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Applied Microbiology 2018: Towards the Development of New Integrated Ultrasonic Filtration Systems for Monitoring Waterborne Pathogens- Abdelfateh Kerrouche- Edinburgh Napier University

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The detection of waterborne pathogens mainly relies on sample processing. This process concentrates large volume of water to very small volume that can be used under microscopes. The current approved concentration techniques are slow, very expensive, time-consuming and often results in low recovery rates of pathogens. This research explores the use of high frequency sonication which is attached to two different types of filters to improve the recovery rate and work towards automated filtration/elution systems. Both systems were designed and tested with latex beads and live *Cryptosporidium* parvum oocysts.

Previous research has often focussed on chemical approaches to improve recovery levels during filter elution. But novel physical methods, such as the use of megasonic sonication, offer great potential for effective removal of pathogen from filters. Compared with ultrasound-assisted agitation, megasonic sonication, which operates at a higher frequency of energy excitation, offers more gentleness and during the process more detailed elution process with lower risk of pathogen damage. Megasonic exposure of oocysts with *Cryptosporidium* has been shown to preserve their viability. This elution mode enables pathogen infectivity to be established downstream, because viability and species details can not be extracted from attenuated pathogens.

Here we investigate the use of megasonic elution in two different filtration set-ups to improve *Cryptosporidium* recovery rates: firstly, dead-end filtration using a Rexeed filter and secondly, tangential flow filtration using a Fresenius filter. The results show that recovery levels for both installations are increased by about 50 per cent, demonstrating the capacity of megasonic elution in this application.

Introduction: *Cryptosporidium* is a particularly problematic waterborne pathogen due to resistance to chlorination, low infectious dose and impressive longevity in its environment. Additionally, detection is challenging as recovery rates are often low and the protozoan cannot be cultured in the lab making effective sample processing for concentration essential.

Recently, the use of ultrasonic elution has been shown to significantly improve the rate of recovery for *Cryptosporidium*. Nevertheless, it was also shown that a few minutes of continuous ultrasound destroy *Cryptosporidium* oocysts with more than 90 percent deactivation of the oocyst. As longer exposures significantly impacted viability, the integrated ultrasound filtration system defined by Al-Sabi was limited to 5 s of ultrasonic operation. Deactivation destroys the capacity of the identified pathogens to assess their viability status. In addition, DNA degradation may be incompatible with species-determining molecular methods.

By comparison, megasonic sonication offers a way to elude undamaged and potentially viable oocysts from filters and membranes, by minimizing the time needed for bubble growth and the resulting low cavitation energy. This work also demonstrated *Cryptosporidium* 's viability even after 120 min of megasonic exposure, with an excystation assay reporting an excystation rate of 96 percent with a sporoziote / shell ratio of 2.26 (compared to 97 percent and 2.4 for control)

Here, we explore the use of megasonic sonication for pathogen elution in an automated filtration system with different filter types and suggest how the integration of megasonic transducers could boost the set-up efficiency. Typically, protozoan pathogen recovery rates from the detection protocol filtration stages are low; therefore, the key focus of this study was to determine whether megasonic elution would lead to an increase in recovery rates.

Objective: The goal was to investigate whether megasonic elution could increase *Cryptosporidium* 's recovery levels using filters included in automated filtration systems.

Results & Discussion: Experiments were undertaken to explore the potential of megasonic elution with different filter types using two different systems. The Rexeed 25AX filter used a dead-end filtration system and a tangential flow set-up was introduced with a Fresenius FX1000 filter. These filters have been selected from previous literature because they were used in automated set-ups and based on data, i.e. previous demonstration of good filtration efficiency with *Cryptosporidium* oocysts. Before the filter is back-flushed to elute pathogens from the filter, the sample is first passed through the filter and pathogens captured in the dead-end operation.

The tangential flow system was part of a fully automated system to replace the previously mentioned *Cryptosporidium* regulatory control process, developed by Shaw Water Company. No pre-treatments of chemical filters or elution buffers were used for this study focusing solely on the impact of the method of physical elution. Normal operating procedures have been contrasted with the use of the megasonic transducer (for the dead-end filter during the back-flush process and for the tangential flow filter in operation).

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Importantly, however, both increases were statistically significant for the tangential flow and dead-end flow filters with a student T-value of 4,064 and 3,077 respectively, both larger than the T-test value of 2,776 using a confidence interval of 95 per cent. This suggests waterborne protozoa monitoring systems should consider including megasonic elution. Previous work with the Filta-Max filter did not demonstrate such a large increase in recovery rates, and rather advantages were observed and emphasized in terms of automated and more efficient elution into smaller volumes.

Conclusions: Megasonic elution provides a major increase in *Cryptosporidium* recovery levels from automated filtration setups.