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Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions

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Printed March 1997

Sandia National Laboratories

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Distribution
Category UC-721

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ABSTRACT

Gas generation from the microbial degradation of the organic constituents of transuranic waste under conditions expected at the Waste Isolation Pilot Plant (WIPP) repository was investigated at Brookhaven National Laboratory. These laboratory studies were part of the Sandia National Laboratories (SNL) WIPP Gas Generation Program. The biodegradation of mixed cellulosics (various types of paper) and electron-beam irradiated plastic and rubber materials (polyethylene, polyvinylchloride, neoprene, hypalon, and leaded hypalon) was examined. The effects were determined of starting atmosphere (air or nitrogen), water content (humid (~70% relative humidity) and brine inundated), nutrients (nitrogen, phosphate, yeast extract, and excess nitrate), and potential backfill material (bentonite) on microbial gas generation. Total gas production was analyzed as well as the gases CO_2, H_2, N_2O, O_2, CH_4, and H_2S in the headspace of sample bottles. The rate of gas production from cellulose biodegradation in inundated samples incubated for 1228 days at 30°C was biphasic, with an initial rapid rate up to approximately 600 days incubation, followed by a slower rate. More gas was produced in samples containing nutrients, especially excess nitrate, relative to those without a nutrient addition. The rate of total gas production in anaerobic samples containing mixed inoculum was as follows: 0.002 mL g^{-1} cellulose day^{-1} without nutrients; 0.004 mL g^{-1} cellulose day^{-1} with nutrients; and 0.01 mL g^{-1} cellulose day^{-1} in the presence of excess nitrate. Carbon dioxide production proceeded at a rate of 0.009 μmol g^{-1} cellulose day^{-1} in anaerobic samples without nutrients, 0.05 μmol g^{-1} cellulose day^{-1} in the presence of nutrients, and 0.2 μmol g^{-1} cellulose day^{-1} with excess nitrate. Adding nutrients and excess nitrate stimulated denitrification, as evidenced by the accumulation of N_2O in the headspace (220 μmol g^{-1} cellulose). The addition of the potential backfill bentonite increased the rate of CO_2 production to 0.3 μmol g^{-1} cellulose day^{-1} in anaerobic samples with excess nitrate. Analysis of the solution showed that lactic, acetic, propionic, butyric, and valeric acids were produced due to cellulose degradation. Samples incubated under anaerobic humid conditions for 415 days produced CO_2 at a rate of 0.2 μmol g^{-1} cellulose day^{-1} in the absence of nutrients, and 1 μmol g^{-1} cellulose day^{-1} in the presence of bentonite and nutrients. There was no evidence of biodegradation of electron-beam irradiated plastic and rubber.

Performed under Contract No. 67-8602 for Sandia National Laboratories, Waste Isolation Pilot Plant Gas Generation Program

ACKNOWLEDGMENTS

The authors thank Drs. L. H. Brush, M. A. Molecke, and Y. Wang, Program Managers, Sandia National Laboratories, for their support and guidance; and P. Carr and V. Gutierrez for their assistance with the Quality Assurance aspects of the project.

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1.0 EXECUTIVE SUMMARY

Gas generation from the biodegradation of the organic constituents (cellulose or plastic and rubber materials) of transuranic (TRU) waste under conditions expected at the Waste Isolation Pilot Plant (WIPP) repository was examined at Brookhaven National Laboratory (BNL). These laboratory studies were part of the Sandia National Laboratories (SNL) - WIPP Gas Generation Program. Experiments were designed to determine the effects of multiple variables on the biodegradation of the simulated TRU waste and the resultant microbial production of gas. Appropriate controls were included to account for gas production due to abiotic processes. Variables that were examined included the composition of the initial atmosphere (air or nitrogen), moisture content (brine-inundated or humid), the presence of nutrients (including excess nitrate), the presence of microbes, and the addition of a proposed backfill (bentonite). Total gas production was measured and the gases CO_2, N_2O, H_2, O_2, CH_4, and H_2S. In addition, the dissolved products of cellulose biodegradation were quantified.

- Samples were prepared with and without cellulose (5 g) and inundated with G-Seep brine (a natural brine from the repository underground workings) and incubated as follows:

(i.) uninoculated (no microbial inoculum added but the microbial populations in the brine from the repository were present), unamended (no nutrient added); (ii.) inoculated (repository-relevant microorganisms added as part of a "mixed inoculum" obtained from the WIPP surface and subsurface environment), unamended; (iii.) inoculated, amended (nutrients added including ammonium nitrate (0.1% w/v), potassium phosphate (0.1% w/v), and yeast extract (0.05% w/v); and, (iv.) inoculated, amended plus excess nitrate as potassium nitrate (0.5% w/v, 5-fold excess to simulate contact of the cellulosics with nitrate-bearing sludge waste). All treatments were prepared with either an initial atmosphere of nitrogen or of air (simulating the initial repository atmosphere, and expected to be consumed by aerobic microbes and eventually render the treatments anaerobic). Treatments also were prepared with bentonite to determine its influence on gas generation. Samples were incubated at 30°C. The quantity of gas produced due to microbial activity over 1228 days depended upon the nutrients present and the microbial inoculum. Gas production was significant in the presence of nutrients relative to treatments without them. The rate of gas production was relatively rapid up to 600 days, and then leveled off considerably up to 1228 days. Average gas production rates are presented as the linear extrapolation of gas production from 69 to 1228 days. Aerobic inoculated amended samples produced 0.001 mL total gas g^{-1} cellulose day^{-1} and 0.01 μmol CO_2 g^{-1} cellulose day^{-1} up to 1228 days. The rate was higher in anaerobic inoculated amended samples, which produced 0.004 mL total gas g^{-1} cellulose day^{-1} and 0.05 μmol CO_2 g^{-1} cellulose day^{-1}. Gas production was stimulated by adding excess nitrate: aerobic inoculated samples produced 0.008 mL total gas g^{-1} cellulose day^{-1} and 0.1 μmol CO_2 g^{-1} cellulose day^{-1} up to 1228 days. Anaerobic inoculated samples containing excess nitrate produced 0.01 mL total gas g^{-1} cellulose day^{-1} and 0.2 μmol CO_2 g^{-1} cellulose day^{-1}. Adding bentonite resulted in an even higher generation of gas in anaerobic inoculated amended samples which produced 0.01 mL total gas g^{-1} cellulose day^{-1} and 0.3 μmol CO_2 g^{-1} cellulose day^{-1} over 1228 days. Soluble organic acid metabolites such as lactic, acetic, and propionic acid, were detected in the brine of inoculated unamended and amended samples. Denitrification, an important microbial process under anaerobic conditions, was stimulated by the addition of excess nitrate (220 μmol N_2O g^{-1} cellulose accumulated over 1228 days in anaerobic inoculated samples with excess nitrate in contrast to 19.1 μmol N_2O in samples without excess).

Gas production was limited in the absence of nutrients, and depended upon the addition of microbial inoculum. The amount of total gas detected in anaerobic uninoculated unamended samples containing cellulose was indistinguishable from background. Anaerobic inoculated unamended samples produced 0.002 mL total gas g^{-1} cellulose day^{-1} and 0.009 μmol CO_2 g^{-1} cellulose day^{-1} over 1228 days. The gases O_2, H_2, CH_4, and H_2S were also analyzed; H_2 was detected in anaerobic inoculated amended samples due to fermentation; CH_4, and H_2S were not detected.

- Gas production from cellulose biodegradation was examined under humid (~70% relative humidity) conditions. Samples were prepared with and without 1 g cellulose as follows: (i.) uninoculated, unamended; (ii.) uninoculated, amended (the nutrients ammonium nitrate, potassium phosphate, and yeast extract were added); (iii.) inoculated, unamended; and, (iv.) inoculated, amended. Crushed salt from the repository (muck-pile salt) or a crushed salt/bentonite mix was added to simulate a proposed backfill. Samples were prepared with an initial atmosphere of air or nitrogen. Biotic gas produced from samples containing cellulose as the sole carbon source ranged from 0.03 μmol CO_2 g^{-1} cellulose day^{-1} over 804 days in initially aerobic inoculated unamended samples (with concurrent consumption of O_2) to 0.2 μmol g^{-1} cellulose day^{-1} in anaerobic inoculated unamended samples over 415 days incubation. Total gas production was negligible. Uninoculated and inoculated amended samples produced no total gas and very little CO_2. Adding the backfill material bentonite significantly increased CO_2 production. Initially aerobic inoculated amended samples produced 1.8 μmol CO_2 g^{-1} cellulose day^{-1} over 804 days, and anaerobic inoculated amended samples produced 1 μmol CO_2 g^{-1} cellulose day^{-1} over 415 days. However, total gas production was negligible.

- Plastic (polyethylene and polyvinylchloride) and rubber (neoprene, hypalon, and leaded hypalon) were irradiated in a linear accelerator resulting in absorbed doses from 500 (low) to 5,850 (high) Mrad. Unirradiated and electron-beam irradiated material (0.1 to 0.2 g) was inundated with brine and incubated with mixed inoculum without nutrients (unamended) or with nutrients (amended with ammonium nitrate, potassium phosphate, and yeast extract). Gas production (total gas, O_2, CO_2, H_2, N_2O, CH_4, and H_2S) was analyzed up to 840 days in samples incubated with an initial atmosphere of air or nitrogen. Gas production was insignificant, and radiation damage did not affect it.

- A comprehensive Quality Assurance Program (QAP) was instituted for the laboratory research at BNL. It ensured that the data generated were valid, accurate, repeatable, protected, and could withstand critical peer and other reviews.

2.0 INTRODUCTION

This report summarizes laboratory work at Brookhaven National Laboratory (BNL) from 1991 to 1995, funded by the Sandia National Laboratories (SNL) Waste Isolation Pilot Plant (WIPP) Gas Generation Program. A previous report summarized the progress of work from initiation (1991) through the end of 1992 (Francis and Gillow, 1994). The initial effort gave laboratory-based data on gas generation due to biodegradation of cellulose under WIPP repository-relevant conditions. From the inception of work in June 1991 through the end of that year, short-term tests (< 6 months) were completed to examine the potential for microbial gas production in repository-relevant samples. More specifically, gas production due to aerobic and anaerobic microbial activity, including cellulose degradation, was observed in brine from the repository, in samples that were amended with nutrients, such as nitrogen and phosphorus. Brine from the underground workings, a mixture of brine and sediment from the surficial environment, and brine from the subsurface contained aerobes, anaerobes, cellulose degraders, denitrifiers, and sulfate reducers. All these experiments focused on verifying the presence of microorganisms in WIPP repository-relevant sources and quantifying their potential for generating gas.

A long-term (> 2 years) experiment was started in January, 1992 to obtain data on microbial gas production from samples inundated with G-Seep brine from the repository and in the presence and absence of cellulose, microbial inoculum, oxygen, nutrients, and the proposed backfill material. The data collected included quantification of total gas, CO_2, and N_2O production over multiple sampling periods. The data up to 200 days were presented in the previous report (Francis and Gillow, 1994). Data in this report extend to 1228 days (3.4 years) of incubation at 30°C, collected during 1993 and 1994, and includes quantification of the total gas, H_2, O_2, CO_2, N_2, N_2O, CH_4, and H_2S produced. In addition, we analyzed dissolved organic and inorganic constituents in the supernatant.

The production of microbial gas under repository-relevant humid conditions (74% relative humidity) was examined, with cellulose as the major organic carbon source, and variables including the presence and absence of microbial inoculum, nutrients, and proposed backfill material. Assessments of microbial activity under humid, initially aerobic conditions were started in 1993, with humid anaerobic experiments initiated in 1994. Gas analysis included total gas, H_2, O_2, CO_2, N_2, N_2O, H_2S, and CH_4, with data presented here up to 804 days incubation at 30 ± 2°C for initially aerobic samples, and to 415 days incubation for anaerobic samples.

A major effort during 1993 focused on the designing experiments to examine gas production due to the biodegradation of radiation-damaged plastic and rubber materials. We examined the effects of electron-beam (e-beam) irradiation on the biodegradability of polyethylene, polyvinylchloride, neoprene, hypalon, and leaded hypalon under WIPP repository-relevant conditions. Alpha-irradiation from actinides in the repository induces high linear-energy transfer (LET) radiation damage that is confined to the surface of the material, with minimal penetration. E-beam irradiated materials serve as an "overtest" for radiation damage, simulating long-term radiolytic damage to the polymers with low LET radiation damage induced by the e-beam throughout the material. Samples were prepared according to a test-matrix to examine multiple WIPP repository-relevant variables, and analyses completed through 840 days incubation, including quantification of total gas, H_2, O_2, CO_2, N_2, N_2O, H_2S, and CH_4.

Brookhaven National Laboratory developed a Quality Assurance Program (QAP) for the laboratory research that complied with the requirements of Sandia National Laboratories. This QAP was fully implemented during the work at BNL and was reviewed by SNL in formal on-site audits. The QAP ensured that the data generated were valid, accurate, repeatable, protected, and could withstand critical peer and other reviews.

3.0 OBJECTIVE

The objective of the SNL-WIPP Gas Generation project at BNL was to quantify microbial gas production due to biodegradation of the organic constituents of TRU waste under WIPP repository-relevant conditions, which include hypersaline inundated and humid, initially aerobic and anaerobic conditions.

4.0 BACKGROUND

The WIPP is a U.S. Department of Energy facility located in southeastern New Mexico, approximately 656 m (2150 ft) below the surface in a bedded salt, Permian evaporite formation. A mined geologic repository, WIPP is designed to demonstrate the safe, permanent disposal of radioactive TRU wastes from defense-related activities. TRU wastes contain alpha-emitting transuranium nuclides with half-lives greater than twenty years at concentrations greater than 100 nCi per gram. These wastes were generated from nuclear-weapons production and related nuclear processing and include various organics, adsorbed liquids, sludges, cellulosics, plastics, rubber, leaded rubber, a variety of metals, and cemented materials containing the following radionuclides: ^{232}Th, ^{233}U, ^{235}U, ^{238}U, ^{237}Np, ^{238}Pu, ^{239}Pu, ^{240}Pu, ^{241}Pu, ^{242}Pu, ^{241}Am, ^{244}Cm, ^{252}Cf.

Containers of TRU waste will be emplaced inside 3,640m^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 (Brush, 1990). 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, and leaded hypalon). Wastes consisting of inorganic process sludges from secondary waste treatment, containing ~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). With a significant portion of the waste scheduled for disposal consisting of biodegradable material, there is concern about the effects of microbial activity on the performance of the repository. In addition, radiolytic damage of plastic and rubber due to contact with alpha-emitting radionuclides in the waste may make them more susceptible to biodegradation.

The repository is located in a Permian halite deposit and, therefore, is designed to entomb the TRU waste in salt when the waste containers are in place and the facility is closed. During the initiation of work at BNL, backfill material was being considered for placement around the containers as the disposal rooms are filled; the proposed composition of this material was salt, or a mixture of 70% salt and 30% bentonite. To simulate a variety of expected repository-relevant conditions under which microbiological degradation of cellulose might occur, a sequence of scenarios for WIPP performance was proposed according to what was reasonably expected at the time.

Initially, the repository will be ventilated, but after sealing, humid conditions will develop; the ambient humidity is expected to be 18 to 27 g/m^3 (about 74% relative humidity), and the temperature 30°C (Brush, 1990). Over time, the conditions in the repository may change from humid to brine-inundated; possibly, the repository may be partially inundated and partially humid. Brine formations exist above the repository's horizon (as undersaturated brines), at or near the horizon (intergranular brine from the Salado Formation), and below it (isolated brine reservoirs of the Castille Formation). Presently, these brines are seeping into the WIPP underground workings through the ribs (walls) and the floor, but do not accumulate because the mine is ventilated (Brush, 1990). After sealing, with the elevated humidity in the facility, these brines may accumulate and eventually inundate the facility. Therefore, the cellulosic waste material, which will most likely be exposed when the waste containers breach after closure or the

container's integrity decays over time, will be in contact with the surrounding backfill under humid or inundated conditions.

Microorganisms, which can grow under hypersaline conditions (halotolerant, and moderate and extreme halophiles), will be present in the facility 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 the WIPP from several sources, including (i.) association with the TRU waste, (ii.) the surface environment via the mine ventilation systems and human intrusion, and (iii.) as resident populations in the salt crystals and brine formations. Alpha-radiation from TRU waste is not expected to significantly affect microbial activity (Barnhart et al., 1980; Francis, 1990). 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., 1980a,b; Francis, 1985). Halotolerant and halophilic microorganisms, including aerobic, nitrate-reducing, and anaerobic bacteria were detected in the WIPP surficial environment and underground workings (Francis and Gillow, 1994). Cellulose-degrading extreme halophiles from the underground workings also have been isolated. Introduced microorganisms, as well as resident or indigenous halotolerant and halophilic bacteria, can metabolize organic compounds and nitrate in the waste, and may generate metabolic byproducts, such as organic acids, alcohols, carbon dioxide, nitrous oxide, nitrogen, hydrogen, hydrogen sulfide, and methane.

Gas generation due to biodegradation of the organic constituents of TRU is expected to be one of the two most important gas producing processes in the repository, the other being H_2 generation due to anoxic corrosion (Telander and Westerman, 1993), followed by brine radiolysis (Brush, 1990). Experiments at BNL were designed to quantify gas generation due to microbial activity under repository-relevant conditions.

5.0 EXPERIMENTAL RATIONALE AND APPROACH

The WIPP disposal room gave us the framework within which to develop the experimental test conditions for gas generation due to microbial activity. The disposal room scenarios 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.

6.0 MATERIALS AND METHODS

6.1 Cellulose Biodegradation under Inundated Repository Relevant Conditions

Figures 1 through 4 show the sample treatments to assess the biodegradation of cellulose.

6.1.1 Substrate

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 × 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.).

6.1.2 WIPP Brines

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 1a gives the chemical composition of G-Seep brine; it contains $10^4 - 10^6$ bacterial cells mL^{-1} (Francis and Gillow, 1994).

Table 1a. Composition of G-Seep brine (Brush, 1990)

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

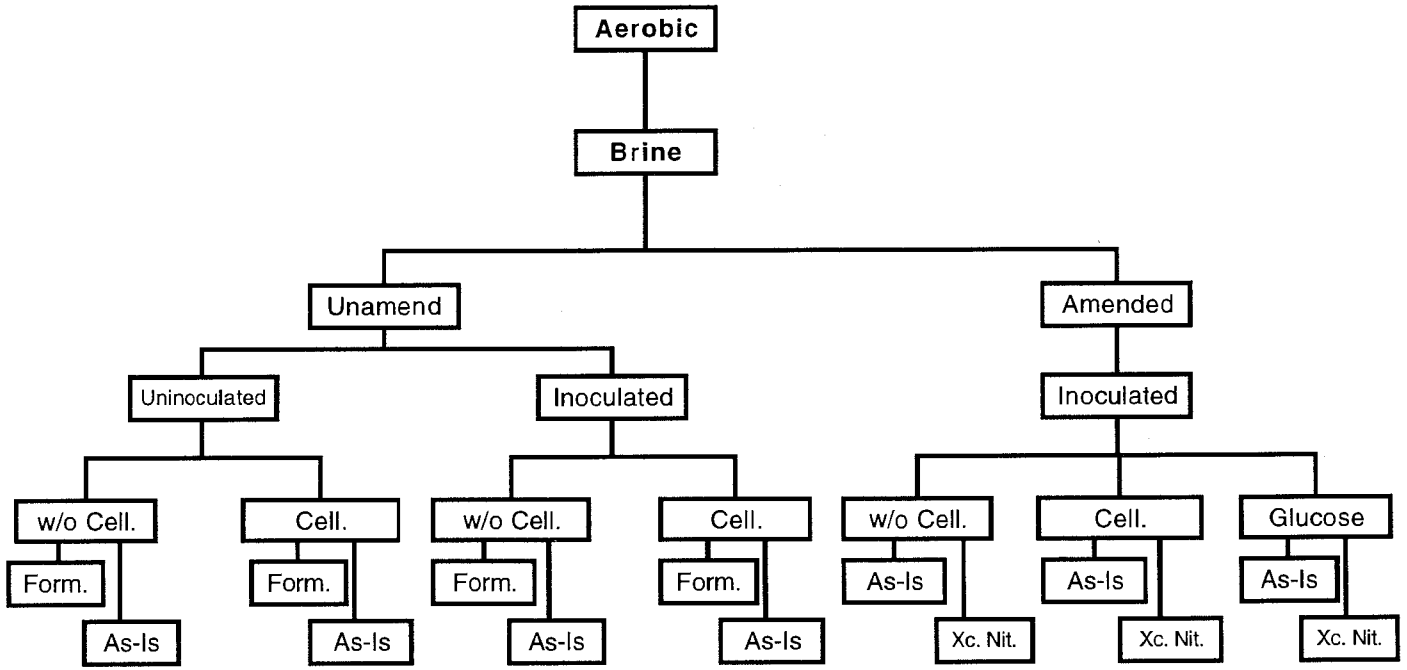


Figure 1. Treatment matrix for the examination of gas production due to cellulose biodegradation (aerobic samples).

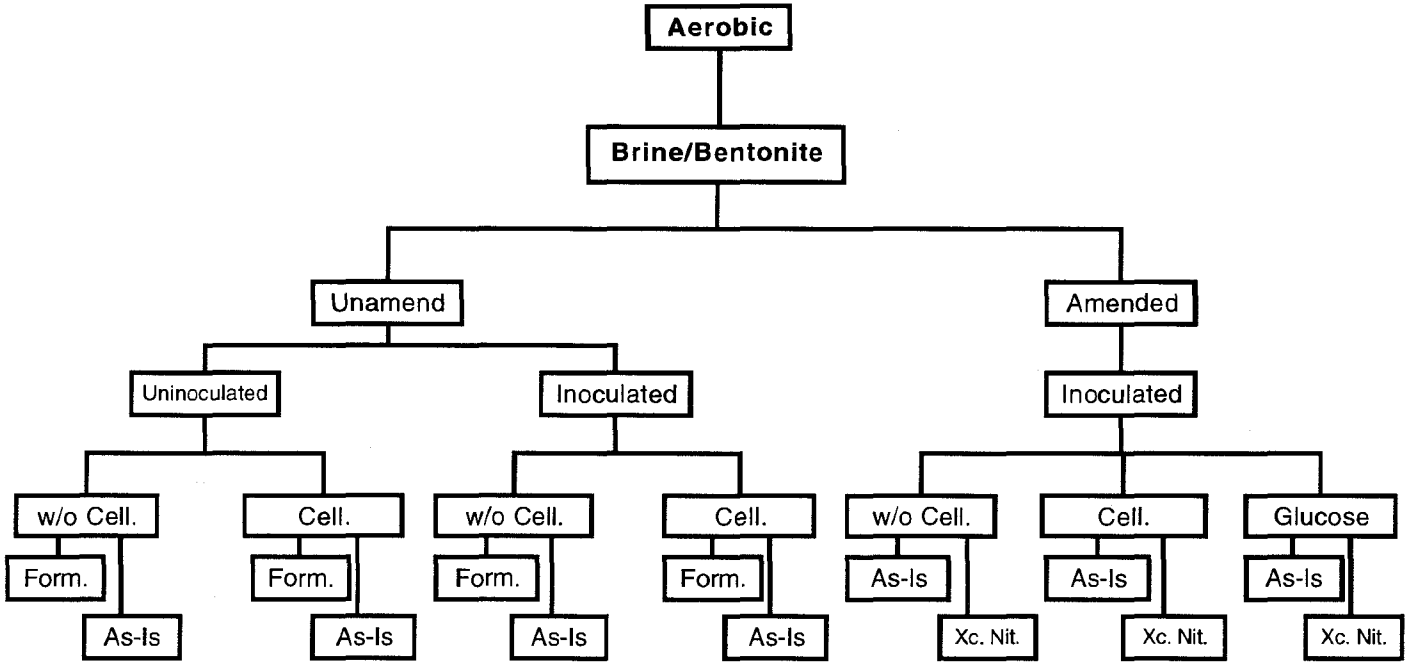


Figure 2. Treatment matrix for the examination of gas production due to cellulose biodegradation (aerobic samples containing bentonite).

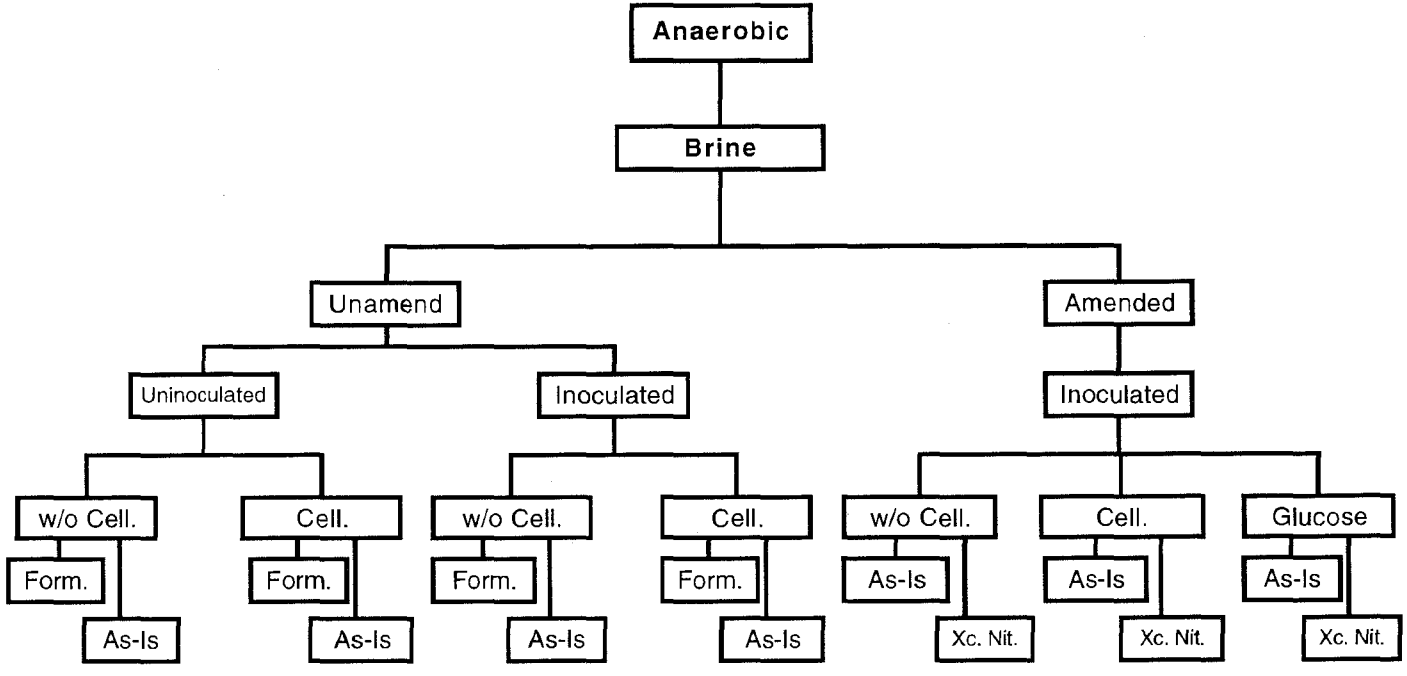


Figure 3. Treatment matrix for the examination of gas production due to cellulose biodegradation (anaerobic samples).

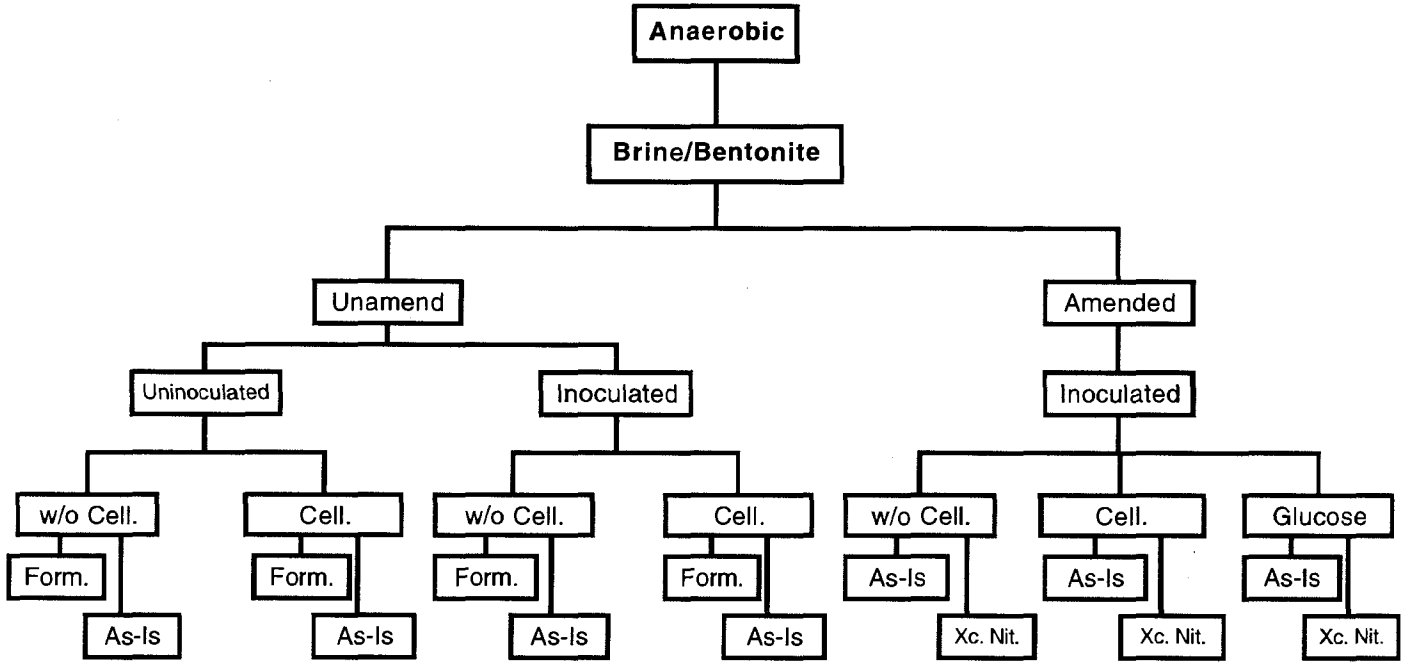


Figure 4. Treatment matrix for the examination of gas production due to cellulose biodegradation (anaerobic samples containing bentonite).

6.1.3 Bentonite

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. Table 1b shows its chemical composition.

Table 1b. 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 (Milliequivalents/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

6.1.4 Mixed Inoculum

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 2.

(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.

Table 2. Surficial Lake Brine and Sediment

Sediment and Brine Source	Brine, mL	Sediment, mL
Laguna Quatro	60	40
Laguna Cinco	35	40
Laguna Tres South	13	40
Lindsey Lake	50	40
Surprise Springs	25	40
Totals	**183**	**200**

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.

6.1.5 Treatments

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 0.22 mm syringe filter units (Millipore Corp.).

6.1.6 Alternate Electron Acceptors

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.

6.1.7 Anaerobic Sample Preparation

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.

6.1.8 Aerobic Sample Preparation

Aerobic samples were prepared as described above except that brine solutions were not purged with UHP N2. 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).

6.1.9 Incubation

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 t=0 (January 29, 1992), and at 18 intervals thereafter, up to 1228 days of incubation. The incubator's temperature was monitored weekly with Hg-filled thermometers calibrated by the manufacturer (Princo Instruments) 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.

6.2 Gas Generation from the Biodegradation of Cellulose under Humid Conditions

6.2.1 Relative Humidity Measurements

Cellulose samples were incubated in serum bottles with a headspace of air or N_2. To maintain the desired relative humidity of approximately 70-74%, 3 mL of G-Seep brine (a_w = 0.73) in a 5 mL glass tube (1.0×7.5 cm) was placed inside the 160 mL serum bottle containing 1 g of mixed cellulose (Figure 5). The serum bottles were closed with butyl rubber stoppers and incubated with air or N_2 as the initial atmosphere. 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 BNL procedure BNL-ACW-HU-1, Rev. 0, 7/18/94, describes the procedure used to measure humidity; the target relative humidity remained constant throughout the experiment (Figure 6).

6.2.2 Sample 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).

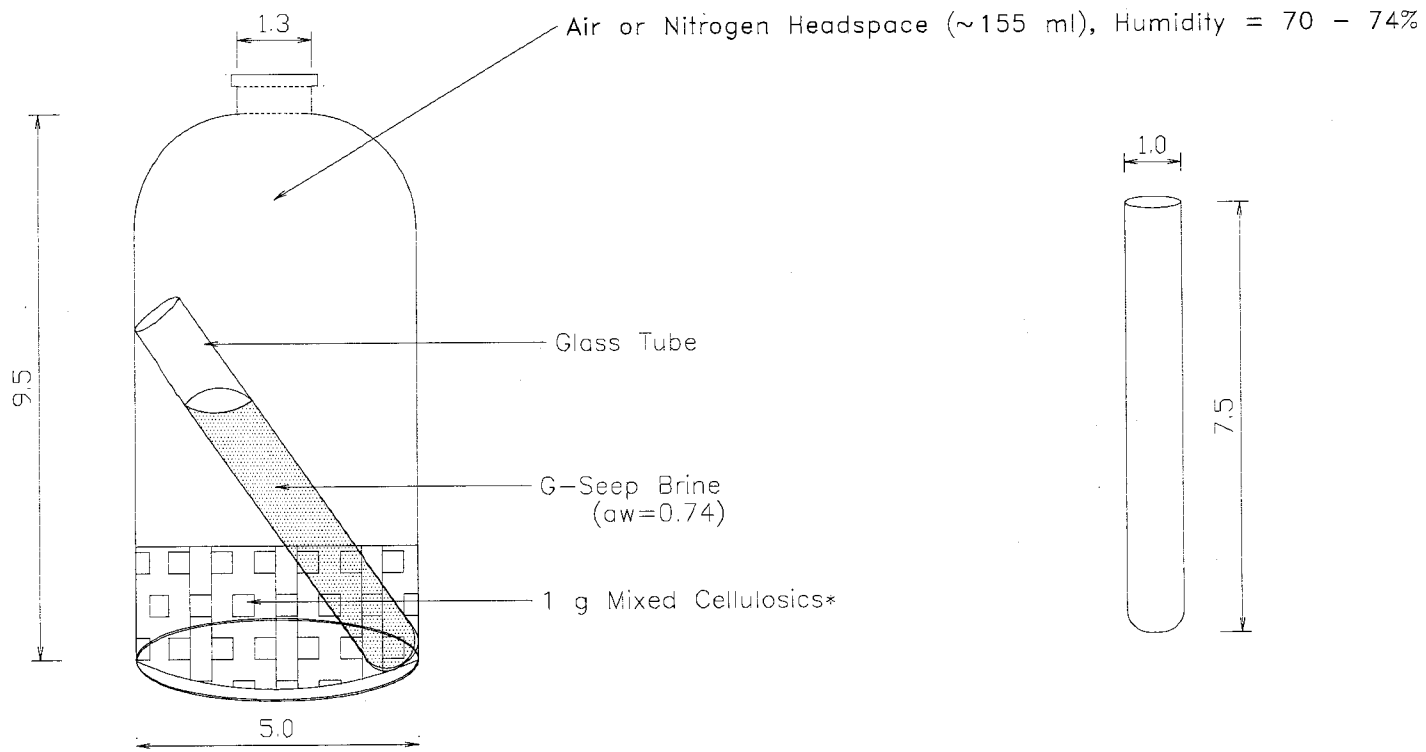
Samples were prepared with and without added nutrients. The nutrients added (amended samples) consisted of a 0.50 mL solution containing nitrogen (ammonium nitrate, 0.1% w/v), phosphorus (potassium phosphate, 0.1% w/v), and yeast extract (0.05% w/v). Unamended samples received 0.50 mL of a filtered,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

6.2.3 Inoculum

Mixed inoculum was prepared as described previously (see Section 6.1.4), 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.

6.2.4 Controls

Because WIPP crushed salt contains viable bacteria (Brush et al., 1991), 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.



* Mixed cellulosics to contain crushed salt or crushed salt/bentonite, with or without mixed inoculum.

Figure 5. Experimental methodology for the humid experiments.

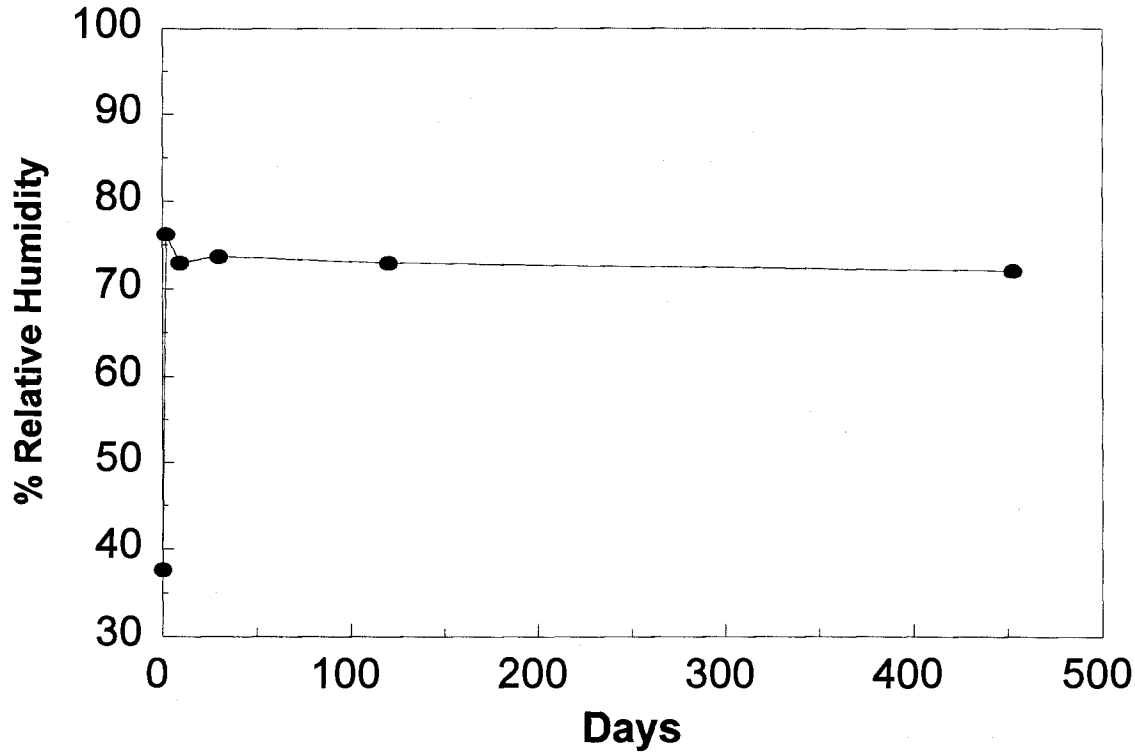


Figure 6. Measurement of humidity in a sample containing cellulose over 453 days showed a constant 72% relative humidity.

6.2.5 Atmosphere

6.2.5.1 INITIALLY AEROBIC (SEALED)

Samples were sealed with butyl rubber stoppers and aluminum crimp seals in an air atmosphere.

6.2.5.2 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.

6.2.6 Microbial Activity Measurements under Humid Conditions

In addition to the above treatments, 1% succinate or glucose was added with the nutrient amendment to certain samples to determine microbial gas generation under humid conditions in the presence of a readily metabolizable source of carbon. The ability of specific microorganisms (i.e., denitrifiers) to grow under such low-moisture conditions was examined. Note 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.

6.2.7 Incubation

Seventy-two samples were incubated at 30 ± 2°C for 804 days (initially aerobic experiment) and for 415 days (anaerobic experiment), after which the experiments were terminated. Total gas volume and carbon dioxide production were monitored as described in Section 7.0. At each time, 0.8 mL of gas was removed from the 160 mL bottle for analysis.

6.3 Gas Generation from the Biodegradation of Irradiated Plastic and Rubber Materials

6.3.1 Objective

Plastic and rubber materials consist of long repeating single bonded carbon chains and are usually quite resistant to biodegradation (Krupp and Jewell, 1992; Low et al., 1992; Cameron et al., 1988). Irradiation causes the polymer to break down due to free radical formation. Irradiation experiments also have shown that there is cross-linking of the polymer chain after free radicals are formed, thereby reducing the molecular mass (Collett et al., 1989). Although the effects of radiolysis on the mechanical and chemical properties of plastics have been evaluated by Argonne National Laboratory (ANL), the extent of their biodegradability and their overall contribution to the total gas production in the WIPP is not known. In this study, we determined the rate and extent of gas production due to biodegradation of electron-beam irradiated plastic and rubber materials under conditions relevant to the WIPP repository. 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.

6.3.2 E-Beam Irradiated Plastic and Rubber Materials

The plastics examined were polyethylene and polyvinylchloride; the rubber materials were neoprene and hypalon (leaded and unleaded). These materials were exposed to electron-beam irradiation at the linear accelerator (LINAC) at Argonne National Laboratory by Dr. D. Reed, Chemical Technology Division. The polymer samples received an absorbed dose of either 500-700 Mrad (low-dose) or 4000-6000 Mrad (high dose), see Table 3. Tests with unleaded and leaded hypalon did not include a high-dose irradiation because it caused extensive degradation (melting) of the leaded sample.

Table 3. Irradiation Conditions and Material Characteristics (data from Dr. D. Reed, ANL)

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
Polyethlyene	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

After irradiation, the weight changes in the plastic and rubber samples were recorded by Dr. D. Reed, ANL and the samples were shipped to BNL via overnight express. At BNL, the characteristics of the materials were recorded and the polymer films were photographed.

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.

6.3.3 Mixed Inoculum/Inundation Fluid

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

6.3.4 Sample Treatments

Samples were incubated either unamended (without added nutrients) or amended (with nutrients). Table 4 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 4. 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.

6.2.5 Incubation

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

6.3.6 Mixed Inoculum Activity Measurements

The denitrification activity of the mixed inoculum was determined to confirm that viable microorganisms were present in the mixed inoculum, and to determine whether dissolved organic carbon was introduced into the samples through adding the inoculum. Five mL of the mixed inoculum was dispensed into 10 mL serum bottles in a N_2-filled glove box and incubated without additions (unamended (U)), with succinate (carbon amended (C)), with potassium nitrate (nitrate amended (N)), and with carbon and nitrate (C & N) in quadruplicate. Denitrification was monitored by the acetylene blockage technique (Yoshinari and Knowles, 1976). One sample from each treatment was treated with formalin as a control.

7.0 ANALYTICAL METHODOLOGY

7.1 Gas Analysis

7.1.1 Total Gas

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

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

7.1.2 Carbon Dioxide and Hydrogen Sulfide

A Perkin Elmer 900 gas chromatograph (GC) fitted with a porous polymer (Porapak QS™, Alltech Corp.) packed column (6-ft. stainless steel, 100°C) was used to determine CO_2 with a thermal conductivity detector (250°C, 225 mA). It was quantified by linear regression using calibration standards traceable to NIST (Scott Specialty Gases, NJ [gravimetric and certified master gases, as well as EPA Protocol™ gas standards]). Concentrations of CO_2 ranged from 100 – 500000 ppm. High concentrations (>100 ppm) of N_2O and H_2S (>500 ppm) were determined using this GC.

7.1.2 Nitrous Oxide

A Shimadzu GC-9A gas chromatograph equipped with a porous polymer (Porapak QS™, Alltech Corp.) packed column (6-ft stainless steel, 70°C) and a ^{63}Ni electron capture detector (250°C, 2 nA) was used to determine N_2O (0.01 to 100 ppm); it was calibrated using N_2O working standards (Sotty II™, Scott Specialty Gases).

7.1.3 Hydrogen, Oxygen, Nitrogen, and Methane

A Varian 3400 gas chromatograph equipped with a molecular sieve 3A packed column (Alltech Corp., 6-ft, 100°C) was used to determine H_2 (1000 to 20000 ppm), O_2 (1000 to 210000 ppm), N_2 (100 to 800000 ppm), and CH_4 (0.01 to 100 ppm) with a thermal conductivity detector in series to a flame ionization detector. It was calibrated using gas standards traceable to NIST (gravimetric master gases, Scott Specialty Gases).

7.1.4 Data Reduction

The concentration of each gas in the headspace gas was quantified by calculating the total volume of headspace gas in the sample, which was obtained from the pressure measurement, and using this volume to scale-up the results from the GC component gas analyses. A "headspace gas

mass balance," was prepared for some samples as a quality-control check for completeness of the GC analysis, by comparing the total amount of gas in the headspace (moles) determined by pressure measurement, and the total amount of gas in the headspace (moles) determined by summing the results from the component-gas analyses. Typically, the two values agreed within ±2%, with a deviation <10%. The data were not corrected for gas dissolution in the brine.

Gas production was assessed by comparing increase in total gas volume over time, as well as the increase in the concentration of each component (usually CO_2, a product of cellulose biodegradation). The values were measured against the baseline (t=0), or against control values. In this study, we prepared several control samples: (i.) formalin-treated samples (abiotic gas production); (ii.) unamended, uninoculated samples; (iii.) and samples without cellulose substrate. The gas data in this report are cumulative from t=0.

7.2 Liquid Analysis

Aliquots from initially aerobic and anaerobic treatments were removed and analyzed for pH, dissolved organic and inorganic carbon, total carbohydrates, organic acids, nitrate, iron (Fe^{2+} and Fe^{3+}), sulfate, and total bacterial counts. Samples were removed from bottles reserved specifically for this purpose, after which the samples were not used further for gas quantification.

Samples from the following treatments were analyzed: (i.) unamended, uninoculated without cellulose (control); (ii.) unamended, inoculated without cellulose (control); (iii.) amended, inoculated without cellulose (control); (iv.) amended, inoculated plus excess nitrate without cellulose (control); (v.) unamended, uninoculated with cellulose, (vi.) unamended, inoculated with cellulose; (vii.) amended, inoculated with cellulose; and, (viii.) amended, inoculated plus excess nitrate with cellulose.

Before liquid analysis, complete gas analysis (total gas, CO_2, N_2O, H_2, O_2, N_2, H_2S, CH_4) was performed on each sample. The sample bottles were transferred into an anaerobic glovebox, and a 16 mL aliquot was removed from each sample using a 22-gauge sterile needle and 20 mL syringe. One milliliter of the sample was removed and immediately fixed with 1% formalin for direct bacterial counts. An aliquot was removed for pH determination, while the remaining liquid was filtered (0.22 μm (Millipore Corp.) and stored in precleaned vials (acid-washed, rinsed, dried) at 4°C. The samples were analyzed within 48 hours of collection. All manipulations and preparations were performed in an anaerobic glovebox.

7.2.1 pH

The pH of each sample was analyzed with a Sentron pH meter (Model 1001) and solid-state probe. This was an "operational pH", i.e., not corrected for ionic strength.

7.2 Organic Analysis

Total dissolved organic and inorganic carbon were analyzed using a Beckman 915B total organic carbon analyzer, as described in BNL-ACW-TC-1. Total soluble carbohydrates were analyzed spectrophotometrically using Dreywood's Anthrone reagent (BNL-ACW-TCH-1). Organic acids were analyzed by high-performance liquid chromatography according to the procedure described in BNL-ACW-OA-1. Known quantities of acids (standard additions) were added to selected samples to confirm the presence of certain acids.

7.2.3 Inorganic Analysis

Nitrate was analyzed spectrophotometrically by the brucine method (BNL-ACW-NI-1). Total iron, Fe^{2+}, and Fe^{3+} were analyzed by the phenanthroline method (BNL-ACW-Fe-1). Sulfate was determined turbidimetrically (BNL-ACW-SF-1).

7.2.4 Bacterial Counts

Bacterial counts were performed on the anaerobic samples without bentonite. One mL aliquots were removed from unmixed sample bottles and preserved with 10% v/v formalin. Aliquots were stained with the DNA-specific stain 4' 6-diamidino-2-phenylindole (DAPI, Polysciences, Inc.) and vacuum filtered onto black polycarbonated filters (0.2 μm, Poretics). The filter was examined by epi-fluorescence microscopy at 1875× magnification using a Zeiss Axiophot microscope and UV light source. The DAPI-DNA complex fluoresces blue, while unbound DAPI or DAPI bound to non-DNA material fluoresces yellow. The analysis was performed according to procedure BNL-EPI.

8.0 RESULTS AND DISCUSSION

8.1 Gas Generation from Cellulose Biodegradation under Inundated Conditions

We determined total gas, H_2, O_2, N_2, CO_2, N_2O, H_2S, and CH_4 produced in samples containing cellulose incubated up to 1228 days. The treatments consisted of cellulose and brine:

(i.) uninoculated,

(ii.) inoculated with a mixed inoculum,

(iii.) inoculated and amended with nutrients (yeast extract (0.05% w/v), potassium phosphate (0.1% w/v), and ammonium nitrate (0.1% w/v), and

(iv.) inoculated and amended with nutrients plus excess nitrate (0.5% w/v potassium nitrate).

Figures 7 through 18 show the total gas, CO_2, and N_2O production on a per gram cellulose basis over the entire experiment, with corrections made for gas production in the absence of cellulose (the data for these figures are shown in Appendix A, Tables 1 through 12, together with a description of the procedure used to calculate the results). In addition, the complete data set is given there on a per sample basis (gross data, Tables 1(a)-4(c)). Data for control samples containing cellulose (formalin-treated uninoculated and inoculated; both without nutrients) are presented in Appendix A, Tables 1 through 12. Error bars represent the standard error of the mean of the analysis of triplicate samples (error bars appear to be absent when they do not exceed the boundary of the symbol used to represent the mean).

8.1.1 Aerobic Treatments without Bentonite

8.1.1.1 TOTAL GAS PRODUCTION

Figure 7 shows the total gas produced in samples incubated with an initial atmosphere of air (aerobic). In addition, total gas production in uninoculated and inoculated samples that did not receive nutrients (unamended) fluctuated between a gas loss and slight increase to 0.30 mL g^{-1} cellulose at 1228 days. In contrast, inoculated amended samples produced 1.42 mL of gas g^{-1} cellulose over 1228 days, and the inoculated amended samples with excess nitrate showed a maximum gas production at 1034 days (12.2 mL of gas g^{-1} cellulose). Adding excess nitrate to the sample stimulated the rate and extent of gas production, with an initial rate of 0.023 mL g^{-1} cellulose day^{-1} from 69 to 200 days, and 0.006 mL g^{-1} cellulose day^{-1} from 200 to 1228 days. The rate was calculated from the linear slope of gas production from 69 to 200 days, and from 200 to 1228 days. The marked difference in the two rates may be due to (i.) the establishment of anaerobic conditions after 200 days as oxygen is consumed, (ii.) nutrient limitation, (iii.) use of the electron acceptor, NO_3^-, or (iv.) buildup of inhibitory metabolic products. Prior to 69 days, there was little gas production. Formalin-treated control samples showed no increase in total gas production over 1228 days (Table 1, Appendix A).

