Sandia National Laboratories
Waste Isolation Pilot Plant
Test Plan TP-99-01

Re-Evaluation of Microbial Gas Generation under Expected Waste Isolation Pilot Plant Conditions

WBS 1.1.09.3.1

Revision 1

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1.0 REVISION HISTORY

Previous experimental work on microbial gas generation was conducted under a test plan for laboratory and modeling studies of repository and radionuclide chemistry for the Waste Isolation Pilot Plant (Brush, 1990). Test Plan TP 99-01 is based on the findings of the earlier work and provides specifications for further studies of microbial gas generation under expected Waste Isolation Pilot Plant (WIPP) conditions. Revision 1 of the plan updates quality assurance (QA) requirements and milestones to reflect WIPP budgetary and QA changes since the previous revision became effective on 02/04/99.

2.0 DEFINITION OF ACRONYMS

ATCC	American Type Culture Collection
AWWA	American Water Works Association
BNL	Brookhaven National Laboratory
CCA	Compliance Certification Application
DAS	Department of Applied Science
DOE	Department of Energy
GC	Gas Chromatography
LSC	Liquid scintillation counting
MPN	Most probable number technique
PA	Performance assessment
PCR	Polymerase chain reaction
PPE	Personal protective equipment
PQR	Project quality representative
QA	Quality Assurance
QAPD	Quality Assurance Program Document
QAPP	Quality Assurance Program Plan
RWP	Radiological work permit
SNL	Sandia National Laboratories
SOP	Standard Operating Procedure
SWCF	SNL WIPP Central File
TOP	Technical Operating Procedure
WBS	Work Breakdown Structure
WIPP	Waste Isolation Pilot Plant

3.0 INTRODUCTION

The transuranic wastes to be emplaced in the WIPP contain a large quantity of cellulosics, plastics, and rubbers. These materials will potentially be degraded sequentially by microorganisms in the repository via the following reactions (Wang and Brush, 1996):

$$C_6H_{10}O_5 + 4.8H^+ + 4.8NO_3^- \rightarrow 7.4H_2O + 6CO_2 + 2.4N_2 \quad (1)$$

$$C_6H_{10}O_5 + 6H^+ + 3SO_4^{2-} \rightarrow 5H_2O + 6CO_2 + 3H_2S \quad (2)$$

$$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2. \quad (3)$$

Microbial gas generation will pressurize the WIPP repository and increase spalling release of radionuclides in the event of human intrusion. The CO_2 generated from microbial reactions will also acidify the repository and increase actinide mobility in WIPP brines. To mitigate the effect of microbial gas generation on brine chemistry, a MgO backfill system has been designed to sequester CO_2 from aqueous and gaseous phases in the repository. The quantity of MgO to be added to the backfill system is directly determined by the maximum amount of CO_2 to be generated in the repository.

Previous work at Brookhaven National Laboratory (BNL) on microbial gas generation was focused on the denitrification pathway (Francis and Gillow, 1994; Francis et al., 1997). The work shows that rates of cellulose degradation in the presence of an excess nitrate amendment are significantly higher than those without excess nitrate. This points out the possibility that the microbial gas generation rate may decrease as biodegradation shifts from an energetically-favorable pathway to a less energetically-favorable one, with the rate of methanogenesis being the lowest. The gas generation rates used in the WIPP Compliance Certification Application (CCA) are derived mainly from excess nitrate-amended experiments. Since more than 90% of organic materials in the repository are expected to be degraded via anaerobic pathways, specifically by methanogenesis (Wang and Brush, 1996), the CCA has overestimated gas generation rates. Direct measurements of the rate of methanogenesis will certainly reduce this conservatism in the CCA models.

It was observed in the previous experiments that the rate of cellulose degradation was high during the first 400 days and diminished with time. The decrease in the rate could be attributed to nutrient depletion, metabolite accumulation, or the increase of refractory fraction in remaining organic materials; the actual cause still needs to be determined. Cellulose materials are generally composed of amorphous and crystalline fractions. The amorphous fraction is more liable to biodegradation than crystalline fraction (e.g. Leschine, 1995). Natural cellulose can contain 60-90% of crystalline fraction (Leschine, 1995). In the WIPP combustible materials, the crystalline fraction can be even higher. To further reduce the conservatism in the CCA, scoping experiments on the effect of crystallinity on cellulose degradation are necessary.

