Pathogen Removal and Inactivation in Reclamation Plants—Study Design

WateReuse Foundation

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About the WateReuse Foundation

The mission of the WateReuse Foundation is to conduct and promote applied research on the reclamation, recycling, reuse, and desalination of water. The Foundation's research advances the science of water reuse and supports communities across the United States and abroad in their efforts to create new sources of high-quality water through reclamation, recycling, reuse, and desalination while protecting public health and the environment.

The Foundation sponsors research on all aspects of water reuse, including emerging chemical contaminants, microbiological agents, treatment technologies, salinity management and desalination, public perception and acceptance, economics, and marketing. The Foundation's research informs the public of the safety of reclaimed water and provides water professionals with the tools and knowledge to meet their commitment of increasing reliability and quality.

The Foundation's funding partners include the U.S. Bureau of Reclamation, the California State Water Resources Control Board, and the Southwest Florida Water Management District. Funding is also provided by the Foundation's Subscribers, water and wastewater agencies, and other interested organizations. The Foundation also conducts research in cooperation with two water research coalitions—the Global Water Research Coalition and the Joint Water Reuse & Desalination Task Force.

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Principal Investigator

Jeannie Darbie, Ph.D., P.E.
University of California, Davis

Co-Principal Investigators

Adam W. Olivieri, Ph.D., P.E
EOA, Inc.

Chi-Chung Tang, Ph.D., P.E.
Sanitation Districts of Los Angeles County

Andrew Salveson, P.E.
Carollo Engineers

Cosponsors

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For more information, contact:
WateReuse Foundation
1199 North Fairfax St., Suite 410
Alexandria, VA 22314
703-548-0880
703-548-5085 (fax)
www.WateReuse.org/Foundation

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FOREWORD

The WateReuse Foundation, a nonprofit corporation, sponsors research that advances the science of water reclamation, recycling, reuse, and desalination. The Foundation funds projects that meet the water reuse and desalination research needs of water and wastewater agencies and the public. The goal of the Foundation's research is to ensure that water reuse and desalination projects provide high-quality water, protect public health, and improve the environment.

A Research Plan guides the Foundation's research program. Under the plan, a research agenda of high-priority topics is maintained. The agenda is developed in cooperation with the water reuse and desalination communities, including water professionals, academics, and Foundation Subscribers. The Foundation's research focuses on a broad range of water reuse research topics, including the following:

- Defining and addressing emerging contaminants;
- Public perceptions of the benefits and risks of water reuse;
- Management practices related to indirect potable reuse;
- Groundwater recharge and aquifer storage and recovery;
- Evaluating methods for managing salinity and desalination; and
- Economics and marketing of water reuse.

The Research Plan outlines the role of the Foundation's Research Advisory Committee (RAC), Project Advisory Committees (PACs), and Foundation staff. The RAC sets priorities, recommends projects for funding, and provides advice and recommendations on the Foundation's research agenda and other related efforts. PACs are convened for each project and provide technical review and oversight. The Foundation's RAC and PACs consists of experts in their fields and provide the Foundation with an independent review, which ensures the credibility of the Foundation's research results. The Foundation's Project Managers facilitate the efforts of the RAC and PACs and provide overall management of projects.

The Foundation's primary funding partners are the U.S. Bureau of Reclamation, the California State Water Resources Control Board, the Southwest Florida Water Management District, the California Department of Water Resources, Foundation Subscribers, water and wastewater agencies, and other interested organizations. The Foundation leverages its financial and intellectual capital through these partnerships and funding relationships. The Foundation is also a member of two water research coalitions: the Global Water Research Coalition and the Joint Water Reuse & Desalination Task Force (JWR&DTF).

This publication is the result of a study sponsored by the Foundation and is intended to communicate the results of this research project. The goals of this project were to design a monitoring study to evaluate the removal and inactivation of pathogens in effluent from reclamation facilities where the recycled water is intended for either public access or (indirect) potable reuse.

Ronald E. Young
President
WateReuse Foundation

G. Wade Miller
Executive Director
WateReuse Foundation

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Principal Investigators

Jeannie Darbie, Ph.D., P.E., *University of California, Davis*
Adam W. Olivieri, Dr.PH, P.E, *EOA, Inc., Oakland, CA*
Chi-Chung Tang, Ph.D., P.E., *Sanitation Districts of Los Angeles County, Whittier, CA*
Andrew Salveson, P.E., *Carollo Engineers, Walnut Creek, CA*

Project Team

Mary Kay Anuskiewics, M.S., *University of California, Davis*
Stephan Wuertz, Ph.D., *University of California, Davis*
Don M. Eisenberg, Ph.D., P.E., *EOA, Inc*.
Erica Mahar, *Carollo Engineers*

Project Advisory Committee

William Bellamy, *CH2M Hill*
Malcolm Castor, *Southwest Florida Water Management District*
Glenn Howard, *U.S. Bureau of Reclamation*
Bob Hultquist, *California Department of Health Services*
Rich Mills, *California State Water Resources Control Board*
Terri Slifko, *Orange County Utilities Laboratory*
George Tchobanoglous, *University of California, Davis*
William A. Yanko, *Environmental Microbiology Consultant*

Workshop Participants

Anthony Andrade, *Southwest Florida Water Management District*
Takashi Asano, *University of California, Davis*
Michael Baker, *California State Water Resources Control Board*
William Blomquist, *Indiana University–Purdue University, Indianapolis*
James Crook, *Environmental Consultant*
Kathy Cupps, *Washington State Department of Ecology*
Richard Danielson, *BioVir Laboratories, Inc.*
Joseph Eisenberg, *University of California, Berkeley*
Debra Huffman, *University of South Florida*
Bob Hultquist, *California Department of Health Services*
Paul Kinshella, *City of Phoenix Water Services Department*
Mark LeChevallier, *American Water*

Jeff Mosher, *WateReuse Association/WateReuse Foundation*
Margie Nellor, *Sanitation Districts of Los Angeles County*
Dave Requa, *Dublin San Ramon Services District*
Alan Rimer, *Black & Veatch*
Joan Rose, *Michigan State University*
Rick Sakaji, *California Department of Health Services*
Terri Slifko, *Orange County Utilities Laboratory*
Robert Spear, *University of California, Berkeley*
Jeff Stone, *California Department of Health Services*
Chi-Chung Tang, *Sanitation Districts of Los Angeles County*
George Tchobanoglous, *University of California, Davis*
Rhodes Trussell, *Trussell Technologies, Inc.*
Mike Wehner, *Orange County Water District*
William A. Yanko, *Environmental Microbiology Consultant*
David York, *Florida Department of Environmental Protection*

EXECUTIVE SUMMARY

PROJECT BACKGROUND AND OBJECTIVES

The WateReuse Foundation identified a need for research to investigate the removal and inactivation of pathogens in effluent from reclamation facilities where the recycled water is intended for either public access or (indirect) potable reuse. The purpose of this work is to design a monitoring study.

