New England Water Treatment Technology Assistance Center
University of New Hampshire • Durham, New Hampshire
PROJECT SUMMARY REPORT

Effectiveness of *Aeromonas* Removal in Water Treatment Processes Based on Quantitative Molecular Analysis

Objectives

The overall goal of this research was to assess the effectiveness of drinking water treatment processes for *Aeromonas* removal. The objective of this study was to use a quantitative and sensitive molecular meth-od to quantify total *Aeromonas* spp. and virulence-gene-containing *Aeromonas* spp. in source, intermedi-ate and finished water of several selected water utilities and thus determine the treatment effectiveness of various processes.

Methodology

Development of real-time PCR assay for quantifying total Aeromonas spp.
The region of 16S rDNA of *Aeromonas* spp. was used as the target region for designing the SYBR®Green real-time PCR assay for quantifying total *Aeromonas* spp. A set of primers, a forward primer Aer66f (5'-GCGGCAGCGGGAAAGTAG-3') and a reverse primer Aer613r (5'-GCTTTCACATCTAACTTATCCAAC-3'), were used for amplifying 16S rDNA of *Aeromonas* spp.

Development of real-time PCR assay for quantifying pathogenic *Aeromonas* spp. In this study, aerolysin gene was used as a biomarker of pathogenic *Aeromonas* spp. A SYBR®Green real-time PCR assay using a forward primer AHCF1 (5'-GAGAAGGTGACCACCAAGAACA-3') and a reverse primer AHCR1M (5'-ARCTGACATCGGCCTTGAACTC-3') was developed for quantifying aerolysin gene-containing *Aeromonas* spp.

Drinking water treatment plants and sampling locations.
Water samples were collected from different treatment units of seven drinking water treatment plants and one pilot plant. Two sampling events were conducted between April 2004 and August 2005. Selected plants are located in four different states: Plants 1-4 are located in Tennessee; Plant 5 is located in Georgia; Plant 6 is located in Vermont; Plant 7 and pilot plant are located in Maine. Sampling loca-tions and process flow diagrams of each plant are shown in Figure 1. Procedures for sample collection, preservation, and storage were followed as described in EPA Method 1605. The removal effectiveness of *Aeromonas* by coagulation/flocculation/sedimentation, filtration, and disinfection processes were evalu-ated by using results from molecular analyses and from EPA culture-based method 1605.

Enumeration of culturable *Aeromonas.*
Aeromonas bacteria were enumerated by using ampicillin-dextrin agar with vancomycin (ADA-V) according to EPA Method 1605.

Results

Results of this study indicated that the combination of drinking water treatment processes, conventional and unconventional, could effectively remove *Aeromonas* from treated water. While most of the *Aeromonas* in source water was removed by filtration process (Plants 1-3, except one sample collected in Plant 2), *Aeromonas* was detected in water after filtration (Plants 5-7). One major difference in the treat-ment processes among these plants was prechlorination. Since the free *Aeromonas* cells are susceptible to chlorine-based disinfectants, prechlorination of source water before other treatment processes might improve the removal of *Aeromonas* as observed in Plants 1-3. In addition, ozonation of source water as employed by the pilot plant was also effective. Unlike the traditional treatment combination (prechlor-ination/coagulation/sedimentation/rapid sand filtration used by Plants 1-3), membrane filtration alone (Plant 4) was sufficient to remove *Aeromonas* effectively. Despite the low turbidity of the source water used by Plant 6, 16S rDNA gene of *Aeromonas* was detected in samples collected after slow sand filtra-tion, suggesting that slow sand filtration alone might not be sufficient to remove *Aeromonas* effectively. The removal efficiencies for *Aeromonas* by different disinfection processes were unable to determine in this study due to the undetectable *Aeromonas* concentrations in the samples collected before and after disinfection processes in all plants surveyed.

Conclusions

The traditional treatment combination (prechlorination/coagulation/sedimentation/ rapid sand filtration) and membrane filtration alone could remove *Aeromonas* effectively. Slow sand filtration alone might not be effective for *Aeromonas* removal. The removal efficiencies for *Aeromonas* by different disinfec-tion processes were unable to determine in this study due to the undetectable *Aeromonas* concentrations in the samples collected before and after disinfection processes in all plants surveyed. No *Aeromonas* concentrations were detected by three methods when turbidities of water samples below 0.06 NTU. This value, NTU<0.06, might be used as a criteria for producing *Aeromonas*-free water.

