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**CRADA Final Report
for
CRADA Number ORNL94-0323**

**EFFICIENT ON-SITE DEGRADATION OF
HIGH CONCENTRATION OF SPEND
DEICING FLUIDS: A LABORATORY STUDY**

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MASTER

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C/ORNL94-0323

**CRADA Final Report
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CRADA Number ORNL94-0323**

**Efficient On-site Degradation of High Concentration of Spent Deicing Fluids:
*A Laboratory Study***

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Abstract

The on-site treatment of antifreeze compounds and aircraft deicing fluids (ethylene glycol and propylene glycol) will reduce disposal costs, decrease environmental impact, minimize the potential for additional spills/contamination and meet the goals of pollution prevention by reducing the amount of hazardous materials generated. We have identified bacteria that can degrade 1-10% glycol waste at room temperatures of ca. 23C. A second subculture was isolated that could degrade glycol waste at ca. 4C.

Objectives and Discussion

The overall objectives of this project were to isolate bacteria that could degrade glycol wastes and identify a potentially cost effective disposal method for spent deicing fluids. This project was a collaboration between ORNL and REMTECH.

Millions of gallons of deicing fluids (EG/PG) mixtures are used each year. One airline carrier in Minneapolis alone (1992-93) used 1.5 million gallons of deicing fluid. During use this material is diluted to 7-8%. Thus, 19 million gallons of spent fluid required disposal. Typically this material is transferred into wastewater treatment facilities where it is degraded. The airlines pay for the disposal of deicing fluids based on the glycol concentration. As the concentration goes up the oxygen demand for degradation goes up. Thus, we examined the potential for on-site degradation of the glycol from approximately 10% to below 5%. This 50% decrease in glycol concentration has the potential to decrease the disposal costs by 50-65%.

This project identified bacteria capable of degrading both propylene glycol and ethylene glycol and were active at degrading high concentrations of glycol (>2000 mg/l/d). Previous reports had shown that propylene glycol is more resistant to microbial degradation than ethylene glycol and literature values report degradation rates of low concentration of deicing fluids at about 66 mg/kg/day. The microbial community assayed in this study was isolated from soils that had a long history of glycol contamination.

This laboratory work was carried out at ORNL and included personnel from REMTECH who worked at ORNL. REMTECH was also responsible for identifying the airline facility that could be approached for pilot scale testing of a small bioreactor for on site glycol degradation. REMTECH has expertise in the design and implementation of remediation technology and has completed a variety of successful soil and groundwater remediation projects.

Laboratory experiments identified what additional inorganic nutrients were necessary for the degradation of 7-8% deicing fluids in typical rain water. Spent deicing fluid was obtained from 2 northern airports. A commercially available fertilizer mixture was capable of supplying nutrients necessary for contaminant degradation. The main limiting factor to glycol degradation is typically oxygen availability.

Experiments also identified a community of microorganisms that appeared to have elevated metabolic activity at low ambient temperatures (ca. 4 to 10C). Although not part of the initial CRADA, these bacteria were characterized with regard to their ability to degrade various glycol concentrations and nutrient requirements. This information would be useful to airport facilities that have open ponds where spent deicing fluids are held prior to transfer to waste water treatment facilities. It is possible that these bacteria could be added to the ponds to accelerate the degradation of high glycol concentrations of 10% to below 5%.

Concurrent with the laboratory experiments described above, negotiations with USAir were initiated to determine if they would be interested in a pilot scale demonstration. Initially ORNL and

REMTECH were invited to set-up a reactor in Pittsburgh, however, during the restructuring of the airport support contractor this decision was reversed.

Work from this CRADA produced 2 communities of bacteria that are capable to degrading high concentrations of glycol, both propylene and ethylene at temperatures from 4 to 25C. The efforts to scale-up this work were stopped due to obstacles of identifying a facility within the time frame of this project. REMTECH will continue to pursue the pilot-scale demonstration.

This project has been presented at several scientific meetings and has been published as a peer-reviewed manuscript. Copies are attached to this report.

Benefits to DOE

The bacteria isolated as part of this project are capable of degrading high concentrations of glycol based waste. Experiments were also able to determine that degradation can occur at low environmental temperatures. These bacteria could be used to meet not only the needs of DOE remediation and pollution prevention but could be transferred to DoD to address their needs for the degradation of spent wing and runway deicers.