The rate of microbial degradation under humid conditions is important for the prediction of gas generation in the cases where the repository is relatively dry. The rate used in the CCA was derived from the “humid” experiments in which small amounts of liquid inoculum were directly added to cellulose samples (Francis et al., 1997). The addition of the inoculum might increase the water content of the humid experiments. The emplacement of MgO backfill in the WIPP can possibly reduce the water vapor pressure in the repository down to $10^{-6.3}$ atm (Wang, 1998). Based on these considerations, the gas generation rate under relatively dry repository conditions may be overestimated in the CCA.

4.0 REGULATORY REQUIREMENTS

There are no specific regulatory requirements applicable to the conduct of this work. All experimental work will be performed at Brookhaven National Laboratory under contract AT-8739 according to Quality Assurance procedures set forth in Sandia National Laboratories Nuclear Waste Management Program Procedures (NPs) and the BNL Standards Based Management System (SBMS) procedures for calibration and purchasing items.

5.0 COMPLIANCE JUSTIFICATION

40 CFR 194 § 194.14 and § 194.24 specifically require considering microbial degradation and gas generation of combustible materials. Microbial gas generation is expected to have two major impacts on WIPP performance. First, microbial gas generation together with metal corrosion will increase repository pressure and therefore enhance spalling and direct brine release of radionuclides. Second, CO_2 generated from microbial degradation will lower pH and increase carbonate concentration in the WIPP brines and therefore significantly increase the solubility of actinides. To mitigate this detrimental effect, MgO will be added to the repository as a backfill to provide additional assurance for WIPP containment. The maximum CO_2 to be generated thus directly determines the amount of MgO to be emplaced in the repository. The work documented in this Test Plan will help to derive more realistic gas generation parameters. Therefore, it will allow us to further demonstrate the robustness of the WIPP repository and even possibly to reduce the amount of MgO needed.

6.0 PURPOSE AND SCOPE

This work documented in this Test Plan will provide additional information on gas generation due to microbial degradation of cellulosic materials under WIPP conditions. Combined with the previous experimental data, this information will help to constrain a more realistic set of gas generation parameter values than those used in the CCA calculations. The new set of gas generation data will allow DOE to further demonstrate the robustness of the WIPP repository and even possibly to reduce the amount of MgO needed. This work consists of the following major activities:

1. Re-evaluation of the existing microbial gas data and develop appropriate technical approaches to reducing the conservatism in the current gas generation model.
2. Scoping experiments to test the effect of crystallinity on cellulose degradation under hypersaline conditions and to clarify the factors that caused a diminishing microbial gas generation rate with time in the previous experiments.
3. Re-examination and improvement of the experiment for cellulose degradation under humid conditions to derive a more realistic rate for humid microbial degradation.
4. Experiment to determine the rate and extent of methanogenesis by halophilic microorganisms. Determine the effect of MgO on the rate and extent of methanogenesis.

7.0 EXPERIMENTAL PROCESS DESCRIPTION

The research necessary to address the above objectives of this work is described in the following sections. The general experimental provisions and requirements apply to all of the experiments. These include: (i) where an understanding of the precision of a measurement can be obtained by replicates of an individual measurement, those measurements shall be performed in triplicate, and (ii) record in the scientific notebook the source of all reagents, their lot number (if available), and the purity (if known).

7.1 Re-Evaluation of the Existing Microbial Gas Generation Data and Develop Appropriate Technical Approaches to Reducing the Conservatism in the Current Gas Generation Model

Previous studies provided data on gas generation due to cellulose biodegradation under repository-relevant test conditions. The results were published in two reports, entitled “Effects of Microbial Processes on Gas Generation Under Expected WIPP Repository Conditions, SAND93-7036” (Francis and Gillow, 1994) and “Microbial Gas Generation Under Expected Waste Isolation Pilot Plant Repository Conditions, SAND96-2582” (Francis et al., 1997). The reports discuss data on: (i) gas generation from unamended samples; (ii) gas generation from nutrient amended samples; (iii) gas generation from amended samples with excess nitrate simulating nitrate bearing TRU waste. The results show differences in the rate and extent of gas generation among the treatments. The reasons for the variations in gas production in the above treatments will be examined as described in this Test Plan.

