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POTENTIAL *CRYPTOSPORIDIUM* SURROGATES AND EVALUATION OF COMPRESSIBLE OOCYSTS

Sylvana Y. Li, James A. Goodrich, James H. Owens, and Robert M. Clark
Drinking Water Research Division, US EPA
26 W. Martin Luther King Dr., Cincinnati, OH 45268

Gene E. Willeke
Institute of Environmental Sciences
Miami University, Oxford, OH 45056,

Frank W. Schaefer III
EMSL, US EPA
26 W. Martin Luther King Dr., Cincinnati, OH 452683

INTRODUCTION

Cryptosporidium has been recognized as an important waterborne agent of gastroenteritis and a biological contaminant in drinking water. The widespread presence of *Cryptosporidium* in surface source water and either untreated or insufficiently treated drinking water has led to *Cryptosporidium* outbreaks in the United States and worldwide.

Cryptosporidium is highly resistant to commonly used drinking water disinfectants. Typical dosages of chlorine or monochloramine currently used in water plants appear to have no significant inactivation effect on *Cryptosporidium*.[1] High-dose ozonation may constitute an effective treatment technology to inactivate *Cryptosporidium* in drinking water.[2] Among the conventional control practices, filtration[3] and high temperature distillation[4] appear to be the potentially viable technologies for protection against *Cryptosporidium* in drinking water.

As employed in many water plants, filtration is likely to be the most practical treatment technology utilized for *Cryptosporidium* removal in the near future. Consequently, accurate and reliable methods for evaluation of *Cryptosporidium* removal rates for filtration-based systems are necessary to assist States in determining drinking water quality and complying with the up-coming national standard for *Cryptosporidium* in drinking water. Furthermore, searching for reliable and non-hazardous surrogates for evaluation of treatment plant efficiency has been intensified because of the potential health risk associated with *Cryptosporidium*. Additionally, during the filtration procedure *Cryptosporidium* may squeeze and fold through pores size of the filtration systems that are smaller than the diameter of the organism; a fraction of these *Cryptosporidium* oocysts may still remain a certain degree of viability. These uncertainties are critical for the evaluation and optimization of filtration-based physical treatment systems.

The in-house research studies described below consist of two parts. One is a potential surrogate study using bag filtration systems at the US EPA Test & Evaluation Facility in Cincinnati, Ohio. The second is *Cryptosporidium* compressibility and viability investigation.

METHODOLOGY

Part 1. Potential Surrogate Studies

The potential surrogates for the non-preserved *Cryptosporidium* tested include 4.5 μm polystyrene beads, 1-25 μm particle counts, 4-6 μm particle counts, turbidity, and preserved *Cryptosporidium*. A given volume of influent solution spiked with either polystyrene beads or *Cryptosporidium* was injected into the raw water ahead of the inlet pump of the bag filter system. An influent sample was collected from the spiked solution before each test. An effluent sampling consisted of collecting approximately 5% of the total effluent volume using a manifold and a 1.0 μm polycarbonate membrane filter. The effluent sample was concentrated to

approximately 8 ml using centrifugation (1200 rg) to a given small volume. A flow meter was used to determine the total effluent volume of each test. The duration of each test was also recorded. Both collected influent and effluent samples were scanned and examined under a UV microscope to determine the number of beads or *Cryptosporidium* oocysts using a hemacytometer. The preserved *Cryptosporidium* oocysts were from neonate male holstein calves and stored in dichromate. Indirect fluorescent antibody was used to label the effluent *Cryptosporidium* oocysts. Particle count analyses were conducted using a Met One® particle counter equipped with a light scattering liquid 211 sensor. The turbidity measurements were performed with a Hach® 2100P portable turbidimeter.

Three bag filter systems were used in the studies. Bag filter #1 was supplied by Strainrite Inc. Lewiston, Maine, and Bag filter #2 was manufactured by Filtration Systems, a division of Mechanical Mfg. Co. at Sunrise, Florida. Bag Filter #3 was obtained from the 3M Company. The studies were performed using 50 psi inlet pressure as recommended by the manufacturers, at varying pressure drops across the bag filter and influent flow rates of 12.5, 25 and 40 gpm.