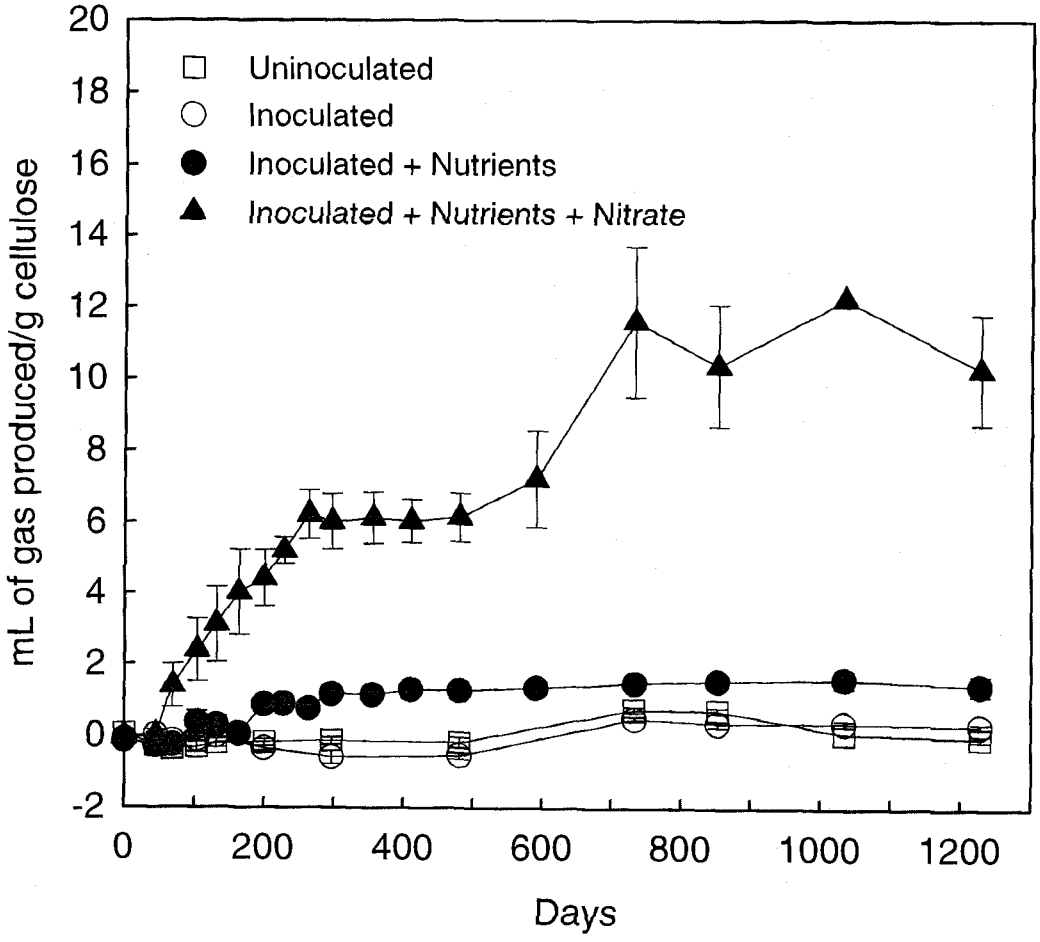


Figure 7. Total gas produced in samples incubated with an initial atmosphere of air.

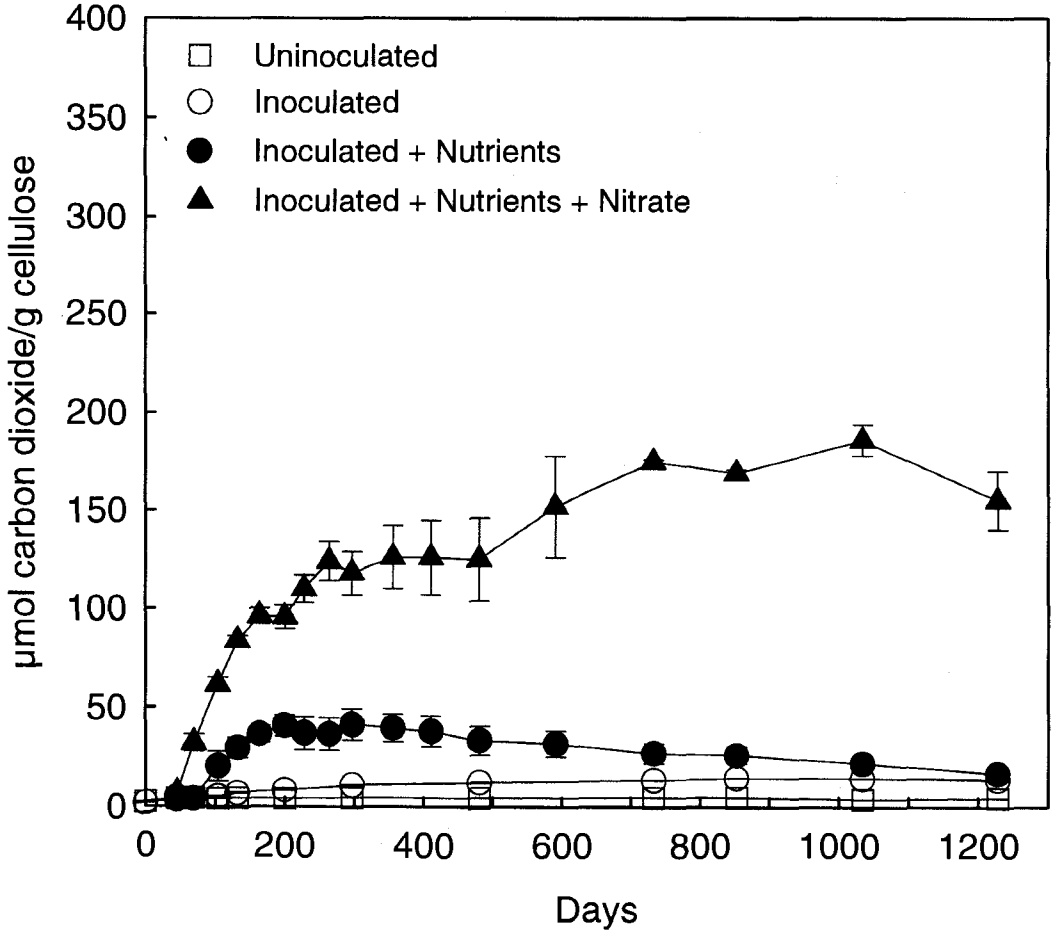


Figure 8. Carbon dioxide produced in samples incubated with an initial atmosphere of air.

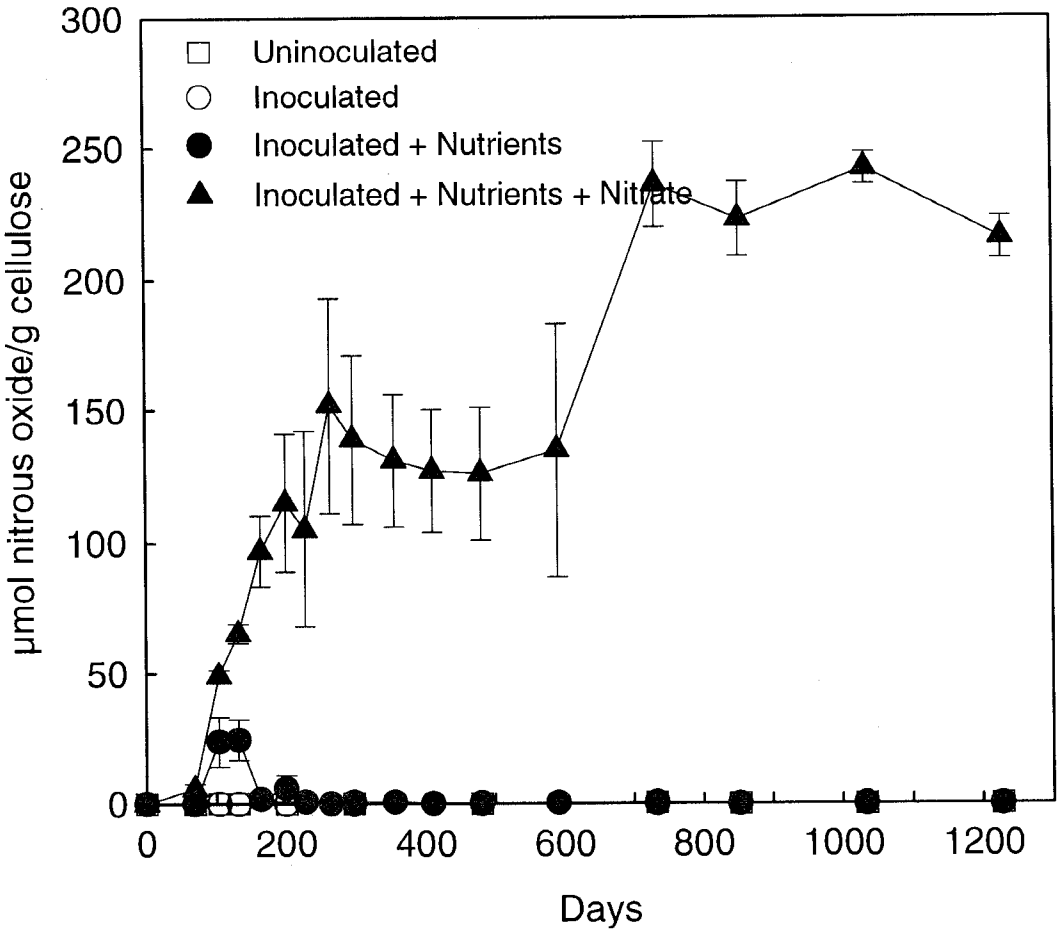


Figure 9. Nitrous oxide produced in samples incubated with an initial atmosphere of air.

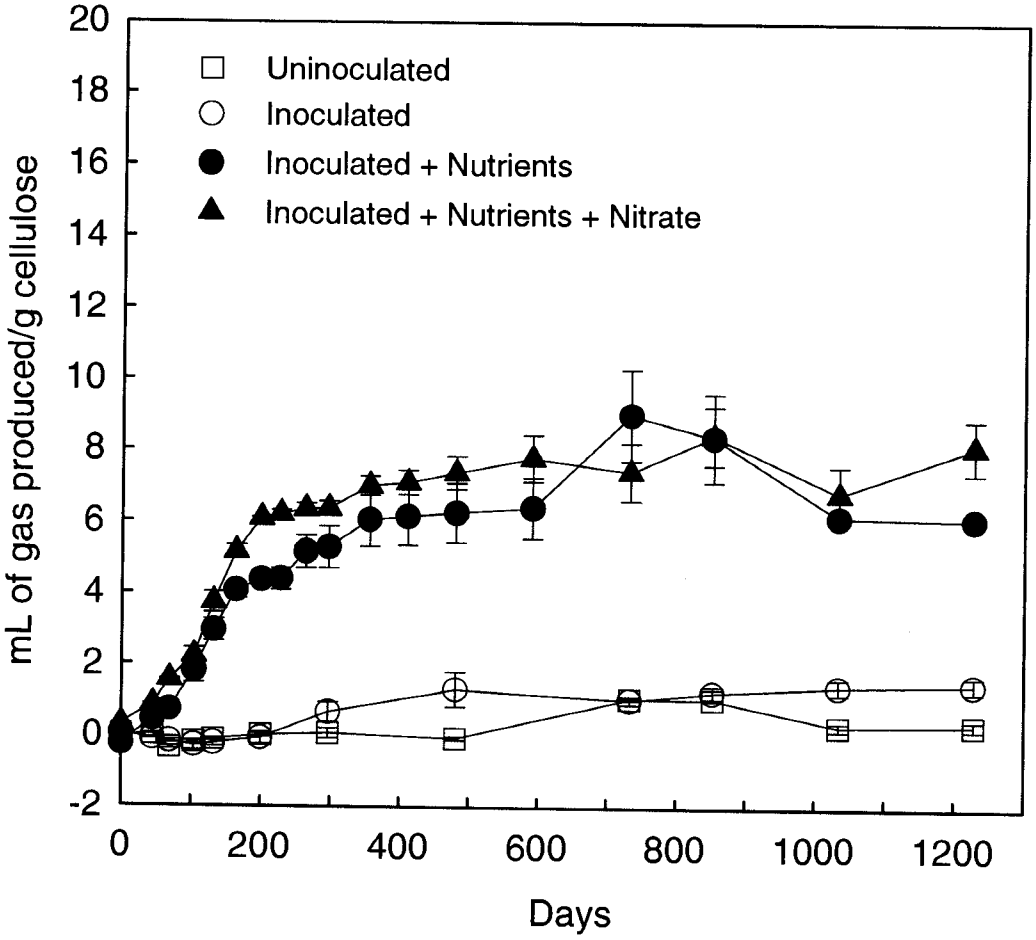


Figure 10. Total gas produced in samples containing bentonite incubated with an initial atmosphere of air.

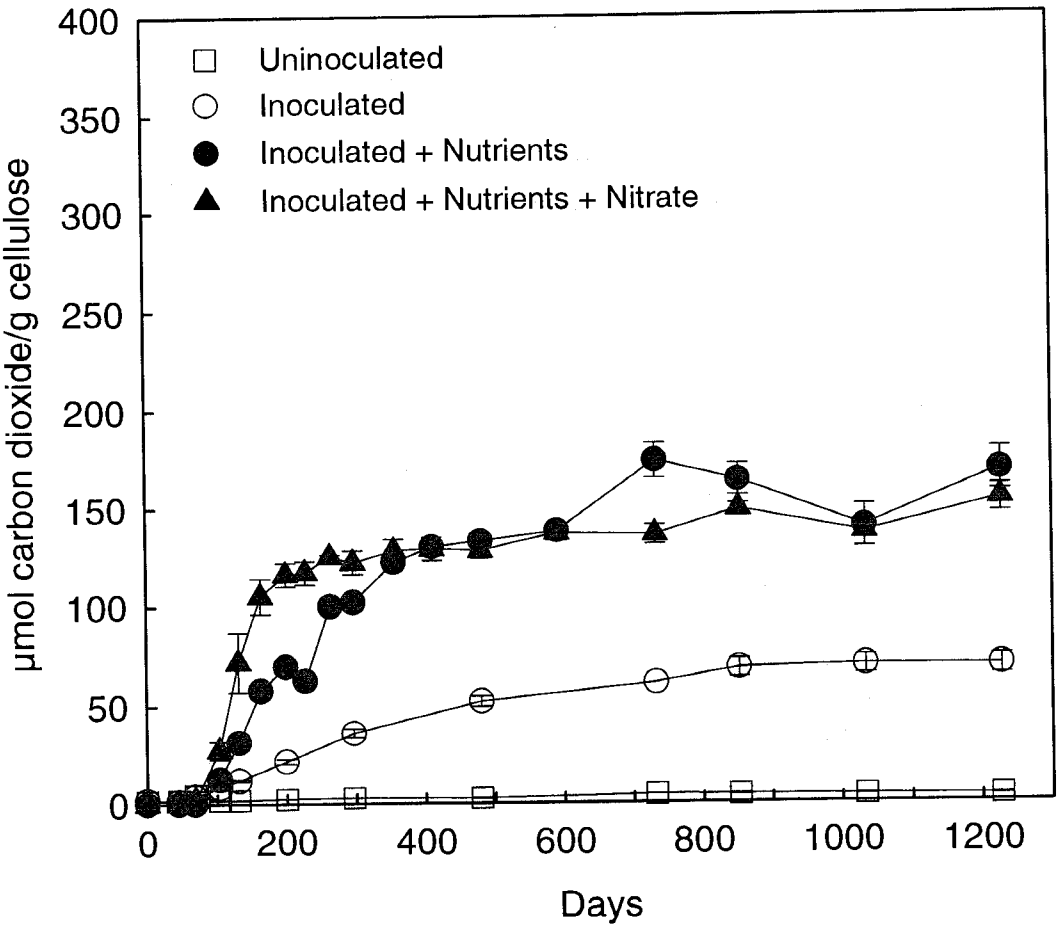


Figure 11. Carbon dioxide produced in samples containing bentonite incubated with an initial atmosphere of air.

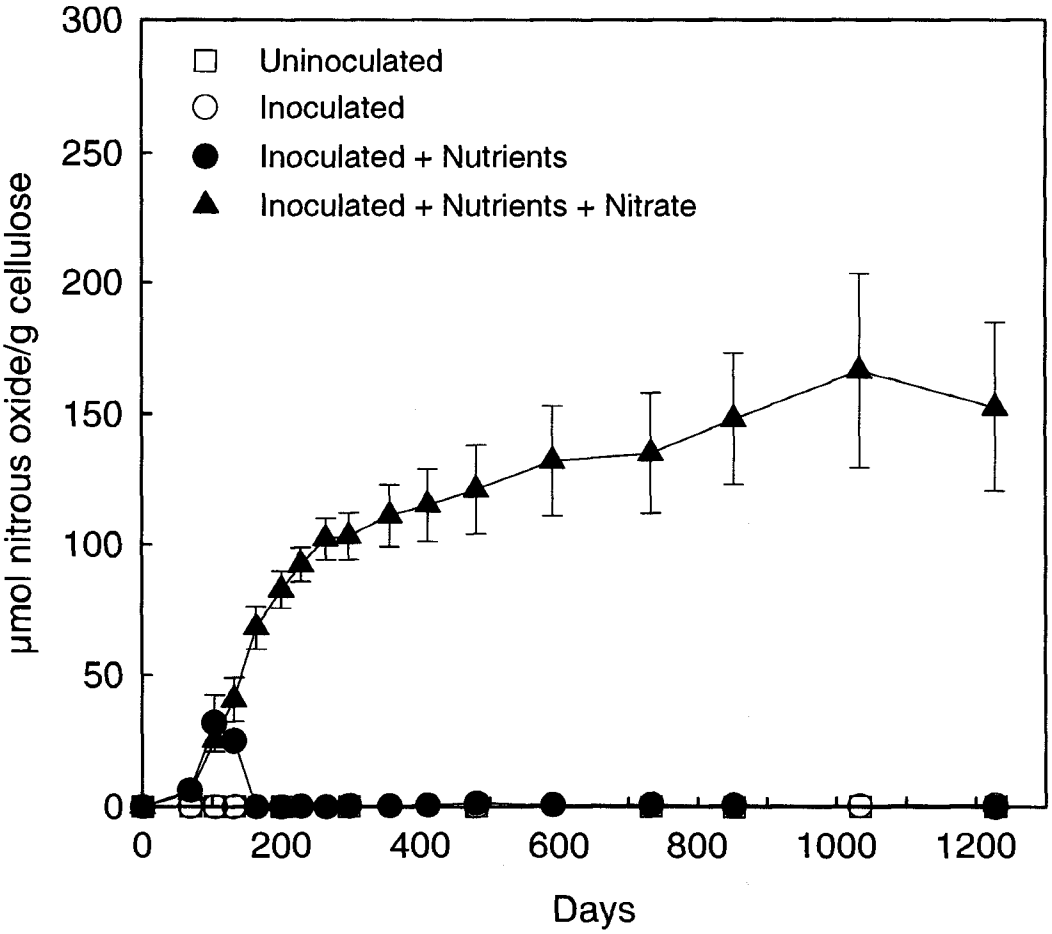


Figure 12. Nitrous oxide produced in samples containing bentonite incubated with an initial atmosphere of air.

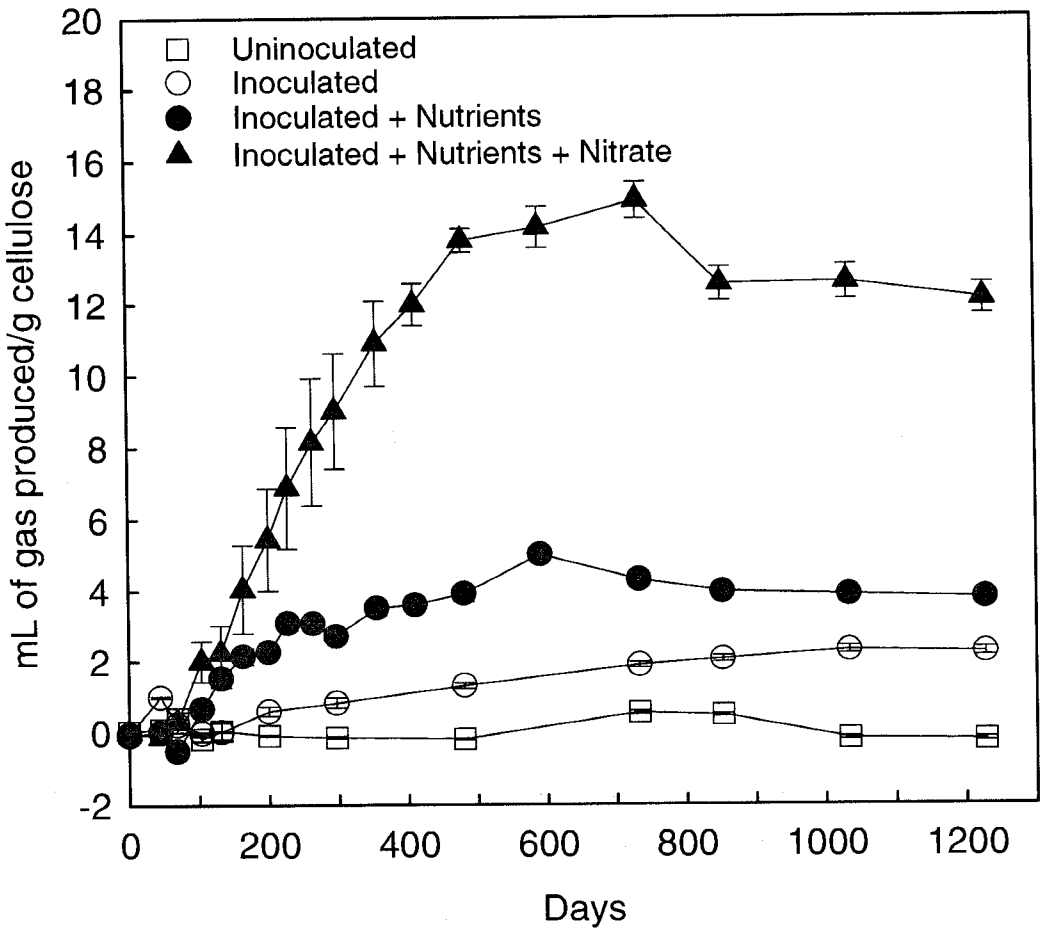


Figure 13. Total gas produced in anaerobic samples.

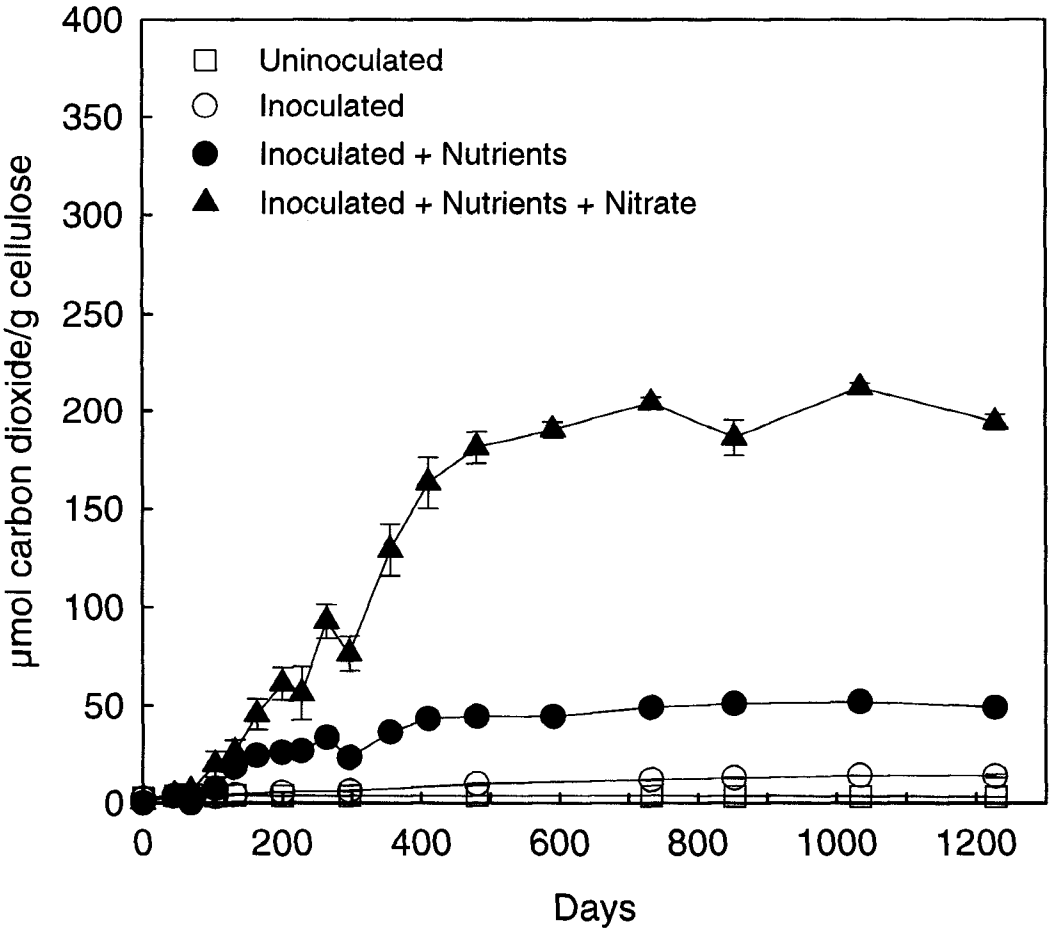


Figure 14. Carbon dioxide produced in anaerobic samples.

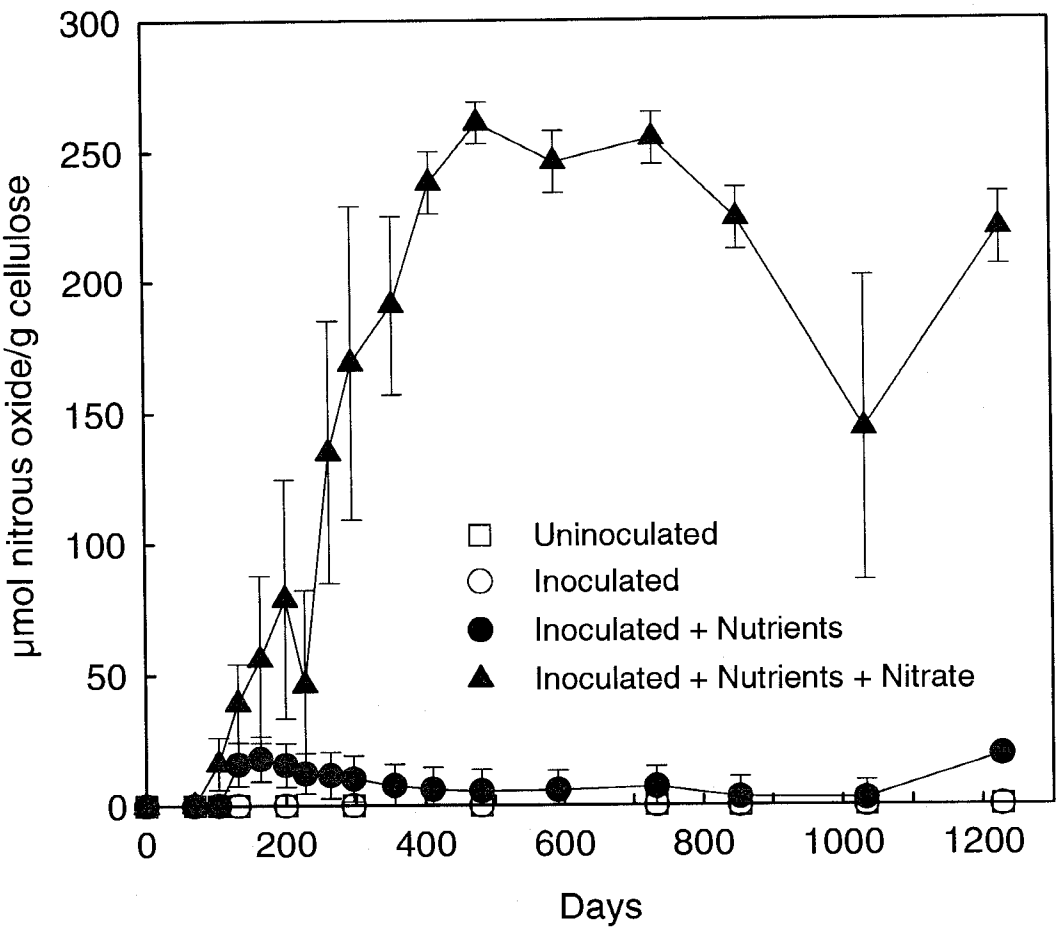


Figure 15. Nitrous oxide produced in anaerobic samples.

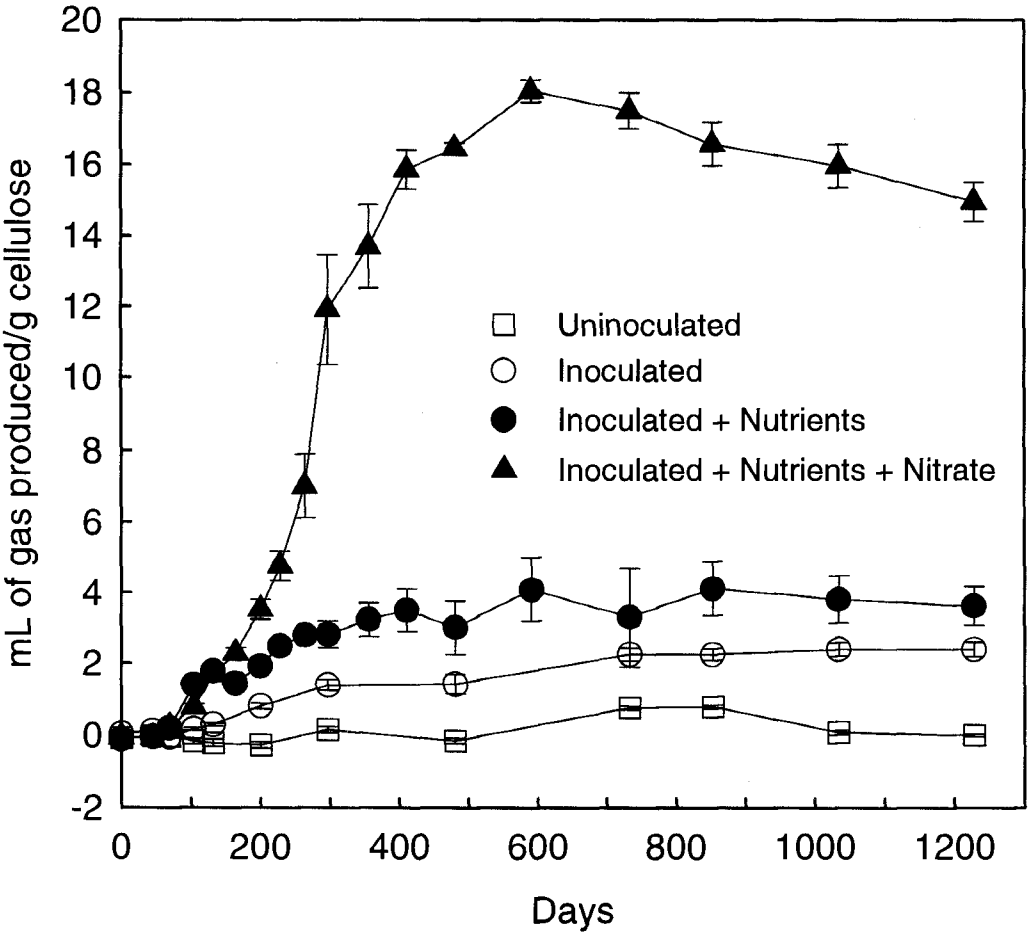


Figure 16. Total gas produced in anaerobic samples containing bentonite.

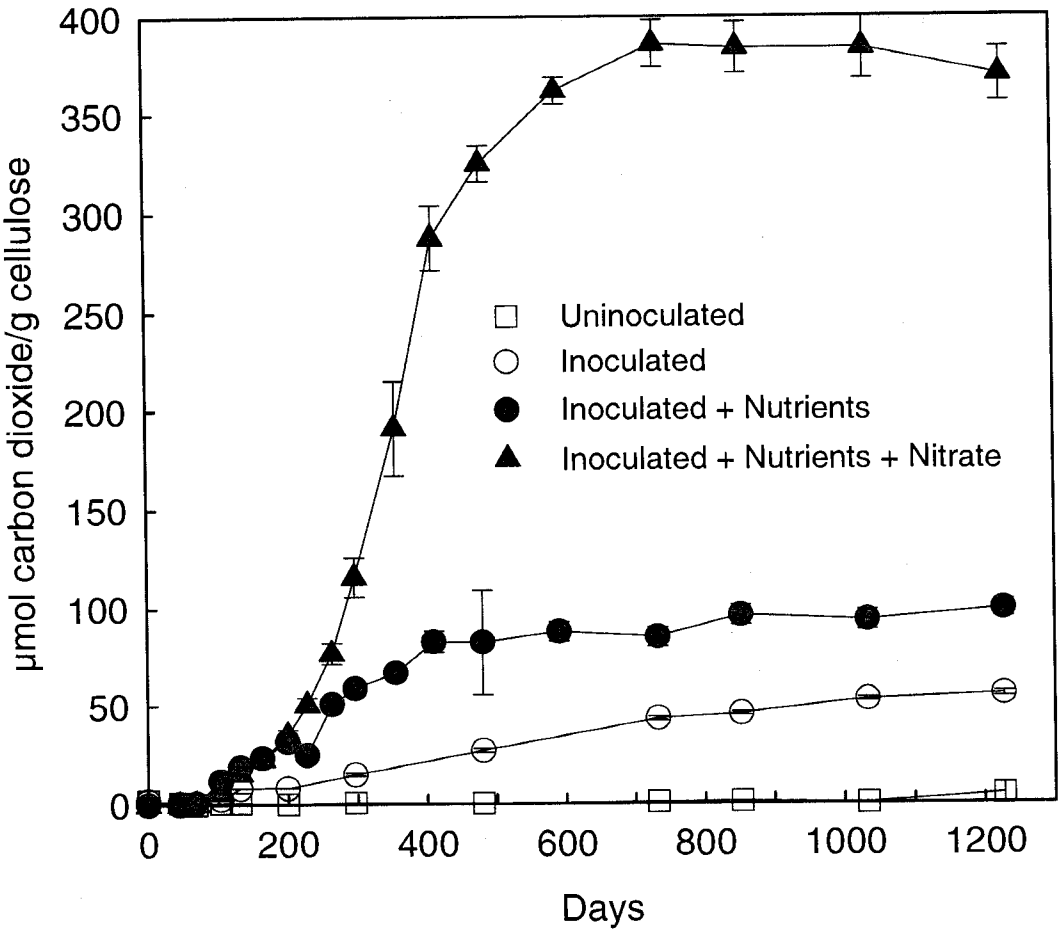


Figure 17. Carbon dioxide produced in anaerobic samples containing bentonite.

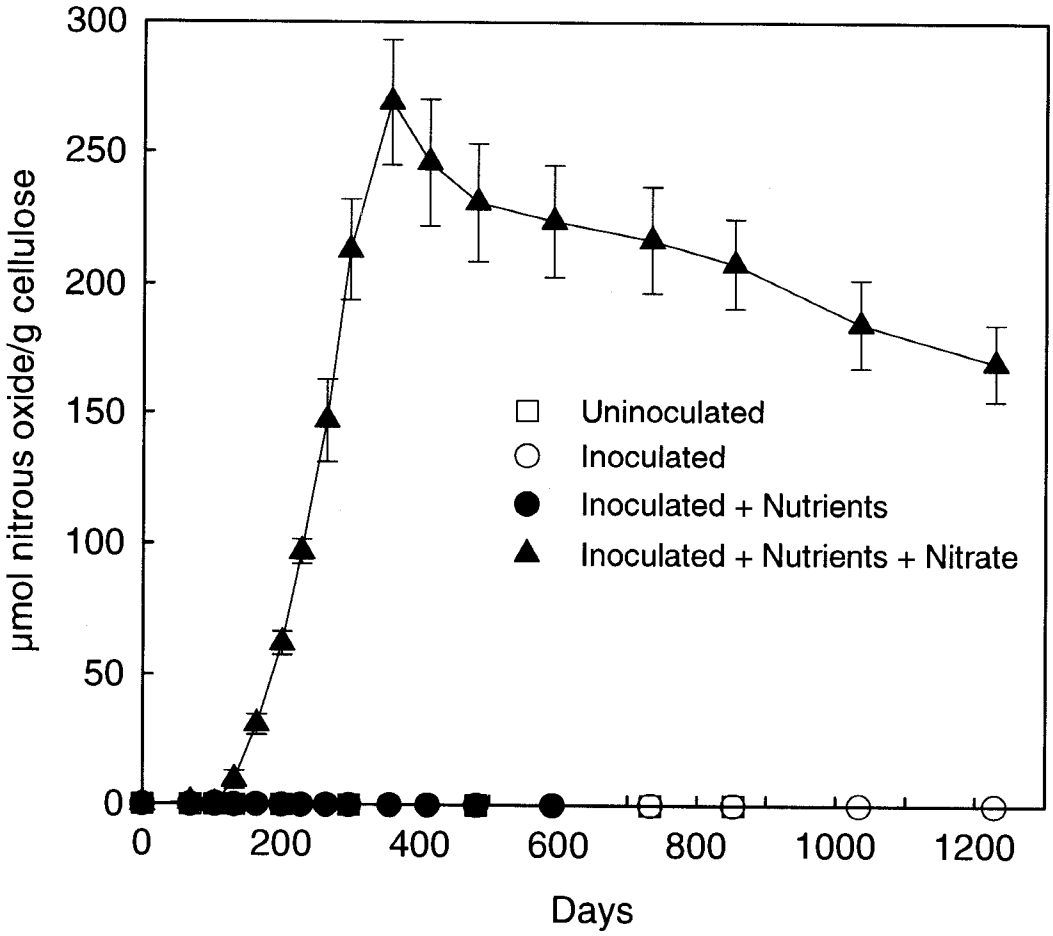


Figure 18. Nitrous oxide produced in anaerobic samples containing bentonite.

8.1.1.2 CARBON DIOXIDE PRODUCTION

Figure 8 shows that the production of CO_2 followed the same trend as total gas production. Uninoculated samples generated 4.43 μmol of CO_2 g^{-1} cellulose up to 1228 days, which was slightly less than that of the formalin-treated control (Table 2, Appendix A). Inoculated unamended samples produced 13.8 μmol CO_2, whereas in samples containing nutrients CO_2 reached a maximum of 41.0 μmol g^{-1} cellulose at 200 days which gradually decreased to 17.0 μmol CO_2 g^{-1} cellulose at 1228 days. Excess nitrate, however, stimulated both the rate and extent of CO_2 production. Treated samples reached a maximum of 186 μmol CO_2 over 1034 days, which declined thereafter. The rate of CO_2 production was 0.484 μmol g^{-1} cellulose day^{-1} from 69 to 200 days, and 0.057 μmol g^{-1} cellulose day^{-1} from 200 to 1228 days. The formalin-treated control produced 9.32 μmol g^{-1} cellulose, twice as high as uninoculated samples. Apparently, adding formalin caused chemical changes in the samples resulting in the liberation of CO_2, although there was no increase in total gas production (Table 1, Appendix A).

8.1.1.3 NITROUS OXIDE PRODUCTION

Nitrous oxide is an intermediary gaseous species produced by denitrifying bacteria that can use nitrate as an electron acceptor in the absence of oxygen; the end-product is nitrogen gas. Nitrous oxide accumulates due to (i.) circumstances in which the rate of the conversion of nitrate to N_2O is faster than that of N_2O to N_2, (ii.) the lack of production of the N_2O-reductase enzyme by the halophilic bacteria, or, (iii.) inhibition of N_2O-reductase. We did not investigate the reasons for the accumulation of N_2O. However, quantifying the production of N_2O is useful, even though, under ideal conditions, it does not accumulate and is rapidly converted to N_2 gas. The production of N_2O under our experimental conditions was not expected to be due to any process other than a biological one. Therefore, it was a useful "biomarker" for microbiological activity in the treatments; this was illustrated by the fact that it was not produced in any of the formalin-treated controls (Table 3, 6, 9, and 12, Appendix A). In addition, N_2O did not accumulate to a significant extent in any of the treatments without nutrients (Figure 9, and Tables 3, 6, 9, and 12, and 1(c), 2(c), 3(c) and 4(c), Appendix A).

Denitrification was expected to be an important gas-producing microbial process in treatments to which nitrate and excess nitrate was added. Nutrient-amended samples contained 250 μmol nitrate g^{-1} cellulose, while such samples with excess nitrate contained 1240 μmol nitrate g^{-1} cellulose. In the inoculated nutrient-amended samples, the maximum concentration of N_2O, 24.4 μmol g^{-1} cellulose , was reached at 132 days and then declined to less than 1 μmol g^{-1} cellulose from 228 days onwards (Table 3, Appendix A). With excess nitrate, 152 μmol N_2O g^{-1} cellulose had accumulated from 69 to 264 days, reaching an overall total of 216 μmol g^{-1} cellulose at 1228 days (Figure 9). Nitrate was used as an alternate electron acceptor in these samples as oxygen was depleted; the excess nitrate significantly stimulated denitrification.

8.1.2 Aerobic Samples Containing Bentonite

8.1.2.1 TOTAL GAS PRODUCTION

Figure 10 shows total gas production in initially aerobic samples containing bentonite. Uninoculated and inoculated samples, with no added nutrients, produced less than 1.5 mL gas g^{-1} cellulose over 1228 days (Table 4, Appendix A). Inoculated samples containing nutrients

produced a maximum of 9.0 mL gas g^{-1} cellulose at 733 days. The rate of gas production from 69 to 200 days was 0.028 mL g^{-1} cellulose day^{-1} and, from 200 to 1228 days, 0.001 mL gas g^{-1} cellulose day^{-1}. In the presence of excess nitrate, 8.10 mL of gas g^{-1} cellulose was produced. The maximum amount was detected at 853 days (8.41 mL gas g^{-1} cellulose), with a production rate of 0.034 mL g^{-1} cellulose day^{-1} from 69 to 200 days, and 0.002 mL g^{-1} cellulose day^{-1} from 200 to 1228 days. The effect of adding the proposed backfill bentonite on total gas production under initially aerobic conditions was that it (i.) enhanced the initial (69 to 200 days) rate of gas production, (ii.) enhanced the amount of total gas produced in unamended samples, and (iii.) enhanced the rate and extent of total gas production in inoculated samples with nutrients (without excess nitrate; compare Figures 7 and 10).

8.1.2.2 CARBON DIOXIDE PRODUCTION

The addition of bentonite generated a substantial amount of abiotically produced carbon dioxide in samples without cellulose. Approximately 40.0 μmol CO_2 $sample^{-1}$ was produced over 1228 days in the absence of cellulose, inoculum, or nutrients (Appendix A, Table 2(b), Treatments 4(NC)-a and 4(NC)-a). Formalin-treated control samples showed the same trend. Figure 11 shows CO_2 production in initially aerobic samples containing bentonite. Uninoculated-unamended samples produced 3.30 μmol CO_2 g^{-1} cellulose and inoculated-unamended samples produced 69.6 μmol CO_2 g^{-1} cellulose over 1228 days (Table 5, Appendix A); both have been corrected for abiotic gas production. Inoculated-unamended samples produced just 13.8 μmol CO_2 g^{-1} cellulose over 1228 days in the absence of bentonite; nutrient-amended inoculated samples produced 169 μmol CO_2 g^{-1} cellulose over 1228 days at the following rates: 0.533 μmol g^{-1} cellulose day^{-1} from 69 to 200 days, and 0.096 μmol g^{-1} cellulose day^{-1} from 200 to 1228 days. Nutrient-amended inoculated samples with excess nitrate produced at total of 154 μmol CO_2 g^{-1} cellulose over 1228 days at a rate of 0.869 μmol g^{-1} cellulose day^{-1} from 69 to 200 days, and 0.036 μmol g^{-1} cellulose day^{-1} from 200 to 1228 days. Although there were differences in the initial rate of CO_2 production between the amended samples and samples containing excess nitrate, the final extent of CO_2 production was similar in both.

8.1.2.3 NITROUS OXIDE PRODUCTION

In the presence of bentonite, very little N_2O was detected in uninoculated and inoculated unamended samples (Table 6, Appendix A, sample 4(C)-a and 5(C)-a). Figure 12 shows N_2O production in initially aerobic samples containing bentonite. Nutrient addition to inoculated samples stimulated N_2O production from 69 to 104 days, resulting in the accumulation of a maximum of 32 μmol N_2O g^{-1} cellulose; this then rapidly declined to zero due to its conversion to N_2 gas. In the presence of excess nitrate, the conversion of N_2O to N_2 did not occur as quickly, so that 152 μmol g^{-1} cellulose accumulated over 1228 days, with a maximum of 166 μmol g^{-1} cellulose at 1034 days. The large error bars for this data set are due to the simultaneous production and conversion of N_2O, and variation in this rate between samples.

8.1.3 Anaerobic Treatments without Bentonite

8.1.3.1 TOTAL GAS PRODUCTION

Figure 13 shows the total gas produced in samples incubated anaerobically (in the presence of N_2). Uninoculated-unamended samples produced a maximum of 0.53 mL g^{-1}

cellulose over 733 days (Table 7, Appendix A). Inoculated-unamended samples produced 2.23 mL g^{-1} cellulose up to 1228 days incubation; the same treatment under initially aerobic conditions produced 1.47 mL g^{-1} cellulose. The inoculated nutrient-amended samples generated 4.00 mL g^{-1} cellulose up to 591 days at a rate of 0.004 mL g^{-1} cellulose day^{-1}; production then leveled off to 3.78 mL g^{-1} cellulose at 1228 days. Inoculated samples with nutrients plus excess nitrate produced 14.2 mL g^{-1} cellulose at 591 days at a rate of 0.027 mL g^{-1} cellulose day^{-1}; then it leveled off and declined slightly thereafter to 12.1 mL g^{-1} cellulose at 1228 days. Total gas production in inoculated anaerobic treatments with and without nutrient additions was higher than in treatments under initially aerobic conditions.

8.1.3.2 CARBON DIOXIDE PRODUCTION

Carbon dioxide production followed the same trend as total gas production (Figure 14); uninoculated-unamended samples produced very little CO_2 over 1228 days (3.13 μmol g^{-1} cellulose) and inoculated-unamended samples produced 13.9 μmol g^{-1} cellulose (Table 8, Appendix A). However, inoculated samples amended with nutrients gave 49.2 μmol g^{-1} cellulose over 1228 days at the initial rate of 0.085 μmol g^{-1} cellulose day^{-1} from 69 to 591 days. Excess nitrate stimulated CO_2 production to 190 μmol g^{-1} cellulose at 591 days at a rate of 0.352 μmol g^{-1} cellulose day^{-1}; thereafter production leveled off considerably to 0.006 μmol g^{-1} cellulose day^{-1} after 1228 days, resulting in the accumulation of only 4 μmol more, to 194 μmol g^{-1} cellulose, at 1228 days.

8.1.3.3 NITROUS OXIDE PRODUCTION

The rate of N_2O accumulation in anaerobic samples is shown in Figure 15. Nitrous oxide was not detected in uninoculated or inoculated samples without nutrients (Table 9, Appendix A). In the inoculated, nutrient-amended samples, N_2O first accumulated to a maximum of 17.9 μmol g^{-1} cellulose up to 164 days, and then gradually decreased to 2.54 μmol g^{-1} cellulose at 1034 days, with a subsequent increase to 19.1 μmol g^{-1} cellulose at 1228 days. In the presence of excess nitrate, N_2O accumulated significantly reaching a maximum of 261 μmol g^{-1} cellulose up to 481 days, then gradually decreased to 220 μmol g^{-1} cellulose at 1228 days. The initial high rate of N_2O accumulation was maintained for approximately 591 days; this correlates well with the duration of the initially high rates of total gas and CO_2 evolution described above.

8.1.4 Anaerobic Treatments Containing Bentonite

8.1.4.1 TOTAL GAS PRODUCTION

Figure 16 shows the total gas produced in anaerobic samples containing bentonite. Uninoculated-unamended samples produced only 0.79 mL gas g^{-1} cellulose over 853 days, with a net loss at 1228 days to zero (Table 10, Appendix A). In contrast, inoculated-unamended samples produced 2.39 mL g^{-1} cellulose up to 1228 days. Bentonite had no effect on total gas production in inoculated nutrient-amended samples, and, in fact, resulted in a smaller amount of gas over 1228 days (3.62 mL g^{-1} cellulose) than did the same treatment initially incubated aerobically (6.09 mL g^{-1} cellulose). In contrast, bentonite increased gas production in inoculated samples containing excess nitrate, to a maximum of 18.1 mL g^{-1} cellulose at 591 days at a rate of 0.034 mL g^{-1} cellulose day^{-1} from 69 to 591 days. Thereafter, gas production decreased, and a net loss to 14.9 mL g^{-1} cellulose was detected at 1228 days.

8.1.4.2 CARBON DIOXIDE PRODUCTION

Figure 17 shows CO_2 production in anaerobic samples containing bentonite. Its addition enhanced the background (abiotic) CO_2 concentration by about 40.0 μmol (Table 4(b), Appendix A) similar to initially aerobic samples containing bentonite.

Uninoculated-unamended samples produced a maximum of 1.00 μmol CO_2 g^{-1} cellulose up to 843 days; this increased to 4.70 μmol CO2 g^{-1} cellulose from 1034 to 1228 days. Inoculated-unamended samples produced 55.2 μmol CO_2 g^{-1} cellulose over 1228 days; this finding is important because it was the greatest quantity of CO_2 detected in inoculated samples without added nutrients (unamended). Inoculated samples with nutrients produced 99.4 μmol CO_2 g^{-1} cellulose over 1228 days, with the highest rate of production from 69 to 411 days (0.242 μmol g^{-1} cellulose day^{-1}). Inoculated samples with nutrients plus excess nitrate had a maximum of 386 μmol CO_2 at 733 days incubation at an initial rate of 0.581 μmol g^{-1} cellulose day^{-1}, the highest rate detected in any of the treatments.

8.1.4.3 NITROUS OXIDE PRODUCTION

Accumulation of N_2O in anaerobic samples containing bentonite is shown in Figure 18. Nitrous oxide was not detected in uninoculated and inoculated-unamended samples (Table 12, Appendix A), and only traces were found in inoculated samples containing nutrients at 69, 104, and 164 days. In inoculated samples with excess nitrate, N_2O was produced at a rate of 0.857 μmol g^{-1} cellulose day^{-1} up to 356 days resulting in the accumulation of 269 μmol N_2O g^{-1} cellulose. The concentration decreased thereafter from 356 to 1228 days, with 170 μmol g^{-1} cellulose detected at 1228 days; a complete reduction of N_2O to N_2 was not observed.

