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MICROBIAL GAS GENERATION UNDER EXPECTED WASTE ISOLATION PILOT PLANT REPOSITORY CONDITIONS: FINAL REPORT

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Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions: Final Report

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ABSTRACT

Gas generation from the microbial degradation of the organic constituents of transuranic (TRU) waste under conditions expected in the Waste Isolation Pilot Plant (WIPP) was investigated. The biodegradation of mixed cellulosic materials and electron-beam irradiated plastic and rubber materials (polyethylene, polyvinylchloride, hypalon, leaded hypalon, and neoprene) was examined. We evaluated the effects of environmental variables such as initial atmosphere (air or nitrogen), water content (humid (~70% relative humidity, RH) and brine inundated), and nutrient amendments (nitogen phosphate, yeast extract, and excess nitrate) on microbial gas generation. Total gas production was determined by pressure measurement and carbon dioxide (CO_2) and methane (CH_4) were analyzed by gas chromatography; cellulose degradation products in solution were analyzed by high-performance liquid chromatography. Microbial populations in the samples were determined by direct microscopy and molecular analysis.. The results of this work are summarized below.

- Over 10.8 years, under initially aerobic conditions 0.84 ± 0.10 mL of total gas was produced per g cellulose without a nutrient amendment, while samples with a nutrient amendment supplemented without and with excess nitrate produced 1.71 ± 1.03 mL and 12.2 ± 0.0 mL total gas g^{-1} cellulose, respectively. Over the same period, 16.3 ± 1.3 μmol CO_2 was produced g^{-1} cellulose in the absence of a nutrient amendment; 41.4 ± 7.8 μmol CO_2 g^{-1} cellulose with nutrient amendment, and 186 μmoles CO_2 g^{-1} cellulose when excess NO_3^- was present. The overall rate of total gas production from these treatments was 2.3×10^{-4}, 4.0×10^{-4}, and 1.9×10^{-3} mL total gas g^{-1} cellulose day^{-1}, respectively, and CO_2 production was 3.7×10^{-3}, 4.5×10^{-3}, and 4.1×10^{-2} μmol CO_2 g^{-1} cellulose day^{-1}, respectively.

- Under anaerobic conditions, 2.48 ± 0.31 mL total gas g^{-1} cellulose was produced without a nutrient amendment, 4.12 ± 0.76 mL total gas g^{-1} cellulose with nutrients, and 18.1 ± 0.38

mL total gas g^{-1} cellulose with excess NO_3^-. Carbon dioxide production under anaerobic conditions was as follows: 27.4 ± 5.8 μmol CO_2 g^{-1} cellulose in the absence of nutrients, 66.9 ± 1.1 μmol CO_2 g^{-1} cellulose with them, and 251 ± 5 μmol CO_2 g^{-1} cellulose with excess NO (after 6 years of incubation $2.24 \pm 0.24 \times 10^8$ bacterial cells mL^{-1} were detected in these samples). The overall rate of total gas production in anaerobic samples was 6.7×10^{-4}, 6.7×10^{-4}, and 2.5×10^{-3} mL g^{-1} cellulose day^{-1}, respectively, and for CO_2 production it was 6.4×10^{-3}, 1.4×10^{-2}, and 5.6×10^{-2} μmol CO_2 g^{-1} cellulose day^{-1}, respectively.

- Cellulose degradadation products deterred in solution include fumaric, lactic, oxalic, oxalacetic, propionic, and succinic acids indicating fermentative microbial activity.

- Methane was first detected after 7.4 years incubation in brine inundated samples. The amount of methane detected at 9.5 years under anaerobic conditions were without nutrients 5.89 ± 1.30 nmol g^{-1} cellulose; 2.74 ± 0.90 nmol g^{-1} cellulose with nutrients, and 2.57 ± 0.79 nmol g^{-1} cellulose with excess NO_3^-. In samples when the initial conditions were aerobic the amount of CH_4 detected was very low: 1.34 ± 0.03 nmol g^{-1} cellulose without nutrients, 0.84 ± 0.05 nmol g^{-1} cellulose with nutrients, and 1.27 ± 0.37 nmol g^{-1} cellulose with excess NO_3^-.

- The presence of bentonite, once proposed as a backfill additive, enhanced the total gas production, concentration of gaseous and aqueous metabolites by several fold.

- Under humid conditions, total gas and CO_2 production was much lower than smples incubated under iundated conditions. Adding nutrients lowered gas production; under initially aerobic conditions: 6.09 ± 2.41 μmol CO_2 was produced g^{-1} cellulose after ~9 years without nutrients while 0.48 ± 0.29 μmol CO_2 was produced g^{-1} cellulose with a nutrient amendment. The same held true under anaerobic conditions: unamended samples produced 115 ± 20 μmol CO_2 g^{-1} cellulose while nutrient-amended ones generated 21.9 ± 3.3 μmol CO_2 g^{-1} cellulose after ~9 years incubation. Bentonite enhanced gas production: anaerobic unamended samples produced 591 ± 135 μmol CO_2 g^{-1} cellulose, and amended samples produced 673 ± 49 μmoles CO_2 g^{-1} cellulose. Methane (32.6 ± 9.3 nmoles g^{-1} cellulose) was detected only in bentonite containing samples.

- Plastic and rubber-materials subjected to an absorbed radiation dose of up to 4,000 Mrad to determine if radiation damage could affect biodegradability of the polymers and gas generation. Microbial gas production was not observed after ~7 years incubation in polyethylene or polyvinylchloride samples, whereas irradiated rubber materials neoprene and hypalon showed enhanced CO_2 production.

- After 6 years of incubation, the microbial population in anaerobic brine-inundated samples were enumerated by direct microscopy: unamended uninoculated samples contained $5.12 \pm 3.41 \times 10^5$ bacterial cells mL^{-1}, unamended inoculated samples contained $1.59 \pm 0.15 \times 10^7$ cells ml^{-1}, amended inoculated samples contained $1.62 \pm 0.07 \times 10^8$ cells mL^{-1}, and amended inoculated samples with excess NO_3^- contained $2.24 \pm 0.24 \times 10^8$ cells mL^{-1}. DNA analyses showed a diverse assemblage of bacteria and archae in unamended and nutrient-amended inundated cellulose samples.

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1.0 INTRODUCTION

The Waste Isolation Pilot Plant (WIPP) is a U.S. Department of Energy facility located in southeastern New Mexico, approximately 656 m (2150 ft.) below ground surface in a bedded salt, Permian evaporite formation. This mined geologic repository has been receiving transuranic (TRU) waste from defense-related and environmental- management activities since March 1999. TRU waste contains alpha-emitting transuranium nuclides with half-lives greater than twenty years at concentrations greater than 100 nCi gram. These wastes were generated from nuclear-weapons production and related processing activities. They include various organics, adsorbed liquids, sludges, cellulosics, plastics, rubber, leaded rubber, and a variety of metals and cemented materials containing the following radionuclides: ^{232}Th, ^{233}U, ^{235}U, ^{237}Np, ^{238}Pu, ^{239}Pu, ^{240}Pu, ^{241}Pu, ^{242}Pu, ^{241}Am, ^{244}Cm, and ^{252}Cf. Estimates suggest that the total volume of TRU waste managed by the DOE through 2034 will be approximately 171,000 m^3; the WIPP's total capacity for contact-handled (CH) and remote-handled TRU waste is set at 176,000 m^3 (U.S. DOE, 2001). Remote-handled TRU waste has radiation levels ≥200 millirem hr^{-1}; the majority of TRU waste is classified as CH. The total radioactive content of CH-TRU waste in the DOE's inventory at the end of 1996 was 2.5 x 10^6 curies, predominantly from Pu and Am. The TRU waste will be shipped to WIPP from ten major sites throughout the United States. Containers of TRU waste will be emplaced inside 3,640 m^3 disposal rooms in the repository (Brush, 1990). Fifty-six rooms are planned or under construction, each able to hold approximately 6,800 55-gallon waste containers. The waste contains a large quantity of cellulosic material, 70% of which is paper (Brush, 1990). Thus, an average drum of TRU waste will contain 10 kg of cellulosic material, or ~70,000 kg per disposal room. In addition, the TRU waste inventory will contain plastics (polyethylene and polyvinylchloride) and rubber materials (neoprene, hypalon, leaded hypalon). Approximately 3 million moles of nitrate and a much smaller amount of phosphate also will be placed in the WIPP (Brush, 1990, Brush et al., 1991). The U.S. Environmental Protection Agency certified that the U.S. DOE's plans to operate WIPP complies with laws governing the long-term disposal of radioactive waste, 40 CFR 191 and 40 CFR 194 (U.S. EPA, 1998). Part of this certification relied on the DOE demonstrating an understanding of chemical processes in the repository over the 10,000 years of performance dictated by 40 CFR 194. Gas will be generated in the repository primarily by metal corrosion and microbial processes. It could result in

pressurization of the repository after it is sealed, causing anhydrite interbeds in the Salado formation to fracture and contribute to spalling and direct brine releases (U.S. EPA, 1998). In addition, microbially produced CO_2 could lower the pH of the repository if it were to become inundated with brine which, in turn, could increase the solubility of actinides.

Microorganisms that can grow under hypersaline conditions (halotolerant, and moderate and extreme halophiles) will be present in the WIPP from underground and surficial sources, and may become active under a variety of conditions over the repository's lifetime (Francis and Gillow, 1994). Microorganisms can enter WIPP from several sources, including (i) association with TRU waste from generator sites, (ii) the surface environment via the mine ventilation system and human intrusion, and (iii) as resident populations in the salt crystals and brine formations (Francis et al., 1997). Previous studies of low-level radioactive wastes and waste leachates showed that microbes can metabolize a variety of organic carbon compounds that are present in the wastes (Francis et al., 1980 a, b; Francis, 1985).

From 1992 to 2003, researchers at Brookhaven National Laboratory (BNL) undertook long-term experiments designed to examine gas generation due to biodegradation of the organic fraction of transuranic (TRU) wastes under WIPP repository-relevant conditions. Francis et al. (1997) summarized these experiments from 1991 to 1996. After a gap of 4 years, analyses of microbial gas generation from these experiments were resumed in 1999 until 2003. Table 1 summarizes the status of these experiments.

Table 1. Summary of long-term experiments designed to examine gas generation due to biodegradation of the organic fraction of transuranic wastes under WIPP repository-relevant conditions.

Experiment	Start Date	**SAND93-7036[1] (Days) (1994)**	**SAND96-2582[2] Days(Years) (1996)**	Analyses Completed Through Period Ending July 2003 (Days/Years)
Long-term Inundated Cellulose	1/29/92	200	1228 (3.4)	2718 / 7.4; total gas, CO_2 and CH_4 3462 / 9.5; total gas, CO_2 and CH_4 3561 / 9.9; aqueous metabolite analysis 3929 / 10.75; total gas and CO_2
Initially Aerobic Humid Cellulose	4/7/93	na	804 (2.3)	2553 / 7.0; total gas, CO_2 3009 / 8.2; total gas and CO_2 3334 / 9.1; total gas and CO_2
Anaerobic Humid Cellulose	5/4/94	na	415 (1.1)	2156 / 5.9; total gas, CO_2 2616 / 7.2; total gas, CO_2 and CH_4 (2623) 2945 / 8.1; total gas and CO_2
Inundated PE, PVC, and Neoprene	3/9/93	na	840 (2.3)	2612 / 7.2; total gas, CO_2 and CH_4 3070 / 8.4; CH_4
Inundated Hypalon	8/3/93	na	664 (1.8)	2464 / 6.8; total gas, CO_2 and CH_4 2926 / 8.0; CH_4

na = not applicable
1. Sandia National Laboratories Report #93-7036 (Francis and Gillow, 1994)
2. Sandia National Laboratory Report #96-2582 (Francis et al., 1997)

We used the test plan titled “Re-evaluation of Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Conditions, TP-99-01” for studies subsequent to publication of SAND96-2582. In addition, Brookhaven National Laboratory developed a Quality Assurance Program (QAP) for this research that complied with Sandia National Laboratories (SNL) requirements. This QAP was fully implemented during the work at BNL and was reviewed by SNL during formal on-site audits. It ensured that the data generated were valid, accurate, repeatable, protected and could withstand critical peer reviews and other reviews.

2.0 EXPERIMENTAL RATIONALE AND APPROACH

The expected conditions within the WIPP disposal rooms before 1996 gave us the framework within which to develop the experimental test conditions for microbial generation of gases (Brush, 1990). The disposal room scenarios developed by SNL dictated the following: i) substrates for biodegradation; ii) environmental conditions, including atmosphere and moisture content, and iii) alternate electron acceptors for biological activity.

Laboratory experiments were designed to determine the potential gas generation due to biodegradation of organic constituents of TRU waste under conditions expected in the WIPP repository after emplacing waste. The organic constituents include cellulose, plastic and rubber materials, specifically polyethylene (PE), polyvinylchloride (PVC), neoprene (NEO), hypalon (HYP), and leaded hypalon. The PE and PVC are predominantly used as liner and bagging materials for steel waste-containers. While the plastics are the most abundant polymers in the WIPP inventory, the rubbers NEO and HYP make up a sizable portion of the materials. In the repository, the plastic and rubber materials undergo continuous alpha-irradiation (radiolysis) from the radionuclides in the waste that may change their structural properties, potentially rendering them more susceptible to biodegradation.

Successions of microbial processes will occur under the changing environmental conditions inside the repository. Changes from aerobic to anaerobic, and humid to inundated conditions (and possibly back to humid) will regulate the activities of (i) microbes present in the waste, and (ii) resident and indigenous halotolerant or halophilic bacteria in the brine and salt. The following additional influencing variables were identified in the disposal-room scenario, the presence or absence of which might affect microbial gas generation: i) oxygen, ii) substrates (cellulose, plastic, or rubber), iii) brine, iv) bentonite, v) microbes, vi) nutrients, and vii) alternate electron acceptors. Evaluating the effects of these variables on microbial gas generation was the basis of our experimental methodology.

3.0 MATERIALS AND METHODS

3.1 Inundated Treatments

3.1.1 Cellulose

Four types of paper were used to simulate TRU cellulosic waste material: (i) filter paper (Whatman #1™); (ii) white paper towel (Fort Howard); (iii) brown paper towel; and (iv) Kimwipes™ (Kimberly-Clark, lintless tissue wipers). These types comprise the typical cellulosic wastes resulting from laboratory and process work. They were shredded into strips in a large paper shredder, and then cut into 1 cm x 1 cm squares in a small portable shredder. Each type of paper was weighed (1.25 g), mixed thoroughly, and transferred to 160 ml serum bottles that had been acid-washed (10% HCl) and sterilized (autoclaved at 120°C, 20 psi for 20 min.).

3.1.2 Brine

Sandia National Laboratories provided fifteen liters of brine from G-Seep, SNL #9 (the identifier is part of SNL's brine cataloging system) via overnight express delivery, on ice; it was stored at 4°C until used. G-Seep is a natural brine source that was slowly accumulating underground in the WIPP and was collected by SNL in 1991. Table 2 gives its chemical composition; it contains 10^4 - 10^6 bacterial cells ml^{-1} (Francis and Gillow, 1994).

Table 2. Composition of G-Seep brine (Brush, 1989).

Major Ion	g/L	M
Na^+	95.0	4.11
Cl^-	181	5.10
Mg^{2+}	15.3	0.63
K^+	13.7	0.35
Ca^+	0.32	0.01
SO_4^{2-}	29.1	0.30
HCO_3^-	0.73	0.01

3.1.3 Bentonite

Sandia National Laboratories also provided bentonite clay in two one-liter containers. It is a granular MX-80 Volclay bentonite, available from the American Colloid Company of Belle Fourche, South Dakota. At the time when these experiments were begun, bentonite was being considered as a potential backfill for the waste in WIPP to control the mobility of actinides. Table 3 shows its chemical composition.

Table 3. Composition of Bentonite*

Chemical Composition	$(NaCa)_{0.35}(Al_{1.60}Fe_{0.15}Mg_{0.25})(Si_{3.90}Al_{0.10})O_{10}(OH)_2$	
Montmorillonite Content	90%	
Typical Chemical Analysis, %	Silica	63.02 SiO_2
	Alumina	21.08 Al_2O_3
	Iron (Ferric)	3.25 Fe_2O_3
	Iron (Ferrous)	0.35 FeO
	Magnesium	2.67 MgO
	Sodium	2.57 Na_2O
	Calcium	0.67 CaO
	Crystal Water	5.64 H_2O
	Trace Elements	0.72
Exchangeable Ions (Milli-equivalents/100g)	Sodium	55-65
	Calcium	15-25
	Magnesium	10-15
Moisture Content	10% Maximum as Shipped	
pH	8.5 - 10.5	

*Data provided by the American Colloid Company, Skokie, IL

3.1.4 Microbial Inoculum

A microbial inoculum was prepared from a mixture of several WIPP repository-relevant samples. Microorganisms are expected to enter and reside in the repository from various sources (see Section 1.0) that may harbor microorganisms able to use different substrates for growth via numerous metabolic pathways. To eliminate the possibility of biasing the experiments toward one type of microorganism (i.e., selecting one pure halophilic microbial strain), we used a mixture of brine and sediment from the repository surficial and subterranean environments to obtain a consortium of microorganisms (mixed inoculum). This would allow these microorganisms to become active in the experiment based upon the environmental conditions and available electron donors and acceptors. The mixed inoculum was composed of the following:

Samples were collected on 12/12/91 from surficial lakes adjacent to the WIPP site in an area called Nash Draw. Brine was collected in sterile glass serum bottles, and sediment was collected from the lake bottom using 2" x 6" steel core samplers (AMS Inc., American Falls, ID). The sediment was stored anoxically in serum bottles, on ice and shipped to BNL overnight and then stored at 4°C. Before adding to the mixed inoculum, the sediment samples were filtered through sterile cotton in an O_2-free N_2-filled (anaerobic) glove box to remove large particulates. Lake brine and sediment were combined together in the anaerobic glove box in the proportions listed in Table 4.

Table 4. Surficial lake brine and sediment.

Sediment and Brine Source	Brine, ml	Sediment, ml
Laguna Quattro	60	40
Laguna Cinco	35	40
Laguna Tres South	13	40
Lindsey Lake	50	40
Surprise Springs	25	40
Total	183	200

The mixed inoculum was comprised of (i) sediment and brine from Nash Draw, (ii) Brine from the WIPP underground workings (G-Seep collected December 12, 1991, 200 ml), and (iii) inocula from a non-sterile laboratory environment (dust gathered from laboratories in Bldg. 318 (BNL) for non-halophilic microorganisms, 2.5 grams).

The sediment, brine, and dust samples were then mixed together in a sterile beaker in the anaerobic glove box. The total volume of the mixed inoculum was 583 ml. The viability of microorganisms in the mixed inoculum was examined by incubating subsamples under aerobic and anaerobic conditions in the presence of a simple carbon source (glucose) and nutrients (phosphate, ammonium, and nitrate). The activity results are reported elsewhere (Francis and Gillow (1994). In addition, most probable number (MPN) analysis of the mixed inoculum showed the presence of aerobes, denitrifiers, fermenters, sulfate reducers, and methanogens.

The treatments consisted of (a) 100 ml of brine, and (b) 100 ml of brine and 5 g mixed cellulosic papers. The samples were incubated with and without nutrients. The nutrients consisted of yeast extract (Difco, 0.05% w/v), K_2HPO_4 (potassium phosphate dibasic, Aldrich reagent grade, 0.1% w/v), and NH_4NO_3 (ammonium nitrate, Aldrich reagent grade, 0.1% w/v). All nutrient solutions were sterilized by filtration through 0.22mm syringe filter units (Millipore Corp.).

Some nutrient-amended samples received excess nitrate as potassium nitrate (Aldrich reagent grade, 0.5%). Nitrate can serve as an alternate electron acceptor in the absence of oxygen, reducing nitrate to nitrogen gas and perhaps nitrous oxide (an intermediate end-product). Bentonite MX-80, which contained approximately 3.25% ferric iron, also was a potential alternate electron acceptor for microbial activity under anaerobic conditions (iron reduction). In addition, sulfate, a natural constituent of the brine, can be used as an electron acceptor. In this process, sulfate is reduced to sulfide, liberating H_2S gas and precipitating metals as metal sulfides.

Anaerobic samples were prepared by flushing the serum bottles containing the mixed cellulosic paper were flushed with ultra-high purity (UHP) nitrogen. The bottles were placed inside the glove box for 24 hours before inoculation to allow any trapped air to escape. Ten liters of G-Seep brine #9 were removed from storage at 4°C and equilibrated overnight at room temperature. One hundred milliliters of the brine solutions with and without nutrients or excess nitrate were added to sample bottles with and without bentonite. Brine was measured in a sterile 100 ml graduated cylinder (KIMAX™, Kimble Glass Co., tolerance = ± 0.6 ml at 20°C). Bentonite (6.00 ± 0.10 g) was added to separate sample bottles inside the glove box and distributed by gently mixing the sample.

The mixed microbial inoculum prepared in the anaerobic glove box was mixed continuously and 4 ml added to specific samples using a calibrated continuously adjustable pipette (Pipetteman™, Rainin Instrument Co.). The samples were gently swirled to blend in the inoculum, the bottles then were capped with butyl rubber stoppers, and crimped with aluminum seals. Uninoculated samples were similarly set up. Three ml of 37% formaldehyde (to give a final concentration of 1% formaldehyde) were added to control samples to kill the bacteria and so measure abiotic gas production.

Aerobic samples were prepared as described above except that brine solutions were not purged with UHP N_2. Brine was added to the bottles with a sterile 100 ml graduated cylinder, the samples were inoculated, capped with butyl rubber stoppers, and sealed with aluminum crimp seals. This was done outside the glove box, thereby sealing air in the headspace. A detailed description is given elsewhere of all of the sample treatments (aerobic and anaerobic) and the number of replicate samples is listed in Appendix ?? (also see Francis and Gillow, 1994, Appendix C).

One hundred and eighty-four sample bottles were incubated under static (unshaken) conditions in a 30 ± 2°C incubator (Precision Scientific, Inc.). Headspace gas was analyzed at 21 intervals starting in 1992 up to 3929 days of incubation. The incubator's temperature was monitored weekly with thermometers calibrated by the manufacturer to standards traceable to the National Institute of Standards and Technology (NIST). The incubators also were continually monitored by electronic temperature sensors to provide immediate notification of a power failure or temperature deviation (± 2°C). The incubator's temperature did not deviate from the established range during the experiment. The treatment matrix is provided in Figures 1-4.

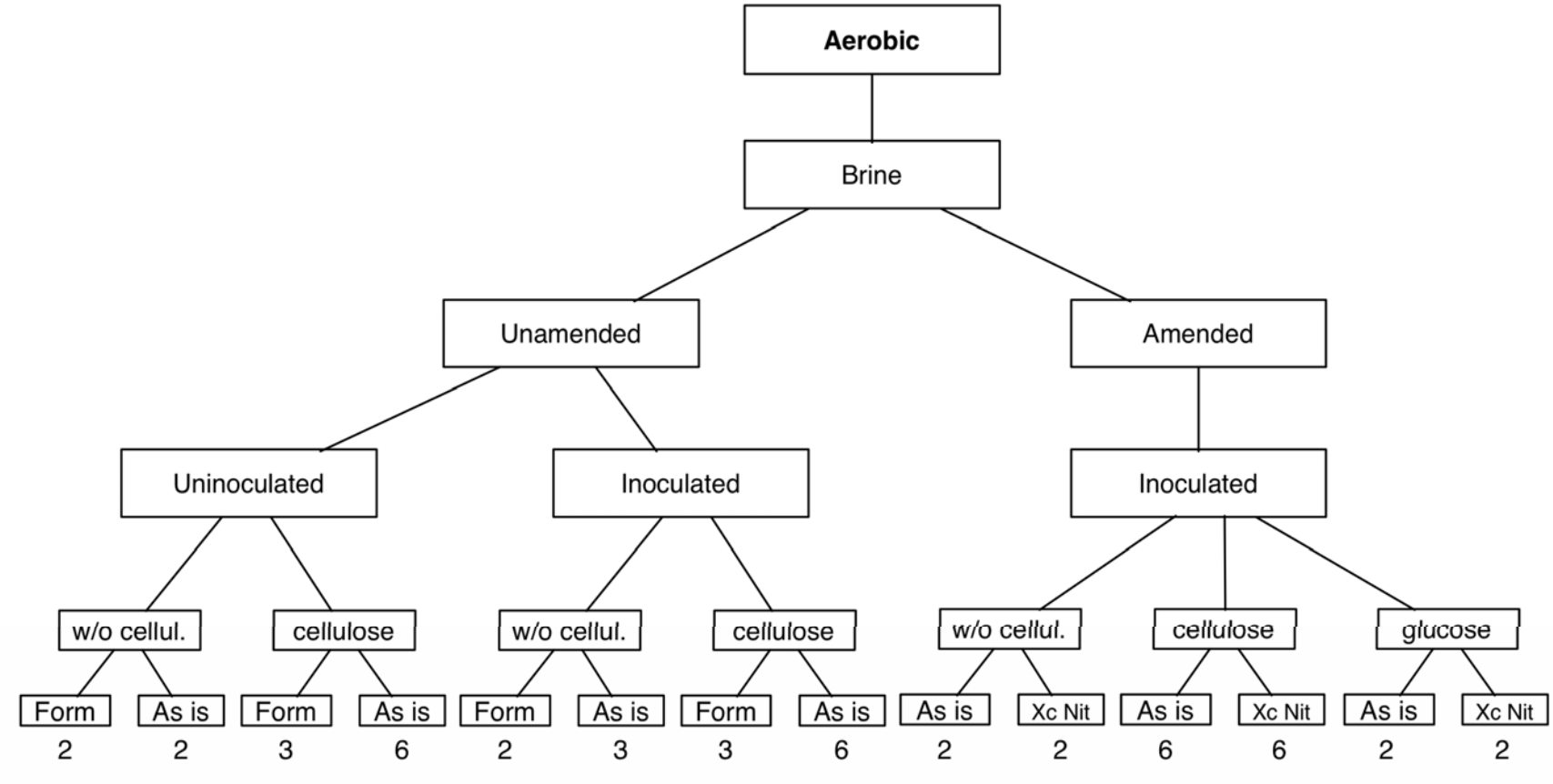

Figure 1. Long-term inundated experiment treatment matrix (aerobic samples); number of sample bottles is provided.

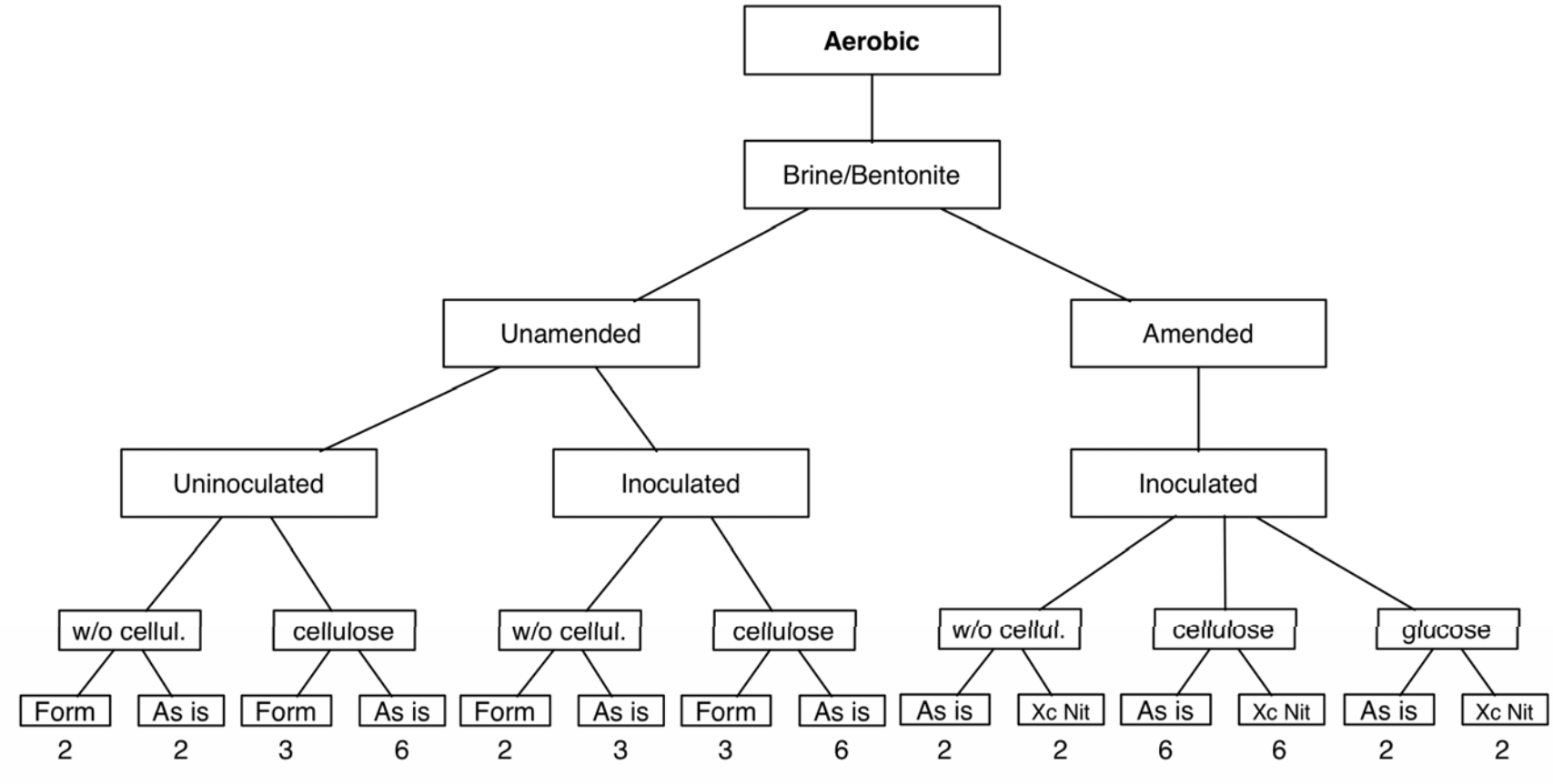

Figure 2. Long-term inundated experiment treatment matrix (aerobic samples containing bentonite); number of sample bottles is provided.

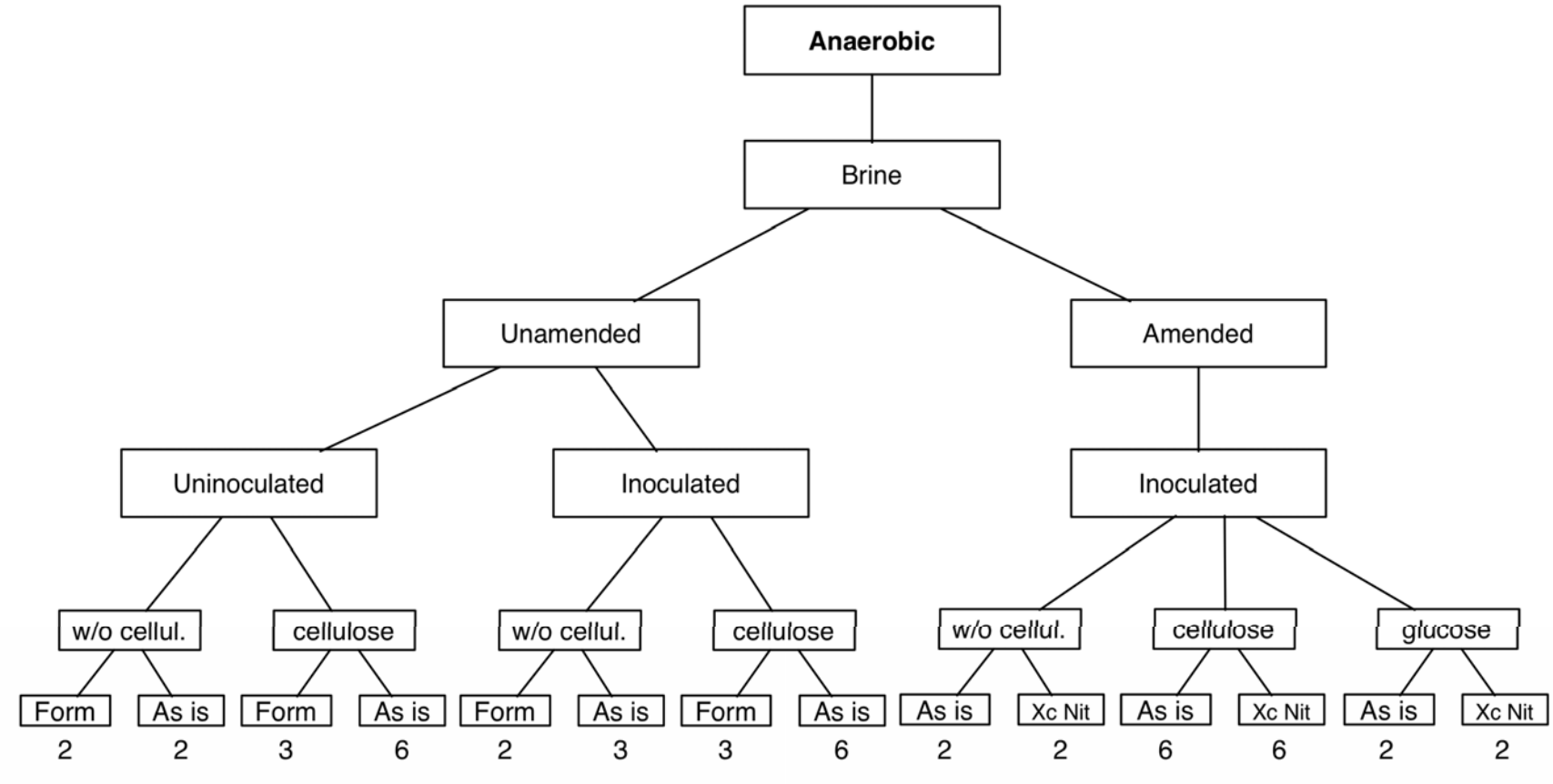

Figure 3. Long-term inundated experiment treatment matrix (anaerobic samples); number of sample bottles is provided.

