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ORIGINAL ARTICLE

Investigation of quaternary ammonium silane-coated sand filter for the removal of bacteria and viruses from drinking water

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Abstract

Aims: To develop an anti-microbial filter media using an attached quaternary ammonium compound (QAC) and evaluate its performance under conditions relevant to household drinking water treatment in developing countries.

Methods and Results: Silica sand was coated with dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride via covalent silane chemistry. Filter columns packed with coated media were challenged with micro-organisms under different water quality conditions. The anti-bacterial properties were investigated by visualizing *Escherichia coli (E. coli)* attachment to coated media under fluorescence microscopy combined with a live/dead stain. A 9-cm columns with a filtration velocity of 18 m h^{-1} achieved $\log_{10}$ removals of 1·7 for *E. coli*, 1·8 for MS2 coliphage, 1·9 for Poliovirus type 3 and 0·36 for Adenovirus type 2, compared to 0·1–0·3 $\log_{10}$ removals of *E. coli* and MS2 by uncoated sand. Removal scaled linearly with column length and decreased with increasing ionic strength, flow velocity, filtration time and humic acid presence. *Escherichia coli* attached to QAC-coated sand were observed to be membrane-permeable, providing evidence of inactivation.

Conclusions: Filtration with QAC-coated sand provided higher removal of bacteria and viruses than filtration with uncoated sand. However, major limitations included rapid fouling by micro-organisms and natural organic matter and low removal of viruses PRD1 and Adenovirus 2.

Significance and Impact of the Study: QAC-coated media may be promising for household water treatment. However, more research is needed on long-term performance, options to reduce fouling and inactivation mechanisms.

Introduction

Unsafe drinking water, sanitation and hygiene (WASH) practices contribute to the global disease burden, increasing the number and severity of infectious diseases. Low-income countries bear the largest disease burden from unsafe WASH, which is the 2nd leading risk factor for disability-adjusted life years (DALYs) and 4th leading risk factor for mortality (WHO 2009). The greatest concern with unsafe water and sanitation practices is highly endemic diarrhoea (Clasen *et al.* 2007). Diarrhoea is particularly dangerous for children under five, accounting for nearly 17% of overall deaths (WHO 2009). The United Nations is addressing unsafe WASH related DALYs through the Millennium Development Goals (MDGs), targeting a 50% reduction in people without access to improved water sources and sanitation by 2015 (1990 baseline). In 2008, however, 884 million people still lacked access to improved water sources, a figure that does not account for the water quality of the supply. In

all, 84% of this population currently resides in rural settings beyond the reach of urban centralized water treatment (WHO/UNICEF 2010).

Household water treatment and safe storage (HWTS) has been introduced as part of the solution to provide safe drinking water around the globe. HWTS seeks to provide users with the means to treat unsafe water at the point of use (POU) and prevent recontamination during storage. In 2007, HWTS was estimated to reach 18·8 million users, employing boiling, chlorination, ceramic filtration, solar disinfection (SODIS) and biosand filtration (Clasen 2009). Several meta-analyses evaluating the effectiveness of water quality interventions have reported HWTS to be effective in reducing the overall incidence of diarrhoea by about 30% (Fewtrell *et al.* 2005; Arnold and Colford 2007; Clasen *et al.* 2007; Waddington *et al.* 2009), and 42% in children under five (Waddington *et al.* 2009). Due to the effectiveness of water quality interventions, the World Health Organization (WHO) has called for a scale-up of HWTS (Clasen 2009). While current HWTS technology has proven effective at reducing diarrheal disease incidence, further technology development and consumer preference research are still necessary to improve pathogen removal and increase adoption. The current HWTS options are not effective against all types of pathogens (e.g. chlorination against protozoan cysts, ceramic and biosand filters against viruses), may leave undesirable aftertastes (chlorination), or require extensive time (SODIS) (Sobsey *et al.* 2008). An ideal HWTS would provide high levels of removal or inactivation of waterborne viruses, bacteria and protozoan cysts (WHO 2011), be affordable and readily available, reflect user preferences and require little user input and training.

The purpose of this research was to investigate the ability of quaternary ammonium compounds (QACs) immobilized on granular media to remove bacteria and viruses. QACs are a group of chemicals known for their anti-microbial properties. These compounds have been studied and used as disinfectants and antiseptics in hospitals for nearly a century, are available off patent and cost very little (Fraise *et al.* 2008). QACs are surfactants consisting of a positively charged nitrogen atom with four alkyl chain substituents, providing both hydrophilic and hydrophobic regions. In solution, QACs' anti-microbial activity is attributed to membrane/envelope disruption by the charged nitrogen atom and hydrophobic alkyl chain (Gilbert and Moore 2005). The efficiency is dependent upon the alkyl chain length (Daoud *et al.* 1983; Gilbert and Al-taae 1985), micro-organism (Maillard 2002) and contact time (Nakagawa *et al.* 1984).

Quaternary ammonium silanes (QAS) are a silanized version of QAC that can covalently bond to hydroxylated surfaces such as silica sand; the coatings are reported to be further stabilized by cross-links between QAS molecules (Witucki 1993). Prior research reports that while tethered QAS molecules have limited movement, anti-microbial activity still occurred without entrance into the cell (Isquith *et al.* 1972), and surface immobilization did not impede the ability of QAS molecules to disrupt cell walls (Brizzolara and Stamper 2007). High concentrations of surface bound QAS molecules were capable of causing cell membrane disruption by inducing cation exchange with calcium ions (Kügler *et al.* 2005; Murata *et al.* 2007). In addition, the positively charged nitrogen atom and hydrophobic chains facilitate removal of micro-organisms by attachment to the coated surfaces through hydrophobic and electrostatic interactions. It is expected that these enhanced interactions, however, may be compromised under the water quality conditions in typical water supplies. Prior studies have shown promising results for the removal of *Escherichia coli* (*E. coli*), MS2 coliphage and *Cryptosporidium parvum* oocysts with QAS-coated zeolite filters (Abbaszadegan *et al.* 2006).

