

**TECHNICAL REPORT  
(40751R09)**

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## ABSTRACT

Under this USDOE-NETL contract, the bacterium *Pseudomonas fluorescens* is being developed as a biocontrol agent for zebra mussels. The specific purpose of the contract is to identify biotic and abiotic factors that affect mussel kill. Ingestion of these bacteria by zebra mussels is required to achieve kill, and tests evaluating factors that relate to mussel feeding are contained in this report. Specifically the impact of the following two factors were investigated: 1) mussel siphoning behavior; 2) naturally suspended particle loads.

**1. Mussel siphoning behavior:** In nature, zebra mussels typically have their two shells spread apart and their inhalant siphon tube extended from between their shells for taking food particles into their mantle cavities (Fig. 1). Our tests indicated that there is a direct correlation between mussel siphoning activity and mussel mortality achieved by a bacterial treatment. Therefore, to encourage mussel feeding on bacteria, future pipe treatments within power plants should be carried out using procedures which minimize disturbance to mussel siphoning.

**2. Particle load:** Since bacterial cells are lethal only if ingested by mussels, waters containing very high levels of naturally suspended particles might reduce the mortality that can be achieved by a bacterial treatment. If true, this inhibition might occur as a result of particle exclusion, i.e., there could be reduced ingestion of bacterial cells since they represent a reduced percentage of all particles ingested. Our tests indicated that a range of particle concentrations that might naturally exist in a turbid river did not inhibit mussel kill by the bacterial cells, but that an artificially high load of natural particles was capable of causing a reduction in kill. To be conservative, therefore, future pipe treatments should be timed to occur when intake waters have relatively low quantities of naturally suspended particulate matter.

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## EXECUTIVE SUMMARY

The purpose of this research project is to identify factors that affect the efficacy of the bacterium *Pseudomonas fluorescens* as a zebra mussel control agent. This report contains information on the impact of the following two factors: 1) mussel siphoning behavior and 2) naturally suspended particle load.

**1. Mussel Siphoning Behavior:** In nature, a zebra mussel typically spreads its two shells spread apart and extends its inhalant siphon tube from between its shells (Fig. 1). In addition to bringing in oxygen for respiration, water passing into this tube brings suspended food particles (e.g., algae and bacteria). The laboratory trials reported herein indicate that the more active this siphoning behavior is (as defined by length of the tube), the higher the mortality that will likely be achieved by a bacterial treatment. Specific information gained in these trials includes:

- Observations made during the first 6 hr of exposure indicated that mean siphoning length and percentage of mussels siphoning were both significantly greater among dying versus surviving mussels.
- The longer a mussel's siphon was extended, the more likely it was to die.
- Any mussel seen siphoning at any time during the first 6 hr of the exposure, had an increased chance of dying.
- Since some mussels that were observed to be siphoning survived the test and some that were not observed to be siphoning during the first 6 hr of the exposure eventually died, one cannot look at an individual mussel during

the initial 6 hr of a 24 hr exposure and predict with 100% confidence that the mussel will or will not die based solely on its siphoning behavior.

In conclusion, any stimulus that causes mussels to close their shells (and thus terminate siphoning) during a bacterial treatment will likely reduce feeding and thus lower the chances for its kill. Thus, to ensure maximum kill in pipes, normal mussel siphoning should not be disturbed. Potential disturbances might include pipe vibrations and rapid changes in water velocity – both of which are known to disturb mussel siphoning.

**2. Particle Load:** Since bacterial cells are presumptively lethal only when ingested by mussels, it was hypothesized that waters containing high levels of naturally suspended particles might reduce the mortality that can be achieved by a bacterial treatment. Our tests indicated that whereas a range of normal particle load concentrations in the Mohawk River (12 to 67 ppm) did not inhibit mussel kill by the bacterial cells, an artificially high particle load of 113 ppm (created in the laboratory from Mohawk water) did show evidence of inhibiting mussel kill (mortality was reduced from 95% to 75%). We suggest that this latter inhibition might have occurred as a result of particle exclusion, i.e., there might have been reduced ingestion of bacterial cells since they represented a reduced percentage of all particles ingested. To be conservative, therefore, future pipe treatments should be timed to occur when waters have relatively low quantities of naturally suspended particulate matter.

## REPORT

### 1. MUSSEL SIPHONING ACTIVITY

#### Introduction

In nature, a zebra mussel typically spreads its two shells apart and extends its inhalant siphon tube from between its shells (Fig. 1). In addition to bringing in oxygen for respiration, water passing into this tube brings suspended particles (e.g., algae and bacteria) for ingestion. It would only seem logical, therefore, that the more active this siphoning behavior is in a mussel (as defined by length of the tube), the higher the mortality that might be achieved by a bacterial treatment. We examined this hypothesis in laboratory tests.