8.1.5 Other Gases (H_2, O_2, N_2, CH_4, H_2S)

Tables 5 through 8 give the concentration of all of the gases analyzed from initially aerobic and anaerobic samples at 853 days of incubation. Hydrogen production was due to fermentation (Colberg, 1988); the largest quantities were detected in unamended and amended-inoculated samples containing cellulose incubated anaerobically.

The amended-inoculated anaerobic samples without bentonite contained 19.4% H_2 at 853 days (Table 8). Samples incubated under initially aerobic conditions contained less H_2 because anaerobic microbial processes, specifically fermentation, were not as advanced as in anaerobic samples. Hydrogen production was less significant in aerobic (initial air atmosphere) and in samples containing excess nitrate due to the presence of O_2 and NO_3^- as electron acceptors.

Oxygen was not detected in anaerobic samples. Initially, aerobic unamended-inoculated samples with cellulose contained up to 3.7% O_2 at 853 days. Oxygen was not completely consumed due to the lack of available inorganic nutrients, whereas nutrient amended samples had only 0.7 to 1.3% O_2 remaining. Hydrogen sulfide and methane were not detected in any samples analyzed at 853 days nor at 1228 days (data not shown).

Table 5. Composition of Headspace Gas of Initially Aerobic Samples at 853 Days

Sample	Gas Volume (ml)	Hydrogen	Oxygen	Nitrogen	Carbon Dioxide	Nitrous Oxide	Methane	Total
		----------	----------	----------	µmol/sample	----------	----------	----------
without Cellulose								
Unamended/Inoculated	49.9	ND	360	1665	7.8	ND	ND	2032
Amended/Inoculated	47.5	ND	77.2	1718	146	ND	ND	1941
Amended Inoculated/Exc. Nitrate	54.5	ND	263	1868	89.5	ND	ND	2220
with Cellulose								
Unamended/Inoculated	41.7	ND	54.8	1559	80.2	ND	ND	1694
Amended Inoculated	45.2	153	24.3	1411	276	ND	ND	1865
Amended Inoculated/Exc. Nitrate	95.5	66.1	21.2	1937	936	1116	ND	4076
without Cellulose/with Bentonite								
Unamended/Inoculated	52.4	ND	330	1759	39.2	0.1	ND	2128
Amended/Inoculated	48.3	ND	72.3	1718	177	7.6	ND	1974
Amended Inoculated/Exc. Nitrate	49.1	ND	177	1732	89.9	0.9	ND	2000
with Cellulose/with Bentonite								
Unamended/Inoculated	47.1	242	70.3	1316	381	3.8	ND	2013
Amended Inoculated	71.1	284	19.1	1799	998	10.0	ND	3110
Amended Inoculated/Exc. Nitrate	80.9	ND	24.9	1835	836	740	ND	3437

Values are the mean of the analysis of triplicate samples.
ND = none detected

Table 6. Composition of Headspace Gas (%) of Aerobic (Sealed) Samples at 853 Days

Sample	Gas Volume (ml)	Hydrogen	Oxygen	Nitrogen	Carbon Dioxide (%)	Nitrous Oxide	Methane	Total	Unaccounted
without Cellulose									
Unamended/Inoculated	49.9	0.0	17.6	81.5	0.4	0.0	0.0	99.5	0.5
Amended/Inoculated	47.5	0.0	4.0	88.4	7.5	0.0	0.0	99.9	0.1
Amended Inoculated/Exc. Nitrate	54.5	0.0	11.8	83.8	4.0	0.0	0.0	99.6	0.4
with Cellulose									
Unamended/Inoculated	41.7	0.0	3.2	91.4	4.7	0.0	0.0	99.3	0.7
Amended Inoculated	45.2	8.3	1.3	76.3	14.9	0.0	0.0	100.8	-0.8
Amended Inoculated/Exc. Nitrate	95.5	1.7	0.5	49.6	24.0	28.6	0.0	104.4	-4.4
without Cellulose/with Bentonite									
Unamended/Inoculated	52.4	0.0	15.4	82.0	1.8	0.0	0.0	99.2	0.8
Amended/Inoculated	48.3	0.0	3.7	86.9	8.9	0.4	0.0	99.9	0.1
Amended Inoculated/Exc. Nitrate	49.1	0.0	8.8	86.2	4.5	0.0	0.0	99.5	0.5
with Cellulose/with Bentonite									
Unamended/Inoculated	47.1	12.5	3.7	68.3	19.8	0.2	0.0	104.5	-4.5
Amended Inoculated	71.1	9.8	0.7	61.9	34.3	0.3	0.0	106.9	-6.9
Amended Inoculated/Exc. Nitrate	80.9	0.0	0.8	55.5	25.3	22.4	0.0	103.9	-3.9

Values are the mean of the analysis of triplicate samples.

Table 7. Composition of Headspace Gas of Anaerobic Samples at 853 Days

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

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

Table 8. Composition of Headspace Gas (%) of Anaerobic Samples at 853 Days

Sample	Gas Volume (ml)	Hydrogen	Oxygen*	Nitrogen	Carbon Dioxide (%)	Nitrous Oxide	Methane	Total	Unaccounted
without Cellulose									
Unamended/Inoculated	52.3	1.0	<0.5	94.5	0.2	0.0	0.0	97.0	3.0
Amended/Inoculated	60.7	0.0	<0.5	91.4	5.2	1.3	0.0	98.1	1.9
Amended Inoculated/Exc. Nitrate	57.6	0.2	<0.5	92.4	5.3	0.0	0.0	98.1	1.9
with Cellulose									
Unamended/Inoculated	52.5	17.5	<0.5	76.3	3.1	0.0	0.0	97.1	2.9
Amended Inoculated	70.5	19.4	<0.5	65.8	13.3	1.6	0.0	100	-0.3
Amended Inoculated/Exc. Nitrate	110	8.0	<0.5	44.8	23.4	24.9	0.0	101.2	-1.2
without Cellulose/with Bentonite									
Unamended/Inoculated	53.0	0.0	<0.5	94.7	1.6	0.0	0.0	97.1	2.9
Amended/Inoculated	59.7	0.0	<0.5	90.8	7.2	0.6	0.0	98.8	1.2
Amended Inoculated/Exc. Nitrate	62.4	0.5	<0.5	90.0	7.7	1.2	0.0	99.6	0.4
with Cellulose/with Bentonite									
Unamended/Inoculated	54.3	13.1	<0.5	76.3	11.8	0.0	0.0	101	-1.4
Amended Inoculated	70.3	10.8	<0.5	68.2	22.9	0.0	0.0	102	-2.0
Amended Inoculated/Exc. Nitrate	135	5.2	<0.5	41.8	38.2	19.3	0.0	105	-4.7

Values are the mean of the analysis of triplicate samples.
**Detection limit = 0.5%*

8.1.6 Analysis of Liquid from Inundated Samples

Tables 9 through 12 give the values for total dissolved organic carbon, soluble carbohydrates, and organic acid metabolites produced from cellulose degradation after 885 and 1228 days of incubation. In general, the amended inoculated samples showed an increase in dissolved organic carbon. The initially aerobic samples had the least dissolved organic carbon, while anaerobic samples showed the most indicating the activity of fermenters. The concentration of soluble carbohydrates and organic acids followed a similar trend. The following organic acids were detected: acetic, butyric, formic, lactic, methyl malonic, propionic, valeric, and isovaleric. The major ones were lactic (1860 $\mu g\ mL^{-1}$), acetic (754 $\mu g\ mL^{-1}$), and propionic (335 $\mu g\ mL^{-1}$). Following the same trend as total soluble organics, the acid concentration had decreased by 1228 days (1880 $\mu g\ mL^{-1}$ total organic acids). Overall, a very consistent trend in soluble organic compounds was observed between treatments that contained cellulose: amended-inoculated plus excess nitrate > amended-inoculated > unamended-inoculated > unamended-uninoculated. In addition, in initially aerobic samples, the quantities were always highest in samples of the same treatment without bentonite, while the reverse was true in anaerobic samples. Total soluble organic carbon provides an indication of the extent of cellulose degradation. These data correlate with trends in gas production (anaerobic samples produced the most gas, specifically those containing inoculum and nutrients). These results also show microbial activity in unamended samples; total organic acids detected in unamended-inoculated anaerobic samples with and without bentonite at 1228 days were 889 and 127 $\mu g\ mL^{-1}$, respectively, Tables 12 and 11, values which correlated with their CO_2 production (Figures 14 and 17).

Analyses of pH, nitrate, iron (total and Fe^{2+}), and sulfate are presented in Tables 13 through 16. The initial pH was 6.1; in samples containing cellulose, this dropped to a low of 5.52 in initially aerobic amended-inoculated samples (Table 14), and to 5.64 in anaerobic unamended-inoculated samples (16). By 1228 days, nitrate was completely consumed in all samples containing cellulose (Tables 13-16) (Note: the initial concentration of nitrate was 775 $\mu g\ mL^{-1}$ in amended samples and 3,825 $\mu g\ mL^{-1}$ in amended samples with excess nitrate). Ferrous iron was detected at 853 and 1228 days in initially aerobic inoculated-unamended (19.0 $\mu g\ mL^{-1}$) and inoculated-amended (22.5 $\mu g\ mL^{-1}$) samples containing bentonite (Table 14). Most iron was in the reduced form, Fe^{2+}; there was very little as Fe^{3+}. Iron reduction was due to anaerobic microbial activity; this assertion is further supported by the detection of ferrous iron in anaerobic samples with bentonite (13.6 $\mu g\ mL^{-1}$ in inoculated-unamended samples, 26.9 $\mu g\ mL^{-1}$ in inoculated-amended samples, and 32.1 $\mu g\ mL^{-1}$ in inoculated-amended samples with excess nitrate). No ferrous iron was detected in the absence of inoculum or cellulose. The source of iron was bentonite, which contains 3.25% w/w Fe_2O_3 (see Table 1b, Section 6.1.3). Sulfate concentrations were relatively constant (approximately 28,000 to 33,000 $\mu g\ mL^{-1}$ or ~310 mM), correlating with the measured SO_4^{2-} concentration in G-Seep (303 mM) reported by Brush (1990). This constancy in the concentrations supports the gas data, which did not show any H_2S production, and therefore sulfate reduction was not detected.

The formation of low-molecular-weight organic compounds should stimulate the activity of sulfate reducers, resulting in the precipitation and immobilization of radionuclides and toxic metals. Although these samples contained a large amount of sulfate, we did not observe the activity of sulfate reducers, but we expect that they will be active in the natural environment and in the WIPP repository.

Table 9. Organic analyses of aerobic samples without bentonite at 885 and 1228 days.

a. Total dissolved organics

Treatment	Total Dissolved Organic Carbon		Total Soluble Carbohydrates		Total Organic Acids	
	885	1228	885	1228	885	1228
	µg/ml					
Unam.-Uninoc. w/o Cell.	78.3 ± 17.7	45.0 ± 4.0	nd	39.9 ± 6.2	4.12 ± 0.58	41.0 ± 10.0
Unam.-Uninoc. w/ Cell.	269 ± 5	206 ± 4	52.0 ± 0.0	25.0 ± 3.0	75.3 ± 2.7	195 ± 88
Unam.-Inoc. w/o Cell.	113 ± 15	120 ± 16	nd	73.0 ± 26.3	6.76 ± 1.16	52.0 ± 17.0
Unam.-Inoc. w/ Cell.	287 ± 10	182 ± 6	nd	40.0 ± 16.0	75.0 ± 5.0	170 ± 76
Amend.-Inoc. w/o Cell.	189 ± 16	197 ± 16	78.7 ± 13	50.9 ± 10.6	26.4 ± 4.1	63.0 ± 16.0
Amend.-Inoc. w/ Cell.	545 ± 8	554 ± 25	332 ± 2	241 ± 6	393 ± 8	469 ± 71
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	186 ± 21	147 ± 13	95.6 ± 9.7	115 ± 4	18.9 ± 2.2	140 ± 36
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	594 ± 16	677 ± 18	455 ± 12	470 ± 3	616 ± 66	577 ± 91

b. Organic acids

Treatment	Formic		Acetic		Propionic		Butyric	
	885	1228	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	nd	15.0 ± 4.0	1.70 ± 0.2	26.0 ± 6.0	nd	nd	nd	nd
Unam.-Uninoc. w/ Cell.	18.0 ± 0.15	85.0 ± 26.0	10.9 ± 0.4	78.0 ± 43.0	8.53 ± 0.63	nd	nd	nd
Unam.-Inoc. w/o Cell.	1.10 ± 0.34	17.0 ± 5.0	3.06 ± 0.5	33.0 ± 10.0	nd	nd	nd	nd
Unam.-Inoc. w/ Cell.	1.68 ± 0.61	40.0 ± 6.0	4.34 ± 0.1	121 ± 66	5.97 ± 2.55	nd	nd	nd
Amend.-Inoc. w/o Cell.	1.30 ± 0.65	23.0 ± 4.0	16.8 ± 0.6	37.0 ± 11.0	nd	nd	4.74 ± 1.33	nd
Amend.-Inoc. w/ Cell.	12.0 ± 1.4	116 ± 7	103 ± 1	267 ± 36	22.1 ± 1.3	15.0 ± 13.0	4.32 ± 0.25	nd
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	2.95 ± 0.16	73.0 ± 22.0	11.2 ± 0.7	51.0 ± 13.0	nd	nd	nd	nd
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	15.2 ± 0.1	157 ± 4	73.9 ± 5.7	266 ± 66	61.0 ± 13.1	9.00 ± 8.00	8.16 ± 0.68	nd

b. Organic acids (continued)

Treatment	Isobutyric		Valeric		Succinic		Lactic	
	885	1228	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	2.42 ± 0.36	nd	nd	nd	nd	nd	nd	nd
Unam.-Uninoc. w/ Cell.	13.4 ± 0.4	nd	2.72 ± 0.4	nd	1.70 ± 0.01	nd	8.03 ± 0.19	32.0 ± 18.0
Unam.-Inoc. w/o Cell.	2.60 ± 0.37	nd	nd	nd	nd	nd	nd	2.00 ± 2.00
Unam.-Inoc. w/ Cell.	13.6 ± 1.3	nd	nd	nd	nd	nd	46.8 ± 0.1	8.00 ± 4.00
Amend.-Inoc. w/o Cell.	nd	nd	nd	nd	1.25 ± 0.04	nd	nd	3.00 ± 1.00
Amend.-Inoc. w/ Cell.	nd	10.0 ± 7.0	73.9 ± 0.0	nd	61.1 ± 0.1	nd	90.5 ± 0.9	62.0 ± 9.0
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	4.74 ± 1.33	nd	nd	nd	nd	nd	nd	16.0 ± 1.0
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	51.1 ± 2.3	13.0 ± 8.0	324 ± 13	nd	7.38 ± 5.22	4.00 ± 2.00	26.7 ± 2.9	141 ± 12

nd = none detected

Table 10. Organic analyses of aerobic samples with bentonite at 885 and 1228 days.

a. Total dissolved organics

Treatment	Total Dissolved Organic Carbon		Total Soluble Carbohydrates		Total Organic Acids	
	885	1228	885	1228	885	1228
	µg/ml					
Unam.-Uninoc. w/o Cell.	101 ± 51	45.0 ± 4.0	nd	nd	20.7 ± 8.7	97.0 ± 21.0
Unam.-Uninoc. w/ Cell.	132 ± 25	230 ± 13	nd	nd	18.3 ± 2.9	167 ± 117
Unam.-Inoc. w/o Cell.	47.6 ± 42.4	120 ± 16	nd	nd	4.88	41.0 ± 10.0
Unam.-Inoc. w/ Cell.	214 ± 13	456 ± 32	58.7 ± 4.1	69.0 ± 0.7	366 ± 18	325 ± 108
Amend.-Inoc. w/o Cell.	65.2 ± 13.9	197 ± 16	73.6 ± 5.0	111 ± 13	17.6 ± 9.1	63.0 ± 8.0
Amend.-Inoc. w/ Cell.	335 ± 10	952 ± 31	nd	384 ± 1	386 ± 7	505 ± 56
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	116 ± 31	147 ± 13	163 ± 1	179 ± 12	29.7 ± 17.0	84.0 ± 14.0
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	449 ± 8	261 ± 14	358 ± 2	100 ± 15	62.0 ± 1.7	101 ± 29

b. Organic acids

Treatment	Formic		Acetic		Propionic		Butyric	
	885	1228	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	8.60 ± 6.08	15.0 ± 4.0	10.8 ± 1.7	26.0 ± 6.0	nd	nd	nd	nd
Unam.-Uninoc. w/ Cell.	7.39 ± 1.16	92.0 ± 61.0	5.56 ± 0.6	65.0 ± 51.0	nd	nd	nd	nd
Unam.-Inoc. w/o Cell.	nd	17.0 ± 5.0	4.88	33.0 ± 10.0	nd	nd	nd	nd
Unam.-Inoc. w/ Cell.	10.6 ± 0.6	93.0 ± 46.0	237 ± 6	190 ± 62	nd	nd	nd	nd
Amend.-Inoc. w/o Cell.	8.67 ± 6.13	23.0 ± 4.0	7.70 ± 2.1	37.0 ± 11.0	nd	nd	nd	nd
Amend.-Inoc. w/ Cell.	11.0 ± 1.2	118 ± 9	277 ± 1	220 ± 37	nd	nd	17.4 ± 0.7	nd
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	15.4 ± 10.8	73.0 ± 22.0	12.6 ± 5.0	51.0 ± 13.0	nd	nd	nd	nd
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	nd	18.0 ± 1.0	18.4 ± 0.9	78.0 ± 27.0	nd	nd	nd	nd

b. Organic acids (continued)

Treatment	Methyl Malonic		Valeric		Succinic		Lactic	
	885	1228	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	nd
Unam.-Uninoc. w/ Cell.	nd	nd	nd	nd	nd	nd	3.20 ± 0.21	9.00 ± 5.00
Unam.-Inoc. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	2.00 ± 2.00
Unam.-Inoc. w/ Cell.	24.9 ± 5.4	nd	nd	nd	nd	nd	77.1 ± 5.3	42.0 ± 0.0
Amend.-Inoc. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	3.00 ± 1.00
Amend.-Inoc. w/ Cell.	13.2 ± 0.9	nd	nd	nd	nd	nd	59.4 ± 0.0	167 ± 10
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	16.0 ± 1.0
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	nd	nd	43.6 ± 0.8	nd	nd	nd	nd	5.00 ± 1.00

nd = none detected

Table 11. Organic analyses of the anaerobic samples without bentonite at 885 and 1228 days.

a. Total dissolved organics

Treatment	Total Dissolved Organic Carbon		Total Soluble Carbohydrates		Total Organic Acids	
	885 d	1228 d	885 d	1228 d	885 d	1228 d
	µg/ml					
Unam.-Uninoc. w/o Cell.	101 ± 10	102	nd	85.0 ± 8.0	23.8 ± 11.8	68.0 ± 17.0
Unam.-Uninoc. w/ Cell.	104 ± 25	215 ± 16	43.8 ± 4.9	67.0 ± 5.0	43.3 ± 4.0	176 ± 66
Unam.-Inoc. w/o Cell.	127 ± 20	130	24.4 ± 4.1	65.0 ± 8.0	14.1 ± 2.1	51.0 ± 17.0
Unam.-Inoc. w/ Cell.	126 ± 28	537 ± 70	18.7 ± 6.5	79.0 ± 7.0	127 ± 8	349 ± 132
Amend.-Inoc. w/o Cell.	278 ± 19	235 ± 10	36.1 ± 8.6	149 ± 2	52.4 ± 20.6	59.0 ± 12.0
Amend.-Inoc. w/ Cell.	417 ± 9	620 ± 6	43.8 ± 3.2	103 ± 1	350 ± 8	320 ± 50
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	282 ± 21	273 ± 36	211 ± 13	222 ± 15	222 ± 9	107 ± 27
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	726 ± 26	1047 ± 37	662 ± 2	742 ± 14	58.0 ± 4.6	515 ± 23

b. Organic acids

Treatment	Formic		Acetic		Propionic		Butyric	
	885	1228	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	1.01 ± 0.08	32.0 ± 7.0	19.7 ± 11	29.0 ± 7.0	nd	nd	nd	nd
Unam.-Uninoc. w/ Cell.	10.6 ± 0.4	80.0 ± 28.0	16.8 ± 0.1	83.0 ± 31.0	2.16 ± 0.26	nd	nd	nd
Unam.-Inoc. w/o Cell.	1.13 ± 0.03	20.0 ± 2.0	8.93 ± 1.6	29.0 ± 14.0	nd	nd	nd	nd
Unam.-Inoc. w/ Cell.	18.4 ± 1.4	117 ± 73	63.6 ± 0.6	209 ± 56	4.02 ± 0.95	nd	nd	nd
Amend.-Inoc. w/o Cell.	4.70 ± 1.91	26.0 ± 5.0	25.5 ± 9.6	29.0 ± 5.0	1.83 ± 0.42	nd	3.26 ± 0.62	nd
Amend.-Inoc. w/ Cell.	22.0 ± 0.1	47.0 ± 1.0	224 ± 1	234 ± 45	7.70 ± 0.07	nd	14.4 ± 6.5	nd
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	1.78 ± 0.42	43.0 ± 13.0	63.8 ± 2.0	59.0 ± 11.0	8.54 ± 0.37	nd	44.8 ± 1.5	nd
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	nd	274 ± 15	nd	114 ± 1	13.1 ± 4.2	nd	nd	nd

b. Organic acids (continued)

Treatment	Isovaleric		Valeric		Succinic		Lactic	
	885	1228	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	6.00 ± 3.00
Unam.-Uninoc. w/ Cell.	1.81 ± 1.28	nd	3.74 ± 0.1	nd	nd	nd	4.56 ± 0.09	13.0 ± 7.0
Unam.-Inoc. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	1.00 ± 1.00
Unam.-Inoc. w/ Cell.	2.47 ± 1.74	nd	3.44 ± 0.1	nd	nd	nd	26.3 ± 0.6	23.0 ± 4.0
Amend.-Inoc. w/o Cell.	nd	nd	nd	nd	nd	nd	2.37 ± 1.16	3.00 ± 1.00
Amend.-Inoc. w/ Cell.	nd	nd	nd	nd	nd	nd	60.5 ± 0.3	40.0 ± 3.0
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	95.3 ± 4.6	nd	nd	nd	nd	nd	nd	6.00 ± 2.00
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	nd	nd	44.9 ± 0.4	nd	nd	nd	nd	127 ± 7

nd = none detected

Table 12. Organic analyses of anaerobic samples with bentonite at 885 and 1228 days.

a. Total dissolved organics

Sample	Total Dissolved Organic Carbon		Total Soluble Carbohydrates		Total Organic Acids	
	885 d	1228 d	885 d	1228 d	885 d	1228 d
	µg/ml					
Unam.-Uninoc. w/o Cell.	163 ± 10	135 ± 4	nd	93.5 ± 10.2	13.1 ± 0.3	81.0 ± 12.0
Unam.-Uninoc. w/ Cell.	36.9 ± 22.7	192 ± 14	18.4 ± 5.5	nd	37.3 ± 2.8	74.0 ± 22.0
Unam.-Inoc. w/o Cell.	163 ± 15	114 ± 10	nd	35.9 ± 14.3	16.2 ± 1.8	25.0 ± 9.0
Unam.-Inoc. w/ Cell.	582 ± 16	372 ± 5	372 ± 3	25.8 ± 0.7	889 ± 20	199 ± 14
Amend.-Inoc. w/o Cell.	168 ± 11	173 ± 15	30.9 ± 3.4	129 ± 12	23.6 ± 3.5	123 ± 41
Amend.-Inoc. w/ Cell.	463 ± 29	416 ± 8	80.5 ± 2.4	79.5 ± 6.8	708 ± 26	271 ± 93
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	204 ± 17	175 ± 14	171 ± 3	186 ± 21	16.4 ± 2.5	51.0 ± 11.0
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	2410 ± 70	1884 ± 21	2850 ± 30	1294 ± 36	3808 ± 232	1880 ± 283

b. Organic acids

Treatment	Formic		Acetic		Propionic		Butyric	
	885	1228	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	1.80 ± 0.12	30.0 ± 11.0	7.73 ± 0.1	51.0 ± 1.0	nd	nd	nd	nd
Unam.-Uninoc. w/ Cell.	5.98 ± 0.50	24.0 ± 3.0	11.9 ± 0.4	24.0 ± 11.0	nd	nd	nd	22.0 ± 7.0
Unam.-Inoc. w/o Cell.	1.46 ± 0.02	8.00 ± 5.00	7.49 ± 0.3	15.0 ± 2.0	nd	nd	nd	nd
Unam.-Inoc. w/ Cell.	25.0 ± 0.7	30.0 ± 5.0	467 ± 13	145 ± 7	12.8 ± 0.3	nd	6.60 ± 0.16	nd
Amend.-Inoc. w/o Cell.	1.49 ± 0.01	45.0 ± 12.0	8.04 ± 0.3	76.0 ± 28.0	nd	nd	1.57 ± 1.11	nd
Amend.-Inoc. w/ Cell.	44.9 ± 0.5	83.0 ± 41.0	385 ± 7	153 ± 42	24.0 ± 6.8	nd	51.9 ± 3.8	nd
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	nd	23.0 ± 7.0	8.51 ± 0.5	25.0 ± 3.0	nd	nd	1.34 ± 0.64	nd
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	161 ± 21	713 ± 102	754 ± 17	502 ± 27	335 ± 36	10.0 ± 5.0	85.6 ± 20.1	106 ± 17

b. Organic acids (continued)

Treatment	Methyl Malonic		Valeric		Succinic		Lactic	
	885 d	1228 d	885	1228	885	1228	885	1228
	µg/ml							
Unam.-Uninoc. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	nd
Unam.-Uninoc. w/ Cell.	2.01 ± 0.68	nd	5.86 ± 0.0	nd	nd	nd	8.44 ± 0.46	5.00 ± 1.00
Unam.-Inoc. w/o Cell.	nd	nd	nd	nd	nd	nd	nd	2.00 ± 2.00
Unam.-Inoc. w/ Cell.	51.4 ± 0.8	nd	22.8 ± 0.5	nd	35.2 ± 0.3	nd	218 ± 3	23.0 ± 2.0
Amend.-Inoc. w/o Cell.	nd	nd	nd	nd	nd	nd	1.77 ± 1.16	2.00 ± 1.00
Amend.-Inoc. w/ Cell.	nd	nd	14.9 ± 2.2	nd	nd	2.00 ± 2.00	183 ± 3	17.0 ± 1.0
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	nd	nd	nd	nd	nd	nd	0.23 ± 0.16	3.00 ± 2.00
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	120 ± 85	nd	161 ± 5	nd	278 ± 3	92 ± 80	1860 ± 40	441 ± 47

nd = none detected

Table 13. Inorganic analyses of aerobic samples without bentonite at 885 and 1228 days.

Sample	pH		Nitrate		Iron						Sulfate	
					Fe(II)		Fe(III)		Total			
	885	1228	885	1228	885	1228	885	1228	885	1228	885	1228
			µg/ml									
Unam.-Uninoc. w/o Cell.	5.89	6.07	nd	nd	nd	nd	nd	nd	nd	nd	27800 ± 1300	31700 ± 800
Unam.-Uninoc. w/ Cell.	5.96	6.08	nd	nd	nd	nd	nd	nd	nd	nd	30500 ± 200	32600 ± 600
Unam.-Inoc. w/o Cell.	5.88	6.03	nd	nd	nd	nd	nd	nd	nd	nd	27800 ± 300	31100 ± 300
Unam.-Inoc. w/ Cell.	5.98	6.01	nd	nd	nd	nd	nd	nd	nd	nd	27800 ± 100	30300 ± 0
Amend.-Inoc. w/o Cell.	5.85	5.88	534 ± 93	nd	nd	nd	nd	nd	nd	nd	27900 ± 100	32700 ± 1200
Amend.-Inoc. w/ Cell.	5.88	5.54	nd	nd	1.06 ± 0.19	nd	0.55	1.50 ± 0.00	1.61 ± 0.19	1.50 ± 0.00	29300 ± 100	29800 ± 300
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	5.83	6.06	2710 ± 120	4150 ± 130	nd	nd	nd	nd	nd	nd	28200 ± 300	31200 ± 600
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	6.07	6.08	1950 ± 30	nd	nd	nd	nd	nd	nd	nd	29400 ± 500	31500 ± 500

Table 14. Inorganic analyses of aerobic samples with bentonite at 885 and 1228 days.

Sample	pH		Nitrate		Iron						Sulfate	
					Fe(II)		Fe(III)		Total			
	885	1228	885	1228	885	1228	885	1228	885	1228	885	1228
			µg/ml									
Unam.-Uninoc. w/o Cell.	6.04	6.09	nd	nd	nd	nd	nd	nd	nd	nd	27900 ± 300	31400 ± 1000
Unam.-Uninoc. w/ Cell.	6.00	6.08	nd	nd	nd	nd	nd	nd	nd	nd	28700 ± 1100	30300 ± 300
Unam.-Inoc. w/o Cell.	6.03	6.16	nd	nd	nd	nd	nd	nd	nd	nd	27400 ± 1100	29800 ± 600
Unam.-Inoc. w/ Cell.	5.65	5.54	nd	nd	3.49 ± 0.00	19.0 ± 0.1	0.54	0.00 ± 0.30	4.03 ± 0.00	18.3 ± 0.2	27300 ± 1100	26100
Amend.-Inoc. w/o Cell.	6.02	5.96	522 ± 10	nd	nd	nd	nd	nd	nd	nd	26600 ± 200	29700 ± 400
Amend.-Inoc. w/ Cell.	5.57	5.52	nd	nd	22.6 ± 0.1	22.5 ± 0.1	0.95	1.00 ± 0.40	23.6 ± 0.1	23.5 ± 0.3	27300 ± 400	29700 ± 300
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	5.96	6.09	3780 ± 30	4480 ± 220	nd	nd	nd	nd	nd	nd	27900 ± 400	29900 ± 500
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	6.14	6.16	552 ± 144	nd	nd	nd	nd	nd	nd	nd	26800 ± 500	30600

nd = none detected

Table 15. Inorganic analyses of anaerobic samples without bentonite at 885 and 1228 days.

Sample	pH		Nitrate		Iron						Sulfate	
					Fe(II)		Fe(III)		Total			
	885	1228	885	1228	885	1228	885	1228	885	1228	885	1228
			µg/ml									
Unam.-Uninoc. w/o Cell.	5.90	6.08	nd	nd	nd	nd	nd	nd	nd	nd	30500 ± 600	31000 ± 600
Unam.-Uninoc. w/ Cell.	5.96	6.10	nd	nd	nd	nd	nd	nd	nd	nd	30300 ± 600	31200 ± 800
Unam.-Inoc. w/o Cell.	5.91	6.11	nd	nd	nd	nd	nd	nd	nd	nd	28800 ± 200	32400 ± 1000
Unam.-Inoc. w/ Cell.	5.98	5.92	nd	nd	nd	nd	nd	nd	nd	nd	29600 ± 400	32000 ± 400
Amend.-Inoc. w/o Cell.	6.08	6.09	476 ± 9	nd	nd	nd	nd	nd	nd	nd	27500 ± 100	31800 ± 700
Amend.-Inoc. w/ Cell.	6.11	5.89	nd	nd	nd	nd	nd	nd	nd	nd	29100 ± 300	34000 ± 1300
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	5.97	6.06	2640 ± 100	3900 ± 190	nd	nd	nd	nd	nd	nd	27800 ± 0	29800 ± 800
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	6.21	6.12	1630 ± 50	nd	nd	nd	nd	nd	nd	nd	28200 ± 600	30700 ± 2300

Table 16. Inorganic analyses of anaerobic samples with bentonite at 885 and 1228 days.

Sample	pH		Nitrate		Iron						Sulfate	
					Fe(II)		Fe(III)		Total			
	885	1228	885	1228	885	1228	885	1228	885	1228	885	1228
			µg/ml									
Unam.-Uninoc. w/o Cell.	6.04	6.08	nd	nd	nd	nd	nd	nd	nd	nd	28900 ± 200	28700 ± 900
Unam.-Uninoc. w/ Cell.	6.00	6.27	nd	nd	nd	nd	nd	nd	nd	nd	28700 ± 100	29200 ± 1600
Unam.-Inoc. w/o Cell.	6.03	6.14	nd	nd	nd	nd	nd	nd	nd	nd	28700 ± 1200	29700 ± 0
Unam.-Inoc. w/ Cell.	5.64	5.71	nd	nd	3.14 ± 0.08	13.6 ± 0.00	0.06	3.5 ± 0.20	3.2 ± 0.08	17.1 ± 0.20	28200 ± 100	30500 ± 0
Amend.-Inoc. w/o Cell.	5.92	6.05	294 ± 4	nd	nd	nd	nd	nd	nd	nd	27300 ± 200	26400 ± 1800
Amend.-Inoc. w/ Cell.	5.74	5.83	nd	nd	31.4 ± 0.00	26.9 ± 0.20	0.00	6.7 ± 0.50	31.4 ± 0.00	33.6 ± 0.30	27600 ± 500	29100 ± 200
Amend.-Inoc.+ Ex. Nitr. w/o Cell.	6.09	6.11	3030 ± 50	4500	nd	nd	nd	nd	nd	nd	27700 ± 100	29600 ± 1200
Amend.-Inoc.+ Ex. Nitr. w/ Cell.	6.19	5.84	nd	nd	34.8 ± 0.02	32.1 ± 0.3	0.89		35.7 ± 0.02		26000 ± 200	29500 ± 900

nd = none detected

Bacterial counts were as follows (anaerobic samples without bentonite only): 4.5 $\pm 1.42\times10^4$ bacterial cells mL^{-1} in unamended and uninoculated formalin-treated samples, 7.38 $\pm 2.08\times10^5$ bacterial cells mL^{-1} in unamended and uninoculated samples, 9.66 $\pm 2.40\times10^4$ cells mL^{-1} in unamended and inoculated formalin-treated samples, 8.84 $\pm 0.67\times10^6$ cells mL^{-1} in unamended inoculated samples, 7.04 $\pm 0.41\times10^7$ cells mL^{-1} in amended inoculated samples, and 1.14 $\pm 0.06\times10^8$ cells mL^{-1} in amended inoculated samples with excess nitrate. The standard error reported here is of the mean of duplicate counting preparations of one aliquot taken from each sample bottle. These results show that formalin arrested the growth of the mixed inoculum (10^4 cells mL^{-1}), while the addition of excess nitrate resulted in the largest bacterial population (10^8 cells mL^{-1}) of the samples tested.

8.2 Gas Generation from Cellulose Biodegradation under Humid Conditions: Initially Aerobic Incubation

Total gas, H_2, O_2, N_2, CO_2, N_2O, CH_4, and H_2S production were analyzed in samples incubated under initially aerobic, humid conditions; H_2, CH_4, and H_2S were not detected during incubation. In Tables 17 through 24, total gas, CO_2, and N_2O data are presented. The tables are arranged so that control values are presented first, followed by data for treatments containing cellulose alone, then cellulose plus glucose, and finally cellulose plus succinate. The treatments most relevant to the expected WIPP repository conditions were those with cellulose alone. Treatments with additional carbon sources (glucose or succinate) were made to evaluate the effects of humid conditions on microbial activity, i.e., they were test systems for microbial activity in the presence of an easily metabolized carbon source, hence the label "activity measurement treatments." The absence of growth in these treatments would most likely indicate that the halophilic microorganisms in the mixed inoculum cannot grow under humid conditions tested in this study.

Data are the mean of triplicate analyses, with the standard error of the mean (SEM). Gas production data on a per sample basis are presented in Tables 17 through 24. In Table 25, "corrected" data are given, with control samples subtracted to yield CO_2 production on a per gram cellulose basis. We noted during the analyses that slight fluctuations in temperature resulted in large fluctuations in pressure, as measured with the pressure gauge apparatus. This is a direct result of the large headspace volume (~155 mL) and negligible total gas production in these samples. To account for fluctuations due to temperature changes, accurate temperature readings were recorded during sampling and gas production was normalized to 30°C. Gas analyses at t=0 were actually performed after 6 days incubation.

8.2.1 Cellulose Degradation

Total gas production, in general, was negligible in samples incubated for 804 days (Tables 17 and 18), and no consistent trend was observed in any sample. Carbon dioxide was not produced in any of the samples without bentonite (Table 19); however, CO_2 was produced in unamended-inoculated and in amended-inoculated samples with bentonite (Table 20); a small amount of N_2O was also detected in the latter (Table 22). The most convincing evidence of microbial activity was provided by O_2 consumption data (Tables 23 and 24). Oxygen consumption was not detected in samples without bentonite (Table 23); slight consumption was detected in unamended-inoculated samples with bentonite, and there was a substantial depletion

Table 17. Total Volume of Gas Produced in Initially Aerobic Humid Treatments Without Bentonite.

Treatments (without bentonite)	Volume of Gas Produced (ml/sample) Incubation Time (Days)					
	6	120	317	399	593	804
Control						
Empty bottle	7.15	-0.22	0.28	1.08	1.19	2.51
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52	0.32
No cellulose (salt/ inoculum/ tube+brine)	6.23 ± 0.09	-2.36 ± 0.04	-0.21 ± 0.07	0.73 ± 0.07	0.23 ± 0.04	3.01 ± 0.22
Carbon Source: Cellulose Only						
Unamended uninoculated	6.87 ± 0.11	-0.03 ± 1.85	-0.41 ± 0.09	-0.20 ± 0.14	0.12 ± 0.03	1.10 ± 0.17
Unamended inoculated	7.50 ± 0.33	-0.31 ± 1.62	0.19 ± 0.33	-0.61 ± 0.25	0.31 ± 0.05	1.29 ± 0.25
Amended uninoculated	6.98 ± 0.18	-0.03 ± 1.68	-0.23 ± 0.10	-0.29 ± 0.13	0.20 ± 0.10	0.50 ± 0.21
Amended inoculated	7.39 ± 0.11	-0.21 ± 1.57	-0.02 ± 0.18	-0.39 ± 0.07	0.13 ± 0.17	0.77 ± 0.18
Carbon Source: Cellulose + Glucose						
Amended uninoculated	6.45 ± 0.11	-2.08	0.75 ± 0.00	-0.06 ± 0.21	0.02 ± 0.14	0.13 ± 0.28
Amended inoculated	7.03 ± 0.07	-1.92 ± 0.11	0.79 ± 0.33	0.35 ± 0.23	0.15 ± 0.04	0.50 ± 0.22
Amended uninoculated (RG salt)	NA	3.12	1.99 ± 1.90	-0.80 ± 0.11	-0.34 ± 0.33	0.18 ± 0.40
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	19.5	NA	0.64	-0.10	1.66	-0.10
Amended uninoculated (w/o acetylene)	5.15	-2.08	0.98	-0.37	-0.08	0.72
Amended inoculated (w/ acetylene)	12.9	NA	1.17	0.35	-0.34	-0.10
Amended inoculated (w/o acetylene)	5.88	-2.29	1.27	0.05	0.17	0.72

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

Table 18. Total Volume of Gas Produced in Initially Aerobic Humid Treatments With Bentonite.

Treatments (with bentonite)	Volume of Gas Produced (ml/sample) Incubation Time (Days)					
	6	120	317	399	593	804
Control						
Empty bottle	7.15	-0.22	0.28	1.08	1.19	2.51
Blank (tube+brine only)	5.74	-2.27	-0.68	0.14	0.52	0.32
No cellulose (salt/ inoculum/ tube+brine)	7.25 ± 0.03	-2.42 ± 0.08	-0.42 ± 0.07	0.52 ± 0.18	0.33 ± 0.04	1.68 ± 0.95
Carbon Source: Cellulose Only						
Unamended uninoculated	5.67 ± 0.00	1.03 ± 1.41	-0.62 ± 0.17	-0.39 ± 0.15	0.31 ± 0.05	-0.01 ± 0.10
Unamended inoculated	6.35 ± 0.48	-0.59 ± 1.52	0.11 ± 0.13	-0.40 ± 0.08	0.06 ± 0.12	0.02 ± 0.32
Amended uninoculated	6.09 ± 0.00	0.08 ± 1.85	0.01 ± 0.13	-0.15 ± 0.13	0.11 ± 0.05	0.19 ± 0.27
Amended inoculated	7.81 ± 0.26	0.78 ± 1.56	0.35 ± 0.31	0.02 ± 0.24	0.11 ± 0.14	0.51 ± 0.19
Carbon Source: Cellulose + Glucose						
Amended uninoculated	6.35 ± 0.04	-1.98	-1.45 ± 0.29	-0.09 ± 0.25	0.07 ± 0.07	1.03 ± 0.76
Amended inoculated	7.29 ± 0.11	-1.45 ± 0.07	-0.42 ± 0.07	0.23 ± 0.11	0.20 ± 0.04	1.28 ± 0.83
Amended uninoculated (RG salt)	NA	2.60	1.78 ± 1.57	-0.82 ± 0.21	0.13 ± 0.04	1.59 ± 0.76
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	18.7	NA	0.74	-0.15	0.07	-0.63
Amended uninoculated (w/o acetylene)	5.56	-1.98	1.71	-0.76	0.27	-0.33
Amended inoculated (w/ acetylene)	18.0	NA	2.00	0.05	0.10	0.55
Amended inoculated (w/o acetylene)	6.82	-2.29	2.30	0.67	-0.11	1.16

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

Table 19. Production of Carbon Dioxide in Initially Aerobic Humid Treatments Without Bentonite.

Treatments (without bentonite)	Carbon Dioxide (µmoles/sample) Incubation Time (Days)					
	6	120	317	399	593	804
Control						
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71
Blank (tube+brine only)	4.18	4.64	4.54	4.63	3.00	2.76
No cellulose (salt / inoculum/ tube+brine)	7.93 ± 0.19	14.0 ± 0.1	10.7 ± 0.3	9.21 ± 0.06	6.28 ± 0.22	3.61 ± 0.18
Carbon Source: Cellulose Only						
Unamended uninoculated	7.45 ± 0.21	10.7 ± 0.2	12.2 ± 0.7	12.2 ± 0.9	11.2 ± 1.5	8.96 ± 1.82
Unamended inoculated	11.7 ± 0.1	56.0 ± 4.4	72.6 ± 11.4	65.5 ± 11.5	45.3 ± 8.1	27.6 ± 5.3
Amended uninoculated	14.0 ± 1.1	28.1 ± 0.8	24.1 ± 1.8	22.9 ± 2.6	17.4 ± 3.1	12.2 ± 2.7
Amended inoculated	35.9 ± 1.3	42.4 ± 1.5	31.1 ± 2.4	24.8 ± 2.9	14.7 ± 2.4	8.21 ± 1.75
Carbon Source: Cellulose + Glucose						
Amended uninoculated	12.7 ± 0.4	32.7	39.7 ± 0.6	38.6 ± 1.2	35.0 ± 3.07	26.5 ± 4.5
Amended inoculated	28.3 ± 1.6	183 ± 98	236 ± 140	166 ± 96	79.8 ± 39.8	28.2 ± 9.0
Amended uninoculated (RG salt)	NA	36.0	44.8 ± 0.1	46.5 ± 0.1	47.4 ± 2.6	39.4 ± 5.6
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	15.1	NA	28.8	27.7	21.0	16.8
Amended uninoculated (w/o acetylene)	15.7	26.0	22.7	19.7	14.4	7.06
Amended inoculated (w/ acetylene)	14.5	NA	1384	1450	1470	1270
Amended inoculated (w/o acetylene)	15.8	42.4	40.0	38.2	29.5	23.6

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

Table 20. Production of Carbon Dioxide in Initially Aerobic Humid Treatments With Bentonite.

Treatments (with bentonite)	Carbon Dioxide (µmoles/sample) Incubation Time (Days)					
	6	120	317	399	593	804
Control						
Empty bottle	4.05	4.97	4.96	4.94	4.87	2.71
Blank (tube+brine only)	4.18	4.64	4.54	4.63	3.00	2.76
No cellulose (salt / inoculum/ tube+brine)	34.2 ± 0.8	164 ± 1	168 ± 8	144 ± 4	89.1 ± 0.8	42.3 ± 3.0
Carbon Source: Cellulose Only						
Unamended uninoculated	9.15 ± 0.58	12.1 ± 0.6	13.2 ± 0.6	13.1 ± 0.3	11.0 ± 0.5	9.82 ± 0.15
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61	184 ± 76
Amended uninoculated	15.2 ± 0.9	52.2 ± 1.8	49.9 ± 1.1	45.1 ± 2.4	33.2 ± 4.2	23.1 ± 5.5
Amended inoculated	53.7 ± 2.4	1030 ± 80	1620 ± 30	1600 ± 40	1520 ± 40	1470 ± 40
Carbon Source: Cellulose + Glucose						
Amended uninoculated	14.8 ± 0.5	46.3	590 ± 364	625 ± 394	694 ± 438	631 ± 401
Amended inoculated	44.9 ± 2.6	1590 ± 40	1240 ± 20	1250 ± 160	1240 ± 240	816 ± 355
Amended uninoculated (RG salt)	NA	39.5	50.9 ± 1.3	54.6 ± 2.4	55.7 ± 6.7	45.7 ± 8.6
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	22.9	NA	50.0	50.8	46.1	38.9
Amended uninoculated (w/o acetylene)	21.7	47.7	50.4	46.8	43.6	37.3
Amended inoculated (w/ acetylene)	38.5	NA	1430	1470	1540	1460
Amended inoculated (w/o acetylene)	52.8	1130	1460	1500	1520	1400

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

Table 21. Production of Nitrous Oxide in Initially Aerobic Humid Treatments Without Bentonite.

Treatments (without bentonite)	Nitrous Oxide (µmoles/sample) Incubation Time (Days)					
	6	120	317	399	593	804
Control						
Empty bottle	ND	ND	ND	ND	ND	ND
Blank (tube+brine only)	ND	ND	ND	ND	ND	ND
No cellulose (salt/ inoculum/ tube+brine)	ND	ND	ND	ND	ND	ND
Carbon Source: Cellulose Only						
Unamended uninoculated	ND	ND	ND	ND	ND	ND
Unamended inoculated	ND	ND	ND	ND	ND	ND
Amended uninoculated	ND	ND	ND	ND	ND	ND
Amended inoculated	ND	ND	ND	ND	ND	ND
Carbon Source: Cellulose + Glucose						
Amended uninoculated	ND	ND	ND	ND	ND	ND
Amended inoculated	ND	ND	ND	ND	ND	ND
Amended uninoculated (RG salt)	NA	ND	ND	ND	ND	ND
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	ND	NA	ND	ND	ND	ND
Amended uninoculated (w/o acetylene)	ND	ND	ND	ND	ND	ND
Amended inoculated (w/ acetylene)	ND	NA	86.7	62.3	22.0	12.4
Amended inoculated (w/o acetylene)	ND	ND	ND	ND	ND	ND

RG salt = reagent-grade NaCl was used in place of WIPP salt
NA=not analyzed
ND=not detected

Table 22. Production of Nitrous Oxide in Initially Aerobic Humid Treatments With Bentonite.

Treatments (with bentonite)	Nitrous Oxide (μmoles/sample) Incubation Time (Days)					
	6	120	317	399	593	804
Control						
Empty bottle	ND	ND	ND	ND	ND	ND
Blank (tube+brine only)	ND	ND	ND	ND	ND	ND
No cellulose (salt/ inoculum/ tube+brine)	ND	ND	ND	ND	ND	ND
Carbon Source: Cellulose Only						
Unamended uninoculated	ND	ND	ND	ND	ND	ND
Unamended inoculated	ND	ND	ND	ND	ND	ND
Amended uninoculated	ND	ND	ND	ND	ND	ND
Amended inoculated	ND	18.5 ± 4.6	16.4 ± 0.3	16.6 ± 0.5	ND	12.5 ± 0.4
Carbon Source: Cellulose + Glucose						
Amended uninoculated	ND	ND	5.51 ± 3.81	5.97 ± 4.22	ND	ND
Amended inoculated	ND	15.7 ± 0.6	12.7 ± 0.5	ND	ND	ND
Amended uninoculated (RG salt)	NA	ND	ND	ND	ND	ND
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	ND	NA	ND	ND	ND	ND
Amended uninoculated (w/o acetylene)	ND	ND	ND	ND	ND	ND
Amended inoculated (w/ acetylene)	ND	NA	82.2	44.6	ND	12.4
Amended inoculated (w/o acetylene)	ND	43.8	14.1	16.0	ND	11.6

RG salt = reagent-grade NaCl was used in place of WIPP salt
NA=not analyzed
ND=not detected

Table 23. Consumption of Oxygen in Initially Aerobic Humid Treatments Without Bentonite.

Treatments (without bentonite)	Oxygen (µmoles/sample) Incubation Time (days)					
	6	120	317	399	593	804
Control						
Empty bottle	1383	1387	1343	1359	1339	1376
Blank (tube+brine only)	1338	1337	1291	1307	1296	1312
No cellulose (salt/ inoculum/ tube+brine)	1307	1291	1251 ± 4	1272 ± 0	1255	1302
Carbon Source: Cellulose Only						
Unamended uninoculated	1298	1308	1239 ± 3	1243 ± 3	1239 ± 6	1268 ± 11
Unamended inoculated	1298	1251	1184 ± 16	1192 ± 14	1223 ± 7	1266 ± 6
Amended uninoculated	1298	1299	1244 ± 2	1246 ± 0	1243 ± 5	1271 ± 7
Amended inoculated	1298	1295	1238 ± 5	1246 ± 3	1246 ± 3	1252 ± 2
Carbon Source: Cellulose + Glucose						
Amended uninoculated	1298	1269	1224 ± 11	1222 ± 6	1210 ± 1	1213 ± 11
Amended inoculated	1298	1110 ± 116	1039 ± 142	1121 ± 94	1187 ± 35	1250 ± 9
Amended uninoculated (RG salt)	1298	1313	1224 ± 24	1213 ± 0	1190 ± 7	1208 ± 17
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	1298	NA	1180	1185	1211	1210
Amended uninoculated (w/o acetylene)	1298	1275	1258	1248	1252	1265
Amended inoculated (w/ acetylene)	1298	NA	64.8	68.3	75	177
Amended inoculated (w/o acetylene)	1298	1272	1250	1240	1221	1228

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

Table 24. Consumption of Oxygen in Initially Aerobic Humid Treatments With Bentonite.