We evaluated the ability of QAS-coated sand media to remove bacteria and viruses under conditions relevant to POU water treatment. We report on experiments demonstrating the micro-organism removal performance under different solution chemistries, flow rates and the anti-bacterial activity of QAS-coated sand.

Materials and methods

A quaternary ammonium silane (QAS) was coated onto silica sand and evaluated as a filtration media in column experiments. Columns were packed with the QAS-coated media and challenged to evaluate the removal of bacteria (*E. coli*), bacteriophage (MS2 and PRD1) and human viruses (poliovirus and adenovirus). In addition, the columns were challenged to remove *E. coli* or MS2 under different ionic strengths and in the presence of natural organic matter (NOM). Prefilters of powdered activated carbon (PAC) were tested with source water containing NOM to determine the effectiveness of a multistage filter.

Bacteria

Escherichia coli F_{amp} (ATCC no. 700891) were grown the night before each experiment in tryptic soy broth containing 0·015 g l^{-1} of ampicillin and streptomycin. A frozen *E. coli* F_{amp} culture was grown in a 37°C incubator overnight. This overnight culture was then re-propagated the morning of an experiment to an optical density of 1·0 at a wavelength of 600 nm. *Escherichia coli* were concentrated by centrifugation at 8000 ***g*** for 5 min. The

supernatant was discarded and the pellet was rinsed three times in a 1 × phosphate buffer solution (PBS) solution. The spread plate technique on selective agar containing 0·015 g l^{-1} of ampicillin and streptomycin was used to enumerate *E. coli* concentrations in duplicate as colony forming units (CFU) in 100 μl inocula from each sample during column experiments.

MS2 and PRD1 bacteriophage

MS2 and PRD1 were propagated using broth enrichment (EPA Standard Method 1601) in *E. coli* F_{amp} and *Salm. typhimurium* LT2, respectively. The bacteriophage plaque assay was conducted using the double agar layer (DAL) method with 100 μl inocula to determine plaque forming unit (PFU) concentrations in duplicate from influent and effluent samples during column studies.

Human virus and host cells

Adenovirus type 2 was obtained from Mark Sobsey (University of North Carolina, Chapel Hill) and Poliovirus type 3 was provided by Ali Boehm (Stanford University). Adenovirus type 2 and Poliovirus type 3 were cultured in A549 cells (ATCC CCL-185) and HeLa cells (ATCC CCL-2), respectively. Virus plaque assays were conducted in 6-well plates with 100 μl inocula. PFU concentrations from column studies were enumerated in duplicate after 6 days of incubation for adenovirus and 3 days of incubation for poliovirus. Details of human virus propagation and enumeration are provided in the Supporting Information.

Coating procedure of QAS-coated sand

Silica sand (50–70 mesh, Sigma-Aldrich, St Louis, MO) was coated with the QAS compound dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride (42% w/w methanol solution, Sigma-Aldrich). To remove metal impurities, the sand was soaked in 12 N HCl overnight, and rinsed in MilliQ water until the pH returned to 5·5. Next, the sand was soaked for 8 h in 1 mol l^{-1} NaOH to hydroxylize the SiO_2 surface, and rinsed with MilliQ water until the pH returned to 5·5. The sand was then mixed for 24 h in the coating solution at a 2 : 3 volume ratio of sand : coating solution. The coating solution was a 1·2% v/v QAS solution in ethanol at pH 3 (pH adjusted with 1 N HCl). After 24 h, the coating solution was drained and the sand was placed in a 100°C oven overnight, rinsed in ethanol for 5 min, and then rinsed three times in DI water for 5 min each. Following the last rinse, the sand was placed in the 100°C oven overnight.

Leaching assays (microbial and bromophenol blue methods)

Two approaches, one microbiological and one chemical, were employed to quantify QAS leaching from each batch of coated sand. The leachate solution used in leaching experiments was created by mixing 1 g of QAS-coated sand with 10 ml of MilliQ water in a 15-ml centrifuge vial for 48 h, then recovering the liquid supernatant after centrifugation at 5000 ***g*** for 15 min. In the microbiological approach, equal volumes of *E. coli* (at 10^5 CFU ml^{-1}) and leachate solution were mixed by vortexing five times and allowed to rest for 10 min. A mixture of uncoated sand with the *E. coli* solution was used as a control. The mixture was then diluted and plated for counting. A decrease in the culturable concentration of *E. coli* in the QAS-coated sand mixture greater than the decrease observed in the control was interpreted as evidence of QAS leaching.

The chemical approach involved mixing dilutions of bromophenol blue (BPB) with the leachate solution as an electrostatic complex occurs between QAS and BPB molecules. A total of 2·5 ml of the supernatant was mixed with 2·5 ml of diluted BPB solutions (BPB concentrations of 6×10^{-5}, 6×10^{-6}, and 6×10^{-7} mol l^{-1}). A mixture of uncoated sand with each BPB dilution was used as a control. The mixture was vortexed, the absorbance measured on a UV-VIS spectrometer at 600 nm and the peak absorbance wavelength recorded. A 1 : 1 ratio of BPB : QAS was observed to cause a shift in the peak absorption wavelength, such that a BPB solution without QAS had a peak absorption wavelength around 592 nm while a BPB solution with QAS had a peak absorption wavelength near 605 nm. Thus, a shift in the peak absorption wavelength from 592 to 605 nm was interpreted as evidence of QAS leaching from the sand batch, and the QAS concentration was estimated to be similar to the minimum BPB concentration necessary to cause a shift in the peak absorption wavelength. QAS-coated sand batches were used in experiments if they: (i) did not cause inactivation of *E. coli* greater than the plain sand control and (ii) did not cause a shift in the peak absorption wavelength in the BPB solution containing 6×10^{-6} mol l^{-1} or higher. It should be noted that there was evidence of leaching in some sand batches that were not used for further experiments. We were not able to determine what step(s) in the coating process were responsible for producing leaching *vs* nonleaching coatings. The QAS compound we used has been tested extensively and found to exhibit low toxicity. Mutagenicity and teratogenicity tests in albino rats were negative, and the LD50 was found to be 12·3 g kg^{-1} body weight (Du Pont de Nemours and Co. 1992). Assuming this LD50 applies to humans, if the compound

was present in water at the detection limit of our BPB leaching assay, a user would need to drink >200 000 l in one sitting to reach the reported LD50. Future research should examine whether long-term use of the QAS-coated sand will cause leaching at higher levels.

