Pergamon

Wat. Sci. Tech. Vol. 40, No. 4–5, pp. 247–252, 1999

Published by Elsevier Science Ltd on behalf of the IAWQ

0273–1223/99 $20.00 + 0.00

PII: S0273-1223(99)00576-4

PUBLIC HEALTH EVALUATION OF ADVANCED RECLAIMED WATER FOR POTABLE APPLICATIONS

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ABSTRACT

The City of Tampa in the Southwest portion of Florida is examining the development of an advanced treatment facility for supplementing their potable water supply with highly treated reclaimed water. Full-scale microbiological monitoring of a similar treatment train demonstrated that the high-pH chemical treatment process was an effective barrier for removal and inactivation of viruses (99.99%), bacteria (99.9999%) and enteric protozoa (99.9%). A constructed pilot plant and bench-scale studies of the high-pH chemical process demonstrated that the removal of *Cryptosporidium* oocysts was due to physical removal and no inactivation was shown with up to 180 minutes exposure at a pH of 11.2. Combination of high-pH chemical treatment and chlorination were also ineffective at kill of *Cryptosporidium* oocysts. © 1999 Published by Elsevier Science Ltd on behalf of the IAWQ. All rights reserved

KEYWORDS

Cryptosporidium; inactivation; chemical lime high-pH treatment.

INTRODUCTION

Communities in the Southwest portion of Florida are facing a water shortage because of population growth, overdraft of groundwater, seasonal rainfall and drought. There are over 400 reuse projects throughout the State that utilize reclaimed water for non-potable applications. Tampa, however is considering an indirect potable reuse project that will include chemical lime high-pH treatment, sand filtration, carbon adsorption and ozonation. The removal of bacteria, viruses and protozoa was evaluated through full-scale monitoring of the Upper Occoquan Sewage Authority. Reductions were 99.99999%, 99.999% and 99.9%, respectively, with the greatest reductions seen after the chemical lime high-pH treatment. *Cryptosporidium* is the most significant cause of waterborne disease in the U.S. today. The occurrence in surface waters has been reported in 4 to 100% of the samples examined at levels between 0.1 to 10,000/100L depending on the impact from sewage and animals (Lisle and Rose, 1995). *Cryptosporidium* is of particular concern for three reasons: The oocyst is extremely resistant to disinfection and can not be killed with routine water disinfection procedures, the disease is not treatable and the risk of mortality ranges between 50 and 85% in the immunocompromised populations (Hoxie, *et al.*, 1997; Rose, 1997). Therefore, the removal and

inactivation of *Cryptosporidium* in any reclaimed water or sewage system that may impact potable water supplies is of interest.

The objectives of this study included:

- Evaluation of high-pH chemical treatment process for the inactivation of viruses and *Cryptosporidium*
- Evaluation of high-pH chemical clarification for the physical removal of *Cryptosporidium*.

MATERIALS AND METHODS

Two pilot study experiments were conducted, September 26, 1995 and March 5, 1996 where secondary effluent was seeded with two bacterial viruses (MS2 coliphage and PRD1 bacteriophage) fluorescent beads (3 μm in diameter) and *Cryptosporidium parvum* oocysts. Two seeded trials on each date were conducted at two flows: 7 gpm and 3 gpm.

Pilot plant description and operations

A schematic of the pilot plant is presented in Figure 1. The pilot plant consisted of two 50 gallon round polyethylene tanks to serve as chemical feed tanks for calcium hydroxide slurry and polymer; one 10 gallon square polyethylene tank equipped with a variable-speed mixer to serve as a rapid-mix tank where calcium hydroxide was mixed with secondary effluent; and two 50 gallon round polyethylene tanks equipped with variable-speed mixers to serve as two-stage tapered flocculation basins. A 450 gallon circular fiberglass tank functioned as a clarifier to settle lime solids and collect supernatant. The 52 inch high, 4.2 feet diameter clarifier was placed inside a shallow 5 feet diameter circular basin, a plastic pool, to collect clarifier effluent.

