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ABSTRACT

Under this USDOE-NETL contract, the bacterium *Pseudomonas fluorescens* strain CL145A (*Pf*-CL145A) is being developed as a biocontrol agent for zebra mussels (*Dreissena polymorpha* and *Dreissena bugensis*) that infest water pipes in power plants. Progress was made in the following areas during this reporting period:

- The cost of the fermentation medium to mass produce the bacterium was reduced from \$2.32/L to \$0.26/L, a 89% reduction. This is a major advancement for the project as it significantly reduces production cost and thus increases the likelihood of commercial success of this bacterial approach for zebra mussel control.
- A comprehensive series of dosage trials (varying treatment duration and bacterial concentration) indicated that a 6-hr treatment at 50 ppm (i.e., 50 mg dry weight of bacterial cells per liter of power plant intake water) is optimal to achieve mussel kill. This is a much shorter treatment duration than any commercial mussel control method currently in use in power plants, e.g., chlorination is typically several weeks in duration (~100X longer). Therefore, in addition to reducing power plant concerns/liabilities about the handling of hazardous materials (e.g., materials for chlorination), the use of this environmentally safe, bacterial approach should also lower plant personnel costs required for treatment. The challenge that now remains is to increase the toxicity of each bacterial cell so that less bacterial product (e.g., <50 ppm) is needed to achieve mussel control during the 6-hr treatment. Efforts are now underway to sequence the bacterium's DNA and then manipulate it to produce more toxin per cell.
- Cell toxicity stabilization is a critically important aspect to successfully developing our commercial product. When the bacterial cells are cultured and harvested, they must be processed in a manner that maintains their toxicity to zebra mussels. We have submitted a \$100,000 request to the National Science Foundation (NSF) to work collaboratively with a St. Louis company, Particle and Coatings Technologies, to develop a method to dry the bacteria so that loss of toxicity when stored as a commercial product is minimal. This NSF grant program is specifically designed to advance the commercialization of biotechnological products by funding joint industry-governmental research collaborations. A decision on this request should be made shortly.

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EXECUTIVE SUMMARY

Use of the bacterium *Pseudomonas fluorescens* strain CL145A (*Pf*-CL145A) represents a potential alternative to the current use of polluting biocides for control of zebra mussel infestations in power plant water pipes. The purpose of this DOE-NETL project is to accelerate research toward commercialization of this biocontrol agent so that it will be feasible to use it against the two zebra mussel species infesting North American power plants, *Dreissena bugensis* and *D. polymorpha*. During the last six months, research efforts focused on the following activities:

1. REDUCTION IN THE COST OF THE *Pf*-CL145A CELL-BASED BIOCIDES THROUGH THE DEVELOPMENT OF AN INEXPENSIVE FERMENTATION MEDIUM.

The sole purpose of previous fermentation medium development was to increase the toxicity of the harvested *Pf*-CL145A cell product, without seriously considering of cost of the media. Having successfully accomplished this goal and characterizing key components in the medium required for high cell toxicity, as previously reported, our goal in the current reporting period was to reduce the cost of the fermentation medium without reducing biomass production or efficacy to zebra mussels. Our target goal was to reduce the cost of the medium by at least 50%. We report herein, that through a systematic series of tests, the cost of the medium has been reduced from \$2.32/L to \$0.26/L, a reduction of 89%, thus exceeding our targeted goal.

2. DEMONSTRATING DOSAGE EFFICACY IN KILLING *D. BUGENSIS* IN POWER PLANT INTAKE WATER.

A treatment array containing various dosages of *Pf*-CL145A was carried out in the Rochester Gas and Electric (RG&E) Russell Power Station environmental trailer to evaluate their ability to kill *D. bugensis* in Lake Ontario water under flow-through conditions. Testing was performed exclusively on *D. bugensis* which accounts for approximately 95% of the fouling zebra mussels inside the service water of power plants located along Lake Ontario. Treatment with *Pf*-CL145A at concentrations between 25 and 200-ppm produced moderately high levels of mortality among *D. bugensis* held under 1 L/min flow-through conditions at 23°C regardless of the duration of treatment.

Thus, this comprehensive series of dosage trials (varying treatment duration and bacterial concentration) indicated that a 6-hr treatment at 50 ppm (i.e., 50 mg dry weight of bacterial cells per liter of power plant intake water) is optimal to achieve mussel kill. This is a much shorter treatment duration than any commercial mussel control method currently in use in power plants (e.g., chlorination is typically several weeks in duration or ~100X longer). Therefore, in addition to reducing power plant concerns/liabilities about the handling of hazardous materials (e.g., as for chlorination), the use of this environmentally safe, bacterial approach should also lower plant personnel costs required for treatment.