Treatments (with bentonite)	Oxygen (µmoles/sample) Incubation Time (days)					
	6	120	317	399	593	804
Control						
Empty bottle	1383	1387	1343	1359	1339	1377
Blank (tube+brine only)	1338	1337	1291	1307	1296	1312
No cellulose (salt/ inoculum/ tube+brine)	1307	1155	1124 ± 13	1165 ± 8	664 ± 379	1265 ± 12
Carbon Source: Cellulose Only						
Unamended uninoculated	1298	1319 ± 14	1241 ± 2	1249 ± 0	1244 ± 5	1270 ± 9
Unamended inoculated	1298	1122 ± 15	986 ± 27	1003 ± 41	1059 ± 59	1113 ± 78
Amended uninoculated	1298	1290 ± 20	1235 ± 1	1241 ± 3	1241 ± 6	1842 ± 482
Amended inoculated	1298	285 ± 75	67.8 ± 0.4	66.0 ± 1.2	75 ± 2	69 ± 2
Carbon Source: Cellulose + Glucose						
Amended uninoculated	1298	1269	667 ± 390	664 ± 399	643 ± 390	679 ± 370
Amended inoculated	1298	NA	346 ± 65	332 ± 185	290 ± 154	542 ± 334
Amended uninoculated (RG salt)	1298	1307	1242 ± 13	1221 ± 5	1205 ± 7	1241 ± 19
Carbon Source: Cellulose + Succinate						
Amended uninoculated (w/ acetylene)	1298	NA	1180	1179	1180	1192
Amended uninoculated (w/o acetylene)	1298	1271	1274	1241	1229	1229
Amended inoculated (w/ acetylene)	1298	NA	73.6	66.8	73	66
Amended inoculated (w/o acetylene)	1298	70.8	75.8	64.9	72	102

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

Table 25. Summary of Carbon Dioxide Production per gram Cellulose in Initially Aerobic Humid Treatments, Iincluding Corrected Data.

Treatments ***without bentonite***	Carbon Dioxide (µmoles/ gram cellulose) Incubation Time (Days) 6	120	317	399	593	804
Control						
No cellulose (salt/ inoculum/ tube+brine	7.93 ± 0.19	14.0 ± 0.1	10.7 ± 0.3	9.21 ± 0.06	6.38 ± 0.22	3.61 ± 0.18
Carbon Source: Cellulose						
Unamended inoculated	11.7 ± 0.1	56.0 ± 4.4	72.6 ± 11.4	65.5 ± 11.5	45.3 ± 8.1	27.6 ± 5.3
Amended inoculated	35.9 ± 1.3	42.4 ± 1.5	31.1 ± 2.4	24.8 ± 2.9	14.7 ± 2.4	8.21 ± 1.75
*Unamended inoculated (corrected)**	3.8 ± 0.0	42.1 ± 3.3	62.0 ± 9.8	56.3 ± 9.9	38.9 ± 7.0	24.0 ± 4.6
*Amended inoculated (corrected)**	28.0 ± 1.0	28.5 ± 1.0	20.5 ± 1.6	15.6 ± 1.8	8.32 ± 1.4	4.60 ± 0.98

Treatments ***with bentonite***	Carbon Dioxide (µmoles/ gram cellulos Incubation Time (Days) 6	120	317	399	593	804
Control						
No cellulose (salt/ inoculum/ tube+brine	34.2 ± 0.8	164 ± 1	168 ± 8	144 ± 4	89.1 ± 0.8	42.3 ± 3
Carbon Source: Cellulose						
Unamended inoculated	20.7 ± 0.0	172 ± 5	273 ± 25	268 ± 44	219 ± 61	184 ± 76
Amended inoculated	53.7 ± 2.4	1033 ± 76	1623 ± 26	1600 ± 44	1520 ± 40	1470 ± 40
*Unamended inoculated (corrected)**	-13.5 ± -0.0	8 ± 0.2	105 ± 9.6	124 ± 20.4	130 ± 36.2	142 ± 58.5
*Amended inoculated (corrected)**	19.5 ± 0.9	869 ± 63.9	1455 ± 23.7	1456 ± 40.0	1431 ± 37.7	1428 ± 38.8

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

of O_2 in amended-inoculated samples with bentonite (from an initial concentration of 1298 μmol sample^{-1} to 69 μmol sample^{-1} over 798 days, Table 24). In Table 25, CO_2 production is summarized; in amended-inoculated samples with bentonite 1428 μmol of CO_2 g^{-1} cellulose was produced over 798 days incubation at a rate of 1.77 μmol CO_2 g^{-1} cellulose day^{-1}. Oxygen consumption was concomitant with CO_2 production in these samples. All the active samples showed marked discoloration of the cellulosic material (fading of the brown paper towel), and much of the material was covered with a red pigment, characteristic of bacterioruberin, a C_{50}-carotenoid produced by many extremely halophilic bacteria. A small portion of some of the paper in the amended inoculated samples containing bentonite had been noticeably reduced to fibers, indicative of cellulose biodegradation.

8.2.2 Activity Measurement Treatments

Total gas production was not significant in samples which received glucose or succinate over 804 days incubation, and generally showed a net loss (Table 17 and 18). Gas production in the absence of bentonite was detected in inoculated samples with succinate and C_2H_2 (Table 19), with a total of 1270 μmol CO_2 sample^{-1} over 804 days. In addition, inoculated samples with glucose showed slight activity. With bentonite, all of the inoculated samples produced CO_2 (Table 20). The uninoculated samples with glucose produced 631 μmol CO_2 sample^{-1} over 804 days (Table 20). In the presence of reagent-grade (RG) NaCl, the same treatment did not show gas production. Inoculated samples with glucose produced 1590 μmol CO_2 sample^{-1} within 120 days incubation, whereas inoculated samples with succinate produced 1130 μmol CO_2 sample^{-1} reaching a maximum of 1500 μmol CO_2 sample^{-1} (Table 20). In activity measurement treatments, In the absence of bentonite, only inoculated samples were active, and O_2 consumption correlated with CO_2 production. With bentonite, uninoculated samples showed activity with glucose, as did all of the inoculated samples. These results demonstrate that halophilic microbes in the mixed inoculum and those associated with the WIPP salt are active under humid conditions. In all active samples, some of the cellulose was colored red, indicating the development of a halophilic biomass, as described above. We note that although there was visual evidence of cellulose biodegradation in the glucose- and succinate-amended samples, no distinction was made between gas production due to their metabolism and that due to biodegradation of cellulose alone. Therefore, these treatments served only to examine the potential for microbial activity under the given test conditions.

Microbial activity in an initially aerobic, humid (relative humidity ~ 70%) environment produced CO_2 and N_2O with concurrent consumption of O_2 in specific samples, most of the production occurring within 120 days. Enhanced microbial activity was observed in samples containing bentonite. In the absence of bentonite, microbial activity was detected in unamended inoculated samples with cellulose, and inoculated samples amended with glucose or succinate only. Hydrogen, CH_4, and H_2S were not detected in any of the samples tested.

8.3 Gas Generation from Cellulose Biodegradation under Humid Conditions: Anaerobic Incubation

The results presented in Tables 26 through 29 for the anaerobic humid experiment contain four sets of gas data arranged so that control values are presented first, followed by data for treatments containing cellulose alone, and cellulose plus glucose or succinate. The most WIPP-

relevant treatments are those with cellulose alone; treatments with additional carbon sources (i.e., glucose or succinate) were used to evaluate the effects of humid conditions on microbial activity in the presence of an easily metabolized carbon source (activity measurement treatments). The data are the mean of triplicate analyses, with the standard error of the mean (SEM). Tables 26 through 29 present gas data on a per sample basis; Table 30 shows the "corrected" data for CO_2 production, with control samples subtracted to yield gas production on a per gram cellulose basis.

8.3.1 Cellulose Degradation

There was no detectable total gas production in any of the samples tested (Tables 26 and 27). However, in the absence of bentonite, the unamended-inoculated samples produced 89.0 μmol CO_2 sample^{-1} over 415 days and the amended-uninoculated and amended-inoculated samples produced 32.3 and 34.7 μmol CO_2 sample^{-1}, respectively (Table 28). Adding bentonite generated more CO_2: 434 μmol sample^{-1} in unamended-inoculated samples, 442 μmol sample^{-1} in amended-inoculated samples, and 251 μmol sample^{-1} in amended-inoculated samples to which C_2H_2 was added (Table 29). The other treatments did not produce CO_2. The final quantities of gas produced, corrected with the appropriate control for gas production in the absence of cellulose, were (i.) without bentonite: 83.1 μmol g^{-1} cellulose in unamended-inoculated samples and 28.8 μmol g^{-1} cellulose in amended-inoculated samples and (ii.) with bentonite: 397 μmol g^{-1} cellulose in unamended-inoculated samples and 405 μmol g^{-1} cellulose in amended-inoculated samples (Table 30). The rate of gas production in amended-inoculated samples with bentonite was, therefore, 0.98 μmol g^{-1} cellulose day^{-1}. The most active samples, all containing bentonite, showed marked discoloration of the cellulosic material (specifically, fading of the brown paper towel), and a very small portion of the material was covered with a red pigment, indicative of the growth of halophilic bacteria.

8.3.2 Activity Measurement Treatments

Total gas production did not show any consistent trend (Tables 26 and 27). Carbon dioxide was produced in glucose-amended treatments without bentonite within 100 days incubation. Uninoculated samples produced 23.5 μmol sample^{-1} by 100 days, which increased to only 38.6 μmol sample^{-1} by 415 days (Table 28). Inoculated samples produced 39.8 μmol sample^{-1} by 100 days, which increased to only 41.8 μmol sample^{-1} by 415 days. Therefore, the major activity was confined to the first 100 days incubation. In succinate amended treatments, limited gas production occurred in uninoculated samples up to 100 days. However, the inoculated samples continued to produce gas over 415 days, giving 328 μmol sample^{-1} at 415 days (Table 28). The addition of bentonite markedly increased the rate and extent of CO_2 production in uninoculated and inoculated samples amended with glucose (Table 29). Uninoculated samples produced 64.3 μmol sample^{-1} over 415 days, 70% within 100 days, whereas the controls produced 51.6 μmol sample^{-1} over 415 days; therefore, only slightly more was produced which could be attributed to microbial activity in uninoculated samples. Inoculated samples produced 584 μmol sample^{-1} over 415 days at a rate of 3.96 μmol sample^{-1} day^{-1}, 70% of which was detected in the headspace at 100 days. Added succinate had a similar effect, with 54.0 μmol sample^{-1} produced in uninoculated samples over 415 days, and 324 to 516 μmol sample^{-1} in inoculated samples. Table 24 summarizes CO_2 production in samples containing glucose and succinate. Halophilic microbial activity was apparent visually in samples that produced significant quantities of CO_2 as described in Section 8.3.1.

Table 26. Total Gas Production in Anaerobic Humid Samples Without Bentonite.

Treatments (without bentonite)	Total Gas Produced (ml/sample)*			
	Days			
	6	100	140	415
Control				
Empty bottle	nd	nd	nd	nd
Blank (tube+brine only)	nd	nd	nd	nd
Salt / inoculum/ tube+brine (no cellulose)	nd	nd	nd	nd
Carbon Source: Cellulose Only				
Unamended uninoculated	nd	nd	nd	nd
Unamended inoculated	nd	nd	nd	nd
Amended uninoculated	nd	nd	nd	nd
Amended inoculated	nd	nd	nd	nd
Amended inoculated (w/ acetylene)	nd	nd	nd	nd
Carbon Source: Cellulose + Glucose				
Amended uninoculated	nd	nd	nd	nd
Amended inoculated	nd	nd	nd	0.54 ± 1.50
Amended uninoculated (RG salt)	nd	nd	nd	nd
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	nd	nd	nd	2.72 ± 1.94
Amended uninoculated (w/o acetylene)	nd	nd	5.68 ± 2.15	nd
Amended inoculated (w/ acetylene)	nd	nd	nd	nd
Amended inoculated (w/o acetylene)	nd	nd	0.33 ± 0.12	0.69 ± 0.36

*Data reported are net gas production at each time (gross (raw) data not shown)
RG salt = reagent-grade NaCl was used in place of WIPP salt
nd=not detected

Table 27. Total Gas Production in Anaerobic Humid Samples With Bentonite.

Treatments (with bentonite)	Total Gas Produced (ml/sample)*			
	Days			
	6	100	140	415
Control				
Empty bottle	nd	nd	nd	nd
Blank (tube+brine only)	nd	nd	nd	nd
Salt / inoculum/ tube+brine (no cellulose)	nd	nd	nd	nd
Carbon Source: Cellulose Only				
Unamended uninoculated	nd	nd	nd	nd
Unamended inoculated	nd	nd	nd	1.03 ± 1.22
Amended uninoculated	nd	nd	1.83 ± 0.71	0.94 ± 0.79
Amended inoculated	nd	3.59 ± 0.59	0.65 ± 0.30	nd
Amended inoculated (w/ acetylene)	nd	nd	nd	nd
Carbon Source: Cellulose + Glucose				
Amended uninoculated	nd	nd	nd	3.45 ± 3.33
Amended inoculated	nd	2.82 ± 1.07	nd	1.15 ± 0.51
Amended uninoculated (RG salt)	nd	nd	nd	nd
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	nd	nd	nd	nd
Amended uninoculated (w/o acetylene)	nd	nd	6.36 ± 2.53	nd
Amended inoculated (w/ acetylene)	nd	nd	nd	nd
Amended inoculated (w/o acetylene)	nd	3.42 ± 0.10	nd	nd

*Data reported are net gas production at each time period (gross (raw) data not presented)
RG salt = reagent-grade NaCl was used in place of WIPP salt
nd=not detected

Table 28. Production of Carbon Dioxide in Anaerobic Humid Samples Without Bentonite.

Treatments (without bentonite)	Carbon dioxide (µmoles/sample)			
	Days			
	6	100	140	415
Control				
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00
Salt / inoculum/ tube+brine (no cellulose)	3.60 ± 0.01	5.90 ± 0.11	7.63 ± 1.08	16.4 ± 0.6
Carbon Source: Cellulose Only				
Unamended uninoculated	4.07 ± 0.09	5.44 ± 0.10	6.22 ± 0.82	8.05 ± 0.18
Unamended inoculated	11.3 ± 0.12	25.9 ± 3.8	36.1 ± 7.0	89.0 ± 24.4
Amended uninoculated	3.34 ± 0.22	34.3 ± 1.44	39.8 ± 0.9	32.3 ± 1.5
Amended inoculated	16.9 ± 1.15	36.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9
Amended inoculated (w/ acetylene)	13.7 ± 1.3	38.5 ± 2.2	42.7 ± 2.5	61.0 ± 16.9
Carbon Source: Cellulose + Glucose				
Amended uninoculated	3.34 ± 0.27	23.5 ± 1.6	31.3 ± 0.0	38.6 ± 2.1
Amended inoculated	17.7 ± 0.47	39.8 ± 0.2	42.2 ± 0.9	41.8 ± 4.2
Amended uninoculated (RG salt)	4.07 ± 0.37	19.8 ± 2.4	28.9 ± 0.6	26.3 ± 2.9
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	3.21 ± 0.04	22.5 ± 0.8	29.4 ± 2.5	28.8 ± 3.0
Amended uninoculated (w/o acetylene)	3.19 ± 0.18	21.4 ± 0.2	27.9 ± 0.5	34.1 ± 2.5
Amended inoculated (w/ acetylene)	13.5 ± 0.7	78.1 ± 33.4	123 ± 63	308 ± 175
Amended inoculated (w/o acetylene)	14.8 ± 0.2	60.5 ± 16.0	106 ± 21	328 ± 78

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

Table 29. Production of Carbon Dioxide in Anaerobic Humid Samples With Bentonite.

Treatments (with bentonite)	Carbon dioxide (µmoles/sample) Days 6	100	140	415
Control				
Empty bottle	0.00 ± 0.00	0.68 ± 0.48	1.34 ± 0.95	0.00 ± 0.00
Blank (tube+brine only)	0.00 ± 0.00	0.32 ± 0.22	0.00 ± 0.00	0.00 ± 0.00
Salt / inoculum/ tube+brine (no cellulose)	14.2 ± 0.51	36.6 ± 6.1	39.8 ± 5.5	51.6 ± 3.4
Carbon Source: Cellulose Only				
Unamended uninoculated	5.04 ± 0.15	12.1 ± 3.2	14.4 ± 3.6	26.5 ± 8.9
Unamended inoculated	20.3 ± 0.2	93.7 ± 2.6	186 ± 6	434 ± 39
Amended uninoculated	6.65 ± 0.80	39.2 ± 1.5	45.5 ± 1.5	49.6 ± 1.6
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152
Amended inoculated (w/ acetylene)	26.8 ± 0.7	94.0 ± 18.6	123 ± 30	251 ± 92
Carbon Source: Cellulose + Glucose				
Amended uninoculated	6.71 ± 0.12	44.5 ± 0.2	53.1 ± 0.4	64.3 ± 1.0
Amended inoculated	31.4 ± 0.7	396 ± 13	487 ± 1	584 ± 28
Amended uninoculated (RG salt)	5.28 ± 0.45	45.9 ± 0.7	55.1 ± 1.4	74.9 ± 2.2
Carbon Source: Cellulose + Succinate				
Amended uninoculated (w/ acetylene)	5.77 ± 0.60	0.00 ± 0.00	41.5 ± 3.1	36.7 ± 0.9
Amended uninoculated (w/o acetylene)	8.58 ± 0.74	44.9 ± 1.6	51.5 ± 1.0	54.0 ± 2.0
Amended inoculated (w/ acetylene)	27.7 ± 0.27	70.3 ± 2.7	114 ± 0	324 ± 30
Amended inoculated (w/o acetylene)	28.0 ± 0.82	237 ± 2	317 ± 6	516 ± 0

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

Table 30. Summary of Carbon Dioxide Production per gram Cellulose in Anaerobic Humid Samples

Treatments ***without bentonite***	Carbon dioxide (µmoles/ gram cellulose) Days			
	6	100	140	415
Control				
No cellulose (salt/ inoculum/ tube+brine	3.60 ± 0.01	5.9 ± 0.1	7.64 ± 1.08	16.4 ± 0.6
Carbon Source: Cellulose				
Unamended inoculated	11.3 ± 0.1	25.9 ± 3.8	36.1 ± 7	89 ± 24.4
Amended inoculated	16.9 ± 1.2	36.4 ± 0.8	40.4 ± 0.8	34.7 ± 0.9
*Unamended inoculated (corrected)**	7.70 ± 0.08	20.0 ± 2.9	32.5 ± 6.3	83.1 ± 22.8
*Amended inoculated (corrected)**	13.3 ± 0.9	30.5 ± 0.7	36.8 ± 0.7	28.8 ± 0.7

Treatments ***with bentonite***	Carbon dioxide (µmoles/ gram cellulose) Days			
	6	100	140	415
Control				
No cellulose (salt/ inoculum/ tube+brine	14.2 ± 0.5	36.6 ± 6.1	39.8 ± 5.5	51.6 ± 3.4
Carbon Source: Cellulose				
Unamended inoculated	20.3 ± 0.2	94 ± 3	186 ± 6	434 ± 39
Amended inoculated	32.2 ± 1.1	250 ± 30	473 ± 25	442 ± 152
*Unamended inoculated (corrected)**	6.10 ± 0.06	57.1 ± 1.6	171.8 ± 5.5	397.4 ± 35.7
*Amended inoculated (corrected)**	18.0 ± 0.6	213.4 ± 25.6	458.8 ± 24.2	405.4 ± 139.4

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

Microbial activity in an anaerobic, humid environment (relative humidity=70%) over 415 days resulted in the production of total gas and CO_2 in specific samples. Bentonite stimulated gas production from cellulose degradation in both unamended and amended samples. As with aerobic humid samples, the total quantity of gas produced in anaerobic samples containing cellulose alone was similar to those containing cellulose and an easily metabolized carbon source.

8.4 Gas Generation from Biodegradation of Irradiated Plastic and Rubber Materials

Gas production data for samples containing polyethylene (PE), polyvinylchloride (PVC), and neoprene (NEO) up to 840 days of incubation are given in Appendix B Figures 1–24 and Tables 1–16. The tables include total volume, CO_2, O_2, N_2O, H_2, and CH_4 production. The figures present mean total gas and CO_2 production data (refer to Tables 1–16, Appendix B, for data for the standard error of the mean [SEM]). We analyzed the final headspace gas of samples containing hypalon (HYP) at 664 days; these data are presented in Appendix B Figures 25–40 and Tables 17–27. All the figures show gas production in control samples without plastic or rubber ("no polymer"). Oxygen data are given on a percent basis (Appendix B, Tables 1 and 17). Qualitative information on H_2S production is also included. The figures and tables in Appendix B are divided up into five sections: I. Polyethylene; II. Polyvinylchloride; III. Neoprene; IV. Unleaded hypalon; V. Leaded hypalon.

8.4.1 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 the 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, NEO, HYP or leaded-HYP to determine if unirradiated or irradiated polymer stimulated more production.

8.4.2 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. There was evidence of the formation of a biofilm on the surface of the PE; a reddish tint, indicative of the presence of bacterioruberin, a C_{50} carotenoid produced by extreme halophiles, was noted at 30 days in initially aerobic treatments. However, over 840 days, the concentration of O_2 (Table 1, Appendix B), total gas (Figures 1, 3, Appendix B), and CO_2 (Figures 2, 4, Appendix B) did not vary significantly in initially aerobic unamended and amended samples with and without PE. Nitrous oxide did not accumulate in any of the treatments, however, CH_4 and H_2S were detected (Table 5, Appendix B), indicating that anaerobic conditions were established in the brine. In the presence of irradiated PE, total gas (Figures 5, 7, Appendix B), CO_2 (Figures 6, 8, Appendix B), CH_4, and H_2S (Table 5, Appendix B) production did not differ significantly from anaerobic samples containing unirradiated PE.

8.4.3 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. Initially aerobic unamended samples showed enhanced CO_2 production in the presence of low-dose irradiated PVC (Figure 10, Appendix B); however, the opposite was observed in amended samples (Figures 11 and 12, Appendix B). Nitrous oxide (Table 9, Appendix B), CH_4, and H_2S were not detected in initially aerobic samples containing PVC. The presence of low- and high-dose irradiated PVC in anaerobic unamended and amended samples caused a significant decrease in total gas (Figures 13, 15, Appendix B), and CO_2 production (Figures 14, 16, Appendix B). In addition, N_2O accumulated to the greatest extent of all treatments in samples containing low- and high-dose irradiated PVC (Table 9); this probably was due to partial inhibition of denitrification by the soluble products of irradiation. Very small quantities of CH_4 and H_2S were produced in the presence of irradiated PVC compared to unirradiated PVC (Table 10, Appendix B). Hydrogen was detected only in the presence of irradiated PVC under anaerobic conditions (Table 11, Appendix B).

8.4.4 Neoprene

The total volume of gas produced in samples containing unirradiated, low- and high-dose irradiated NEO did not differ significantly (Figures 17, 19, 21, 23; Table 12, Appendix B); most gas was produced in the first 30 days of incubation and decreased thereafter. This trend was consistent in most treatments and indicates that gas production was due to metabolism of readily available carbon in the brine. Carbon dioxide followed a similar trend (Figures 18, 20, 22, and 24; Table 12, Appendix B); however, CO_2 production was enhanced over 840 days in the presence of high-dose irradiated NEO under both aerobic and anaerobic conditions in the presence of nutrients (55.2 and 48.7 μmoles CO_2 $sample^{-1}$, respectively). Nitrous oxide was not detected (Table 14, Appendix B); CH_4 and H_2S were detected, primarily in amended samples (Table 15, Appendix B). Hydrogen was detected at 334 days in unamended and unamended anaerobic samples containing high-dose irradiated NEO, but was not subsequently observed (Table 16, Appendix B).

8.4.5 Unleaded Hypalon

Oxygen was consumed in initially aerobic samples to the same extent in the presence and absence of HYP (Table 17, Appendix B). Generally, very little total gas (Figures 25, 27, 29, and 31, Appendix B) and CO_2 production (Figures 26, 28, 30, and 32, Appendix B) was detected in samples containing unleaded hypalon. In initially aerobic nutrient-amended samples containing irradiated HYP, 40.9 μmol CO_2 $sample^{-1}$ was produced over 157 days versus 27.9 μmol CO_2 $sample^{-1}$ in samples containing unirradiated HYP (Table 19, Appendix B). Nitrous oxide did not accumulate (Table 20, Appendix B), H_2 was not produced (Table 21, Appendix B), and CH_4 was produced in anaerobic samples (Table 22, Appendix B). The presence or absence of these gases did not correlate with the presence or absence of irradiated HYP.

8.4.6 Leaded Hypalon

Irradiated leaded HYP did not stimulate total gas production (Figures 33, 35, 37, and 39, Appendix B) or CO_2 production (Figures 34, 36, 38, and 40). The largest difference in CO_2

production between samples containing unirradiated and irradiated leaded HYP was in initially aerobic amended samples (37.4 μmol CO_2 $sample^{-1}$ detected in samples containing unirradiated HYP vs. 20.4 μmol CO_2 $sample^{-1}$ detected in samples containing irradiated HYP). This difference, however, suggests that the irradiated leaded HYP had an inhibitory effect. Nitrous oxide did not accumulate (Table 25, Appendix B), H_2 was not produced (Table 26, Appendix B), and CH_4 was produced in anaerobic samples (Table 27, Appendix B).

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APPENDIX A: NET (PER GRAM CELLULOSE) AND GROSS (PER SAMPLE) GAS DATA FROM THE INUNDATED CELLULOSE BIODEGRADATION EXPERIMENT

APPENDIX A: NET (PER GRAM CELLULOSE) AND GROSS (PER SAMPLE) GAS DATA FROM THE INUNDATED CELLULOSE BIODEGRADATION EXPERIMENT

i. Data Reduction

Total gas, carbon dioxide, and nitrous oxide produced by aerobic and anaerobic samples up to 1228 days of incubation are presented in Tables 1-12 on a per gram cellulose basis (net gas data). Tables 1(a) to 12(c) present the same data on a per sample basis (gross gas data). The gross gas data were used to prepare Tables 1-12 in which total gas, carbon dioxide, and nitrous oxide production data from aerobic and anaerobic treatments are corrected for gas production in the absence of cellulose by subtracting the measured gas values in respective treatments without cellulose. As an example, total gas production in aerobic samples was corrected (Table 1) by subtracting total gas production in treatments without cellulose (designated "NC," Table 1(a)) from samples with cellulose (designated "C," Table 1(a)) at each time period. The resultant value was divided by the total amount of cellulose in each sample bottle (5 grams) to arrive at a value that represents the total gas produced per gram of cellulose. Tables 1 to 12 represent the gas produced strictly due to the presence of cellulose in the samples, and are therefore corrected for gas produced due to metabolism of dissolved organic carbon that may be present in the brine, nutrient addition, or mixed inoculum. Carbon dioxide was produced in certain samples in the absence of cellulose, specifically before 69 days incubation. Gas produced at time 0 was not subtracted from later values. This was not done because time 0 measurements were taken 3 days after sample preparation; therefore gas present at time 0 is due to evolution of dissolved gases and equilibration of the headspace gas and brine. These processes contribute to the overall gas production.

Table 1. Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days)					
		0	45	69	104	132	164
Unamended/Uninoculated (1)							
Formalin	1(C)-f	0.24 ± 0.06	0.89 ± 0.03	0.85 ± 0.02	0.42 ± 0.21	0.27 ± 0.19	NA
As is	1(C)-a	0.05 ± 0.02	-0.29 ± 0.19	-0.36 ± 0.18	-0.31 ± 0.14	-0.20 ± 0.12	NA
Unamended/Inoculated (2)							
Formalin	2(C)-f	-0.01 ± 0.01	-0.03 ± 0.08	-0.35 ± 0.11	-0.32 ± 0.08	-0.41 ± 0.11	NA
As is	2(C)-a	-0.08 ± 0.01	0.01 ± 0.04	-0.15 ± 0.03	-0.11 ± 0.04	0.00 ± 0.05	NA
Amended/Inoculated (3)							
As is	3(C)-a	-0.12 ± 0.03	-0.27 ± 0.14	-0.25 ± 0.06	0.36 ± 0.31	0.29 ± 0.24	0.07 ± 0.13
Exc. nitrate	3(C)-x	-0.02 ± 0.01	0.02 ± 0.14	1.39 ± 0.61	2.38 ± 0.87	3.10 ± 1.06	4.02 ± 1.21

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

Table 1 (continued). Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose					
		Incubation Time (Days)					
		200	228	264	297	356	411
					(ml)	(ml)	
Unamended/Uninoculated (1)							
Formalin	1(C)-f	0.42 ± 0.18	NA	NA	0.44 ± 0.17	NA	A
As is	1(C)-a	-0.18 ± 0.10	NA	NA	-0.13 ± 0.10	NA	A
Unamended/Inoculated (2)							
Formalin	2(C)-f	-0.11 ± 0.09	NA	NA	-0.11 ± 0.06	NA	A
As is	2(C)-a	-0.34 ± 0.07	NA	NA	-0.58 ± 0.18	NA	A
Amended/Inoculated (3)							
As is	3(C)-a	0.86 ± 0.08	0.89 ± 0.04	0.75 ± 0.03	1.16 ± 0.04	1.14 ± 0.08	1.28 ± 0.13
Exc. nitrate	3(C)-x	4.42 ± 0.80	5.20 ± 0.38	6.21 ± 0.68	6.01 ± 0.77	6.12 ± 0.72	6.03 ± 0.60

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

Table 1 (continued). Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days)					
		481	591	733	853	1034	1228
Unamended/Uninoculated (1)							
Formalin	1(C)-f	0.03 ± 0.08	NA	-0.31 ± 0.05	NA	NA	-0.38 ± 0.14
As is	1(C)-a	-0.17 ± 0.14	NA	0.74 ± 0.08	0.71 ± 0.08	0.08 ± 0.00	-0.04 ± 0.08
Unamended/Inoculated (2)							
Formalin	2(C)-f	-0.08 ± 0.04	NA	0.39 ± 0.08	NA	NA	-0.05 ± 0.12
As is	2(C)-a	-0.54 ± 0.09	NA	0.48 ± 0.07	0.35 ± 0.09	0.36 ± 0.08	0.30 ± 0.07
Amended/Inoculated (3)							
As is	3(C)-a	1.26 ± 0.14	1.34 ± 0.10	1.47 ± 0.00	1.53 ± 0.14	1.59 ± 0.24	1.42 ± 0.28
Exc.nitrate	3(C)-x	6.14 ± 0.67	7.20 ± 1.34	**11.6 ± 2.1	10.4 ± 1.7	12.2 ± 0.0	10.3 ± 1.5

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

**Analysis at this time point is of a reserved replicate sample

NA = not analyzed

Table 2. Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (1)							
Formalin	1(C)-f	5.58 ± 0.54	7.66 ± 0.02	8.20 ± 0.04	7.46 ± 0.16	7.64 ± 0.06	NA
As is	1(C)-a	2.48 ± 0.10	3.92 ± 0.06	4.21 ± 0.02	3.93 ± 0.02	4.25 ± 0.02	NA
Unamended/Inoculated (2)							
Formalin	2(C)-f	5.91 ± 0.16	6.24 ± 0.00	6.80 ± 0.14	5.88 ± 0.12	6.04 ± 0.02	NA
As is	2(C)-a	1.96 ± 0.06	3.3 ± 0.16	3.94 ± 0.18	4.98 ± 0.18	6.87 ± 0.20	NA
Amended/Inoculated (3)							
As is	3(C)-a	-0.01 ± 0.02	4.62 ± 0.28	3.78 ± 1.18	20.4 ± 7.4	29.6 ± 5.0	36.6 ± 4.2
Exc.nitrate	3(C)-x	-0.04 ± 0.02	6.88 ± 0.00	32.2 ± 4.2	61.4 ± 3.6	83.6 ± 2.6	96.2 ± 4.0

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

Table 2 (continued). Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (1)							
Formalin	1(C)-f	7.62 ± 0.10	NA	NA	9.08 ± 0.08	NA	NA
As is	1(C)-a	4.00 ± 0.02	NA	NA	4.44 ± 0.06	NA	NA
Unamended/Inoculated (2)							
Formalin	2(C)-f	5.65 ± 0.22	NA	NA	6.0 ± 0.34	NA	NA
As is	2(C)-a	8.30 ± 0.28	NA	NA	10.9 ± 0.3	NA	NA
Amended/Inoculated (3)							
As is	3(C)-a	40.8 ± 5.4	37.0 ± 8.2	36.6 ± 8.2	41.4 ± 7.8	39.6 ± 7.0	38.0 ± 7.6
Exc.nitrate	3(C)-x	95.6 ± 6.0	110 ± 7	124 ± 10	118 ± 11	126 ± 16	126 ± 19

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

Table 2 (continued). Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 481	591	773	853	1034	1228
Unamended/Uninoculated (1)							
Formalin	1(C)-f	8.46 ± 0.22	NA	8.30 ± 0.11	NA	NA	9.32 ± 0.10
As is	1(C)-a	4.20 ± 0.06	NA	4.56 ± 0.05	4.67 ± 0.04	3.83 ± 0.03	4.43 ± 0.06
Unamended/Inoculated (2)							
Formalin	2(C)-f	5.61 ± 0.60	NA	5.40 ± 0.99	NA	NA	4.89 ± 1.38
As is	2(C)-a	12.1 ± 0.2	NA	13.4 ± 0.2	14.5 ± 0.1	14.4 ± 0.1	13.8 ± 0.2
Amended/Inoculated (3)							
As is	3(C)-a	33.3 ± 7.2	31.8 ± 6.6	26.8 ± 5.4	26.2 ± 4.3	22.0 ± 2.9	17.0 ± 2.4
Exc.nitrate	3(C)-x	125 ± 21	152 ± 26	**175 ± 1	169 ± 2	186 ± 8	155 ± 15

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

**Analysis at this time point is of a reserved replicate sample

NA = not analyzed

Table 3. Nitrous Oxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (1)							
Formalin	1(C)-f	ND	NA	ND	0.001 ± 0.000	0.003 ± 0.000	NA
As is	1(C)-a	ND	NA	ND	0.001 ± 0.000	0.003 ± 0.000	NA
Unamended/Inoculated (2)							
Formalin	2(C)-f	ND	NA	ND	ND	0.003 ± 0.000	NA
As is	2(C)-a	0.002 ± 0.000	NA	ND	ND	ND	NA
Amended/Inoculated (3)							
As is	3(C)-a	0.001 ± 0.000	NA	0.024 ± 0.029	23.6 ± 9.6	24.4 ± 7.8	1.76 ± 1.20
Exc.nitrate	3(C)-x	ND	NA	5.64 ± 0.01	49.0 ± 2.2	65.2 ± 3.6	96.8 ± 13.4

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

Table 3 (continued). Nitrous Oxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (1)							
Formalin	1(C)-f	0.001 ± 0.000	NA	NA	ND	NA	NA
As is	1(C)-a	ND	NA	NA	0.001 ± 0.000	NA	NA
Unamended/Inoculated (2)							
Formalin	2(C)-f	0.001 ± 0.000	NA	NA	ND	NA	NA
As is	2(C)-a	ND	NA	NA	-0.110 ± 0.000	NA	NA
Amended/Inoculated (3)							
As is	3(C)-a	5.83 ± 5.04	0.750 ± 0.380	0.003 ± 0.002	0.650 ± 0.070	0.600 ± 0.050	0.040 ± 0.180
Exc.nitrate	3(C)-x	115 ± 26	105 ± 37	152 ± 41	139 ± 32	131 ± 25	127 ± 23

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

Table 3 (continued). Nitrous Oxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 481	591	773	853	034	228
Unamended/Uninoculated (1)							
Formalin	1(C)-f	ND	NA	NA	NA	NA	ND
As is	1(C)-a	-0.004 ± 0.000	NA	ND	ND	ND	ND
Unamended/Inoculated (2)							
Formalin	2(C)-f	ND	NA	ND	NA	NA	ND
As is	2(C)-a	-0.001 ± 0.000	NA	ND	ND	ND	ND
Amended/Inoculated (3)							
As is	3(C)-a	0.250 ± 0.180	0.220 ± 0.190	0.500 ± 0.240	ND	0.180 ± 0.150	ND
Exc.nitrate	3(C)-x	126 ± 25	135 ± 48	236 ± 16	223 ± 14	242 ± 6	216 ± 8

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

**Analysis at this time point is of a reserved replicate sample

NA = not analyzed

Table 4. Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (4)							
Formalin	4(C)-f	0.13 ± 0.03	0.63 ± 0.03	0.47 ± 0.03	0.15 ± 0.03	0.02 ± 0.01	NA
As is	4(C)-a	-0.05 ± 0.02	0.02 ± 0.02	-0.36 ± 0.04	-0.20 ± 0.05	-0.13 ± 0.04	NA
Unamended/Inoculated (5)							
Formalin	5(C)-f	0.11 ± 0.02	0.21 ± 0.01	0.31 ± 0.00	0.29 ± 0.00	0.07 ± 0.03	NA
As is	5(C)-a	0.03 ± 0.01	-0.10 ± 0.02	-0.18 ± 0.04	-0.30 ± 0.13	-0.23 ± 0.09	NA
Amended/Inoculated (6)							
As is	6(C)-a	-0.25 ± 0.03	0.43 ± 0.01	0.71 ± 0.15	1.82 ± 0.35	2.96 ± 0.30	4.07 ± 0.22
Exc.nitrate	6(C)-x	0.30 ± 0.01	0.85 ± 0.02	1.56 ± 0.02	2.23 ± 0.24	3.74 ± 0.29	5.15 ± 0.18

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

Table 4 (continued). Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (4)							
Formalin	4(C)-f	0.04 ± 0.01	NA	NA	0.04 ± 0.18	NA	NA
As is	4(C)-a	0.00 ± 0.05	NA	NA	0.05 ± 0.14	NA	NA
Unamended/Inoculated (5)							
Formalin	5(C)-f	0.16 ± 0.02	NA	NA	0.21 ± 0.13	NA	NA
As is	5(C)-a	-0.08 ± 0.16	NA	NA	0.65 ± 0.28	NA	NA
Amended/Inoculated (6)							
As is	6(C)-a	4.38 ± 0.20	4.40 ± 0.30	5.15 ± 0.45	5.28 ± 0.58	6.04 ± 0.74	6.13 ± 0.79
Exc.nitrate	6(C)-x	6.07 ± 0.04	6.19 ± 0.11	6.33 ± 0.16	6.35 ± 0.20	6.98 ± 0.27	7.08 ± 0.34

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

Table 4 (continued). Total Volume of Gas Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days) 481	591	733	853	034	228
Unamended/Uninoculated (4)							
Formalin	4(C)-f	-0.09 ± 0.07	NA	-0.17 ± 0.17	NA	NA	0.12 ± 0.16
As is	4(C)-a	-0.08 ± 0.05	NA	1.06 ± 0.03	1.04 ± 0.03	0.29 ± 0.11	0.33 ± 0.13
Unamended/Inoculated (5)							
Formalin	5(C)-f	-0.09 ± 0.04	NA	-0.17 ± 0.03	NA	NA	-0.20 ± 0.02
As is	5(C)-a	1.30 ± 0.49	NA	1.02 ± 0.12	1.24 ± 0.18	1.41 ± 0.22	1.47 ± 0.22
Amended/Inoculated (6)							
As is	6(C)-a	6.24 ± 0.82	6.38 ± 0.84	**9.0 ± 1.34	8.36 ± 1.24	6.14 ± 0.10	6.09 ± 0.04
Exc.nitrate	6(C)-x	7.35 ± 0.46	7.77 ± 0.65	7.35 ± 0.77	8.41 ± 0.82	6.79 ± 0.77	8.10 ± 0.75

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

**Analysis at this time point is of a reserved replicate sample

NA = not analyzed

Table 5. Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	32	64
Unamended/Uninoculated (4)							
Formalin	4(C)-f	4.16 ± 0.24	1.30 ± 0.04	2.24 ± 0.00	2.60 ± 0.00	2.40 ± 0.20	NA
As is	4(C)-a	1.52 ± 0.30	1.76 ± 0.06	4.48 ± 2.36	1.76 ± 0.14	1.82 ± 0.10	NA
Unamended/Inoculated (5)							
Formalin	5(C)-f	4.92 ± 0.18	4.78 ± 0.00	5.58 ± 0.04	3.2 ± 0.12	4.80 ± 0.00	NA
As is	5(C)-a	2.04 ± 0.04	1.38 ± 0.08	4.00 ± 0.80	8.32 ± 0.42	11.9 ± 0.6	NA
Amended/Inoculated (6)							
As is	6(C)-a	-0.54 ± 0.00	-6.12 ± 0.20	-2.60 ± 1.00	12.4 ± 2.2	31.4 ± 3.8	57.8 ± 1.2
Exc.nitrate	6(C)-x	-0.32 ± 0.02	-3.32 ± 0.24	2.20 ± 0.60	27.2 ± 4.6	72.0 ± 15.0	105 ± 9

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

Table 5 (continued). Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (4)							
Formalin	4(C)-f	3.20 ± 0.20	NA	A	31.8 ± 0.2	NA	NA
As is	4(C)-a	2.32 ± 0.02	NA	A	2.74 ± 0.12	NA	NA
Unamended/Inoculated (5)							
Formalin	5(C)-f	4.60 ± 0.00	NA	A	4.9 ± 0.20	NA	NA
As is	5(C)-a	21.5 ± 1.2	NA	A	35.9 ± 2.2	NA	NA
Amended/Inoculated (6)							
As is	6(C)-a	69.8 ± 1.2	62.8 ± 1.6	100 ± 2	102 ± 2	122 ± 1	130 ± 2
Exc.nitrate	6(C)-x	116 ± 6	117 ± 6	125 ± 1	122 ± 6	128 ± 6	129 ± 6

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

Table 5 (continued). Carbon Dioxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (4)							
Formalin	4(C)-f	6.20 ± 0.20	NA	8.45 ± 0.32	NA	NA	19.20 ± 1.20
As is	4(C)-a	2.50 ± 0.40	NA	4.03 ± 0.21	3.91 ± 0.27	3.54 ± 0.34	3.30 ± 0.32
Unamended/Inoculated (5)							
Formalin	5(C)-f	7.20 ± 0.00	NA	7.89 ± 0.04	NA	NA	11.00 ± 0.20
As is	5(C)-a	52.0 ± 2.6	NA	60.7 ± 3.0	68.4 ± 4.6	69.9 ± 5.0	69.6 ± 4.8
Amended/Inoculated (6)							
As is	6(C)-a	133 ± 2	138 ± 2	**174 ± 9	164 ± 8	140 ± 11	169 ± 11
Exc.nitrate	6(C)-x	128 ± 4	137 ± 5	136 ± 5	149 ± 3	137 ± 1	154 ± 7

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

**Analysis at this time point is of a reserved replicate sample

NA = not analyzed

Table 6. Nitrous Oxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (4)							
Formalin	4(C)-f	ND	NA	0.001 ± 0.000	-0.042 ± 0.000	0.002 ± 0.000	NA
As is	4(C)-a	ND	NA	0.145 ± 0.098	0.037 ± 0.011	0.041 ± 0.014	NA
Unamended/Inoculated (5)							
Formalin	5(C)-f	ND	NA	-0.012 ± 0.000	ND	-0.028 ± 0.000	NA
As is	5(C)-a	ND	NA	-0.006 ± 0.001	-0.038 ± 0.000	-0.042 ± 0.000	NA
Amended/Inoculated (6)							
As is	6(C)-a	ND	NA	6.30 ± 2.60	32.0 ± 11.0	25.0 ± 2.0	-0.890 ± 0.090
Exc.nitrate	6(C)-x	ND	NA	5.40 ± 1.30	25.0 ± 4.0	41.0 ± 8.0	68.0 ± 8.0

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

Table 6 (continued). Nitrous Oxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (4)							
Formalin	4(C)-f	0.001 ± 0.000	NA	NA	0.001 ± 0.000	NA	NA
As is	4(C)-a	0.048 ± 0.020	NA	NA	0.066 ± 0.029	NA	NA
Unamended/Inoculated (5)							
Formalin	5(C)-f	-0.013 ± 0.000	NA	NA	-0.009 ± 0.001	NA	NA
As is	5(C)-a	-0.019 ± 0.005	NA	NA	0.350 ± 0.160	NA	NA
Amended/Inoculated (6)							
As is	6(C)-a	-0.850 ± 0.000	0.110 ± 0.020	-0.850 ± 0.000	0.440 ± 0.010	0.390 ± 0.070	0.690 ± 0.030
Exc.nitrate	6(C)-x	83.0 ± 7.0	92.0 ± 6.0	102 ± 8	103 ± 9	111 ± 12	115 ± 14

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

Table 6 (continued). Nitrous Oxide Produced in Aerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (4)							
Formalin	4(C)-f	ND	NA	ND	NA	NA	ND
As is	4(C)-a	0.048 ± 0.017	NA	0.200 ± 0.030	ND	0.244 ± 0.058	0.238 ± 0.000
Unamended/Inoculated (5)							
Formalin	5(C)-f	-0.001 ± 0.000	NA	ND	NA	NA	ND
As is	5(C)-a	0.180 ± 0.170	NA	0.720 ± 0.050	0.750 ± 0.050	0.530 ± 0.046	0.824 ± 0.000
Amended/Inoculated (6)							
As is	6(C)-a	1.49 ± 0.03	0.850 0.030	1.07** ± 0.010	0.480 ± 0.100	-0.670 ± 0.170	ND
Exc.nitrate	6(C)-x	121 ± 17	132 21	135 ± 23	148 ± 25	166 ± 37	152 ± 32

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

**Analysis at this time point is of a reserved replicate sample

NA = not analyzed

ND = not detected

Table 7. Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (7)							
Formalin	7(C)-f	0.26 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.17 ± 0.01	0.14 ± 0.01	NA
As is	7(C)-a	0.03 ± 0.05	0.10 ± 0.02	0.40 ± 0.01	-0.18 ± 0.07	0.04 ± 0.23	NA
Unamended/Inoculated (8)							
Formalin	8(C)-f	0.02 ± 0.01	0.22 ± 0.02	0.16 ± 0.03	0.16 ± 0.00	0.12 ± 0.01	NA
As is	8(C)-a	-0.04 ± 0.01	1.00 ± 0.03	0.22 ± 0.03	-0.02 ± 0.03	0.02 ± 0.01	NA
Amended/Inoculated (9)							
As is	9(C)-a	-0.08 ± 0.02	0.02 ± 0.03	-0.52 ± 0.01	0.66 ± 0.09	1.52 ± 0.25	2.15 ± 0.24
Exc.nitrate	9(C)-x	0.01 ± 0.03	-0.12 ± 0.08	0.29 ± 0.29	2.00 ± 0.58	1.01 ± 0.78	4.04 ± 1.24

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

Table 7 (continued). Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days)					
		200	228	264	297	356	411
Unamended/Uninoculated (7)							
Formalin	7(C)-f	0.26 ± 0.00	NA	NA	0.20 ± 0.02	NA	NA
As is	7(C)-a	-0.09 ± 0.03	NA	NA	-0.15 ± 0.04	NA	NA
Unamended/Inoculated (8)							
Formalin	8(C)-f	0.22 ± 0.01	NA	NA	0.18 ± 0.02	NA	NA
As is	8(C)-a	0.59 ± 0.13	NA	NA	0.82 ± 0.14	NA	NA
Amended/Inoculated (9)							
As is	9(C)-a	2.27 ± 0.13	3.09 ± 0.20	3.08 ± 0.20	2.72 ± 0.16	3.51 ± 0.21	3.60 ± 0.19
Exc.nitrate	9(C)-x	5.44 ± 1.43	6.88 ± 1.69	8.14 ± 1.77	9.00 ± 1.61	10.89 ± 1.20	11.98 ± 0.59

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

Table 7 (continued). Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose

Treatments [Brine]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days)					
		481	591	733	853	1034	1228
Unamended/Uninoculated (7)							
Formalin	7(C)-f	0.17 ± 0.01	NA	0.22 ± 0.01	NA	NA	0.02 ± 0.06
As is	7(C)-a	-0.20 ± 0.03	NA	0.53 ± 0.05	0.46 ± 0.04	-0.20 ± 0.04	-0.24 ± 0.05
Unamended/Inoculated (8)							
Formalin	8(C)-f	0.14 ± 0.01	NA	0.04 ± 0.12	NA	NA	0.10 ± 0.06
As is	8(C)-a	1.30 ± 0.08	NA	1.87 ± 0.08	2.04 ± 0.09	2.19 ± 0.11	2.23 ± 0.12
Amended/Inoculated (9)							
As is	9(C)-a	3.91 ± 0.22	4.00 ± 0.17	4.32 ± 0.20	3.96 ± 0.16	3.87 ± 0.13	3.78 ± 0.09
Exc.nitrate	9(C)-x	13.80 ± 0.33	14.16 ± 0.57	14.87 ± 0.53	12.55 ± 0.48	12.60 ± 0.49	12.12 ± 0.44

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

Table 8. Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (7)							
Formalin	7(C)-f	5.89 ± 0.10	7.22 ± 0.02	7.16 ± 0.02	6.55 ± 0.04	6.81 ± 0.02	NA
As is	7(C)-a	2.38 ± 0.08	3.74 ± 0.02	3.92 ± 0.02	3.63 ± 0.02	3.83 ± 0.04	NA
Unamended/Inoculated (8)							
Formalin	8(C)-f	5.54 ± 0.14	6.08 ± 0.08	5.92 ± 0.04	5.5 ± 0.00	5.70 ± 0.10	NA
As is	8(C)-a	2.11 ± 0.04	3.41 ± 0.04	3.34 ± 0.02	3.01 ± 0.14	3.97 ± 0.10	NA
Amended/Inoculated (9)							
As is	9(C)-a	-0.06 ± 0.00	3.79 ± 0.04	-3.28 ± 0.34	7.22 ± 0.60	18.2 ± 1.4	24.2 ± 0.6
Exc.nitrate	9(C)-x	0.47 ± 0.00	4.29 ± 0.04	6.10 ± 3.42	19.7 ± 6.6	25.8 ± 6.4	45.4 ± 8.0

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

Table 8 (continued). Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (7)							
Formalin	7(C)-f	6.78 ± 0.02	NA	NA	-0.51 ± 0.00	NA	NA
As is	7(C)-a	3.59 ± 0.04	NA	NA	3.53 ± 0.04	NA	NA
Unamended/Inoculated (8)							
Formalin	8(C)-f	5.77 ± 0.08	NA	NA	6.8 ± 0.14	NA	NA
As is	8(C)-a	5.47 ± 0.34	NA	NA	6.14 ± 0.30	NA	NA
Amended/Inoculated (9)							
As is	9(C)-a	26.0 ± 0.8	26.6 ± 2.0	33.6 ± 0.4	23.2 ± 0.6	36.2 ± 0.2	43.2 ± 0.4
Exc.nitrate	9(C)-x	61.4 ± 8.2	56.2 ± 13.6	92.8 ± 8.6	76.4 ± 8.8	129.2 ± 13.0	162.6 ± 13.0

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

Table 8 (continued). Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (7)							
Formalin	7(C)-f	6.57 ± 0.05	NA	6.49 ± 0.01	NA	NA	0.70 ± 0.01
As is	7(C)-a	3.61 ± 0.04	NA	3.45 ± 0.04	3.39 ± 0.04	3.31 ± 0.04	3.13 ± 0.02
Unamended/Inoculated (8)							
Formalin	8(C)-f	5.48 ± 0.04	NA	5.33 ± 0.13	NA	NA	5.46 ± 0.02
As is	8(C)-a	9.68 ± 0.24	NA	11.8 ± 0.3	12.8 ± 0.5	14.0 ± 0.5	13.9 ± 1.0
Amended/Inoculated (9)							
As is	9(C)-a	44.4 ± 0.6	44.4 ± 1.0	49.1 ± 0.6	51.1 ± 0.5	52.0 ± 1.0	49.2 ± 0.8
Exc.nitrate	9(C)-x	181 ± 8	190 ± 4	204 ± 3	186 ± 9	212 ± 2	194 ± 4