Secondary effluent was pumped at the designated flow rate (3 gpm or 7 gpm) into the rapid-mix tank. Calcium hydroxide slurry from the chemical feed tank was added using a variable speed peristaltic pump adjusted to maintain a pH of 11.2 in the rapid-mix tank. After exiting rapid-mix, the flow passed through the two flocculation basins. After passing through tapered flocculation, anionic polymer was added using a variable speed peristaltic pump to achieve a 0.3 mg/L dosage and the flow entered the circular clarifier.

The seed stock solution containing beads, MS2, and PRD1 stocks was added to 1 L of secondary effluent in a 3-L carboy. The seeded solution was injected into the pilot-plant infuent over a 5 minute period. Influent samples were withdrawn from the influent sample port every 30 to 60 seconds. Effluent samples were collected in 500-mL bottles.

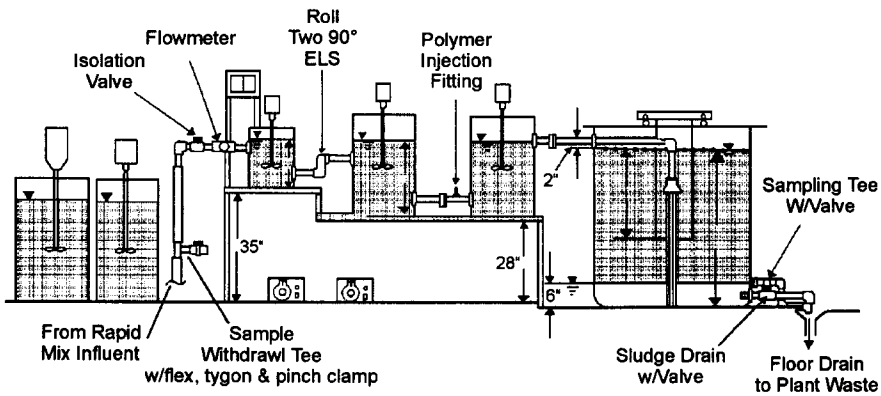

Figure 1. Schematic of the pilot plant.

Seed preparation and assays

MS2 coliphage (9ATCC catalog number 15597-B1) was propagated for use in the pilot studies by inoculating a one litre flask containing 200 mL of tryptone yeast extract (TYE) with 2 mL of the host bacteria *Escherichia coli* (*E. coli*) (ATCC catalog number 15597). The culture flask was placed in a shaking incubator maintained at 37°C. When the bacterial density reached approximately $1x10^8$ cfu/mL (derived density was previously determined), an aliquot of the virus stock (approximately 10^{12} pfu/mL), was added to provide a multiplicity of infection (MOI) of 0.1. The culture was shaken continuously until the host cells lysed. Then 0.02g of lysozyme and 6 mL of sterile 0.2M ethylenediaminetetraacetic acid (EDTA) was added to the culture to further lyse the host cells and release the virus (bacteriophage) and the sample was incubated for an additional 30 minutes in a shaking incubator at 37°C. The propagated virus and cellular debris were then centrifuged for 20 minutes at 3600 rpm and filter sterilized with a sterile 0.45 um filter. The resultant stock was titered by the agar overlay technique and refrigerated at 4°C until needed. PRD1 bacteriophage was propagated in the same manner using a *Salmonella* host. Pilot plant seeded influent samples were diluted with phosphate buffer saline (PBS) solution. The diluted influent samples and the effluent samples were assayed directly for the bacteriophage using an agar overlay technique with each of the respective hosts specific for each bacteriophage.

Fluorescent bead and formalinized oocyst preparations

Fluorescent beads, 3 μm in diameter and a concentration of approximately 1 x 10^9/mL were obtained from Polysciences, Inc. (Warrington PA). Formalin fixed *Cryptosporidium parvum* oocysts, approximately 1 x 10^8/mL, were obtained from Waterborne Inc. (Tulane LA). Beads and oocysts were assayed directly in influent samples (0.1 to 1.0 mL) and effluent samples were concentrated by centrifuging 50 mL of the sample or by filtering 10 to 20 mL directly onto a cellulose acetate membrane filter. Oocysts were stained with monoclonal antibodies on membrane filters and counted under the microscope, while the beads (which were already labeled) could be read directly under the microscope.