7.1.1 **Re-examine Gas Production in Samples With and Without Nutrient Amendments**

The gas generation rates used in the CCA are derived mainly from excess nitrate-amended experiments. While these rates are most relevant to a fraction of the TRU waste containing nitrate, it is important to examine the rate and extent of gas generation from samples containing no nutrient additions and samples amended with a lower concentration of nutrients. First, in order to place the gas generation rates from experiments at BNL into perspective, an in-depth review of the published literature relevant to biodegradation of cellulose will be performed. The gas production data will be compared to other studies done on simulated and actual waste forms under varying nutrient conditions. While it is expected that in most cases studies in the literature will have little direct relevance to conditions in WIPP, it will be useful to identify if similar trends in rates have been observed. For example, whether denitrification is typically a transient process followed by a lowered gas production rate due to fermentation, sulfate reduction and methanogenesis by natural microbial communities in the presence of a high loading of cellulose. This will lend support to a defense of less conservative gas production if there is a recommendation for this as a result of the re-evaluation of the data.

The degradation products of cellulose (organic acids), sulfate concentration, and pH will be examined in order to determine the cause for the diminished rates of gas production. Additional information on the distribution of microorganisms in the samples will be obtained. These include the presence and abundance of denitrifiers, fermenters, sulfate reducers, and methanogens by the most probable number technique (MPN) and if possible by molecular techniques (polymerase chain reaction, or PCR, of 16s ribosomal-RNA from existing samples followed by sequence analysis for specific microbial species). This will allow us to determine the causes(s) of cessation of gas generation and specifically if it is due to the absence of specific groups of microbes. This critical analysis has not been performed to date and will allow the assignment of a reaction pathway to the gas production data used by the WIPP performance assessment (PA). This may result in a recommendation that less conservative gas production data be considered.

7.1.2 Sulfate Reduction and Methanogenic Activity

The samples that have been incubated for 7 years at 30 ± 2°C to date will be selected and analyzed for the gases hydrogen sulfide and methane. These two gases are products of important reaction pathways relative to WIPP (Wang and Brush, 1996). The reason why these gases were not detected by analysis performed over 3.4 years is not clear. They may not have been detected because testing stopped prior to their production. Gas analysis of the selected samples incubated for 7 years at 30±2°C include measurement of total gas using a digital pressure gauge fitted to a 22 gage needle, and withdrawal of a sample of gas using a Pressure-lok™ gas-tight syringe. Carbon dioxide will be analyzed by gas chromatography (GC) with thermal conductivity detection. Methane will be analyzed by GC using a Varian 3400 gas chromatograph with flame ionization detection. Hydrogen sulfide will be analyzed by either wet chemistry to detect sulfide (Methylene Blue Method, American Water Works Association (AWWA) method 4500D) or gas chromatography. Liquid samples will be analyzed for the presence of sulfate reducing and methanogenic bacteria by most probable number technique (MPN) or by molecular methods if possible as described in Section 7.1.1.

7.2 Scoping Experiments to Test the Effect of Crystallinity on Cellulose Degradation Under Hypersaline Conditions: A Diminishing Microbial Gas Generation Rate with Time May Be Related to the Degree of Crystallinity of Cellulose

7.2.1 Determination of the Effect of Crystallinity on Cellulose Degradation Under Hypersaline Conditions

The effect of crystallinity on cellulose degradation under hypersaline conditions, which may be a factor that caused a diminishing microbial gas generation rate with time, will be assessed by a review of the literature followed by limited laboratory experiments. The initial experiments will involve enzymatic assay of degradation of amorphous and crystalline forms of ^{14}C-labeled cellulose by the enzyme cellulase in water and in brine. If warranted, further laboratory experiments will consist of incubation of the ^{14}C-labeled amorphous and crystalline forms of cellulose in brine with and without a nutrient amendment, in the presence of denitrifiers, fermenters, sulfate reducers, or methanogens. The resultant $^{14}CO_2$, or $^{14}CH_4$, produced due to biodegradation of the labeled fraction, will be quantified by liquid scintillation counting (LSC). An EG&G Guardian 1414 Environmental Monitoring System or EG&G Wallac 1410 β-LSC will be used to quantify $^{14}CO_2$ produced after trapping in NaOH using standard procedures. The comparison of gas production from amorphous cellulose relative to crystalline cellulose will provide insight into the potential effect that the physicochemical characteristics of cellulose may have on gas production rates in the WIPP.