The monitoring study that will be carried out based on this Study Plan has two purposes. First, it is intended to collect the appropriate data to characterize the pathogen removal and inactivation performance of a wastewater treatment and reclamation facility. Second, the data analysis is intended to characterize the risk associated with waterborne pathogens in this treated effluent in a manner that can be used for comparison with water treated by different unit processes or different combinations of unit processes.

The study that is described in this Study Design Report will have three components.

1) *Laboratory or bench-scale studies* are included to investigate the effectiveness of chlorine disinfection on specific pathogens and/or on selected indicator organisms that are shown to respond similarly to such pathogens. The most important area for bench-scale investigation is the kinetics of inactivation of different types of viruses by free chlorine and chloramines. An understanding of the impact of organics on disinfection by chlorine is also desired.
2) *Pilot-scale studies* are necessary to provide a realistic simulation of variability in treatment process performance and interaction between processes in series, at a scale that is small enough for spiking studies to remain feasible.
3) *Full-scale studies* are necessary to verify that performance is not significantly worse and variability is not significantly higher, when the processes studied at the pilot scale are constructed and operated at the much larger capacities that are typical of actual wastewater treatment and reuse facilities.

The unit processes to be investigated include secondary treatment followed by direct filtration and chlorine disinfection with 90-min modal contact time and CT[1] of approximately 450 mg-min/L. Pilot- and bench-scale studies will also include testing at shorter contact times and lower chlorine doses. Indigenous microorganisms will be monitored at selected points in the treatment process with emphasis on enteric viruses and bacteriophage indicator organisms. Spiking studies with at least one type of enteric virus will also be implemented at the bench scale and, if feasible, also at the pilot scale. Monitoring results will be analyzed by time–series and consequence frequency data analysis. Implementation of the study design will result in

[1] CT is defined as the concentration of total chlorine residual (free and combined) measured in milligrams per liter times the contact time in minutes. California's Water Recycling Criteria require a minimum CT of 450 mg-min/L and a minimum 90-min modal contact time for chlorine disinfection.

quantitative, transparent, and reproducible characterization of the pathogen removal and/or inactivation performance associated with such facilities. The study design may also serve as a sample methodology for future studies characterizing and comparing the performance of other disinfection technologies, including UV radiation and other potential alternatives to chlorine disinfection.

STUDY DESIGN

1 PURPOSE OF STUDY DESIGN

The purpose of this work is to design a monitoring study to evaluate the removal and inactivation of pathogens in effluent from reclamation facilities where the recycled water is intended for either public access or (indirect) potable reuse.

2 PURPOSE OF MONITORING STUDY

The monitoring study itself has two purposes. First, it is intended to collect the appropriate data to characterize the pathogen removal and inactivation performance of a wastewater treatment and reclamation facility. Second, the data analysis is intended to characterize the risk associated with waterborne pathogens in this treated effluent in a manner that can be used for comparison with water treated by different unit processes or different combinations of unit processes.

The facility that is the subject of the initial study design will consist of a series of treatment processes that are commonly accepted to produce reclaimed water of a quality such that its use in the designated manner does not represent an unacceptable risk to the health of the potentially exposed population. The unit processes to be investigated include secondary treatment followed by direct filtration and chlorine disinfection with 90-min modal contact time and a CT[1] of approximately 450 mg-min/L. Pilot studies will also include testing at shorter contact times and lower chlorine doses, resulting in a CT of approximately 15 mg-min/L. Bench-scale studies will also include samples collected at interim time intervals to characterize at least one CT value that is between these two extremes. Implementation of the study design will result in quantitative, transparent, and reproducible characterization of the pathogen removal and/or inactivation performance associated with such facilities.

In practical terms, it would appear that the level of treatment required for unrestricted irrigation reuse under California's Water Recycling Criteria represents the high and relatively stringent end of the range of accepted treatment technology that has been shown by many years of application to be adequately protective of human health. The lower end of the generally accepted treatment range appears to be represented by direct filtration of secondary effluent followed by chlorine with a CT as low as 15 mg-min/L, which is the minimum CT allowed for unrestricted irrigation reuse under State of Florida water reuse regulations (subject to also meeting specified fecal coliform standards and other design requirements—Florida Administrative Code Chapter 62-600, Section 440(5)(b)). These conditions can be used in identifying a representative "baseline" range of treatment processes for the pathogen inactivation study.

UV disinfection is the other generally accepted disinfection process for these uses. Removal of pathogens in a tertiary treatment plant utilizing UV disinfection could be measured and

[1] CT is defined as the concentration of total chlorine residual (free and combined) measured in milligrams per liter times the contact time in minutes. California's Water Recycling Criteria require a minimum CT of 450 mg-min/L and a minimum 90-min modal contact time for chlorine disinfection.

characterized by an investigation almost identical to the one described here. It is anticipated that such a study will be carried out at some point, but this investigation is limited to chlorine disinfection to simplify and focus the study design and to maximize the number of data points for data analysis.

The above characterization serves as a baseline against which to compare similar assessments from a new unit process or different process combinations. The study design includes measurement of both removal and inactivation, as appropriate and feasible, for a range of pathogens and/or indicators representative of viruses, bacterial pathogens, and protozoa. The data analysis will incorporate and characterize variability of performance over time, which allows a comparison of processes that includes performance reliability as well as effectiveness of treatment.

To compare public health protection provided by different treatment processes or combinations of processes, it is necessary to have directly comparable measurements or reliable estimates of the effectiveness and reliability of treatment in each system. For pathogens in wastewater, it might at first appear necessary to identify all potential pathogens in the wastewater, characterize their occurrence in wastewater, and, for each process, characterize the effectiveness for the range of possibilities. However, there is a significant history of safe water reuse and generally accepted processes and process combinations for achieving acceptable treatment. Therefore, if it is possible to establish a range of baseline performance for such processes by using pathogenic microorganisms or indicator organisms that are determined to be representative and ubiquitous, it will then be possible to compare various new or alternative systems and combinations to those that have already been shown to protect public health.

3 ELEMENTS OF THE PATHOGEN INACTIVATION AND REMOVAL STUDY

The proposed study will have three components.

1) *Laboratory or bench-scale studies* are included to investigate the effectiveness of chlorine disinfection on specific pathogens and/or on selected indicator organisms that are shown to respond similarly to such pathogens. The most important area for bench-scale investigation is the kinetics of inactivation of different types of viruses by free chlorine and chloramines.[2] An understanding of the impact of organics on disinfection by chlorine is also desired.
2) *Pilot-scale studies* are necessary to provide a realistic simulation of variability in treatment process performance and interaction between processes in series, at a scale that is small enough for spiking studies to remain feasible.
3) *Full-scale studies* are necessary to verify that performance is not significantly worse and that variability is not significantly higher when the processes studied at the pilot scale are constructed and operated at the much larger capacities that are typical of actual wastewater treatment and reuse facilities.