The challenge that now remains is to increase the toxicity of each bacterial cell so that less bacterial product (e.g., <50 ppm) is needed to achieve mussel control during the 6-hr treatment. Efforts are now underway to sequence the bacterium's DNA and then manipulate it to produce more toxin per cell.

3. GRANT APPLICATION TO NATIONAL SCIENCE FOUNDATION.

Cell toxicity stabilization is a critically important aspect to successfully developing our commercial product. After our bacterial cells are cultured and harvested from fermentation units, they must be processed to a commercial product in a manner that maintains their toxicity to zebra mussels. We have submitted a \$100,000 request to the National Science Foundation (NSF) to work collaboratively with a St. Louis company, Particle and Coatings Technologies, to develop a method to dry the bacteria so that loss of toxicity when stored as a commercial product is minimal. This NSF grant program is specifically designed to advance the commercialization of biotechnological products by funding joint industry-governmental research collaborations. A decision on this NSF funding request should be made shortly.

INTRODUCTION

Coal-fired power plants within North America need an effective, economical, and non-polluting technique for managing infestations of zebra mussels within their facilities. Due to a lack of options, many facilities have relied on the use of broad-spectrum, chemical biocides for control of these freshwater mussels. However, biocide treatments, such as continuous chlorination for three weeks, are widely regarded as environmentally unacceptable because they can result in the formation of potentially carcinogenic substances. Use of the bacterium *Pseudomonas fluorescens* strain CL145A (*Pf*-CL145A) represents a potential alternative to the use of polluting biocide treatments and is the leading candidate in the world for the biological control of these macrofouling mussels. The purpose of this DOE-NETL project is to accelerate research toward commercialization of this biocontrol agent so that it will be feasible to use against the two zebra mussel species infesting North American power plants, *Dreissena bugensis* and *D. polymorpha*. During the last six months, progress was made in the following areas:

1. REDUCTION IN THE COST OF THE *PF*-CL145A CELL-BASED BIOCIDES THROUGH THE DEVELOPMENT OF AN INEXPENSIVE FERMENTATION MEDIUM.

In the development of a cost-effective fermentation process, there are two main areas in which significant reductions in overall cost can be achieved. These are: 1) development of a fermentation medium composed of low-cost components, and 2) modification of the fermentation protocol and component concentrations to increase biomass production. The sole purpose of previous fermentation medium development was to increase the toxicity of the harvested *Pf*-CL145A cell product, without serious concern over the cost of the media. This latter period of research was aimed at characterizing some of the nutritional and environmental requirements of CL145A that encourage high levels of toxin production. Having accomplished this goal, as previously reported, our goal in the current reporting period was to reduce the cost of the fermentation medium without reducing biomass production within the cultures or efficacy of the harvested cells to zebra mussels. Our target goal was to reduce the cost of the medium by at least 50%. We report herein, that through a systematic series of tests, the cost of the medium has been reduced from \$2.32/L to \$0.26/L, a total cost reduction of 89%, thus, far exceeding our original goal. We are continuing to develop this medium and protocol for commercial-scale production since the cheaper we can make the entire *Pf*-CL145A production process, the more likely this product will be to succeed as a realistic alternative to the economically lower-cost, yet environmentally higher-cost chemical controls currently employed to control zebra mussels within power plant pipes. The developments in fermentation achieved now will likely be effective for the large-scale production of the genetically enhanced strain of *Pf*-CL145A that is in progress with the completion of the whole genome sequence scheduled for later this year.

2. DEMONSTRATING DOSAGE EFFICACY IN KILLING *D. BUGENSIS* IN POWER PLANT INTAKE WATER.

Traditional laboratory testing is centered on experiments in aerated containers with recirculating water because they are easily managed, can evaluate many variables at once, and are much less time consuming to conduct. Mussels in power plants, however, are under flow-through conditions in pipes, so it is important to demonstrate that *Pf*-CL145A cells are capable of achieving high mussel kill under more realistic, i.e., flow-through, pipe conditions. Toward this end, an experiment was conducted in the intake water system at RG&E's Russell Station environmental trailer to determine the effects of various *Pf*-CL145A dosages on zebra mussel mortality. Determining the ideal treatment dosage for large-scale plant treatments will be an important cost-cutting tool and will help ensure maximum efficacy.

3. GRANT APPLICATION TO NATIONAL SCIENCE FOUNDATION.

Cell toxicity stabilization is a critically important aspect to successfully developing our commercial product. After our bacterial cells are cultured and harvested from fermentation units, they must be processed to a commercial product in a manner that maintains their toxicity to zebra mussels. We have submitted a \$100,000 request to the National Science Foundation (NSF) to work collaboratively with a St. Louis company, Particle and Coatings Technologies, to developing a method to dry the bacteria so that loss of toxicity when stored as a commercial product is minimal. This NSF grant program is specifically

designed to advance the commercialization of biotechnological products by funding joint industry-governmental research collaborations. A decision on this request should be made shortly.

