TECHNICAL REPORT
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Report Title:	Impact of Water pH on Zebra Mussel Mortality
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DISCLAIMER:

ABSTRACT:

The experiments conducted this past quarter have suggested that the bacterium *Pseudomonas fluorescens* strain CL0145A is effective at killing zebra mussels throughout the entire range of pH values tested (7.2 to 8.6). Highest mortality was achieved at pH values characteristic of preferred zebra mussel waterbodies, i.e., hard waters with a range of 7.8 to 8.6. In all water types tested, however, ranging from very soft to very hard, considerable mussel kill was achieved (83 to 99% mean mortality), suggesting that regardless of the pH or hardness of the treated water, significant mussel kill can be achieved upon treatment with *P. fluorescens* strain CL0145A. These results further support the concept that this bacterium has significant potential for use as a zebra mussel control agent in power plant pipes receiving waters with a wide range of physical and chemical characteristics.

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EXECUTIVE SUMMARY:

Subtask 2.2 of the Statement of Work requires that the Contractor examine the effect of the pH of the treatment water on the efficacy of treatment with *Pseudomonas fluorescens* strain CL0145A. To address this subtask, tests were conducted in a variety of synthetic fresh water types with pH ranges of 7.2 to 7.8 (very soft water), 7.5 to 7.9 (soft water), 7.8 to 8.1 (moderately hard water), 8.1 to 8.4 (hard water), and 8.4 to 8.6 (very hard water). Strain CL0145A was effective at killing zebra mussels in all pH ranges from 7.2 to 8.6, in these five synthetic water types.

EXPERIMENTAL MATERIALS AND METHODS:

Culturing *Pseudomonas fluorescens* strain CL0145A:

Under shaken conditions, 250-ml Erlenmeyer flasks containing 25 ml buffered tryptic soy broth (bTSB) were each inoculated with 0.4 ml stock frozen bacterial culture. Flasks were then incubated at 26(±1)°C on orbital shakers at 200 rpm for 24 hr. These shake-flask seed cultures were subsequently used to inoculate 250-ml Erlenmeyer flasks (1 ml/flask) containing 100 ml of bTSB and incubated at 26(±1)°C under static conditions for ca. 72 hr.

Obtaining the Cell Fraction Inoculum:

Final whole cultures were centrifuged (30 min at ca. 1,450 x g) in 50-ml batches, supernatants were discarded, and cell pellets were resuspended in dilution water (80 ppm KH_2PO_4, 405.5 ppm $MgCl_2 \bullet 6H_2O$ in deionized water, pH adjusted to 7.2 with NaOH). The optical density of the cell fraction (CF) was determined by taking one absorbance reading (spectrophotometer, λ = 660 nm) from each of 3 separate CF samples. Based on an absorbance equation developed during previous trials (author, unpublished data), the optical density of the CF was then used to calculate the volume of CF required to treat mussels at the target concentration of 200 ppm (dry bacterial cell mass/unit volume). Two 1-ml samples of CF were air dried in a dessicator then weighed on a Denver Instruments balance to determine actual treatment concentration.

Production of Different Synthetic Fresh Waters:

Natural waters tend to exhibit a range of pH values characteristic of their water chemistries. The specific pH range is particularly due to the water's alkalinity, which is often described as a water's buffering capacity or ability to withstand changes in pH, particularly acidification (Cole, 1994). Generally (but not always), waters having higher alkalinity display characteristically higher pH values. In the series of experiments described in this report, five types of synthetic fresh waters were produced following Peltier and Weber (1985) which varied in chemical composition defined by their relative hardness (i.e., very soft to very hard) and exhibited a range of pH. In the case of these five water types, hardness and alkalinity were directly proportional. Chemicals were added to deionized water to produce very soft, soft, moderately hard, hard and very hard waters as indicated in Table 1.

Table 1: Five types of synthetic fresh waters and their theoretical and actual pH ranges.