Technical Discussion

Soil Enrichment. Soil samples were received from a site that had a long history of glycol contamination. Soils (5 gm) were set up in 100 ml of minimal salt medium (Little et al. 1988) in 250 ml milk bottles with varying carbon contamination. Propylene and ethylene glycol were analytical grade (J.T. Baker, Inc., Phillipsburg, NJ), the commercial antifreeze was ethylene glycol based, and the deicing fluids were an unused ethylene glycol based product (NDEG) and a spent propylene glycol based product (PPG).

All solutions were filter sterilized (0.2 um filter) and kept at 4°C prior to use. The ethylene and propylene glycol, the commercial antifreeze, and the concentrated deicing fluid (ethylene glycol-NDEG) were handled as pure solutions (100%). The propylene glycol based deicing fluid was a spent solution with a concentration of ca. 7% propylene glycol (PPG) in rain water. Enrichments were set up in duplicate at 1, 5, and 10 % solutions with the exception of the 7% spent deicing fluid (1, 5, and 7%). Fresh transfers were made weekly (100 ul supernatant:100 ml minimal salts medium). Bottles were incubated at room temperature on an orbital shaker.

Culture Identification. Supernatant samples (100 ul) were plated onto nonspecific nutrient agar (Difco) plates and noble agar plates containing 1% ethylene glycol or 1% propylene glycol. Plates were incubated at room temperature for 3-7 days. Pure cultures were isolated and gram stains performed.

Analytical Assay. Quantitatively degradation was verified using gas chromatography. Supernatant samples were filtered using a 0.8/0.2 um stacked Acrodisc filter. Liquid injections of 1 ul were run on a Perkin-Elmer Sigma 2000 gas chromatograph equipped with a flame ionizing detector using a 30 m J & W Scientific DBWAX column (0.53 megabore, 1 micron film). Run conditions were oven temperature 160°C, injector temperature 250°C and detector 230°C with a nitrogen gas carrier flow of 5 ml/min.

Results and Discussion

A microbial consortia isolated from soil enrichments was shown to degrade a variety of glycol-based products at high concentrations. The consortia initially consisted of 5-10 phenotypically

different microbes, however, it was reduced to 3 isolates by maintaining colonies on plates containing only ethylene glycol or propylene glycol as the sole carbon source. The dominant isolate produced a water-soluble yellow pigment (EG-y), however, biochemical tests and lipid analyses have not provided a conclusive identification for any of the gram negative isolates. We are currently using the Biolog identification system (Hayward, CA) in conjunction with phospholipid fatty acid analyses (PLFA) (Microbial Insights, Knoxville, TN) to identify these organisms. The presence of gram negative rods is consistent with the report that *Pseudomonas*, *Flavobacterium*, *Mycobacterium*, and *Xanthomonas* (all gram negative rods) were found in an industrial activated sludge unit treating ethylene glycol wastes (Grabinska-Loniewska 1974).

Microbial growth and respiration indicated different patterns of glycol usage. The greatest increase in optical density was for cultures grown on propylene glycol, followed by cultures grown on ethylene glycol, antifreeze and deicing fluids (Figure 1). Results of respirometry experiments (Figure 2) showed that growth on the spent deicing fluid (PPG), resulted in the greatest amount of oxygen consumed followed by the ethylene glycol deicing fluid (NDEG), pure ethylene glycol and the commercial antifreeze and last, pure propylene glycol. Hall and Pfaender (1993) were investigating the of mixed PG and EG wastes and reported that PG was predominantly respired but a larger portion of EG was incorporated into biomass.

Quantification of glycol degradation was confirmed using the gas chromatography (e.g. Figure 3). Ethylene glycol was reduced from 10% to <5% within 7 days, the commercial antifreeze (propylene glycol based) was reduced from 5% to <2% with propylene glycol being the most recalcitrant. Typically, 10-40% of the initial concentration was degraded over a 7-day period which corresponded to an average glycol metabolism of 1,000-4,000 mg/L/7-day period. Degradation of 1-5% glycol was not as significant as the 10% solutions. However, reducing a 7-10% concentration to <4% is often acceptable to waste water treatment facilities.

The biodegradation of these glycol wastes may be a cost effective disposal method for a variety of industries. Yet to have cost effective degradation other conditions must be evaluated. For example, we have begun to measure the degradation of deicing fluids at reduced ambient temperatures (4°C). This is important for the potential to bioremediate spent deicing fluids at Northern waste water treatment facilities during winter months.