Fig. 1. Lateral view of a well extended inhalant siphon that would be typical of a mussel that is feeding.

### Siphoning Test – Methods

In two replicate bacterial exposure tests, observations of mussel siphoning activity were made in recirculating testing chambers to see if a correlation existed between siphoning behavior and mussel mortality. In both tests, siphoning activity of individual mussels was recorded immediately following treatment, at 0.5 hr, and then hourly during the first 6 hr of a total 48-hr bacterial exposure. Siphoning observation data were based on a numerical ranking system of ranging from 0 to 3, where 0 = shells completely closed (no siphoning), 1 = shells slightly open (i.e., siphon likely slightly extended but not visible under test conditions), 2 = moderately extended siphon, and 3 = siphon well extended (Fig. 1). Following the 48-hr bacterial exposure, mussels were then individually held in freshwater for an additional 8 days in individually labeled plastic dishes. (This and all other tests in this entire report were conducted at  $23\pm1^{\circ}\text{C}$ .)

### Siphoning Test #1 – Results

- The siphon length of all the mussels that died in the test had a mean value of 1.2 during the 6-hr observation period, while mean siphoning length of the mussels that survived the test was 0.9 (significantly different using Scheffe Test,  $p<0.05$ ).
- The mean percentage of mussels that died in the test and that had been observed siphoning at some point during the 6-hr observation period was 55%, while those that survived treatment was 37% (significantly different using Scheffe Test,  $p<0.05$ ).

### Siphoning Test #2 – Results

- The siphon length of all the mussels that died in the test had a mean value of 1.4 during the 6-hr observation period, while mean siphoning length of the mussels that lived was 1.0 (significantly different using Scheffe Test,  $p<0.05$ ).
- The mean percentage of mussels that died in the test and that had been observed siphoning at some point during the 6-hr observation period was 71%, while those that survived treatment was 51% (significantly different using Scheffe Test,  $p<0.05$ ).

### Siphoning Tests – Discussion and Conclusions

- In both Test #1 and #2, observations made during the initial 6 hr of exposure indicated that mean siphoning length and percentage of mussels siphoning were both significantly greater among dying than surviving mussels. Thus, the longer a mussel's siphon, the more likely it was to die. Likewise, any mussel seen siphoning at any time during the first 6 hr of the exposure, had an increased chance of dying.
- Since some mussels that were observed to be siphoning lived and some that were not observed to be siphoning during the first 6 hr of the exposure eventually died, one cannot look at an individual mussel during the initial 6 hr of a 48-hr exposure and predict with 100% confidence that the mussel will or will not die based solely on its siphoning behavior. Because the bacteria are lethal via ingestion, any mussel that died must have ingested cells at some point during the 48-hr exposure. Mussels were observed at 8 points in time (not continuously) during the first 6 hr of exposure. Thus, mussels recorded as "closed" at all the observation times, may actually have been siphoning and ingesting cells between observation periods or could have started to siphon (i.e., feed) following the end of the 6-hr observation period.
- These data suggest that any factor that might cause mussels to close their shells or reduce the length of their siphon during treatment will likely reduce the mortality that can be achieved. Thus, to ensure maximum kill in pipes, normal mussel feeding should not be disturbed. Potential disturbances to be avoided during a pipe treatment would include pipe vibrations and sudden changes in water velocity – both of which are known to disturb mussel siphoning.

## 2. PARTICLE LOAD

### Introduction

Since bacterial cells are lethal only when ingested by a mussel, it was hypothesized that waters containing high levels of naturally suspended particles might reduce the mortality that can be achieved by a bacterial treatment. If this were true, this might be the result of particle exclusion, i.e., reduced ingestion of bacterial cells since they represent a reduced percentage of all particles ingested. The timing of when mussels experience particle loads was also investigated. For example, would it make a difference if water was relatively clear before treatment and then had a high load of naturally occurring particles during and after treatment? Or just during treatment, etc.? To address these questions, the impact of particle load on mortality was investigated in the following series of three laboratory tests.

### Particle Test #1 – Methods

Water from a local mussel-infested waterbody, the Mohawk River (Crescent, New York), which contained a natural particle load of 12 ppm was used for the test. To examine the impact of particle load either before, during or after treatment, tests were conducted under a variety of exposure scenarios (Table 1).