Synthetic fresh water type	Amount of each chemical added to deionized water (mg/L)				Theoretical pH range	Actual initial pH range
	$NaHCO_3$	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	KCl		
Very Soft Water	12.0	7.5	7.5	0.5	6.4 - 6.8	7.18 - 7.25
Soft Water	48.0	30.0	30.0	2.0	7.2 - 7.6	7.58 - 7.66
Moderately Hard Water	96.0	60.0	60.0	4.0	7.4 - 7.8	7.88 - 8.01
Hard Water	192.0	120.0	120.0	8.0	7.6 - 8.0	8.18 - 8.31
Very Hard Water	384.0	240.0	240.0	16.0	8.0 - 8.4	8.41 - 8.55

Upon production of these five synthetic fresh water types for these experiments, the actual measured pH of the different water types was higher than the theoretical pH we anticipated to achieve (Table 1). The deionized water produced in our laboratory and used to produce these synthetic fresh waters may be somehow different than that used in the literature reference from which the method was obtained. However, we did achieve distinct ranges of pH that overlapped most of our targeted pH range which included the pH values in which zebra mussels are typically found in natural waterbodies, e.g., pH of 7.6 to 8.8.

Treatment of Zebra Mussels:

Two separate tests were conducted using zebra mussels collected from the Mohawk River (Crescent, NY) having mean lengths (±SD) of 8.87±1.82 (test #1), and 10.05±1.26 (test #2).

Prior to treatment, mussels were transferred from 7°C unchlorinated tap water to an aquarium in the laboratory containing 7°C unchlorinated tap water (pH ≅ 8), and allowed to warm slowly over a period of two days to the ambient laboratory temperature of 23°C. Four to six days before treatment, mussels were transferred from the

aquarium containing unchlorinated tap water to aquaria containing their respective synthetic water type. The mussels remained in these aquaria until one day before treatment, when 100 mussels were placed in 975-ml testing jars containing 100 ml of the respective water type to a depth of 3 cm. The morning of treatment, unattached mussels were removed and replaced with attached mussels, the testing jars were filled with 495 ml of the appropriate aerated synthetic fresh water type, and fit with airstones set at gentle aeration.

In each test, for each synthetic fresh water type, 3 replicate testing jars were treated with bacteria and one untreated control testing jar, each containing 100 mussels. Mussels were exposed for a period of 24 hr at an initial concentration of 114 and 195 ppm (bacterial dry mass per unit volume) in tests #1 and #2, respectively. Following treatment in the testing jars, mussels were transferred to 7.6x7.6 cm plastic dishes in ca. 100 ml of their respective water type and dishes were submersed in 40-L aquaria containing the appropriate synthetic fresh water type. To reduce the risk of elevated ammonia levels, water in these aquaria was completely replaced with respective synthetic fresh water type once during the post-treatment period. Mussel mortality was scored daily for an additional 9 days following treatment.

The pH of each water type was measured before, during and after mussel treatment using a pH probe (Accumet #13-620-287) and meter (Corning model 420).

All binomial mortality data were analyzed following angular transformation (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION:

In both tests the pH values in the holding tanks were relatively consistent throughout the testing period (Figs. 1 and 2). Therefore, by holding mussels in dishes submersed in a relatively large volume of water (i.e., 40-L aquaria), we were able to maintain a relatively constant range of pH for each type of synthetic fresh water. The pH range throughout the tests were: very soft water – 7.2 to 7.8, soft water – 7.5 to 7.9, moderately hard water – 7.8 to 8.1, hard water – 8.1 to 8.4, and very hard water – 8.4 to 8.6 (Table 2).

Fig. 1: pH of 5 types of synthetic fresh water in test #1 with mussels held post-treatment in dishes submersed in aquaria. The vertical lines represent the 24-hr treatment period.

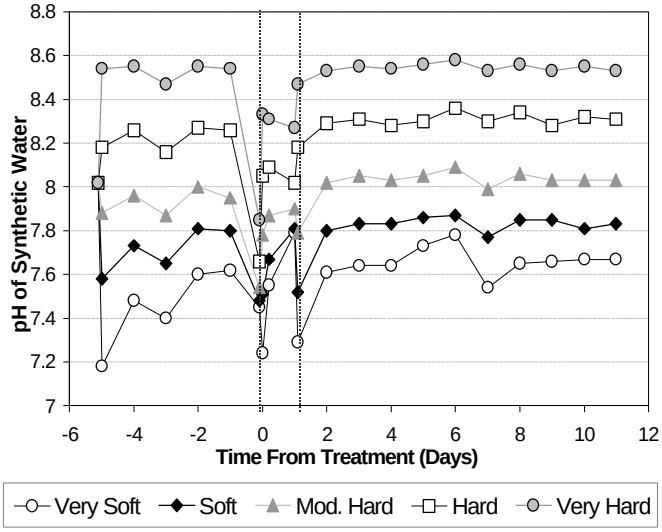

Fig. 2: pH of 5 types of synthetic fresh water in test #2 with mussels held post-treatment in dishes submersed in aquaria. The vertical lines represent the 24-hr treatment period.