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

Table 9. Nitrous Oxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (7)							
Formalin	7(C)-f	ND	NA	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.001	NA
As is	7(C)-a	ND	NA	ND	ND	ND	NA
Unamended/Inoculated (8)							
Formalin	8(C)-f	ND	NA	ND	ND	0.003 0.000	NA
As is	8(C)-a	ND	NA	ND	ND	ND	NA
Amended/Inoculated (9)							
As is	9(C)-a	ND	NA	-0.003 ± 0.029	-4.50 ± 2.00	15.8 ± 8.3	17.9 ± 8.6
Exc.nitrate	9(C)-x	ND	NA	0.158 ± 0.084	16.1 ± 10.0	39.0 ± 15.0	56.0 ± 32.0

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

Table 9 (continued). Nitrous Oxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (7)							
Formalin	7(C)-f	ND	NA	NA	ND	NA	NA
As is	7(C)-a	ND	NA	NA	ND	NA	NA
Unamended/Inoculated (8)							
Formalin	8(C)-f	0.002 ± 0.000	NA	NA	-0.002 ± 0.000	NA	NA
As is	8(C)-a	ND	NA	NA	ND	NA	NA
Amended/Inoculated (9)							
As is	9(C)-a	15.5 ± 8.4	12.3 ± 7.7	11.6 ± 8.9	10.2 ± 8.8	7.50 ± 8.10	6.10 ± 8.40
Exc.nitrate	9(C)-x	79.0 ± 46.0	46.0 ± 36.0	135 ± 50	169 ± 60	191 ± 34	238 ± 12

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

Table 9 (continued). Nitrous Oxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine]	Sample Designation	xide (µmoles/gram cellulose) cubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (7)							
Formalin	7(C)-f	ND	NA	ND	NA	NA	0.024 ± 0.000
As is	7(C)-a	-0.040 ± 0.001	NA	0.030 ± 0.020	ND	ND	ND
Unamended/Inoculated (8)							
Formalin	8(C)-f	ND	NA	ND	NA	NA	ND
As is	8(C)-a	-0.002 ± 0.000	NA	ND	ND	0.000 ± 0.000	ND
Amended/Inoculated (9)							
As is	9(C)-a	5.40 ± 8.30	5.68 ± 7.64	7.04 ± 7.77	3.04 ± 7.72	2.54 ± 6.80	19.1 ± 0.0
Exc.nitrate	9(C)-x	261 ± 8	246 ± 12	255 ± 10	224 ± 12	144 ± 58	220 ± 14

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

Table 10. Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine /Bentonite]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (10)							
Formalin	10(C)-f	0.06 ± 0.01	0.01 ± 0.01	0.37 ± 0.00	-0.09 ± 0.03	-0.14 ± 0.03	NA
As is	10(C)-a	-0.08 ± 0.10	-0.04 ± 0.03	-0.04 ± 0.05	-0.17 ± 0.08	-0.22 ± 0.10	NA
Unamended/Inoculated (11)							
Formalin	11(C)-f	-0.10 ± 0.01	-0.10 ± 0.00	-0.05 ± 0.01	-0.22 ± 0.23	-0.28 ± 0.22	NA
As is	11(C)-a	0.03 ± 0.01	0.11 ± 0.01	-0.06 ± 0.03	0.16 ± 0.04	0.29 ± 0.04	NA
Amended/Inoculated (12)							
As is	12(C)-a	-0.11 ± 0.05	-0.05 ± 0.02	0.19 ± 0.09	1.39 ± 0.09	1.78 ± 0.08	1.44 ± 0.07
Exc.nitrate	12(C)-x	-0.06 ± 0.02	-0.09 ± 0.03	0.23 ± 0.06	0.78 ± 0.09	1.68 ± 0.10	2.19 ± 0.14

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

Table 10 (continued). Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (10)							
Formalin	10(C)-f	-0.06 ± 0.03	NA	NA	0.04 ± 0.01	NA	NA
As is	10(C)-a	-0.28 ± 0.09	NA	NA	0.15 ± 0.08	NA	NA
Unamended/Inoculated (11)							
Formalin	11(C)-f	-0.06 ± 0.24	NA	NA	-0.13 ± 0.22	NA	NA
As is	11(C)-a	0.81 ± 0.08	NA	NA	1.39 ± 0.14	NA	NA
Amended/Inoculated (12)							
As is	12(C)-a	1.92 ± 0.08	2.48 ± 0.14	2.79 ± 0.19	2.81 ± 0.38	3.23 ± 0.48	3.50 ± 0.61
Exc.nitrate	12(C)-x	3.52 ± 0.28	4.76 ± 0.41	7.01 ± 0.89	11.9 ± 1.5	13.7 ± 1.2	15.9 ± 0.6

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

Table 10 (continued). Total Volume of Gas Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Milliliters of Gas Produced/Gram Cellulose Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (10)							
Formalin	10(C)-f	0.23 ± 0.04	NA	0.04 ± 0.02	NA	NA	0.10 ± 0.02
As is	10(C)-a	-0.16 ± 0.08	NA	0.76 ± 0.06	0.79 ± 0.05	0.08 ± 0.06	0.00 ± 0.04
Unamended/Inoculated (11)							
Formalin	11(C)-f	0.07 ± 0.20	NA	-0.08 ± 0.21	NA	NA	-0.31 ± 0.04
As is	11(C)-a	1.42 ± 0.27	NA	2.25 ± 0.18	2.25 ± 0.17	2.39 ± 0.18	2.39 ± 0.20
Amended/Inoculated (12)							
As is	12(C)-a	3.00 ± 0.76	4.08 ± 0.90	3.33 ± 1.42	4.12 ± 0.76	3.81 ± 0.67	3.62 ± 0.56
Exc.nitrate	12(C)-x	16.4 ± 0.2	18.1 ± 0.3	17.5 ± 0.5	16.6 ± 0.6	15.9 ± 0.6	14.9 ± 0.6

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

Table 11. Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (10)							
Formalin	10(C)-f	1.94 ± 0.20	0.52 ± 0.04	0.62 ± 0.08	-0.90 ± 0.06	-0.82 ± 0.18	NA
As is	10(C)-a	2.04 ± 0.10	0.98 ± 0.04	0.92 ± 0.08	0.64 ± 0.04	0.66 ± 0.10	NA
Unamended/Inoculated (11)							
Formalin	11(C)-f	2.28 ± 0.26	-0.12 ± 0.10	-0.32 ± 0.04	-0.3 ± 0.38	-1.02 ± 0.32	NA
As is	11(C)-a	1.86 ± 0.12	0.62 ± 0.04	0.84 ± 0.02	2.56 ± 0.50	8.06 ± 0.14	NA
Amended/Inoculated (12)							
As is	12(C)-a	-0.40 ± 0.16	-1.04 ± 0.06	0.84 ± 1.00	11.8 ± 0.4	48.7 ± 1.6	23.6 ± 2.0
Exc.nitrate	12(C)-x	-0.72 ± 0.12	-2.36 ± 0.34	0.20 ± 0.60	5.80 ± 1.00	15.6 ± 1.2	22.6 ± 1.4

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

Table 11(continued). Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (10)							
Formalin	10(C)-f	-0.94 ± 0.06	NA	NA	-0.48 ± 0.20	NA	NA
As is	10(C)-a	0.22 ± 0.04	NA	NA	0.84 ± 0.06	NA	NA
Unamended/Inoculated (11)							
Formalin	11(C)-f	-0.62 ± 0.16	NA	NA	7.7 ± 0.04	NA	NA
As is	11(C)-a	8.28 ± 0.20	NA	NA	15.0 ± 0.6	NA	NA
Amended/Inoculated (12)							
As is	12(C)-a	31.8 ± 2.0	25.0 ± 2.2	50.8 ± 2.0	58.8 ± 2.8	66.8 ± 3.0	82.8 ± 5.4
Exc.nitrate	12(C)-x	35.0 ± 2.8	50.6 ± 3.4	76.8 ± 5.4	116 ± 10	191 ± 24	288 ± 16

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

Table 12. Nitrous Oxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (10)							
Formalin	10(C)-f	ND	NA	ND	ND	ND	NA
As is	10(C)-a	ND	NA	ND	-0.019 ± 0.000	-0.200 ± 0.000	NA
Unamended/Inoculated (11)							
Formalin	11(C)-f	ND	NA	ND	0.001 ± 0.000	0.005 ± 0.001	NA
As is	11(C)-a	ND	NA	ND	ND	-0.007 ± 0.000	NA
Amended/Inoculated (12)							
As is	12(C)-a	ND	NA	0.019 ± 0.011	0.360 ± 0.380	ND	0.060 ± 0.050
Exc.nitrate	12(C)-x	0.001 ± 0.000	NA	0.760 ± 0.480	-0.012 ± 0.004	9.40 ± 3.60	31.0 ± 4.0

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

Table 11(continued). Carbon Dioxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Carbon Dioxide (µmoles/gram cellulose) Incubation Time (Days) 481	591	733	843	1034	1228
Unamended/Uninoculated (10)							
Formalin	10(C)-f	-1.04 ± 0.10	NA	-1.47 ± 0.05	NA	NA	-2.60 ± 0.00
As is	10(C)-a	0.42 ± 0.00	NA	0.80 ± 0.08	1.00 ± 0.03	0.28 ± 0.12	4.70 ± 4.90
Unamended/Inoculated (11)							
Formalin	11(C)-f	-1.34 ± 0.10	NA	-2.09 ± 0.10	NA	NA	-3.40 ± 0.20
As is	11(C)-a	27.0 ± 1.0	NA	42.9 ± 0.8	45.5 ± 0.8	52.6 ± 1.0	55.2 ± 1.4
Amended/Inoculated (12)							
As is	12(C)-a	82.4 ± 5.4	87.8 ± 5.0	85.1 ± 5.3	96.2 ± 5.1	93.6 ± 5.2	99.4 ± 4.4
Exc.nitrate	12(C)-x	325 ± 9	362 ± 8	386 ± 12	384 ± 13	384 ± 16	370 ± 14

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

Table 12(continued). Nitrous Oxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (10)							
Formalin	10(C)-f	ND	NA	NA	ND	NA	NA
As is	10(C)-a	-0.340 ± 0.000	NA	NA	-0.410 ± 0.000	NA	NA
Unamended/Inoculated (11)							
Formalin	11(C)-f	ND	NA	NA	0.001 ± 0.001	NA	NA
As is	11(C)-a	-0.001 ± 0.000	NA	NA	-0.002 ± 0.000	NA	NA
Amended/Inoculated (12)							
As is	12(C)-a	-0.670 ± 0.000	-0.430 ± 0.160	-0.900 ± 0.230	-0.890 ± 0.040	-1.30 ± 0.00	-2.80 ± 0.30
Exc.nitrate	12(C)-x	62.0 ± 5.0	97.0 ± 5.0	147 ± 16	213 ± 19	269 ± 24	246 ± 24

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

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

Treatments* [Brine]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	-1.44 ± 0.00	NA	NA	-0.36 ± 0.10	NA	NA
Formalin treated, with cellulose	1(C)-f	0.67 ± 0.90	NA	NA	1.83 ± 0.83	NA	NA
As is, w/o cellulose	1(NC)-a	-0.61 ± 0.75	NA	NA	-1.28 ± 0.64	NA	NA
As is, with cellulose	1(C)-a	-1.53 ± 0.51	NA	NA	-1.94 ± 0.48	NA	NA
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	0.07 ± 0.25	NA	NA	0.22 ± 0.28	NA	NA
Formalin treated, with cellulose	2(C)-f	-0.50 ± 0.44	NA	NA	-0.32 ± 0.29	NA	NA
As is, w/o cellulose	2(NC)-a	-2.86 ± 0.04	NA	NA	-3.28 ± 0.11	NA	NA
As is, with cellulose	2(C)-a	-4.57 ± 0.34	NA	NA	-6.18 ± 0.89	NA	NA
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	-4.53 ± 0.46	-4.72 ± 0.42	-5.14 ± 0.30	-6.88 ± 0.23	-6.40 ± 0.30	-7.92 ± 0.19
As is, with cellulose	3(C)-a	-0.22 ± 0.41	-0.25 ± 0.22	-1.38 ± 0.13	-1.08 ± 0.19	-0.72 ± 0.38	-1.53 ± 0.63
As is, with glucose	3(G)-a	-1.83 ± 1.30	-0.53 ± 0.23	-0.53 ± 0.34	1.46 ± 0.23	1.41 ± 1.49	2.51 ± 0.15
Excess nitrate, w/o cellulose	3(NC)-x	-2.82 ± 0.27	-2.40 ± 0.08	-2.86 ± 0.65	-1.68 ± 0.04	-2.29 ± 0.42	-3.85 ± 0.69
Excess nitrate, with cellulose	3(C)-x	19.3 ± 4.0	23.6 ± 1.9	28.2 ± 3.4	28.4 ± 3.9	28.3 ± 3.6	26.3 ± 3.0
Excess nitrate, with glucose	3(G)-x	2.90 ± 2.02	6.82 ± 1.14	11.3 ± 4.9	20.2 ± 8.8	27.3 ± 11.9	12.6 ± 0.5

*Dissolved gas concentration not included
NA = not analyzed.

Table 12(continued). Nitrous Oxide Produced in Anaerobic Samples in the Presence of Cellulose*

Treatments [Brine/ Bentonite]	Sample Designation	Nitrous Oxide (µmoles/gram cellulose) Incubation Time (Days) 481	591	733	843	1034	1228
Unamended/Uninoculated (10)							
Formalin	10(C)-f	ND	NA	ND	NA	NA	ND
As is	10(C)-a	-0.340 ± 0.040	NA	ND	ND	-0.150 ± 0.090	-0.260 ± 0.000
Unamended/Inoculated (11)							
Formalin	11(C)-f	ND	NA	ND	NA	NA	ND
As is	11(C)-a	ND	NA	ND	ND	ND	ND
Amended/Inoculated (12)							
As is	12(C)-a	-2.40 ± 0.10	-1.69 ± 0.05	-2.86 ± 0.07	-2.74 ± 0.00	-2.32 ± 0.03	-2.78 ± 0.04
Exc.nitrate	12(C)-x	231 ± 23	224 ± 21	217 ± 20	208 ± 17	185 ± 17	170 ± 15

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

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

Treatments* [Brine]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	4.22 ± 0.13	-0.35 ± 0.13	-0.06 ± 0.45	-0.26 ± 0.06	-0.13 ± 0.13	NA
Formalin treated, with cellulose	1(C)-f	5.41 ± 0.32	4.09 ± 0.16	4.19 ± 0.10	2.08 ± 1.06	1.37 ± 0.93	NA
As is, w/o cellulose	1(NC)-a	4.39 ± 0.03	3.71 ± 0.03	3.84 ± 0.03	1.90 ± 0.61	1.33 ± 0.85	NA
As is, with cellulose	1(C)-a	4.63 ± 0.10	2.28 ± 0.95	2.04 ± 0.92	0.34 ± 0.68	0.34 ± 0.61	NA
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	4.96 ± 0.02	2.88 ± 0.0	3.50 ± 0.00	2.49 ± 0.04	2.45 ± 0.14	NA
Formalin treated, with cellulose	2(C)-f	4.89 ± 0.06	2.72 ± 0.38	1.76 ± 0.56	0.91 ± 0.41	0.41 ± 0.53	NA
As is, w/o cellulose	2(NC)-a	3.56 ± 0.02	2.90 ± 0.15	2.21 ± 0.30	0.53 ± 0.11	-0.91 ± 0.04	NA
As is, with cellulose	2(C)-a	3.16 ± 0.03	2.97 ± 0.22	1.44 ± 0.16	-0.44 ± 0.22	-1.28 ± 0.25	NA
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	3.20 ± 0.04	1.37 ± 0.04	0.30 ± 0.00	0.11 ± 0.04	-1.49 ± 0.15	-2.48 ± 0.38
As is, with cellulose	3(C)-a	2.60 ± 0.16	0.03 ± 0.69	-0.94 ± 0.31	1.91 ± 1.53	1.44 ± 1.19	0.34 ± 0.63
As is, with glucose	3(G)-a	3.73 ± 0.08	2.29 ± 0.15	1.83 ± 0.38	-1.79 ± 0.34	-1.22 ± 1.37	1.87 ± 2.63
Excess nitrate, w/o cellulose	3(NC)-x	3.16 ± 0.08	0.84 ± 0.46	-1.30 ± 0.08	-0.78 ± 0.30	-1.14 ± 0.04	-1.80 ± 0.18
Excess nitrate, with cellulose	3(C)-x	3.04 ± 0.06	0.94 ± 0.72	5.66 ± 3.04	11.9 ± 4.4	15.5 ± 5.3	18.3 ± 6.1
Excess nitrate, with glucose	3(G)-x	3.52 ± 0.02	2.74 ± 0.50	0.88 ± 0.76	-1.49 ± 0.27	-1.30 ± 0.04	-0.86 ± 0.72

*Dissolved gas concentration not included
NA = not analyzed.

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

Treatments* [Brine]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	-0.70 ± 0.03	NA	-1.29 ± 0.03	NA	NA	-0.61 ± 0.00
Formalin treated, with cellulose	1(C)-f	-0.54 ± 0.38	NA	-2.86 ± 0.26	NA	NA	-2.53 ± 0.70
As is, w/o cellulose	1(NC)-a	-2.48 ± 0.48	NA	-5.39 ± 2.20	-5.51 ± 2.07	-2.45	-2.31
As is, with cellulose	1(C)-a	-3.33 ± 0.71	NA	-1.69 ± 0.38	-1.96 ± 0.42	-2.03 ± 0.00	-2.52 ± 0.40
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	0.11 ± 0.22	NA	-2.54 ± 0.37	NA	NA	-2.42 ± 0.41
Formalin treated, with cellulose	2(C)-f	-0.29 ± 0.18	NA	-0.59 ± 0.41	NA	NA	-2.68 ± 0.59
As is, w/o cellulose	2(NC)-a	-4.88 ± 0.04	NA	-5.73 ± 0.15	-6.11 ± 0.12	-6.25	-6.17
As is, with cellulose	2(C)-a	-7.60 ± 0.47	NA	-3.31 ± 0.37	-4.36 ± 0.46	-4.44 ± 0.39	-4.65 ± 0.36
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	-7.50 ± 0.08	-7.31 ± 0.27	-8.08 ± 0.27	-8.61 ± 0.00	-8.23	-7.89
As is, with cellulose	3(C)-a	-1.19 ± 0.69	-0.59 ± 0.50	-0.72 ± 0.01	-0.95 ± 0.68	-0.29 ± 1.22	-0.77 ± 1.40
As is, with glucose	3(G)-a	2.29 ± 2.02	1.30 ± 1.64	-1.05 ± 0.12	-1.24 ± 0.26	-0.25 ± 0.07	-1.70 ± 0.66
Excess nitrate, w/o cellulose	3(NC)-x	-4.00 ± 0.95	-2.25 ± 0.19	-4.08 ± 1.70	-1.49 ± 0.00	-0.14	-0.65
Excess nitrate, with cellulose	3(C)-x	26.7 ± 3.4	33.8 ± 6.7	**53.8 ± 10.4	50.4 ± 8.5	61.0 ± 0.0	50.7 ± 7.7
Excess nitrate, with glucose	3(G)-x	43.7 ± 12.7	53.5 ± 9.8	10.3 ± 0.9	16.1 ± 0.8	15.1 ± 2.1	17.1 ± 3.4

*Dissolved gas concentration not included

**Analysis at this time point is of a reserved replicate sample

NA = not analyzed.

Table 1(b). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	2.59 ± 0.01	2.50 ± 0	3.40 ± 0.40	3.32 ± 0.00	3.61 ± 0.00	NA
Formalin treated, with cellulose	1(C)-f	30.5 ± 2.7	40.8 ± 0.1	44.4 ± 0.2	40.6 ± 0.8	41.8 ± 0.3	NA
As is, w/o cellulose	1(NC)-a	1.38 ± 0.01	1.49 ± 0	0.94 ± 0.00	1.66 ± 0.01	1.83 ± 0.06	NA
As is, with cellulose	1(C)-a	13.8 ± 0.5	21.1 ± 0.3	22.0 ± 0.1	21.3 ± 0.1	23.1 ± 0.1	NA
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	4.97 ± 0.45	4.38 ± 0.3	4.70 ± 0.11	4.88 ± 0.3	5.02 ± 0.30	NA
Formalin treated, with cellulose	2(C)-f	34.5 ± 0.8	35.6 ± 0.0	38.7 ± 0.7	34.3 ± 0.6	35.2 ± 0.1	NA
As is, w/o cellulose	2(NC)-a	2.30 ± 0.02	2.97 ± 0	2.92 ± 0.09	5.88 ± 0.08	6.56 ± 0.11	NA
As is, with cellulose	2(C)-a	12.1 ± 0.3	19.7 ± 0.8	22.6 ± 0.9	30.8 ± 0.9	40.9 ± 1.0	NA
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	2.87 ± 0.03	27.8 ± 1.2	72.9 ± 2.6	113 ± 5	130 ± 2	150 ± 1
As is, with cellulose	3(C)-a	2.80 ± 0.10	50.9 ± 1.4	91.8 ± 5.9	215 ± 37	278 ± 25	333 ± 21
As is, with glucose	3(G)-a	2.41 ± 0.03	4.03 ± 0.1	30.8 ± 2.1	94.9 ± 7.5	167 ± 32	325 ± 108
Excess nitrate, w/o cellulose	3(NC)-x	2.79 ± 0.03	17.2 ± 1.9	48.9 ± 0.2	92.2 ± 0.6	115 ± 3	131 ± 2
Excess nitrate, with cellulose	3(C)-x	2.60 ± 0.10	51.6 ± 0.0	210 ± 21	399 ± 18	533 ± 13	612 ± 20
Excess nitrate, with glucose	3(G)-x	2.51 ± 0.01	4.05 ± 0.3	24.1 ± 5.9	72.0 ± 7.7	121 ± 4	211 ± 6

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 1(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	3.68 ± 0.01	NA	NA	0.00 ± 0.00	NA	NA
Formalin treated, with cellulose	1(C)-f	41.8 ± 0.5	NA	NA	45.4 ± 0.4	NA	NA
As is, w/o cellulose	1(NC)-a	2.18 ± 0.04	NA	NA	0.00 ± 0.00	NA	NA
As is, with cellulose	1(C)-a	22.2 ± 0.1	NA	NA	22.2 ± 0.3	NA	NA
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	4.94 ± 0.27	NA	NA	5.21 ± 0.3	NA	NA
Formalin treated, with cellulose	2(C)-f	33.2 ± 1.1	NA	NA	35.3 ± 1.7	NA	NA
As is, w/o cellulose	2(NC)-a	7.01 ± 0.11	NA	NA	6.91 ± 0.15	NA	NA
As is, with cellulose	2(C)-a	48.5 ± 1.4	NA	NA	61.6 ± 1.3	NA	NA
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	142 ± 1	144 ± 1	159 ± 1	153 ± 1	159 ± 2	147 ± 0
As is, with cellulose	3(C)-a	346 ± 27	329 ± 41	342 ± 41	360 ± 39	357 ± 35	337 ± 38
As is, with glucose	3(G)-a	323 ± 95	361 ± 104	378 ± 104	413 ± 83	517 ± 44	573 ± 56
Excess nitrate, w/o cellulose	3(NC)-x	126 ± 2	137 ± 1	138 ± 0	132 ± 3	133 ± 0	118 ± 0
Excess nitrate, with cellulose	3(C)-x	604 ± 30	686 ± 37	757 ± 50	724 ± 54	764 ± 82	748 ± 95
Excess nitrate, with glucose	3(G)-x	296 ± 20	453 ± 20	602 ± 69	722 ± 104	852 ± 142	740 ± 127

Dissolved gas concentrations not included.
NA = not analyzed.

Table 1(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	4.70 ± 0.20	NA	3.87 ± 0.3	NA	NA	3.31 ± 0.46
Formalin treated, with cellulose	1(C)-f	47.0 ± 1.1	NA	45.4 ± 0.5	NA	NA	49.9 ± 0.5
As is, w/o cellulose	1(NC)-a	2.00 ± 0.10	NA	2.17 ± 0.2	2.22 ± 0.09	7.25 ± 0	2.04
As is, with cellulose	1(C)-a	23.0 ± 0.3	NA	25.0 ± 0.3	25.6 ± 0.2	26.4 ± 0.15	24.2 ± 0.3
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	5.84 ± 0.27	NA	5.55 ± 0.3	NA	NA	5.67 ±
Formalin treated, with cellulose	2(C)-f	33.9 ± 3.0	NA	32.6 ± 4.9	NA	NA	30.1 ± 6.9
As is, w/o cellulose	2(NC)-a	7.11 ± 0.10	NA	7.52 ± 0.2	7.8 ± 0.0	8.57 ± 0	7.77
As is, with cellulose	2(C)-a	67.4 ± 1.2	NA	74.4 ± 0.9	80.2 ± 0.5	80.8 ± 0.7	76.9 ± 1.1
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	146 ± 1	140 ± 0	147 ± 1	146	145	140
As is, with cellulose	3(C)-a	313 ± 36	299 ± 33	281 ± 27	276 ± 21	255 ± 14.5	225 ± 12
As is, with glucose	3(G)-a	607 ± 39	633 ± 19	584 ± 32	580 ± 45	540 ± 15	511 ± 13
Excess nitrate, w/o cellulose	3(NC)-x	108 ± 1	94.9 ± 3.1	85.5 ± 4.8	90	79.7	69
Excess nitrate, with cellulose	3(C)-x	735 ± 105	855 ± 132	**963 ± 3	936 ± 8	1010 ± 40	845 ± 76
Excess nitrate, with glucose	3(G)-x	1150 ± 140	1340 ± 120	851 ± 85	1110 ± 120	1060 ± 120	1020 ± 130

* Dissolved gas concentrations not included.
**Analysis at this time point is of a reserved replicate sample
NA = not analyzed.

Table 1(c). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (μmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	0.000 ± 0.000	NA	0.003 ± 0.002	0.000 ± 0.000	0.000 ± 0.000	NA
Formalin treated, with cellulose	1(C)-f	0.000 ± 0.000	NA	0.003 ± 0.001	0.003 ± 0.001	0.013 ± 0.001	NA
As is, w/o cellulose	1(NC)-a	0.000 ± 0.000	NA	0.162 ± 0.110	0.000 ± 0.000	0.000 ± 0.000	NA
As is, with cellulose	1(C)-a	0.000 ± 0.000	NA	0.003 ± 0.001	0.006 ± 0.001	0.015 ± 0.001	NA
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	0.000 ± 0.000	NA	0.025 ± 0.018	0.000 ± 0.000	0.000 ± 0.000	NA
Formalin treated, with cellulose	2(C)-f	0.000 ± 0.000	NA	0.001 ± 0.000	0.002 ± 0.000	0.014 ± 0.002	NA
As is, w/o cellulose	2(NC)-a	0.003 ± 0.000	NA	0.001 ± 0.001	0.000 ± 0.000	0.016 ± 0.011	NA
As is, with cellulose	2(C)-a	0.014 ± 0.000	NA	0.002 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	NA
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	0.000 ± 0.000	NA	0.061 ± 0.043	0.087 ± 0.061	0.024 ± 0.003	0.000 ± 0.000
As is, with cellulose	3(C)-a	0.004 ± 0.000	NA	0.182 ± 0.147	118 ± 48.0	122 ± 39.0	8.81 ± 5.98
As is, with glucose	3(G)-a	0.000 ± 0.000	NA	0.056 ± 0.010	0.252 ± 0.122	5.64 ± 3.40	114 ± 80.0
Excess nitrate, w/o cellulose	3(NC)-x	0.000 ± 0.000	NA	0.001 ± 0.000	0.004 ± 0.002	0.006 ± 0.004	0.006 ± 0.001
Excess nitrate, with cellulose	3(C)-x	0.000 ± 0.000	NA	28.2 ± 8.80	245 ± 11.0	326 ± 18.0	484 ± 67.0
Excess nitrate, with glucose	3(G)-x	0.000 ± 0.000	NA	0.000 ± 0.000	2.97 ± 2.10	2.99 ± 2.08	11.2 ± 7.30

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 1(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (μmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	0.001 ± 0.001	NA	NA	0.000 ± 0.000	NA	NA
Formalin treated, with cellulose	1(C)-f	0.005 ± 0.002	NA	NA	0.001 ± 0.001	NA	NA
As is, w/o cellulose	1(NC)-a	0.051 ± 0.035	NA	NA	0.000 ± 0.000	NA	NA
As is, with cellulose	1(C)-a	0.008 ± 0.000	NA	NA	0.006 ± 0.002	NA	NA
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	0.000 ± 0.000	NA	NA	0.002 ± 0.001	NA	NA
Formalin treated, with cellulose	2(C)-f	0.004 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
As is, w/o cellulose	2(NC)-a	0.062 ± 0.007	NA	NA	0.058 ± 0.005	NA	NA
As is, with cellulose	2(C)-a	0.009 ± 0.006	NA	NA	0.005 ± 0.000	NA	NA
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	0.050 ± 0.033	0.134 ± 0.065	0.000 ± 0.000	0.015 ± 0.001	0.170 ± 0.048	1.00 ± 0.57
As is, with cellulose	3(C)-a	29.2 ± 25.2	3.87 ± 1.92	0.015 ± 0.012	3.27 ± 0.36	3.18 ± 0.25	1.19 ± 0.89
As is, with glucose	3(G)-a	105 ± 74.0	109 ± 77.0	105 ± 61.0	142 ± 25.0	236 ± 60.0	213 ± 44.0
Excess nitrate, w/o cellulose	3(NC)-x	0.023 ± 0.006	0.06 ± 0.007	0.26 ± 0.057	0.176 ± 0.045	0.135 ± 0.066	0.155 ± 0.039
Excess nitrate, with cellulose	3(C)-x	577 ± 129	523 ± 186	760 ± 207	694 ± 160	655 ± 125	635 ± 114
Excess nitrate, with glucose	3(G)-x	66.0 ± 41.9	155 ± 40.0	199 ± 83.0	231 ± 155	299 ± 201	248 ± 157

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 1(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (μmoles/sample) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (1)							
Formalin treated, w/o cellulose	1(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
Formalin treated, with cellulose	1(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
As is, w/o cellulose	1(NC)-a	0.040 ± 0.028	NA	0.170 ± 0.120	0.150 ± 0.110	0.000 ± 0.000	0.000 ± 0.000
As is, with cellulose	1(C)-a	0.018 ± 0.000	NA	0.350 ± 0.290	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Unamended/Inoculated (2)							
Formalin treated, w/o cellulose	2(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
Formalin treated, with cellulose	2(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
As is, w/o cellulose	2(NC)-a	0.006 ± 0.004	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
As is, with cellulose	2(C)-a	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Amended/Inoculated (3)							
As is, w/o cellulose	3(NC)-a	0.066 ± 0.047	1.06 ± 0.75	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
As is, with cellulose	3(C)-a	1.32 ± 0.90	2.15 ± 0.930	2.52 ± 1.22	0.000 ± 0.000	0.900 ± 0.740	0.000 ± 0.000
As is, with glucose	3(G)-a	180 ± 29.0	136 ± 2.00	101 ± 14.0	82.6 ± 22.9	62.6 ± 19.4	52.9 ± 19.3
Excess nitrate, w/o cellulose	3(NC)-x	0.007 ± 0.005	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Excess nitrate, with cellulose	3(C)-x	631 ± 125	674 ± 241	1180 ± 80.0	1120 ± 70.0	1210 ± 30.0	1080 ± 42.0
Excess nitrate, with glucose	3(G)-x	169 ± 63.0	14.9 ± 0.000	26.1 ± 11.7	32.1 ± 14.1	28.7 ± 13.0	614 ± 404

* Dissolved gas concentrations not included.
**Analysis at this time point is of a reserved replicate sample
NA = not analyzed.

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

Treatments* [Brine/Bentonite]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	5.93 ± 0.04	1.24 ± 2.02	1.43 ± 1.74	1.09 ± 1.05	-0.31 ± 0.00	NA
Formalin treated, with cellulose	4(C)-f	6.59 ± 0.16	4.38 ± 0.13	3.77 ± 0.13	1.82 ± 0.13	0.10 ± 0.03	NA
As is, w/o cellulose	4(NC)-a	5.43 ± 0.04	3.63 ± 0.53	4.78 ± 0.78	2.65 ± 0.45	1.92 ± 0.20	NA
As is, with cellulose	4(C)-a	5.17 ± 0.10	3.74 ± 0.10	2.99 ± 0.20	1.67 ± 0.24	1.26 ± 0.20	NA
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	5.08 ± 0.36	2.56 ± 0.83	1.19 ± 1.73	0.76 ± 0.94	0.54 ± 0.65	NA
Formalin treated, with cellulose	5(C)-f	5.65 ± 0.12	3.63 ± 0.03	2.72 ± 0.00	2.22 ± 0.00	0.88 ± 0.15	NA
As is, w/o cellulose	5(NC)-a	3.66 ± 0.30	3.62 ± 0.04	2.78 ± 0.08	1.64 ± 0.15	1.14 ± 0.11	NA
As is, with cellulose	5(C)-a	3.79 ± 0.06	3.13 ± 0.09	1.88 ± 0.19	0.16 ± 0.66	-1.94 ± 0.47	NA
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	3.85 ± 0.04	0.84 ± 0.84	1.52 ± 0.84	1.56 ± 0.80	0.11 ± 0.53	-1.49 ± 0.34
As is, with cellulose	6(C)-a	2.60 ± 0.16	3.00 ± 0.06	5.07 ± 0.75	10.7 ± 1.8	14.9 ± 1.5	18.9 ± 1.1
As is, with glucose	6(G)-a	1.60 ± 0.04	1.10 ± 0.69	0.69 ± 0.50	-0.34 ± 0.19	-1.07 ± 0.27	-1.62 ± 0.19
Excess nitrate, w/o cellulose	6(NC)-x	1.41 ± 0.08	-1.45 ± 0.04	-0.99 ± 0.08	0.00 ± 0.00	-0.27 ± 0.08	-1.30 ± 0.04
Excess nitrate, with cellulose	6(C)-x	2.91 ± 0.06	2.82 ± 0.09	6.79 ± 0.09	11.2 ± 1.2	18.7 ± 1.5	24.4 ± 0.9
Excess nitrate, with glucose	6(G)-x	2.29 ± 0.23	2.51 ± 1.71	0.69 ± 0.57	-0.38 ± 0.50	-0.53 ± 0.11	-1.49 ± 0.15

NA = not analyzed.

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

Treatments* [Brine/Bentonite]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	-2.17 ± 0.00	NA	NA	-0.5 ± 0.12	NA	NA
Formalin treated, with cellulose	4(C)-f	-1.98 ± 0.03	NA	NA	-0.3 ± 0.88	NA	NA
As is, w/o cellulose	4(NC)-a	-0.82 ± 0.20	NA	NA	-0.41 ± 0.29	NA	NA
As is, with cellulose	4(C)-a	-0.82 ± 0.27	NA	NA	-0.14 ± 0.71	NA	NA
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	-2.02 ± 0.36	NA	NA	-1.33 ± 0.86	NA	NA
Formalin treated, with cellulose	5(C)-f	-1.23 ± 0.12	NA	NA	-0.28 ± 0.63	NA	NA
As is, w/o cellulose	5(NC)-a	-2.06 ± 0.34	NA	NA	-1.88 ± 0.23	NA	NA
As is, with cellulose	5(C)-a	-2.47 ± 0.78	NA	NA	1.38 ± 1.41	NA	NA
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	-3.47 ± 0.15	-2.40 ± 0.50	-4.08 ± 0.15	-3.70 ± 0.27	-5.52 ± 0.30	-6.40 ± 0.30
As is, with cellulose	6(C)-a	18.4 ± 1.0	19.6 ± 1.5	21.7 ± 2.3	22.7 ± 2.9	24.7 ± 3.7	24.3 ± 3.9
As is, with glucose	6(G)-a	-1.52 ± 0.61	-1.87 ± 0.72	-2.55 ± 0.91	-3.70 ± 0.27	-1.56 ± 0.61	-3.31 ± 0.61
Excess nitrate, w/o cellulose	6(NC)-x	-3.05 ± 0.08	-2.51 ± 0.34	-2.48 ± 0.57	-3.64 ± 0.48	-4.15 ± 0.53	-5.56 ± 0.42
Excess nitrate, with cellulose	6(C)-x	27.3 ± 0.2	28.4 ± 0.6	29.2 ± 0.8	28.1 ± 1.0	30.8 ± 1.4	29.9 ± 1.7
Excess nitrate, with glucose	6(G)-x	-1.94 ± 0.69	-2.59 ± 0.99	-2.82 ± 0.99	-1.87 ± 1.46	-4.38 ± 1.52	-2.78 ± 0.04

NA = not analyzed.

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

Treatments* [Brine/Bentonite]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	-0.81 ± 0.08	NA	-1.61 ± 0.04	NA	NA	-3.30 ± 1.40
Formalin treated, with cellulose	4(C)-f	-1.25 ± 0.35	NA	-2.48 ± 0.85	NA	NA	-2.72 ± 0.79
As is, w/o cellulose	4(NC)-a	-2.82 ± 0.45	NA	-7.22 ± 2.40	-7.82 ± 2.32	-4.41	-4.33
As is, with cellulose	4(C)-a	-3.20 ± 0.24	NA	-1.94 ± 0.14	-2.60 ± 0.16	-2.94 ± 0.53	-2.69 ± 0.63
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	-2.34 ± 1.08	NA	-4.06 ± 1.75	NA	NA	-3.70 ± 1.57
Formalin treated, with cellulose	5(C)-f	-2.78 ± 0.20	NA	-4.90 ± 0.13	NA	NA	-4.68 ± 0.08
As is, w/o cellulose	5(NC)-a	-3.20 ± 0.57	NA	-4.23 ± 0.65	-5.24 ± 0.55	-5.98	-5.90
As is, with cellulose	5(C)-a	3.32 ± 2.44	NA	0.89 ± 0.59	0.97 ± 0.92	1.06 ± 1.11	1.43 ± 1.11
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	-6.36 ± 0.42	-6.13 ± 0.42	-7.70 ± 0.11	-8.00 ± 0.00	-7.35	-6.93
As is, with cellulose	6(C)-a	24.9 ± 4.1	25.8 ± 4.2	**37.1 ± 6.7	33.8 ± 6.2	23.3 ± 0.5	23.5 ± 0.2
As is, with glucose	6(G)-a	-1.14 ± 0.30	-1.94 ± 0.46	-1.96 ± 0.23	-2.42 ± 0.20	-3.68 ± 0.36	-2.88 ± 0.63
Excess nitrate, w/o cellulose	6(NC)-x	-5.68 ± 0.38	-6.82 ± 0.04	-7.05 ± 0.19	-7.09 ± 0.00	0.00	-2.44
Excess nitrate, with cellulose	6(C)-x	31.1 ± 2.3	32.0 ± 3.3	29.7 ± 3.9	34.9 ± 4.1	34.0 ± 3.9	38.1 ± 3.7
Excess nitrate, with glucose	6(G)-x	-0.88 ± 0.04	-2.10 ± 0.46	-1.89 ± 0.20	-2.02 ± 0.08	-3.64 ± 0.07	-1.81 ± 0.50

**Analysis at this time point is of a reserved replicate sample
NA = not analyzed.

Table 2(b). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Aerobic Samples

Treatments [Brine/Bentonite]	Sample Designation	Carbon Dioxide (μmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	50.3 ± 0.4	88.8 ± 1.0	98.8 ± 0.4	101 ± 1	115 ± 2	NA
Formalin treated, with cellulose	4(C)-f	71.1 ± 1.2	95.3 ± 0.2	110 ± 0	114 ± 0	127 ± 1	NA
As is, w/o cellulose	4(NC)-a	17.7 ± 0.3	38.8 ± 0.4	44.5 ± 0.4	40.2 ± 0.3	42.7 ± 0.2	NA
As is, with cellulose	4(C)-a	25.3 ± 1.5	47.6 ± 0.3	66.9 ± 12	49.0 ± 0.7	51.8 ± 0.5	NA
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	37.3 ± 10.1	59.6 ± 16	66.1 ± 18	77.4 ± 18	76.0 ± 26.5	NA
Formalin treated, with cellulose	5(C)-f	61.9 ± 0.9	83.5 ± 0.0	94.0 ± 0.2	93.2 ± 0.6	100 ± 0	NA
As is, w/o cellulose	5(NC)-a	13.5 ± 2.9	36.7 ± 0.7	41.3 ± 0.2	38.7 ± 0.7	41.4 ± 0.7	NA
As is, with cellulose	5(C)-a	23.7 ± 0.2	43.6 ± 0.4	61.3 ± 4.0	80.3 ± 2.1	101 ± 3	NA
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	11.3 ± 0.1	103 ± 3	169 ± 3	185 ± 2	201 ± 2	203 ± 0
As is, with cellulose	6(C)-a	8.60 ± 0.00	72.4 ± 1.0	156 ± 5	247 ± 11	358 ± 19	492 ± 6
As is, with glucose	6(G)-a	8.50 ± 2.80	67.1 ± 0.2	73.1 ± 0.4	72.9 ± 0.2	75.8 ± 0.4	79.5 ± 1.0
Excess nitrate, w/o cellulose	6(NC)-x	10.5 ± 0.4	84.6 ± 0.0	153 ± 1	171 ± 3	139 ± 37	184 ± 4
Excess nitrate, with cellulose	6(C)-x	8.90 ± 0.10	68.0 ± 1.2	164 ± 3	307 ± 23	499 ± 75	711 ± 46
Excess nitrate, with glucose	6(G)-x	9.40 ± 0.90	65.0 ± 2.0	71.4 ± 1.2	71.2 ± 0.6	76.5 ± 0.2	80.1 ± 1.5

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 2(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Aerobic Samples

Treatments [Brine/Bentonite]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	121 ± 4	NA	NA	0.00 ± 0.00	NA	NA
Formalin treated, with cellulose	4(C)-f	137 ± 1	NA	NA	159 ± 1	NA	NA
As is, w/o cellulose	4(NC)-a	40.0 ± 0.1	NA	NA	42.5 ± 0.6	NA	NA
As is, with cellulose	4(C)-a	51.6 ± 0.1	NA	NA	56.2 ± 0.6	NA	NA
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	79.0 ± 33.0	NA	NA	93.5 ± 43.9	NA	NA
Formalin treated, with cellulose	5(C)-f	102 ± 0	NA	NA	118 ± 1	NA	NA
As is, w/o cellulose	5(NC)-a	37.6 ± 0.7	NA	NA	41.4 ± 0.7	NA	NA
As is, with cellulose	5(C)-a	145 ± 6	NA	NA	221 ± 11	NA	NA
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	181 ± 1	199 ± 1	196 ± 0	202 ± 1	197 ± 1	184 ± 1
As is, with cellulose	6(C)-a	530 ± 6	513 ± 8	697 ± 8	713 ± 10	805 ± 7	835 ± 12
As is, with glucose	6(G)-a	75.7 ± 1.7	79.0 ± 1.9	80.7 ± 2.5	80.7 ± 2.2	81.2 ± 3.0	79.1 ± 2.1
Excess nitrate, w/o cellulose	6(NC)-x	159 ± 4	168 ± 4	171 ± 3	150 ± 4	144 ± 4	129 ± 4
Excess nitrate, with cellulose	6(C)-x	741 ± 30	752 ± 28	797 ± 4	759 ± 30	785 ± 28	776 ± 28
Excess nitrate, with glucose	6(G)-x	81.1 ± 5.0	87.1 ± 6.8	92.5 ± 10	92.0 ± 12	94.6 ± 13.2	98.2 ± 15.5

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 2(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Aerobic Samples

Treatments [Brine/Bentonite]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	149 ± 3	NA	154 ± 2	NA	NA	129 ± 2
Formalin treated, with cellulose	4(C)-f	180 ± 1	NA	196 ± 2	NA	NA	225 ± 6
As is, w/o cellulose	4(NC)-a	39.5 ± 0.1	NA	37.7 ± 0.1	38.9 ± 0.2	42.1 ± 0	40.4 ± 0
As is, with cellulose	4(C)-a	52.0 ± 2.0	NA	57.9 ± 1.1	58.4 ± 1.4	59.8 ± 1.7	56.9 ± 1.6
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	102 ± 54	NA	106 ± 60	NA	NA	114 ± 67
Formalin treated, with cellulose	5(C)-f	138 ± 0	NA	146 ± 0	NA	NA	169 ± 1
As is, w/o cellulose	5(NC)-a	37.8 ± 0.7	NA	39.6 ± 0.9	39.2 ± 0.9	42.6 ± 0	40 ±
As is, with cellulose	5(C)-a	298 ± 13	NA	343 ± 15	381 ± 23	392 ± 25	388 ± 24
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	179 ± 0	180 ± 1	177 ± 0	177	177	11
As is, with cellulose	6(C)-a	846 ± 11	870 ± 8	*1050 ± 50	998 ± 42	875 ± 56	855 ± 54
As is, with glucose	6(G)-a	85.7 ± 4.5	84.2 ± 5.3	88.7 ± 5.3	94.2 ± 5.5	99.4 ± 5.5	100 ± 5
Excess nitrate, w/o cellulose	6(NC)-x	117 ± 5	97.5 ± 6.3	89.9 ± 7.7	89.9	91.3	69
Excess nitrate, with cellulose	6(C)-x	759 ± 22	782 ± 25	772 ± 25	836 ± 13	776 ± 4.6	838 ± 34
Excess nitrate, with glucose	6(G)-x	106 ± 18	107 ± 18	114 ± 20	124 ± 20	126 ± 21	131 ± 19

* Dissolved gas concentrations not included.
**Analysis at this time point is of a reserved replicate sample
NA = not analyzed.

Table 2(c). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	0.000 ± 0.000	NA	0.002 ± 0.002	0.231 ± 0.146	0.010 ± 0.007	NA
Formalin treated, with cellulose	4(C)-f	0.000 ± 0.000	NA	0.008 ± 0.002	0.001 ± 0.001	0.019 ± 0.000	NA
As is, w/o cellulose	4(NC)-a	0.000 ± 0.000	NA	0.006 ± 0.001	0.003 ± 0.002	0.017 ± 0.000	NA
As is, with cellulose	4(C)-a	0.000 ± 0.000	NA	0.731 ± 0.489	0.188 ± 0.054	0.222 ± 0.070	NA
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	0.000 ± 0.000	NA	0.070 ± 0.049	0.009 ± 0.002	0.166 ± 0.160	NA
Formalin treated, with cellulose	5(C)-f	0.000 ± 0.000	NA	0.012 ± 0.001	0.011 ± 0.002	0.025 ± 0.001	NA
As is, w/o cellulose	5(NC)-a	0.000 ± 0.000	NA	0.038 ± 0.024	0.195 ± 0.065	0.210 ± 0.066	NA
As is, with cellulose	5(C)-a	0.000 ± 0.000	NA	0.010 ± 0.006	0.000 ± 0.000	0.000 ± 0.000	NA
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	0.000 ± 0.000	NA	0.002 ± 0.001	1.21 ± 0.360	6.06 ± 4.21	4.96 ± 3.51
As is, with cellulose	6(C)-a	0.000 ± 0.000	NA	31.6 ± 13.2	160 ± 53.0	132 ± 8.00	0.528 ± 0.459
As is, with glucose	6(G)-a	0.000 ± 0.000	NA	0.003 ± 0.000	0.003 ± 0.000	0.028 ± 0.001	0.001 ± 0.001
Excess nitrate, w/o cellulose	6(NC)-x	0.000 ± 0.000	NA	0.000 ± 0.000	14.6 ± 2.7	13.9 ± 5.2	13.8 ± 5.4
Excess nitrate, with cellulose	6(C)-x	0.000 ± 0.000	NA	27.1 ± 6.50	142 ± 22	217 ± 41	354 ± 41
Excess nitrate, with glucose	6(G)-x	0.000 ± 0.000	NA	0.005 ± 0.001	0.010 ± 0.001	0.022 ± 0.003	0.092 ± 0.063

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 2(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
Formalin treated, with cellulose	4(C)-f	0.006 ± 0.001	NA	NA	0.006 ± 0.002	NA	NA
As is, w/o cellulose	4(NC)-a	0.005 ± 0.003	NA	NA	0.008 ± 0.003	NA	NA
As is, with cellulose	4(C)-a	0.245 ± 0.101	NA	NA	0.339 ± 0.147	NA	NA
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	0.076 ± 0.049	NA	NA	0.047 ± 0.004	NA	NA
Formalin treated, with cellulose	5(C)-f	0.009 ± 0.002	NA	NA	0.003 ± 0.003	NA	NA
As is, w/o cellulose	5(NC)-a	0.161 ± 0.050	NA	NA	0.184 ± 0.065	NA	NA
As is, with cellulose	5(C)-a	0.064 ± 0.027	NA	NA	1.93 ± 0.800	NA	NA
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	0.427 ± 2.55	4.28 ± 2.89	4.25 ± 2.76	4.35 ± 2.92	4.60 ± 1.97	3.02 ± 2.00
As is, with cellulose	6(C)-a	0.000 ± 0.000	4.83 ± 0.10	0.000 ± 0.000	6.54 ± 0.06	6.53 ± 0.33	6.48 ± 0.16
As is, with glucose	6(G)-a	0.015 ± 0.004	0.014 ± 0.003	0.028 ± 0.004	0.120 ± 0.008	0.468 ± 0.331	0.269 ± 0.04
Excess nitrate, w/o cellulose	6(NC)-x	11.6 ± 4.9	11.2 ± 4.9	10.7 ± 4.5	9.02 ± 3.88	6.95 ± 3.01	5.58 ± 2.27
Excess nitrate, with cellulose	6(C)-x	425 ± 35	472 ± 32	520 ± 41	526 ± 45	562 ± 60	582 ± 69
Excess nitrate, with glucose	6(G)-x	0.011 ± 0.001	0.014 ± 0.003	0.022 ± 0.006	0.034 ± 0.015	0.599 ± 0.423	0.000 ± 0.000

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 2(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 481	592	733	853	1034	1228
Unamended/Uninoculated (4)							
Formalin treated, w/o cellulose	4(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
Formalin treated, with cellulose	4(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
As is, w/o cellulose	4(NC)-a	0.021 ± 0.015	NA	0.000 ± 0.000	0.800 ± 0.290	0.200 ± 0.000	0.000 ± 0.000
As is, with cellulose	4(C)-a	0.259 ± 0.085	NA	1.01 ± 0.150	0.200 ± 0.160	1.42 ± 0.290	1.19 ± 0.000
Unamended/Inoculated (5)							
Formalin treated, w/o cellulose	5(NC)-f	0.003 ± 0.002	NA	0 ± 0.000	NA	NA	0 ± 0.000
Formalin treated, with cellulose	5(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
As is, w/o cellulose	5(NC)-a	0.112 ± 0.079	NA	0.000 ± 0.000	0.060 ± 0.040	0.940 ± 0.000	0.000 ± 0.000
As is, with cellulose	5(C)-a	1.02 ± 0.830	NA	3.60 ± 0.230	3.81 ± 0.260	3.59 ± 0.230	4.12 ± 0.000
Amended/Inoculated (6)							
As is, w/o cellulose	6(NC)-a	0.000 ± 0.000	4.56 ± 0.610	6.35 ± 0.380	7.55	9.09	0.000 ± 0.000
As is, with cellulose	6(C)-a	7.44 ± 0.14	8.79 ± 0.17	**11.7 ± 0.00	9.97 ± 0.50	5.75 ± 0.85	0.00 ± 0.000
As is, with glucose	6(G)-a	0.206 ± 0.031	NA	0.000 ± 0.000	0.000 ± 0.000	1.31 ± 0.000	0.000 ± 0.000
Excess nitrate, w/o cellulose	6(NC)-x	0.010 ± 0.004	2.00 ± 1.24	1.76 ± 0.460	0.890	1.60	0.000 ± 0.000
Excess nitrate, with cellulose	6(C)-x	606 ± 86.0	661 ± 104	676 ± 116	740 ± 126	833 ± 183	0 ± 0.000
Excess nitrate, with glucose	6(G)-x	0.101 ± 0.019	NA	0.000 ± 0.000	0.000 ± 0.000	75.1 ± 52.0	0.000 ± 0.000

* Dissolved gas concentrations not included.
**Analysis at this time point is of a reserved replicate sample
NA = not analyzed.