7.2.2 Determination of the Extent of Cellulose Degradation from the Long-Term Gas Generation Study

The solids from selected samples (previously incubated for ~7 years) will be recovered by centrifugation, washed with deionized water, and dried in a drying oven at 90°C to a constant weight. The loss in dry weight of the cellulose will be determined by subtracting from the initial dry weight. The weight loss between treatments will be compared. If possible, the degree of crystallinity of the cellulose sample before and after microbial action will be determined and compared with the degradation rates.

7.3 Re-examination and Improvement of the Experiment for Cellulose Degradation Under Humid Conditions to Derive a More Realistic Rate for Humid Microbial Degradation

A review of the literature on the effect of water activity on microbial activity will be conducted. Magnesium oxide was not considered when the BNL Gas Generation Experiment began to examine biodegradation of cellulose under humid conditions. Addition of MgO as backfill will further reduce water activity for humid cases. More relevant water activities (bounded) provided by Sandia National Laboratories will be used to determine the effects of humid conditions on microbial activity. To avoid introducing additional water into samples, microbial inoculum consisting of nearly-dry cell pellets of denitrifiers, fermenters or methanogens, or a mixture of these, will be added to a test system containing ^{14}C-labeled substrate. This system will be assayed for $^{14}CO_2$ production, indicative of microbial activity, by LSC. Gas production rates under extremely low water vapor pressures may be provided, with extrapolation to even lower vapor pressures than actually measured, using this method, with extremely low limits of detection afforded by the analysis of radiolabeled end-products.

7.4 Determination of the Rate and Extent of Methanogenesis by Halophilic Microorganisms and the Effect of MgO on Methanogenesis

Two possible reaction pathways of methanogenesis may exist in the WIPP. In the first reaction pathway, cellulosic materials will first break down into acetate or other low-molecular weight organic compounds, which will be then used by microbes to produce methane. This process has been grossly represented by Reaction (3) in Section 3.0. In the second reaction pathway, given the fact that metal corrosion in the repository will generate a significant quantity of H_2, methanogenic microbes may use both CO_2 and H_2 as substrates to produce methane: $CO_2 + 4H_2 = CH_4 + H_2O$. This reaction pathway is basically a gas consuming process and, if it occurs, it will have a significant impact on repository pressure.

This experiment is designed to determine the rate and extent of methane production in brine in the presence of (i) acetate or other low-molecular weight organic compounds resulting from cellulose breakdown which may predominate in the WIPP over the long-term, or (ii) in the presence of CO_2 and H_2. The experiment will use either the cultures isolated from the gas generation experiments or the known methanogenic halophiles obtained from the American Type Culture Collection (ATCC), Rockville, MD. Methane production by the two reaction pathways will be evaluated. The effect of MgO on the rate and extent of methane production by both pathways will be tested. Methane production will be determined by gas chromatography using flame-ionization detector.

8.0 LICENSE/PERMIT REQUIREMENTS

There are no licenses or permits required for the conduct of experiments under this Test Plan.

9.0 INSTRUMENTATION/TEST EQUIPMENT/FACILITY

All laboratory work will be performed at BNL in Building 490A, the Environmental Microbiology Group. It is expected that the following instrumentation will be used.

1. Varian Liberty 150 Inductively Coupled Plasma Atomic Emission Spectrometer. Serial No. 95081637. Used for elemental analysis. Calibrated before each use with NIST-traceable standards. Analysis range from 0.05 to 15 ppm. Accuracy = 2%, Precision = 2%.

Technical Operating Procedure: BNL-ICP-1 "Procedure for the Analysis of Uranium and Thorium in Brine by ICP-AES." 1/5/95, Authored by Jeff Gillow and Kirk Mantione.

2. Hewlett Packard 8453 Scanning UV/Vis Spectrophotometer. Serial No. DE52400431. Used for optical density measurements of biomass. Calibrated (with blank) before each use. Analysis range from 200 to 800 nm. Accuracy = 1%, Precision = 1%.

Technical Operating Procedure: BNL-UV/Vis-1 "Procedure for Analysis of Optical Density of Biomass by Spectrophotometry." 1/14/98, Authored by Jeff Gillow.