Current laboratory results have identified propylene glycol as the easiest to degrade (10% reduced to 4%) followed by the commercial antifreeze (propylene glycol based) pure ethylene glycol and the NDEG. The necessary inorganic nutrients required for efficient degradation are being investigated. Pilot scale bioreactor experiments are characterizing the degradation efficiency of antifreeze and spent deicing fluids at 25 and 4°C.

Degradation of antifreeze and deicing fluids has the potential to significantly reduce the glycol concentration fed into waste water treatment plants which is currently the single most common compound during the winter months at numerous northern waste water treatment facilities. Furthermore, municipal airports could significantly reduce their waste disposal costs by reducing the concentration of glycol fed to a treatment facility. Information from this work will be applied to a field scale demonstration of the on site degradation of spent deicing fluids.

Inventions

No invention disclosures were filed as part of this project.

Commercialization

No commercialization plans have been written at this time.

Figure 1: An increase in optical density over time represents an increase in bacterial numbers. MS-minimal salts solution only (control); PG-propylene glycol; EG-ethylene glycol; PG/EG-mixture of propylene glycol and ethylene glycol; AF-commercial antifreeze.

Figure 2: An increase in oxygen consumption over time represents increased microbial metabolic activity. The most readily available carbon source was spent deicing fluid (2 years old), followed by new deicing fluid (unused), ethylene glycol and a commercial antifreeze compound. The most recalcitrant compound was propylene glycol. NATE was the minimal salts media (control).

Figure 3: The degradation of commercial antifreeze was further quantified by using gas chromatography. The line represents the standard curve for 1, 2.5 and 5% glycol (v/v) in a minimal salts solution. The symbols represent the amount of glycol remaining in solution following a 1 week incubation.

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Degradation of High Concentrations of Glycols, Antifreeze, and Deicing Fluids

*Janet M. Strong-Gunderson, Susan Wheelis,
Susan L. Carroll, Michael D. Waltz,
and Anthony V. Palumbo*

ABSTRACT

A microbial consortium (EG-c) capable of degrading high concentrations of glycol-based waste was isolated from soil enrichments. The isolate primarily responsible for glycol degradation was a gram-negative rod (EG-y) that produced a water-soluble pigment. Initial laboratory experiments measured the degradation of ethylene glycol (EG) and propylene glycol (PG) at 25°C. Cell biomass optical densities (660 nm) increased from 0.01 to 0.5 within 48 h and reached a maximum of 0.73 at 72 h. Respirometry experiments showed oxygen consumption rates of ca. 1,000 mg/L/day with glycol degraded at a rate of ca. 2,000 mg/L/day (confirmed with gas chromatography). Laboratory tests were expanded to evaluate the degradation of a commercial antifreeze (primarily EG-based) and two deicing fluids (one PG- and one EG-based) at both 25 and 4°C (a more realistic winter temperature).

INTRODUCTION

Glycol-based products are used in numerous industries. EG is a primary component of equipment coolants and aircraft- and runway-deicing fluids and is used in the pharmaceutical-manufacturing industry. Approximately 4.93 billion pounds (2.2 billion kg) were produced in 1991, making it the 30th most-produced chemical in the United States (American Chemical Society 1992). PG is an antifreeze additive, as well as a preservative and emulsifier in food and bath products. More than 745 million pounds (335 million kg) were produced in 1991 (American Chemical Society 1992).

One significant source of glycol waste is generated from spent deicing fluids. Millions of gallons of deicing fluids (EG/PG mixtures) are used each year at northern aircraft facilities, with estimates greater than 25,000 gal (95,000 L)/y for one small military air base and 1.5 million gal (5.7 million L) for one

commercial airline (1992-1993) (Airline representative, Minnesota, personal communication). Use of these deicing compounds results in large amounts of spent fluid discharged into sewer systems or collected for treatment at off-site facilities (Anon 1989).

On-site degradation of high concentrations of deicing fluids and antifreezes may prove to be a cost-effective method for glycol disposal. Glycol waste is currently diluted to <10% before municipal facilities will accept it for treatment (Airline representative, Minnesota, personal communication). Wastewater treatment facilities usually specify 1 to 5% glycol as the maximum concentration for efficient microbial degradation and acceptable oxygen demands. Thus, the degradation of glycol to concentrations <5% can significantly reduce the volume of material discarded to municipal facilities.

This work focused on the isolation of bacteria capable of degrading a >10% concentration of glycol to <5% glycol. Preliminary results also showed that contaminant-degradation rates at 4°C were only slightly slower than at 25°C.