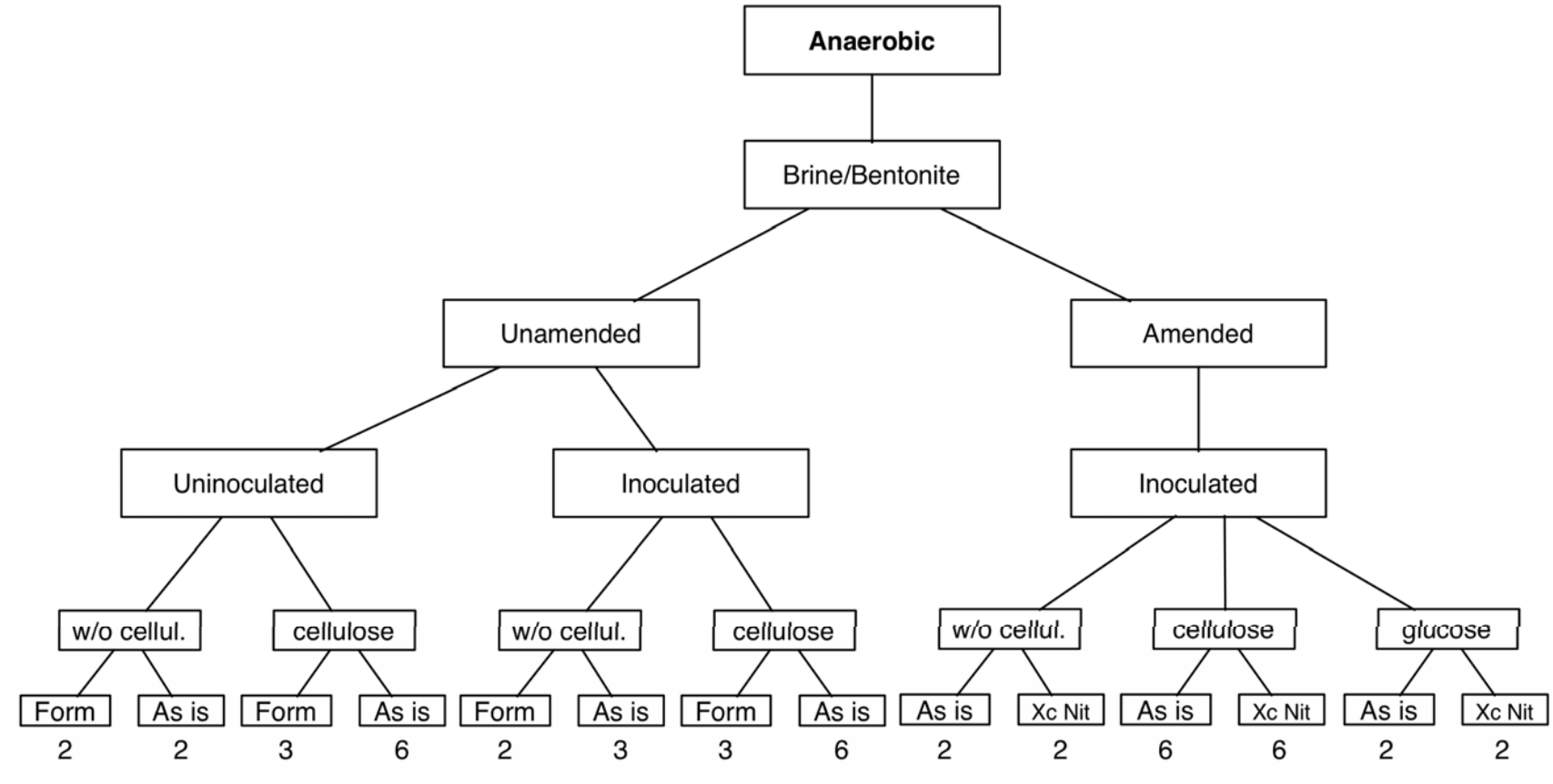

Figure 4. Long-term inundated experiment treatment matrix (anaerobic samples containing bentonite); number of sample bottles is provided.

3.2 Humid Treatments

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).

Triplicate samples were prepared with and without added nutrients. The nutrients (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).

Mixed inoculum was prepared as described above 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 a further, 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.

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.

After sealing the sample bottles, the relative humidity was measured with a Hygroskop GT™ (Rotronic, Zurich) portable humidity meter, the probe of which was fitted with a rubber seal so that measurements could be taken inside 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 by this method.

Initially aerobic 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.

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. We examined the ability of specific microorganisms (i.e., denitrifiers) to grow under such low-moisture conditions. 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. Seventy-two samples were incubated at 30 ± 2°C.

3.3 Treatments Containing Plastic and Rubber Materials

In this study, we attempted to determine the rate and extent of gas production due to the biodegradation of unirradiated and electron-beam irradiated plastic- and rubber-materials under conditions relevant to the WIPP repository. These were deemed accelerated tests because the entire structure of the irradiated 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 the overall radiation dose. The influence of adding nutrients (nitrogen, phosphorus, and yeast extract) on the extent of biodegradation also was evaluated.

The plastics examined were polyethylene and polyvinylchloride; the rubber materials were neoprene and hypalon (leaded and unleaded). They were exposed to electron-beam irradiation at the linear accelerator (LINAC) at Argonne National Laboratory by Dr. D. Reed, Chemical Technology Division. The polymers received an absorbed dose of either 500-700 Mrad (low-dose) or 4000-6000 Mrad (high dose), see Table 5. Tests with unleaded and leaded hypalon did not include a high-dose irradiation because it extensively degradaded (melted) the leaded sample.

Table 5. 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, weighed, and the pieces placed in acid-washed sterilized (autoclaved) 70 ml glass serum bottles. The mean weights (22 samples for each

polymer) were as follows: Polyethylene (86.1 mg), Polyvinylchloride (134.6 mg), Neoprene (257.5 mg).

Every sample bottle containing plastic or rubber was filled with 50 ml of a mixture 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 a fluid composed 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 additional "overtest" was done 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.

Unamended samples (without added nutrients) and amended ones (with nutrients) were incubated. Table 6 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 6. 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.

Samples were incubated initially under 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.

3.4 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). To sample the headspace gas pressure, the fitted butyl rubber stopper of the sample bottle was pierced with a sterile 22-gauge needle (Becton Dickinson, Franklin Lakes, NJ) attached to a digital pressure gauge (-5.00 to 35.00 psi (calibrated to NIST by the manufacturer (Wallace and Tiernan, Vineland, NJ): 0.00 to 35.00 psi), to calculate total gas production. At the same time, the room temperature was recorded with a thermometer calibrated to NIST.

Immediately after this, a gas-tight syringe (Pressure-Lok™, Precision Sampling Corp., Baton Rouge, LA) 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). Using a gas-sample valve (Valco Instrument Co., Houston, TX) equipped with a 100 μl stainless-steel sample loop we introduced reproducible quantities of gas from the syringe into the gas chromatograph. All analyses were performed according to written procedures prepared as part of the BNL Quality Assurance Program (QAP).

Carbon dioxide, oxygen, hydrogen, nitrogen, and nitrous oxide were 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 was quantified 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; and, ii) and samples without organic substrate (cellulose or plastic/rubber material). The gas data in this report are cumulative from t=0.

Methane was determined using a Varian 3400 gas chromatograph equipped with a 6' column (1/8" o.d. x 0.085" i.d.) packed with Porapak QS 80/100 (Alltech) to routinely separate CH_4 from headspace gases, with a thermal conductivity detector (TCD) in-line before the FID. Three- or four-point calibrations were made using external standards consisting of methane gas standards certified traceable to NIST (Scott Specialty Gases). Using this arrangement, the minimum detectable quantity of methane was 0.2 nmol CH_4 g^{-1} cellulose dry wt.

3.4.1 Gas Production Rates

Data from the long-term inundated was processed to determine gas production rates. The overall rate was calculated as follows:

$$rate_{overall} = \frac{dC}{dt} = \frac{C_{end} - C_{initial}}{3929 \text{ days}}$$

Where *C* is concentration and *t* is time. The overall rate (~11 years) may be relevant within the context of the 10,000-year regulatory time period for the WIPP repository. However, because the experiments were performed in a closed-system ("batch mode") the evolution of gas due to microbial metabolism of cellulose occurred in distinct phases.

3.5 Aqueous Analytes

Samples from the inundated experiments reserved at 885, 1228, and 3561 days incubation for aqueous chemical analysis were analyzed for organic acids by high-performance liquid chromatography (HPLC; Shimadzu LC-10ATVP and SCL-10A system controller/SIL-10A autoinjector). A BioRad Aminex HPX-87H ion exclusion column was used to separate the organic acid metabolites; the eluent was 0.008 N H_2SO_4, at 0.7 ml/min. Organic acids were quantified at 210 nm (Francis et al., 1991). The presence of these aqueous metabolites, produced by bacterial metabolism of cellulose, provides insight into the effect of various nutrient treatments on the succession of microbial processes. These metabolites may accumulate and disappear depending upon microbial activity (they accumulate from the fermentation of glucose, and are consumed as electron-donor substrates for iron-reduction, sulfate-reduction, and methanogenesis). The pH was measured with a Mettler MP220 pH meter and DG111SC electrode filled with 3M KCl. Dissolved organic carbon was measured on 0.2 µm filtered aliquots using a Beckman 915B total carbon analyzer and according to the method of Hansel et al., 1993. Total dissolved carbohydrates were quantified in filtered aliquots using Dreywood's anthrone reagent (Morris, 1948). Iron (Fe(II) and Fe(III)) was determined by spectrophotometry using o-phenanthroline (APHA, 1995). Nitrate was determined using brucine-sulfanilic acid (APHA, 1995). Sulfate was quantified by turbidimetric method by reaction with BaCl2 forming BaSO4 precipitate (absorbance measured at 420 nm)(APHA, 1995).

3.6 Microbiological Characterization

Select samples from the inundated cellulose experiment were analyzed for DNA by the polymerase chain reaction (PCR) to identify the predominant microorganisms and also differences in community to help to explain differences in gas generation rates (Pancost et al., 2001; Petsch et al., 2001; Lehman et al., 2001). Bacterial and archaeal 16S rRNA gene fragments were used. The PCR products were then run through denaturing gradient gel electrophoresis (DGGE) to separate fragments according to their melting properties. Bands on the fragment that were stained with ethidium bromide were excised and re-amplified by PCR. The PCR product was sequenced using an automated sequencer and the sequences were identified using the BLASTN facility of the National Center for Biotechnology Information (NCBI) or the Ribosomal Database Project (RDP). Four sub-samples were taken at 3447 days incubation from anaerobic inundated samples: (i) unamended uninoculated, (ii) unamended inoculated, (iii) amended inoculated, and, (iv) amended and inoculated and excess nitrate. A "QA mix" was prepared containing known halophilic isolates to validate the analysis. This work was done by a commercial company (Microbial Insights, Knoxville, TN) able to perform the DGGE and PLFA analyses. Samples of well-mixed supernatant also were taken after ~2190 days (6 years) incubation and enumerated by direct microscopy using the DNA-specific fluorochrome 1,4-diamidino-2-phenylindole (DAPI) (Kepner and Pratt, 1994).

4.0 RESULTS AND DISCUSSION

4.1 Gas Produced in Inundated Cellulose Treatments

Total gas and CO_2 produced in the initially aerobic and anaerobic inundated samples are presented in Appendix A; Table 1-8. Figure 5 provides photographs of the initially aerobic samples at the end of the experiment and gas production data are summarized in Figures 6 and 7 which present total gas and CO_2, respectively. Figure 8 provides photographs of the initially anaerobic samples at the end of the experiment and Figures 9 and 10 show total gas and CO_2 production, respectively, in these samples. Tables 7 and 8 present the gas generation rates for all treatments in the inundated experiment. The rates are calculated from single point data at each time over four different phases: i) Phase 1, an acclimation phase when microbial populations adjust to the nutrient conditions prior to the onset of metabolic activity, ii) Phase 2, an initially rapid rate of gas production when easily metabolized dissolved organic carbon was available and cellulose is degraded, iii) Phase 3, a cessation phase (the period over which gas generation was sustained but the rate began to diminish, easily metabolized cellulose became less abundant, or a limiting nutrient was exhausted or inhibitory condition developed), and iv) overall gas production rate. These phases are similar in profile to that observed for the microbial degradation of cellulosic materials (e.g., leaf litter (Kuesel and Drake, 1999; Gulis and Suberkropp, 2003)): Over 10 years of data is represented in the rate calculated from 0-3929 days incubation. Using the overall rate considerably smoothes the data between the beginning and end of the experiment. This smoothing can be justified in terms of the 10,000 years of repository performance, but equally, may be no less applicable than the shorter periods. Examining the four rates provides a means to compare gas generation in the various treatments.

Figure 5(a). Photograph of aerobic samples at 3929 days (10.8 years) incubation: unamended uninoculated (U-3) , unamended inoculated (UI-3), amended inoculated (AI-1), and amended inoculated plus excess nitrate (AINO3-2). Degradation of the cellulose is evident in AI-1 and AINO3-2 samples as shown by pulping of the squares of paper.

Figure 5(b). Photograph of aerobic samples with bentonite at 3929 days (10.8 years) incubation: unamended uninoculated (BU-3), unamended inoculated (BUI-3), amended inoculated (BAI-1), and amended inoculated plus excess nitrate (BAINO3-1). Blackening in BUI-3 and BAINO3-1 is due to iron sulfide formation. The brown paper towel in BU-3 is clearly evident while BAI-1 shows loss of color.

4.1.1 Aerobic Treatments

Figure 5(a) shows the aerobic samples without bentonite at 3929 days (10.8 years) incubation. Cellulose biodegradation is noticeable by the squares of paper turning to pulp in the samples containing nutrients (AI-1 and AINO3-1). Figure 5(b) shows the aerobic samples containing bentonite at the end of the experiment. This photograph shows that blackening of the paper occurred in the unamended inoculated and amended inoculated plus excess nitrate samples. The blackening is due to iron sulfide formed by the reaction of sulfide produced due to sulfate reduction with iron oxide in the bentonite. Cellulose biodegradation is also evident in the photograph; of note is the loss of color from the brown paper in all of the samples except the unamended uninoculated (as seen in BU-3).

4.1.1.1 TOTAL GAS PRODUCTION IN SAMPLES WITHOUT BENTONITE

Aerobic samples incubated with air in the headspace showed rapid consumption of oxygen (0.5% v/v O_2 detected at 853 days incubation in samples with excess nitrate). Over the course of the experiment, unamended uninoculated samples produced a maximum of 0.74 ± 0.45 ml of gas g^{-1} cellulose at 733 days incubation; however, there was only 0.11 ml of gas g^{-1} cellulose left at 3929 days incubation (Table 1(c), Appendix A, Figure 6). The unamended uninoculated treatment produced gas at a rate of 3.6 x 10^{-3} ml gas g^{-1} cellulose day^{-1} during the second phase and at a rate of 1.5 x 10^{-5} ml gas g^{-1} cellulose averaged over the entire incubation period (3929 days (10.8 years))(Table 7). Not unexpectedly, this was the lowest gas production rate of all of the initially aerobic treatments because this treatment contained the lowest population of bacteria (G-Seep contains 1.24 ± 0.13 x 10^5 bacterial cells ml^{-1} (Francis et al., 1998)) and nutrients were not added. Unamended inoculated samples produced 0.84 ± 0.10 ml gas g^{-1} cellulose over 3929 days incubation; this is the maximum produced in this treatment at a rate of 4.1 x 10^{-3} ml gas g^{-1} cellulose day^{-1} over 252 days after an initial acclimation phase for 481 days, and 2.3 x 10^{-4} ml gas g^{-1} cellulose day^{-1} over the entire incubation period (Figure 6). The extended acclimation phase was probably due to adjustment of the inoculum to the

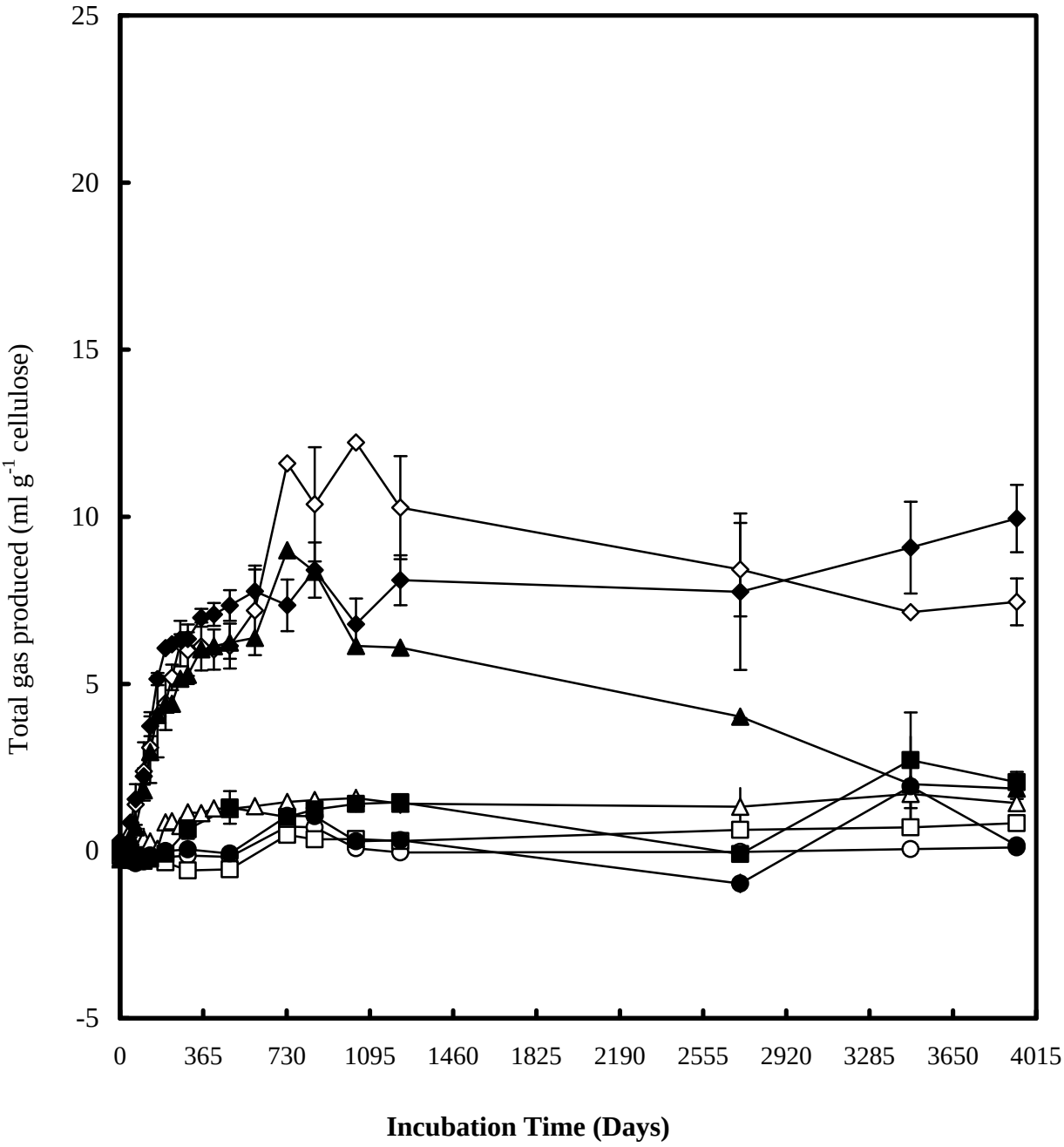

Figure 6. Total gas produced in initially aerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

low-nutrient conditions. The mixed inoculum contained $3.89 \pm 0.08 \times 10^6$ bacterial cells ml^{-1} and 4 ml of this was added to each inoculated sample. Adding the mixed inoculum had the effect in inoculated samples of doubling the bacterial population relative to uninoculated samples (the population increased from 1.24×10^5 bacterial cells ml^{-1} (in G-Seep) to 2.7×10^5 cells ml^{-1} afterwards). The maximum volume of gas produced in amended inoculated samples was at 3464 days incubation (1.71 ± 1.03 ml gas g^{-1} cellulose (Table 1(c), Appendix A)). The highest rate of gas production was 3.4×10^{-3} ml gas g^{-1} cellulose day^{-1}); the absence of an acclimation phase indicated that the mixed inoculum was immediately able to take advantage of the added nutrients for metabolism and growth (Figure 6). Finally, the amended inoculated samples containing excess nitrate produced up to 12.2 ± 0.0 ml gas g^{-1} cellulose at 1034 days incubation. This treatment had the highest rate of gas generation throughout (1.6×10^{-2} ml gas g^{-1} cellulose day^{-1} during phase 2, and 1.9×10^{-3} ml gas g^{-1} cellulose day^{-1} overall). Gas production was not sustained over the long-term; there was a gradual diminishment after 1034 days incubation (Figure 6) and the rate during phase 3 was -1.3×10^{-3} ml gas g^{-1} cellulose day^{-1} (Table 7). This trend correlated with CO_2 production.

4.1.1.2 TOTAL GAS PRODUCTION IN SAMPLES WITH BENTONITE

Aerobic samples with added bentonite had consistently higher rates of total gas production through the second phase, or period of maximum gas production (Figure 6). Unamended uninoculated samples produced gas at an overall rate of 5.6×10^{-5} ml gas g^{-1} cellulose day^{-1} with a maximum of 1.94 ± 0.21 ml gas g^{-1} cellulose produced at 3464 days (Table 2(c), Appendix A). Unamended inoculated samples produced gas at an overall rate of 5.2×10^{-4} ml gas g^{-1} cellulose day^{-1} with a maximum of 2.72 ± 1.43 ml gas g^{-1} cellulose at 3464 days. Amended inoculated samples produced gas at an overall rate of 5.2×10^{-4} ml gas g^{-1} cellulose day^{-1} with a maximum of 8.96 ml gas g^{-1} cellulose at 733 days. Samples containing excess nitrate produced gas at an overall rate of 1.9×10^{-3} ml gas g^{-1} cellulose day^{-1}. The yield of gas in this treatment was 9.95 ± 1.01 ml g^{-1} cellulose at 3929 days incubation. The predominant effect of bentonite on the samples with excess

nitrate treatment was to sustain gas production; these samples did not show a decrease in total gas volume at the end of the experiment as seen in all the other aerobic treatments (Figure 6 and Table 2(c), Appendix A).

4.1.1.3 CARBON DIOXIDE PRODUCTION IN INITIALLY AEROBIC SAMPLES WITHOUT BENTONITE

Unamended uninoculated samples produced 5.19 ± 0.18 μmol CO_2 g^{-1} cellulose after 3929 days incubation (Table 5(c), Appendix A) at an overall rate of 6.9 x 10^{-4} μmol CO_2 g^{-1} cellulose day^{-1} (Table 8). There wasn't any acclimation phase, however while the rate during phase 2 (3.2 x 10^{-2} μmol CO_2 g^{-1} cellulose day^{-1}) was quite a lot higher than the overall rate, it was very short lived (45 days) indicating that the consumption of O_2 could not be sustained by the microbial population without the addition of nutrients (Figure 7). Unamended inoculated samples produced 16.3 ± 1.3 μmol CO_2 g^{-1} cellulose after 3929 days incubation at an overall rate of 3.7 x 10^{-3} μmol CO_2 g^{-1} cellulose day^{-1}. Here the rate during phase 2 was the same as the unamended uninoculated samples (3.2 x 10^{-2} μmol CO_2 g^{-1} cellulose day^{-1}) however it lasted for 200 days and a net positive CO_2 production sustained at 2.2 x 10^{-3} μmol CO_2 g^{-1} cellulose day^{-1} for 3729 days during the cessation phase. The overall rate was only slightly higher (4.5 x 10^{-3} μmol CO_2 g^{-1} cellulose day^{-1}) for amended inoculated samples and 41.4 ± 7.8 μmoles CO_2 g^{-1} cellulose was produced after 297 days incubation and 17.7 ± 1.8 μmoles CO_2 g^{-1} cellulose at the end (3929 days). There wasn't any net positive CO_2 production during the cessation phase, however. The overall rate of CO_2 production in amended inoculated samples containing excess nitrate was almost 10x higher than amended inoculated samples, and 186 ± 8 μmol CO_2 g^{-1} cellulose was produced at 1034 days incubation (Figure 7). These samples contained 162 ± 39 μmol CO_2 g^{-1} cellulose at 3929 days, approximately 10x more CO_2 than unamended inoculated or amended inoculated samples at the end of the experiment. The excess nitrate amendment supported a consistently high rate of CO_2 production throughout the experiment (4.1 x 10^{-2} μmoles g^{-1} cellulose day^{-1} overall (Table 8 and Figure 7).

4.1.2.2. CARBON DIOXIDE PRODUCTION IN INITIALLY AEROBIC SAMPLES WITH BENTONITE.

The addition of bentonite significantly increased the rate and total amount of CO_2 produced in aerobic samples over the course of the experiment (Figure 7). The maximum amount of CO_2 produced in unamended uninoculated samples was 11.7 ± 0.8 µmol CO_2 g^{-1} cellulose at 2718 days (Table 6(c), Appendix A); this is double that produced in the absence of bentonite (5.19 ± 0.18 µmol CO_2 g^{-1} cellulose; Table 5(c), Appendix A) and the greatest amount of CO_2 produced by any of the uninoculated treatments (aerobic or anaerobic). The rate of CO_2 production in unamended uninoculated samples peaked at 5.6 x 10^{-3} µmol CO_2 g^{-1} cellulose day^{-1} and this rate was maintained for 1490 days. The rate of CO_2 production in unamended inoculated samples peaked at 1.0 x 10^{-1} µmol CO_2 g^{-1} cellulose day^{-1}, with a maximum of 77.9 ± 13.1 µmol CO_2. The maximum amount and overall rate of CO_2 production by amended inoculated samples was 175 ± 10 µmol CO_2 g^{-1} cellulose and 2.4 x 10^{-1} µmol g^{-1} cellulose day^{-1}, respectively; this is twice the values for unamended inoculated samples. The highest rate of CO_2 production in any treatment, aerobic or anaerobic, was seen in aerobic amended inoculated samples containing excess nitrate plus bentonite: 5.3 x 10^{-1} µmol CO_2 g^{-1} cellulose day^{-1} during phase 2, although this was maintained for only 200 days (shortest phase 2 of all of the treatments)(Figure 7). This treatment also induced the third highest overall rate, 5.4 x 10^{-2} µmol CO_2 g^{-1} cellulose day^{-1}, with a maximum of 233 µmol CO_2 g^{-1} cellulose at 2718 days incubation.

4.1.3. SUMMARY OF AEROBIC TREATMENTS

Tables 7 and 8 show that the trend in the rate of gas production increased according to treatment in aerobic samples with and without bentonite. Unamended uninoculated samples showed the lowest rate of total gas and CO_2 production and excess nitrate amended samples showing the highest rate. After the phase in gas production that had the highest rate (phase 2) the cessation phase is most likely the period when the more recalcitrant carbon was beginning to be metabolized. Cellulose hydrolysis probably started during phase 2 and continued into phase 3; the result was a significant drop in the

highest rates when the most easily metabolized dissolved organic carbon leached from the cellulose (see Tables 7 and 8). At the beginning of the cessation phase, the amorphous cellulose was probably being depleted, priming metabolism of more crystalline cellulose during the remainder of the stationary phase when the rates dropped considerably. Bentonite had a significant effect on total gas and CO_2 production: the maximum overall rate of total gas production by samples without bentonite was seen in inoculated plus excess nitrate treatment and was 1.6×10^{-2} ml gas g^{-1} cellulose day^{-1}, while with bentonite the rate was 2.2×10^{-2} ml gas g^{-1} cellulose day^{-1}. The same held for CO_2 production in these samples: 4.7×10^{-1} μmol CO_2 g^{-1} cellulose day^{-1} was produced without bentonite and 5.3×10^{-1} μmol CO_2 g^{-1} cellulose day^{-1} with bentonite. Even in the absence of a nutrient amendment, bentonite stimulated gas production: without bentonite, unamended inoculated samples produced 3.2×10^{-2} μmol CO_2 g^{-1} cellulose day^{-1} during phase 2, while with bentonite 1.0×10^{-1} μmol CO_2 g^{-1} cellulose day^{-1} was produced (3 x faster).

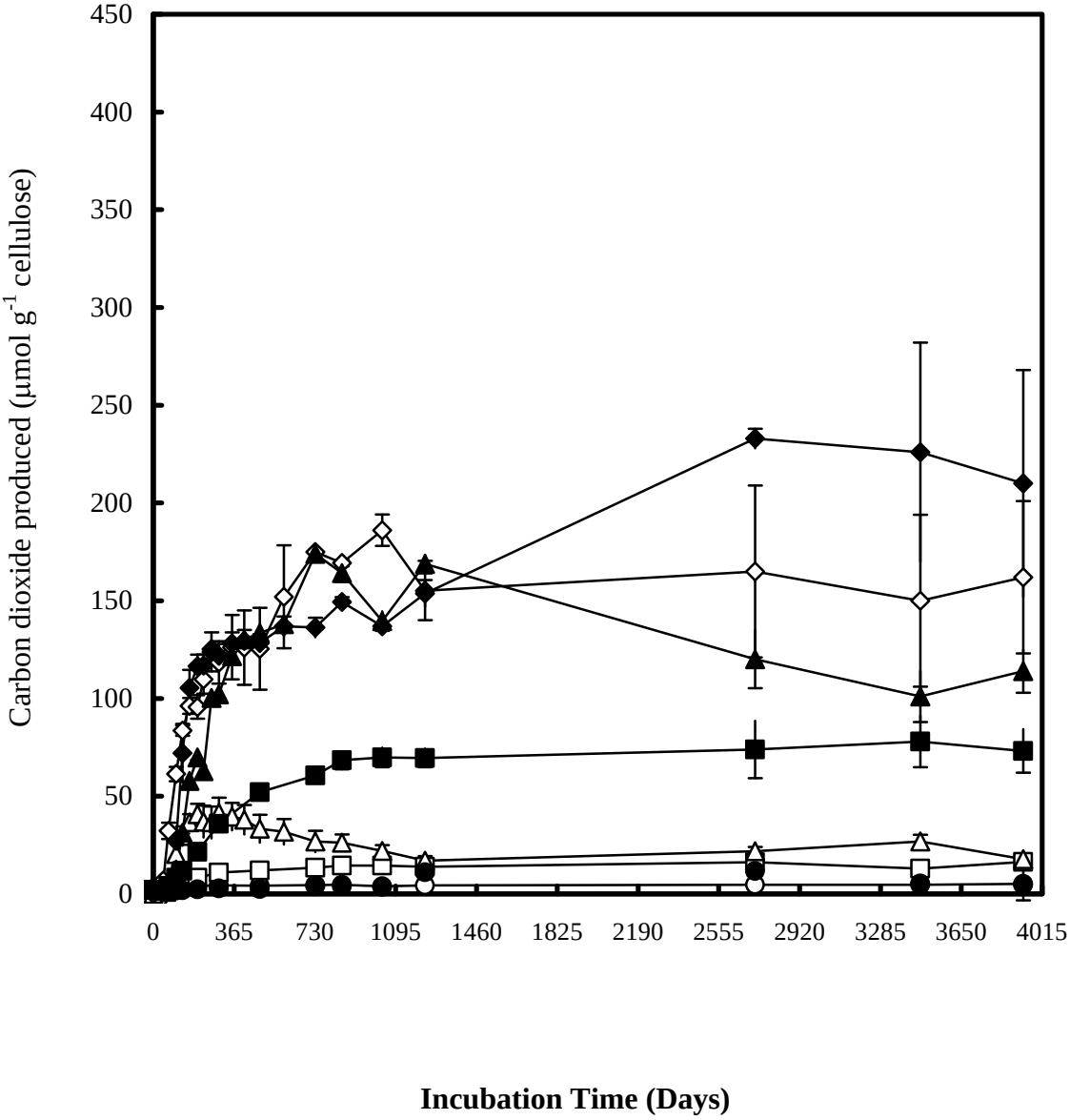

Figure 7. Carbon dioxide produced in initially aerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

4.1.4. Anaerobic Treatments

Figure 8(a) shows the anaerobic samples at 3929 days (10.8 years) incubation. Discoloration of the paper is clearly evident in this photograph, as is the disintegration of the paper in nutrient amended samples. Figure 8(b) shows the anaerobic samples containing bentonite at 10.8 years incubation. Blackening, due to iron sulfide formation, is evident in BUI-2 and BAINO3-3 samples.

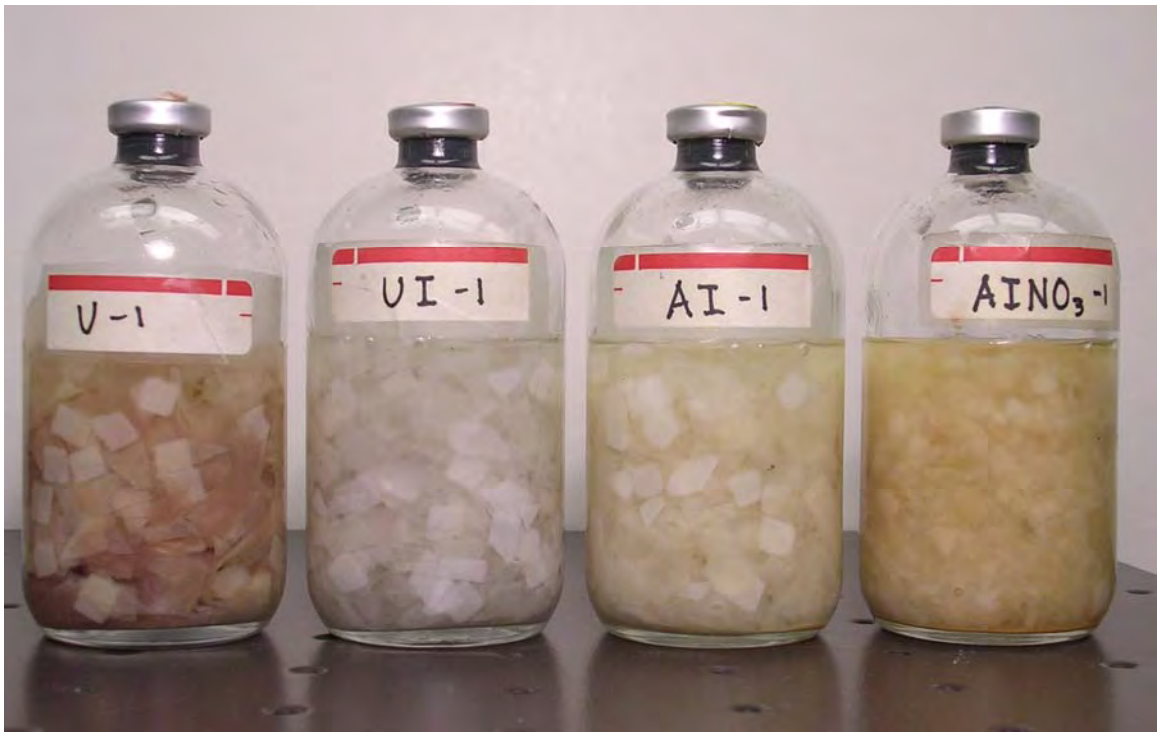

Figure 8(a). Photograph of anaerobic samples at 3929 days (10.8 years) incubation: unamended uninoculated (U-1), unamended inoculated (UI-1), amended inoculated (AI-1) and amended, inoculated plus excess nitrate (AINO3-1). Discoloration and pulping of the paper due to cellulose biodegradation is evident in UI-1, AI-1 and AINO3-1.

