MICROBIAL CHARACTERIZATION FOR THE SOURCE-TERM WASTE TEST PROGRAM (STTP) AT LOS ALAMOS

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ABSTRACT:

Microbes have been found to inhabit almost any subsurface environment, including areas once thought to be too hostile for any life forms to exist. The effects of microbial activity on the performance of the proposed underground nuclear waste repository, the Waste Isolation Pilot Plant (WIPP) at Carlsbad, New Mexico are being studied at Los Alamos National Laboratory (LANL) as part of an *ex situ* large-scale experiment. Actual actinide-containing waste is being used to predict the effect of potential brine inundation in the repository in the distant future. The study conditions are meant to simulate what might exist should the underground repository be flooded hundreds of years after closure as a result of inadvertent drilling into brine pockets below the repository. The Department of Energy (DOE) selected LANL to conduct the Actinide Source-Term Waste Test Program (STTP) to confirm the predictive capability of computer models being developed at Sandia National Laboratory. The project consists of 15 drum-scale test vessels containing heterogeneous wastes, 33 liter-scale vessels with homogeneous wastes, and six pressurized containers. Testing of the vessel contents has included assays of the actinide elements and measurements of the headspace gases generated by the transuranic waste immersed in brines that are chemically similar to those found in the salt formation in which the WIPP repository is located. The waste is representative of inventories that currently are stored temporarily at several DOE sites nationwide. In addition, the test containers were inoculated with a microbial population obtained from the hypersaline brine environment near the WIPP site. We have focused on three major activities with regard to this microbial population: 1) viability of the organisms in the liter-scale containers more than two years after inoculation, 2) microbial contribution to gas generation, and 3) the toxic effects of magnesium oxide, a material being considered for use as a backfilled barrier at the repository. Our studies of recent brine samples revealed little or no growth under the conditions chosen, but the absence of viable, but nonculturable cells could not be ruled out. Microbial cells are surmised to be present as a biofilm coating the surface of the sludge material in the test containers, which therefore were not sampled in the liquid column to any great extent. We utilized fluorescent staining techniques that detect viable, respiring organisms. Low levels of microbes were detected with these staining procedures in the liquid column of vessels prepared with waste organics. Other experiments investigated the effect of radiolysis on the generation of gases in the test containers. Large amounts of nitrous oxide detected in

test containers containing high concentrations of nitrate ion were thought to be the result of microbial denitrification or of a nitrate radiolysis process. Our results show that radiolysis plays a very minor role in the generation of N_2O under the conditions of our tests. Reports in the literature state that magnesium oxide inhibits microbial activity by formation of superoxide ions on the oxide surface. In order to discover its effects on WIPP-relevant populations, we cultivated our microorganisms in brine-based cultures that contained increasing concentrations of MgO. Toxic effects included decreased cell numbers and lack of pigment production at higher levels of MgO (above 0.5 g/L). It is vital for safe disposal of transuranic waste at the WIPP site that we understand microbial processes occurring in this unique environment.

INTRODUCTION:

The Waste Isolation Pilot Plant (WIPP) located at Carlsbad, New Mexico and operated by the Department of Energy (DOE) is a deep geologic repository that was designed to be the permanent disposal site for defense-related transuranic waste (TRU). It lies in an ancient, stable salt formation 2,150 feet underground. The DOE has the responsibility for storage and management of defense-related TRU waste (1). The cleanup of weapon sites and dismantling of aging weapons will generate even more radioactive material that will require storage space. Much of TRU waste (containing elements heavier than uranium, and having a half-life of more than 20 years, such as plutonium) consists of contaminated lab gloves, tools, clothing, plastics, and dried sludge. This material will remain radioactive for many thousands of years, thus creating a need for a permanent storage area for these materials in an isolated location in order to prevent human exposure for present and future generations.