[2] Bench-scale testing in this study is limited to a few focused tests of the effectiveness of chlorine and chloramines on several types of viruses in order to facilitate interpretation of the full-scale and pilot-scale testing conditions and results. Similar tests on bacteria and on protozoan cysts are not included because chlorine disinfection of those pathogens is better characterized. Also, for protozoa it is generally accepted that filtration rather than chlorination is the critical treatment process for controlling risk.

a. Bench-Scale Studies

The purpose of the bench-scale studies is to characterize the disinfection effectiveness of free and combined chlorine for representative virus pathogens and indicator organisms. In the context of the current investigation, the objective is to identify "worst-case" combinations of disinfection conditions (e.g., free chlorine and chloramine species concentrations and contact times) and organisms that might reasonably be encountered in the treatment system that is selected as representative of currently accepted (and commonly used) treatment technology. A series of spiking studies will be conducted in which the inactivation rate of selected organisms will be measured versus time for a range of concentrations of free chlorine and chloramines.

Application of the CT concept based on total chlorine residual does not differentiate between the effectiveness of different forms of chlorine used as a disinfectant. Hypochlorous acid (commonly referred to as "chlorine") reacts with ammonia to form monochloramine, dichloramine, and trichloramine in the well-understood breakpoint reactions. As the number of chlorine atoms attached to the amine or ammonia molecule increases, the effectiveness of the oxidant decreases. These reactions have been well-characterized, and drinking water disinfection requirements differentiate between free chlorine and combined chlorine. However, a similar approach has not been used in establishing disinfection process requirements for wastewater reclamation. Wastewater contains high concentrations of organics that react with chlorine to produce organochloramines, which are weaker oxidants than the chloramines. Further, the addition of chlorine to wastewater produces a myriad of reactions resulting in a variety of oxidants, all of which act as disinfectants to various degrees. Ammonia concentration, organic nitrogen concentration, chlorine dose, and distribution of combined chlorine forms also affect the efficacy of disinfection (Soller et al., 2004).

A series of spiking studies will be conducted using the filter effluent from one full-scale tertiary treatment facility. In these bench-scale tests, the inactivation rate of at least one selected type of virus will be measured versus time for a range of concentrations of free chlorine and chloramines. These results will be compared against the results of an equivalent set of tests conducted with indigenous coliphage and seeded MS2 coliphage, the most commonly used virus indicator organism. The bench-scale studies will include spiking with an enteric virus (poliovirus, for example, if available) and comparison with coliphage under similar test conditions. This bench-scale comparison will be useful in interpreting the results of pilot- and full-scale measurements for which poliovirus spiking may no longer be allowed and no practical substitute has yet been identified. If poliovirus can be used in the pilot-scale work or a poliovirus substitute is identified and if the necessary additional budget is also available, then the spiking studies will be carried out with such an organism as well as with MS2 for both bench- and pilot-scale testing.

The methods to be used for the spiking study will be similar to those used in microbial challenge studies carried out at the City of San Diego's Aqua 2000 Research Center (Soller et al., 1997). Similar to the chlorination studies described in that report, bench-scale batch tests will be carried out by using filtered (5-µm-pore-size nucleopore filter or other filter to be determined), secondary effluent. For combined chlorine experiments, ammonia will be added prior to the addition of the chlorine dose sufficient to assure that the chlorine-to-ammonia ratio will be greater than 3.0 by weight. Chlorine doses will be sufficient to consistently achieve a chlorine residual of 5 mg/L after 90 min of contact time.

To investigate the impact of organics, the BOD_5 and TOC and humic substances will be measured, and the organics will be concentrated and fractionated such that the amount and proportion of hydrophilic and hydrophobic organics can be estimated for the subject-treated wastewater that is used in each set of bench-scale tests. Detailed protocols will be finalized prior to implementation.

Organisms to be investigated will include at least one type of virus that is related to pathogens of public health concern and are found in wastewater. One set of tests will be performed where MS2 coliphage will be spiked and monitored. The second set of tests will be performed on at least one of the following organisms: attenuated poliovirus (if available), echovirus, coxsackievirus, or an enteric virus such as adenovirus. For at least these two types of organism (e.g., MS2 and a selected enteric virus), inactivation will be tested for a minimum of three chlorine doses and three chloramine doses at different contact times. Three replicate tests will be conducted for each combination.

b. Post-Secondary Pilot Filtration and Disinfection Studies

The purpose of the pilot studies is to establish a baseline for tertiary treatment with chlorine disinfection, demonstrating and quantifying the performance of each treatment unit process, which in combination are generally accepted to provide adequate protection of public health. A key objective in utilizing a pilot-scale facility is the potential to seed large concentrations of organisms at selected points in the process train and measure detectable levels of those organisms in the treatment unit effluent.

The pilot plant will operate 24 h per day throughout the 50-week study period. It will be equipped to accept, regulate, and meter a continuous flow of secondary effluent from the full-scale water reuse plant that is selected for use in this study. The pilot system will provide tertiary treatment with unit processes similar to those of the full-scale plant, consisting of coagulant injection with flash mixing, dual-medium filtration, and chlorination followed by two chlorine contact basins designed to be operated in parallel, one with a modal contact time of 90 min and a chlorine dose sufficient to result in a CT of 450 mg-min/L, the other with a contact time of 15 min and a CT of 15 mg-min/L. To the extent that it is feasible and practical, the pilot study treatment system that includes chlorination with the longer contact time will be operated with flow patterns, mean cell residence time, and disinfection contact time that are similar to the full-scale facility that is chosen as the subject for the investigation.

Details of the monitoring program for the pilot studies are described below. Also see Table 1 below.

The objectives are to

1. characterize the influent flow and water quality to the filters; and
2. characterize the performance of the filters and the disinfection units.