Figure 8(b). Photograph of anaerobic samples with bentonite at 3929 days (10.8 years) incubation: unamended uninoculated (BU-1), unamended inoculated (BUI-2), amended inoculated (BAI-3), and amended inoculated plus excess nitrate (BAINO3-3). Blackening in BUI-2 and BAINO3-3 is due to iron sulfide. Discoloration of brown paper is evident in BU-1.

4.1.4.1 TOTAL GAS PRODUCTION IN ANAEROBIC SAMPLES WITHOUT BENTONITE.

Anaerobic unamended uninoculated samples without bentonite produced the least amount of total gas of all of the experimental treatments, aerobic or anaerobic (-0.32 ml gas g^{-1} cellulose at 3929 days incubation (Figure 9; Table 3(c), Appendix A), and overall rate of -8.9 x 10^{-5} ml gas g^{-1} cellulose day^{-1} (Table 7). This result indicates that the microorganisms in G-Seep were unable to metabolize cellulose to any significant degree in the absence oxygen or nutrients. The microorganisms in the mixed inoculum, however, could metabolize organic carbon in the samples, and possibly even degrade some of the cellulose, as evidenced by the production of 2.60 ± 0.46 ml of gas at 3929 days incubation, and an overall rate of 5.7 x 10^{-4} ml gas g^{-1} cellulose day^{-1} in unamended inoculated samples. The nutrient amendment shortened the acclimation phase and raised gas production to 4.32 ± 0.34 ml of gas g^{-1} cellulose at 733 days incubation in amended inoculated samples; the highest rate was 8.3 x 10^{-3} ml gas g^{-1} cellulose day^{-1} 5.2 x 10^{-4} ml gas g^{-1} cellulose day^{-1} overall (Figure 9). The highest rate of total gas production of all treatments was in the samples with excess nitrate: 2.9 x 10^{-2} ml gas g^{-1} cellulose day^{-1} (Table 7). This rate was sustained for only 481 days; a total of ~15 ml of gas was produced g^{-1} of cellulose by 733 days (Figure 9).

4.1.5.1 TOTAL GAS PRODUCTION IN ANAEROBIC SAMPLES WITH BENTONITE.

Bentonite promoted gas production in unamended uninoculated samples, and total gas production peaked at 733 days with 0.762 ± 0.492 ml gas produced g^{-1} cellulose at an overall rate of 8.7 x 10^{-5} ml gas g^{-1} cellulose day^{-1} (Figure 10; Table 8). Unamended inoculated samples produced a maximum of 2.48 ± 0.31 ml gas g^{-1} cellulose at an overall rate of 3.8 x 10^{-4} ml gas g^{-1} cellulose day^{-1}. Amended inoculated samples produced a maximum of 4.12 ± 0.76 ml gas g^{-1} cellulose at 853 days incubation at an overall rate of 7.3 x 10^{-4} ml gas g^{-1} cellulose day^{-1}. Anaerobic amended inoculated samples containing excess nitrate and bentonite produced gas at an overall rate of 2.6 x 10^{-3} ml gas g^{-1} cellulose day^{-1} with a maximum of 18.1 ml gas produced g^{-1} cellulose at 591 days (Figure 9).

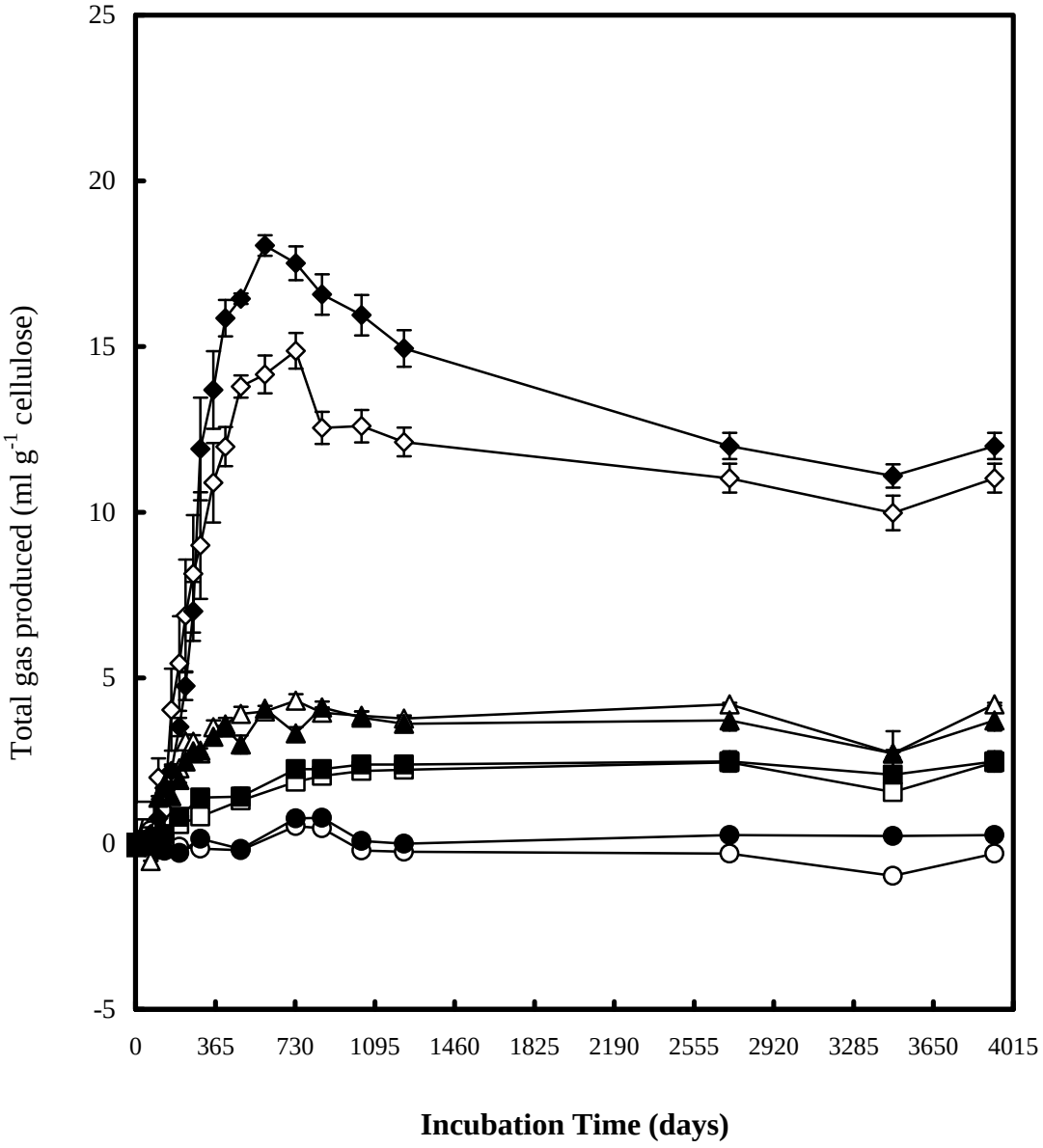

Figure 9. Total gas produced in anaerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

4.1.4.2 CARBON DIOXIDE PRODUCTION IN ANAEROBIC SAMPLES WITHOUT BENTONITE.

The rate of carbon dioxide production was lowest in unamended uninoculated samples, correlating with total gas production (Table 8; Figure 10). Gas production in unamended uninoculated samples peaked at 8.29 ± 3.77 µmol CO_2 g^{-1} cellulose by 2718 days but the overall rate of production was 1.6 x 10^{-4} µmol CO_2 g^{-1} cellulose. In the absence of nutrients, anaerobic (unamended) inoculated samples were able to produce 27.4 ± 5.8 µmol CO_2 g^{-1} cellulose by the end of the experiment (3929 days) at an overall rate of 6.4 x 10^{-3} µmol CO_2 g^{-1} cellulose day^{-1}. Amended inoculated samples produced 66.9 ± 1.1 µmol CO_2 g^{-1} cellulose by 2718 days at an overall rate of 1.4 x 10^{-2} µmol CO_2 g^{-1} cellulose day^{-1}, and samples containing excess nitrate produced 251 ± 5 µmol CO_2 g^{-1} cellulose over the same period at an overall rate of 5.6 x 10^{-2} µmol CO_2 g^{-1} cellulose day^{-1}. The amount of CO_2 produced was correlated well with the size of the microbial populations in these samples (Table 11).

4.1.5.2 CARBON DIOXIDE PRODUCTION IN ANAEROBIC SAMPLES WITH BENTONITE.

Anaerobic unamended uninoculated samples containing bentonite produced the greatest amount of CO_2 of any of the samples of this treatment (aerobic or anaerobic); 10.1 ± 8.0 µmol CO_2 g^{-1} cellulose were produced after 3929 days incubation (Table 8 (c), Appendix A), with CO_2 production fairly steady and sustained over the course of the experiment (5.0 x10^{-3} µmol g^{-1} cellulose day^{-1} during phase 2 maintained for 1684 days, and almost the same overall rate (2.1 x 10^{-3}))(Table 8; Figure 10). Unamended inoculated samples produced 59.0 ± 7.1 µmol CO_2 g^{-1} cellulose at 2718 days incubation, over an extended phase 2 period of 1228 days at 4.5 x 10^{-2} µmol g^{-1} cellulose day^{-1} and at an overall rate of 1.5 x 10^{-2} µmol g^{-1} cellulose day^{-1}. Carbon dioxide production in amended inoculated samples peaked at 99.4 ± 4.4 µmol CO_2 g^{-1} cellulose at 1228 days, at 2.2 x 10^{-2} µmol CO_2 g^{-1} cellulose day^{-1} overall. Finally, excess nitrate amended samples had the highest rate of CO_2 production of any of the samples in the experiment (5.3 x 10^{-1} µmol CO_2 g^{-1} cellulose day^{-1})(Figure 10). The maximum amount of CO_2 produced was at 733

days (397 ± 12 µmol CO_2 g^{-1} cellulose) with 266 µmol CO_2 g^{-1} cellulose detected at 3929 days at a rate of 6.8 x 10^{-2} µmol CO_2 g^{-1} cellulose day^{-1} overall.

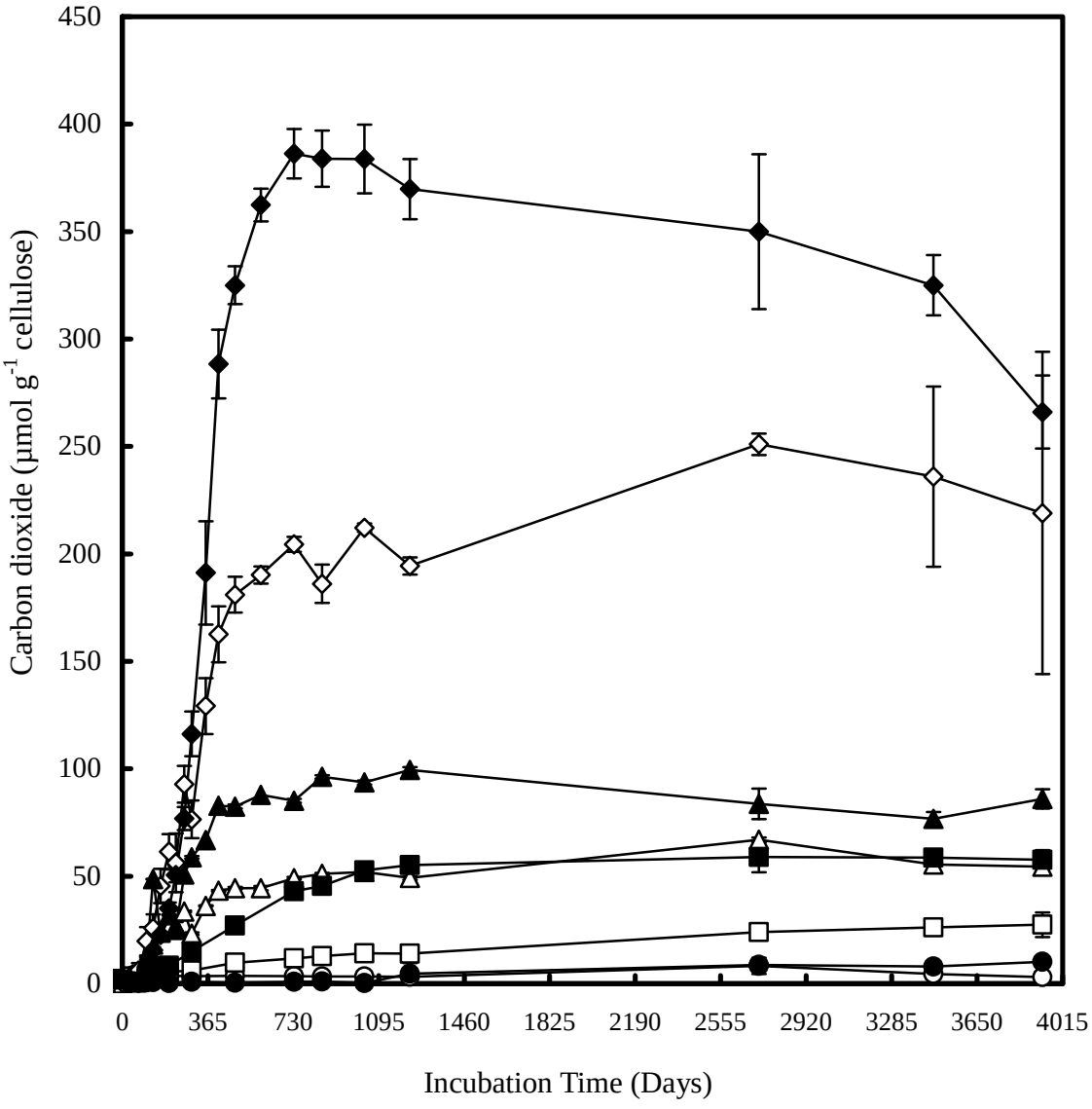

Figure 10. Carbon dioxide produced in anaerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

4.1.6. Summary of Anaerobic Samples

Similar to aerobic samples, anaerobic samples with and without bentonite showed an increasing trend in the rate and total amount of gas and CO_2 produced depending upon the addition of inoculum or nutrients (Tables 7 and 8). There was a very slight stimulatory effect of bentonite on unamended inoculated samples during the period of the highest rate of gas production: 2.9×10^{-3} ml gas g^{-1} cellulose day^{-1} vs. 3.1×10^{-3} ml gas g^{-1} cellulose day^{-1} in the absence of bentonite. Overall there wasn't much effect because of the decreased gas volume at the end of the experiment in unamended inoculated samples (gas production peaked at 2.48 ± 0.31 ml gas g^{-1} cellulose at 2718 days but then dropped to 1.54 ± 0.41 ml gas g^{-1} cellulose at 3929 days). Whereas under aerobic conditions bentonite served to increase gas production rates and yields initially, under anaerobic conditions the opposite was true. This may be due to bentonite serving as a pH buffer and source of trace elements under initially aerobic conditions resulting in an increased rate of gas production, while under anaerobic conditions the ferric iron in the bentonite was utilized thus initially lowering the rate of CO_2 production due to a diversion of electrons to ferrous iron. This is also supported by the fact that nitrate didn't have much of a stimulatory effect on total gas production. The highest rate of gas production in anaerobic amended inoculated samples containing excess nitrate and bentonite was 3.1×10^{-2} ml gas g^{-1} cellulose day^{-1} while it was 2.9×10^{-2} ml gas g^{-1} cellulose day^{-1} for the same treatment without bentonite (Table 7). Over the long term, and with respect to total gas volume yields, bentonite had a stimulatory effect probably owing to the presence of ferric iron as an electron acceptor and ferrous iron as a potential trace element nutrient. The total gas volume peaked at 18.1 ± 0.38 ml gas g^{-1} cellulose at 591 days in samples containing excess nitrate and this dropped to 10.2 ± 0.3 ml gas g^{-1} cellulose at 3929 days incubation (Table 4(c), Appendix A).

Table 7. Rate of total gas production by inundated samples

Treatment	Rate of Total Gas Production			
	Phase 1	Phase 2	Phase 3	Overall
	ml g^{-1} cellulose day^{-1}			
Aerobic				
Unamended/Uninoculated	-4.57×10^{-4} (481)*	3.61×10^{-3} (252)	-1.97×10^{-4} (3196)	1.53×10^{-5}
Unamended/Inoculated	-9.56×10^{-4} (481)	4.05×10^{-3} (252)	1.13×10^{-4} (3196)	2.34×10^{-4}
Amended/Inoculated	na	3.41×10^{-3} (411)	4.55×10^{-5} (3518)	3.97×10^{-4}
Inoculated + Excess Nitrate	na	1.58×10^{-2} (733)	-1.30×10^{-3} (3196)	1.90×10^{-3}
Anaerobic				
Unamended/Uninoculated	-4.78×10^{-4} (481)	2.90×10^{-3} (252)	-2.66×10^{-4} (3196)	-8.91×10^{-5}
Unamended/Inoculated	4.55×10^{-4} (132)	2.41×10^{-3} (902)	1.42×10^{-4} (2895)	6.72×10^{-4}
Amended/Inoculated	na	8.30×10^{-3} (481)	-3.97×10^{-4} (3448)	6.67×10^{-4}
Inoculated + Excess Nitrate	na	2.87×10^{-2} (481)	-1.11×10^{-3} (3448)	2.54×10^{-3}
Aerobic+Bentonite				
Unamended/Uninoculated	-6.24×10^{-5} (481)	4.52×10^{-3} (252)	-2.78×10^{-4} (3196)	5.60×10^{-5}
Unamended/Inoculated	-5.50×10^{-4} (200)	4.91×10^{-3} (281)	2.20×10^{-4} (3448)	5.17×10^{-4}
Amended/Inoculated	na	1.30×10^{-2} (733)	-2.22×10^{-3} (3196)	5.24×10^{-4}
Inoculated + Excess Nitrate	na	2.21×10^{-2} (200)	8.15×10^{-4} (3729)	1.90×10^{-3}
Anaerobic+Bentonite				
Unamended/Uninoculated	-1.66×10^{-4} (481)	3.66×10^{-3} (252)	-1.57×10^{-4} (3196)	8.65×10^{-5}
Unamended/Inoculated	na	3.07×10^{-3} (733)	-2.22×10^{-4} (3196)	3.84×10^{-4}
Amended/Inoculated	na	8.78×10^{-3} (411)	-2.13×10^{-4} (3518)	7.28×10^{-4}
Inoculated + Excess Nitrate	na	3.07×10^{-2} (591)	-2.37×10^{-3} (3338)	2.61×10^{-3}

*Values in parentheses are the number of days over which the rate is calculated at each phase.
na = not applicable

Table 8. Rate of carbon dioxide production by inundated samples

Treatment	Rate of Carbon Dioxide Production			
	Phase 1	Phase 2	Phase 3	Overall
	---------- μmoles g^{-1} cellulose day^{-1} ----------			
Aerobic				
Unamended/Uninoculated	na	3.20×10^{-2} (45)*	3.27×10^{-4} (3884)	6.90×10^{-4}
Unamended/Inoculated	na	3.17×10^{-2} (200)	2.23×10^{-3} (3729)	3.65×10^{-3}
Amended/Inoculated	na	2.04×10^{-1} (200)	-6.19×10^{-3} (3729)	4.51×10^{-3}
Inoculated + Excess Nitrate	na	4.70×10^{-1} (264)	1.03×10^{-2} (3665)	4.12×10^{-2}
Anaerobic				
Unamended/Uninoculated	6.11×10^{-4} (1228)	3.46×10^{-3} (1490)	4.26×10^{-3} (1211)	1.58×10^{-4}
Unamended/Inoculated	na	1.57×10^{-2} (481)	5.14×10^{-3} (3448)	6.44×10^{-3}
Amended/Inoculated	na	1.05×10^{-1} (411)	3.18×10^{-3} (3518)	1.39×10^{-2}
Inoculated + Excess Nitrate	na	3.76×10^{-1} (481)	1.10×10^{-2} (3448)	5.57×10^{-2}
Aerobic+Bentonite				
Unamended/Uninoculated	1.45×10^{-3} (1228)	5.64×10^{-3} (1490)	-5.82×10^{-3} (1211)	7.97×10^{-4}
Unamended/Inoculated	na	1.04×10^{-1} (481)	6.15 x 10-3 (3448)	1.18×10^{-2}
Amended/Inoculated	na	2.39×10^{-1} (733)	-1.91×10^{-2} (3196)	2.91×10^{-2}
Inoculated + Excess Nitrate	na	5.25×10^{-1} (200)	2.82×10^{-2} (3729)	5.35×10^{-2}
Anaerobic+Bentonite				
Unamended/Uninoculated	-1.70×10^{-3} (1034)	5.01×10^{-3} (1684)	1.14×10^{-3} (1211)	2.05×10^{-3}
Unamended/Inoculated	na	4.49×10^{-2} (1228)	9.40×10^{-4} (2701)	1.47×10^{-2}
Amended/Inoculated	na	2.02×10^{-1} (411)	9.10×10^{-4} (3518)	2.19×10^{-2}
Inoculated + Excess Nitrate	na	5.28×10^{-1} (733)	-3.79×10^{-2} (3196)	6.77×10^{-2}

*Values in parentheses are the number of days over which the rate is calculated at each phase.
na = not applicable

4.1.7. Other Gases (Oxygen, Hydrogen, Nitrous Oxide, Nitrogen)

At 853 days incubation (2.3 years) the gases oxygen, hydrogen, nitrous oxide, and nitrogen were quantified in all samples, along with total gas and CO_2. This data is presented in Appendix E.

4.2 Aqueous Metabolite Analysis

The results of HPLC of initially aerobic and anaerobic inundated samples are given in Figures 11-14 and Appendix B, Tables 1-4. Concentrations of acids are reported as mM and are scaled from acid concentrations detected in 100 ml of brine containing 5 g of mixed cellulosics. These values can be converted organic acid produced per gram cellulose by dividing the concentrations by 5.

4.2.1 Aerobic Treatments

Organic acids produced included acetic, butyric, formic, fumaric, lactic, oxalic, oxalacetic, propionic, and succinic acids (Figure 11 and 12). The propionic, succinic, formic, and lactic acids produced at 885 and 1228 days were metabolized by 3561 days (Figures 7 and 8). Formic acid was consumed in nutrient-amended samples, dropping from 2.52 and 3.41 mM at 1228 days to undetectable at 3561 days in amended inoculated samples and samples with excess nitrate, respectively (Table 1, Appendix B). Metabolism of formic acid was not complete and it was still detected in unamended samples at 3561 days. Acetic acids accumulated to a significant extent in amended inoculated samples and samples with excess nitrate, with an increase from 4.45 to 6.91 mM in the former and 4.43 to 11.0 mM in the latter (Table 1, Appendix B). Linear regression of the entire excess nitrate data set shows that acetate is produced at a rate of 3.1 $\mu M\ day^{-1}$; this rate was sustained in amended inoculated samples only until 1228 days and then leveled off.

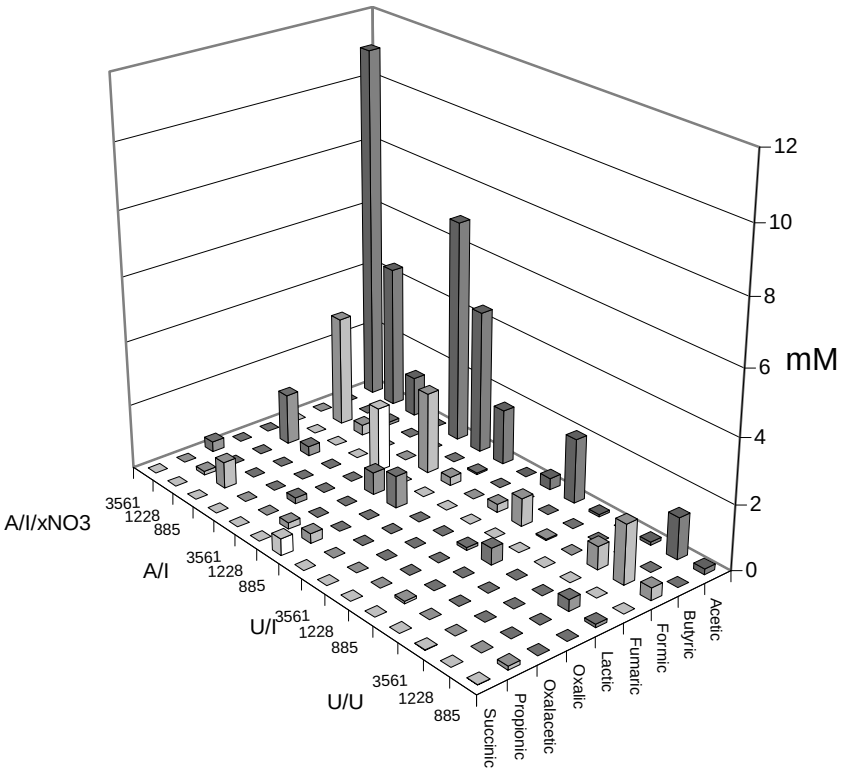

Figure 11. Organic acids produced at 885, 1228, and 3561 days incubation in **aerobic treatments without bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

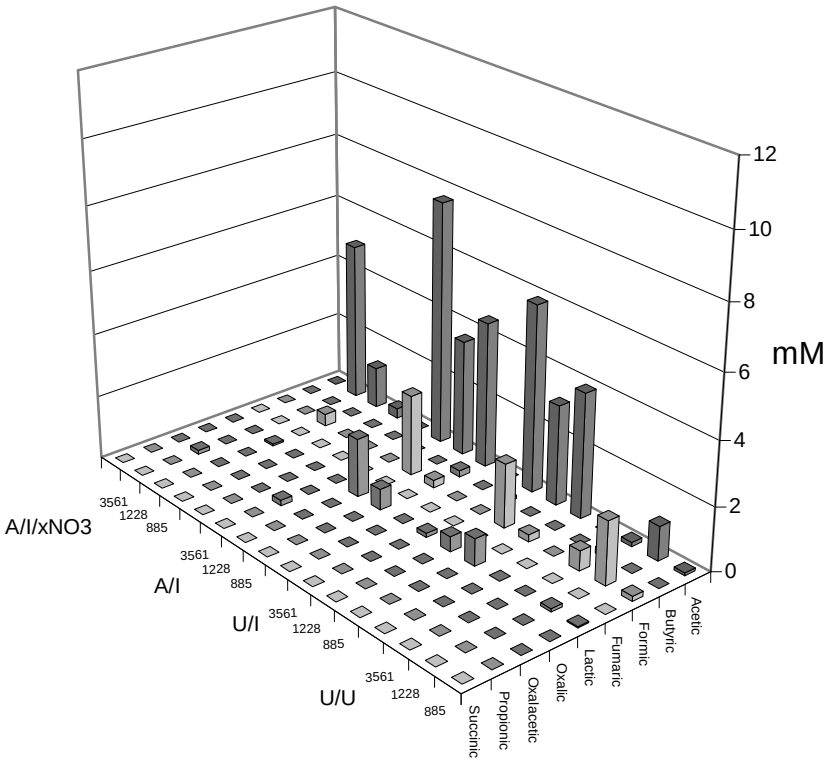

Figure 12. Organic acids produced at 885, 1228, and 3561 days incubation in **aerobic treatments with bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

The predominant effect of bentonite on aerobic samples was to stimulate the production of acetate in unamended inoculated samples and decrease the rate and extent of accumulation in samples with excess nitrate (Figure 12 and Table 2, Appendix B). The organic-acid content of initially aerobic samples was generally lower than that of anaerobic samples,

4.2.2. Anaerobic Treatments.

Organic-acid production in anaerobic samples followed similar trends as in aerobic samples; however lactic, fumaric, formic, and butyric acids accumulated and were present at 3561 days (Figure 13 and 14). Acetate production in unamended inoculated samples was steady over 3561 days at a rate of 1.7 μM day^{-1}, this is significantly greater relative to aerobic samples (1.6 μM day^{-1} up to 1228 days, 0.15 μM day^{-1} thereafter) although similar to aerobic samples with bentonite (1.7 μM day^{-1}). Therefore, both anaerobic conditions and bentonite stimulated acetate production by the mixed inoculum in the absence of added nutrients. Both acetic (6.99 and 5.21 mM) and butyric acid (6.38 and 5.49 mM) were detected at 3561 days incubation in amended inoculated samples and samples with excess nitrate, respectively. The accumulation of butyric acid is indicative of established fermentative microbial processes. Bentonite had a profound effect on acetic and butyric acid production in amended inoculated samples: 38.6 and 49.8 mM respectively, were detected at 3561 days (Figure 10 and Table 4, Appendix B). Significant amounts of isobutyric (50mM) and valeric (39mM), and other unidentified metabolites were also detected in amended inoculated samples with bentonite. Bentonite, a source of iron oxyhydroxides, also stimulated the production of minor amounts of oxalic and oxalacetic acids (Table 4, Appendix B) as well as formic and fumaric acids in anaerobic samples.

Overall, the presence of bentonite resulted in a more rapid accumulation of acetic acid in initially aerobic samples but otherwise did not have much of an effect on the identity and final quanitity of organic acids produced. In the case of anaerobic samples,

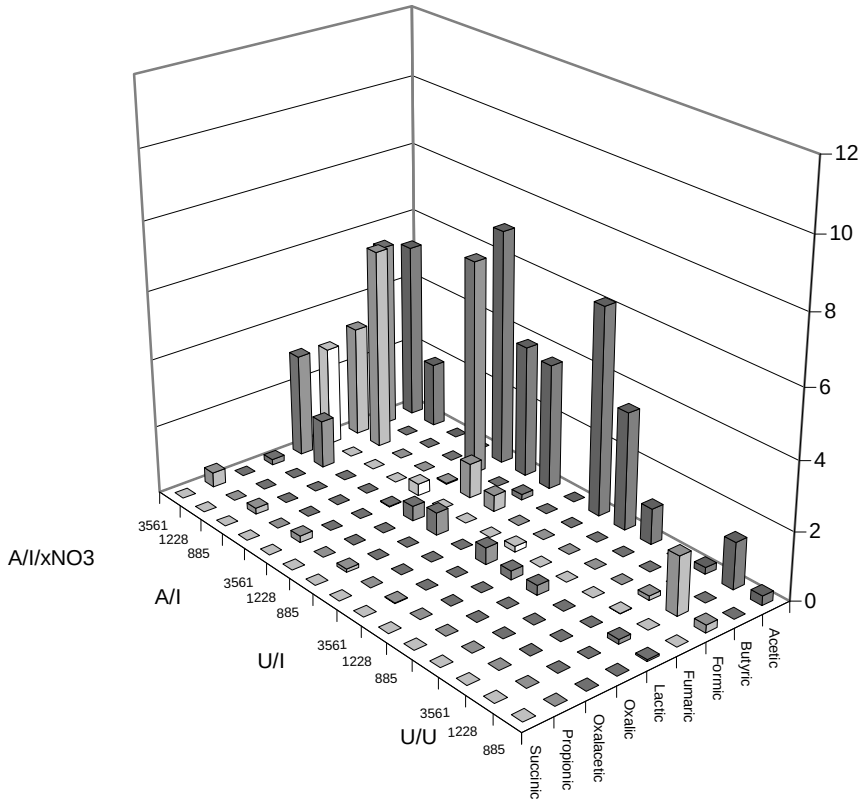

Figure 13. Organic acids produced at 885, 1228, and 3561 days incubation in **anaerobic treatments without bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

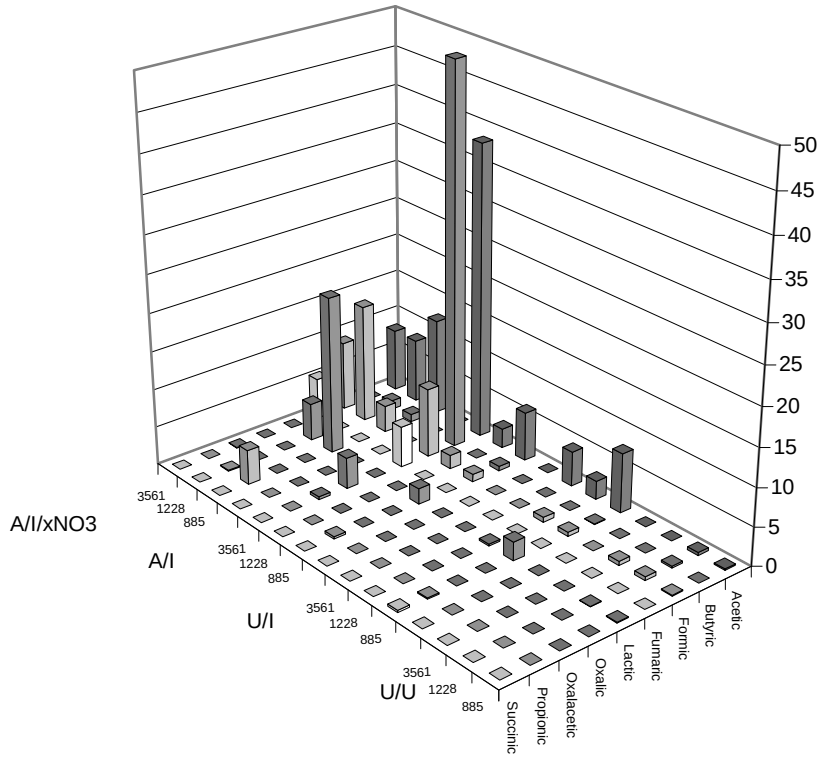

Figure 14. Organic acids produced at 885, 1228, and 3561 days incubation in **anaerobic treatments with bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

however, the presence of bentonite resulted in a significant increase in lactic, acetic, and butyric acids in amended samples.