Part 2. Compressibility and Viability Tests

A unique compressibility test device was developed which made it possible to examine *Cryptosporidium* passage through a membrane of designated pore size using a manifold under various inlet pressures. The non preserved *Cryptosporidium* oocysts that we used for this study came from neonate male holstein calves. A given number of *Cryptosporidium* oocysts were suspended in 0.01% Tween 20 (v/v) contained in a 5 L pressure reservoir. The influent *Cryptosporidium* oocysts passed through the system equipped with a 25 mm polycarbonate membrane filter. The collected effluent was centrifuged to approximately 200 μl and then was scanned and counted under the microscope. The membrane filter used in this study was made of polycarbonate with a pore size of 3 μm, which is smaller than the *Cryptosporidium* average size 4-6 μm. Polystyrene beads were used to demonstrate membrane integrity; no beads were detected. This advantageous filter characteristic allows precise determination of removal rates for both the beads and *Cryptosporidium* at various inlet pressures.

Three blank tests without a filter were performed under 50 psi inlet pressures in order to evaluate *Cryptosporidium* loss in the system. Four tests were conducted under 50 psi inlet pressure and one test under 25 psi with the 3 μm filter. Non-preserved *Cryptosporidium* was used in the blank tests. In order to compare the system loss difference between non-preserved and preserved *Cryptosporidium*, one blank test using preserved *Cryptosporidium* was performed under 50 psi inlet pressure.

For the compression test using the 50 psi inlet pressure conditions, a viability test was performed on the non-preserved *Cryptosporidium* recovered from the effluent. *Cryptosporidium* concentrated from the effluent was fed to neonatal mice at two dosages, 340 and 3400 *Cryptosporidium* oocysts per animal. A control sample consisted of 18 mice fed with 63 unfiltered non-preserved *Cryptosporidium* oocysts per animal.

RESULTS

The initial bag filter testing results indicated that the removal rates for 4.5 μm polystyrene beads, turbidity, and particle counts in the 1-25 μm and 4-6 μm size ranges, vary significantly among the three bag filters. The log reduction ranges from 0.14 to 3.42 for the 4.5 μm beads, from 0.04 to 1.89 for turbidity, from 0.09 to 2.84 for 1-25 μm particle counts, and 0.06 to 2.99 for 4-6 μm particle counts. Initial bag filter testing for preserved *Cryptosporidium* reveals a log reduction of 1.8.

Inspection of the bag filter testing results reveals varying relationships between the 4.5 μm beads, 4-6 μm particle counts, 1-25 μm particle counts, turbidity and non-preserved *Cryptosporidium*. The correlations between log reductions for beads and turbidity or particle counts for three bag filters at varying pressure drops and inflow rates, are consistent with the linear model:

$$\text{Log (beads)} = \alpha \text{ Log (turbidity or particle count)} + \beta$$

where α is the slope constant of the linear correlation, and β is the regression constant. The 4.5 μm beads and turbidity have a linear correlation with α and β of 0.52±0.15 and 0.04±0.24, respectively. The squared coefficient of determination (R^2) is 0.79. The beads and 4-6 μm particle count have a 1:1 correlation in removal rates as shown by the linear regression results: α and β are 0.97±0.35 and -0.29±0.54 respectively, and a squared coefficient of determination (R^2) is 0.79. On the other hand, 1-25 μm and particle counts display the correlation departured from 1:1 relationship and closer to the correlation exhibited between beads and turbidity. The linear correlation between 4.5 μm beads and 1-25 μm particle counts has the slope constant (α) of 0.73 and coefficient of determination (R^2) of 0.55.

Detailed size analyses show that the size distribution of non-preserved *Cryptosporidium*, 4.5 μm beads, and particle counts, primarily determines their similarities and dissimilarities in the removal rates for bag filters. The similar removal rates between 4.5 μm beads and non-preserved *Cryptosporidium* are consistent with their similar size distributions. *Cryptosporidium* has the size ranging 3.50-6.50 μm with an abundance peak at 4.01-4.50 μm, whereas the 4.5 μm beads range in size from 3.50-7.00 μm with the peak at 4.51-5.00 μm. Furthermore, the 4-6 μm particle counts have a similar size distribution with 4.5 μm beads. In contrast, the 1-25 μm particle counts have two abundance peaks at 5.01-6.00 μm and <2.00 μm. This size distribution is significantly different from those of the *Cryptosporidium* and 4.5 μm beads. The difference appears to be the explanation for the distinct removal rates of 1-25 μm particle counts from those of 4.5 μm beads and non-preserved *Cryptosporidium*.