In 1992, based on recommendations by the National Academy of Science and the New Mexico Environmental Evaluation Group, DOE selected Los Alamos to conduct the WIPP Actinide Source Term Waste Test Program (STTP) to confirm predictive capabilities of computer models concerning the fates of the waste and the suitability of WIPP to house waste safely and permanently (1,2). To date, the DOE has invested about 40 million dollars in the project, including about four million for capital equipment. Actual sampling and analysis of the test containers began in March 1995. The STTP is a project designed to measure time-dependent and total concentration of actinides in actual waste immersed in brines that are chemically similar to those found in the salt formations in which the WIPP underground is located. The experimental model consists of 15 drum scale containers with heterogeneous wastes (combustibles, laboratory wastes, metals), 33 liter scale containers with homogeneous wastes (sludges, cemented or solidified waste, pyrochemical salts), and six pressurized liter-scale containers at 60 bars (870 psig) pressure with CO_2. The containers are made of titanium metal which is resistant to brine corrosion. All containers are maintained in an environment that is temperature-controlled to 30^{o}C and are rotated 360 degrees once a week. The containers are sampled on a regular

basis. The samples are filtered and analyzed for parameters that will be needed to understand the chemistry of actinides in the WIPP environment over the long term. The test protocol has included assays of the actinide elements and the headspace gases generated by the transuranic waste. This waste is representative of inventories that are being stored temporarily above the ground or in shallow burial sites at several DOE facilities nationwide.

The STTP test vessels were inoculated with a consortium, or mixture, of at least five dominant species of halophilic or "salt-loving" microorganisms obtained from salt ponds at the WIPP Site. The effects of the presence of indigenous organisms as well as the consequences of the introduction of "foreign" microorganisms to the WIPP environment must be evaluated (3,4). Besides degrading wastes and producing gas, these organisms could have a role in enhancing the release and migration of radionuclides to the biosphere. Additional effects might be corrosion or degradation of the metal storage containers or the immobilization of the matrix materials inside them such as the cement, thus facilitating the release of radionuclides. Corrosion might also occur from inside a container that contains contaminated waste or from microbes present in backfill material. We have been following this microbial population over time in the STTP vessels, taking samples from the containers for microbial analyses. These studies were of three basic types: 1) viability of the organisms in the liter-scale containers, 2) gas generation, and 3) the toxic effects of magnesium oxide, a potential backfill material for the waste in storage at WIPP. Any program that purports to assess the long term safety of a nuclear storage facility must include studies on the microbiological effects on the particular environment under study.

EXPERIMENTAL METHODS:

Viability studies.

Indigenous or introduced microorganisms could be important in actinide transport or retardation, transformation of organics or inorganics in radioactive waste containers, or gas generation due to metabolic activity. In order to demonstrate the potential for microbially-influenced transformations of TRU waste in the STTP test vessels, a consortium consisting of many different species of microorganisms was added to all containers. A stable consortium isolated from the inoculum and designated BAB, contained halophilic species with a broad range of metabolic capabilities. It was derived from a culture prepared at Brookhaven National Laboratory from muck pile salt, hypersaline lake brine, and sediment slurry taken from the WIPP environs.

Detection of active microorganisms can be difficult since no single analytical method will identify all physiological types (5). Initial viability studies based on growth measurements of specific types of bacteria utilizing different anaerobic metabolic processes were designed to correlate the gas generation observations noted in the STTP

test containers with the specific types of microbial activity. Microbiological media selected for cultivation of the microorganisms included two different formulations: 1) one for the detection and enhancement of nitrate-reducing organisms (denitrifiers) and 2) one for glucose fermenters. Samples from both the drum-scale and liter-scale test vessels were cultured in both types of media. One ml aliquots of each test sample were inoculated into media in glass serum vials that contained inverted glass (Durham) tubes. The vials were incubated in a 30^{o}C incubator for approximately two months. They were inspected weekly for signs of growth: turbidity and gas evolution in the form of bubbles in the Durham tubes. At one to two week intervals, aliquots of the cultures were collected by needle and syringe and processed for total, but not necessarily viable, cell counts by the DAPI (4'-6-diamidino-2-phenylindole dihydrochloride) staining procedure (6), utilizing epifluorescence microscopy. Since these growth experiments showed little or no growth under the conditions chosen and did not rule out the presence of viable cells, a different approach was required.