The following approach is proposed for the pilot testing:

- Small-scale filtration and disinfection systems will be mounted on transportable units and will be deployed to the selected full-scale wastewater reclamation plant monitoring site for 50 weeks.
- Fully operational pilot-scale granular-medium filtration technology will process up to 100 gpm (total) of clarified secondary effluent.
- Two parallel systems of chlorine disinfection reactors will disinfect the filtered effluent.
- For chlorine disinfection, two different CTs will be tested, including a CT of 450 mg-min/L with 90 min of modal contact time and a CT of 15 mg-min/L with 15 min of contact time.
- Filter flux and chemical usage rates of the filtration–disinfection testing will include filter operation and disinfection dosages (for the higher-CT disinfection unit) similar to the filtration–disinfection strategies at the full-scale treatment plant.
- Monitoring of physical/chemical and microbiological sampling will be concurrent whenever feasible.
- Sample locations will include secondary effluent, filter effluent, and effluent from each of the two disinfection units; this selection constitutes four samples per sampling event.
- Samples will be taken twice per week for 50 weeks and analyzed for the constituents as specified below.

i. Physical/Chemical Monitoring in Pilot Studies

Table 1. Physical/chemical parameters to measure in the pilot studies

Parameter	Secondary effluent	Filter effluent (granular media)	Chlorination effluent (one from each disinfection unit)
BOD_5	X		
COD	X		
TOC	X		
NH_3	X	X	X[d]
NO_3	X	X	X[d]
TDS	X		
Turbidity[a]	X	X	
TSS[b]	X	X	
PSD[e]	X	X	
Zeta potential[c]	X		
UVT (filtered and unfiltered)	X	X	
Humic substances	X	X	
Hydrophilic and hydrophobic organics by fractionation	X	X	
Flow and variability	X	X	
Temp	X		

[a]Continuous logging turbidity meters will be installed pre- and post-filters.

[b]Continuous logging TSS meters will be installed pre- and post-granular medium filter.

[c]PSD and zeta potential meters will be utilized, as needed, as part of the pilot-scale filtration effort to characterize particle size distribution and particle charge.

[d]Parallel chlorine contact basins will be operated at different chlorine dose and contact time. Therefore, two postchlorination samples will be collected for each test.

ii. Microbiological Monitoring in Pilot Studies

Both indigenous and spiked microorganisms will be monitored in the pilot filtration and disinfection studies. Microbiological monitoring will include organisms similar to those measured in a recent WERF study (Rose et al., 2004). In that study, microbial indicators (coliforms, enterococci, and coliphages) as well as bacterial (*Clostridium*), viral (culturable enteric viruses), and protozoan (*Cryptosporidium* and *Giardia*) pathogens were monitored by using classical microbiological assays in six wastewater reclamation plants at the full-scale level. Samples were taken at least four times from the influent, secondary effluent, filtered effluent, and disinfected reclaimed effluent from each plant. The authors concluded that pathogens were always present in the influent and that the percentage removal of target organisms varied widely among the six plants. Microbial techniques will be utilized that yield numerical values for concentration or most probable number of organisms. Where available, methods will be consistent with the standard methods (American Public Health Association, 2005).

Potential alternative indicators for pathogen removal monitoring were discussed extensively at a recent WERF workshop (in San Antonio, Texas [December 2003]). A number of alternative indicators were suggested as having the potential to represent various categories of pathogens, but the general consensus was that the indicators proposed herein are currently useful (to various degrees) and widely used and readily available. Other alternative indicators would require more research before widespread implementation is feasible and useful. Such research is not the topic of this study, which is focused on characterizing treatment plant pathogen removal and inactivation performance with the best indicators that are currently proven and widely used.

Indigenous monitoring. Indigenous microorganisms to be analyzed twice per week at each of the four pilot sample locations include:

- Total and fecal coliform;
- *Escherichia coli;*
- Enterococci;
- *Clostridum perfringens;*
- Coliphage (indigenous somatic and MS2);
- Enteric culturable viruses;
- Adenovirus;
- *Cryptosporidium* and viable *Cryptosporidium;*
- *Giardia; and*
- Heterotrophic plate count bacteria.

Spiking studies. Spiking studies will be performed once per month of testing (a total of 12 tests). Prior to the start of each test, MS2 will be added immediately upstream of the pilot filtration facilities and, as necessary, immediately upstream of the disinfection facilities. Samples will be collected for MS2 analysis at each of the four pilot sample locations as described above. Samples will be collected at each of the four sample locations 30 min after addition of MS2 and also at the three downstream sample points (postfilter and two postchlorination) at 30-min intervals for an additional 2 h thereafter.

Measurement of the microorganisms listed above will be sufficient to characterize and compare the effectiveness of treatment processes and process trains. However, direct measurement of the prevalence of specific pathogenic organisms in the wastewater and/or

treated water would improve the capability for interpretation of significance of these performance results. Little information is currently available on actual measured prevalence of pathogens in wastewater and on their removal during passage through the treatment train. If funding were available, spiking studies could also include:

- Attenuated poliovirus (if available);
- Echovirus and coxsackievirus;
- Adenovirus;
- Enteric viruses
- *E. coli* (*Aeromonas* and *C. perfringens* have also been suggested);
- *Giardia; and*
- *Cryptosporidium.*

c. Full-Scale Monitoring at Water Reclamation Plant

The purpose of the full-scale monitoring is to verify that the effectiveness and variability of pathogen removal and inactivation measured at the pilot scale are, in fact, representative of full-scale performance. A full-scale wastewater treatment and reclamation plant will be identified and selected to be used as a location and source of partially treated wastewater for the pilot testing and also for full-scale verification monitoring. The pilot filtration and disinfection testing, described above, will be conducted at the full-scale plant selected.

The facility selected shall be representative of "accepted technology" relative to processes included, process design, and sizing. It will also be representative of typical wastewater sources and characteristics and of process operation. Specifically, the capacity of the plant will be at least 2 million gallons per day. The selected plant will have treatment processes that consist of activated sludge (with or without) nitrification, followed by secondary sedimentation that produces an effluent with consistently less than 10 nephelometric turbidity units (NTU) of turbidity, followed by coagulant addition and dual-medium filtration at not more than 5 gpm/ft^2 of filter surface, which consistently produces an effluent with not more than 2 NTU of turbidity, followed in turn by chlorine disinfection in a contact basin with a modal contact time of approximately (but not less than) 90 min and a CT of approximately (but not less than) 450.

The following approach is proposed for the monitoring of full-scale wastewater reclamation plants:

- Monitoring of the full-scale facility will occur twice per week for 50 weeks.
- At a minimum, five locations at each plant will be sampled, including treatment plant influent, primary effluent, secondary clarified effluent, filter effluent, and disinfection effluent.
- Mechanical reliability will be tracked and analyzed for the full-scale plant. For this purpose, process units and components will be defined and mechanical failures and availability will be documented throughout the study.
- Physical/chemical and microbiological parameters measured at the full-scale plant will be identical to the pilot-scale investigation, except that zeta potentials will not be measured at the full scale and that no spiking studies or special testing will be conducted at the full

scale. A summary of the locations at which the samples for physical/chemical analysis will be collected is presented in Table 2 below.

- Data analysis for the full- and pilot-scale operations will also be identical, and probability plots and whisker plots for indicators and conventional pollutants and/or chemical constituents will be overlaid for comparison of treatment performance distributions. Based on comparisons of design and loading information for the pilot- and full-scale facilities, combined with the treatment and performance results, conclusions will be made about the applicability of pilot-scale test results to full-scale facilities and operations.