3. Weighing Equipment

Sartorius 1213 MP Top Loading Balance, Serial 3001028, Range 0.01 to 300 g
Ohaus AP1105 Analytical Plus Balance, Serial 1113203581, Range 0.0001 to 110 g
Mettler H31AR Analytical Balance, Serial 661431, Range 0.0001 to 160 g

Mettler PB303 Analytical Balance, Serial 1116340934, Range 0.001 to 310 g

Balances calibrated yearly by calibration service. Accuracy and precision checked at calibration and periodically by using Troemner 1 g ± 0.051 mg Ultra Class Weight (NVLAP Certified (Certificate 53267)), and routine Ohaus check weights.

Technical Operating Procedure: On-The-Job (OJT) Checklist for Balance Training and Use.

4. Liquid Measuring Equipment

Rainin 20µl Pipetman, Serial N16716B, Range 2 - 20µl, Specification for Accuracy ±7% at 2µl, ±1% at 20µl, Precision 0.04 to 0.06 µl across range. Note: Pipette may perform better than specifications, this is noted on cert.

Rainin 200µl Pipetman, Serial D-83-14191, Range 50 to 200µl, Accuracy ±1%, Precision 0.2 to 0.3µl across range.

Rainin 1000µl Pipetman, Serial N18534A, Range 100 to 1000µl, Accuracy 0.8% at 1000µl, Precision 0.6 to 1.3µl across range.

Rainin 5000µl Pipetman, Serial M15038A, Range 500 to 5000µl, Accuracy 0.6% at 5000µl, Precision 3 to 8µl across range.

Digital Pipette Rainin EDP2-25, Serial D402474, Range 2.5 to 25µl, Accuracy 6% at 2.5µl, 1% at 25µl, Precision 0.05 to 0.075µl across range.

Digital Pipette Rainin EDP2-250, Serial C500558, Range 25 to 250µl, Accuracy 2% at 25µl, 0.8% at 250µl, Precision 0.15 to 0.38µl across range.

Digital Pipette Rainin EDP2-2500, Serial C501767, Range 250 to 2500µl, Accuracy 3.2% at 250µl, 0.8% at 2500µl, Precision 2 to 3µl across range.

Pipettes are calibrated annually by the manufacturer (Rainin Instrument Corp., Woburn, MA). Calibration is checked routinely against calibrated balance.

Technical Operating Procedure: On-The-Job (OJT) Checklist for Pipette Training and Use.

5. Beckman phi-11 pH meter, Serial 226316 and Mettler MP220 pH meter, Serial 026987, are used for pH measurements. Range pH 0.00 to 12.00. Calibrated before each use or daily (which ever is less frequent) with pH 4.0, 7.0, 10.0 (typically 2-point) buffers. pH buffers manufactured by Fisher Scientific with unique lot numbers and expiry; Traceable to NIST. Accuracy ±0.01 pH units.

Technical Operating Procedure: BNL-ACW-2 "Procedure for pH Analysis of WIPP Samples." 7/15/95, Authored by Jeff Gillow.

6. Zeiss Axioskop Optical Microscope, Serial No. G-120805. Used for observation of microbe preparations and for counting/sizing. Counting grid standardized with Wild stage micrometer Serial 2660, calibrated every 5 years by Klarman Rulings (NIST-traceable). Sizing accuracy checked using Bangs Laboratories NIST-traceable uniform microspheres (0.538 and 1.900 µm).

Technical Operating Procedure: BNL-SIZE "Measurement of Bacterial Size and Cell Biovolume Calculations using 2-Dimensional Digital Images." Authored by Maureen Dunn, 5/21/96.

Technical Operating Procedure: BNL-EPI "The Direct Count Method for Enumerating Bacteria." Authored by Maureen Dunn, 5/21/96.

7. Gas Chromatographs: Shimadzu Gas Chromatograph, Serial No.65455YS. Used for analysis of gases from denitrification. Calibrated with nitrous oxide standard certified traceable to NIST by Scott Specialty Gases, NJ. Calibrated Before Each Use. Accuracy and precision specified in TOP.

Technical Operating Procedure: BNL-GCA-1 "Gas Chromatographic Analysis of Gases from Microbial Activity." Authored by Jeff Gillow.