QAS coverage of silica sand media measurement

One gram of coated silica sand was placed in a 15-ml centrifuge tube with 10 ml of bromophenol blue (BPB) dye. The sand/BPB mixture was vortexed five times and rotated overnight to fully coat the sand. The next day, the BPB liquid was discarded and 10 ml of DI water was added, vortexed 6–10 times and discarded. The DI water rinse was repeated five times to remove any excess dye. A sum of 10 ml of a soap buffer solution (1·8 l DI water, 0·2 l 1×phosphate buffer solution (PBS), and 40 g dissolved Fischer Sparkleen soap no. 1) was then mixed with the dyed sand. The soap buffer solution weakened the BPB : QAS complex on the sand surface, releasing BPB back into solution. The sample was centrifuged for 15 min at 8000 ***g*** to remove colloids, and the solution absorbance measured using a UV-VIS spectrometer. The measured absorbance was related to BPB molarity through a standard curve. Each mole of BPB was assumed to correspond to one mole of N^+. We attempted to measure the silica sand's surface area by obtaining nitrogen adsorption isotherms on an Autosorb 1 (Quantachrome) and then analysing the data using the Brunauer-Emmet-Teller (BET) method. The coverage data were then converted to surface charge density (N^+ cm^{-2}).

QAS-coated sand column experiments

Filtration columns packed with QAS-coated sand were challenged with various micro-organisms and water qualities. The columns were created from 25-ml pipette tubes (~1·2 cm diameter) that were cut to the desired length (4·5–30 cm depending on the experiment). The column diameter (1·2 cm) to average sand grain diameter (0·25 mm) ratio was 48, a value above a threshold of 30 found to minimize wall effects (Cohen and Metzner 1981). The columns were packed by wetting the QAS-coated sand with ethanol and then gradually pouring the sand into the column. A hand-held massager was used to enable tight packing. A Masterflex peristaltic pump delivered influent at the desired flow rate (15 and 36 ml min^{-1}). Once packed, the column and pump tubing were rinsed with a 70% ethanol solution for 10 min to remove any prechallenge contamination, followed by 30 min rinse with MilliQ water and 30 min rinse with 1 mmol l^{-1} NaCl. The challenge water was spiked with micro-organisms (~10^5 CFU or PFU ml^{-1}) and then introduced for 60–120 min with samples taken every 20–30 min. Various influent conditions, such as ionic strength (1 mmol l^{-1}, 10 mmol l^{-1} and 100 mmol l^{-1} NaCl), flow velocity (3·6 m h^{-1}, 7·2 m h^{-1} and 18 m h^{-1}) and NOM (20 mg l^{-1} Fluka humic acid), were then altered to evaluate the effectiveness of the QAS-coated sand media. An equivalent sized column of powdered activated carbon (PAC) was placed before the QAS-coated sand column to evaluate the effectiveness of a dual media filter when NOM was present in the source water (20 mg l^{-1} of Fluka humic acid).

Filtration model parameters

The parameters used in the filtration model to determine the predicted column removal are included in Table 1 (Tufenkji and Elimelech 2004).

Bacteria membrane damage/death from QAS-coated sand exposure

Loss of culturability from exposure to QAS-coated sand was assessed with the following assay. A total of 2 g of QAS-coated sand was exposed to 5 ml of a stationary phase *E. coli* suspension. The mixture was vortexed five times and then placed in a 37°C incubator for 24 h. After 24 h, the supernatant was plated to determine whether *E. coli* in the solution had adhered to the sand. The remaining sand was then rinsed with 50 ml of a 170 mmol l^{-1} NaCl and 0·5 mmol l^{-1} $NaHCO_3$ salt solution at pH 7. The rinse step with the salt solution was repeated two times. In the final rinse step, a small sample of rinse solution was plated to determine whether the final rinsate contained unbound *E. coli*. Finally, the 2 g of QAS-coated sand was placed in growth media and incubated to observe whether adhered bacteria were culturable. A sample of uncoated sand was used as an experimental control.

Table 1 Parameters used in the filtration model to predict column removal (Tufenkji and Elimelech 2004)

Description	Symbol	Units	Value
Diameter of collector	d_c	mm	0·25
Fluid approach velocity	U	m h^{-1}	18
Particle density	p_p	kg m^{-3}	1050
Fluid temperature	T	k	298
Porosity	f	–	0·36
Fluid density	p_w	kg m^{-3}	997
Boltzmann constant	K	J k^{-1}	$1{\cdot}38 \times 10^{-23}$
Dynamic viscosity	u	Ns m^{-2}	0·001002
Hamaker's constant	A	J	1×10^{-20}

Fluorescence microscopy

Fluorescence microscopy was used to observe bacterial membrane damage from QAS-coated sand exposure. An *E. coli* suspension was exposed to QAS-coated sand for 24 h and then stained with the BacLight Live/Dead kit (Invitrogen, Grand Island, NY) to image cell membrane permeability. Sand grains were placed on a microscope slide for viewing. Cells stained with SYTO-9 were interpreted as membrane-impermeable (potentially viable) and cells stained with propidium iodide (PI) were interpreted as membrane-permeable (dead or damaged) cells.

