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Subject: SRNL Environmental Sciences and Biotechnology Support of WIPP

(R.L. Brigmon and C. E. Milliken)

The Environmental Sciences and Biotechnology (ESB) group was consulted concerning the potential for biological activity, e.g., microbial heating and gas production, contributing to the Waste Isolation Pilot Plant (WIPP) Drum 68660 incident. Available data on the incident was provided along with a sample of the same wheat based packing material (Swheat™) that was used in the WIPP drum for analysis. Biological heating and gas production requires a microbial inoculum, nutrients, and specific environmental conditions to occur. A small series of laboratory tests were performed to facilitate a rapid assessment of microbial heating in the drum.

METHODS

Microscopy.

The Swheat™ received was a subsample from a previously opened container that appeared dry and powdery on reception. ESB microscopic inspection of a sterile filtered Phosphate Buffer Saline (PBS) rinse of the Swheat™ revealed numerous microorganisms (Figure 1). An overnight culture of the PBS Swheat™ rinse in minimal medium (Lura Broth, Sigma-Aldrich) revealed a viable indigenous microbial population including bacteria, yeast, and fungi (Figure 2). The main nutrient for the microorganisms in this Swheat™ was wheat, containing food and enzymes for microbial metabolism. Observations of microbial populations in processed wheat products are not unusual, even when dried.

Microbiological Analysis

An inoculum was removed from the Swheat™ sample provided and transferred into 4 different media and then serially diluted. The cultures were incubated for the appropriate amount of time (approximately 2 weeks) according to the manufacturer’s recommendations (1MICkit® 3 by BTI products). This microbiological test kit was employed for initial sample analysis since the cause of the WIPP drum incident was unknown and microbial induced corrosion (MIC) could have been a factor. The MICkit® includes media for growing Aerobes (Aero), Anaerobes (Ana), Acid-producing Bacteria (APB), and Sulfate-reducing Bacteria (SRB). The right combination of environmental factors and number of bacteria must be present over time to cause corrosion. Keep in mind that 99% of bacteria are non-viable, and this assay is designed to determine the risk of microbially-induced corrosion.
Anaerobe cultures came up the fastest within 1-2 days. Results are shown in Table 1. Highest densities were demonstrated in the SRB and ANA cultures with up to 10,000 cells/gram Swheat™. Some hydrogen production was detected in the anaerobic cultures during the second week. The gas tight bottles were also used for analysis of gas and other metabolite production. All microbial procedures were done at ambient temperatures (i.e., 75°F).

Based on the culture results over time, biological conversion of methane to carbon dioxide could have produced large amounts of heat in the drum. The amount of microbial activity, including byproduct such as gas production, would have been dependent on environmental factors including nutrient availability, moisture content, temperature, and pH. In these conditions with nitrate salts, microbial denitrification was also a viable process. This denitrification of the nitrate might generate both heat and gases (both carbon dioxide from respiration and nitrogen from denitrification) to increase pressure. The temperature of the WIPP storage facility was 80 °F at the time of the incident where Drum 68660 had been stored for 14 days, which would have been ideal for microbial growth.

Current 10-gallon drum simulation studies at Sandia National Laboratory (SNL) have shown carbon dioxide and nitrous oxide production in parallel with oxygen consumption providing evidence for microbial respiration over time. Swheat™ + H2O only mixtures were found to exhibit no N2O generation. Automatic Pressure Tracking Adiabatic Calorimeter (APTACTM) testing showed ignition with select mixtures of constituents. Similarly with microbial activity, different metabolic patterns can occur resulting in heat generation depending on the biological growth limiting factor (e.g., nitrate). Under nitrate limited growth, two distinctly separated biphasic periods have been observed representing the quantitative reduction of nitrate to nitrite in the first phase and a complete reduction of the accumulated nitrite in the second phase, presumably to nitrous oxide as observed in the ongoing SNL tests. Nitrous oxide can be the end product of the denitrification process of the microorganisms. As the system was oxygen limited, and aerobic activity limited as proven here, the facultative anaerobes and anaerobic microbial activity most likely predominated and would not have contributed to excessive heating (>176°F).

