ± 0.64
Irradiated (High-Dose)	0.84	1.94 ± 0.22	1.52 ± 0.14	1.73 ± 0.57	1.95 ± 0.61	2.97 ± 0.56	1.99
Polyethylene - Anaerobic							
Unamended							
Unirradiated	1.21	1.44	1.19	2.34	2.09	2.40	3.47
Irradiated (Low-Dose)	1.14	1.35	1.22	2.24	2.10	2.51	3.46
Irradiated (High-Dose)	1.22	1.41	0.59	1.98	2.32	2.67	3.51
Amended							
Unirradiated	1.15	5.09 ± 0.06	3.33 ± 0.92	3.73 ± 0.91	3.33 ± 0.45	3.48 ± 0.58	3.15
Irradiated (Low-Dose)	1.26	5.61 ± 0.21	4.99 ± 0.58	4.84 ± 0.81	4.30 ± 0.61	3.76 ± 0.14	4.05 ± 0.06
Irradiated (High-Dose)	1.08	5.41 ± 0.19	4.37 ± 0.81	4.75 ± 0.74	4.54 ± 0.85	4.69 ± 0.83	4.02

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 12. Total Volume of Gas Produced in Samples Containing Polyvinylchloride.

Sample	Milliliters of Gas Produced/Sample						
	Days						
	0	30	189	334	488	840	2612
No Plastic or Rubber							
Aerobic							
Unamended	0.93	0.97 ± 0.13	-1.09 ± 0.63	0.45 ± 0.50	0.76 ± 0.52	1.70 ± 0.35	3.29 ± 0.37
Amended	0.85	1.74 ± 0.17	1.56 ± 0.03	0.90 ± 0.48	1.73 ± 0.57	2.69 ± 0.59	2.86 ± 0.49
Anaerobic							
Unamended	1.07	1.17 ± 0.05	0.98 ± 0.08	0.66 ± 0.37	1.59 ± 0.42	2.48 ± 0.34	2.31 ± 0.4
Amended	0.93	4.96 ± 0.24	3.13 ± 1.19	3.13 ± 1.15	3.66 ± 0.98	4.24 ± 0.82	5.27
Polyvinylchloride - Aerobic							
Unamended							
Unirradiated	1.06	0.64	-1.99	1.39	1.13	2.08	3.36
Irradiated (Low-Dose)	0.90	0.92	0.59	1.59	1.02	2.29	3.38
Irradiated (High-Dose)	1.12	1.18	-2.05	1.40	1.09	1.34	1.97
Amended							
Unirradiated	0.89	1.90 ± 0.23	1.67 ± 0.13	1.67 ± 0.29	1.60 ± 0.32	2.57 ± 0.37	3.23 ± 0.36
Irradiated (Low-Dose)	0.90	-0.47 ± 0.31	-0.05 ± 0.23	0.17 ± 0.18	0.49 ± 0.15	1.37 ± 0.17	2.65 ± 0.2
Irradiated (High-Dose)	0.87	-1.08 ± 0.14	2.81 ± 0.71	2.05 ± 0.04	2.48 ± 0.10	3.00 ± 0.17	3.81 ± 0.12
Polyvinylchloride - Anaerobic							
Unamended							
Unirradiated	1.06	1.66	1.70	2.12	2.14	3.08	3.55
Irradiated (Low-Dose)	1.24	1.86	1.61	1.09	0.96	1.66	2.66
Irradiated (High-Dose)	1.09	1.53	1.53	1.34	1.54	1.72	3.97
Amended							
Unirradiated	1.02	5.10 ± 0.19	3.89 ± 1.08	4.07 ± 0.94	4.01 ± 0.80	4.69 ± 0.58	4.72 ± 0.42
Irradiated (Low-Dose)	0.99	1.32 ± 0.06	3.82 ± 0.92	5.01 ± 0.30	4.78 ± 0.23	4.94 ± 0.16	4.75 ± 0.20
Irradiated (High-Dose)	0.96	2.73 ± 0.79	5.34 ± 0.11	5.24 ± 0.11	5.31 ± 0.09	5.19 ± 0.03	5.27 ± 0.02

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 13. Total Volume of Gas Produced in Samples Containing Neoprene.