EXPERIMENTAL

The following is an overview of the materials and methods used in above-mentioned research projects:

1. REDUCTION IN THE COST OF THE *PF-CL145A* CELL-BASED BIOCIDES THROUGH THE DEVELOPMENT OF AN INEXPENSIVE FERMENTATION MEDIUM.

- Shaken seed cultures: 250-mL Erlenmeyer flasks containing 25 mL of buffered tryptic soy broth (bTSB) were inoculated with 0.4 mL of stock culture and shaken at 200 rpm at 26±1°C for 24 hr.
- Experimental media: The design and composition of experimental media progressed in three steps:
 - 1) Identification of currently present medium components that could be reduced or omitted. The presence and concentrations of six components were tested.
 - 2) Replacement of current high-cost medium components with low-cost alternatives. Twenty-five different economical peptone sources were screened in experimental media formulations.
 - 3) Modification of presence and/or concentration of components using factorial designs and analysis (Minitab 14.0).
- Shaken flask cultures: Replicate flasks containing 35 mL of the experimental culture medium were inoculated at a 1% (v:v) concentration with the 24-hr seed culture. Flasks were shaken at 200 rpm at 26±1°C for 24 hr (Fig. 1).
- Biomass production: Biomass production in shake-flask cultures was determined from optical density readings ($A_{660\text{ nm}}$; Genesys 20 Spectrophotometer) of the 24-hr final whole culture (FWC), using sterile media as a blank (Fig. 2).
- Production of cell fraction (CF): The final whole culture (FWC) from each culture was centrifuged and the pellets were combined to produce a common pellet. Cell pellets were resuspended in dilution water (80 ppm KH_2PO_4 , 405.5 ppm $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in deionized water).
- Preparation of CF: Mean dry bacterial cell mass/mL for each CF was calculated from two 1.0 mL desiccated subsamples using a Denver Instruments balance. The amount of inoculum needed to treat at the targeted concentration was based on the mean dry bacterial cell mass/mL.
- Preparation of mussels: Zebra mussels were collected from various locations, brought to the testing site, sieved, and stored until testing began. Mussels were collected from the Mohawk River (Crescent, NY).
- Standard laboratory bioassays of mussels with CF: The day before treatment, mussels were picked, placed into testing containers (either micro-chambers or testing jars) containing 5 or 100 mL of aerated hard water (Peltier and Weber, 1985), respectively, and allowed to attach overnight. The morning of treatment, unattached mussels were replaced with attached mussels from an extra dish. At least one hour before treatment, the testing containers were filled with fresh aerated hard water (10 mL for micro-chambers, 500 mL for testing jars), set up with aeration, and labeled (Figs. 3 and 4). Mussels were exposed for the treatment period (24 hr), then the fluid was poured off and mussels were collected in clean plastic dishes with fresh, oxygenated hard water to be examined for mortality. Mussels were held in the dishes for an additional 6 (micro-chambers) or 9 (testing jars) days changing the water and scoring mortality each day. All binomial mortality data were analyzed following angular transformation (Sokal and Rohlf, 1995).
- Calculation of relative biomass production and relative toxicity: The relative values for biomass production and toxicity were calculated as the ratio of the value, optical density or angular transformed mortality, from each experimental medium compared to the value achieved from the original fermentation medium (FM2) in each experiment.



Figure 1: Shake-flask cultures containing experimental media for cost reduction tests.



Figure 2: Characterization of biomass production in cultures by measuring optical density.



Figure 3: Three replicate micro-chambers containing zebra mussels with aeration.



Figure 4: Micro-chamber bioassay to characterize toxicity of CL145A cells harvested from experimental media.

2. DEMONSTRATING DOSAGE EFFICACY IN KILLING *D. BUGENSIS* IN POWER PLANT INTAKE WATER.

The following is a general outline of the methodology employed in the trial conducted within the RG&E Russell Power Station environmental trailer to assess the ability of *Pf*-CL145A to kill zebra mussels in service water piping under various treatment dosages:

- Bacterial production: *Pf*-CL145A was cultured at the University of Iowa's Center for Biocatalysis and Bioprocessing within a 100-L fermentation unit following our standardized protocol. The harvested bacterial mass was subsequently frozen in blocks at -80°C and irradiated to kill the cells. Frozen blocks were thawed within 48 hr prior to the test and the aqueous cell suspension was held at ~1°C until ready to use.
- Preparation of mussels: Prior to testing, all of the mussels were held in acrylic pipes under flow-through conditions within a research trailer on the power plant grounds (Fig. 5). The trailer receives water diverted from the plant's intake pipe and mussels were held at ambient Lake Ontario water temperatures. Zebra mussels at this power station are almost exclusively *D.*

bugensis; therefore they were exclusively used to evaluate the effectiveness of Pf-CL145A dosages in this test (Fig. 6). To obtain *D. bugensis* for use in the test, individuals of that species were selected from those that naturally had populated the flow-through water system within the trailer.