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

Treatments* [Brine]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	2.99 ± 0.10	2.65 ± 0.10	2.01 ± 0.03	1.09 ± 0.06	1.66 ± 0.00	NA
Formalin treated, with cellulose	7(C)-f	4.28 ± 0.06	3.49 ± 0.06	3.10 ± 0.03	1.95 ± 0.03	2.37 ± 0.03	NA
As is, w/o cellulose	7(NC)-a	3.20 ± 0.34	2.96 ± 0.07	0.99 ± 0.14	2.24 ± 0.00	2.07 ± 0.10	NA
As is, with cellulose	7(C)-a	3.37 ± 0.27	3.47 ± 0.10	2.99 ± 0.07	1.33 ± 0.34	2.28 ± 1.17	NA
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	3.79 ± 0.04	2.31 ± 0.07	1.73 ± 0.18	0.76 ± 0.11	1.55 ± 0.11	NA
Formalin treated, with cellulose	8(C)-f	3.89 ± 0.03	3.39 ± 0.09	2.54 ± 0.15	1.58 ± 0.00	2.14 ± 0.06	NA
As is, w/o cellulose	8(NC)-a	3.66 ± 0.08	-1.37 ± 0.99	1.41 ± 0.23	1.87 ± 0.08	2.10 ± 0.08	NA
As is, with cellulose	8(C)-a	3.47 ± 0.06	3.63 ± 0.13	2.53 ± 0.16	1.78 ± 0.13	2.22 ± 0.06	NA
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	3.77 ± 0.04	3.35 ± 0.08	6.63 ± 0.30	7.96 ± 1.33	9.07 ± 0.88	8.41 ± 0.48
As is, with cellulose	9(C)-a	3.35 ± 0.09	3.44 ± 0.16	4.04 ± 0.03	11.3 ± 0.5	16.7 ± 1.3	19.2 ± 1.2
As is, with glucose	9(G)-a	2.97 ± 0.08	2.59 ± 0.15	2.36 ± 0.15	1.64 ± 0.15	1.52 ± 0.27	0.18 ± 0.16
Excess nitrate, w/o cellulose	9(NC)-x	3.24 ± 0.04	2.86 ± 0.08	4.30 ± 0.23	6.59 ± 0.76	9.18 ± 0.23	8.65 ± 0.67
Excess nitrate, with cellulose	9(C)-x	3.29 ± 0.13	2.28 ± 0.38	5.76 ± 1.47	16.6 ± 2.9	14.2 ± 3.9	28.9 ± 6.2
Excess nitrate, with glucose	9(G)-x	3.01 ± 0.11	0.61 ± 0.15	0.19 ± 0.11	0.46 ± 0.04	0.46 ± 0.61	1.33 ± 2.08

NA = not analyzed.

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

Treatments* [Brine]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	0.06 ± 0.03	NA	NA	-0.99 ± 0.08	NA	NA
Formalin treated, with cellulose	7(C)-f	1.37 ± 0.00	NA	NA	0.00 ± 0.08	NA	NA
As is, w/o cellulose	7(NC)-a	0.65 ± 0.31	NA	NA	1.22 ± 0.27	NA	NA
As is, with cellulose	7(C)-a	0.20 ± 0.17	NA	NA	0.48 ± 0.20	NA	NA
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	-0.07 ± 0.11	NA	NA	-0.97 ± 0.07	NA	NA
Formalin treated, with cellulose	8(C)-f	1.02 ± 0.03	NA	NA	-0.08 ± 0.12	NA	NA
As is, w/o cellulose	8(NC)-a	-0.50 ± 0.00	NA	NA	0.16 ± 0.04	NA	NA
As is, with cellulose	8(C)-a	2.44 ± 0.63	NA	NA	4.28 ± 0.68	NA	NA
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	8.04 ± 0.46	4.80 ± 0.27	5.87 ± 0.08	8.74 ± 0.23	5.37 ± 0.42	3.85 ± 0.76
As is, with cellulose	9(C)-a	19.4 ± 0.6	20.3 ± 1.0	21.3 ± 1.0	22.3 ± 0.8	22.9 ± 1.0	21.9 ± 0.9
As is, with glucose	9(G)-a	-1.26 ± 0.30	-2.06 ± 0.15	-2.51 ± 0.23	-1.41 ± 0.16	-3.43 ± 0.15	-5.60 ± 0.23
Excess nitrate, w/o cellulose	9(NC)-x	6.93 ± 0.27	4.42 ± 0.30	3.77 ± 0.19	4.30 ± 0.19	3.12 ± 0.04	1.68 ± 0.23
Excess nitrate, with cellulose	9(C)-x	34.1 ± 7.1	38.8 ± 8.5	44.5 ± 8.9	49.3 ± 8.0	57.6 ± 6.0	61.6 ± 3.0
Excess nitrate, with glucose	9(G)-x	1.14 ± 2.90	1.41 ± 3.01	1.56 ± 2.82	2.83 ± 2.74	3.62 ± 0.61	-1.26 ± 2.44

NA = not analyzed.

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

Treatments* [Brine]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	-1.12 ± 0.19	NA	-2.40 ± 0.05	NA	NA	-2.14 ± 0.52
Formalin treated, with cellulose	7(C)-f	-0.29 ± 0.03	NA	-1.31 ± 0.07	NA	NA	-2.05 ± 0.29
As is, w/o cellulose	7(NC)-a	0.78 ± 0.24	NA	-3.93 ± 2.27	-4.27 ± 2.13	-2.01	-1.43
As is, with cellulose	7(C)-a	-0.20 ± 0.17	NA	-1.28 ± 0.23	-1.95 ± 0.19	-3.02 ± 0.22	-2.65 ± 0.23
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	-1.15 ± 0.04	NA	-2.49 ± 0.10	NA	NA	-3.50 ± 0.03
Formalin treated, with cellulose	8(C)-f	-0.44 ± 0.06	NA	-2.31 ± 0.60	NA	NA	-2.98 ± 0.31
As is, w/o cellulose	8(NC)-a	-0.91 ± 0.04	NA	-2.80 ± 0.01	-3.68 ± 0.09	-4.38	-3.73
As is, with cellulose	8(C)-a	5.60 ± 0.41	NA	6.53 ± 0.40	6.52 ± 0.46	6.59 ± 0.55	7.42 ± 0.58
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	4.46 ± 0.84	3.58 ± 1.56	3.64 ± 1.41	4.72 ± 0.00	4.23	4.65
As is, with cellulose	9(C)-a	24.0 ± 1.1	23.6 ± 0.8	25.2 ± 1.0	24.5 ± 0.8	23.6 ± 0.6	23.6 ± 0.5
As is, with glucose	9(G)-a	-3.54 ± 0.00	-4.84 ± 0.04	-4.84 ± 0.16	-5.81 ± 0.20	-7.03 ± 0.12	-7.05 ± 0.19
Excess nitrate, w/o cellulose	9(NC)-x	2.02 ± 0.23	1.03 ± 0.76	-3.81 ± 4.26	1.60 ± 0.00	1.22	1.60
Excess nitrate, with cellulose	9(C)-x	71.0 ± 1.7	71.9 ± 2.9	70.6 ± 2.7	64.3 ± 2.4	64.2 ± 2.4	62.2 ± 2.2
Excess nitrate, with glucose	9(G)-x	0.91 ± 2.48	-0.38 ± 2.48	-0.10 ± 2.57	-2.91 ± 1.39	-2.55 ± 2.53	-2.46 ± 2.55

NA = not analyzed.

Table 3(b). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Anaerobic Samples

Treatments [Brine]	Sample Designation	Carbon Dioxide (μmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	2.26 ± 0.01	1.92 ± 0	2.20 ± 0.20	2.24 ± 0.04	2.33 ± 0.01	NA
Formalin treated, with cellulose	7(C)-f	31.7 ± 0.5	38.0 ± 0.1	38.0 ± 0.1	35.0 ± 0.2	36.4 ± 0.1	NA
As is, w/o cellulose	7(NC)-a	1.12 ± 0.01	0.58 ± 0.1	0.90 ± 0.00	1.37 ± 0.01	1.35 ± 0.01	NA
As is, with cellulose	7(C)-a	13.0 ± 0.4	19.3 ± 0.1	20.5 ± 0.1	19.5 ± 0.1	20.5 ± 0.2	NA
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	4.42 ± 0.01	3.88 ± 0.1	3.89 ± 0.1	3.88 ± 0	3.99 ± 0.03	NA
Formalin treated, with cellulose	8(C)-f	32.1 ± 0.7	34.3 ± 0.4	33.5 ± 0.2	31.2 ± 0.0	32.5 ± 0.5	NA
As is, w/o cellulose	8(NC)-a	1.96 ± 0.02	1.37 ± 0	2.29 ± 0	2.75 ± 0	2.86 ± 0.01	NA
As is, with cellulose	8(C)-a	12.5 ± 0.2	18.4 ± 0.2	19.0 ± 0.1	17.8 ± 0.7	22.7 ± 0.5	NA
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	3.02 ± 0.00	6.86 ± 0	58.9 ± 3.1	95.9 ± 9.5	109 ± 4	119 ± 3
As is, with cellulose	9(C)-a	2.70 ± 0.00	25.8 ± 0.2	42.5 ± 1.7	132 ± 3	200 ± 7	240 ± 3
As is, with glucose	9(G)-a	2.25 ± 0.01	3.36 ± 0	2.96 ± 0	3.50 ± 0.1	3.88 ± 0.06	4.26 ± 0.09
Excess nitrate, w/o cellulose	9(NC)-x	3.07 ± 0.03	5.17 ± 0.3	35.3 ± 5.3	94.5 ± 4.4	120 ± 1	125 ± 1
Excess nitrate, with cellulose	9(C)-x	5.40 ± 0.00	26.6 ± 0.2	65.8 ± 17	193 ± 33	249 ± 32	352 ± 40
Excess nitrate, with glucose	9(G)-x	1.81 ± 0.27	2.88 ± 0.2	2.65 ± 0.3	8.64 ± 3.6	23.6 ± 13.3	53.6 ± 31.4

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 3(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Anaerobic Samples

Treatments [Brine]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	2.39 ± 0.01	NA	NA	2.55 ± 0.08	NA	NA
Formalin treated, with cellulose	7(C)-f	36.3 ± 0.1	NA	NA	0.00 ± 0.00	NA	NA
As is, w/o cellulose	7(NC)-a	1.46 ± 0.03	NA	NA	1.67 ± 0.07	NA	NA
As is, with cellulose	7(C)-a	19.4 ± 0.2	NA	NA	19.3 ± 0.2	NA	NA
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	3.97 ± 0.03	NA	NA	4.93 ± 0.0	NA	NA
Formalin treated, with cellulose	8(C)-f	32.8 ± 0.4	NA	NA	38.7 ± 0.7	NA	NA
As is, w/o cellulose	8(NC)-a	2.74 ± 0.01	NA	NA	3.38 ± 0	NA	NA
As is, with cellulose	8(C)-a	30.1 ± 1.7	NA	NA	34.1 ± 1.5	NA	NA
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	120 ± 1	111 ± 0	121 ± 0	111 ± 0	121 ± 1	131 ± 1
As is, with cellulose	9(C)-a	250 ± 4	244 ± 10	289 ± 2	227 ± 3	302 ± 1	347 ± 2
As is, with glucose	9(G)-a	4.31 ± 0.07	4.11 ± 0.3	4.55 ± 0.4	5.48 ± 0.6	5.02 ± 0.55	4.88 ± 0.5
Excess nitrate, w/o cellulose	9(NC)-x	123 ± 1	117 ± 1	119 ± 0	120 ± 0	120 ± 1	130 ± 0
Excess nitrate, with cellulose	9(C)-x	430 ± 41	398 ± 68	583 ± 43	502 ± 44	766 ± 65	943 ± 65
Excess nitrate, with glucose	9(G)-x	74.9 ± 40.9	75.8 ± 37	82.9 ± 38	92.0 ± 42	88.2 ± 40.7	83.2 ± 38.3

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 3(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Anaerobic Samples

Treatments [Brine]	Sample Designation	Carbon Dioxide (μmoles/sample) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	2.64 ± 0.02	NA	2.75 ± 0	NA	NA	3.5 ± 0.03
Formalin treated, with cellulose	7(C)-f	35.5 ± 0.2	NA	35.2 ± 0.0	NA	NA	36.7 ± 0.1
As is, w/o cellulose	7(NC)-a	1.96 ± 0.16	NA	1.68 ± 0.2	2.12 ± 0.13	1.87	1.13
As is, with cellulose	7(C)-a	20.0 ± 0.2	NA	19.0 ± 0.2	19.1 ± 0.2	18.4 ± 0.2	16.8 ± 0.1
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	4.29 ± 0.02	NA	4.37 ± 0	NA	NA	5.19 ± 0.04
Formalin treated, with cellulose	8(C)-f	31.7 ± 0.2	NA	31.0 ± 0.7	NA	NA	32.5 ± 0.1
As is, w/o cellulose	8(NC)-a	3.58 ± 0.04	NA	3.14 ± 0.1	3.30 ± 0.10	3.86	2.83 ± 0
As is, with cellulose	8(C)-a	52.0 ± 1.2	NA	62.3 ± 1.3	67.5 ± 2.3	74.0 ± 2.7	72.4 ± 4.8
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	126 ± 1	126 ± 0	132 ± 1	129	133	118
As is, with cellulose	9(C)-a	348 ± 3	348 ± 5	377 ± 3	384 ± 3	393 ± 5	364 ± 4
As is, with glucose	9(G)-a	6.19 ± 0.47	5.93 ± 0.3	6.96 ± 0.3	7.33 ± 0.06	8.17 ± 0.34	8.38 ± 0.03
Excess nitrate, w/o cellulose	9(NC)-x	125 ± 1	119 ± 0	113 ± 2	124	129	108
Excess nitrate, with cellulose	9(C)-x	1030 ± 40	1070 ± 20	1140 ± 20	1060 ± 40	1190 ± 10	1080 ± 20
Excess nitrate, with glucose	9(G)-x	89.3 ± 40.6	84.3 ± 38	57.4 ± 17.8	85.1 ± 38.2	85.7 ± 37.4	87.5 ± 38.1

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 3(c). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	NA
Formalin treated, with cellulose	7(C)-f	0.000 ± 0.000	NA	0.009 ± 0.007	0.004 ± 0.001	0.007 ± 0.005	NA
As is, w/o cellulose	7(NC)-a	0.000 ± 0.000	NA	0.003 ± 0.002	0.001 ± 0.000	0.000 ± 0.000	NA
As is, with cellulose	7(C)-a	0.000 ± 0.000	NA	0.002 ± 0.002	0.003 ± 0.002	0.000 ± 0.000	NA
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	NA
Formalin treated, with cellulose	8(C)-f	0.000 ± 0.000	NA	0.002 ± 0.001	0.002 ± 0.000	0.014 ± 0.001	NA
As is, w/o cellulose	8(NC)-a	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	NA
As is, with cellulose	8(C)-a	0.000 ± 0.000	NA	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	NA
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	0.000 ± 0.000	NA	0.367 ± 0.262	35.5 ± 25.1	7.55 ± 5.34	0.000 ± 0.000
As is, with cellulose	9(C)-a	0.000 ± 0.000	NA	0.359 ± 0.145	13.2 ± 10.1	86.5 ± 41.3	89.3 ± 43.0
As is, with glucose	9(G)-a	0.000 ± 0.000	NA	0.002 ± 0.001	0.000 ± 0.000	0.005 ± 0.003	0.005 ± 0.000
Excess nitrate, w/o cellulose	9(NC)-x	0.000 ± 0.000	NA	0.081 ± 0.021	5.13 ± 2.48	0.014 ± 0.002	0.000 ± 0.000
Excess nitrate, with cellulose	9(C)-x	0.000 ± 0.000	NA	0.870 ± 0.420	85.7 ± 50.2	196 ± 75	280 ± 160
Excess nitrate, with glucose	9(G)-x	0.000 ± 0.000	NA	0.028 ± 0.020	3.84 ± 2.72	0.523 ± 0.006	0.539 ± 0.381

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 3(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
Formalin treated, with cellulose	7(C)-f	0.002 ± 0.002	NA	NA	0.002 ± 0.002	NA	NA
As is, w/o cellulose	7(NC)-a	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
As is, with cellulose	7(C)-a	0.002 ± 0.002	NA	NA	0.002 ± 0.001	NA	NA
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	0.000 ± 0.000	NA	NA	0.013 ± 0.004	NA	NA
Formalin treated, with cellulose	8(C)-f	0.008 ± 0.000	NA	NA	0.003 ± 0.002	NA	NA
As is, w/o cellulose	8(NC)-a	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
As is, with cellulose	8(C)-a	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	0.359 ± 0.235	3.16 ± 2.19	6.81 ± 4.83	9.10 ± 6.40	15.1 ± 10.7	21.3 ± 15.0
As is, with cellulose	9(C)-a	77.8 ± 42.2	64.9 ± 38.6	64.8 ± 44.4	60.1 ± 44.1	52.8 ± 40.7	51.7 ± 42.2
As is, with glucose	9(G)-a	0.015 ± 0.010	0.204 ± 0.14	0.265 ± 0.180	0.332 ± 0.224	0.266 ± 0.178	0.228 ± 0.151
Excess nitrate, w/o cellulose	9(NC)-x	0.000 ± 0.000	0.000 ± 0.00	0.000 ± 0.000	0 ± 0	0.000 ± 0.000	0.007 ± 0.005
Excess nitrate, with cellulose	9(C)-x	395 ± 228	231 ± 181	675 ± 249	845 ± 299	953 ± 170	1190 ± 60
Excess nitrate, with glucose	9(G)-x	13.800 ± 0.500	31.2 ± 1.00	32.6 ± 6.20	34.2 ± 6.70	32.4 ± 6.70	30.8 ± 6.40

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 3(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (μmoles/sample) Incubation Time (Days) 481	592	733	853	1034	1228
Unamended/Uninoculated (7)							
Formalin treated, w/o cellulose	7(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
Formalin treated, with cellulose	7(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.120 ± 0.000
As is, w/o cellulose	7(NC)-a	0.205 ± 0.142	NA	0.000 ± 0.000	0.650 ± 0.060	0.000 ± 0.000	0.000 ± 0.000
As is, with cellulose	7(C)-a	0.004 ± 0.003	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Unamended/Inoculated (8)							
Formalin treated, w/o cellulose	8(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
Formalin treated, with cellulose	8(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
As is, w/o cellulose	8(NC)-a	0.012 ± 0.008	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
As is, with cellulose	8(C)-a	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Amended/Inoculated (9)							
As is, w/o cellulose	9(NC)-a	23.8 ± 16.8	20.1 ± 14.2	16.3 ± 11.6	32.1	32.8	30.4
As is, with cellulose	9(C)-a	50.9 ± 41.5	48.6 ± 38.2	51.5 ± 38.9	47.3 ± 38.6	45.5 ± 34.0	126 ± 0
As is, with glucose	9(G)-a	0.287 ± 0.165	0.200 ± 0.140	0.290 ± 0.210	0.000 ± 0.000	0.370 ± 0.260	0.530 ± 0.060
Excess nitrate, w/o cellulose	9(NC)-x	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Excess nitrate, with cellulose	9(C)-x	1310 ± 40	1230 ± 60	1280 ± 50	1120 ± 60	719 ± 292	1100 ± 70.0
Excess nitrate, with glucose	9(G)-x	31.4 ± 6.1	29.0 ± 5.3	29.4 ± 4.4	28.4 ± 4.4	27.1 ± 4.3	24.5 ± 4.1

* Dissolved gas concentrations not included.
NA = not analyzed.

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

Treatments* [Brine/Bentonite]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	4.65 ± 0.23	4.61 ± 0.47	2.37 ± 1.47	3.41 ± 0.19	4.07 ± 0.04	NA
Formalin treated, with cellulose	10(C)-f	4.96 ± 0.06	4.64 ± 0.03	4.22 ± 0.00	2.94 ± 0.16	3.39 ± 0.16	NA
As is, w/o cellulose	10(NC)-a	4.24 ± 0.33	4.04 ± 0.04	3.51 ± 0.69	2.86 ± 0.08	3.27 ± 0.20	NA
As is, with cellulose	10(C)-a	3.84 ± 0.48	3.84 ± 0.14	3.33 ± 0.27	2.01 ± 0.41	2.18 ± 0.48	NA
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	5.16 ± 0.07	5.01 ± 0.14	3.89 ± 0.22	2.85 ± 0.25	3.57 ± 0.25	NA
Formalin treated, with cellulose	11(C)-f	4.65 ± 0.03	4.53 ± 0.00	3.66 ± 0.03	1.73 ± 1.14	2.16 ± 1.08	NA
As is, w/o cellulose	11(NC)-a	4.23 ± 0.11	3.62 ± 0.08	3.47 ± 0.19	2.06 ± 0.19	2.44 ± 0.30	NA
As is, with cellulose	11(C)-a	4.38 ± 0.03	4.19 ± 0.06	3.19 ± 0.16	2.88 ± 0.22	3.88 ± 0.19	NA
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	3.85 ± 0.08	4.53 ± 0.15	6.97 ± 0.15	9.90 ± 0.08	10.9 ± 0.1	11.1 ± 0.3
As is, with cellulose	12(C)-a	3.32 ± 0.25	4.29 ± 0.09	7.92 ± 0.44	16.8 ± 0.4	19.8 ± 0.4	18.3 ± 0.3
As is, with glucose	12(G)-a	3.50 ± 0.04	5.52 ± 0.34	9.49 ± 2.40	24.7 ± 3.3	33.9 ± 0.6	38.8 ± 1.8
Excess nitrate, w/o cellulose	12(NC)-x	3.66 ± 0.00	4.19 ± 0.15	8.19 ± 0.69	11.8 ± 0.3	12.4 ± 0.0	12.8 ± 0.1
Excess nitrate, with cellulose	12(C)-x	3.38 ± 0.09	3.76 ± 0.16	9.36 ± 0.31	15.7 ± 0.4	20.8 ± 0.5	23.8 ± 0.7
Excess nitrate, with glucose	12(G)-x	3.09 ± 0.08	2.29 ± 0.19	2.21 ± 0.46	23.4 ± 0.6	52.3 ± 6.7	77.4 ± 20.2

NA = not analyzed.

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

Treatments* [Brine/Bentonite]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 200	228	264	(297	(356	411
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	2.33 ± 0.00	NA	NA	0.86 ± 0.00	NA	NA
Formalin treated, with cellulose	10(C)-f	2.05 ± 0.16	NA	NA	1.08 ± 0.03	NA	NA
As is, w/o cellulose	10(NC)-a	1.55 ± 0.08	NA	NA	0.12 ± 0.04	NA	NA
As is, with cellulose	10(C)-a	0.14 ± 0.44	NA	NA	0.86 ± 0.41	NA	NA
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	1.23 ± 0.22	NA	NA	0.89 ± 0.40	NA	NA
Formalin treated, with cellulose	11(C)-f	0.94 ± 1.20	NA	NA	0.23 ± 1.11	NA	NA
As is, w/o cellulose	11(NC)-a	0.34 ± 0.34	NA	NA	-0.42 ± 0.06	NA	NA
As is, with cellulose	11(C)-a	4.38 ± 0.38	NA	NA	6.96 ± 0.68	NA	NA
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	9.87 ± 0.80	7.35 ± 0.42	6.67 ± 0.91	7.73 ± 0.86	6.55 ± 0.88	5.26 ± 0.88
As is, with cellulose	12(C)-a	19.5 ± 0.4	19.8 ± 0.7	20.6 ± 0.9	21.8 ± 1.9	22.7 ± 2.4	22.8 ± 3.0
As is, with glucose	12(G)-a	37.8 ± 2.3	37.8 ± 2.7	39.4 ± 2.2	40.9 ± 2.4	41.0 ± 2.4	37.5 ± 2.1
Excess nitrate, w/o cellulose	12(NC)-x	11.2 ± 0.1	9.33 ± 0.04	8.91 ± 0.27	8.86 ± 0.30	8.30 ± 0.19	6.74 ± 0.30
Excess nitrate, with cellulose	12(C)-x	28.9 ± 1.4	33.1 ± 2.1	44.0 ± 4.4	68.4 ± 7.7	76.8 ± 5.9	86.0 ± 2.8
Excess nitrate, with glucose	12(G)-x	80.7 ± 21.1	65.5 ± 11.3	67.5 ± 12.8	88.5 ± 12.6	65.9 ± 13.4	60.3 ± 12.7

NA = not analyzed.

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

Treatments* [Brine/Bentonite]	Sample Designation	Volume of Gas Produced (ml) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	-0.39 ± 0.00	NA	-0.39 ± 0.11	NA	NA	-1.32 ± 0.11
Formalin treated, with cellulose	10(C)-f	0.74 ± 0.19	NA	-0.21 ± 0.12	NA	NA	-0.82 ± 0.10
As is, w/o cellulose	10(NC)-a	-0.41 ± 0.00	NA	-5.16 ± 2.44	-6.04 ± 2.25	-3.63	-2.98
As is, with cellulose	10(C)-a	-1.22 ± 0.41	NA	-1.35 ± 0.32	-2.11 ± 0.26	-3.22 ± 0.31	-2.96 ± 0.21
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	-0.36 ± 0.00	NA	-0.67 ± 0.50	NA	NA	-1.42 ± 0.47
Formalin treated, with cellulose	11(C)-f	0.00 ± 1.02	NA	-1.08 ± 1.05	NA	NA	-2.96 ± 0.21
As is, w/o cellulose	11(NC)-a	-1.14 ± 0.27	NA	-2.11 ± 0.28	-2.97 ± 0.27	-3.73	-3.28
As is, with cellulose	11(C)-a	5.95 ± 1.35	NA	9.14 ± 0.92	8.28 ± 0.87	8.22 ± 0.90	8.65 ± 1.02
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	9.18 ± 0.19	6.06 ± 0.84	4.46 ± 0.32	3.73 ± 0.00	3.73	3.92
As is, with cellulose	12(C)-a	24.2 ± 3.8	26.4 ± 4.5	21.1 ± 7.1	24.3 ± 3.8	22.8 ± 3.3	22.0 ± 2.8
As is, with glucose	12(G)-a	0.91 ± 2.48	17.7 ± 12.5	17.1 ± 12.1	15.3 ± 11.5	12.9 ± 10.7	12.3 ± 10.3
Excess nitrate, w/o cellulose	12(NC)-x	9.18 ± 0.19	5.94 ± 1.07	6.91 ± 0.44	6.40 ± 0.00	6.10	6.25
Excess nitrate, with cellulose	12(C)-x	91.4 ± 0.8	96.2 ± 1.6	94.5 ± 2.6	89.3 ± 3.1	85.8 ± 3.1	81.0 ± 2.8
Excess nitrate, with glucose	12(G)-x	62.8 ± 13.0	58.9 ± 12.5	57.1 ± 11.9	54.0 ± 11.6	50.3 ± 10.9	48.1 ± 10.5

NA = not analyzed.

Table 4(b). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Anaerobic Samples

Treatments [Brine/Bentonite]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	55.8 ± 0.6	82.9 ± 1.1	85.5 ± 0.8	86.4 ± 1.7	92.8 ± 2.0	NA
Formalin treated, with cellulose	10(C)-f	65.5 ± 1.0	85.5 ± 0.2	88.6 ± 0.4	81.9 ± 0.3	88.7 ± 0.9	NA
As is, w/o cellulose	10(NC)-a	16.4 ± 0.4	37.7 ± 0.0	38.4 ± 0.1	38.1 ± 0.3	39.8 ± 0.3	NA
As is, with cellulose	10(C)-a	26.6 ± 0.5	42.6 ± 0.2	43.0 ± 0.4	41.3 ± 0.2	43.1 ± 0.5	NA
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	53.0 ± 0.2	76.9 ± 0.6	80.2 ± 0.4	76.5 ± 0.0	83.7 ± 0.6	NA
Formalin treated, with cellulose	11(C)-f	64.4 ± 1.3	76.3 ± 0.5	78.6 ± 0.2	75.1 ± 1.9	78.6 ± 1.6	NA
As is, w/o cellulose	11(NC)-a	20.8 ± 0.0	35.0 ± 0.1	36.3 ± 0.1	35.2 ± 0.2	19.6 ± 11.9	NA
As is, with cellulose	11(C)-a	30.1 ± 0.6	38.1 ± 0.2	40.5 ± 0.1	48.0 ± 2.5	59.9 ± 0.7	NA
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	14.0 ± 0.0	60.2 ± 0.2	99.8 ± 4.7	150 ± 4	16.6 ± 0.4	177 ± 1
As is, with cellulose	12(C)-a	12.0 ± 0.8	55.0 ± 0.3	104 ± 5	209 ± 2	260 ± 8	295 ± 10
As is, with glucose	12(G)-a	15.2 ± 0.6	76.0 ± 2.4	144 ± 28	375 ± 36	590 ± 15	691 ± 20
Excess nitrate, w/o cellulose	12(NC)-x	13.9 ± 0.3	69.2 ± 0.0	121 ± 3	166 ± 1	186 ± 2	196 ± 2
Excess nitrate, with cellulose	12(C)-x	10.3 ± 0.6	57.4 ± 1.7	122 ± 3	195 ± 5	264 ± 6	309 ± 7
Excess nitrate, with glucose	12(G)-x	15.1 ± 0.0	69.4 ± 1.2	111 ± 4	422 ± 12	916 ± 35	1370 ± 20

Dissolved gas concentrations not included.
NA = not analyzed.

Table 4(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Anaerobic Samples

Treatments [Brine/Bentonite]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	94.9 ± 1.1	NA	NA	100 ± 1	NA	NA
Formalin treated, with cellulose	10(C)-f	90.2 ± 0.3	NA	NA	97.6 ± 1.0	NA	NA
As is, w/o cellulose	10(NC)-a	40.0 ± 0.4	NA	NA	39.5 ± 0.7	NA	NA
As is, with cellulose	10(C)-a	41.1 ± 0.2	NA	NA	43.7 ± 0.3	NA	NA
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	83.5 ± 0.2	NA	NA	45.1 ± 32	NA	NA
Formalin treated, with cellulose	11(C)-f	80.4 ± 0.8	NA	NA	83.4 ± 0.2	NA	NA
As is, w/o cellulose	11(NC)-a	36.2 ± 0.2	NA	NA	37.1 ± 0.1	NA	NA
As is, with cellulose	11(C)-a	77.6 ± 1.0	NA	NA	112 ± 3	NA	NA
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	179 ± 0	172 ± 0	178 ± 1	185 ± 2	173 ± 1	189 ± 0
As is, with cellulose	12(C)-a	338 ± 10	297 ± 11	432 ± 10	479 ± 14	507 ± 15	603 ± 27
As is, with glucose	12(G)-a	786 ± 25	823 ± 33	983 ± 21	1070 ± 24	1100 ± 30	1130 ± 20
Excess nitrate, w/o cellulose	12(NC)-x	196 ± 2	191 ± 2	195 ± 3	202 ± 2	194 ± 4	208 ± 4
Excess nitrate, with cellulose	12(C)-x	371 ± 14	444 ± 17	579 ± 27	783 ± 52	1150 ± 120	1650 ± 80
Excess nitrate, with glucose	12(G)-x	1610 ± 40	1460 ± 60	1720 ± 90	1730 ± 110	1650 ± 120	1690 ± 110

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 4(b)(continued). Long-Term Inundated Experiment: Production of Carbon Dioxide* in Anaerobic Samples

Treatments [Brine/Bentonite]	Sample Designation	Carbon Dioxide (µmoles/sample) Incubation Time (Days) 481	591	733	853	1034	1228
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	105 ± 2	NA	114 ± 1	NA	NA	136 ± 2
Formalin treated, with cellulose	10(C)-f	99.8 ± 0.5	NA	107 ± 0	NA	NA	123 ± 0
As is, w/o cellulose	10(NC)-a	40.0 ± 0.0	NA	36.6 ± 0.2	34.94 ± 0.17	38.8 ± 0	35.5
As is, with cellulose	10(C)-a	42.1 ± 0.0	NA	40.6 ± 0.4	39.95 ± 0.15	40.2 ± 0.6	59.0 ± 24.5
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	94.0 ± 0.0	NA	104 ± 1	NA	NA	124 ± 0
Formalin treated, with cellulose	11(C)-f	87.3 ± 0.5	NA	93.8 ± 0.5	NA	NA	107 ± 1
As is, w/o cellulose	11(NC)-a	36.0 ± 0.0	NA	35.6 ± 0.2	34.0 ± 0.2	17.8 ± 12.6	32.2 ± 0
As is, with cellulose	11(C)-a	171 ± 5	NA	250 ± 4	262 ± 4	281 ± 5	308 ± 7
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	190 ± 1	182 ± 1	189 ± 5	177	182	164
As is, with cellulose	12(C)-a	602 ± 27	621 ± 25	615 ± 26	658 ± 26	650 ± 26	661 ± 22
As is, with glucose	12(G)-a	678 ± 0	879 ± 54	994 ± 63	964 ± 57	921 ± 45	928 ± 40
Excess nitrate, w/o cellulose	12(NC)-x	201 ± 2	194 ± 1	205 ± 1	196	101	181
Excess nitrate, with cellulose	12(C)-x	1830 ± 40	2010 ± 40	2140 ± 60	2120 ± 70	2020 ± 80	2030 ± 70
Excess nitrate, with glucose	12(G)-x	1740 ± 120	1640 ± 110	1690 ± 100	1660 ± 100	1530 ± 150	1540 ± 150

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 4(c). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 0	45	69	104	132	164
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	NA
Formalin treated, with cellulose	10(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	0.001 ± 0.000	0.000 ± 0.000	NA
As is, w/o cellulose	10(NC)-a	0.000 ± 0.000	NA	0.004 ± 0.003	0.102 ± 0.065	1.04 ± 0.490	NA
As is, with cellulose	10(C)-a	0.000 ± 0.000	NA	0.005 ± 0.002	0.008 ± 0.004	0.021 ± 0.009	NA
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	NA
Formalin treated, with cellulose	11(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	0.006 ± 0.002	0.024 ± 0	NA
As is, w/o cellulose	11(NC)-a	0.000 ± 0.000	NA	0.001 ± 0.001	0.000 ± 0.000	0.037 ± 0.03	NA
As is, with cellulose	11(C)-a	0.000 ± 0.000	NA	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000	NA
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	0.000 ± 0.000	NA	0.016 ± 0.006	0.534 ± 0.378	0.000 ± 0.000	0.000 ± 0.000
As is, with cellulose	12(C)-a	0.000 ± 0.000	NA	0.111 ± 0.056	2.34 ± 1.92	0.000 ± 0.000	0.300 ± 0.250
As is, with glucose	12(G)-a	0.000 ± 0.000	NA	1.56 ± 1.10	40.1 ± 28.3	0.780 ± 0.560	0.016 ± 0.011
Excess nitrate, w/o cellulose	12(NC)-x	0.000 ± 0.000	NA	0.007 ± 0.001	0.081 ± 0.057	0.000 ± 0.000	0.003 ± 0.002
Excess nitrate, with cellulose	12(C)-x	0.004 ± 0.000	NA	3.80 ± 2.39	0.022 ± 0.018	47.1 ± 17.8	154 ± 20.0
Excess nitrate, with glucose	12(G)-x	0.005 ± 0.000	NA	0.077 ± 0.049	2.31 ± 1.59	261 ± 184	616 ± 428

Dissolved gas concentrations not included.
NA = not analyzed.

Table 4(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 200	228	264	297	356	411
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
Formalin treated, with cellulose	10(C)-f	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
As is, w/o cellulose	10(NC)-a	1.73 ± 0.100	NA	NA	2.06 ± 0.110	NA	NA
As is, with cellulose	10(C)-a	0.031 ± 0.011	NA	NA	0.035 ± 0.004	NA	NA
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	0.002 ± 0.001	NA	NA	0.000 ± 0.000	NA	NA
Formalin treated, with cellulose	11(C)-f	0.000 ± 0.000	NA	NA	0.004 ± 0.003	NA	NA
As is, w/o cellulose	11(NC)-a	0.007 ± 0.005	NA	NA	0.011 ± 0.008	NA	NA
As is, with cellulose	11(C)-a	0.000 ± 0.000	NA	NA	0.000 ± 0.000	NA	NA
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	3.35 ± 2.19	4.42 ± 2.83	5.93 ± 2.68	8.67 ± 2.29	11.2 ± 1.5	15.4 ± 0.800
As is, with cellulose	12(C)-a	0.000 ± 0.000	2.29 ± 0.80	1.43 ± 1.16	4.23 ± 0.18	4.61 ± 0.11	1.54 ± 1.26
As is, with glucose	12(G)-a	0.000 ± 0.000	6.94 ± 0.30	3.84 ± 2.71	9.59 ± 0.19	9.51 ± 0.20	8.61 ± 0.16
Excess nitrate, w/o cellulose	12(NC)-x	0.481 ± 0.340	1.78 ± 1.11	2.08 ± 0.780	3.20 ± 0.260	6.49 ± 1.35	11.4 ± 203
Excess nitrate, with cellulose	12(C)-x	311 ± 23	487 ± 23	736 ± 82	1070 ± 94	1350 ± 120	1240 ± 120
Excess nitrate, with glucose	12(G)-x	585 ± 406	480 ± 331	494 ± 349	484 ± 331	436 ± 300	441 ± 303

* Dissolved gas concentrations not included.
NA = not analyzed.

Table 4(c)(continued). Long-Term Inundated Experiment: Production of Nitrous Oxide* in Aerobic Samples

Treatments* [Brine]	Sample Designation	Nitrous Oxide (µmoles/sample) Incubation Time (Days) 481	592	733	853	1034	1228
Unamended/Uninoculated (10)							
Formalin treated, w/o cellulose	10(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
Formalin treated, with cellulose	10(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
As is, w/o cellulose	10(NC)-a	2.02 ± 0.080	NA	2.03 ± 0.080	1.85 ± 0.030	1.80 ±	1.28
As is, with cellulose	10(C)-a	0.338 ± 0.217	NA	0.340 ± 0.150	0.640 ± 0.310	1.05 ± 0.450	0.000 ± 0.000
Unamended/Inoculated (11)							
Formalin treated, w/o cellulose	11(NC)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
Formalin treated, with cellulose	11(C)-f	0.000 ± 0.000	NA	0.000 ± 0.000	NA	NA	0.000 ± 0.000
As is, w/o cellulose	11(NC)-a	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
As is, with cellulose	11(C)-a	0.000 ± 0.000	NA	0.000 ± 0.000	0.000 ± 0.000	2.64 ± 0.040	0.000 ± 0.000
Amended/Inoculated (12)							
As is, w/o cellulose	12(NC)-a	17.2 ± 3.1	14.8 ± 6.50	20.4 ± 6.80	13.7	17.2	19.4
As is, with cellulose	12(C)-a	5.10 ± 0.26	6.40 ± 0.25	6.08 ± 0.34	0.000 ± 0.000	5.58 ± 0.15	5.50 ± 0.22
As is, with glucose	12(G)-a	5.75 ± 0.00	7.45 ± 0.52	9.73 ± 0.61	10.3 ± 0.10	7.54 ± 0.33	7.46 ± 0.31
Excess nitrate, w/o cellulose	12(NC)-x	16.1 ± 2.30	19.8 ± 2.20	38.2 ± 6.40	30.8	32.6	33.1
Excess nitrate, with cellulose	12(C)-x	1170 ± 110	1140 ± 110	1120 ± 100	1070 ± 90	956 ± 84	883 ± 75
Excess nitrate, with glucose	12(G)-x	442 ± 303	407 ± 277	414 ± 282	393 ± 267	357 ± 245	319 ± 218

* Dissolved gas concentrations not included.
NA = not analyzed.

APPENDIX B: GAS PRODUCTION DATA FOR THE ELECTRON-BEAM IRRADIATED PLASTIC AND RUBBER BIODEGRADATION EXPERIMENT

SECTION 1: POLYETHYLENE
SECTION 2: POLYVINYLCHLORIDE
SECTION 3: NEOPRENE
SECTION 4: UNLEADED HYPALON
SECTION 5: LEADED HYPALON

APPENDIX B
SECTION 1: POLYETHYLENE

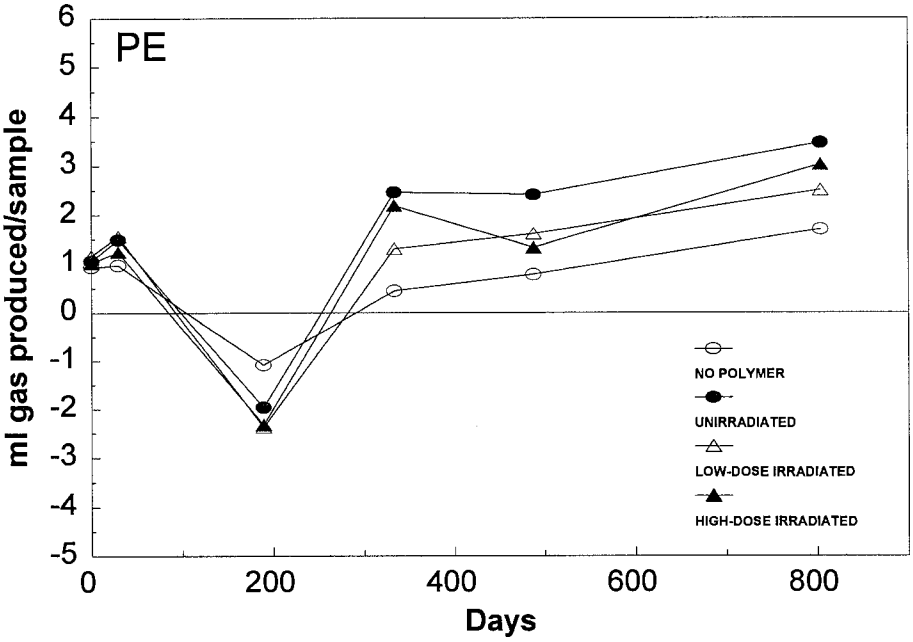

Figure 1. Total gas produced in aerobic unamended samples containing polyethylene.

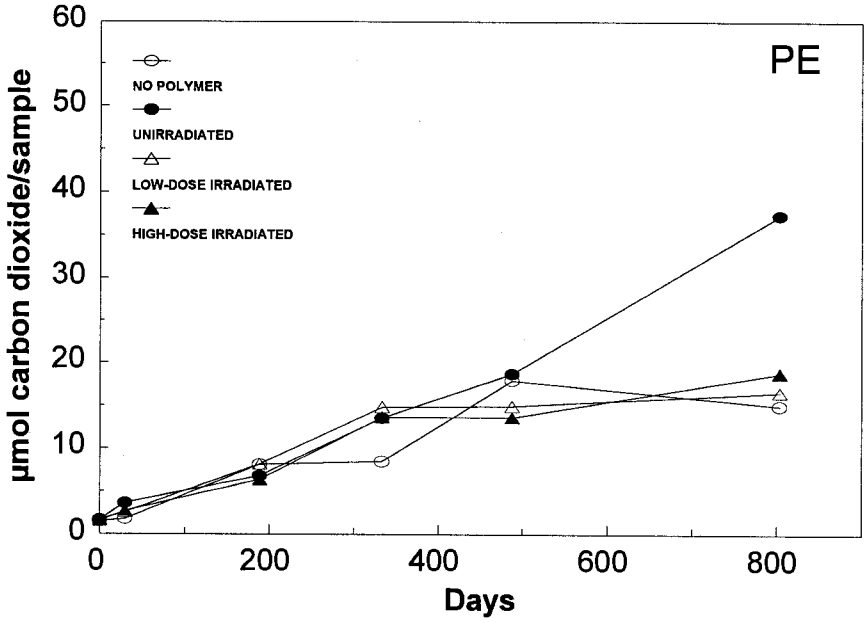

Figure 2. Carbon dioxide produced in aerobic unamended samples containing polyethylene.

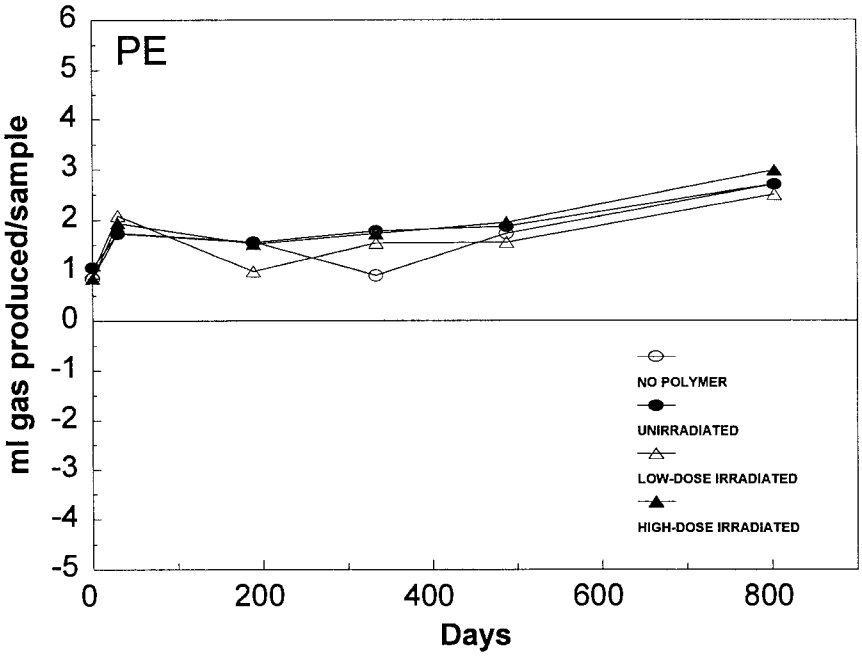

Figure 3. Total gas produce in aerobic amended samples containing polyethylene.

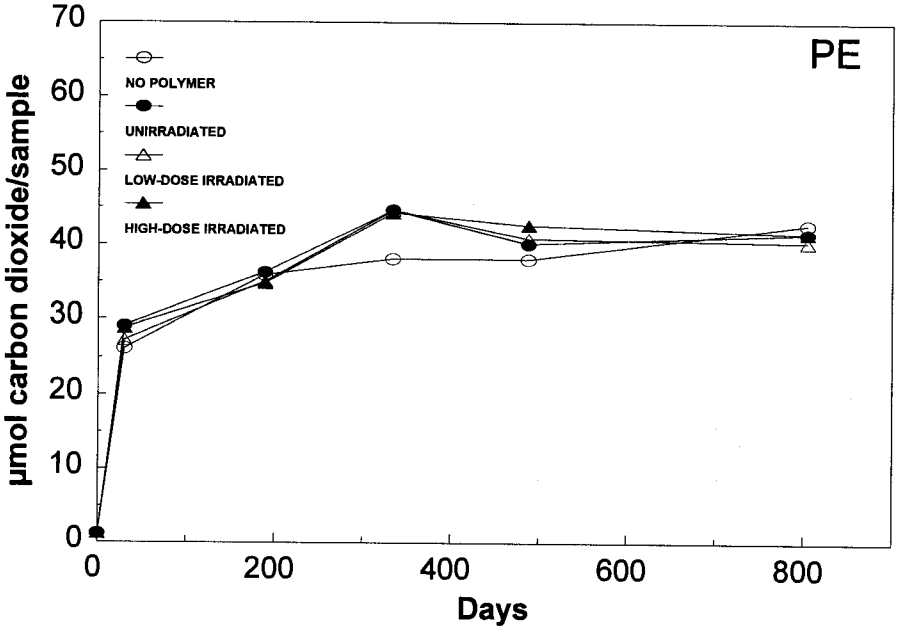

Figure 4. Carbon dioxide produced in aerobic amended samples containing polyethylene.

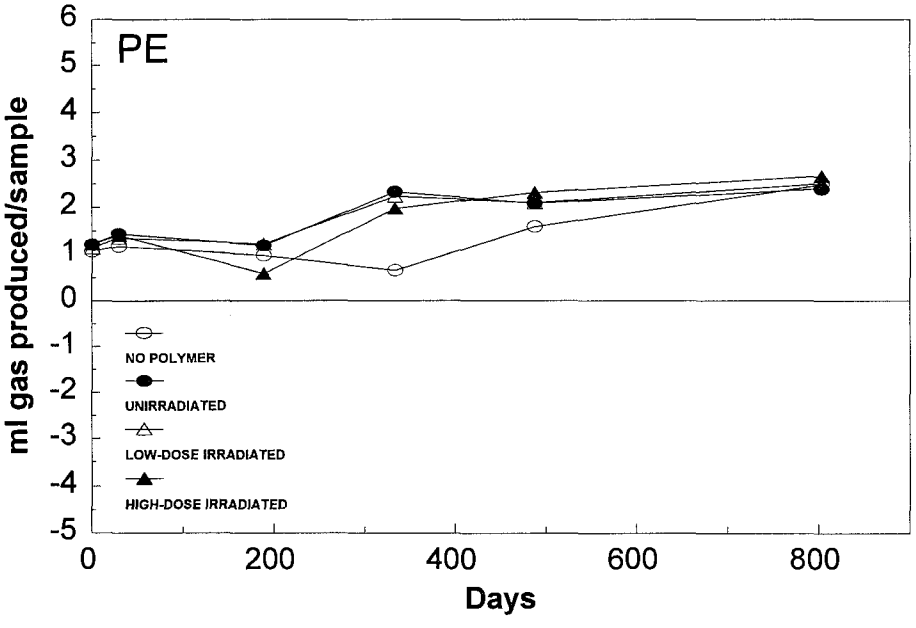

Figure 5. Total gas produced in anaerobic unamended samples containing polyethylene.

