TECHNICAL REPORT
(40751R08)

Report Title:	**Analysis of Materials in an Experimental Testing Pipe System for an Inhibitor of Mussel Kill**
Type of Report:	**Quarterly**
Reporting Period Start Date:	**January 1, 2003**
Reporting Period End Date:	**March 31, 2003**
Principal Author:	**Daniel P. Molloy**
Date Report Was Issued:	**June 4, 2003**
DOE Award No.:	**DE-FC26-00NT40751**
Name and Address of Submitting Organization:	**New York State Education Department State Education Building - Room 125 Albany, NY 12234**

DISCLAIMER

ABSTRACT

A comprehensive series of 16 laboratory experiments demonstrated that the presence of vinyl tubing within a recirculating pipe system was responsible for lowering zebra mussel kill following treatment with the bacterium *Pseudomonas fluorescens*. All vinyl tubing was replaced in all testing units with silicone tubing, and high mussel kill (>95%) was then obtained.

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EXECUTIVE SUMMARY

The bacterium *Pseudomonas fluorescens* is being developed as a biocontrol agent for zebra mussels. The purpose of this USDOE-NETL project is to identify biotic and abiotic factors that affect mussel kill. In the course of performing laboratory pipe tests using recirculating water, it became evident that one or more materials within the experimental testing pipe apparatus was responsible for lowering zebra mussel kill. A comprehensive series of tests demonstrated that the presence of vinyl tubing within the recirculating pipe system was responsible. The short lengths of vinyl tubing present within the test pipe systems did not actually kill mussels, but rather in some undetermined way (possibly by inhibiting feeding on bacteria), reduced overall mussel mortality following *P. fluorescens* treatment. Further trials in which mussels were exposed to larger quantities of vinyl tubing in the absence of any bacteria resulted in actual mussel mortality – clearly indicating that the vinyl tubing had some type of toxin leaching from it. Phthalate, a known hazardous compound, is added to vinyl during manufacture to make it flexible, and we suspect that this compound leaches out from the tubing into the water over an extended period. Vinyl tubing was replaced in all testing units with silicone tubing, and high mussel kill (>95%) was finally obtained, allowing the research project to move forward again.

REPORT

The bacterium *P. fluorescens* strain is being developed as a biocontrol agent for zebra mussels. The purpose of this USDOE-NETL project is to identify biotic and abiotic factors that affect mussel kill. Various testing vessels are used in the course of this multi-year project to run experiments. One advanced piece of laboratory testing equipment is the recirculating pipe system (Fig. 1).

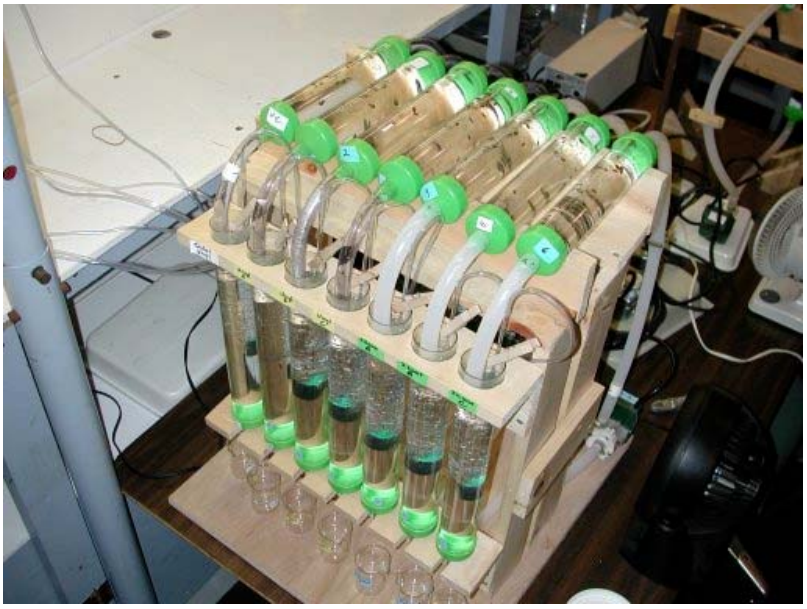

Fig. 1. Seven clear acrylic pipes with zebra mussels are an integral part of the recirculating pipe system. Each pipe has a pump and connecting hoses.