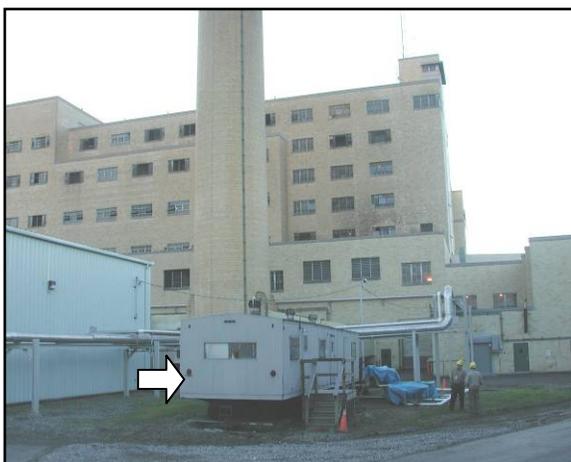


Figure 5. The Rochester Gas & Electric Company has provided use of a research trailer with continuously-flowing intake water on the grounds of their Russell Power Station. The trailer contains areas for small-scale pipe tests as well as holding areas for maintaining mussels both before and after conducting experiments.

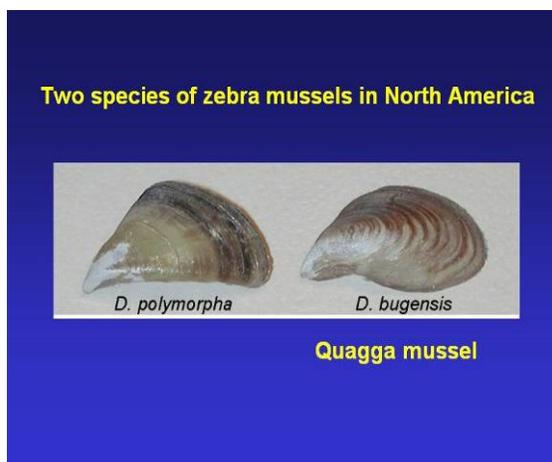


Figure 6. Comparison of *Dreissena polymorpha* and *D. bugensis*. A vast majority of the mussels at the Rochester Gas & Electric Russell Power Station are *D. bugensis*, often referred to as the “Quagga mussel”.

- **Bacterial treatment:** Acrylic pipes containing mussels were placed along their longest side inside a cascading system of 4 liter plastic biotanks held under flow-through conditions at 1.0 L/min directly from the intake water system inside the environmental trailer 24 hr before the experimental treatment (Fig. 7). A pair of peristaltic pumps was used to inject a 1°C aqueous suspension of Pf-CL145A directly into the stream of flowing intake water (Fig. 8). Treated water cascaded from one tank to the next, with each subsequent tank also receiving additional bacterial inoculum to effectively double the dosage of the previous tank. The bacterial injection resulted in 3, 6, 12, 25, 50, 100 and 200-ppm treatment concentrations (Fig. 9). The duration of exposure varied from 6-96 hr. The control mussels were contained in an identical biotank at the head of the system, upstream to bacterial injection. After treatment, mussels were removed from the power plant, placed back into flow-through conditions inside the research trailer and checked for mortality. All binomial data were analyzed following angular transformation (Sokal and Rohlf, 1995).



Figure 7. A picture of one of the cascading biotanks with mussels. A natural rock was used to weigh down the pipes to avoid disturbance and movement. The bacterial inoculum for the subsequent biotank (12-ppm) is seen dripping from the two hoses on the right into the 6-ppm discharge pipe.



Figure 8. The peristaltic pump is delivering inoculum that is contained in an ice-cold, stirred beaker to the 3, 6, 12 and 25-ppm biotanks. An identical setup was used to deliver additional inoculum to the 50, 100 and 200-ppm tanks.