Results

QAS coverage and coating stability on silica sand media

The QAS coverage of the silica sand media, represented as the surface charge density (N^+ cm^{-2}), is shown in Fig. 1. The charge density was calculated by dividing the total moles of N^+ by the surface area of the sand. However, the measured surface area was below the detection limit for the BET analyser; thus, the approximate detection limit of 0·1 m^2 g^{-1} was used for the calculations. The different sands labelled in Fig. 1 (Q206, Q208, Q213, Q215 and Q216) refer to the batches of QAS-coated sand used in the experiments reported here. The surface charge density in the batches ranged from 6×10^{13} to 1×10^{14} N^+ cm^{-2}. A surface charge density threshold for bactericidal activity has been suggested at 10^{12} N^+ cm^{-2} for *E. coli* in a dividing state and 10^{14} N^+ cm^{-2} for *E. coli* in a stationary state (Kügler *et al.* 2005). 10^{15} N^+ cm^{-2} has also been reported as a surface charge density threshold for anti-bacterial surfaces (Murata *et al.* 2007). Thus, we estimate that the QAS-coated sands exhibited charge densities at or just below threshold values determined by Kügler *et al.* (2005), and below the value determined by Murata *et al.* (2007). However, the actual charge density is likely underestimated because the surface area of the sand may be lower than what was assumed for the calculation.

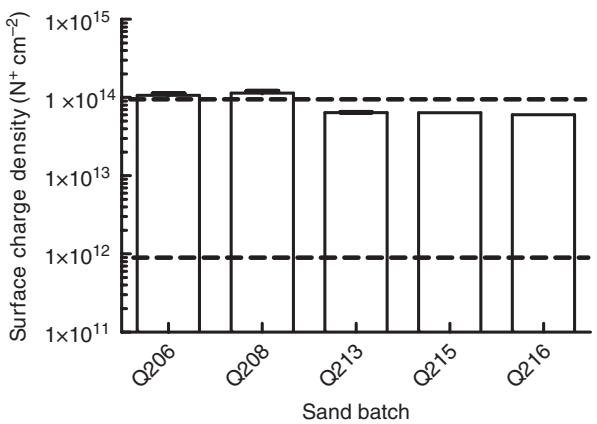

Figure 1 Estimated surface charge density of QAS-coated sand batches used in column experiments. Dotted lines show the charge density thresholds reported by Kügler *et al.* (2005). Error bars report two standard deviations and reflect only the variability in the measurement of the coating concentration. As described in the text, it was not possible to estimate the variability of the surface area of the sand.

In addition to verifying the coating density of each batch of sand used for experiments (Fig. 1), each batch was tested for leaching using an *E. coli* bioassay and a bromophenol blue assay, and only sand batches that did not leach were used for experiments (see Supporting Information for details of the leaching assays).

QAS-coated sand column studies

The results from clean bed filtration experiments using QAS-coated sand challenged with *E. coli* and several viruses are summarized in Table 2. Clean bed filtration refers to the initial micro-organism removal of a packed column before the surface was altered by significant accumulation of particles. Samples for quantifying clean bed removal were collected within four pore volumes of starting a challenge. The $\log_{10}$ removal of *E. coli* and MS2 was similar, and increased approximately linearly as the height of the coated sand columns increased, consistent with filtration theory. For example, a 9-cm column achieved 1·7-$\log_{10}$ removal of *E. coli* while the 18-cm column achieved roughly double this $\log_{10}$ removal value, or 4·1-$\log_{10}$ removal. No difference in $\log_{10}$ removal of *E. coli* was observed between the 18-cm and 30-cm columns because *E. coli* were removed to the detection limit in both experiments. The clean bed removals of PRD1, Poliovirus 3 and Adenovirus 2 were investigated with a single column length. Poliovirus 3 removal in a 9-cm column was similar to the removal of MS2. In contrast, clean bed removals of bacteriophage PRD1 and Adenovirus 2 were much lower, with observed removals of 1·2-$\log_{10}$ in an 18-cm column and 0·36-$\log_{10}$ in a 9-cm column, respectively.

In Fig. 2, the observed clean bed removal from column experiments was compared to the predicted clean bed removal using the filtration model presented by Tufenkji and Elimelech (2004). In the model, the attachment coefficient (α) was assumed to be one. The comparison was conducted with observed clean bed removal data from 18-cm columns for *E. coli,* MS2 and PRD1. For *E. coli* and MS2, the predicted clean bed removals were significantly lower than the observed removals (0·37-$\log_{10}$ *vs* 4·1-$\log_{10}$ for *E. coli*, and 1·5-$\log_{10}$ *vs* 4·4-$\log_{10}$ for MS2).

Table 2 Clean bed removal of *Escherichia coli,* MS2, PRD1, Poliovirus 3 and Adenovirus 2 by QAS-coated sand columns at an ionic strength of 1 mmol l^{-1} NaCl and filtration velocity of 18 m h^{-1}. Dashes in the standard deviation column indicate the microorganisms were removed to the detection limit in each experiment. The standard deviation was calculated based on duplicate influent and effluent samples taken from a single column experiment at 2 min. Column tests conducted with different batches of sand resulted in similar $\log_{10}$ removal levels