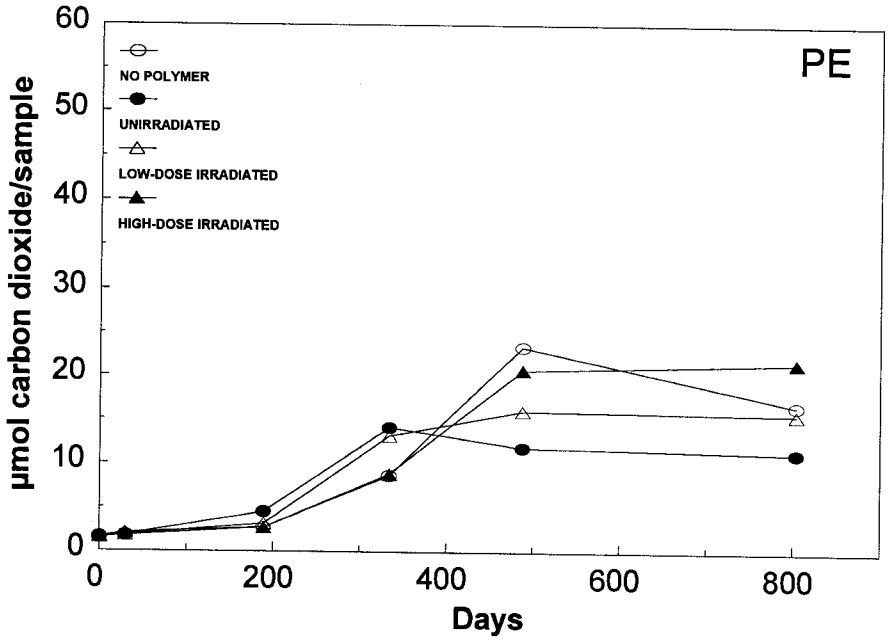

Figure 6. Carbon dioxide produced in anaerobic unamended samples containing polyethylene.

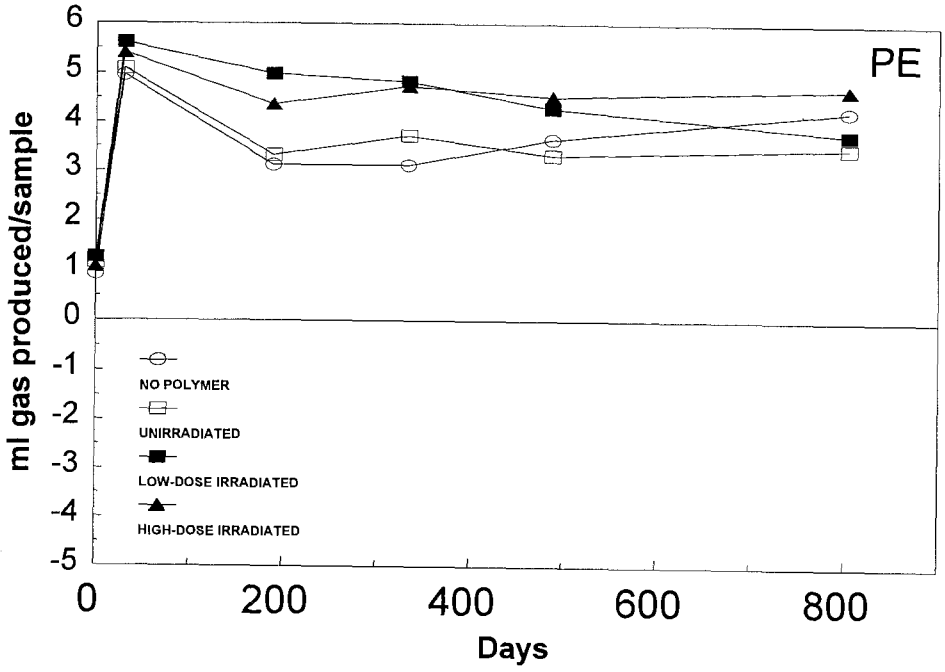

Figure 7. Total gas produced in anaerobic amended samples containing polyethylene.

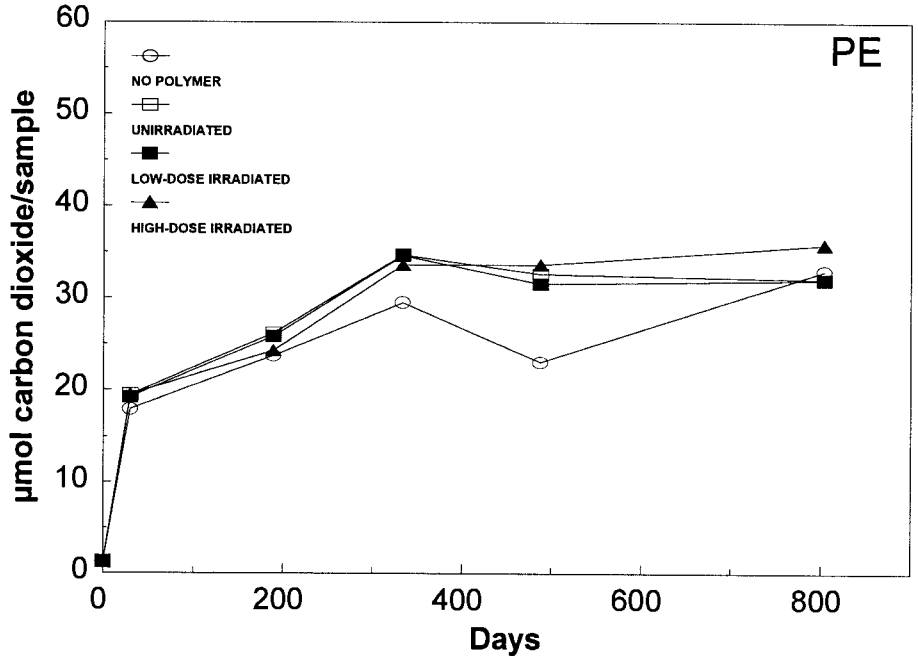

Figure 8. Carbon dioxide produced in anaerobic amended samples containing polyethylene.

Table 1. Oxygen Consumption in Aerobic/Sealed Samples Containing Polyethylene, Polyvinylchloride and Neoprene.

Sample	Oxygen (%)					
	0	30	189	334	488	840
No Plastic or Rubber						
Unamended	21.00	20.18	6.83 ± 0.53	9.97 ± 0.61	7.59 ± 1.28	5.83 ± 1.60
Amended	21.00	6.43	1.29 ± 0.15	2.15 ± 0.37	2.41 ± 0.18	2.34 ± 0.09
Polyethylene						
Unamended						
Unirradiated	21.00	16.50	4.07	7.25	8.21	5.17
Irradiated (Low-Dose)	21.00	19.27	1.71	3.32	3.09	3.11
Irradiated (High-Dose)	21.00	18.51	2.24	4.60	3.62	3.42
Amended						
Unirradiated	21.00	4.93	1.09 ± 0.00	1.89 ± 0.10	2.97 ± 0.10	4.54 ± 1.39
Irradiated (Low-Dose)	21.00	6.72	1.19 ± 0.03	3.68 ± 0.70	5.49 ± 0.70	4.17 ± 1.09
Irradiated (High-Dose)	21.00	6.05	1.14 ± 0.02	2.75 ± 0.25	5.40 ± 0.25	11.53 ± 0.16
Polyvinylchloride						
Unamended						
Unirradiated	21.00	2.82	2.51	4.35	3.37	3.23
Irradiated (Low-Dose)	21.00	19.39	1.44	2.69	3.19	2.90
Irradiated (High-Dose)	21.00	NA	1.86	2.93	3.24	4.03
Amended						
Unirradiated	21.00	5.56	1.13 ± 0.01	1.77 ± 0.12	1.75 ± 0.09	2.38 ± 0.11
Irradiated (Low-Dose)	21.00	11.47	3.90 ± 1.09	5.16 ± 0.76	4.94 ± 0.76	5.09 ± 0.62
Irradiated (High-Dose)	21.00	1.59	1.23 ± 0.00	2.28 ± 0.74	2.03 ± 0.62	2.08 ± 0.43
Neoprene						
Unamended						
Unirradiated	21.00	NA	2.87	5.29	4.75	3.35
Irradiated (Low-Dose)	21.00	NA	2.44	3.45	3.09	2.28
Irradiated (High-Dose)	21.00	NA	NA	3.70	5.44	2.29
Amended						
Unirradiated	21.00	6.48	1.16 ± 0.03	1.68 ± 0.22	2.02 ± 0.22	2.28 ± 0.12
Irradiated (Low-Dose)	21.00	5.35	1.15 ± 0.01	3.53 ± 1.07	5.05 ± 1.07	6.35 ± 2.28
Irradiated (High-Dose)	21.00	5.98	1.16 ± 0.00	2.22 ± 0.05	3.67 ± 0.05	2.88 ± 0.17

NA = Not Analyzed

Table 2. Total Volume of Gas Produced in Samples Containing Polyethylene.

Sample	Milliliters of Gas Produced/Sample Days 0	30	189	334	488	840
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
Amended	0.85	1.74 ± 0.17	1.56 ± 0.03	0.90 ± 0.48	1.73 ± 0.57	2.69 ± 0.59
Anaerobic						
Unamended	1.07	1.17 ± 0.05	0.98 ± 0.08	0.66 ± 0.37	1.59 ± 0.42	2.48 ± 0.34
Amended	0.93	4.96 ± 0.24	3.13 ± 1.19	3.13 ± 1.15	3.66 ± 0.98	4.24 ± 0.82
Polyethylene - Aerobic						
Unamended						
Unirradiated	1.06	1.50	-1.97	2.47	2.42	3.46
Irradiated (Low-Dose)	1.17	1.56	-2.37	1.30	1.61	2.51
Irradiated (High-Dose)	1.02	1.25	-2.32	2.19	1.33	3.02
Amended						
Unirradiated	1.06	1.73 ± 0.05	1.55 ± 0.34	1.78 ± 0.49	1.87 ± 0.44	2.70 ± 0.25
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
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
Polyethylene - Anaerobic						
Unamended						
Unirradiated	1.21	1.44	1.19	2.34	2.09	2.40
Irradiated (Low-Dose)	1.14	1.35	1.22	2.24	2.10	2.51
Irradiated (High-Dose)	1.22	1.41	0.59	1.98	2.32	2.67
Amended						
Unirradiated	1.15	5.09 ± 0.06	3.33 ± 0.92	3.73 ± 0.91	3.33 ± 0.45	3.48 ± 0.58
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
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

Table 3. Carbon Dioxide Produced in Samples Containing Polyethylene.

Sample	Carbon Dioxide (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	1.50	1.76 ± 0.13	8.11 ± 0.33	8.48 ± 0.39	17.87 ± 0.69	14.96 ± 1.67
Amended	1.21	26.11 ± 0.17	35.86 ± 0.39	38.04 ± 0.85	64.15 ± 2.25	42.68 ± 2.14
Anaerobic						
Unamended	1.52	1.76 ± 0.05	2.71 ± 0.08	8.60 ± 0.50	23.31 ± 0.31	16.59 ± 1.92
Amended	1.21	17.95 ± 0.24	23.67 ± 0.05	29.48 ± 0.58	50.33 ± 0.97	32.94 ± 0.69
Polyethylene - Aerobic						
Unamended						
Unirradiated	1.70	3.63	6.81	13.59	18.67	37.32
Irradiated (Low-Dose)	1.67	2.57	8.16	14.80	14.90	16.51
Irradiated (High-Dose)	1.56	2.70	6.37	13.68	13.62	18.73
Amended						
Unirradiated	1.29	29.09 ± 0.26	36.27 ± 0.16	44.59 ± 0.73	40.11 ± 1.02	41.48 ± 2.69
Irradiated (Low-Dose)	1.23	27.26 ± 0.33	34.95 ± 0.29	44.59 ± 0.72	40.79 ± 1.64	40.31 ± 2.19
Irradiated (High-Dose)	1.25	28.84 ± 0.06	34.77 ± 0.42	44.26 ± 1.31	42.58 ± 0.16	41.52 ± 0.26
Polyethylene - Anaerobic						
Unamended						
Unirradiated	1.66	1.83	4.53	14.00	11.74	11.21
Irradiated (Low-Dose)	1.58	1.82	3.15	13.07	15.92	15.58
Irradiated (High-Dose)	1.63	2.10	2.71	8.80	20.60	21.54
Amended						
Unirradiated	1.29	19.49 ± 0.09	26.13 ± 0.10	34.67 ± 0.44	32.58 ± 0.44	32.02 ± 2.27
Irradiated (Low-Dose)	1.35	19.23 ± 0.24	25.80 ± 0.50	34.59 ± 0.86	31.52 ± 1.06	31.95 ± 0.66
Irradiated (High-Dose)	1.23	19.51 ± 0.21	24.25 ± 0.31	33.59 ± 0.06	33.60 ± 1.27	35.80 ± 2.22

Table 4. Nitrous Oxide Produced in Samples Containing Polyethylene.

Sample	Nitrous Oxide (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	0.006	ND	0.33 ± 0.27	0.32 ± 0.16	ND
Anaerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Polyethylene - Aerobic						
Unamended						
Unirradiated	ND	0.002	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	0.001	ND	ND	ND	ND
Amended						
Unirradiated	ND	0.001	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	0.002	ND	ND	ND	ND
Irradiated (High-Dose)	ND	0.003	ND	ND	ND	ND
Polyethylene - Anaerobic						
Unamended						
Unirradiated	0.001	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	0.001	ND	ND	ND	ND	ND
Irradiated (High-Dose)	0.001	ND	ND	ND	ND	ND
Amended						
Unirradiated	0.001	0.001	ND	ND	ND	ND
Irradiated (Low-Dose)	0.001	ND	ND	ND	ND	ND
Irradiated (High-Dose)	0.001	ND	ND	ND	ND	ND

ND = None Detected

Table 5. Methane Produced in Samples Containing Polyethylene.

Sample	Methane (µmoles/sample) Days 0	30	189	334	488	804
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	0.10 ± 0.06	0.30 ± 0.14
Anaerobic						
Unamended	ND	ND	*0.48 ± 0.03	*0.69 ± 0.08	*0.77 ± 0.09	0.91 ± 0.14
Amended	ND	ND	*3.85 ± 0.14	*3.97 ± 0.16	*3.75 ± 0.16	4.03 ± 0.17
Polyethylene - Aerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	0.01	ND	ND	0.02
Irradiated (High-Dose)	ND	ND	0.01	ND	ND	0.01
Amended						
Unirradiated	ND	ND	0.11 ± 0.09	0.44 ± 0.07	*0.41 ± 0.11	0.43 ± 0.21
Irradiated (Low-Dose)	ND	ND	0.01 ± 0.01	0.19 ± 0.15	*0.23 ± 0.18	0.44 ± 0.31
Irradiated (High-Dose)	ND	ND	0.04 ± 0.02	0.31 ± 0.08	0.16 ± 0.05	0.05 ± 0.01
Polyethylene - Anaerobic						
Unamended						
Unirradiated	ND	ND	*0.57	*0.77	*0.79	0.85
Irradiated (Low-Dose)	ND	ND	*0.55	*0.79	*0.85	1.01
Irradiated (High-Dose)	ND	ND	*0.64	*0.72	*0.88	1.02
Amended						
Unirradiated	ND	ND	*3.93 ± 0.19	*4.11 ± 0.24	*3.58 ± 0.13	2.14 ± 1.52
Irradiated (Low-Dose)	ND	ND	*4.17 ± 0.11	*4.44 ± 0.15	*3.97 ± 0.12	4.13 ± 0.02
Irradiated (High-Dose)	ND	ND	*4.17 ± 0.12	*4.35 ± 0.10	*4.03 ± 0.12	4.29 ± 0.13

ND = None Detected

** = Hydrogen Sulfide was detected but not quantified.*

Table 6. Hydrogen Produced in Samples Containing Polyethylene.

Sample	Hydrogen (µmoles/sample) Days					
	0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Anaerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Polyethylene - Aerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	3.15	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Polyethylene - Anaerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND

ND = None Detected

APPENDIX B
SECTION 2: POLYVINYLCHLORIDE

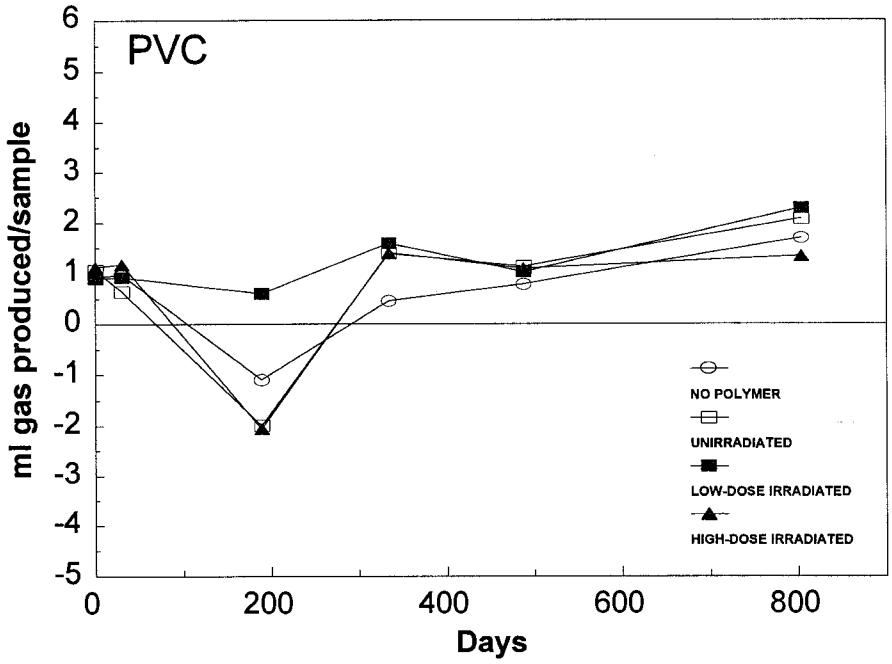

Figure 9. Total gas produced in aerobic unamended samples containing polyvinylchloride.

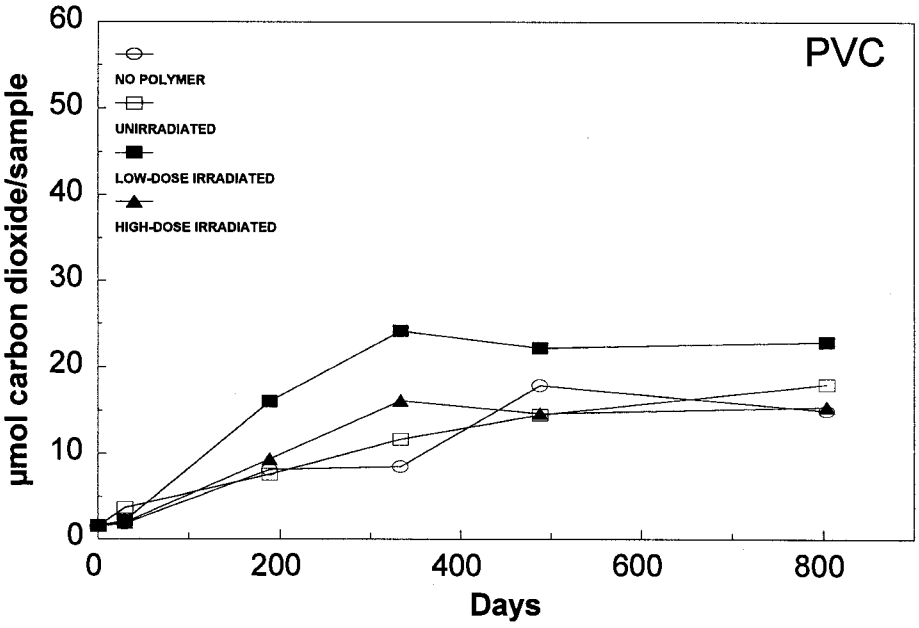

Figure 10. Carbon dioxide produced in aerobic unamended samples containing polyvinylchlorid

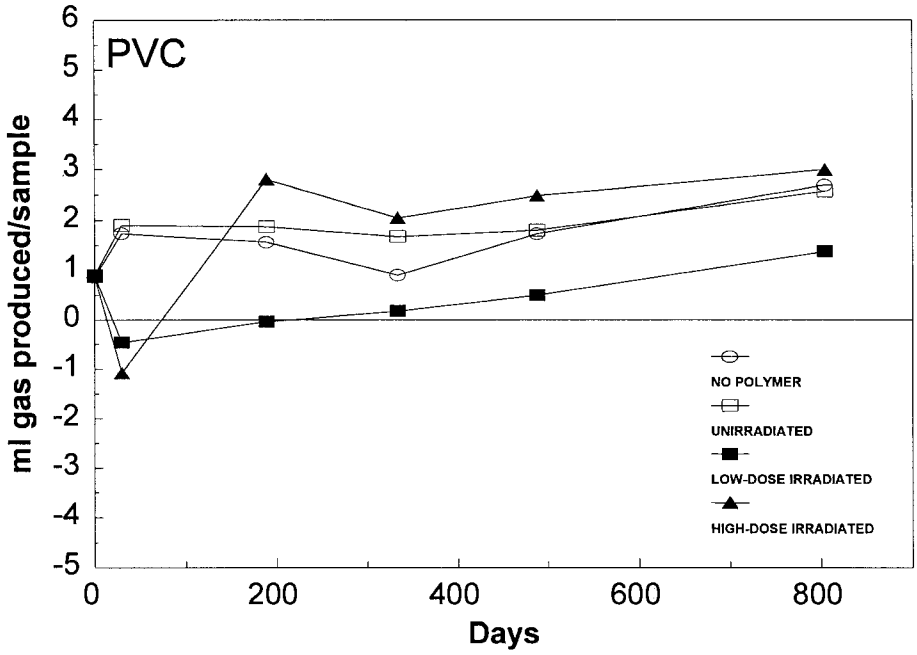

Figure 11. Total gas produced in aerobic amended samples containing polyvinylchloride.

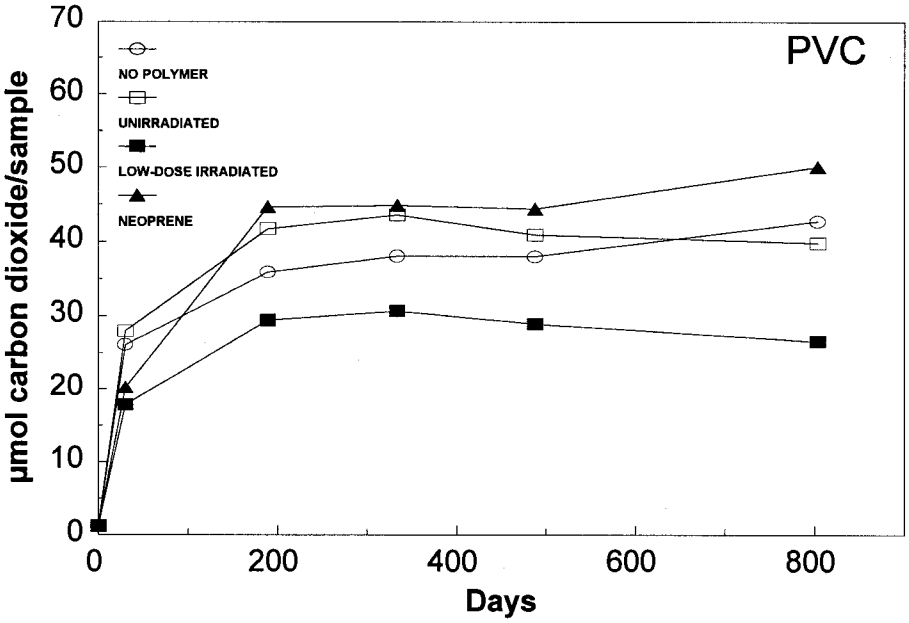

Figure 12. Carbon dioxide produced in aerobic amended samples containing polyvinylchloride.

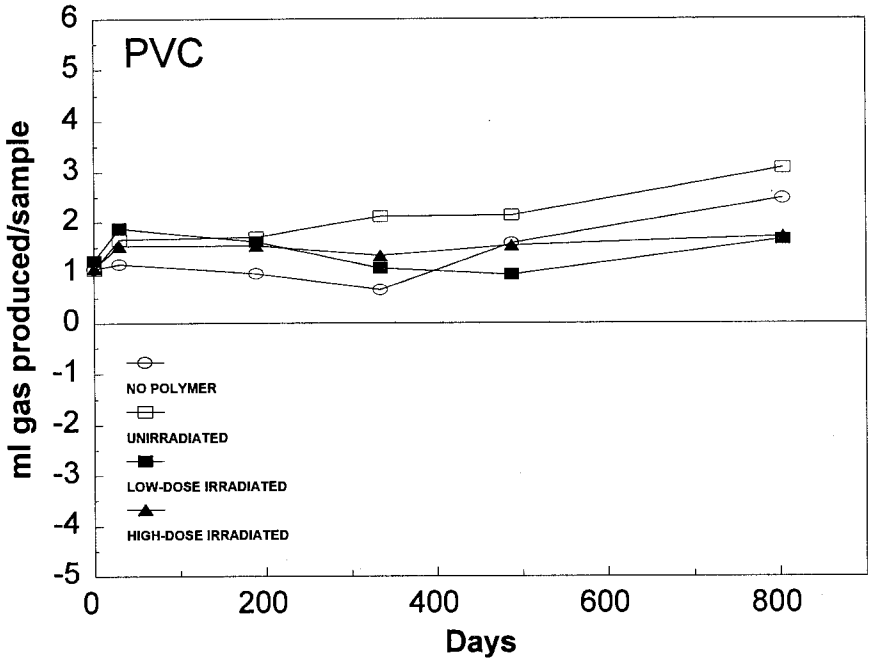

Figure 13. Total gas produced in anaerobic unamended samples containing polyvinylchloride.

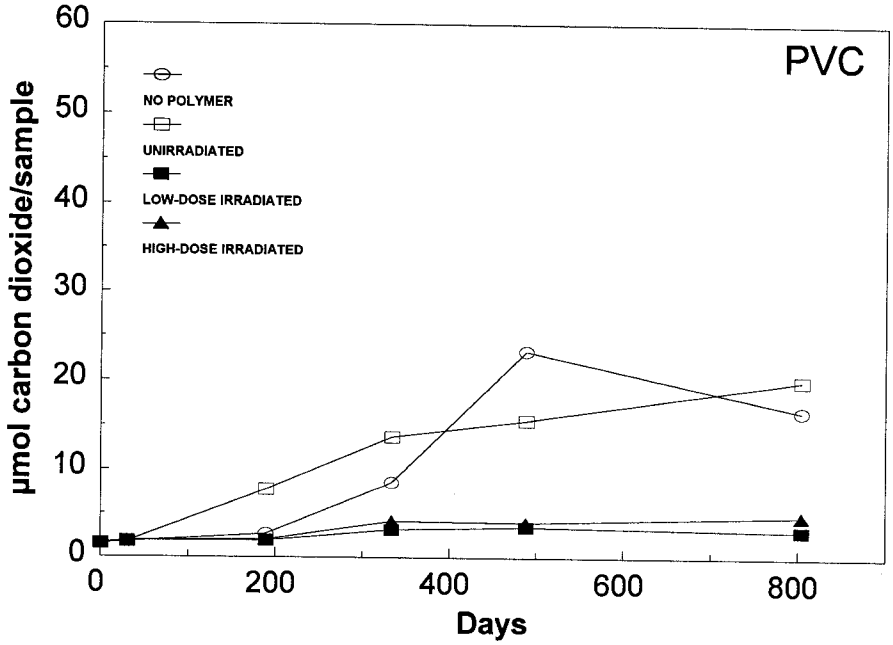

Figure 14. Carbon dioxide produced in anaerobic unamended samples containing polyvinylchloride.

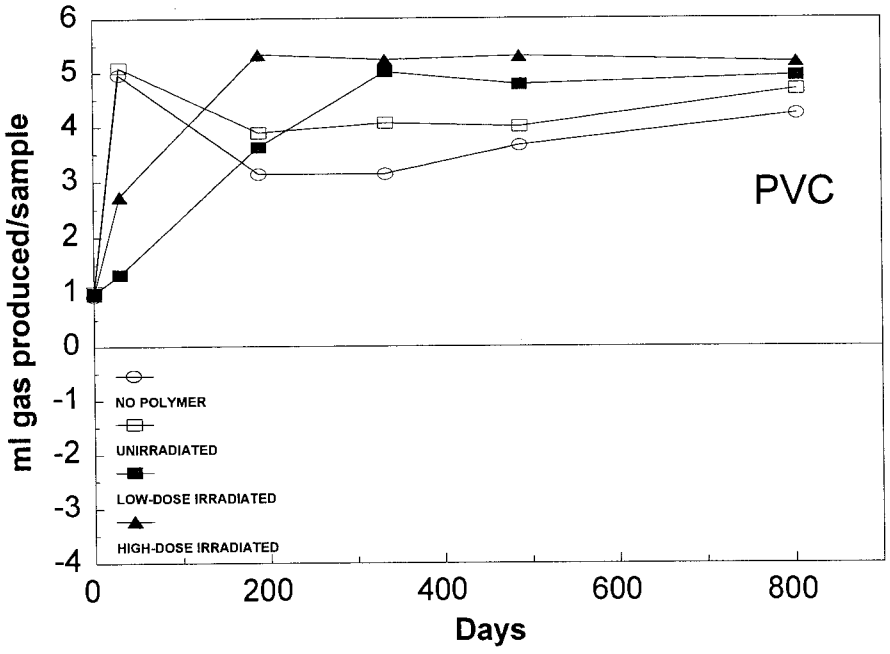

Figure 15. Total gas produced in anaerobic amended samples containing polyvinylchloride.

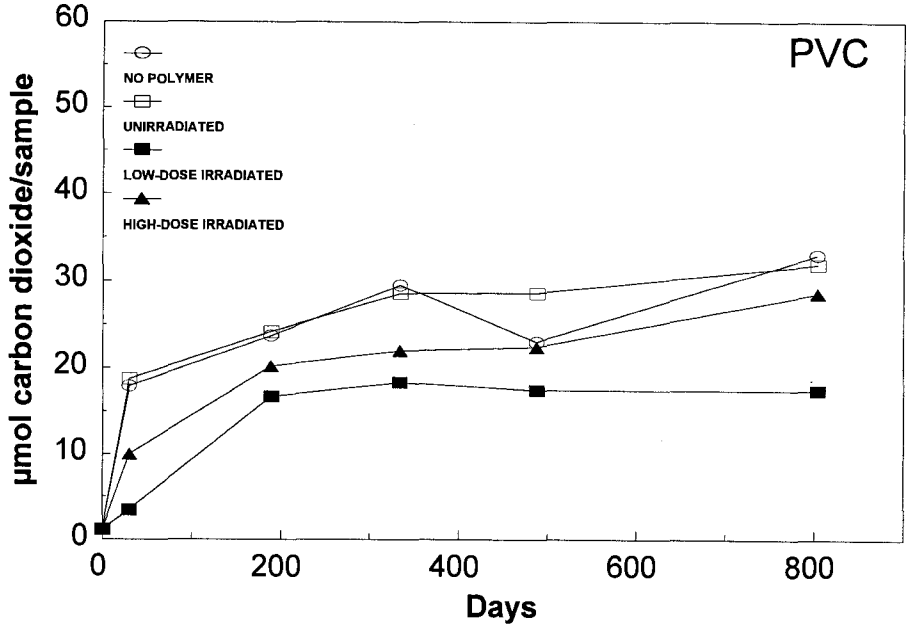

Figure 16. Carbon dioxide produced in anaerobic amended samples containing polyvinylchloride.

Table 7. Total Volume of Gas Produced in Samples Containing Polyvinylchloride.

Sample	Milliliters of Gas Produced/Sample Days 0	30	189	334	488	840
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
Amended	0.85	1.74 ± 0.17	1.56 ± 0.03	0.90 ± 0.48	1.73 ± 0.57	2.69 ± 0.59
Anaerobic						
Unamended	1.07	1.17 ± 0.05	0.98 ± 0.08	0.66 ± 0.37	1.59 ± 0.42	2.48 ± 0.34
Amended	0.93	4.96 ± 0.24	3.13 ± 1.19	3.13 ± 1.15	3.66 ± 0.98	4.24 ± 0.82
Polyvinylchloride - Aerobic						
Unamended						
Unirradiated	1.06	0.64	-1.99	1.39	1.13	2.08
Irradiated (Low-Dose)	0.90	0.92	0.59	1.59	1.02	2.29
Irradiated (High-Dose)	1.12	1.18	-2.05	1.40	1.09	1.34
Amended						
Unirradiated	0.89	1.90 ± 0.23	1.87 ± 0.13	1.67 ± 0.29	1.80 ± 0.32	2.57 ± 0.37
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
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
Polyvinylchloride - Anaerobic						
Unamended						
Unirradiated	1.06	1.66	1.70	2.12	2.14	3.08
Irradiated (Low-Dose)	1.24	1.88	1.61	1.09	0.96	1.66
Irradiated (High-Dose)	1.09	1.53	1.53	1.34	1.54	1.72
Amended						
Unirradiated	1.02	5.10 ± 0.19	3.89 ± 1.08	4.07 ± 0.94	4.01 ± 0.80	4.69 ± 0.58
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
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

Table 8. Carbon Dioxide Produced in Samples Containing Polyvinylchloride.

Sample	Carbon Dioxide (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	1.50	1.76 ± 0.13	8.11 ± 0.33	8.48 ± 0.39	17.87 ± 0.69	14.96 ± 1.67
Amended	1.21	26.11 ± 0.17	35.86 ± 0.39	38.04 ± 0.85	64.15 ± 2.25	42.68 ± 2.14
Anaerobic						
Unamended	1.52	1.76 ± 0.05	2.71 ± 0.08	8.60 ± 0.50	23.31 ± 0.31	16.59 ± 1.92
Amended	1.21	17.95 ± 0.24	23.67 ± 0.05	29.48 ± 0.58	50.33 ± 0.97	32.94 ± 0.69
Polyvinylchloride - Aerobic						
Unamended						
Unirradiated	1.50	3.63	7.58	11.65	14.49	18.00
Irradiated (Low-Dose)	1.54	2.11	16.06	24.13	22.15	22.84
Irradiated (High-Dose)	1.57	1.89	9.38	16.16	14.69	15.42
Amended						
Unirradiated	1.25	28.00 ± 0.51	41.71 ± 0.23	43.57 ± 0.30	40.87 ± 0.30	39.79 ± 0.09
Irradiated (Low-Dose)	1.15	17.83 ± 1.21	29.44 ± 0.85	30.69 ± 0.44	28.91 ± 0.34	26.54 ± 0.12
Irradiated (High-Dose)	1.22	20.25 ± 0.13	44.64 ± 0.03	44.83 ± 0.33	44.37 ± 0.58	50.07 ± 3.42
Polyvinylchloride - Anaerobic						
Unamended						
Unirradiated	1.54	1.76	7.77	13.69	15.55	20.02
Irradiated (Low-Dose)	1.59	1.85	1.95	3.20	3.50	3.12
Irradiated (High-Dose)	1.56	1.88	2.03	4.18	4.02	4.79
Amended						
Unirradiated	1.19	18.76 ± 0.28	24.13 ± 0.38	28.53 ± 0.75	28.57 ± 0.88	31.86 ± 0.68
Irradiated (Low-Dose)	1.20	3.44 ± 0.08	16.73 ± 0.47	18.34 ± 0.22	17.44 ± 0.10	17.38 ± 0.34
Irradiated (High-Dose)	1.18	10.02 ± 3.77	20.21 ± 2.28	21.99 ± 3.01	22.40 ± 3.65	28.52 ± 7.09

Table 9. Nitrous Oxide Produced in Samples Containing Polyvinylchloride.

Sample	Nitrous Oxide (µmoles/Sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	0.006	ND	0.33 ± 0.27	0.32 ± 0.16	ND
Anaerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Polyvinylchloride - Aerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	0.31 ± 0.20	1.37 ± 0.32	1.60 ± 0.41	1.39 ± 0.50	1.53 ± 0.65
Irradiated (High-Dose)	ND	0.22 ± 0.12	1.00 ± 0.71	1.06 ± 0.75	0.61 ± 0.43	0.83 ± 0.26
Polyvinylchloride - Anaerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	0.001	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	10.02 ± 7.09	ND	ND	ND	ND

ND = None Detected

Table 10. Methane Produced in Samples Containing Polyvinylchloride.

Sample	Methane (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	0.10 ± 0.06	0.30 ± 0.14
Anaerobic						
Unamended	ND	ND	*0.48 ± 0.03	*0.69 ± 0.08	*0.77 ± 0.09	0.91 ± 0.14
Amended	ND	ND	*3.85 ± 0.14	*3.97 ± 0.16	*3.75 ± 0.16	4.03 ± 0.17
Polyvinylchloride - Aerobic						
Unamended						
Unirradiated	ND	ND	0.01	ND	ND	0.51
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	0.01
Amended						
Unirradiated	ND	ND	1.24 ± 0.12	*1.53 ± 0.10	*1.71 ± 0.08	2.06 ± 0.04
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	0.01 ± 0.00
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Polyvinylchloride - Anaerobic						
Unamended						
Unirradiated	ND	ND	*0.61	*0.84	*0.95	1.27
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	*1.3 ± 0.00	*4.88 ± 0.18	*4.66 ± 0.19	4.88 ± 0.11
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	*ND	0.03 ± 0.02

ND = None Detected

** = Hydrogen Sulfide was detected but not quantified.*

Table 11. Hydrogen Produced in Samples Containing Polyvinylchloride.

Sample	Hydrogen (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Anaerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Polyvinylchloride - Aerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Polyvinylchloride - Anaerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	9.25	ND	5.93
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	16.42 ± 0.54	ND	13.33 ± 0.74
Irradiated (High-Dose)	ND	ND	ND	8.24 ± 5.83	ND	6.76 ± 4.78

ND = None Detected

APPENDIX B
SECTION 3: NEOPRENE

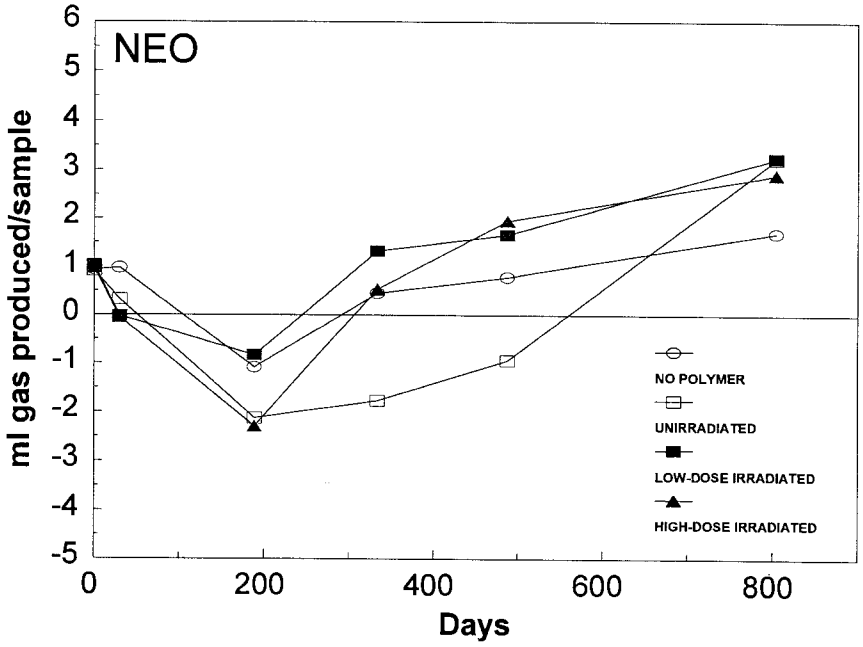

Figure 17. Total gas produced in aerobic unamended samples containing neoprene.

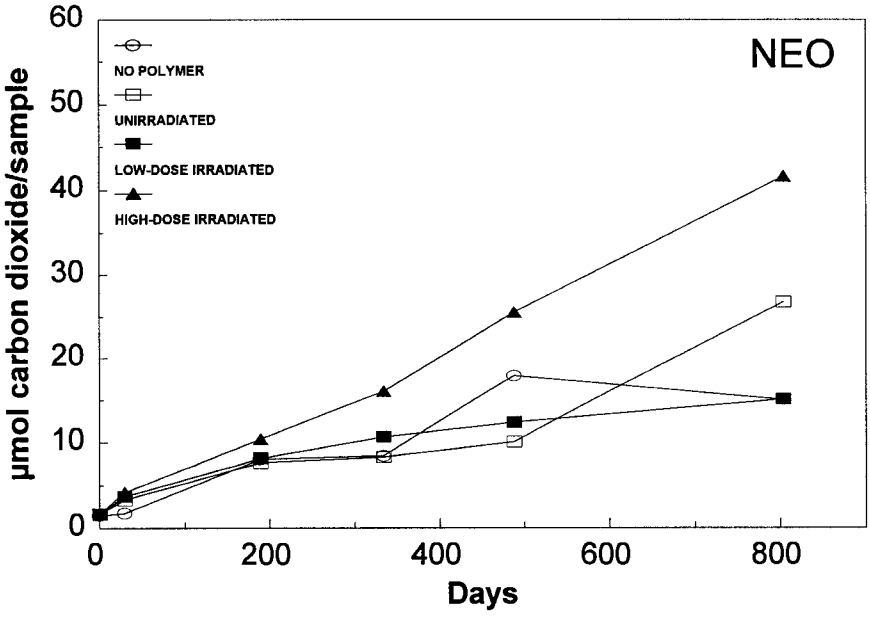

Figure 18. Carbon dioxide produced in aerobic unamended samples containing neoprene.

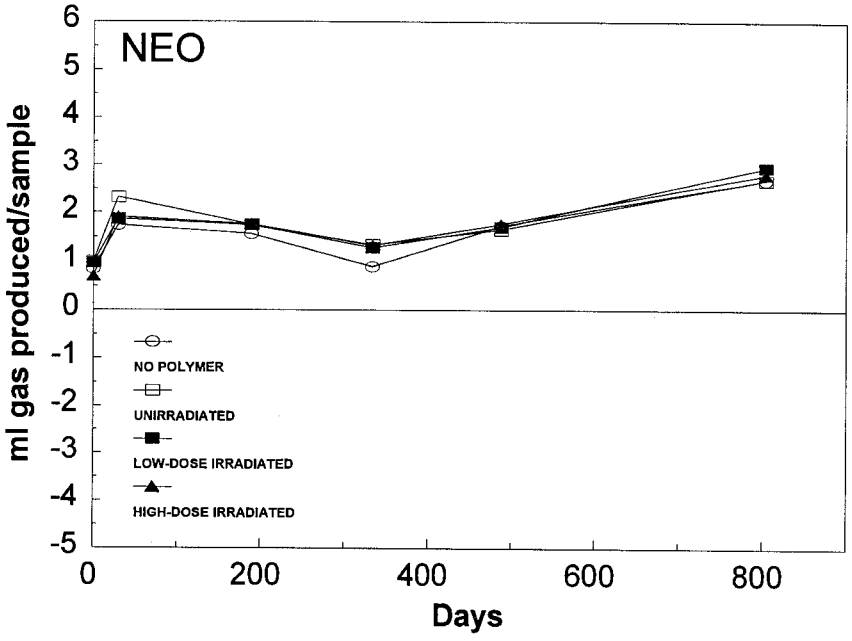

Figure 19. Total gas produced in aerobic amended samples containing neoprene.

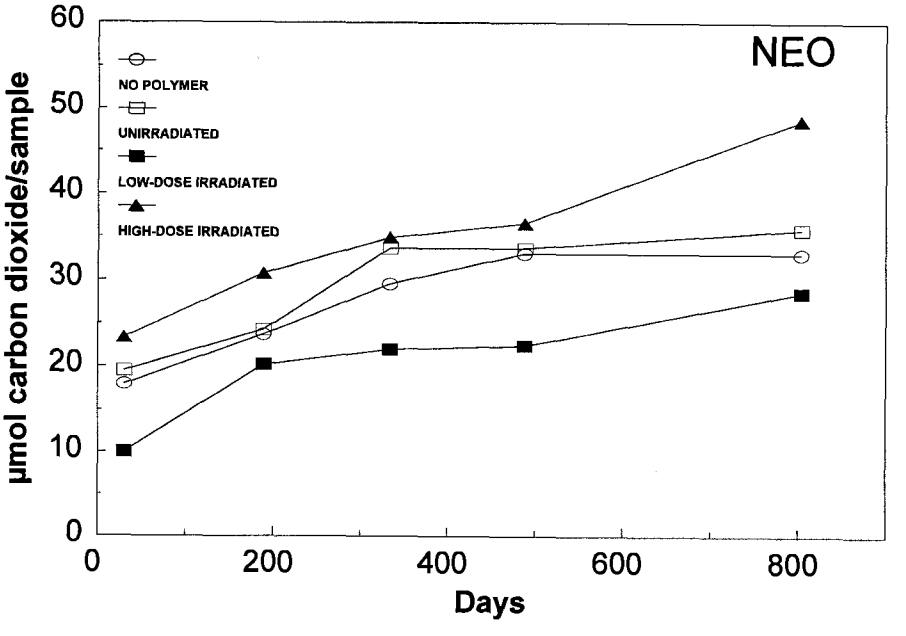

Figure 20. Carbon dioxide produced in aerobic amended samples containing neoprene.

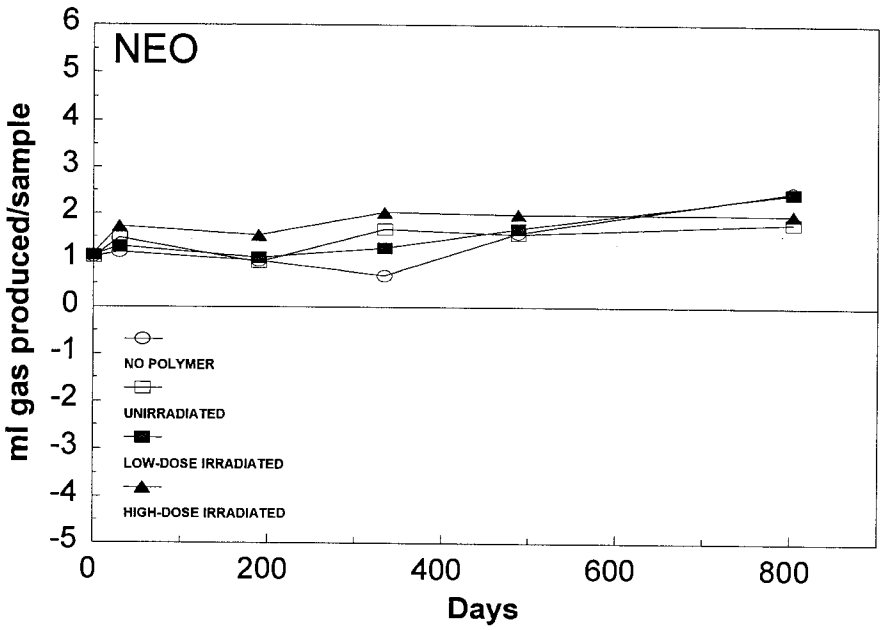

Figure 21. Total gas produced in anaerobic unamended samples containing neoprene.

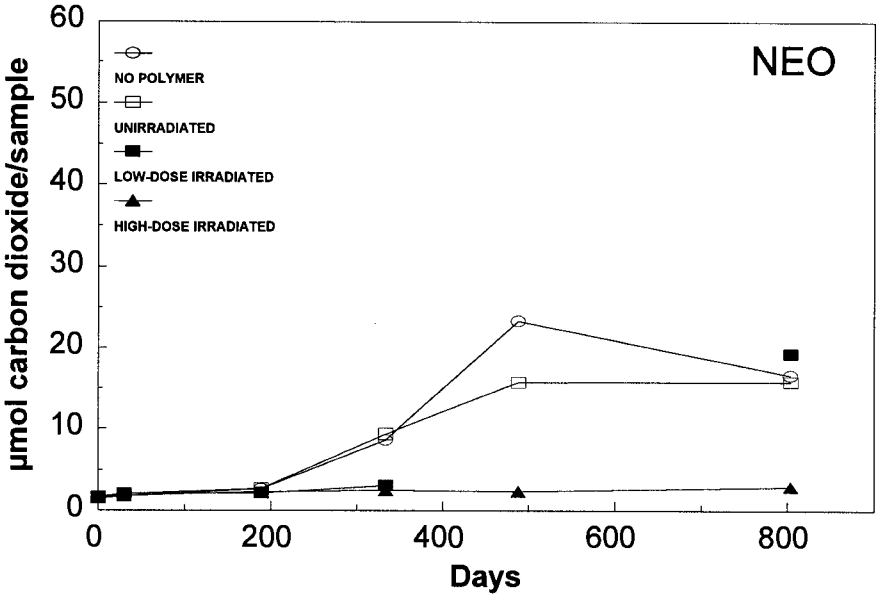

Figure 22. Carbon dioxide produced in anaerobic unamended samples containing neoprene.

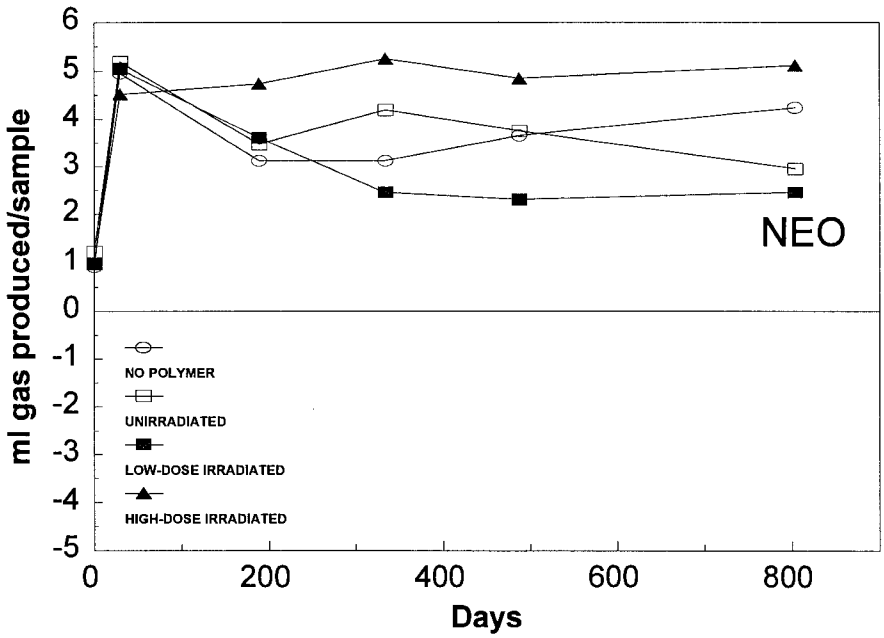

Figure 23. Total gas produced in anaerobic amended samples containing neoprene.