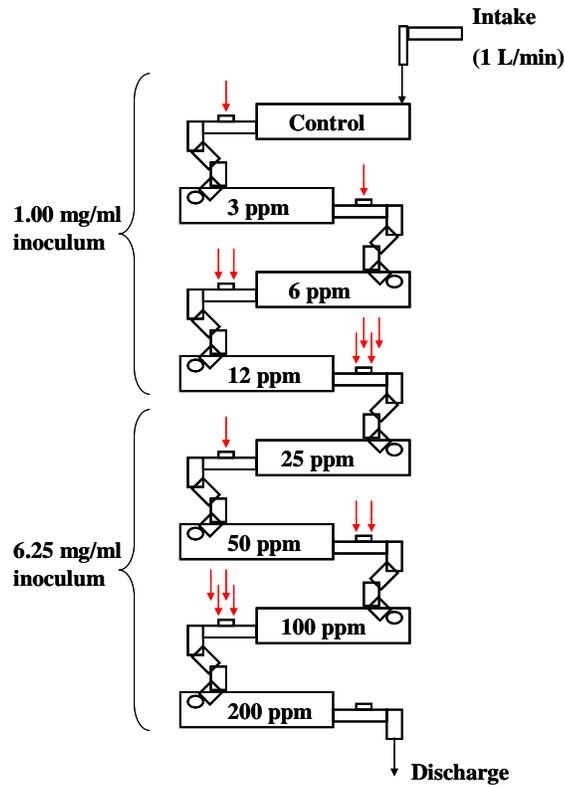


Figure 9. The cascading apparatus schematic. Red arrows indicate inoculum delivery hoses.

Water quality monitoring: During the treatment, the optical density of the treated service water was monitored to confirm that the bacterial treatment concentrations were near the target ppm. According to periodic monitoring the mean water temperature during the treatments was approximately 23°C, the mean pH was 7.9, and mean oxygen concentration was 7.5 ppm (~90% saturated).

RESULTS

This section gives an overview of the progress made during the six-month reporting period in both planning and conducting of the research experiments:

1. REDUCTION IN THE COST OF THE *Pf*-CL145A CELL-BASED BIOCIDES THROUGH THE DEVELOPMENT OF AN INEXPENSIVE FERMENTATION MEDIUM.

The progressive reduction in the cost of the fermentation medium was achieved in three general steps. The first step involved the examination of the cost of individual components present in the current medium to identify components that would affect the total medium cost to the greatest degree, i.e., the most expensive components, then assessing whether the concentrations of these components could be reduced or if they could be completely omitted. Two components were omitted in this first step, reducing the cost of the medium by 56%, yet maintaining biomass production and the toxicity of the harvested cells (Fig. 11). In the second step, media were designed in which the high-cost peptone source was replaced with one of 25 different lower-cost peptone sources. One peptone component resulted in higher biomass production and toxicity than the original peptone, i.e., relative values greater than 1.0 (Fig. 10). The use of peptone source #18 was repeatedly tested as a new medium component and its most effective concentration in the medium was resolved. Replacement of the previous peptone with the lower cost peptone resulted in a total cost reduction of 76% from the original fermentation medium (Fig. 11). In the third stage of testing, multiple components in the medium were tested using factorial designs to analyze their individual and combined effects on biomass production and toxicity. Component presence and concentrations were further modified resulting in a total cost reduction of 89% by the end of this reporting period (Fig. 11).

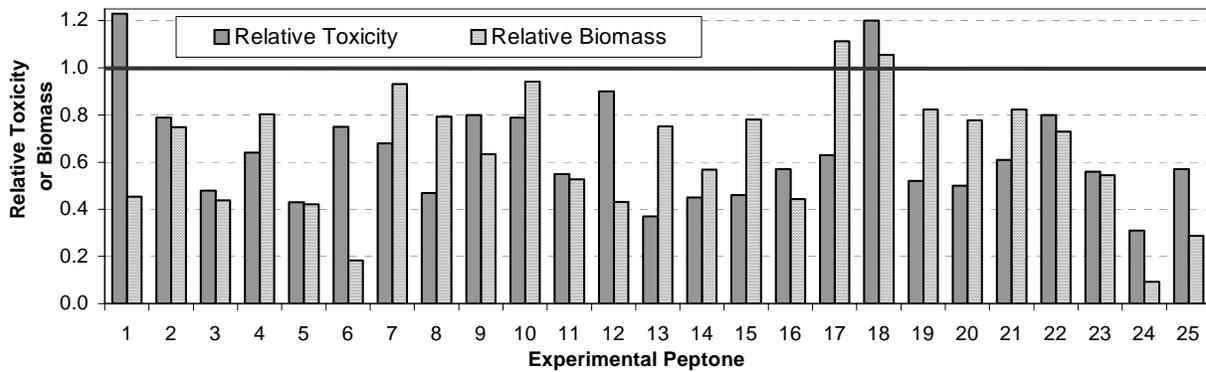


Figure 10: Relative biomass production and toxicity of cells from experimental media in which 25 different economical peptone sources were screened. The solid horizontal line represents a relative value of 1.0, i.e., values equal to the original fermentation medium.