In the course of performing pipe tests using this equipment, it became evident that a material within the experimental testing pipe apparatus was responsible for lowering the zebra mussel kill that could be obtained following treatment with the bacteria. We report here that a comprehensive series of 16 tests were conducted to address this problem and that they demonstrated that the presence of vinyl tubing within the recirculating pipe system was identified as the problem. (Tests conducted with other materials from the testing system, such as silicone tubing, acrylic pipe, and stainless steel fittings indicated they were nontoxic.) The short lengths of vinyl tubing present within the test pipe systems did not actually kill mussels, but rather in some undetermined way (possibly by inhibiting feeding on bacteria), reduced overall mussel mortality resulting from *P. fluorescens* treatment. Further trials in which mussels were exposed to larger quantities of vinyl tubing in the absence of any bacteria actually killed mussels - clearly indicating that the vinyl tubing had some type of toxin leaching from it. Phthalate, a known hazardous compound, is added to vinyl during manufacture to make it flexible, and we suspect that this compound leached out from the tubing into the water during our tests. Vinyl tubing was replaced in all testing units with silicone tubing, and high mussel kill (>95%) was finally obtained, allowing the research project to move forward again.

PLANS FOR NEXT REPORTING PERIOD

Tests evaluating the following abiotic and biotic factors will be reported on:

1. Mussel siphoning behavior
   - In nature, a zebra mussel typically has its two shells spread apart and extends an inhalant siphon tube from between its shells for taking food particles, including bacteria, into its mantle cavity (Fig 2).
   - We will report on whether there is a correlation between the activity of this siphoning behavior and the mussel mortality that is achieved by a bacterial treatment.

2. Particle concentration
   - Waters containing very high levels of naturally suspended particles may reduce efficacy of bacteria during treatment, due possibly to reduced ingestion of bacterial cells (Fig. 3).
   - We will report on whether this is true for the application of *P. fluorescens* cells against zebra mussels. If true, then treatments should be timed to occur when waters have relatively low quantities of suspended matter.

Fig. 2. Zebra mussels feeding with extended siphons. In order for *P. fluorescens* bacteria to be lethal they must be ingested by the mussels.

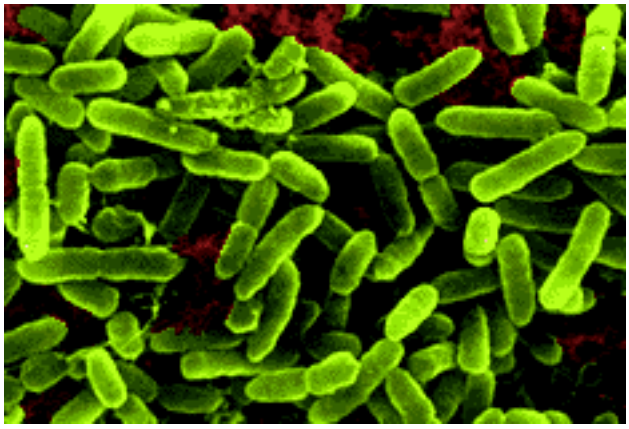

Fig. 3. Cells of *P. fluorescens*.

TECHNOLOGY AND INFORMATION TRANSFER

This project was highlighted in the following conference presentation:

- Molloy, D. P. David versus Goliath: Controlling zebra mussels with a tiny microbe. March 6, 2003. Great Lakes Conference, Michigan State University, East Lansing, Michigan. (Invited speaker.)

This project was highlighted in an article in the leading trade magazine of the North American hydroelectric industry:

- Fulton, E. 2003. Common bacterium shows promise in controlling zebra mussels. *Hydro Review* 22(1):74-75.