Sample	Milliliters of Gas Produced/Sample Days 0	30	189	334	488	840	2612
No Plastic or Rubber							
Aerobic							
Unamended	0.93	0.97 ± 0.13	-1.09 ± 0.63	0.45 ± 0.50	0.78 ± 0.52	1.70 ± 0.35	3.29 ± 0.37
Amended	0.85	1.74 ± 0.17	1.56 ± 0.03	0.90 ± 0.48	1.73 ± 0.57	2.69 ± 0.59	2.86 ± 0.49
Anaerobic							
Unamended	1.07	1.17 ± 0.05	0.98 ± 0.08	0.66 ± 0.37	1.59 ± 0.42	2.48 ± 0.34	2.31 ± 0.40
Amended	0.93	4.96 ± 0.24	3.13 ± 1.19	3.13 ± 1.15	3.66 ± 0.98	4.24 ± 0.82	5.27
Neoprene - Aerobic							
Unamended							
Unirradiated	0.91	0.32	-2.13	-1.77	-0.94	3.23	2.70
Irradiated (Low-Dose)	1.03	-0.02	-0.84	1.32	1.66	3.25	3.55
Irradiated (High-Dose)	0.97	-0.05	-2.30	0.53	1.95	2.91	2.74
Amended							
Unirradiated	1.00	2.32 ± 0.09	1.75 ± 0.12	1.34 ± 0.12	1.65 ± 0.21	2.69 ± 0.34	2.66 ± 0.25
Irradiated (Low-Dose)	0.97	1.87 ± 0.20	1.74 ± 0.30	1.28 ± 0.37	1.70 ± 0.26	2.96 ± 0.22	3.13 ± 0.43
Irradiated (High-Dose)	0.70	1.91 ± 0.15	1.76 ± 0.38	1.33 ± 0.37	1.77 ± 0.24	2.80 ± 0.06	3.16 ± 0.40
Neoprene - Anaerobic							
Unamended							
Unirradiated	1.06	1.48	0.95	1.67	1.56	1.80	2.15
Irradiated (Low-Dose)	1.10	1.29	1.05	1.26	1.66	2.44	1.90
Irradiated (High-Dose)	1.14	1.73	1.54	2.03	1.99	1.98	3.44
Amended							
Unirradiated	1.23	5.19 ± 0.14	3.48 ± 1.00	4.19 ± 0.93	3.78 ± 0.73	2.96 ± 0.54	3.64 ± 0.31
Irradiated (Low-Dose)	0.98	5.05 ± 0.11	3.61 ± 0.64	2.46 ± 0.33	2.31 ± 0.39	2.46 ± 0.36	2.79 ± 0.35
Irradiated (High-Dose)	1.00	4.53 ± 0.09	4.74 ± 0.24	5.26 ± 0.20	4.86 ± 0.04	5.12 ± 0.07	4.58 ± 0.06

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 14. Total Volume of Gas Produced in Samples Containing Unleaded Hypalon.

