EGU21-6583, updated on 20 Oct 2021
https://doi.org/10.5194/egusphere-egu21-6583
EGU General Assembly 2021

Development of new pathogen surrogates and synthetic DNA tracers for water applications

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In recent years, we have conducted research into developing new pathogen surrogates and synthetic DNA tracers for water applications. Biomolecule-modified particles have been used to mimic *Cryptosporidium*, rotavirus and adenovirus with respect to their filtration removal and transport in porous media. Additionally, we have developed new DNA tracers as free DNA molecules or DNA-encapsulated biopolymer microparticles to track water contamination. DNA markers are also used to label some surrogates to facilitate their sensitive detection by using qPCR.

The surrogates have been validated in laboratory conditions alongside the actual pathogens. The *Cryptosporidium* surrogates have been satisfactorily validated in alluvial sand, in limestone sand, in coagulation and rapid sand filtration studies. The rotavirus surrogates have been successfully validated in coastal sand aquifer media, in unmodified and hydrophobically modified quartz sand, and in stony alluvial soils under on-site wastewater applications. The research findings have demonstrated that these new surrogates significantly outperform the most commonly used existing surrogates, namely, unmodified microspheres for *Cryptosporidium* oocysts and MS2 phage for viruses. Working with the water industry, we have applied the *Cryptosporidium* surrogate to pilot-scale rapid sand filters and point-of-use domestic filters and determined its removal efficiencies in water filtration systems commonly used in New Zealand. The artificial DNA tracers have been validated in surface water, groundwater and soils, and they were readily trackable in a surface stream for up to 1 km.

Our proof-of-concept studies suggest that the new pathogen surrogates and synthetic DNA tracers we have developed show great promise as new tools for water applications. The 'micro mimics' approach has opened up a new avenue for assessing pathogen removal and transport in water systems without the risk and expense that accompany work with actual pathogens. With further validation, the new surrogates could be used to study pathogen removal and transport in subsurface media after the disposal of effluent and biosolids to land, and to assess the performance of filtration processes in water and wastewater treatment. With future up-scaling validation of the new synthetic DNA tracers, these tracers could be useful for concurrently tracking multiple pollution sources and pathways in freshwater environments.