RESULTS

Table 1 and Figure 2 show the oocyst, phage and beads in the influent and effluent and the removals. The high-pH chemical treatment pilot study data show that bacteriophage were inactivated by the high-pH and that MS2 coliphage was more sensitive to inactivation, with 99.9999% kill compared to the PRD1 where between 94 to 96% inactivation was observed. There was little difference in the removal of the formalinized oocysts or beads at either flow rate with each being removed on average by 99%.

Table 1. Pilot scale study on the reduction of beads, *Cryptosporidium* and phage by chemical lime high-pH treatment

	Beads*	***Cryptosporidium****	**MS2****	**PRD1****
Influent #s	6.5×10^5	2,072	$5.6x10^8$	$3.6x10^7$
Effluent #s	3,020	7.2	574	$1.64x10^6$
Percent Reductions	99.5	99.6	99.9999	95.4

* Removal

** Inactivation

Bench-scale studies on *Cryptosporidium* oocyst viability

In April 1996, bench-scale tests were conducted at USF to test the high pH chemical treatment and chlorination effects on the removal/inactivation of surrogates such as fluorescent beads and viable *Cryptosporidium* oocysts in a controlled laboratory environment. Two trials were conducted to determine the high pH effect on removals. One trial used the surrogate, fluorescent beads and the other trial used live oocysts. Viability was determined under three sets of conditions: after high-pH treatment, after chlorine addition, and after high-pH treatment followed by chlorination.

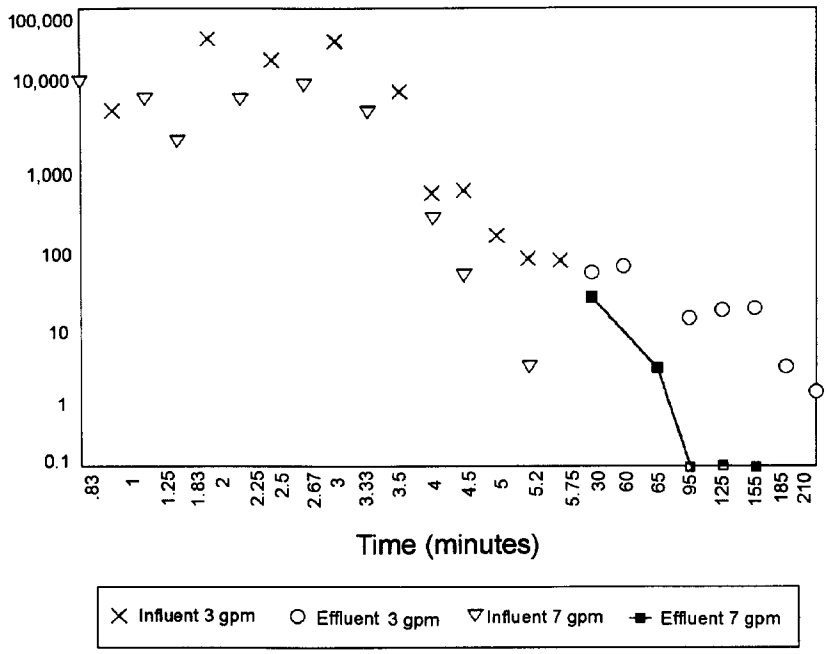

Figure 2. Removal of *Cryptosporidium* oocysts associated with chemical lime high-pH treatment.

A paddle stirrer (Phipps & Bird ™) equipped with 6 2 L jars was used for this study. All jars were placed in an ice bath to maintain a sample temperature of approximately 8-10°C for the duration of each test. One of the six jars was designated as a control. The adjustable-speed paddle stirrer was positioned above the jars to provide the appropriate initial rapid mixing followed by a tapered flocculation period. Secondary effluent was seeded with a stock culture of approximately 3 x 10^8 oocysts/mL and this was used in each experiment. Oocysts were obtained from Waterborne Inc. (Tulane LA) and Dr. Steve Upton's laboratory (Kansas State University). A 1000 mg/L stock of calcium hydroxide was prepared to dose the seeded effluent and maintain pH at 11.2. A 67 mg/L sodium hypochlorite solution was used for disinfection and a 1-mg/L sodium thiosulfate stock solution was used to dechlorinate samples prior to analysis in the experiments. Cell culture was used for assessing *Cryptosporidium* oocyst infectivity (Slifko *et al.*, 1997) as well as excystation (a laboratory surrogate for viability).