Sample	Milliliters of Gas Produced/Sample Days 0	157	332	664	2464
No Plastic or Rubber					
Aerobic					
Unamended	1.08	0.88 ± 0.08	0.33 ± 0.09	0.38 ± 0.15	1.45 ± 0.27
Amended	1.00	-0.21 ± 0.07	-0.04 ± 0.09	0.51 ± 0.07	1.37 ± 0.07
Anaerobic					
Unamended	0.65	1.47 ± 0.04	0.88 ± 0.17	1.07 ± 0.08	1.51 ± 0.08
Amended	0.76	4.30 ± 0.11	2.45 ± 0.95	3.09 ± 0.81	3.58 ± 0.74
Unleaded Hypalon - Aerobic					
Unamended					
Unirradiated	1.12	1.05	0.14	0.34	0.82
Irradiated (Low-Dose)	1.06	-0.24	0.21	1.18	0.87
Amended					
Unirradiated	1.14	-0.60 ± 0.06	-0.25 ± 0.15	0.49 ± 0.09	1.40 ± 0.35
Irradiated (Low-Dose)	1.11	0.54 ± 0.91	1.07 ± 0.89	1.90 ± 0.88	1.68 ± 0.15
Unleaded Hypalon - Anaerobic					
Unamended					
Unirradiated	0.84	1.45	0.94	1.55	2.21
Irradiated (Low-Dose)	0.77	1.39	0.91	1.08	1.36
Amended					
Unirradiated	0.82	4.04 ± 0.04	2.92 ± 0.92	3.49 ± 0.89	3.29 ± 0.78
Irradiated (Low-Dose)	0.86	2.92 ± 0.69	2.67 ± 0.98	3.41 ± 0.90	2.99 ± 0.67

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 15. Total Volume of Gas Produced in Samples Containing Leaded Hypalon.

Sample	Milliliters of Gas Produced/Sample Days 0	157	332	664	2464
No Plastic or Rubber					
Aerobic					
Unamended	1.08	0.86 ± 0.08	0.33 ± 0.09	0.36 ± 0.15	1.45 ± 0.27
Amended	1.00	-0.21 ± 0.07	-0.04 ± 0.09	0.51 ± 0.07	1.37 ± 0.07
Anaerobic					
Unamended	0.65	1.47 ± 0.04	0.86 ± 0.17	1.07 ± 0.08	1.51 ± 0.08
Amended	0.76	4.30 ± 0.11	2.45 ± 0.95	3.09 ± 0.81	3.58 ± 0.74
Leaded Hypalon - Aerobic					
Unamended					
Unirradiated	1.06	-0.13	-0.41	-0.58	0.86
Irradiated (Low-Dose)	1.02	-0.26	-1.04	-1.36	-1.07
Amended					
Unirradiated	1.17	-1.11 ± 0.67	1.40 ± 0.93	1.81 ± 0.93	2.67 ± 0.79
Irradiated (Low-Dose)	1.08	-0.72 ± 0.08	-0.17 ± 0.14	0.57 ± 0.16	2.23 ± 0.25
Leaded Hypalon - Anaerobic					
Unamended					
Unirradiated	0.31	1.00	1.09	1.49	1.85
Irradiated (Low-Dose)	0.29	1.06	1.01	1.01	1.34
Amended					
Unirradiated	0.94	3.85 ± 0.02	2.96 ± 0.78	3.30 ± 1.12	3.60 ± 0.93
Irradiated (Low-Dose)	1.06	3.83 ± 0.10	3.77 ± 0.14	4.45 ± 0.05	3.97 ± 0.38

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 16. Carbon Dioxide Produced in Samples Containing Polyethylene.

