TECHNICAL REPORT
(40751R010)

Report Title:	**Impact of Five Treatment Factors on Mussel Mortality**
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DISCLAIMER

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 factors that affect mussel kill. Test results reported herein indicate that mussel kill should not be affected by: 1) air bubbles being carried by currents through power plant pipes; 2) pipe orientation (e.g., vertical or horizontal); 3) whether the bacterial cell concentration during a treatment is constant or slightly varying; 4) whether a treatment is between 3 hr and 12 hr in duration, given that the total quantity of bacteria being applied to the pipe is a constant; 5) whether the water temperature is between 13C and 23C.

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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 examined the following five factors:

1. Effect on Mussel Kill of Air Bubble Disturbance within a Pipe

Could air bubbles passing through a pipe effect mussel kill? Test results were encouraging since they suggested that if air bubbles were being carried by currents through power plant pipes, mussel kill should not be adversely effected during a treatment.

2. Effect on Mussel Kill of Pipe Orientation

Might mussel kill be different inside a vertical *versus* horizontal pipe? Test results were encouraging since they suggested that the orientation of a pipe should not effect mussel kill.

3. Effect on Mussel Kill of Constant *versus* Pulsating Bacterial Concentration

Is it critically important to maintain a very constant bacterial concentration during a treatment? Test results were encouraging since they suggested that it was not important whether the bacterial concentration was constant or pulsating during a treatment, as long as the total mass of bacteria applied was the same to all pipes over the same time period.

4. Effect on Mussel Kill of Varying Both Treatment Concentration and Duration - Warm & Cold Waters

The purpose of these tests was to determine the most effective method of treating zebra mussels with a given amount of inoculum in warm and cold waters. Could higher mortality be achieved by exposing mussels to lower concentrations of inoculum over a longer period of time *versus* higher concentrations over a shorter period of time?

- The results of the warm water tests (ca. 23C) suggested that as long as the total quantity of bacteria applied was the same, similar mussel mortality would be achieved in treatments lasting 1.5 hr to 12.0 hr. It was noteworthy that 1.5 hr treatments consistently achieved high mussel kill at 23C (i.e., >90%) as no other zebra mussel control method has been reported that can achieve such high kill following such a short treatment period.
- The results of the cold water tests (ca. 13C) suggested that when treating with a fixed amount of bacteria, higher mortality will most likely be achieved by treating for at least 3 hr.

- These warm and cold water tests also indicated that the total percentage of mussel mortality achieved should be the same at 13C and 23C. This confirmed similar test results achieved last year. This is significant because the development of a zebra mussel control method that is equally efficacious in such a wide range of temperatures broadens its usefulness as a potential commercial product. Moreover, the cold water tests indicated that this bacterial control agent is actually more effective at lower temperatures than currently commercialized chemical molluscicides that are used for zebra mussel control. The latter commercialized products, such as chlorine, are not able to achieve high mussel kill below ca. 18C.

5. Power Plant Trials: Achieving High Mussel Kill under Once-Through, Service-Water Conditions

Could high kill also be achieved in service water inside a power plant? Tests in small acrylic pipes (ca. 80 ppm for 6 hr) were conducted twice over a one-month period within a New York Power Authority hydrostation. Mean mussel mortalities achieved were 97.2% and 99.7%. These results were encouraging since they demonstrated that, based on the knowledge we had gained from laboratory tests to date, we were able to design treatment protocols which successfully achieved very high mussel kill in service water within a power plant.

REPORT

The following laboratory and power plant tests were conducted using acrylic minipipes (L x D - 25 x 5 cm) (Fig. 1). In all tests, pipes were each seeded with 100 mussels on the day prior to treatment and then were treated with strain CL0145A *P. fluorescens* cells while maintaining a constant water flow rate (4L/hr) within each pipe. Identical untreated control pipes with 100 mussels were also maintained. Streamwater and service waters were used, respectively, in the laboratory and power plant tests. Following the treatment period, mussels continued to be held in pipes and received fresh water under once-through conditions. Except where noted, all testing was conducted at 23(±1)C.

