# Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions

Final Report

Revision 0, October 6, 2003

Jeff Gillow and A.J. Francis
Brookhaven National Laboratory

## Executive Summary

Gas generation from the microbial degradation of the organic constituents of transuranic waste under conditions expected at the Waste Isolation Pilot Plant (WIPP) repository was investigated at Brookhaven National Laboratory from 1992-2003. The biodegradation of mixed cellulosics and electron-beam irradiated plastic and rubber materials (polyethylene, polyvinylchloride, neoprene, hypalon, and leaded hypalon) was examined. The effects of environmental variables such as initial atmosphere (air or nitrogen), water content (humid (~70% relative humidity) and brine inundated), and nutrient amendments (nitrogen, phosphate, yeast extract, and excess nitrate) on microbial gas generation was evaluated. Total gas volume was determined by pressure measurement and $CO_2$ and $CH_4$ were analyzed by gas chromatography. Soluble cellulose degradation products were analyzed by high-performance liquid chromatography (HPLC). Microbial populations were determined by direct microscopy and denaturing gradient gel electrophoresis (DGGE). Results showed that the addition of a mixed inoculum composed of sources of salt, brine, and sediment from the WIPP underground and surficial environments led to the biodegradation of cellulose under brine inundated and humid (70% relative humidity) conditions, especially when nutrients were added, and to the greatest extent when anaerobic conditions were established from the start, as follows:

- Over a 10.8 year period, under initially aerobic conditions (oxygen was consumed after 2 years incubation) 0.84 ± 0.10 ml of total gas was produced per gram cellulose without a nutrient amendment, while samples with a nutrient amendment produced 1.71 ± 1.03 ml total gas $g^{-1}$ celluose, and 12.2 ± 0.00 ml total gas $g^{-1}$ celluose with excess nitrate. 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 a nutrient amendment, and 186 µmoles $CO_2$ $g^{-1}$ cellulose when excess nitrate was added. The overall rate of total gas production from the start of the experiment in these treatments was 0.0003, 0.0004, and 0.0016 ml total gas $g^{-1}$ cellulose $day^{-1}$, respectively, and $CO_2$ production was

0.003, 0.004, and 0.034 μmoles $CO_2$ $g^{-1}$ cellulose $day^{-1}$, respectively. Under anaerobic conditions, 2.48 ± 0.31 ml total gas $g^{-1}$ cellulose was produced in the absence of 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 nitrate. Carbon dioxide was produced under anaerobic conditions as follows: 27.4 ± 5.8 μmoles $CO_2$ $g^{-1}$ cellulose in the absence of nutrients, 66.9 ± 1.1 μmoles $CO_2$ $g^{-1}$ cellulose with nutrients, and 251 ± 5 μmoles $CO_2$ $g^{-1}$ cellulose with excess nitrate (after 6 years of incubation 2.24 ± 0.24 x $10^8$ bacterial cells $ml^{-1}$ were detected in these samples). The overall rate of total gas production in anaerobic samples was 0.0006, 0.0008, and 0.0025 ml $g^{-1}$ cellulose $day^{-1}$, respectively, and for $CO_2$ production it was 0.018, 0.030, and 0.054 μmoles $CO_2$ $g^{-1}$ cellulose $day^{-1}$, respectively.

- Organic acids, predominantly formate and acetate, and smaller amounts of butyric, fumaric, lactic, oxalic, oxalacetic, propionic, and succinic acids were detected in solution indicating fermentative microbial activity.
- Methane was first detected at ~7.4 years incubation in brine inundated samples, and 5.89 ± 1.30 nmol $g^{-1}$ cellulose was detected at ~9.5 years under anaerobic conditions without nutrients; 2.74 ± 0.90 nmol $g^{-1}$ celluose with nutrients, and 2.57 ± 0.79 nmol $g^{-1}$ cellulose with excess nitrate. The amount of methane detected at ~9.5 years was smaller under initially aerobic conditions: 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 nitrate.
- Bentonite, once a potential backfill additive for WIPP, enhanced the concentration of gaseous and aqueous metabolites; 387 ± 12 μmoles $CO_2$ $g^{-1}$ cellulose was produced under anaerobic conditions with excess nitrate and bentonite (1.5x more $CO_2$ than without bentonite).
- Under humid conditions, a nutrient amendment resulted in lower gas production than without; under initially aerobic conditions 6.09 ± 2.41 μmoles $CO_2$ was produced $g^{-1}$ cellulose after ~9 years while 0.48 ± 0.29 μmoles $CO_2$ was produced $g^{-1}$ cellulose in the presence of a nutrient amendment. The same held under anaerobic conditions: unamended samples produced 115 ± 20 μmoles $CO_2$ $g^{-1}$ cellulose while nutrient amended samples produced 21.9 ± 3.3 μmoles $CO_2$ $g^{-1}$ cellulose after ~9 years incubation. Bentonite greatly enhanced gas production under humid conditions as well (anaerobic unamended samples produced 591 ± 135 μmoles $CO_2$ $g^{-1}$ cellulose, and amended samples produced 673 ± 49 μmoles $CO_2$ $g^{-1}$ cellulose). Methane was detected, up to 32.6 ± 9.3 nmoles $g^{-1}$ cellulose, only when bentonite was present.

- Plastic and rubber materials were subjected to an absorbed radiation dose of up to 4,000 Mrad in order to determine if radiation damage could affect polymer biodegradability and gas generation. After ~7 years incubation microbial gas production did not increase when the plastics polyethylene or polyvinylchloride were present. Inhibitory reactants to microbial activity were formed after irradiation of polyvinylchloride. Irradiation of rubber materials neoprene and hypalon resulted in 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 nitrate contained $2.24 \pm 0.24 \times 10^8$ cells $ml^{-1}$. Through analysis of DNA, a diverse assemblage of bacterial and archael microorganisms, well populated with extreme halophiles, were detected in unamended and nutrient amended inundated cellulose samples.

## 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. A mined geologic repository, WIPP 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 and 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 $^{252}Cf$. The total volume of TRU waste managed by the DOE through 2034 is estimated to be approximately 171,000 $m^3$; WIPP's total capacity for contact-handled and remote-handled TRU waste is set at 176,000 $m^3$ (U.S. DOE, 2001). Remote-handled TRU waste possesses 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 inventory at the end of 1996 was $2.5 \times 10^6$ curies, predominantly from

Pu and Am. The TRU waste will be shipped to WIPP from 10 major sites throughout the U.S. 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 room 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). An average drum of TRU waste will contain 10 kg of cellulosic material, or ~70,000 kg of cellulosic material 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 will also be placed in the WIPP (Brush, 1990, Brush et al., 1991). The U.S. Environmental Protection Agency has certified that the U.S. DOE plans to operate WIPP complies with laws governing the long-term disposal of radioactive waste, 40 CFR 191 and 40 CFR 194 (Federal Register, 1998). Part of this certification relied on the U.S. DOE demonstrating an understanding of chemical processes in the repository over the 10,000 year period of performance dictated by 40 CFR 194. Gas will be generated in the repository primarily by metal corrosion and microbial processes. Gas production could result in pressurization of the repository after it is sealed causing fracturing of anhydrite interbeds in the Salado formation, and contribute to spalling and direct brine releases (Federal Register, 1998). In addition, microbially-produced $CO_2$ could decrease the pH of the repository if it were to become inundated with brine which in turn could increase actinide solubility.

Microorganisms, which 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 have shown that microbes in the wastes can metabolize a variety of organic carbon compounds that are present (Francis et al., 1980 a, b; Francis, 1985).

Long-term experiments designed to examine gas generation due to biodegradation of the organic fraction of transuranic (TRU) wastes under WIPP repository-relevant conditions were performed at Brookhaven National Laboratory (BNL) from 1992-2003. A summary of these experiments for the period 1991 to 1996 was published in Francis et al., 1997. After a hiatus of 4 years, the experiments to quantify gas generation due to biodegradation of simulated TRU wastes were again analyzed in 1999 through to 2003. Table 1 provides the status of these experiments as of 2003 (at the end of the experiment).

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

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

The test plan titled "Re-evaluation of Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Conditions, TP-99-01" was used 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 the requirements of Sandia National Laboratories (SNL). This QAP was fully implemented during the work at BNL and was reviewed by SNL during formal on-site audits. The QAP ensured that the data generated were valid, accurate, repeatable, protected and could withstand critical peer and other reviews.

## 2.0 EXPERIMENTAL RATIONALE AND APPROACH

The expected conditions within the WIPP disposal rooms prior to 1996 gave us the framework within which to develop the experimental test conditions for gas generation due to microbial activity. 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 the waste is emplaced. 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, NEO and HYP make up a sizable portion of the rubber materials. In the repository, the plastic and rubber materials will 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. Additional influencing variables are identified in the disposal room scenario described in the previous section, the presence or absence of which may affect microbial gas generation, including the following: i) oxygen, ii) substrates (cellulose, plastic, or rubber), iii) brine, iv) bentonite, v) microbes, vi) nutrients, and vii) alternate electron acceptors. The evaluation of the effects of these variables on microbial gas generation formed the basis for our experimental methodology.

## 3.0 MATERIALS AND METHODS

### 3.1 Inundated Treatments

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 activities. 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 together 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.).

Fifteen liters of brine from G-Seep (SNL #9) were provided by Sandia National Laboratories' brine laboratory (the identifier is part of SNL's brine cataloging system) via overnight express delivery, on ice, and 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 the chemical composition of G-Seep brine; 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 |

Bentonite clay in two one-liter containers was provided by Sandia National Laboratories. It was a granular MX-80 Volclay bentonite, available from the American Colloid Company of Belle Fourche, South Dakota. At the time these experiments were begun, bentonite was considered a potential backfill for the waste in WIPP to be used to control actinide mobility. 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

A microbial inoculum was prepared from a mixture of a variety of WIPP repository-relevant samples. Microorganisms are expected to enter and reside in the repository from several sources (see Section 4.0). These sources may harbor microorganisms that can use various 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:

(i) Sediment and Brine from Nash Draw: 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 steel cores. The sediment was stored anoxically in serum bottles. All of the samples were stored on ice and shipped to BNL overnight and then stored at 4°C until use. 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 in to remove large particulate material. 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 |

(ii) Brine from the WIPP underground workings: G-Seep collected December 12, 1991, 200 ml.

(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 results of activity measurements were presented earlier (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, 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 first due to the need to make the mixed inoculum in an anaerobic ($N_2$-filled) glove box to maintain the viability of the anaerobic bacteria. The serum bottles containing the mixed cellulosic paper were flushed with ultra-high purity (UHP) nitrogen and 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 with 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 to determine its influence on gas production 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 the inoculum, capped with butyl rubber stoppers, and crimped with aluminum seals. Uninoculated samples were similarly set up. Control samples to measure abiotic gas production received 3 ml of 37% formaldehyde to give a final concentration of 1% formaldehyde to kill the bacteria present.

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 listed (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.

### 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).

Samples were prepared with and without added nutrients. The nutrients added (amended samples) consisted of a 0.50 ml solution containing nitrogen (ammonium nitrate, 0.1% w/v), phosphorus (potassium phosphate, 0.1% w/v), and yeast extract (0.05% w/v). Unamended samples received 0.50 ml of a filtered, sterilized reagent-grade salt solution (20% w/v). All samples were prepared in triplicate.

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 an additional, but integral, source of inoculum. Samples containing WIPP salt but without inoculum are not true "abiotic" controls. Therefore, reagent-grade NaCl was added to specific uninoculated samples to serve as abiotic controls.

In order to maintain the desired relative humidity of approximately 70-74%, 3 ml of G-Seep brine ($a_W$ (water activity of the brine) = 0.73) in an unsealed 5 ml glass tube (1.0 x 7.5 cm) was placed inside the 160 ml serum bottle containing 1 g of mixed

cellulose. Upon sealing the sample bottles, the relative humidity was measured using a Hygroskop GT™ (Rotronic, Zurich) portable humidity meter, the probe of which was fitted with a rubber seal to allow measurements to be taken inside of an uncapped serum bottle. The meter was calibrated before use with a standard solution (80% relative humidity) according to the manufacturer's specifications. The relative humidity in the sample bottles (72%) was verified using this method.

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. The ability of specific microorganisms (i.e., denitrifiers) to grow under such low-moisture conditions was examined. We point out that WIPP halophiles can function under low-moisture conditions because they can grow in highly concentrated brine, which has a low water activity.

Two of the inoculated, succinate-amended treatments (one with bentonite, the other without bentonite) were incubated with 0.1 atm of acetylene to examine $N_2O$ production from denitrification. Seventy-two samples were incubated at 30 ± 2°C.

### 3.3 Treatments Containing Plastic and Rubber Materials

In this study, we attempt to determine the rate and extent of gas production due to biodegradation of unirradiated and electron-beam irradiated plastic and rubber materials under conditions relevant to the WIPP repository. In the case of irradiated materials, these were accelerated tests because the entire structure of the polymer was altered as opposed to the effects of alpha-irradiation, which alter only the surface of the polymer. These samples, therefore, represented "overtest" conditions in terms of overall radiation

dose. The influence of adding nutrients (nitrogen, phosphorus, and yeast extract) on the extent of biodegradation also was determined.

The plastics examined were polyethylene and polyvinylchloride; the rubber materials were neoprene and hypalon (leaded and unleaded). These materials were exposed to electron-beam irradiation at the linear accelerator (LINAC) at Argonne National Laboratory by Dr. D. Reed, Chemical Technology Division. The polymer samples received an absorbed dose of either 500-700 Mrad (low-dose) or 4000-6000 Mrad (high dose), see Table 5. Tests with unleaded and leaded hypalon did not include a high-dose irradiation because it caused extensive degradation (melting) of 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, the weights were recorded, and the pieces placed in acid-washed sterilized (autoclaved) 70 ml glass serum bottles. Mean weights (22 samples for each polymer) were as follows: Polyethylene (86.1 mg), Polyvinylchloride (134.6 mg), Neoprene (257.5 mg).

Every sample bottle containing plastic or rubber was filled with 50 ml of a mixture consisting of 56% G-Seep Brine #10 (collected 12/13/89-1/10/90), 27% WIPP muck pile salt slurry, and 17% surficial lake brine/sediment slurry. The salt slurry and brine/sediment slurry were prepared as previously described. The inundation fluid differed from that added to the sample bottles containing cellulose; the sample bottles containing plastic or rubber material were inundated with fluid comprised of 100% mixed inoculum. The mixed inoculum was used without dilution to increase the proportion of potential plastic/rubber degrading microorganisms in the experiment. This was done to provide an additional "overtest" because we expected at the outset that biodegradation rates potentially would be very low, especially if the same concentration of mixed inoculum (3.8% v/v) was used as in the cellulose experiment.

Samples were incubated either unamended (without added nutrients) or amended (with nutrients). 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 under initially aerobic and anaerobic conditions in serum bottles fitted with butyl rubber stoppers and sealed with aluminum crimps. Anaerobic samples were prepared in a glove box and incubated under a $N_2$ atmosphere, whereas aerobic samples were prepared on the lab bench. We expected that the aerobic samples would eventually become anaerobic due to consumption of oxygen by aerobic microorganisms in the sealed bottle. All samples were incubated unshaken (static) at 30 ± 2°C.

### 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). For each sampling, the serum bottle fitted with a butyl rubber septum was pierced with a sterile 22-gauge needle (Becton Dickenson) attached to a digital pressure gauge (-5.00 to 35.00 psi (calibrated to NIST by the manufacturer (Wallace and Tiernan): 0.00 to 35.00 psi), to measure the headspace gas pressure to calculate total gas production. At the same time, the room temperature was recorded with a thermometer calibrated to NIST.

Immediately after this, a gas-tight syringe (Pressure-Lok™, Precision Instrument Corp.) fitted with a stainless-steel side-port needle was used to remove 0.3 ml of headspace gas to determine the various gases quantitatively by gas chromatography (GC). A gas-sample valve (Valco Instrument Corp.) equipped with a 100 µl stainless-steel sample loop was used to introduce 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 was analyzed using a Varian 3400 gas chromatograph according to methods detailed in SAND96-2582. Gas production was assessed by examining the increase in total gas volume over time, in addition $CO_2$ is quantitated as an indicator of microbial activity. The values were measured against the baseline (t=0), or against control values. For these experiments we prepared the following control samples: i) unamended, uninoculated samples; ii) and samples without organic substrate (cellulose or plastic/rubber material). The gas data in this report are cumulative from t=0.

Methane was determined using a Varian 3400 gas chromatograph equipped with a flame-ionization detector (FID). Initially, a 6' stainless-steel column (1/8" o.d. x 0.085" i.d.) packed with molecular sieve 5A (Alltech Chromatography Corp.) was used to resolve $CH_4$ from the mixture of gases in the headspace. Additional analyses were performed using a 10' stainless-steel column (1/8" o.d. x 0.085" i.d.) packed with Hayesep D (Alltech) in order to confirm separation and quantitation of $CH_4$. Finally, a 6' column (1/8" o.d. x 0.085" i.d.) packed with Porapak QS 80/100 (Alltech) was used for routine separation of CH4 from headspace gases, with a thermal conductivity detector (TCD) in-line prior to the FID. Three or four-point calibrations were performed 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 determined to be 0.2 nmol $CH_4\ g^{-1}$ cellulose dry wt.

### 3.5 Aqueous Metabolite Analysis

Samples from the inundated experiments reserved at t=3561 days incubation for aqueous chemical analysis were analyzed for organic acids and alcohols by high-performance liquid chromatography (HPLC; Shimadzu LC-10ATVP and SCL-10A system controller/SIL-10A autoinjector). 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 as a result of fermentation of glucose and they are consumed as electron-donor substrates for iron-reduction, sulfate-reduction, and methanogenesis). Finally, quantification of these metabolites provides important information relative to the carbon-balance in the samples, since cellulose hydrolysis and subsequent metabolism results in both aqueous and gaseous intermediates and end-products. A 0.25 ml sample was withdrawn from select samples and diluted to 1.0 ml with deionized water. Analytes were separated by HPLC using ion-exclusion chromatography where 1) strong and weak electrolytes (NaCl, $KNO_3$) are eluted unseparated at the beginning of the elution and 2) the retention times of the organic acids and alcohols are proportional to their dissociation constant values. A sulfonated macroporous styrene HPLC-column (Biorad Aminex HPX-87H (300 mm x 7.8 mm) was used where analytes with higher pKa values are retained longer on the column. Acids with larger pKa values and molecular weights than butyric (pKa=4.85, MW=88.11) are separated by a secondary mechanism, hydrophobic adsorption, which is a size-exclusion phenomenon. Low-molecular weight carboxylic acids of the form $CH_3(CH_2)_nCOOH$ were principally quantified using UV detection at 210 nm (Shimadzu SPD-10A); alcohols (ethanol, propanol, butanol) and glucose were quantified by refractive index detection (Shimadzu RID-6A). Retention times using both detection methods were compared to commercially-prepared standard mixtures (Supelco), and both detection methods were used for positive identification of analytes. The standards included the following: 1. volatile acids (formic, acetic, propionic, butyric, isobutyric, valeric, isocaproic, isovaleric, hexanoic, heptanoic), 2. non-volatile acids (pyruvic, lactic, oxalacetic, oxalic, methyl malonic, malonic, fumaric, succinic), 3. alcohols (butanol, pentanol, propanol, ethanol), and 4. glucose.