Table 2. Physical/chemical parameters to measure in the full-scale studies

Parameter	Plant influent	Primary effluent	Secondary effluent	Filter effluent	Chlorination effluent
BOD_5	X		X		
COD	X		X		
TOC	X		X		
NH_3	X		X	X	
NO_3	X		X	X	
TDS	X		X		
Turbidity[a]			X	X	
TSS[b]			X	X	
PSD[c]			X	X	
UVT (filtered and unfiltered)			X	X	
Humic substances	X	X	X	X	X
Hydrophilic and hydrophobic organics by fractionation	X	X	X	X	X
Flow and variability	X	X	X	X	X

[a]Continuous logging turbidity meters will be installed pre- and postfilters.

[b]Continuous logging TSS meters will be installed pre- and post-granular medium filter.

[c]A PSD meter will be utilized, as needed, as part of the full-scale filtration effort to characterize particle size distribution.

4. DATA INTERPRETATION

a. Performance Distributions

Characterization of treatment effectiveness requires data on the occurrence and amount of the constituent of interest in the influent and effluent of the treatment process or series of processes. To be meaningful, a statistically significant number of measurements must be

made of the influent and effluent over time. If the influent concentration remains constant and if measurable concentrations are present in the effluent, then the effluent variability can be used to characterize the variability of process effectiveness.

The pathogen removal performance, as well as performance related to selected indicators and relevant physical and chemical constituents, will be evaluated by summarizing observed effluent quality by using the basic statistics associated with frequency analysis, i.e., mean values, standard deviations, etc.

Simple time–series plots will be constructed first, in order to identify temporal trends or mechanical and maintenance issues that may require separation and grouping of sets of data that arise from the same unit process. A sample time–series plot is shown in Figure 1.

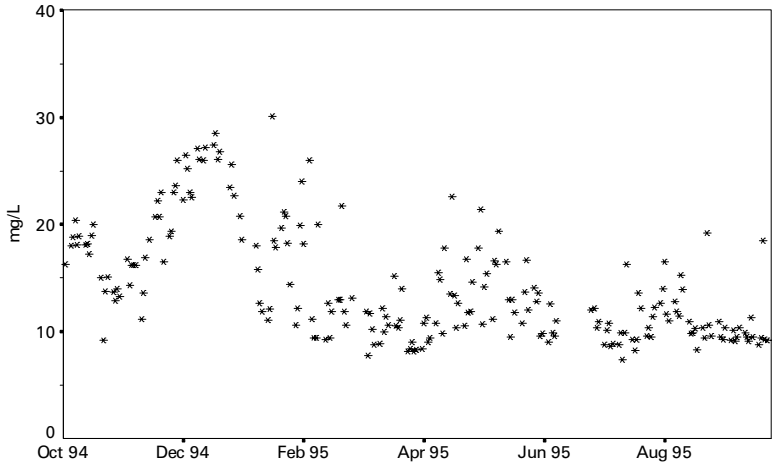


Figure 1. Time–Series Plot for TOC in Secondary Effluent

The performance of unit processes and of the overall system will next be characterized by estimating the cumulative probability distributions associated with enteric virus and coliform measured at key treatment units through both the pilot- and full-scale facility and for selected test microorganisms through a series of spiking studies. These probability distributions will explicitly characterize the variability of treatment performance and allow estimation of the probability that pathogen removal goals will be met or exceeded. To carry out such an analysis, effluent data must be fit to a distribution by using one of several techniques (Ott, 1995). In many cases, constituents in effluent from a treatment facility may be well-characterized by using a lognormal distribution (*Technical Support,* 1991).

For example, Figure 2 presents COD concentration data observed in raw wastewater, secondary effluent, tertiary effluent, and reverse osmosis effluent from a pilot-scale test advanced wastewater treatment facility operated for approximately a one-year test period. Plots such as Figure 2 are generated by ranking observed data from lowest to highest,

computing the proportion of samples less than a given sample using Blom's transformation or equivalent (SPSS, 1993), and plotting that proportion versus the observed concentration.

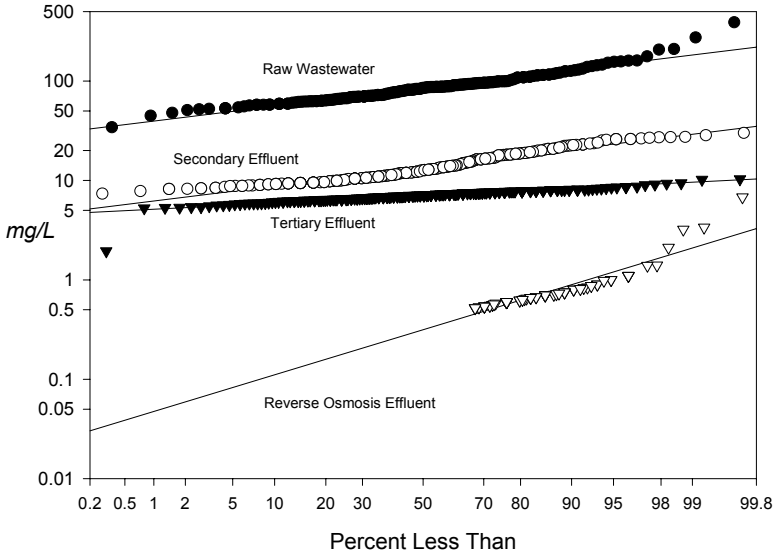


Figure 2. Lognormal Probability Plot for TOC, October 1994–September 1995

The reverse osmosis effluent data presented in Figure 2 demonstrate the use of probit analysis to estimate the distribution of treatment plant performance when a large percentage of data is reported below detectable limits. It should be noted that use of this procedure requires a minimum of detected data and that those data are considered highly reliable. The benefit of using this type of procedure is that summary statistics such as (geometric) mean values and (geometric) standard deviations may be estimated from the plots, even though a large proportion of the data may not have been quantifiable.

In this initial phase of data evaluation, the data will also be reviewed to identify any potential subsets of data that may represent significantly different operating conditions such as unusually high influent flow rates or significantly different influent physical or chemical characteristics. If such conditions are identified, those data subsets will be evaluated separately and results will be compared. Such evaluation of subsets will be in addition to the analysis that is described for the entire aggregated data set, which will remain useful as a realistic characterization of the overall treatment effectiveness and variability that are measured, given the actual variability of the influent wastewater stream.