4.2.3. Dissolved organic carbon, carbohydrates, total organic acid content, ph, nitrate, sulfate, and iron.

At 885 and 1228 days incubation, total dissolved organic carbon, carbohydrates, pH, nitrate, sulfate, and iron concentration was determined in all of the samples. This data is presented in Appendix F, Tables 1-6. In addition, the total organic acid content is provided at 885, 1228, and 3561 days incubation.

4.3. Methane Production in Inundated Samples.

Tables 9-10 provide data from the methane analysis of inundated cellulose samples up to 3462 days incubation. Methane was analyzed by gas chromatography using flame- ionization detection. The minimum detectable quantity was 0.2 nmol CH_4 g^{-1} cellulose dry wt. After 2178 days (7.4 years) incubation methane was detected in initially aerobic unamended uninoculated and unamended inoculated samples without bentonite (Table 9). At 3462 days (9.5 years) all of the initially aerobic samples showed methane production, up to 2.16 nmol g^{-1} cellulose in the unamended inoculated treatment with bentonite. In contrast to initially aerobic samples, methane was first detected in small quantities in almost all anaerobic samples except those with excess nitrate (Table 10, 2718 days (7.4 years)). At 3462 days (9.5 years), methane was still found in greatest quantity in samples that were not amended with any nitrogen (NH_4NO_3, KNO_3); specifically in the unamended/inoculated samples. However, methane was detected in samples that initially contained excess nitrate (2.57 ± 0.79 nmol CH_4 g^{-1} cellulose (w/o bentonite) and 2.81 ± 0.16 nmol CH_4 g^{-1} cellulose (w/ bentonite)). Although there was a long lag time, these samples eventually produced methane at a relatively rapid rate: 2.5 pmol CH_4 g^{-1} cellulose d^{-1} in unamended and amended inoculated samples, and 3.5 pmol

CH_4 g^{-1} cellulose d^{-1} in samples containing excess nitrate (over 744 days between time 2718 and 3462). Overall, the slow rate of CH_4 accumulation, relative to CO_2, may reflect the extreme difficulty halophilic methanogens have in metabolizing the available substrates, such as acetate, CO_2, and H_2 under hypersaline conditions due to bioenergetic constraints (Oren, 1999). Their preferred substrate is a methylated amine, such as trimethylamine, commonly found in saline surface waters. Methane was detected in initially aerobic samples at 3462 days, with those samples that were not amended producing the largest initial quantities. Production rates ranged from 0.7 to 1.7 CH_4 pmol g^{-1} cellulose d^{-1}.

Table 9. Methane analysis of initially aerobic inundated cellulose samples.

Sample	Incubation Time (d) 1228	2718	3462
	---------- (nmol g^{-1} cellulose) ----------		
Initially Aerobic			
Unamended	nd	1.25 ± 0.29	1.82 ± 0.05
Unamended/Inoculated	nd	1.10 ± 0.13	1.34 ± 0.03
Amended/Inoculated	nd	nd	0.84 ± 0.05
Amended/Inoc. + Exc. Nitrate	nd	nd	1.27 ± 0.37
Initially Aerobic + Bentonite			
Unamended	nd	nd	1.59 ± 0.47
Unamended/Inoculated	nd	nd	2.16 ± 0.07
Amended/Inoculated	nd	nd	0.64 ± 0.06
Amended/Inoc. + Exc. Nitrate	nd	nd	1.45 ± 0.26

nd = not detected

Table 10. Methane produced in anaerobic 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	4.51 ± 0.06
Unamended/Inoculated	nd	3.52 ± 0.20	4.06 ± 0.15
Amended/Inoculated	nd	1.12 ± 0.03	3.41 ± 0.13
Amended/Inoc. + Exc. Nitrate	nd	nd	2.81 ± 0.16

nd = not detected

4.4. Microbiological Characterization

The microbial community was identified in one of the triplicate reserve samples (not used for periodic gas analysis) from the following anaerobic inundated cellulose treatments: i) unamended, uninoculated, ii) unamended, inoculated, iii) nutrient amended and inoculated, and iv) nutrient amended, inoculated, plus excess nitrate. A fifth sample, consisting of three "known" halophiles (*Halobacterium salinarium*, *Haloanaerobium praevalens*, and *Halomonas* sp.) was analyzed to verify and validate the method. A culture-independent method was used to quantify and identify microorganisms, specifically, denaturing gradient gel electrophoresis (DGGE) analysis (Muyzer et al., 1993). Figure 15 presents the findings. Each lettered band in the figure corresponds to a unique bacterial species; the greater the number of bands, the more species in the samples. Higher diversity, as determined by a greater number of microbial species, was correlated with nutrient amendment and concomitant gas production. Table 11 gives data from enumerating the bacteria in the treatments after 6 years incubation (from Francis et al., 1998). One gram-positive spore-forming, fermentative microorganism (genus *Clostridium*, band A, Figure 15) was detected in the anaerobic unamended uninoculated treatment; this is of interest because almost all halophiles are gram-negative. This treatment is characterized by a low starting biomass and a lack of abundant electron acceptors. Introducing a mixed inoculum, but not nutrients, resulted in dominance by one genus, *Halobacter utahensis* (bands B, M, N, and O, Figure 15). In general, abundant nutrient availability lowers microbial diversity, as was found in non-saline, low-carbon environments. Samples from the inundated cellulose experiment are analogous to environments loaded with highly complex carbohydrates. Diverse cellulolytic microbial populations were found in the animal rumen, a very high carbon-loading environment (Cho and Kim, 2000). Besides availability of organic carbon, Roling et al. (2001) showed that microbial community structure in a benzene-impacted groundwater environment was determined by the available electron acceptors. *Halobacterium, Haloarcula, Halobacter, and Natranobacterium* were found in the nutrient-amended, inoculated samples ((Four genera) bands C, D, E, P, Q, R, Figure 15); there was a fairly high diversity and they were unique in containing *Natranobacterium*. This genus consists of species adapted to

life under hypersaline, extremely alkaline conditions (pH 9-10 such as soda lakes). Excess nitrate resulted in the establishment of five genera: *Halobacterium*, *Halobacter*, *Halococcus*, *Natranobacterium*, *Natranomonas* (bands F, G, H, S, T, U, and V, Figure 15)). Unidentified archaea (bands S and V) were also found. Three genera were identified in the known sample, thus verifying the applicability of this technique to halophilic bacteria: bands I, J (*Halomonas* sp.); K, L (*Haloanaerobium praevalens*); and the archaea, *Halobacterium salinarium* (band W). A serious limitation of the technique, however, is the size of the bacterial databases; they generally are less populated with environmentally relevant isolates, especially extremophiles, and, in some instances, a positive identification could not be made (e.g. bands S and V, Figure 15).

Table 11. Number of bacteria in anaerobic inundated cellulose treatments after 6 years incubation (Francis et al., 1998).

Treatment	**Number of bacteria/ml**
Unamended/Uninoculated	$5.12 \pm 3.41 \times 10^5$
Unamended/Inoculated	$1.59 \pm 0.15 \times 10^7$
Amended/Inoculated	$1.62 \pm 0.07 \times 10^8$
Amended/Inoculated + Excess Nitrate	$2.24 \pm 0.24 \times 10^8$

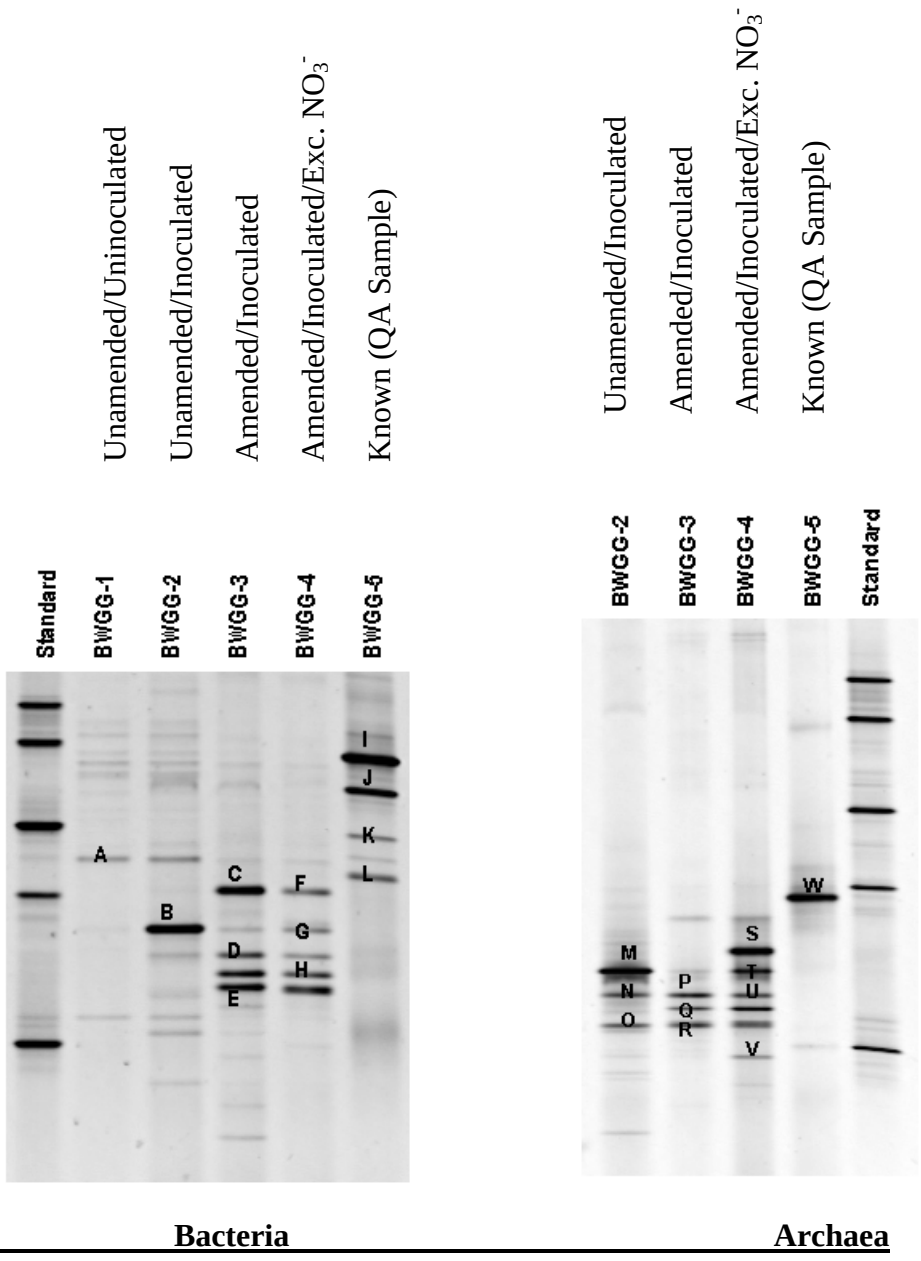

Figure 15. DGGE gel image of amplified primers from a conserved region of bacterial and archaeal 16S rDNA from the anaerobic inundated cellulose treatments. Banding patterns and relative intensities of the recovered bands provide a measure of differences among the communities. Dominant species must constitute at least 1-2% of the total bacterial community to form a visible band. The labeled bands were excised and sequenced.

4.5 Gas Produced in Humid Cellulose Treatments

Tables 1-4, Appendix C, show total gas and CO_2 produced per sample in initially aerobic humid cellulose samples incubated for 3334 days (9.1 years). All samples contain 1 g of cellulosic material. Table 5 gives data that were corrected to account for CO_2 produced in the absence of cellulose due to the metabolism of any dissolved organic carbon in the mixed inoculum; the results are reported as CO_2 produced per gram cellulose. Tables 6-9 provide data for total gas and CO_2 produced in anaerobic humid samples incubated for 2945 days (8.1 years); Table 10 has the corrected data for CO_2 production in these samples. All data are reported as gas produced sample^{-1} or g^{-1} cellulose, and are the mean ± standard error of the mean of the analysis of triplicate samples. Samples prepared to determine the viability of the inoculum (succinate or glucose amended treatments) were not analyzed during every time period. Figures 16(a,b) provide photographs of the initially aerobic samples. Carbon dioxide concentrations are the best indicator of microbial activity under humid conditions; they are given in Figures 17-18 for initially aerobic samples. Photographs of the anaerobic samples are provided in Figure 19(a,b), and CO_2 data in Figure 19-20. Data in the figures are corrected for CO_2 produced in the absence of cellulose, with data provided in Tables 9 and 10, Appendix C. Rates discussed in the following are calculated from single point data at the beginning and end of the experiment and a linear extrapolation between them.

4.5.1 Initially Aerobic Treatments

Figure 16 shows samples from the initially aerobic experiment at 3334 days (9.1 years); U-5 was unamended inoculated while A-2 was nutrient amended and inoculated. The brown paper of sample U-5 has faded while in A-2 the color remains intact. Figure 12(b) shows the initially aerobic samples with bentonite. Disintegration of the paper and a bright red color was observed on the bentonite particles in sample BA-1 (and all of the amended inoculated samples containing bentonite). The red coloration was most likely due to the presence of bacterioruberin, a 50-carbon carotenoid produced by extremely

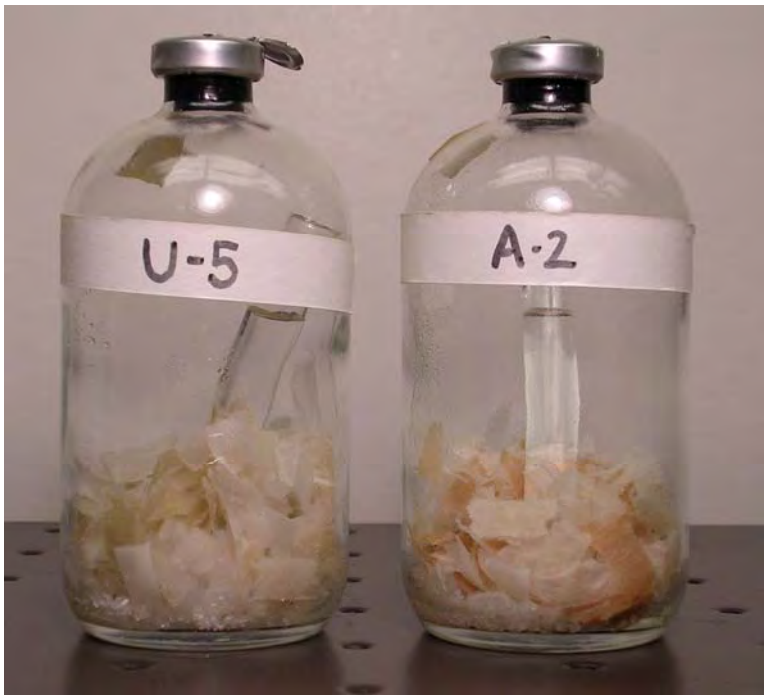

Figure 16(a). Initially aerobic humid samples at 3334 days (9.1 years) incubation: unamended inoculated (U-5) and amended inoculated (A-2). Some discoloration of the paper is evident in U-5.

Figure 16(b). Initially aerobic humid samples containing bentonite at 3334 days (9.1 years) incubation : unamended inoculated (BU-3) and amended inoculated (BA-1). The paper in BA-1 has compacted and a biofilm has formed on the paper as indicated by a red color.

halophilic bacteria (McGenity et al., 2000; Oren et al., 2001). By the end of the experiment at 3334 days, initially aerobic humid treatments with and without bentonite generally had ceased to produce gas (Figure 17 and 18). In the absence of bentonite, CO_2 production in unamended inoculated samples peaked at 317 days incubation at 62.0 ± 11.4 μmoles CO_2 g^{-1} cellulose (Table 9(a), Appendix C) and 0.19 ± 0.33 ml total gas $sample^{-1}$ (Table 1(a), Appendix C)(Figure 17). The linearized overall rate of CO_2 production was 0.0007 μmol CO_2 g^{-1} cellulose day^{-1} over 3334 days. In amended inoculated samples, CO_2 production peaked at 120 days incubation at 28.5 ± 1.3 μmoles CO_2 g^{-1} cellulose (Table 9(a), Appendix C) and -0.21 ± 1.57 ml total gas $sample^{-1}$ (Table 1(a), Appendix C). The overall rate of CO_2 production was -0.008 μmol CO_2 g^{-1} cellulose day^{-1}. Oxygen was consumed during the very early stages of the experiment; this accounts for a loss in total gas in the samples. Total gas volume and CO_2 concentrations were corrected for gas lost due to sampling (removal of ~1 ml of gas at each period, or 9.0 ml overall from a ~155 ml headspace volume). Even with this correction, the profile for CO_2 production shows a decrease after the early peaks in production (Figure 13). Some of this loss results from correcting the data due to CO_2 production in the mixed inoculum in the absence of cellulose (see "Control" treatments, Table 5(a,b), Appendix C). Finally, the 3 ml of G-Seep brine that was placed in a glass tube in the samples (see Figure 16) was not acidified and CO_2 could have been absorbed from the headspace as it reacted with the brine to form carbonic acid and bicarbonate. Nevertheless, the gas production profiles for samples without bentonite show that the bacteria had a limited capability for growth on cellulose under initially aerobic humid conditions.

Similar to its effect on inundated samples, bentonite enhanced gas production under humid conditions. Figure 18 shows this enhancement; the amended inoculated samples containing bentonite peaked at 399 days incubation at 1456 ± 44 μmoles CO_2 g^{-1} cellulose (Table 9(a), Appendix C) and 0.02 ± 0.24 ml total gas $sample^{-1}$ (Table 2(a), Appendix C). The overall rate of CO_2 production was 0.18 μmol CO_2 g^{-1} cellulose day^{-1}. This was ~50x more CO_2 produced g^{-1} cellulose than in the same treatment without bentonite. The bentonite alone produced 144 ± 4 μmoles of CO_2 per sample at 399 days incubation (Control treatment (salt/inoculum/tube+brine) Table 6(a), Appendix C). After

this point, CO_2 was lost gradually over time, both in the presence and absence of cellulose. Only unamended samples containing bentonite continued to produce gas from the start of the experiment at a continuous overall rate of 0.09 μmoles CO_2 g^{-1} cellulose day^{-1}. The activity in this treatment shows the viability of the microbial community over 9.1 years; therefore, the lack of gas production in samples without bentonite, which are relevant to the WIPP repository environment, is not due to a loss of microbial viability.

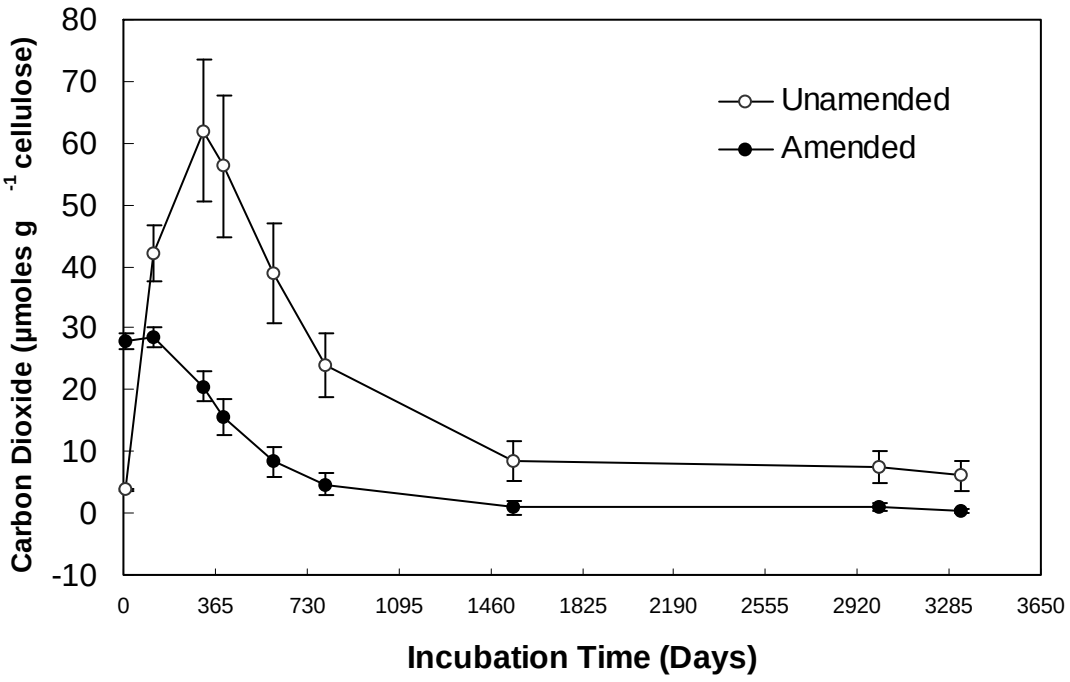

Figure 17. Carbon dioxide produced in initially aerobic humid samples without bentonite.

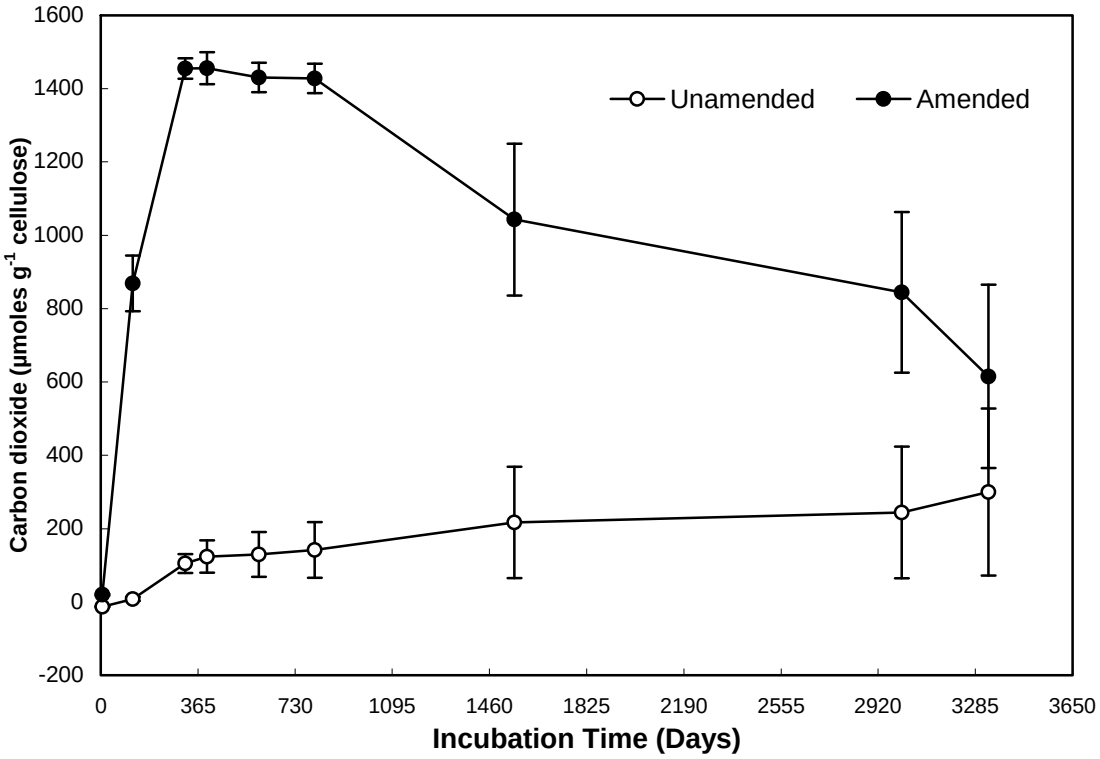

Figure 18. Carbon dioxide produced in initially aerobic humid samples with bentonite.

4.5.2 Anaerobic Treatments

Figure 19(a) shows the anaerobic humid samples at the end of the experiment (2945 days (8.1 years)). Paper discoloration is evident in this photograph in sample U-3. Figure 19(b) shows the anaerobic humid samples with bentonite at the end of the experiment. Here both samples show paper discoloration, and there was a red color on the bentonite at the bottom of the bottles. After correcting for gas production in the absence of cellulose (Table 10, Appendix C), the unamended inoculated samples without bentonite showed 115 ± 20 μmol CO_2 g^{-1} cellulose at 2945 days and amended inoculated samples showed 21.9 ± 3.3 μmol CO_2 g^{-1} cellulose (Figure 20). These values were close to their maximum CO_2 production of 155 ± 36 μmol CO_2 g^{-1} cellulose at 2156 days and 32.8 ± 1.3 μmol CO_2 g^{-1} cellulose at 140 days incubation, respectively. Total gas volumes at these times were 2.00 ± 1.02 ml $sample^{-1}$ (unamended inoculated at 2156 days) and -0.54 ± 1.03 ml $sample^{-1}$ (amended inoculated at 140 days). Unamended inoculated samples produced CO_2 at a rate of 0.037 μmol CO_2 g^{-1} cellulose day^{-1}. Amended inoculated samples produced CO_2 at a rate of 0.003 μmol CO_2 g^{-1} cellulose day^{-1}. About 8x more CO_2 was produced in the absence of nutrients than in their presence (Figure 20). Including bentonite enhanced CO_2 production under anaerobic humid conditions, with 28x more CO_2 produced in amended inoculated samples with bentonite than in the same treatment without it. While nutrients had a detrimental effect on the mixed inoculum under anaerobic humid conditions, bentonite nullified this effect. In fact, whether or not nutrients were present, CO_2 production proceeded similarly (Figure 21). Unamended inoculated samples with bentonite generated 541 ± 135 μmol CO_2 g^{-1} cellulose at 2945 days at an overall rate of 0.18 μmol CO_2 g^{-1} cellulose day^{-1}. Amended inoculated samples produced 618 ± 125 μmol CO_2 g^{-1} cellulose by 2945 days at an overall rate of 0.20 μmol CO_2 g^{-1} cellulose day^{-1}. We note that samples that show a larger deviation from the mean generally show evidence of microbial activity (trending toward positive gas production). The larger spread in the data suggests that microbial processes that may be occurring at

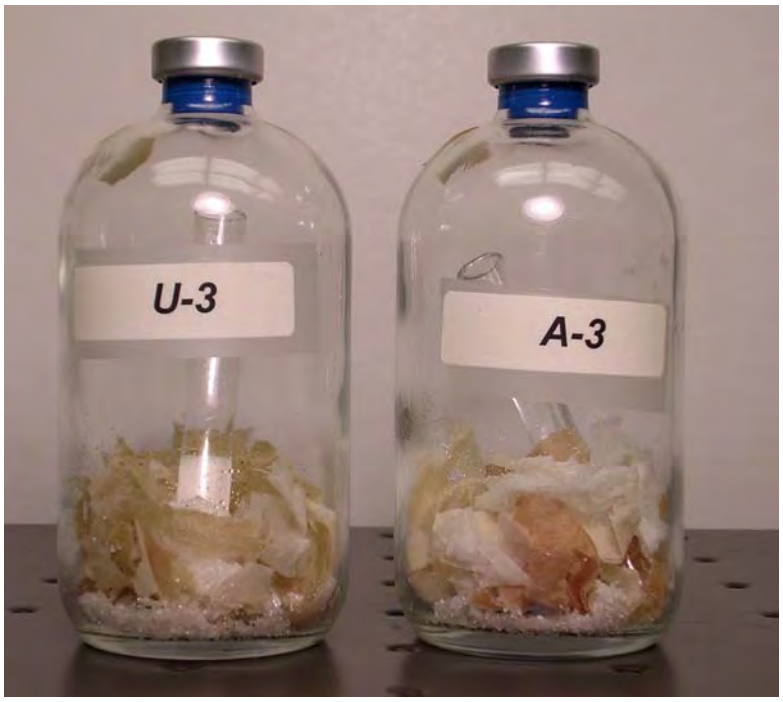

Figure 19 (a). Anaerobic humid samples at 2945 days (8.1 years) incubation: unamended inoculated (U-3) and amended inoculated (A-3). The brown paper in U-3 has become discolored.

Figure 19 (b). Anaerobic humid samples with bentonite at 2945 days (8.1 years) incubation: unamended inoculated (BU-4) and amended inoculated (BA-3). Discoloration of the paper is evident in both treatments, as well as a red biofilm in the bentonite at the bottom of the bottles.

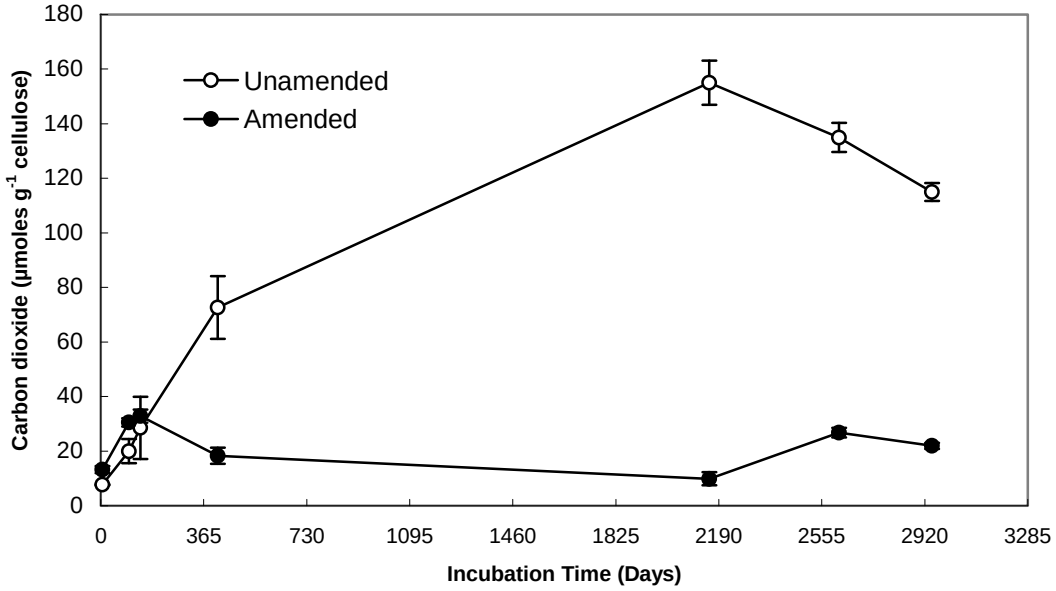

Figure 20. Carbon dioxide produced in anaerobic humid samples without bentonite.

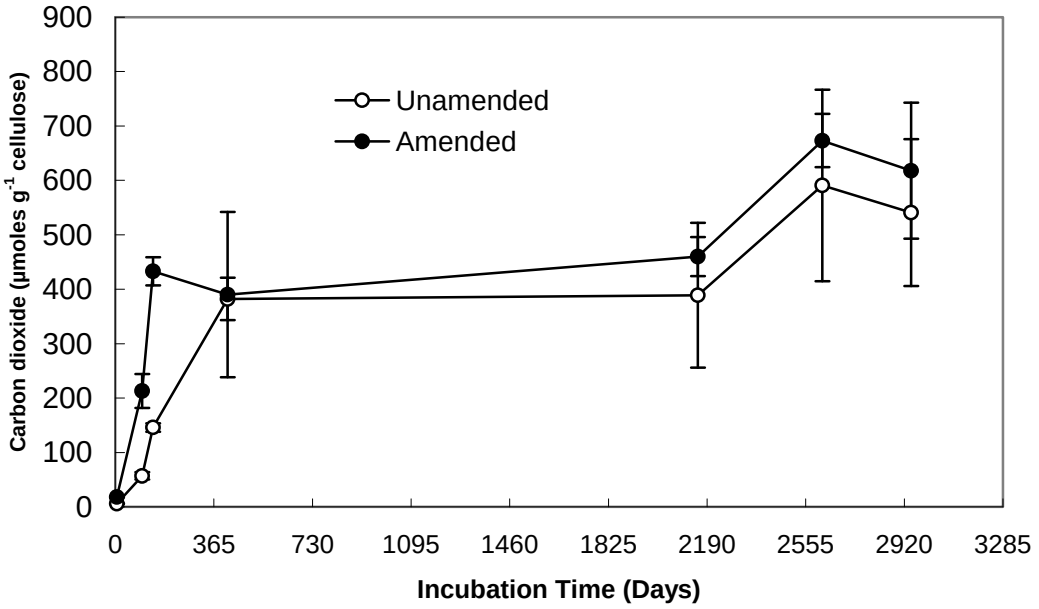

Figure 21. Carbon dioxide produced in anaerobic humid samples with bentonite.

slightly different rates in the active samples, due to differences in the overall microbial population or their metabolic capability. Samples with a smaller variation in values between triplicate bottles generally failed to show additional microbial gas production.

4.6. Analysis of Methane in Anaerobic Humid Samples

Methanogenesis is a potential CO_2-consuming microbial process that may occur under repository conditions (Lehmann-Richter et al., 1999). In addition, methanogenic bacteria are extremely sensitive to changes in pH, Eh, the presence of oxygen, and seldom have been found to metabolize acetate, CO_2, and H_2 under hypersaline conditions (Oren, 1999). The entire set of samples from the anaerobic humid cellulose biodegradation experiment was analyzed for the presence of methane at 2653 days (7.3 years) incubation. Methane was detected in unamended inoculated samples with bentonite (25.5 ± 1.2 nmol g^{-1} cellulose) and amended inoculated samples with bentonite (32.6 ± 9.3 nmol g^{-1} cellulose), but was below detectable (<0.1 nmol ml^{-1}) in all other samples. Section 4.5 of this report discusses data on methane produced under inundated conditions; in these samples at 3462 days incubation (9.5 years), unamended inoculated sample with bentonite had 4.51 ± 0.06 nmol CH_4 g^{-1} cellulose, and amended inoculated samples with bentonite 3.41 ± 0.13 nmol CH_4 g^{-1} cellulose. The production of methane appears more favorable under humid conditions, and in the presence of bentonite. In unamended samples, methane production was 6x greater than under inundated conditions and almost 10x greater for amended humid samples. This is further evidence of bentonite's stimulatory effect on microbial activity. In addition, methanogenic bacterial activity verified that strictly anaerobic conditions were established since it cannot occur even in the presence of traces of oxygen (Ramakrishnan et al., 2000). Methanogenic bacterial activity might account for the loss of CO_2 under initially aerobic conditions with and without bentonite (Figures 13 and 14), although methane was not detected in these samples.