### 3.6 Microbiological Characterization

The predominant microorganisms in select samples from the inundated cellulose experiment, and differences in community structure which may help to explain difference in gas generation rates, was assessed by DNA analysis (Pancost et al., 2001; Petsch et al., 2001; Lehman et al., 2001). For the DNA analysis, polymerase chain reaction (PCR) of bacterial and archaeal 16S rRNA gene fragments was performed. The PCR products were then run through denaturing gradient gel electrophoresis (DGGE) to separate fragments according to their melting properties. Ethidium bromide stains the fragment bands 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 analysis was provided by a commercial source (Microbial Insights, Knoxville, TN) capable of performing the DGGE and PLFA analyses. Samples of well-mixed supernatant were also taken after 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 inundated experiment are presented in Appendix A, Table 1-8. A summary of these data is provided in the following figures: i) Figures 1 and 2 present total gas and $CO_2$, respectively, produced in initially aerobic inundated cellulose samples over the course of the experiment, and ii) Figures 3 and 4 present the same data for anaerobic samples. Table 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 period over 4 different incubation periods: (A) 69-200 days correlates with the initially rapid rate of gas production (see Figure 1); (B) 200-1228 days incubation correlates with a period over which the rate began to diminish and is the latest time period summarized in Francis et al., 1997; (C) 1228-3929 days is the period for which new data is presented in this report and is the longest-term data and represents the lowest gas generation rates; finally (D) 69-3929 is the overall gas production rate calculated as a linear extrapolation between these two time periods. Over 10 years of data is represented in the rate calculated from 69-3929 days incubation. The overall rate considerably smoothes the data between the beginning and end of the experiment. This smoothing can be justified by the 10,000 year repository performance period and therefore may be no less applicable than the shorter periods. The four rates are provided for comparison of gas generation in the various treatments.

### 4.1.1 AEROBIC TREATMENTS WITHOUT BENTONITE

#### *4.1.1.1 Total Gas Production*

Aerobic samples are more correctly "initially aerobic samples" since air was sealed in the headspace of the sample bottles however this was rapidly consumed; only 0.5% v/v $O_2$ was detected in excess nitrate amended samples at 853 days incubation and it is expected that this was fully consumed soon after in this treatment and in all of the others (Table 6,

Francis et al., 1997). Unamended uninoculated samples produced a maximum of 0.74 ± 0.45 ml of gas $g^{-1}$ cellulose at 733 days incubation, however there was 0.11 ml of gas $g^{-1}$ at 3929 days incubation. The unamended uninoculated treatment produced gas at a rate of 0.001 ml gas $g^{-1}$ cellulose $day^{-1}$ from 69-200 days (the time period of maximum gas production in all of the inundated samples); and a rate of 0.0001 ml gas $g^{-1}$ cellulose averaged over the entire incubation period (3860 days (10.6 years)). This is in fact the lowest gas production rate of all of the initially aerobic treatments; this is as expected since this treatment contains the lowest population of bacteria (G-Seep contains 1.24 ± 0.13 x $10^5$ bacterial cells $ml^{-1}$ (Francis et al., 1998)) and was not amended with nutrients. Unamended inoculated samples produced 0.84 ± 0.10 ml gas $g^{-1}$ cellulose over 3929 days incubation; this is the maximum that was produced in this treatment at a rate of 0.001 ml gas $g^{-1}$ cellulose $day^{-1}$ over 1028 days between 200 and 1228 days incubation, and 0.0003 ml gas $g^{-1}$ cellulose $day^{-1}$ over the entire incubation period. The highest rate of gas production was detected in this treatment during period B (Table 7) indicating a lag period prior to 200 days due to acclimation of the inoculum to the low-nutrient conditions in the samples. The mixed inoculum contained 3.89 ± 0.08 x $10^6$ bacterial cells $ml^{-1}$ and 4 ml of this was added to each inoculated sample. The addition of the mixed inoculum had the effect in inoculated samples of doubling the bacterial population relative to uninoculated samples (the population increased from 1.24 x $10^5$ bacterial cells $ml^{-1}$ (in G-Seep) to 2.7 x $10^5$ cells $ml^{-1}$ due to the addition of the mixed inoculum). 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 during period A (0.008 ml gas $g^{-1}$ cellulose $day^{-1}$), indicating the mixed inoculum was able to immediately take advantage of the added nutrients for metabolism and growth. 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 (0.023 ml gas $g^{-1}$ cellulose $day^{-1}$ during period A, 0.006 ml gas $g^{-1}$ cellulose $day^{-1}$ during period B, and 0.0016 ml gas $g^{-1}$ cellulose $day^{-1}$ overall). Gas production was not sustained over the long-term, however, with a gradual diminishment after 1034 days incubation (Figure 1). This trend correlated with $CO_2$ production in this treatment.

*4.1.1.2 Carbon Dioxide Production*

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 0.0003 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$ (Table 8). This treatment had the lowest rate of $CO_2$ production and correlated with total gas production. Unamended inoculated samples produced 16.3 ± 1.3 μmol $CO_2$ $g^{-1}$ cellulose after 3929 days incubation at an overall rate of 0.003 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$. The overall rate of $CO_2$ production was only slightly higher (0.004 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$) for amended inoculated samples, however the rate during period A (0.283 μmol $g^{-1}$ cellulose $day^{-1}$) was 10x higher than unamended inoculated samples during this same period. This is further evidence of the stimulatory effect of nutrients on the mixed inoculum population. 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. 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 provided a consistently high rate of $CO_2$ production throughout the experiment (Figure 2).

### 4.1.2 AEROBIC TREATMENTS WITH BENTONITE

*4.1.2.1 Total Gas Production*

Bentonite provided consistently higher rates of total gas production through periods A and B in aerobic samples (Table 7). Unamended uninoculated samples produced gas at a rate of 0.003 ml gas $g^{-1}$ cellulose $day^{-1}$ through period A, with 1.94 ml gas $g^{-1}$ cellulose produced at 3464 days. Unamended inoculated samples overall produced gas at a higher rate in the presence of bentonite (0.0006 ml gas $g^{-1}$ cellulose $day^{-1}$ vs. 0.0003 ml gas $g^{-1}$ cellulose $day^{-1}$ without bentonite). Bentonite increased the rate of gas production greater than 3-fold in amended inoculated samples during period A (0.028 ml gas $g^{-1}$ cellulose $day^{-1}$ vs. 0.008 ml gas $g^{-1}$ cellulose $day^{-1}$ without bentonite) resulting in a maximum of 8.96 ml of gas produced $g^{-1}$ cellulose at 733 days. The effect of bentonite was not as

profound in samples containing excess nitrate, with the rate increased by ~1.5-fold during period A, and 1.4-fold overall (0.0022 ml gas $g^{-1}$ cellulose $day^{-1}$ vs. 0.0016 ml gas $g^{-1}$ cellulose $day^{-1}$ (Table 7)). The yield of gas was 9.95 ± 1.01 ml $g^{-1}$ cellulose at 3929 days incubation. The predominant effect of bentonite on the excess nitrate treatment was to sustain gas production; this treatment did not experience a loss in total gas volume as seen in all of the other aerobic treatments at the end of the experiment (Figure 1 and Table 2(c), Appendix A).

*4.1.2.2. Carbon Dioxide Production*

The addition of bentonite resulted in a significant increase in the rate and total amount of $CO_2$ produced in aerobic samples over the course of the experiment. 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 2x more than 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 during period B and was 0.001 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$. The rate of $CO_2$ production in unamended inoculated samples peaked during period A, at 0.134 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 0.030 μmol $g^{-1}$ cellulose $day^{-1}$, respectively; this is 2x the same 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: 0.869 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$ during period A (Figure 1). This treatment also had the third highest overall rate, 0.054 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$, with a maximum of 233 μmol $CO_2$ $g^{-1}$ cellulose produced at 2718 days incubation.

### 4.1.3. ANAEROBIC SAMPLES WITHOUT BENTONITE

*4.1.3.1 Total Gas Production*

Unamended uninoculated samples without bentonite produced the least amount of total gas of all of the treatments, aerobic or anaerobic, in the experiment (-0.32 ml gas $g^{-1}$ cellulose at 3929 days incubation (Table 3(c), Appendix A), and overall rate of –0.0002 ml gas $g^{-1}$ cellulose $day^{-1}$ (Table 7). This result indicates that the microorganisms in G-Seep were not able to metabolize cellulose to any significant degree in the absence oxygen or nutrients. The microorganisms in the mixed inoculum, however, were able to 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 0.0006 ml gas $g^{-1}$ cellulose $day^{-1}$ in unamended inoculated samples. The nutrient amendment resulted in the production of 4.32 ± 0.34 ml of gas $g^{-1}$ cellulose at 733 days incubation in amended inoculated samples, at a rate of 0.021 ml gas $g^{-1}$ cellulose $day^{-1}$ during period A, and 0.0008 ml gas $g^{-1}$ cellulose $day^{-1}$ overall. The highest rate of total gas production of all treatments was seen in excess nitrate amended samples: 0.039 ml gas $g^{-1}$ cellulose $day^{-1}$ during period A (Table 7, Figure 3). This rate was not sustained throughout the experiment, however a total of ~15 ml of gas was produced $g^{-1}$ of cellulose by 733 days (Figure 3).

*4.1.3.2 Carbon Dioxide Production*

The rate of carbon dioxide production was lowest in unamended uninoculated samples, correlating with total gas production (Table 8). 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), almost 2x more than aerobic samples of the same treatment. Although the rate of $CO_2$ generation during period A was lower for anaerobic unamended inoculated samples (0.016 µmol $CO_2$ $g^{-1}$ cellulose day-1 (Table 8) than aerobic unamended inoculated samples (0.033 µmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$) showing the stimulatory effect of oxygen on the metabolism of dissolved organic carbon in the samples. Amended inoculated samples produced 66.9 ± 1.1 µmol $CO_2$ $g^{-1}$ cellulose by 2718 days, and samples containing excess nitrate produced 251 ± 5 µmol $CO_2$ $g^{-1}$ cellulose over the same time period. The amount of $CO_2$ produced was well correlated with microbial populations in these samples (Table 9).

## 4.1.4 ANAEROBIC SAMPLES WITH BENTONITE

### *4.1.4.1 Total Gas Production*

Bentonite provided nutrients or alternate electron acceptors for gas production in unamended uninoculated samples, and total gas production peaked at 733 days with $0.762 \pm 0.492$ ml gas produced $g^{-1}$ cellulose. This amount was lower than that produced in the same aerobic treatment, but higher than the same anaerobic treatment without bentonite. There was a very slight stimulatory effect of bentonite on unamended inoculated samples during period A (0.007 ml gas $g^{-1}$ cellulose $day^{-1}$ vs. 0.003 ml gas $g^{-1}$ cellulose $day^{-1}$ in the absence of bentonite), but overall there wasn't much effect because of the decreased gas volume at the end of the experiment (gas production peaked at $2.48 \pm 0.31$ ml gas $g^{-1}$ cellulose at 2718 days but then dropped to $1.54 \pm 0.41$ ml gas $g^{-1}$ cellulose at 3929 days). Of interest is the decrease in the rate of gas production in amended inoculated samples with bentonite compared to the same treatment without bentonite during period A: 0.013 ml gas $g^{-1}$ cellulose $day^{-1}$ vs. 0.021 ml gas $g^{-1}$ cellulose $day^{-1}$ respectively (Table 7). Whereas under aerobic conditions bentonite served to increase gas production rates and yields early on, 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 early-on: anaerobic amended inoculated samples containing excess nitrate and bentonite produced gas at a rate of 0.025 ml gas $g^{-1}$ cellulose $day^{-1}$ during period A vs. 0.039 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 $18.1 \pm 0.38$ 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).

*4.1.4.2 Carbon Dioxide Production*

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 (0.002 μmol $g^{-1}$ cellulose $day^{-1}$ during period C, and the same rate overall)(Table 8). Unamended inoculated samples produced 59.0 ± 7.1 μmol $CO_2$ $g^{-1}$ cellulose at 2718 days incubation, at a rate of 0.057 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$ during the initial period (A), followed by gradual leveling off to 0.001 μmol $CO_2$ $g^{-1}$ cellulose day at period C. Carbon dioxide production in amended inoculated samples peaked at 99.4 ± 4.4 μmol $CO_2$ $g^{-1}$ cellulose at 1228 days, with an initial rate (A) of 0.236 μmol CO2 $g^{-1}$ cellulose $day^{-1}$, and 0.022 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$ overall. This rate was less than the same aerobic treatment and further confirms that bentonite had a greater stimulatory effect on initially aerobic samples than anaerobic samples. Finally, excess nitrate amended samples had the longest sustained rapid rate of $CO_2$ production as evidence by the high rate of $CO_2$ production during periods A and B: 0.266 and 0.326 μmol $CO_2$ $g^{-1}$ cellulose $day^{-1}$, respectively (Table 8). The was the highest rate of $CO_2$ production during period B of any of the samples in the experiment. 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.

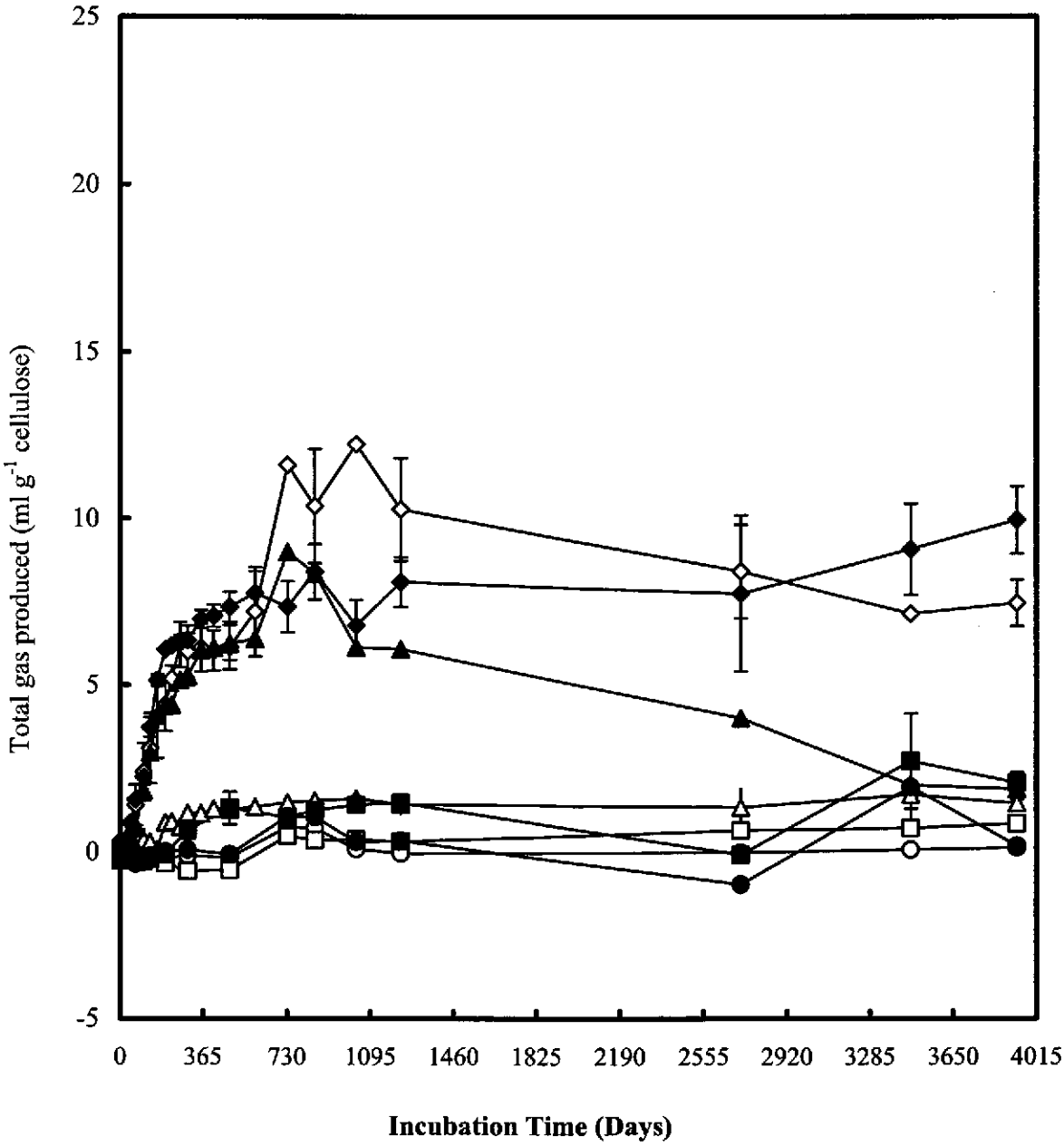


Figure 1. Total gas produced in initially aerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