	Column height (cm)	Challenge concentration (CFU ml^{-1} or PFU ml^{-1})	Effluent concentration (CFU ml^{-1} or PFU ml^{-1})	$\log_{10}$ removal (CFU ml^{-1} or PFU ml^{-1})	Standard Deviation of $\log_{10}$ removal
E. coli	4·5	$5{\cdot}2 \times 10^4$	$2{\cdot}2 \times 10^3$	1·4	0·045
	9	$6{\cdot}9 \times 10^4$	$1{\cdot}5 \times 10^3$	1·7	0·087
	18	$1{\cdot}1 \times 10^5$	<9·0	>4·1	–
	30	$1{\cdot}1 \times 10^5$	<9·0	>4·1	–
MS2	9	$3{\cdot}8 \times 10^7$	$5{\cdot}9 \times 10^5$	1·8	0·058
	18	$2{\cdot}4 \times 10^5$	<9·0	>4·4	–
PRD1	18	$3{\cdot}0 \times 10^5$	$2{\cdot}0 \times 10^4$	1·2	0·011
Poliovirus 3	9	$1{\cdot}3 \times 10^5$	$1{\cdot}5 \times 10^3$	1·9	0·10
Adenovirus 2	9	$1{\cdot}8 \times 10^6$	$7{\cdot}8 \times 10^5$	0·36	0·092

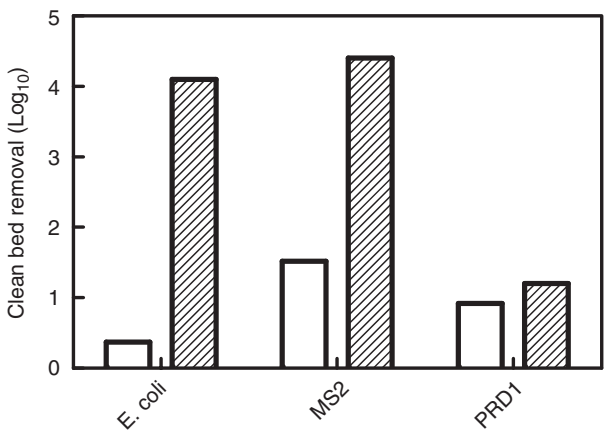

Figure 2 Comparison of the predicted removal from the filtration model (Tufenkji and Elimelech 2004) and the observed clean bed removal in QAS-coated sand columns. Columns for the *Escherichia coli*, MS2, and PRD1 experiments were 18 cm. □ – Predicted removal; ▨ – observed removal.

The predicted and observed removals of PRD1, however, were much closer to the observed removal (0·92-$\log_{10}$ *vs* 1·2-$\log_{10}$).

The removal of *E. coli* and MS2 by QAS-coated sand as a function of ionic strength (IS), flow rate (*E. coli* only) and in the presence of NOM (Fluka humic acid) is shown in Fig. 3. The removal of both *E. coli* and MS2 was higher in 1 and 10 mmol l^{-1} NaCl solutions compared to 100 mmol l^{-1} NaCl (Fig. 3a). A decrease in removal over time was observed for both *E. coli* and MS2, likely because the positively charged sites on the QAS-coated media became covered by micro-organisms. The removal of *E. coli* increased as the filtration velocity decreased from 18 to 3·6 m h^{-1} (Fig. 3b). This trend follows expected behaviour from filtration theory. Slower flow velocity is expected to increase removal due to longer transport times and lower hydrodynamic forces. In addition, *E. coli* removal decreased over time more quickly at 18 m h^{-1} than at 7·2 m h^{-1} (it is not possible to compare to 3·6 m h^{-1} because four of the data points were at the detection limit). The removals of *E. coli* and MS2 in the presence of 20 mg l^{-1} of humic acid dropped dramatically within 20 min in the QAS-coated columns, to levels comparable to uncoated silica sand columns challenged without humic acid (Fig. 3c). The humic acid likely competes with *E. coli* and MS2 for positively charged attachment sites on the media's surface. In addition, the humic acid may coat the micro-organisms, altering their surface characteristics and further decreasing attachment (Yuan *et al.* 2008; Pham *et al.* 2009). By preceding the QAS-coated sand column with a PAC prefilter, the dual media column was capable of removing *E. coli* in 20 mg l^{-1} of humic acid near the detection limit for 120 min. For MS2, the PAC prefilter and QAS-coated sand column fouled completely within 120 min when challenged with 20 mg l^{-1} of humic acid. However, this performance was greatly improved over a single QAS-coated sand column challenged with humic acid, which failed within 30 min.

Loss of culturability and membrane damage due to QAS-coated sand exposure

The qualitative results from the *E. coli* anti-bacterial assay are reported in Table 3. Following exposure to 10^4 CFU ml^{-1} of *E. coli* for 24 h and rinsing with a salt buffer, uncoated sand that was placed in a growth media showed growth of *E. coli*, whereas no growth was observed for the coated sand. The observed growth after exposure to the uncoated sand may be attributed to