TABLE I: Media Formulations for Cultivation of Denitrifiers and Fermenters

Nutrient Specific To:	**Components**	**Quantity (g/L)**
Denitrifers (Stock solution, 20x concentrate)	sodium succinate	100
	KNO_3	20
	K_2HPO_4	5
	yeast extract (Difco)	10
Fermenters (Stock solution, 20x concentrate)	glucose	100
	$(NH_4)_2SO_4$	10
	K_2HPO_4	5
	yeast extract (Difco)	10

Viability studies based on use of other fluorescent dyes were undertaken. Instead of culturing aliquots from the test vessels, stains for cell counts were prepared directly from the aliquots. The first stain used was CTC (5-cyano-2,3-di-4-tolyl-tetrazolium chloride) (5). It was necessary to modify this particular stain for use with extreme halophiles because it did not dissolve very readily in the brine. This technique does not stain a particular cellular component, but is dependent on the metabolic respiration of living cells to reduce the non-fluorescent CTC compound by means of electron transport activity, to the fluorescent reduced CTC-formazan that accumulates intracellularly. Only actively respiring organisms are stained by this technique. The CTC staining procedure requires a four hour incubation period, thus ensuring that respiration can occur. As with the DAPI procedure, the cells are then filtered from the solution onto a black filter and observed by epifluorescence microscopy.

Use of a viability staining kit called Live/Dead^{R} Baclight ^{TM} (Molecular Probes, Inc, Eugene, OR) (7) proved superior to the CTC method in that the need for the four hour incubation period was eliminated. This staining process utilizes a mixture of SYTO 9^{R} green fluorescent nucleic acid stain and a red fluorescent nucleic acid stain, propidium iodide. Live cells are stained fluorescent green and the nonviable cells are red with this method. Sixty-six raw samples (some duplicates) from liter-scale STTP vessels have been received and processed for cell counts for enumeration of total and viable cells.

Gas generation.

Our microbial studies explored the issue of gas generation in the test vessels in two ways. The first set of experiments addressed the effect of radiolysis on the generation of primarily, nitrous oxide and hydrogen, in the presence of various waste matrix materials and nitrate in order to determine the major input sources for the large amounts of gases that were noted in the STTP test vessels. As hydrogen is known to be a product of radiolysis, our emphasis was placed more heavily on nitrous oxide generation in the presence of nitrate. Microbial production of hydrogen can also occur under appropriate anaerobic conditions. The test system contained various combinations of an alpha source (plutonium), Brine A , Envirostone, melamine (an organic constituent of Envirostone), and nitrate. The test solutions in glass serum vials were outgassed using argon in an anaerobic glove box in order to achieve negligible initial levels of oxygen, carbon dioxide, and nitrogen. The concentrations of nitrate and plutonium used in this experiment represented the maximum levels measured in the STTP test vessels, while the amounts of Envirostone and melamine were approximated based on average amounts in the test vessels. All vials were stored in an incubator at 30^{o}C and were mixed frequently by inversion. Sampling of the headspace gases produced in the vials was performed in the glove box at times t = 0, 5, and 10 months. No-Con gas tight syringes were used for the sampling. The filled syringes were transported to a different facility for analysis by gas chromatography-mass spectrometry (GC-MS). The following table depicts the matrix of the experiment, showing the contents of the ten test vials.

TABLE II: N_2O Gas Generation from Radiolytic Processes Test Matrix

Vial No.	Contents	Vol. Brine A (ml)	Wt. Envirostone (gm)	Wt. Melamine (gm)	Wt. x $NaNO3^-$ (gm)	Vol. 7.78E-5M ^{239}Pu (ml)
1	Only Brine A	30	0	0	0	0
2	Envirostone	30	2 - 3	0	0	0
3	Envirostone, Pu	28.7	2 - 3	0	0	1.3
4	Envirostone, NO_3^-, Pu	28.7	2 - 3	0	1.2	1.3
5	NO_3^-	30	0	0	1.2	0
6	NO_3^-, Pu	28.7	0	0	1.2	1.3
7	Pu, Melamine, NO_3^-	28.7	0	2 - 3	1.2	1.3
8	Pu, Melamine	28.7	0	2 - 3	0	1.3
9	Melamine	30	0	2 - 3	0	0
10	Pu	28.7	0	0	0	1.3

Confirmatory samples were taken at time points in excess of one year and analyzed as described. There was no evidence of any microbial activity (growth) at the end of the incubation period.

Aside from radiolysis, the most likely source of the N_2O in the STTP test vessels is through the microbial reduction of nitrate, an enzymatic process known as denitrification, which produces nitrous oxide as an endpoint of microbial reduction in some species and as an intermediate prior to conversion to nitrogen gas in others. The assessment of microorganisms as the source of nitrous oxide production from nitrate reduction was incorporated into the magnesium toxicity experiments described below where the control container was observed for evidence of N_2O production.