4.7 Gas Produced in Samples Containing Plastic and Rubber Materials

Plastic and rubber materials are major constituents of TRU waste and consist of long repeating single-bonded carbon chains, and usually are quite resistant to biodegradation. Irradiation causes the polymer to break down due to free radical formation; in addition there may be cross-linking of the polymer chain after free radical formation, and a reduction of the molecular mass of the polymer (Woods and Pikaev, 1994). Figures 22-24 show the PE, PVC, and NE before and after irradiation; signs of radiation damage were obvious and include shrinkage, embrittlement, and extreme discoloration (the opaque plastic films became yellow, brown, or even black). Our experiments sought to examine the effect of irradiation on the biodegradability of plastic and rubber materials, with total gas, CO_2, or CH_4 production providing evidence of their biodegradation. Total gas volume and the concentration of CO_2 or CH_4 in samples containing low- and high-dose irradiated polymer were compared to baseline concentrations for control samples without polymers and to samples containing unirradiated polymer. Variables that can influence biodegradation, including atmosphere (air) or nutrients, were tested for each irradiation dose and type of polymer material type.

Tables 1-10, Appendix D show total gas and carbon dioxide produced in samples containing plastic (polyethylene (PE) and polyvinylchloride (PVC)) and rubber materials (neoprene (NE), hypalon (HY), and leaded-hypalon (LHY)). This data is represented graphically in Figures 25-33 (data are the mean of duplicate or triplicate analyses (see Section 3.3)).

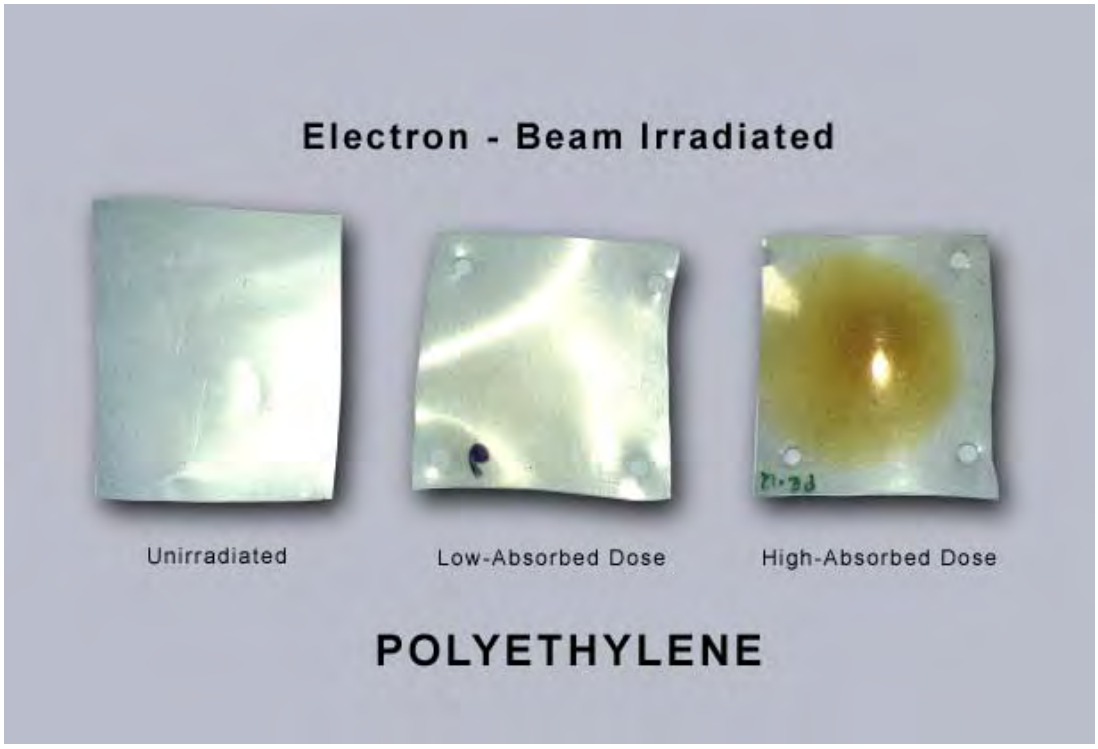

Figure 22. Unirradiated and irradiated (low-absorbed dose, and high-absorbed dose) polyethylene. Radiation damage is evident by embrittlement and darkening.

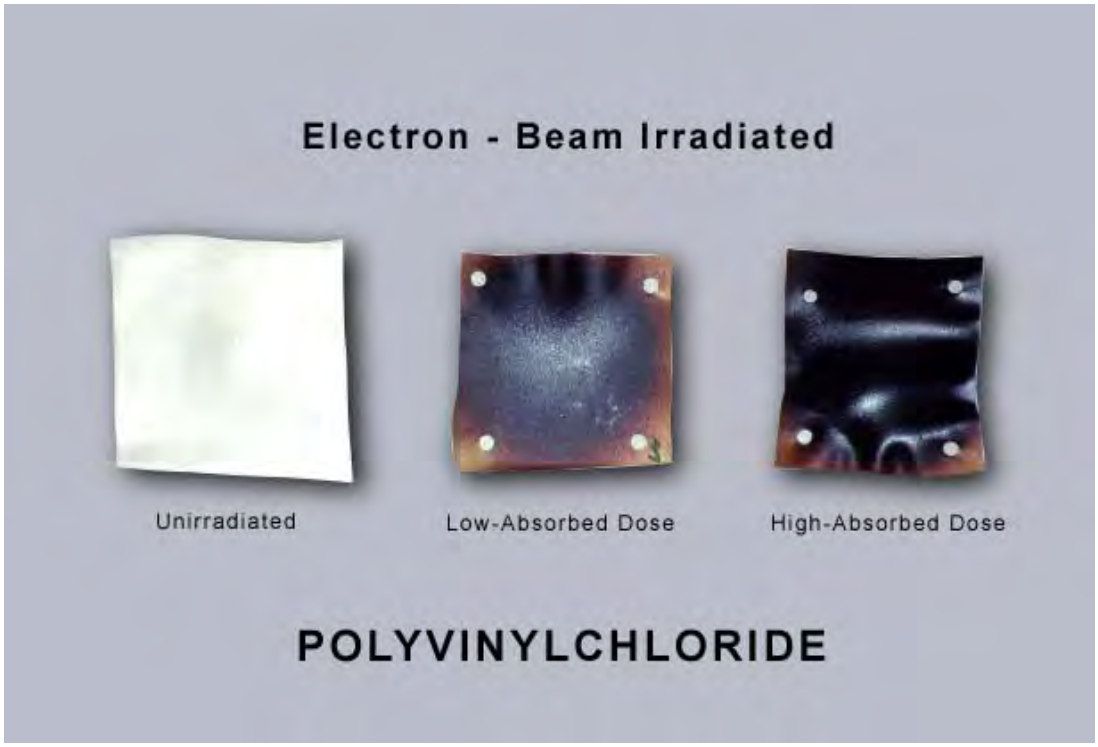

Figure 23. Unirradiated and irradiated (low-absorbed dose, and high-absorbed dose) polyvinylchloride. Radiation caused extreme discoloration and embrittlement.

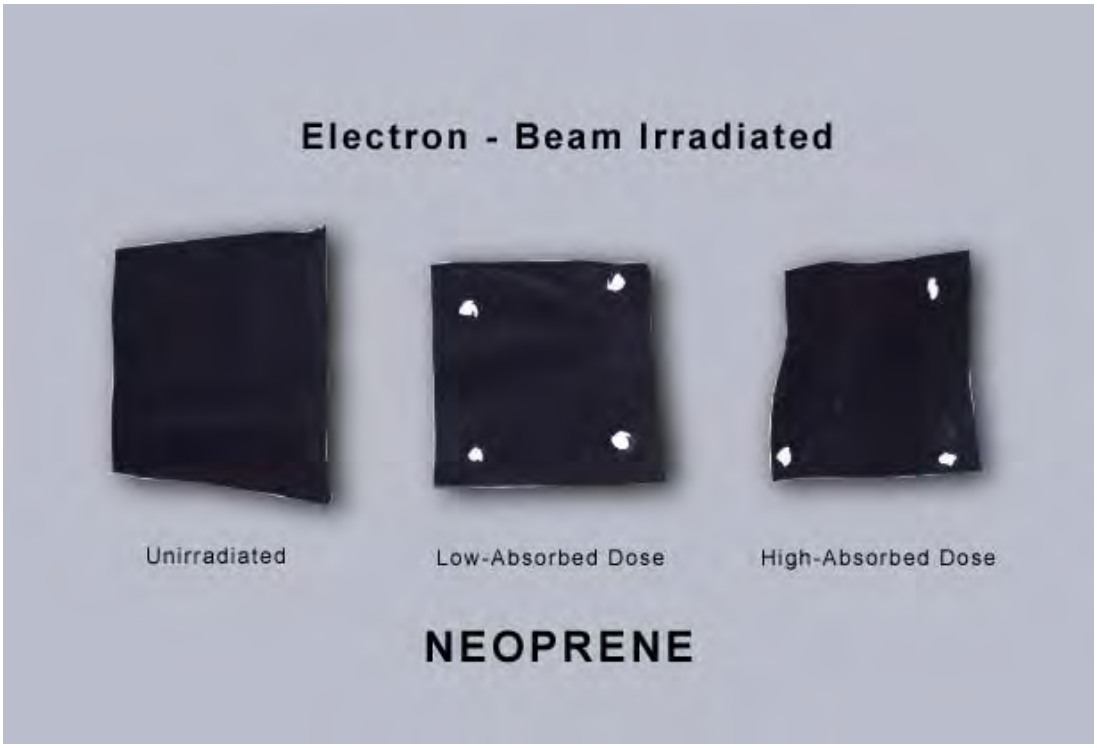

Figure 24. Unirradiated and irradiated (low-absorbed dose, and high-absorbed dose) neoprene. Low- and high-absorbed doses caused embrittlement.

4.7.1 Control (No Polymer)

Samples incubated without plastic or rubber material served as controls; they are referred to as "no polymer," and contained 50 mL of brine composed of 56% v/v G-Seep, 27% v/v 200 g/L WIPP salt solution, and 17% v/v Nash Draw lake brine/sediment slurry. The samples were incubated with added nutrients (amended) or without them (unamended). Microbial gas production was detected in both, and was due to metabolism of dissolved organic carbon and trace inorganic nutrients in the brine inoculum. Gas analysis of these samples provided the background gas production to compare with samples containing PE, PVC, NE, HY, or LHY to determine if the unirradiated or irradiated polymer stimulated more production.

4.7.2 Polyethylene

In most cases, total gas production was slightly higher in the presence of PE than in its absence, regardless of nutrient amendment, radiation dose, or initial atmosphere (Figures 25 and 26). Polyethylene may serve as a substrate for the attachment of a biofilm community (Schwartz et al., 1998), thereby allowing a more effective utilization of dissolved organic carbon and trace nutrients in the samples; this is evident in total gas and CO_2 production in unamended samples (Figure 25 (A), and (C); Figure 26 (A)). Under initially aerobic conditions, unamended samples containing unirradiated PE produced 64.2 μmoles CO_2 sample^{-1}, while in those without PE only 19.9 ± 1.2 μmoles CO_2 sample^{-1} was produced. There is no evidence for degradation of PE as indicated by the production of total gas or CO_2 in these samples.

4.7.3 Polyvinylchloride

Irradiated PVC showed the most obvious changes in characteristics. A viscous residue was present on the surfaces of low-dose irradiated PVC, but less prominent on the high-

dose ones. Figure 27 (B) shows the inhibitory effect of irradiated PVC on total gas production under initially aerobic nutrient-amended conditions; this correlates with Figure 28 (B), where low-dose irradiated PVC had a marked effect on CO_2 production, lowering it by 30% compared to samples without polymer. This same effect was seen under anaerobic conditions, with total gas and CO_2 production suppressed in samples containing low-dose irradiated PVC (Figure 27 (C) and 28 (C and D)). The inhibitory effect of the presence of high-dose irradiated PVC was overcome in unamended anaerobic samples between 840 and 2612 days incubation (4.9 years)(Figure 28 (C), Table 7, Appendix D). Although this observation is based upon one data point, over this period of time the amount of CO_2 in sample containing high-dose irradiated PVC increased 10x, and was 65% greater than the same treatment without polymer. This phenomenon is difficult to explain based upon gas production alone; however, it is likely that irradiation created a substrate at the surface of the PVC film that while initially toxic, over the long-term and in the absence of nutrients a microbial population was able exploit. Other than this finding, there is no evidence for degradation of PVC as indicated by the production of total gas or CO_2 in these samples.

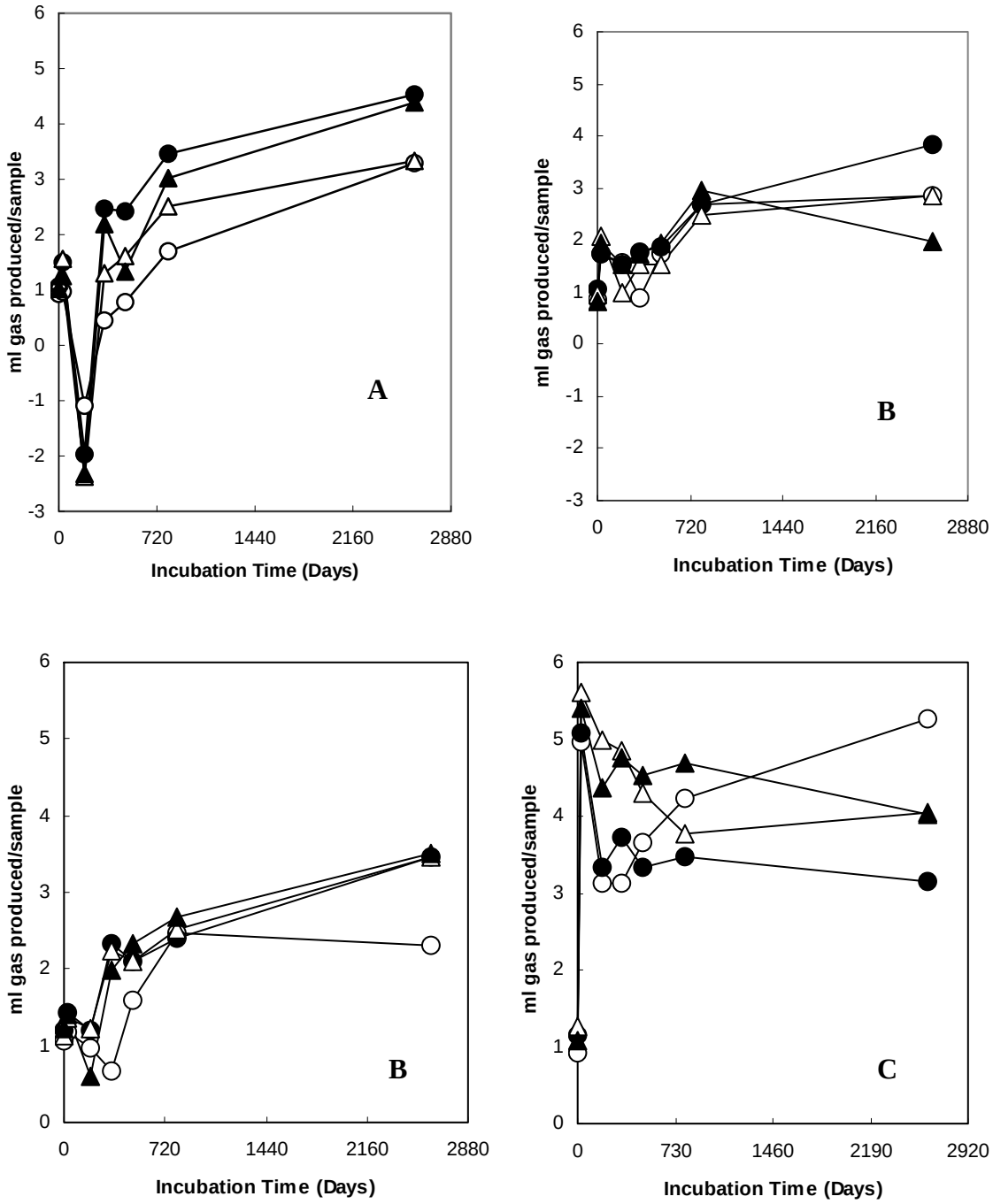

Figure 25. Total gas produced in samples containing polyethylene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D). No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

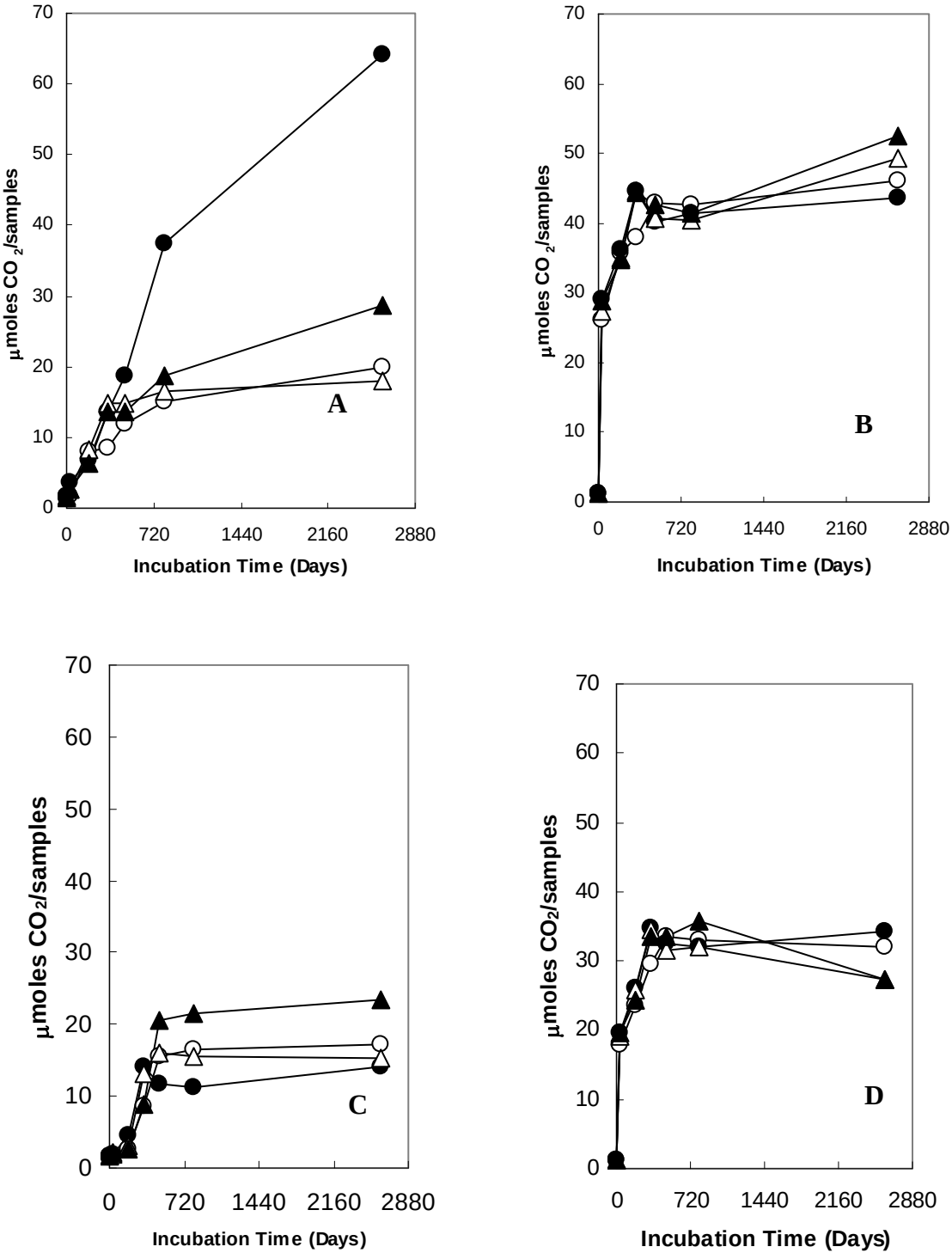

Figure 26. Carbon dioxide produced in samples containing polyethylene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

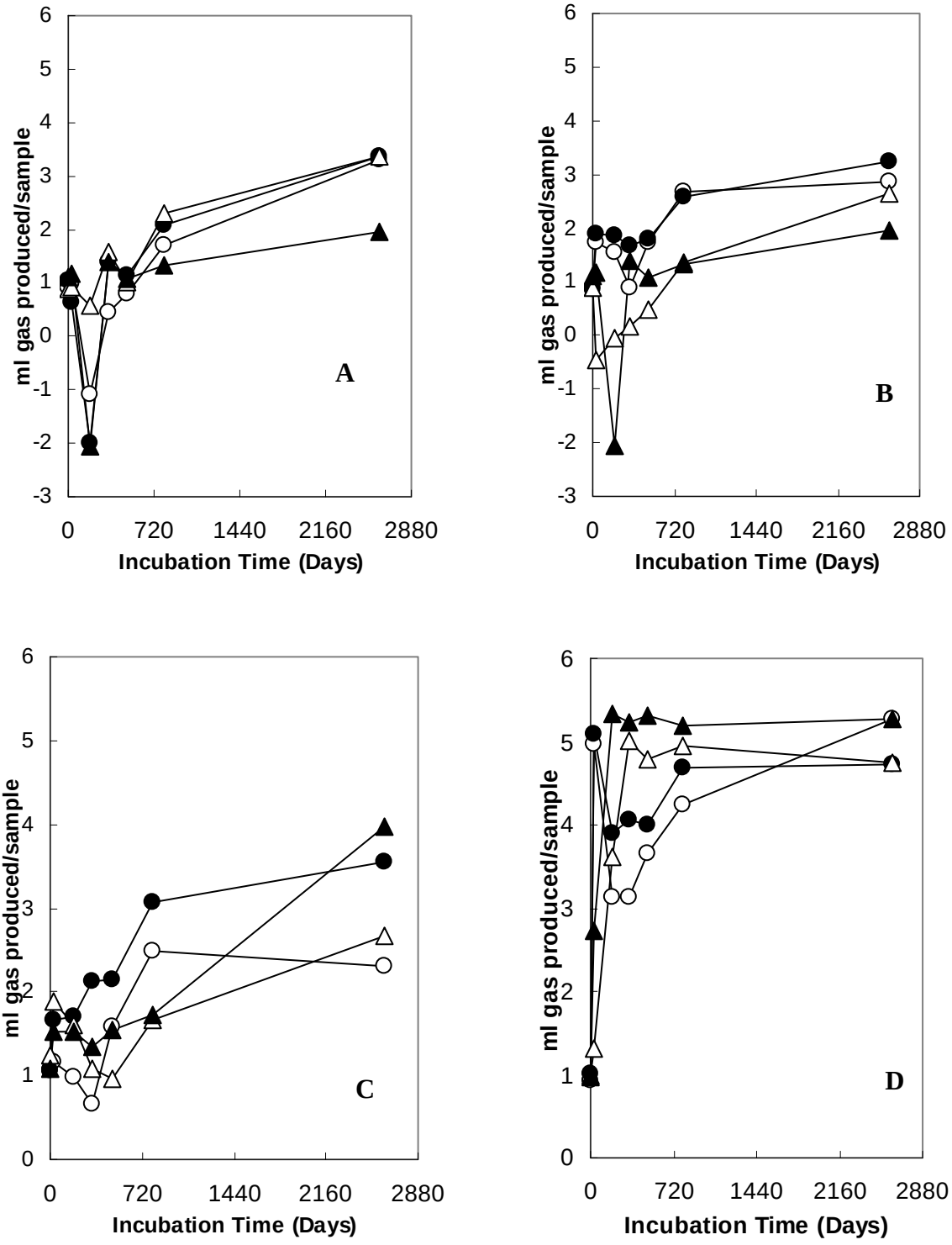

Figure 27. Total gas produced in samples containing polyvinylchloride: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

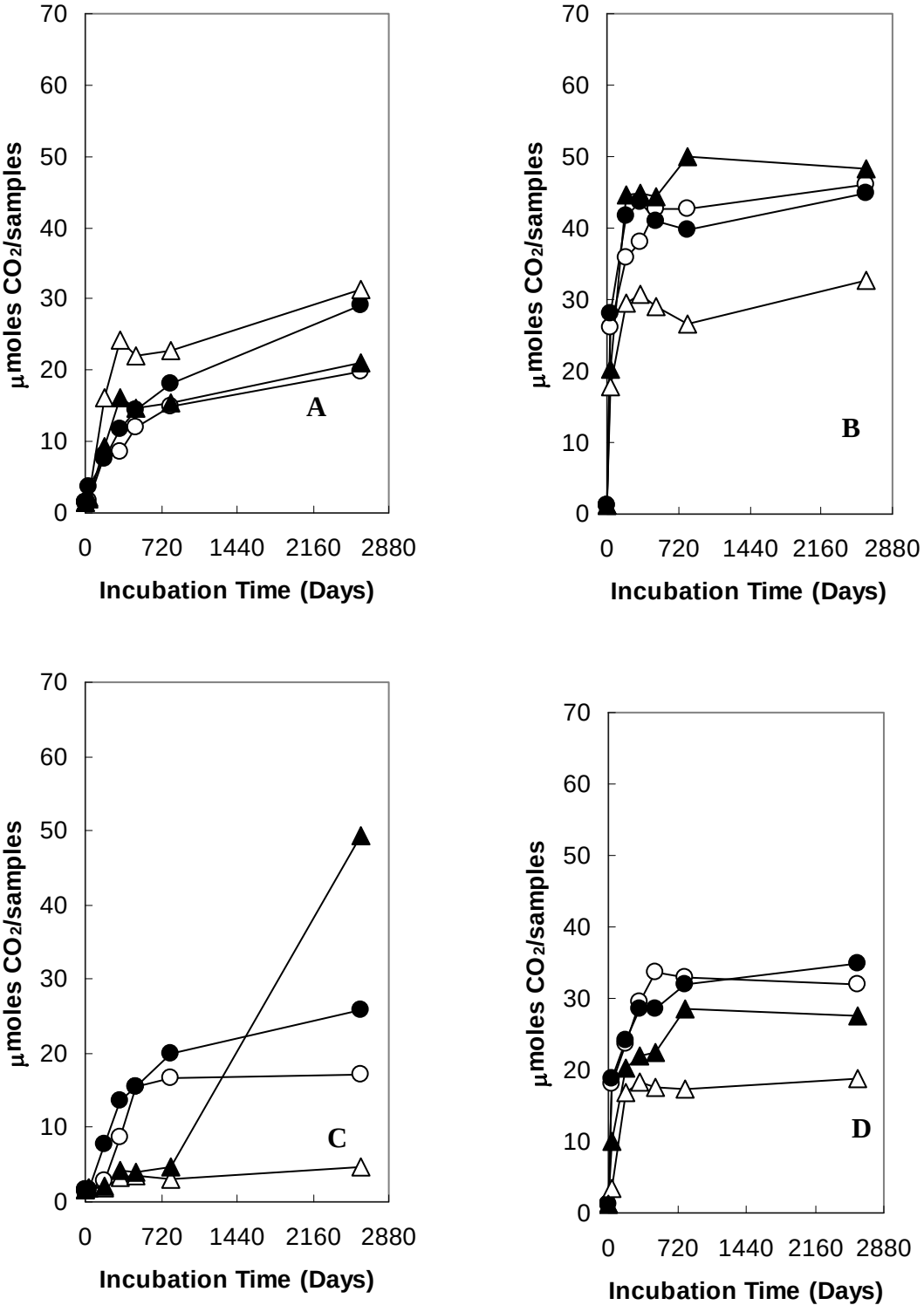

Figure 28. Carbon dioxide produced in samples containing polyvinylchloride: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

4.7.4 Neoprene

The data for total gas and CO_2 production in initially aerobic samples containing neoprene, unamended and amended, show that low- and high-dose irradiated neoprene supported sustained gas production over the long term (Figure 29 (A and B); Figure 30 (A and B)). Up to 74.6 μmoles CO_2 sample^{-1} was detected at 2612 days incubation in initially aerobic amended samples containing high-dose irradiated neoprene, with 46.2 μmoles sample^{-1} produced in the same treatment containing unirradiated polymer (Table 8, Appendix D). In samples containing low-dose irradiated neoprene, the effect on CO_2 production was similar, although not as great: at 2612 days incubation there was 55.8 μmoles CO_2 sample^{-1}. (17% more CO_2 than samples with unirradiated polymer). Under anaerobic conditions, CO_2 production was initially inhibited in unamended samples containing high-dose irradiated neoprene (Figure 30 (C), closed triangles). Evidence of inhibition is provided by the fact that over 720 days very little CO_2 was produced. However after 840 days CO_2 production recovered to the same levels as those samples containing unirradiated polymer and without it. The reason for the delayed onset of CO_2 produciton is unkown, however in the case of neoprene it was only detected in the anaerobic unamended samples containing high-absorbed dose neoprene. Unamended samples containing low-dose irradiated neoprene showed slightly more CO_2 production after 2612 days incubation (Figure 30 (C), open triangles). The nutrient amendment increased CO_2 production when high-dose irradiated neoprene was present under anaerobic conditions; the rate of CO_2 production was significant early on, and was 47.8 μmoles CO_2 sample^{-1} at 2612 days vs. 31.7 μmoles CO_2 sample^{-1} when unirradiated neoprene was present. It is difficult to determine if the enhanced gas generation in the presence of irradiated neoprene is due to biodegradation of the polymer or the creation of readily-metabolizeable organic material released onto its surface due to irradiation.

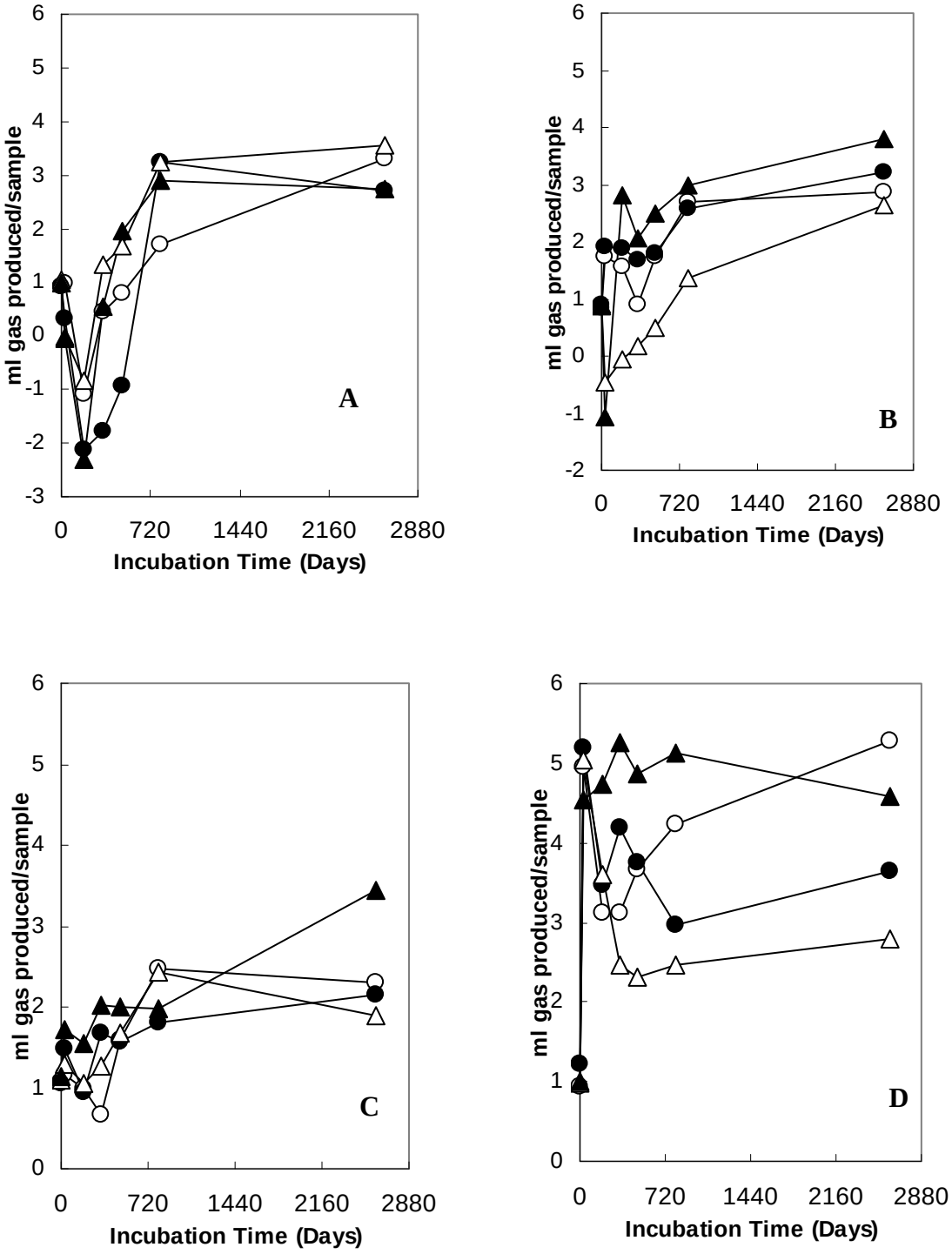

Figure 29. Total gas produced in samples containing neoprene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

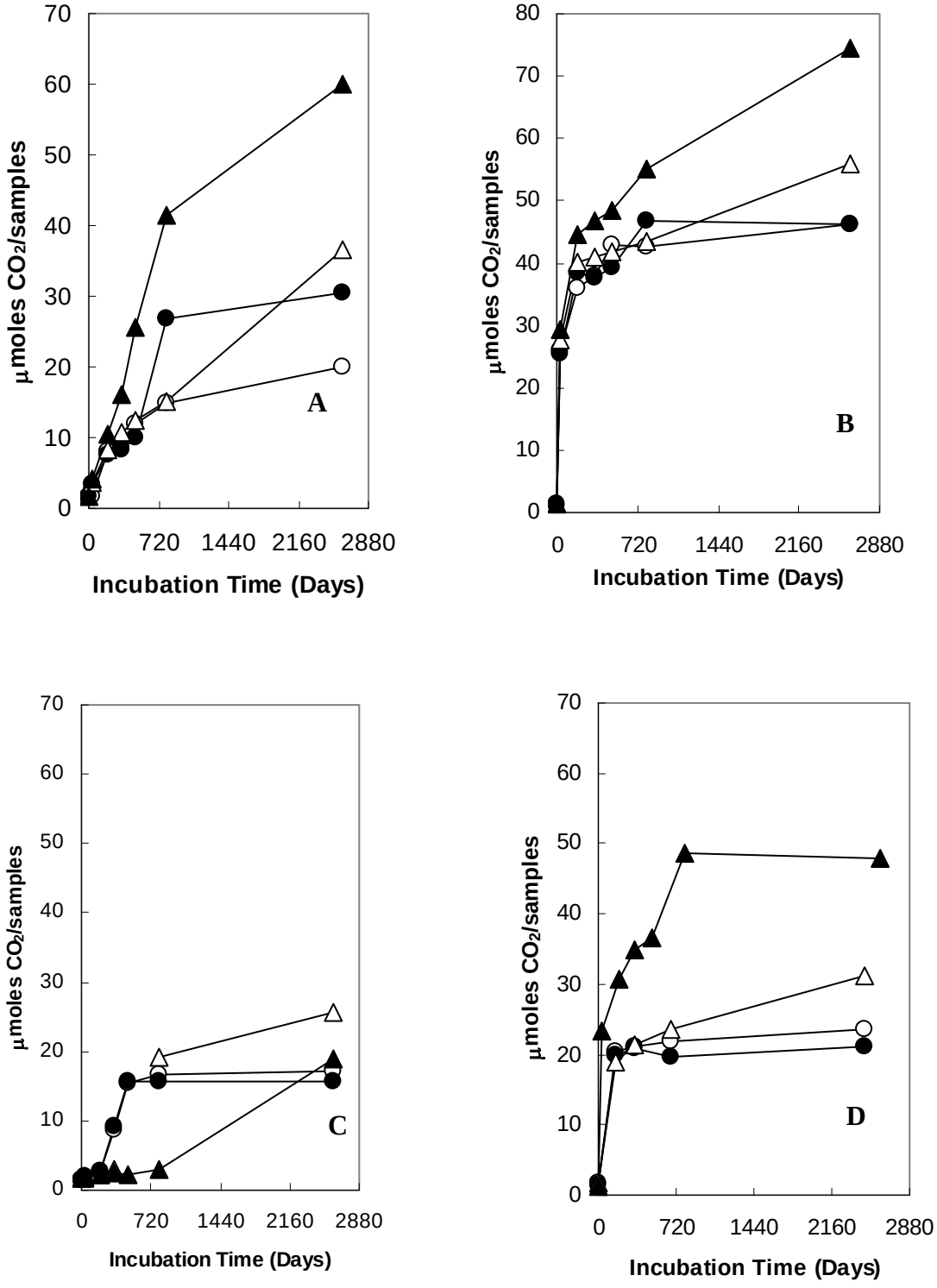

Figure 30. Carbon dioxide produced in samples containing neoprene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

The evidence of an inhibitory effect (Figure 30 (C), high-dose irradiated neoprene) suggests that a film of material was deposited on the surface of the polymer that readily interfered with the metabolism of dissolved organic carbon in the sample. A similar phenomenon was shown for low-dose irradiated PVC (Figure 28 (C)). Over time, however, this material was metabolized. Furthermore, the inhibitory effect of this material decreased when nutrients were added (Figure 30 (C)). The radiation dose to the neoprene was different from the expected irradiation conditions in the WIPP repository; hence, data obtained with this material are not easily extrapolated to conditions expected in the WIPP. However, the rationale for choosing electron-beam radiation, rather than alpha-irradiation, was to accelerate damage to the polymers to simulate long-term radiation damage in WIPP.