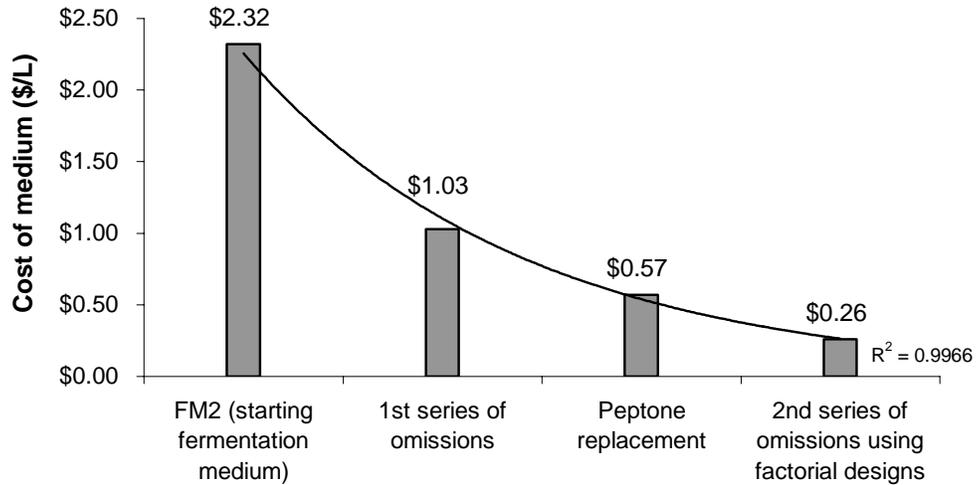


Figure 11: Reduction in the cost of fermentation medium in each progressive step of testing. The line represents the exponential trendline of the data ($R^2=0.9966$).

2. DEMONSTRATING DOSAGE EFFICACY IN KILLING *D. BUGENSIS* IN POWER PLANT INTAKE WATER.

The bacterial suspension of *Pf*-CL145A was successfully delivered to the cascading apparatus resulting in a variety of dosage treatments. Treatment at 50-ppm for 6 hr killed $85.0 \pm 9.9\%$ of the exposed mussels, the second highest average mortality from two replicates. Only the 100-ppm for 12 hr treatment resulted in slightly higher mortality ($89.0 \pm 7.1\%$) although it also used 4 times more bacteria (Table 1). The difference in mortality was not significant and thus a 50-ppm, 6 hr treatment maximizes efficacy while minimizing both bacterial and personnel costs. Data indicated that the use of additional inoculum above 18g did not increase mortality among zebra mussels treated in RG&E intake water flowing at 1 L/min. Treatments performed with a set amount of bacteria should be done at shorter durations at higher concentrations rather than long durations at low concentrations to maximize efficacy (Fig. 12).

Table 1. Summary of mussel mortalities scored over 36 days.

Treatment	Mean % Mortality (\pm SD)	Mean Angular Mortality (\pm SD)
24 hr Control	$2.0 \pm 0.0\%$	0.142 ± 0.000
48 hr Control	$2.0 \pm 2.8\%$	0.101 ± 0.142
96 hr Control	$2.0 \pm 2.8\%$	0.101 ± 0.142
03 ppm-96 hr	$41.0 \pm 4.2\%$	0.695 ± 0.043
06 ppm-48 hr	$45.0 \pm 9.9\%$	0.735 ± 0.100
06 ppm-96 hr	$50.0 \pm 5.7\%$	0.785 ± 0.057
12 ppm-24 hr	$51.0 \pm 7.1\%$	0.795 ± 0.071
12 ppm-48 hr	$64.0 \pm 8.5\%$	0.928 ± 0.089
12 ppm-96 hr	$59.0 \pm 1.4\%$	0.876 ± 0.014
25 ppm-12 hr	$72.0 \pm 0.0\%$	1.013 ± 0.000
25 ppm-24 hr	$80.0 \pm 19.8\%$	1.136 ± 0.265
25 ppm-48 hr	$66.5 \pm 2.1\%$	0.954 ± 0.022
50 ppm-06 hr	$85.0 \pm 9.9\%$	1.183 ± 0.142
50 ppm-12 hr	$85.0 \pm 4.2\%$	1.175 ± 0.060
50 ppm-24 hr	$78.0 \pm 2.8\%$	1.083 ± 0.034
100 ppm-6 hr	$82.0 \pm 2.8\%$	1.133 ± 0.037
100 ppm-12 hr	$89.0 \pm 7.1\%$	1.241 ± 0.116
200 ppm-6 hr	$77.0 \pm 1.4\%$	1.071 ± 0.017