Magnesium oxide toxicity.

The inhibitory and toxic effects of magnesium oxide on WIPP-derived organisms are of interest because of the possibility the MgO may be used as a backfill in the repository (8). Addition of MgO to selected STTP test vessels in order to determine at the bench scale the optimal amount needed in the repository has been proposed for the project. For these studies, we investigated the toxic or inhibitory effects of MgO using

cultures of BAB, our consortium of halophilic microorganisms indigenous to WIPP. At least one of the species produces a reddish carotenoid pigment (9) which was a useful marker for growth in cultures otherwise turbid due to the presence of MgO. The basic experiment was performed in two different ways. In the first two experiments, the microbial inoculum was added to bottles of media containing MgO, and in the third, the MgO was added to actively growing cultures. The cultures were prepared in a brine-based medium containing nitrate (Brine A/Denitrifying medium) as an electron acceptor, in a series containing seven different amounts of MgO: 0.9 mg, 7.6, 28, 61.7, 105, 295.4, and 473.7 mg per 50 ml culture, plus another control culture without MgO. The cultures were sampled every seven days for a six week period and then stained with the DAPI procedure and analyzed for total cell counts.

In another set of experiments, we determined the threshold toxicity level, i.e. the level at which 100% of the microbial growth is inhibited by using amounts of MgO in 5 mg increments between 5 and 35 mg. In addition, we measured headspace gas production by GC-MS in the presence of the MgO. Cultures were prepared in duplicate in sealed glass serum vials, each containing 50 ml of Brine A/Denitrifying medium. One vial without added MgO served as a growth control. One milliliter aliquots of an actively growing culture of BAB were used as the inoculum for each vial. Cultures were incubated for more than 30 days at 30^{o}C. At weekly intervals, all cultures were sampled for total and viable cell counts. Gas samples were taken on days 0, 14, and 30.

A pH-controlled experiment was also devised where Brine A/Denitrifying medium was buffered with 0.2M Tris buffer, giving a final pH of 7.0. A set of six BAB cultures was prepared that contained 0, 5, 10, 15, 20, and 25 mg MgO, respectively. These cultures were incubated at 30^{o}C for three weeks. Cultures were sampled and the aliquots stained for total cell counts.

RESULTS AND DISCUSSION:

The STTP containers have been under investigation for more than two years, but microbial growth studies of the samples were discontinued in early 1997 as there was no evidence of further growth. In addition, cell counts as determined by microscopy had declined to extremely low numbers, almost undetectable with the sample sizes used. Reports in the literature indicate that more than 99 per cent of all bacterial species that exist in the environment are viable, but not culturable (10). Since gases have continued to be evolved in the STTP vessels, we changed our method for demonstrating viable organisms to the use of two fluorescent stains, CTC and the Live/Dead^{R} procedure. Small numbers of viable microorganisms were detected in samples of the brine column taken from three vessels that contained organic wastes. As attachment and growth on surfaces is vital to the survival of many microorganisms, and biofilm formation is important in many degradation processes (10), it is conceivable that halophilic microorganisms have migrated to the sludge in the vessels. The sludge would provide a nutrient source as well as an attachment site. Biofilm formation can occur under anaerobic conditions where a series of electron acceptors and donors are used in a chain of different reactions through a symbiotic relationship beneficial to all members of the biofilm. With time, the biofilm may become very tenacious through production of extracellular polymers that will be

cemented in place. This scenario could have occurred in the STTP vessels so that even though the vessels are rotated and mixed, the microorganisms remain aggregated and settle rapidly before the samples of the brine are taken.

Several of the STTP test vessels with high concentrations of nitrate ion have generated large amounts of N_2O. The possibility for microbial denitrification processes and/or radiolysis as the cause of the gas production were both investigated. In the natural environment, in niches where oxygen is scarce or absent, denitrification occurs where nitrate is used as an alternate electron acceptor during microbial anaerobic respiration, and it is converted into more reduced forms of nitrogen.The principle products of dissimilatory nitrate reduction, or denitrification (11) are nitrous oxide and dinitrogen gas (N_2). The conversion of nitrate to these products by this process is illustrated as follows with the oxidation state of nitrogen shown:

$$\underset{+5}{NO_3^-} \text{ ---> } \underset{+3}{NO_2^-} \text{ ---> } \underset{+2}{[NO]} \text{ ---> } \underset{+1}{N_2O} \text{ ---> } \underset{0}{N_2}$$