4.7.5 Unleaded Hypalon

Figures 31-32 show the results of gas analysis of samples containing unleaded hypalon under various conditions; the material was not irradiated at high doses. Experiments involving hypalon, unleaded and leaded, were started later than those with the other polymers. Therefore, a new mixed inoculum was prepared for the hypalon experiments; the dissolved organic carbon content of this inoculum was most likely lower than the previous one as evidenced by the smaller amount of total gas and, in some cases CO_2 produced in samples without polymer or unirradiated material vs. the same treatment for PE, PVC, or NE (e.g., compare no polymer (open circles) treatment in Figure 32 (A, B) and Figure 21 (A,B)). Nevertheless, the inoculum was viable because gas, including CO_2, was generated. A greater amount of CO_2 was produced when low-dose irradiated hypalon was present under initially aerobic conditions without a nutrient amendment (Figure 32(A)) or with a nutrient amendment (Figure 32(B)). Only 25% more CO_2 (43.8 $\pm$ 7.1 μmoles CO_2 sample^{-1}) was produced under initially aerobic nutrient amended conditions compared to samples containing unirradiated hypalon or without polymer; while 33% more CO_2 (31.1 $\pm$ 5.9 μmoles CO_2 sample^{-1}) was produced in samples

containing low-dose irradiated hypalon under anaerobic nutrient amended conditions (Figure 32 (D)).

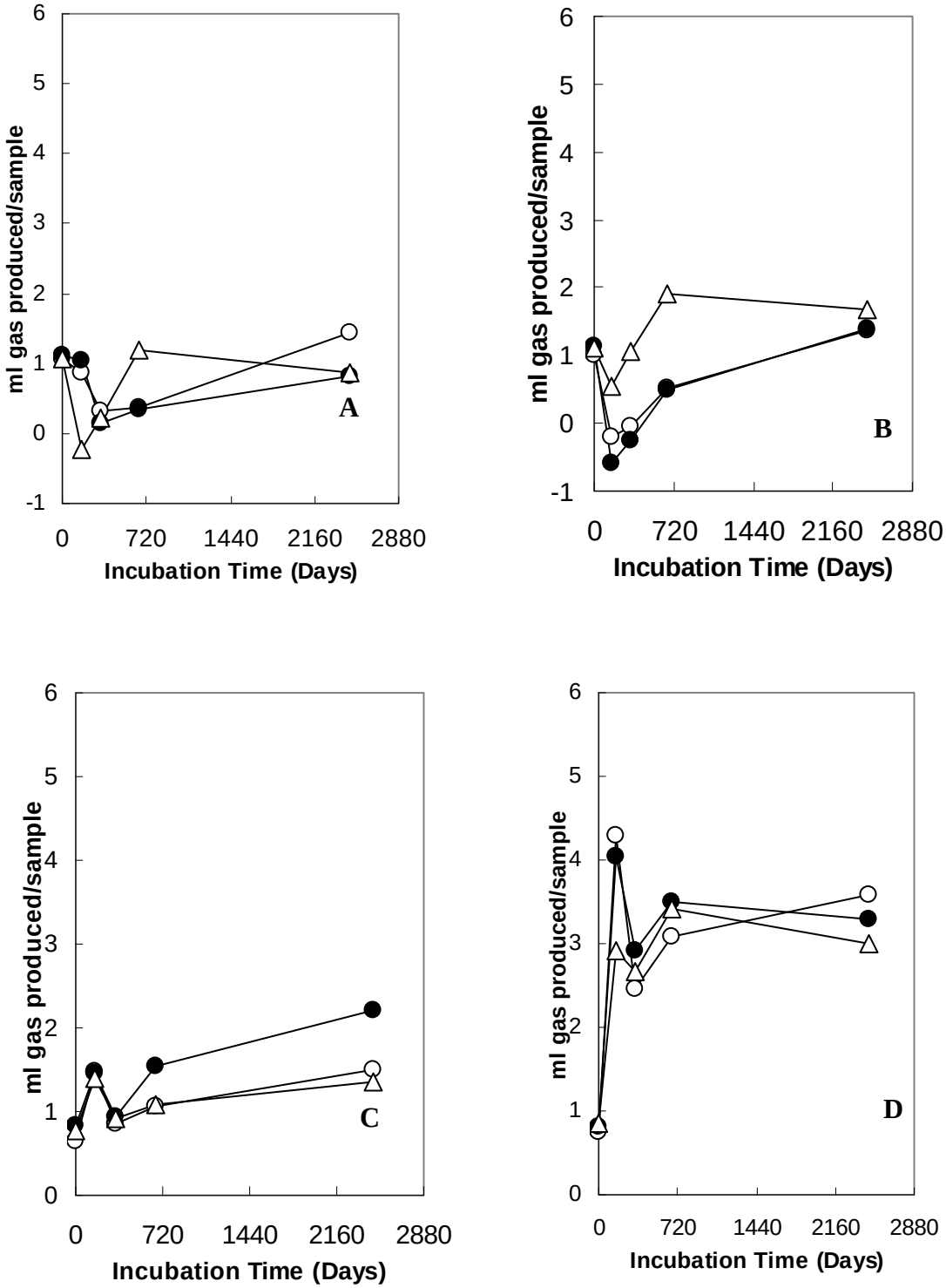

Figure 31. Total gas produced in samples containing unleaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△).

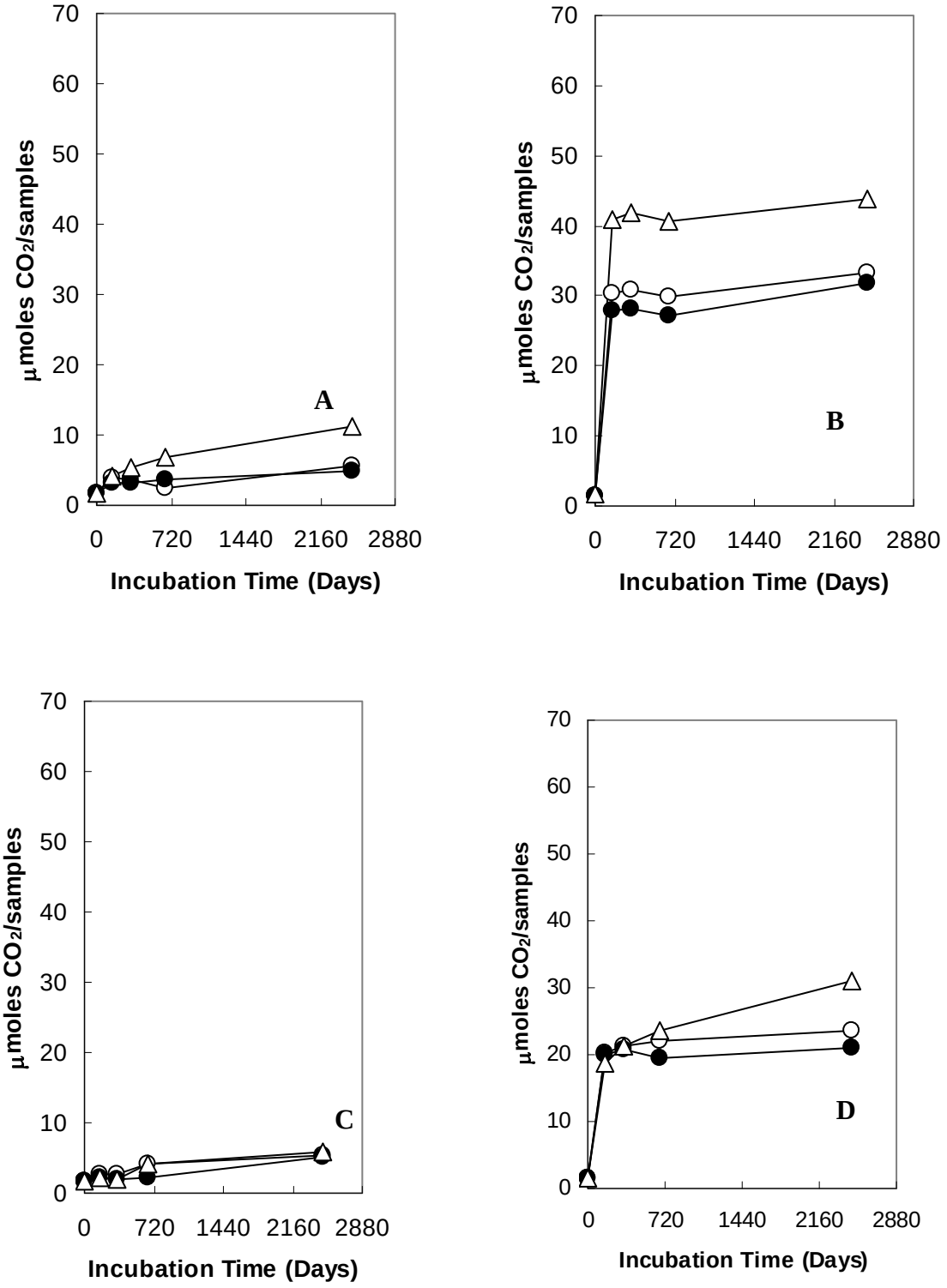

Figure 32. Carbon dioxide produced in samples containing unleaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△).

4.7.6 Leaded Hypalon

Figures 33-34 show the results of gas analysis of samples containing leaded hypalon. Absorbed radiation doses >4,000 Mrad resulted in heating and complete destruction of the leaded hypalon samples; for this reason, only low-dose irradiated leaded hypalon was studied. Low-dose irradiated leaded hypalon affected total gas and CO_2 production under initially aerobic conditions, with and without nutrients (Figure 33 (A,B) and Figure 34 (A, B)). Conversely, the presence of unirradiated leaded hypalon stimulated total gas and CO_2 production when nutrients were present (closed circles, Figure 33 (B) and Figure 34 (B)). In fact, CO_2 production in samples containing unirradiated leaded hypalon was on par with samples containing low-dose irradiated unleaded hypalon (Figure 32 (B)). Irradiated leaded hypalon had no significant effect on gas production under anaerobic conditions (Figure 33 (C,D) and Figure 34 (C,D).

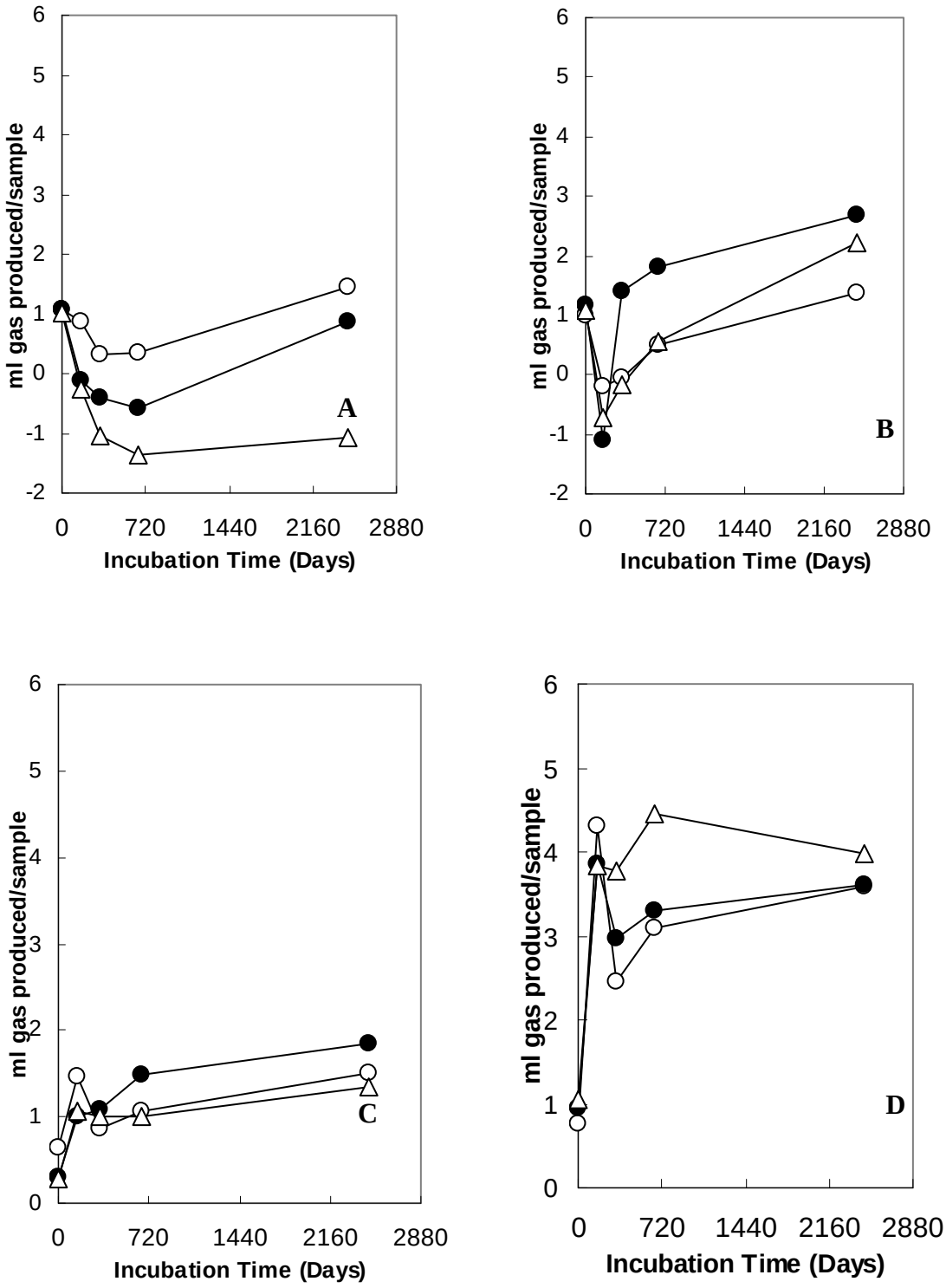

Figure 33. Total gas produced in samples containing leaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△).

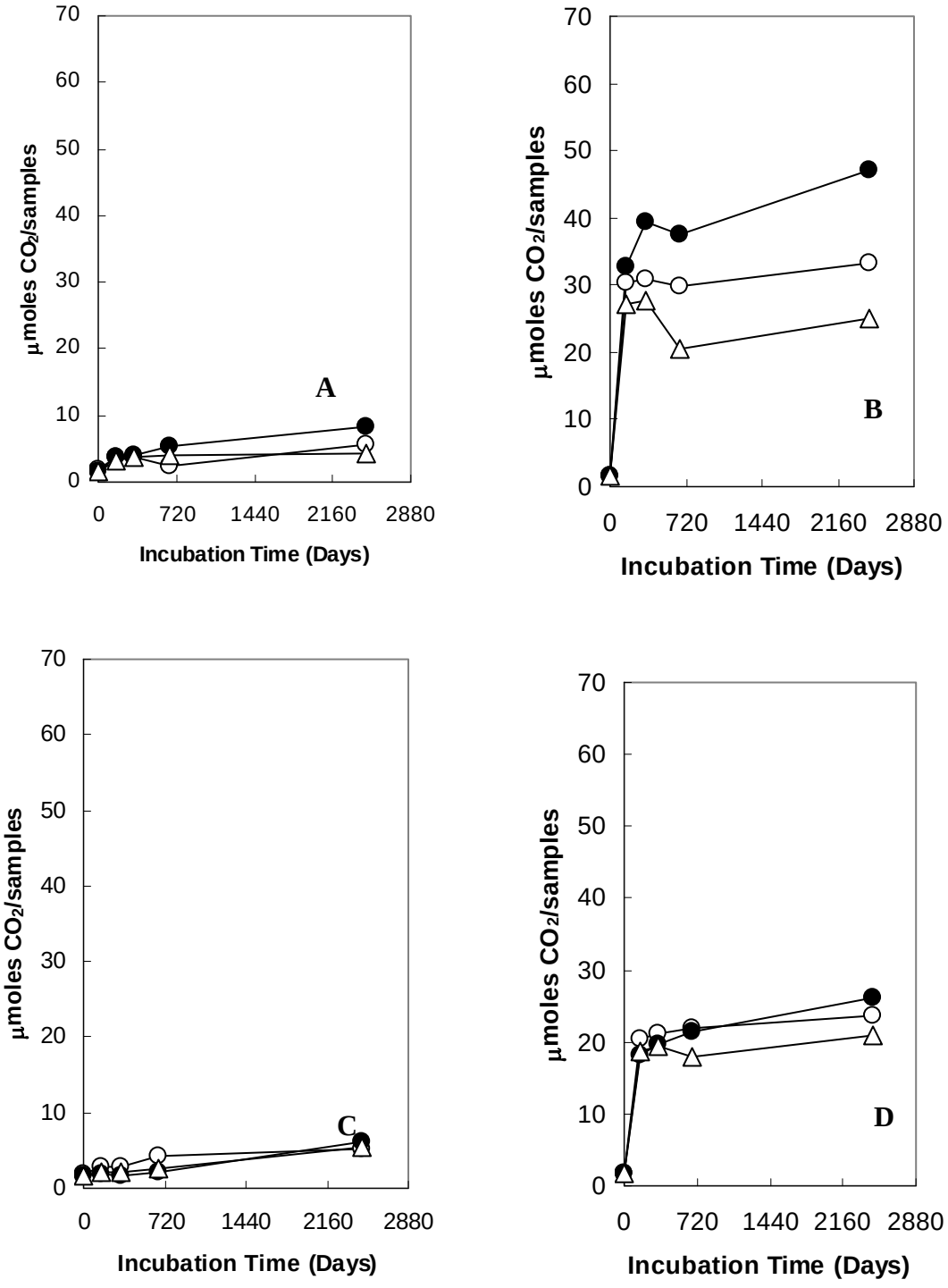

Figure 34. Carbon dioxide produced in samples containing leaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△).

4.8 Analysis of Methane Production in Samples Containing Plastic or Rubber Materials

Table 13 summarizes the results of methane analyses up to 3070 days (8.4 years) incubation for PE, PVC, and NE and 2926 (8 years) for HY (anaerobic treatments only). Over 2230 days incubation (6.1 years) the concentration of methane in almost all samples containing polyethylene, polyvinylchloride, and neoprene did not increase but remained nearly equal to that measured at 840 days incubation. The exception was the unirradiated polyethylene, in which production increased from 2.14 ± 1.52 μmol sample^{-1} at 840 days incubation to 2.50 ± 0.26 μmol sample^{-1} at 3070 days. In addition, samples containing hypalon did not show any increase in methane generation over 2262 days incubation (6.2 years). The concentrations of methane detected at 664 and 840 days and at 2926 and 3070 days are consistent, indicating that no further methanogenesis occurred in these samples. The methane detected most likely reflects the metabolism of dissolved organic carbon in the mixed inoculum/inundation fluid; additional production due to biodegradation of the polymer is not evident. The inhibitory effect of irradiated PVC persisted at 6.1 years, indicating that the degradation products produced by irradiation continued to affect gas production.

Table 13. Analysis of methane in samples containing plastic and rubber materials.

Anaerobic Treatment	Incubation Time (Days) 840	3070
	Methane (µmol sample^{-1})	
Samples without polymer (control)		
Unamended	0.91 ± 0.14	0.99 ± 0.20
Amended	4.03 ± 0.17	3.65 ± 0.11
Polyethylene		
Unirradiated – Unamended	0.85	0.53
Amended	2.14 ± 1.52	2.50 ± 0.26
Low-Dose- Unamended	1.01	0.72
Amended	4.13 ± 0.02	3.04 ± 0.11
High-Dose – Unamended	1.02	0.70
Amended	4.29 ± 0.13	1.73 ± 1.20
Polyvinylchloride		
Unirradiated – Unamended	1.27	1.00
Amended	4.88 ± 0.11	3.50 ± 0.37
Low-Dose-Unamended	nd	nd
Amended	nd	0.004 ± 0.004
High-Dose – Unamended	nd	0.01
Amended	0.03 ± 0.02	0.04 ± 0.04
Neoprene		
Unirradiated – Unamended	0.03	0.02
Amended	4.03 ± 0.22	2.64 ± 0.34
Low-Dose- Unamended	nd	0.01
Amended	3.87 ± 0.23	3.05 ± 0.14
High-Dose – Unamended	nd	2.79
Amended	4.91 ± 0.04	3.71 ± 0.01
Unleaded Hypalon	*664*	*2926*
Unirradiated – Unamended	nd	0.01
Amended	0.02 ± 0.00	0.02 ± 0.01
Low-Dose- Unamended	nd	0.02
Amended	0.01 ± 0.00	0.02 ± 0.01

5.0 References

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Appendix A

Gas Produced in Inundated Cellulose Treatments

Total gas and CO_2 produced in the inundated experiment are presented in Table 7-14 as follows: The data are presented as gas volume or CO_2 produced per gram cellulose, values were corrected for gas production in the absence of cellulose by subtracting out control data; data were not corrected for dissolved CO_2 and are for headspace (gaseous) CO_2 only; errors are ± 1 standard deviation with errors on control data summed with errors on sample data according to the following: reported standard deviation $= \sqrt{(\sigma_a^2 + \sigma_b^2)}$, where a and b are the standard deviation of control and sample data:

Tables 1(a)-(c): Total volume of gas produced in initially aerobic samples.

Tables 2(a)-(c): Total volume of gas produced in initially aerobic samples with bentonite.

Tables 3(a)-(c): Total volume of gas produced in anaerobic samples.

Tables 4(a)-(c): Total volume of gas produced in anaerobic samples with bentonite.

Tables 5(a)-(c): Carbon dioxide produced in initially aerobic samples.

Tables 6(a)-(c): Carbon dioxide produced in initially aerobic samples with bentonite.

Tables 7(a)-(c): Carbon dioxide produced in anaerobic samples.

Tables 8(a)-(c): Carbon dioxide produced in anaerobic samples with bentonite.

Table 1(a). Long-Term Inundated Experiment: Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	0	45	69	104	132	164	200
Unamended/ Uninoculated	0.05 ± 0.02	-0.29 ± 0.19	-0.36 ± 0.18	-0.31 ± 0.18	-0.20 ± 0.21	NA	-0.18 ± 0.18
Unamended/ Inoculated	-0.08 ± 0.01	0.01 ± 0.05	-0.15 ± 0.07	-0.19 ± 0.05	-0.07 ± 0.05	NA	-0.34 ± 0.07
Amended/ Inoculated	-0.12 ± 0.03	-0.27 ± 0.14	-0.25 ± 0.06	0.36 ± 0.31	0.59 ± 0.24	0.56 ± 0.15	0.86 ± 0.12
Amended/Inoc. + Exc. Nitrate	-0.02 ± 0.02	0.02 ± 0.17	1.39 ± 0.61	2.54 ± 0.87	3.33 ± 1.06	4.02 ± 1.21	4.42 ± 0.80

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 1(b). Long-Term Inundated Experiment: Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	NA	NA	-0.13 ± 0.16	NA	NA	-0.17 ± 0.17	NA
Unamended/ Inoculated	NA	NA	-0.58 ± 0.18	NA	NA	-0.54 ± 0.09	NA
Amended/ Inoculated	0.89 ± 0.09	0.75 ± 0.07	1.16 ± 0.06	1.14 ± 0.10	1.28 ± 0.13	1.26 ± 0.14	1.34 ± 0.11
Amended/Inoc. + Exc. Nitrate	5.20 ± 0.38	6.21 ± 0.7	6.01 ± 0.77	6.12 ± 0.72	6.03 ± 0.62	6.14 ± 0.70	7.20 ± 1.34

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 1(c). Long-Term Inundated Experiment: Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	0.74 ± 0.45	0.71 ± 0.4	0.08 ± 0.00	-0.04 ± 0.08	-0.02 ± 0.00	0.06 ± 0.01	0.11 ± 0.04
Unamended/ Inoculated	0.48 ± 0.08	0.35 ± 0.1	0.36 ± 0.1	0.30 ± 0.07	0.64 ± 0.04	0.71 ± 0.04	0.84 ± 0.10
Amended/ Inoculated	1.47 ± 0.054	1.53 ± 0.1	1.59 ± 0.24	1.42 ± 0.28	1.33 ± 0.56	1.71 ± 1.03	1.44 ± 0.29
Amended/Inoc. + Exc. Nitrate	11.6 ± 2.11	10.4 ± 1.71	12.2 ± 0.00	10.3 ± 1.54	8.42 ± 1.40	7.15	7.46 ± 0.70

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 2(a). Long-Term Inundated Experiment: Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	0	45	69	104	132	164	200
Unamended/ Uninoculated	-0.05 ± 0.02	0.02 ± 0.11	-0.36 ± 0.16	-0.20 ± 0.10	-0.13 ± 0.06	NA	0.00 ± 0.07
Unamended/ Inoculated	0.03 ± 0.06	-0.10 ± 0.02	-0.18 ± 0.04	-0.30 ± 0.14	-0.62 ± 0.10	NA	-0.08 ± 0.17
Amended/ Inoculated	-0.25 ± 0.03	0.43 ± 0.17	0.71 ± 0.23	1.82 ± 0.38	2.96 ± 0.32	4.07 ± 0.23	4.38 ± 0.20
Amended/Inoc. + Exc. Nitrate	0.30 ± 0.02	0.85 ± 0.02	1.56 ± 0.02	2.23 ± 0.24	3.79 ± 0.29	5.15 ± 0.18	6.07 ± 0.05

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 2(b). Long-Term Inundated Experiment: Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	NA	NA	0.05 ± 0.15	NA	NA	-0.08 ± 0.10	NA
Unamended/ Inoculated	NA	NA	0.65 ± 0.29	NA	NA	1.30 ± 0.50	NA
Amended/ Inoculated	4.40 ± 0.32	5.15 ± 0.45	5.28 ± 0.58	6.04 ± 0.74	6.13 ± 0.79	6.24 ± 0.82	6.38 ± 0.84
Amended/Inoc. + Exc. Nitrate	6.19 ± 0.13	6.33 ± 0	6.35 ± 0.22	6.98 ± 0.29	7.08 ± 0.35	7.35 ± 0.46	7.77 ± 0.65

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 2(c). Long-Term Inundated Experiment: Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	1.06 ± 0.48	1.04 ± 0.47	0.29 ± 0.11	0.33 ± 0.13	-0.97 ± 0.26	1.94 ± 0.21	0.17 ± 0.15
Unamended/ Inoculated	1.02 ± 0.18	1.24 ± 0.21	1.41 ± 0.2	1.47 ± 0.22	-0.09 ± 0.04	2.72 ± 1.43	2.06 ± 0.31
Amended/ Inoculated	8.96 ± 1.34	8.36 ± 1.24	6.14 ± 0.10	6.09 ± 0.04	4.02	2.00 ± 0.50	1.87 ± 0.81
Amended/Inoc. + Exc. Nitrate	7.35 ± 0.77	8.41 ± 0.82	6.79 ± 0.77	8.10 ± 0.75	7.76 ± 2.34	9.08 ± 1.37	9.95 ± 1.01

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 3(a). Long-Term Inundated Experiment: Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	0	45	69	104	132	164	200
Unamended/ Uninoculated	0.03 ± 0.09	0.10 ± 0.02	0.40 ± 0.03	-0.18 ± 0.07	0.04 ± 0.23	NA	-0.09 ± 0.07
Unamended/ Inoculated	-0.04 ± 0.02	1.00 ± 0.20	0.22 ± 0.06	-0.02 ± 0.03	0.02 ± 0.02	NA	0.59 ± 0.13
Amended/ Inoculated	-0.08 ± 0.02	0.02 ± 0.04	-0.52 ± 0.06	0.66 ± 0.28	1.52 ± 0.31	2.15 ± 0.25	2.27 ± 0.16
Amended/Inoc. + Exc. Nitrate	0.01 ± 0.03	-0.12 ± 0.08	0.29 ± 0.30	2.00 ± 0.60	1.01 ± 0.78	4.04 ± 1.24	5.44 ± 1.43

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 3(b). Long-Term Inundated Experiment: Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	NA	NA	-0.15 ± 0.07	NA	NA	-0.20 ± 0.06	NA
Unamended/ Inoculated	NA	NA	0.82 ± 0.14	NA	NA	1.30 ± 0.08	NA
Amended/ Inoculated	3.09 ± 0.21	3.08 ± 0.20	2.72 ± 0.16	3.51 ± 0.22	3.60 ± 0.24	3.91 ± 0.28	4.00 ± 0.35
Amended/Inoc. + Exc. Nitrate	6.882 ± 1.6911	8.14 ± 1.8	9.00 ± 1.61	10.89 ± 1.2	12.0 ± 0.6	13.8 ± 0.34	14.2 ± 0.59

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 3(c). Long-Term Inundated Experiment: Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	0.53 ± 0.4563	0.46 ± 0.4	-0.20 ± 0.04	-0.24 ± 0.05	-0.30 ± 0.08	-0.97 ± 0.23	-0.32 ± 0.18
Unamended/ Inoculated	1.866 ± 0.08	2.04 ± 0.1	2.19 ± 0.1	2.23 ± 0.12	2.45 ± 0.27	1.56 ± 0.26	2.60 ± 0.46
Amended/ Inoculated	4.318 ± 0.3434	3.96 ± 0.2	3.87 ± 0.1	3.78 ± 0.09	4.21 ± 0.04	2.72 ± 0.11	2.54 ± 0.69
Amended/Inoc. + Exc. Nitrate	14.87 ± 1.0	12.5 ± 0.5	12.6 ± 0.5	12.12 ± 0.4	11.0 ± 0.43	9.98 ± 0.52	9.97 ± 0.79

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 4(a). Long-Term Inundated Experiment: Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	0	45	69	104	132	164	200
Unamended/ Uninoculated	-0.08 ± 0.12	-0.04 ± 0.03	-0.04 ± 0.15	-0.17 ± 0.08	-0.22 ± 0.10	NA	-0.28 ± 0.09
Unamended/ Inoculated	0.03 ± 0.02	0.11 ± 0.02	-0.06 ± 0.05	0.16 ± 0.06	0.29 ± 0.07	NA	0.81 ± 0.10
Amended/ Inoculated	-0.11 ± 0.05	-0.05 ± 0.03	0.19 ± 0.09	1.39 ± 0.09	1.78 ± 0.08	1.44 ± 0.10	1.92 ± 0.18
Amended/Inoc. + Exc. Nitrate	-0.06 ± 0.02	-0.09 ± 0.04	0.23 ± 0.15	0.78 ± 0.10	1.68 ± 0.10	2.19 ± 0.14	3.52 ± 0.28

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 4(b). Long-Term Inundated Experiment: Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	NA	NA	0.15 ± 0.08	NA	NA	-0.16 ± 0.08	NA
Unamended/ Inoculated	NA	NA	1.48 ± 0.14	NA	NA	1.42 ± 0.28	NA
Amended/ Inoculated	2.48 ± 0.16	2.79 ± 0.26	2.81 ± 0.41	3.23 ± 0.51	3.50 ± 0.63	3 ± 0.76	4.08 ± 0.92
Amended/Inoc. + Exc. Nitrate	4.756 ± 0.4141	7.01 ± 0.9	11.9 ± 1.5	13.69 ± 1.17	15.86 ± 0.55	16.4 ± 0.16	18.1 ± 0.38