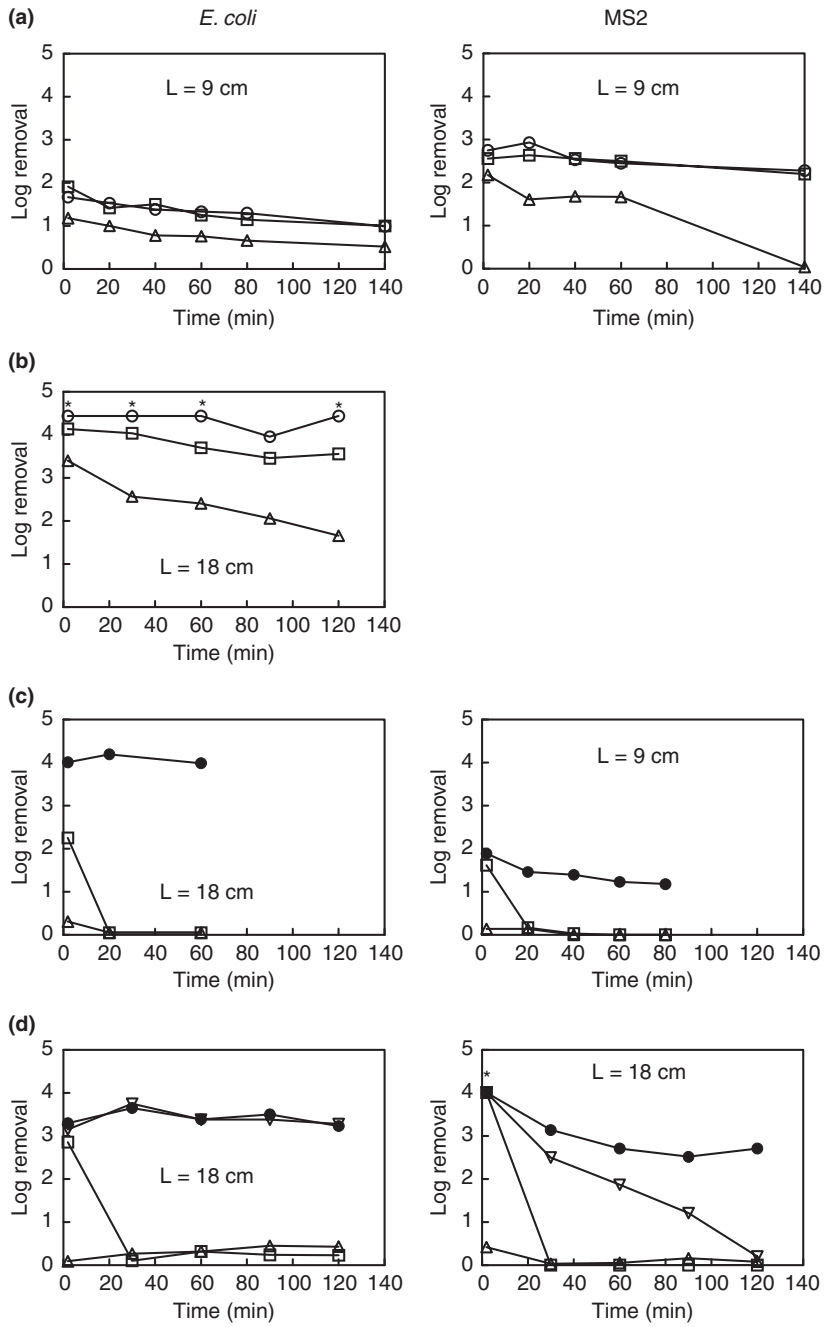

Figure 3 Removal of *Escherichia coli* and MS2 by QAS-coated sand as a function of: (a) ionic strength (b) flow velocity (c) presence of 20 mg l^{-1} humic acid (in 1 mmol l^{-1} NaCl) and (d) presence of powdered activated carbon (PAC) prefilter to remove Fluka humic acid. The flow velocity in each column test was 18 m h^{-1} unless otherwise indicated. Column lengths for each experiment are indicated on each graph. An asterisk indicates that the removal was equal to or greater than the detection limit of the assay. (a) ○ – 1 mmol l^{-1} NaCl; □ – 10 mmol l^{-1} NaCl; △ – 100 mmol l^{-1} NaCl. (b) ○ – 3.6 m h^{-1}; □ – 7.2 m h^{-1}; △ – 18 m h^{-1}. (c) ● – QAS; □ – QAS (+HA); △ – Silica. (d) ● – QAS; □ – QAS (+HA); △ – PAC (+HA); ▽ – PAC + QAS (+HA).

residual *E. coli* in the pore water, as *E. coli* were present in the supernatant after the final rinsing step. Conversely, no active *E. coli* were present in the pore water or rinsate after exposure to QAS-coated sand. These results suggest that the *E. coli* adhered to the QAS-coated sand media were inactivated.

Table 3 *Escherichia coli* in pore water and re-growth on sand grains. Sands were exposed to *E. coli* by tumbling for 24 h, followed by rinsing. Rinsed sand was re-introduced to growth media to detect *E. coli* re-growth

Media	*E. coli* in exposed PBS (CFU ml^{-1})	*E. coli* in supernatant after rinsing (CFU ml^{-1})	*E. coli* re-growth in media with rinsed, pre-exposed sand		
			A	B	C
Uncoated sand (control)	590	120	+	+	+
QAS-coated sand	0	0	–	–	–

Further qualitative evidence of bacterial membrane damage is presented in Fig. 4. A representative field of view is shown in which the sand grains are visible under bright field microscopy and stained bacteria are visible using fluorescence microscopy. No bacteria were visible on the uncoated sand, suggesting that minimal adherence of *E. coli* occurred, consistent with the column studies. On the coated sand, however, bacteria stained with both PI and SYTO-9 were visible, indicating that adhesion occurred, and that some *E. coli* became membrane permeable. The presence of these dead or damaged *E. coli* cells on the QAS-sand surface provides evidence that the QAS coated surface has anti-microbial properties. We confirmed that the vast majority of bacteria in the *E. coli* stock solution before exposure to QAS-coated and uncoated sands were intact and not permeable to PI.

Discussion

The results from this study demonstrate the effectiveness of fresh QAS-coated silica sand as a filtration medium for treating pure water solutions containing viruses and bacteria. However, the results also highlight major shortcomings of the media that need to be addressed before it can be recommended for use in a HWTS device.

The surface charge density measurements seem to return reasonable values (6×10^{13}–1×10^{14} N^+ cm^{-2}; Fig. 1). Assuming the Si-O-Si bond is ~0·161 nm (Baur 1977) each QAS molecule would occupy approximately 0·026 nm^2 if cross-linking was complete, and the theoretical charge density limit for a homogeneous monolayer would be around 4×10^{15} N^+ cm^{-2}. This calculation assumes that the quaternary ammonium groups do not limit the coating density due to steric hindrance or electrostatic repulsion. The experimentally determined coating density of γ–aminopropyltrimethoxysilane, a primary amine QAS with a one-carbon alkyl chain, was found to be on the order of ~10^{14} N^+ cm^{-2} when deposited in the vapor phase (Ek *et al.* 2003). Vapor phase deposition is known to yield well-formed, dense monolayers of methoxy silanes. Thus, our estimate of the coating density on sand suggests that it is at the upper limit for a monolayer. However, the coating on sand may not exist as a monolayer. We attempted to characterize QAS coatings using the same coating chemistry on planar surfaces with AFM, contact angle measurements and ellipsometry. These measurements suggested that the coatings were very heterogeneous and included clumps (Alper 2010). Thus, it is possible that the surface coatings on sand were characterized by areas of high charge density where the QAS existed in clumps with cross-linking chains extending from the surface, and other areas with low or no coverage.