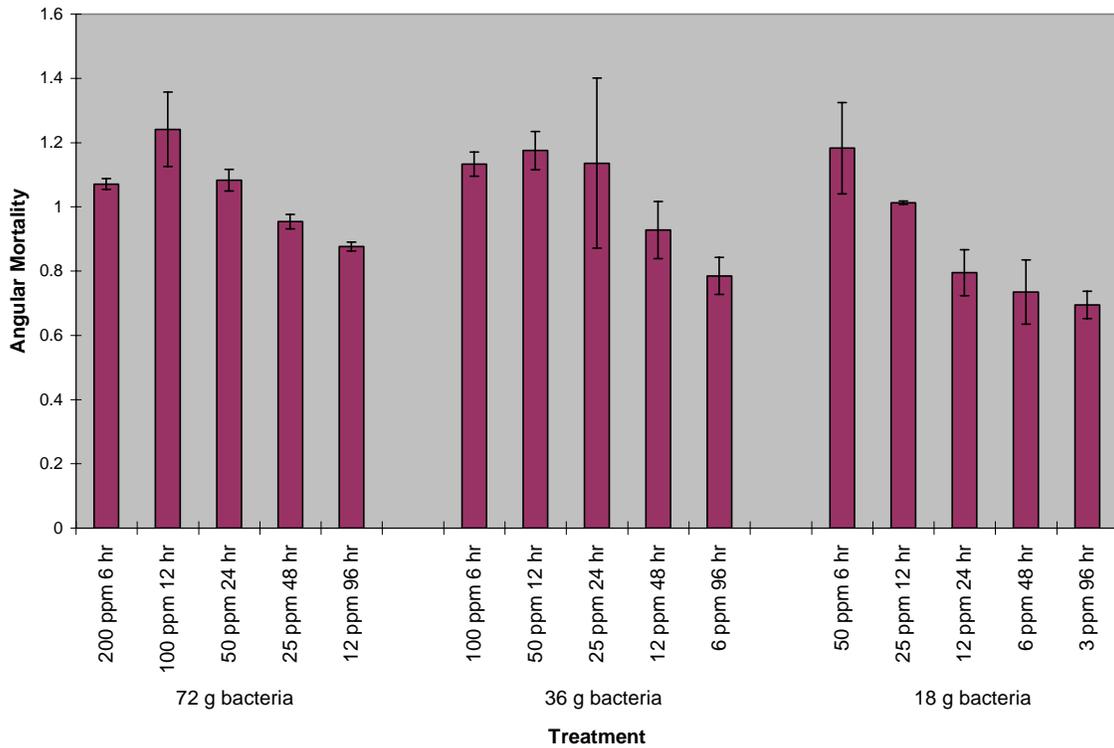


Figure 12. Summary of pipe mortality data. Red bars represent angular transformed mortality for each treatment labeled along the x-axis including standard deviation bars. The 5 treatments grouped to the left used a total of 72 g of bacteria, the middle group 36 g and the group on the right 18 g.

DISCUSSION

1. REDUCTION IN THE COST OF THE *Pf*-CL145A CELL-BASED BIOCIDES THROUGH THE DEVELOPMENT OF AN INEXPENSIVE FERMENTATION MEDIUM.

During this reporting period, the cost of the fermentation medium was reduced from \$2.32/L to \$0.26/L (89%). This is a very important advancement for this project as it contributes to the likelihood of commercial success of a CL145A cell-based product for zebra mussel biocontrol, particularly considering the scale of commercial fermentations, i.e., up to 100,000-L. This reduction in medium cost cannot be understated. The cheaper we can design the entire *Pf*-CL145A production process, the more likely this product will be to succeed as a realistic alternative to the environmentally higher-cost chemical controls currently employed to control zebra mussels within power plant pipes. Production costs will continue to be reduced through alterations of the fermentation protocol within fermentors and genetic enhancement of strain CL145A. The advancements made in understanding the requirements of strain *Pf*-CL145A for biomass and toxin production now will likely be applicable to culturing the genetically enhanced strain in the future.

2. DEMONSTRATING DOSAGE EFFICACY IN KILLING *D. BUGENSIS* IN POWER PLANT INTAKE WATER.

Determining the ideal treatment dosage for large-scale plant treatments will be an important cost-cutting tool and will help insure maximum efficacy. A majority of the bacteria used to treat mussels in flowing water passes through the system and is discharged without having ever been ingested, especially at high flow rates. Discovering the treatment concentration and duration that minimizes bacterial waste

and maximizes efficacy is essential. In this experiment a 50-ppm treatment for 6 hr was the leading candidate for an ideal treatment dosage. Additional dosage testing centered on the 50-ppm 6 hr treatment could further resolve an ideal testing dosage. Previous dosage testing in 2003 against *D. polymorpha* suggested that treating with 18 g of bacteria for shorter durations is similarly advantageous against that species. In that experiment, treating at 200-ppm for 1.5 hr was just as efficacious as treating at 25-ppm for 12 hr (Fig. 13). Shorter treatment periods minimize personnel costs.

The challenge that now remains is to increase the toxicity of each bacterial cell so that less bacterial product (e.g., <50 ppm) is needed to achieve mussel control during the 6-hr treatment. Efforts are now underway to sequence the bacterium's DNA and then manipulate it to produce more toxin per cell.