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 4(c). Long-Term Inundated Experiment: Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Milliliters of Gas Produce/Gram Cellulose						
	Incubation Time (Days)						
	733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	0.762 ± 0.4922	0.79 ± 0.5	0.08 ± 0.06	0.00 ± 0.04	0.26 ± 0.06	0.23 ± 0.05	0.26 ± 0.14
Unamended/ Inoculated	2.25 ± 0.19	2.25 ± 0.2	2.39 ± 0.18	2.386 ± 0.20	2.48 ± 0.31	2.08 ± 0.68	1.54 ± 0.41
Amended/ Inoculated	3.33 ± 1.42	4.12 ± 0.76	3.81 ± 0.67	3.618 ± 0.56	3.72 ± 0.63	2.72 ± 0.11	2.75 ± 0.17
Amended/Inoc. + Exc. Nitrate	17.51 ± 0.5	16.6 ± 0.6	15.9 ± 0.6	14.94 ± 0.6	12.0 ± 0.40	11.1 ± 0.4	10.2 ± 0.3

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 5(a). Long-Term Inundated Experiment: Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164	200
Unamended/ Uninoculated	2.48 ± 0.10	3.92 ± 0.06	4.21 ± 0.02	3.93 ± 0.02	4.25 ± 0.02	0.00 ± 0.00	4.00 ± 0.02
Unamended/ Inoculated	1.96 ± 0.06	3.35 ± 0.16	3.94 ± 0.18	4.98 ± 0.18	6.87 ± 0.20	0.00 ± 0.00	8.30 ± 0.28
Amended/ Inoculated	-0.01 ± 0.02	4.62 ± 0.37	3.78 ± 1.29	20.4 ± 7.5	29.6 ± 5.0	36.6 ± 4.2	40.8 ± 5.4
Amended/Inoc. + Exc. Nitrate	-0.04 ± 0.02	6.88 ± 0.38	32.2 ± 4.2	61.4 ± 3.6	83.6 ± 2.7	96.2 ± 4.0	95.6 ± 6.0

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 5(b). Long-Term Inundated Experiment: Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	0.00 ± 0.00	NA	4.44 ± 0.06	NA	NA	4.20 ± 0.06	NA
Unamended/ Inoculated	0.00 ± 0.00	NA	10.94 ± 0.26	NA	NA	12.1 ± 0.2	NA
Amended/ Inoculated	37.0 ± 8.2	36.6 ± 8.2	41.4 ± 7.8	39.6 ± 7.0	38.0 ± 7.6	33.3 ± 7.2	31.8 ± 6.6
Amended/Inoc. + Exc. Nitrate	110 ± 7	124 ± 10	118 ± 11	126 ± 16.4	126 ± 19	125 ± 21	152 ± 26

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 5(c). Long-Term Inundated Experiment: Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	4.56 ± 0.07	4.67 ± 0	3.83 ± 0.03	4.43 ± 0.06	4.61 ± 0.14	4.70 ± 0.16	5.19 ± 0.18
Unamended/ Inoculated	13.4 ± 0.2	14.5 ± 0.1	14.4 ± 0.1	13.8 ± 0.2	16.2 ± 0.1	12.9 ± 0.5	16.3 ± 1.3
Amended/ Inoculated	26.8 ± 5.4	26.2 ± 4.3	22.0 ± 2.90	17.0 ± 2.4	21.9 2.1	26.8 ± 3.5	17.7 ± 1.8
Amended/Inoc. + Exc. Nitrate	176 ± 1	169 ± 2	186 ± 8	155 ± 15	165 ± 44	150 44	162 ± 39

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 6(a). Long-Term Inundated Experiment: Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days)																								
	0			45			69			104			132			164			200						
Unamended/ Uninoculated	1.52	±	0.31	1.76	±	0.10	4.48	±	2.36	1.76	±	0.15	1.82	±	0.11	NA			2.32	±	0.03				
Unamended/ Inoculated	2.04	±	0.58	1.38	±	0.16	4.00	±	0.80	8.32	±	0.44	11.9	±	0.6	NA			21.5	±	1.2				
Amended/ Inoculated	-0.54	±	0.02	-6.12	±	0.63	-2.60	±	1.17	12.4	±	2.2	31.4	±	3.8	57.8	±	1.2	69.8	±	1.2				
Amended/Inoc. + Exc. Nitrate	-0.32	±	0.08	-3.32	±	0.24	2.20	±	0.63	27.2	±	4.6	72.0	±	16.7	105	±	9	116	±	6				

*All values have been corrected with specific controls for gas production in the absence of cellulose

NA = not analyzed

Table 6(b). Long-Term Inundated Experiment: Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	NA	NA	2.74 ± 0.17	NA	NA	2.50 ± 0.40	NA
Unamended/ Inoculated	NA	NA	35.9 ± 2.2	NA	NA	52.0 ± 2.6	NA
Amended/ Inoculated	62.8 ± 1.6	100 ± 2	102 ± 2	122 ± 1	130 ± 2	133 ± 2.2	138 ± 2
Amended/Inoc. + Exc. Nitrate	116.8 ± 6	125 ± 1	122 ± 6	128.2 ± 6	129 ± 6	128 ± 4.51	137 ± 5

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 6(c). Long-Term Inundated Experiment: Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	4.026 ± 0.2138	3.91 ± 0.3	3.54 ± 0.34	3.30 ± 0.32	11.70 ± 0.80	5.23 ± 0.19	4.65 ± 0.56
Unamended/ Inoculated	60.72 ± 3.0	68.4 ± 4.6	69.9 ± 5.0	69.6 ± 4.80	73.9 ± 14.7	77.9 ± 13.1	73.2 ± 11.1
Amended/ Inoculated	175 ± 10	164 ± 8	140 ± 11	168.8 ± 11	101 ± 11	101 ± 11	114 ± 16
Amended/Inoc. + Exc. Nitrate	136.3 ± 5	149 ± 3	137 ± 1	154 ± 7	233 ± 5	226 ± 56	210 ± 58

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 7(a). Long-Term Inundated Experiment: Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164	200
Unamended/ Uninoculated	2.38 ± 0.08	3.74 ± 0.03	3.92 ± 0.02	3.63 ± 0.02	3.83 ± 0.04	NA	3.59 ± 0.04
Unamended/ Inoculated	2.11 ± 0.04	3.41 ± 0.04	3.34 ± 0.02	3.01 ± 0.14	3.97 ± 0.10	NA	5.47 ± 0.34
Amended/ Inoculated	-0.06 ± 0.00	3.79 ± 0.04	-3.28 ± 0.71	7.22 ± 1.99	18.2 ± 1.6	24.2 ± 0.8	26.0 ± 0.8
Amended/Inoc. + Exc. Nitrate	0.47 ± 0.01	4.29 ± 0.07	6.10 ± 3.58	19.7 ± 6.7	25.8 ± 6.4	45.4 ± 8.0	61.4 ± 8.2

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 7(b). Long-Term Inundated Experiment: Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Carbon Dioxide (µmoles/gram cellulose)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	NA	NA	3.53 ± 0.04	NA	NA	3.61 ± 0.05	NA
Unamended/ Inoculated	NA	NA	6.14 ± 0.30	NA	NA	9.68 ± 0.24	NA
Amended/ Inoculated	26.6 ± 2.0	33.6 ± 0.4	23.2 ± 0.6	36.2 ± 0.3	43.2 ± 0.4	44.4 ± 0.63	44.4 ± 1.0
Amended/Inoc. + Exc. Nitrate	56.2 ± 13.6	92.8 ± 8.6	76.4 ± 8.8	129 ± 13	163 ± 13	181 ± 8	190 ± 4

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 7(c). Long-Term Inundated Experiment: Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine]	Carbon Dioxide (µmoles/gram cellulose)						
	733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	3.45 ± 0.06	3.39 ± 0.0	3.31 ± 0.04	3.13 ± 0.02	8.29 ± 3.77	4.56 ± 0.26	3.00 ± 0.15
Unamended/ Inoculated	11.8 ± 0.3	12.8 ± 0.5	14.0 ± 0.5	13.9 ± 1.0	24.0 ± 1.7	26.1 ± 2.2	27.4 ± 5.8
Amended/ Inoculated	49.1 ± 0.6	51.1 ± 0.5	52.0 ± 1.0	49.2 ± 0.8	66.9 ± 1.1	55.4 ± 2.6	54.4 ± 3.5
Amended/Inoc. + Exc. Nitrate	205 ± 4	187 ± 8	212 ± 2	194 ± 4	251 ± 5	236 ± 42	219 ± 75

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 8(a). Long-Term Inundated Experiment: Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164	200
Unamended/ Uninoculated	2.04 ± 0.13	0.98 ± 0.04	0.92 ± 0.08	0.64 ± 0.07	0.66 ± 0.12	NA	0.22 ± 0.09
Unamended/ Inoculated	1.86 ± 0.12	0.62 ± 0.04	0.84 ± 0.03	2.56 ± 0.50	8.06 ± 2.38	NA	8.28 ± 0.20
Amended/ Inoculated	-0.40 ± 0.16	-1.04 ± 0.07	0.84 ± 1.37	11.8 ± 0.9	48.7 ± 1.6	23.6 ± 2.0	31.8 ± 2.0
Amended/Inoc. + Exc. Nitrate	-0.72 ± 0.13	-2.36 ± 0.34	0.20 ± 0.85	5.80 ± 1.02	15.60 ± 1.26	22.6 ± 1.5	35.0 ± 2.8

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 8(b). Long-Term Inundated Experiment: Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Carbon Dioxide (µmoles/gram cellulose)						
	228	264	297	356	411	481	591
Unamended/ Uninoculated	NA	NA	0.84 ± 0.15	NA	NA	0.42 ± 0.00	NA
Unamended/ Inoculated	NA	NA	15.0 ± 0.6	NA	NA	27.0 ± 1.0	NA
Amended/ Inoculated	25.0 ± 2.20	50.8 ± 2.0	58.8 ± 2.8	66.8 ± 3.0	82.8 ± 5.4	82.4 ± 5.4	87.8 ± 5.0
Amended/Inoc. + Exc. Nitrate	50.6 ± 3.4	76.8 ± 5.4	116 ± 10	191 ± 24	288 ± 16	326 ± 8	363 ± 8

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Table 8(c). Long-Term Inundated Experiment: Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*.

Treatments* [Brine/Bentonite]	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 733	853	1034	1228	2718	3464	3929
Unamended/ Uninoculated	0.80 ± 0.0859	1.00 ± 0	0.28 ± 0.1	4.70 ± 4.90	8.72 ± 0.55	8.05 ± 4.49	10.1 ± 8.0
Unamended/ Inoculated	42.9 ± 0.8	45.5 ± 0.8	52.6 ± 2.7	55.16 ± 1.40	59 ± 7.1	58.6 ± 3.2	57.7 ± 4.5
Amended/ Inoculated	85.1 ± 5.4	96.2 ± 5.1	93.6 ± 5.2	99.4 ± 4.4	83.6 ± 8.2	76.7 ± 3.0	86.0 ± 5.6
Amended/Inoc. + Exc. Nitrate	387 ± 12	385 ± 14	384 ± 16	370 ± 14	350 ± 36	325 ± 14	266 ± 17

*All values have been corrected with specific controls for gas production in the absence of cellulose
NA = not analyzed

Appendix B

Organic Acids Produced in Inundated Cellulose Samples

Table 1. Organic acids detected in anaerobic inundated cellulose samples (latest data is in bold (3561 days incubation)).

Anaerobic Treatment & Incubation Time (days)	Organic Acid (mM)								
	Acetic	Butyric	Formic	Fumaric	Lactic	Oxalic	Oxalacetic	Propionic	Succinic
Unamended									
885	0.28	nd	0.23	nd	0.05	nd	nd	nd	nd
1228	1.38	nd	1.74	nd	0.14	nd	nd	nd	nd
3561	**0.20**	**nd**	**0.13**	**0.01**	**nd**	**nd**	**nd**	**nd**	**nd**
Unamended/Inoculated									
885	1.06	nd	nd	nd	0.29	nd	nd	nd	nd
1228	3.48	nd	nd	nd	0.26	nd	nd	nd	nd
3561	**6.17**	**nd**	**nd**	**0.17**	**0.50**	**nd**	**nd**	**0.02**	**nd**
Amended/Inoculated									
885	3.73	0.16	0.48	nd	0.67	nd	nd	0.10	nd
1228	3.90	nd	1.02	nd	0.44	nd	nd	nd	nd
3561	**6.99**	**6.38**	**0.03**	**0.35**	**0.02**	**nd**	**nd**	**0.20**	**nd**
Amended/Inoculated + Excess Nitrate*									
885	nd	nd	nd	nd	nd	nd	nd	0.18	nd
1228	1.90	nd	5.95	nd	1.41	nd	nd	nd	nd
3561	**5.21**	**5.49**	**3.26**	**2.94**	**3.03**	**0.163**	**nd**	**0.43**	**nd**

*Isocaproic acid and two unknown acids with pKa, MW > butyric were detected at significant quantities at 3561 days.

Table 2. Organic acids detected in anaerobic inundated cellulose samples w/ bentonite (latest data is in bold (3561 days incubation)).

Anaerobic + Bentonite	Organic Acid (mM)								
Treatment & Incubation Time (days)	Acetic	Butyric	Formic	Fumaric	Lactic	Oxalic	Oxalacetic	Propionic	Succinic
Unamended									
885	0.20	nd	0.13	nd	0.10	nd	nd	nd	nd
1228	0.40	0.25	0.52	nd	0.06	nd	nd	nd	nd
3561	**nd**	**nd**	**0.54**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Unamended/Inoculated									
885	7.78	0.07	0.54	nd	2.42	nd	nd	0.17	0.30
1228	2.41	nd	0.65	nd	0.26	nd	nd	nd	nd
3561	**4.55**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Amended/Inoculated*									
885	6.41	0.59	0.98	nd	2.03	nd	nd	0.32	nd
1228	2.54	nd	1.80	nd	nd	nd	nd	nd	0.02
3561	**38.6**	**49.8**	**9.05**	**5.35**	**nd**	**4.04**	**0.38**	**nd**	**nd**
Amended/Inoculated + Excess Nitrate									
885	12.6	0.97	3.50	nd	20.64	nd	nd	4.52	nd
1228	8.36	1.20	15.5	nd	4.90	nd	nd	0.13	nd
3561	**8.22**	**nd**	**9.05**	**5.35**	**nd**	**nd**	**0.06**	**nd**	**nd**

*Isobutyric acid (50 mM), valeric (39 mM), glucose, and three unknown acids with pKa, MW > butyric were detected at significant quantities at 3561 days.

Table 3. Organic acids detected in initially aerobic inundated cellulose samples (latest data is in bold (3561 days incubation)).

Initially Aerobic Treatment & Incubation Time (days)	Organic Acid (mM)								
	Acetic	Butyric	Formic	Fumaric	Lactic	Oxalic	Oxalacetic	Propionic	Succinic
Unamended									
885	0.18	nd	0.39	nd	0.10	nd	nd	0.12	0.01
1228	1.30	nd	1.85	nd	0.36	nd	nd	nd	nd
3561	**0.10**	**nd**	**0.72**	**nd**	**nd**	**nd**	**nd**	**nd**	**0.01**
Unamended/Inoculated									
885	0.07	nd	0.04	nd	0.52	nd	nd	0.08	nd
1228	2.01	nd	0.87	nd	0.09	nd	nd	nd	nd
3561	**0.36**	**nd**	**0.26**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Amended/Inoculated*									
885	1.72	0.05	0.26	nd	1.00	nd	nd	0.30	0.52
1228	4.45	nd	2.52	nd	0.69	nd	nd	0.20	nd
3561	**6.91**	**nd**	**nd**	**1.99**	**nd**	**nd**	**0.18**	**nd**	**nd**
Amended/Inoculated + Excess Nitrate**									
885	1.23	0.09	0.33	nd	0.30	nd	nd	0.82	nd
1228	4.43	nd	3.41	nd	1.57	nd	nd	0.12	nd
3561	**11.0**	**nd**	**nd**	**nd**	**nd**	**nd**	**0.32**	**nd**	**nd**

*Malonic acid was detected at 3561 days (1.13 mM) and a significant acid (unknown) with pKa, MW>butyric.
**Malonic acid was detected at 3561 days (4.72 mM) and valeric acid (8.82 mM) as well as two acids of unknown identity (pKa > butyric).

Table 4. Organic acids detected in initially aerobic inundated cellulose samples w/ bentonite (latest data is in bold (3561 days incubation)).

Initially Aerobic + Bentonite Treatment & Incubation Time (days)	Organic Acid (mM)								
	Acetic	Butyric	Formic	Fumaric	Lactic	Oxalic	Oxalacetic	Propionic	Succinic
Unamended									
885	0.09	nd	0.16	nd	0.04	nd	nd	nd	nd
1228	1.08	nd	2.00	nd	0.10	nd	nd	nd	nd
3561	**0.13**	**0.21**	**0.63**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Unamended/Inoculated*									
885	3.95	nd	0.23	nd	0.86	nd	nd	nd	nd
1228	3.16	nd	2.02	nd	0.47	nd	nd	nd	nd
3561	**5.91**	**0.11**	**nd**	**nd**	**0.13**	**nd**	**nd**	**nd**	**nd**
Amended/Inoculated**									
885	4.61	0.20	0.24	nd	0.66	nd	nd	nd	nd
1228	3.66	nd	2.56	nd	1.85	nd	nd	nd	nd
3561	**7.70**	**nd**	**nd**	**nd**	**nd**	**nd**	**0.17**	**nd**	**nd**
Amended/Inoculated + Excess Nitrate***									
885	0.31	nd	nd	nd	nd	nd	nd	nd	nd
1228	1.30	nd	0.39	nd	0.06	nd	nd	nd	nd
3561	**5.00**	**nd**	**nd**	**nd**	**nd**	**nd**	**0.13**	**nd**	**nd**

*Malonic acid was detected at 3561 days (0.45 mM); ** 3561 days - malonic acid, 2.56 mM; *** 3561 days - malonic acid, 0.33 mM

Appendix C

Gas Produced in Humid Cellulose Treatments

Total gas and carbon dioxide produced in the humid experiment is presented in Table 1-10 as follows:

Tables 1(a,b): Total gas produced in initially aerobic humid samples without bentonite.

Tables 2(a,b): Total gas produced in initially aerobic humid samples with bentonite.

Table 3: Total gas produced in anaerobic samples without bentonite.

Table 4: Total gas produced in anaerobic samples with bentonite.

Tables 5(a,b): Carbon dioxide produced in initially aerobic humid samples without bentonite.

Tables 6(a,b): Carbon dioxide produced in initially aerobic humid samples with bentonite.

Table 7: Carbon dioxide produced in anaerobic samples without bentonite.

Table 8: Carbon dioxide produced in anaerobic samples with bentonite.

Tables 9(a,b): Carbon dioxide produced in initially aerobic samples with values corrected by control samples (corrected for gas production in the absence of cellulose).

Table 10: Carbon dioxide produced in anaerobic samples with values corrected by control samples (corrected for gas production in the absence of cellulose).

Table 1(a). 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
Control					
Empty bottle	7.15	-0.22	0.28	1.08	1.19
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52
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
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
Unamended inoculated	7.50 ± 0.33	-0.31 ± 1.62	0.19 ± 0.33	-0.61 ± 0.25	0.31 ± 0.05
Amended uninoculated	6.98 ± 0.18	-0.03 ± 1.68	-0.23 ± 0.10	-0.29 ± 0.13	0.20 ± 0.10
Amended inoculated	7.39 ± 0.11	-0.21 ± 1.57	-0.02 ± 0.18	-0.39 ± 0.07	0.13 ± 0.17
Carbon Source: Cellulose + Glucose					
Amended uninoculated	6.45 ± 0.11	-2.08	0.75 ± 0.00	-0.06 ± 0.21	0.02 ± 0.14
Amended inoculated	7.03 ± 0.07	-1.92 ± 0.11	0.79 ± 0.33	0.35 ± 0.23	0.15 ± 0.04
Amended uninoculated (RG salt)	NA	3.12	1.99 ± 1.90	-0.80 ± 0.11	-0.34 ± 0.33
Carbon Source: Cellulose + Succinate					
Amended uninoculated (w/ acetylene)	19.5	NA	0.64	-0.10	1.66
Amended uninoculated (w/o acetylene)	5.15	-2.08	0.98	-0.37	-0.08
Amended inoculated (w/ acetylene)	12.9	NA	1.17	0.35	-0.34
Amended inoculated (w/o acetylene)	5.88	-2.29	1.27	0.05	0.17

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

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

Treatments (without bentonite)	Volume of Gas Produced (ml/sample)			
	Incubation Time (Days)			
	804	2553	3009	3334
Control				
Empty bottle	2.51	0.73	3.37	1.24
Blank (tube+brine only)	0.32	-0.89	1.88	-1.18
No cellulose (salt/ inoculum/ tube+brine)	3.01 ± 0.22	-0.48 ± 0.87	0.20 ± 0.02	-0.62 ± 0.05
Carbon Source: Cellulose Only				
Unamended uninoculated	1.10 ± 0.17	0.77 ± 0.16	3.84 0.38	-0.73 ± 0.12
Unamended inoculated	1.29 ± 0.25	1.15 ± 0.39	2.91 ± 0.49	-0.96 ± 0.14
Amended uninoculated	0.50 ± 0.21	1.26 ± 0.24	2.12 ± 0.36	-0.73 ± 0.07
Amended inoculated	0.77 ± 0.18	0.91 ± 0.12	1.33 ± 0.27	-0.46 ± 0.40
Carbon Source: Cellulose + Glucose				
Amended uninoculated	0.13 ± 0.28	1.05 ± 0.22	1.10 ± 0.77	NA
Amended inoculated	0.50 ± 0.22	1.15 ± 0.00	1.31 ± 0.40	NA
Amended uninoculated (RG salt)	0.18 ± 0.40	2.87 ± 0.99	2.09 ± 0.29	NA
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	-0.10	1.98	1.05	NA
Amended uninoculated (w/o acetylene)	0.72	0.74	0.22	NA
Amended inoculated (w/ acetylene)	-0.10	NA	NA	NA
Amended inoculated (w/o acetylene)	0.72	2.18	1.25	NA

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

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

Treatments (with bentonite)	Volume of Gas Produced (ml/sample)				
	Incubation Time (Days)				
	6	120	317	399	593
Control					
Empty bottle	7.15	-0.22	0.28	1.08	1.19
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52
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
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
Unamended inoculated	6.35 ± 0.48	-0.59 ± 1.52	0.11 ± 0.13	-0.40 ± 0.08	0.06 ± 0.12
Amended uninoculated	6.09 ± 0.00	0.08 ± 1.85	0.01 ± 0.13	-0.15 ± 0.13	0.11 ± 0.05
Amended inoculated	7.81 ± 0.26	0.78 ± 1.56	0.35 ± 0.31	0.02 ± 0.24	0.11 ± 0.14
Carbon Source: Cellulose + Glucose					
Amended uninoculated	6.35 ± 0.04	-1.98	-1.45 ± 0.29	-0.09 ± 0.25	0.07 ± 0.07
Amended inoculated	7.29 ± 0.11	-1.45 ± 0.07	-0.42 ± 0.07	0.23 ± 0.11	0.20 ± 0.04
Amended uninoculated (RG salt)	NA	2.60	1.78 ± 1.57	-0.82 ± 0.21	0.13 ± 0.04
Carbon Source: Cellulose + Succinate					
Amended uninoculated (w/ acetylene)	18.7	NA	0.74	-0.15	0.07
Amended uninoculated (w/o acetylene)	5.56	-1.98	1.71	-0.76	0.27
Amended inoculated (w/ acetylene)	18.0	NA	2.00	0.05	0.10
Amended inoculated (w/o acetylene)	6.82	-2.29	2.30	0.67	-0.11

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

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

Treatments (with bentonite)	Volume of Gas Produced (ml/sample)			
	Incubation Time (Days)			
	804	2553	3009	3334
Control				
Empty bottle	2.51	0.73	3.37	1.24
Blank (tube+brine only)	0.32	-0.89	1.88	-1.18
No cellulose (salt/ inoculum/ tube+brine)	1.68 ± 0.95	1.47 ± 0.51	1.11 ± 0.48	-0.80 ± 0.14
Carbon Source: Cellulose Only				
Unamended uninoculated	-0.01 ± 0.10	1.36 ± 0.25	4.67 ± 0.34	2.21 ± 0.16
Unamended inoculated	0.02 ± 0.32	1.05 ± 0.30	2.39 ± 0.69	0.76 ± 0.15
Amended uninoculated	0.19 ± 0.27	2.05 ± 0.99	1.36 ± 0.29	-0.46 ± 0.03
Amended inoculated	0.51 ± 0.19	1.15 ± 0.18	0.43 ± 0.48	0.02 ± 0.00
Carbon Source: Cellulose + Glucose				
Amended uninoculated	1.03 ± 0.76	1.41 ± 0.40	3.38 ± 0.76	NA
Amended inoculated	1.28 ± 0.83	1.20 ± 0.04	NA	NA
Amended uninoculated (RG salt)	1.59 ± 0.76	1.26 ± 0.37	4.06 ± 0.22	NA
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	-0.63	1.46	2.18	NA
Amended uninoculated (w/o acetylene)	-0.33	0.84	2.30	NA
Amended inoculated (w/ acetylene)	0.55	NA	NA	NA
Amended inoculated (w/o acetylene)	1.16	0.74	-0.19	NA

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

Table 3. Total Volume of Gas Produced in Anaerobic Humid Treatments (without bentonite)

Treatments (without bentonite)	Total Volume of Gas Produced (ml/sample) Days						
	6	100	140	415	2156	2616	2945
Control							
Empty bottle	7.98 ± 0.59	4.62 ± 0.54	3.61 ± 0.66	2.01 ± 1.04	0.72	0.29	2.51 ± 0.46
Blank (tube+brine only)	6.85 ± 0.38	3.81 ± 0.34	2.80 ± 0.27	0.37 ± 1.02	-0.89	NA	-0.85 ± 0.11
No cellulose (salt/ inoculum/ tube+brine)	6.49 ± 0.04	3.07 ± 0.07	1.56 ± 0.63	2.76 ± 0.88	5.53	2.33	-0.57 ± 0.93
Carbon Source: Cellulose Only							
Unamended uninoculated	7.33 ± 0.80	1.59 ± 1.25	0.01 ± 1.07	-2.26 ± 0.17	0.09 ± 0.18	2.51 ± 0.59	-0.64 ± 0.73
Unamended inoculated	9.49 ± 0.45	2.40 ± 1.23	1.17 ± 1.39	-0.28 ± 1.23	2.00 ± 1.02	1.42 ± 0.56	-0.50 ± 0.31
Amended uninoculated	7.50 ± 0.13	0.93 ± 1.25	-0.92 ± 1.12	-1.87 ± 0.24	1.70 ± 1.05	1.86 ± 1.01	-0.57 ± 0.74
Amended inoculated	7.64 ± 0.37	0.89 ± 0.69	-0.54 ± 1.03	-1.07 ± 1.15	0.43 ± 0.00	0.19 ± 0.15	1.48 ± 1.14
Amended inoculated (w/ acetylene)	20.4 ± 0.1	16.6 ± 0.6	14.95 ± 0.48	7.15 ± 5.15	0.32 ± 0.08	0.25 ± 0.23	NA
Carbon Source: Cellulose + Glucose							
Amended uninoculated	6.55 ± 0.63	3.82 ± 0.73	2.07 ± 0.66	-0.51 ± 0.44	2.50 ± 0.62	1.57 ± 0.62	NA
Amended inoculated	7.18 ± 0.04	4.83 ± 0.11	1.77 ± 1.10	0.68 ± 1.90	3.27 ± 1.74	2.34 ± 1.89	NA
Amended uninoculated (RG salt)	6.60 ± 0.00	2.35 ± 1.90	0.18 ± 2.28	0.09 ± 1.48	3.83 ± 0.51	1.27 ± 0.15	NA
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	18.9 ± 0.1	10.8 ± 4.1	3.66 ± 1.90	8.11 ± 5.24	NA	1.60	NA
Amended uninoculated (w/o acetylene)	6.30 ± 0.19	4.50 ± 0.29	4.21 ± 0.37	2.49 ± 1.80	8.69	NA	NA
Amended inoculated (w/ acetylene)	18.7 ± 0.1	7.27 ± 6.63	6.83 ± 6.43	6.46 ± 4.32	5.70 ± 3.19	3.25	NA
Amended inoculated (w/o acetylene)	5.67 ± 0.04	1.70 ± 1.72	0.67 ± 1.71	2.46 ± 1.61	7.05	NA	NA

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

Table 4. Total Volume of Gas Produced in Anaerobic Humid Treatments (with bentonite)

Treatments (with bentonite)	Total Volume of Gas Produced (ml/sample)						
	Days						
	6	100	140	415	2156	2616	2945
Control							
Empty bottle	7.98 ± 0.59	4.62 ± 0.54	3.61 ± 0.66	2.01 ± 1.04	0.72	0.29	2.51 ± 0.46
Blank (tube+brine only)	6.85 ± 0.38	3.81 ± 0.34	2.80 ± 0.27	0.37 ± 1.02	-0.89	n/a	-0.85 ± 0.11
No cellulose (salt/ inoculum/ tube+brine)	6.18 ± 0.19	4.60 ± 0.37	0.87 ± 1.85	1.93 ± 0.37	-1.79	0.78	-0.83 ± 0.11
Carbon Source: Cellulose Only							
Unamended uninoculated	7.22 ± 0.25	2.91 ± 0.90	1.40 ± 1.22	-0.65 ± 1.05	0.98 ± 0.52	-1.04 ± 0.28	0.00 ± 0.79
Unamended inoculated	6.63 ± 0.03	6.36 ± 1.22	5.86 ± 3.11	11.22 ± 5.42	6.37 ± 2.06	-0.59 ± 0.62	-3.09 ± 0.50
Amended uninoculated	6.18 ± 0.08	3.72 ± 0.51	1.57 ± 1.11	-0.79 ± 1.06	1.05 ± 0.47	2.92 ± 0.56	-1.24 ± 0.63
Amended inoculated	6.81 ± 0.12	10.4 ± 1.7	15.31 ± 1.70	8.60 ± 2.97	2.58 ± 1.49	1.52 ± 0.20	-2.19 ± 1.18
Amended inoculated (w/ acetylene)	18.2 0.3	17.2 ± 0.3	15.54 ± 0.74	7.32 ± 5.11	8.16 ± 4.20	6.22 ± 2.44	NA
Carbon Source: Cellulose + Glucose							
Amended uninoculated	7.18 ± 0.04	3.18 ± 1.10	-0.39 ± 0.77	-1.91 ± 0.00	0.19	-0.43	NA
Amended inoculated	6.97 ± 0.11	9.79 ± 3.73	7.87 ± 4.78	7.46 ± 6.62	7.73 ± 4.82	7.73 ± 4.53	NA
Amended uninoculated (RG salt)	7.18 ± 0.14	5.51 ± 0.04	3.27 ± 0.29	2.43 ± 0.95	6.23 ± 1.15	5.01 ± 0.94	NA
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	19.9 ± 0.4	8.36 ± 2.14	4.75 ± 3.05	-1.54 ± 0.03	2.34 ± 0.62	1.51 ± 0.10	NA
Amended uninoculated (w/o acetylene)	7.91 ± 0.48	4.26 ± 1.10	3.20 ± 1.03	3.86 ± 0.24	3.37 ± 2.03	2.86 ± 1.60	NA
Amended inoculated (w/ acetylene)	19.6 ± 0.1	16.7 ± 0.5	8.59 ± 4.01	5.36 ± 5.00	10.04	1.46	NA
Amended inoculated (w/o acetylene)	6.76 ± 0.18	10.2 ± 0.3	10.41 ± 1.22	3.84 ± 1.94	-0.53	0.50	NA

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

Table 5(a). 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
Control					
Empty bottle	4.05	4.97	4.96	4.94	4.87
Blank (tube+brine only)	4.18	4.64	4.54	4.63	3.00
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
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
Unamended inoculated	11.7 ± 0.1	56.0 ± 4.4	72.6 ± 11.4	65.5 ± 11.5	45.3 ± 8.1
Amended uninoculated	14.0 ± 1.1	28.1 ± 0.8	24.1 ± 1.8	22.9 ± 2.6	17.4 ± 3.1
Amended inoculated	35.9 ± 1.3	42.4 ± 1.5	31.1 ± 2.4	24.8 ± 2.9	14.7 ± 2.4
Carbon Source: Cellulose + Glucose					
Amended uninoculated	12.7 ± 0.4	32.7	39.7 ± 0.6	38.6 ± 1.2	35.0 ± 3.07
Amended inoculated	28.3 ± 1.6	183 ± 98	236 ± 140	166 ± 96	79.8 ± 39.8
Amended uninoculated (RG salt)	NA	36.0	44.8 ± 0.1	46.5 ± 0.1	47.4 ± 2.6
Carbon Source: Cellulose + Succinate					
Amended uninoculated (w/ acetylene)	15.1	NA	28.8	27.7	21.0
Amended uninoculated (w/o acetylene)	15.7	26.0	22.7	19.7	14.4
Amended inoculated (w/ acetylene)	14.5	NA	1384	1450	1470
Amended inoculated (w/o acetylene)	15.8	42.4	40.0	38.2	29.5

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

Table 5(b). Production of Carbon Dioxide in Initially Aerobic Humid Treatments (without bentonite)

Treatments (without bentonite)	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 804	2553	3009	3334
Control				
Empty bottle	2.71	2.68	2.94	3.07
Blank (tube+brine only)	2.76	2.74	3.50	3.48
No cellulose (salt / inoculum/ tube+brine)	3.61 ± 0.18	3.55 ± 0.20	2.89 ± 0.08	2.87 ± 0.00
Carbon Source: Cellulose Only				
Unamended uninoculated	8.96 ± 1.82	8.73 ± 2.43	7.40 ± 1.66	5.99 ± 1.14
Unamended inoculated	27.6 ± 5.3	12 ± 3.25	10.4 ± 2.68	8.96 ± 2.41
Amended uninoculated	12.2 ± 2.7	6.08 ± 1.78	6.23 ± 1.88	5.94 ± 1.88
Amended inoculated	8.21 ± 1.75	4.48 ± 1.09	3.96 ± 0.56	3.35 ± 0.29
Carbon Source: Cellulose + Glucose				
Amended uninoculated	26.5 ± 4.5	29.83 ± 5.84	28.4 ± 10	NA
Amended inoculated	28.2 ± 9.0	9.1 ± 1.46	8.41 ± 2.77	NA
Amended uninoculated (RG salt)	39.4 ± 5.6	56.81 ± 3.99	61.0 ± 5.8	NA
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	16.8	22.12	NA	NA
Amended uninoculated (w/o acetylene)	7.06	4.75	3.25	NA
Amended inoculated (w/ acetylene)	1270	NA	NA	NA
Amended inoculated (w/o acetylene)	23.6	16.86	11.3	NA