A previous DOE report entitled “An Assessment of the Flammability and Explosion Potential of Transuranic Waste” stated that methane levels may increase in waste forms that are readily biodegradable as a potential risk factor. While the drum was vented, anaerobic pockets may have occurred due to the packing, settling, and layering of contents. Hydrogen, not measured here, may have also been produced both through biological activity as well as potential generation due to radiolysis and corrosion.

**Liquid Chromatography**

A sample was taken from the Swheat™ culture and tested; saccharides, acetate and ethanol were detected in the Swheat™ with an Agilent 1200 Liquid Chromatograph equipped with a refractive index detector. The column was a Bio-Rad Aminex HPX-87H (300 x 7.8mm) with a Biorad Cation H micro-guard column (30 x 4.6mm) and the mobile phase was aqueous 5mM Sulfuric acid. Baseline separation was achieved with a flow rate of 0.6ml/min, sample volume was 5ul; and 3 point calibration standards were used to determine concentrations of saccharides.

The pulverized Swheat™ was found to contain approximately 8 g cellobiose/kg Swheat™, 0.8g glucose/kg Swheat™ and 0.8g Xylose/kg Swheat™, which are food, and 6mM acetate and 0.4 g ethanol/kg Swheat™, which are products of microbial metabolism. The quantities of acetate and ethanol produced were similar to other reports in the literature, depending on the conditions. Microbially-produced volatile fatty acids like acetate can provide a readily biodegradable substrate for other bacteria. For example, methane production from acetate can
proceed through syntrophic acetate oxidation. The drum also contained fiberboard, which when wet can serve a
cellulosic microbial substrate. There was likely mixing of the drum’s contents during shipment and placement that
accelerated biological activity. However, the moisture levels, nitrate salts, and oxalates, could also be factors in
the metabolic events leading up to the physical/chemical cause of incident. Although the drum was vented, this
appears to have been an oxygen limited environment due to the layering and packing of the materials. The actual
pH of the drum’s contents after packing was not determined.

CONCLUSIONS

Observations of diverse microbial populations as described here in processed wheat products is a common
occurrence. No microbially-produced heat measurements were made at SRNL. From our knowledge of the
Drum 68660 contents, it may have contained both aerobic and anaerobic environments due to the layering,
packaging, and settling of materials. We did demonstrate aerobic and anaerobic microbial activity in the
Swheat™. For most anaerobic processes, heat production is usually small. Some hydrogen production was
observed in the anaerobic cultures. Hydrogen production by anaerobic microbial communities using organic
waste as the substrate has drawn attention because of its ability to produce an energy source, while simultaneously
stabilizing waste. In conclusion, from these results a combination of Swheat™ and associated
microorganisms could have provided the substrate and inoculum for microbial activity in WIPP Drum 68660.
REFERENCES


FIGURES AND TABLE

Figure 1. Bacteria in Phosphate buffered saline (PBS) rinse of Swheat™

Figure 2. Bacteria, yeast, and fungi in overnight culture of Swheat™ cultured with minimal medium (Lura Broth).
Table 1. Bacteria densities in Swheat™ as measured by MICkit®

<table>
<thead>
<tr>
<th>Bacteria type</th>
<th>Cells/gram Swheat™*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobes ANA)</td>
<td>9,000-10,000</td>
</tr>
<tr>
<td>Aerobes (AERO)</td>
<td>1-100</td>
</tr>
<tr>
<td>Sulfate-reducing (SRB)</td>
<td>9,000-10,000</td>
</tr>
<tr>
<td>Acid Producing (APB)</td>
<td>1-100</td>
</tr>
</tbody>
</table>

*Range

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