Table 1: Summary of water treatment scenarios before, during, and after the treatment period.

Water treatment scenario*	Before treatment (2 days)	During treatment (1 day)	After treatment (9 days)
HW → <u>HW</u> → TW	HW	HW	TW
UMW → <u>UMW</u> → UMW	UMW	UMW	UMW
UMW → <u>UMW</u> → FMW	UMW	UMW	FMW
FMW → <u>FMW</u> → FMW	FMW	FMW	FMW
UMW → <u>FMW</u> → UMW	UMW	FMW	UMW

\* Underlined letters represent the 24-hr treatment period. Abbreviations are: unchlorinated tap water (TP, 0 ppm particles); standard laboratory-prepared hard water (HW, 0 ppm particles); unfiltered Mohawk water (UMW, 12 ppm particles); and filtered Mohawk water (FMW, 3 ppm particles, 0.45- $\mu$ m pore-size filter).

### Particle Test #1 – Results and Discussion

All water scenarios tested produced mean mussel mortalities in the range of 82.3 to 88.7% (Table 2). There were no significant differences in the mortalities achieved from any of the treatment scenarios ( $p > 0.05$ ). The data from this test suggest that 12 ppm of naturally occurring particulate material will not affect the efficacy of bacterial treatments at 115 ppm. Thus, irrespective of whether there is a natural particle load of 12 ppm before, during or after a 115 ppm bacterial treatment, mortality should be the same. These results suggest that this bacterium has a realistic potential for use as a biological control agent on power plant pipes.

Table 2: Zebra mussel mortality after 24-hr exposure to 115 ppm of bacterial cells in different water treatment scenarios.

Water treatment scenario*	Bacterial treatment concentration (ppm)	10-day mortality (%)	Mean mortality $\pm$ SD (%)
HW → <u>HW</u> → TW	0	0	NA
UMW → <u>UMW</u> → UMW	0	5	NA
FMW → <u>FMW</u> → FMW	0	0	NA
HW → <u>HW</u> → TW	115.4	93, 88, 85	88.7 $\pm$ 4.0
UMW → <u>UMW</u> → UMW	115.4	85, 89, 89	87.7 $\pm$ 2.3
UMW → <u>UMW</u> → FMW	115.4	88, 83, 94	88.4 $\pm$ 5.4
FMW → <u>FMW</u> → FMW	115.4	86, 83, 83	84.0 $\pm$ 1.7
UMW → <u>FMW</u> → UMW	115.4	76, 85, 86	82.3 $\pm$ 5.7

\* See footnote Table 1.

### Particle Test #2 – Methods

Test protocols were similar to Test #1. Mohawk River water used for the this test contained 27 ppm of naturally suspended particles, and filtration of this water (0.45- $\mu$ m pore-size) reduced it to a 2 ppm particle load.

### Particle Test #2 – Results and Discussion

Water treatment scenarios in which mussels were held before and during treatment in HW or UMW produced high kill (HW→HW→TW = 99.3%, UMW→UMW→UMW = 98.0%, UMW→UMW→FMW = 98.3%, Table 3). However, the water treatment scenario in which mussels were treated in FMW achieved significantly less kill (FMW→FMW→FMW = 69.4%, UMW→FMW→UMW = 91.0%, Table 3) than the HW or UMW treatments. (It is somewhat anomalous that mussels that were treated in filtered water but “fed” particles before and after treatment appeared to have been more susceptible to kill by CL0145A cells than mussels that were in filtered water the entire time; these results, however, are of academic interest since water flowing through a power plant will not be filtered before, during or after treatment.)

Table 3: Zebra mussel mortality after 24-hr exposure to 89.7 ppm CF in different water treatment scenarios.

Water treatment scenario*	Bacterial treatment concentration (ppm)	10-day mortality (%)	Mean mortality $\pm$ SD (%)
HW $\rightarrow$ <u>HW</u> $\rightarrow$ TW	0	0	NA
UMW $\rightarrow$ <u>UMW</u> $\rightarrow$ UMW	0	0	NA
FMW $\rightarrow$ <u>FMW</u> $\rightarrow$ FMW	0	1	NA
HW $\rightarrow$ <u>HW</u> $\rightarrow$ TW	89.7	99, 99, 100	99.3 $\pm$ 0.6
UMW $\rightarrow$ <u>UMW</u> $\rightarrow$ UMW	89.7	99, 98, 97	98.0 $\pm$ 1.0
UMW $\rightarrow$ <u>UMW</u> $\rightarrow$ FMW	89.7	99, 98, 98	98.3 $\pm$ 0.6
FMW $\rightarrow$ <u>FMW</u> $\rightarrow$ FMW	89.7	68, 70, 70	69.4 $\pm$ 1.3
UMW $\rightarrow$ <u>FMW</u> $\rightarrow$ UMW	89.7	95, 88, 90	91.0 $\pm$ 3.6

\* See footnote Table 1.