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

Table 6(a). 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
Control					
Empty bottle	4.05	4.97	4.96	4.94	4.87
Blank (tube+brine only)	4.18	4.64	4.54	4.63	3.00
No cellulose (salt / inoculum/ tube+brine)	34.2 ± 0.8	164 ± 1	168 ± 8	144 ± 4	89.1 ± 0.8
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
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61
Amended uninoculated	15.2 ± 0.9	52.2 ± 1.8	49.9 ± 1.1	45.1 ± 2.4	33.2 ± 4.2
Amended inoculated	53.7 ± 2.4	1030 ± 80	1620 ± 30	1600 ± 40	1520 ± 40
Carbon Source: Cellulose + Glucose					
Amended uninoculated	14.8 ± 0.5	46.3	590 ± 364	625 ± 394	694 ± 438
Amended inoculated	44.9 ± 2.6	1590 ± 40	1240 ± 20	1250 ± 160	1240 ± 240
Amended uninoculated (RG salt)	NA	39.5	50.9 ± 1.3	54.6 ± 2.4	55.7 ± 6.7
Carbon Source: Cellulose + Succinate					
Amended uninoculated (w/ acetylene)	22.9	NA	50.0	50.8	46.1
Amended uninoculated (w/o acetylene)	21.7	47.7	50.4	46.8	43.6
Amended inoculated (w/ acetylene)	38.5	NA	1430	1470	1540
Amended inoculated (w/o acetylene)	52.8	1130	1460	1500	1520

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

Table 6(b). Production of Carbon Dioxide in Initially Aerobic Humid Treatments (with bentonite)

Treatments (with bentonite)	Carbon Dioxide (µmoles/sample)			
	Incubation Time (Days)			
	804	2553	3009	3334
Control				
Empty bottle	2.71	2.68	2.94	3.07
Blank (tube+brine only)	2.76	2.74	3.50	3.48
No cellulose (salt / inoculum/ tube+brine)	42.3 ± 3.0	16.13 ± 4.52	13.6 ± 4	10.6 ± 2.5
Carbon Source: Cellulose Only				
Unamended uninoculated	9.82 ± 0.15	9.98 ± 1.15	10.5 ± 0.3	10.2 ± 0.3
Unamended inoculated	184 ± 76	233 ± 152	258 ± 180	311 ± 228
Amended uninoculated	23.1 ± 5.5	22.1 ± 6.29	15.1 ± 6.9	12.0 ± 6.0
Amended inoculated	1470 ± 40	1059 ± 207	858 ± 219	626 ± 250
Carbon Source: Cellulose + Glucose				
Amended uninoculated	631 ± 401	53.8 ± 26.3	50.5 ± 27.5	NA
Amended inoculated	816 ± 355	964 ± 230	n/a ±	NA
Amended uninoculated (RG salt)	45.7 ± 8.6	82.0 ± 37.0	90.7 ± 45.3	NA
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	38.9	27.8	27.7	NA
Amended uninoculated (w/o acetylene)	37.3	34.0	30.3	NA
Amended inoculated (w/ acetylene)	1460	NA	NA	NA
Amended inoculated (w/o acetylene)	1400	631	320	NA

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

Table 7. Production of Carbon Dioxide in Anaerobic Humid Samples (without bentonite)

Treatments (without bentonite)	µmoles CO_2/Sample						
	Days						
	6	100	140	415	2156	2616	2945
Control							
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00	4.13	1.84	1.80 ± 0.09
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14	2.39	2.37 ± 0.04
Salt / inoculum/ tube+brine (no cellulose)	3.60 ± 0.01	5.90 ± 0.11	7.63 ± 1.08	16.4 ± 0.6	8.35	6.81	5.38 ± 1.97
Carbon Source: Cellulose Only							
Unamended uninoculated	4.07 ± 0.09	5.44 ± 0.10	6.22 ± 0.82	8.05 ± 0.18	15.8 ± 0.46	17.7 ± 0.3	16.5 ± 0.8
Unamended inoculated	11.3 ± 0.12	25.9 ± 3.8	36.1 ± 7.0	89.0 ± 24.4	163 ± 36	142 ± 28	120 ± 20
Amended uninoculated	3.34 ± 0.22	34.3 ± 1.44	39.8 ± 0.9	32.3 ± 1.5	13.5 ± 2.76	31.2 ± 7.0	25.1 ± 8.0
Amended inoculated	16.9 ± 1.15	36.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9	18.2 ± 1	33.6 ± 1.0	27.3 ± 2.7
Amended inoculated (w/ acetylene)	13.7 ± 1.3	38.5 ± 2.2	42.7 ± 2.5	61.0 ± 16.9	47.3 ± 17	76.5 ± 27.0	n/a
Carbon Source: Cellulose + Glucose							
Amended uninoculated	3.34 ± 0.27	23.5 ± 1.6	31.3 ± 0.0	38.6 ± 2.1	42.9 ± 5.2	54.9 ± 8.9	NA
Amended inoculated	17.7 ± 0.47	39.8 ± 0.2	42.2 ± 0.9	41.8 ± 4.2	52.8 ± 10.8	58.9 ± 12.2	NA
Amended uninoculated (RG salt)	4.07 ± 0.37	19.8 ± 2.4	28.9 ± 0.6	26.3 ± 2.9	47.8 ± 12.3	48.2 ± 19.7	NA
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	3.21 ± 0.04	22.5 ± 0.8	29.4 ± 2.5	28.8 ± 3.0	NA	33.8 ± 7.2	NA
Amended uninoculated (w/o acetylene)	3.19 ± 0.18	21.4 ± 0.2	27.9 ± 0.5	34.1 ± 2.5	984	NA	NA
Amended inoculated (w/ acetylene)	13.5 ± 0.7	78.1 ± 33.4	123 ± 63	308 ± 175	99.8	133 ± 79	NA
Amended inoculated (w/o acetylene)	14.8 ± 0.2	60.5 ± 16.0	106 ± 21	328 ± 78	1034	NA	NA

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

Table 8. Production of Carbon Dioxide in Anaerobic Humid Samples (with bentonite)

Treatments (with bentonite)	µmoles CO_2/Sample						
	Days						
	6	100	140	415	2156	2616	2945
Control							
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00	4.13	1.84	1.80 ± 0.09
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00	2.14	2.39	2.37 ± 0.04
Salt / inoculum/ tube+brine (no cellulose)	14.2 ± 0.51	36.6 ± 6.1	39.8 ± 5.5	51.6 ± 3.4	93.8	59.21 ± 14.1	63.9 ± 11.8
Carbon Source: Cellulose Only							
Unamended uninoculated	5.04 ± 0.15	12.1 ± 3.2	14.4 ± 3.6	26.5 ± 8.9	37.6 ± 19.1	70.5 ± 36.4	80.7 ± 40.6
Unamended inoculated	20.3 ± 0.2	93.7 ± 2.6	186 ± 6	434 ± 39	483 ± 133	650 ± 175	605 ± 134
Amended uninoculated	6.65 ± 0.80	39.2 ± 1.5	45.5 ± 1.5	49.6 ± 1.6	41.7 ± 3.2	70.3 ± 4.3	67.1 ± 10.1
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7	732 ± 47	682 ± 124.0
Amended inoculated (w/ acetylene)	26.8 ± 0.7	94.0 ± 18.6	123 ± 30	251 ± 92	558 ± 270	609 ± 273	NA
Carbon Source: Cellulose + Glucose							
Amended uninoculated	6.71 ± 0.12	44.5 ± 0.2	53.1 ± 0.4	64.3 ± 1.0	177	201 ± 4	NA
Amended inoculated	31.4 ± 0.7	396 ± 13	487 ± 1	584 ± 28	754 ± 94	641 ± 16	NA
Amended uninoculated (RG salt)	5.28 ± 0.45	45.9 ± 0.7	55.1 ± 1.4	74.9 ± 2.2	178 ± 3	209 ± 1	NA
Carbon Source: Cellulose + Succinate							
Amended uninoculated (w/ acetylene)	5.77 ± 0.60	0.00 ± 0.00	41.5 ± 3.1	36.7 ± 0.9	48.5 ± 0.5	75.0 ± 6.3	NA
Amended uninoculated (w/o acetylene)	8.58 ± 0.74	44.9 ± 1.6	51.5 ± 1.0	54.0 ± 2.0	79.4 ± 3.4	44.8 ± 0.6	NA
Amended inoculated (w/ acetylene)	27.7 ± 0.27	70.3 ± 2.7	114 ± 0	324 ± 30	447	568	NA
Amended inoculated (w/o acetylene)	28.0 ± 0.82	237 ± 2	317 ± 6	516 ± 0	1356	944 ± 110	NA

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

Table 9(a). Carbon Dioxide Produced in Initially Aerobic Humid Treatments

Treatments ***without bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 6	120	317	399	593
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
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
Amended inoculated	35.9 ± 1.3	42.4 ± 1.5	31.1 ± 2.4	24.8 ± 2.9	14.7 ± 2.4
*Unamended inoculated (corrected)**	3.77 ± 0.22	42.1 ± 4.4	62.0 ± 11.4	56.3 ± 11.5	38.9 ± 8.1
*Amended inoculated (corrected)**	28.0 ± 1.3	28.5 ± 1.5	20.5 ± 2.4	15.6 ± 2.9	8.32 ± 2.41

Treatments ***with bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 6	120	317	399	593
Control					
No cellulose (salt/ inoculum/ tube+brine)	34.2 ± 0.8	164 ± 1	168 ± 8	144 ± 4	89.1 ± 0.8
Carbon Source: Cellulose					
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61
Amended inoculated	53.7 ± 2.4	1033 ± 76	1623 ± 26	1600 ± 44	1520 ± 40
*Unamended inoculated (corrected)**	-13.5 ± 0.8	8.00 ± 5.41	105 ± 26	124 ± 44	130 ± 61
*Amended inoculated (corrected)**	19.5 ± 2.5	869 ± 76	1455 ± 28	1456 ± 44	1431 ± 40

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

Table 9(b). Carbon Dioxide Produced in Initially Aerobic Humid Treatments

Treatments ***without bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 804	2553	3009	3334
Control				
No cellulose (salt/ inoculum/ tube+brine)	3.61 ± 0.18	3.55 ± 0.2	2.89 ± 0.08	2.87 ± 0
Carbon Source: Cellulose				
Unamended inoculated	27.6 ± 5.3	12 ± 3.25	10.4 ± 2.68	8.96 ± 2.41
Amended inoculated	8.21 ± 1.75	4.48 ± 1.09	3.96 ± 0.56	3.35 ± 0.29
*Unamended inoculated (corrected)**	23.99 ± 5.303	8.45 ± 3.256	7.51 ± 2.681	6.09 ± 2.41
*Amended inoculated (corrected)**	4.6 ± 1.759	0.93 ± 1.108	1.07 ± 0.566	0.48 ± 0.29

Treatments ***with bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 804	2553	3009	3334
Control				
No cellulose (salt/ inoculum/ tube+brine)	42.3 ± 3	16.13 ± 4.52	13.6 ± 4	10.6 ± 2.52
Carbon Source: Cellulose				
Unamended inoculated	184 ± 76	233 ± 152	258 ± 180	311 ± 228
Amended inoculated	1470 ± 40	1059 ± 207	858 ± 219	626 ± 250
*Unamended inoculated (corrected)**	141.7 ± 76.06	216.9 ± 152.1	244.4 ± 180	300.4 ± 228
*Amended inoculated (corrected)**	1428 ± 40.11	1043 ± 207	844.4 ± 219	615.4 ± 250

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

Table 10. Carbon Dioxide Produced in Anaerobic Humid Samples

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

Treatments ***with bentonite***	Carbon dioxide (µmoles/ gram cellulose) Days						
	6	100	140	415	2156	2616	2945
Control							
No cellulose (salt/ inoculum/ tube+brine)	14.2 ± 0.5	36.6 ± 6.1	39.8 ± 5.5	51.6 ± 3.4	93.8	59.2 ± 14.1	63.9 ± 11.8
Carbon Source: Cellulose							
Unamended inoculated	20.3 ± 0.2	94 ± 3	186 ± 6	434 ± 39	483 ± 133	650 ± 175	605 ± 134
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152	554 ± 35.7	732 ± 47	682 ± 124
*Unamended inoculated (corrected)**	6.10 ± 0.55	57.1 ± 6.6	146 ± 8	382 ± 39	389 ± 133	591 ± 176	541 ± 135
*Amended inoculated (corrected)**	18.0 ± 1.2	213 ± 31	433 ± 26	390 ± 152	460 ± 36	673 ± 49	618 ± 125

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

Appendix D

Gas Produced in Samples Containing Plastic and Rubber Materials.

Total gas and carbon dioxide produced in samples containing plastic and rubber materials is presented in tables 1-10 as follows (values are not corrected for dissolved CO_2 and are headspace (gaseous) CO_2 only; values are total gas or CO_2 produced per sample):

Table 1: Total gas produced in samples containing polyethylene.

Table 2: Total gas produced in samples containing polyvinylchloride.

Table 3: Total gas produced in samples containing neoprene.

Table 4: Total gas produced in samples containing unleaded hypalon.

Table 5: Total gas produced in samples containing leaded hypalon.

Table 6: Carbon dioxide produced in samples containing polyethylene.

Table 7: Carbon dioxide produced in samples containing polyvinylchloride.

Table 8: Carbon dioxide produced in samples containing neoprene.

Table 9: Carbon dioxide produced in samples containing unleaded hypalon.

Table10: Carbon dioxide produced in samples containing leaded hypalon.

Table 1. Total Volume of Gas Produced in Samples Containing Polyethylene.

	Milliliters of Gas Produced/Sample						
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 ± 0.4
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 ± 0.61	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 2. Total Volume of Gas Produced in Samples Containing Polyvinylchloride.

	Milliliters of Gas Produced/Sample						
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 ± 0.4
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	1.87 ± 0.13	1.67 ± 0.29	1.80 ± 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	1.88	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	3.62 ± 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 3. Total Volume of Gas Produced in Samples Containing Neoprene.

	Milliliters of Gas Produced/Sample						
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	1.68	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	3.76 ± 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 4. Total Volume of Gas Produced in Samples Containing Unleaded Hypalon.

	Milliliters of Gas Produced/Sample				
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
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 5. 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 ± 0.06	-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 6. Carbon Dioxide Produced in Samples Containing Polyethylene.

	μmoles CO_2/Sample						
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	1.76 ± 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
Polyethylene - Aerobic							
Unamended							
Unirradiated	1.70	3.63	6.81	13.6	18.7	37.3	64.2
Irradiated (Low-Dose)	1.67	2.57	8.16	14.8	14.9	16.5	18.0
Irradiated (High-Dose)	1.56	2.70	6.37	13.7	13.6	18.7	28.8
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	40.8 ± 1.6	40.3 ± 2.2	49.4 ± 2.6
Irradiated (High-Dose)	1.25	28.8 ± 0.1	34.8 ± 0.4	44.3 ± 1.3	42.6 ± 0.2	41.5 ± 0.3	52.4
Polyethylene - Anaerobic							
Unamended							
Unirradiated	1.66	1.83	4.53	14.0	11.7	11.2	14.0
Irradiated (Low-Dose)	1.58	1.82	3.15	13.1	15.9	15.6	15.2
Irradiated (High-Dose)	1.63	2.10	2.71	8.80	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	25.8 ± 0.5	34.6 ± 0.9	31.5 ± 1.1	32.0 ± 0.7	27.4 ± 2.8
Irradiated (High-Dose)	1.23	19.5 ± 0.2	24.3 ± 0.3	33.6 ± 0.1	33.6 ± 1.3	35.8 ± 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 7. Carbon Dioxide Produced in Samples Containing Polyvinylchloride.

	μmoles CO_2/Sample						
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	1.76 ± 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	16.1	24.1	22.2	22.8	31.3
Irradiated (High-Dose)	1.57	1.89	9.38	16.2	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	44.6 ± 0.0	44.8 ± 0.3	44.4 ± 0.6	50.1 ± 3.4	48.4 ± 3.4
Polyvinylchloride - Anaerobic							
Unamended							
Unirradiated	1.54	1.76	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	16.7 ± 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.

Table 8. Carbon Dioxide Produced in Samples Containing Neoprene.

	μmoles CO_2/Sample						
Sample							
	0	**30**	**189**	**334**	**488**	**840**	**2612**
No Plastic or Rubber				**Days**			
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	46.2 ± 1.1
Anaerobic							
Unamended	1.52	1.76 ± 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
Neoprene - Aerobic							
Unamended							
Unirradiated	1.60	3.34	7.68	8.33	10.1	26.8	30.5
Irradiated (Low-Dose)	1.66	3.69	8.18	10.7	12.3	15.1	36.6
Irradiated (High-Dose)	1.64	4.21	10.4	16.0	25.5	41.6	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	27.6 ± 0.3	40.2 ± 0.7	40.9 ± 0.9	41.8 ± 1.6	43.5 ± 3.1	55.8 ± 1.8
Irradiated (High-Dose)	1.30	29.3 ± 0.2	44.5 ± 1.1	46.7 ± 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	2.16	3.09	NA	19.2	25.7
Irradiated (High-Dose)	1.67	1.81	2.28	2.50	2.36	2.92	19.0
Amended							
Unirradiated	1.24	18.3 ± 0.1	22.7 ± 0.3	32.9 ± 0.6	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 9. Carbon Dioxide Produced in Samples Containing Unleaded Hypalon.

	μmoles CO_2/Sample				
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 10. Carbon Dioxide Produced in Samples Containing Leaded Hypalon.

	μmoles CO_2/Sample				
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	29.84 ± 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	18.6 ± 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.

Appendix E

Other Gases (Oxygen, Hydrogen, Nitrous Oxide, Nitrogen) Produced at 853 Days Incubation in the Long-Term Inundated Cellulose Experiment.

Table 1: Other gases produced in initially aerobic samples.

Table 2: Other gases produced in anaerobic samples.

Table 1. Composition of headspace gas of initially aerobic samples at 853 days.

Sample	Total Gas Volume	Hydrogen	Oxygen*	Nitrogen	Carbon Dioxide	Nitrous oxide	Methane	Total
	(ml)	----------µmol/sample----------						
Without Cellulose								
Unamended/Inoculated	49.9	ND	360	1665	7.8	ND	ND	2032
Amended/Inoculated	47.5	ND	77.2	1718	146	ND	ND	1941
Amended/Inoculated/Exc. Nitrate	54.5	ND	163	1868	89.5	ND	ND	2220
With Cellulose								
Unamended/Inoculated	41.7	ND	54.8	1559	80.2	ND	ND	1694
Amended/Inoculated	45.2	153	24.3	1411	276	ND	ND	1865
Amended/Inoculated/Exc. Nitrate	95.5	66.1	21.2	1937	936	1116	ND	4076
Without Cellulose/With Bentonite								
Unamended/Inoculated	52.4	ND	330	1759	39.2	0.1	ND	2128
Amended/Inoculated	48.3	ND	72.3	1718	177	7.6	ND	1974
Amended/Inoculated/Exc. Nitrate	49.1	ND	177	1732	89.9	0.9	ND	2000
With Cellulose/With Bentonite								
Unamended/Inoculated	47.1	242	70.3	1316	381	3.8	ND	2013
Amended/Inoculated	71.1	284	19.1	1799	998	10.0	ND	3110
Amended/Inoculated/Exc. Nitrate	80.9	ND	24.9	1835	836	740	ND	3437

Values are the mean of the analysis of triplicate samples.
ND = none detected
*Detection limit = 10 µmoles/sample

Table 2. Composition of headspace gas of anaerobic samples at 853 days.

Sample	Total Gas Volume	Hydrogen	Oxygen*	Nitrogen	Carbon Dioxide	Nitrous oxide	Methane	Total
	(ml)	µmol/sample						
Without Cellulose								
Unamended/Inoculated	52.3	20.5	<10	2022	3.3	ND	ND	2076
Amended/Inoculated	60.7	ND	<10	2271	129	32.1	ND	2437
Amended/Inoculated/Exc. Nitrate	57.6	5.1	<10	2177	124	ND	ND	2311
With Cellulose								
Unamended/Inoculated	52.5	376	<10	1638	67.5	ND	ND	2086
Amended/Inoculated	70.5	560	<10	1896	384	47.3	ND	2892
Amended/Inoculated/Exc. Nitrate	110	361	<10	2022	1055	1122	ND	4565
Without Cellulose/With Bentonite								
Unamended/Inoculated	53.0	ND	<10	2055	34.0	ND	ND	2106
Amended/Inoculated	59.7	ND	<10	2218	177	13.7	ND	2413
Amended/Inoculated/Exc. Nitrate	62.4	11.8	<10	2297	196	30.8	ND	2541
With Cellulose/With Bentonite								
Unamended/Inoculated	54.3	292	<10	1694	262	ND	ND	2252
Amended/Inoculated	70.3	310	<10	1963	658	ND	ND	2935
Amended/Inoculated/Exc. Nitrate	135	288	<10	2314	2116	1070	ND	5793

Values are the mean of the analysis of triplicate samples.
ND = none detected
*Detection limit = 10 µmoles/sample

Appendix F

Total Dissolved Organic Carbon, Carbohydrates, Organic Acids in Long-Term Inundated Cellulose Experiment. Also pH, Nitrate, Sulfate, Iron (II), Iron (III), and Total Iron.

Table 1: Total dissolved organic carbon, carbohydrates, and organic acids in initially aerobic samples at 885, 1228 days incubation (and 3561 days for organic acids).

Table 2: pH, nitrate, and sulfate in initially aerobic samples at 885 and 1228 days incubation.

Table 3: Iron speciation in initially aerobic samples at 885 and 1228 days incubation.

Table 4: Total dissolved organic carbon, carbohydrates, and organic acids in anaerobic samples at 885, 1228 days incubation (and 3561 days for organic acids).

Table 5: pH, nitrate, and sulfate in anaerobic samples at 885 and 1228 days incubation.

Table 6: Iron speciation in anaerobic samples at 885 and 1228 days incubation.

Table 1. Total dissolved organic carbon, carbohydrates, and organic acids in initially aerobic samples.

Treatment	Total Dissolved Organic Carbon		Total Soluble Carbohydrates		Total Organic Acids		
	885	1228	885	1228	885	1228	3561
Without Cellulose	----------µg/ml----------						
Unamended/Uninoculated	78.3 ± 17.7	45.0 ± 4.0	ND	39.9 ± 6.2	4.12 ± 0.58	41.0 ± 10.0	NA
Unamended/Inoculated	113 ± 15	120 ± 16	ND	73.0 ± 26.3	6.76 ± 1.16	52.0 ± 17.0	NA
Amended/Inoculated	189 ± 16	197 ± 16	78.7 ± 13.0	50.9 ± 10.6	26.4 ± 4.1	63.0 ± 16.0	NA
Amended/Inoculated/Exc. Nitrate	186 ± 21	147 ± 13	95.6 ± 9.7	115 ± 4	18.9 ± 2.2	140 ± 36	NA
With Cellulose							
Unamended/Uninoculated	269 ± 5	206 ± 4	52.0 ± 0.0	25.0 ± 3.0	75.3 ± 2.7	195 ± 88	40.3
Unamended/Inoculated	287 ± 10	182 ± 6	ND	40.0 ± 16.0	75.0 ± 5.0	170 ± 76	33.6
Amended/Inoculated	545 ± 8	554 ± 25	332 ± 2	241 ± 6	393 ± 8	469 ± 71	670
Amended/Inoculated/Exc. Nitrate	594 ± 16	677 ± 18	455 ± 12	470 ± 3	616 ± 66	577 ± 91	703
Without Cellulose/With Bentonite							
Unamended/Uninoculated	101 ± 51	45.0 ± 4.0	ND	ND	20.7 ± 8.7	97.0 ± 21.0	NA
Unamended/Inoculated	47.6 ± 42.4	120 ± 16	ND	ND	4.88	41.0 ± 10.0	NA
Amended/Inoculated	65.2 ± 13.9	197 ± 16	73.6 ± 5.0	111 ± 13	17.6 ± 9.1	63.0 ± 8.0	NA
Amended/Inoculated/Exc. Nitrate	116 ± 31	147 ± 13	163 ± 1	179 ± 12	29.7 ± 17.0	84.0 ± 14.0	NA
With Cellulose/With Bentonite							
Unamended/Uninoculated	132 ± 25	230 ± 13	ND	ND	18.3 ± 2.9	167 ± 117	55.3
Unamended/Inoculated	214 ± 13	456 ± 32	58.7 ± 4.1	69.0 ± 0.7	366 ± 18	325 ± 108	376
Amended/Inoculated	335 ± 10	952 ± 31	ND	384 ± 1	386 ± 7	505 ± 56	485
Amended/Inoculated/Exc. Nitrate	449 ± 8	261 ± 14	358 ± 2	100 ± 15	62.0 ± 1.7	101 ± 29	318

ND = none detected
NA = not analyzed

Table 2. pH, nitrate and sulfate in aerobic samples at 885 and 1228 days incubation.

Treatment	pH		Nitrate		Sulfate	
	885	1228	885	1228	885	1228
Without Cellulose			----------mg/ml----------			
Unamended/Uninoculated	5.89	6.07	ND	ND	27.8 ± 1.3	31.7 ± 0.8
Unamended/Inoculated	5.88	6.03	ND	ND	27.8 ± 0.3	31.1 ± 0.3
Amended/Inoculated	5.85	5.88	0.534 ± 0.93	ND	27.9 ± 0.1	32.7 ± 1.2
Amended/Inoculated/Exc. Nitrate	5.83	6.06	2.71 ± 0.12	4.15 ± 0.13	28.2 ± 0.3	31.2 ±0.6
With Cellulose						
Unamended/Uninoculated	5.96	6.08	ND	ND	30.5 ± 0.2	32.6 ± 0.6
Unamended/Inoculated	5.98	6.01	ND	ND	27.8 ± 0.1	30.3 ± 0.0
Amended/Inoculated	5.88	5.54	ND	ND	29.3 ± 0.1	29.8 ± 0.3
Amended/Inoculated/Exc. Nitrate	6.07	6.08	1.95 ± 0.03	ND	29.4 ± 0.5	31.5 ± 0.5
Without Cellulose/With Bentonite						
Unamended/Uninoculated	6.04	6.09	ND	ND	27.9 ± 0.3	31.4 ±1.0
Unamended/Inoculated	6.03	6.16	ND	ND	27.4 ± 1.1	29.8 ± 0.6
Amended/Inoculated	6.02	5.96	0.522 ± 0.010	ND	26.6 ± 0.2	29.7 ± 0.4
Amended/Inoculated/Exc. Nitrate	5.96	6.09	3.78 ± 0.03	4.48 ± 0.22	27.9 ± 0.4	29.9 ± 0.5
With Cellulose/With Bentonite						
Unamended/Uninoculated	6.00	6.08	ND	ND	28.7 ± 1.1	30.3 ± 0.3
Unamended/Inoculated	5.65	5.54	ND	ND	27.3 ± 1.1	26.1
Amended/Inoculated	5.57	5.52	ND	ND	27.3 ± 0.4	29.7 ± 0.3
Amended/Inoculated/Exc. Nitrate	6.14	6.16	0.552 ± 0.144	ND	26.8 ± 0.5	30.6

ND = none detected
NA = not analyzed

Table 3. pH, nitrate and sulfate in aerobic samples at 885 and 1228 days incubation.

Treatment	Fe (II)		Fe (III)		Total Fe	
	885	1228	885	1228	885	1228
Without Cellulose	----------µg/ml----------					
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND
With Cellulose						
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated	1.06 ± 0.19	ND	0.55	1.50 ± 0.00	1.61 ± 0.19	1.50 ± 0.00
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND
Without Cellulose/With Bentonite						
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND
With Cellulose/With Bentonite						
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	3.49 ± 0.00	19.0 ± 0.1	0.54	ND	4.03 ± 0.00	19.0 ± 0.1
Amended/Inoculated	22.6 ± 0.1	22.5 ± 0.1	0.95	1.00 ± 0.40	23.6 ± 0.1	23.5 ± 0.3
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND

ND = none detected
NA = not analyzed

Table 4. Total dissolved organic carbon, carbohydrates, and organic acids in initially aerobic samples.

Treatment	Total Dissolved Organic Carbon		Total Soluble Carbohydrates		Total Organic Acids		
	885	1228	885	1228	885	1228	3561
Without Cellulose	----------µg/ml----------						
Unamended/Uninoculated	101 ± 10	102	ND	85.0 ± 8.0	23.8 ± 11.8	68.0 ± 17.0	NA
Unamended/Inoculated	127 ± 20	130	24.4 ± 4.1	65.0 ± 8.0	14.1 ± 2.1	51.0 ± 17.0	NA
Amended/Inoculated	278 ± 19	235 ± 10	36.1 ± 8.6	149 ± 2	52.4 ± 20.6	59.0 ± 12.0	NA
Amended/Inoculated/Exc. Nitrate	282 ± 21	273 ± 36	211 ± 13	222 ± 15	222 ± 9	107 ± 27	NA
With Cellulose							
Unamended/Uninoculated	104 ± 25	215 ± 16	43.8 ± 4.9	67.0 ± 5.0	43.3 ± 4.0	176 ± 66	19.2
Unamended/Inoculated	126 ± 28	537 ± 70	18.7 ± 6.5	79.0 ± 7.0	127 ± 8	349 ± 132	437
Amended/Inoculated	417 ± 9	620 ± 6	43.8 ± 3.2	103 ± 1	350 ± 8	320 ± 50	1041
Amended/Inoculated/Exc. Nitrate	726 ± 26	1047 ± 37	662 ± 2	742 ± 14	58.0 ± 4.6	515 ± 23	1607
Without Cellulose/With Bentonite							
Unamended/Uninoculated	163 ± 10	135 ± 4	ND	93.5 ± 10.2	13.1 ± 0.3	81.0 ± 12.0	NA
Unamended/Inoculated	163 ± 15	114 ± 10	ND	35.9 ± 14.3	16.2 ± 1.8	25.0 ± 9.0	NA
Amended/Inoculated	168 ± 11	173 ± 15	30.9 ± 3.4	129 ± 12	23.6 ± 3.5	123 ± 41	NA
Amended/Inoculated/Exc. Nitrate	204 ± 17	175 ± 14	171 ± 3	186 ± 21	16.4 ± 2.5	51.0 ± 11.0	NA
With Cellulose/With Bentonite							
Unamended/Uninoculated	36.9 ± 22.7	192 ± 14	18.4 ± 5.5	ND	37.3 ± 2.8	74.0 ± 22.0	24.9
Unamended/Inoculated	582 ± 16	372 ± 5	372 ± 3	25.8 ± 0.7	889 ± 20	199 ± 14	273
Amended/Inoculated	463 ± 29	416 ± 8	80.5 ± 2.4	79.5 ± 6.8	708 ± 26	271 ± 93	8156
Amended/Inoculated/Exc. Nitrate	2410 ± 70	1884 ± 21	2850 ± 30	1294 ± 36	3808 ± 232	1880 ± 283	1539

ND = none detected
NA = not analyzed

Table 5. pH, nitrate and sulfate in anaerobic samples at 885 and 1228 days incubation.

Treatment	pH		Nitrate		Sulfate	
	885	1228	885	1228	885	1228
Without Cellulose			----------mg/ml----------			
Unamended/Uninoculated	5.90	6.08	ND	ND	30.5 ± 0.6	31.0 ± 0.6
Unamended/Inoculated	5.91	6.11	ND	ND	28.8 ± 0.2	32.4 ± 1.0
Amended/Inoculated	6.08	6.09	0.476 ± 0.009	ND	27.5 ± 0.1	31.8 ± 0.7
Amended/Inoculated/Exc. Nitrate	5.97	6.06	2.64 ± 0.1	3.90 ± 0.19	27.8 ± 0.0	29.8 ± 0.8
With Cellulose						
Unamended/Uninoculated	5.96	6.10	ND	ND	30.3 ± 0.6	31.0 ± 0.6
Unamended/Inoculated	5.98	5.92	ND	ND	29.6 ± 0.4	32.0 ± 0.4
Amended/Inoculated	6.11	5.89	ND	ND	29.1 ± 0.3	34.0 ± 1.3
Amended/Inoculated/Exc. Nitrate	6.21	6.12	1.63 ± 0.05	ND	28.2 ± 0.6	30.7 ± 2.3
Without Cellulose/With Bentonite						
Unamended/Uninoculated	6.04	6.08	ND	ND	28.9 ± 0.2	28.7 ±0.9
Unamended/Inoculated	6.03	6.14	ND	ND	28.7 ± 1.2	29.7 ± 0.0
Amended/Inoculated	5.92	6.05	0.294 ± 0.004	ND	27.3 ± 0.2	26.4 ± 1.8
Amended/Inoculated/Exc. Nitrate	6.09	6.11	3.03 ± 0.05	4.5	27.7 ± 0.1	29.6 ± 1.2
With Cellulose/With Bentonite						
Unamended/Uninoculated	6.00	6.27	ND	ND	28.7 ± 0.1	29.2 ± 0.9
Unamended/Inoculated	5.64	5.71	ND	ND	28.2 ± 0.1	30.5 ± 0.0
Amended/Inoculated	5.74	5.83	ND	ND	27.6 ± 0.5	29.1 ± 0.2
Amended/Inoculated/Exc. Nitrate	6.19	5.84	ND	ND	26.0 ± 0.2	29.5 ± 0.9

ND = none detected
NA = not analyzed

Table 6. pH, nitrate and sulfate in aerobic samples at 885 and 1228 days incubation.

Treatment	Fe (II)		Fe (III)		Total Fe	
	885	1228	885	1228	885	1228
Without Cellulose	----------µg/ml----------					
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND
With Cellulose						
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated	1.06 ± 0.19	ND	0.55	1.50 ± 0.00	1.61 ± 0.19	1.50 ± 0.00
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND
Without Cellulose/With Bentonite						
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated	ND	ND	ND	ND	ND	ND
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND
With Cellulose/With Bentonite						
Unamended/Uninoculated	ND	ND	ND	ND	ND	ND
Unamended/Inoculated	3.49 ± 0.00	19.0 ± 0.1	0.54	ND	4.03 ± 0.00	19.0 ± 0.1
Amended/Inoculated	22.6 ± 0.1	22.5 ± 0.1	0.95	1.00 ± 0.40	23.6 ± 0.1	23.5 ± 0.3
Amended/Inoculated/Exc. Nitrate	ND	ND	ND	ND	ND	ND

ND = none detected
NA = not analyzed