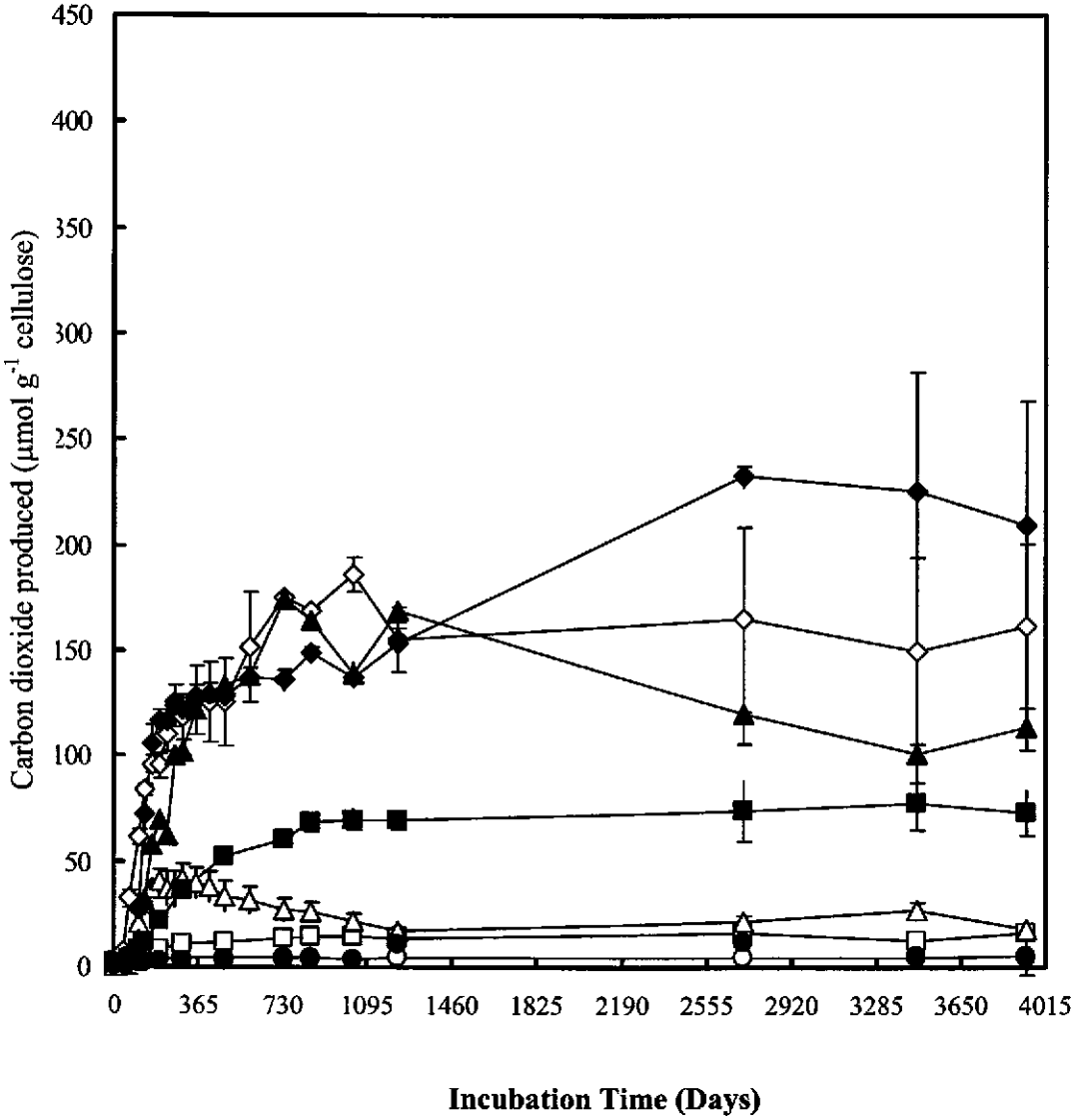


Figure 2. Carbon dioxide produced in initially aerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

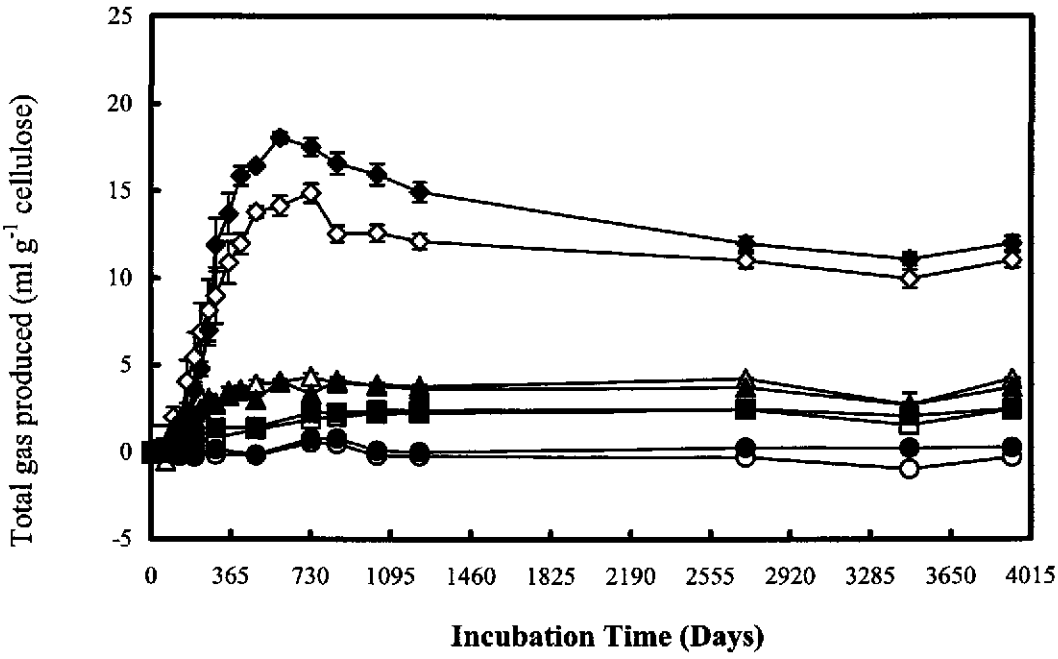


Figure 3. Total gas produced in initially aerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

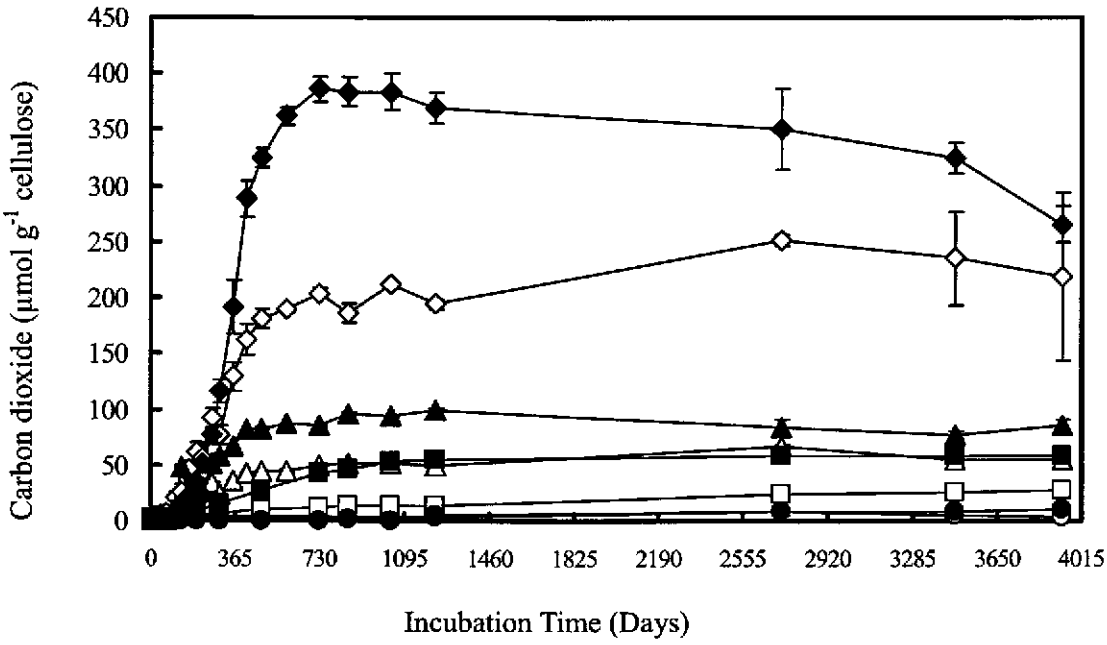


Figure 4. Carbon dioxide produced in anaerobic samples inundated with brine: unamended (○); unamended and inoculated (□); amended and inoculated (△); amended, inoculated, plus excess nitrate (◇). Closed symbols are samples with bentonite.

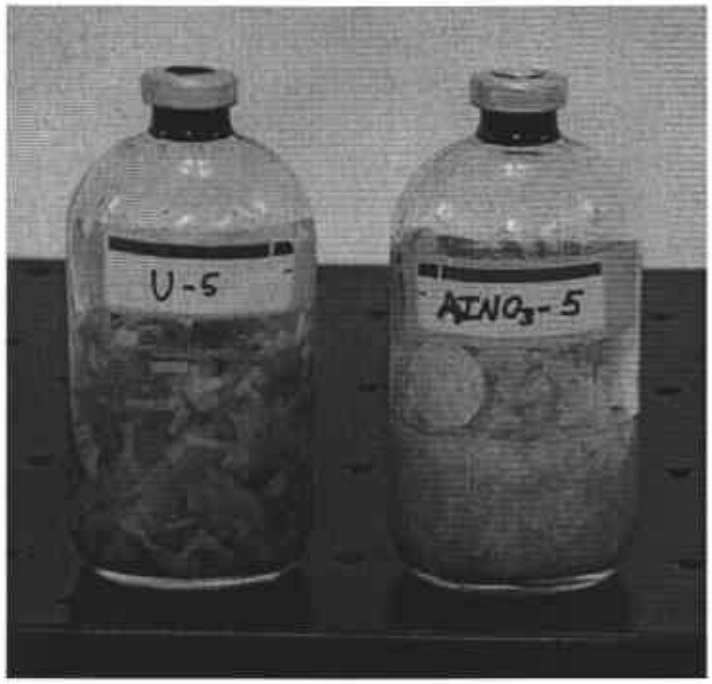


Figure 5. Photograph of anaerobic samples at 885 days incubation: unamended uninoculated (U-5) and amended, inoculated + excess nitrate (AINO3-5, liquid was removed for aqueous metabolite analyses).


Figure 6. Photograph of anaerobic samples containing bentonite at 885 days incubation: unamended uninoculated (BU-4) and amended inoculated (BAI-4, liquid was removed for aqueous metabolite analyses)

Table 7. Rate of total gas production by inundated samples

| Treatment | Rate calculated over incubation period (days): 69 - 200 (131) A | 200 - 1228 (1028) B | 1228 - 3929 (2701) C | 69-3929 (3860) D |
|---|---|---|---|---|
| | ml $g^{-1}$ cellulose $day^{-1}$ | | | |
| Aerobic | | | | |
| Unamended/Uninoculated | 0.001 | 0.0001 | 0.00006 | 0.0001 |
| Unamended/Inoculated | -0.001 | 0.001 | 0.0002 | 0.0003 |
| Amended/Inoculated | 0.008 | 0.001 | 0.000007 | 0.0004 |
| Inoculated + Excess Nitrate | 0.023 | 0.006 | -0.001 | 0.0016 |
| Anaerobic | | | | |
| Unamended/Uninoculated | -0.004 | -0.0001 | -0.00003 | -0.0002 |
| Unamended/Inoculated | 0.003 | 0.002 | 0.0001 | 0.0006 |
| Amended/Inoculated | 0.021 | 0.001 | -0.001 | 0.0008 |
| Inoculated + Excess Nitrate | 0.039 | 0.006 | -0.001 | 0.0025 |
| Aerobic+Bentonite | | | | |
| Unamended/Uninoculated | 0.003 | 0.0003 | -0.00006 | 0.0001 |
| Unamended/Inoculated | 0.001 | 0.002 | 0.0002 | 0.0006 |
| Amended/Inoculated | 0.028 | 0.002 | -0.002 | 0.0003 |
| Inoculated + Excess Nitrate | 0.034 | 0.002 | 0.001 | 0.0022 |
| Anaerobic+Bentonite | | | | |
| Unamended/Uninoculated | -0.002 | 0.0003 | 0.0001 | 0.0001 |
| Unamended/Inoculated | 0.007 | 0.002 | -0.0003 | 0.0004 |
| Amended/Inoculated | 0.013 | 0.002 | -0.0003 | 0.0007 |
| Inoculated + Excess Nitrate | 0.025 | 0.011 | -0.002 | 0.0030 |

Table 8. Rate of carbon dioxide production by inundated samples

| Treatment | Rate calculated over incubation period (days): 69 - 200 (131) A | 200 - 1228 (1028) B | 1228 - 3929 (2701) C | 69-3929 (3860) D |
|---|---|---|---|---|
| | ---------- μmoles $g^{-1}$ cellulose $day^{-1}$ ---------- | | | |
| Aerobic | | | | |
| Unamended/Uninoculated | -0.002 | 0.0004 | 0.0003 | 0.0003 |
| Unamended/Inoculated | 0.033 | 0.005 | 0.001 | 0.003 |
| Amended/Inoculated | 0.283 | -0.023 | 0.0003 | 0.004 |
| Inoculated + Excess Nitrate | 0.484 | 0.058 | 0.003 | 0.034 |
| Anaerobic | | | | |
| Unamended/Uninoculated | -0.003 | -0.0004 | -0.00005 | -0.0002 |
| Unamended/Inoculated | 0.016 | 0.008 | 0.005 | 0.006 |
| Amended/Inoculated | 0.224 | 0.023 | 0.002 | 0.015 |
| Inoculated + Excess Nitrate | 0.422 | 0.129 | 0.009 | 0.055 |
| Aerobic+Bentonite | | | | |
| Unamended/Uninoculated | -0.016 | 0.001 | 0.0005 | 0.00004 |
| Unamended/Inoculated | 0.134 | 0.047 | 0.001 | 0.018 |
| Amended/Inoculated | 0.553 | 0.096 | -0.02 | 0.030 |
| Inoculated + Excess Nitrate | 0.869 | 0.037 | 0.021 | 0.054 |
| Anaerobic+Bentonite | | | | |
| Unamended/Uninoculated | -0.005 | 0.004 | 0.002 | 0.002 |
| Unamended/Inoculated | 0.057 | 0.046 | 0.001 | 0.015 |
| Amended/Inoculated | 0.236 | 0.066 | -0.005 | 0.022 |
| Inoculated + Excess Nitrate | 0.266 | 0.326 | -0.039 | 0.069 |

Table 9. Enumeration of bacteria in anaerobic inundated cellulose treatments at 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 | $2.24 \pm 0.24 \times 10^8$ |

## 4.4 Aqueous Metabolite Analysis.

Results of HPLC of initially aerobic and anaerobic inundated samples are presented in Figures 7-10 and Appendix B, Tables 1-4 (data are the mean of duplicate analyses; relative standard error was generally <0.5% and is not reported in order to provide a more organized depiction of the data (i.e., a less lengthy table)). Previous data reported in SAND96-2582 (1997) are included for comparison. 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. Conversion of values to organic acid produced per gram cellulose is accomplished by dividing the concentrations by 5.

### 4.4.1 AEROBIC TREATMENTS.

The propionic, succinic, formic and lactic acids that were produced in early stages of the experiment 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 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 later (Table 1, Appendix B). Linear regression of the entire excess nitrate data set shows that acetate is produced at a rate of 3.1 $\mu M\ day^{-1}$, while this rate was sustained in amended inoculated samples only until 1228 days and has since leveled off. 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 8 and Table 2, Appendix B). The organic acid content of initially aerobic samples was generally lower than anaerobic samples; this is consistent with less mature fermentative processes in these samples due to the initial bias toward aerobic respiration.

### 4.4.2. 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 9 and 10). Acetate production in unamended inoculated samples was steady over 3561 days at a rate of 1.8 $\mu M\ day^{-1}$, this is significant relative to aerobic samples, although similar to aerobic samples with bentonite. Therefore both anaerobic conditions and bentonite stimulated the activity of the mixed inoculum in the absence of a nutrient amended. 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 in these samples. 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.

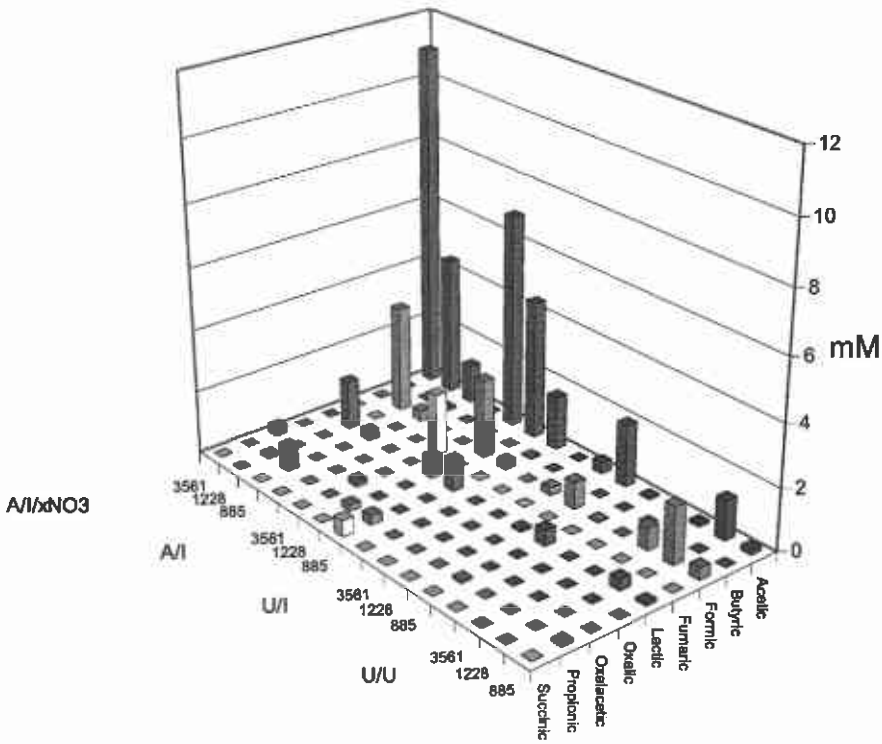


Figure 7. Organic acids produced at 885, 1228, and 3561 days incubation in **aerobic treatments without bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

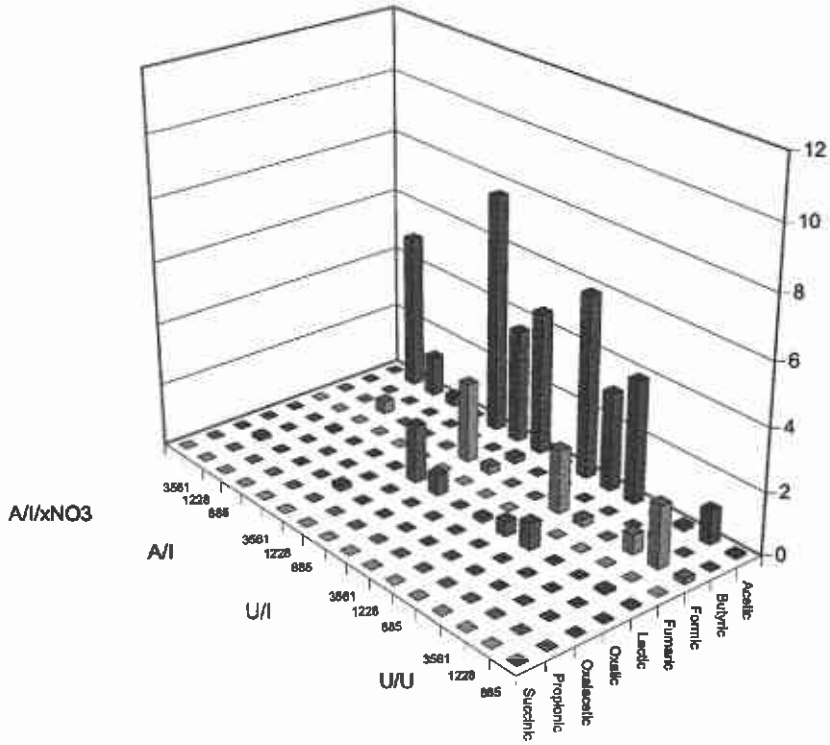


Figure 8. Organic acids produced at 885, 1228, and 3561 days incubation in **aerobic treatments with bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