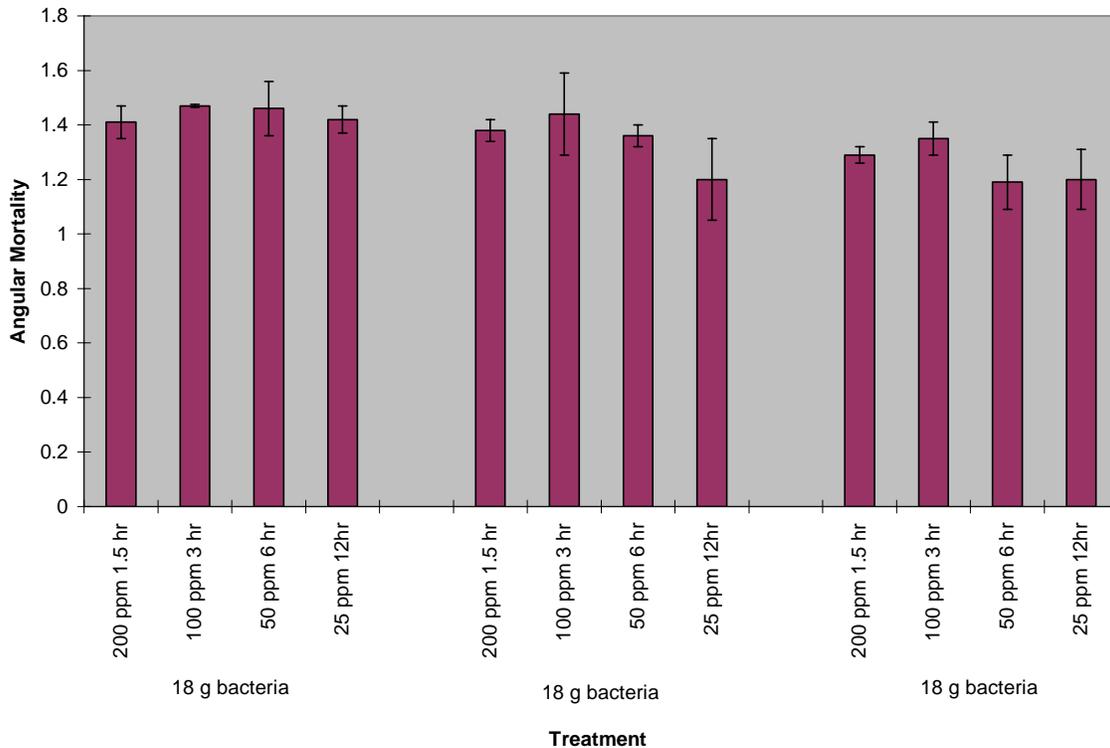


Figure 13. Summary of pipe mortality data from 2003. Red bars represent angular transformed mortality for each treatment labeled along the x-axis including standard deviation bars. The 3 treatment groups all used 18 g of bacteria.

CONCLUSIONS

1. REDUCTION IN THE COST OF THE *Pf*-CL145A CELL-BASED BIOCIDES THROUGH THE DEVELOPMENT OF AN INEXPENSIVE FERMENTATION MEDIUM.

The fermentation medium advancements achieved during this reporting period are essential for the commercial success of *Pf*-CL145A as a biocontrol agent against zebra mussels. At a per liter cost of only \$0.26, further significant cost reductions of the medium are not realistic. Therefore, we will focus on production costs that will develop new methods that could increase biomass production and other procedural components (i.e., seed culture cost) rather than the fermentation medium alone.

2. DEMONSTRATING DOSAGE EFFICACY IN KILLING *D. BUGENSIS* IN POWER PLANT INTAKE WATER.

Additional dosage testing against *D. bugensis* under flow-through conditions centered on 50-ppm for 6 hr could further resolve the ideal *Pf*-CL145A treatment scenario. Determining a treatment dosage that minimizes bacterial use and personnel costs is essential to the development of *Pf*-CL145A as a biocontrol agent. Research to date has indicated that treatment at 50-ppm for 6 hr under flow-through conditions is

adequate for achieving moderately high mortality between both species of zebra mussel. Additional testing may show that the use of fewer bacteria per treatment may produce similar results.

3. GRANT APPLICATION TO NATIONAL SCIENCE FOUNDATION.

If approved, the \$100,000 request to the National Science Foundation (NSF) to work collaboratively with Particle and Coatings Technologies would be a major advance for the project. Achievement of its research goal, i.e., developing a method to dry the bacteria without loss of toxicity, would accelerate commercialization of this bacterium for use in power plants. A decision on this NSF funding request should be made shortly.

REFERENCES

- Peltier, W. H. and Weber, C. I. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Third edition. U. S. EPA Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. 216 pp.
- Sokal, R. R. and Rohlf, F. J. 1995. Biometry: The Principles and Practice of Statistics in Biological Research. Third edition. W. H. Reeman and Company, New York. 887 pp.