Fig. 1. Examples of the acrylic minipipes used in the tests described in this report. The pipe with clear water on left is an untreated control, whereas water in other pipes has a grayish color due to presence of suspended bacteria during treatment. Mussels are visible attached to walls of pipe.

1. Lab Tests: Effect on Mussel Kill of Air Bubble Disturbance within a Pipe

Could air bubbles passing through a pipe effect mussel kill? Two identical tests (3 hr exposures at ca. 50 ppm, 3 replicates per treatment) using vertical minipipes flowing on once-through conditions determined that there was no statistical difference in mortality whether mussels were in relatively calmly-moving unaerated water *versus* turbulently-moving aerated water. Mortalities in these tests were respectively: Test 1: 72% *versus* 77% and Test 2, 87% *versus* 91%. These results were encouraging since they suggested that if air bubbles were being carried by currents through power plant pipes, mussel kill should not be adversely effected.

2. Lab Tests: Effect on Mussel Kill of Pipe Orientation

Might mussel kill be different inside a vertical *versus* horizontal pipe? Two identical tests (3 hr exposures at ca. 100 ppm, 3 replicates per treatment) using minipipes on once-through flow conditions determined that there was no statistical difference in mortality whether mussels were inside vertical *versus* horizontal pipes. Mortalities in these tests were, respectively: Test 1: 96% *versus* 95% and Test 2, 97% *versus* 94%. These results were encouraging since they suggested that the orientation of a pipe should not effect the mussel kill.

3. Lab Tests: Effect on Mussel Kill of Constant *versus* Pulsating Bacterial Concentration

Is it critically important to maintain a precise bacterial concentration during a treatment? Two identical tests (3 hr exposures, 3 replicates per treatment) using minipipes on once-through conditions suggested that there was no statistical difference in mortality whether mussels were exposed to a constant concentration of bacteria *versus* pulsating concentrations as long as the total mass of bacteria the mussels were exposed to and the total treatment duration were the same. During the tests, the constant and pulsating concentrations were achieved by delivering the bacteria into pipes, respectively, by pump *versus* by 5-min, hand-delivered injections. Mortalities in these tests were, respectively: Test 1: 88% *versus* 88% and Test 2, 100% *versus* 100%. These results were encouraging since they suggested that it was not so critically important to maintain a steady bacterial concentration during a treatment, as long as the total mass of bacteria applied was the same to all pipes over the same time period.

4. Lab Tests: Effect on Mussel Kill of Varying Both Treatment Concentration and Duration - Warm and Cold Waters

The purpose of these tests was to determine the most effective method of treating zebra mussels with a given amount of inoculum. Could higher mortality be achieved by exposing mussels to lower concentrations of inoculum over a longer period of time or higher concentrations over a shorter period of time? Once-through minipipes were treated (3 replicates per treatment) at concentrations of 25, 50, 100 or 200 ppm, respectively, for 12, 6, 3, or 1.5 hr – thereby providing the same total quantity of bacteria to each pipe, irrespective of treatment scenario.

4a. Warm Water Tests - 23C (Table 1)

The results of the three tests run suggested that as long as the total quantity of bacteria applied to a pipe over the entire treatment was the same, similar mussel mortality would be achieved. In all three tests, there was no significant difference in the mortality received in any of the four treatment scenarios. It was noted, however, that although not significantly different, the mean mortality achieved from the 3-hr treatments was the highest in all three tests. Thus, these tests suggested that given a defined quantity of bacteria to treat water at 23C, it is not critically important whether inoculum is added to a pipe slowly over a 12 hr period or relatively quickly over a 1.5 hr period. This is good news in that it indicates that there is some flexibility in choosing a treatment duration and concentration that might be used within power plant pipes. What is also noteworthy is that the 1.5 hr treatments achieved such high mussel kill (i.e., >90% in all three tests). We know of no other zebra mussel control method that can achieve such high kill following such a short treatment period.