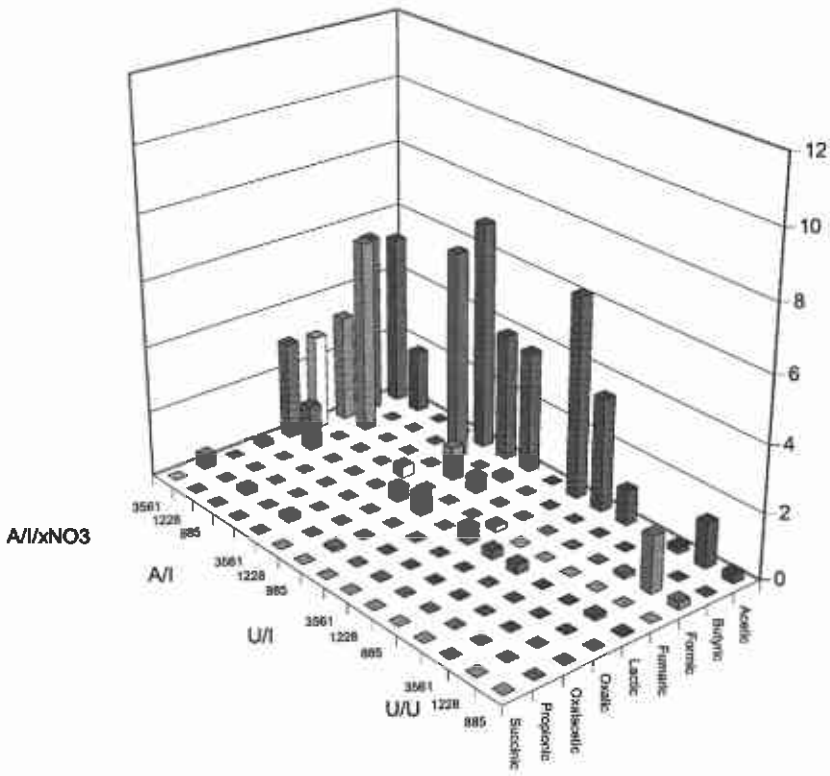


Figure 9. Organic acids produced at 885, 1228, and 3561 days incubation in **anaerobic treatments without bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

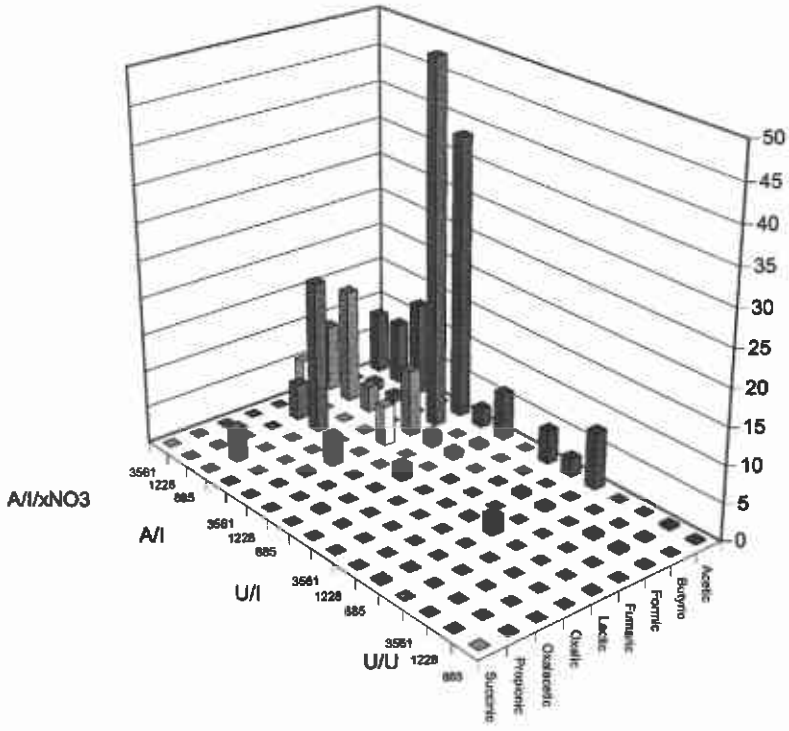


Figure 10. Organic acids produced at 885, 1228, and 3561 days incubation in **anaerobic treatments with bentonite**: U/U= unamended, uninoculated; U/I=unamended, inoculated; A/I=amended, inoculated; A/I/xNO3=amended, inoculated + excess nitrate.

### .4.5. Methane Analysis of Inundated Samples.

Tables 10-11 provide data for 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 is 0.2 nmol $CH_4$ $g^{-1}$ cellulose dry wt. Methane was first detected in small quantities in most anaerobic samples except those with excess nitrate (Table 10, 2718 days (7.4 years)). At 3462 days (9.5 years) methane was still detected in greatest quantity in samples that were not amended with any nitrogen-containing compounds ($NH_4NO_3$, $KNO_3$) specifically the unamended/inoculated samples. However, for the first time, 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 the time to initial production was lengthy, these samples eventually accumulated methane at a relatively rapid rate: the rate of methane production was 2.5 pmol $CH_4$ $g^{-1}$ cellulose $d^{-1}$ in unamended and amended inoculated samples and 3.45 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 be due to the extreme difficulty methanogens have in metabolizing the substrates such as acetate, $CO_2$, and $H_2$ (the presence of $H_2$ was reported in SAND96-2582, $CO_2$ concentrations are given in Section 4.1 of this report, and acetate concentrations are reported in section 4.4) under hypersaline conditions due to bioenergetic constraints (Oren, 1999). The preferred substrate is 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 range from 0.7 to 1.7 $CH_4$ pmol $g^{-1}$ cellulose $d^{-1}$.

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

Table 11. 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

## 4.6. Microbiological Characterization

One of the triplicate reserve samples (not used for periodic gas analysis) from the following anaerobic inundated cellulose treatments were analyzed to identify the microbial community: i) unamended, uninoculated, ii) unamended, inoculated, iii) nutrient amended and inoculated, 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. Culture-independent methods were used to quantify and identify microorganisms, specifically denaturing gradient gel electrophoresis (DGGE) analysis (Muyzer et al., 1993). Figure 11 presents the results of microbiological analysis. Each lettered band in the figure corresponds to a unique bacterial species; the greater the number of bands the greater number of bacterial species in the samples. Higher diversity, as determined by a greater number of microbial species, was correlated with nutrient amendment and concomitant gas production. Data for the enumeration of bacteria in the treatments after 6 years incubation is presented in Table 9 (data from Francis et al., 1998). One gram-positive microorganism (genus *Clostridium,* band A, Figure 9) was detected in the anaerobic unamended uninoculated treatment; this is of interest given that almost all halophiles are gram-negative. This treatment is characterized by a low starting biomass and continual stress induced by lack of abundant electron acceptors. Introduction of mixed inoculum, but not nutrients, also resulted in dominance by one genus, *Halobacter utahensis* (bands B, M, N, and O, Figure 9). In general, abundant nutrient availability lowers microbial diversity, as has been found in non-saline, low-carbon environments. Samples from the inundated cellulose experiment are analogous to environments loaded with highly complex-carbohydrates. Cellulolytic microbial populations associated with the animal rumen, a very high carbon-loading environment, have been shown to be diverse (Cho and Kim, 2000). Besides organic carbon availability, Roling et al. (2001) showed that microbial community structure in a benzene-impacted groundwater environment was determined by available electron acceptor. *Halobacterium, Haloarcula, Halobacter, and Natranobacterium* were found in the nutrient amended, inoculated treatment ((Four genera) bands C, D, E, P, Q, R, Figure 9); a fairly high diversity and

unique due to the presence of *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 *Halobacterium*, *Halobacter*, *Halococcus*, *Natranobacterium*, *Natranomonas* ((Seven genera) bands F, G, H, S, T, U, and V, Figure 9), and unidentified archaea (bands S and V). The known sample resulted in the identification of three genera, thus verifying the applicability of this technique to halophilic bacteria: bands I, J (*Halomonas* sp.); K, L (*H. praevalens*); and the archaea, *H. salinarium* (band W). An obvious limitation of the technique, however, is the size of the bacterial databases; these are generally less populated with environmentally-relevant isolates, especially extremophiles, and in some instances a positive identification is not possible (e.g. bands S and V, Figure 5).

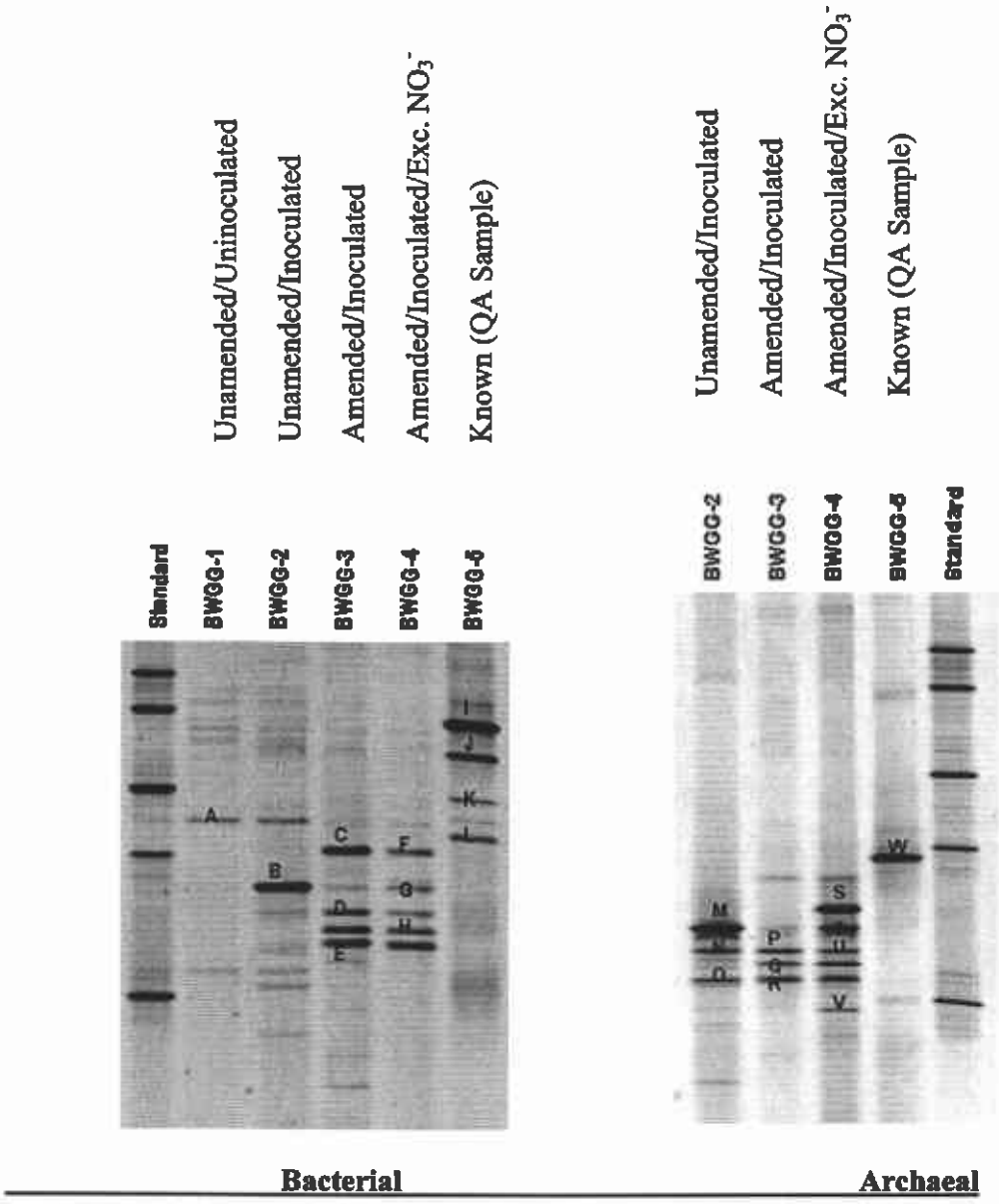


Figure 11. DGGE gel image of amplified primers from a conserved region of bacterial and archaeal 16S rDNA from the anaerobic inundated cellulose treatments. Banding patterns and relative intensities of the recovered bands provide a measure of differences among the communities. Dominant species must constitute at least 1-2% of the total bacterial community to form a visible band. Labeled bands were excised and sequenced.

## 4.6 Gas Produced in Humid Cellulose Treatments

Tables 1-4, Appendix C, provide data for 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 provides data that has been corrected to account for $CO_2$ produced in the absence of cellulose due to metabolism of any dissolved organic carbon in the mixed inoculum; the resultant data is reported as $CO_2$ produced per gram cellulose. Table 6-9 provide data for total gas and $CO_2$ produced in anaerobic humid samples incubated for 2945 days (8.1 years); Table 10 provides corrected data for $CO_2$ production 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 inoculum viability (succinate or glucose amended treatments) were not analyzed during every time period. Carbon dioxide concentrations are the best indicator of microbial activity under humid conditions and are therefore provided in Figures 13-16. 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.

### 4.6.1 INITIALLY AEROBIC TREATMENTS

Figure 12 shows samples from the initially aerobic experiment at 399 days incubation; A-2 was amended with nutrients while BA-3 was amended with nutrients and also contained bentonite. All samples of the later treatment reached their maximum gas production at 399 days and a bright red biomass was observed on the bentonite particles. By the end of the experiment at 3334 days, initially aerobic humid treatments with and without bentonite had generally entered a period of cessation in gas production (Figure 13 and 14). 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 11). In amended inoculated samples, CO2 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). 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 time period, or 9.0 ml overall from a ~155 ml headspace volume). Even with this correction, the profile for $CO_2$ production shows decreasing concentrations past the early peaks in production (Figure 13). Some of this loss is also 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 12) was not acidified and $CO_2$ could have been absorbed from the headspace due to reaction with the brine to form carbonic acid and bicarbonate. Nevertheless, the gas production profiles for samples without bentonite show that a limited capability for microbial growth on cellulose under initially aerobic humid conditions (relative humidity = 70%). Similar to it's effect on inundated samples, bentonite enhanced gas production under humid conditions. Figure 14 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). This was ~50x more $CO_2$ produced $g^{-1}$ cellulose than in the same treatment than without bentonite. The bentonite alone did not provide enough organic carbon to account for this excess; in the absence of cellulose, 144 ± 4 μmoles of $CO_2$ was produced 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 since the start of the experiment at a fairly steady 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.

### 4.6.2 ANAEROBIC TREATMENTS

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 15). 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 time periods 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). The nutrient amendment had a major impact on $CO_2$ production in anaerobic samples without bentonite, with ~8x more $CO_2$ produced in the absence of nutrients than in their presence (Figure 15). The addition of bentonite enhanced $CO_2$ production under anaerobic humid conditions, with 28x more $CO_2$ produced in amended inoculated samples with bentonite than the same treatment without bentonite. While nutrients had a detrimental effect on the mixed inoculum under anaerobic humid conditions, the addition of bentonite served to nullify this effect. In fact, whether or not nutrients were present, $CO_2$ production proceeded similarly (Figure 16). Unamended inoculated samples with bentonite showed 541 ± 135 μmol $CO_2$ $g^{-1}$ cellulose at 2945 days and amended inoculated samples showed 618 ± 125 μmol $CO_2$ $g^{-1}$ cellulose. It is important to note that samples that show a larger deviation from the mean generally show evidence of microbial activity (trending toward positive gas production). The larger spread in the data is indicative of microbial processes that may be occurring at slightly different rates in the active samples due to differences in overall microbial population or metabolic capability. Samples with a smaller variation in values between triplicate bottles have generally ceased to show additional microbial gas production.

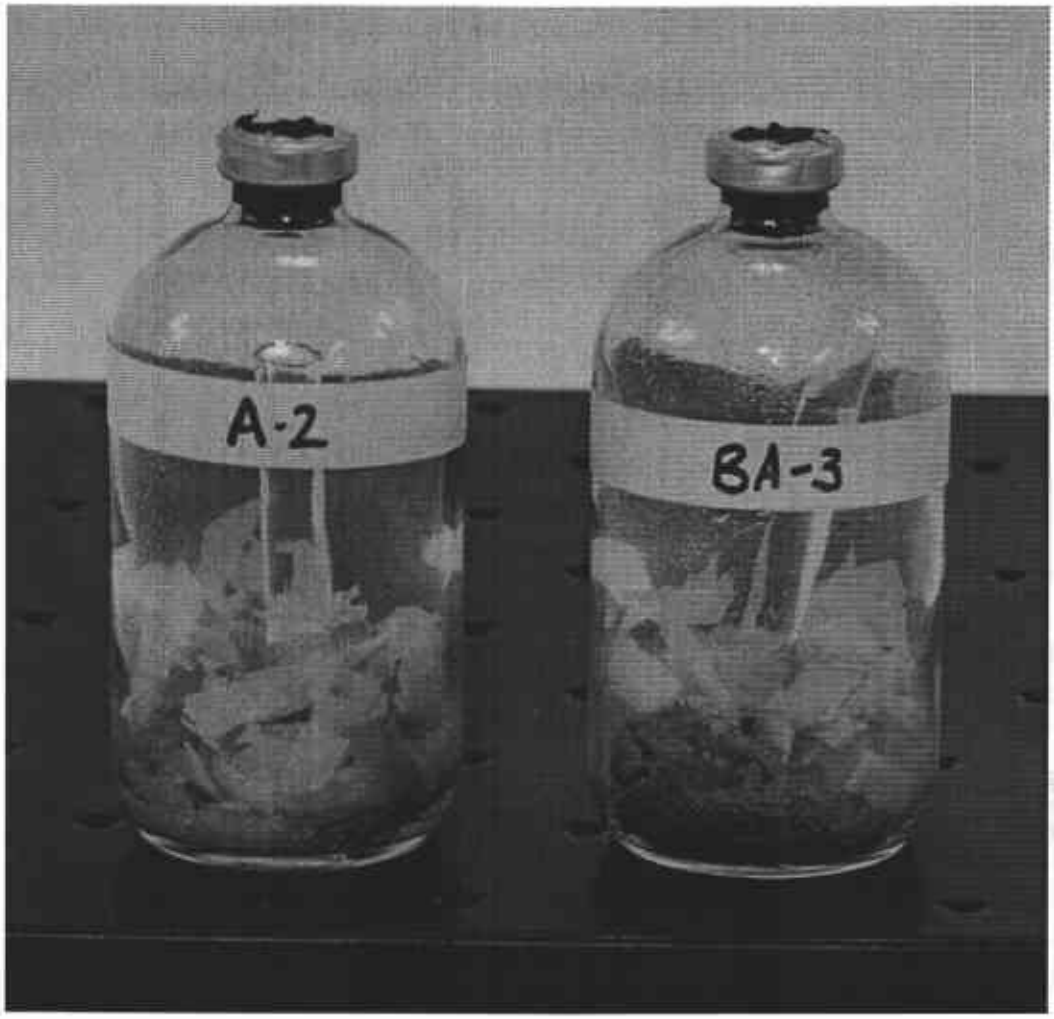


Figure 12. Initially aerobic humid samples at 399 days incubation : amended (A-2), amended plus bentonite (BA-3).