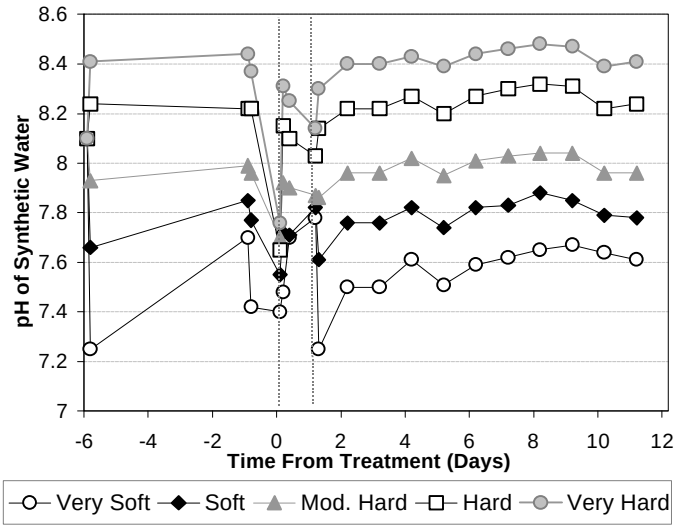

Table 2: Zebra mussel mortality in different synthetic fresh water types after 24-hr exposure to 114 and 195 ppm (tests #1 and #2, respectively) treating 100 musses/testing jar containing 500 ml of treatment water.

Synthetic fresh water type	Actual pH range	Test #	Mean zebra mussel mortality ± SD (% mortality in untreated control)
Very Soft Water	7.2 – 7.8	1	83.0 ± 4.6% (3%)
		2	88.7 ± 2.3% (1%)
Soft Water	7.5 – 7.9	1	96.7 ± 0.6% (1%)
		2	81.0 ± 3.5% (0%)
Moderately Hard Water	7.8 – 8.1	1	97.7 ± 1.2% (2%)
		2	97.0 ± 1.0% (0%)
Hard Water	8.1 – 8.4	1	98.3 ± 1.2% (0%)
		2	97.0 ± 2.6% (2%)
Very Hard Water	8.4 – 8.6	1	98.0 ± 1.7% (1%)
		2	99.0 ± 1.0% (2%)

Experiments, using different types of synthetic water to maintain relatively constant and distinct pH ranges, resulted in lower mussel mortalities in the softer water types, including very soft and soft water, which displayed pH ranges from 7.2 to 7.9. At the same time, treating mussels in the harder water types, including moderately hard, hard and very hard waters, which displayed pH ranges from 7.8 to 8.6, resulted in higher mussel mortality. These results appear to suggest that, due to pH alone, treating mussels in waters with pH values ranging from 7.8 to 8.6 will give us the best chance to achieve the highest mussel kill. Since the chemical make-up of the synthetic water types used in this test were different, however, we cannot exclude the possibility that the differences in mortality seen in these tests were due to a combination of factors, including pH, hardness, and alkalinity. Therefore, in the final analysis, direct evidence concerning the exclusive impact of pH on the efficacy of CL0145A cells during zebra mussel treatment could not be conclusively assessed. However, since zebra mussels tend to flourish in harder waters as opposed to softer waters (due to their requirement for shell-building components, including calcium carbonate which contributes to hardness and alkalinity), it is likely that future field applications in power plants will occur in naturally hard waters, thus offering the best opportunity for high mussel kill using this strain. In all water types tested, ranging from very soft to very hard, considerable mussel kill was achieved (83 to 99% mean mortality), suggesting that regardless of the pH or hardness of the treatment water, significant mussel kill can be achieved upon treatment with *P. fluorescens* strain CL0145A.

CONCLUSIONS:

Treating zebra mussels in waters having a pH in the range of 7.8 to 8.6 should give us the best chance for the highest mussel kill, while we may expect slightly lower mussel kill when treating mussels in waters with a pH lower than 7.8. In all water types tested, ranging from very soft to very hard, considerable mussel kill was achieved (83 to 99% mean mortality), suggesting that regardless of the pH or hardness of the treatment water, significant mussel kill can be achieved upon treatment with *P. fluorescens* strain CL0145A. These results further support the concept that this bacterium has significant potential for use as a zebra mussel control agent in power plant pipes receiving waters with a wide range of physical and chemical characteristics.

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