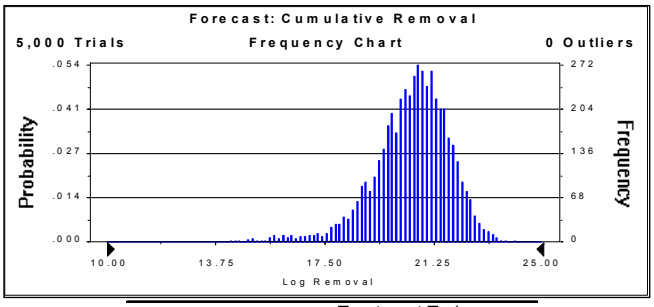
b. Consequence Frequency Assessment

Consequence frequency assessment methodology will be used to characterize the removal of pathogens through the selected pilot- and full-scale treatment system. A detailed description of using consequence frequency assessment to evaluate the performance of an advanced

wastewater treatment facility is summarized in a number of references (National Research Council, 1998; Olivieri et al., 1999; Soller et al., 1997).

The concentration of indicator or pathogen organisms spiked at each stage of treatment may be described mathematically as a conditional probability density function. Formally, the probability distribution of the plant effluent may be expressed as a multiple integral (one integral for each unit process) (Stuart and Ord, 1987). However, the resulting integral is difficult or impossible to evaluate. As a practical alternative, a Monte Carlo simulation will be applied (Finkel, 1990; Haas et al., 1993; Burmaster and Anderson, 1994), fitting distributions to the removal of a particular constituent across each treatment unit, sampling each distribution repeatedly, and computing the final concentration for each set of random samples. Using this procedure, it is possible to quantitatively describe plant performance in a probabilistic manner that explicitly acknowledges uncertainty and reflects the variability of the underlying data.

To estimate process train performance, probability distributions of removal through each of the unit processes will be identified by using a maximum likelihood estimate approach (Ott, 1995). The identified probability distributions are then used in a Monte Carlo simulation model to estimate the distribution of removals and associated variability across the integrated treatment system (Soller et al., 1999). An example based on the results from a previous investigation is presented in Figure 3.



		Treatment Train Removal Statistics (log removal)
Trials		5000
Mean		20.5
Median		20.6
Standard Deviation		1.3
Variance		1.8
Skewness		-1.0
Kurtosis		5.1
Range Minimum		13.7
Range Maximum		24.0
Percentile	2.5%	17.2
	5.0%	18.2
	50.0%	20.6
	95.0%	22.3
	97.5%	22.6

Figure 3. Summary of Consequence Frequency Assessment MS2 Removal through an Advanced Wastewater Treatment Plant

Inspection of Figure 3 reveals that this method results in a description of plant performance in terms of both effectiveness and expected variability, and it is evident that this description can be used as a common basis to compare the performance of different process units or process trains, as well as to characterize the "baseline" or currently accepted level of pathogen removal performance. The consequence frequency assessment methodology summarized above has been used previously to estimate the removal of MS2 bacteriophage through an advanced wastewater treatment facility (Soller et al., 1999). This type of methodology was also endorsed by the National Research Council in a 1998 publication reporting on the viability of augmenting drinking water supplies with reclaimed water (National Research Council, 1998).

c. MRA

The results of the consequence frequency assessment could be used to develop a microbial risk assessment (MRA) for the treatment system that is the subject of the pilot-scale and full-scale investigations. The objective would be to provide a risk-level "baseline" against which future tests of systems containing proposed or existing alternative treatment processes could be directly compared. This would ensure a common risk assessment approach for any alternative treatment configurations that may be implemented or evaluated. For example, agencies are considering the use of UV light in lieu of chlorine for disinfection. MRA could be used to ensure that the regulations are similar when considering the standard technology as compared to a proposed alternative.

However, it does not appear to be feasible to use MRA in the short term for the current study, nor does it appear necessary. There is difficulty in applying MRA at this time directly because there is a lack of understanding of the relationship (correlation) between the indicators and water quality parameters that are to be monitored and the pathogens of public health concern. Unfortunately, such a relationship is, at least currently, quantitatively tenuous (Soller et al., 2004). MRA may be used in the future to determine if currently accepted and proposed alternative treatment methods provide a common level of public health protection, but doing so would require significant effort including new research (some of which is being planned by WERF [Workshop, 2003]) to characterize and document the relationship between the indicators and water quality parameters and the pathogens of concern.

In previous work where MRA was used to inform regulatory decision-making, a combination of conservative and realistic assumptions was employed so that the results of the assessment would protect health yet be practical (Soller et al., 2003; Soller et al., 2004). A model enteric virus was employed to characterize, conservatively and representatively, the risk to public health that may be associated with exposure to the epidemiologically important enteric viruses via recreational activities in a river. For the purposes of that assessment, it was assumed that the model virus possessed the clinical features of rotavirus, as rotavirus is the most infectious virus for which human dose–response data are available, and environmental features of male-specific (MS) coliphage, as MS coliphages are of size, shape, and environmental persistence similar to those of several pathogenic viruses of public health concern and have been reported to exhibit similar or greater resistance to conventional wastewater treatment as viruses of public health concern. Under these assumptions, it may be feasible to use monitoring from indicator organisms to express reclamation plant performance in terms of relative public health risk. However, in the context of this study, that approach would result in additional effort that may not be necessary and could increase the uncertainty of the results.

Fortunately, the comparison can also be based directly on performance as described by the consequence frequency assessment results. The recent WERF pathogen removal study (Rose et al., 2004) found that the "reclaimed water as monitored in (that) study ... is not pathogen free and exposure of the public to these waters carries some risk, albeit this level may be very low and quite acceptable to most populations." The implication of this finding is that there will be some level of detectable pathogens and/or indicators in the effluent that will be measured directly. If the treatment performance for the full range of microorganisms proposed to be measured in this study is characterized rigorously by using consequence frequency assessment, this characterization based on performance alone will be directly useful for comparison with the performance of other existing or proposed systems.

5 DISCUSSION

Reliability evaluation as described in the data analysis section above is an important tool for comparing processes or combinations of processes. The means for determining equivalent treatment unit or process performance for new technologies with limited pilot- and small-scale performance data are limited. Estimating treatment reliability provides one means for evaluating and comparing treatment trains comprised of accepted conventional technology to proposed alternative trains containing innovative technology. The data analysis described above allows an evaluation to determine if the quality of the product from two processes or process trains would be the same. If one examines the range of quality produced by the two processes or process trains, either they should match perfectly or the process train containing the alternative technology should show less variability in product quality.

As long as the effectiveness is at least equivalent and the variability in product quality for the alternative technology is similar to the variability of the "accepted" or conventional train, the process train containing the alternative may be considered equivalent. The monitoring and data analysis described above will provide the necessary quantitative description of the effectiveness and variability of the accepted conventional technology. These results and methods will then be available for comparison in the testing of proposed alternative technology.