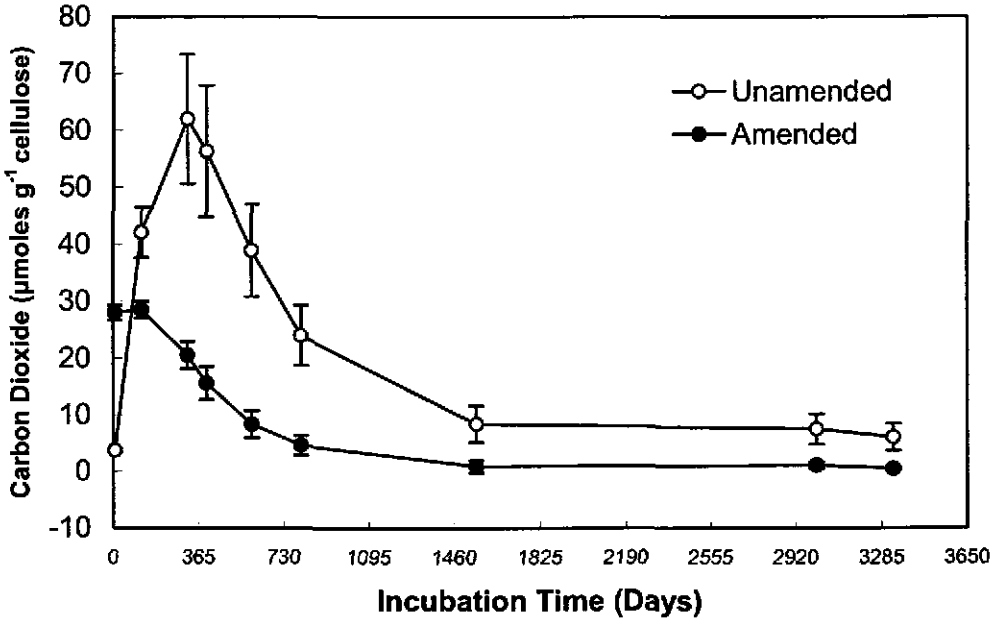


Figure 13. Carbon dioxide produced in initially aerobic humid samples without bentonite.

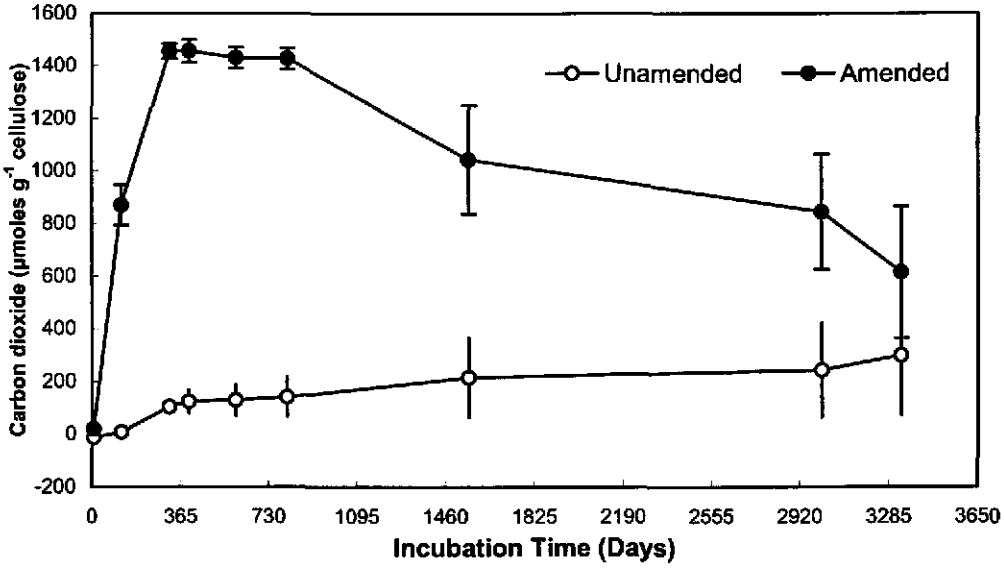


Figure 14. Carbon dioxide produced in initially aerobic humid samples with bentonite.

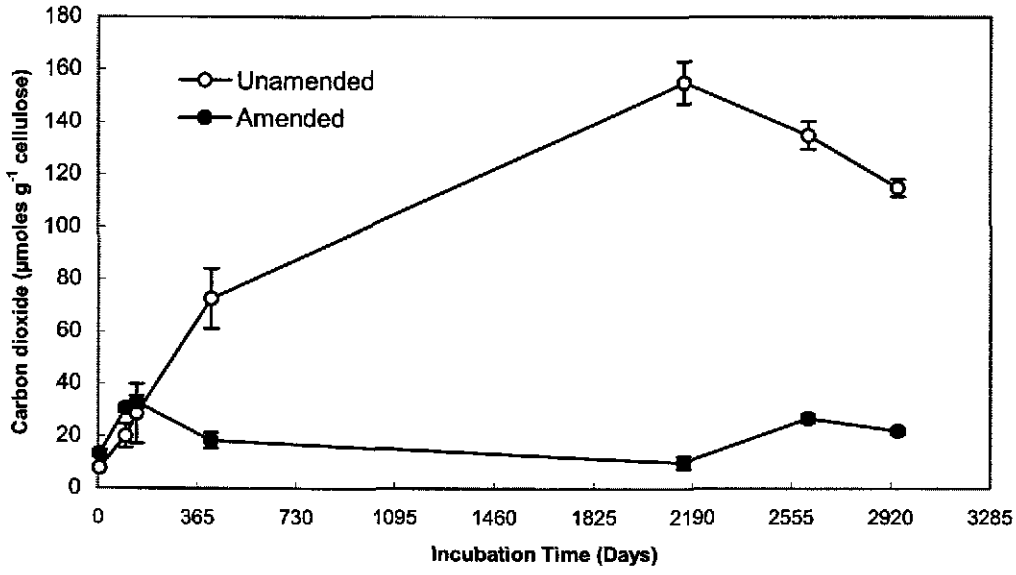


Figure 15. Carbon dioxide produced in anaerobic humid samples without bentonite.

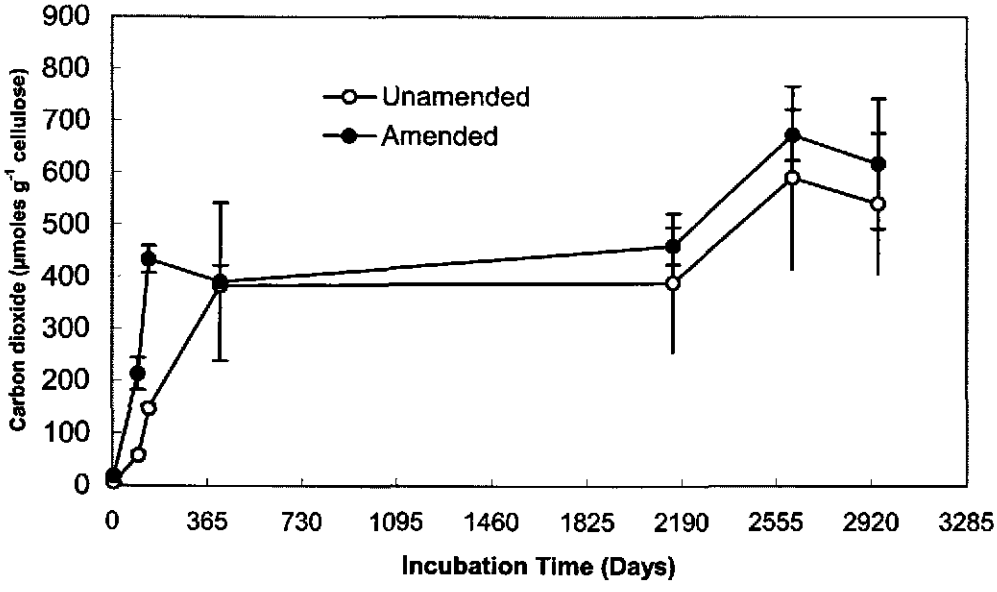


Figure 16. Carbon dioxide produced in anaerobic humid samples with bentonite.

**4.7. Analysis of Methane in Anaerobic Humid Samples.** Methanogenesis is a potential gas-consuming microbial process that may occur under repository conditions. In addition, methanogenic bacteria are extremely sensitive to changes in pH, Eh, the presence of oxygen, and have seldom been found to metabolize complex organic substrates under hypersaline conditions (Oren, 1999). The entire set of samples from the anaerobic humid cellulose biodegradation experiment was analyzed for the presence of methane at 2653 days (7.3 years) incubation. Methane was detected in unamended inoculated samples with bentonite and amended inoculated samples with bentonite, but was below detectable (<0.1 nmol $ml^{-1}$) in all other samples. Table 12 summarizes the results of this analysis.

Table 12. Methane analysis of anaerobic humid samples at 2653 days incubation.

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

Section 4.5 of this report provides data for methane produced under inundated conditions; in these samples at 3462 days incubation ( 9.5 years), unamended inoculated sample with bentonite showed 4.51 ± 0.06 nmol $CH_4$ $g^{-1}$ cellulose and amended inoculated samples with bentonite showed 3.41 ± 0.13 nmol $CH_4$ $g^{-1}$ cellulose. Under humid conditions, and in the presence of bentonite, the production of methane appears more favorable; in unamended samples methane production was 6x greater than under inundated conditions and almost 10x greater for amended humid samples. This is further evidence of the stimulatory effect of bentonite on microbial activity and also verifies the presence of viable methanogenic bacteria which should be capable of methanogenesis in all of the samples if conditions are favorable. In addition, the maintenance of strictly anaerobic conditions is verified by methanogenic bacterial activity which cannot occur even in the presence of trace oxygen (Ramakrishnan et al., 2000) It is possible that methanogenic

bacterial activity could account for the loss of $CO_2$ as seen under initially aerobic conditions with and without bentonite (Figures 13 and 14), although methane was not detected in these samples.

## 4.8 Gas Produced in Samples Containing Plastic and Rubber Materials.

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)) is presented in Tables 1-10, Appendix D. This data is presented graphically in Figures 20-29 (data are the mean of duplicate or triplicate analyses (see Section 3.3)).

Plastic and rubber materials consist of long repeating single bonded carbon chains and are usually quite resistant to biodegradation. Irradiation causes the polymer to break down due to free radical formation, in addition there can be cross-linking of the polymer chain after free radical formation and reduction of the molecular mass of the polymer (Woods and Pikaev, 1994). Results of experiments presented here 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 polymer biodegradation. Total gas volume and the concentration of $CO_2$ or $CH_4$ in samples containing low- and high-dose irradiated polymer are compared to baseline concentrations for control samples without polymer or samples containing unirradiated polymer. Variables that can influence biodegradation, *including atmosphere (air) or nutrients were tested* for each irradiation dose and polymer material type.

### 4.8.1 CONTROL (NO POLYMER)

Samples incubated without plastic or rubber material served as controls. These samples 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 without added nutrients (unamended) or with them (amended). 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 unirradiated or irradiated polymer stimulated more production.

### 4.8.2 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 20 and 21). Polyethylene may serve as a substrate for attachment of a biofilm community thereby providing for 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 20 (A), and (C); Figure 21 (A)). Under initially aerobic conditions, unamended samples containing unirradiated PE produced 64.2 μmoles $CO_2$ sample$^{-1}$, while in the absence of 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.

### 4.8.3 POLYVINYLCHLORIDE

Irradiated PVC showed the most obvious changes in characteristics. A viscous residue was present on the low-dose irradiated PVC, but less prominent on the high-dose irradiated PVC. Figure 22 (B) shows the inhibitory effect of irradiated PVC on total gas production under initially aerobic nutrient-amended conditions; this correlates with Figure 23 (B), where low-dose irradiated PVC had a marked effect on $CO_2$ production, lowering production by 30% relative 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 22 (C) and 23 (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 23 (C), Table 7, Appendix D). While 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.

### 4.8.4 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 24 (A and B); Figure 25 (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 the same, 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 25 (C), closed triangles). After 840 days incubation, however, this inhibition was overcome and $CO_2$ production recovered to levels of samples containing unirradiated and without polymer. Unamended samples containing low-dose irradiated neoprene showed slightly more $CO_2$ production after 2612 days incubation (Figure 25 (C), open triangles). The nutrient amendment resulted in increased and $CO_2$ production when high-dose irradiated neoprene was present under anaerobic conditions; the rate of CO2 production was significant early-on, and this resulted in the production of 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 back-bone or the creation of readily-metabolizeable organic material released onto the surface of the neoprene due to the irradiation process. The evidence of an inhibitory effect (Figure 25 (C), high-dose irradiated neoprene) suggests that a film of material was deposited on the surface of the polymer that readily interfered with

metabolism of dissolved organic carbon in the sample. A similar phenomenon was shown for low-dose irradiated PVC (Figure 23 (C)). Over time, however, this material was metabolized; the toxicity of this material was decreased when a nutrient amendment was present (Figure 25 (C)). The radiation dose that the neoprene received was different from the expected irradiation conditions in the WIPP repository; it is difficult to directly extrapolate data obtained with this material 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 in order to simulate long-term radiation damage in WIPP.

### 4.8.5 UNLEADED HYPALON

Figures 26-27 show the results of gas analysis of samples containing unleaded hypalon under various conditions; irradiation at high-dose rates were not performed. Experiments involving hypalon, unleaded and leaded, were started at a later date than experiments with the other polymers. A new mixed inoculum was prepared for the experiments involving hypalon; the dissolved organic carbon content of this inoculum was most likely lower than the previous inoculum 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 27 (A, B) and Figure 21 (A,B)). Nevertheless, the inoculum was viable as indicated by gas and $CO_2$ production. A greater amount of $CO_2$ was produced when low-dose irradiated hypalon was present under initially aerobic conditions without a nutrient amendment (Figure 27(A)) or with a nutrient amendment (Figure 27(B)). Only 25% more $CO_2$ ($43.8 \pm 7.1$ μmoles $CO_2$ $sample^{-1}$) was produced under initially aerobic nutrient amended conditions relative 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. In the later case, the effect of the presence of low-dose irradiated hypalon may become significant only after 6.75 years incubation if $CO_2$ production continues to be sustained (Figure 27 (D)).

### 4.8.6 LEADED HYPALON

Figures 28-29 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. There was an inhibitory effect of the presence of low-dose irradiated leaded hypalon on total gas and CO2 production under initially aerobic conditions, with and without nutrients (Figure 28 (A,B) and Figure 29 (A, B)). Conversely, the presence of unirradiated leaded hypalon stimulated gas and $CO_2$ production when nutrients were present (closed circles, Figure 28 (B) and Figure 29 (B)). In fact, $CO_2$ production in samples containing unirradiated leaded hypalon was on par with samples containing low-dose irradiated unleaded hypalon (Figure 27 (B)). There was no significant effect on gas production when irradiated leaded-hypalon was present under anaerobic conditions (Figure 28 (C,D) and Figure 29 (C,D).

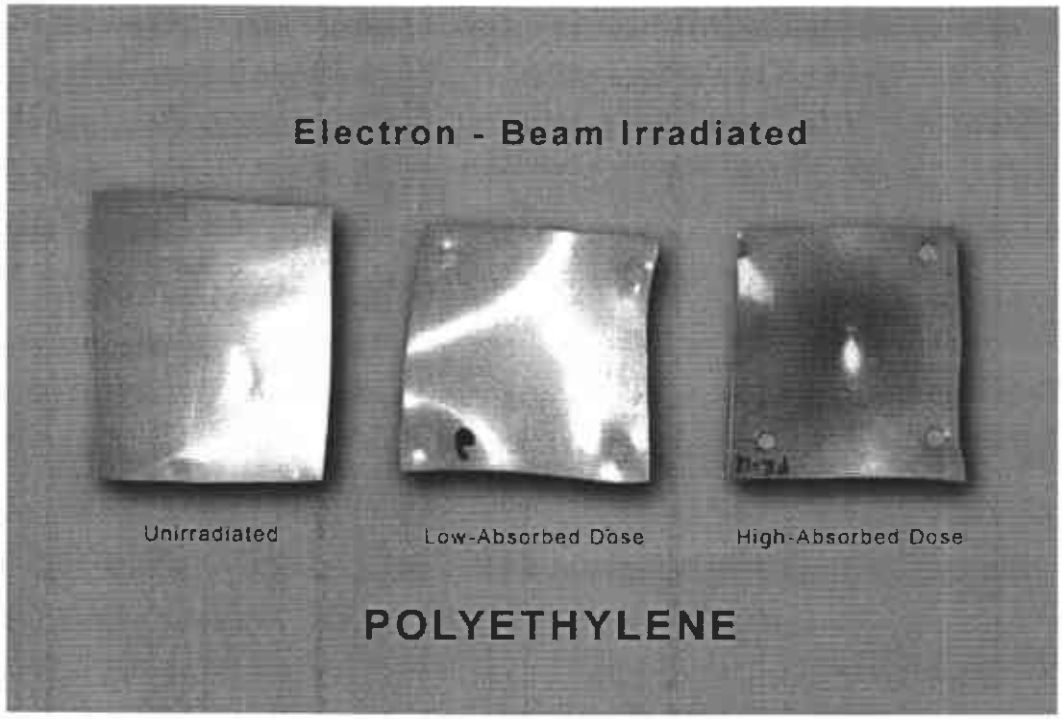


Figure 17. Unirradiated and irradiated (low-absorbed dose, and high-absorbed dose) polyethylene.