Table 1. Warm water tests.

Test No.	hr	ppm	% Mean Mortality
1	12	25	98
	6	50	98
	3	100	99
	1.5	200	97
2	12	25	86
	6	50	95
	3	100	97
	1.5	200	96
3	12	25	86
	6	50	86
	3	100	95
	1.5	200	92

4b. Cold Water Tests - 13C (Table 2)

The purpose of these two tests was identical to the above-mentioned warm-water tests, but specifically sought to evaluate if a lower temperature would provide different test results.

- Test 1: Mussel mortalities following treatments for 12, 6, and 3 hr at ca. 25, 50 and 100 ppm, respectively, were not significantly different (Table 2). Mortality following treatment at 1.5 hr for 200 ppm, however, was significantly lower than 3 hr at 100 ppm.
- Test 2: Treatments for 12 hr at 25 ppm and 6 hr at 50 ppm achieved the highest mean mortalities and were not statistically different from each other. The mortality in pipes treated for 6 hr at 50 ppm was also significantly higher than pipes treated for 3 hr at 100 ppm and 1.5 hr at 200 ppm.

Based on the significantly lower mortality in the 1.5 hr treatment in one of the tests (i.e., Test 1), treatments in cold waters (i.e., near 13C) should be for a minimum of 3 hr.

Table 2. Cold water tests.

Test No.	hr	ppm	% Mean Mortality
1	12	25	94
	6	50	95
	3	100	96
	1.5	200	89
2	12	25	97
	6	50	99
	3	100	94
	1.5	200	95

4c. Effect of Temperature on Mussel Mortalities - Confirmation of Previous Results

A previous laboratory test (reported in Technical Report R40751R05) examining the effect of temperature on mortality indicated that that there was no statistical difference in mussel kill following treatment at 23C *versus* 12C water. The warm water (23C, Table 1) and cold water (13C, Table 2) data within this current report again support this same conclusion, i.e., that almost identical mortalities are achievable in such warm and cold waters. This is significant because the development of a zebra mussel control method that is efficacious in such a wide range of temperatures broadens its usefulness as a potential commercial product. Moreover, these tests indicated that this bacterium is more effective at lower temperatures than currently commercialized chemical molluscicides that are used for zebra mussel control. The latter commercialized products, such as chlorine, are not able to achieve high mussel kill below ca. 18C.

5. Power Plant Trials: Achieving High Mussel Kill under Once-Through, Service-Water Conditions

As indicated in the above tests, high mussel kill in minipipe tests was repeatedly achieved in the laboratory, but could such high kill be achieved in service water inside a power plant? Tests were conducted within a New York Power Authority hydrostation on the Mohawk River (Crescent, NY). Four minipipes were treated at ca. 80 ppm throughout a 6-hr period. The entire test was conducted twice over a one-month period. Mean (±SD) mussel mortalities in these two tests were 97.2 (±1.5)% and 99.7 (±0.6)%. In contrast, mean (±SD) mussel mortalities in untreated control pipes were, respectively, 2.0 (±1.7)% and 3.7 (±1.5)%. These results were encouraging since they demonstrated that exposure to bacterial strain CL0145A could result in high zebra mussel mortality in service-water within a hydropower station.

PLANS FOR NEXT REPORTING PERIOD

Tests assessing the impact of mussel density and water velocity on mussel mortality will be reported, as well as higher-flow, service-water tests in bigger acrylic pipes inside power plants.

TECHNOLOGY AND INFORMATION TRANSFER

This project was highlighted in the following conference presentation:

Mayer, D. A., Molloy, D. P., Gaylo, M. J., and Presti, K. T. Small-scale flow-through application of *Pseudomonas fluorescens* strain CL0145A in the service water of a hydropower facility for the biological control of zebra mussels. Annual Meeting of the Society for Industrial Microbiology. August 11, 2003. Minneapolis, Minnesota. (Submitted poster.)