Sample	µmoles CO_2/Sample Days 0	30	189	334	488	840	2612
No Plastic or Rubber							
Aerobic							
Unamended	1.50	1.76 ± 0.13	6.11 ± 0.33	8.46 ± 0.39	11.9 ± 0.5	15.0 ± 1.7	19.9 ± 1.2
Amended	1.21	26.1 ± 0.2	35.9 ± 0.4	38.0 ± 0.9	42.6 ± 1.5	42.7 ± 2.1	46.2 ± 1.1
Anaerobic							
Unamended	1.52	1.76 ± 0.05	2.71 ± 0.06	6.60 ± 0.50	15.5 ± 0.2	16.6 ± 1.9	17.2 ± 1.4
Amended	1.21	16.0 ± 0.2	23.7 ± 0.1	29.5 ± 0.6	33.6 ± 0.7	32.9 ± 0.7	31.9
Polyethylene - Aerobic							
Unamended							
Unirradiated	1.70	3.63	6.61	13.6	16.7	37.3	64.2
Irradiated (Low-Dose)	1.67	2.57	6.16	14.6	14.9	16.5	16.0
Irradiated (High-Dose)	1.58	2.70	6.37	13.7	13.6	16.7	26.8
Amended							
Unirradiated	1.29	29.1 ± 0.3	36.3 ± 0.2	44.6 ± 0.7	40.1 ± 1.0	41.5 ± 2.7	43.7 ± 5.6
Irradiated (Low-Dose)	1.23	27.3 ± 0.3	35.0 ± 0.3	44.6 ± 0.7	40.6 ± 1.6	40.3 ± 2.2	49.4 ± 2.6
Irradiated (High-Dose)	1.25	26.8 ± 0.1	34.6 ± 0.4	44.3 ± 1.3	42.6 ± 0.2	41.5 ± 0.3	52.4
Polyethylene - Anaerobic							
Unamended							
Unirradiated	1.66	1.63	4.53	14.0	11.7	11.2	14.0
Irradiated (Low-Dose)	1.58	1.62	3.15	13.1	15.9	15.6	15.2
Irradiated (High-Dose)	1.63	2.10	2.71	6.80	20.6	21.5	23.4
Amended							
Unirradiated	1.29	19.5 ± 0.1	26.1 ± 0.1	34.7 ± 0.4	32.6 ± 0.4	32.0 ± 2.3	34.2
Irradiated (Low-Dose)	1.35	19.2 ± 0.2	25.6 ± 0.5	34.6 ± 0.9	31.5 ± 1.1	32.0 ± 0.7	27.4 ± 2.6
Irradiated (High-Dose)	1.23	19.5 ± 0.2	24.3 ± 0.3	33.6 ± 0.1	33.6 ± 1.3	35.6 ± 2.2	27.3

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 17. Carbon Dioxide Produced in Samples Containing Polyvinylchloride.

Sample	µmoles CO_2/Sample Days 0	30	189	334	488	840	2612
No Plastic or Rubber							
Aerobic							
Unamended	1.50	1.76 ± 0.13	8.11 ± 0.33	8.48 ± 0.39	11.9 ± 0.5	15.0 ± 1.7	19.9 ± 1.2
Amended	1.21	26.1 ± 0.2	35.9 ± 0.4	38.0 ± 0.9	42.8 ± 1.5	42.7 ± 2.1	46.2 ± 1.1
Anaerobic							
Unamended	1.52	1.78 ± 0.05	2.71 ± 0.08	8.60 ± 0.50	15.5 ± 0.2	16.6 ± 1.9	17.2 ± 1.4
Amended	1.21	18.0 ± 0.2	23.7 ± 0.1	29.5 ± 0.6	33.6 ± 0.7	32.9 ± 0.7	31.9
Polyvinylchloride - Aerobic							
Unamended							
Unirradiated	1.50	3.63	7.58	11.7	14.5	18.0	29.1
Irradiated (Low-Dose)	1.54	2.11	18.1	24.1	22.2	22.8	31.3
Irradiated (High-Dose)	1.57	1.89	9.38	18.2	14.7	15.4	21.1
Amended							
Unirradiated	1.25	28.0 ± 0.5	41.7 ± 0.2	43.6 ± 0.3	40.9 ± 0.3	39.8 ± 0.1	44.9 ± 0.4
Irradiated (Low-Dose)	1.15	17.8 ± 1.2	29.4 ± 0.9	30.7 ± 0.4	28.9 ± 0.3	26.5 ± 0.1	32.7 ± 0.3
Irradiated (High-Dose)	1.22	20.3 ± 0.1	44.8 ± 0.0	44.8 ± 0.3	44.4 ± 0.6	50.1 ± 3.4	48.4 ± 3.4
Polyvinylchloride - Anaerobic							
Unamended							
Unirradiated	1.54	1.78	7.77	13.7	15.6	20.0	25.9
Irradiated (Low-Dose)	1.59	1.85	1.95	3.20	3.50	3.12	4.70
Irradiated (High-Dose)	1.56	1.88	2.03	4.18	4.02	4.79	49.4
Amended							
Unirradiated	1.19	18.8 ± 0.3	24.1 ± 0.4	28.5 ± 0.8	28.6 ± 0.9	31.9 ± 0.7	34.8 ± 1.7
Irradiated (Low-Dose)	1.20	3.44 ± 0.08	18.7 ± 0.5	18.3 ± 0.2	17.4 ± 0.1	17.4 ± 0.3	18.7 ± 0.4
Irradiated (High-Dose)	1.18	10.0 ± 3.8	20.2 ± 2.3	22.0 ± 3.0	22.4 ± 3.7	28.5 ± 7.1	27.5 ± 6.3