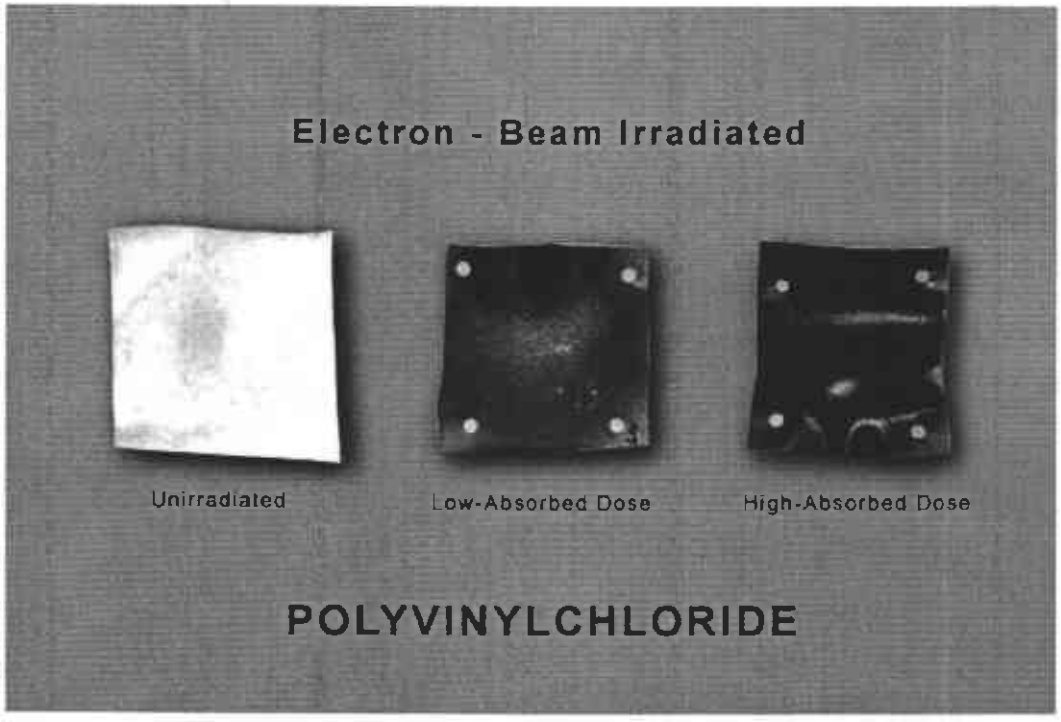


Figure 18. Unirradiated and irradiated (low-absorbed dose, and high-absorbed dose) polyvinylchloride.

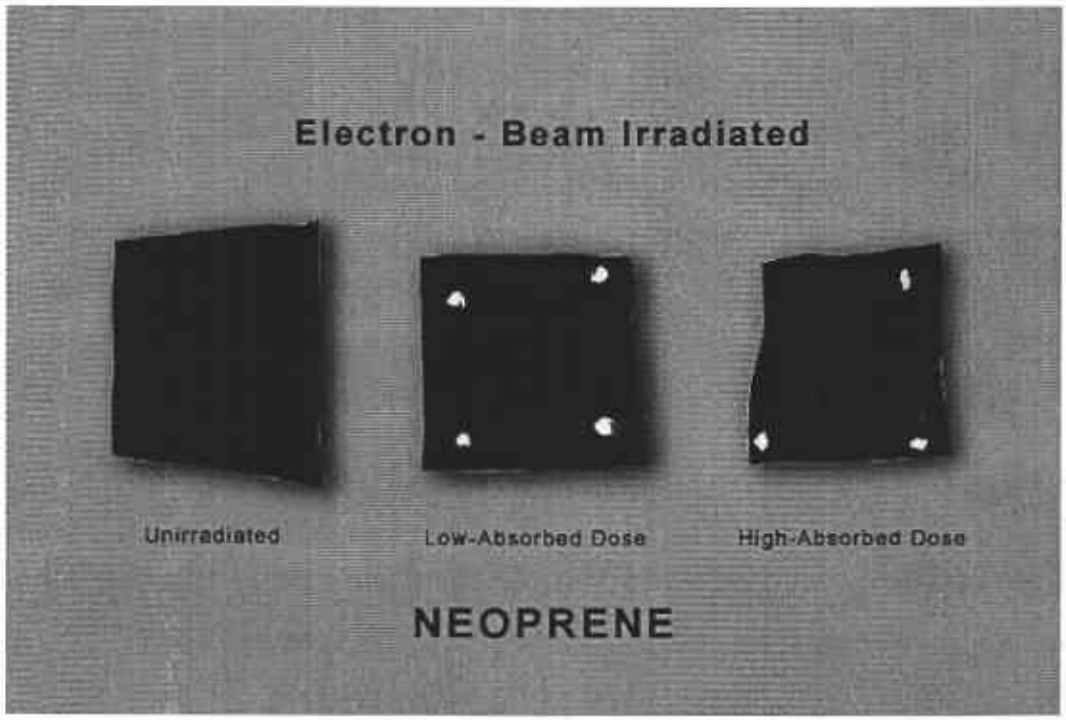


Figure 19. Unirradiated and irradiated (low-absorbed dose, and high-absorbed dose) neoprene.

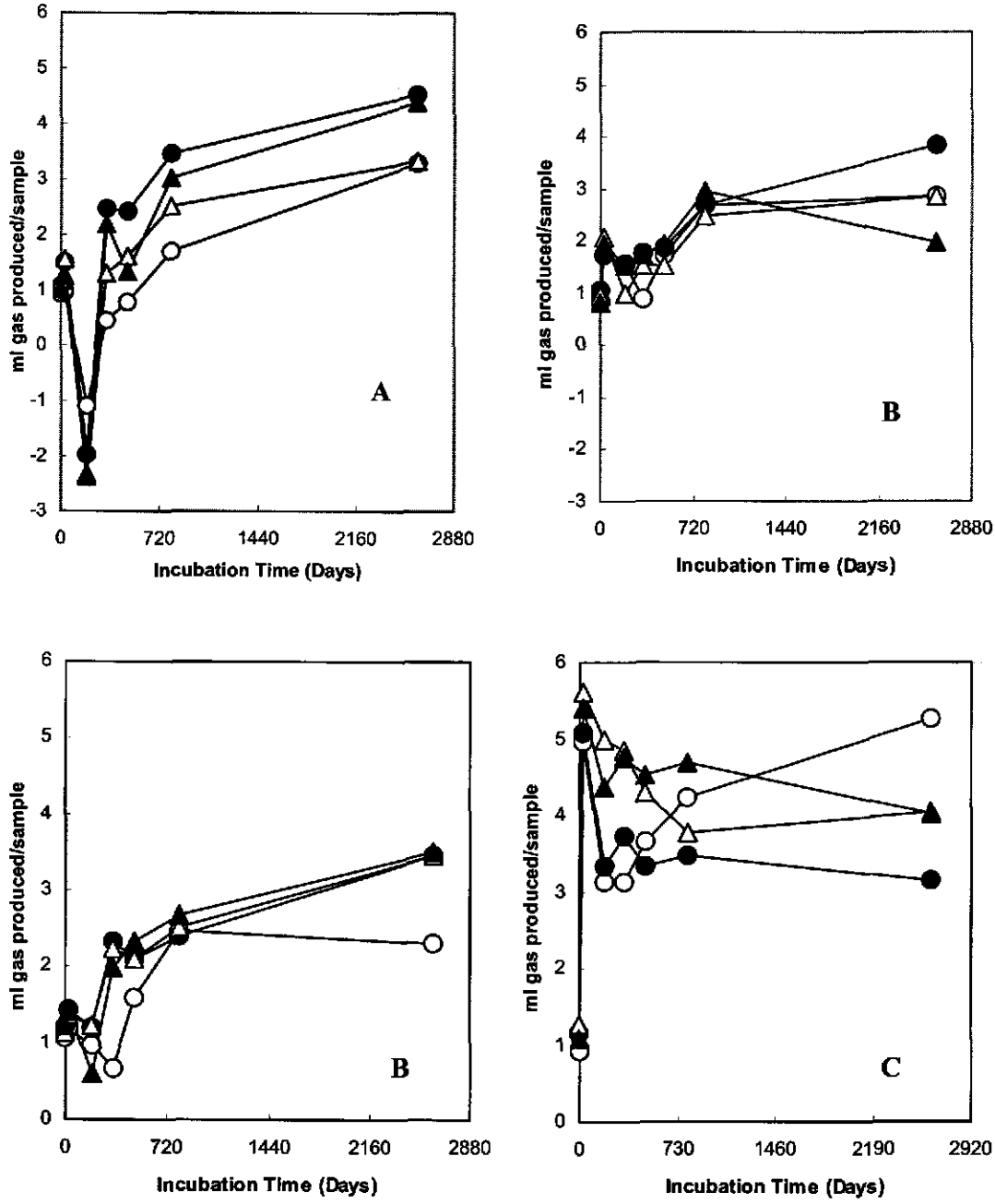


Figure 20. Total gas produced in samples containing polyethylene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D). No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

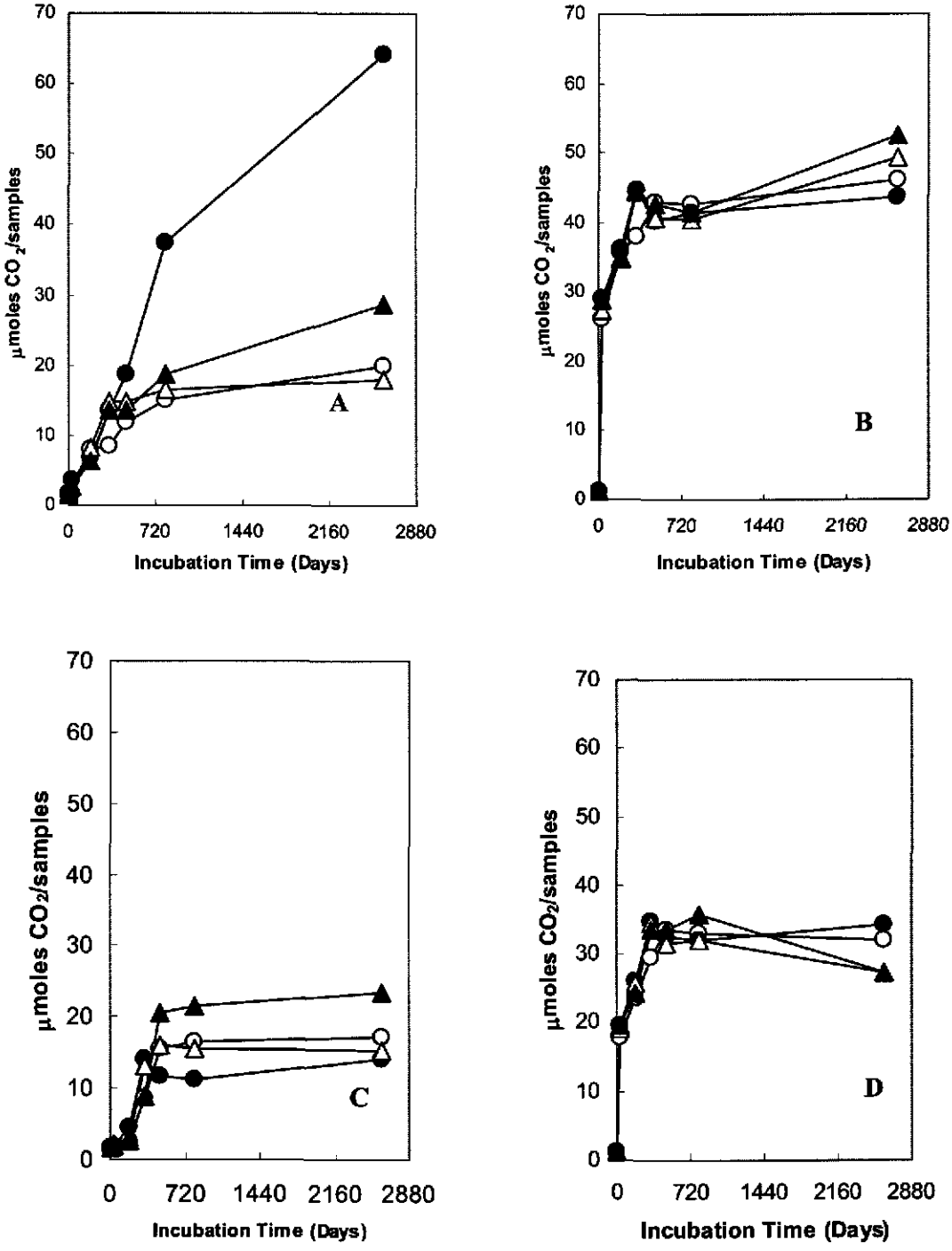


Figure 21. Carbon dioxide produced in samples containing polyethylene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

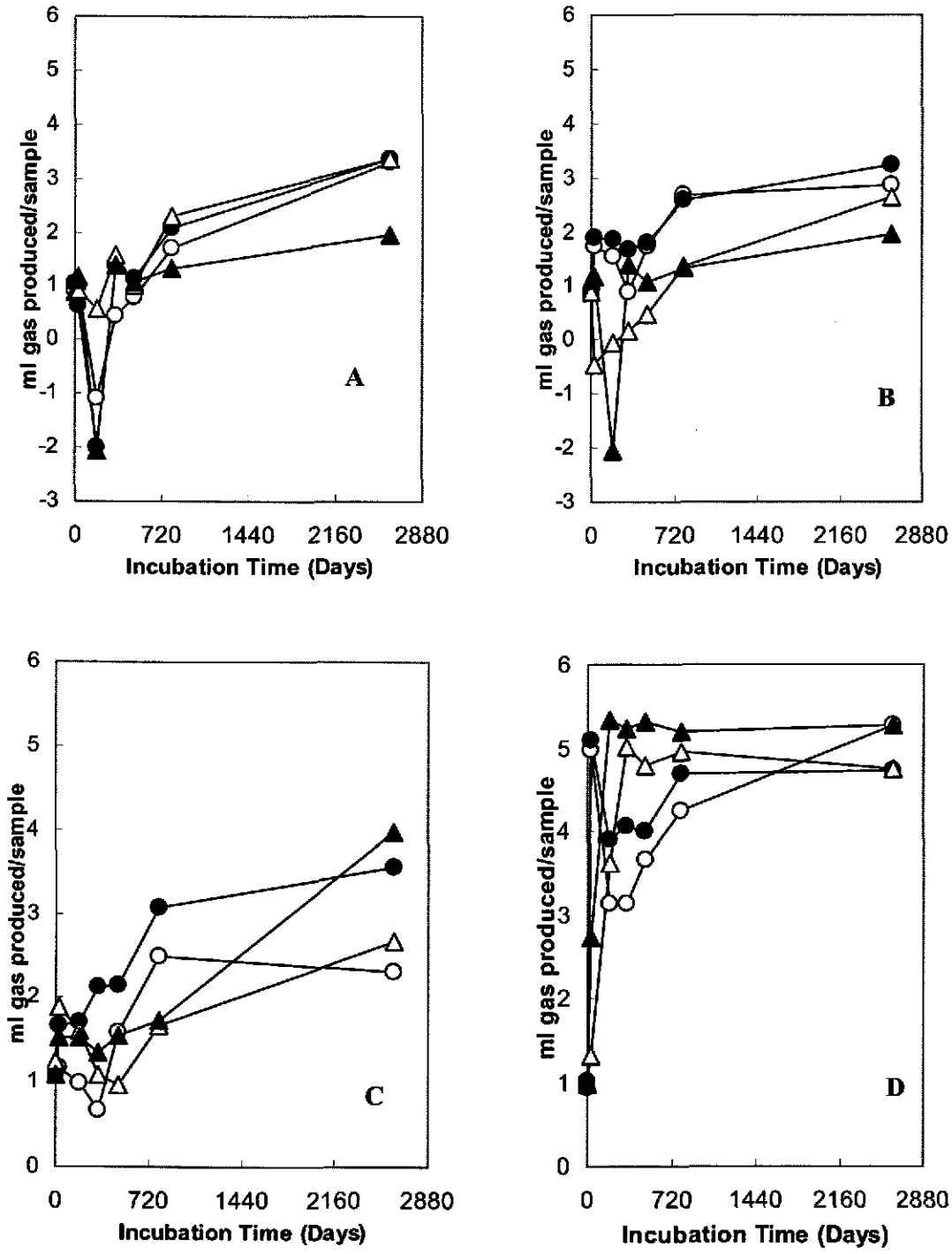


Figure 22. Total gas produced in samples containing polyvinylchloride: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (O), unirradiated (●), low-dose (△), high-dose (▲).