Varian Gas Chromatograph, Serial No.15612. Used for analysis of methane, carbon dioxide, hydrogen, and hydrogen sulfide. Calibrated with gas mixture certified traceable to NIST by Scott Specialty Gases, NJ. Calibrated Before Each Use.

Technical Operating Procedure: BNL-GCA-1 "Gas Chromatographic Analysis of Gases from Microbial Activity." Authored by Jeff Gillow.

10.0 TEST/EQUIPMENT REQUIREMENTS

Requirements for the calibration, operation, and environmental parameters are provided in Technical Operating Procedures.

11.0 DATA ACQUISITION PLAN

Data acquisition and analysis will be performed according to NP 9-1 (Analyses). The means to collect the data varies for each individual instrument being used. The various data printouts will be attached directly to the scientific notebook(s). For the instruments that do not have direct data printout, the instrument reading will be directly recorded into the scientific notebook. For details of the data acquisition for a particular instrument, see the specific TOP for that instrument. Data collected directly onto magnetic media will be uploaded into an Excel spreadsheet.

From the scientific notebook(s), the numerical data will be transferred into a Microsoft Excel spreadsheet. A second person will check the data as they exist in the Excel spreadsheet to verify that no data transcription errors have occurred. This verification will be documented via memorandum with a copy of the spreadsheet attached. The data will be directly analyzed from the spreadsheet.

12.0 DATA IDENTIFICATION AND USE

The data resulting from these activities will be recorded directly into the scientific notebook(s) being used for these Tests. In cases where an instrument provides a printed output, that output will be attached to a page within the scientific notebook.

The method for recording data, whether it is a scientific notebook, data sheet, or some other document, shall be identified in the test procedure. Data transfer and reduction shall be performed in such a way to ensure that data transfer is accurate; that no information is lost in the transfer; and that the input is completely recoverable. Data transfer and reduction shall be controlled to permit independent reproducibility by another qualified individual.

13.0 CONCEPT AND HARDWARE DESIGN ANALYSIS

No prototypes or hardware are being designed for this test plan. No separate design review is required for this work. Experiments that are designed will be based upon published literature and their review will be part of the experiment review required for each experiment procedure.

14.0 PROVISION FOR SIGNIFICANT EVENTS

There are no significant events anticipated for the work described in this Test Plan. If significant nonconformances occur they will be documented and appropriate action taken according to NP 16-1 (Corrective Action).

15.0 QUALITY ASSURANCE

All experimental work will be performed at Brookhaven National Laboratory under contract AT-8739 according to the Sandia National Laboratories Nuclear Waste Management Program Procedures (NPs) and the BNL Standards Based Management System (SBMS) procedures for calibration and purchasing items

16.0 TRAINING

All personnel involved in the experiments described in this Test Plan will be trained and qualified for their assigned work. Requirements are implemented by NP 2-1 (Qualification and Training). SNL Form NP 2-1-1 will be used to document qualification and training.

17.0 PROCUREMENT DOCUMENT CONTROL

Documentation related to procurement of items or services in support of the proposed experiments will be done in accordance with the BNL SBMS subject areas "Procurement Operations Manual," "Procurement of and Approval to Use Radioactive Materials," "Purchase Requisition Review for Quality-related Requirements," "Evaluation of Seller Quality Assurance (QA) Programs," "Inspections and Acceptance," and "Graded Approach for Quality Requirements."

18.0 DOCUMENT CONTROL

The preparation and issuance of this Test Plan, and any major changes to it in the future will be controlled through SNL NP 20-1 (Test Plans). Any other document control issues relative to the performance of proposed experimentation will be governed by NP 6-2 (Document Control).

19.0 CONTROL OF PURCHASED ITEMS AND SERVICES

Procurement of items and services required for the proposed experiments will be done in accordance with the BNL SBMS subject areas "Procurement Operations Manual," "Procurement of and Approval to Use Radioactive Materials," "Purchase Requisition Review for Quality-related Requirements," "Evaluation of Seller Quality Assurance (QA) Programs," "Inspections and Acceptance," and "Graded Approach for Quality Requirements." This provides QA controls and guidelines for the following, as appropriate:

-source evaluation and selection;
-evaluation of objective evidence of quality furnished by the supplier;
-source inspection
-audit; and
-examination of items or services upon delivery or completion.