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Teble 18. Carbon Dioxide Produced in Samples Containing Neoprene.

Sample	μmoles CO_2/Sample						
	0	30	189	334	488	840	2612
	Days						
No Plastic or Rubber							
Aerobic							
Unamended	1.50	1.76 ± 0.13	8.11 ± 0.33	8.48 ± 0.39	11.91 ± 0.46	15.0 ± 1.7	19.9 ± 1.2
Amended	1.21	26.1 ± 0.2	35.9 ± 0.4	38.0 ± 0.9	42.8 ± 1.5	42.7 ± 2.1	48.2 ± 1.1
Anaerobic							
Unamended	1.52	1.78 ± 0.05	2.71 ± 0.08	8.60 ± 0.50	15.5 ± 0.2	18.6 ± 1.9	17.2 ± 1.4
Amended	1.21	18.0 ± 0.2	23.7 ± 0.1	29.5 ± 0.8	33.6 ± 0.7	32.9 ± 0.7	31.9
Neoprene - Aerobic							
Unamended							
Unirradiated	1.60	3.34	7.68	8.33	10.1	28.8	30.5
Irradiated (Low-Dose)	1.66	3.69	8.18	10.7	12.3	15.1	36.8
Irradiated (High-Dose)	1.64	4.21	10.4	18.0	25.5	41.8	60.0
Amended							
Unirradiated	1.27	25.4 ± 0.4	38.4 ± 0.5	37.7 ± 0.3	39.4 ± 0.9	46.8 ± 2.7	46.2 ± 2.7
Irradiated (Low-Dose)	1.32	27.8 ± 0.3	40.2 ± 0.7	40.9 ± 0.9	41.8 ± 1.8	43.5 ± 3.1	55.8 ± 1.8
Irradiated (High-Dose)	1.30	29.3 ± 0.2	44.5 ± 1.1	48.7 ± 2.3	48.5 ± 3.2	55.2 ± 7.1	74.6 ± 0.0
Neoprene - Anaerobic							
Unamended							
Unirradiated	1.58	2.01	2.75	9.34	15.7	15.7	15.7
Irradiated (Low-Dose)	1.65	2.09	2.18	3.09	NA	19.2	25.7
Irradiated (High-Dose)	1.67	1.81	2.28	2.50	2.38	2.92	19.0
Amended							
Unirradiated	1.24	18.3 ± 0.1	22.7 ± 0.3	32.9 ± 0.8	33.1 ± 0.8	33.5 ± 1.0	31.7 ± 0.1
Irradiated (Low-Dose)	1.32	19.0 ± 0.4	22.5 ± 0.2	28.3 ± 0.9	31.3 ± 1.0	31.7 ± 0.8	33.9 ± 0.5
Irradiated (High-Dose)	1.35	23.4 ± 0.9	30.7 ± 1.3	34.8 ± 1.0	36.5 ± 0.7	48.7 ± 1.7	47.8 ± 2.2

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 19. Carbon Dioxide Produced in Samples Containing Unleaded Hypalon.