EXPERIMENTAL PROCEDURES AND MATERIALS

Soil Enrichment

Soil samples were received from a site with a long history of glycol contamination. Soils (5 g) were established in 100 mL minimal salt medium (Little et al. 1988) in 250-mL milk bottles with varying carbon contamination. PG and EG were analytical grade (J.T. Baker, Inc., Phillipsburg, New Jersey), the commercial antifreeze was EG-based, and the deicing fluids were a concentrated, unused EG-based product (NDEG) and a dilute, spent PG-based product (PPG).

All solutions were filter sterilized (0.2 μ m filter) and kept at 4°C prior to use. The EG, PG, commercial antifreeze, and concentrated deicing fluid were handled as pure solutions (100%). PG-based deicing fluid was a spent solution with a concentration of almost 7% PPG in rain water. Enrichments were established in duplicate at 1, 5, and 10% solutions with the exception of the spent deicing fluid (1, 5, and 7%). Fresh transfers were made weekly (100 μ L supernatant: 100 mL minimal salts medium). Bottles were incubated at room temperature on an orbital shaker.

Culture Identification

Supernatant samples (100 μ L) were plated onto nonspecific nutrient agar (Difco) plates and Noble agar plates containing 1% EG or 1% PG. Plates were incubated at room temperature for 3 to 7 days. Pure cultures were isolated, and gram stains were performed.

Analytical Assay

Quantitative degradation was verified using gas chromatography. Supernatant samples were filtered using a 0.8/0.2- μ m stacked Acrodisc filter.

Liquid injections of 1 μL were analyzed using a Perkin-Elmer Sigma 2000 gas chromatograph equipped with a flame ionization detector using a 30-m J & W Scientific DBWAX column (0.53 megabore, 1- μ film). Method conditions were oven temperature 160°C, injector temperature 250°C, and detector 230°C, with a nitrogen gas carrier flow of 5 mL/min.

RESULTS AND DISCUSSION

The microbial consortium (EG-c) isolated from soil enrichments initially consisted of 5 to 10 phenotypically different organisms and was able to degrade a variety of glycol-based products at high concentrations. The consortium was reduced to 3 isolates by maintaining colonies on plates containing only EG or PG as the sole carbon source. The dominant isolate (EG-y) produced a water-soluble yellow pigment; however, biochemical tests and lipid analyses have not provided conclusive identification for any of the gram-negative isolates.

Microbial growth and respiration indicated different patterns of glycol metabolism. The greatest increase in optical density occurred in cultures grown on PG, followed by cultures grown on PPG, EG, antifreeze, and NDEG (Figure 1). However, respirometry results (Figure 2) showed that growth on

FIGURE 1. Qualitative measure of glycol degradation by increased optical densities. All carbon compounds were at 5% glycol in NATE (minimal salts media).

FIGURE 2. Oxygen-consumption rates for various carbon compounds at 5% glycol.

the spent deicing fluid (PPG) resulted in the greatest amount of oxygen consumed, followed by the EG deicing fluid (NDEG), pure EG, the commercial antifreeze, and, finally, pure PG. Quantification for all test compounds was confirmed using gas chromatography (Figure 3). EG was reduced from 10% to <6% within 7 days, and the commercial antifreeze was reduced from 5% to <2%. PG was the most recalcitrant. Typically, 10 to 40% of the initial concentration was degraded over a 7-day period, which corresponded to an average glycol metabolism of 1,000 to 4,000 mg/L/7 days. Overall, the degradation of 1 to 5% glycol was not as significant as that of the 10% solutions.

The on-site biodegradation of glycol wastes may be a cost-effective disposal method for a variety of industries. However, to have cost-effective degradation, other operating conditions must be evaluated and optimized. For example, we have begun to measure the degradation of deicing fluids at reduced ambient temperatures ($\geq 4^{\circ}\text{C}$). This is important for the potential to bioremediate spent deicing fluids at northern glycol-waste-generating facilities during winter months.

Degradation of antifreeze and deicing fluids has the potential to significantly reduce the glycol concentration released to treatment plants, where it is the single most common compound during the winter months at numerous northern wastewater treatment facilities. Municipal airports could significantly

FIGURE 3. Verification of glycol degradation using the gas chromatograph. The dotted line represents the standard curve. Individual data points ($n = 2$) represent the glycol concentration after a 7-day incubation at 25°C.

reduce their waste-disposal costs by reducing the concentration of glycol fed to a treatment facility. Information from this work will be applied to a field-scale demonstration addressing on-site degradation of spent deicing fluids.

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