20.0 IDENTIFICATION AND CONTROL OF ITEMS

The requirements for identification and control of materials, parts, samples, and specimens are provided in NP 13-1 (Sample Control). All test samples for experiments will have unique identifiers which will include as a minimum the treatment name and replicate number. Sample coding documentation will be maintained as part of the experimental protocol and project records. Chain-of-Custody forms will be used for all samples transferred off-site.

21.0 CONTROL OF PROCESSES

Scientific research, experimentation, and analyses will be controlled by the BNL QAPP. Section 14.0 (Scientific Investigations) of the QAPP provides guidance.

Experiments will be conducted with protocols that provide sufficient detail to repeat them. Test media (samples prepared for experiments) will be controlled in accordance with implementing procedures for the control of samples and processes.

22.0 INSPECTION

In addition to periodic QA surveillances covering any or all aspects of the experiments being performed for this Test Plan, routine safety inspections will be performed as per BNL policy on a quarterly basis.

23.0 TEST CONTROL

Experimental activities will be controlled and conducted in accordance with the approved Test Plan, a copy of which will be maintained in the laboratory(ies) where the work is being performed, utilizing approved TOPs and scientific notebooks.

24.0 CONTROL OF MEASURING AND TESTING EQUIPMENT

The calibration requirements for measuring and test equipment used in the experiments will be controlled according to BNL SBMS Subject Area “Calibration.” The calibration procedures for each instrument are described in the various TOPs. These procedures will be followed and the calibration data will be kept in the data package for this activity.

25.0 HANDLING, STORAGE, SHIPMENT

Handling, storage, and shipment of laboratory equipment and materials related to the proposed experiments will be done in accordance with NP 13-1 (Sample Control). Methods and facilities will be established to identify test data and control their handling and storage. Tags/labels will identify test equipment status. Handling of radioactive materials may be required for the proposed experiments; this will be done in accordance with BNL policy and applicable Radiological Work Permits (RWPs) approved by Environment, Safety and Health professionals and Health Physics personnel.

26.0 INSPECTION, TEST, AND OPERATING STATUS

Instruments to be used in the proposed experiments will require periodic inspections and testing for their satisfactory operation. This will be done in accordance with applicable TOPs and manufacturer’s specifications. Equipment requiring external standards for calibration will be labeled “Calibrate Before Each Use.” Equipment requiring biannual or annual calibration will be labeled with calibration date and due date. Equipment that does not perform according to specifications or fails calibration will be labeled “Hold, Do Not Use,” and the failure investigated according to NP 16-1 (Corrective Action).

The status of these experiments will be provided periodic written progress reports, conveyed verbally to SNL WIPP Project Management, SNL reports, through presentations at meetings, workshops, and conferences, and through peer-reviewed journal publications.

27.0 CORRECTIVE ACTIONS

Conditions adverse to data quality such as power failures during data acquisition, instrument malfunctions or failures, and defective items and their root cause will be immediately evaluated to assess their impact on the experiments and to determine what corrective actions are needed. This evaluation and any corrective actions taken will be performed in accordance with NP 16-1 (Corrective Action).

28.0 QUALITY ASSURANCE RECORDS

Records documenting actions taken during the conduct of experimentation described in this Test Plan will be maintained by the BNL Project Quality Representative (PQR) and listed in the Master Index for the project. These will include:

-Test Plan
-Training and qualification records
-Procurement documents
-ES&H SOPs and Experiment Reviews
-Logbooks and scientific notebooks
-Data printouts from instrumentation and on magnetic media
-Inspections/testing documents
-Technical Operating Procedures (TOPs)
-Assessment reports
-Calibration/certification reports
-Technical progress reports and SAND reports
-Nonconformance and corrective action documentation

Records will be maintained according to guidance provided NP 17-1 (Records). Any data collected using an automated data acquisition system will be downloaded and backed up regularly.

29.0 COMPUTER SOFTWARE

Specialized computer software will not be developed as part of the work under this Test Plan. Off-the-shelf software will be used (Microsoft Word, Excel). A list of these software packages will be maintained in the Master Index. Spreadsheet software adapted to perform data reduction, manipulation, or analysis will be verified by hand calculation followed by independent review of the calculations. Guidance for use of software is provided in NP 19-1 (Software).