6 COST ESTIMATE

Based upon the proposed monitoring study plant outlined in Section 3 of this report, an estimated project cost was developed and is presented in this section. As described in Section 3, the monitoring study will include a series of bench-scale tests of chlorine and chloramine inactivation of virus and pilot-scale testing of dual-granular-medium filtration and disinfection by chlorination or chloramination at a single water reclamation facility. The study will also include a full-scale monitoring program at the same location that is selected to provide secondary effluent to the pilot facility.

a. Bench-Scale Testing

i. Microbial Analysis

Following the study plan presented in Section 3 of this report, nine free-chlorine disinfection tests and nine chloramine disinfection tests will be conducted for each of two spiked organisms and for indigenous coliphage. One set of tests will be conducted by using MS2 coliphage. The second set will be conducted on a second virus for the purpose of comparison with the results from the coliphage tests. For the purposes of this estimate, it is assumed that

the second organism will be adenovirus. The occurrence of indigenous coliphage through chlorination and chloramination will be monitored in a third set of tests. Additional organisms could be tested as described in Section 3 of this report, but the cost associated with monitoring additional organisms is not included here.

For each disinfectant, three different doses will be evaluated and each dose will be replicated three times (for a total of nine tests for each disinfectant). For each test, four samples will be collected for determining the concentration of the target organism. One sample will be collected immediately following the addition of the organism to the filtered secondary effluent sample. Three additional samples will be collected at various times after the disinfectant has been added to determine the impact of different contact times on disinfection efficacy.

Based on this study plan, a total of 72 samples (nine tests per disinfectant times four samples per test times two disinfectants) will be analyzed for each organism (in this case, MS2 coliphage, adenovirus, and indigenous coliphage). MS2 analysis costs approximately $110 per sample, while adenovirus analysis costs approximately $715 per sample. The cost for indigenous coliphage analysis is approximately $28 per sample. These costs, in addition to stock solution costs for MS2 and adenovirus as well as shipping costs, total approximately $60,000.

ii. Physical/Chemical Analysis

In order to characterize the water used in the bench-scale study so it can be compared with the water used in the pilot- and full-scale studies, BOD_5, TOC, humic substances, and hydrophilic and hydrophobic organics will be measured for the filtered secondary effluent provided for the bench-scale study. These parameters will be measured a total of three times during the bench-scale study at a total cost of approximately $1,500.

b. Pilot- and Full-Scale Monitoring

For both the full-scale monitoring program and the pilot-scale testing program, the same physical/chemical and microbial constituents will be measured with two exceptions as follows:

- Zeta potential measurements will not be collected as a part of the full-scale monitoring program.
- No spiking studies with MS2 coliphage will occur during the full-scale monitoring.

i. Post-Secondary Pilot Filtration and Disinfection Studies

Microbial analyses. Following the study plan presented in Section 3 of this report, 100 sampling events will be conducted at both the full-scale and the pilot-scale facilities (twice per week for 50 weeks). For each, four samples will be collected for each of the indigenous organisms detailed in Section 3. This protocol assumes one secondary effluent sample, one filter effluent sample, and two postdisinfection samples (one for each of two disinfection pilot systems).

In addition to monitoring indigenous organisms, MS2 coliphage will be spiked ahead of the filters 12 times during the 12-month test period. Four samples for each test will be collected for the spiked MS2 as well. It is assumed that 1.5 L of MS2 stock solution will be required for each test at approximately $550/L.

The microbiological costs are based upon estimates from various commercial and utility laboratories. Presented in Table 3 are the unit costs for each organism as well as the cost reference, analysis methods, and the projected pilot-scale microbiological analysis cost. Note that no viable *Giardia* test costs were included in this estimate.

Based upon the number of samples collected for each monitored organisms, the cost associated with the microbiological analysis portion of the pilot testing is approximately $1.2 million. These costs include the cost of shipping samples to laboratories for analysis.

Physical/chemical analyses. For each of the 100 tests performed at each water reclamation plant, 13 physical/chemical constituents will be measured according to the schedule outlined in Table 1. The physical/chemical analysis costs are based upon estimates from various commercial and utility laboratories.

The estimated costs associated with the pilot-scale physical/chemical parameter analysis are presented in Table 4. These costs are based upon the number of locations for physical/chemical sampling detailed in Section 3. Based on these assumptions, the pilot-scale physical/chemical monitoring plan will cost a total of approximately $100,000. These costs include the cost of shipping samples to laboratories for analysis.

Pilot trailer. The pilot testing equipment will be trailer-mounted for easy transportation to the water reclamation facility and is intended to provide detailed pathogen removal and disinfection data for a range of secondary effluent quality and for a range of filtration rates and disinfection doses.

Our goal was to develop a fully operational and transportable pilot-scale filtration and chlorination disinfection treatment system. Table 5 details the items included in the treatment "trailer."

The estimated cost for the trailer does not include trailer-shipping costs. A gross estimate of shipping costs is approximately $20,000 for transportation to a single location. Further, the above costs do not include electrical and water costs, which are assumed to be covered by the host water reclamation plant. Our team did encounter a number of equipment vendors who would likely supply the pilot equipment for minimal to no cost. Further, it is assumed that most of the project teams that would propose on this project already have the essential bench-top monitoring and UV equipment specified here that could be offered at little or no cost to the project. However, the costs developed here do not assume such generosity.

Table 3. Summary of pilot-scale microbiological analysis costs

Organism	Cost per sample ($)	No. of sample locations	Cost per test ($)	Total no. of tests	Total cost ($)	Test method	Laboratory
Enteric virus	825	4	3,300	100	330,000	SM 9510 F&G	OCU
E. coli	17	4	68	100	6,800	9222B/D	OCU
Giardia/Cryptosporidium	605	4	2,420	100	242,000	USEPA 1623	OCU
Viable *Cryptosporidium*	704	4	2,816	100	281,600	Slifko et al., 1999	OCU
TC/FC	17	4	68	100	6,800	SM 9221	MWH
Enterococci	8	4	32	100	3,200	SM 9230/EPA 1600	MWH/OCU
C. perfringens	22	4	88	100	8,800	EPA/600/R-95/178 - Mod	BioVir
Coliphage (indigenous somatic and MS2)	28	4	112	100	11,200	U.S. EPA 1602	OCU
Adenovirus	715	4	2,860	100	286,000		OCU
HPC	28	4	112	100	11,200	SM 9215	MWH
MS2 (spiked)	110	16	1,760	12	21,120		BioVir
Subtotal					1,208,720		
MS2 stock solution	550	1.5	12		9,900		
Shipping					5,000		
Total					1,223,840		

Table 4. Summary of pilot-scale physical/chemical analysis costs

Measured parameter	Cost per sample ($)	No. of sample locations	Cost per test ($)	Total no. of tests	Total cost ($)	Test method	Laboratory
BOD_5	55	1	55	100	5,500	SM 5210B	MWH
COD	39	1	39	100	3,900	EPA 410.4	MWH
TOC	44	1	44	100	4,400	ML/SM5310C	MWH
NH_3	33	4	132	100	13,200	EPA 350.1	MWH
NO_3	13	4	53	100	5,300	EPA 300.0	MWH
TDS	17	1	17	100	1,700	ML/S254OC	MWH
Turbidity	0	2	0	100	0	EPA 180.1	MWH
TSS	0	2	0	100	0	EPA 160.2	MWH
PSD	0	2	0	100	0	Meter	
Zeta potential	0	1	0	100	0	Meter	
UVT	0	2	0	100	0	Meter	
				100			
Humic substances	110	2	220	100	22,000		est
Hydrophilic and hydrophobic organics by fractionation	220	2	440	100	44,000		est
Subtotal					100,000		
Shipping					2,500		
Total					102,500		