We also used a conservative measurement of the sand's surface area. The BET analysis conducted to determine the silica sand's surface area returned the result that the surface area was <0·1 m^2 g^{-1}. A surface area of 0·1 m^2 g^{-1} was used in the estimate, but the sand's actual surface area may be lower. A perfect sphere of sand with diameter 0·25 mm has a theoretical surface area of 0·014 m^2 g^{-1} (Blott *et al.* 2004). Therefore, the average surface charge density of the QAS-coated sand may actually be higher than our estimate of ~10^{14} N^+ cm^{-2}. A higher coating density, or patches with higher density, would contribute to greater bactericidal activity according to reports in the literature on threshold charge densities, which range from 10^{12} to 10^{15} N^+ cm^{-2} (Kügler *et al.* 2005; Murata *et al.* 2007). It should be noted that these threshold values were determined for quaternary ammonium architectures employing polymer brushes, which may have other characteristics that contribute to their bactericidal properties as well.

The results from the *E. coli* re-growth test in Table 3 demonstrate that the coated sand has at least some bactericidal activity. The preexposed QAS-coated sand did not allow bacteria re-growth in a growth media, implying the bacteria adhered to the surface were inactivated. More qualitative evidence of anti-bacterial activity was observed with the PI stain. Some bacteria adhered to the QAS-coated sand particle were shown to be permeable to PI, suggesting the cells were dead and/or damaged. A similar experiment could not be conducted, however, on viruses. Not much is known about the effectiveness of QAS compounds on nonenveloped viruses, although some efficacy

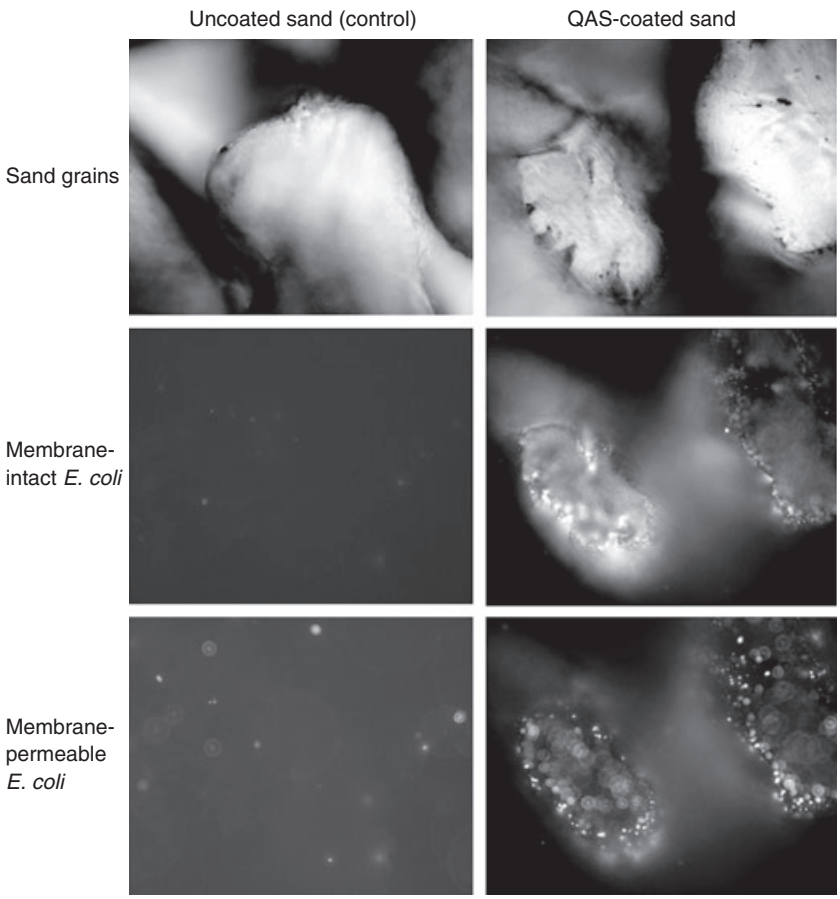

Figure 4 Fluorescence microscopy images of QAS-coated sand exposed to *Escherichia coli. E. coli* were stained with SYTO-9 to image membrane-impermeable (live) cells and propidium iodide to image membrane-permeable (dead) cells.

was observed against MS2 in solution. While a QAS concentration as low as 10^{-5} mol l^{-1} was observed to cause a 0·91-$\log_{10}$ reduction of MS2, it remains unclear whether these results were from inactivation or aggregation (data not shown). Natural iron oxide surfaces on sand (which are also positively charged) have been reported to cause inactivation of MS2 and PRD1, but the time scale (~30 days) was much longer than that used in our experiments (Ryan *et al.* 2002).