Sample	µmoles CO_2/Sample Days 0	157	332	664	2464
No Plastic or Rubber					
Aerobic					
Unamended	1.78	3.84 ± 0.15	3.69 ± 0.06	2.52 ± 0.52	5.55 ± 0.08
Amended	1.56	30.3 ± 0.5	30.8 ± 0.4	29.8 ± 0.2	33.3 ± 0.7
Anaerobic					
Unamended	1.78	2.76 ± 0.01	2.76 ± 0.01	4.15 ± 1.44	5.26 ± 0.15
Amended	1.65	20.4 ± 0.2	21.2 ± 0.1	22.0 ± 0.1	23.6 ± 0.5
Unleaded Hypalon - Aerobic					
Unamended					
Unirradiated	1.78	3.21	3.18	3.67	4.90
Irradiated (Low-Dose)	1.77	4.08	5.33	6.77	11.2
Amended					
Unirradiated	1.51	27.9 ± 0.3	28.1 ± 0.3	27.1 ± 0.6	31.8 ± 0.3
Irradiated (Low-Dose)	1.64	40.9 ± 8.6	41.8 ± 8.4	40.6 ± 6.4	43.8 ± 7.1
Unleaded Hypalon - Anaerobic					
Unamended					
Unirradiated	1.79	2.10	1.9	2.23	5.10
Irradiated (Low-Dose)	1.79	2.22	1.97	4.04	5.80
Amended					
Unirradiated	1.56	19.9 ± 0.2	20.8 ± 0.2	19.6 ± 0.3	21.1 ± 0.1
Irradiated (Low-Dose)	1.65	18.8 ± 0.6	21.3 ± 0.4	23.5 ± 1.8	31.1 ± 5.9

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.

Table 20. Carbon Dioxide Produced in Samples Containing Leaded Hypalon.

Sample	µmoles CO_2/Sample Days 0	157	332	664	2464
No Plastic or Rubber					
Aerobic					
Unamended	1.76	3.84 ± 0.15	3.69 ± 0.06	2.52 ± 0.52	5.55 ± 0.08
Amended	1.56	30.3 ± 0.5	30.8 ± 0.4	29.84 ± 0.22	33.3 ± 0.7
Anaerobic					
Unamended	1.78	2.76 ± 0.01	2.76 ± 0.01	4.15 ± 1.44	5.26 ± 0.15
Amended	1.65	20.4 ± 0.2	21.2 ± 0.1	22.0 ± 0.1	23.6 ± 0.5
Leaded Hypalon - Aerobic					
Unamended					
Unirradiated	1.72	3.77	4.03	5.33	8.27
Irradiated (Low-Dose)	1.71	3.30	3.72	4	4.33
Amended					
Unirradiated	1.53	32.8 ± 3.9	39.5 ± 8.2	37.4 ± 9.4	47.2 ± 3.2
Irradiated (Low-Dose)	1.59	27.3 ± 0.2	27.6 ± 0.1	20.4 ± 6.6	25.1 ± 1.7
Leaded Hypalon - Anaerobic					
Unamended					
Unirradiated	1.71	1.80	1.66	2.12	6.08
Irradiated (Low-Dose)	1.74	2.05	2.12	2.60	5.39
Amended					
Unirradiated	1.69	18.1 ± 0.1	19.6 ± 0.2	21.5 ± 0.8	26.1 ± 4.4
Irradiated (Low-Dose)	1.72	18.8 ± 0.1	19.4 ± 0.2	18.0 ± 1.7	20.9 ± 0.1

Amended: NH_4NO_3 (0.5 g/L), K_2HPO_4 (0.5 g/L), yeast extract (0.25 g/L); Unamended: no nutrient addition.