NO is shown in parentheses because there is some controversy concerning the formation of NO in this process. In any case, it is rapidly converted to N_2O, making it difficult to detect, and to determine what role it plays in the process. Many organisms have the enzyme systems (i.e., the first two in the chain, nitrate reductase and nitrite reductase) to produce only N_2O, while other organisms have enzyme systems to carry out the reduction to completion (i.e. nitric oxide reductase and nitrous oxide reductase), producing N_2 as the final gaseous product. In organisms which produce N_2 from nitrate, N_2O will be an intermediate in the denitrification process. Under typical environmental conditions, denitrification is the only process whereby nitrate is reduced to N_2O or N_2.

In environments such as radioactive waste repositories where radiolysis of material can occur, the possibility for the reduction of nitrate to form N_2O also exists. This process is not a direct radiolytic process where the +5 state of nitrogen in nitrate is reduced down to +1 state in nitrous oxide entirely by radiolysis. Instead the reduction will be secondary to a process in which nitrate is first reduced to nitrite as a result of radiolytic processes. Several studies on the formation of nitrite from the radiolysis of nitrate are available in the scientific literature (12,13,14). In all of these studies, N_2O generation is postulated to be dependent on the concentration of nitrate, nitrite and organic material, although an unambiguous understanding of the exact mechanisms involved has not been achieved. Once nitrite is formed, other chemically driven redox processes can occur. For instance, the following half-reaction illustrates how nitrite can be reduced to nitrous oxide:

$$2NO_2^- + 3H_2O + 4e^- \text{ ---> } N_2O + 6OH^- \quad (E^o = 0.15 \text{ V})$$

The electrode potential for this process is much lower than that required for biological denitrification ($E^o = 0.40$ V) (15). In all likelihood, the test containers hold material that can be readily oxidized during nitrite reduction. Although feasible, no studies have been

found to date in the literature in which this particular scenario for N_2O production has been investigated.

In the STTP samples, large rates of N_2O gas generation have been measured in test vessels holding Envirostone and high concentrations of nitrate. The organic compound melamine is a constituent (15-20% by wt.) of Envirostone. It is hypothesized that, in addition to microbial denitrification: 1) N_2O generation may be tied to the organic constituent in Envirostone, perhaps concurrent with nitrate reduction to nitrite, or 2) chemical redox processes come into play once nitrite formation occurs. In order to investigate both of these hypotheses, we have performed scoping experiments to determine if indeed N_2O generation occurs in test mixtures containing combinations of Pu (the alpha source), Brine A, Envirostone, melamine, and/or nitrate.The radiolysis experiment demonstrated indirectly and under artificial experimental conditions that radiolysis plays a minor role in the N_2O generation that is occurring in the STTP test containers. Only one sample, one that contained Brine A, Envirostone, and plutonium, produced nitrous oxide after five months. There was no evidence of N_2O production in any sample after ten months. Hydrogen gas generation was also extremely low and not significant. Since generation of large amounts of gas in the WIPP repository over time would be highly undesirable, the experiments conducted to pinpoint the major sources of these gases are relevant.

TABLE III: Concentrations of Headspace Gases* at 5 and 10 months, in ppm

Samples Taken 1/28/98	**H_2**	**N_2**	**CO_2**	**N_2O**
Brine A + Envirostone + Pu	44	30475	834	23
Brine A + Envirostone + NO_3^- + Pu	0	Off scale	360	0
Brine A + NO_3^- + Pu	0	Off scale	441	0
Brine A + NO_3^- + Pu + Melamine	12	25922	327	0
Brine A + Pu + Melamine	33	25730	367	0
Brine A + Pu	19	27125	328	0
Final Samples Taken 5/26/98				
Brine A + Envirostone + Pu	10	12417	568	0
Brine A + Envirostone + NO_3^- + Pu	10	25610	918	0
Brine A + NO_3^- + Pu	0	14790	372	0
Brine A + NO_3^- + Pu + Melamine	0	15235	342	0
Brine A + Pu + Melamine	0	17615	681	0
Brine A + Pu	0	17861	483	0