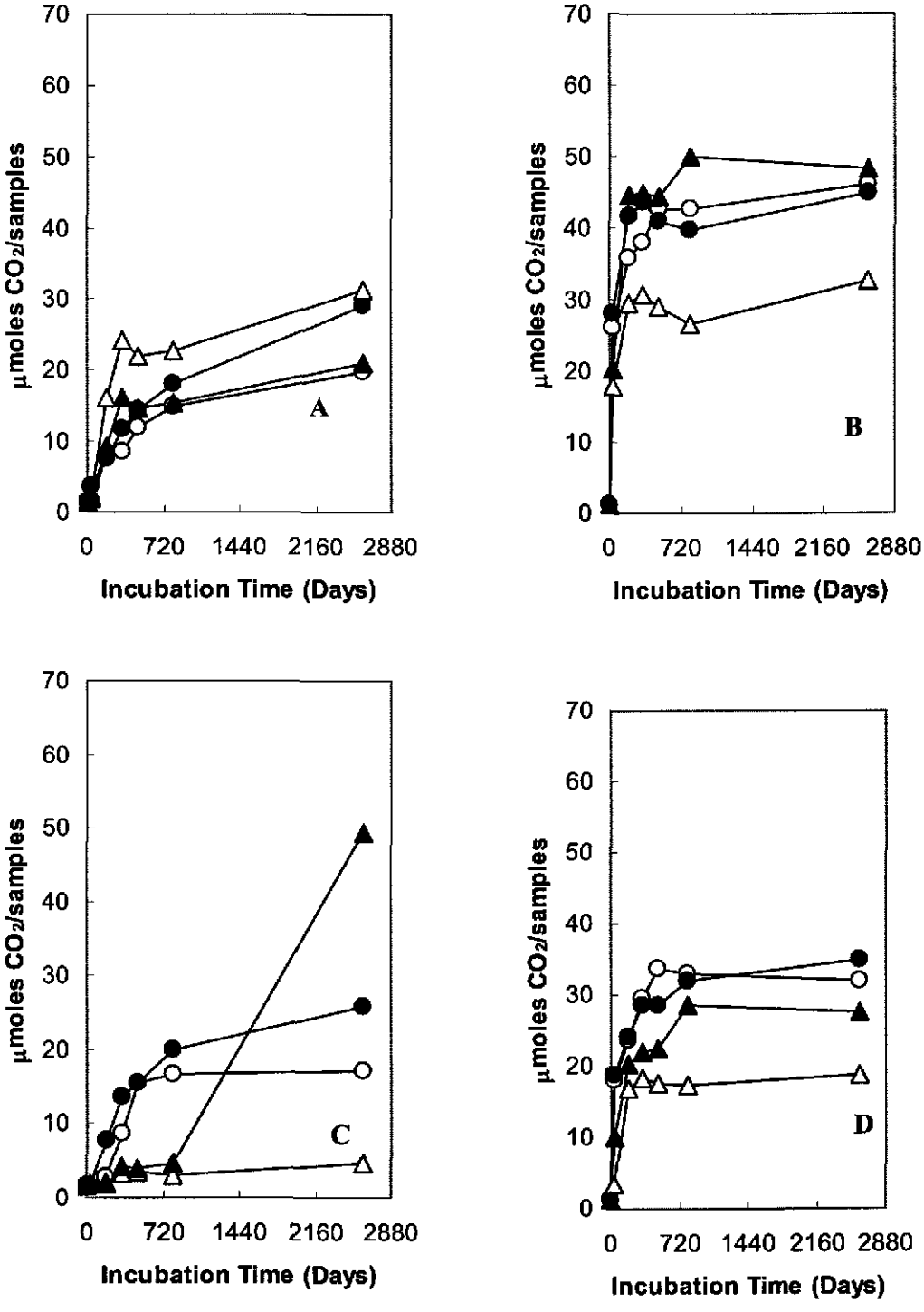


Figure 23. Carbon dioxide produced in samples containing polyvinylchloride: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

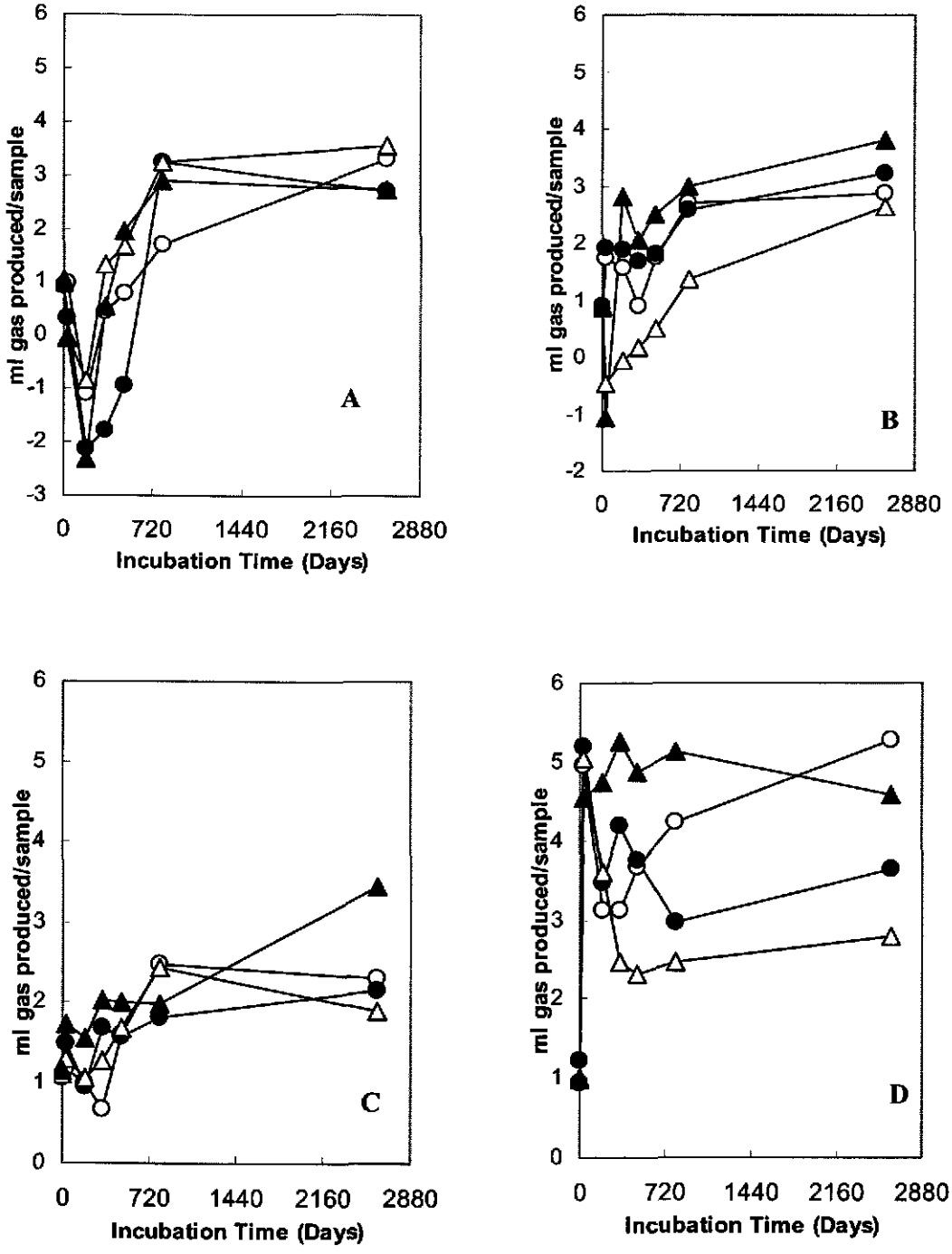


Figure 24. Total gas produced in samples containing neoprene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

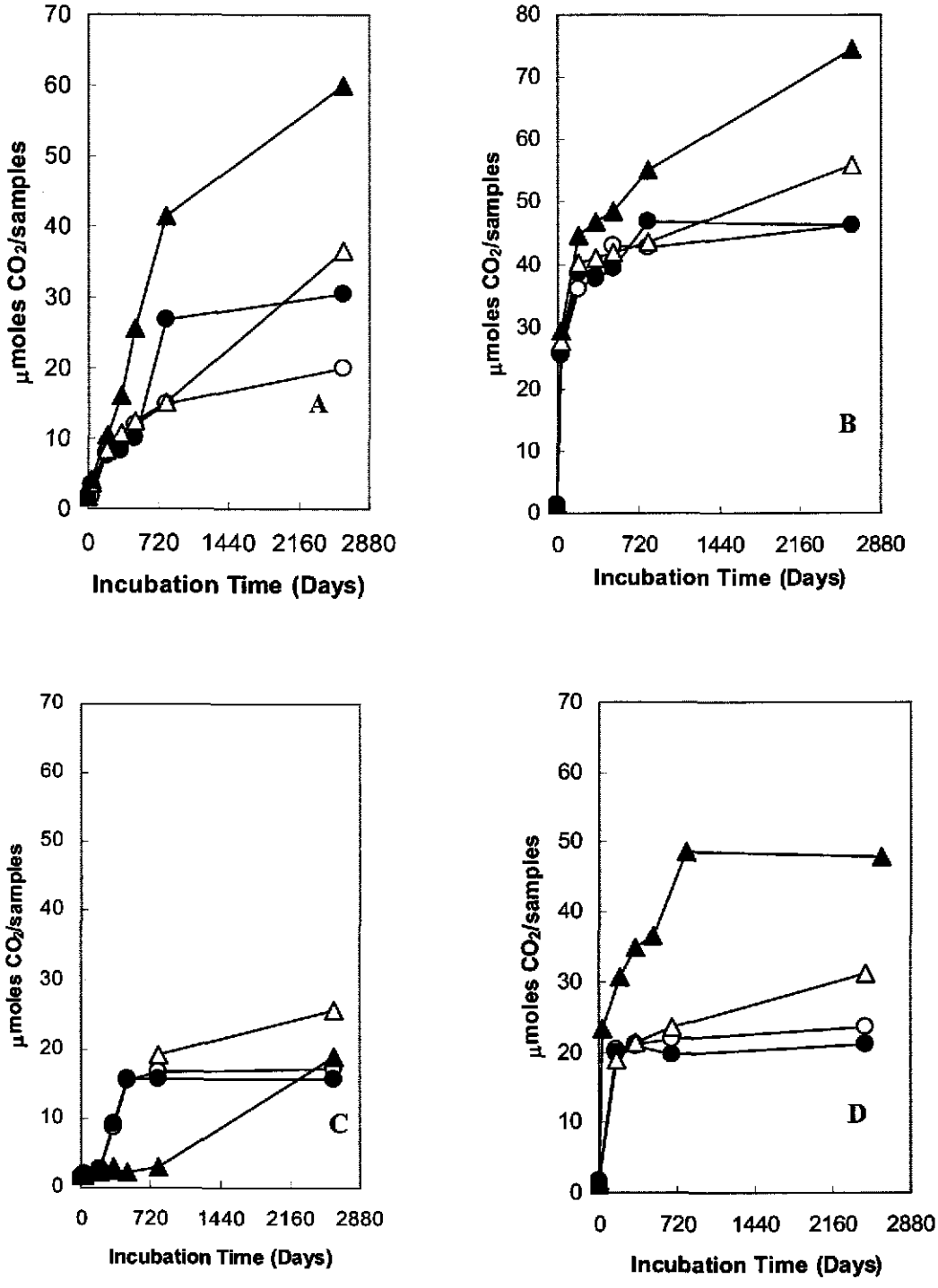


Figure 25. Carbon dioxide produced in samples containing neoprene: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

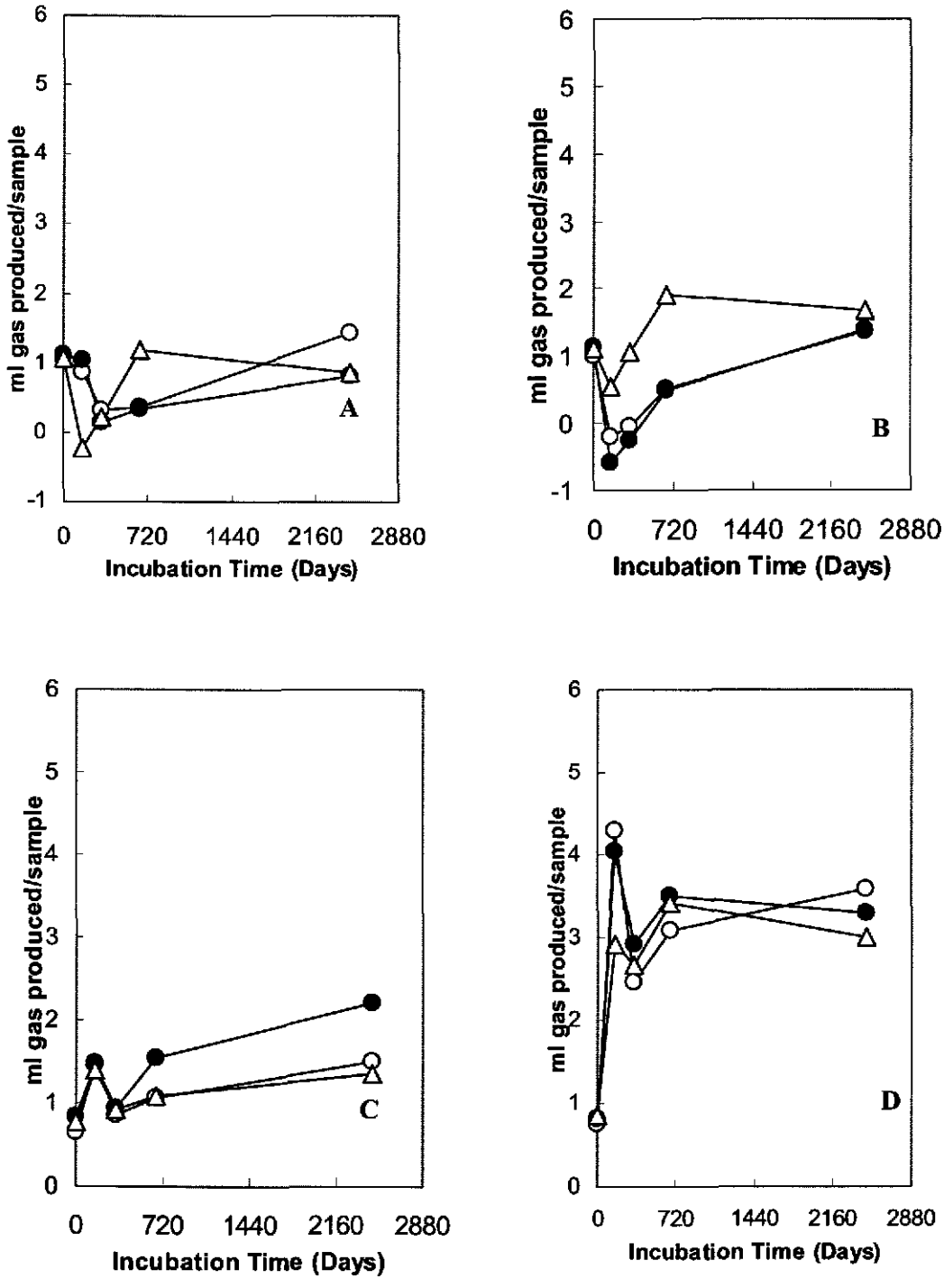


Figure 26. Total gas produced in samples containing unleaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

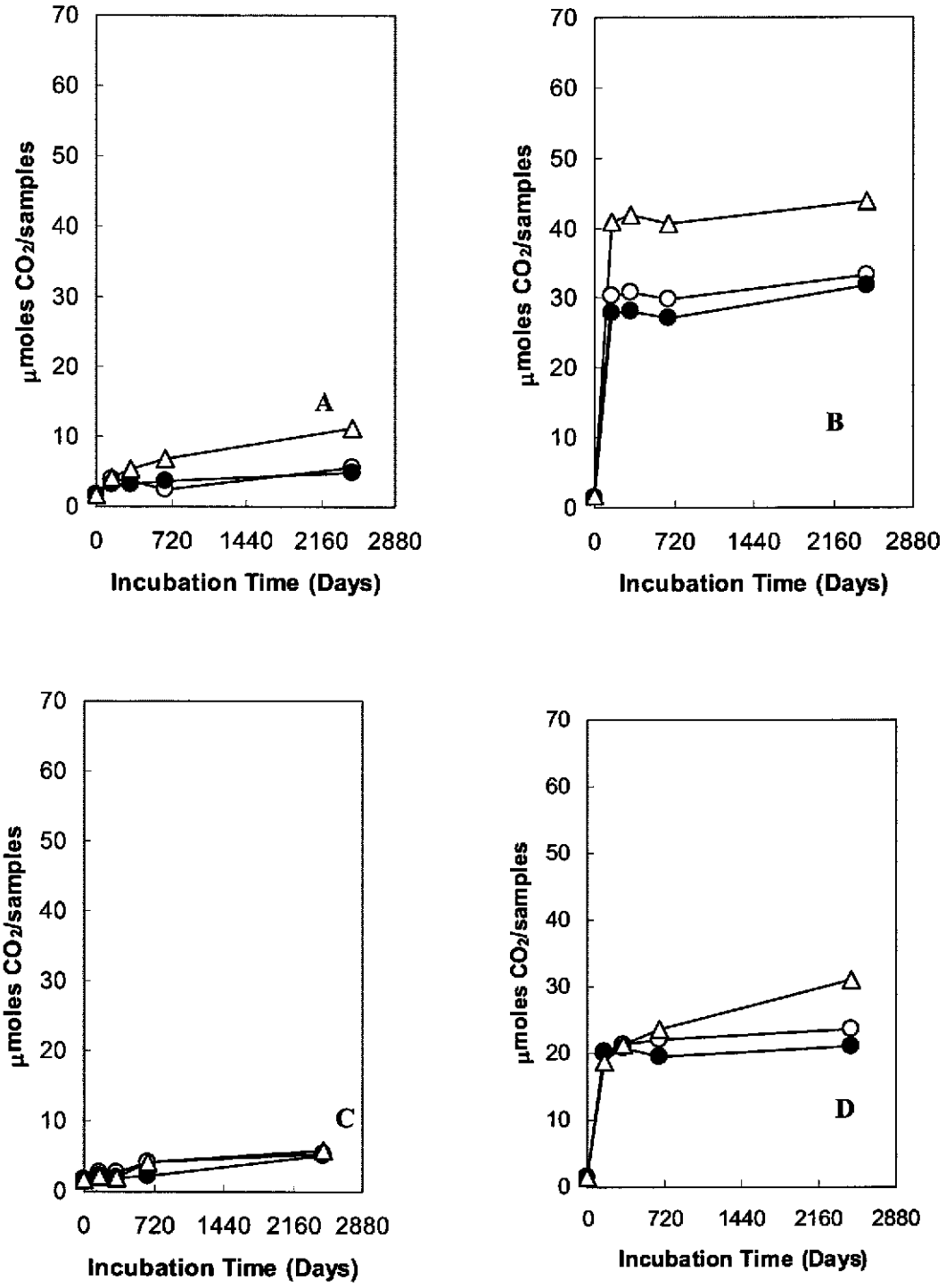


Figure 27. Carbon dioxide produced in samples containing unleaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

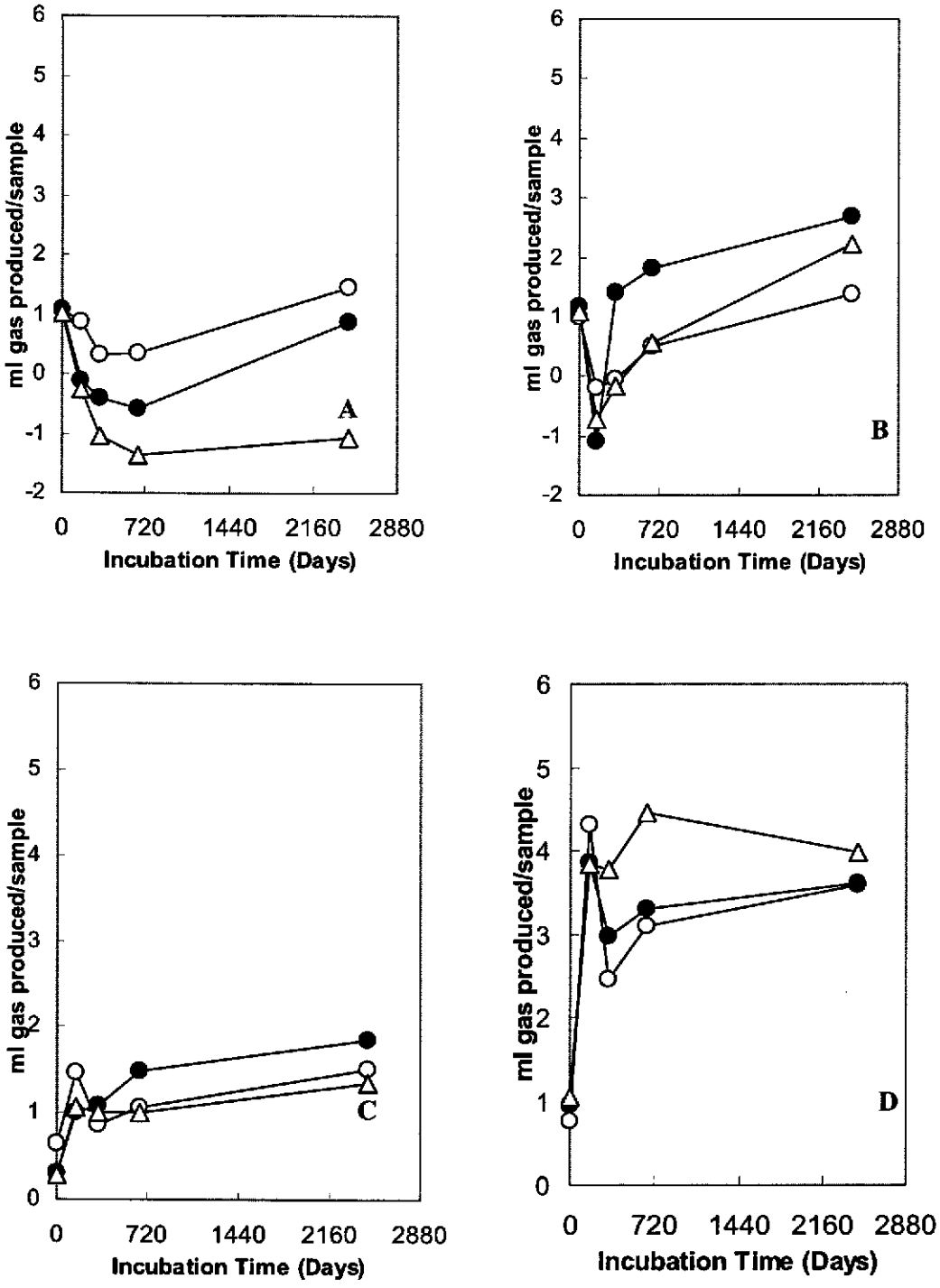


Figure 28. Total gas produced in samples containing leaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (○), unirradiated (●), low-dose (△), high-dose (▲).