The QAS-coated sand media demonstrated high removal of *E. coli,* MS2 and Poliovirus 3. Importantly, the removal was much higher than the predicted values from the filtration model for *E. coli* and MS2 (Tufenkji and Elimelech 2004). This increased removal was likely due to attractive double layer interactions between the sand media and microbe. At low ionic strength, the attractive double layer extends over larger distances and is responsible for the increased transport of colloids onto the collector surface. In fact, small particles are capable of changing their trajectory in a filter toward collectors when encountering attractive electrostatic forces (Elimelech and Song 1992). At higher ionic strengths, removal was lower due to the effect of charge shielding at the highest IS. Based on the results of a theoretical modelling, Elimelech and Song (1992) reported that deposition rates of particles onto an oppositely charged collector exhibit a strong dependence on IS and suggested a critical IS value of 1 mmol l^{-1}. Below this critical value, the electric double layer's range expands with decreasing IS. Thus, attractive electrostatic interactions would be expected to enhance transport of the net negatively charged micro-organisms to the net positively charged coating. Above this critical IS value, however, the electric double layer is compressed considerably and electrostatic attraction is not expected to enhance transport (Elimelech and Song 1992). In experimental studies with ionic strengths ranging from 20 to 200 mmol l^{-1}, Yuan *et al.* (2008) found no change in the deposition rate of MS2 onto a positively charged poly-ι-lysine (PLL) layer. In addition, Pham *et al.* observed no increase in the

deposition rate of MS2 onto a PLL layer in solutions with IS between 0·3 and 3 mmol l^{-1}. Together, these studies suggest removal should be similar throughout an IS from 0·3 to 200 mmol l^{-1} (2009). In contrast, we observed lower removal of *E. coli* and MS2 at 100 mmol l^{-1} than at 1 and 10 mmol l^{-1}. One important difference is that our experiments were conducted with a monovalent cation (Na^+), whereas the Yuan *et al.* and Pham *et al.* studies were conducted with divalent cations (Ca^{2+} and Mg^{2+}). Pham *et al.* (2009) suggest that divalent cations may undergo specific interactions with carboxylate groups on virus surfaces, so that the effect of the cations cannot be explained simply by electrostatic interactions. The Yuan *et al.* and Pham *et al.* studies were also conducted with single layer, planar, positively charged surfaces, whereas our experiments used columns packed with net positively charged sand. In the coated porous media the micro-organisms experience a complex and heterogeneous environment, in which ions may alter many types of surface interactions.

In this study, removals of PRD1 and Adenovirus 2 were much lower than removals of MS2 and Poliovirus 3. The relative removals of these viruses are roughly consistent with that reported for their removal by coagulation and filtration (simulating conventional drinking water treatment), but the reported removals also varied considerably for different conditions (Abbaszadegan *et al.* 2007, 2008; Mayer *et al.* 2008). The differences in removal are likely due to differences in the capsid properties of the viruses. A comparison of isoelectric points (IEP) does not provide much insight, as MS2, PRD1 and Adenovirus 2 all have IEPs significantly below the pH used in our experiments (reported values are 3·9, 4 and 2·6, respectively), (Dowd *et al.* 1998; Michen and Graule 2010), whereas poliovirus strains can exist in two interconvertible states with IEPs around 4·5 and 7·1 (Michen and Graule 2010). However, IEP does not provide information about net surface charge. Based on electrophoretic mobility measurements, the net surface charge of MS2 is less negative at neutral pH than PRD1, and thus cannot explain the fourfold greater removal of MS2 than PRD1 observed in our setup (Redman *et al.* 1997; Abudalo *et al.* 2005). Information on the electrophoretic mobility of adenovirus has not been published. Other capsid features are likely to contribute, for example the Adenovirus 2 capsid has been shown to include a receptor-binding site formed by five copies of a penton base. Lower than expected removal of viruses (PhiX174) in ceramic depth filters has been attributed to steric interactions between similar viral capsid features (knobs) and media surface (Michen *et al.* 2011). The PRD1 capsid does not have a similar penton base structure, which could account for different interactions at the coated sand surface (Rydman *et al.* 1999).

The major limitation to the effectiveness of QAS-coated media is the fouling of columns. As seen in the results in Fig. 3, *E. coli* and MS2 removal decreased throughout each experiment. In the experiments using MilliQ water, the reduction in removal was presumably due to biofouling from cells or virus themselves, or their debris as they disintegrated. The reduction in removal was much more pronounced when the challenge water included NOM. If the laboratory columns are scaled up to a size typical for a media cartridge in a POU filter (~9·5 cm diameter), our results suggest that the cartridge would foul after treating <68 l, which is clearly unacceptable performance. When a PAC prefilter was used, the fouling due to NOM was eliminated in the *E. coli* experiment, illustrating that the main role that NOM played was competing for attachment sites on the coated media. The PAC prefilter did not completely prevent fouling when the column was challenged with MS2; one possible explanation is that NOM coated the surface of the MS2 particles, reducing their attachment efficiency (Pham *et al.* 2009). As NOM is present in significant concentrations in most surface waters, it is clear that more research is needed to develop approaches for increasing the lifetime of the QAS-coated media, and/or regenerating it, if QAS coated sand is to be a feasible option for HWTS.

Overall, the findings from this study demonstrate the ability of filtration using QAS-coated media to remove bacteria and viruses from water. However, this study also identified major limitations and barriers to use of the coated media in a POU filter. These major limitations include: (i) rapid fouling due to the micro-organisms or NOM, which contributed to an unacceptable filter lifetime and (ii) low removal of bacteriophage PRD1 (a model virus) and Adenovirus 2. A two-stage device with a PAC column followed by QAS-coated sand had improved removal of bacteria and viruses from water containing NOM. This idea could be extended to other multi-stage designs that included other media, such as metal oxide surfaces, which may further improve removal of NOM as well as viruses. QAS-coated media may also be a useful polishing step to remove viruses from other HWTS filters that target bacterial removal. However, more research is needed on the long-term performance and possible leaching of QAS-coated media, additional options to reduce fouling and regenerate the media, and the inactivation mechanisms of bacteria and viruses by QAS-coatings.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1 Adenovirus type 2 and poliovirus type 3 methods.

Data S2 Microbial and bromophenol blue leach assay results.

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