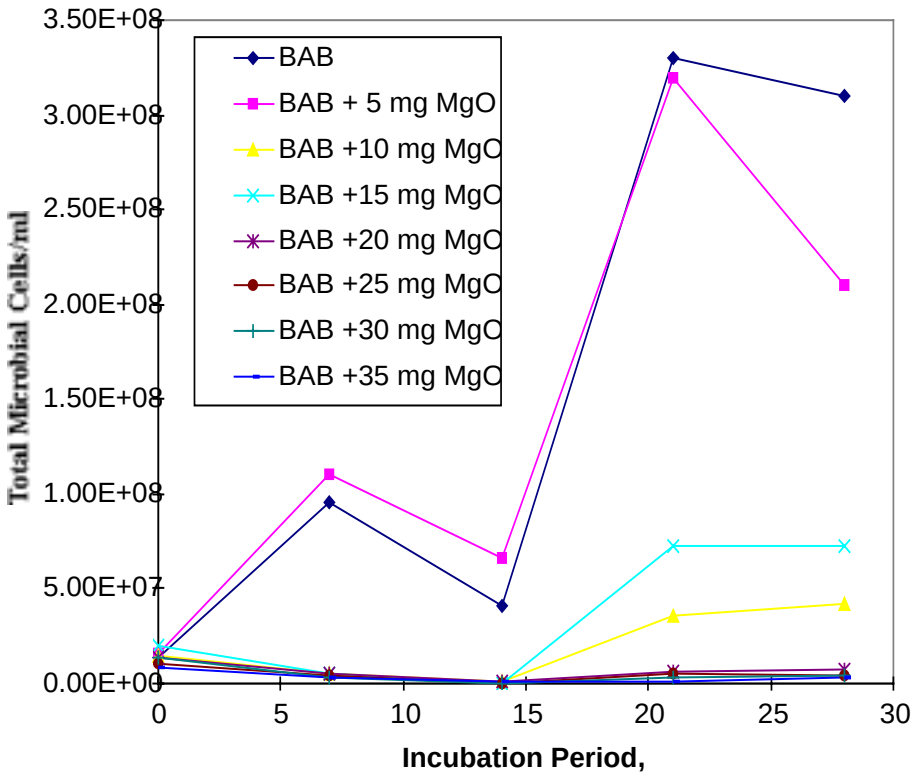
*O_2/argon, and CH_4 levels were also measured but were not found to be significant.

Nitrous oxide production has been measured in several studies of the radiolysis of nitrate-containing material (16,17,18,19) at Oak Ridge National Laboratory, Argonne National Laboratory and Westinghouse Savannah River Co. From all of these studies, it was clear that N_2O generation is directly related to the radiolysis of nitrate, however, the amounts generated have consistently been extremely low, except in the presence of hydroxylamine nitrate (18). The results from one of the studies at Argonne on the radiation chemistry of synthetic waste suggest that organic additives greatly affect the amount of N_2O produced in solutions of nitrate during radiolytic irradiation (19).

Our experimental conditions were designed to emulate the STTP test containers and did contain some solid material, however the bulk of the sludge was not present. A large amount of radiolysis could take place in the sludge, which contains most of the actinide material. It is difficult to simulate the sludge. Therefore, radiolysis may have a greater effect than our results showed. It still appears to be less significant than the microbial component.

Magnesium oxide has been shown to inhibit microbial activity, possibly by formation of superoxide ions on the oxide surface. Superoxides are free radicals and may have a lethal effect on microorganisms that lack the enzymes superoxide dismutase and catalase (20,21). The pH of a magnesium oxide slurry is very high, potentially more alkaline than most microorganisms can tolerate. Our initial studies of MgO toxicity revealed definite signs of inhibition with increasing amounts of MgO. The major toxic effects observed were decreased cell numbers and lack of pink pigment production in cultures with higher levels of MgO. Assessment of growth of the cultures became increasingly difficult to interpret with the naked eye as the amounts of MgO were increased, due to the presence of insoluble MgO particles. Also, many cells appeared on the stained preparations to have been removed from solution while attached to the MgO particles, thus distributing them unevenly on the slides. We repeated the initial experiment, and at the same time, tested for toxicity both at the time of addition of the inoculum ($t = 0$), and by addition of the MgO to cells that had been growing for about three weeks. Toxicity indicators were present in all cultures that contained 28 mg of MgO or more/ 50 ml medium. As there was a considerable gap between the amounts of MgO used in these first studies (7.6 mg vs. 28 mg) the threshold amount for complete inhibition could well be much lower than 28 mg/50 ml. Therefore, the experiment was repeated using 5 mg increments up to 35 mg. Graph 1 shows the results of this study; the threshold amount of MgO is between 15 and 20 mg/50 ml media.