The determined correlations in removal rates among *Cryptosporidium* and its potential surrogates do not appear to be the artifacts of influences from operational parameters including pressure drop, flow rate, or spiked influent level of surrogates. Testing results for the beads at varying pressure drops and flow rates for bag filter #1 show that the variation in log reduction is approximately 0.5, within the range of experimental variations determined with duplicate testing. Similarly, the number of influent beads does not appear to have a statistically significant influence on the removal rates.

Compression study results lead to two important observations regarding non-preserved *Cryptosporidium* removal efficiency of the filtration process and the reliability of surrogates. First, about 0.7% of the non-preserved *Cryptosporidium* at 3.50-5.00 μm size passed through the 3 μm polycarbonate filter at 50 psi inlet pressure. In contrast, the polystyrene beads of the same size range were completely removed. Different removal rates of the non-preserved *Cryptosporidium* and the beads are believed to reflect the ability of *Cryptosporidium* to squeeze and fold through the pressurized membrane filter. Furthermore, compressibility tests at 25 psi inlet pressures show an increased removal rate of *Cryptosporidium*. Only about 0.0021% non-preserved *Cryptosporidium* passed through the filter. It is possible that the *Cryptosporidium* squeezing and folding effect on the removal rate of the filter may diminish at lower inlet pressures.

Secondly, 4 blank tests on non-preserved *Cryptosporidium* at 50 psi inlet pressure lead to the observation that 53.61±5.30% of *Cryptosporidium* in oocysts in the influent was lost in the system. However, the result from one blank test on preserved *Cryptosporidium* at the same experimental conditions show the system loss less than 1%. Such a large difference in system loss between preserved and non-preserved *Cryptosporidium* indicate that the non-preserved *Cryptosporidium* not only can squeeze and fold through membrane filter, but also adhere to physical surfaces at applied inlet pressure. Both characteristics of the *Cryptosporidium* have substantial influence on apparent removal rates.

Initial viability test results show that the non-preserved *Cryptosporidium* collected from effluent of compression study are still viable. Five of seven mice were infected with a dose of 340 *Cryptosporidium* oocysts per animal. Ten of ten mice were infected with a dose of 3400 *Cryptosporidium* oocysts per animal. Control samples of 63 *Cryptosporidium* oocysts per dose infected 13 out of 18 animals.

CONCLUSIONS

The *Cryptosporidium* surrogate investigation, compressibility study and viability test, lead to the following conclusions:

Accuracy and reliability of the *Cryptosporidium* surrogates are determined by similarities of size

distribution between *Cryptosporidium* and its surrogates. The 4.5 μm polystyrene beads have a similar size distribution as the *Cryptosporidium* with a peak distribution in the 4-5 μm range. Consequently, the 4.5 μm beads and *Cryptosporidium* show the closest removal rates. This similarity will be further characterized in future studies. Studies on surrogate interchangeability show that the 4-6 μm particle counts and 4.5 μm beads have a 1:1 correlation in removal rate. On the other hand, 1-25 μm particle counts and turbidity are not directly interchangeable surrogates with 4.5 μm beads. As the latter most closely resemble the *Cryptosporidium*, both 1-25 μm particle counts and turbidity are less reliable surrogates for *Cryptosporidium* removal evaluation.

Compression test results demonstrate that unlike the 4.5 μm beads, a small fraction of 3.50-5.00 μm *Cryptosporidium* oocysts squeeze and fold through smaller pore sizes of a membrane filter. This unique characteristic of *Cryptosporidium* leads to apparently lower removal rates than that of the 4.5 μm beads. However, the influence of *Cryptosporidium* size change at the membrane filter appears diminished at low inlet pressures. A large fraction of *Cryptosporidium* oocysts that passed through membrane filter remain viable. Therefore, a high removal rate might be required for filtration-based treatment technology to ensure safety of the treated drinking water.

It is noted that both surrogate and compressibility studies are presently on-going. Results from future studies will be supplemental to the conclusions presented here.

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For more information:

Sylvana Y. Li, Systems & Field Evaluation Branch
Drinking Water Research Division, 26 W Martin Luther King Dr.
Cincinnati, OH 45268, (513)-569-7074, (513)-569-7185(Fax)