In summary, the data from this test suggested that 27 ppm of naturally occurring particulate material will not inhibit the efficacy of treatments with 90 ppm of bacteria. These results further suggest that this bacterium has a realistic potential for use as a biological control agent on power plant pipes.

### Particle Test #3 – Methods

Test protocols were similar to Test #1 and #2. Mohawk River water used for the this test contained 67 ppm of naturally suspended particles, and filtration of this water (0.45- $\mu$ m pore-size) reduced it to a 9 ppm particle load. In addition, an artificially high particle load of 113 ppm was created in the laboratory from concentrating particles and resuspending them in Mohawk water.

### Particle Test #3 – Results and Discussion

In this test similar mussel mortality was achieved in all water types, except for the artificially concentrated Mohawk water (CMW) that had a particle concentration of 113 ppm (Table 4). Mean mussel mortality in CMW was 72.7%, while all other water treatments ranged from 88.7 to 96.0% (Table 5 and Fig. 1). It may be that in very high particle load situations, i.e., 113 ppm in CMW, the particles in the water out compete the toxic bacterial particles (98 ppm) for mussel ingestion (evaluated by mussel mortality). These results are in agreement with Test# 1, but still leave the question of why we got significantly lower mortality in FMW-FMW-FMW in Test# 2 unanswered.

Table 4: Zebra mussel mortality after 24-hr exposure to 97.6 ppm CF in different water treatment scenarios.

Water treatment scenario*	Bacterial treatment concentration (ppm)	10-day mortality (%)	Mean mortality $\pm$ SD (%)
HW $\rightarrow$ <u>HW</u> $\rightarrow$ TW	0	0	NA
UMW $\rightarrow$ <u>UMW</u> $\rightarrow$ UMW	0	0	NA
FMW $\rightarrow$ <u>FMW</u> $\rightarrow$ FMW	0	0	NA
CMW $\rightarrow$ <u>CMW</u> $\rightarrow$ CMW	0	0	NA
HW $\rightarrow$ <u>HW</u> $\rightarrow$ TW	97.6	97, 95, 95	95.7 $\pm$ 1.2
UMW $\rightarrow$ <u>UMW</u> $\rightarrow$ UMW	97.6	95, 93, 90	92.7 $\pm$ 2.5
UMW $\rightarrow$ <u>UMW</u> $\rightarrow$ FMW	97.6	75, 98, 93	88.7 $\pm$ 12.1
FMW $\rightarrow$ <u>FMW</u> $\rightarrow$ FMW	97.6	99, 93, 96	96.0 $\pm$ 3.0
UMW $\rightarrow$ <u>FMW</u> $\rightarrow$ UMW	97.6	92, 95, 95	94.0 $\pm$ 1.2
CMW $\rightarrow$ <u>CMW</u> $\rightarrow$ CMW	97.6	79, 75, 64	72.7 $\pm$ 7.8

\* See footnote Table 1.

### **Particle Tests #1-3 – Conclusions**

Whereas a range of normal particle load concentrations in the Mohawk River (12 to 67 ppm) did not inhibit mussel kill by the bacterial cells, an artificially high particle load of 113 ppm (created in the laboratory from Mohawk water) did show evidence of inhibiting mussel kill. This may have occurred as a result of competitive displacement, i.e., lower feeding by mussels on the suspended bacteria *versus* naturally occurring particles. To be conservative, therefore, future pipe treatments should be timed to occur when waters have relatively low quantities of naturally suspended particulate matter.

### **PLANS FOR NEXT REPORTING PERIOD**

All laboratory work to date has been conducted in vessels (jars, bottles, pipes) in which water is recirculated either by aerators or pumps. Tests conducted under flow-through (once-through) conditions will be reported on in the next quarterly report.

### **TECHNOLOGY AND INFORMATION TRANSFER**

This project was highlighted in the following conference presentation:

Molloy, D. P., Mayer, D. A., and Presti, K. T. Biological control of zebra mussels with microbial toxin: Small-scale once-through pipe tests. Twelfth International Conference on Aquatic Invasive Species. June 10, 2003. Windsor, Ontario. (Submitted paper.)