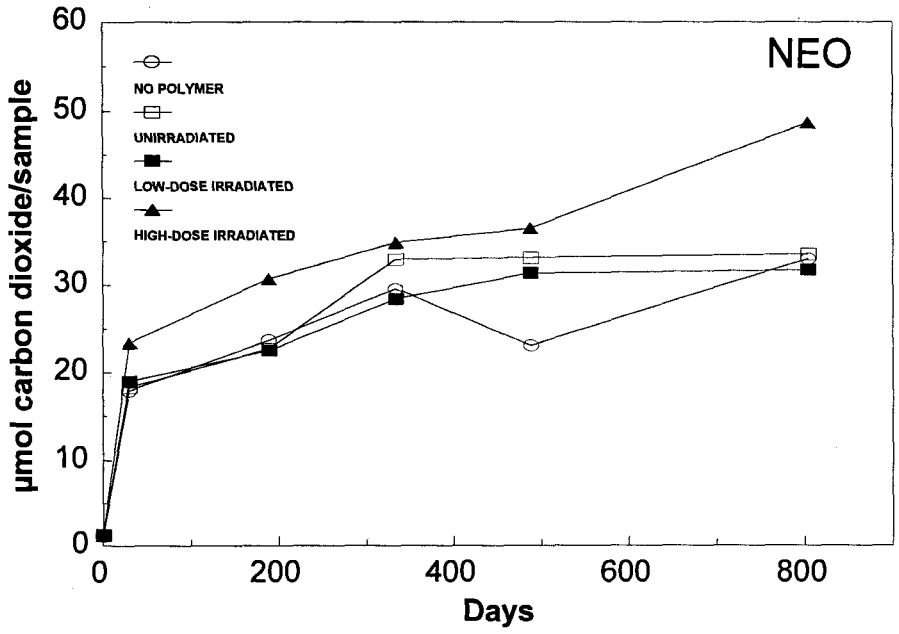

Figure 24. Carbon dioxide produced in anaerobic amended samples containing neoprene.

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

Sample	Milliliters of Gas Produced/Sample Days 0	30	189	334	488	840
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
Amended	0.85	1.74 ± 0.17	1.56 ± 0.03	0.90 ± 0.48	1.73 ± 0.57	2.69 ± 0.59
Anaerobic						
Unamended	1.07	1.17 ± 0.05	0.98 ± 0.08	0.66 ± 0.37	1.59 ± 0.42	2.48 ± 0.34
Amended	0.93	4.96 ± 0.24	3.13 ± 1.19	3.13 ± 1.15	3.66 ± 0.98	4.24 ± 0.82
Neoprene - Aerobic						
Unamended						
Unirradiated	0.91	0.32	-2.13	-1.77	-0.94	3.23
Irradiated (Low-Dose)	1.03	-0.02	-0.84	1.32	1.66	3.25
Irradiated (High-Dose)	0.97	-0.05	-2.30	0.53	1.95	2.91
Amended						
Unirradiated	1.00	2.32 ± 0.09	1.75 ± 0.12	1.34 ± 0.12	1.65 ± 0.21	2.69 ± 0.34
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
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
Neoprene - Anaerobic						
Unamended						
Unirradiated	1.06	1.48	0.95	1.67	1.56	1.80
Irradiated (Low-Dose)	1.10	1.29	1.05	1.26	1.68	2.44
Irradiated (High-Dose)	1.14	1.73	1.54	2.03	1.99	1.98
Amended						
Unirradiated	1.23	5.19 ± 0.14	3.48 ± 1.00	4.19 ± 0.93	3.76 ± 0.73	2.96 ± 0.54
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
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

Table 13. Carbon Dioxide Produced in Samples Containing Neoprene.

Sample	Carbon Dioxide (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	1.50	1.76 ± 0.13	8.11 ± 0.33	8.48 ± 0.39	17.87 ± 0.69	14.96 ± 1.67
Amended	1.21	26.11 ± 0.17	35.86 ± 0.39	38.04 ± 0.85	64.15 ± 2.25	42.68 ± 2.14
Anaerobic						
Unamended	1.52	1.76 ± 0.05	2.71 ± 0.08	8.60 ± 0.50	23.31 ± 0.31	16.59 ± 1.92
Amended	1.21	17.95 ± 0.24	23.67 ± 0.05	29.48 ± 0.58	50.33 ± 0.97	32.94 ± 0.69
Neoprene - Aerobic						
Unamended						
Unirradiated	1.60	3.34	7.68	8.33	10.11	26.76
Irradiated (Low-Dose)	1.66	3.69	8.18	10.66	12.34	15.06
Irradiated (High-Dose)	1.64	4.21	10.44	16.04	25.53	41.57
Amended						
Unirradiated	1.27	25.43 ± 0.39	38.37 ± 0.50	37.67 ± 0.30	39.35 ± 0.87	46.83 ± 2.74
Irradiated (Low-Dose)	1.32	27.56 ± 0.27	40.24 ± 0.71	40.92 ± 0.87	41.81 ± 1.55	43.52 ± 3.14
Irradiated (High-Dose)	1.30	29.29 ± 0.24	44.50 ± 1.14	46.72 ± 2.27	48.48 ± 3.18	55.21 ± 7.06
Neoprene - Anaerobic						
Unamended						
Unirradiated	1.58	2.01	2.75	9.34	15.73	15.74
Irradiated (Low-Dose)	1.65	2.09	2.16	3.09	NA	19.24
Irradiated (High-Dose)	1.67	1.81	2.28	2.50	2.36	2.92
Amended						
Unirradiated	1.24	18.29 ± 0.07	22.67 ± 0.30	32.88 ± 0.55	33.10 ± 0.77	33.47 ± 1.02
Irradiated (Low-Dose)	1.32	18.98 ± 0.39	22.45 ± 0.18	28.33 ± 0.88	31.33 ± 0.99	31.68 ± 0.75
Irradiated (High-Dose)	1.35	23.38 ± 0.85	30.70 ± 1.26	34.84 ± 0.99	36.50 ± 0.72	48.65 ± 1.65

*NA = Not Available

Table 14. Nitrous Oxide Produced in Samples Containing Neoprene.

Sample	Nitrous Oxide (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	0.006	ND	0.33 ± 0.27	0.32 ± 0.16	ND
Anaerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Neoprene - Aerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Neoprene - Anaerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	0.60 ± 0.42	ND	ND	ND	ND

ND = None Detected

Table 15. Methane Produced in Samples Containing Neoprene.

Sample	Methane (µmoles/sample) Days 0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	0.10 ± 0.06	0.30 ± 0.14
Anaerobic						
Unamended	ND	ND	*0.48 ± 0.03	*0.69 ± 0.08	*0.77 ± 0.09	0.91 ± 0.14
Amended	ND	ND	*3.85 ± 0.14	*3.97 ± 0.16	*3.75 ± 0.16	4.03 ± 0.17
Neoprene - Aerobic						
Unamended						
Unirradiated	ND	ND	0.01	ND	ND	0.01
Irradiated (Low-Dose)	ND	ND	0.01	ND	0.01	0.23
Irradiated (High-Dose)	ND	ND	ND	0.14	0.09	0.07
Amended						
Unirradiated	ND	ND	ND	0.30 ± 0.08	0.86 ± 0.25	1.42 ± 0.27
Irradiated (Low-Dose)	ND	ND	0.01 ± 0.00	0.11 ± 0.08	0.32 ± 0.25	0.41 ± 0.32
Irradiated (High-Dose)	ND	ND	0.14 ± 0.06	0.48 ± 0.26	0.42 ± 0.00	0.58 ± 0.26
Neoprene - Anaerobic						
Unamended						
Unirradiated	ND	ND	ND	*ND	*0.02	0.03
Irradiated (Low-Dose)	ND	ND	ND	ND	*ND	ND
Irradiated (High-Dose)	ND	ND	ND	0.36	1.85	ND
Amended						
Unirradiated	ND	ND	4.90	*4.83 ± 0.25	*4.20 ± 0.25	4.03 ± 0.22
Irradiated (Low-Dose)	ND	ND	2.19	*3.72 ± 0.11	*3.56 ± 0.14	3.87 ± 0.23
Irradiated (High-Dose)	ND	ND	2.87	4.89 ± 0.01	*4.47 ± 0.01	*4.91 ± 0.04

ND = None Detected

** = Hydrogen Sulfide was detected but not quantified.*

Table 16. Hydrogen Produced in Samples Containing Neoprene.

Sample	Hydrogen (µmoles/sample) Days					
	0	30	189	334	488	840
No Plastic or Rubber						
Aerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Anaerobic						
Unamended	ND	ND	ND	ND	ND	ND
Amended	ND	ND	ND	ND	ND	ND
Neoprene - Aerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND
Neoprene - Anaerobic						
Unamended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	13.46	ND	ND
Amended						
Unirradiated	ND	ND	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND	ND	ND
Irradiated (High-Dose)	ND	ND	ND	ND	ND	ND

ND = None Detected

APPENDIX B
SECTION 4: UNLEADED HYPALON

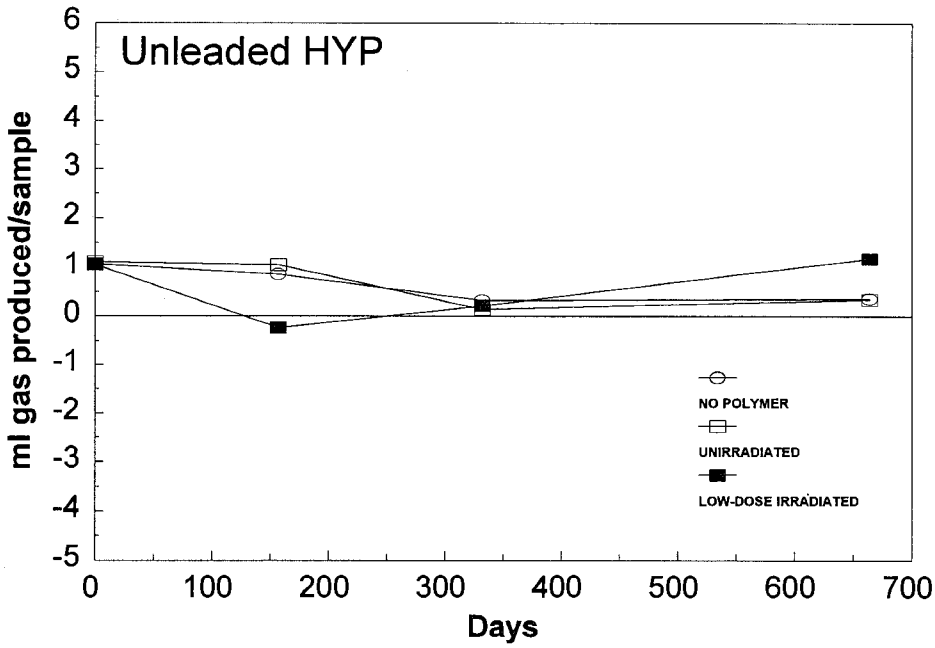

Figure 25. Total gas produced in aerobic unamended samples containing unleaded hypalon.

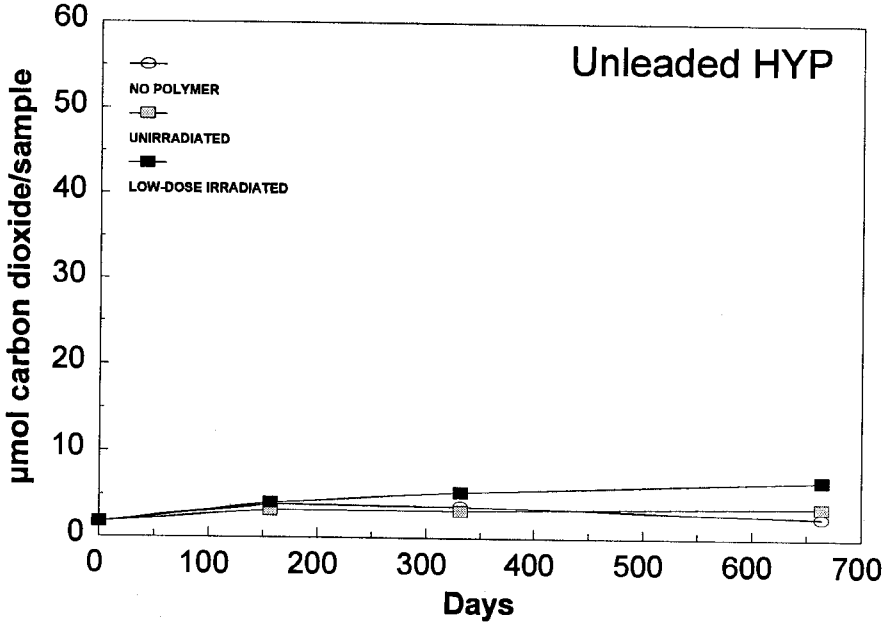

Figure 26. Carbon dioxide produced in aerobic unamended samples containing unleaded hyp

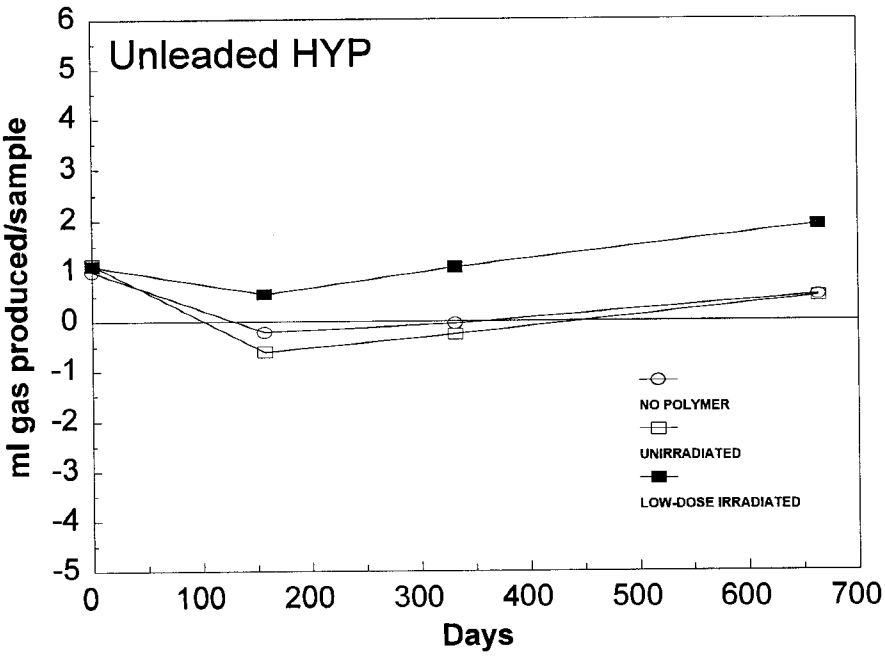

Figure 27. Total gas produce in aerobic amended samples containing unleaded hypalon.

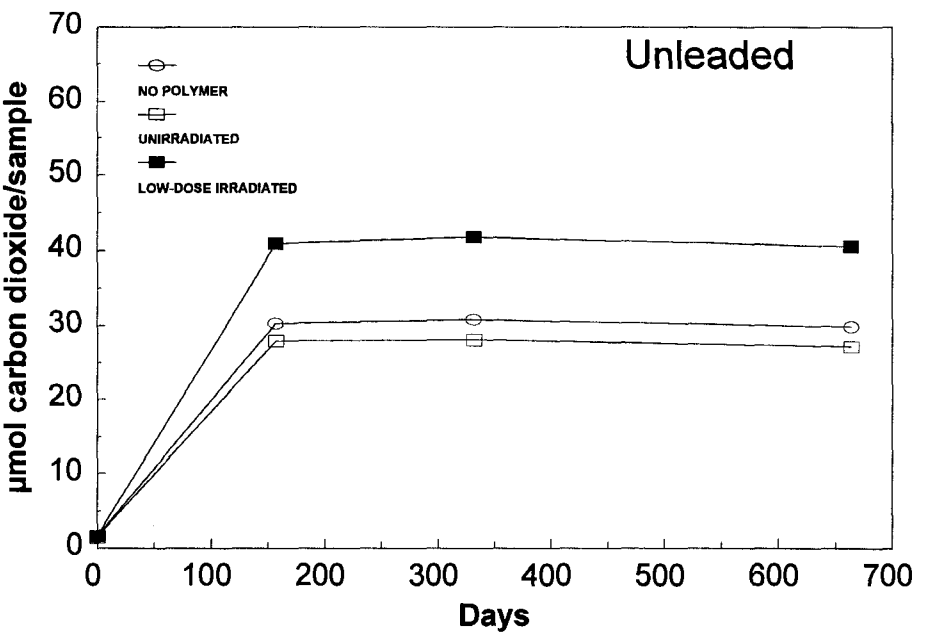

Figure 28. Carbon dioxide produced in aerobic amended samples containing unleaded hypal

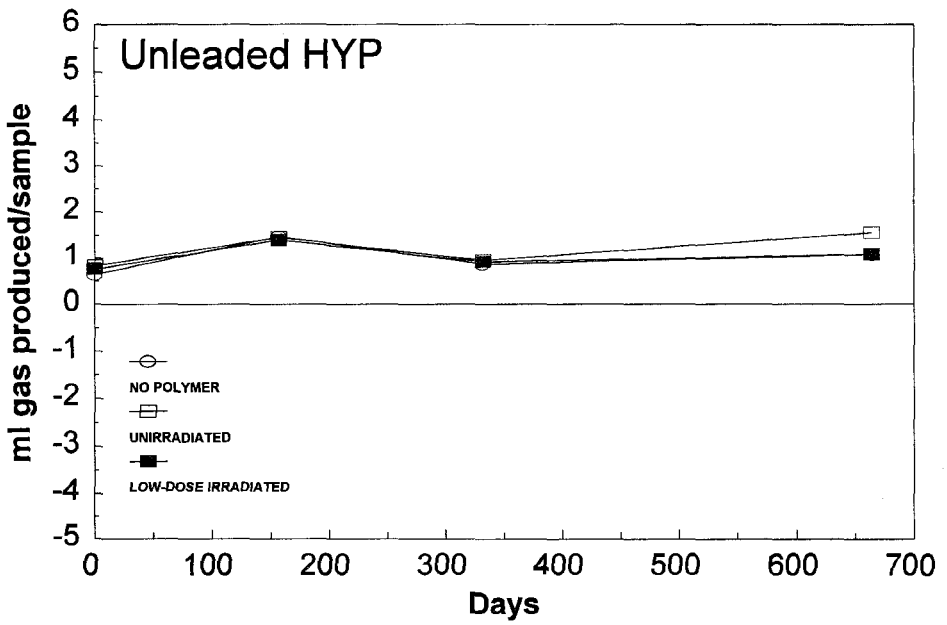

Figure 29. Total gas produced in anaerobic unamended samples containing unleaded hypal

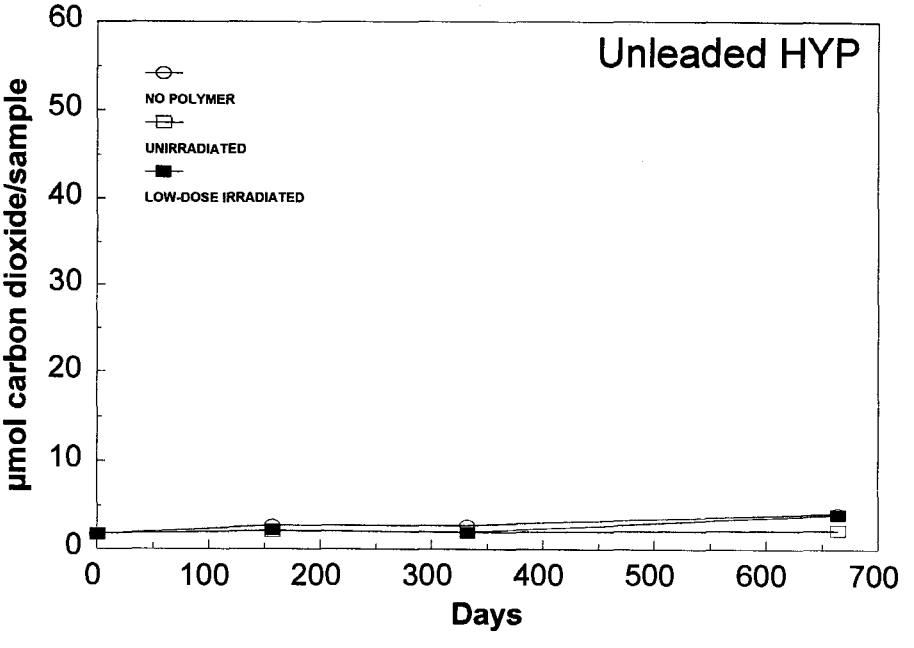

Figure 30. Carbon dioxide produced in anaerobic unamended samples unleaded hypalon.

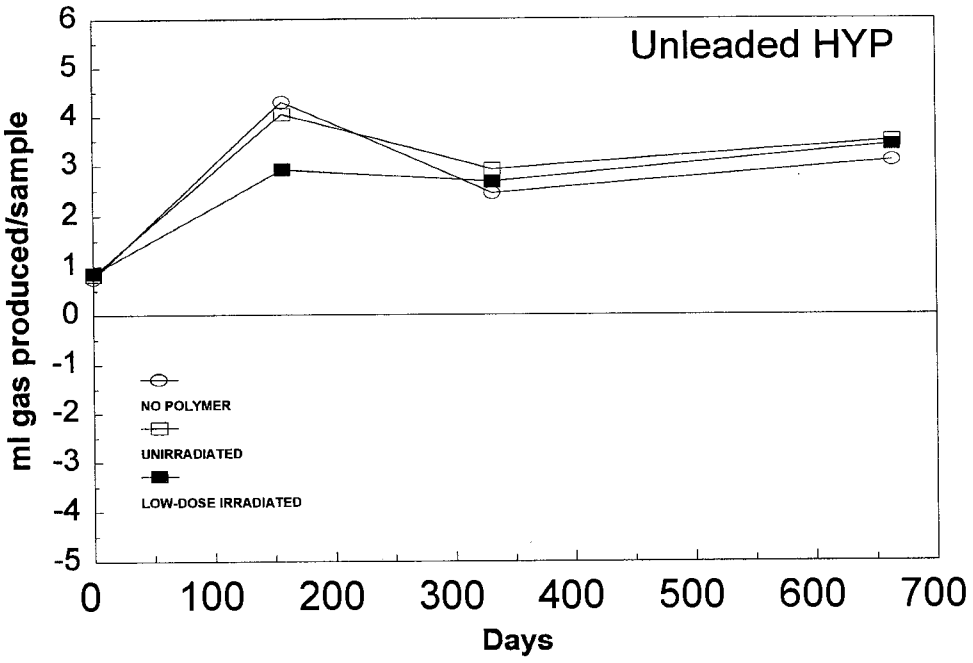

Figure 31. Total gas produced in anaerobic amended samples containing unleaded hypalon.

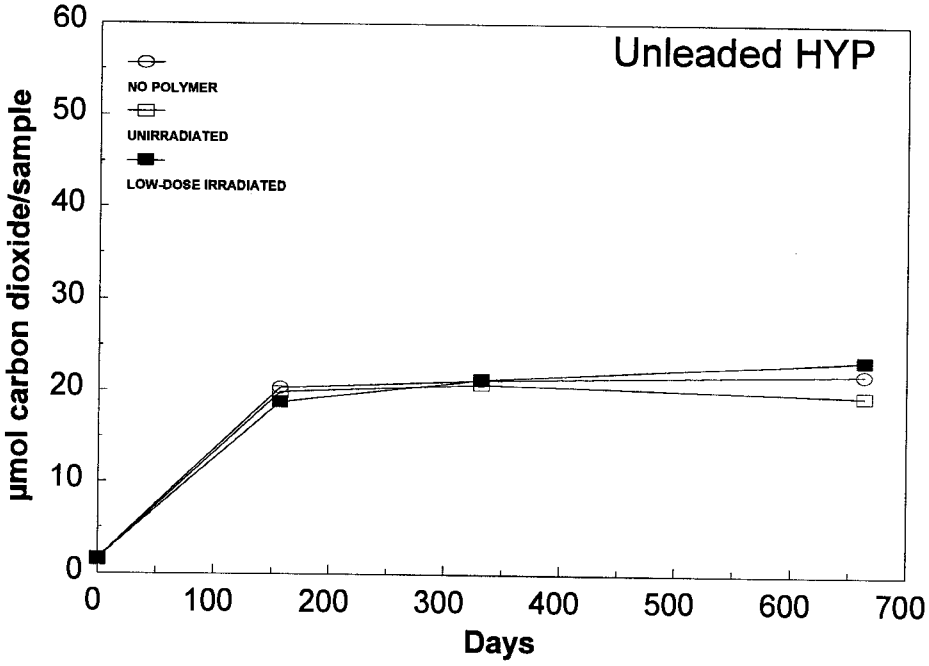

Figure 32. Carbon dioxide produced in anaerobic amended samples containing unleaded hypalon.

Table 17. Oxygen Consumption in Aerobic Samples Containing Hypalon.

Sample	%/Sample Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	21.00	16.95 ± 0.09	15.91 ± 0.07	14.9 ± 0.16
Amended	21.00	2.37 ± 0.12	2.34 ± 0.08	2.58 ± 0.13
Unleaded Hypalon				
Unamended				
Unirradiated	21.00	17.33	16.87	15.54
Irradiated (Low-Dose)	21.00	12.25	12.62	14.83
Amended				
Unirradiated	21.00	2.15 ± 0.10	2.95 ± 0.06	2.68 ± 0.06
Irradiated (Low-Dose)	21.00	1.77 ± 0.30	2.10 ± 0.46	1.87 ± 0.37
Leaded Hypalon				
Unamended				
Unirradiated	21.00	15.23	14.05	11.26
Irradiated (Low-Dose)	21.00	14.64	11.56	8.35
Amended				
Unirradiated	21.00	1.72 ± 0.29	1.87 ± 0.37	2.35 ± 0.59
Irradiated (Low-Dose)	21.00	2.28 ± 0.14	2.28 ± 0.09	2.68 ± 0.04

Table 18. Total Volume of Gas Produced in Samples Containing Unleaded Hypalon.

Sample	Milliliters of Gas Produced/Sample Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	1.08	0.86 ± 0.08	0.33 ± 0.09	0.36 ± 0.15
Amended	1.00	-0.21 ± 0.07	-0.04 ± 0.09	0.51 ± 0.07
Anaerobic				
Unamended	0.65	1.47 ± 0.04	0.86 ± 0.17	1.07 ± 0.08
Amended	0.76	4.30 ± 0.11	2.45 ± 0.95	3.09 ± 0.81
Unleaded Hypalon - Aerobic				
Unamended				
Unirradiated	1.12	1.05	0.14	0.34
Irradiated (Low-Dose)	1.06	-0.24	0.21	1.18
Amended				
Unirradiated	1.14	-0.60 ± 0.06	-0.25 ± 0.15	0.49 ± 0.09
Irradiated (Low-Dose)	1.11	0.54 ± 0.91	1.07 ± 0.89	1.90 ± 0.88
Unleaded Hypalon - Anaerobic				
Unamended				
Unirradiated	0.84	1.45	0.94	1.55
Irradiated (Low-Dose)	0.77	1.39	0.91	1.08
Amended				
Unirradiated	0.82	4.04 ± 0.04	2.92 ± 0.92	3.49 ± 0.89
Irradiated (Low-Dose)	0.86	2.92 ± 0.69	2.67 ± 0.98	3.41 ± 0.90

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

Sample	Carbon Dioxide (µmoles/sample) Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	1.78	3.84 ± 0.15	3.69 ± 0.06	2.52 ± 0.52
Amended	1.56	30.33 ± 0.52	30.84 ± 0.44	29.84 ± 0.22
Anaerobic				
Unamended	1.78	2.76 ± 0.01	2.76 ± 0.01	4.15 ± 1.44
Amended	1.65	20.38 ± 0.17	21.24 ± 0.06	21.96 ± 0.12
Unleaded Hypalon - Aerobic				
Unamended				
Unirradiated	1.78	3.21	3.18	3.67
Irradiated (Low-Dose)	1.77	4.08	5.33	6.77
Amended				
Unirradiated	1.51	27.91 ± 0.34	28.10 ± 0.32	27.13 ± 0.63
Irradiated (Low-Dose)	1.64	40.91 ± 8.62	41.76 ± 8.42	40.55 ± 6.44
Unleaded Hypalon - Anaerobic				
Unamended				
Unirradiated	1.79	2.10	1.90	2.23
Irradiated (Low-Dose)	1.79	2.22	1.97	4.04
Amended				
Unirradiated	1.56	19.90 ± 0.17	20.81 ± 0.19	19.57 ± 0.27
Irradiated (Low-Dose)	1.65	18.81 ± 0.58	21.32 ± 0.44	23.51 ± 1.84

Table 20. Nitrous Oxide Produced in Samples Containing Unleaded Hypalon.

Sample	Nitrous Oxide (µmoles/sample) Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Anaerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	0.35 ± 0.2	ND
Unleaded Hypalon - Aerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Unleaded Hypalon - Anaerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND

ND = None Detected

Table 21. Hydrogen Produced in Samples Containing Unleaded Hypalon.

Sample	Hydrogen (µmoles/sample) Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Anaerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Unleaded Hypalon - Aerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Unleaded Hypalon - Anaerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND

ND = None Detected

Table 22. Methane Produced in Samples Containing Unleaded Hypalon.

Sample	µmoles/Sample Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Anaerobic				
Unamended	ND	0.42 ± 0.01	0.60 ± 0.02	0.72 ± 0.02
Amended	ND	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Unleaded Hypalon - Aerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	0.01
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Unleaded Hypalon - Anaerobic				
Unamended				
Unirradiated	ND	0.03	0.03	ND
Irradiated (Low-Dose)	ND	0.03	0.03	ND
Amended				
Unirradiated	ND	0.02 ± 0.01	ND	0.02 ± 0.00
Irradiated (Low-Dose)	ND	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00

ND = None Detected

APPENDIX B
SECTION 5: LEADED HYPALON

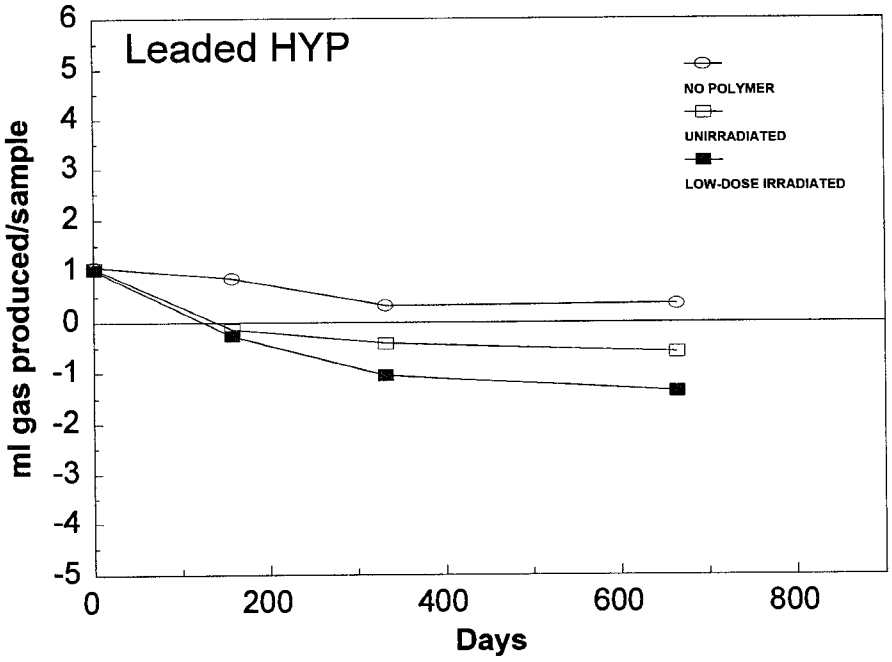

Figure 33. Total gas produced in aerobic unamended samples containing leaded hypalon.

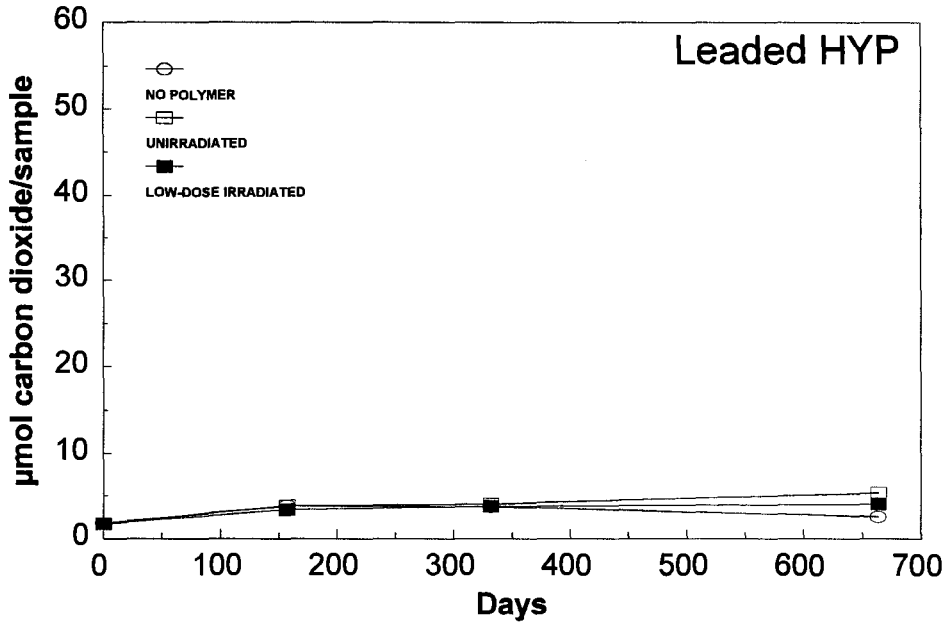

Figure 34. Carbon dioxide produced in aerobic unamended samples containing leaded hypalon.

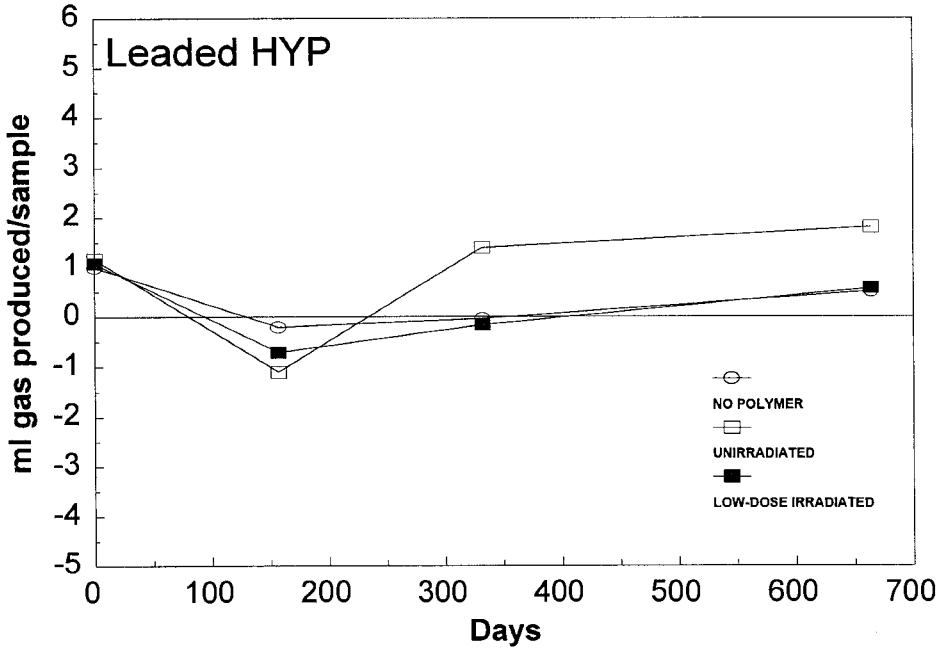

Figure 35. Total gas produced in aerobic amended samples containing leaded hypalon

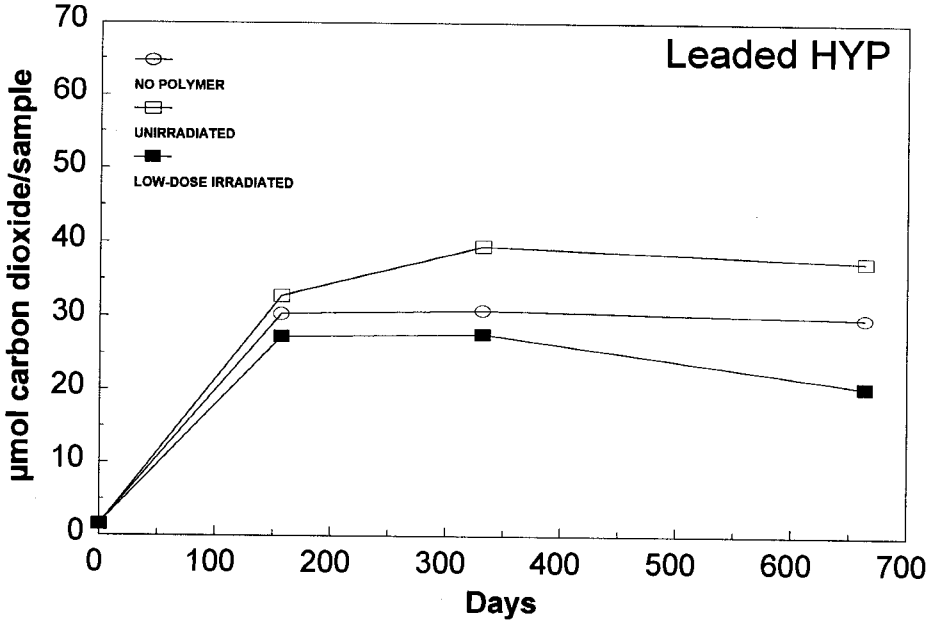

Figure 36. Carbon dioxide produced in aerobic amended samples containing leaded hypalon.

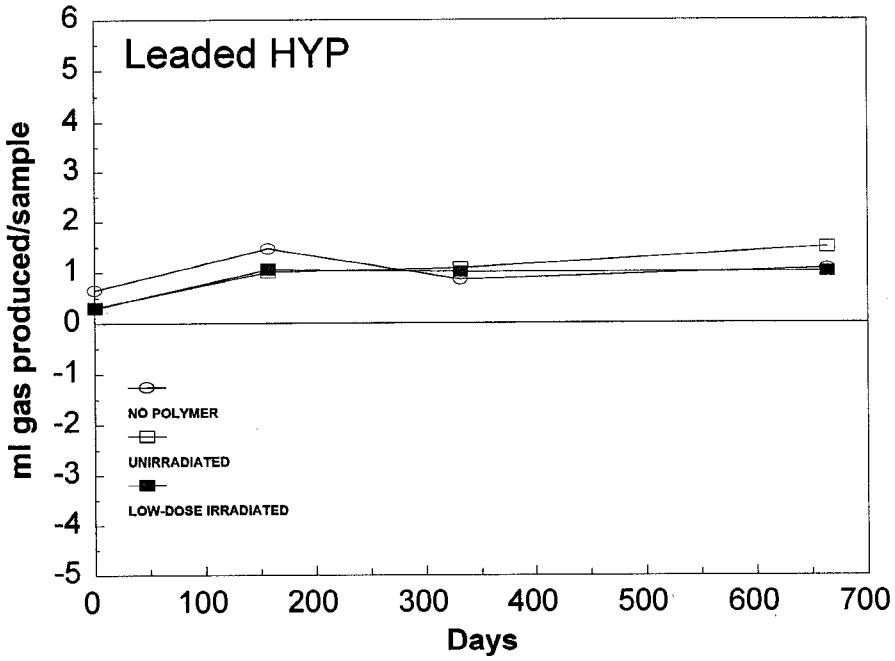

Figure 37. Total gas produced in anaerobic unamended samples containing leaded hypalon.

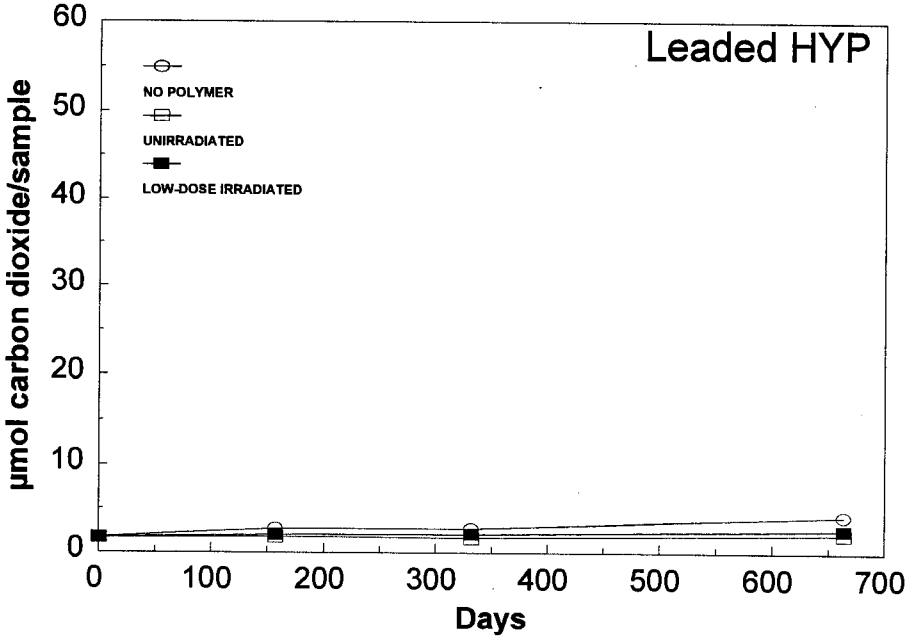

Figure 38. Carbon dioxide produced in anaerobic unamended samples containing leaded hypalon.

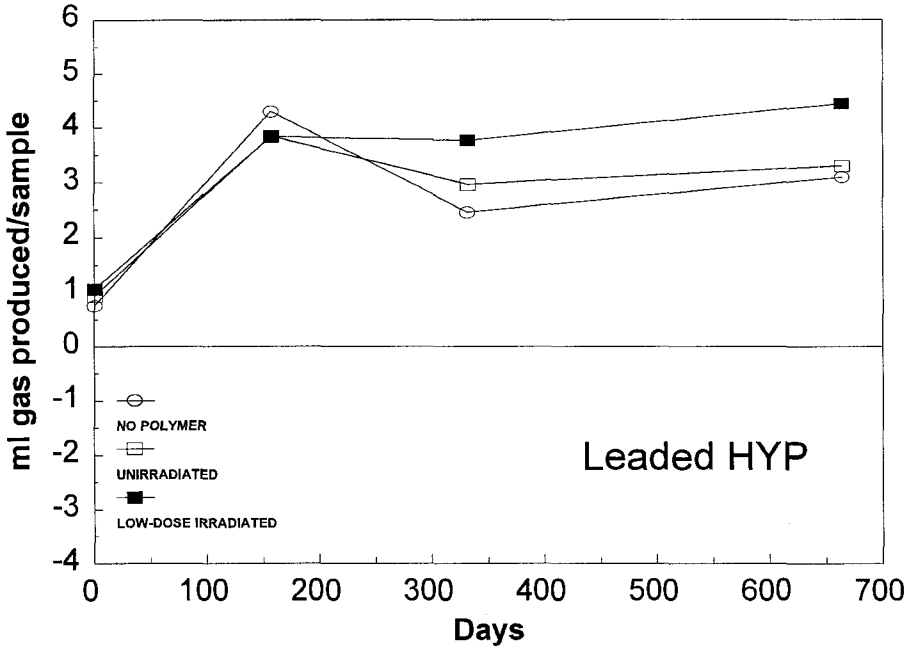

Figure 39. Total gas produced in anaerobic amended samples containing leaded hypalon.

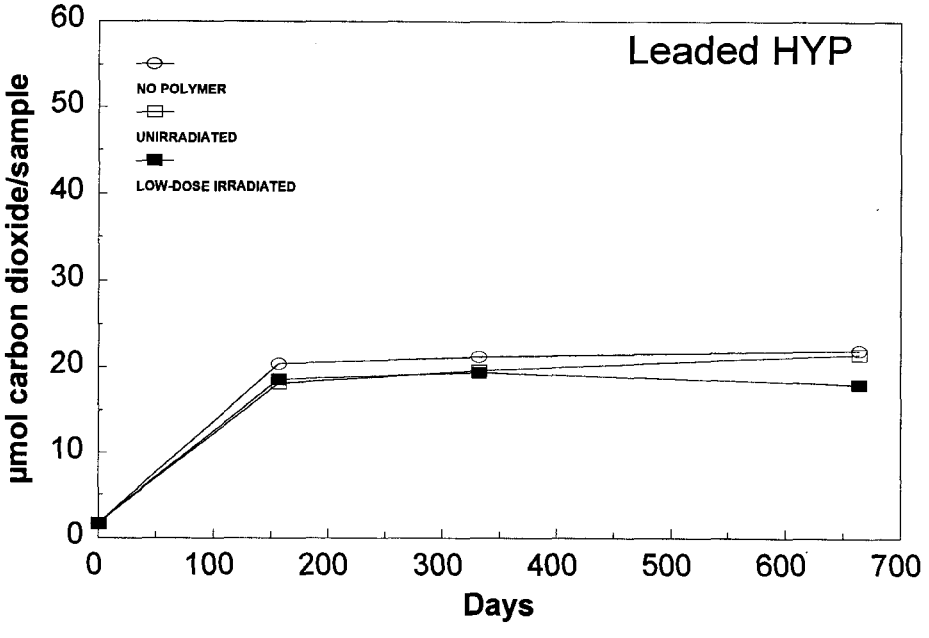

Figure 40. Carbon dioxide produced in anaerobic amended samples containing leaded hypalon.

Table 23. Total Volume of Gas Produced in Samples Containing Leaded Hypalon.

Sample	Milliliters of Gas Produced/Sample			
	Days			
	0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	1.08	0.86 ± 0.08	0.33 ± 0.09	0.36 ± 0.15
Amended	1.00	-0.21 ± 0.07	-0.04 ± 0.09	0.51 ± 0.07
Anaerobic				
Unamended	0.65	1.47 ± 0.04	0.86 ± 0.17	1.07 ± 0.08
Amended	0.76	4.30 ± 0.11	2.45 ± 0.95	3.09 ± 0.81
Leaded Hypalon - Aerobic				
Unamended				
Unirradiated	1.06	-0.13	-0.41	-0.58
Irradiated (Low-Dose)	1.02	-0.26	-1.04	-1.36
Amended				
Unirradiated	1.17	-1.11 ± 0.67	1.40 ± 0.93	1.81 ± 0.93
Irradiated (Low-Dose)	1.08	-0.72 ± 0.06	-0.17 ± 0.14	0.57 ± 0.16
Leaded Hypalon - Anaerobic				
Unamended				
Unirradiated	0.31	1.00	1.09	1.49
Irradiated (Low-Dose)	0.29	1.06	1.01	1.01
Amended				
Unirradiated	0.94	3.85 ± 0.02	2.96 ± 0.78	3.30 ± 1.12
Irradiated (Low-Dose)	1.06	3.83 ± 0.10	3.77 ± 0.14	4.45 ± 0.05

Table 24. Carbon Dioxide Produced in Samples Containing Leaded Hypalon.

Sample	Carbon Dioxide (µmoles/sample) Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	1.78	3.84 ± 0.15	3.69 ± 0.06	2.52 ± 0.52
Amended	1.56	30.33 ± 0.52	30.84 ± 0.44	29.84 ± 0.22
Anaerobic				
Unamended	1.78	2.76 ± 0.01	2.76 ± 0.01	4.15 ± 1.44
Amended	1.65	20.38 ± 0.17	21.24 ± 0.06	21.96 ± 0.12
Leaded Hypalon - Aerobic				
Unamended				
Unirradiated	1.72	3.77	4.03	5.33
Irradiated (Low-Dose)	1.71	3.30	3.72	4.00
Amended				
Unirradiated	1.53	32.79 ± 3.91	39.46 ± 8.15	37.43 ± 9.35
Irradiated (Low-Dose)	1.59	27.26 ± 0.18	27.61 ± 0.10	20.43 ± 6.58
Leaded Hypalon - Anaerobic				
Unamended				
Unirradiated	1.71	1.80	1.66	2.12
Irradiated (Low-Dose)	1.74	2.05	2.12	2.60
Amended				
Unirradiated	1.69	18.11 ± 0.05	19.62 ± 0.18	21.50 ± 0.79
Irradiated (Low-Dose)	1.72	18.59 ± 0.12	19.40 ± 0.22	17.99 ± 1.68

Table 25. Nitous Oxide Produced in Samples Containing Leaded Hypalon.

Sample	Nitrous Oxide (µmoles/sample) Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Anaerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	0.35 ± 0.2	ND
Leaded Hypalon - Aerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Leaded Hypalon - Anaerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND

ND = None Detected

Table 26. Hydrogen Produced in Samples Containing Leaded Hypalon.

Sample	Hydrogen (µmoles/sample) Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Anaerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Leaded Hypalon - Aerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Leaded Hypalon - Anaerobic				
Unamended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND

ND = None Detected

Table 27. Methane Produced in Samples Containing Leaded Hypalon.

Sample	Methane (µmoles/sample) Days 0	157	332	664
No Plastic or Rubber				
Aerobic				
Unamended	ND	ND	ND	ND
Amended	ND	ND	ND	ND
Anaerobic				
Unamended	ND	0.42 ± 0.01	0.60 ± 0.02	0.72 ± 0.02
Amended	ND	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Leaded Hypalon - Aerobic				
Unamended				
Unirradiated	ND	ND	0.01	ND
Irradiated (Low-Dose)	ND	0.01	0.01	0.01
Amended				
Unirradiated	ND	ND	ND	ND
Irradiated (Low-Dose)	ND	ND	ND	ND
Leaded Hypalon - Anaerobic				
Unamended				
Unirradiated	ND	0.03	0.03	0.03
Irradiated (Low-Dose)	ND	0.02	0.02	0.02
Amended				
Unirradiated	ND	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Irradiated (Low-Dose)	ND	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.00

ND = None Detected

APPENDIX C: SAMPLE VOLUMES FOR INUNDATED CELLULOSE BIODEGRADATION EXPERIMENT

APPENDIX C: SAMPLE VOLUMES FOR INUNDATED CELLULOSE BIODEGRADATION EXPERIMENT

The final sample volume (displacement of liquid plus cellulose *or* liquid w/o cellulose) and the headspace folume of each treatment is as follows:

Cellulose Treatments	*sample vol. (mL)*	*headspace vol. (mL)**
U (uninoculated)	110	50
I (inoculated)	114	46
UC (uninoculated control)	113	47
IC (inoculated control)	117	43

"No paper" Treatments	*sample vol. (mL)*	*headspace vol. (mL)**
NU (uninoculated)	100	60
NI (inoculated)	104	56
NUC (uninoculated control)	103	57
NIC (inoculated control)	107	53

*Headspace volume calculated by subtracting sample volume from volume of bottle (160 mL).

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Washington, DC 20460

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