30.0 HEALTH AND SAFETY

Laboratory experimentation performed under this Test Plan will undergo an Experiment Review as dictated by BNL ES&H Procedures. All hazards, both chemical and radiological, will be identified and appropriate training provided to personnel prior to exposure to these hazards. BNL mandated ES&H training includes General Employee Training, General Employee Radiological Training, OSHA Laboratory Standards, Hazardous Waste Generator Training, Radiological Worker I, Dispersable Radioactive Material Training, and Radioactive Waste Generator Training as well as laboratory-specific training. Relevant TOPs provide guidance of personal protective equipment (PPE) as does Experiment Reviews. All employees of BNL have the authority to stop work in the event of imminent danger to personal health and safety.

31.0 MILESTONES

The work will start immediately after this Test Plan becomes effective. The following is a list of Milestones for fiscal year 2001.

January 20, 2001: Report the results of gas analyses of humid cellulose and inundated plastic/rubber materials incubated for 2,514 and 2,610 days respectively.

July 2001: Report the results of new gas analysis and the analyses of the liquid and solids in inundated experiments.

September 2001: Submit for publication a manuscript on i) gas generation studies, ii) methanogenesis under WIPP repository-relevant conditions.

32.0 PROCEDURES AND REFERENCES

32.1 Relevant Technical Procedures

BNL-ICP-1	Procedure for the Analysis of Uranium and Thorium in Brine by ICP-AES. Authored by Jeff Gillow and Kirk Mantione, 1/5/95.
BNL-UV/Vis-1	Procedure for Analysis of Optical Density of Biomass by Spectrophotometry. Authored by Jeff Gillow, 1/14/98
OJT-2	On-The-Job (OJT) Checklist for Balance Training and Use.
OJT-1	On-The-Job (OJT) Checklist for Pipette Training and Use.
OJT-3	On-The-Job (OJT) Checklist for WIPP Sterile Technique.
BNL-ACW-2	Procedure for pH Analysis of WIPP Samples. Authored by Jeff Gillow, 7/15/95

BNL-SIZE	Measurement of Bacterial Size and Cell Biovolume Calculations using 2-Dimensional Digital Images. Authored by Maureen Dunn, 5/21/96.
BNL-EPI	The Direct Count Method for Enumerating Bacteria. Authored by Maureen Dunn, 5/21/96.
BNL-GCA-1	Gas Chromatographic Analysis of Gases from Microbial Activity. Authored by Jeff Gillow.

32.2 Quality Assurance Procedures

Analysis

- NP 9-1 Analyses
- NP 19-1 Software Requirements

Data Collection

- NP 13-1 Sample Control
- NP 19-1 Software Requirements
- NP 20-1 Test Plans
- NP 20-2 Scientific Notebooks

Design

- NP 3-1 Design Control
- NP 19-1 Software Requirements

General (these procedures apply to all work categories)

- NP 1-1 Organization and QA Program
- NP 2-1 Qualification and Training
- NP 5-1 Implementing Procedures
- NP 6-1 Document Review Process
- NP 6-2 Document Control Process
- NP 16-1 Corrective Action
- NP 17-1 Records

- NP 18-1 Audits and Surveillances
- BNL SBMS Subject Areas “Procurement Operations Manual,” “Procurement of and Approval to Use Radioactive Materials,” “Purchase Requisition Review for Quality-related Requirements,” “Evaluation of Seller Quality Assurance (QA) Programs,” “Inspections and Acceptance,” and “Graded Approach for Quality Requirements.”
- BNL SBMS Subject Area “Calibration.”

32.3 References

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Francis A. J. & Gillow J. B. (1994) Effects of Microbial Processes on Gas Generation under Expected WIPP Repository Conditions: Progress Report through 1992. Sandia National Labs., Albuquerque, NM. SAND93-7036.

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Leschine S. B. (1995) Cellulose degradation in anaerobic environments. Annual Review of Microbiology, 49, 399-426.

Wang Y. (1998) Towards a realistic model of gas generation and water budget in the Waste Isolation Pilot Plant. Sandia National Labs., Albuquerque, NM. (memo to P. Vaughn, 5/14/1998).

Wang Y. and Brush L. H. (1996) Estimates of gas-generation parameters for the long-term WIPP performance assessment. Sandia National Labs., Albuquerque, NM. (memo to M. Tierney, 1/26/1996). WPO#30819.