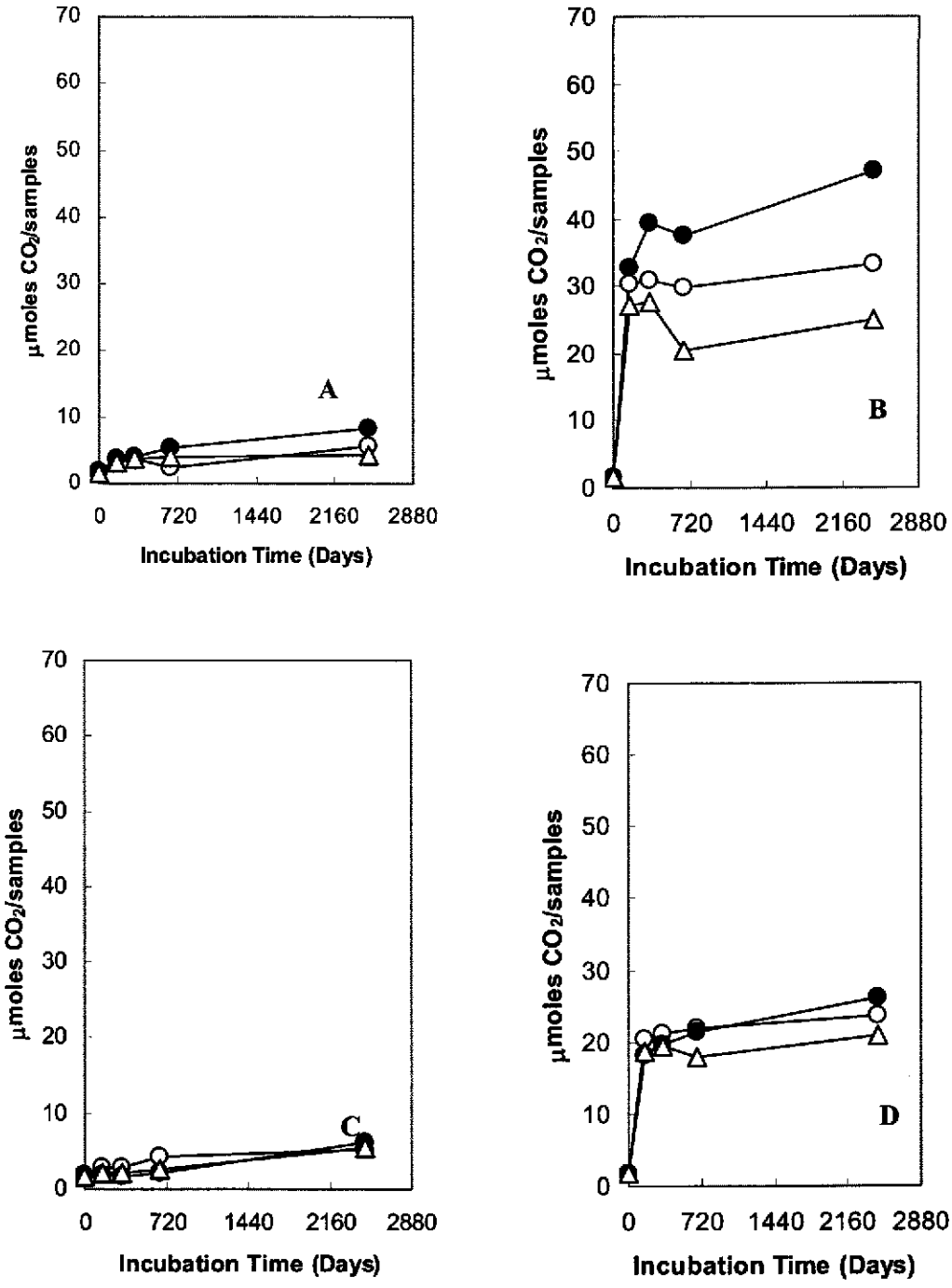


Figure 29. Carbon dioxide produced in samples containing leaded hypalon: aerobic unamended (A); aerobic amended (B); anaerobic unamended (C); anaerobic amended (D).No polymer (O), unirradiated (●), low-dose (△), high-dose (▲).

## 4.9 Analysis of Methane Production in Samples Containing Plastic or Rubber Materials.

Results of methane analyses up to 3070 days (8.4 years) incubation for PE, PVC, and NE and 2926 (8 years) for HY are summarized in Table 13 (anaerobic treatments only). Over a period of 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 to this was the unirradiated polyethylene, which increased from 2.14 ± 1.52 μmol sample$^{-1}$ at 840 days incubation to 2.50 ± 0.26 μmol sample$^{-1}$ at 3070 days, however, this does not appear to be significant. In addition, samples containing hypalon did not show any increase in methane over 2262 days incubation (6.2 years). The concentrations of methane detected at 664 and 840 days and at 2926 and 3070 days are extremely consistent indicating that no further methanogenesis has occurred in these samples. The methane detected is most likely the result of metabolism of dissolved organic carbon in the mixed inoculum/inundation fluid, however, additional methane production due to biodegradation of the polymer is not evident. The inhibitory effect of irradiated PVC remained after 6.1 years indicating that the degradation products produced due to irradiation continue to be toxic to the microbial consortium in the samples.

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

| Anaerobic Treatment | Incubation Time T=840 days | Incubation Time T=3070 days |
|---|---|---|
| | Methane (μmol sample$^{-1}$) | |
| *Samples without polymer (no irradiation)* | | |
| Unamended | 0.91 ± 0.14 | 0.99 ± 0.20 |
| Amended | 4.03 ± 0.17 | 3.65 ± 0.11 |
| *Polyethylene* | | |
| Unirradiated – Unamended | 0.85 | 0.53 |
| Amended | 2.14 ± 1.52 | 2.50 ± 0.26 |
| 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 |

| Anaerobic Treatment | | Incubation Time | |
|---|---|---|---|
| | | T=840 days | T=3070 days |
| | | Methane (μmol sample$^{-1}$) | |
| *Unleaded Hypalon* | | *T=664 days* | *T=2926 days* |
| 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

Barnhart, B.J., E.W. Campbell, E. Martinez, D.E. Caldwell, and R. Hallett. 1980. Potential Microbial Impact on Transuranic Wastes Under Conditions Expected in the Waste Isolation Pilot Plant (WIPP). Annual Report October 1, 1978-September 30, 1979. LA-8297-PR. Los Alamos, NM: Los Alamos Scientific Laboratory.

Brush, L.H. 1990. *Test Plan for Laboratory and Modeling Studies of Repository and Radionuclide Chemistry for the Waste Isolation Pilot Plant*. SAND90-0266. Albuquerque, NM: Sandia National Laboratories.

Brush, L.H., D. Grbic-Galic, D.T. Reed, X. Tong, R.H. Vreeland, and R.E. Westerman. 1991. "Preliminary Results of Laboratory Studies of Repository Chemistry for the Waste Isolation Pilot Plant," *Scientific Basis for Nuclear Waste Management XIV, Materials Research Society Symposium Proceedings*, Boston, MA, November 26-29, 1990. Eds. T.A. Abrajano, Jr. and L.H. Johnson. Pittsburgh, PA: Materials Research Society. Vol. 212, 893-900.

Cho, J-C, and Kim, S-J. 2000. Increase in bacterial community diversity in subsurface aquifers receiving livestock wastewater input. Applied Environ. Microbiol. 66(3): 956-965.

Francis, A.J. 1985. "Low-Level Radioactive Wastes in Subsurface Soils," *Soil Reclamation Processes: Microbiological Analyses and Applications*. Eds. R.L. Tate, III and D.A. Klein. New York, NY: Marcel Dekker, Inc. 279-331.

Federal Register. 1998. *40 CFR Part 194 Criteria for the Certification and Recertification of the Waste Isolation Pilot Plant's Compliance With the Disposal Regulations: Certification Decision; Final Rule*. Vol. 63, No. 5, May 18, 1998, 27353-27406.

Francis, A.J., S. Dobbs, and R.F. Doering. 1980a. "Biogenesis of Tritiated and Carbon-14 Methane from Low-Level Radioactive Waste," *Nuclear and Chemical Waste Management*. Vol. 1, No. 1, 153-159.

Francis, A.J., S. Dobbs, and B.J. Nine. 1980b. "Microbial Activity of Trench Leachates from Shallow-Land, Low-Level Radioactive Waste Disposal Sites," *Applied and Environmental Microbiology*. Vol. 40, No. 1, 108-113.

Francis, A.J., and Gillow, J.B. 1994. *Effects of Microbial Processes on Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions. Progress Report Through 1992*. SAND93-7036. Albuquerque, NM: Sandia National Laboratories.

Francis, A.J., Gillow, J.B., and Giles, M.R. 1997. *Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions*. SAND96-2582. Albuquerque, NM: Sandia National Laboratories.

Francis, A.J., Gillow, J.B., Dodge, C.J., Dunn, M., Mantione, K., Strietelmeier, B.A., Pansoy-Hjelvik, M.E., and Papenguth, H.W. 1998. Role of bacteria as biocolloids in the transport of actinides from a deep underground radioactive waste repository. *Radiochim. Acta* 82: 347-354.

Kepner, R.L., Jr., and J.R. Pratt. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiol. Rev.* 58(4): 603-615.

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

Muyzer, G., De Waal, E.C., and Uitterlinden, A.G. 1993. Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied Environ. Microbiol 59(3): 695-700.

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

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

Roling, W.F.M., Van Breukelen, B.M., Braster, M., Lin, B., and Van Verseveld, H.W. 2001. Relationship between microbial community structure and hydrochemistry in a landfill leachate-polluted aquifer. Applied Environ. Microbiol. 67(10): 4619-4629.

## 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 (Data are presented as gas volume or $CO_2$ produced per gram cellulose, values have been corrected for gas production in the absence of cellulose by subtracting out control data; data have not been 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* Treatment & Incubation Time (days) | Organic Acid (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 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.

| Sample | Milliliters of Gas Produced/Sample | | | | | | |
|---|---|---|---|---|---|---|---|
| | Days | | | | | | |
| | 0 | 30 | 189 | 334 | 488 | 840 | 2612 |
| *No Plastic or Rubber* | | | | | | | |
| **Aerobic** | | | | | | | |
| Unamended | 0.93 | 0.97 ± 0.13 | -1.09 ± 0.63 | 0.45 ± 0.50 | 0.78 ± 0.52 | 1.70 ± 0.35 | 3.29 ± 0.37 |
| Amended | 0.85 | 1.74 ± 0.17 | 1.56 ± 0.03 | 0.90 ± 0.48 | 1.73 ± 0.57 | 2.69 ± 0.59 | 2.86 ± 0.49 |
| **Anaerobic** | | | | | | | |
| Unamended | 1.07 | 1.17 ± 0.05 | 0.98 ± 0.08 | 0.66 ± 0.37 | 1.59 ± 0.42 | 2.48 ± 0.34 | 2.31 ± 0.4 |
| 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.

| Sample | Milliliters of Gas Produced/Sample | | | | | | |
|---|---|---|---|---|---|---|---|
| | Days | | | | | | |
| | 0 | 30 | 189 | 334 | 488 | 840 | 2612 |
| *No Plastic or Rubber* | | | | | | | |
| **Aerobic** | | | | | | | |
| Unamended | 0.93 | 0.97 ± 0.13 | -1.09 ± 0.63 | 0.45 ± 0.50 | 0.78 ± 0.52 | 1.70 ± 0.35 | 3.29 ± 0.37 |
| Amended | 0.85 | 1.74 ± 0.17 | 1.56 ± 0.03 | 0.90 ± 0.48 | 1.73 ± 0.57 | 2.69 ± 0.59 | 2.86 ± 0.49 |
| **Anaerobic** | | | | | | | |
| Unamended | 1.07 | 1.17 ± 0.05 | 0.98 ± 0.08 | 0.66 ± 0.37 | 1.59 ± 0.42 | 2.48 ± 0.34 | 2.31 ± 0.4 |
| 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.

| Sample | Milliliters of Gas Produced/Sample | | | | | | |
|---|---|---|---|---|---|---|---|
| | Days | | | | | | |
| | 0 | 30 | 189 | 334 | 488 | 840 | 2612 |
| *No Plastic or Rubber* | | | | | | | |
| **Aerobic** | | | | | | | |
| Unamended | 0.93 | 0.97 ± 0.13 | -1.09 ± 0.63 | 0.45 ± 0.50 | 0.78 ± 0.52 | 1.70 ± 0.35 | 3.29 ± 0.37 |
| Amended | 0.85 | 1.74 ± 0.17 | 1.56 ± 0.03 | 0.90 ± 0.48 | 1.73 ± 0.57 | 2.69 ± 0.59 | 2.86 ± 0.49 |
| **Anaerobic** | | | | | | | |
| Unamended | 1.07 | 1.17 ± 0.05 | 0.98 ± 0.08 | 0.66 ± 0.37 | 1.59 ± 0.42 | 2.48 ± 0.34 | 2.31 ± 0.40 |
| Amended | 0.93 | 4.96 ± 0.24 | 3.13 ± 1.19 | 3.13 ± 1.15 | 3.66 ± 0.98 | 4.24 ± 0.82 | 5.27 |
| *Neoprene - Aerobic* | | | | | | | |
| **Unamended** | | | | | | | |
| Unirradiated | 0.91 | 0.32 | -2.13 | -1.77 | -0.94 | 3.23 | 2.70 |
| Irradiated (Low-Dose) | 1.03 | -0.02 | -0.84 | 1.32 | 1.66 | 3.25 | 3.55 |
| Irradiated (High-Dose) | 0.97 | -0.05 | -2.30 | 0.53 | 1.95 | 2.91 | 2.74 |
| **Amended** | | | | | | | |
| Unirradiated | 1.00 | 2.32 ± 0.09 | 1.75 ± 0.12 | 1.34 ± 0.12 | 1.65 ± 0.21 | 2.69 ± 0.34 | 2.66 ± 0.25 |
| Irradiated (Low-Dose) | 0.97 | 1.87 ± 0.20 | 1.74 ± 0.30 | 1.28 ± 0.37 | 1.70 ± 0.26 | 2.96 ± 0.22 | 3.13 ± 0.43 |
| Irradiated (High-Dose) | 0.70 | 1.91 ± 0.15 | 1.76 ± 0.38 | 1.33 ± 0.37 | 1.77 ± 0.24 | 2.80 ± 0.06 | 3.16 ± 0.40 |
| *Neoprene - Anaerobic* | | | | | | | |
| **Unamended** | | | | | | | |
| *Unirradiated* | 1.06 | 1.48 | 0.95 | 1.67 | 1.56 | 1.80 | 2.15 |
| Irradiated (Low-Dose) | 1.10 | 1.29 | 1.05 | 1.26 | 1.68 | 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.

| Sample | Milliliters of Gas Produced/Sample<br>Days<br>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.

| Sample | µmoles $CO_2$/Sample Days 0 | 30 | 189 | 334 | 488 | 840 | 2612 |
|---|---|---|---|---|---|---|---|
| *No Plastic or Rubber* | | | | | | | |
| **Aerobic** | | | | | | | |
| Unamended | 1.50 | 1.76 ± 0.13 | 8.11 ± 0.33 | 8.48 ± 0.39 | 11.9 ± 0.5 | 15.0 ± 1.7 | 19.9 ± 1.2 |
| Amended | 1.21 | 26.1 ± 0.2 | 35.9 ± 0.4 | 38.0 ± 0.9 | 42.8 ± 1.5 | 42.7 ± 2.1 | 46.2 ± 1.1 |
| **Anaerobic** | | | | | | | |
| Unamended | 1.52 | 1.76 ± 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** |
| | | | | **Days** | | | |
| *No Plastic or Rubber* | | | | | | | |
| **Aerobic** | | | | | | | |
| Unamended | 1.50 | 1.76 ± 0.13 | 8.11 ± 0.33 | 8.48 ± 0.39 | 11.91 ± 0.46 | 15.0 ± 1.7 | 19.9 ± 1.2 |
| Amended | 1.21 | 26.1 ± 0.2 | 35.9 ± 0.4 | 38.0 ± 0.9 | 42.8 ± 1.5 | 42.7 ± 2.1 | 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.