Table 5. Pilot trailer costs

Description	Unit quantity	Standard unit	Unit price ($)	Item total	Installation factor	Subtotal cost ($)
Trailer w/ counters and cabinets	LS	—	—	32,200	1.00	32,200
Electrical distribution panel	LS	—	—	2,600	1.00	2,600
Lighting/electrical outlets/wiring	LS	—	—	1,600	1.00	1,600
HVAC/roof AC and heater	LS	—	—	1,300	1.00	1,300
Sink/plumbing	LS	—	—	800	1.00	800
License/tags	LS	—	—	1,000	1.00	1,000
Chemical containment	LS	—	—	1,600	1.00	1,600
Chemical feed pumps	EA	2	400	800	1.00	800
Chlorine contact tank (15-min contact time)	LS	1	—	8,300	1.00	8,300
Chlorine contact tank (90-min contact time)	LS	1	—	15,600	1.00	15,600
Static mixer	EA	1	300	300	1.00	300
Polymer	LS	—	—	1,600	1.00	1,600
Coagulant (ferric)	LS	—	—	1,000	1.00	1,000
Submersible pumps	EA	2	500	1,000	1.00	1,000
Flexible hose	LS	—	—	1,000	1.00	1,000
Piping/tubing	LS	—	—	1,600	1.00	1,600
Flow meter	EA	2	2,100	4,200	1.00	4,200
Flocculation tank and stand	EA	1	3,100	3,100	1.00	3,100
Mixer	EA	1	5,200	5,200	1.00	5,200
Dual-medium filter with media, air compressor	LS	—	—	62,000	1.00	62,000
Turbidimeter/TSS meter	EA	3	4,200	12,600	1.00	12,600
Particle sizer	LS	—	—	29,000	1.00	29,000
Zeta potential analyzer	LS	—	—	32,000	1.00	32,000
Titrator	LS	—	—	4,000	1.00	4,000
Calibration standards	LS	—	—	1,000	1.00	1,000
Miscellaneous supplies	LS	—	—	3,000	1.00	3,000
Equipment installation in trailer (labor)	LS	—	—	8,000	1.00	8,000
Subtotal						236,400
Contingency (25%)						59,100
Total						296,000

ii. Full-Scale Monitoring at Wastewater Reclamation Plants

Microbial analyses. As described in Section 3, the same organisms monitored during the pilot study will be observed during the full-scale monitoring program. The only exception is that no spiking studies will occur at the full scale. Therefore, spiked MS2 coliphage will not be measured.

Single samples will be collected at five locations for each of the 100 monitoring events (twice per week for 100 weeks). If one uses the same cost per sample information presented in Table 3 above, the estimated cost for the full-scale microbial monitoring program is approximately $1.5 million.

Physical/chemical analyses. As described in Section 3, the same physical and chemical parameters monitored during the pilot study will be observed during the full-scale monitoring program. The only exception is that zeta potential measurements will not be collected during the full-scale study.

Single samples will be collected for BOD_5, COD, TOC, NH_3, NO_3, TDS, turbidity, TSS, PSD, UVT, humic substances, and hydrophilic or hydrophobic organics from various locations at each facility (as indicated in Table 2) for each monitoring event.

Based on these assumptions and the cost per sample of information presented in Table 4 above, the estimated cost for the full-scale physical/chemical monitoring program is approximately $220,000.

c. Field Staff Costs

The field staffing costs presented herein include staffing for all three phases of this study (bench-top testing, pilot testing, and full-scale testing). While there will be some field staffing requirements for the bench-top study, the most significant portion of the field staffing cost is associated with the pilot study. For the pilot study, it is assumed that the trailer will be deployed for approximately 50 weeks. Two staff-level engineers (one at 100% time and one at 50% time) would be assigned to this project as well as one project-level engineer (for one week of each quarter). For the purposes of this estimate, it is assumed that plant staff from the selected full-scale facility will collect all samples with the assistance of the pilot testing staff during the full-scale study. Therefore, additional staffing costs for the full-scale study are not included in this estimate. Based on these assumptions, the total cost of field staffing for the pilot study is approximately $413,000, as shown in Table 6.

Table 6. Field staff costs

Bench-top study

Staff	No. of h	$/h	Expenses ($)	Subtotal ($)
Staff engineer	40	125	0	5,000
Staff engineer	30	125	0	3,750
Project engineer	10	150	0	1,500
			Total cost of bench-top study staffing	10,250

Pilot study

Staff	No. of h	$/h	Expenses ($)	Subtotal ($)
Staff engineer	2,000	125	0	250,000
Staff engineer	1,000	125	0	125,000
Project engineer	160	150	4,000	28,000
			Total cost of pilot study staffing	403,000
			Total cost for field staff	413,000

d. Reporting and Project Management

The estimated total project time frame is 24 months. This estimate includes 6 months of startup, 12 months of field investigations, and an additional 6 months of data/report compilation.

Accordingly, engineering time needs to be allocated for eight quarterly reports, two draft summary reports, and one final report. Costs are included for four meetings with the project team and the WateReuse Foundation. Costs for project management and reporting (including data analysis) are included in Table 7.

Table 7. Project management and reporting costs

Staff	No. of h	$/h	Expenses ($)	Subtotal ($)
Project manager	420	150	1,000	64,000
Project engineer	1,040	125	1,000	131,000
Production	120	65	2,000	9,800
			Total cost	204,800

e. Cost Summary

Table 8 presents a summary of total estimated cost for the study based on the assumptions and estimates described above. The estimated cost for performing this study is $4 million.

Table 8. Summary of estimated costs

	Cost for a single facility ($)
Bench-scale study	
Microbial analysis	60,000
Physical/chemical analysis	2,000
Pilot-scale study	
Microbial analysis	1,224,000
Physical/chemical analysis	103,000
Pilot equipment	316,000
Full-scale study	
Microbial analysis	1,490,000
Physical/chemical analysis	220,000
Reporting and project management	205,000
Field staffing	413,000
Total cost	4,033,000

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WATEREUSE FOUNDATION

1199 North Fairfax Street, Suite 410
Alexandria, VA 22314 USA
(703) 548-0880
Fax (703) 548-5085
E-mail: Foundation@WateReuse.org
www.WateReuse.org/Foundation

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