Graph I: MgO Toxicity to BAB C



We did not attempt to fully elucidate the mechanism of toxicity in our experiments, but assumed that a combination of factors were operating. In order to separate possible toxic effects and evaluate the role of pH, the controlled pH experiment with Tris-HCl was performed. At the end of the three-week incubation period, all six cultures that contained 0 to 25 mg MgO had maintained a pH of 7, exhibited pink pigment, and had similar cell counts. (See Table IV).

Table IV: Effect of the Addition of Tris-HCl on BAB Cultures

Samples in Series	Cell Counts/ml	pH of Culture	Pigment Present?
BAB + 0 mg MgO	1.60E+08	7.0	+
BAB + 5 mg MgO	2.10E+08	7.0	+
BAB + 10 mg MgO	1.90E+08	7.0	+
BAB + 15 mg MgO	1.80E+08	7.0	+
BAB + 20 mg MgO	1.90E+08	7.0	+
BAB + 25 mg MgO	1.60E+08	7.0	+

Table V displays the gas generation data for the cultures that contained MgO in 5 mg increments. After two weeks of incubation, only three of the cultures, the control containing no MgO and the two with the lowest concentrations of MgO, contained N_2O in the headspace. By the end of four weeks, only two samples contained N_2O, the control and the sample containing 10 mg MgO.

Table V: Toxicity Study, Preliminary Gas Analysis Results, in ppm

Headspace Gas Analysis at Time of Inoculation	**N_2**	**CO_2**	**N_2O**
BAB, no MgO	17334	1034	27
BAB + 5 mg MgO	18631	620	0
BAB + 10 mg MgO	17972	170	0
BAB + 15 mg MgO	19644	166	0
BAB + 20 mg MgO	17435	115	0
Headspace Gas Analysis 2 Weeks after Inoculation			
BAB, no MgO	19461	6690	545
BAB + 5 mg MgO	23743	5026	18
BAB + 10 mg MgO	26428	356	4
BAB + 15 mg MgO	17720	95	0
BAB + 20 mg MgO	15777	85	0
Headspace Gas Analysis 4 Weeks after Inoculation			
BAB, no MgO	20547	6888	7988
BAB + 5 mg MgO	23837	6231	0
BAB + 10 mg MgO	28940	1398	492
BAB + 15 mg MgO	25740	256	0
BAB + 20 mg MgO	23811	222	0

CONCLUSIONS:

The presence of viable cells has been confirmed in certain STTP test vessels, those containing organic waste materials. Samples were taken from the brine column, but not the sludge. As these containers are still generating large amounts of gas, it would be desirable to analyze the surfaces of the sludge contents for microorganisms.

Radiolysis has been shown under our experimental conditions to be an insignificant source for nitrous oxide production in the presence of Envirostone and melamine. Microbial N_2O generation has been shown to be significant through indirect measurements, but not through measurements on the test vessels themselves. The control denitrification culture in the MgO experiment showed a several orders of magnitude increase in N_2O production with growth over time.

The addition of excess magnesium oxide will help to maintain a dry condition in the repository through reaction with water to produce $Mg(OH)_2$ and will retard microbial activity. Magnesium oxide decreases the amounts of gas generated by a mixed culture of actively growing halophilic microorganisms. Toxic effects were seen with levels above 0.5 g/L MgO when pH was not adjusted. The results of the pH-controlled experiment suggest that the major mechanism by which MgO inhibits microbial growth is due to the high pH present, although the alternate mechanism of superoxide formation may also be operating.

Awareness that microbial activity will affect the performance of a system designed for geologic disposal of radioactive waste is universally recognized; many other countries have programs to study and quantify microbial effects on their particular systems. Microbes at WIPP may play roles in actinide retardation or transport, biological transformation of organic or inorganic materials in radioactive waste containers, or gas generation. It is vital for safe disposal at the site that we understand microbial processes occurring in this unique environment.

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