Recommendations

The results of this study provided specific information to regulators and scientists on the effectiveness of *Aeromonas* removal/inactivation with respect to total species and virulence-gene-containing species in each drinking water treatment process. The successful development and applications of real-time PCR assays for quantifying total and pathogenic *Aereomonas* in water samples can be viewed as a model approach for other microbial contaminants listed in EPA Drinking Water Contaminant Candidate List.

Disclaimer

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Mention of specific trade names herein does not imply endorsement on the part of the USEPA, the University of Tennessee, or the Texas A&M University.

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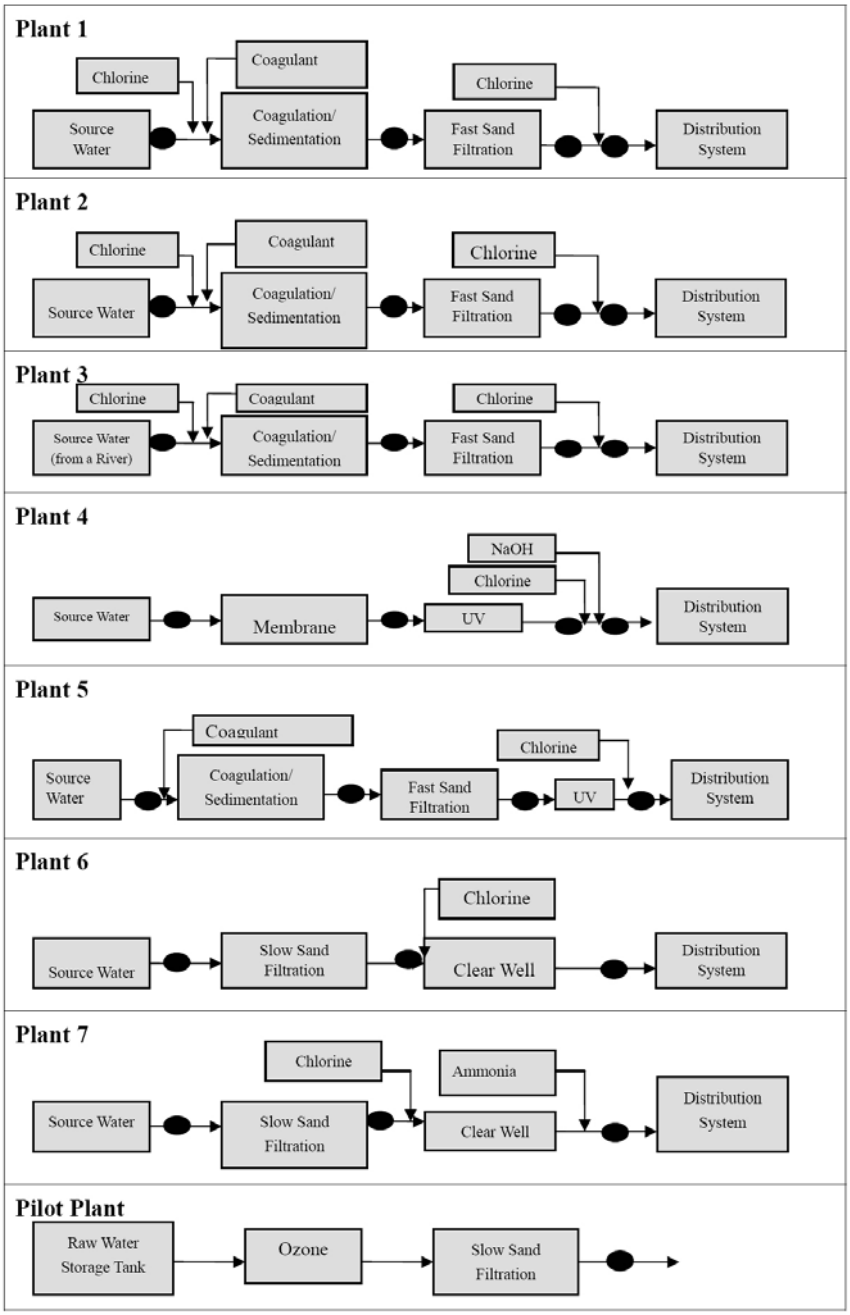

Figure 1 Process flow diagrams of drinking water treatment plants. ● indicates sampling locations.