In the bench-scale studies, beads and oocysts were removed by 86% and 72%, respectively. Viability of the oocyst control was poor in the first of two viability experiments. The poor viability was traced to purification of the stock oocysts. The viability experiment was repeated for a third time. In the second viability experiment, no decrease in oocyst viability as a percentage of the population of oocysts was observed after exposure to high pH or after exposure to high pH treatment and chlorine (Table 2).

Removals or decreases in numbers of oocysts were seen after the chemical lime treatment. The current microscopic method however, does not assess oocyst viability. The bench scale studies demonstrated that those oocysts remaining could be presumed to be viable and the high pH nor the combination of pH extreme and disinfection were effective against the oocyst. Therefore the major mechanism for the decrease in protozoa after high-pH treatment appears to be physical removal.

Table 2. Viability of *Cryptosporidium* oocysts after chemical treatment after high pH and chlorination

	Percent Excystation Exp.1		Percent Excystation Exp.2		Percent Excystation with Chlorination	Excystation with chlorination & high pH*	Cell Infectivity after Chlorination and high pH
	Time 0	Time 180 min.	Time 0	Time 180 min	Time 0/ Time 30 min.	Time 0/ Time 30 min	
Control no lime	16	2	56	45	50/ 79	54/ 49	++
Jar1	9	1	64	50	59/ 92	52/ 78	++
Jar 2	9	3	51	40	64/ 85	77/ 59	++
Jar 3	4	6	57	47	52/ 95	89/ 65	++
Jar 4	1	5	71	42	51/ 90	70/ 63	++
Jar 5	3	9	54	51	50/ 97	66/76	++

Jars 1-5 were replicates, calcium hydroxide dose was 115 mg/L.
* post 180 min. of high pH.

Comparison of pilot-scale and bench scale removals

Table 3 compares the bench scale and pilot scale removals to removals achieved in the indigenous (full-scale plant) monitoring program. The data indicated that the pilot-scale study more closely resembles the removals achieved in the full-scale process. For coliphage, the indigenous monitoring showed a 99% reduction and represents the assay of a heterogenous population of coliphage, some of which (such as the MS2) may be very sensitive to the high-pH and some of which (such as PRD1) are more resistant. More information regarding the resistance of PRD1 should be collected as this may be a more valid surrogate in the high-pH chemical process for human enteroviruses, which are known to be more resistant to high-pH than the coliphage.

Table 3. Removal and inactivation of *Cryptosporidium*, beads, and phage by chemical treatment at pilot, bench-scale and full-scale

Condition Evaluated	**Oocyst Removal (%)**	**Bead Removal (%)**	**MS2 Inactivation (%)**	**PRD1 Inactivation (%)**	**Indigenous Coliphage Inactivation (%)**
Pilot Scale Exp 1	98.99	99.5	99.9999	93.8	NA*
Pilot Scale Exp 2	99.65	98	99.9999	95.4	NA
Bench Scale	72	86	NA	NA	NA
Full-Scale	96.5	NA	NA	NA	98.5

* NA-not applicable

CONCLUSIONS

Reductions of bacteria, viruses and protozoa can be achieved through advanced treatment processes producing waters that are better than ambient water quality. In large-scale wastewater facilities where the discharge may be posing a risk to down stream users, a chemical lime high-pH treatment was shown to be very effective for the inactivation of viruses and the removal of protozoa. No inactivation of *Cryptosporidium* oocysts was found after 180 minutes contact time to pH of 11.2 with or without subsequent chlorination. Therefore, oocyst removal takes place mainly by the precipitation phase prior to recarbonation during chemical lime treatment.

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